Overview of morphological changes in enamel organ cells associated with major events in amelogenesis

CHARLES E. SMITH[1]* and ANTONIO NANCI[2]

[1]Departments of Anatomy and Cell Biology, and Oral Biology, McGill University and
[2]Departments of Anatomy and Stomatology, Université de Montréal, Montreal, Quebec, Canada

ABSTRACT The formation and mineralization of enamel is controlled by epithelial cells of the enamel organ which undergo marked, and in some cases repetitive, alterations in cellular morphology as part of the developmental process. The most dramatic changes are seen in ameloblasts which reverse their secretory polarity during differentiation to allow for extracellular release of large amounts of proteins from plasma membrane surfaces that were originally the embryonic bases of the cells. Secreted enamel proteins at first do not accumulate in a layer but, in part, percolate into the developing predentin and subjacent odontoblast layer. Appositional growth of an enamel layer begins with mineralization of the dentin, and ameloblasts develop a complicated functional apex (Tome's processes) to direct release of matrix proteins, and perhaps proteinases, at interrod and rod growth sites. Once the full thickness of enamel is produced, some ameloblasts degenerate, and the surviving cells shorten in height and spread out at the enamel surface. They reform a basal lamina to cover the immature enamel, and continue producing small amounts of enamel proteins that pass through the basal lamina into the enamel. Ameloblasts also undergo cycles of modulation where apical invaginations enriched in Ca-ATPases and other enzymes are formed and shed on a repetitive basis (ruffle-ended/smooth-ended transitions). As this happens, apatetic crystals seeded earlier expand in volume by gradual layering of new mineral at the surfaces of the preformed crystals. Ameloblasts stop modulating when the crystals almost fill existing volume formerly occupied by protein and water. The ameloblasts then shrink in size and the Golgi apparatus returns to its original position at the pole of the nucleus opposite the enamel. Other cells of the enamel organ show major changes in morphology shortly after ameloblasts begin modulating when they form the papillary layer in intimate association with vascular channels from the adjacent connective tissue layer.

KEY WORDS: *amelogenesis, morphometry, ameloblasts, enamel organ, enamel*

Introduction

Amelogenesis is a complex process which leads to the creation of a fully mineralized layer of enamel on the crowns of teeth. A stratified epithelium, the enamel organ, controls this process. It is widely accepted that cells of the innermost layer of the enamel organ, the ameloblasts, are mainly responsible for producing and secreting various proteins detected within the organic matrix of this hard tissue (reviewed in Boyde, 1989; Deutsch, 1989; Nanci and Smith, 1992). These cells also are responsible for controlling organization of enamel into characteristic rod and interrod patterns (Warshawsky *et al.*, 1981), and they likely influence movement of the large amounts of calcium and phosphate ions required for sustained growth of the unique apatetic crystals which develop in this hard tissue (Bawden, 1989; Takano *et al.*, 1992).

Enamel development traditionally is classified into three main stages called (1) the presecretory stage when the supporting layer of mantle dentin is first formed by odontoblasts and undergoes its initial mineralization, (2) the secretory stage when a partially mineralized enamel layer is formed to its full thickness, and (3) the maturation stage where the mineral content of the enamel layer is increased to its final level (reviewed in Warshawsky, 1985). The same classification usually is applied to the enamel organ cells as a guide to sequential steps in their life cycles (e.g., Warshawsky and Smith, 1974). Hence, for example, presecretory stage ameloblasts have been viewed as cells devoted to differentiation, secretory stage ameloblasts to production and release of enamel proteins, and maturation stage ameloblasts to resorption of matrix proteins and transportation of calcium ions (reviewed in Nanci and Smith, 1992). It is becoming increasingly clear that this view is too simplistic, and cells such as ameloblasts are capable, for example, of secreting proteins throughout amelogenesis and not just during the "secretory" stage. It is mostly a difference relative to the types and the quantities of proteins that are being

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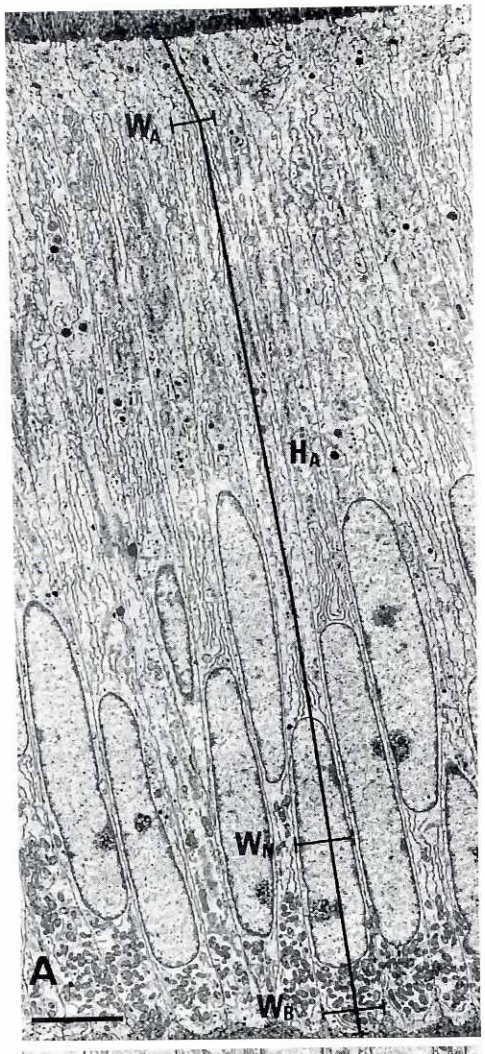
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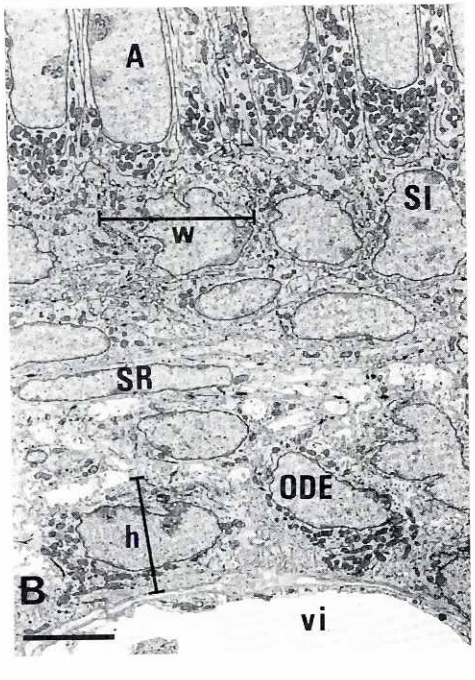
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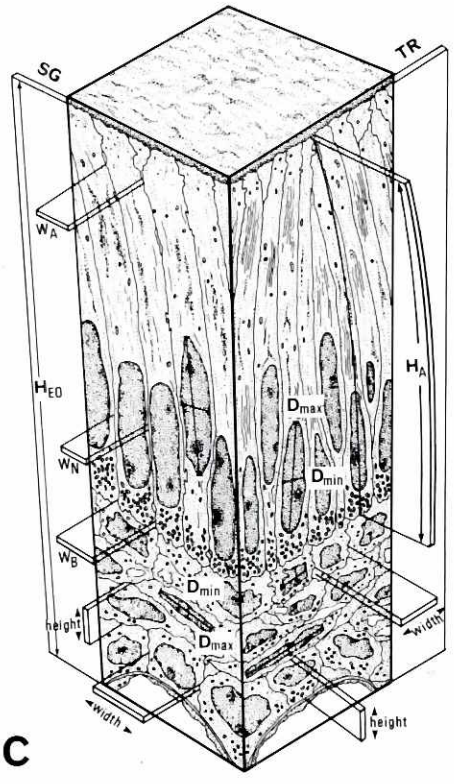
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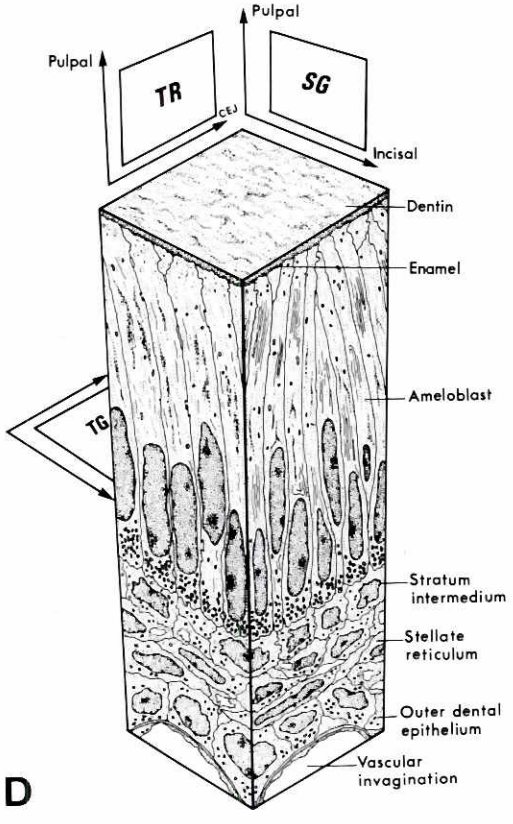
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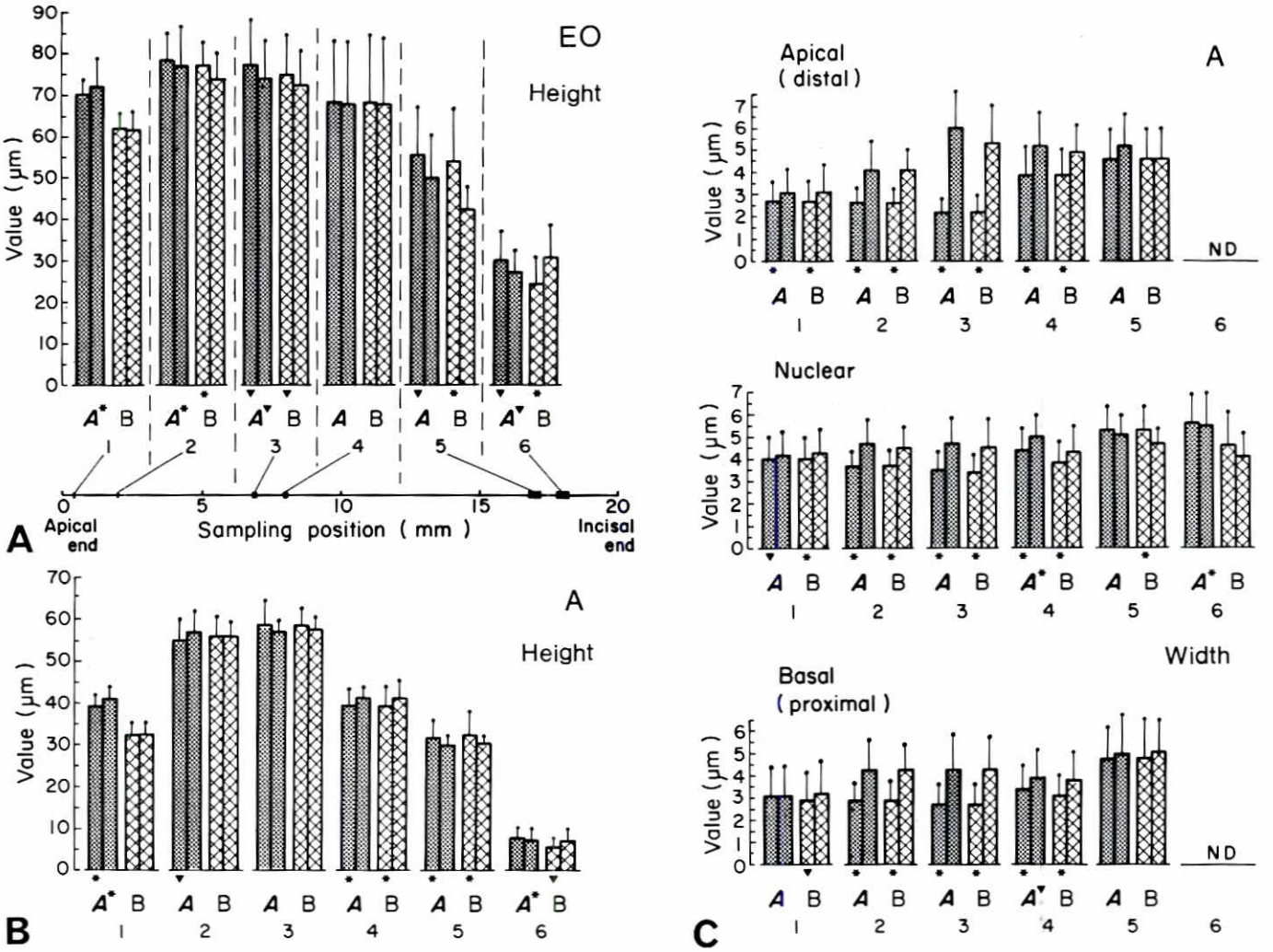

Fig. 2. Morphometry of enamel organ and ameloblasts. (A) *Bar graph illustrating mean profile height of enamel organ (EO)±standard deviation (SD) as measured in electron micrographs at 6 locations along the incisor (1-6; represented by horizontal line and solid rectangles drawn below the graph) for rats perfused with two different fixatives (Method A, first of pair, and Method B, second of pair, at each location). Measurements at each location were done using thin sections cut in the transverse (left-hand bar of each pair) and sagittal (right-hand bar of each pair) planes of the incisor. Significance levels for statistical tests comparing data by plane of section or fixative method are indicated by inverse triangles ($p<0.05$) and asterisks ($p<0.01$) beneath the bars or superscripted to method A, respectively.* **(B)** *Bar graph showing mean profile height of ameloblasts (A)±SD at each sampling position.* **(C)** *Bar graphs showing mean profile width of ameloblasts (A)±SD as measured near the apical junctional complex (top; W_A in Fig. 1C), nuclear compartment (W_N in Fig. 1C), and basal junctional complex (W_B in Fig. 1C). The general arrangement of each graph is explained in the legend to* **A.** *ND, not determined.*

produced at any moment in time. The compositional and structural differences between enamel and other mineralized tissues including bone, dentin, cartilage and cementum appear related in part to the basic origin of enamel as a secretory product of epithelial cells and the unusual non-collagenous matrix proteins these cells express (reviewed in Deutsch, 1989; Nanci and Smith, 1992). In another sense, the uniqueness of enamel appears related to differences in how cells evolve through their life cycles

Fig. 1. Structural organization of enamel organ. (A and B) *Ultrastructural appearance of ameloblasts* **(A)** *and associated epithelial cell layers of the enamel organ (panel B; SI, stratum intermedium; SR stellate reticulum; ODE, outer dental epithelium; vi, vascular invagination) as seen in low power electron micrographs of mandibular rat incisors at a time just prior to when the ameloblasts create the Tomes' processes (sampling position 2 illustrated in Fig. 2A). Height (H_A) and "width" (W_A, W_N, W_B) measurements for ameloblasts (panel A) and other enamel organ cells* **(B)** *h, height; w, width) correspond to measurement parameters as illustrated in the schematic drawing of* ***C****. Bars, 5 μm.* **(C)** *Schematic drawing illustrating measurement parameters for height of the enamel organ (H_{EO}), height (H_A) and "width"» of ameloblasts at their apical junction complex (W_A), nucleus (W_N), and basal junctional complex (W_B), and for height and "width" of SI, SR and ODE cells within two planes of section (TR, transverse, and SG, sagittal; data from each plane shown as paired bars in Figs. 2-5). The terms D_{max} and D_{min} refer to long and short axis measurements of nuclei, the data of which are not included in this report. Note that height measurements for ameloblasts were done along the midline of the cells following their natural curvatures (curvilinear height).* **(D)** *Schematic drawing illustrating organization of enamel organ and adjacent tissues, and the planes of section for rat incisors. The SG plane is equivalent to a labial-lingual cut and the TR plane to a mesial-distal cut on human teeth. Note that cells of the stratum intermedium (SI), stellate reticulum (SR), and outer dental epithelium (ODE)* **(B and D)** *for sampling positions 4, 5, and 6 (Fig. 2A) form part of the papillary layer designated as PL_1, PL_2, and PL_3, respectively, in Figs. 2-5 by positional analogy to the their arrangement at sampling positions 1-3.*

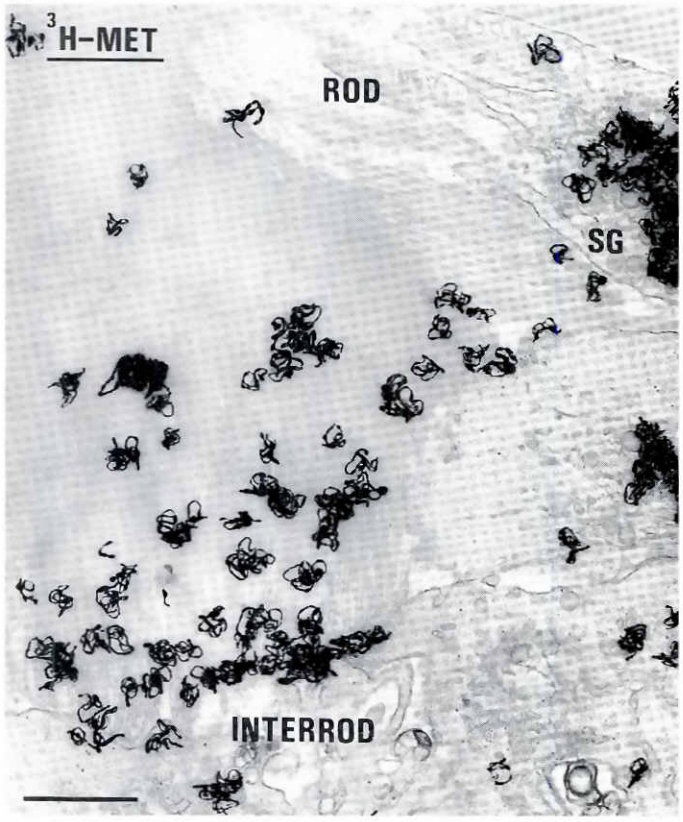

Fig. 3. Micrograph illustrating radioautographic reaction found at the rod and interrod growth sites at 20 min after injection of 3H-methionine. *Numerous silver grains are evident outside the cell over the interrod growth site but they are confined mostly to secretory granules (SG) within the core of the Tomes' process at the rod growth site. Bar, 1 μm.*

in these hard tissues. For example, in bone there are separate cell lineages related to tissue function. Osteoblasts, derived from perivascular stromal cells, form a vascular enclosed matrix of relatively finite thickness. They regulate acquisition of mineral and maintain the bone over a long period of time as entrapped osteocytes (reviewed in Marks, Jr. and Popoff, 1988). Osteoclasts, derived from monocytes, degrade and remove areas of aged bone so it can be renewed as part of general skeletal remodelling and turnover or in response to local stress (reviewed in Marks, Jr. and Popoff, 1988; McKee and Nanci, 1993). In dentin there is only an element of formation; odontoblasts, derived from neural crest cells, create an avascular mineralized tissue of relatively infinite thickness and lifespan (reviewed in Linde and Goldberg, 1994). Each odontoblast permanently maintains the territory of dentin it produces via long cytoplasmic extensions left behind in the matrix as it slowly recedes deeper into the pulp chamber (Ten Cate, 1994). In enamel, there is also only an element of formation, and the ameloblasts, derived from stratified oral epithelium, initially produce a partially mineralized, avascular tissue of finite thickness that is specific, and different, for various locations on the crown of the tooth (reviewed in Warshawsky, 1985; Boyde, 1989). Proteins comprising the organic matrix of the newly formed enamel are then processed and degraded extracellularly, and protein fragments, together with water, are slowly withdrawn and replaced by an equivalent volume of mineral (reviewed in Robinson and Kirkham, 1985). The final completely acellular product has a mineral content that is among the highest of any hard tissue produced biologically and is intended to have infinite lifespan.

Differentiation of ameloblasts

Ameloblasts begin their life cycle as a layer of low columnar, proliferative cells sitting on a basement membrane as established from their origin out of the stratum basal layer of the oral epithelium and maintained across bud and cap stages via inner dental epithelium (Ten Cate, 1994). At this time, there is little specificity in the system and any uncommitted epithelial cell can serve to become ameloblasts in combination with appropriate tooth mesenchyme (Ten Cate, 1994). The cells have their rudimentary Golgi apparatus positioned at the pole of the nucleus on the side away from the basement membrane (facing toward the outer cell layers of the enamel organ) (Matsuo *et al.*, 1992, 1993). Following induction of their differentiation by underlying odontoblasts and/or predentin, the preameloblasts undergo dramatic changes in volume derived mostly from increases in cell height and sagittal width (Figs. 1 and 2). The change in cell height is created mostly within the region between nucleus and basement membrane (future functional apex), and involves large increases in the cytoplasmic volume devoted to endoplasmic reticulum and Golgi apparatus (Smith, 1984). Ameloblasts reverse secretory polarity as they differentiate through movement of the Golgi apparatus around the nucleus (Matsuo *et al.*, 1992). Such reversal of polarity presumably requires reprogramming of control mechanisms employed to receive exportable proteins at apical versus basolateral surfaces since components of the basement membrane which had been targeted basally will be substituted by enamel proteins being targeted apically. Ameloblasts secrete small amounts of enamel proteins from their functional apical surfaces as they differentiate (Inai *et al.*, 1991; Nanci and Smith, 1992; Nakamura *et al.*, 1994). These do not accumulate in a layer but diffuse into the lamina fibroreticularis of the basement membrane separating the differentiating ameloblasts from the predentin and odontoblast (reviewed in Nanci and Smith, 1992). Later, some enamel proteins accumulate as patches within the forming mantle dentin and others percolate through the developing mantle dentin to the level of the cell bodies of odontoblasts and deeper regions of the dental pulp (Inai *et al.*, 1991; Nanci and Smith, 1992; Nakamura *et al.*, 1994). The function of these proteins is known although they could act as morphogens during these early phases of tooth development.

Appositional growth of the enamel layer

Just prior to dentin mineralization, ameloblasts engulf and internalize into their lysosomal system basement membrane components lying at the future dentinoenamel junction (Sawada *et al.*, 1990). The cells then rapidly secrete large amounts of enamel matrix proteins from their functional apical surfaces (Smith *et al.*, 1992). These proteins accumulate as an initial rodless (aprismatic) layer (Warshawsky, 1978) on top of the mantle dentin which is now mineralized (Boyde, 1989; Ten Cate, 1994). As this initial layer forms, the ameloblasts develop cytoplasmic extensions from their apical surfaces called the distal or interdigitating portion of Tomes' processes (Warshawsky, 1978). They presumably do this by secreting enamel proteins in focal regions that will become the future bases of the interdigitating portions of the processes. This ultimately creates a network of interrod cavities continuous with the initial rodless layer of enamel (Warshawsky, 1978). Soon thereaf-

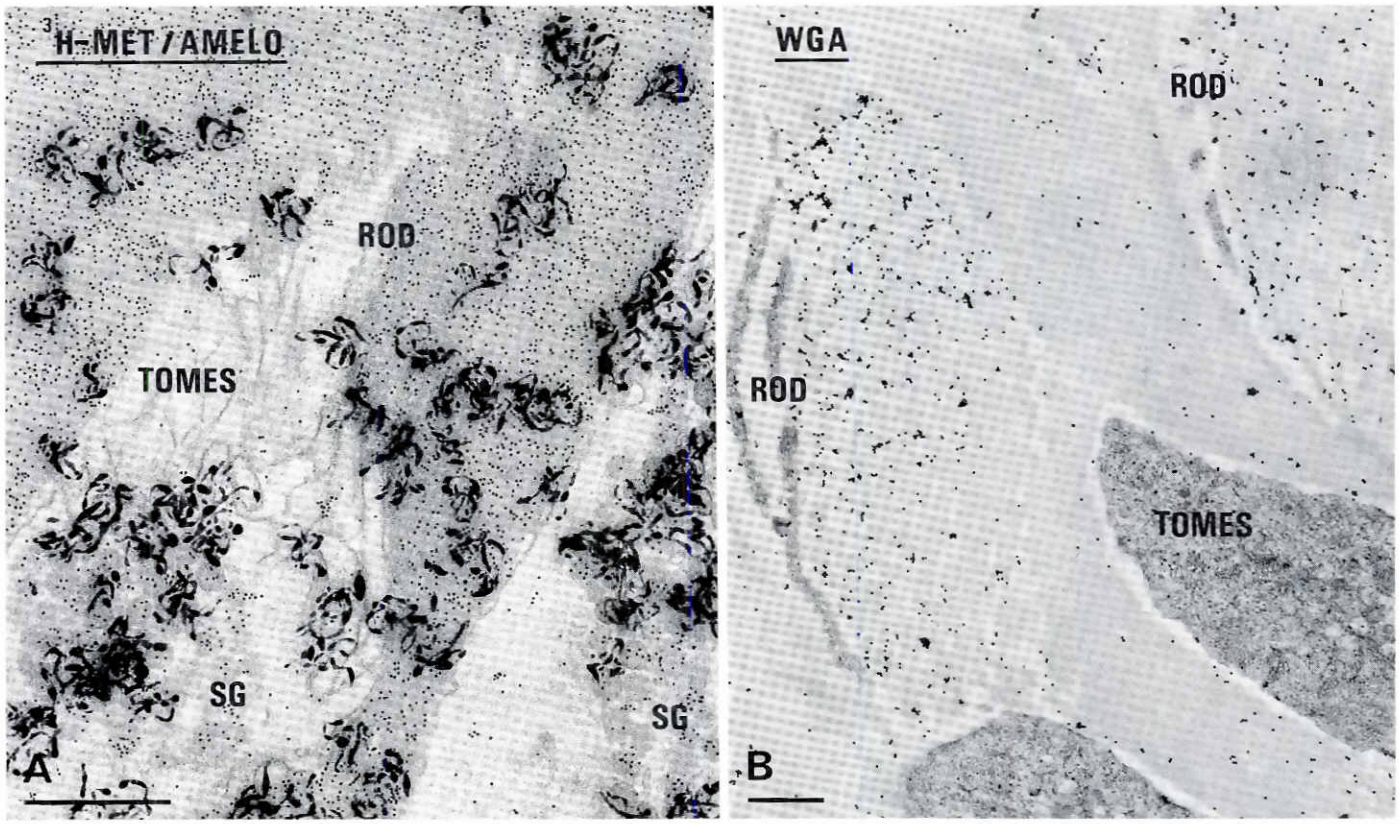

Fig. 4. Combined radioautographic and immunocytochemical preparation (A) and cytochemical preparation (B) illustrating labeling pattern for proteins at rod growth site. (A) *Radioautograph at 1 h after injection of ^{3}H-methionine combined with immunogold labeling using a rabbit anti-mouse amelogenin antibody (AMELO) confirms that newly formed proteins form part of those detected in secretory granules (SG) in Tomes' process and at the rod growth site. Bar, 0.5 μm.* **(B)** *The accumulation of newly formed proteins at secretion sites (rod and interrod) can also be revealed by lectin-gold labeling using wheat germ agglutinin (WGA) which recognizes mainly N- acetyl-D-glucosamine sugar groups. Bar, 0.5 μm.*

ter, a second secretory site becomes active within the Tomes' processes themselves which controls growth of the length and width of the enamel rods which fill the cavities delineated by the rims of interrod enamel (Warshawsky, 1978, 1985; Nanci and Warshawsky, 1984). Each rod, therefore, is the secretory product of a single ameloblast, but interrod enamel requires a cooperative effort of several neighbouring ameloblasts to maintain appositional elongation of the cavity (Nanci and Warshawsky, 1984). It is presently unclear if separate packaging and targeting mechanisms are used for delivery of secretory products at the two growth sites, or if secretory products are all packaged uniformly and then merely targeted for release "apically" on any plasma membrane encountered at this pole of the cell. A slight difference between interrod and rod growth is probably maintained by the fact that secretory granules physically reach the interrod secretory site first in moving apically. Indeed, radioautographic studies suggest that newly formed proteins may be present at the interrod growth site by as early as 10-20 min after injection of a precursor amino acid (e.g., ^{3}H-methionine; Fig. 3) while labeling at rod growth sites is more consistently observed at later time intervals. Radioautographic (Nanci *et al.*, 1989a; Smith *et al.*, 1989) and lectin-gold cytochemical studies (Nanci *et al.*, 1989b) have also established that newly secreted proteins contribute to expansion in the thickness of the enamel layer by "piling up" just outside the membrane of the ameloblast (Figs. 3 and 4) as is typical for appositional growth occurring in other hard tissues such as dentin and bone (Ten Cate, 1994). However, some newly secreted proteins also appear to diffuse rapidly into regions of the enamel layer formed earlier in time by the same ameloblasts (Smith *et al.*, 1989). The biochemical nature of these more mobile proteins is presently unknown but it is possible they may be proteinases (active or latent) or molecules which control the activity of the proteinases, or enamel matrix proteins that have been made more soluble in enamel fluid by C-terminal processing events occurring shortly after they are secreted (Aoba and Moreno, 1991; Fincham *et al.*, 1991; Tanabe *et al.*, 1992, 1994; Moradian-Oldak *et al.*, 1994). As the enamel layer nears its tooth specific absolute thickness, ameloblasts accentuate the difference in secretory activity at rod and interrod growth sites so that the rod cavities become filled and the Tomes' processes are lost (Warshawsky, 1978). The mechanism by which this occurs is unknown. A final layer of interrod enamel is then deposited over the ends of the rods and former prong areas to smoothen out the surface (Boyde, 1989).

Across the period of appositional growth of the enamel layer, the ameloblasts undergo some additional changes in size and dimensions (Fig. 2). There is, of course, an increase followed by a decrease in total cell volume related to the formation, and disappearance, of the Tomes' processes. The curvilinear height of the cell body changes little over this period but there is a 2-3 fold increase in the width of the supranuclear compartment of the cell

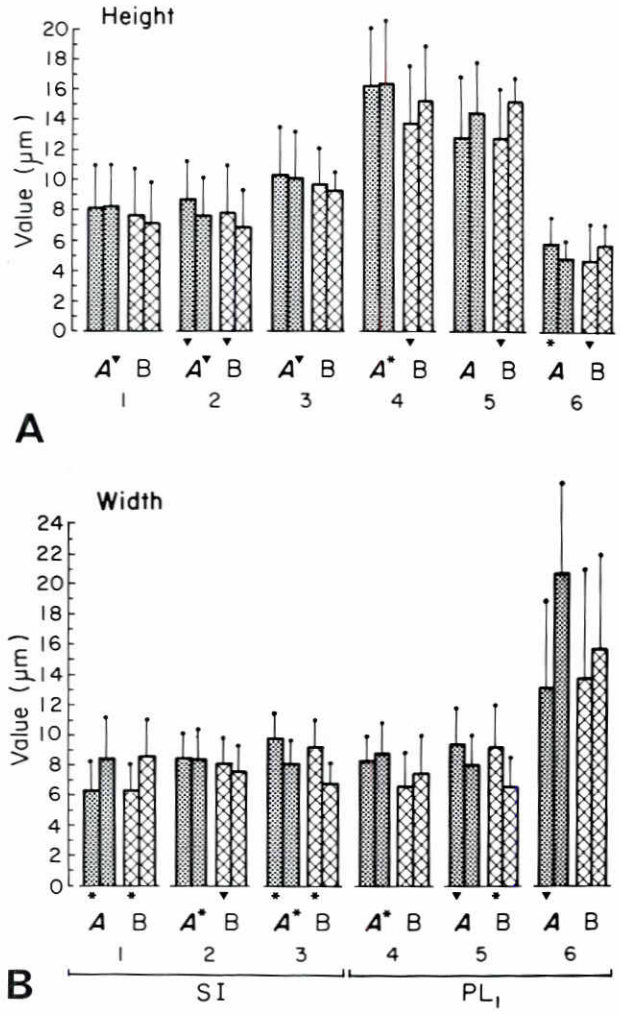

Fig. 5. Bar graphs showing mean profile height±SD (A) and mean profile width±SD (B) for stratum intermedium cells (SI; as PL1 at sampling positions 4-6). *The general arrangement of each graph is explained in the legend to Fig. 2A.*

in a plane running perpendicular to the cell rows (sagittal plane in rodent incisors) (Fig. 2C). Ameloblasts secrete the enamel layer organized in groups of cells interconnected by junctional complexes and desmosomes as opposed to individual cells (Warshawsky, 1978; Nishikawa *et al.*, 1988; Risnes *et al.*, 1989; Nishikawa, 1992). This feature is highly expressed in the alternating rows of ameloblasts typical of rodent incisors (Warshawsky, 1978; Nishikawa *et al.*, 1988; Boyde, 1989; Risnes *et al.*, 1989). The ameloblasts also move through three-dimensional space as they form the enamel (Warshawsky, 1978; Boyde, 1989; Risnes *et al.*, 1989; Nishikawa, 1992). One element of movement is a slow vertical motion away from the dentin which relates to the continuous secretion of proteins at the rod and interrod growth sites. In rat incisors this movement is about 13 μm per day over 7.5 days (Smith and Nanci, 1989). The ameloblasts also move laterally, and in some species circumferentially, to produce the bending (gnarling) patterns typical of some enamel rods (reviewed in Warshawsky, 1985; Boyde, 1989; Ten Cate, 1994). Causative and control mechanisms for the row organization and lateral movement are currently poorly understood (Warshawsky, 1985; Risnes *et al.*, 1989; Nishikawa, 1992). The ameloblasts are attached by junctional complexes and desmosomes to themselves, and by desmosomes to cells of the stratum intermedium positioned at their functional bases (Warshawsky, 1978). The exact role of stratum intermedium cells in amelogenesis remains undefined but these cells differentiate in tandem with the ameloblasts (Ten Cate, 1994), and they expand in cell height as ameloblasts form the enamel layer (Fig. 5). The physical dimensions of stratum intermedium cells in terms of breadth and depth suggests there are probably 2-3 ameloblasts from the same row and at least two ameloblasts from adjacent rows related to the attachment surface of a single stratum intermedium cell (Figs. 2 and 5). Each stratum intermedium cell, therefore, could simultaneously influence at least 6 ameloblasts moving through three-dimensional space (Figs. 2 and 5). Considering their location at the functional bases of ameloblasts, it seems possible that stratum intermedium cells could assist ameloblasts to resolve various vectors of cell motion as enamel rods grow from their Tomes' processes. The vertical motion of ameloblasts away from the dentin surface is likely inherent since this type of movement is the same vertical motion expressed by osteoblasts and odontoblasts when they create their respective hard tissues by appositional growth. However, the lateral, and in some cases twisting, motion of ameloblasts on a scale suggested by the three dimensional shapes of enamel rods is a phenomenon somewhat unique to ameloblasts (Boyde, 1989). The stratum intermedium cells could somehow serve to coordinate this movement so adjacent groups (rows) of

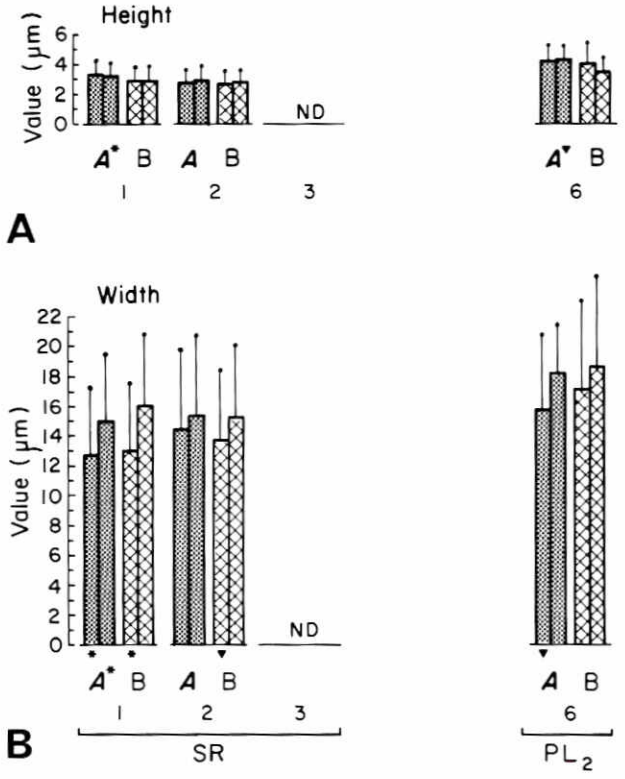

Fig. 6. Bar graphs showing mean profile height±SD (A) and mean profile width±SD (B) for stellate reticulum cells (SR; as PL2 at sampling positions 4-6). *The general arrangement of each graph is explained in the legend to Fig. 2A. ND, not determined (blank space means they were not present as positions 4 and 5).*

ameloblasts form the enamel rods in a controlled way. Stratum intermedium cells are themselves connected by desmosomes to stellate reticulum cells, and indirectly via the variably thick stellate reticulum, to cells of the outer dental epithelium positioned at the connective tissue interface (Kallenbach, 1978). Of these latter two cell populations, the outer dental epithelium is the only layer showing major changes in cell dimensions over the course of amelogenesis (Figs. 6 and 7). This is expressed as a doubling in cell height as the enamel organ enters the maturation stage of amelogenesis (Fig. 7) and the papillary layer develops from blood vessels invaginating deeply toward the stratum intermedium (Sasaki *et al.*, 1984). These cells almost double in cell height as these invaginations are formed (Fig. 7).

Maturation of enamel

Ameloblasts undergo major reorganization in cell size and morphological characteristics once the final enamel layer is formed (Fig. 2). They first undergo postsecretory transition and revert to a height similar to what they showed as inner dental epithelial cells but with slightly broader width dimensions in the supranuclear compartment (Fig. 2). To do this they must shed excess cytoplasmic volume as well as a portion of secretory organelles including rough endoplasmic reticulum and Golgi stacks (Smith, 1984). It is likely for this reason that the lysosomal system of ameloblasts becomes so prominent at this time (Salama *et al.*, 1991; Smid *et al.*, 1992). In addition, as much as 25% of the total ameloblast cell population dies during postsecretory transition and another 25% disappears slowly as the enamel matures (Smith and Warshawsky, 1977). Hence, while there is a direct 1:1 ratio between the number of ameloblasts and the number of enamel rods that are created during appositional growth of the enamel layer, there is initially a 1:1.3 and eventually a 1:2 ratio between the number of ameloblasts and enamel rods that are undergoing maturation. The death of some ameloblasts means that remaining ones must slowly spread out over the enamel surface to occupy twice the surface area as when the enamel layer was first formed (Fig. 2). This likely explains the reason why the width of the ameloblasts enlarges in a direction parallel to the former row axes during the maturation stage (Fig. 2). As this happens, row organization is lost with ameloblasts taking on a more polygonal shape when seen in tangential section (Fig. 1).

Ameloblasts also undergo major reorganizational changes in their secretory functions during postsecretory transition into maturation. These include (1) down regulation, but not complete cessation, of secretory activity for enamel matrix proteins (Smith *et al.*, 1992); (2) upregulation of secretory activity for proteins that are targeted to the functional apex where they reform the 'inner basal lamina' on the surface of immature enamel (Nanci *et al.*, 1993), (3) upregulation of synthetic activity for certain enzymes that are targeted to intracellular sites such as calcium transport ATPases which coat the ruffle-ended apical surface (Borke *et al.*, 1993) and degradative enzymes associated with membranes and/or the contents of the lysosomal system (Salama *et al.*, 1991; Smid *et al.*, 1992); and, (4) perhaps upregulation of secretory activity for enamel proteinases or molecules that activate them. While there appear to be proteolytic and processing enzymes in enamel from the very moment it starts to grow in thickness by appositional growth, certain proteolytic enzymes are detected in maturing enamel which are not seen in forming enamel especially relative to a serine proteinase activity (Moradian-Oldak *et al.*, 1994). It is not yet clear if this represents activation of a latent enzyme(s) or *de novo* secretion of new enzymes following postsecretory transition.

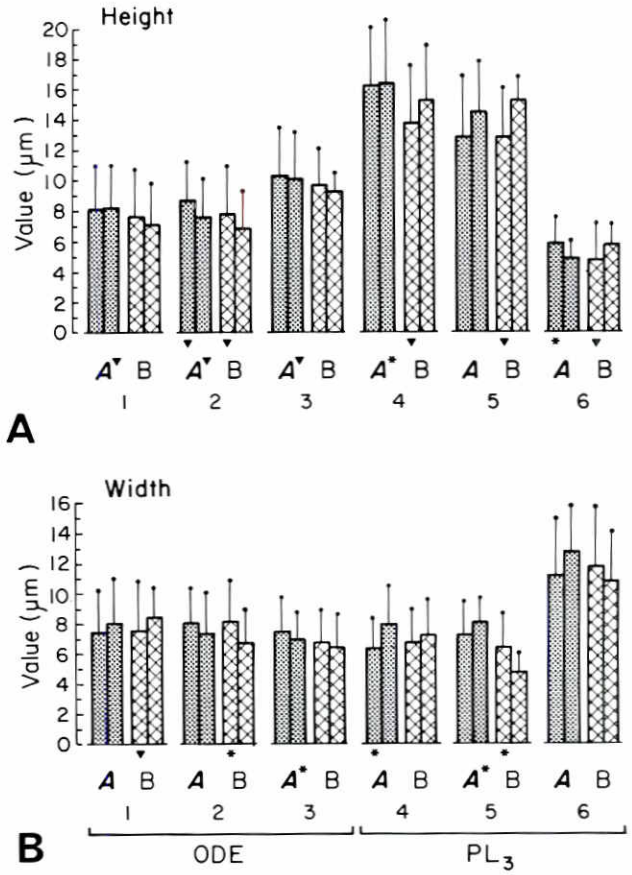

Fig. 7. Bar graphs showing mean profile height±SD (A) and mean profile width±SD (B) for outer dental epithelium cells (ODE; as PL3 at sampling positions 4-6). *The general arrangement of each graph is explained in the legend to Fig. 2A.*

The premiere event of the maturation stage is modulation where ameloblasts rhythmically create, then shed, their ruffled apical membranes which are richly coated with calcium transport ATPases (reviewed in Nanci and Smith, 1992). In some species, modulation is quite rapid and the ameloblasts undergo three ruffle-to-smooth-ended transitions per day (once every 8 h) (Smith *et al.*, 1987). The transition from ruffle-ended state to smooth-ended state occurs almost instantly while the transition from smooth-ended state back into ruffle-end state takes about 2 h in teeth where the ameloblasts modulate once every 8 h (Smith *et al.*, 1987). Ameloblasts, therefore, spend about 50% of the time during maturation exclusively as ruffle-ended cells, about 25% exclusively as smooth-ended, and another 25% recreating the ruffle-ended apical surfaces. Experiments by Sasaki *et al.* (1991) with indicator dyes have suggested that the pH of the enamel beneath smooth- ended ameloblast, which seem more leaky compared to ameloblasts having ruffle-ended apical surfaces (McKee *et al.*, 1986), is near neutral pH. The enamel beneath the ruffle-ended ameloblasts, however, appears considerably more acidic (Sasaki *et al.*, 1991). Some investigators have proposed that the low pH of enamel may be generated by a proton pumping system which could exist because of a co-presence of H^+-ATPases and carbonic anhydrase on the apical sur-

faces of the ruffle-ended ameloblasts (Lin *et al.*, 1994). However, such pumping is most likely unnecessary if we consider that the local pH of the microenvironment immediately around growing enamel crystals would drop naturally as a consequence of creating excess hydrogen ions as the hydroxide ions captured from water are incorporated into new layers of mineral deposited at the surface of the expanding crystals (Aoba and Moreno, 1984; Miake *et al.*, 1993). This is especially likely if the ruffle-ended ameloblasts are as tightly sealed as suggested by their highly developed apical tight junctions (Josephsen and Fejerskov, 1977; Garant *et al.*, 1984). It seems possible that a local "signal" for modulation could reside in lowering of the local pH to some critical level at the apical surfaces of the ruffle-ended ameloblasts which then respond by becoming smooth-ended and leaky to tissues fluids capable of neutralizing the pH so that the growing enamel crystals do not start to demineralize. It is of interest that modulation is among the first events in amelogenesis that is significantly disrupted by long-term (chronic) exposure to fluoride (Smith *et al.*, 1993). This effect could be based in part on a disruption of the local pH "engine" that monitors neutralization of pH within the local environment. That is, if ameloblasts somehow sense that the local pH is more acidic than normal, they would presumably tend to remain in a more smooth-ended like state ("poorly ruffled") for longer periods of time than normal (as seen in ameloblasts of rat exposed to fluoride) in an attempt to keep the pH from dropping even lower.

Lastly, once the enamel is fully mature the ameloblasts stop modulating and they, along with the other cell layers of the enamel organ, undergo regression. The ameloblasts shrink dramatically in height and take on a cuboidal appearance (Fig. 2). All intracellular organelles and general cytoplasm decrease in volume and, interestingly, the remnants of the Golgi apparatus move from the functional apical region back to its original position on the side of the nucleus facing cells of the stratum intermedium. Papillary layer cells occupying the position of cells which originally formed the stratum intermedium (PL1) and outer dental epithelium (PL3) also shrink markedly in height but they expand in width while papillary cells of the former stellate reticulum (PL2) change little in dimensions (Figs. 5-7). It is generally accepted that the cells of the regressed (reduced) enamel organ serve a protective function to keep the mature enamel isolated from the surrounding connective tissue (Ten Cate, 1994). As is the case for afibrillar coronal cementum which is believed to result from premature degeneration of focal areas of the enamel organ in the cervical portion of the tooth (Heritier, 1982; Beertsen and Everts, 1990), exposure of mature enamel to surrounding connective tissue (in humans) would lead to the deposition of large amounts of unwanted acellular cementum over the crown of the tooth.

Materials and Methods

Morphometry

Thirty male Sprague-Dawley rats weighing 162±10 g were anesthetized and perfused intravascularly with either 5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2, (Method A in Figs. 2 and 5-7) or 2.5% glutaraldehyde in 0.08 M sodium cacodylate buffer, pH 7.2 (Method B in Figs. 2 and 5-7). The mandibles were removed, decalcified in isotonic disodium EDTA, and washed in 0.1 M sodium cacodylate buffer containing 5% sucrose. Small segments of the enamel organ were then removed from the mandibular incisors at six sites along the length of the tooth (as illustrated by horizontal line in panel A of Fig. 2) corresponding to (1) early presecretory stage (region of ameloblasts facing pulp), (2) early secretory stage, (3) late secretory stage, (4) early maturation stage, (5) late maturation stage (pigmentation), and (6) regression stage. The segments were postfixed in reduced osmium, dehydrated in graded alcohol, and embedded in Epon for sectioning in the transverse (cross sectional) or sagittal (longitudinal) planes of the tooth (as illustrated as TR and SG in panel D of Fig. 1). Thin sections were cut and grid stained with uranyl acetate and lead citrate. A series of electron micrographs were photographed across the enamel organ for the two planes of section within 2 adjacent fields delineated by a grid bar in a region situated closest to the geometric center of the grid. Prints were made at x6,000 final magnification and montaged to recreate the entire thickness of the enamel organ from inner to outer surfaces. Measurements of cell height and "width" as seen in transverse and sagittal sections were done on the montaged prints using a graphic tablet connected to a computer (data for the planes of section are represented by the paired bars in Figs. 2 and 5-7). Measurements were done exclusively on enamel organ cells which were sectioned through the nucleus and which showed continuous cytoplasm within the height axis of the cell (e.g., only ameloblasts sectioned continuously from apex to base through the nucleus were measured as illustrated in panel A of Fig. 1). An average of 100 cells were measured for each parameter in a given plane of section, stage of amelogenesis, and fixative method. Statistical tests of data were done by analysis of variance comparing results for plane of section and fixative method at each stage examined (significance levels as $p<0.05$= inverse triangle, $p<0.01$= asterisk in Figs. 2 and 5-7).

Other histological methods

Methods for preparing rat incisors for radioautography, lectin-gold cytochemistry, and immunocytochemistry have been described elsewhere (Nanci *et al.*, 1987, 1989a,b; Smith *et al.*, 1989).

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