

## ENAMEL FACTOID

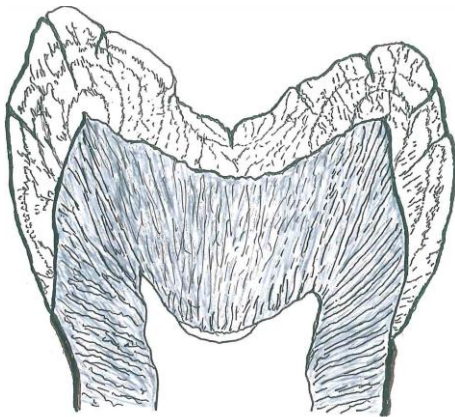
This FACTOID focuses upon the morphology & physiology of human enamel—a non-vital mineralized tissue that cannot repair itself—Craig & Peyton (1958) reported the general hardness of enamel at 343-KHN  $\pm$  23 std dev (Knoop hardness diamond indentation)—the greatest deviation was always noted just below the occlusal surface & an average compressive strength of 55,700 Psi.

In the US, clinicians like H.H. Hayden (1825), C.A. Harris (1839), M.H. Webb (1882) & G.V. Black (1883), J.L. Williams (1896) & T.B. Beust (1911) published their observations of enamel while studying human caries on extracted teeth—a famous 1882 photograph shows Dr. Black sitting in his upstairs laboratory at a sectioning device of his own design. Microscopic tissue fixation & processing for thin section histology (3-7 $\mu$ m) was still in its infancy—many reagents caused shrinkage, distortion & interfered with staining reactions—**enamel** could only be studied by ground section as the mineral substrate is lost with acid demineralization. Europeans—A.P. van Leeuwenhoek (1716), A. Nasmyth (1839), Sir John C. Tomes (1849), R.A. Kölliker (1853), J.E. Oudet (1862), L. Raschkow (1835), A.A. Retzius (1837), E. Magitot (1872), H. Lams (1921) & C.F. Bödecker (1923) also published articles on enamel morphology.

**Enamel** is the only mineralized human tissue that develops from epithelial cells that had proceeded through developmental stages into individual bell (cell) shapes, which invaginated inwards—like pushing your finger into the bottom of a balloon to create an inner layer—that forms a layer of inner enamel epithelial (IEE) cells, which continues to cause reciprocal shifts of proteins to trigger morphodifferentiation of the invaginated layer of IEE into **ameloblasts**. Following the first deposit of a few  $\mu$ m of dentine matrix at the cusp tip, **ameloblasts** then deposit an *enamelin* matrix that rapidly mineralizes at the cusp tip—composed of a unique arrangement of millions of calcium-hydroxyapatite (CaHA) crystals that coalesce into a single rod (prism), whereas rods are least mineralized at the cervical margin. Each **enamel** rod is a long thin prism that projects from the EDJ to the oral surface—mid-crown rod length is about 2-mm & the rod diameter is only about 5 $\mu$ m. Ground sections reveal that enamel is formed about 4 $\mu$ m per day along its rod length. **Enamel** is a dense, rigid cap (99.2% mineral – 0.15%-0.8% organic H.C. Hodge 1938) that ends at the cervical-

gum margin. When enamel formation is complete, IEE cells become part of the epithelial cervical-loop cells, which provides induction to pulp cells to form odontoblasts that continue to form root dentine—these cervical loop cells later split into epithelial rest cells of Hertwig or Cerres, to provide an inductive response that stimulates a thin layer of acellular (epithelial) cementum to cover the root dentine, followed later by cellular cementum deposition of mesenchymal origin (Furseth 1974).

The clinical **enamel** crown—seen below—is the visible part of the tooth that shows in the oral cavity—due to its dense CaHA mineral composition, it varies in color from very white in primary (deciduous) teeth to **light yellow** & dark gray in permanent teeth that continue to become darker as the tooth ages with continued deposition of secondary dentine. **Enamel** is approx. 3.0 mm thick at the cusp tip & tapers off to a thin “feather” edge at the cervical margin where it ends at the enamel-cementum junction. X-ray data by Thewlis (1938) reported a “thin strip of hypercalcified enamel on the surface. . . there is a gradual increase in calcification from the amelo-dentinal junction to the outer hypercalcified layer.”



**Enamel** forms by deposition of CaHA needlelike crystals around an organic *amelogenin* protein scaffold during **enamel** maturation development—adult **enamel** contains several unique morphological features such as **rods, gnarled rods, tufts, lamella, spindles, EDJunction, incremental striae of Retzius & perikymata.**

Each **enamel rod** (prism) has an undulating course from the EDJ to the oral surface & is “keyhole” shaped with a head, neck & tail, being composed of millions of small CaHA needlelike crystals—each being oriented with their long axis parallel to the rod. The peripheral CaHA crystals fan-out towards the tail with nano-amounts of inter-rod

proteins called *amelogenins*—*enamelin*s in mature enamel—are located along each rod periphery, some of these proteins remain as part of the organic lamellar sheath that extends outwards from the EDJ to the oral surface. As the enamel forming ameloblasts project off from the EDJ interface, there is no typical adult type of rod structure—the distal portion of the ameloblast cell has not yet been able to form a terminal “Tomes” process that is responsible to form the adult rod structure. However, after a few days of 5 to 8µm of initial deposition, the Tomes apparatus of each cell begins to define each rod shape. In the last phase of enamel rod formation within 30µm near the oral surface, ameloblasts lose its rod-form—primary tooth enamel is rodless, whereas the cervical 1/3 of enamel in permanent teeth is without any defined rod form.

**Gnarled enamel rods** are seen in all teeth as a dense twisted arrangement of rods that specifically project from the cuspal & incisal EDJ interface to form a complex arrangement of intertwined rods. It has been speculated that the gnarled arrangement provides for an increased degree of rigid support against fracture during biting, chewing & all other masticatory forces that are placed on the enamel.

**Tufts**—different than rod gnarling—are unique structural features of **enamel** that project as intertwined twists of rods from the **EDJ** that project for about 1/3 of the enamel thickness, being most numerous at the cusp tips & contain greater amounts of enamel protein than non-tuft enamel. Beust demonstrated (1911) enamel **tufts** in all human tooth types using an ROH-basic-fuchsin stain & speculated **tuft** form due to abrupt changes of rod direction as they project off the **EDJ** interface. Nakamura (2009) has speculated that enamel **tufts** form when blood proteins became lodged & condense during initiation & could not be degraded by enamel *proteineases*—allowing them to remain in the spirals of **tufts** & to then condense during the crystallization phase.

**Enamel spindles** are random projections of a single odontoblast process that cross the basement membrane at the cuspal **EDJ** during matrix deposition, leaving a dentine tubule-like spindle of mesenchymal tissue—with an organic protein (collagen) content unlike the surrounding enamel. Lester (1967) reported that **enamel spindles** could not be found in the bulk of circumpulpal dentine but speculated that these fiber

bundles penetrate the basement membrane of the **EDJ** as a consequence of a rapid build-up of proliferative cell growth of the dental pulp, speculating that **enamel spindles** are of mesenchymal origin. Several clinical reports have noted that in adult teeth, **enamel spindles** are often associated with areas of extreme hypersensitivity during cavity preparation, as well as that they are potential sites of bacterial penetration into dentine along & through the **EDJ** interface.

**Enamel lamella** were first described in 1838 by Professor Nasmyth as an organic sheath (fin) that course across from labial to lingual in the substance of enamel—generally found at the base of occlusal pits & fissures. Beust (1913) later described lamella as “canals of random shaped fissures that lie between the rods that served as diffusion currents. . . .noting the interrod protein substance was colorable by certain stains & with increasing age the color disappears—indicating further enamel sclerosis after eruption.” **Lamella** have been shown to be developmental remnants of thin sheets of organic proteins that begin at the **EDJ**—coursing throughout the enamel to the enamel surface from the labial cervical margin to the lingual cervical edge—most numerous in the cusp & incisal tips. On the other hand, environmental conditions such as cold water, ice cream & cold drinks can easily create thermal stress of sufficient magnitude to cause cracks in enamel that easily allow biofilm colonization, which may easily lead to caries (Lloyd et al 1978). In essence, these organic lamellar sheaths are pathways for the inward & outward diffusion of not only fluids but also to the proliferation of microorganisms to the **EDJ**, where they may then spread laterally along the **EDJ** interface & penetrate into the dentine tubule complex. Enamel lamella appear to be developmental remnants & often appear to be sites of increased vulnerability to bacterial biofilm invasion & eventual caries.

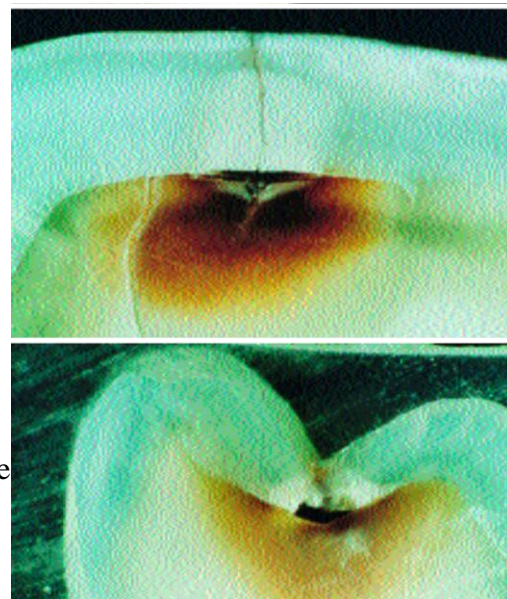
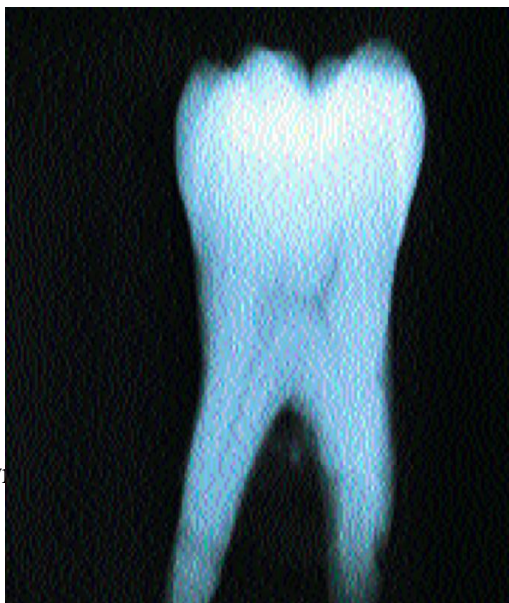
As soon as the individual begins to eat & drink low pH agents, the enamel lamellae protein sheath may become altered or even lost—leaving an open **permeable** space that rapidly becomes colonized with a biofilm of bacteria—some of which colonize the lamellae & other defects to immediately begin to proliferate & migrate towards the **EDJ**. Certain of the acid-producing bacteria may rapidly secrete organic acids e.g. lactic or butyric that etch the lamella walls that widens the space & allows more opportunistic bacteria to occupy the space. If these bacteria are fed sugars in the

absence of proper oral hygiene then a rapid caries response will rapidly result. On the other hand, if caries is halted, many of the normal salivary electrolytes (calcium, potassium, bicarbonate & phosphates) may easily bath the lamella spaces, & easily become a calcified sclerotic mass that serves as a biological plug to seal the leaky space. The person's intake of acidic juices & soft drinks that contains  $H_3PO_4$ , which dissolves the organic lamella & the enamel crystals directly adjacent to the lamellae space. This space permits fluids & bacteria to enter through the open lamellae space to the **EDJ**. A research study by Bartlestone (1947) using radiolabelled Iodine tracers reported a rapid penetration of label through the lamellae cracks to the **EDJ** interface, through the dentine tubule complex & through the vital odontoblast layer to enter into the pulp vasculature & pass throughout the body's bloodstream. These agents became lodged in cells, they were measured in body tissues—only 15-minutes after placement of the tracer onto the enamel surface.

The **EDJunction** is a scalloped interface that is a remnant from the basement membrane between the epithelial & mesenchymal tissues. SEM data have shown the **EDJ** as a series of cratered ridges that increase the adhesion of enamel to dentine due to the increased surface area.

**Striae of Retzius** are seen as incremental growth lines that are most prominent in mature permanent teeth, a lesser amount in postnatal deciduous enamel & uncommon in prenatal enamel. Enamel at birth generally shows an incremental neonatal-line due to metabolic changes during the delivery process.

**Perikymata** are shallow surface furrow manifestations of the striae of Retzius on the oral surface of enamel that run circumferentially across the face of the crown.



The photomicrographs are from a 1998 study by Walker et al. The top left photo is a radiograph from a human molar that failed to show any proximal caries radiolucency of either the enamel or dentine before any staining & sectioning. The same molar was then immersed in a concentrated Orange-G solution for 24-hours, placed in resin, ground sectioned at approximately 100µm & observed under incident light. The photo above right is a ground cross-section through the same human molar tooth that shows a lamella traversing from the oral surface through the entire enamel thickness to the EDJunction. The Orange-G is seen to heavily stain the carious dentine that spreads along the EDJ & deeper into the tubule complex, which was seen with oral transillumination, but was failed to be observed in the initial X-ray.

The bottom right photo is a 100µm Orange-G stained longitudinal ground section through an adult human tooth that shows a carious lesion that has spread through the dentine that is seen directly below the enamel lamella.

The 1998 Walker study reported **“the enamel lamella are shown to be a permeable pathway allowing caries-producing bacteria access to the entire dentine-enamel junction. . . .Caries can thus be established within the tooth without visible evidence at the surface.”**