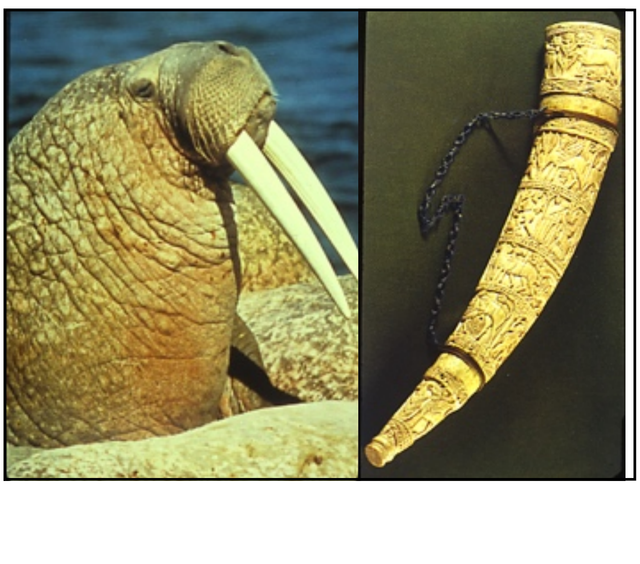
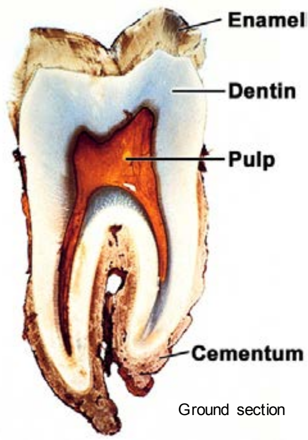
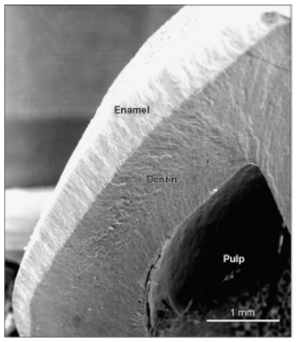
Enamel and Amelogenesis





Ground section



Scanning electron microscopy

Properties of enamel

- Translucent hard tissue, yellow to gray-white
- Up to 2.5 mm in greatest thickness on working surfaces.
- 96% mineral; 4% organic; extremely hard; brittle
- Ca-P (hydroxyapatite) and ions, e.g. Fl, Mg, strontium, lead etc
- Formation of crystals (prisms =old term)
- Complex structural organization
- Dissolution in acid

Ameloblasts & Enamel

- No ameloblasts → No enamel
- Dentin → Enamel
- Ameloblasts are lost after eruption, therefore
  - No enamel regeneration
    - However, there is remineralization…to a certain extent
  - Enamel has to be hardest (but unfortunately most brittle) hard tissue
    - Lack of significant organic component ~ 4%
    - Supported by the more resilient dentin
      - Unsupported enamel fractures easily if dentin gone

Enamel and other hard tissues

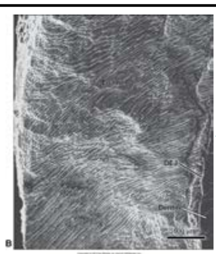
- Fundamental similarities
  - Hydroxyapatite crystals
    - Crystalline calcium phosphate substituted with carbonic ions
- Differences
  - Elasticity
  - Color

Differences from other hard tissues

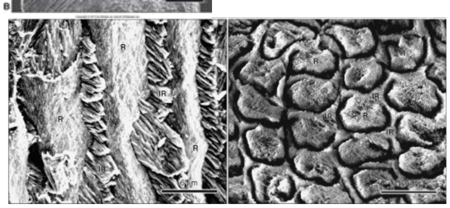
- There is no pre-enamel
- Crystals grow against the the secretory surface of ameloblasts
- Enamel proteins do not play any major structuring function

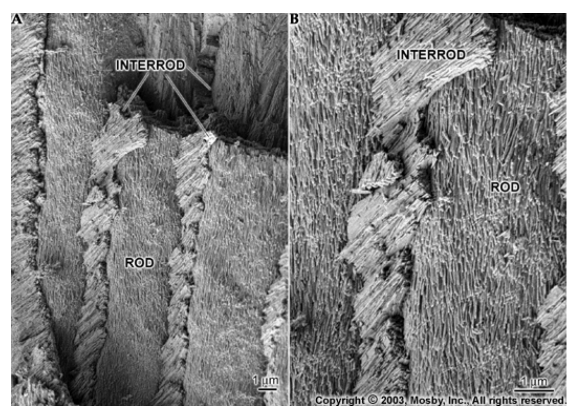
Organizational units

- Crystals form rods (prisms) and interrod (interprismatic substance) enamel
  - Crystals are hexagonal initially (term “prism” is not recommended) in cross-section
  - Irregular because of compaction
  - Crystals run longitudinally along rods
- Rods (prisms)
  - Not a regular geometry
  - Cylindrical
- Interrod enamel (interprismatic enamel)
  - Same crystal morphology, different orientation
- Organic sheath in the interface of rod and interrod enamel
  - When enamel is decalcified the organic sheath is only visible

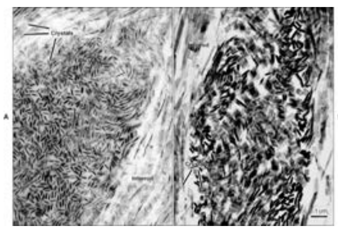


Enamel rods like ribbons





Closely packed and long ribbon-like carbonate-apatite crystals measuring 60 to 70 nm in width and 25 to 30 nm in thickness

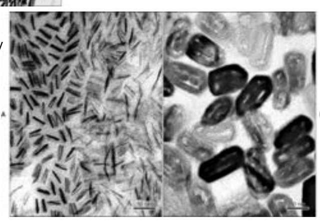


TEM image showing a rod surrounded by interrod enamel. A: younger; B: older

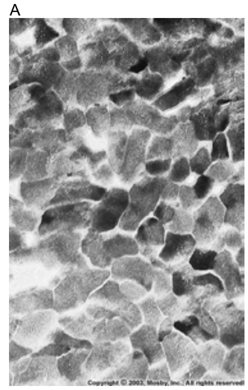
Crystals are long, ribbon-like structures that become thicker as enamel matures. They are similar in structure and composition but have different orientation.

Cross-section of (A) recently formed, secretory stage enamel crystals and (B) older ones from the maturation stage. Crystals are initially thin; and become thicker as they grow in thickness and width becoming more hexagonal (see figure A on next slide).

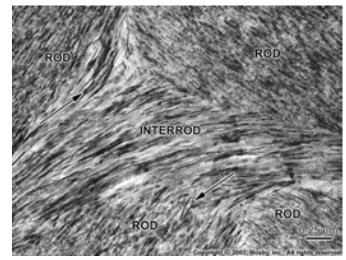
Linear patterns seen in older crystals is due to reflection of their crystalline lattice.



A

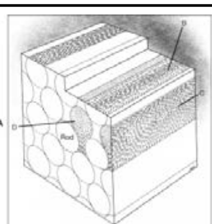


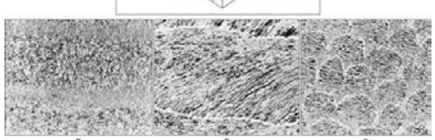
EM showing mature enamel crystals that outline of which is irregular as they press against each other.



Interrod partition associated with four different rod profiles. At certain areas the crystals from the interrod enamel enter the rod (zone of confluence; indicated by arrows)

A



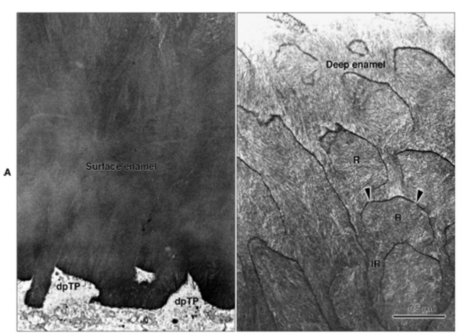


B C D

Structure of human enamel. Crystal orientation along three faces of an enamel block. B to D: TEM of the 3 faces.

Please note the boundary between rod and interrod enamel is delimited by a narrow space containing organic material known as “rod sheath”

A

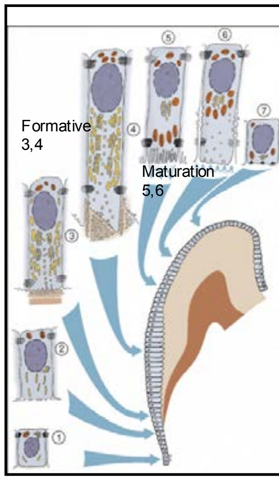


B

Decalcified preparation of cat secretory stage enamel. A: organic matrix near the ameloblasts is younger and shows a uniform texture. No rod sheath is identified.
B: Deeper areas of enamel near the dentin. As enamel matures, matrix accumulates at the interface between rod and interrod to form the rod sheath (arrowheads).

Mineralization

- Two steps
- Initial partial mineralization ~30%
- Crystals grow wider and thicker
  - The process of organic matrix and water removal continues after full thickness of enamel has been formed
- Phenotypic changes of ameloblasts
  - Presecretory (morphogenetic and histodifferentiation)
  - Secretory
  - Maturation



Life cycle of ameloblast is characterized by progressive phenotypic changes

Presecretory

1. Morphogenetic
2. Histodifferentiation

Secretory

3. Initial secretory stage (no Tomes' process)
4. Secretory stage (Tomes' process)

Maturative

5. Ruffle-ended ameloblast
6. Smooth-ended ameloblast

Protective

7. Protective stage (reduced enamel epithelium)

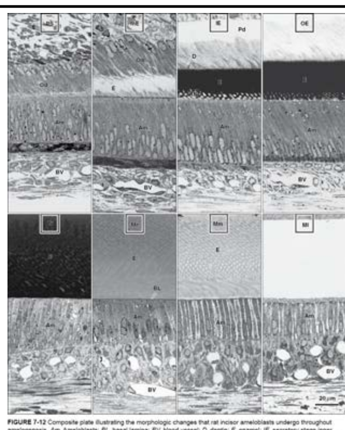


FIGURE 7-12 Composite plate illustrating the morphologic changes that rat incisor ameloblasts undergo throughout amelogenesis. *Am*, Ameloblasts; *BL*, basal lamina; *BV*, blood vessel; *D*, dentin; *E*, enamel; *IE*, secretory stage inner enamel; *InE*, secretory stage initial enamel; *Me*, early maturation stage; *Ml*, late maturation stage; *Mm*, midmaturation stage; *Od*, odontoblasts; *OE*, secretory stage outer enamel; *Pd*, predentin; *PS*, presecretory stage; *TP*, Tomes' process; *Tr*, maturation stage transition.

Amelogenesis

Amelogenesis begins after a few μm of dentin deposition at the dentinoenamel junction

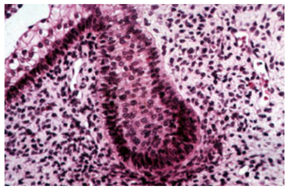
Presecretory stage:

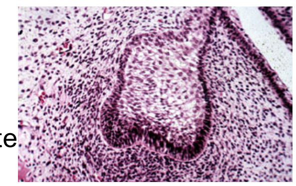
1. Morphogenetic. During this stage the shape of the crown is determined.

2. Histodifferentiation. The cells of the inner dental epithelium is differentiating into ameloblasts. The above two stages are the presecretory stages, where the cells differentiate, acquire phenotype, change polarity, develop an extensive protein synthesis machinery, and prepare to secrete an organic matrix of enamel.

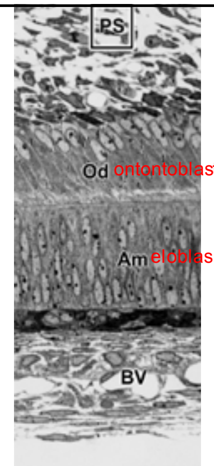
Presecretory
Morphogenetic & Histodifferentiation

- Acquisition of phenotype
- Starts from the cusp tip outlines
- Change in polarity
- Acquire synthesis machinery and preparation to secrete the matrix





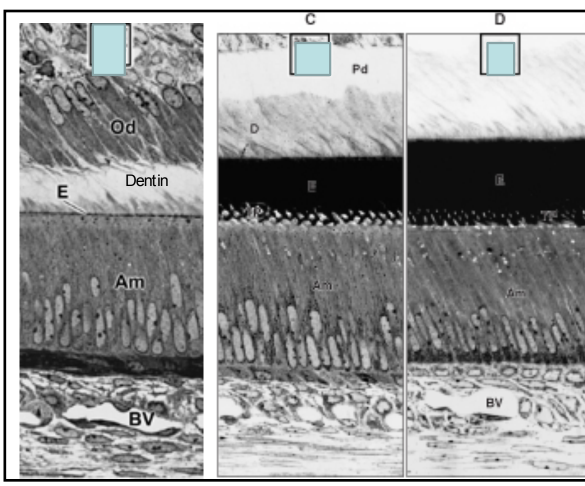
Presecretory



Secretory (formative)
Without & With Tomes processes

- Full thickness formation
- Organization of structure

Ameloblasts elaborate and organize the entire enamel thickness. Short conical processes called Tomes´ processes develop at the apical end of the ameloblasts. The main protein that accumulates is amelogenin.

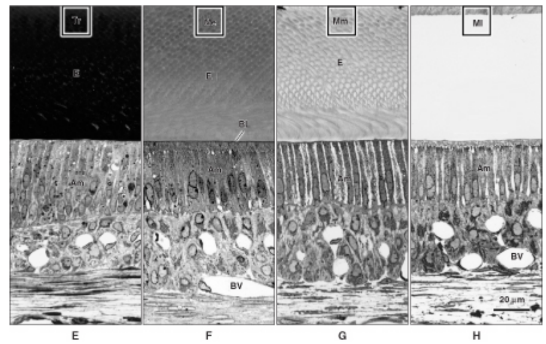


Maturation

Ruffle-ended – Smooth-ended & Protective

- Transportation of ions
- Mineralization

Maturation stage: Ameloblasts modulate and transport specific ions required for the concurrent accretion of mineral. At this stage, ameloblast becomes more active in absorption of the organic matrix and water, which allows mineralization to proceed. After the ameloblasts have completed their contributions to the mineralization phase, they secrete an organic cuticle on the surface of the enamel, which is called developmental or primary cuticle



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Protection: The ameloblasts are shorter and contact the stratum intermedium and outer dental epithelium and fuse to form the reduced dental (enamel) epithelium. The reduced enamel epithelium remains until the tooth erupts. As the tooth erupts and passes through the oral epithelium, the incisal part of the reduced dental epithelium is destroyed but the epithelium present cervically interacts with oral epithelium to become the junctional epithelium

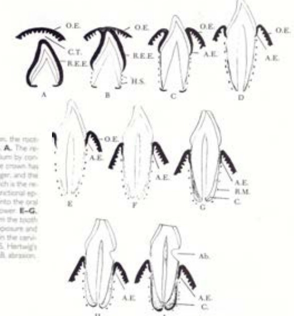
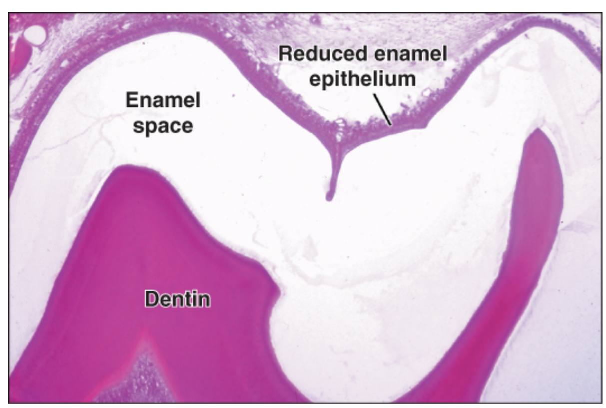


Figure 13–7. Diagrammatic illustration of the process of root formation, tooth eruption, the rootward migration of the attachment epithelium, cementum exposure, and cervical abrasion. **A.** The reduced enamel epithelium covers the tooth crown and is separated from the oral epithelium by connective tissue. The root has not yet begun to form. **B.** Root formation has begun, and the crown has moved incisally. The reduced enamel and oral epithelia are in contact. **C.** The root is longer, and the incisal edge of the crown is exposed in the oral cavity. The reduced enamel epithelium, which is the remains of the enamel organ, is now continuous with the oral epithelium and is called the junctional epithelium. **D.** The length of the root dentin is complete, and the crown has moved farther into the oral cavity. The junctional epithelium is still entirely on the enamel. The apical foramen is narrower. **E–G.** The junctional epithelium grows onto the cementum at its apical border and separates from the tooth surface at its cervical border. Cementum becomes exposed. **H, I.** Increased cementum exposure and improper use of an abrasive dentifrice have resulted in abrasion of cementum and dentin in the cervical area. OE, oral epithelium; CT, connective tissue; REE, reduced enamel epithelium; HS, Hertwig's sheath; AE, attachment (junctional) epithelium; RM, rests of Malassez; C, cementum; and AB, abrasion.



Light microscopic characteristics of ameloblasts

- Tall columnar cells
- Reverse polarization of nuclei
- Initiation of dentin formation
- Ameloblasts secrete enamel proteins
- Initial layer of enamel (rod-less)
- Ameloblasts move away from dental papilla – change in vascular supply
- Development of Tomes' processes (picket-fence appearance)

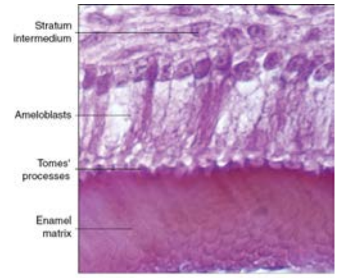
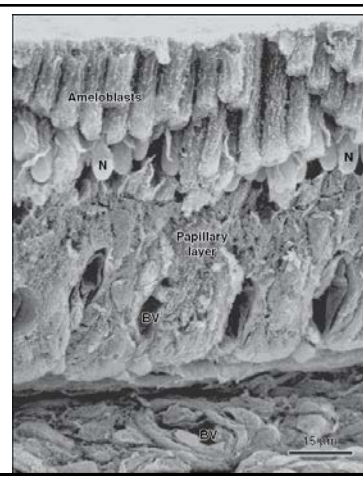


FIGURE 7-17 Enamel matrix formation as seen with the light microscope. The Tomes' processes of ameloblasts jut into the matrix visible after decalcification in certain plane of section, creating a picket-fence appearance in higher mammals.

Light microscopic characteristics

- After full thickness formation, maturation of enamel starts
- Ameloblasts shorten
- Loss of distinct layers of the enamel organ
- Blood vessels invaginate without violating the basement membrane (papillary layer)
- Enamel fully mature*→ ameloblastic layer and layers in the papillary layer zone become reduced enamel epithelium
  - Protects enamel from follicular cells
  - Helps the formation of the junctional epithelium

* Almost fully mature. Composition can still be modified



Ultrastructural Steps in Amelogenesis

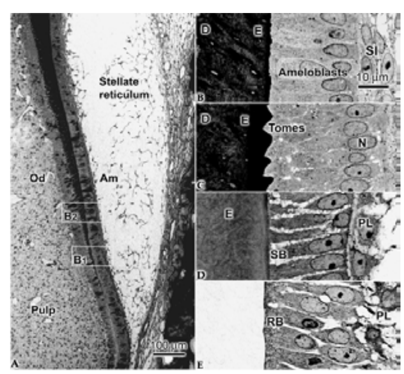
- Presecretory
  - Morphogenetic
    - Cuboidal to low-columnar
    - Centrally placed nuclei
    - Poorly defined Golgi
    - CELLS CAN STILL DIVIDE

Ultrastructural Steps in Amelogenesis

- Presecretory
  - Histodifferentiation
    - Elongation of cells
    - Reverse polarization
      - Some enamel proteins start forming
      - Also secretion of dentin sialoproteins (WHY?!)
    - Break up of the basal lamina
    - Golgi apparatus moves distally
    - RER
    - Development of Tomes' processes (proximal)
    - Junctional complexes and terminal webs
    - Cell division seizes

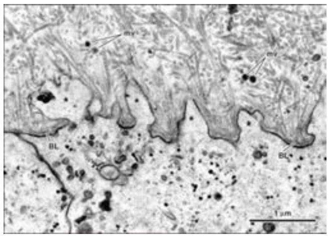
Ultrastructural Steps in Amelogenesis

- Secretory
  - Prominent RER (mRNA translation in ribosomes and translocation in the cisternae) and Golgi (posttranslational modification)
  - Secretory granules
  - Constitutive secretion
    - Continuous, no storage of secretory granules
  - Proteins are released against mantle dentin
    - Amelogenin (90%), ameloblastin, enamelin, etc
  - Mineralization starts concomitantly
  - Development of Tomes' distal processes
    - They become longer and thinner
  - Formation of pit, inter-rod and rod enamel
  - The first and last enamel does not contain rods

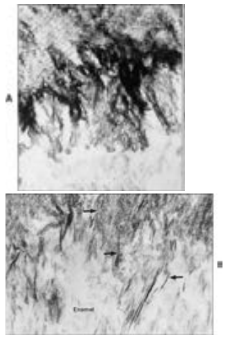


B1

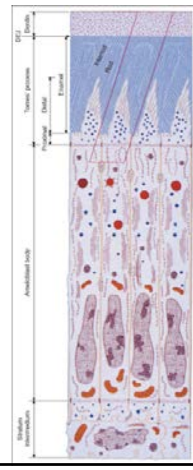
B2

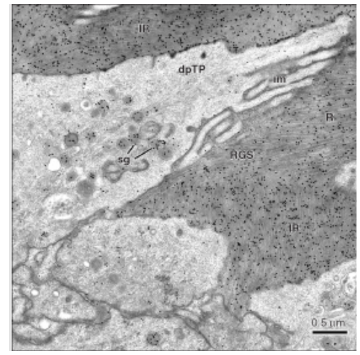


Differentiating ameloblasts extend cytoplasmic projections (*) through the basal lamina (BL), separating them from the forming mantle dentin. The BL is fragmented and is removed before active deposition of enamel matrix.

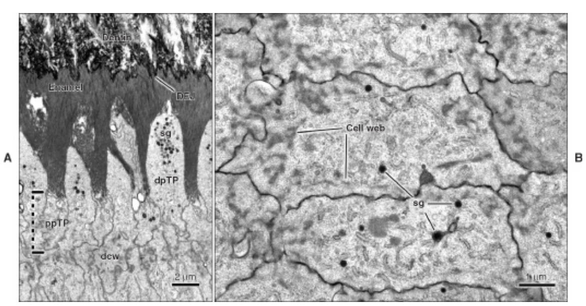


TEM of initial enamel formation. Close intermingling of dentin collagen fibrils with ribbonlike crystals of enamel.
A. Low magnification
B. High magnification

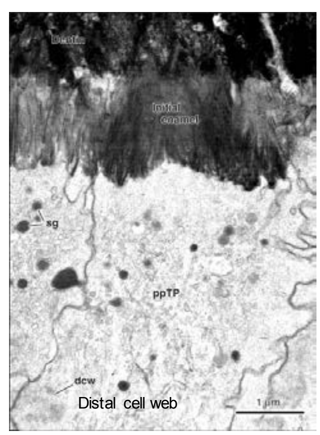




Secretory granules are translocated into Tomes' process and accumulate near secretory surfaces recognized by the presence of membrane infoldings

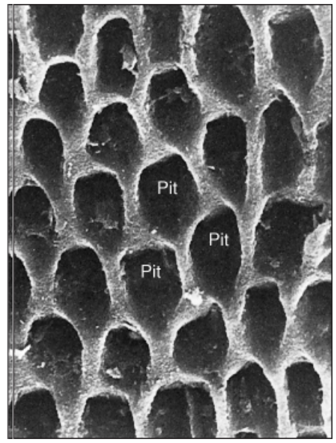


Differentiated ameloblasts develop a junctional complex at their distal extremity. The cell extension above the complex is Tomes' process and is divided into two Parts: proximal (ppTP) extends from the junctional complex to the surface of the enamel layer; and distal (dpTP) that penetrates into enamel.



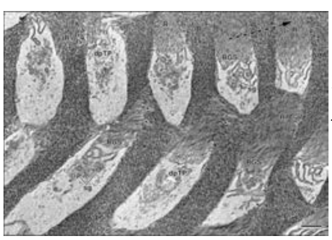
When initial enamel forms, the ameloblast only has a proximal portion of Tomes' process (ppTP). The distal portion develops as an extension of the proximal one slightly later when enamel rods begin forming. Therefore the first enamel does not have rods

Interrod (IR) enamel surrounds the forming rod (R) and the dpTP; this portion is continuation of the ppTP into the enamel layer. The interrod and rod growth sites (IGS and GS) are associated with membrane infoldings (im) on the proximal and distal portions of Tome's process, respectively. These infoldings represent the sites where secretory granules (sg) release enamel proteins extracellularly for growth in length of enamel crystals that results in an increase in thickness if the enamel layer.

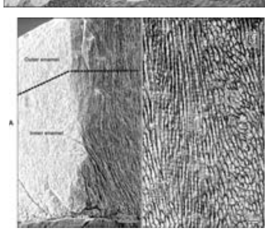


SEM of the surface of a developing human tooth from which ameloblasts have been removed. The surface consists of a series of pits previously filled by Tomes' processes, the walls of which are formed by interrod enamel.

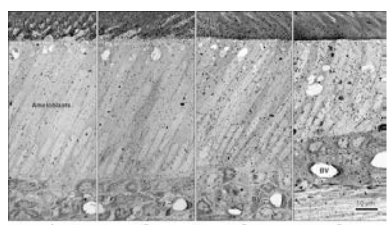
Interrod enamel forms ahead of rod enamel to define the pit



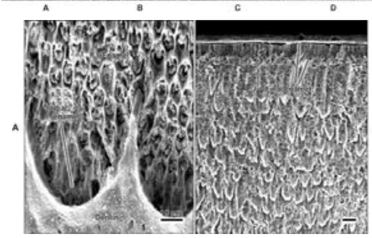
In cross section the dpTP appear as ovoid profiles surrounded by IR enamel. They decrease in size towards the DE junction (dashed arrow). The crystals making up the rod blend with those of IR enamel (small arrows) – zone of confluence.



(A) SEM illustrations showing the complex trajectory of the inner 2/3ds of the enamel layer in human teeth. (B) SEM showing rods organized in groups exhibiting different orientations; this showing 4 adjacent groups.



Light microscopic radioautographic preparations with 3H-methionine labeling of secretory products (black grains over enamel indicates amelogenin).
A and B: secretory
C: transition
D: early maturation



First enamel (A) and last enamel (B) are aprismatic (i.e., they do not contain rods)