Reprinted from the Journal of Cell Biology, 39, 80a, 1968.

Electron Microscope Study of the Incorporation of Epithelial Cells by Developing Dental Cementum:

K.S. Lester, Department of Anatomy,
New York Medical College.

Generally, during tooth root formation, an epithelial sheath

(i) precedes dentine and cementum formation,

(ii) determines the outer extent of the dentine,

(iii) disintegrates to allow cementum deposition upon the dentine surface by differentiated CT cells (cementoblasts).

In contrast, several light microscope studies have suggested that in the latter half of root formation in rat molars, this epithelial sheath is incorporated between the developing dentine and cementum. This subject has been studied by means of thin sections for electron microscopy. It was found that the first-formed "cellular" cementum is constituted by both epithelial cells and cementoblasts embedded singly. Subsequently, epithelial cells and cementoblasts are incorporated en masse at the site of the advancing dentine-cementum junction. The fate of the two incorporated cell-types differs. The cementoblasts surround themselves with a collagenous matrix

which subsequently mineralizes, and give by their ultrastructure every indication of viability at the time of fixation.

Incorporated epithelial cells, however, lose their relation with one another, owing largely to invasion and collagen formation by cementoblasts, and show signs of nuclear and cytoplasmic degeneration (including intracellular mineralization), indicating a gradual process of cell death. It is difficult to reconcile this particular program of cell death with those described elsewhere as accompaniments of morphogenesis.

Reprint from

JOURNAL OF ULTRASTRUCTURE RESEARCH

VOLUME 27, 63–87, 1969

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The Incorporation of Epithelial Cells by Cementum

ACADEMIC PRESS, New York and London

J. ULTRASTRUCTURE RESEARCH 27, 63–87 (1969)

The Incorporation of Epithelial Cells by Cementum[1]

K. S. LESTER

Anatomy Department, New York Medical College, New York, New York 10029

Received November 8, 1968

The formation of cellular cementum was studied by electron microscopy at the advancing edge of developing roots of molars of 25-day-old rats. It was found that the basement lamina about Hertwig's epithelial root sheath first becomes discontinuous on the dental sac side, collagen and cementoblast processes appearing within the formerly intact epithelial compartment. As an increasing bulk of dentinal and cemental collagen is deposited, some sheath cells become reoriented perpendicular to the surface of the dentinal collagen and then migrate from it toward the future periodontal space. Those sheath cells remaining in place after the commencement of cementum mineralization are subsequently embedded there by the mineralizing collagen located external to them. Ultrastructural proof is provided that two cell types, epithelial root sheath cells and cementoblasts, are incorporated by the forming cementum. Rate of formation is discussed as a basis for this nonspecific incorporation of cells by a mineralized connective tissue.

Cementum has been studied by a variety of electron microscopic techniques (*1–3*, *11*, *17*, *24*, *29*, *31*, *35*, *42*, *57–59*, *65*, *69*, *71*). Attention has, however, been focused mainly on the organization of the extracellular component and its comparison with that of dentine and bone. As a result, there remain many unanswered questions concerning the cellular component; among these are problems pertaining to the mechanisms of cementum formation, the fate of embedded cells (cementocytes), and the extent of the continued metabolism and activity of these cells if, indeed, such exists (*5*, *7*, *18–20*, *25–27*, *47*, *67*, *68*).

This paper reports the initial results of a continuing study, by conventional sectioning techniques for transmission electron microscopy, of the process of cell incorporation by developing cementum of the molar teeth of the rat. The procedure has been to survey, at relatively low magnification, the morphological features of a progression of stages so that the sequence of developmental events might be reconstructed.

[1] Supported in part by grants from the Health Research Council of the City of New York (U-1508) and from the United States Public Health Service (DE-02274-04).

MATERIALS AND METHODS

Young Sprague-Dowley rats of two litters were sacrificed at 20, 25, 30, 35, 40, and 45 days after birth. The animals were anesthetized with ether, the lower jaw was removed and placed in phosphate-buffered glutaraldehyde (*54*), and the developing mandibular molars were dissected free from alveolar bone. The individual teeth were postfixed in osmium and dehydrated and Epon-embedded after Luft (*40*) but with prolonged infiltration times. The specimens were not purposefully demineralized at any stage so that the blocks were, of necessity, trimmed to provide relatively small faces for both thick and thin sectioning procedures. The advancing edge of the developing root was included in the block face in every case. Thick sections were examined unstained by phase-contrast microscopy. Thin sections were cut with a diamond knife mounted in a Cambridge-Huxley ultramicrotome. The thin sections were picked up from a collecting trough of distilled water and examined either unstained or after staining with uranyl acetate (*64*) and lead citrate (*53*), these stains being water-based. The thin sections were examined in a Siemens Elmiskop I operated at 80 kV.

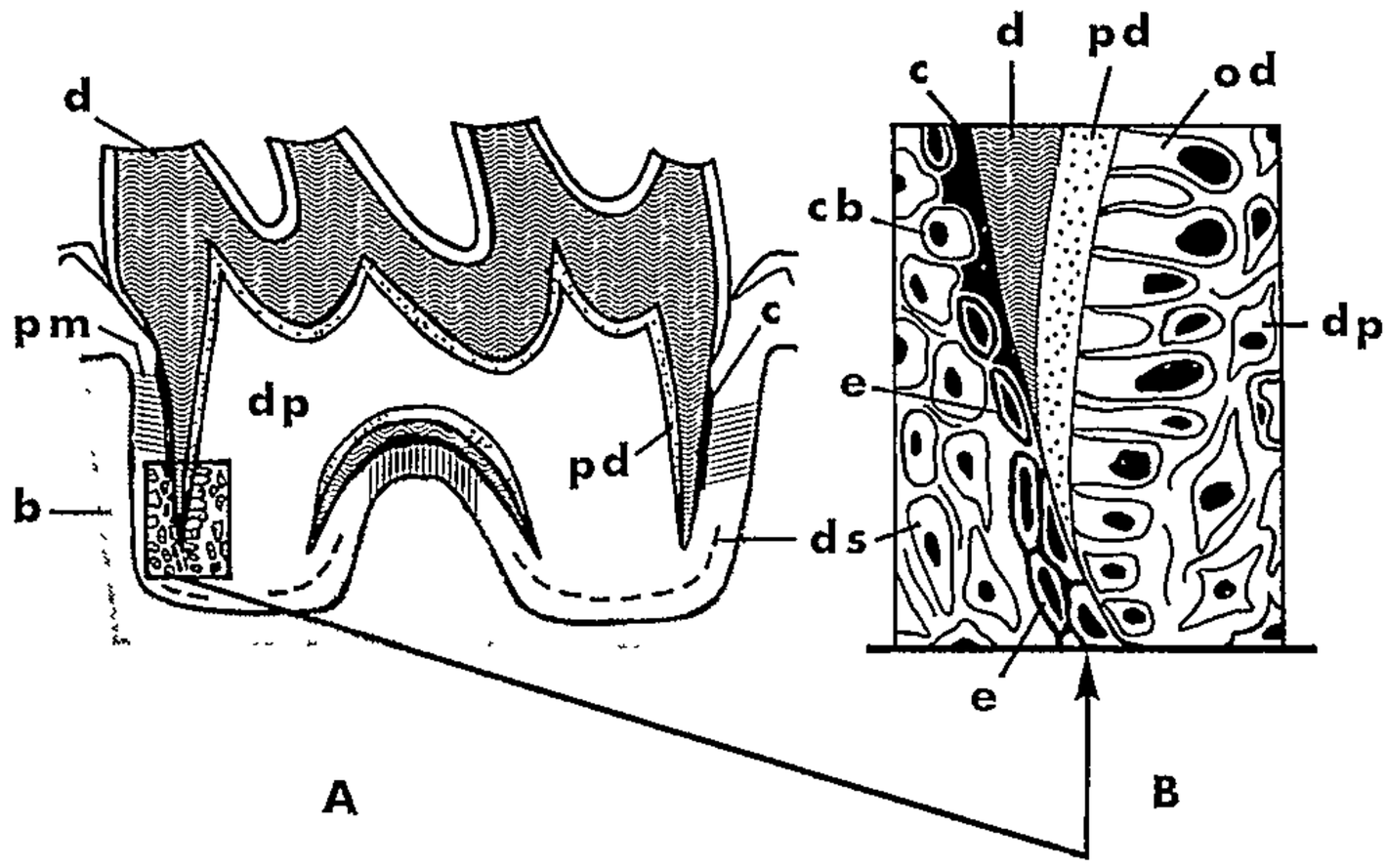
Only some specimens from the 20-day-old animals showed evidence of the formation of cellular cementum (i.e., the inclusion of cells within the forming cementum) when thick sectioned and examined by phase contrast microscopy. In such sections, isolated cells were observed in the process of becoming embedded over the surface of already formed acellular cementum at some distance from the advancing root edge and at a level where a considerable thickness of dentine had formed. In contrast to this, thick sections of the developing root of the 1st mandibular molar of 25-day-old rats characteristically exhibit cells in the process of embeddment at the level of the advancing root edge (see Fig. 1). It is not possible to identify, by phase contrast microscopy, the cell-type involved in either the 20- or 25-day-old material. However, the cells at the advancing root edge more readily meet the requirements of tissue preparation for electron microscopy and, for this reason, the 25-day material was studied more extensively and will be described in detail.

OBSERVATIONS

The embedding of cells at the mineralizing front of the cementum, and the role of Hertwig's epithelial root sheath in this, is best described by considering sections taken transverse to the longitudinal axis of the developing root. This is because transverse sections provide greater lengths of the sheath and surrounding tissue for examination and, as a result, fine transitions in structure illustrating the developmental process are better appreciated. Reconstruction of events is facilitated by cutting these transverse sections first at a level in advance of the developing root (i.e. prior to hard tissue formation) and then from progressively more coronal levels. Fig. 1*b* is a diagrammatic representation of the approximate plane of the transverse section shown in Figs. 2–6.

Intact epithelial sheath

In a section of the soft tissues preceding the advancing root edge (Fig. 2), the epithelial sheath cells are readily identified electron microscopically (Figs. 3 and 4)



Key to abbreviations

b bone
bl basement lamina
bv blood vessel
c cementum
cb cementoblasts (derived from dental sac)
cf collagen fibrils
d dentine
dg dense granule
dj desmosome-like junction (macula adhaerens)
dp cells of the dental papilla (developing pulp)
ds cells of the dental sac (developing periodontal membrane)
e epithelial cells of Hertwig's root sheath
G Golgi apparatus
ger granular endoplasmic reticulum
ij intermediate junction (zonula adhaerens)
is intercellular space
m mitochondrion
mf mineralization foci
mv microvillous process
od odontoblasts (derived from dental papilla)
p polyribosomes
pd predentine
pm periodontal membrane
t tonofilament bundles
tj tight junction (zonula occludens)
x crystals

FIG. 1. (A) Diagram of a longitudinal section of a developing 1st mandibular molar (and related structures) of a 25-day-old rat to show the region of the developing root studied by electron microscopy (boxed area). (B) Enlargement of the area enclosed in A to show the relationships of the various structures at the advancing edge of the developing root. The approximate level of the transverse section shown in Figs. 2–6 is indicated by the heavy line at the lower border of the diagram.

by their typical epithelial sheet arrangement with its minimal amount of intercellular space and with the cells forming junctional complexes. These junctions are of the tight (zonula occludens), intermediate (zonula adhaerens), and desmosomal (macula adhaerens) types and are relatively undeveloped at this point. This applies especially to the predominant desmosomal type and its associated tonofilaments, these junctional complexes becoming more pronounced and numerous in later stages (see below). Granular endoplasmic reticulum is sparse and appears most often in the form of isolated, flattened cisternae (Fig. 4). Polyribosomes are a major feature and abundant throughout the cytoplasm. Mitochondria are numerous, and the Golgi complex is clearly demarcated although membrane-bound dense granules are, as yet, only oc-

casionally observed. The sheath is, on average, two cells thick but may vary locally from one to four cell layers in thickness.

An uninterrupted basement lamina outlines the extent of the epithelium on both sides, the junction with the differentiating cells of the dental papilla (odontoblasts) being the more smooth and the better defined of the two (cf. Figs. 5 and 6). The odontoblasts are relatively further advanced than the dental sac cells in their differentiation —as may be judged by their comparison with the mature cell-types (e.g., *44*, *52*, *65*). The odontoblasts have numerous small processes directed toward the basement lamina, although only a small amount of collagen has been produced in relation to them (Fig. 6). Both differentiating cell-types contain an abundance of granular endoplasmic reticulum, not yet oriented as in the mature forms. The separation of cells on the dental sac side of the sheath (Figs. 3 and 5) is typical of this kind of preparation and may be due, in part at least, to the manipulation required to free the tooth germ from its bony crypt.

Discontinuous epithelial sheath

Sections cut from the same block as those illustrated in Figs. 2–6, but at slightly more coronal levels, are shown in Figs. 7–13. The process of root development is correspondingly advanced and the odontoblasts have progressed in their differentiation to become tall and columnar (Figs. 7, 10, and 11). A small bulk of organized, but as yet unmineralized collagen has been formed in close relation to their terminal processes (Fig. 11). The cementoblasts, too, are further differentiated and a small amount of collagen has been deposited about them (Fig. 8).

The main point, however, for the purposes of this report is that there are the first signs of a loss of integrity of the epithelial sheath compartment. These first indications usually are a loss of clarity of the basement lamina, it becoming diffuse and hazy, and the appearance of collagen fibers within the formerly intact intercellular space (Figs. 8 and 9). The extent of the breakdown of the basement lamina is much greater on the cemental than on the dentinal side of the sheath (Figs. 9 and 12). The subsequent separation which occurs between the sheath cells themselves occurs initially at the expense of those on the dental sac side (the future cemental aspect). This is evidenced, and possibly caused, by the penetration of processes of the

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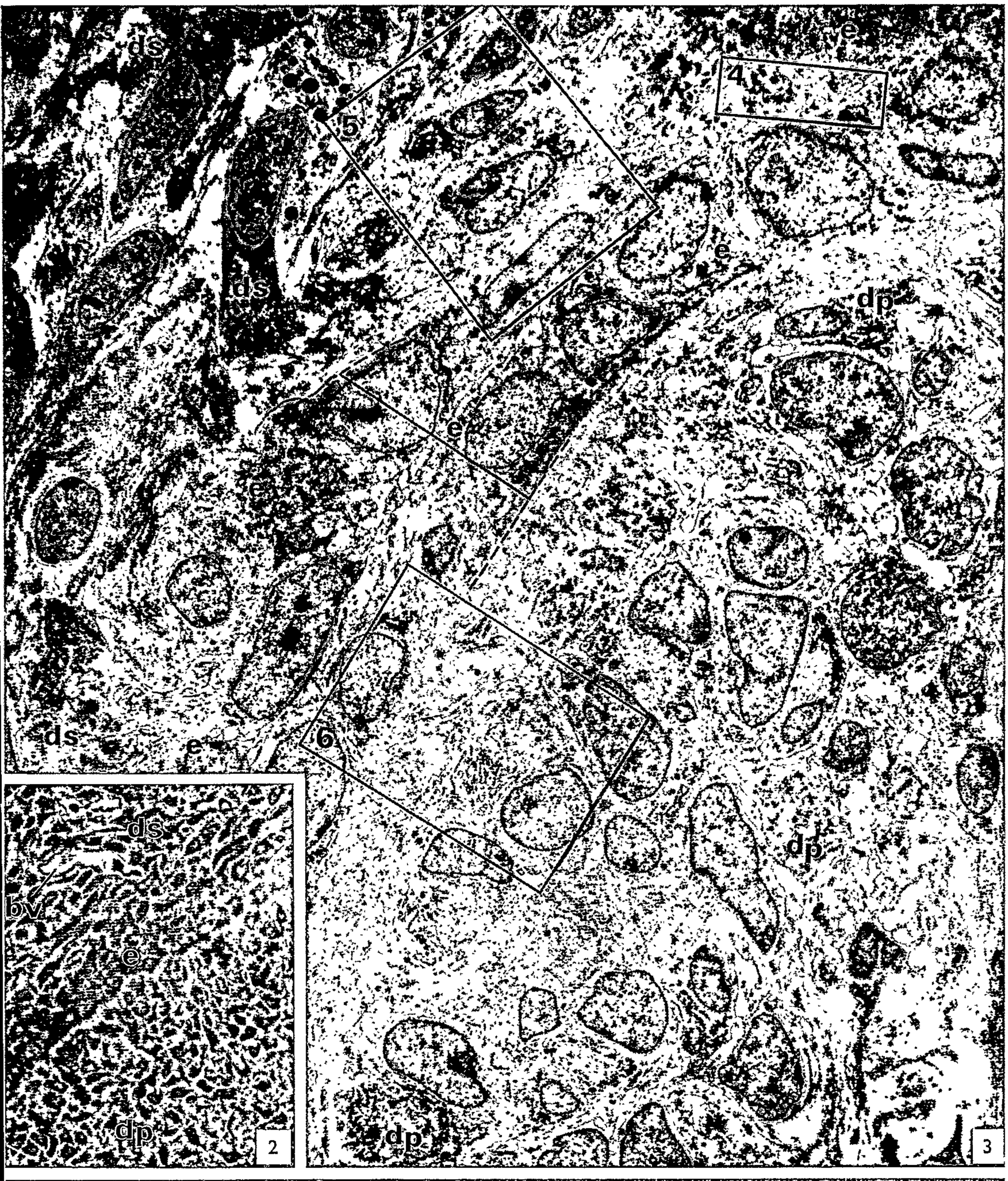
FIG. 2. Transverse section of developing root of 1st mandibular molar of 25-day-old rat. Phase contrast photomicrograph of thick section of an area in advance of hard tissue formation (see Fig. 1) and similar to that illustrated in Figs. 3–6. ×400.

FIG. 3. Electron micrograph survey of an area where the epithelial sheath is intact. Differentiating cells of the dental sac and of the dental papilla lie on either side of the intact epithelial sheath. ×1800.

FIG. 4. Higher magnification of one of the enclosed areas in Fig. 3 to show cytoplasmic organelles of the epithelial sheath cells: polyribosomes; granular endoplasmic reticulum; mitochondria; Golgi apparatus; and two types of cell junctions, the last in early stages of their formation. ×1500.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differentiating cementoblasts between the sheath cells of that side (Fig. 12). Large numbers of collagen fibrils accompany the appearance of these processes in the intercellular spaces of the epithelial sheet (Fig. 12).

Reorientation and migration of epithelial cells

There may also be seen in Figs. 11 and 13 the first indication of a change in orientation of the epithelial sheath cells. This is because contributing to the invasion of the sheath compartment by the cementoblasts, and coincident with it, is the departure or migration of many of the epithelial cells on the outer or cemental side of the sheath (see also Figs. 14 and 15). These epithelial cells move toward the dental sac and away from their previously neighboring epithelial cells, many of the latter remaining apposed, at this point in developmental time, to the outer aspect of the bulk of the dentinal collagen. Prior to migrating, the outer cells of the sheath become reoriented so that the longitudinal axis of both cytoplasm and nucleus comes to lie perpendicular to the dentinal surface (Figs. 14 and 15). Cemental collagen comes to be directly apposed to dentinal collagen where those epithelial cells lining the dentinal surface are also lost to the future periodontal space (Fig. 14). Despite the disorganization of the sheath, the epithelial cells may be readily identified by the increasing development of their tonofilament bundles and the desmosomal junctions with which these are associated (Fig. 15).

Failure of epithelial cells to migrate

Those epithelial sheath cells remaining at the surface of the dentinal collagen (Fig. 16), and virtually surrounded by connective tissue elements (Fig. 17), display a characteristic morphology. They possess, by this time, an abundance of cytoplasmic tonofilaments arranged in bundles (tonofibrils) (Fig. 18). The bundles are oriented circumferentially about, and are in close proximity to, the nucleus except in the Golgi region. There is also an abundance of membrane-bound dense granules, primarily in the vicinity of the prominent Golgi apparatus. Polyribosomes remain relatively

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FIG. 5. Higher magnification of one of the enclosed areas in Fig. 3 showing differentiating cells of the dental sac (future cementoblasts) and their junction with the epithelial sheath. The basement lamina is intact but less clearly demarcated than on the dental papilla side (see Fig. 6). ×4500.
FIG. 6. Higher magnification of one of the enclosed areas in Fig. 3 showing differentiating cells of the dental papilla (future odontoblasts) and their junction with the epithelial sheath. ×5250.
FIG. 7. Phase contrast photomicrograph of a thick transverse section of developing root of 1st mandibular molar of 25-day-old rat taken at a more coronal level than that shown in Fig. 2 and similar to that shown in Figs. 8 and 9. ×600.
FIGS. 8 and 9. Survey electron micrograph (Fig. 8) and enlargment of enclosed area (Fig. 9) to show the loss of definition of the basement lamina on the dental sac side and the appearance of collagen fibrils within the formerly intact intercellular space. The basement lamina is retained on the dental papilla side although there are localized discontinuities through which project microvillous processes of the epithelial cells. Fig. 8, ×3000; Fig. 9, ×7000.


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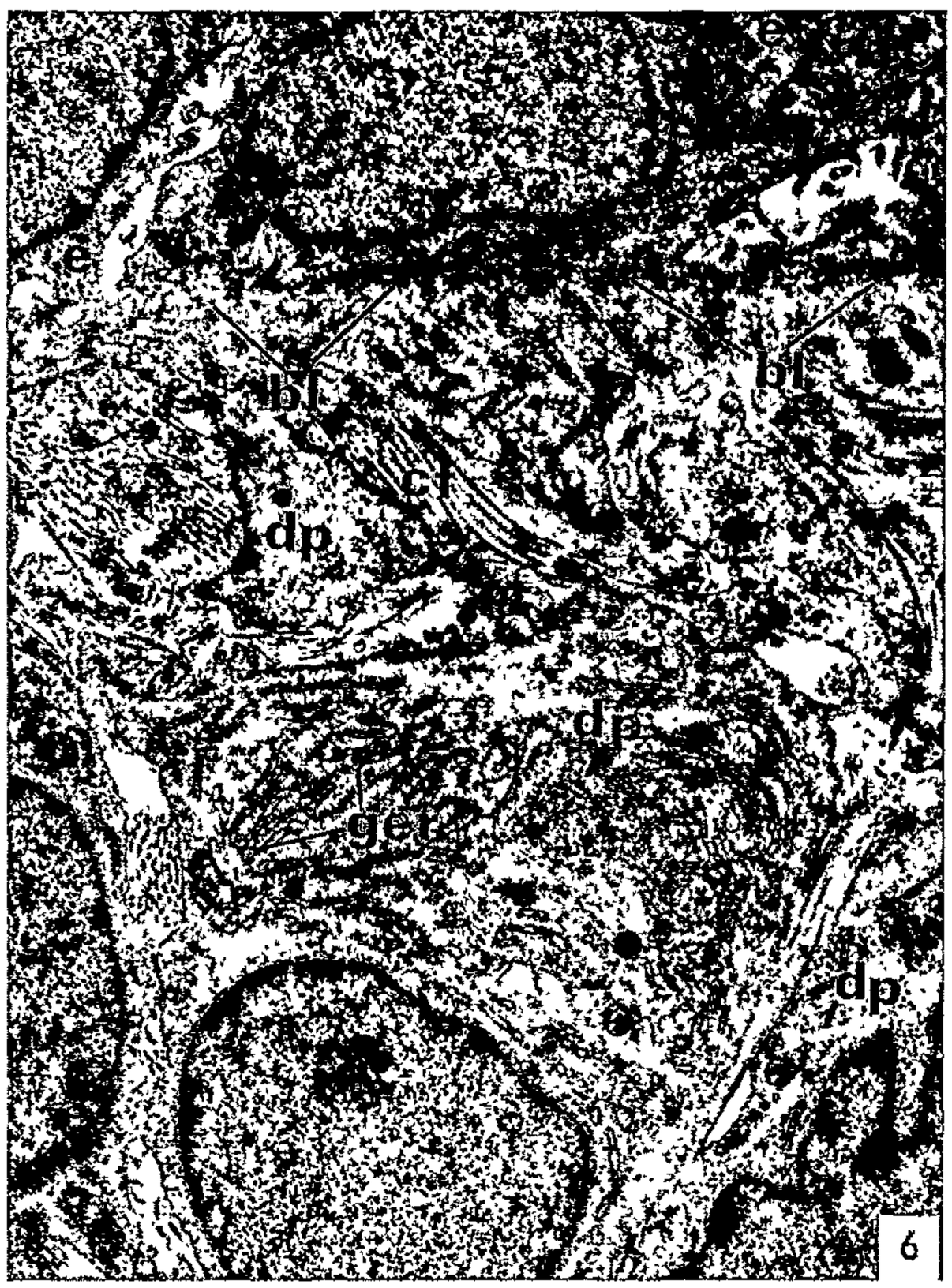
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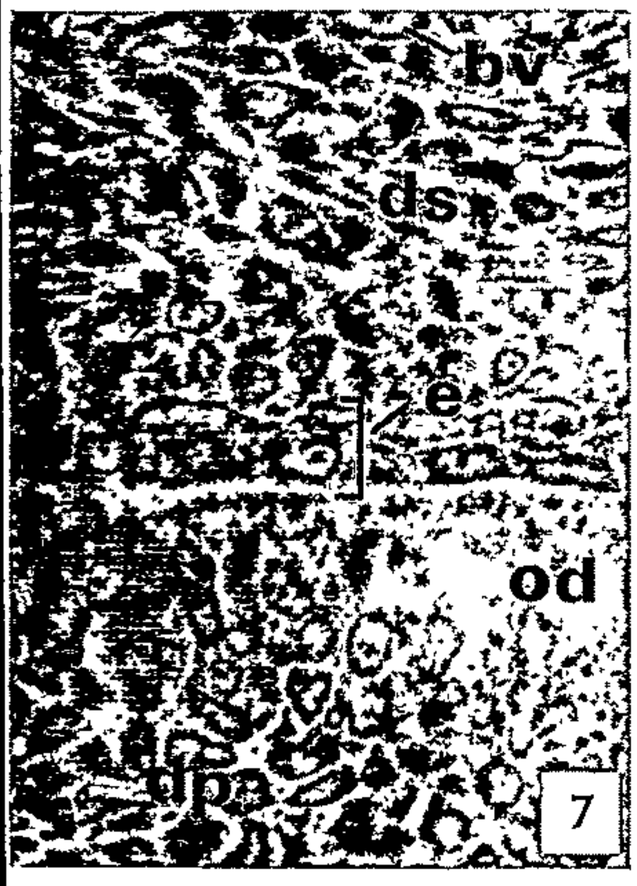
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conspicuous, and the granular endoplasmic reticulum appears in the form of dilated sacs. The cell itself has changed overall to a less-rounded, more elongate, stellate form, and displays many microvillous processes. Despite the irregularity of the epithelial cell surface, traces of basement lamina may remain evident on the dentinal aspect although any such remnants have long since disappeared from the cemental side (Fig. 18).

Mineralization of dentine

In Fig. 18, small foci of mineralization are evident in those collagen fibrils on the odontoblastic side of the sheath. Once this process of mineralization is under way (see Fig. 19) and some bulk of dentinal collagen has mineralized (say 4–5 μ), single epithelial cells, or small groups of them, are found scattered along the lateral aspect of the mineralized dentine (Fig. 20). These cells are separated from the dentine by bundles of collagen fibrils and from each other by occasional cell bodies of cementoblasts. The cementoblasts are readily distinguishable from epithelial sheath cells at this stage. First, cementoblasts do not possess tonofilament bundles or display desmosomal attachments. Second, cementoblasts have a much more extensive granular endoplasmic reticulum in the form of interconnecting, often dilated vesicles and sacs [see also Selvig (*57*) and Stern (*65*)]. Third, the nuclear-cytoplasmic ratio for the cementoblasts is consistently lower than for the epithelial cells. Finally, the cementoblasts possess membrane-bound granules with a striated fine structure, which the epithelial cells do not.

It is impossible to state, at this stage of development, exactly when and where the mineralization of cementum begins and whether or not dentinal collagen consistently contributes to the cementum. It is clear, however, that the dentinal collagen does not become mineralized from the outset up to the level of the basement lamina of that side (Fig. 21).

Some of the epithelial cells remaining at the mineralized surface, at this particular stage of development and level of section, tend to be aligned with their long axis

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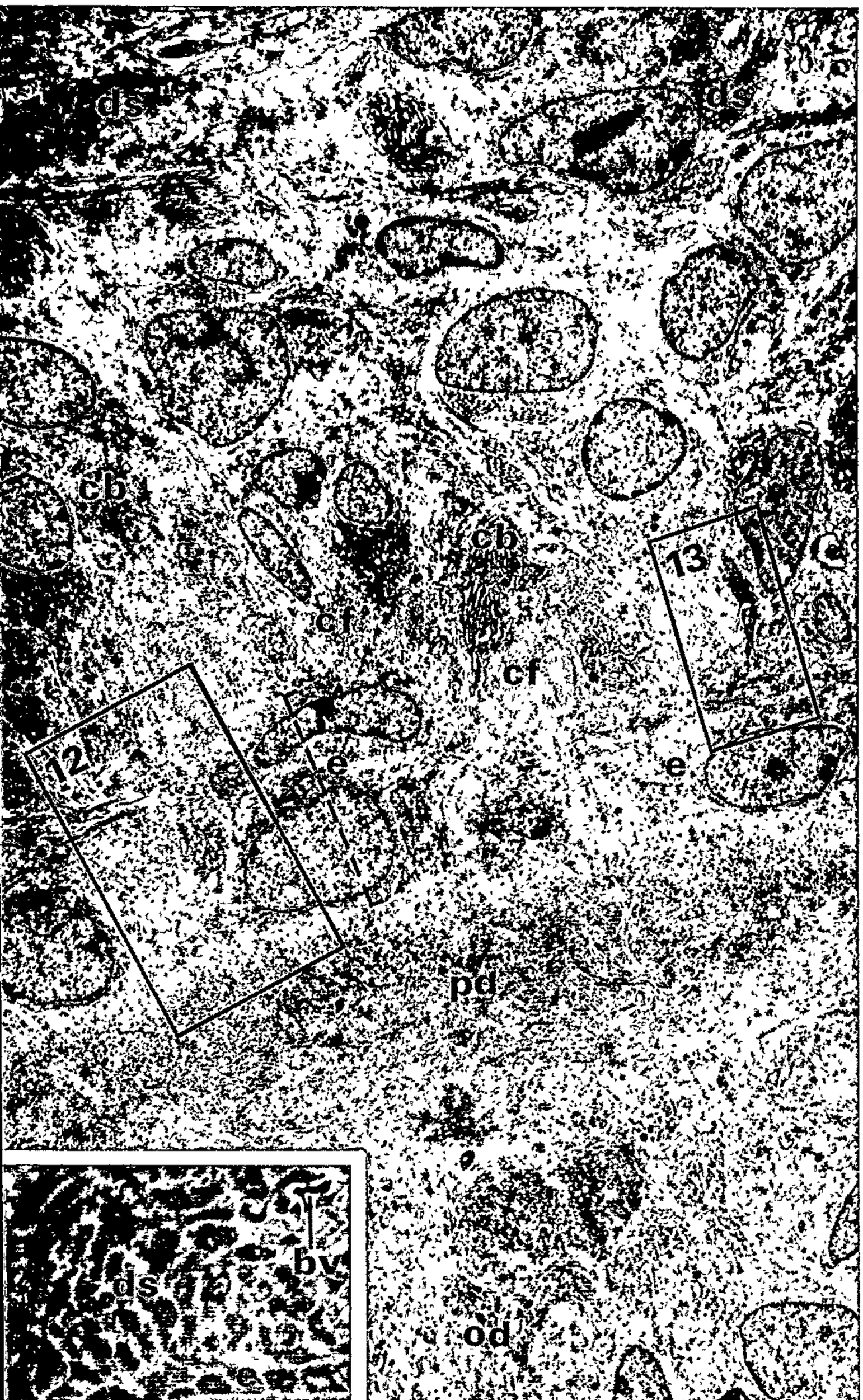
FIG. 10. Transverse section of developing root of 1st mandibular molar of 25-day-old rat. Phase contrast photomicrograph of thick section of an area in which some small bulk of dentinal collagen has formed and similar to that illustrated in Figs. 11–13. × 600.

FIG. 11. Electron micrograph survey of an area in which the epithelial sheath is discontinuous and some bulk of dentinal and cemental collagen has formed. × 1800.

FIG. 12. Higher magnification of one of the areas enclosed in Fig. 11. A basement lamina no longer exists on the cemental side. Collagen (see arrow) and cementoblast cell processes (identifiable by the extensive, dilated cisternae of their granular endoplasmic reticulum) are present within the formerly intact intercellular space. The basement lamina on the dentinal side shows localized discontinuities through which project microvillous processes of the epithelial cells. × 4000.

FIG. 13. Higher magnification of one of the enclosed areas in Fig. 11. Part of a displaced and reoriented epithelial cell may be seen attached by desmosome-like junctions to another sheath cell which has remained in place. × 9000.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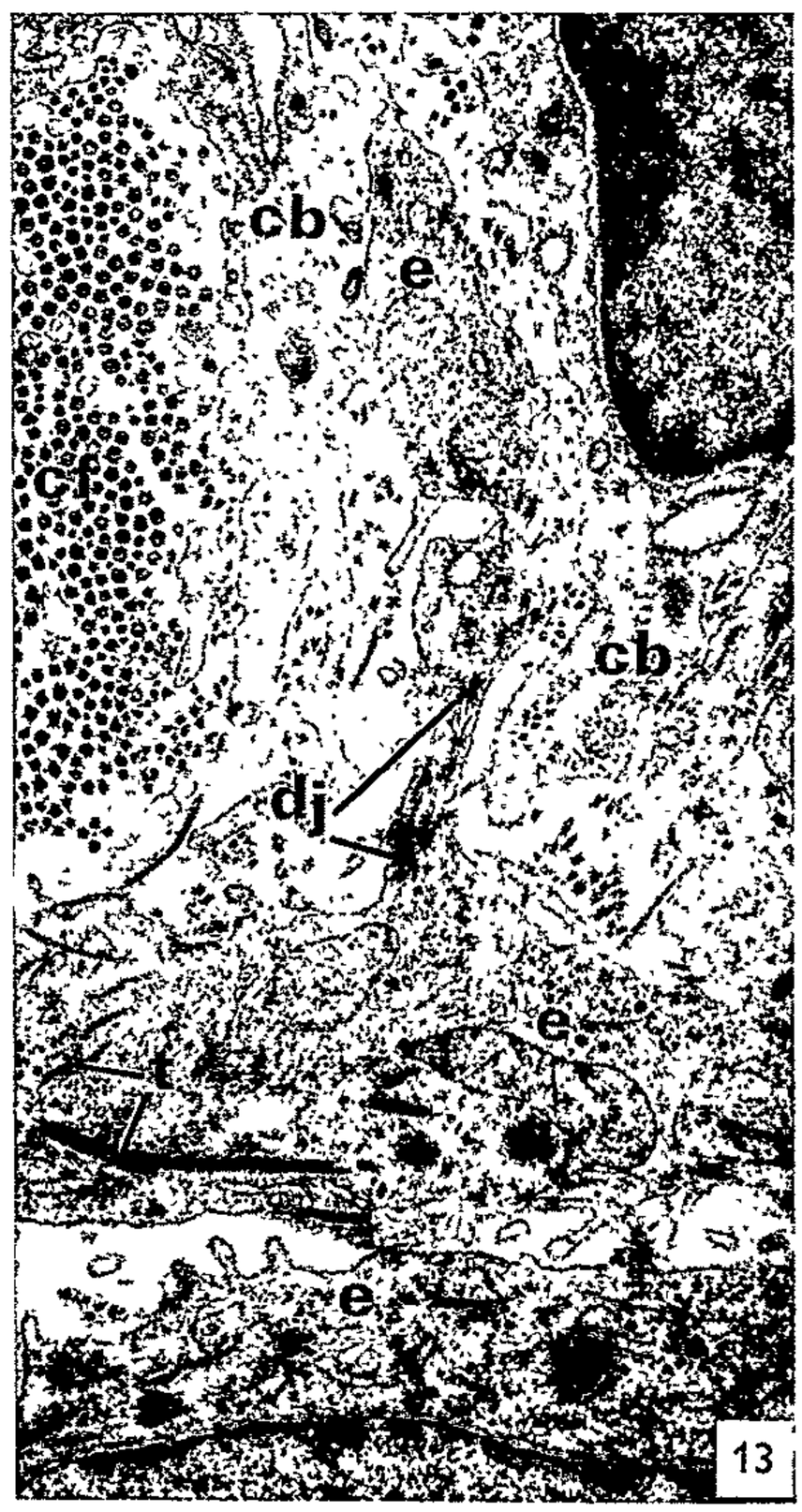
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roughly perpendicular to that surface (Fig. 20). Other epithelial cells become rounded in outline (Figs. 20 and 21) and it appears to be these which fail to migrate and are subsequently embedded by the forming cementum.

Mineralization of cementum and incorporation of epithelial cells

Sections representative of a successive stage in this process, and where a greater bulk of predentine and mineralized dentine have formed, show the mineralizing cementum to be identifiable in its own right by the peaked form of its mineralizing front (Figs. 22 and 23) [for discussion of this, see Albright and Flanagan (*1*), Selvig (*59*), Stern (*65*)]. Many of the epithelial cells which have been retained at the surface of the mineralizing front are surrounded on three sides by mineralized collagen and walled in on the remaining aspect by bundles of collagen fibrils which themselves exhibit foci of mineralization (Fig. 24).

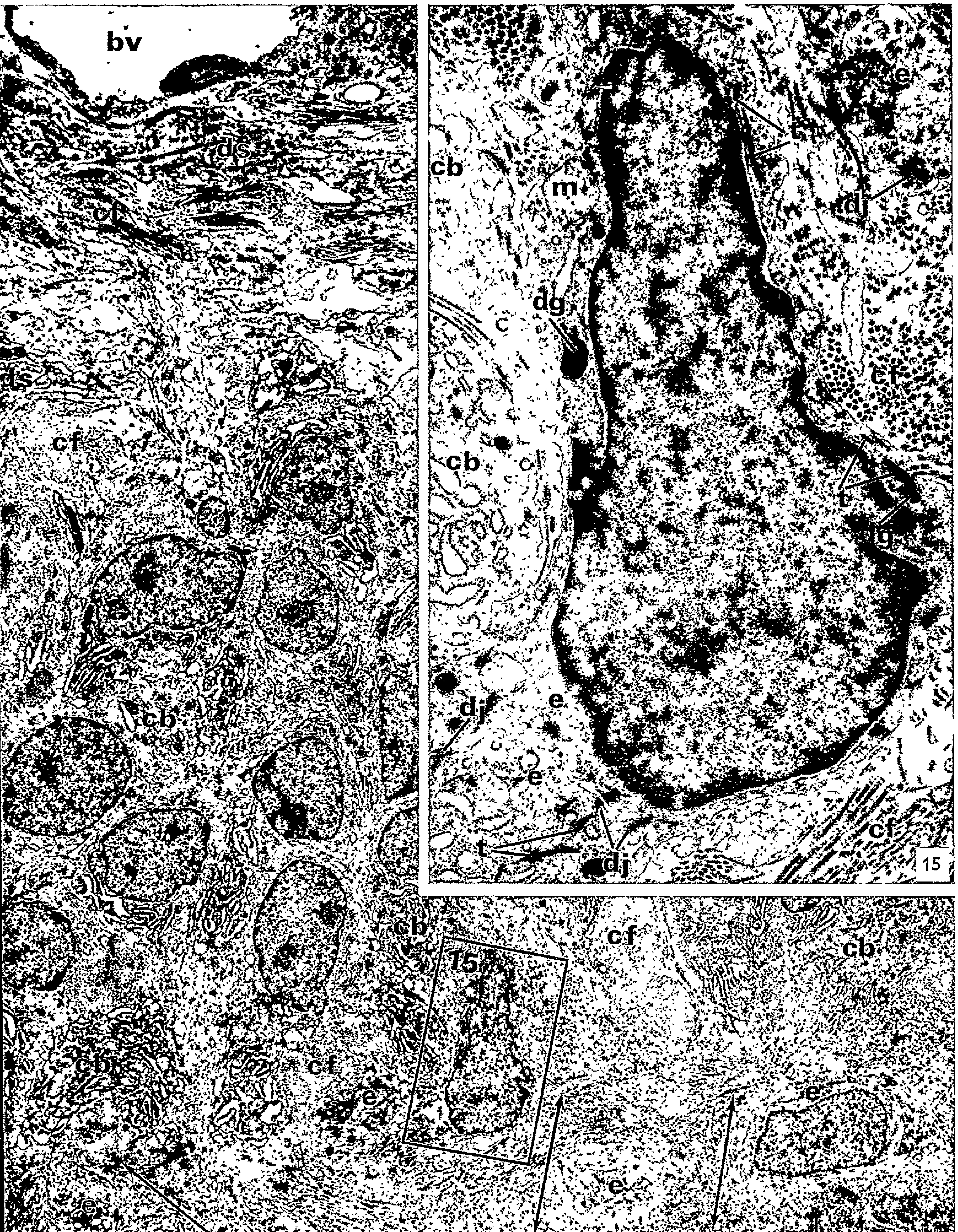
The orientation of the unmineralized collagen adjacent to the cementum is related to the form of the mineralizing front which is itself related to, and dependent upon the cells being embedded within the cementum. Thus, both epithelial cells and cementoblasts occupy bays in the mineralizing front. Those fibrils related to the peaks of the mineralizing front (Sharpey fibers?) appear in longitudinal section, whereas the fibrils on the immediate periodontal or lateral aspect of the cell bodies are sectioned transversely (Figs. 23, 24, and 27). The majority of both epithelial and cementoblast cells are embedded singly at this stage (Figs. 23 and 24), although occasional, paired cells may be found embedded in this plane of section (Figs. 25–27). The latter represent the first signs of a gradual transition to the incorporation of the epithelial sheath whole at the interface which is in the process of forming between the mineralizing fronts of the dentine and the cementum [see Diab and Stallard (*16*) and Paynter and Pudy (*49*)]. Occasionally, traces of basement lamina may be found on the cemental side of epithelial cells being incorporated by the cementum, indicating that cells from the cemental side of the sheath may also be incorporated at this stage (Fig. 28).

Ultrastructural changes in epithelial cells

Changes in the ultrastructure of the epithelial cells which may be interpreted as degenerative are discernible at this early stage of their incorporation by the cementum.

FIG. 14. Survey electron micrograph of an area of the dental sac and of dentinal collagen at a slightly later stage of development than that shown in Figs. 10–13. Individual epithelial cells may be identified, but the sheath is no longer recognizable as such and cemental collagen has beome juxtapposed to dentinal collagen (see arrows). ×2100.

FIG. 15. Higher magnification of enclosed area in Fig. 14 to show desmosome-like junctions and tonofilament bundles in cytoplasm of reoriented sheath cells which appear to be in the process of migrating to the periodontal space. ×10,000.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There is often an increase in the nuclear-cytoplasmic ratio apparently due to a reduction in cytoplasmic volume. Within the cytoplasm, the bundles of tonofilaments appear thicker and more prominent (Fig. 24); the granular endoplasmic reticulum is characteristically less pronounced and assumes the form of small dilated sacs. The Golgi apparatus usually remains prominent although the membranous sacs may be dilated (Fig. 29). Peripheral areas of cytoplasm otherwise devoid of organelles may display accumulations of fine parallel filaments. The nuclear sac commonly appears dilated, and the site of the nuclear pores may become marked (Fig. 30).

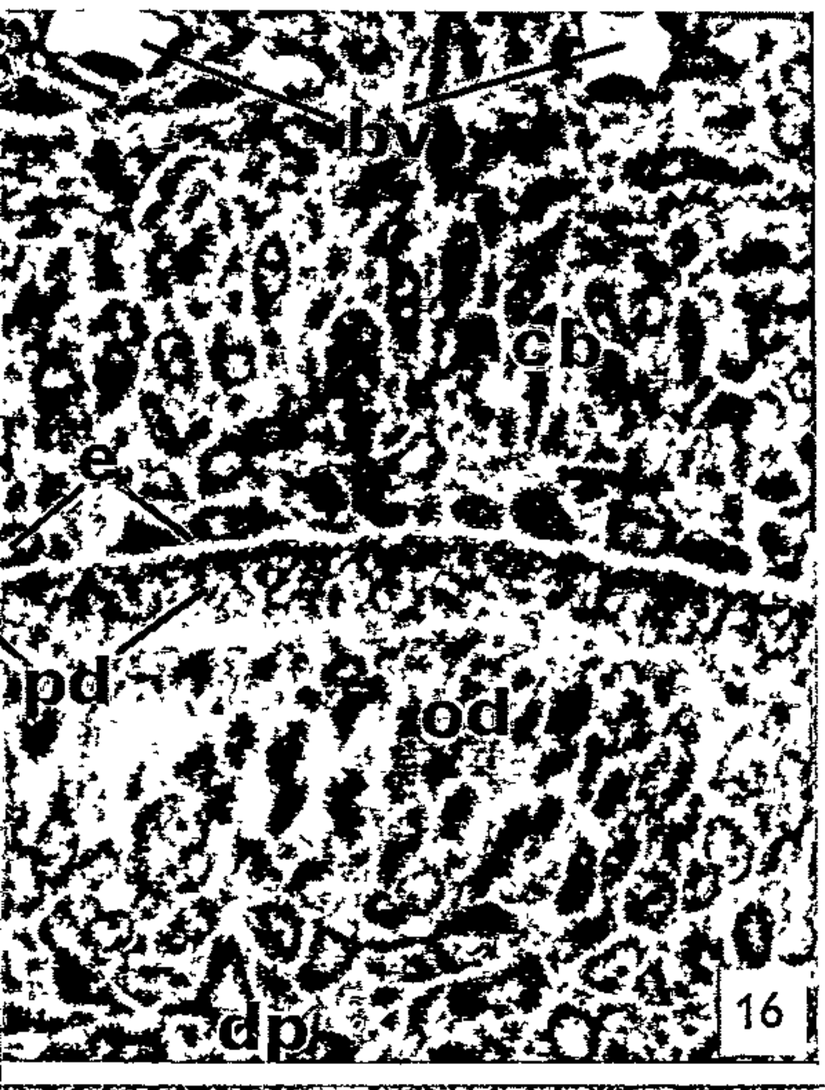
Changes in the ultrastructure of the cementoblasts upon their incorporation are less striking and usually not of a similar degenerative nature to those found in the epithelial cells (Fig. 26). The relative fates of the two cell-types will be discussed elsewhere (Lester, in preparation).

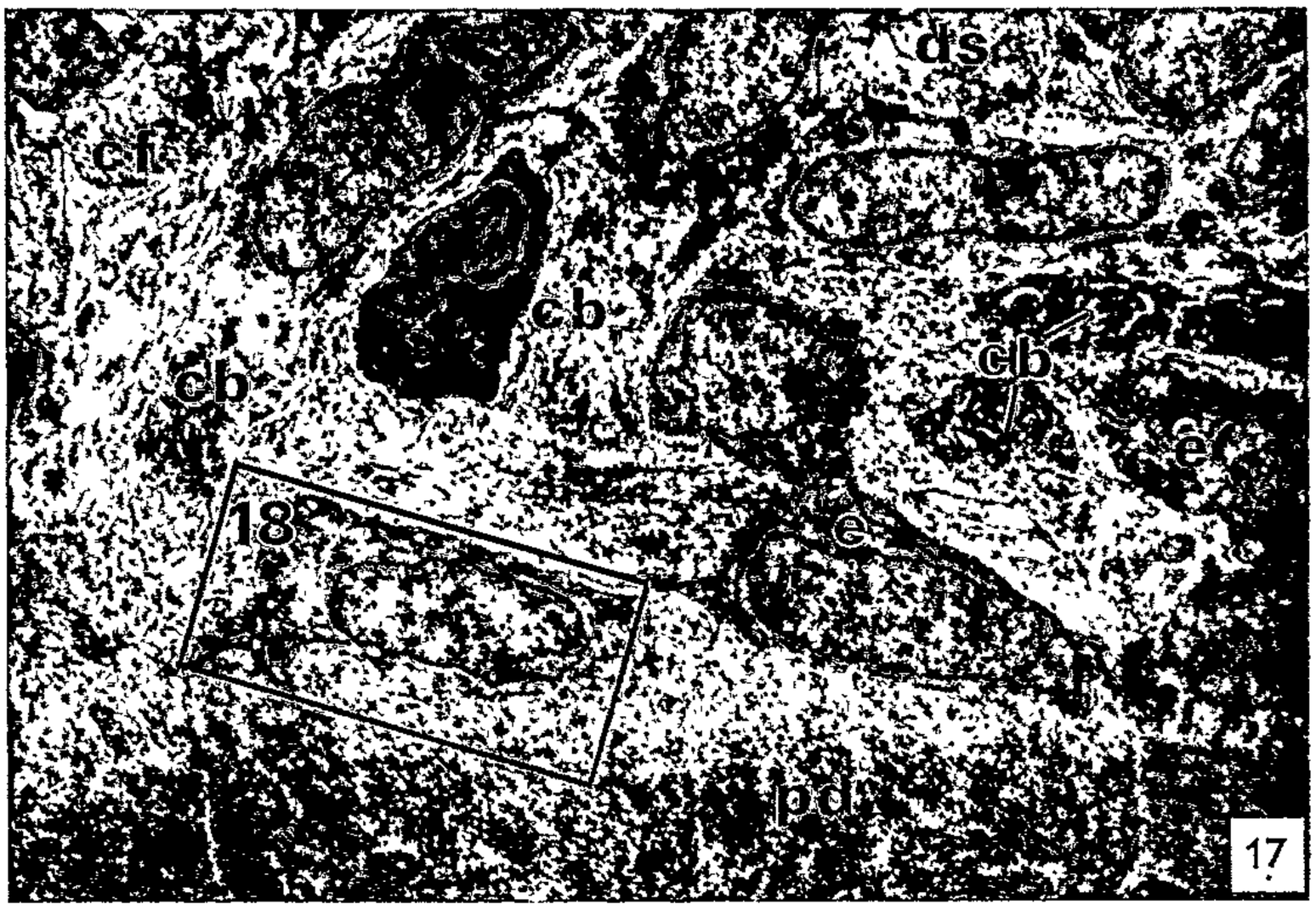
DISCUSSION

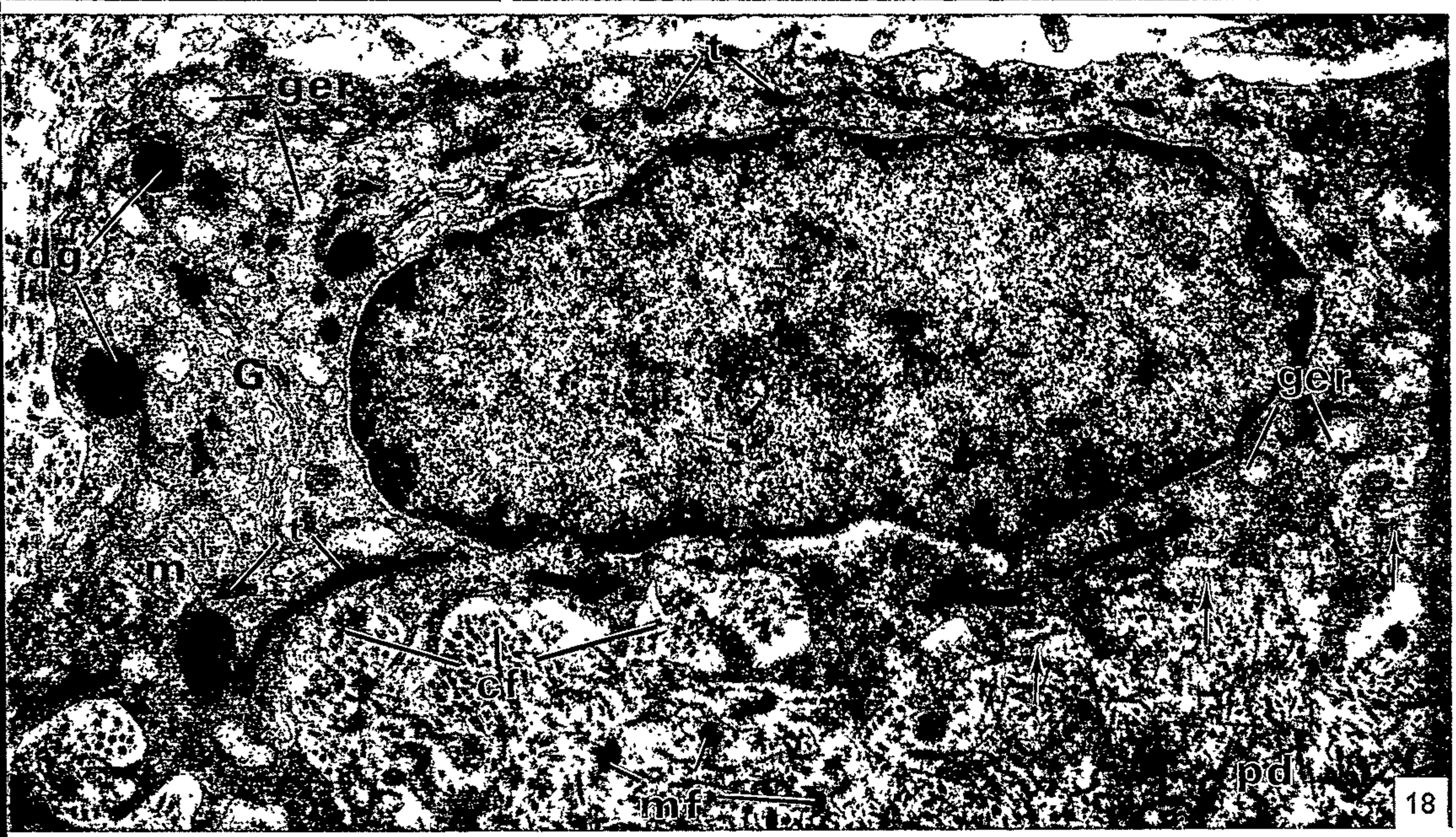
The retention of desmosomal-type junctional complexes and their associated cytoplasmic tonofilaments by cells incorporated at the mineralizing front of the cementum makes it possible to identify these cells as epithelial (*12*, *22*, *37*, *45*, *66*, *73*). Thus, ultrastructural comparison of successive stages of the process of root formation proves beyond doubt that cells of the epithelial root sheath of Hertwig are incorporated singly, or in small groups, by developing cementum at the advancing root edge. Details of the process of embeddment of these cells are relevant to consideration of the fundamentals of root and cementum formation.

Migration of epithelial cells. This study shows that the formation of cellular cementum at the advancing root edge of the rat molar coincides with the failure of the root sheath cells to depart from the surface of the formed dentine. This departure of epithelial cells to the periodontal space, where they are found in the adult and are known as "epithelial rests of Malassez" (*41*, *46*, *51*, *73*), is generally accepted as a normal part of the formative process for both cellular and acellular cementum.

The factors responsible for this migration are quite unknown. Perhaps part of the answer may be found in the changes occurring in their immediate environment. These changes are not inconsiderable for the cells have been: freed of their association with the previously surrounding basement lamina and, in some cases, to each other; effectively blocked by mineralized dentine from one possible source of nutrition, the vessels of the dental papilla; and, as a result of the differentiation of cementoblasts and the production of cemental collagen, placed at a greater distance from the remaining nutritive source, the vessels of the dental sac. Perhaps it is that the sheath cells move toward the remaining nutritive source situated in the least differentiated, more distant cells of the dental sac—the migration of individual epithelial cells is well







G. 16. Transverse section of developing root of 1st mandibular molar of 25-day-old rat. Phase contrast photomicro-aph of thick section of an area similar to that illustrated in Figs. 17 and 18 and in which only a single layer of eath cells remains apposed to the surface of the dentinal collagen. ×300.

GS. 17 and 18. Survey electron micrograph (Fig. 17) and enlargement of enclosed area (Fig. 18) to show epithelial eath cell which has retained its position and orientation at the surface of the dentinal collagen as small foci of mine-lization appear within that collagen. Tonofilament bundles are obvious; cell outline is irregular; and small bays ntaining collagen fibrils appear on the dentinal side. Traces of basement lamina persist on the dentinal aspect (at rows). Fig. 17, ×2100; Fig. 18, ×10,500.

known in experimental systems (e.g., *61*, *72*). Any consideration of the relative effects of microenvironment as against genome on the life cycle of these cells must, however, remain speculative at this time.

Some comparison may be made of the process of root and cementum formation with that of the fusion of palatal processes which, in the mammalian embryo, establishes the definitive oral and nasal cavities. Here, the mid-line epithelial cells degenerate and die, permitting continuity of the connective tissue elements of the two palatal processes (*4*, *74*). In an electron microscopic study, Farbman (*21*) found that the basement lamina underlying the epithelium of the palatal processes was, prior to fusion, discontinuous in places and that microvilli of epithelial cells projected into the connective tissue space (compare this with Figs. 9 and 12). Farbman also suggested that epithelial cells not phagocytosed by their neighbors might migrate away from the middle part of the newly-formed seam and that "... the organism seems to try to discard unwanted cellular debris". Perhaps this is also a possibility when considering the widespread dispersal of sheath elements that may occur during tooth development [e.g., Orban (*46*)].

The breakdown of sheath basement lamina as described in the present paper is apparently necessary to the usual dispersal of the epithelial cells from their "developmental" location at the surface of the dentinal collagen. It has been shown, however, that a basement lamina surrounds the epithelial rests associated with adult teeth (*73*). Apparently, the basement lamina re-forms once the epithelial cells attain their characteristic but rather puzzling "adult" location in the loose, vascular, connective tissue of the periodontal membrane.

Incorporation of epithelial cells by cementum

In the present study, epithelial root sheath cells remaining at the surface of the mineralizing dentine were found to become surrounded by collagen apparently produced by cementoblasts differentiated from cells of the dental sac. This collagen subsequently mineralizes from many separate foci so that the epithelial cells and

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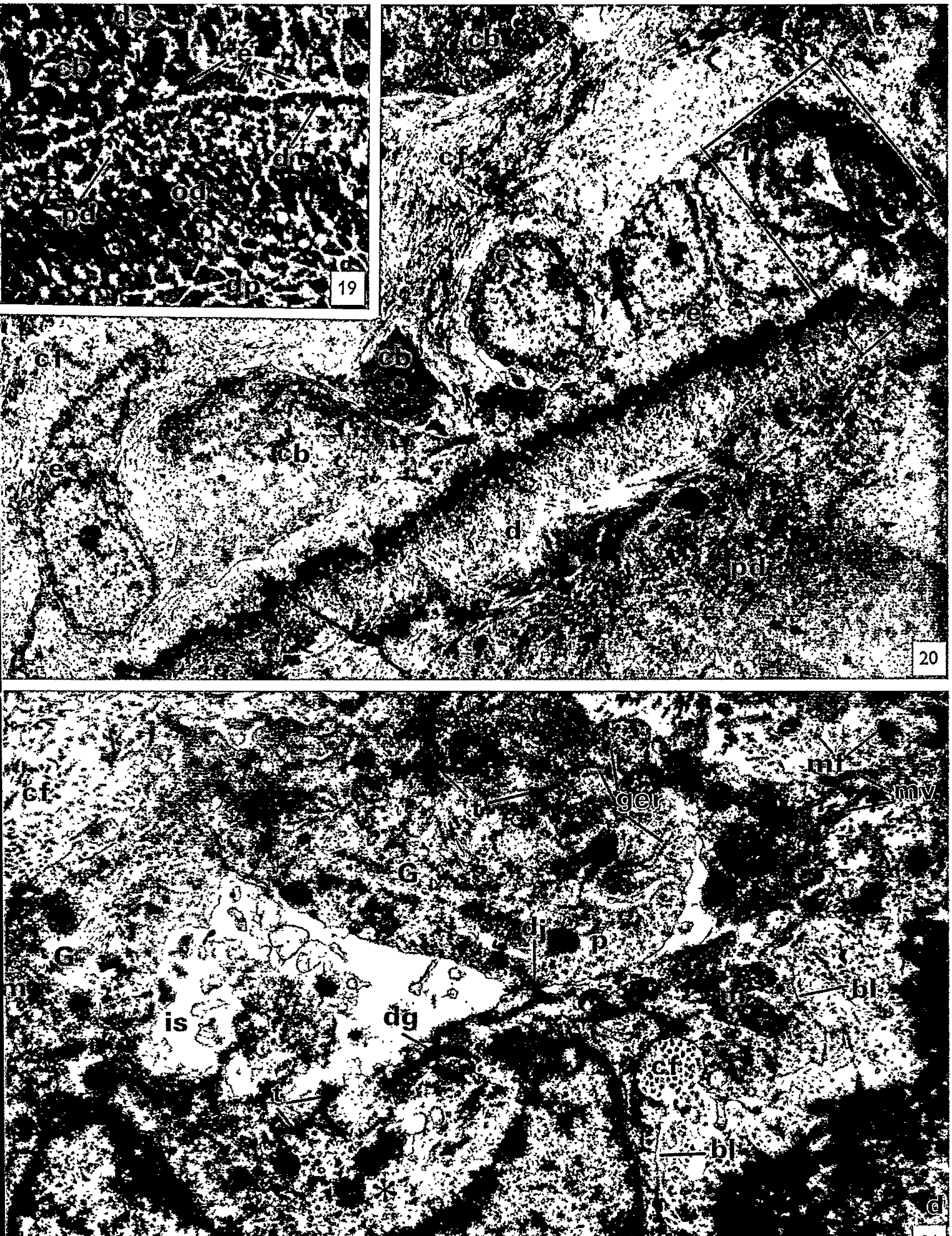
FIG. 19. Transverse thick section of developing root of 1st mandibular molar of 25-day-old rat. Phase contrast photomicrograph of an area in which mineralization of dentinal collagen has commenced and similar to that shown in Figs. 20 and 21. ×450.

FIG. 20. Survey electron micrograph of an area showing the two different cell types, epithelial and cementoblast, at the dentinal surface. Some of the epithelial cells appear rounded (to be embedded?) and others appear elongated and perpendicular to the mineralizing front (to escape to the periodontal space?). ×3000.

FIG. 21. Higher magnification of area enclosed in Fig. 20 showing parts of two epithelial cells remaining at the dentinal surface. These cells have retained some of their intercellular attachments and have assumed a somewhat rounded form. Note the cilium (at asterisk) and the mineralization foci in the collagen ahead of the general mineralization front. ×1200.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cementoblasts become embedded at the advancing mineralizing front of the cementum.

It is generally recognized that an area of "necrotic tissue" (*49*) may reside between dentine and cellular cementum in the apical part of the root of an adult human tooth—this area usually being termed "intermediate cementum". The contents of this area have been variously attributed to cells of the dental sac (*9*, *27*, *55*) and to epithelial sheath cells (*43*, *47*, *56*). Light microscope studies of molar development in the rat have shown that, in this animal, strands of root sheath cells may be embedded between the dentine and the cellular cementum as these tissues are deposited at the advancing root edge (*16*, *49*). As pointed out by Diab and Stallard (*16*), the retention of sheath cells at this site is inconsistent with the generally held view of the sequence of events occurring during root development (e.g., *50*, *55*, *56*, *60*).

The basic question here, in terms of root development, seems to be why the surviving sheath cells should fail to migrate to the future periodontal space. This is difficult even to begin to discuss satisfactorily without first having studied, in a similar way, the behavior of the sheath cells during acellular cementum formation. However, it is known that cellular cementum forms more rapidly than acellular cementum (*30*, *49*). It seems reasonable to assume, therefore, that the more rapid differentiation of cementoblasts, and their subsequent production of collagen at a relatively earlier point in developmental time, leave the sheath with less time to become sufficiently discontinuous so that cementum may form directly against the dentine and so that the sheath cells may escape to the periodontal space. This interpretation accounts for the presence of cells at the dentinal surface, and their availability for embeddment, but does not contribute anything to an explanation of the actual basis for cell incorporation by cementum.

There is general agreement that a relationship exists between the initiation of cellular cementum formation and tooth movement of one kind or another (*14*, *26*, *38*, *49*, *62*, *63*). Thus, it is tempting to think of cell incorporation in entirely mechanical terms and that the parent cementoblasts are simply unable to produce collagen in bulk at the necessary speed, and to mediate its mineralization, without their becoming

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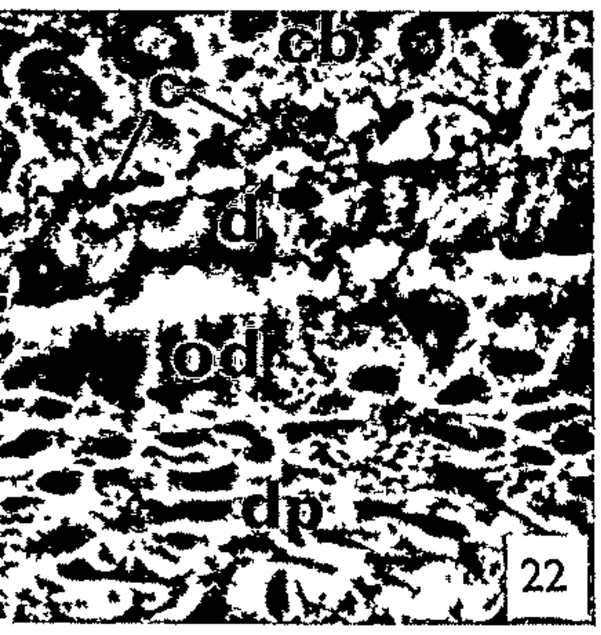
FIG. 22. Oblique transverse section of the developing root of 1st mandibular molar of a 25-day-old rat. Phase contrast photomicrograph of thick section of an area where cells are being incorporated by the mineralizing cementum, and similar to that shown in Figs. 23–28. ×450.

FIG. 23. Electron micrograph of an area where the cementum may be recognized by the form of its mineralizing front. Three epithelial cells are seen in the process of being incorporated by the cementum. ×3750.

FIG. 24. Higher magnification of enclosed area in Fig. 23. Tonofilament bundles, desmosomal junctions, intermediate and tight junctions are exhibited by these cells and characterize them as epithelial. Mineralization foci are present in that cemental collagen which would complete the incorporation of the cell. Note the "intracellular" desmosome-like junction at arrow (Lester, in preparation). ×24,000.



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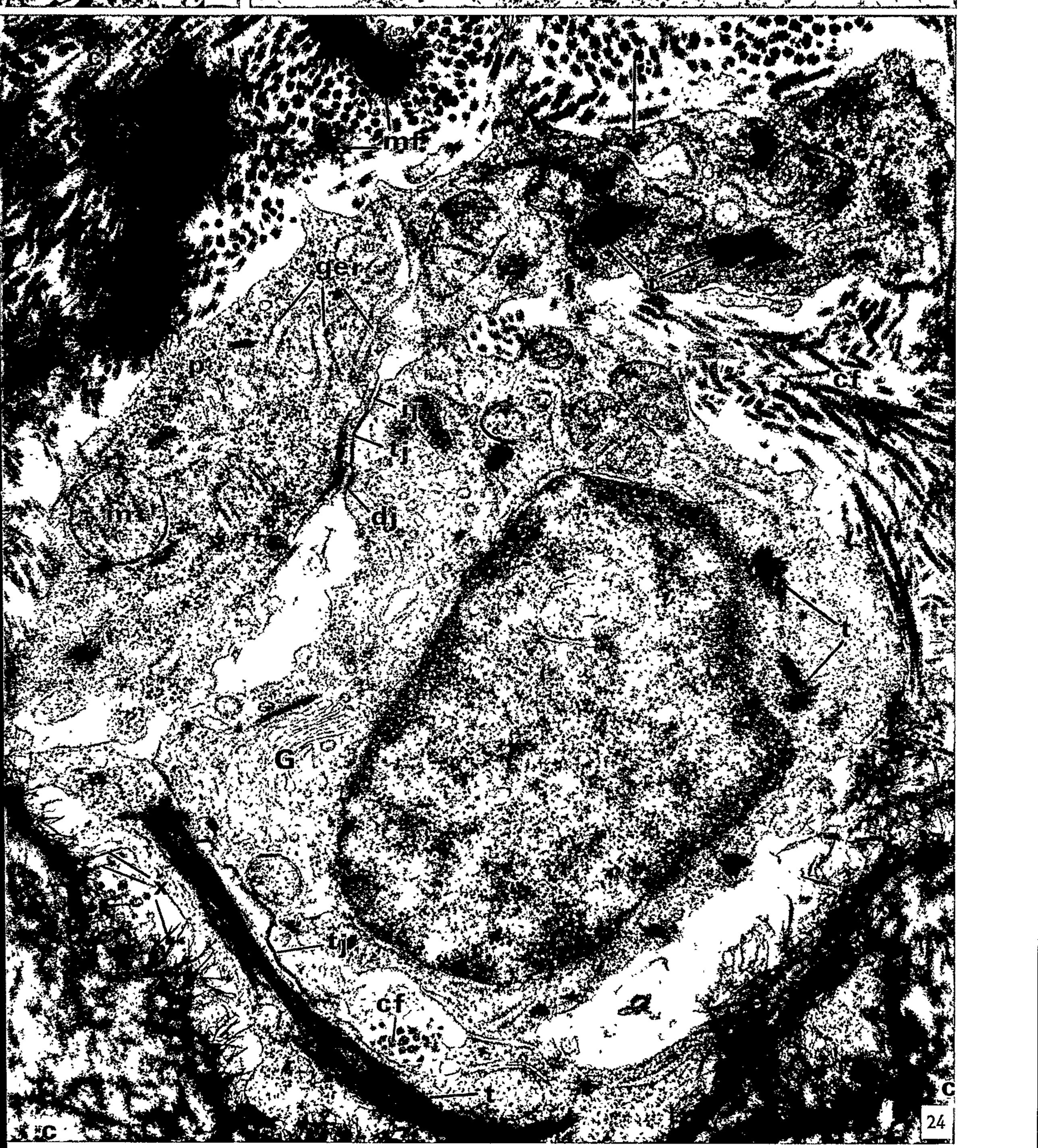

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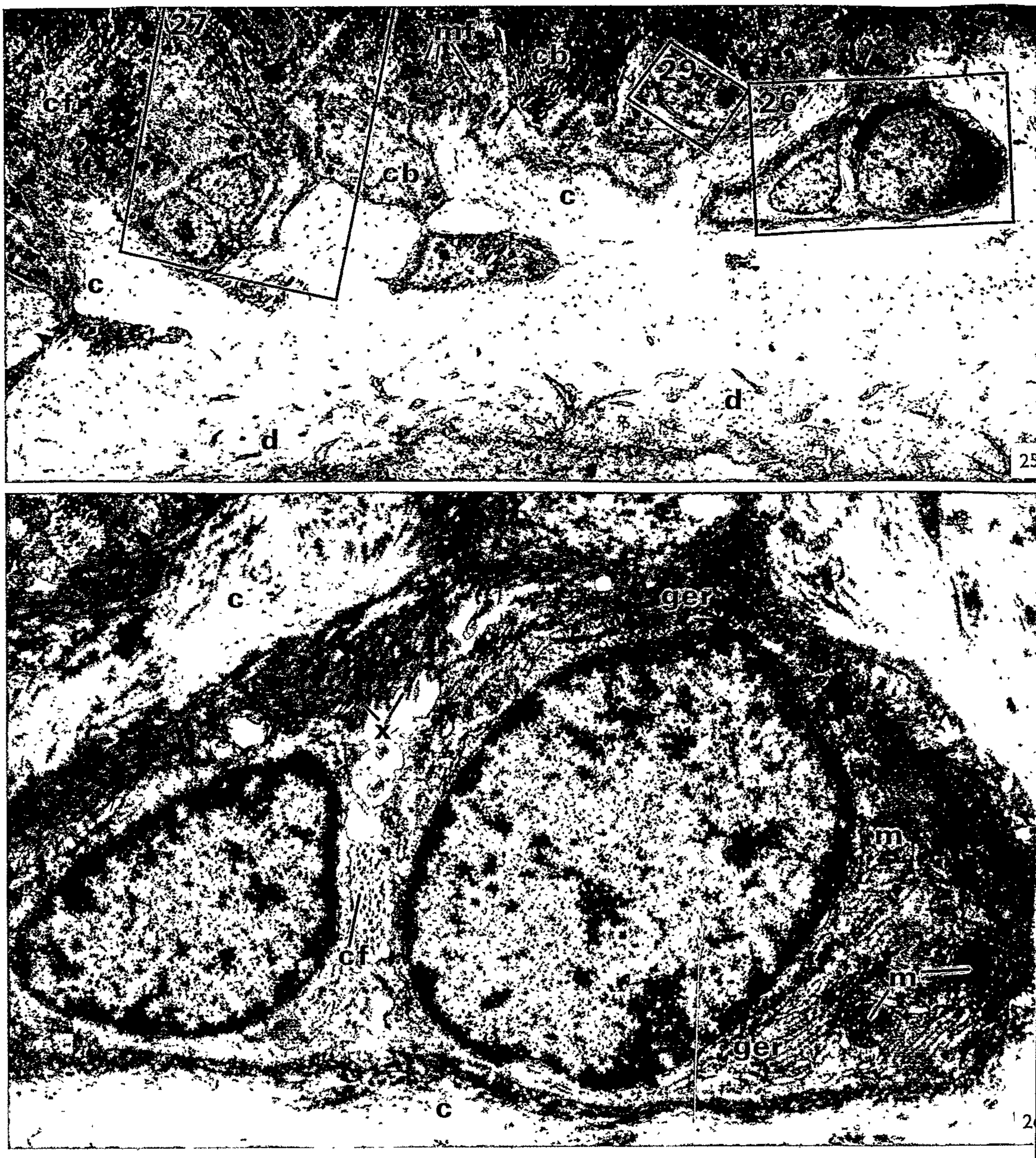
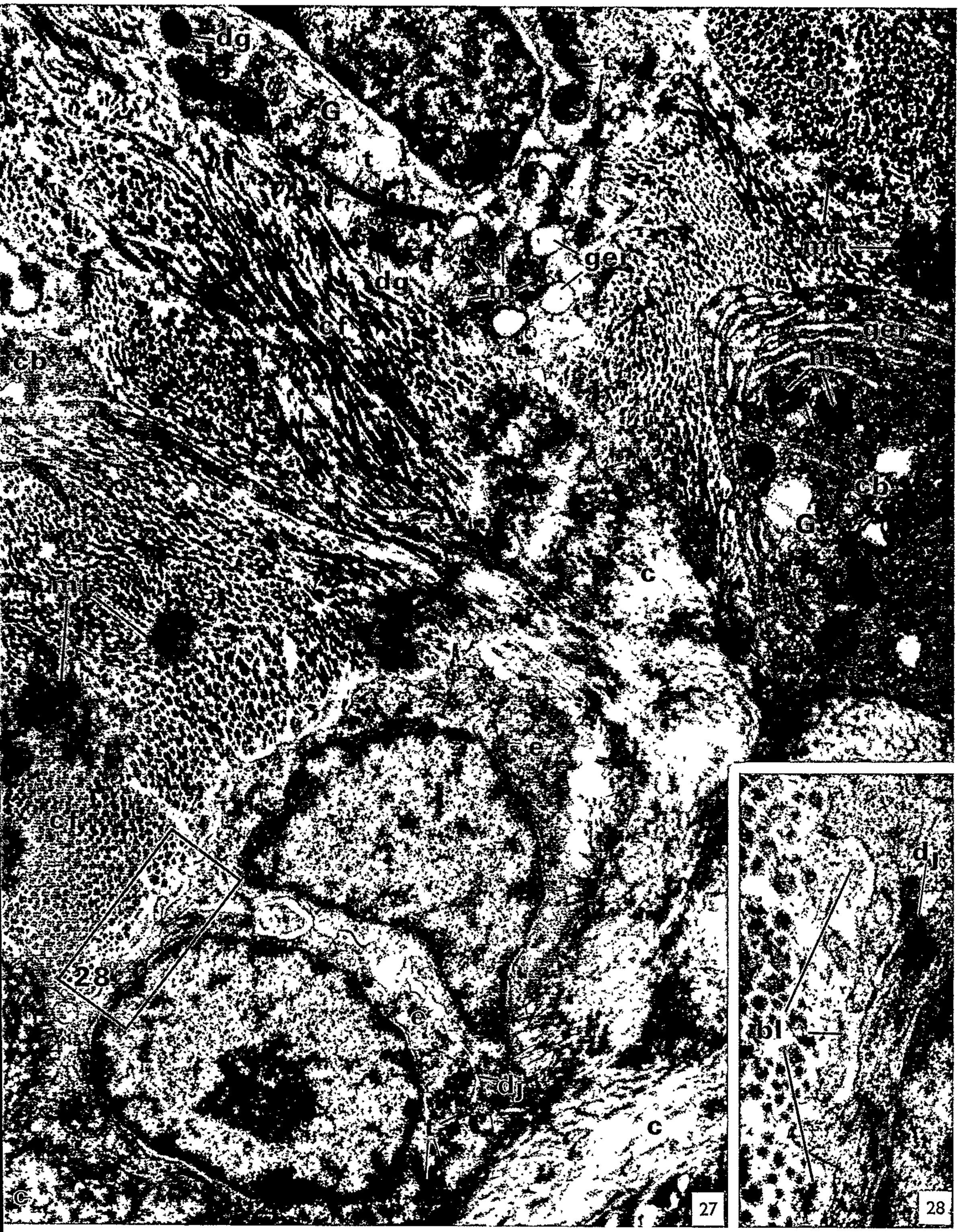


FIG. 25. Electron micrograph of an area similar to that shown in Fig. 22. Numerous cells are shown in various stag of being incorporated by the developing cementum. ×1800.
FIG. 26. Higher magnification of one of the enclosed areas in Fig. 25 showing two recently embedded cementobla (cementocytes). The orientation of the extensive granular endoplasmic reticulum reflects the contour of the lacu wall. ×9000.



G. 27. Higher magnification of one of the enclosed areas in Fig. 25 showing two attached epithelial cells in the process becoming embedded. Note part of a third epithelial cell which is displaced and reoriented and which would very obably have escaped embeddment. ×9000.

G. 28. Higher magnification of part of Fig. 27. The epithelial cells show traces of basement lamina at their surface th the as yet unmineralized collagen suggesting that the cells have originated from the cemental side of the sheath. 30,000.

embedded in the forming tissue [see also Paynter and Pudy (*49*)]. Support for such a concept may be gained by considering the other mammalian mineralized connective tissues. It has been shown that for tooth and alveolar bone formation in the rat the mineralized connective tissues (in comparable areas) may be listed in order of their rate of apposition as: bone (the most rapid); cellular cementum; dentine; and acellular cementum (the slowest) (*30*, *32–34*, *49*). Bone incorporates formative cells and blood vessels; cellular cementum incorporates the formative cells; dentine incorporates only part of the cytoplasm of the formative cell; and acellular cementum incorporates none of these. Thus, it is possible to relate the speed of formation of mineralized connective tissues within the dental environment to the degree to which available cellular elements are incorporated during development. This applies to the rat directly and to the human by association and by similarity of structure. There are, however, many variations in structure of the dental tissues throughout the mammals as a class (e.g., *11*, *48*, *70*) which would require individual consideration.

The fact that epithelial cells are also embedded in cementum when available at the advancing front suggests, at least for cementum, that it is not the need for a specific cell-type that prompts cell incorporation and that this process can reasonably be considered in more mechanical terms. Osteocytes are considered to remain metabolically active in the adult (*6*, *13*, *15*, *28*). The role or fate of odontoblast processes in adult dentine is not at all clear (*23*, *36*, *39*). There is some disagreement as to the extent of the continued viability of cementocytes (*5*, *18–20*, *25–27*, *67*, *68*). It is, nevertheless, reasonable to forecast that epithelial cells, isolated and embedded in an avascular mineralized connective tissue, would not survive. Indeed, some indication of a possibly degenerative change may sometimes be seen prior to their embeddment in the cementum (Figs. 29 and 30).

Relative roles of epithelial sheath and dental sac. Textbook accounts of root formation and cementogenesis are usually written in one of two ways. Either the dental sac cells are assigned a relatively passive role (e.g., *56*, *60*) so that they are described as "coming into contact" with the dentine upon the disintegration of the sheath; or else, and less commonly, they are assigned a more active role so that they "break through" (*55*) or undergo a "migration" (*50*) to the dentinal surface. The results of this investigation suggest that it is extremely difficult to separate these roles and that

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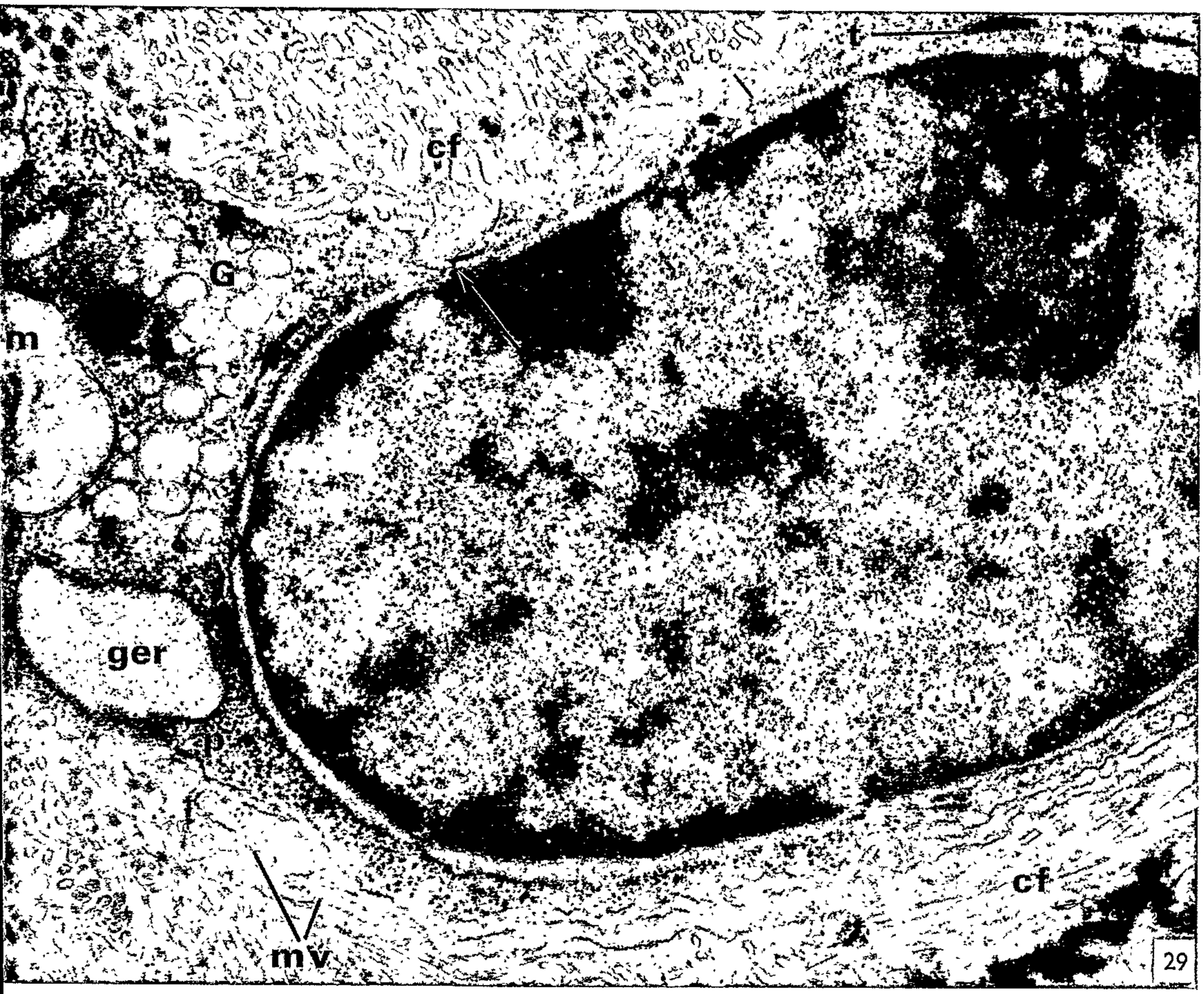
FIG. 29. Higher magnification of one of the enclosed areas in Fig. 25. This incompletely incorporated epithelial cell shows possible signs of degeneration: dilated Golgi sacs; dilated cisternae of the granular endoplasmic reticulum; and apparently discontinuous cytoplasm about the nucleus (at arrow). ×30,000.

FIG. 30. Incompletely incorporated epithelial cell showing possible signs of degeneration: exaggeration of nuclear sac (*ns*) and nuclear pore (*np*) regions; and a generalized decay of polyribosomal structure. ×1800.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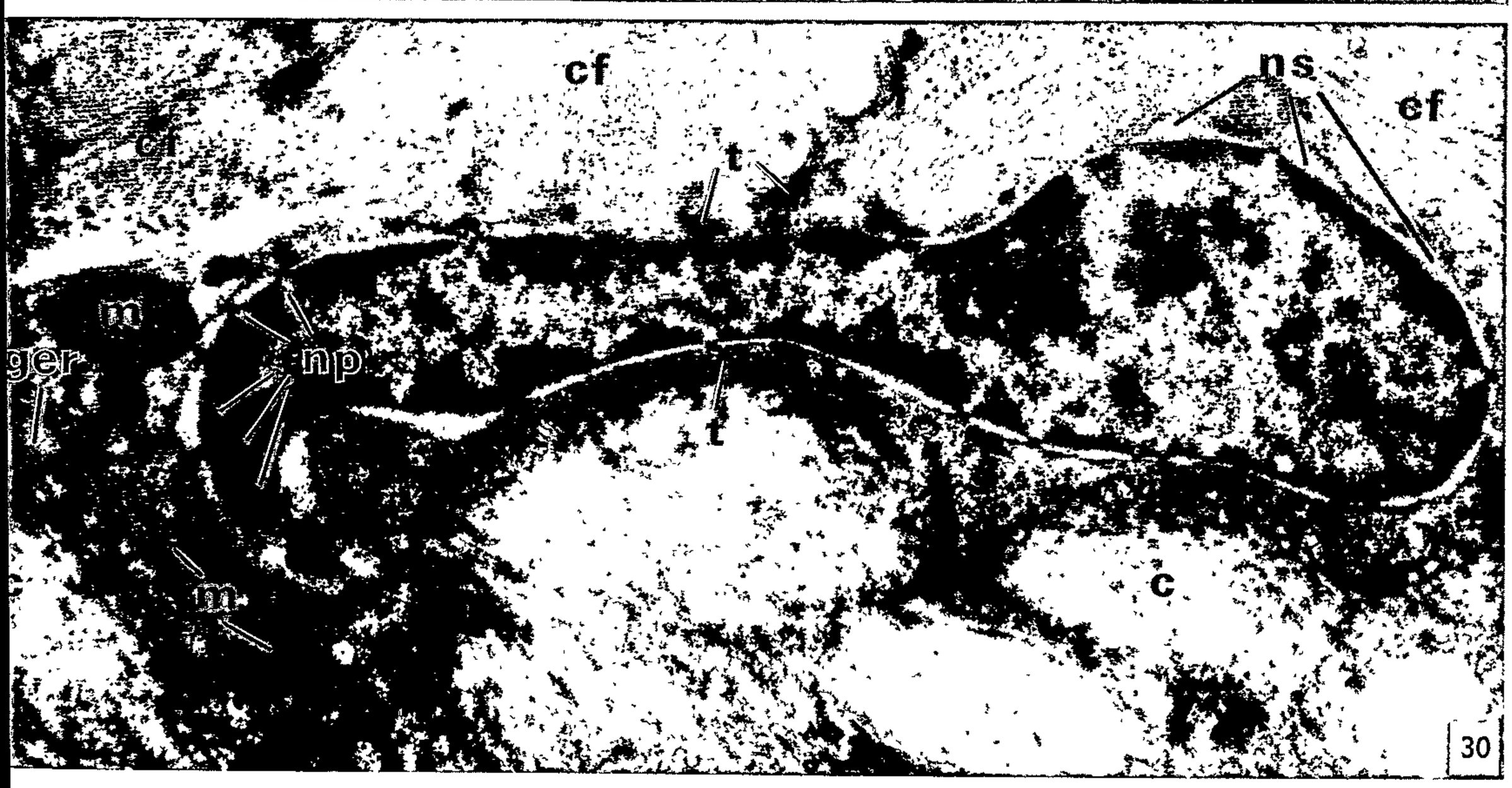
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both epithelial cells and dental sac cells play a part in the disruption of the sheath and the subsequent relative movement of the two cell-types. Further, degeneration of the epithelial sheath compartment was consistently found to be more advanced on the cemental side at any one transverse level, although there was evidence of microvillus formation by epithelial cells on both their cemental and dentinal aspects. These findings do not, however, support those of Bernick and Levy (*8*), who, in studying the molars of the marmoset by light microscopy, concluded that the dental sac cells play no part in the disruption of the epithelial sheath and that the cells of the dental papilla are responsible for this.

It has been suggested that the most peripherally placed collagen fibrils of the coronal dentine are, during the formation of the enamel–dentine junction, mineralized from the enamel side and that this contributes to the intimacy of the junction (*10*). A somewhat analagous situation is seen in the formation of the dentine–cementum junction in the rat molar root. The dentinal collagen does not mineralize initially up to the level of the sheath basement lamina (Fig. 21), and the remaining fibrils on the dentinal side of the sheath cells are mineralized with the cemental collagen. Once this has occurred, the junction between cementum and dentine is indistinguishable in thin sections by electron microscopy (see also *31*). No evidence was found in the mineralized cementum of isolated remnants of basement lamina as described in incisors of the rat (*65*) and mouse (*57*), although remnants of basement lamina were seen associated with the epithelial cells prior to their embeddment (Figs. 21 and 28).

The terms "primary" and "secondary", as applied to cementum and commonly used interchangeably with "acellular" and "cellular" respectively, lose some relevance where cells are incorporated into cementum from the initiation of its formation over a particular area of root surface. These terms do not denote the basic structural and formative difference between the two types of cementum encountered in the rat molar (and elsewhere), and the designations "cellular" and "acellular" have been preferred here for this reason.

Examination of later stages of root development reveal a more complete incarceration of Hertwig's epithelial root sheath by the forming cementum. The sequence of events accompanying this wholesale incorporation, the fate of the embedded cells, and the importance to these considerations of some of the ultrastructural details of the various cell-types as described in the present paper will be discussed elsewhere (Lester, in preparation).

It is a pleasure to acknowledge my gratitude to Miss Anne-Marie Lindgren for her valuable instruction in thin-sectioning technique and for her cheerful assistance with the photographic printing. I am indebted to Drs. E. J. Reith, P. T. Jokelainen, and H. E. Settles for discussing the work and for their critical reading of the manuscript. I am particularly grateful to Dr.

J. A. G. Rhodin and to Dr. E. J. Reith for making the study possible and for their continued support and encouragement. I wish to thank Mrs. B. Stiepock for typing the manuscript.

This study was supported in part by grants from the Health Research Council of the City of New York (U-1508) and from the United States Public Health Service (DE-02274-04).

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UPPSALA 1969

J. ULTRASTRUCTURE RESEARCH **28**, 481–506 (1969)

The Unusual Nature of Root Formation in Molar Teeth of the Laboratory Rat[1]

K. S. LESTER

Anatomy Department, New York Medical College, New York, New York 10029

Received April 14, 1969

The cellular cementum phase of root formation in molar teeth of Sprague-Dawley rats was studied in thin sections for transmission electron microscopy. Once cellular cementum formation is established, cells of Hertwig's epithelial root sheath are embedded en masse between cementum and dentine at the advancing root edge. This developmental event is coincident with an alteration in the rates of formation of cementum and dentine; cellular cementum coming both to precede and to be of greater bulk than dentine at the advancing root edge. This mode of root formation does not seem to have been described for other mammals and should be taken into account in studies utilizing rat molar periodontium. Despite the unusual fate of Hertwig's epithelial root sheath, there is evidence of morphological continuity between epithelial sheath and dental sac compartments prior to cementogenesis. The possible roles of epithelial root sheath and of occlusal function in cellular cementum formation are discussed.

Light microscope studies have suggested that cells of Hertwig's epithelial root sheath become embedded in the developing roots of the molar teeth of the laboratory rat (*15*, *50*). As far as is known, this is not typical of tooth root formation in mammals and appears to contradict some widely held ideas concerning that process. It seems reasonable to expect that consideration of this particular feature in the rat might contribute in some way to our understanding of the process in mammals generally.

A previous electron microscope study showed individual epithelial cells to become embedded in the first-formed cellular cementum of the developing roots of rat molars (*42*). This paper illustrates and describes the succeeding stages of root formation.

MATERIALS AND METHODS

Young Sprague-Dawley rats (*Rattus norwegicus*) of two litters were sacrificed at 20, 25, 30, 35, and 40 days after birth. The animals were anaesthetized with ether and the lower jaw was removed and placed in phosphate-buffered glutaraldehyde (*57*). The developing

[1] Supported in part by grants from the Health Research Council of the City of New York (U-1508) and from the U.S. Public Health Service (DE-02274-04).

mandibular molars were dissected free from the alveolar bone. The individual teeth were postfixed in osmium and dehydrated and Epon-embedded after Luft (*43*), but with prolonged infiltration times. The specimens were not purposefully demineralized at any stage during their preparation. The advancing edge of the developing root was included in the prepared block face in every case. Thick sections were examined unstained by phase contrast microscopy. Thin sections were cut with a diamond knife mounted in a Cambridge-Huxley ultramicrotome. The thin sections, collected on distilled water, were stained with uranyl acetate (*68*) and lead citrate (*53*). The thin sections were examined in a Siemens Elmiskop I operated at 80 kV.

OBSERVATIONS

Early indications of epithelial sheath embedment

The usual disposition of tissues about the advancing edge of a root of a 25-year-old 1st mandibular molar may be seen in longitudinal thick sections examined by phase contrast microscopy (Fig. 1). The dental papilla (future dental pulp), the odontoblasts, and a layer of predentine are located on the inner (medial) aspect of the mineralized dentine. Mineralized cementum showing evidence of cell incorporation covers part of the outer (lateral) surface of the dentine. External to dentine and

Key to abbreviations

bl	basement lamina	*m*	mitochondrion
c	cementum	*mb*	multivesicular body
cb	cementoblast	*mf*	mineralization foci
cc	cementocyte	*mt*	microtubule
cf	collagen fibrils	*mv*	microvillous process
cv	coated vesicle	*n*	nucleus
d	dentine	*od*	odontoblast
dg	dense granule (membrane-bound)	*p*	polyribosomes
dj	desmosome-like junction	*pd*	predentine
dp	cells of the dental papilla	*pl*	plasmalemma
ds	cells of the dental sac	*r*	remnants of degenerating cells
e	cells of Hertwig's epithelial root sheath	*rbc*	extravasated red blood cell
G	Golgi region	*t*	tonofilament bundles
ger	granular endoplasmic reticulum	*tj*	tight junction
is	intercellular space		

FIG. 1. Phase contrast photomicrograph of a longitudinal thick section of the advancing edge of the wall of a developing root of a 1st mandibular molar of a 25-day-old rat. Cellular cementum is forming in bulk close to the level of the advancing edge of the mineralized dentine. Hertwig's epithelial root sheath lies in advance of the developing root. ×400.

FIG. 2. Survey electron micrograph of a thin section of the specimen shown in Fig. 1. There is an obvious alignment of Hertwig's epithelial root sheath with cells lying at the outer surface of the mineralized dentine. Those areas subsequently enlarged in Figs. 3 and 5 are indicated. ×580.

FIG. 3. Higher magnification of one of the areas outlined in Fig. 2. Cementoblasts, an epithelial cell (see also Fig. 4 and cf. Fig. 5), and remnants of cellular degeneration lie at the surface of the mineralized dentine. Mineralization foci are present in the cemental collagen adjacent (external) to the epithelial cell. ×3975.

FIG. 4. Higher magnification of the area outlined in Fig. 3. The cell may be identified as epithelial on the basis of its comparison with cells of the intact sheath (see Fig. 5). Part of a cementoblast is at bottom left. ×13,500.


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cementum is the dental sac (future periodontal membrane). A two-cell-thick sheet, the epithelial root sheath of Hertwig, separates the dental papilla and the dental sac where they lie in advance of the dentine. This sheet of cells is less intact and less clearly demarcated close to the tip of the mineralizing dentine. Compared to what one would normally anticipate for developing mammalian teeth, the apposed cementum is here unusally close to the level of the advancing tip of the mineralizing dentine (Fig. 1).

Low magnification electron micrographs of thin sections of the same material (Fig. 2) illustrate a degree of alignment between cells located at the outer surface of the mineralized dentine and those of the intact epithelial sheath (i.e., where it lies in advance of the mineralizing edge).

Certain characteristics of the epithelial sheath cells will be described briefly at this point in order to provide some basis for identification of individual epithelial cells both in this (Figs. 1–4) and in later, less ordered, less typically mammalian stages of root development (Figs. 6–25). The sheath cells in advance of the developing root edge (see Fig. 2) form a well-defined epithelial sheet (Fig. 5), two cell layers thick and bounded on either side by a continuous, well-defined basement lamina. The cytoplasm of the epithelial cells is small in amount, and the intercellular space is limited. The bundles of cytoplasmic tonofilaments which later characterize and serve as a convenient marker for the cells are, as yet, poorly differentiated. Likewise, desmosomal junctions are few (Fig. 5). Polyribosomes are abundant; there being only few flattened sacs of granular endoplasmic reticulum per cell section. Mitochondria are scattered throughout the cytoplasm and the Golgi region is conspicuous, the latter being characteristically located on that side of the cell away from the basement lamina of the nearest side [see also Reith (*52*)].

On the basis of comparable ultrastructure, some of the cells lining the outer edge of the mineralized dentine in this same specimen may be identified as epithelial (cf. Figs. 3, 4, and 5). Other cells at this site may be identified as cementoblasts (Figs. 3 and 4)—although how they arrive at the dentinal side of the epithelial sheath cells is better described from transverse sections (see below). Significantly, mineralization foci are evident in cemental collagen bundles which are adjacent to the cells on their external (dental sac or periodontal) aspect (Fig. 3).

This specimen (Figs. 1–5) will be shown to be representative of a phase intermediate between the embedment of isolated epithelial cells in the first-formed cellular cementum, as described previously (*41*, *42*), and the subsequent wholesale

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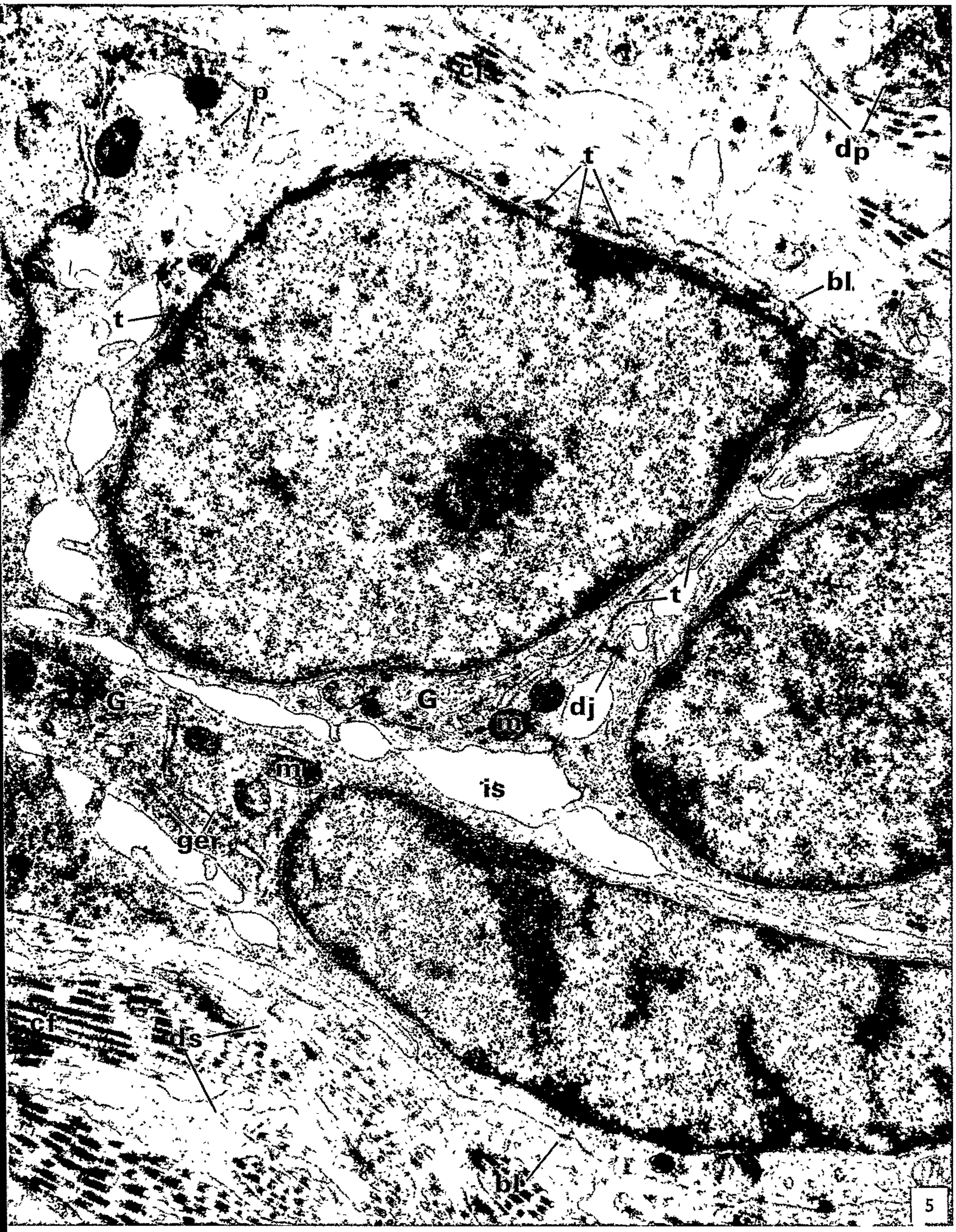
FIG. 5. Higher magnification of one of the areas outlined in Fig. 2 to show part of Hertwig's epithelial root sheath where it lies well-defined in advance of the developing root edge. Tonofilaments and cell attachments are as yet poorly developed; polyribosomes are abundant and granular endoplasmic reticulum minimal. ×15,900.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engulfment of the sheath between the two mineralizing components of the advancing root edge.

Embedment of sheath at cementum-dentine junction

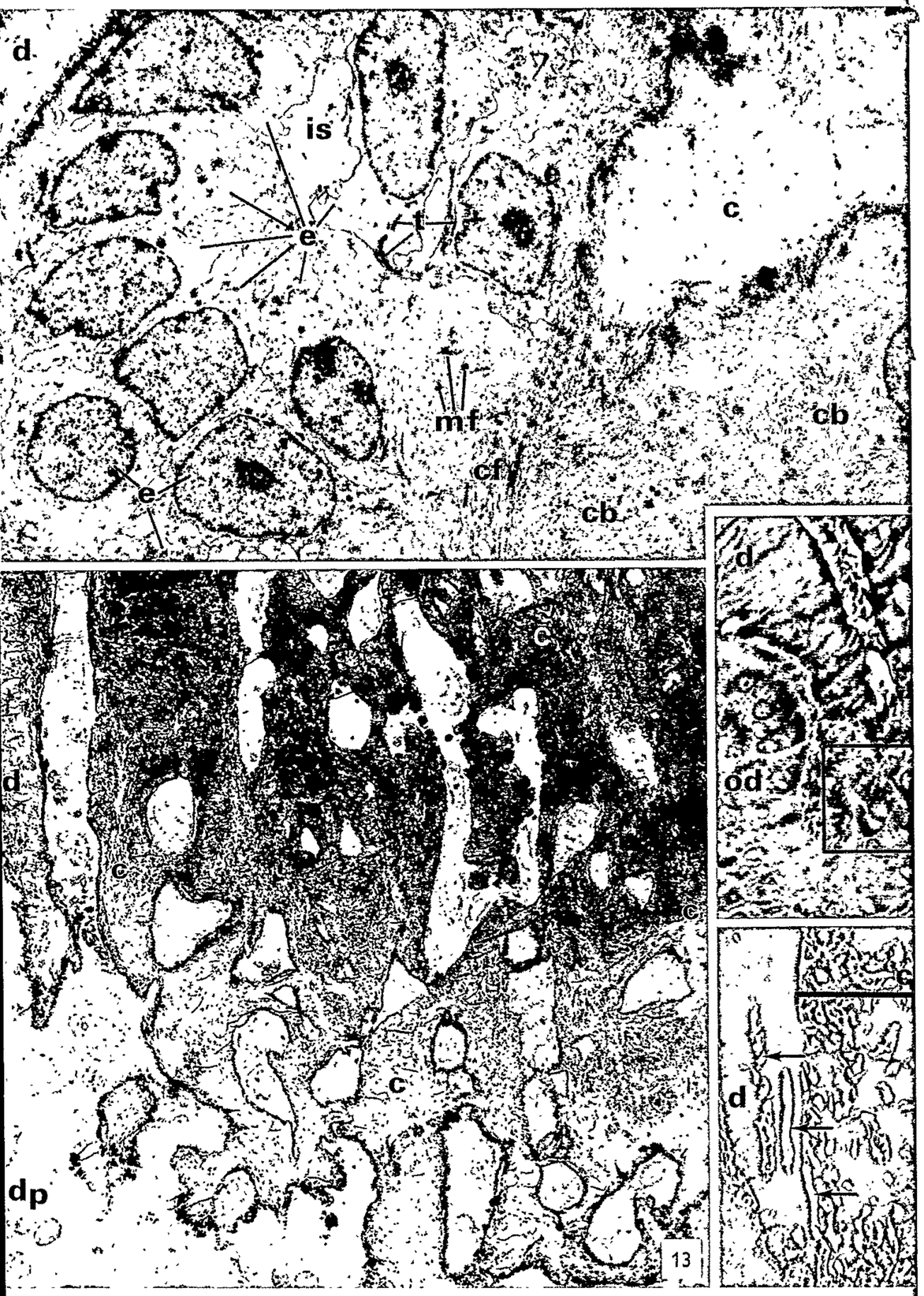
In older animals, the bulk of cementum at the advancing root edge is further increased relative to that of the dentine (cf. Figs. 2 and 6). An exaggerated space exists between the mineralized cementum and dentine, which is occupied to a varying degree by epithelial sheath cells, cementoblasts, and collagen (cf. Figs. 7 and 9). Their nuclear-cytoplasmic ratio, tonofilament bundles, and relationship to one another allow epithelial cells to be distinguished from connective tissue cells even at low magnifications. The collagen incorporated between the cementum and the dentine exhibits mineralization foci.

Cementum comes both to precede, and to be of greater bulk than the dentine at the advancing root edge in later stages of development (Fig. 10). The mineralization fronts of cementum and dentine are here widely separated by a well-compartmented group of epithelial cells constituting part of the root sheath of Hertwig (Fig. 11). Cementoblasts are present at the lateral border of the unincorporated sheath cells and are separated from the latter by cemental collagen (Fig. 11). The degree of invasion of the epithelial compartment by these connective tissue elements appears to decrease as root formation progresses (cf. Figs. 3, 7, and 11).

Irregularity of the cementum-dentine junction in final stages

There is an enormous increase in cemental bulk at the advancing root edge in later stages of root formation (Fig. 12). This cementum is exceedingly porous or "cellular," due in large part to frank cell-containing channels rather than individual lacunae (Fig. 13). Median longitudinal sections of such material often give the impression that the epithelial root sheath has broken up into several longitudinal strands. Thus, epithelial cells are commonly found apparently in the process of becoming incorporated into the root substance at more than one site (Figs. 14–17). Overall, it appears that successive sites of incorporation of these strands are located progressively toward the midline of the tooth as root development proceeds (Figs. 12 and 14).

FIG. 6. Survey electron micrograph of a longitudinal section of the advancing edge of the wall of a developing root of a 2nd mandibular molar of a 30-day-old rat. Cemental bulk at the root tip has increased (cf. Fig. 2) and approximates that of the dentine. ×480.
FIG. 7. Higher magnification of the area outlined in Fig. 6. Epithelial cells, cementoblasts, and mineralizing collagen occupy the area between the mineralized dentine and cementum. ×2100.
FIG. 8. Survey electron micrograph of an oblique longitudinal section of the advancing edge of the wall of a developing root of a 2nd maxillary molar of a 30-day-old rat. A strand of cells lies between the mineralized cementum and dentine. ×580.
FIG. 9. Higher magnification of the area outlined in Fig. 8 showing the epithelial nature of many of the cells situated between dentine and cementum at the advancing root edge. ×1830.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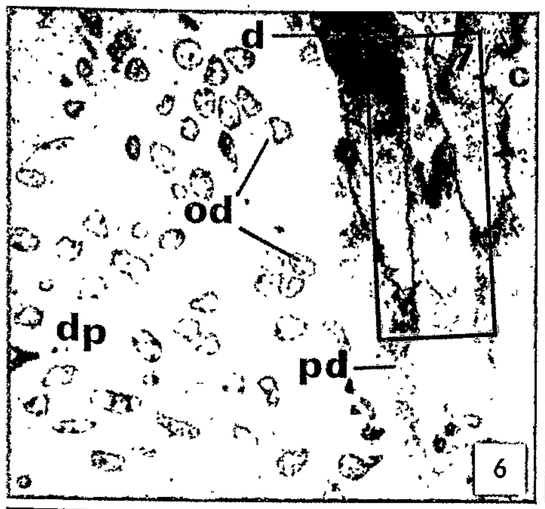
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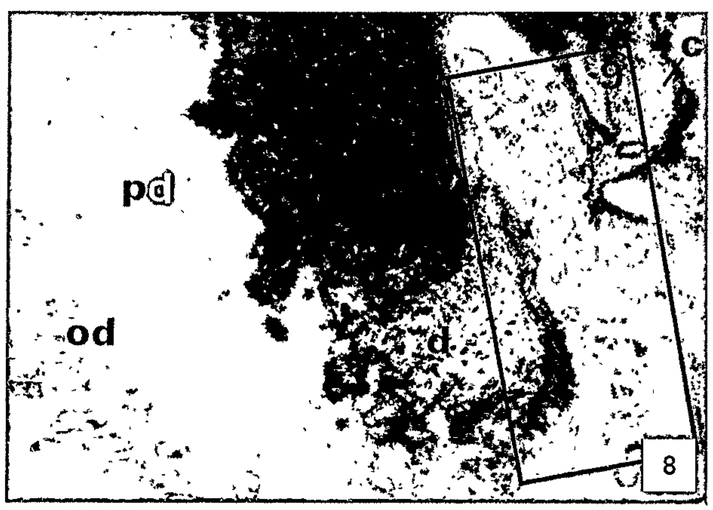
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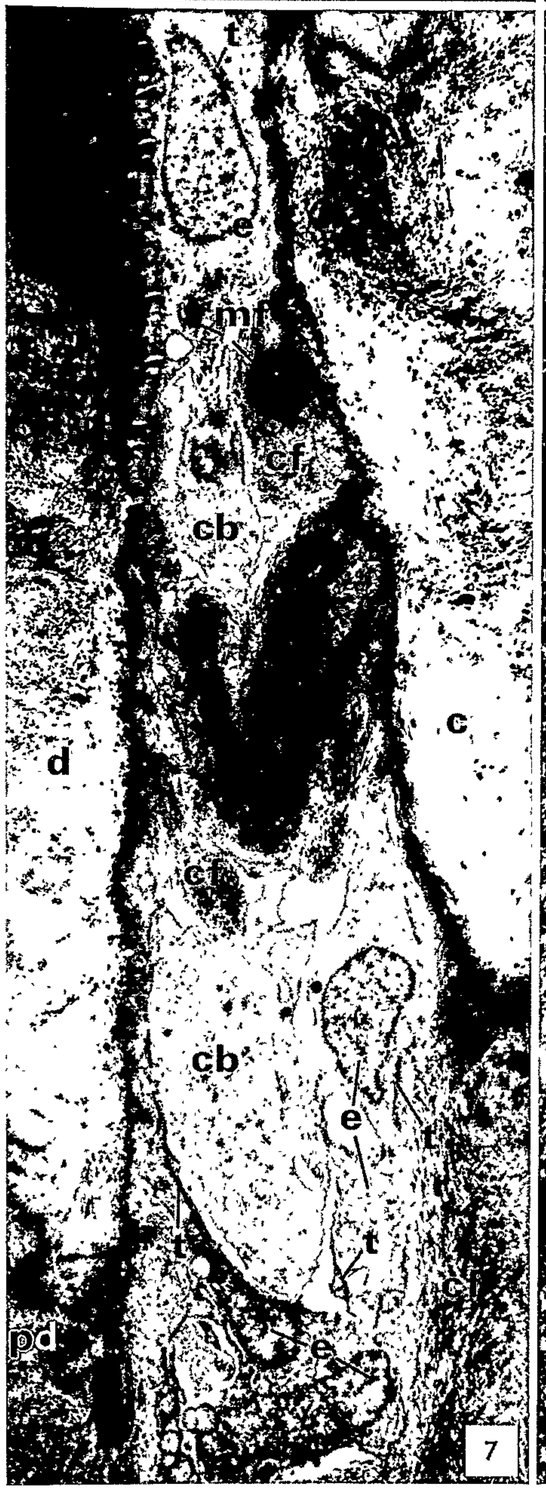
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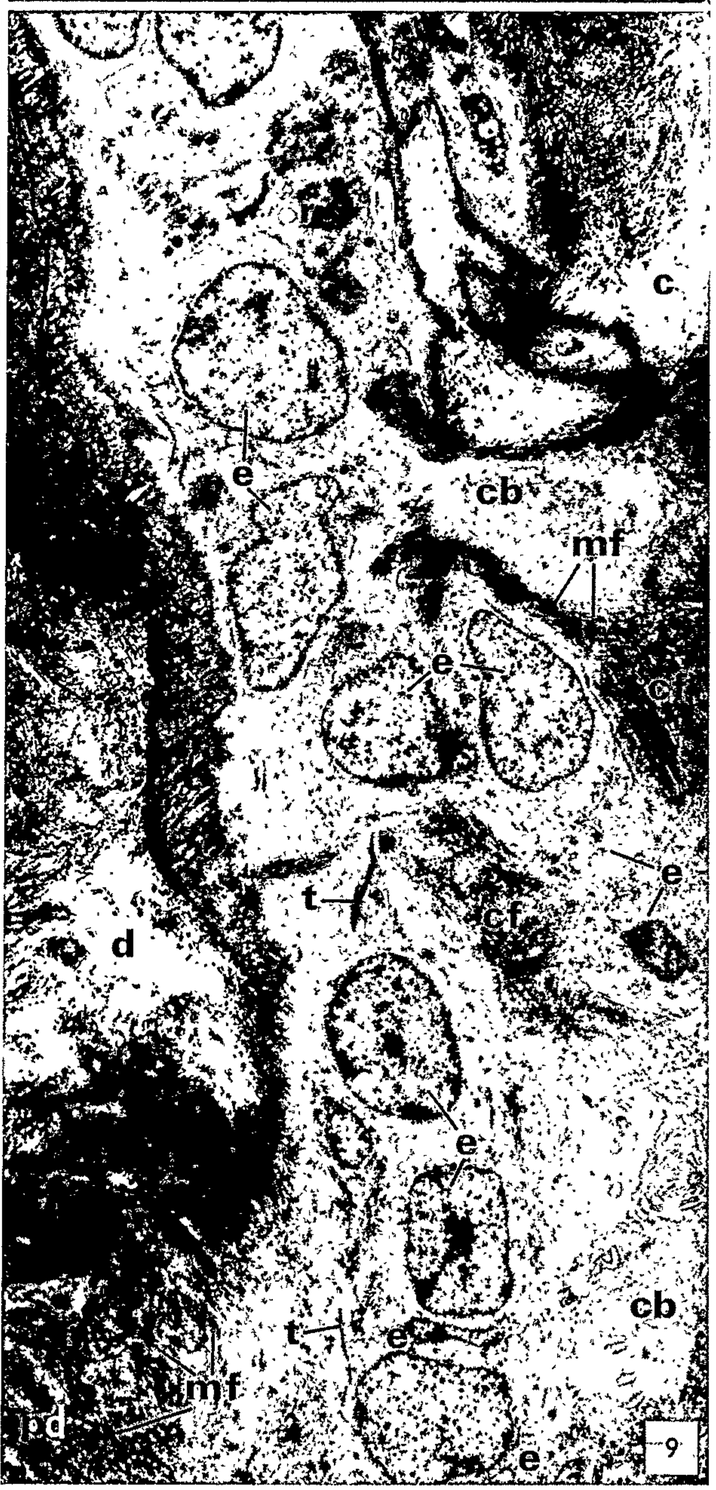
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Cementoblasts accompany the sheath cells in a manner similar to
above and give every appearance of establishing, by their presence
cementum-dentine junction medial to that existing previously (cf. F:

Epithelial sheath-connective tissue relationship

In transverse sections of root primordia. Developmental features
molar root formation, and which lead to the appearances described
illustrated in transverse sections of a developing root taken at a
advance of mineralization. Here the degree of cementoblast differe
cemental collagen production is quite comparable to that of odontob
tion and of dentinal collagen production at any one transverse leve
cells of the epithelial sheath appear to be trapped between these tw
types and the tissues in the process of being elaborated by them. Clea
tion of the cemental collagen would constitute a barrier to what is ge
as the "normal" course of the sheath cells to the periodontal space
cementum formation.

The epithelial sheath cells at this level in the root primordium d
structure significantly different from sheath cells situated at a level
of cementoblast and odontoblast differentiation (see, for example,
differences include an increase in the complexity and number of orga
be interpreted as evidence of a further differentiation of the epitheli
The Golgi region is prominent and may be quite extensive, commonl
vesicular bodies and membrane-bound dense granules associated wi
somes are abundant; elongated profiles of endoplasmic reticulum ar
increased but still moderate degree; and mitochondria are numerou
characteristic cytoplasmic feature is the abundance of tonofilaments.
aggregated into very dense bundles at the periphery of the cell an
appearance of interweaving as if to partially encapsulate the nuc
region [Fig. 20 and cf. Brody (*11*)]. Individual filaments are contri

FIG. 10. Phase contrast photomicrograph of a longitudinal thick section of the
the wall of a developing root of a 2nd maxillary molar of a 35-day-old rat. The bul
at the root tip has come to exceed that of the dentine. ×600.
FIG. 11. Electron micrograph of a thin section of an area similar to that outlin
epithelial sheath has remained relatively intact (cf. Figs. 5, 7, and 11) during its en
the cementum and the dentine. The grouped epithelial cells are readily identified
retained, intact, intercellular space; microvillous processes; and cytoplasmic ton
×2970.
FIG. 12. Phase contrast photomicrograph of a longitudinal thick section of the w
root of a 1st mandibular molar of a 40-day-old rat to show the amount and "poro
tum. The arrowed areas may represent sites of incorporation of epithelial root s
FIG. 13. Low magnification electron micrograph of a thin section of the root tip
The cementum exhibits irregular channels and lacunae, both containing cellular m

bundles to the many sites of desmosome-like junction with neighbouring epithelial cells. Microtubules are present, and well-developed cilia are not uncommon.

There is evidence at this particular level of section and stage of development of a loss of integrity of the epithelial sheath compartment. Early indications of this degeneration are somewhat similar to those described for the initial formation of cellular cementum (*42*). There is, of course, the important difference in the present material (where cellular cementum formation is well established) that the sheath does not contribute to its own discontinuity by migration of its component cells. Thus, the basement lamina consistently exhibits the most obvious signs of discontinuity on the cemental side (Fig. 21). All surfaces of the epithelial cells become more irregular and the intercellular spaces within the sheath become more exaggerated (cf. Fig. 5 and Fig. 21). Cementoblast cell processes may be found projecting into the sheath between separated, previously adjacent epithelial cells (upper arrow in Fig. 18) at sites where the basement lamina of that side is no longer evident. Collagen fibrils, often seen to be continuous with those on the cemental side (Fig. 18 at lower arrow), become evident to varying degrees in the intercellular spaces of the epithelial sheet (Fig. 19).

The basement lamina on the dentinal aspect of the sheath differs in structure from its counterpart on the cemental side. Associated with the basement lamina of the dentinal side are many fine filaments (ca. 180 Å in diameter) aligned approximately perpendicular to it (Fig. 23). These nonstriated filaments may be traced for some distance (up to 1200 Å) in among the dentinal collagen and give the appearance in some areas of being continuous across the basement lamina from the surface of the epithelial cells [and see also (*52*)]. As mentioned above, the basement lamina of the dentinal side [zona lucida ca. 540 Å, and zona densa ca. 360 Å; see Stern (*70*)] is consistently more clearly demarcated and intact than that of the cemental side at any one transverse level in this 35-day-old material. The basement lamina of the dentinal side does, however, become discontinuous as the dentinal collagen mineralizes. At sites where the basement lamina is no longer evident, collagen fibrils and cell processes may be found located in small bays formed in the surface of the epithelial cells. These bays may appear to be closed off by small villous processes (Fig. 24).

FIG. 14. Phase contrast photomicrograph of a median longitudinal thick section of the advancing edge of a developing root of a 1st mandibular molar of a 35-day-old rat. The area from which Figs. 15–17 are taken is indicated. ×450.
FIG. 15. Survey electron micrograph of a thin section of an area similar to that outlined in Fig. 14. There appear to be two sites at which cells are being embedded by the developing root. ×700.
FIG. 16. Higher magnification of one of the areas outlined in Fig. 15 to show epithelial cells being embedded between dentine and cementum. ×3900.
FIG. 17. Higher magnification of an area similar to one of those outlined in Fig. 15 to show epithelial cells and cementoblasts apparently involved in establishing a "new," more medially located cementum-dentine junction. ×5400.


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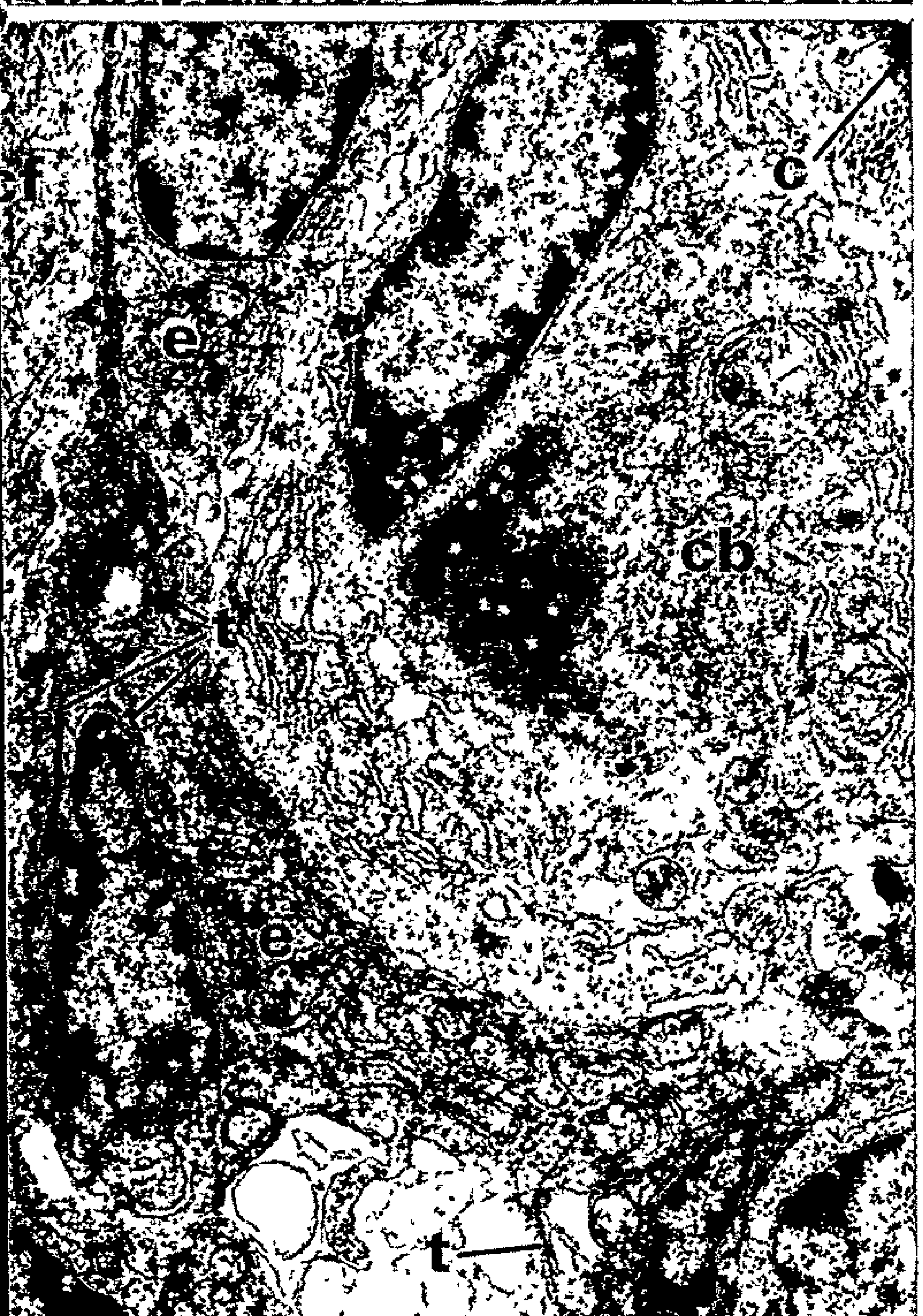
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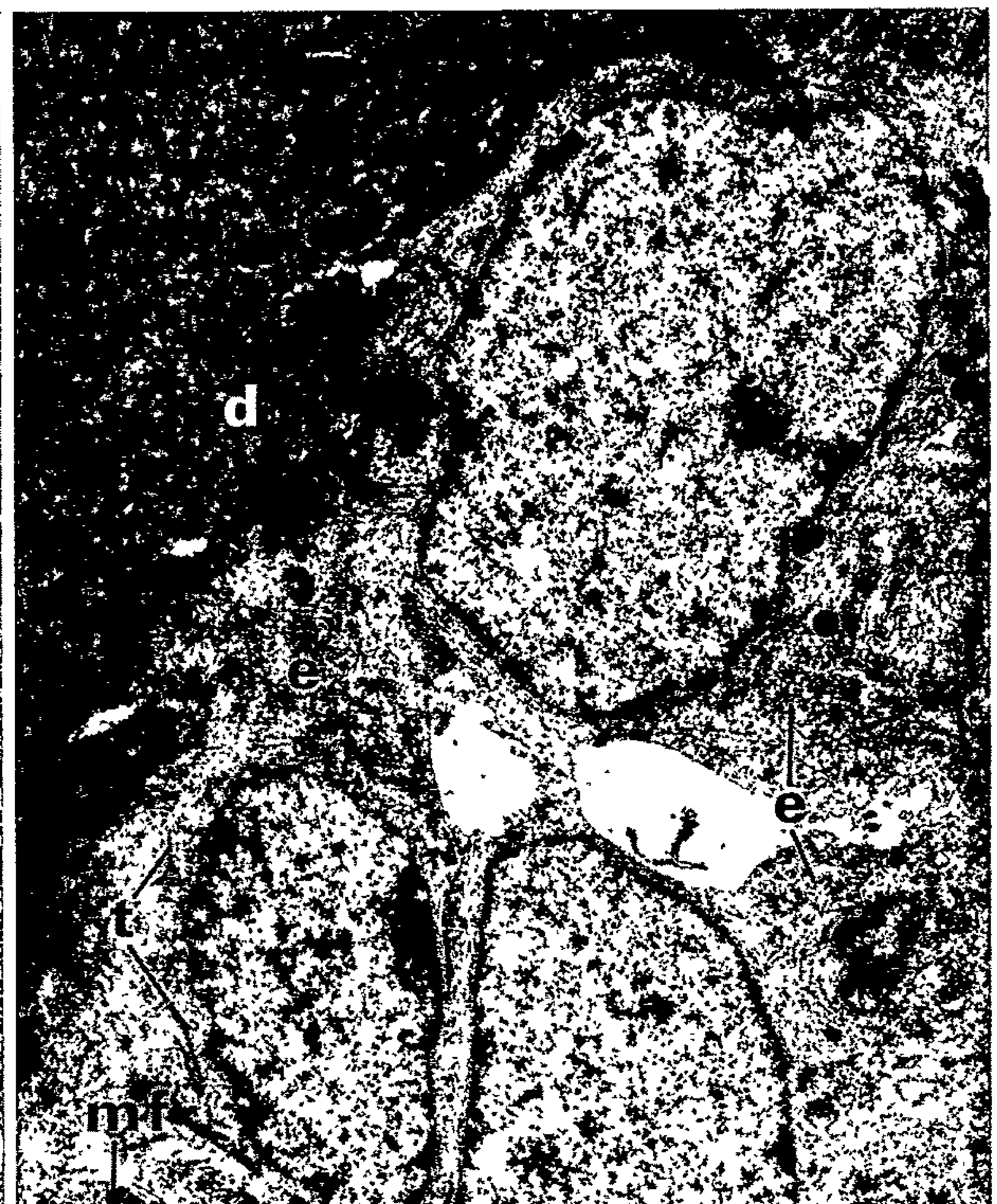
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Tight junctions may be formed at the site of contact between these processes (Fig. 25). Structures resembling coated vesicles [see Roth and Porter (*56*)], some of them open to the intercellular space, also occur along this epithelial-connective tissue interface (Figs. 23 and 24).

In this way, the epithelial-connective tissue interfaces on either side of the sheath become poorly demarcated and irregular. Subsequent mineralization of collagen (i.e., in animals older than 35 days) is commonly found to be more advanced on the cemental side of the developing root at any one transverse level (e.g., Fig. 22).

In longitudinal sections of root primordia. A preliminary attempt was made to determine whether obvious alterations in the ultrastructure of the sheath might be correlated with the cytodifferentiation of the connective tissue occurring on either side. Montages of the sheath and immediately adjacent tissue were prepared from a section of the same 25-day-old specimen illustrated earlier in Figs. 1–5. The images were examined for signs of discontinuity of the sheath basement lamina and for signs of contact between cells of the different compartments.

If one proceeds with such an examination from the apical end of the sheath (see Figs. 26 and 27) toward the root edge, the first positive sign of discontinuity of the sheath on the dental sac (cemental) side is found where a small process of an epithelial cell projects beyond the level of the basement lamina to contact the cytoplasm of a dental sac cell (Figs. 28 and 29). The site within the sheath at which collagen fibrils first appear is located slightly farther toward the advancing root edge, as are the sites of more patent, extensive disintegration of the sheath basement lamina of this side (Fig. 28). These changes occur at about that level where the dental sac cells first exhibit expanded sacs of granular endoplasmic reticulum and a more rounded, bulky cytoplasm (Fig. 28).

On the dental papilla (dentinal) side, the earliest indication of sheath discontinuity is where a small cytoplasmic extension of an epithelial cell projects beyond the level of the basement lamina (Figs. 30 and 31). This interruption of the basement lamina is located in the region where elongation of odontoblast cell bodies is occurring and where the first signs of mineralization of dentinal collagen are found (Fig. 31).

FIG. 18. Survey electron micrograph of a transverse section of the wall of a developing root of a 1st mandibular molar of a 35-day-old rat taken in advance of the level of mineralization. The epithelial sheath is enclosed between differentiating odontoblasts and cementoblasts and the collagen on either side produced by them. A cementoblast process projects between cells of the epithelial sheath (upper arrow). Collagen fibrils project from the cemental side into epithelial territory (lower arrow). ×1660.
FIG. 19. Higher magnification of one of the areas outlined in Fig. 18 showing collagen to be present between adjacent epithelial cells. Note the disintegration of the basal lamina on the cemental aspect (at right). ×1300.
FIG. 20. Higher magnification of one of the areas outlined in Fig. 18 to show a "differentiated" cell of Hertwig's epithelial sheath. Note the increased development of tonofilament bundles and granular endoplasmic reticulum, and the appearance of multivesicular bodies, membrane-bound dense granules, and coated vesicles. ×14,950.


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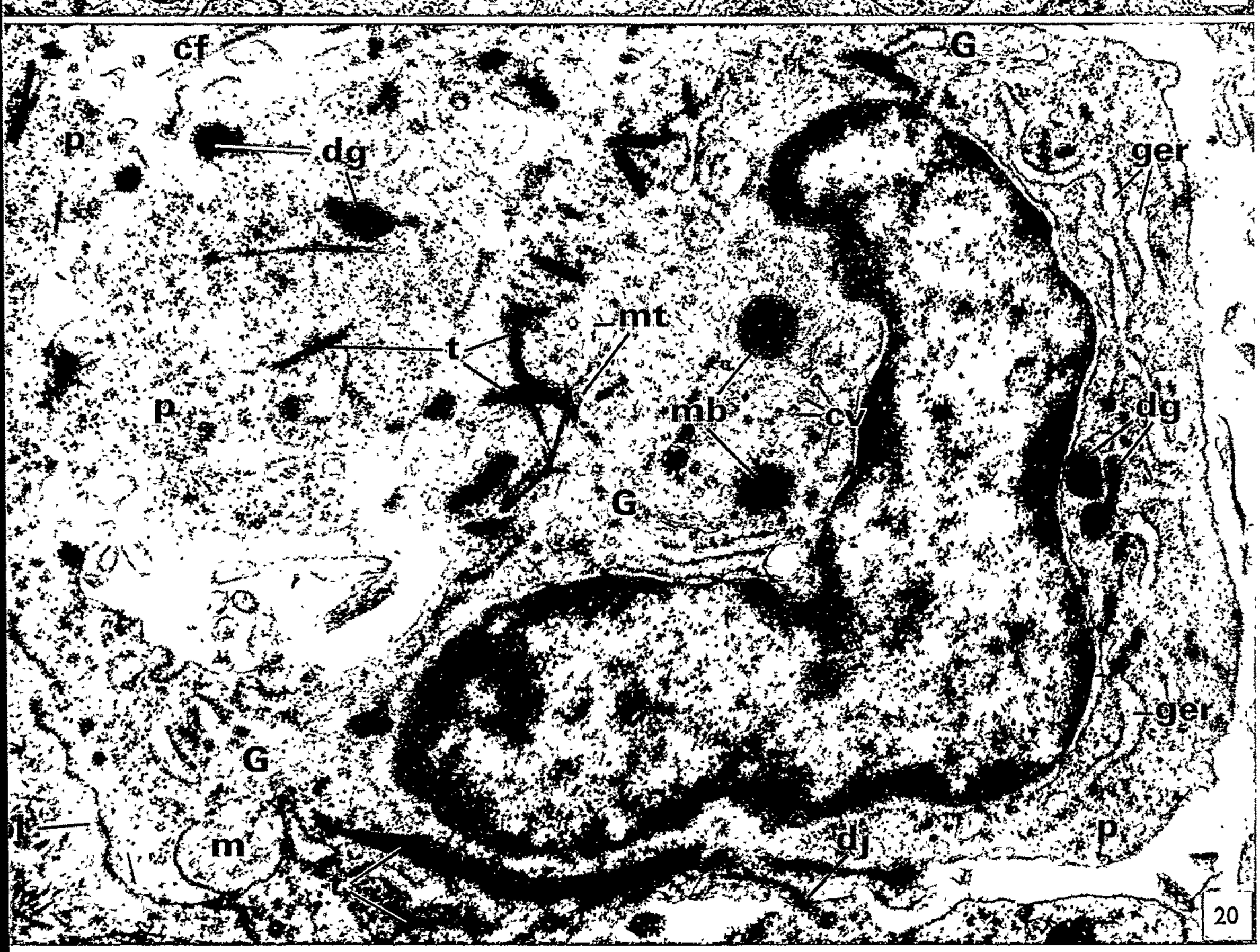
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DISCUSSION

Root and cementum formation

Presently held concepts of the sequence of developmental events occurring during root and cementum formation are based very largely on light microscopy (e.g., *9*, *12*, *16*, *21*, *29*, *47*, *54*, *60*, *65*). The application of electron microscopy to root and cementum formation (e.g., *62*, *63*, *69*) has, until how, confirmed and extended the earlier work. Thus, it is presently held that cementum deposition is dependent upon the prior degeneration of the epithelial sheath of Hertwig, the resulting discontinuity of epithelium permitting the deposition of cementum upon the exposed surface of the mineralized dentine. The normal course of interdependent events in root formation is generally considered to be something as follows (see Fig. 32A): (i) proliferation of epithelial sheath cells; (ii) differentiation of odontoblasts from cells of the dental papilla; (iii) deposition of dentinal matrix and its mineralization; (iv) degeneration of epithelial sheath cells; (v) establishment of contact between mineralized dentine and cells of the dental sac resulting in the differentiation of cementoblasts; (vi) deposition of cementum matrix and its subsequent mineralization.

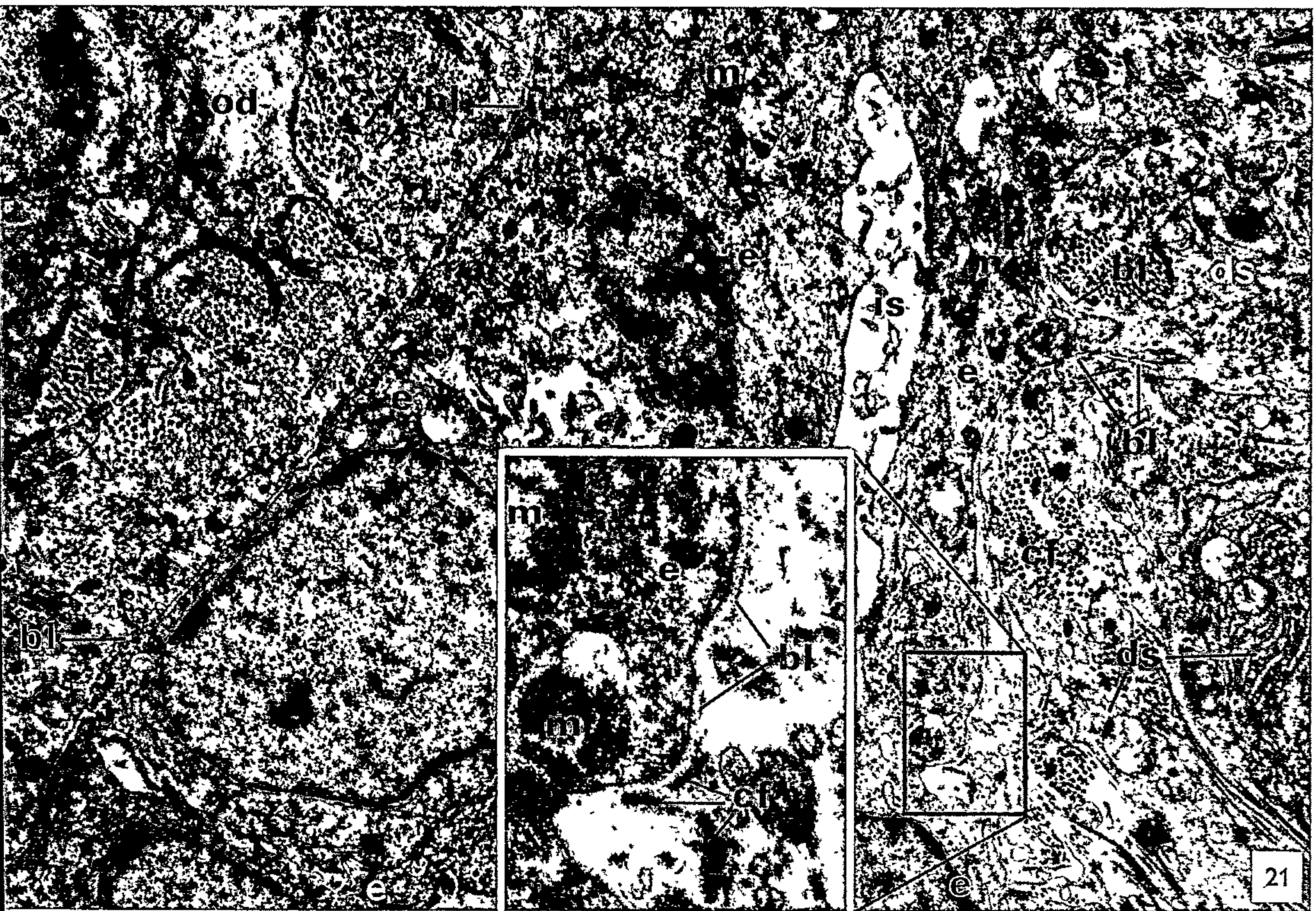
Those epithelial fragments of the sheath which survive the initial breakdown [(iv) above] are generally considered to migrate, or to be displaced, to the periodontal space where they are found in the adult (e.g., *44*, *48*, *49*, *51*, *72*) and are known as the "epithelial rests of Malassez".

It is clear from this and previous studies (*41*, *42*) that a fundamentally different sequence of events occurs in the cellular cementum phase of root formation in the rat molar compared to that generally described for the mammalian tooth. The essential difference is that the epithelial root sheath of Hertwig maintains its topographical relationship to the surface of the dentine while cellular cementum forms external to it (see Fig. 32B). This means that at a certain stage in development cementoblasts differentiate and cemental collagen is deposited (and in later stages mineralized) prior to mineralization of dentine. It follows, for the rat molar at least, that contact between the dental sac cells and the surface of the mineralized dentine is not a prerequisite for cementoblast differentiation and subsequent cementum production.

FIG. 21. Electron micrograph of a transverse section of an area in advance of mineralization taken from the wall of a developing root of a 1st mandibular molar of a 35-day-old rat. The basement lamina is relatively smoothly contoured and intact on the dentinal side (at left), and irregular and discontinuous on the cemental side (at right). ×6300.

Inset: Higher magnification of the area enclosed in Fig. 21 to show detail of discontinuous basement lamina on cemental side of sheath. ×18,900.

FIG. 22. Electron micrograph of a transverse section of the wall of a developing root of a 1st mandibular molar of a 40-day-old rat. Mineralization has commenced and is more advanced on the cemental side. The incorporated sheath has remained relatively intact. ×4000.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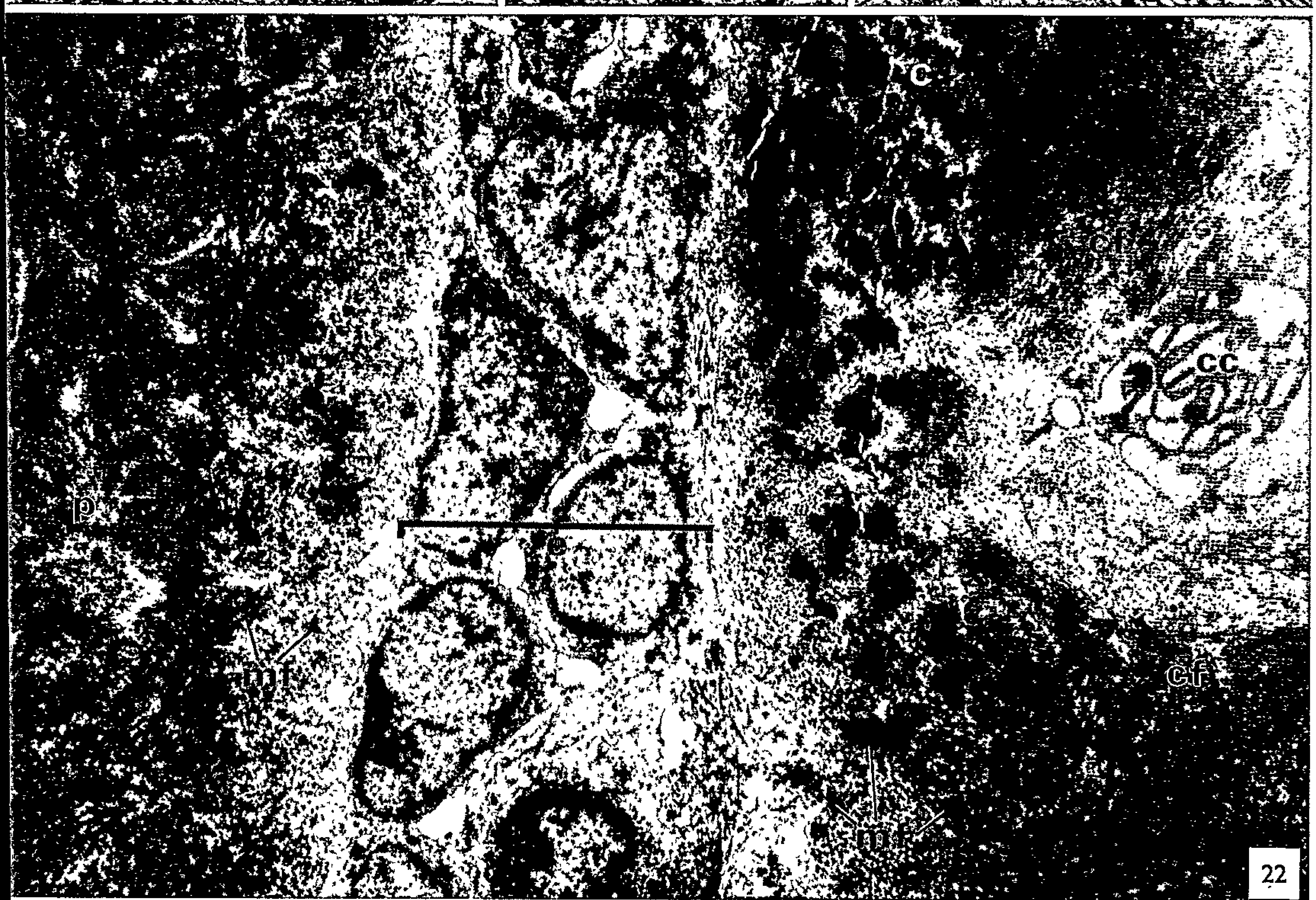
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Epithelial sheath–connective tissue relationship

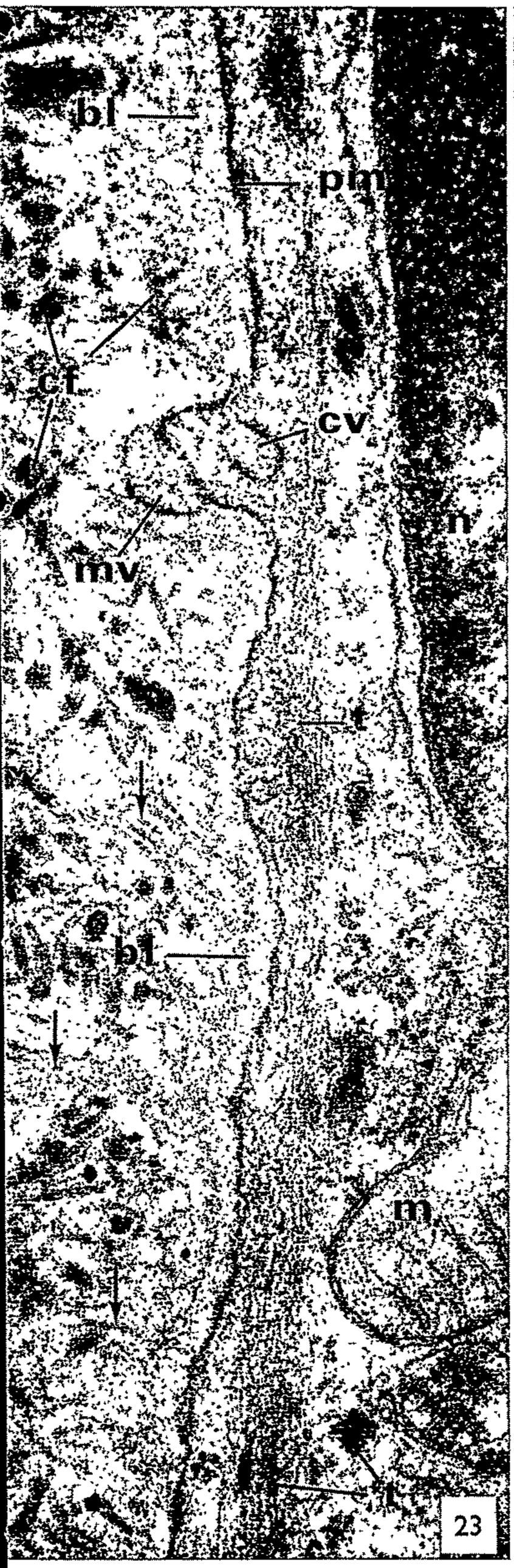
An interdependence of epithelial and mesodermal elements is known to be fundamental to the initiation of tooth development and to the normal formation of enamel and dentine (e.g., *19*, *32*, *37*, *40*, *77*). Morphological details of interfaces existing between the epithelial and connective tissue elements of the root primordium are important in this context and relevant to consideration of the nature of their interaction.

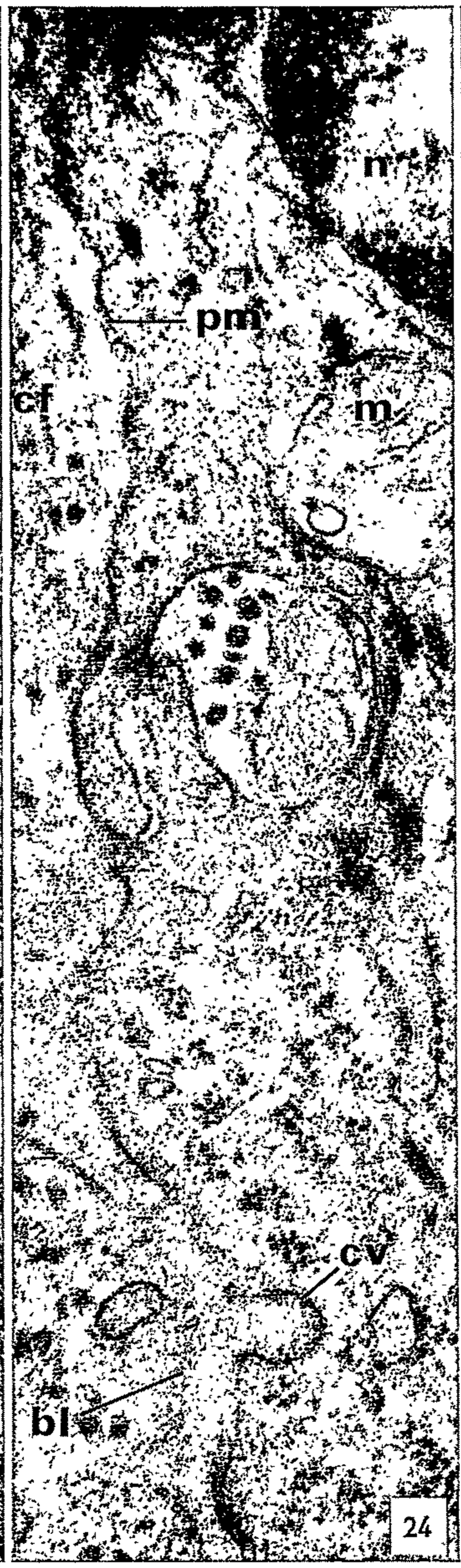
To recapitulate, the pertinent features of the 25- and 35-day-old specimens reported here are as follows. (i) Epithelial root sheath cells reach a previously unattained level of cytodifferentiation at about the time of cementoblast and odontoblast differentiation (Fig. 20). (ii) The basement lamina of the sheath at this same level is often discontinuous, and juxtaposition of epithelial and connective tissue cells may occur (Figs. 18, 28, and 29).

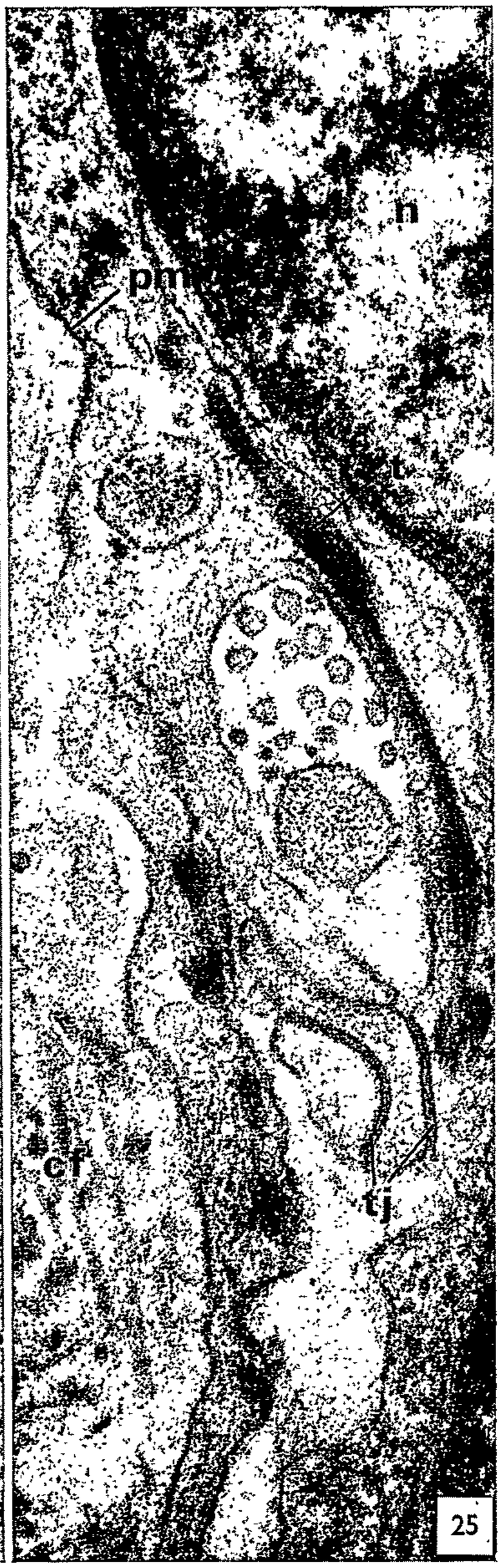
These features suggest to the existence of a possible mechanism for the exchange of information between epithelial and connective tissue compartments. Cell contact during embryogenesis without the formation of organized cell attachments has been demonstrated, *in vitro*, to have a profound effect on cell behavior (e.g., *1*, *73*). Also, materials produced *in vitro* at the interface between interacting mesodermal and epithelial tissues are regarded as likely mediators of inductive influences (*24*, *34*, *35*). It seems reasonable to postulate, therefore, that discontinuation of the normal morphological boundaries between epithelial sheath and dental papilla, and between epithelial sheath and dental sac, might be related to the differentiation of odontoblasts and dentine production; the differentiation of cementoblasts and cementum production; the degeneration and death of epithelial cells; and the overall coordination of these processes.

This kind of interpretation of the possibility of information transfer between morphologically communicating, previously intact, epithelial and connective tissue compartments has been made previously by a number of authors studying different developmental systems. Thus, Salpeter and Singer (*58*), studying limb regeneration in *Triturus*; Frei (*18*), studying epidermal tumours; Reith (*52*), studying enamel formation; and Farbman (*17*), studying fusion of palatal processes, have inferred that some kind of interaction between two tissue compartments influences subsequent differentiation and cellular activity.

Studies of epitheliomesenchymal relationships have, since the work of Grobstein (*22*), been concerned largely with assessing the effect of the mesodermal element on the differentiating epithelial one rather than vice versa, as pointed out by Grobstein (*23*, *26*) and Taderera (*71*). The developing root, however, is possibly unique in that only the mesodermal elements differentiate, producing two specific tissue types, cementum and dentine. The epithelium, for the greater part, degenerates as an ap-







FIGS. 23–25. Electron micrographs of transverse sections of a developing root of a 1st mandibular molar of a 35-day-old rat. Each image shows the interface between an epithelial sheath cell and the predentine.

FIG. 23. Illustrates the structure of the basement lamina on the dentinal side. Note the nonstriated fibrils (at arrows) and the discontinuity of the basement lamina at the site of a microvillous projection from the epithelial sheath cell. × 51,800.

FIG. 24. Shows a bay formed at the surface of the epithelial cell; transversely sectioned fibrils and cell processes lie within it. Note the small processes which appear to close off the bay. × 45,200.

FIG. 25. Similar to Fig. 24, but with the small processes closing off the bay forming tight junctions over their areas of contact. × 52,500.

parently necessary step in the developmental process allowing the formation of an intact cementum-dentine junction.

Some comparison may be made, with respect to the fate of epithelial cells, between root formation and fusion of palatal processes (see *17*, *66*). Farbman (*17*) raises the possibility that epithelial cell degeneration and death occurring with palatal fusion might result from information transfer between previously intact epithelial and connective tissue compartments. Farbman also cites a number of developmental instances where a loss of integrity of the basement lamina may be correlated with a simultaneous, rapid expansion of the part. It is unlikely, however, that a physical "stretching" of the basement lamina is a factor in its disintegration during root development. Indeed, the epithelial sheath seems to act during root formation as a template for the ultimate form of the root.

There is a growing awareness that certain cells may be functionally versatile in the sense of being capable of performing functions other than those classically associated with differentiated cells of their particular tissue type (e.g., *20*, *27*, *55*). For this reason, the possibility exists that the "differentiated" epithelial sheath cells synthesize some of the collagen found in close association with them (Figs. 23–25). Collagen synthesis by the sheath cells would provide the simplest solution to the problem of establishing an intact cementum-dentine junction. However, there is not the physical separation of epithelial and connective tissue elements as in the chick cornea (*20*). Moreover, in no instance were collagen fibrils found within the epithelial compartment in the absence of a nearby discontinuity of the basement lamina. For this reason, and despite the timely "differentiation" of the epithelial cells, the work of Kallman and Grobstein (*35*) should be kept in mind as possibly the more applicable to this particular site.

Epithelial sheath and cementogenesis

It has been proposed previously that the epithelial sheath, in addition to its classically conceived role in the induction of odontoblast differentiation, may be involved in some way in the induction of cementoblast differentiation (*31*, *49*, *59*, *60*). The

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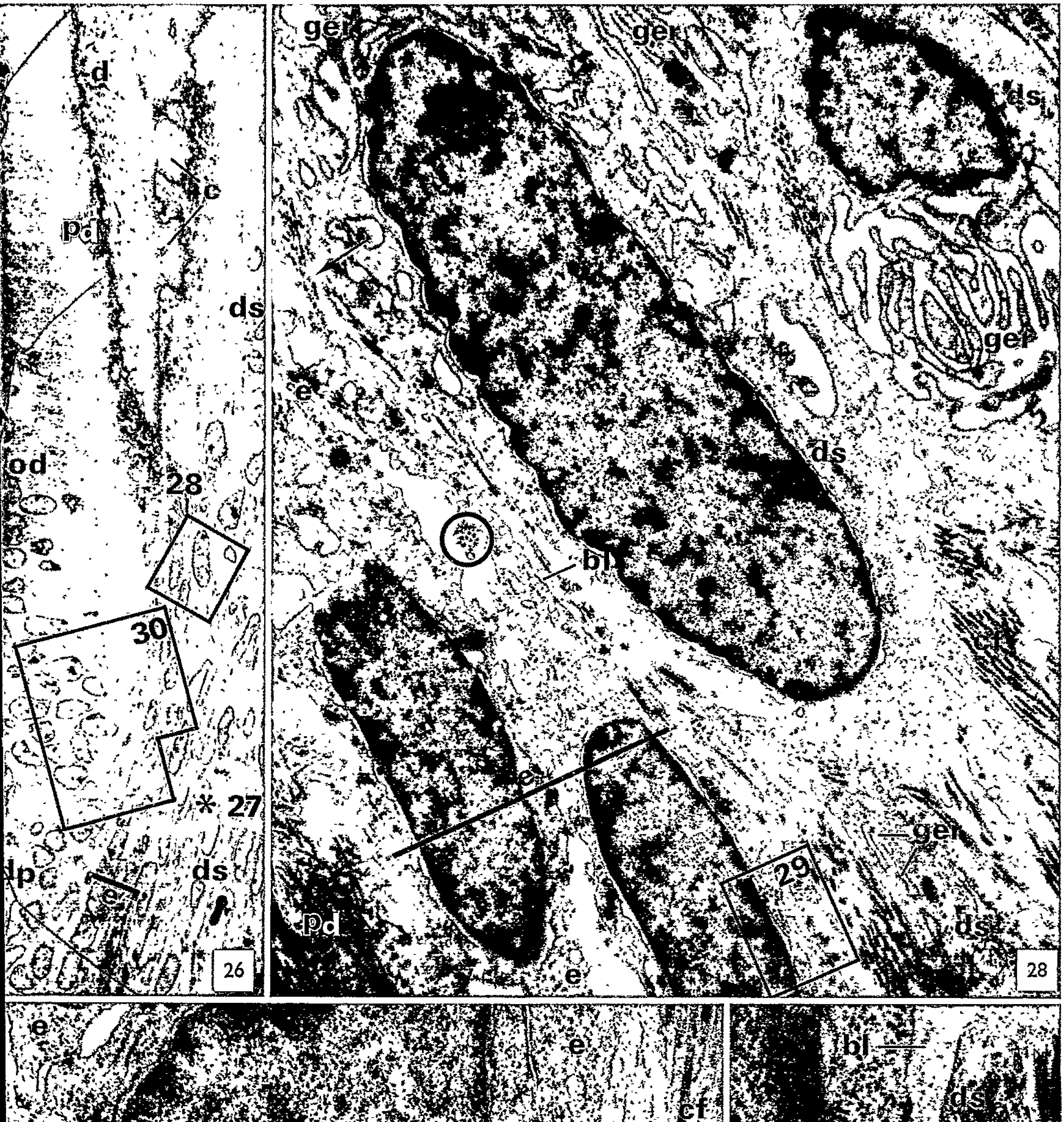
FIG. 26. Low magnification electron micrograph of a longitudinal section of the advancing edge of the wall of a developing root of a 1st mandibular molar of a 25-day-old rat, showing areas subsequently enlarged in Figs. 27, 28, and 30. ×620.

FIG. 27. Higher magnification of an area of Hertwig's epithelial root sheath taken at level of asterisk in Fig. 26. The basement lamina on either side is intact. ×29,000.

FIG. 28. Higher magnification of one of the areas outlined in Fig. 26. Note the microvillous projection extending beyond the level of the basement lamina to contact a cell of the dental sac (enlarged in Fig. 29); the collagen fibrils within the sheath (encircled); the frank discontinuity of the basement lamina (at arrow); and the appearance of numerous expanded sacs of granular endoplasmic reticulum in the differentiating cells of the dental sac (cementoblasts). ×9500.

FIG. 29. See description in Fig. 28 legend. ×39,000.



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present observations lend support to this idea by showing that for the rat molar root: (a) the most commonly invoked "inducer" of cementoblast differentiation, the surface of the mineralized dentine, is not available in the usual way; and (b) there exists a possible mechanism for information exchange, at a relevant stage in development, between epithelial sheath and dental sac.

The unusual nature of root formation in the rat molar (see Fig. 32) may be interpreted in diametrically opposite ways with regard to the question of a possible sheath–cementogenesis relationship. One interpretation is that the formation of cellular cementum is quite independent of the epithelial root sheath. Diab and Stallard (*15*) are of this opinion from their labeling studies. The present paper proposes the alternative view. It is suggested that a change in the relative rates of formation of cementum and dentine is central to the mode of root formation in the rat molar, and that the timetable for gross degeneration of the epithelial sheath cells does not alter correspondingly. The present observations further suggest that the obvious end result, viz, the sheath embedded at the advancing root edge, does not alter the potential for information exchange between sheath and dental sac prior to cementogenesis.

Certain qualifying factors must be kept in mind in considering the question of the relationship between sheath and cementogenesis. First, we have no way of knowing the precise influence of genome on the behavior of either of the two cell-types involved. Second, actual breakdown of the basement lamina may be a necessary step in the subsequent degeneration of epithelial sheath cells, but not in the differentiation of cementoblasts. Further, the relative contribution of these two cell-types to breakdown of the basement lamina is unknown, although the coated vesicles of the sheath cells may be significant here [see Reith (*52*)]. Third, one should be careful not to exaggerate the importance of the phase of "overt differentiation" (*25*)—and what may well be the final link in a long chain of inductive influences [see Jacobson (*33*)]. Finally, there is the possibility of the additional involvement of functional stimuli in the onset of cellular cementum formation. This last is discussed below.

Occlusal function and cementogenesis

As stated above, the peculiarity of root formation in the rat molar may be interpreted as a reversal of the characteristic differential in the rates of formation of dentine and cementum. From a relatively slow (*30*), but typically mammalian, rate of formation during its acellular phase, the cementum comes to overtake and to

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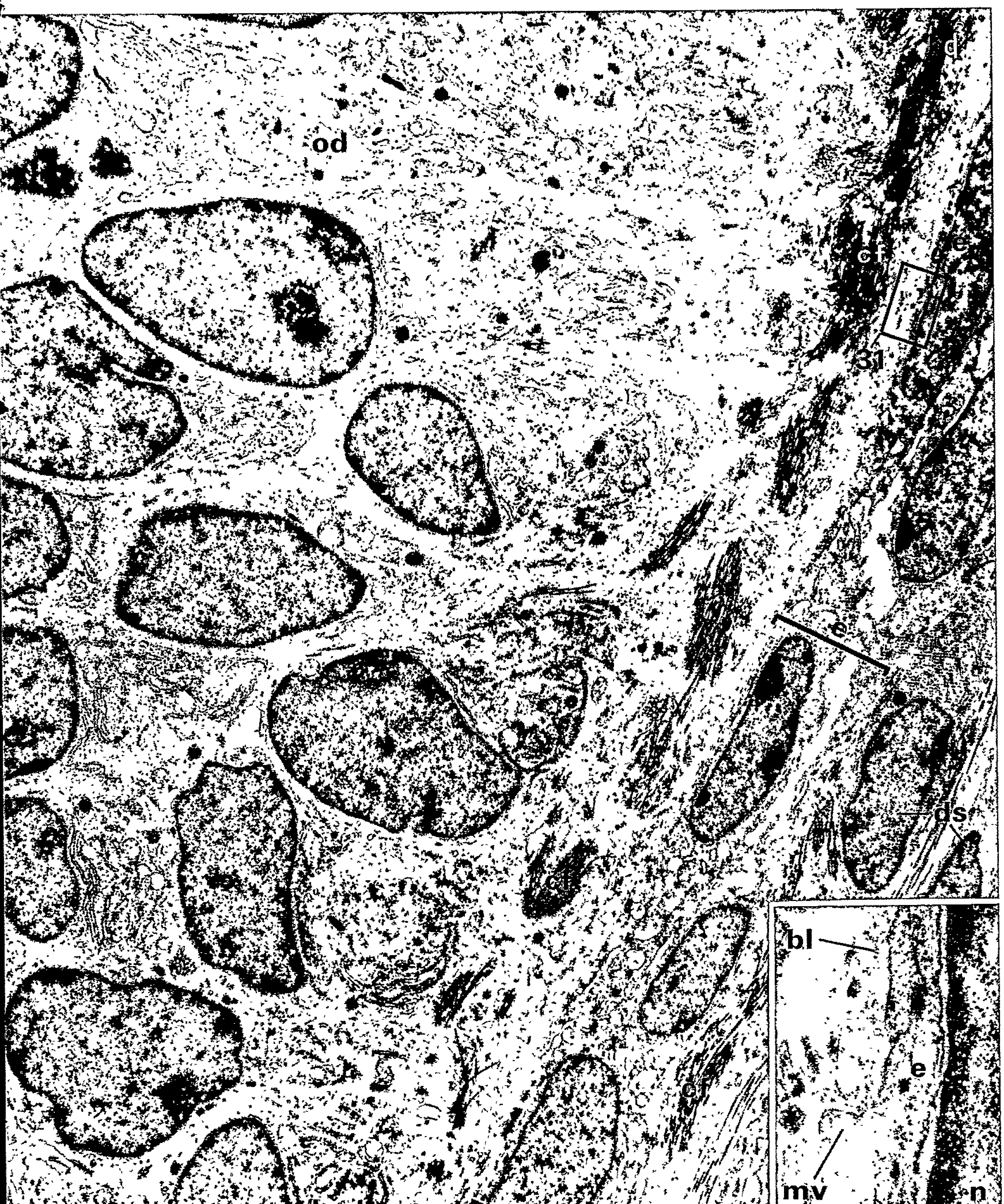
FIG. 30. Higher magnification of one of the areas outlined in Fig. 26. Note the progressive elongation (from bottom to top) of cells of the dental papilla (differentiating odontoblasts); the increasing bulk of collagen produced; and the initial discontinuity of the basement lamina on the dentine side (enlarged in Fig. 31). ×4500.

FIG. 31. See description in Fig. 30 legend. ×30,000.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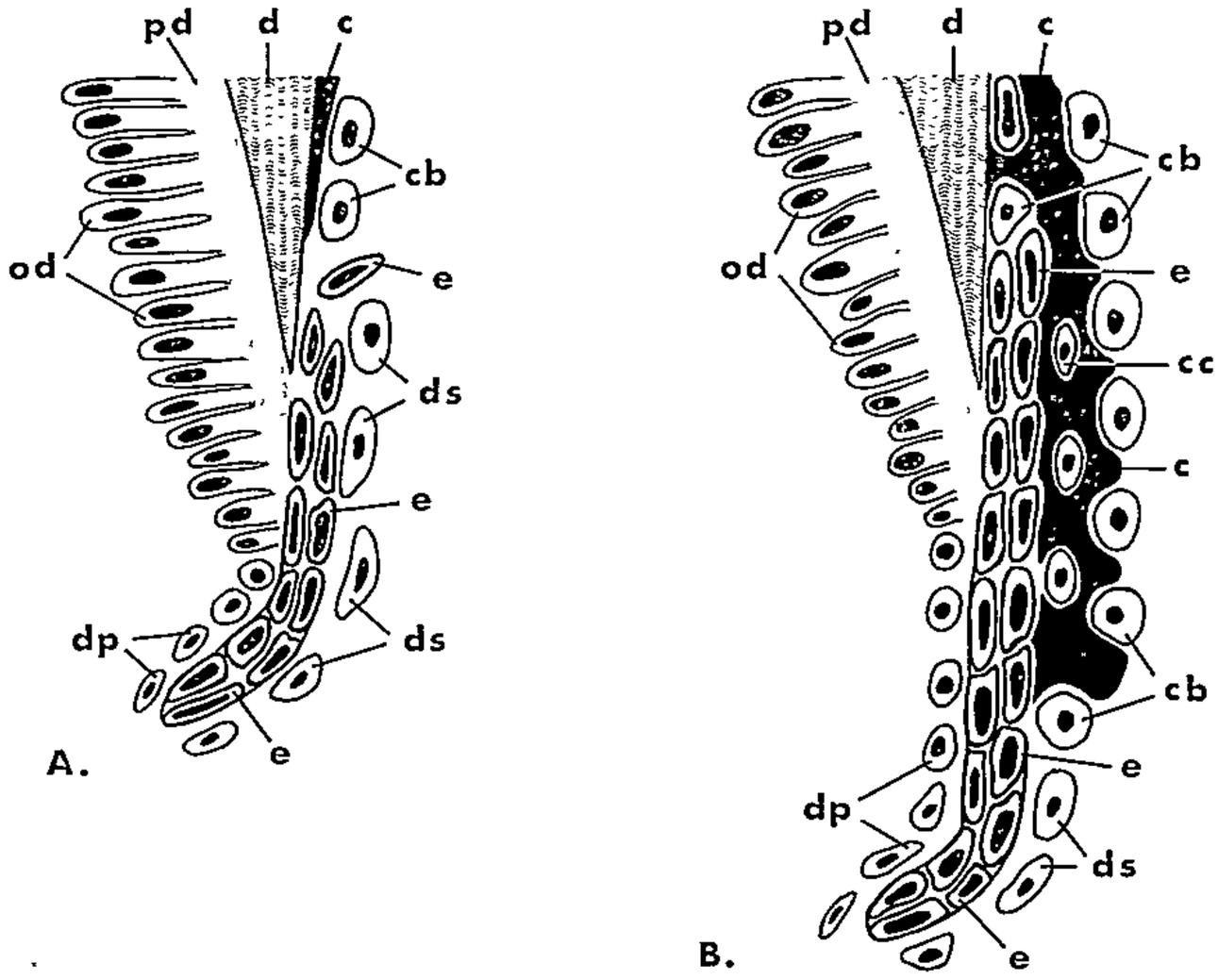

FIG. 32. Diagram representing two hypothetical longitudinal sections from the advancing edge of the wall of a developing root of a rat molar at different stages of development to show two phases of cementum formation. (A) The acellular cementum phase. Diagram shows the disruption of epithelial root sheath; the commencement of epithelial cell migration; and the deposition of cementum by differentiated cells of the dental sac. (B) The cellular cementum phase. Diagram shows cementum forming in advance of dentine and cells of Hertwig's epithelial root sheath becoming embedded between cementum and dentine at the advancing root edge.

precede the dentine at the advancing root edge during its final, rapid (*14*, *28*, *50*, *76*), cellular phase. This reversal of the relative rates of formation takes place, for the first mandibular molar, during the period of from 25 to 35 days after birth, from which time on cellular cementum constitutes the bulk of the developing root.

The actual commencement of cellular cementum formation for the first mandibular molar of the rat has been variously timed at 35 [Hoffman and Schour (*30*)], 30 [O'Brien *et al.* (*46*)], 19 [Paynter and Pudy (*50*)] and 15 [Diab and Stallard (*15*)] days after birth. In this study, cellular cementum formation was found to be under way by 25 days after birth. This is the time at which the first molars are reported either as having established initial occlusal contact (*61*) or as having commenced functional occlusion (*8*, *15*, *46*).

It is known that a relationship exists between continued cementum deposition and tooth function. The exact nature and "directness" of the relationship is not known although it has been the subject of considerable discussion (e.g., *2*, *3*, *36*, *38*, *39*, *45*, *67*, *74*, *76*). The timing, however, suggests that the final or cellular cementum phase of root formation in the rat molar might be a response to initial occlusal contact and directed to the provision of an appropriate supporting apparatus.

Should a direct relationship exist here between functional forces acting on the tooth

crown and an increased rate of cementum production (and this should be amenable to experiment), the theoretical question arises as to mediation of the stimulus and its translation into cementogenesis. It might well be that the cells of the sheath, or of the dental sac, or both, are susceptible in some way to alternating pressures and tensions mediated by the developing root itself. Biological piezoelectricity could possibly provide a mechanism for this [see review by Bassett (*4*)]. This effect has been elicited from dentine (*10*) and from teeth *in situ* (*13*). It has also been postulated to affect cellular activity and to influence osteogenesis and bone remodeling processes (*5*, *6*, *64*), although its relevance to the wet *in vivo* state remains uncertain.

Epithelial sheath and the root apex

It is worth noting that Hertwig's epithelial root sheath is represented during the later stages of root formation (cf. *15*), although the three-dimensional configuration of the sheath is difficult to envision. The irregularity of its form in longitudinal section (Figs. 14–17) may be due to a partial folding of the sheath on itself. The gradual positioning of the sheath, and thereby of the prospective cementum-dentine junction, toward the midline of the tooth contributes to the closing of the root apex. It is because of this relocation, and the greater rate of cementum production relative to the dentine, that the apex comes to be constituted largely by cellular cementum—approximately one-third of the total bulk of the root in the adult [see Schour and Massler (*61*)].

Other evidence of its peculiar mode of development remains with the root in the adult state. Many of the channels and large lacunae are retained in adult rat cellular cementum, especially in the region of the cementum-dentine junction where their localized accumulation is pronounced [see also (*7*)].

Epithelial remnants

There is evidence of cellular degeneration, including intracellular mineralization, in those incorporated epithelial cells located farthest (coronally) from the developing root edge (Lester—in preparation). These degenerative changes are more marked when the epithelial cells come to be completely surrounded by a greater bulk of cementum.

Wentz *et al.* (*75*) studied the distribution of epithelial remnants in the periodontal tissues of the rat molar. They found the number of epithelial remnants to be greatest in the cervical area of the root (44–47 % of the total found) and least in the apical region (5–8 % of the total found). The embedding of Hertwig's epithelial sheath during the formation of the root apex, as described in the present paper, offers an explanation for the low incidence of epithelial rests in the apical area. Certainly, the fate of the epithelial sheath during development of the roots of rat molars should be taken into account in studies involving their periodontium.

I am indebted to and wish to thank Miss Anne-Marie Lindgren for her skilled and kindly technical assistance. I am very grateful to Dr. E. J. Reith for his helpful advice and discussion during the course of the work and for his critical reading of the manuscript. The study was made possible by Dr. J. A. G. Rhodin, who continues to provide encouragement and support. I wish to thank Mrs. Betsey Stiepock and Miss Carol Schuh for typing the manuscript.

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