Odontochronology Course
Masaryk University, Autumn 2015

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Ms Adela Hupková

Course Aims

- At the end of the week you should know more about:
- Dental development
- The structure of hard tissues
- How enamel and dentine act as recording devices
- The principles of odontochronology

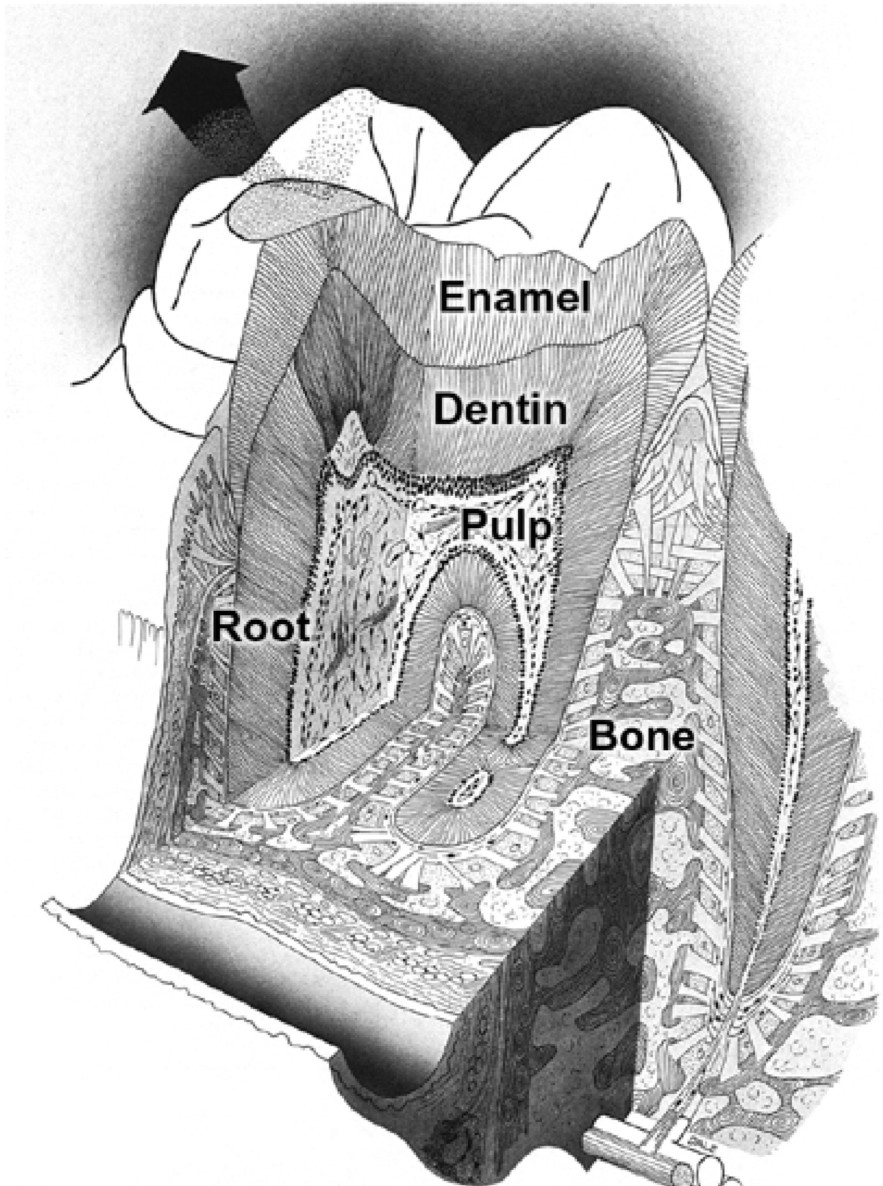
Course stucture

- Day 1:
  - Lecture 1 – Early tooth development and enamel structure; incremental lines
  - Practical 1 – Enamel
  - Lecture 2 – Dentine and cementum; incremental lines
  - Practical 2 – Dentine and cementum
  - Lecture 3 – Introduction to odontochronology and the projects

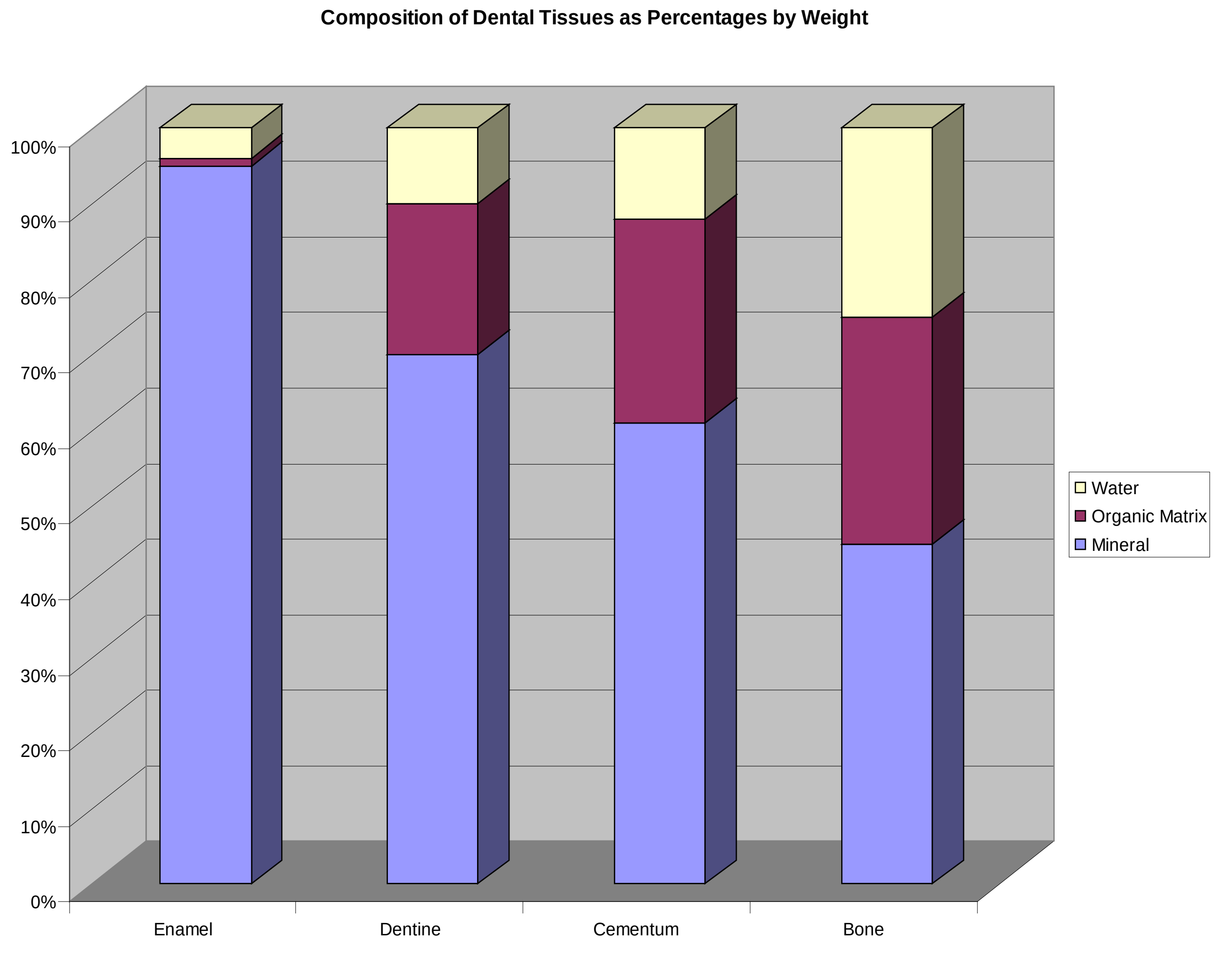
Course structure

- Days 2 & 3 – Work in teams on the research microscope to analyse your teeth
- Day 4 – Preparation of final presentation
- Day 5 – Presentations

Lecture 1: Early tooth development and enamel



General properties of hard tissues



Sagittal section of a three-month old human embryo (H&E stained)

1. <u>Epithelial cells derived from the ectodermal germ layer:</u>

Enamel organ:

a) Inner enamel epithelium (IEE):

b) Stratum intermedium (SI):

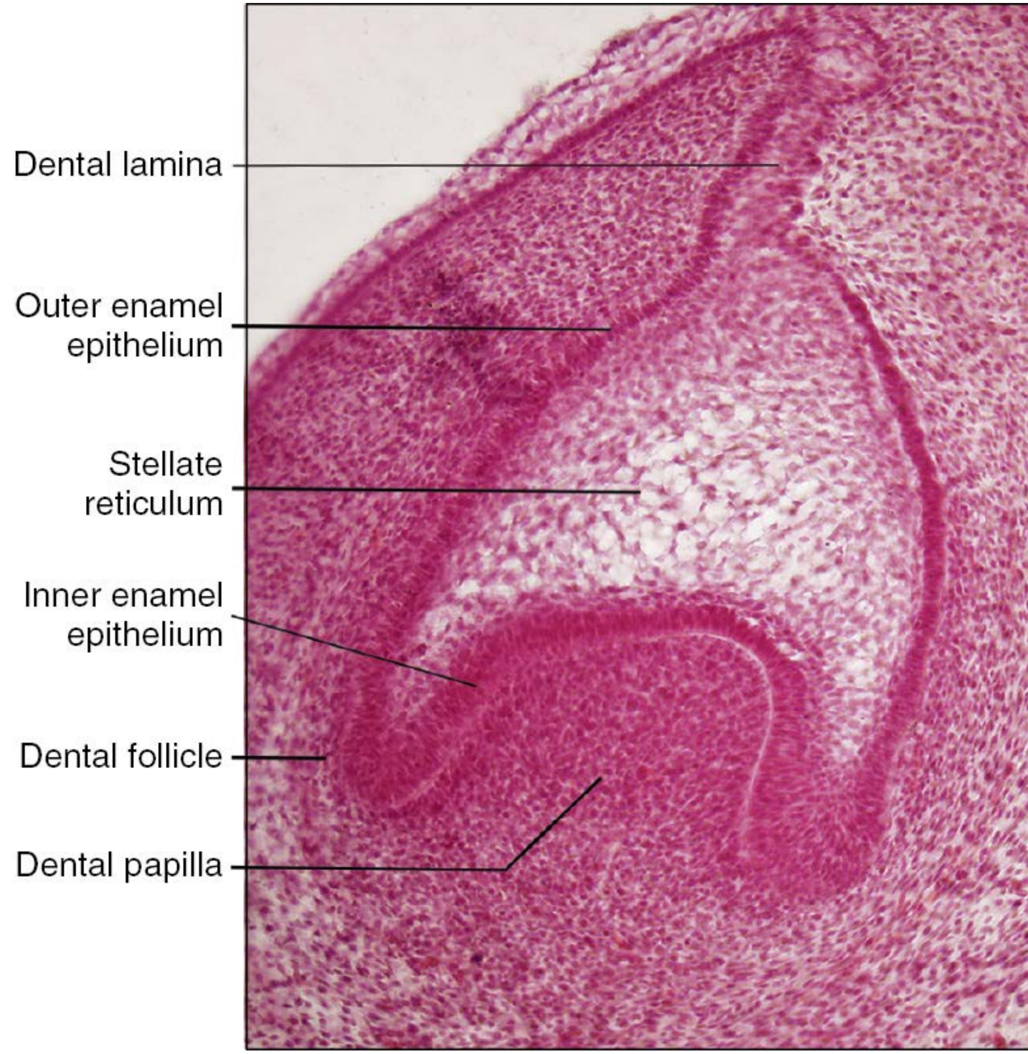
c) Stellate reticulum (SR):

d) Outer enamel epithelium (OEE)

<u>2. Mesenchymal cells derived from neural crest cells:</u>

a) Dental papilla:

b) Dental follicle (DF):



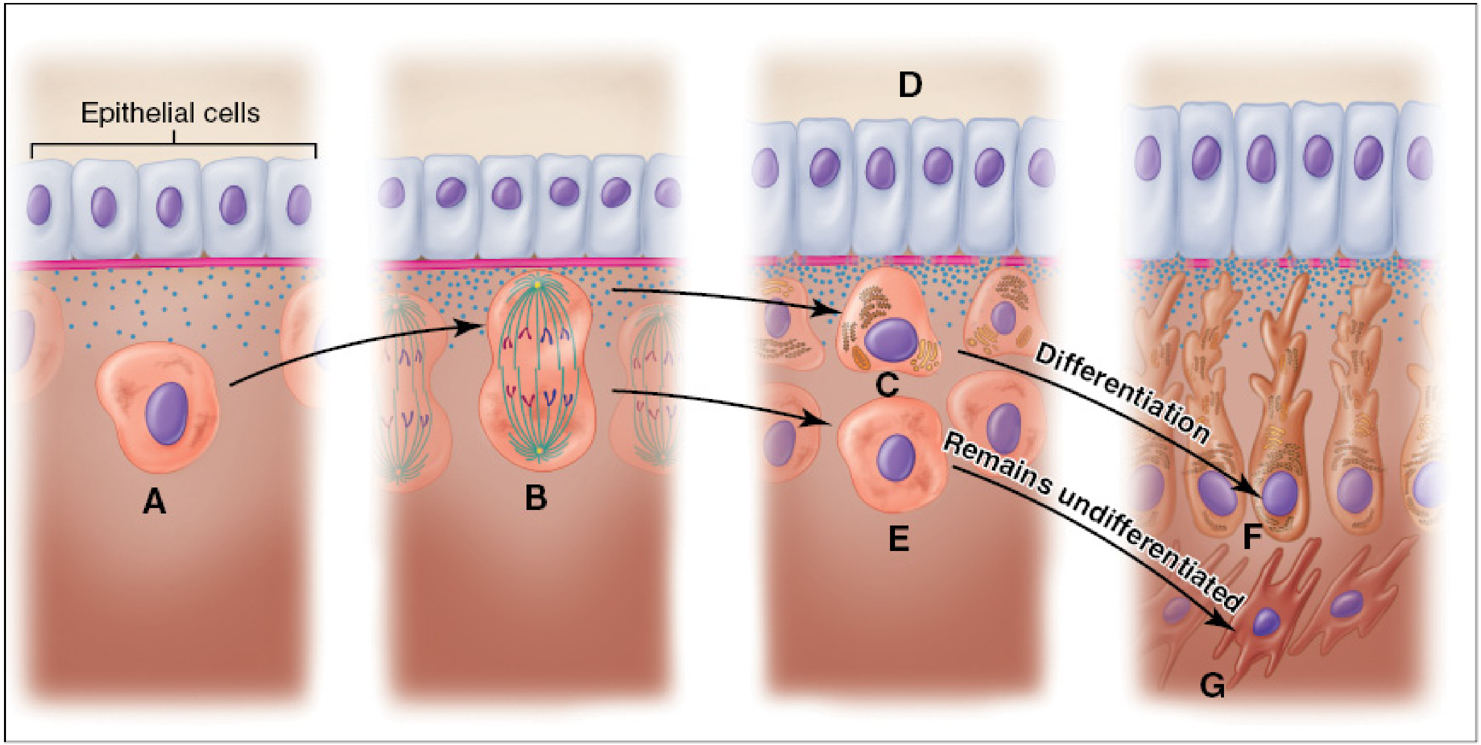


FIGURE 8-11 Odontoblast differentiation. The undifferentiated ectomesenchymal cell *(A)* of the dental papilla divides *(B)*, with its mitotic spindle perpendicular to the basal lamina *(pink line)*. A daughter cell *(C)*, influenced by the epithelial cells and molecules they produce *(D)*, differentiates into an odontoblast *(F)*. Another daughter cell *(E)*, not exposed to this epithelial influence, persists as a subodontoblast cell *(G)*. This cell has been exposed to all the determinants necessary for odontoblast formation except the last.

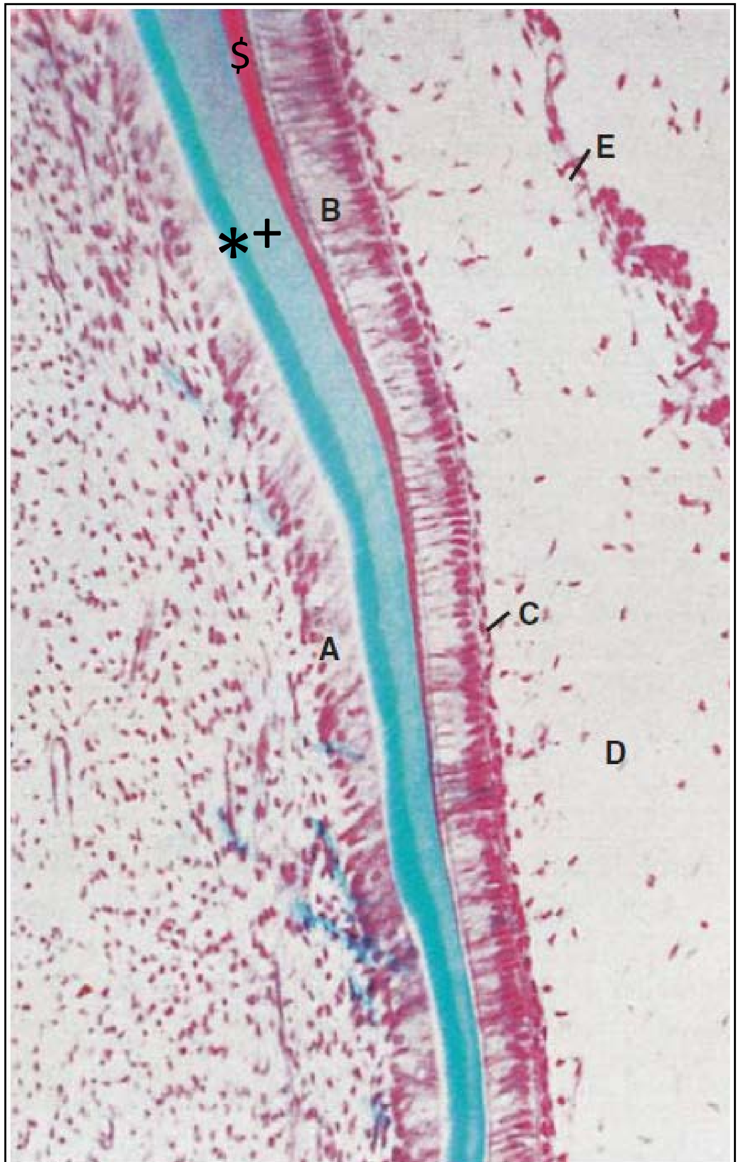


Fig. 21.17: Berkovitz: Oral Anatomy...

Late bell stage: (high power view)

A: **Odontoblasts** (derived from pulp cells)
=> Secrete **predentine** (*) that mineralises to form **dentine** (+).

B: **Ameloblasts** (derived from inner enamel epithelial cells)
=> Secrete **preenamel** ($) that mineralises to form enamel.

C: **Stratum intermedium**
=> Produces alkaline phosphatase (enamel formation); **supports ameloblasts**.

D: **Stellate reticulum**
=> **Protects** and maintains **tooth shape**.

E: **Outer enamel epithelium**
=> **Maintains tooth shape**.
=> **Exchanges substances** with dental follicle.

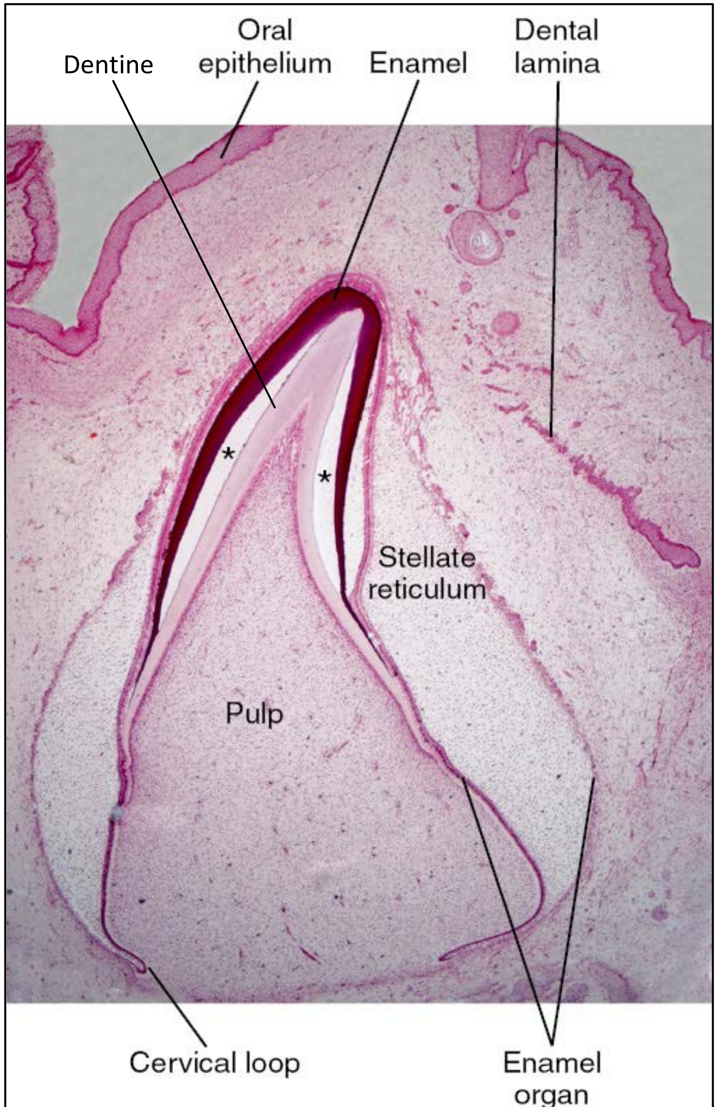
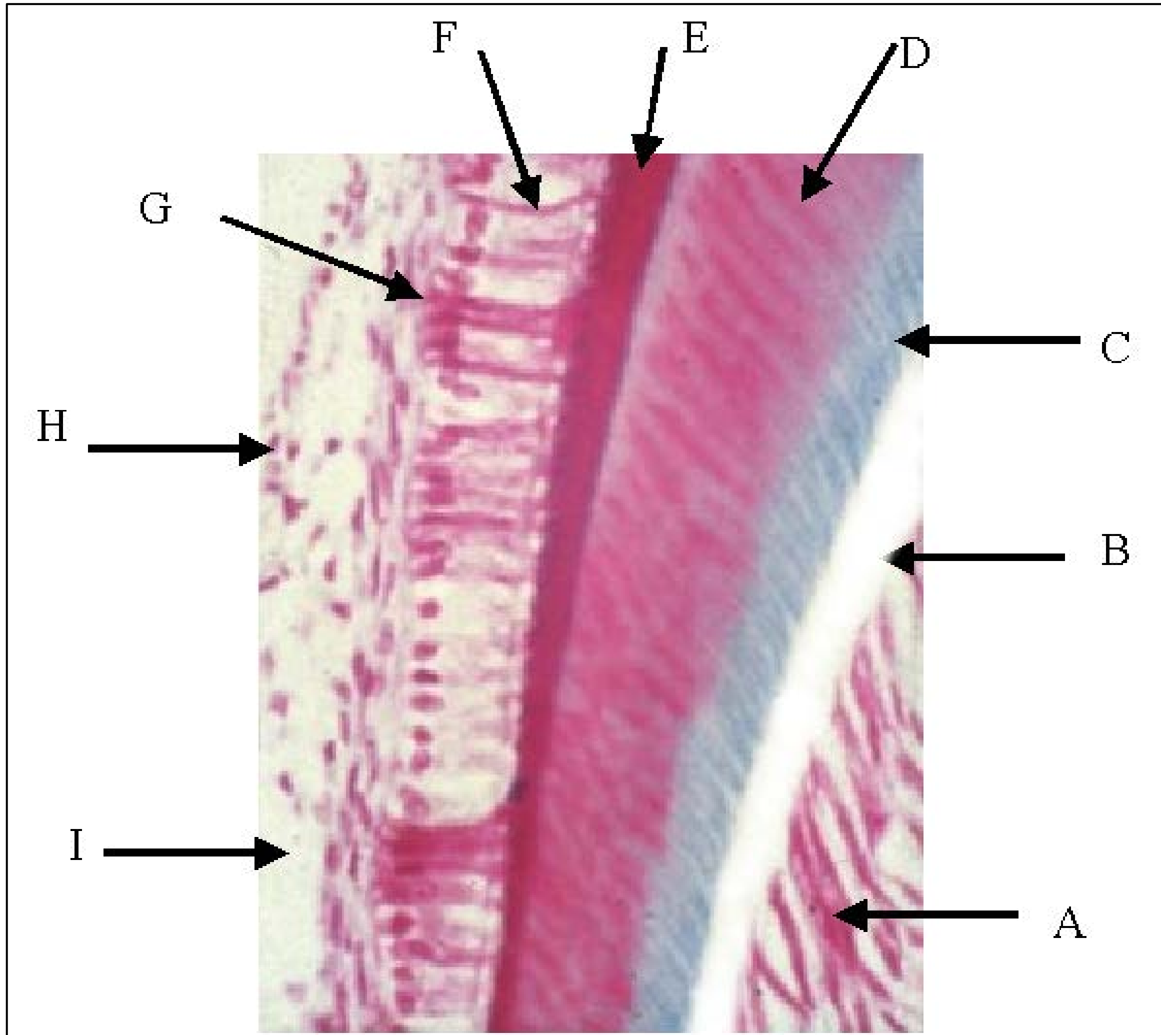


Fig. 5-21: Ten Cate's Oral Histology, 8th ed

Section of a developing tooth of a cat

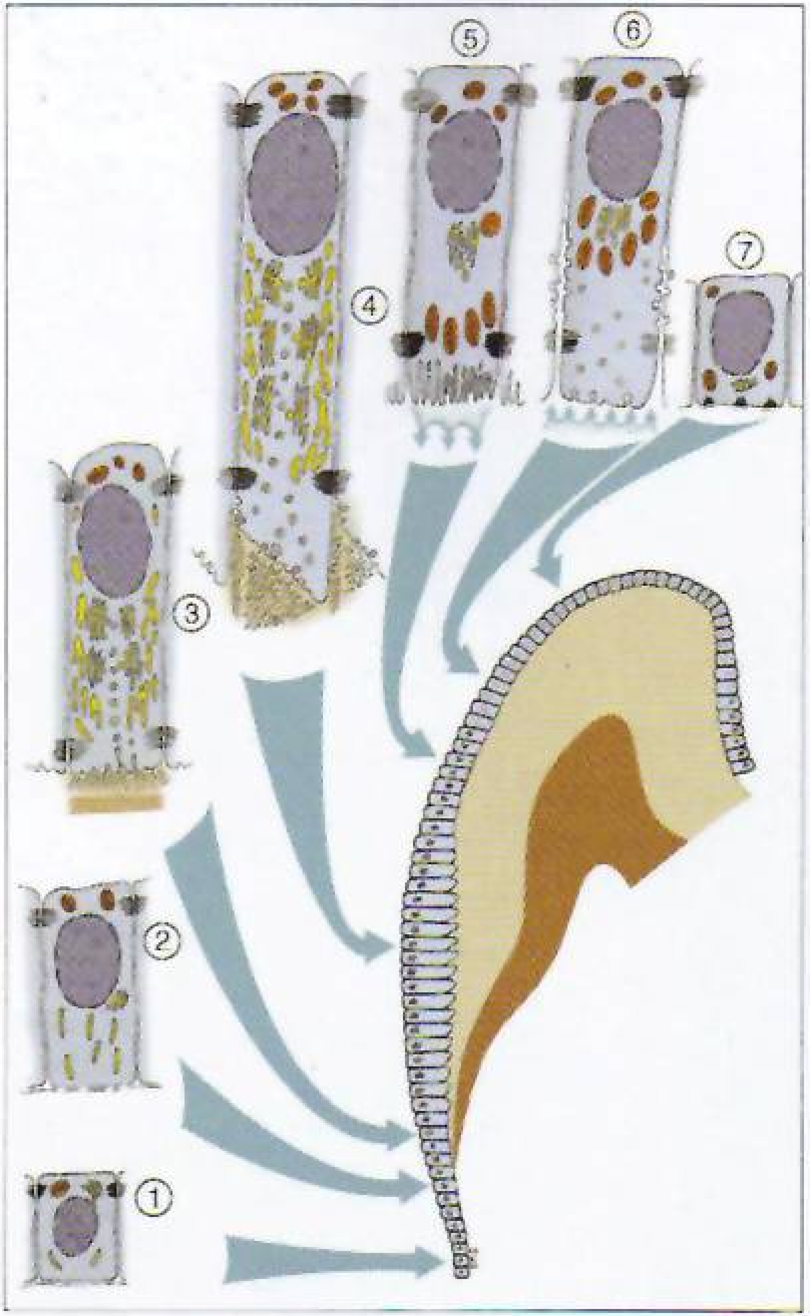


Two Stages of Amelogenesis

- Stage 1 = formation of enamel matrix
  - Enamel = 15% hydroxyapatite, 65% water, 20% organic matter (enamel proteins, 95% amelogenin) after secretion (by weight)
- Stage 2 = enamel maturation
  - Enamel crystals increase in width from 1.5 nm to 25 nm as organic content and water decreases and mineral content increases to 96% (A nanometre is one billionth of a metre.)

The life of an ameloblast

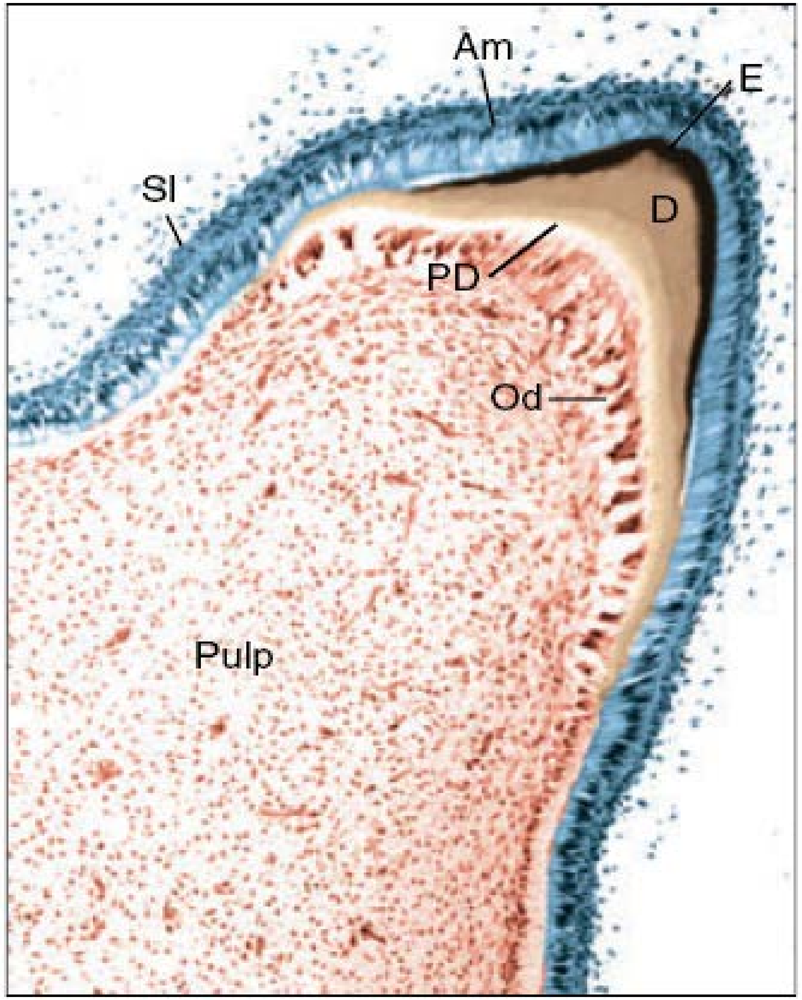
- 1. Presecretory (Morphogenetic & Differentiation phases)
  - Establishment of shape of tooth (cells of inner enamel epithelium)
  - Differentiation of ameloblasts, establishment of polarity, development of ability to synthesize proteins
- 2. Secretory – (initial stage lacks Tome's process; then process develops)
  - Secretion of matrix, initial mineralization, creation and organization of enamel thickness
- 3. Maturation
  - Transitional phase, 50% ameloblasts die and phagocytosed by remainder
  - Transport of ions required for accretion of mineral
  - Removal of water and proteins from matrix
- 4. Protection
  - Re-formation of basal lamina (primary enamel cuticle). Basal lamina and reduced enamel epithelium form Nasmyth's membrane which covers tooth until eruption



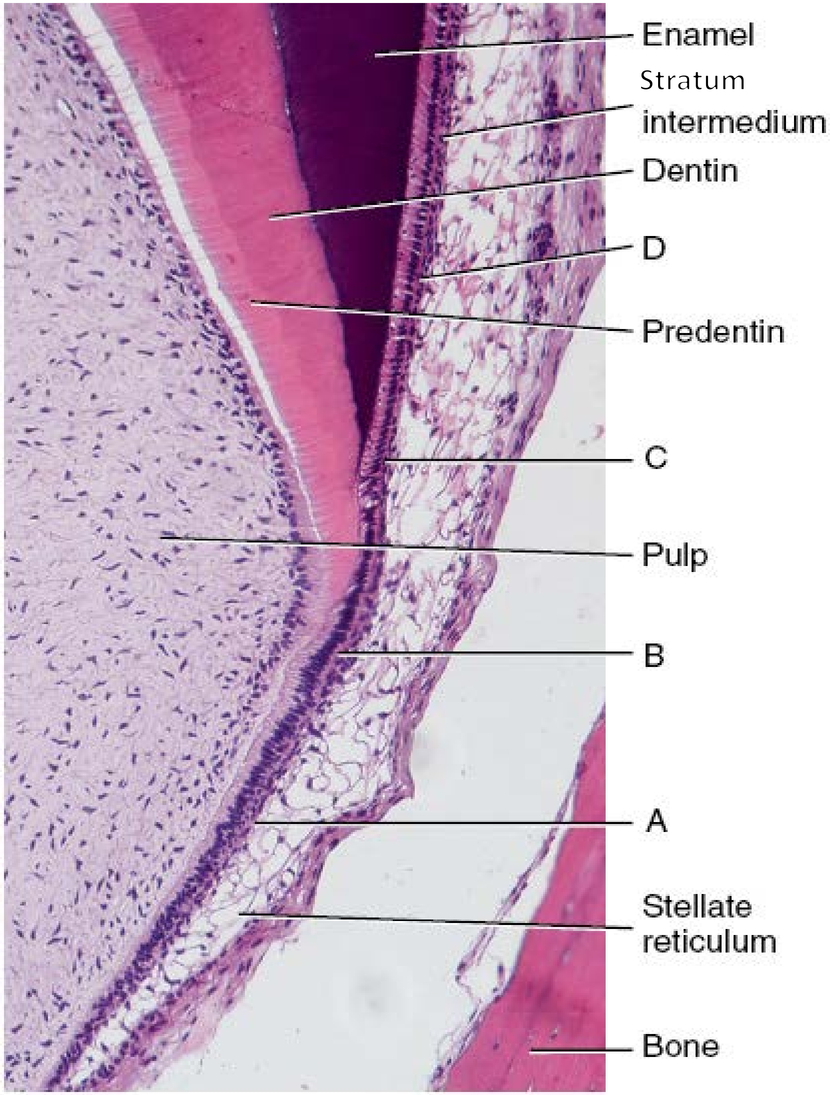
1. Morphogenetic stage
2. Histodifferentiation stage
3. Initial secretory stage without Tome’s process
4. Secretory stage (Tome’s process present)
5. Ruffle-ended ameloblast of maturation stage
6. Smooth ended ameloblast of maturation stage
7. Protective stage

Nanci A (2008) ***Ten Cate’s Oral Histology.*** **St Louis: Mosby Elsevier. Figure 7-14.**

Dentine & enamel have started to form.
Ameloblasts, enamel, stratum intermedium, dentine, predentine and odontoblasts are visible. Photograph from Nanci (2013).



View of developing enamel and dentine seen with the light microscope.
Photograph from Nanci (2013). Letters A-D represent ameloblasts at different stages.

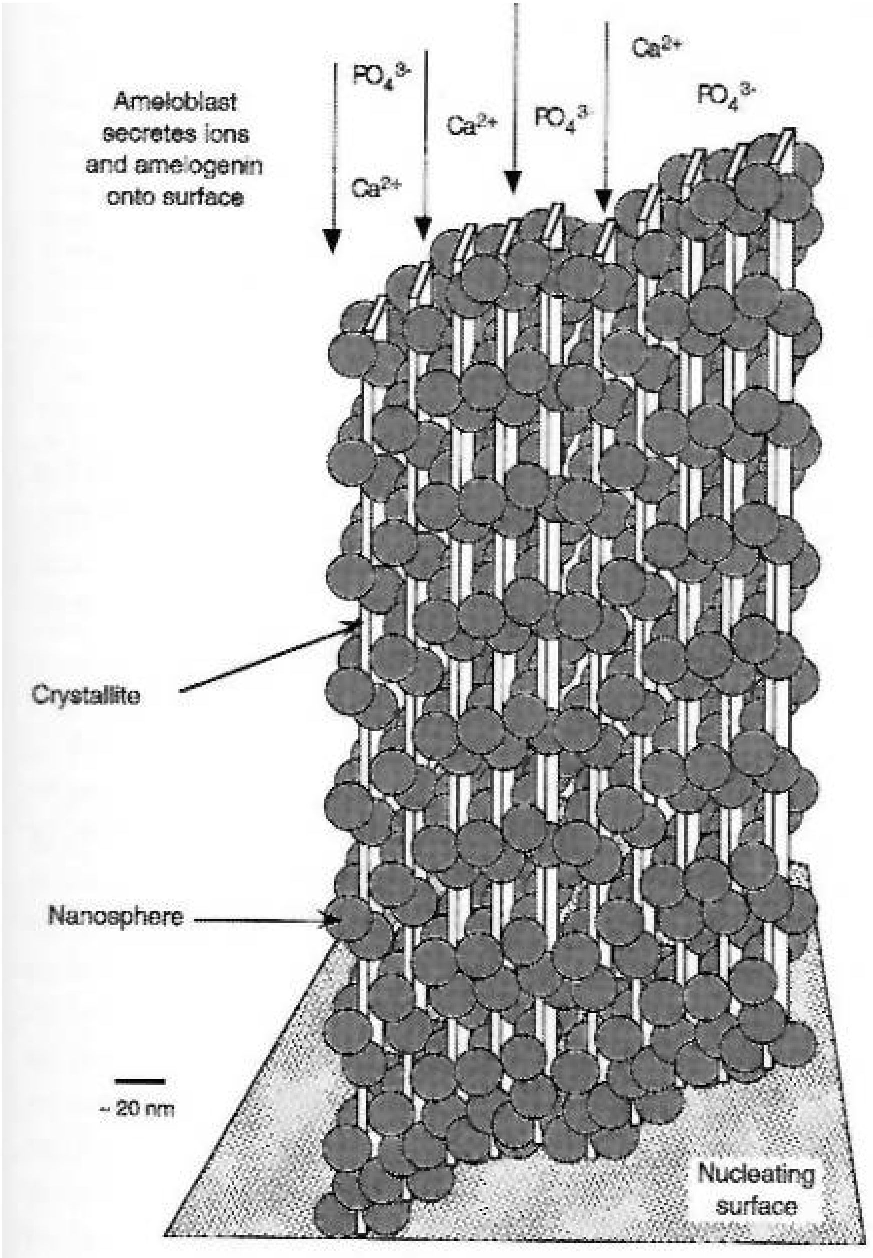


Maturation Stage

- Hardening of enamel due to growth in width and thickness of pre-existing crystals
- Two stages
  - Transitional phase
    - After enamel thickness formed
    - Reduction in height of ameloblasts; decrease in volume
  - Maturation phase

Enamel Biomineralization

- Assembly of enamel matrix occurs extracellularly
  - All relevant information for assembly must reside in structure of the protein
  - Matrix disassembled during maturation phase
- Secretory phase:
  - Amelogenin and mineralized enamel ribbons secreted
    - Ribbons are 2-4 nm wide and 5-10 nm thick
- Amelogenin forms supramolecular aggregates = nanospheres
  - Guide in formation of enamel crystals
  - Restrict growth in thickness and width of crystals, which form between them
- Maturation phase in which cleavage of amelogenin results in collapse of nanosphere and thickening of crystal
- Ameloblasts pump calcium, phosphate and carbonate ions into matrix and remove water and degraded protein



Enamel ribbons surrounded by amelogenin nanospheres

Fincham AG, Luo W, Moradian-Oldak J, Paine ML, Snead ML, Zeichner-David M. (2000) Enamel biomineralization: the assembly and disassembly of the protein extracellular organic matrix. In Teaford M, Meredith Smith M, Fergusion MWJ (eds): *Development, Function and Evolution of Teeth.* Cambridge University Press, pp 37-61.

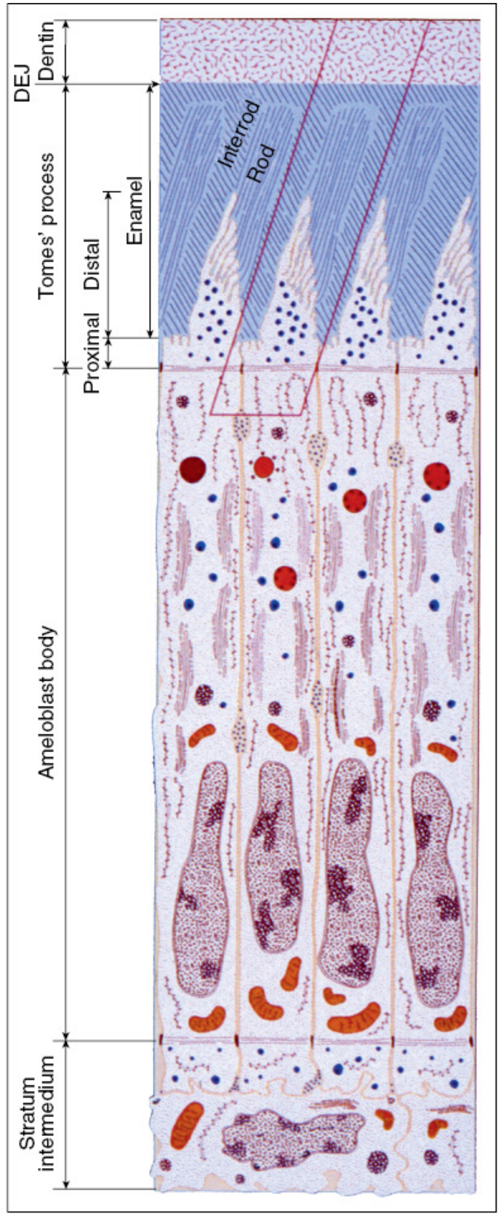
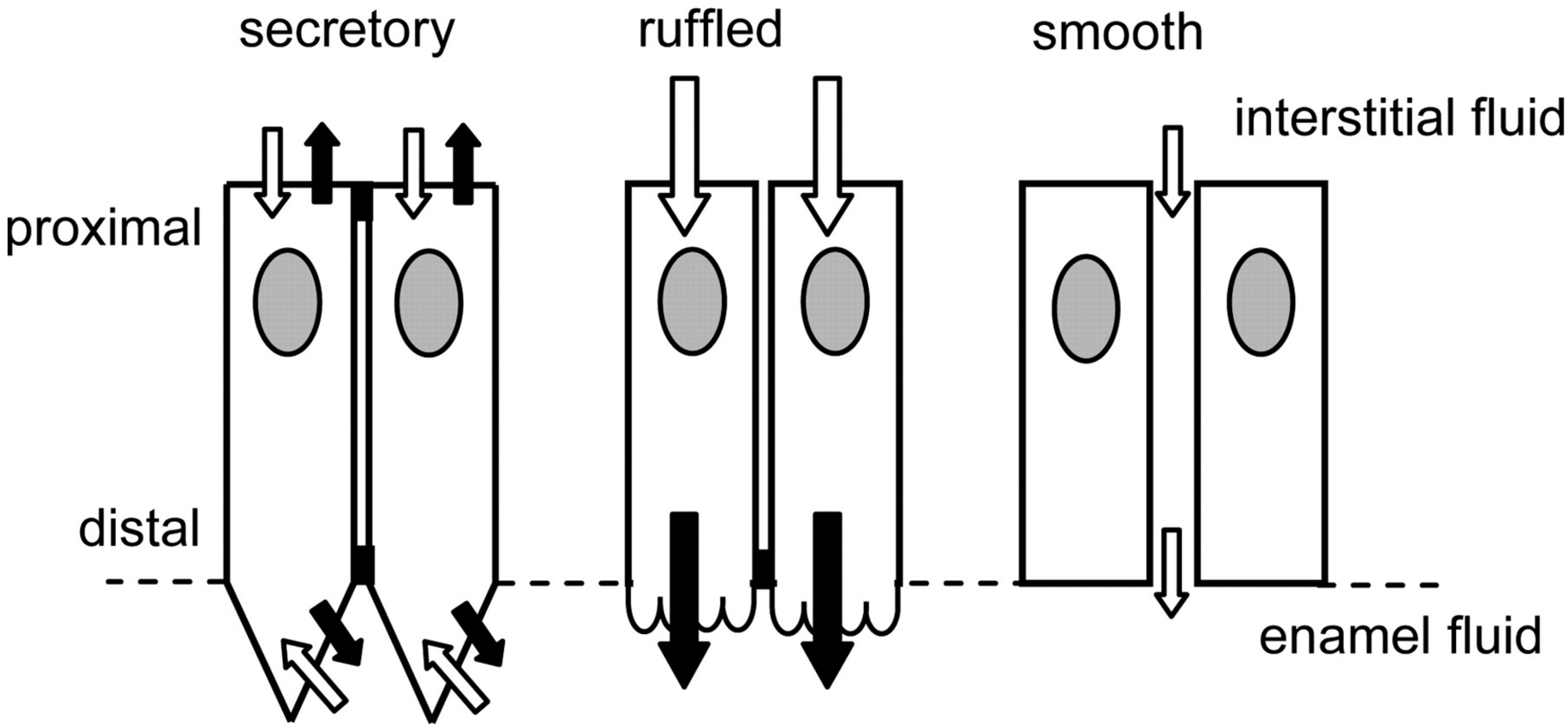


FIGURE 7-27 Schematic representation of the organization of secretory stage ameloblasts as would be revealed in a section along their long axis. *DEJ,* Dentinoenamel junction.

Maturation phase

- Cyclic modulation of ameloblasts:
  - Ruffle ended – leaky junctions between ameloblasts at proximal (basal) end; tight junctions at distal (enamel) end – selective transfer of calcium ions across ameloblast layer
  - Smooth ended – leaky junctions distally – proteins and water exit; interstitial fluid leaks into maturing enamel between ameloblasts - trace elements are incorporated at Ca sites through ion exchange

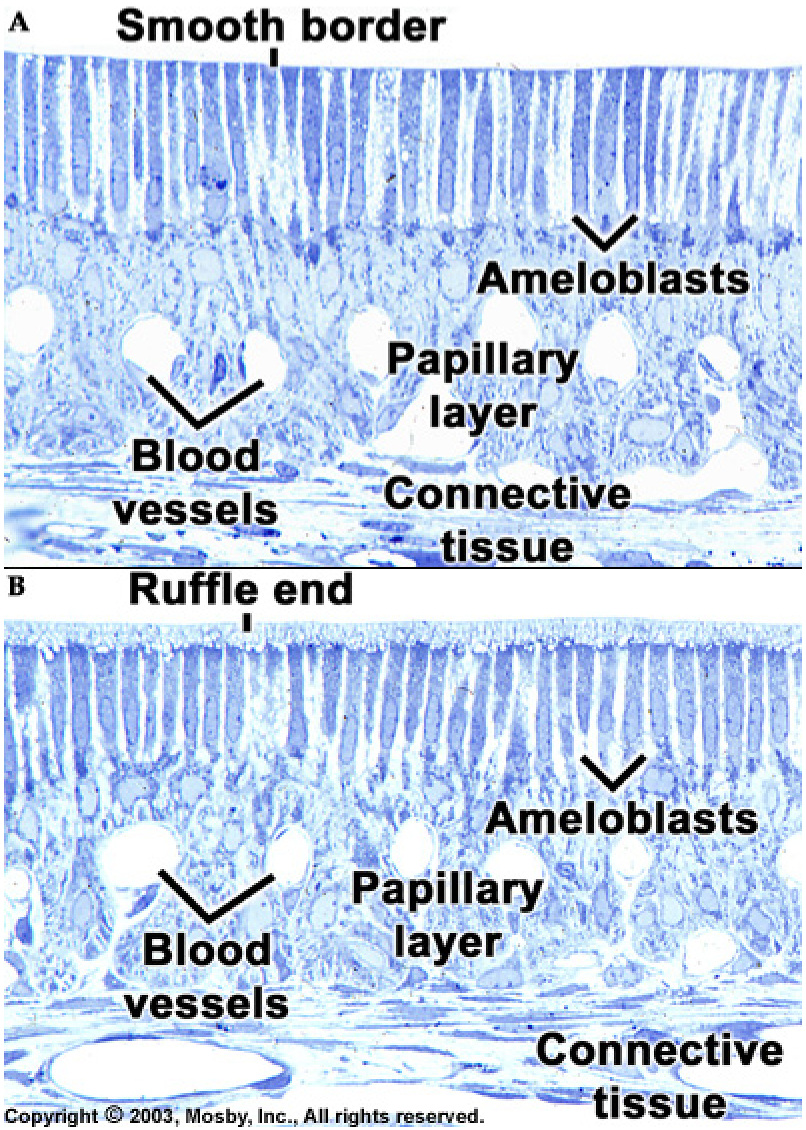
Calcium transport across the ameloblast layer (enamel epithelium) during the secretory stage of enamel formation and the ruffle-ended and smooth-ended phases of the maturation stage. During secretion, the amount of calcium is regulated by Ca-ATPase; calcium is actively pumped into enamel matrix; excess calcium is removed. Active transport of calcium is indicated by black arrows; passive transport of trace elements by white arrows.



Selective transfer of ions across ameloblast layer between interstitial and enamel fluid. Rate of transfer of Ca is 4x higher during maturation phase.

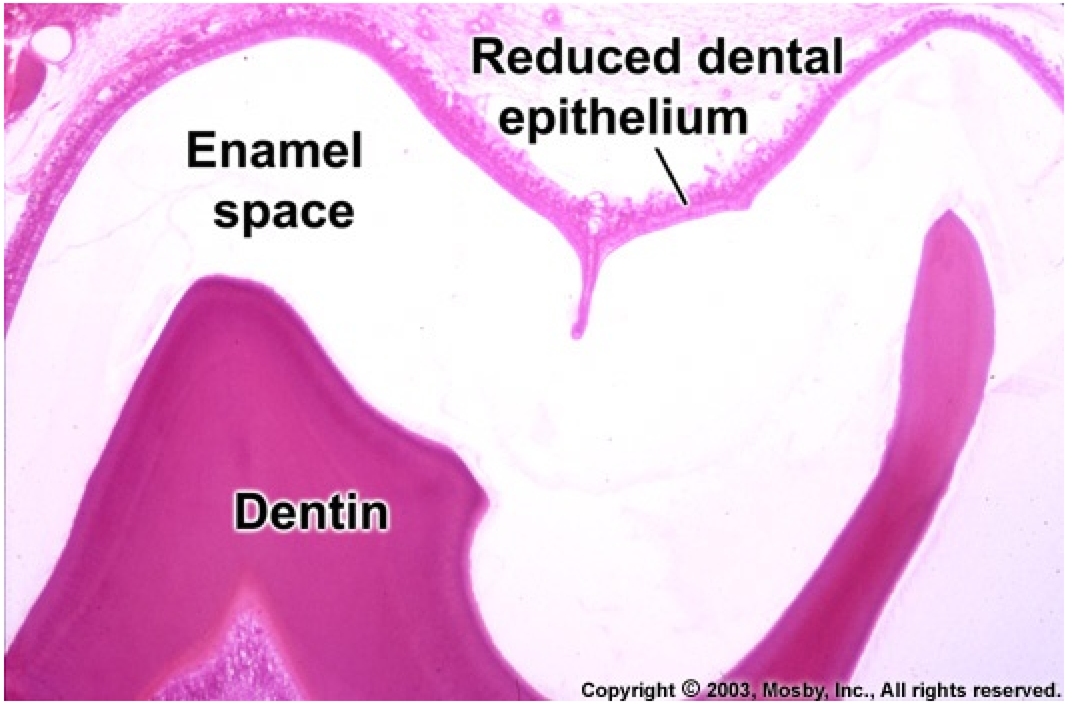
Humphrey L T et al. PNAS 2008;105:6834-6839

Papillary layer formed from remnants of stellate reticulum, stratum intermedium and outer enamel epithelium.



Protective Phase

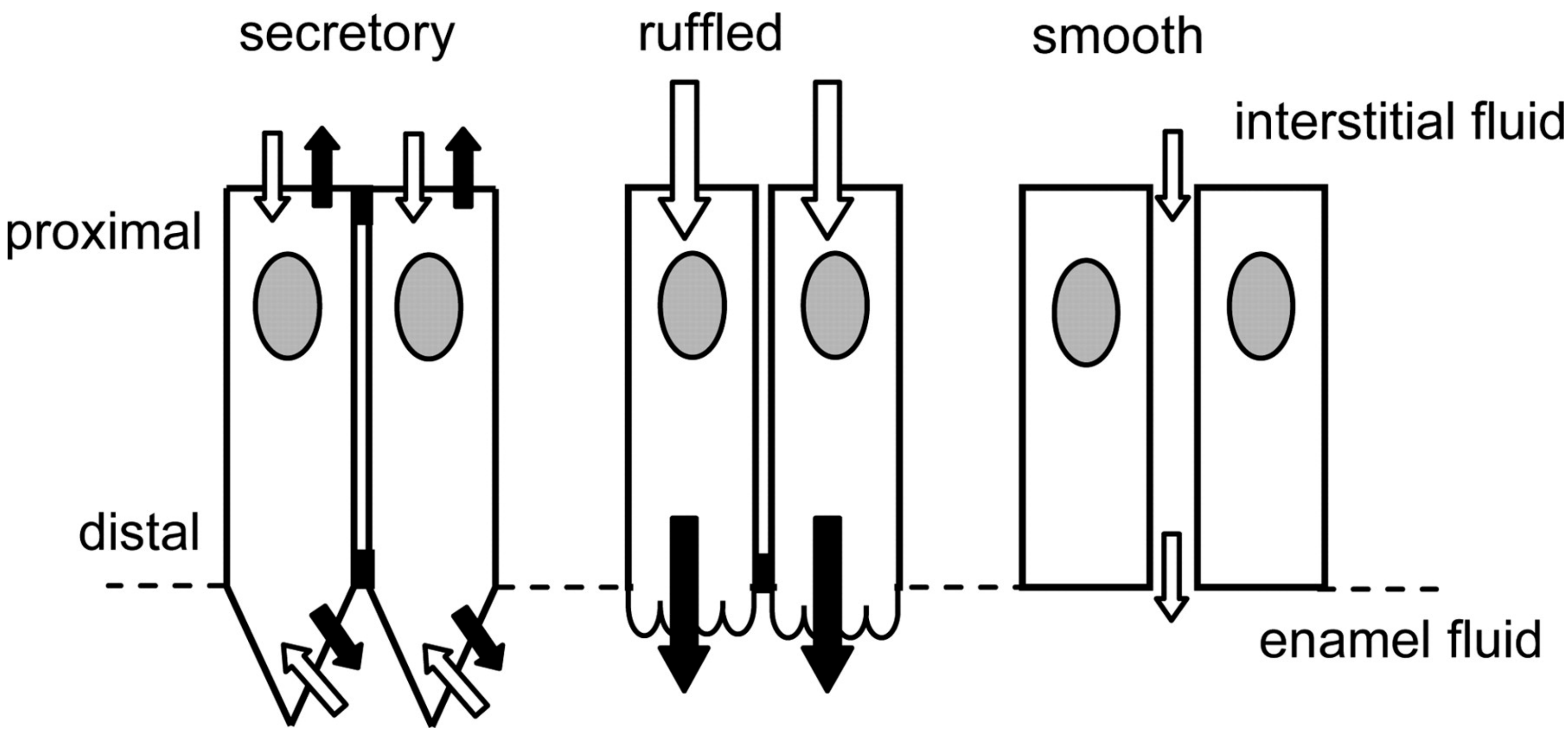
- Ameloblasts secrete a basal lamina called the primary enamel cuticle
- Papillary layer and ameloblast layer regress and form reduced enamel epithelium
- Reduced enamel epithelium and basal lamina (primary enamel cuticle) form Nasmyth’s membrane
- Covers enamel of unerupted teeth; because hemidesmosomes are leaky, fluoride and other trace elements can be incorporated at surface of enamel
- Epithelial layer (REE) of Nasmyth’s membrane fuses with oral epithelium during eruption of teeth, preventing bleeding
- After eruption, primary enamel cuticle (basal lamina) interacts with saliva and becomes acquired pellicle on the exposed crown (later with plaque adhering to it)
- The primary enamel cuticle becomes a layer of the junctional epithelium within the gingival sulcus.


Reduced dental
epithelium
Enamel
space
Dentin

Gradients of mineralisation in enamel

- Mineralisation increases from edj to surface in both primary and permanent teeth
- Mineralisation increases occlusally to cervically in permanent molars, and both permanent and primary incisors.
- Primary molars lack occlusal to cervical gradient of mineralization
- Primary teeth are less mineralised than permanent because less time in maturation phase.

Trace elements are incorporated passively during the maturation phase; during smooth ended phase, allowing components in the interstitial fluid to enter the enamel fluid. Trace elements incorporated this way include strontium, barium, lead, potassium, sodium, magnesium. The most important trace element incorporated into teeth for dentists is fluoride.



Humphrey L T et al. PNAS 2008;105:6834-6839

PNAS

General properties of enamel

- Resembles rock structurally; likely to cleave (split) along crystalline axis
- Extremely hard; 6.8 on Mohs scale; hardest at the surface.
  - (Diamond is 10, Steel is 6.2)
- Lacks resilience (ability to return to absorb stress before plastic deformation occurs) and so likely to shatter
- Dentine is resilient because the crystals are within a collagen lattice
- Enamel is brittle (shatters before plastic deformation), requires support of underlying dentine
- Highly resistant to abrasion
- Structural properties protect crack propagation; resistant to shearing and impact

General properties of enamel

- Enamel thickness varies across the tooth crown; thickest over cusps, thinnest over cervical margin
- Thickness varies from tooth to tooth; increases from first to third molar
- Made up of an impure hydroxyapatite
- Made up of crystallites forming prisms or rods; the crystallite cores are slightly richer in magnesium and carbonate than at the periphery.

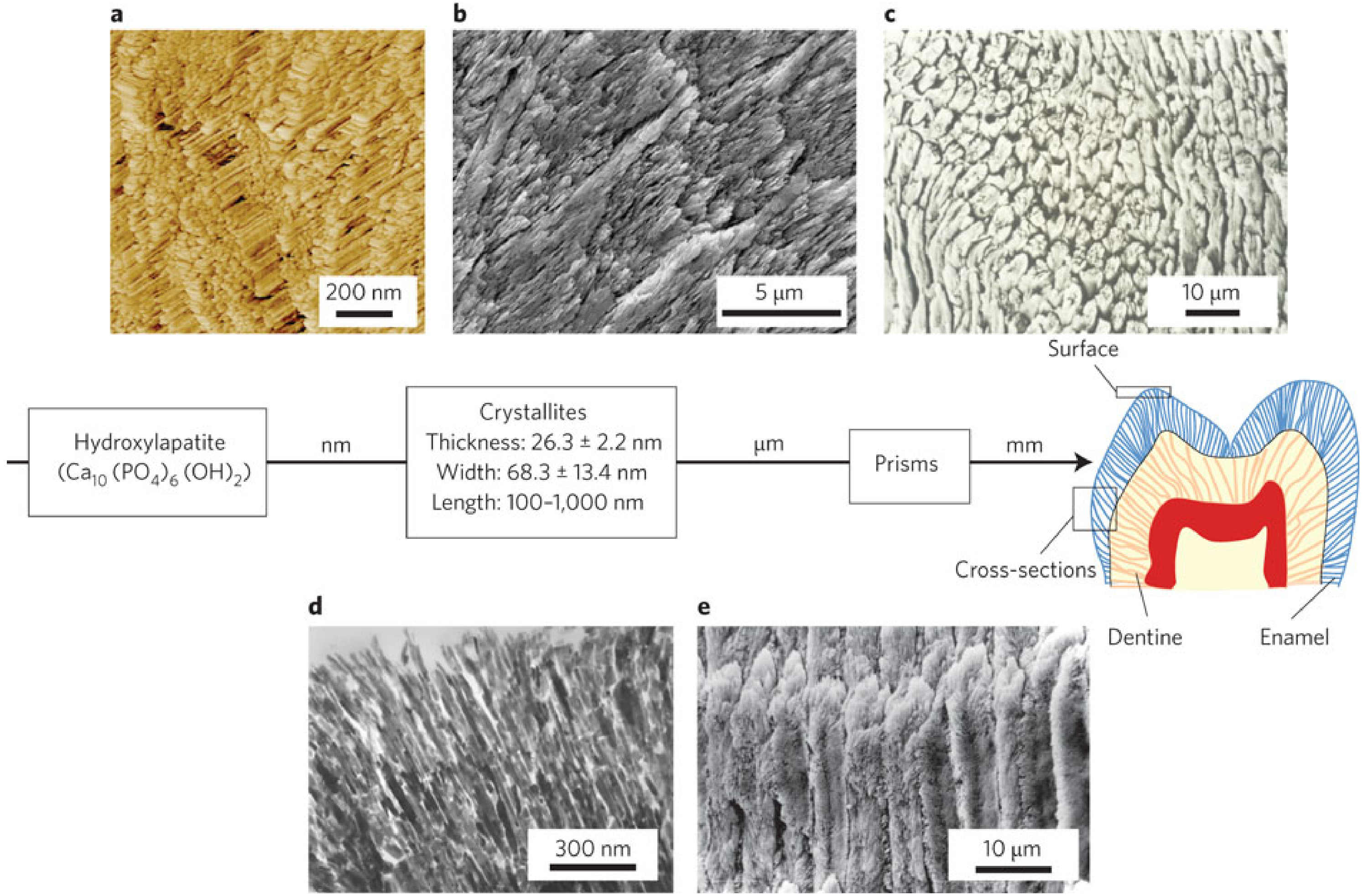
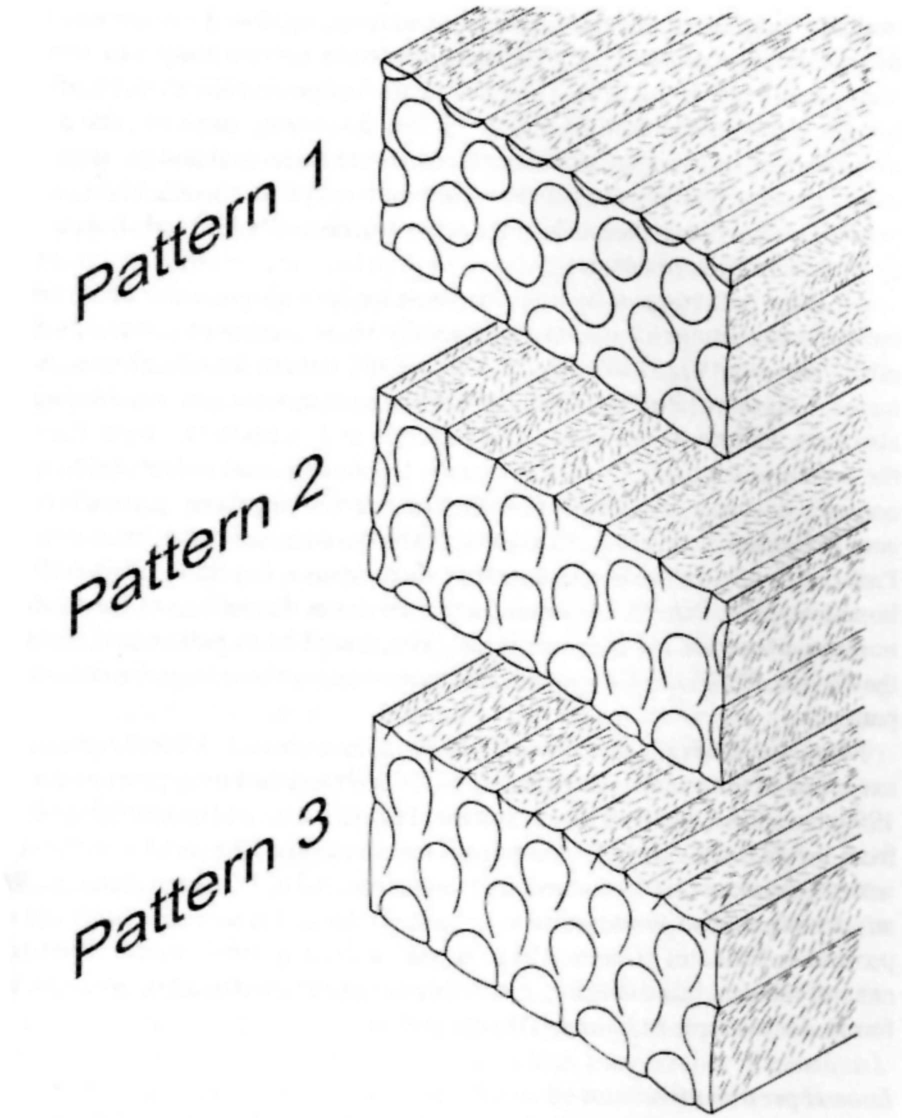


Figure 3 | Hierarchical structure of the dental enamel. Dental enamel is a masterpiece of bioceramics, containing structures at different hierarchical levels from the microscale down to the nanoscale. The enamel is composed of three-dimensionally organized nanosized hydroxyl apatite crystallites (a,b,d) that are arranged into micrometre-sized prisms (c,e). a, Atomic force microscope and b,c, scanning electron microscope images of the enamel surface. d, Transmission electron microscope and e, scanning electron microscope images of a cross-section of the enamel.

Hannig and Hannig (2010) Nanomaterials in preventive dentistry. *Nature Nanotechnology* 5:565-569.

Enamel prisms (Rods)

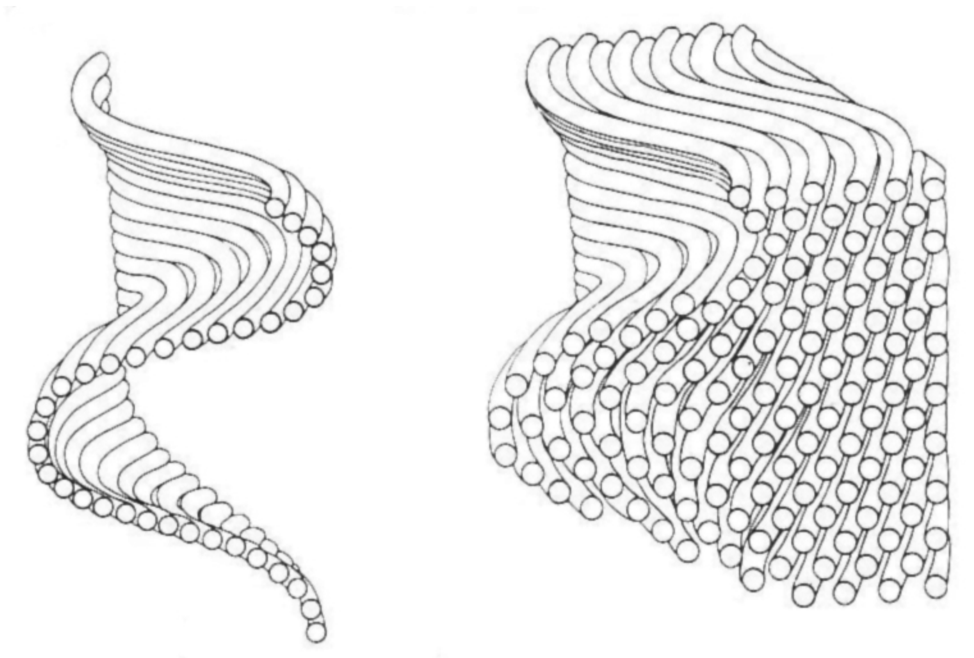
http://dentistry.uic.edu/craniofacialgenetics/researchted.htm



- Pattern 1 is only form with discrete prisms; surrounded by interprismatic enamel
- Pattern 2 discontinuities; prisms in vertical rows with interrow sheets of interprismatic enamel
- Pattern 3 horseshoe shaped; interprismatic enamel attached to tail below head of prism when seen in cross section

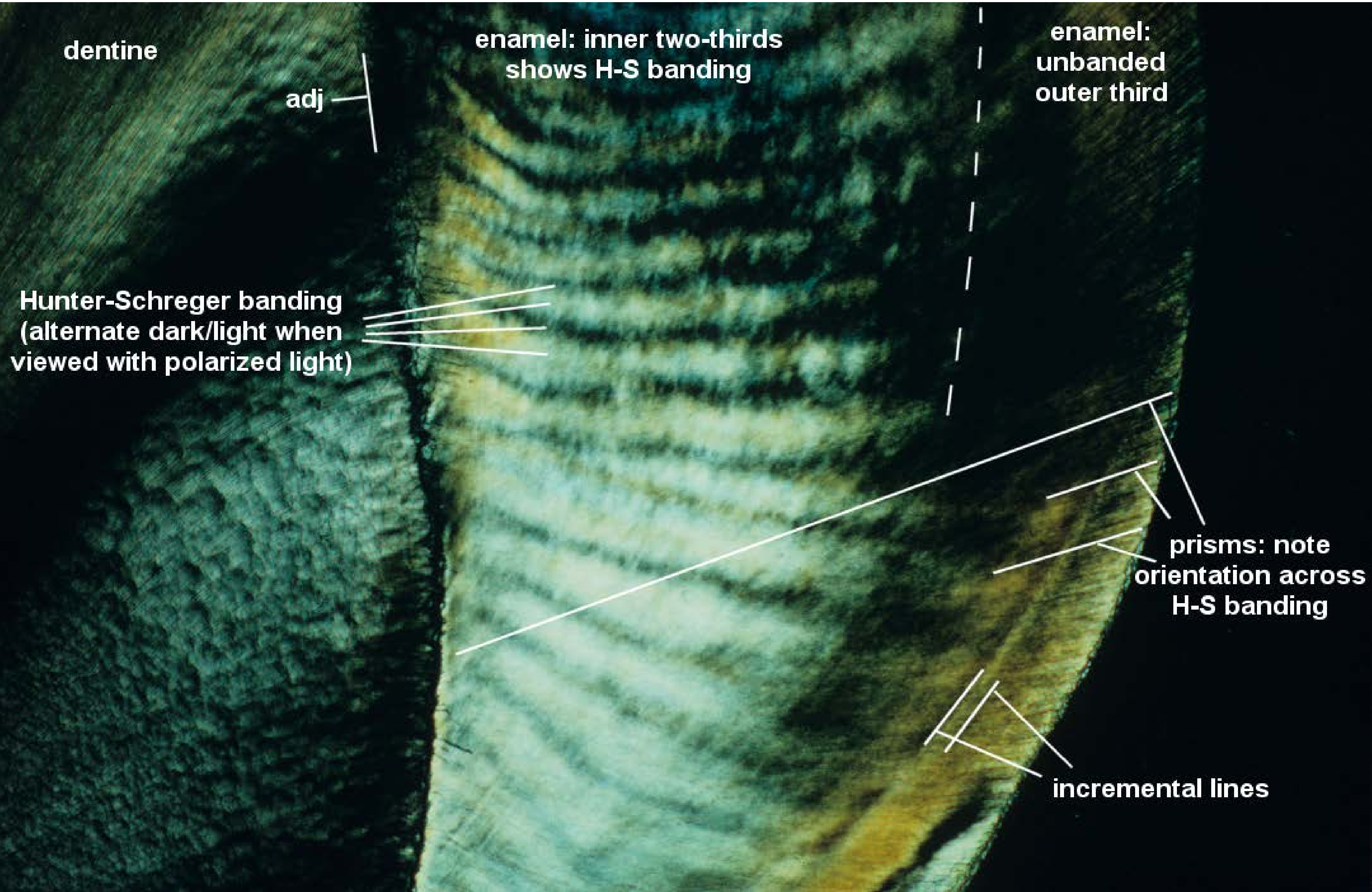
Hillson, 2005: Teeth. 2nd Ed. Cambridge University Press

Prism Decussation

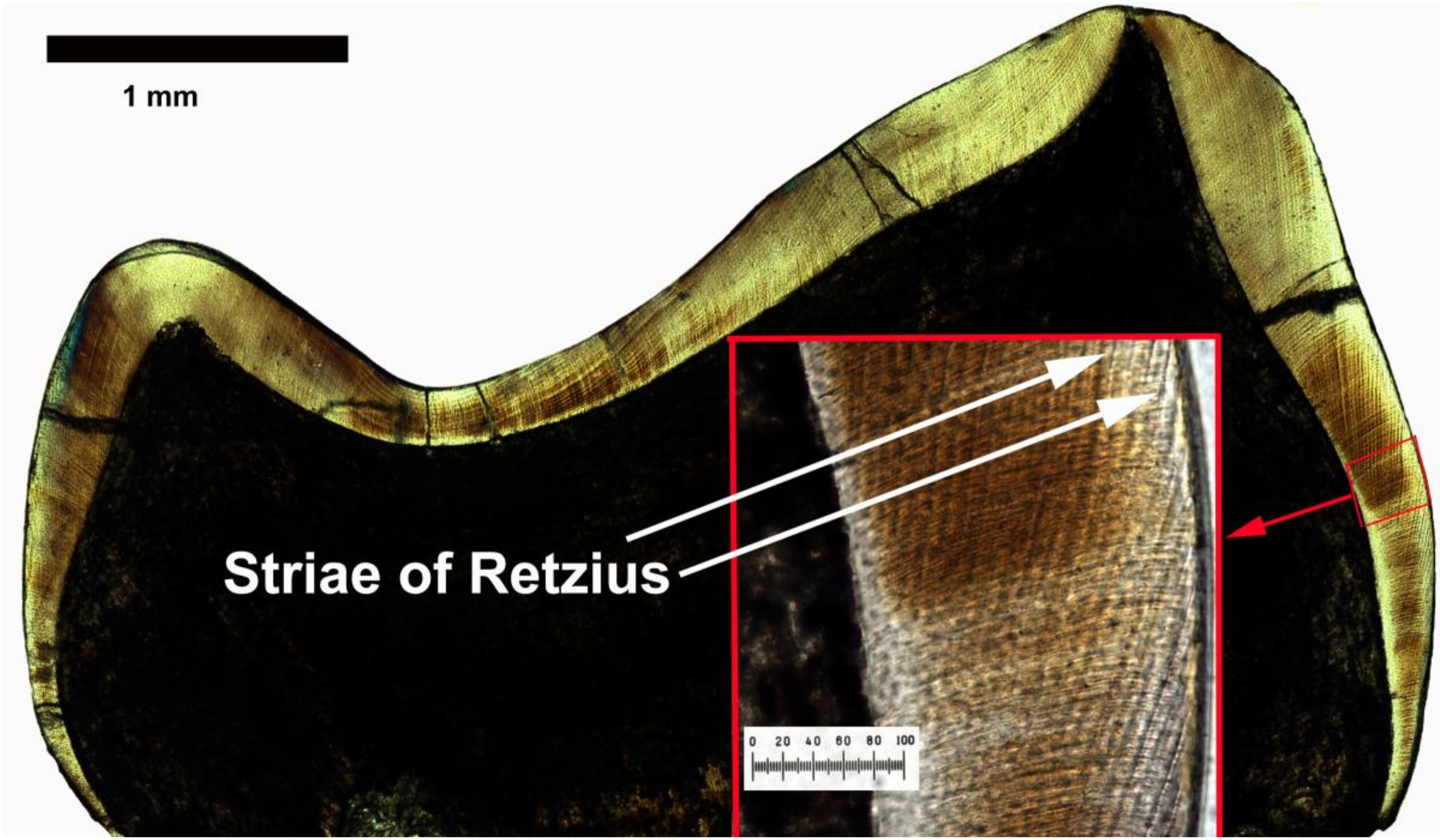


Hillson 1996. Dental Anthropology. Cambridge University Press.

Longitudinal ground section through tooth crown seen with polarized light to display Hunter-Schreger bands in inner two-thirds of enamel

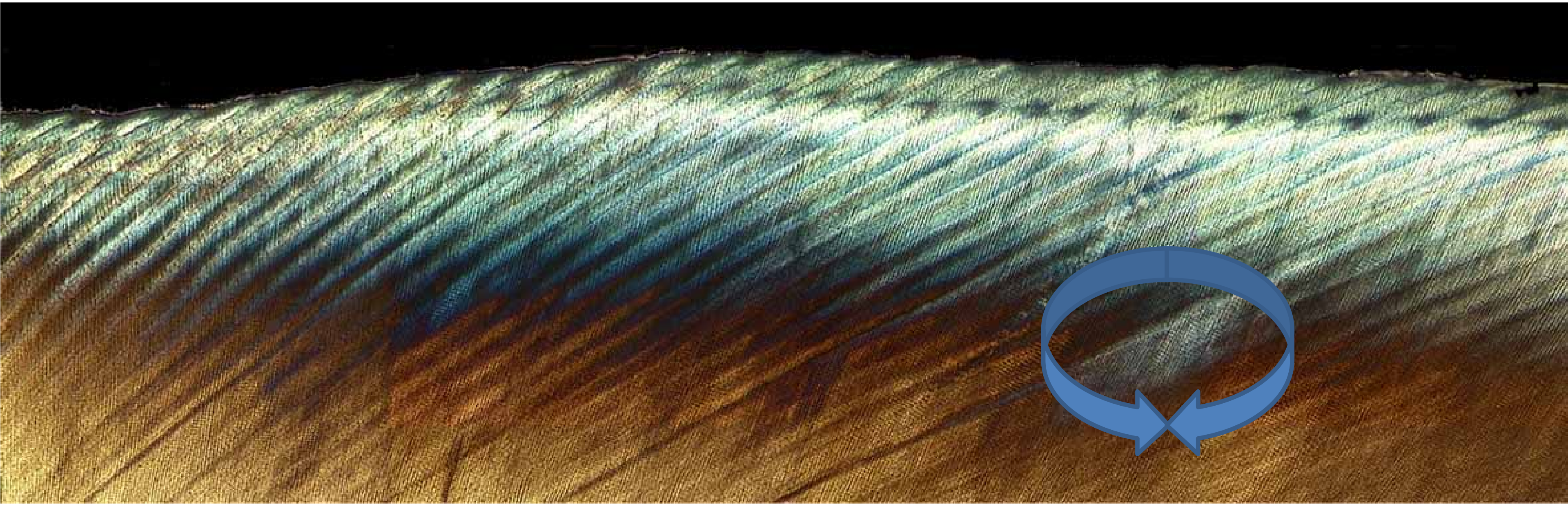


Growth Increments in Enamel

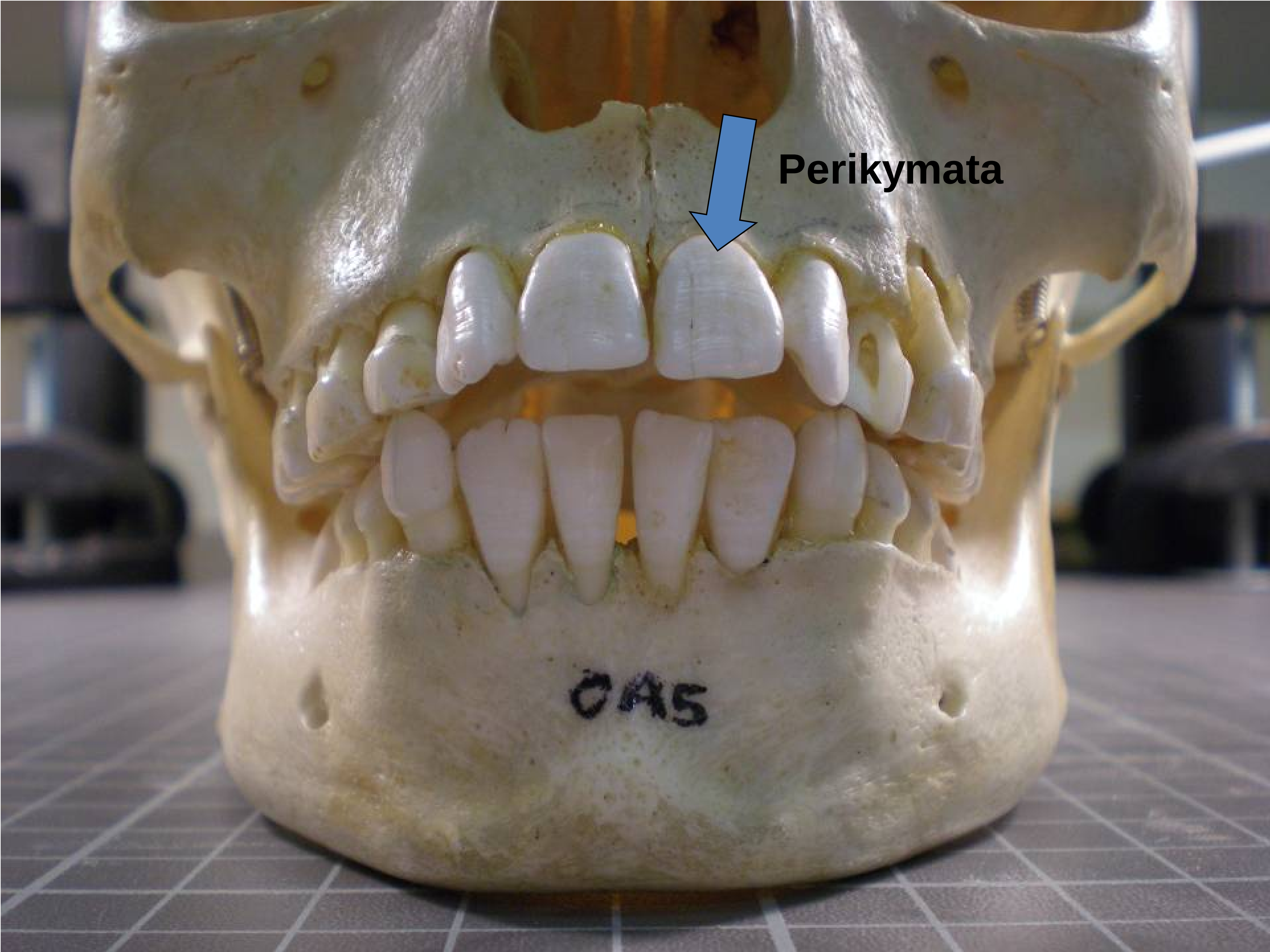


Cantius abditus, Early Eocene

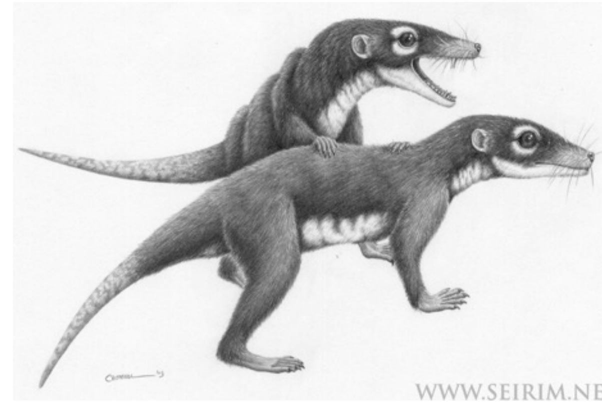
Striae of Retzius at enamel surface; perikymata; human.

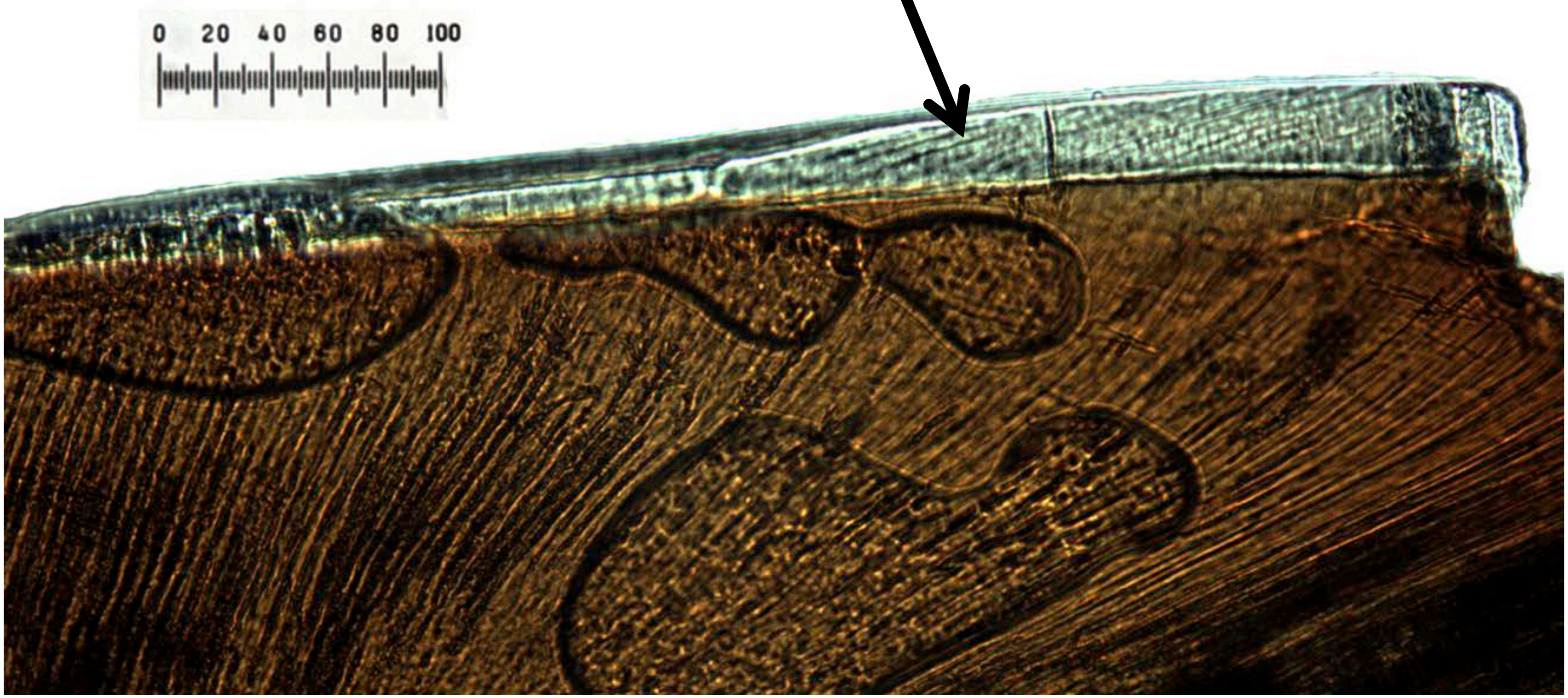


http://hdl.handle.net/2451/14870


Perikymata
0A5

Morganucodon
Late Triassic
27-89 g

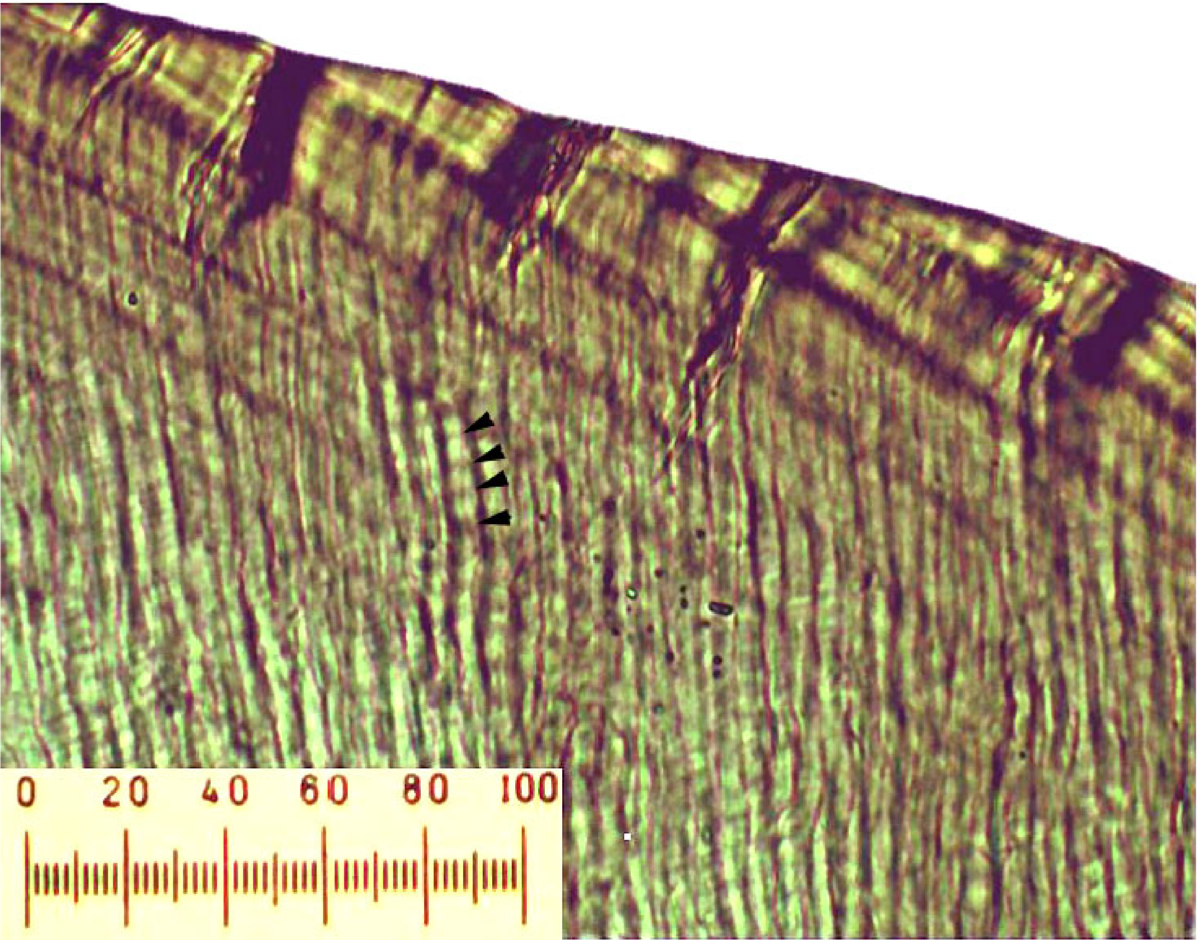




A vervet monkey showing a periodicity of 4 days

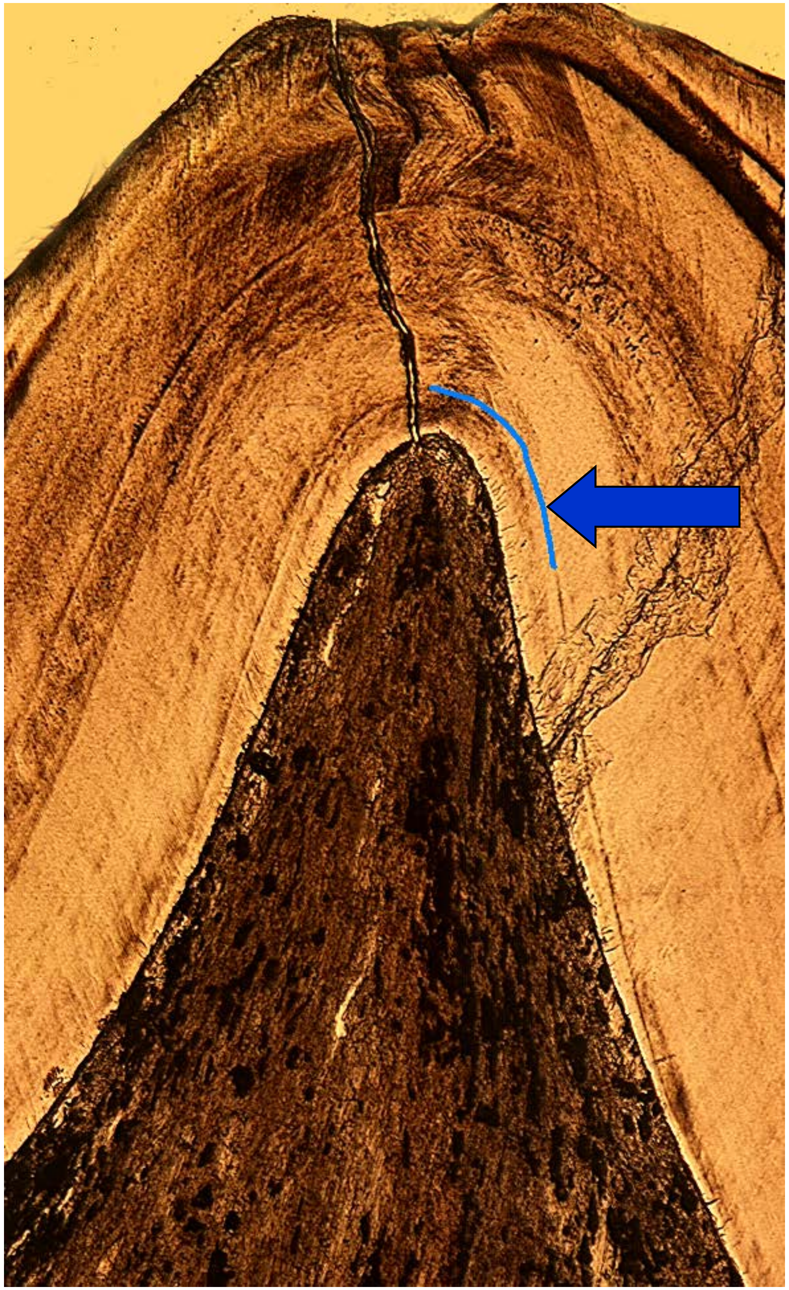
Daily increments = cross striations

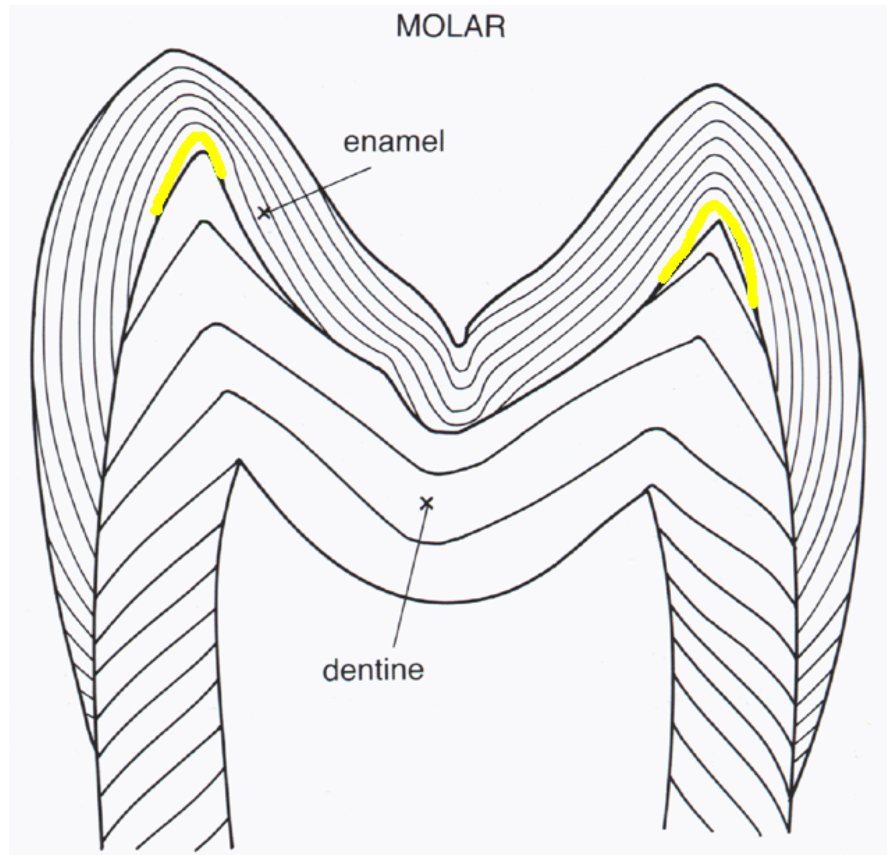
Periodicity = number between striae; dependent on body mass; human mode = 9 days



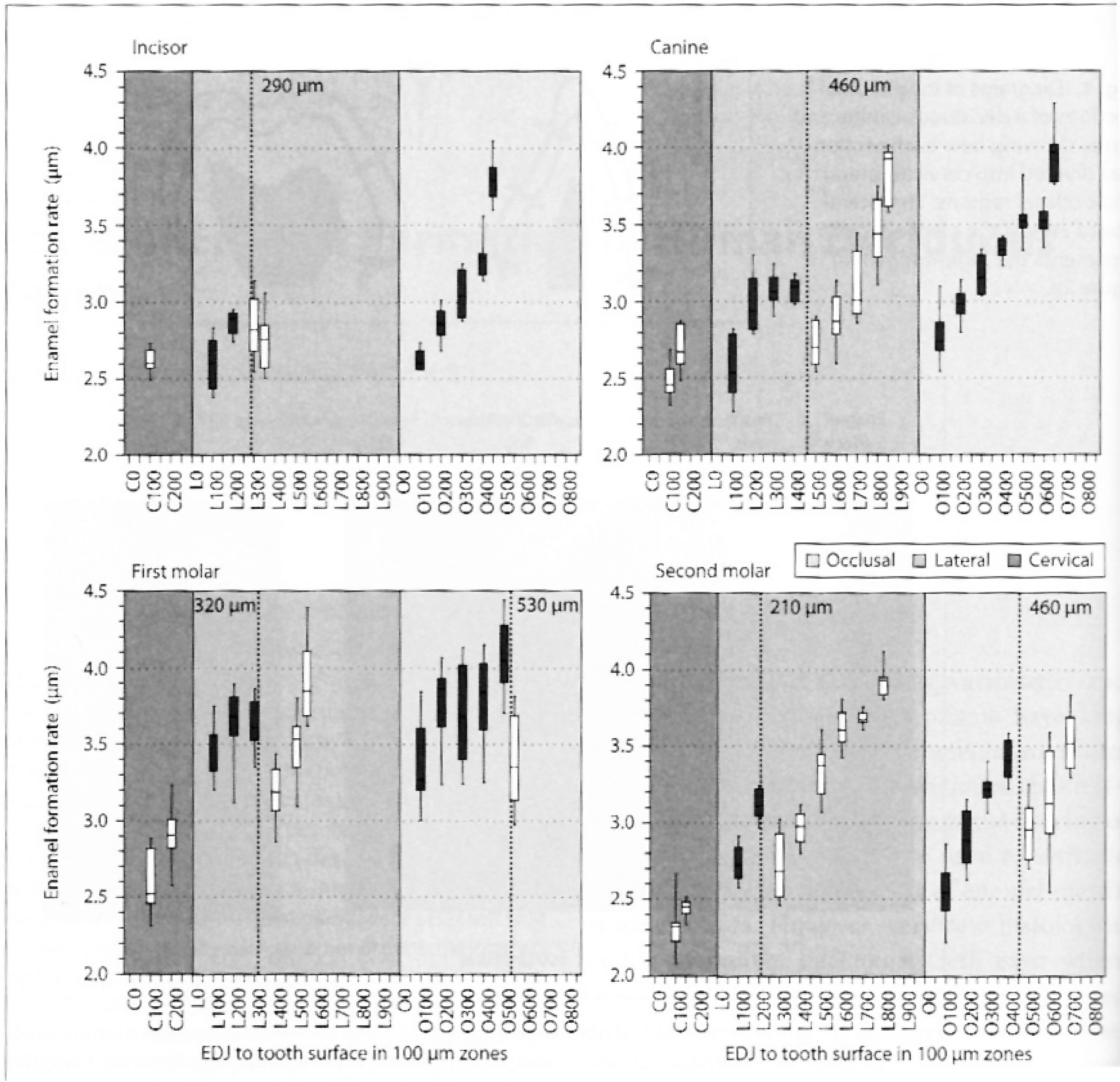
Neonatal line

in M_1





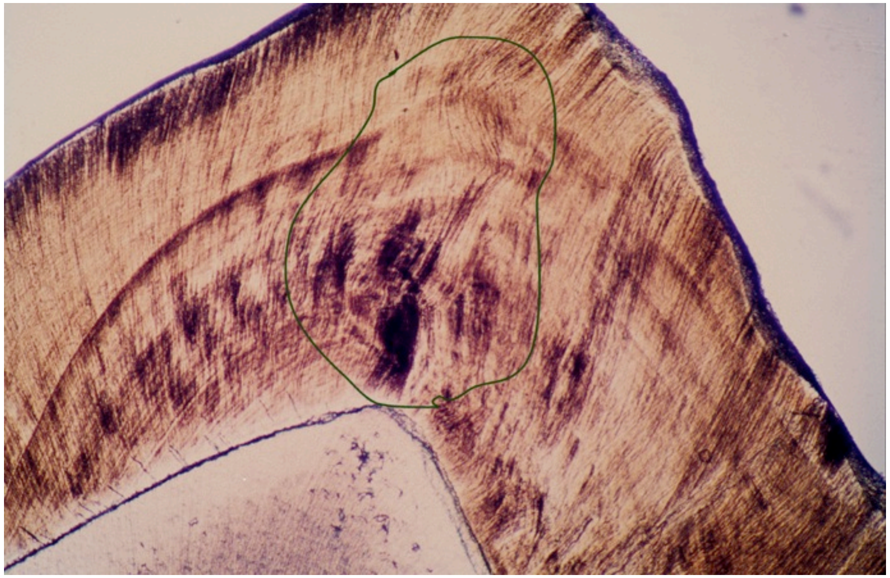
Courtesy Dr Rebecca Ferrell



Birch W & Dean MC (2009). Rates of enamel formation in human deciduous teeth. In *Compartive Dental Morphology,* Koppe T, Meyer G & Alt KW, eds. Basel, Karger: pp 116-120.

Gnarled enamel in human tooth

Green circle encloses an area with exaggerated prism decussation over cusp



Gnarled enamel in *Plesiadapis cookei;*
Around 60 million years old;
note also enamel lamella following prism direction in inner 2/3 enamel.
Longitudinal ground section.