A Surface Manifestation of the Bands of Schreger:

In 1850, Czermak (112) described a surface banding of enamel which was in the form of dark and light lines. He distinguished them from perikymata on account of their greater size and the difficulty involved in their observation in that the tooth must be extensively manoeuvred relative to the light source. He suggested also that the pattern was not a superficial one but rather within the thickness of the enamel as if caused by changes in deeper structural layers. As to their etiology, he suggested the irregular zig-zag movement of rods responsible in that the light was reflected by them at different angles.

This typically exact description from an early German histologist represents, as far as the reviewer can determine, all that is known of the surface bandings to this day. Other German workers have described them (84, 87), Von Ebner depicting them in a line drawing (Fig. 22), but no further details were given.

A photomicrograph of the bands by reflected light is shown (See Fig. 23) in the cervical region of an incisor, where they appear more readily than elsewhere. As maximum contrast was aimed for in this print, the bands do not appear with anything approaching such definition when observed through the microscope. Indeed in some teeth they cannot be seen at all.
Fig. 22.

- taken from J. Lehner and
  H. Plenk (87).

A recently erupted upper bicuspid showing -
  A: Bands of Schreger at surface
  B: Perikymata.
Fig. 23.

From the reviewer's collection.

Surface manifestations of the bands of Schreger as seen in the cervical region of an incisor.

Note: Maximum contrast was gained in this print purposely and in actual fact the bands do not appear with such definition.
ENAMEL TUFTS:

Orban (113) first demonstrated these to be an optical illusion seen in transverse sections, due to the projection into one plane of rods lying in different horizontal planes and passing outwards from the dentino-enamel junction in different directions in these planes (See Fig.22). The rods are said to be twisted, grouped and poorly calcified with abundant cementing substance between.

Others (104, 114) have regarded them as spaces allowing of a flow of nutritive materials to the enamel from a pulpal source via dentinal tubules. Sognaes (114) and Manley (115), also demonstrated unusually dense bundles of organic material in completely decalcified enamel in positions corresponding to the tufts and which he thought "appear to be patent structures". He postulated also a direct relation between the degree of corrugation of the dentino-enamel junction and the appearance of both tufts and Hunter-Schreger bands, in that the discrete organic bands passing out into the enamel appear to take origin from the dentino-enamel junction at the peaks of its corrugations. Permeability experiments (116) and observation of ground sections would tend to indicate a relative absence of other organic matter about or between the rods close to the dentino-enamel junction between these discrete bands (See Fig.24). As a result of these findings an attempt is at
Fig. 24.
- taken from Orban's Oral Histology and Embryology (70).

Horizontal ground section through enamel near the dentino-enamel junction showing enamel tufts.

A and B show change in direction of rods in two adjacent layers of enamel made visible by a change in focus of the microscope. Also relative absence of organic matter may be observed between the tufts.
present being made to enquire into the possibility of a relationship between the enamel tufts and the Hunter-Schreger bands.

It would seem therefore, that co-existent with a change in the direction of rods in different planes, are relatively discrete, longitudinally orientated bands of organic matter which bend to conform with changes in rod direction. The presence of a patent lumen in these organic structures is still a matter of question.
LAMELLAE:

There is less agreement upon this subject than any so far discussed, so little, in fact, that the leader of a symposium of eminent dental histologists on lamellae (117) could only conclude that: "This symposium demonstrates a considerable divergence of opinion which in itself may be a contribution on the subject since it indicates a need for research."

Lamellae can perhaps best be defined as imperfections or discontinuities in the highly calcified arrangement of normal rods, sheaths and inter-rod substance. Such imperfections were first seen by Miller (7) in 1902 as dark areas in otherwise highly refractile, translucent enamel of ground sections. He called them "Fasern". They were named lamellae independently by Bodecker (118) in 1905. Since that time they have been the subject of much controversy. Their significance has ranged from simple enamel fractures (16), to abnormal malformations (119, 120, 121, 122) to important, anatomical channels for metabolism (114) to the central position in one of the theories of the etiology of caries (18).

Sogmaes (16) was one of the first to investigate the matter thoroughly. He showed that up to that time there had been two main schools of thought as to their origin:

1. mechanical injuries such as fractures in
the mouth or cracks occurring during the grinding of the specimen preparatory to examination; 
(ii) developmental defects due to deficient matrix formation or to incomplete calcification of rods and/or inter-rod substance.

By careful decalcification and subsequent preservation of the matrix, Søgmaess was able to eliminate the possibility of cracks formed during grinding of the specimen. The following were his results and the conclusions he drew from them:

(i) Organic bands were found in regions where longitudinal cracks could be observed before decalcification. These longitudinal cracks he attributed to trauma;
(ii) Organic bands, indistinguishable from those just mentioned were found about silicate fillings, that is to say; where

(a) rods are exposed by trauma of cavity preparation,
(b) recurrence of caries is known to be low (a characteristic of the filling material),
(c) leakage is known to be high (also a characteristic of the filling material).

From this he suggested that lamellae may be a crude form of repair and even a nucleus for future
mineralisation. The organic matter, he suggested, originated from the saliva.

(iii) The lamellae neither ran a straight course nor followed any one rod or group of rods. Also as no trace of rod structure could be found in the lamellae it appeared they were not merely poorly calcified rods.

(iv) The lamellae were not found in unerupted teeth with a frequency which came anywhere near that of the erupted teeth. From this, (iii) and (iv), he concluded that lamellae were not of developmental origin.

In spite of Sognnaes' dismissal of lamella formation in unerupted teeth, these are of common occurrence (123, 124, 125). Further, another worker has championed the developmental theory of lamella-formation to the virtual exclusion of all else and claimed to have seen in his electron photo-micrographs of lamellae the faint outlines of rods. Yet only one or two of his series of photo-micrographs are really convincing. He further claimed that some of the incorporated rods showed evidence of extremely fine needle-like crystals while others showed none. He concluded from this that lamellae were indeed incompletely calcified rods. It was also his conviction that traumatic fractures were rare in human beings and practically non-existent in other animals, particularly rabbits of which he examined 671 without seeing lamellae
or fracture lines. However, Awazawa did admit the absence of rods from unusually large lamellae and also that where lamellae were extremely thin, they consisted of inter-rod substance only, indicative of some other etiology besides defective calcification of a pre-existing matrix.

Evabant and Klees (1957) suggested that distinction be made between "brown" and "white" cracks. The former according to these authors extends into the dentine and derives its main organic content from that tissue while the latter are restricted to the enamel and their organic matter is either of salivary origin or the remains of "primitive" lamellae along which these cracks are considered to occur. Primitive lamellae are assumedly those deficiencies occurring in matrix structure before final calcification.

The whole complex question of lamellae would seem one of definition, that definition having to be made with regard to etiology. There seem to be the following types of lamellae:

(i) a normally existing structure of enamel seen and detected on the enamel surface and continuous from it through a variable thickness running towards the dentino-enamel junction. This type corresponds to Orban's type "A" lamellae (70) and consists of poorly calcified
rods and inter-rod substance,
(iii) a developmental defect occurring during matrix formation resulting in discontinuity and which can become filled with many types of organisms and cellular debris. This corresponds to Orban's type "F" lamella. The longitudinal orientation of these two types of lamellae can be explained if, as it would seem, their etiology lies in ameloblast dysfunction. For if one ameloblast or a group of ameloblasts at the cervical loop of the enamel organ become affected their progeny, similarly affected, will form beneath them as the cervical loop proceeds towards the future apex of the tooth. This will result in a longitudinally orientated defect. This classification presumably includes the "brown" lamellae while the "white" lamellae may be included in this or the next.

(iii) A reaction to trauma consisting of unspecified organic material, corresponding to Orban's type "G" lamellae. Why all fractures should be longitudinal is difficult of explanation, however. The organic film concerned is said to originate from the saliva. Chase (75) when investigating Nasmyth's membrane found that a film simulating a membrane could be formed not only on teeth incubated in saliva, but
also on blocks of glass or talc incubated in distilled water and that a film could not be formed if the media were sterile. Therefore, bacteria were necessary for film formation. Could it be that bacteria are necessary also for this type of "reparative" lamellae?

(iv) Elongations of normally existing enamel tufts (84). These can be differentiated from other types because they run from the dentino-enamel junction outwards through one half to one quarter of the width of the enamel.

Much has been made of the possible predisposition of lamellae-affected teeth to caries and of the possibility of the lamellae forming a prepared pathway for cariogenic agents. In a study (126) of 300 replicas of the surfaces of 300 carious lesions it was found that:

(i) Half of the cavities involved lamellae;
(ii) half of the cavities were situated between two lamellae;
(iii) lamellae may cross any part of a carious lesion;
(iv) the carious lesion may be situated anywhere between the lamellae.

It would seem therefore, that from the point of view of inception at least, caries and lamellae are unrelated—unfortunately no attempt was made to determine the type of
lamellae which was involved in the experiment. On the other hand from the point of view of progression of the lesion many workers feel that caries and lamellae are intimately related. For example, Malleson (11) wrote:

"Deeper infection takes place in the form of a fissure. These may be pre-existent....."

Again Frisbie et al (12) stated:

"Following the opening up of the rod core and the inter-rod matrix there is an invasion of the gram-positive spheroid-shaped organisms. The process is observed to extend through the full thickness of the enamel leading to the establishment of foci at the dentino-enamel junction. The pathways of penetration are narrow, irregular tracts and may resemble the classically described lamellae."

Gottlieb (117) went as far as to describe them as:

"the main highways for invasion of the tooth".

For all that, the lamellae themselves are not the complete answer for they are known to occur in the teeth of caries-immune animals - monkey, horse, cow and rat.
STRIAE OF RETZIUS: (Incremental Lines of Retzius):

Retzius in 1837 (71) was the first to prepare ground sections of teeth—which he did apparently with a file—and to study these sections with any degree of magnification. He noted, in the course of an extensive investigation of whole tooth sections the results of which he recorded with great attention to detail in some 80 pages of Muller's Archives, that in enamel longitudinally sectioned were present two kinds of striations. The one was that originally described by Schreger and the other, in his own words (translated literally) "brownish parallel striations which bend around the cuspis ... and at the sides are parallel to the axis." (See Fig. 25). He noted also that these striations were present only in "not-born" teeth, that is to say, teeth formed post-natally. As a result of his description we find the term "brown striae of Retzius" coined (127).

This writer cannot agree with Gustafson (80) that Retzius cites Leeuwenhoek (1678) as the first to see the striae. Certainly Retzius credits Leeuwenhoek with the first observation of perikymata but he also admits of being the first to study ground sections, necessary for observation of the striae.

Once described, the argument was then as to etiology.
Fig. 25.

- taken from A. Retzius (71).

Longitudinal section of an incisor showing -
A : Striae of Retzius.
The following are some of the theories:

(i) Striae due to an abundance of tubes between the rods (84, 112, 128, 129). The majority of these authors wrote at a time when "enamel capillaries" were very fashionable but Beust (129) did not and from his writings one gains the impression that any opaque matter in enamel represented to him an "interrodal capillary", be they tufts, striae or lamellae.

(ii) Striae due to a bending of the enamel rods. (71, 80, 81). There seems little doubt from the published photomicrographs of Anna-Greta Gustafson (see Fig. 26) that within an incremental line the rods change course, although Cape and Kitchin (130) wrote about "the striae of Retzius which can be explained only as an actual difference in the constitution of the enamel material and not ascribed to any change in rod direction."

The Gustafsons (80, 81) would divide the striae of Retzius into two types:

(a) functional striae which are due to bends in rods thought by them to be necessary for the final contour of the tooth — this last the reviewer cannot agree with;

(b) pathological striae which may be due to "increased or decreased mineralization;"
Fig. 26.

- taken from A - G. Gustafson (80).

Retzius line due to bending of the rods (as seen by polarized light).
widening of the inter-rod substance, 
changes in the organic stroma, reduction 
in the height of the sections and variations 
in the inter-section substance or inter-
section sheaths."
Indeed all these possibilities are beautifully 
illustrated (80).

(iii) Striae due to an accumulation of pigment within 
the enamel rods; (10, 79). This was, according to 
Williams (10) quite the normal thing as the "ultimate 
structure of all enamel is granular." The occurrence 
of pigment was disputed by Pickerill in 1913 for the 
following reasons:

(a) striae appear white in reflected light;
(b) truly pigmented areas, such as are seen in 
pathological teeth are obvious, however 
viewed, that is, by reflected or transmitted 
light, but are less intense by transmitted 
than by reflected light. The opposite is the 

case for the striae.

(iv) Striae due to entrance of air between the rows 
of rods (84). Williams disproved this contention 
by demonstrating striae in a section of enamel 
prepared entirely under water.

(v) Striae are a function of the rhythm of secretion 
of the ameloblasts (85, 131, 132, 133, 134, 135, 136, 
137, 138).
Pickerill 1913, first proposed the terms "lines of growth" or "incremental lines", the theory resulting from the observation that the lines conformed to the position of the enamel cusp at different stages of growth and are therefore indicative of intermittent or interrupted calcific deposit. Beust (129) however, remarked in his usual pompous way "The theory of rhythmicality is based on unsupported conjecture. It is incredible that so many well-known histologists have adopted it as a premise for teachings which must fall with their fundament".* His main reason for refuting the incremental nature of the striae was that he attempted to duplicate the system by dipping a plaster model of a tooth alternatively in pure white plaster and then in plaster coloured with lamp black to build up a series of "incremental layers". He then sectioned the hardened result in various planes and demonstrated similarity between these and known appearances of the striae in transverse and radial longitudinal sections but a supposed difference in a tangential longitudinal section. The reviewer feels however, that the tangential section through the actual tooth is not as deeply placed as that through the model, as
exemplified by the likeness of the untouched surface view of the model to that of the tangential section of the tooth.

Definite proof of the relation of the incremental lines to growth of the enamel organ was gained by means of:

(a) injection of a hypoplasia - inducing substance in rats - sodium fluoride (132);
(b) injection of vital dyes in rats for example, Alizarin Red S (135);
(c) histological observation of normal human teeth and correlation with their chronology of development (134, 138).
(d) observation of human teeth affected by acute fluorosis (Schour & Poncher in 1937). It was found by these workers that there is, in human enamel:

(i) a daily appositional rate of 4 microns - at least in permanent teeth but up to 8 microns in deciduous teeth - to result in a transverse rod striation;
(ii) a 4 day appositional cycle amounting to 16 micron increments and resulting in an incremental line of Retzius;
(iii) an accentuated incremental line or "growth ring", the appearance of which is
predictable and corresponds to a
definite chronological pattern.
Although no mention is made of the fact by her, the
"boxes" proposed by Anna-Greta Gustafson (80) would
appear, by their size, to be the daily product of
a fully functional ameloblast.
(vi) Striae due to faulty calcification (12, 87, 139).
Opinion differed however, as to the site of deficient
calcification - rod or inter-rod substance or both.
Noyes, Schour and Noyes stated that as the striae
were due to a lack of calcification of the rod, in a
completely decalcified section they should disappear.
Frisbie, Knuckolls and Saunders did in fact report
such an occurrence:
"The markings of the enamel dependant upon the pattern
of calcification such as incremental and striction lines
have never been observed when the calcium salts are
totally removed, and the description of these features
by other authors leads us to believe that their material
was insufficiently decalcified."
These authors checked for complete decalcification
by the use of the Xanthroproteic reaction, the yellow
colour resulting from the nitration of organic matter
indicating the point at which organic matrix began to
pass into solution. Workers (140, 141, 142) agree
that postnatal enamel exhibits variation in the degree
mineralization in bands parallel to the striae of Retzius (See Fig. 27) and that in developing teeth the neonatal line is marked by lower mineralization than the surrounding enamel of the developing teeth. That variation in degree of calcification is not the complete answer will next be shown.

(vii) Striae due to localized accumulations of the organic matrix (15, 143) Sognnaes (15) demonstrated incremental lines in decalcified enamel, the lines seemingly due to wider zones in the rod sheaths than exist in the surrounding framework. Although no test was made to ensure complete decalcification and this is the critical point here - the authors stated that because of the long decalcification period of 3 weeks in 5% Trichlor-Aetic Acid that it was "... unlikely..." that these organic elements which have been recovered could have been falsely retained due to an incomplete removal of calcium salts..."

As a point of interest, in Sognnaes' photograph (See Fig. 28) of decalcified enamel it is seen that lines similar to the labelled incremental lines in the post-natal enamel are present in the pre-natal enamel. In this regard, Crabb and Darling (144) note that "Linear variations in X-ray absorption
Fig. 27*  
- taken from A. G. Gustafson (30)

Obtained by placing a microradiograph in one microscope and the ground preparation from which the microradiograph was made in a second microscope — in this case a polarizing microscope—combined with each other by a comparison ocular. Suitable adjustment allows of viewing a continuous field. In this case the microradiograph occupies the top half of the figure and shows the striae of Retzius to be less radio-opaque than their surroundings.
Paraffin sections of decalcified deciduous molar.

B.L.: Birth line
R: Retzius line
E.C.: Enamel cuticle
D: Dentine
A: Pre-natal enamel.
in microradiographs of pre-natal enamel are uncommon and, if present, are usually poorly marked; this agree-
ing with the histological observations of Rushton (145, 146) on the "fine contour lines" of deciduous enamel.

It has been proposed (80) that the photomicrographs of Sognnaes (15) and Jansen and Visser (116) could be due rather to an increase in amount of inter-rod substance (See Fig.27), rather than a thickening of the rod sheath which when present is usually smaller in amount than the inter-
rod substance when - it may be added - this itself is present. This thought is not new in that it was propounded by Von Ebner in 1906.

A vast amount of comparative dental anatomy has been done by Japanese workers and from one such a study (147) the following information is gained in regard to the striae of Retzius.

(1) Their appearance is developed to the highest degree in the enamel of the primates especially in the catarrhine apes and man. Other animals show rather faint, irregular parallel lines and an example of one lacking them completely is the rhinoceros - even though the rods possess fairly well developed cross striations.
(ii) The number of actual rod cross striations between two adjacent striae denoting the time interval between their deposition, varies from 2 to 11. The highest number are in the ungulates, proboscidea and catarrhines, while the carnivores, rodents and platyrhines have smaller values.

(iii) The angle between the striae and the dentino-enamel junction is roughly definite for each animal and found to be larger in man than in the carnivores and herbivores. The author must refer either to an actual location or to an overall average, for the angle the striae make with the dentino-enamel junction will of course vary with the position in which it is measured.

(iv) The interval between 2 adjacent striae is specific for each animal.

The author therefore concluded that the anatomical arrangement and periodicity of the growth lines in enamel of the mammalia may be of value in their classification.

To summarise then, as their name implies, the incremental lines are expressions of the normal post-natal rhythm of matrix deposition. This expression takes basically the form of a difference in matrix deposition seemingly both qualitative and quantitative, although precise knowledge as to the nature of the difference is wanting. The other
manifestations, variance in the degree of calcification, difference in optical density and change in the course of the rods are but a function of the basic change in matrix deposition.
PERIKYMATA:

Knowledge of the presence of small, transversely ringed concavities alternating with convexities on the surface of enamel is as old as histology itself in that such structures were first observed by Leeuwenhoek (1632-1723). He supposed them to be indicative of successive stages in the protrusion of the tooth through the gum.

Retzius in 1837 (71) noted "embossed lines" on the surface of enamel. He found them more numerous on the anterior than the posterior teeth and ascribed them to groups of enamel rods laid down in rings about the tooth, each slightly protruding over the next. Czermak (112) described two types of circular grooves; a large one seen in pathological teeth and a smaller one which he described as being a result of the normal process of formation - this last being the perikymata. He was the first to note:

(i) their absence in deciduous teeth - he assumed therefore, a basic difference in enamel deposition between the two dentitions;
(ii) their gradual decrease in density from the cervical to the occlusal areas of the surface.

A little later, Waldeyer (127) related the perikymata to the papillary processes of the enamel organ, but it was
— from reviewer's collection

Perikymata on enamel surface of a bicuspid.
Pickerill (85) who first showed the correspondence of perikymata to the striae of Retzius after precise photomicrography of a ground section in the region of its outer surface. It is his name that is used in conjunction with the convexities between perikymata - the imbrication lines of Pickerill. The relation between perikymata and striae is better illustrated by a more recent photomicrograph of Gustafson (80) (See Fig. 30).

Scott and co-workers (123, 126) by means of optical and electron microscopy of metal-shadowed collodion replicas demonstrated in their now familiar pictures, the minute structure of perikymata. They found in recently erupted teeth pronounced, alternating concavities and convexities, perikymata and imbrication lines of Pickerill respectively, the concavities further characterized by clearly visible rod ends and the convexities by their smoothness. They demonstrated that with age, there was on accessible surfaces - more rapid on buccal and linguval surfaces than on proximal surfaces and more pronounced on anterior than on posterior teeth - a gradual loss of structural detail until the surface was flat and uncharacterized. Wherever pronounced patterns did remain on older teeth, than a cuticle was present and this most often occurred on the proximal surfaces of posterior teeth emphasizing the lack of functional abrasion to that surface.
Fig. 30.

- taken from A.G. Gustafson (80).

Showing relationship between striae of Retzius and the perikymate (by polarized light). The author notes that the white bands at the surface between the perikymate are not due to changes in mineralization but to bends of the prisms, all in the same direction.
These authors also described the differing form of perikymata in different regions of the tooth, noting a decrease in severity of the angulation of the wall closer to the occlusal portion (See Fig.31) and referring to their illustration wrote:

"It has been stated that the perikymata represent an overlapping of sheets of enamel, but the available evidence does not prove or disprove this hypothesis."

Although seemingly apparent the authors did not make a correlation between the angulation of the walls of the perikymata and the angulation of the striae, which are known to meet the surface at an angle which is largest in the cervical region and decreases occlusally until the striae run parallel with the surface in the cuspal region.

The unknown factors to this point would seem to be:
(i) the exact mechanism of their occurrence;
(ii) the reason for the rod ends being visible in the perikymata and not visible in the convexities in just-erupted teeth - certainly not because of wear to the convexities.

Solutions to these problems have been proposed recently by Gustafson (80) after study of ground sections with polarized light and comparison of the results with the various appearances found in replicas of tooth surfaces.
Fig. 31.


Diagrammatic representation of angulation of walls of the perikymata over the surface of the tooth.
Her hypothesis is based on the beliefs that:

(i) rods change course at the level of a striae of Retzius and
(ii) rods are laid down in "sections";

and it is therefore that the perikymata are formed "where the rows of sections marking a Retzius line bend inwards to meet the next Retzius line". This can perhaps best be represented thus:

[Diagram of demarcated rod ends and bending of rods at incremental line, resulting in smooth surface.]

Gustafson indicated also that the change in surface appearance described by Scott and Wyckoff did not occur in all cases and that the surface appearances must be due to deeper structural differences. The reviewer feels however, that there is perhaps another factor involved in that on Gustafson's hypothesis any smooth surface
must have obliquely running enamel rods and any rods perpendicular to the surface—and many such rods are illustrated by Gustafson—should have clearly demarcated rod ends.

To sum up then, present indications are that the changes at enamel surfaces described by Scott and Wyckoff may not be universal and secondly that the surface appearances of perikymata are a function of rod course, itself a function of the occurrence of striae of Retzius.

As a point of interest it has been noted (86) that if two marked striae of Retzius meet at the same position on the enamel surface, the resulting accentuated perikymata may take the form of a hypoplastic spot.
ENAMEL SPINDLES:

Early workers, such as Von Ebner in 1890 and Pickerill (85) regarded enamel spindles as malformations of inter-rod substance resulting in subsequent space formation in the enamel. While others, Römer in 1899 and Mumery (6) saw them as containing nerve fibres originating in the dentine, their continuity with which had been proved by dye diffusion experiments (6).

The spindles are generally regarded now (78) as representing expanded continuations of odontoblastic processes in the enamel, not in the inter-rod substance as previously thought but rather characterized by being tangentially orientated to the enamel rods.

As to their formation, the problem of a soft process expanding across the dentino-enamel junction to become imbedded in a highly calcified substance was realised early. Walshoff in 1901 held that there was an absorption of first-formed dentine and that some of the dentinal tubules escaping absorption persisted as the spindles. While this is, of course, now discredited, information has been gained by electron microscopy of the chronological sequence of events at the dentino enamel junction at the time of odontoblastic induction and initial matrix formation. Quigley (27) showed that the first indication of the dentino-enamel
junction is a very delicate interface between the enamel epithelium and the odontoblasts. An amelo-dentinal membrane then forms on the dentinal side of this interface and odontoblasts differentiate before the ameloblasts become columnar. Forming amelo-blasts are further separated from the fibrillar amelo-dentinal membrane by a complete double cellular membrane. However, and this is the crux of the matter, the boundary is incomplete and Quigley opined that the breaking up of this double membrane at the base of the ameloblasts would allow of a limited penetration of enamel by odontoblastic processes to account for the occurrence of enamel spindles.

Thus it is seen that:

(i) there is a means for odontoblastic processes to enlarge and pass up between the ameloblasts;
(ii) the enamel spindles allow of an obvious diffusion pathway;
(iii) the exact nature, function and formation of the enamel spindles is however, largely undecided.
DENTINO-ENAMEL JUNCTION.

Perhaps the first observations of this region were those of Berzelius— as told by Retzius in 1837 (71). Berzelius found, after immersion of a tooth in acid that the enamel was no longer present and that there was to be found only a "membrane-like tissue" on the dentinal surface. Retzius (1837) concluded from this that the enamel was fixed to a thin membrane on its inner surface. One must disagree then with Volla (1949) who would have that he demonstrated the "dentine-enamel cuticle" in his preparations of decalcified enamel for the first time in 1949.

With regard to the form of the dentinal surface it is found to vary and this was noted as early as 1850 by Czermak who listed three forms that the surface could take, that of:

(i) rounded horizontal ridges
(ii) small multiple papillae, or
(iii) a smooth featureless surface.

These three appearances have been recorded by the reviewer during the course of decalcification experiments (See Figs. 32, 33, 34).

Description of the junction in calcific material (6, 70) is usually that of the papillary type, which as can be imagined from Fig. 32, would result in the convexities facing
Fig. 32.
- from reviewer's collection.
Flat surface at dentino-enamel junction.
Fig. 33.

- from reviewer's collection.

Papillary projections at dentino-enamel junction.
Fig. 34.

- from reviewer's collection.

Transverse ridges at dentino-enamel junction.
dentinewards and sharp peaks projecting out into the enamel. In comparison with other mammalia especially where the stresses to be borne are much greater, the human dentino-enamel junction is a relatively simple one. In the elephant molar for example, (6), which is composed of plates of enamel, dentine and cementum, small thorn-like processes project into the enamel from both dentine and cementum to result in a more complete interlocking of the tissues. (See Fig. 35). Apparently in the wart hog and hippopotamus, however, the thorn-like processes proceed only from the cement and not from the dentine.

It would appear to this writer that the dentino-enamel junction shows least simuousity in the region of the cemento-enamel junction, the extent of the region being greater in deciduous than in permanent teeth as would possibly be expected because of lesser stresses likely to be placed on deciduous teeth.

During recent histochemical studies (124) the dentino-enamel junction has been shown to stain well with alcian blue. This would suggest the presence of an acid mucopoly-saccharide (149). Present concepts (150) see acid mucopolysaccharide as the component responsible for the cementing nature of ground substance and it may well be that it is present in a similar capacity in this location, thus aiding the physical interdigitation already described.
Fig. 35.

- taken from J.H. Mummery (6).

Interlocking of the dental tissues shown in a transverse section of elephant molar.

A : enamel
B : dentine
C : cementum.
Besides simply forming a union of enamel to dentine the dentino-enamel junction has a definite effect on enamel structure. It was Sognnaes (14) who noted that the regularity of the inner part of the enamel organic matrix was a function of the straightness of the amelo-dentinal membrane, in that from the highest points of a markedly scalloped membrane would originate wave-like irregular bundles of organic material, while where the amelo-dentinal membrane was relatively straight there would appear a uniformity in the structure of the organic matrix. Sognnaes further suggested that the morphology of the external dentine surface, which embryologically precedes enamel formation, may also be reflected in the surface texture of the enamel in cases where a scalloped dentine surface is covered with but a thin shell of enamel. While this particular point has yet to be proved, it is certain that the dentino-enamel junction is at least a template for gross enamel morphology as it is the first formed (27) and calcified (144) part of the enamel cap.

Again the dentino-enamel junction has been accorded the role of protecting the dentine during the caries process by Villa (151) and Frisbie, Knuckolls and Saunders (12) who based their assumption on the retro-grade spread of caries on the enamel side before the dentine is affected. Kronfield (152) would, however, disagree with this as it was his observation that once the dentino-enamel junction was broached
there is rapid spread through the "less-resistant dentine".

The dentino-enamel junction then, rather than being just the junction of enamel and dentine:

(i) may determine to some extent the gross morphology of the crown;
(ii) is intimately related to and predetermines the structure of the innermost enamel organic matrix;
(iii) forms perhaps, as well as a physical union, a chemical union between the enamel and dentine;
(iv) may affect the progress of caries by acting as a barrier protecting the less resistant dentine.

A point of interest is that although no statistical analysis has yet been made it was found on examination of fluorozed teeth (153) that the arcade-shape of the dentino-enamel junction was exaggerated, sometimes occurring together with an irregular outer surface and other times with a smooth surface (See Fig. 36).
Fig. 36.
- taken from A.G. Gustafson (153).
Exaggerated arcade-shaped dentino-enamel junction of a fluorozed tooth.
VITALITY OF ENAMEL:

Besides the intrinsic interest of the subject, its discussion is warranted as information concerning enamel histology and physiology and in particular those histological structures allowing of dye diffusion, was gained by experimenters attempting the problem.

Up to the end of the last century dental tissues were regarded as having a lot vitality and were hence considered subject to systemic metabolic changes. Later there was a complete reversal as no cells or vascular exchange could be demonstrated in the enamel. Much evidence has been gained however of physical and chemical changes taking place in enamel. This evidence and the means by which it was gained will now be discussed:

1. Permeability Experiments:

Enamel was shown to be permeable to relatively large molecules, by dye diffusion experiments (41, 154, 155, 156), by electro-endosmotic means (157, 158) and by osmosis (159, 160). As to the dye diffusion experiments these were originally carried out in an effort to establish the circulation of a dental lymph but because of the varying use of vital and non-vital teeth, experiments in vivo and in vitro, the different techniques of dye application, the varying dyes and the dissimilar results obtained, a vast amount of heterogeneous material is at our disposal. From the early
experiments, however, it would seem that both enamel and dentine are permeable, the degree of permeability depending on the nature of the dye (with regard to its molecular size) and the time of application.

It is interesting that Bodecker and Lefkowitz found after placing powdered dye in a dentinal cavity that the enamel of just pulpectomized teeth was more permeable than that of vital teeth but that the permeability decreased with the time elapsed after pulpectomy. As a result, these workers stated that dye diffusion in enamel of non-vital teeth was "entirely different" — this last in italics — from that observed in vital teeth as here only the enamel over the "affected tubules" was stained whereas in non-vital teeth the whole enamel cap was stained. Now firstly the entire dentine is stained in non-vital teeth and secondly when a cavity is cut in the dentine of a vital tooth in the position these were (see Fig. 37) for the placement of dye, surely the tubules so severed from the pulp are as "non-vital" as any in a pulpectomized tooth. The point being that the enamel above any group of "non-vital" dentinal tubules is permeable to the dyes used by these workers.

The specific structures affected in stained enamel were the rod sheaths, often accentuated along the bands of Schreger. The dyes differed in their ability to diffuse
Fig. 37.


Illustrating the technique of vital staining. Cavity drilled into labial surface and dye deposited in base of cavity.
through the enamel, for example both methylene blue and
eosin staining while Congo Red did not. Also in one
investigation (41) a decrease in permeability through
dog, monkey and human enamel was observed.

Conclusion in early experiments such as these are
difficult of justification because of the often gross
damage to the tooth, for example, the slicing of the
crown and subsequent injection of dye into the pulp or
even the cutting of a cavity for dye placement. As any
such traumatic procedures must affect the "normal" permea-
bility of involved dentinal tubules.

An example of a later work is that of Jansen and
Vissner (116), who in a valuable review pointed out that
one must not confuse information on the permeability of
microanatomical parts gained by means of a randomly selected
dye with information on physiological processes in the
enamel cap as a whole. They realised the need for care-
fully controlled experiments, in vivo, with physiological
indicators, the ideal being, of course, radio-isotopes,
or otherwise with dyes, the molecular size of which was
taken into account. Their experiment utilized a fluoro-
escent dye "Trypanflavin" using vital teeth in vivo and with
saliva the solvent. After removal of the tooth great
pains were taken to ensure no further displacement of the
dye occurred, freeze-drying, rapid dehydration and infil-
tration with plastic being utilized. Results showed
penetration of the enamel which varied in certain well-defined zones and also in the specific structures stained in these zones. (See Fig. 38).

It may be seen that their results corroborate other observations of organic matter distribution:

(i) the additional accumulations of dyestuff in the rod sheaths corresponding to the striae of Retzius;

(ii) the most densely staining part - the middle third - the region where Hunter-Schreger bands are most prominent;

(iii) the lack of diffuse organic matter in the innermost region of the enamel close to the dentino-enamel junction;

(iv) the dense staining of the lamellae;

(v) the homogeneous nature of the outer enamel surface.

The guarded conclusion of Jensen and Vissar emphasises the need for care in the interpretation of results of this kind of experiment:

"It may be assumed that substances which behave physico-chemically in a way similar to Trypaflavin and consist of molecules of equal or smaller size will permeate the enamel under normal circumstances."

That more than consideration of just the molecular weight of the dye is necessary may be illustrated by
Fig. 38.
- taken from Jansen and Visser (116).

Transverse section of enamel (E) and dentine (D) of a dog's permanent canine, dyed with fluorescent dye for two days.

- o.s.: outer surface of enamel.
- h.d.l.: homogeneously dyed outer layer of enamel.
- c.z.: central dyed zone of enamel.
- o.z.: less intensely dyed outer zone of enamel.
- i.z.: less intensely dyed inner zone of enamel.

Retzius lines seen in outer enamel. The prism sheaths of adjacent prisms have been represented by one single line only.
examining some physical characteristics of two of the
dyes used by Bodecker and Lefkowitz. One of these was
eosin, a xanthene derivative of formula:

\[
\text{Na}^+ - \overset{0}{\text{Br}} \overset{0}{\text{Br}} \overset{0}{\text{C}} \overset{0}{\text{N}} ^+ \text{Na}
\]

The molecular weight is 647.73. It is seen from
examination of Baker's text concerning biological micro-
techniques (161) that:

"A special advantage of eosin is that the dye is
able to penetrate red blood corpuscles and other close
textured components of tissues. This is so despite the
large size of the dyeing ion. The capacity to penetrate
well must be ascribed to the tendency of the ions to
remain separate instead of aggregating."

Another dye was Congo Red. This is an "azo" deriv-
ative, a "diazo" in fact:

\[
\text{SO}_3
\]
The molecular weight is 696.67.

Of the diazo dyes, Baker states:

"Those that exist as large molecules or ions — notably the diazo and triazo dyes — have a tendency to clump, that is flocculate into particles of colloidal dimension. Even though the molecular weights of the two dyes are very similar it is seen that consideration of their physical properties when in solution is at least as important when considering their behaviour.

Results of osmotic experiments indicated (159, 160):

(a) the tooth, by means of the organic constituents of the enamel can act as an osmotic membrane;

(b) there is a decrease in tooth permeability with age;

(c) in deciduous teeth, there is concomitant with resorption a loss of permeability until the time of shedding, at which time the tooth is virtually impermeable;

(d) in extracted deciduous teeth, the permeable structures are the rod sheaths and the lamellae.

It would seem then that there is a difference in permeability between shed and extracted unresorbed deciduous teeth the nature of which the authors could not determine. The reason could possibly lie in the effects of resorption as mediated by the giant cells — these were demonstrated in
enamel by Sognnaes, 1955, in depressions on the resorbing surface similar to Howship's lacunae found in bone. In bone it is a well-known fact (162) that both organic and inorganic constituents are removed simultaneously. If it is a similar process in the case of enamel resorption then the loss of permeability due to any imbalance of organic-inorganic relation is unlikely, that is, the portion of tooth remaining should have the same relative composition as before resorption. This rather suggests that there is blocking of the micro-anatomical spaces necessary for permeability by some means, possibly the products of autolysis.

En passant Atkinson (157) showed that applications of silver nitrate which were supposed to render teeth impermeable to bacteria (163) affected in no way the permeability of the enamel cap, but that the permeability may be prevented by an application of methyl methacrylate in chloroform.

2. Radio-isotope Experiments

An ionic exchange was found to occur in enamel (3, 164, 165, 166). The knowledge gained from radio-isotope experiments not only emphasized the permeability of enamel but has been interpreted (3) as bringing the dental hard tissues closer into the orbit of mineral metabolism. For it was found that there was a graded uptake of radio-active
phosphate (P32) from the saliva, but that when the blood alone was rendered radio active, the innermost layer of enamel did show some activity, the radio-active material reaching it presumably by way of the dentinal tubules, the dentino-enamel junction and possibly the enamel spindles and tufts.

Unfortunately little or nothing is known of the turnover of organic elements and even though in one experiment (167) radio-active carbon was the indicator, no attempt was made by the authors to distinguish between its organic and inorganic origin.

It is interesting that enamel exhibits a physiological similarity to other mineralized tissues during the resorptive process, in that resorption of all hard tissues bone, cementum, dentine and enamel follows a comparable histomorphological pattern to result in all elements of these resorbable tissues disintegrating without any sub-surface demineralization beyond the depth of one micron.

To sum up with regard to the question of vitality it would appear one of definition for, "although most biologically trained observers do not doubt the vitality of any normal tissue retained permanently in the body" (41), if by vitality (to paraphrase Churchill) is meant the presence of phenomena characteristic of cell life such as metabolism, respiration, growth, regeneration, reproduction, irritability, contractility and conductivity, one must regard enamel as non-vital.
To conclude in a lighter vein, I wish to present some findings of Dr. Sten Forshuvud (104). I was interested to note not long ago in the review section of a "Sydney Morning Herald" (168) that he was the author of a biography on Napoleon which purported to prove that Napoleon had been the victim of arsenical poisoning. The reviewer was prompted to suggest that the author had overstated his case and had not admitted one contrary fact to his theme. For example, Forshuvud stated that Napoleon had not had one single sick day in his life before he was sent to St. Helena. He stated also that Napoleon's corpse was found to be hairless—proof for his arsenic theory—and yet later said that chemical examination of a hair from the body showed unmistakable signs of arsenic poisoning.

Unfortunately, his dental efforts would seem to show the same signs of over-enthusiasm for a particular scheme. In 1946-1947 he injected Indian ink particles and gelatin, separately and together, into young dogs. He found that the Indian ink not only stained walls and partly filled the lumen of the blood vessels in the omentum but stained also connective tissue fibres between the vessels and a fairly large number of the tissue cells, the histiocytes. The author concluded that these fibres were "ultracapillaries" and further that they were identical with the reticular
fibres and further that they were identical with argyrophile fibres. The author proceeded, that as the organic matrix was argyrophile then these were also reticular fibres and also ultrastructural capillaries, these ultrastructural capillaries being responsible for blood flow in the enamel. The author supported his argument with what he interpreted as specific staining of nerve fibres and fibrin in a lamella.

If one can perhaps disregard his conclusions (and the usual prognosis for those associating themselves with Napoleon) to concentrate on his results they appear striking:

(i) After injection with gelatin the enamel became more resistant to acid decalcification and the enamel organic matrix was preserved even by the usual decalcification procedures;
(ii) it seemed, macroscopically, that lamellae stained with the Indian ink particles, although any such particles could not be demonstrated after decalcification and sectioning;
(iii) after injecting haemolyzed red blood cells into a young dog, regular pigmented banding occurs corresponding to the Hunter-Schreger bands, the colouration changing to a typical haematin brown colour on boiling.
Stimulated by his apparent findings of a blood supply to enamel, Forshuvud set out to prove that enamel could "repair" given a structural basis on which to elaborate. He denatured pieces of ox teeth and placed them in prepared cavities. He claimed that although there were many failures, sometimes the ox material, at first dull and lustreless, would in a period of weeks become bright and shining and indistinguishable from the surrounding tooth substance. More important in such cases, it was impossible to dislodge the fragment with the force which would normally remove any metal or porcelain restoration. While the clinical photograph of the restoration seemed as described, the photomicrographs he published purporting to show an organic connection of reticular fibres between the graft and the adult structure were unconvincing. He gave little detail but awaited further results.

Although nothing more seems to have been published by him, the prospect is needless to say, an exciting one.
SUMMARY:

(i) Organic Matrix: (a) The importance of the organic matrix is realised in that it can account for a great number of the histological characteristics of enamel. More than this, on a sub-microscopic scale, it may be seen to be a determining factor in enamel structure ranging from the initiation of its calcification to the onset and development of the caries process.

(b) Difficulties concerned with the preservation and histological demonstration of the organic matrix and means of overcoming some of the difficulties are discussed.

(c) The acid-insoluble protein of the mature organic matrix is now thought to be a keratin peculiar to enamel.

(ii) Inorganic Component: (a) Enamel apatite is now suggested to contain its carbonate as an integrated impurity of the lattice structure.

(b) Advanced techniques allow of precise three-dimensional definition of crystallite orientation.

(iii) Enamel Rods: (a) The idea of regular hexagonal rods with complete sheaths and a definite inter-rod substance is no longer tenable. Instead, the "fish-scale"
appearance is seen to be a true one with irregularity of rods, sheaths, and inter-rod substance the rule rather than the exception.

(b) Knowledge of rod course in human enamel is inadequate. Rod course in some other mammalia is discussed.

(c) Rod dimension is discussed, especially with reference to spatial problems of enamel morphology, and need for careful reassessment of previous work is suggested.

(iv) Bands of Schreger: So many investigators, using different material and observing this material under different conditions, have nominated observed bandings as Hunter-Schreger bands that it would be of interest to determine whether these bandings are all identical with those originally described by Schreger. Difficulties arising from the most commonly proposed theory of their etiology, based on the section of bands of rods running in alternating directions, are discussed.

(v) Enamel tufts: Although their basic etiology has been carefully explained, exact knowledge of the relative amount of organic matter and the presence or absence or spaces in their structure is wanting.

(vi) Lamellae: The problem appears one of definition and yet such
definition must be made with regard to etiology and structural characteristics, about which there is more dissention than any other aspect of enamel histology.

(vii) Striae of Retzius: These are doubtless of an incremental nature and involve many variations of structure. The exact mechanism responsible for the change at a cellular level is yet unknown.

(viii) Perikymata: Recent work sees these as superficial expressions of deeper structural differences.

(ix) Enamel Spindles: Their precise nature, function and formation are largely undecided.

(x) Dentino-enamel junction: The form may vary considerably and is a definite factor in the determination of enamel structure.

(xi) Permeability: Enamel allows of a physical and chemical interchange with its environment and if not "vital" in the strictest sense it is certainly not without physiological activity.
It may be seen that in enamel structural research there is the need, perhaps more than is usual, for a correlation of findings from all applicable techniques. The reason for this lies in the singular nature of enamel namely the magnitude of the calcific content, the minuteness of the organic matter and the intimacy of their relation. These factors soon realise the limitations of any one particular technique.

Although many of the unsolved problems of enamel histology will doubtless be solved by electron microscopy, electron diffraction and other such ultrastructural tools, it is this reviewer's opinion that there are yet many areas of application for optical microscopy, its modifications and refinements.
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