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BASIS OF THE STRUCTURE AND DEVELOPMENT OF MAMMALIAN ENAMEL AS SEEN BY SCANNING ELECTRON MICROSCOPY

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Abstract

Mature enamel is the most mineralized of mammalian tissues, contains the least water and therefore does not present problems of shrinkage on preparation for SEM. However, the developing enamel is highly hydrated and presents severe problems in preparation.

The structure of enamel is determined by the activity of its individual formative cells and their group behaviour. The peculiar, unequal secretion of matrix at the distal pole of the ameloblast leads to the presence of characteristically shaped pits in the surface of the formative tissue. Crystals grow in a special relationship to this surface. Sharp changes in orientation of the surface are reflected in abrupt changes in orientation of neighbouring crystals beneath it, leading to the formation of structural discontinuities at prism boundaries or junctions. Several different patterns of prism cross section have arisen in mammalian enamel. Inequalities in the rate of production of the tissue lead to the formation of features known as varicosities or cross striations. Exaggerations of this presumed daily incremental rhythm lead to the formation of the more major incremental lines which can also be visualized by scanning electron microscopy. Differences in the course of the ameloblasts throughout their life history, in the nature of a translatory motion over the surface which they are secreting, lead to the development of prism decussation, which shows characteristic patterns in different mammalian groups of probable functional significance. One largely ignored area in the study of comparative histology concerns the enamel-dentine junction. Particularly in the marsupial mammals, dentine tubules cross the junction and are continuous with enamel tubules. Methacrylate casting of these features has given new insights into these structures.

KEYWORDS: Enamel, ameloblasts, mineralization, prism patterns, decussation, cross-striations, microhardness, enamel-dentine junction, tubules.

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Introduction

This paper is a very brief introduction to some of the themes and the terminology used in the other contributions concerning the comparative micro-anatomy of mammalian dental enamel presented at a special symposium held in New Orleans LA in May 1986 in honour of the late Edward J Reith.

Mature, dental enamel is the hardest mammalian mineralized tissue, contains the least water and the least organic matrix. It is no surprise, therefore, that it was the first biological tissue to be examined by scanning electron microscopy (SEM). The number of papers studying the structure of this tissue, if one includes clinical studies, now certainly exceeds a thousand. Restricting our attention to those which have contributed to our understanding of the structure, developmental mechanics, evolution or functions of this tissue still leaves too many to catalogue here.

Although limited by resolution, light microscopy has made important contributions to enamel histology. The pattern of enamel prism packing in man was described by Nasmyth [35] and Smreker [40]. On the comparative front, it was early realized by Tomes [41, 42] that certain structural features of enamel were different in different groups of mammals. In two classical papers, he pointed first to the existence of enamel tubules continuous with the dentine tubules in the marsupials with the exception of the wombat [41]. He also showed that the principal sub-ordinal groups of the rodents showed different patterns of decussation of the enamel prisms [42]. Particularly important later papers from the light microscopic era were those of Korvenkontio [29] who greatly expanded Tomes' work on the order Rodentia, and Shobusawa [39] who used an etching and staining technique to examine the cross-sectional shape of enamel prisms. An extensive bibliography of the earlier and light microscopic literature concerning enamel structure and development will be found in reference 2.

The SEM was introduced into the study of enamel in 1959 and extensive studies were made of its applicability to the study of both the developing and the mature tissue in 1962 and 1963 [2,3,18].


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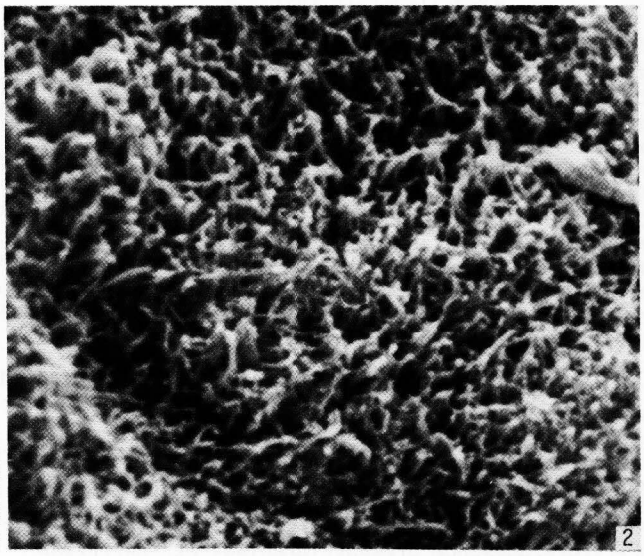
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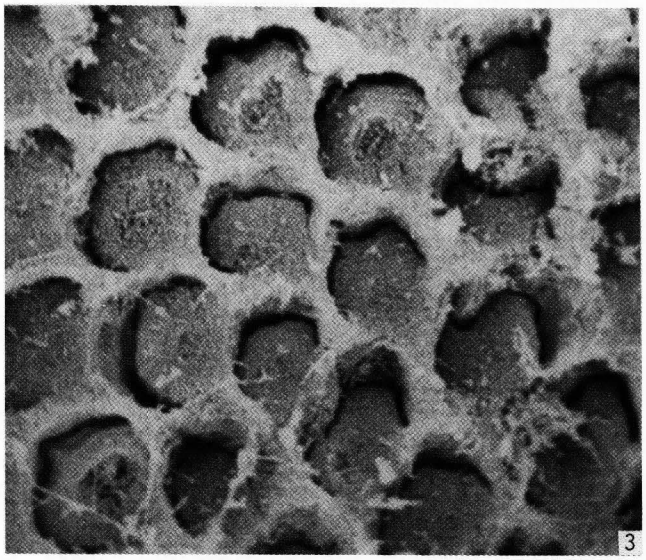
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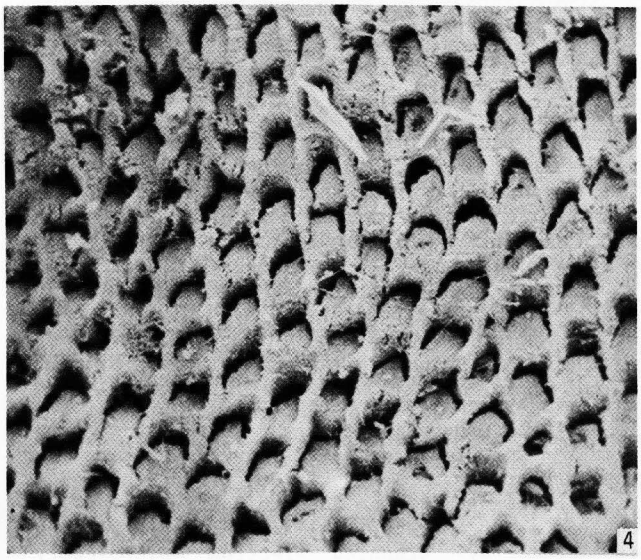
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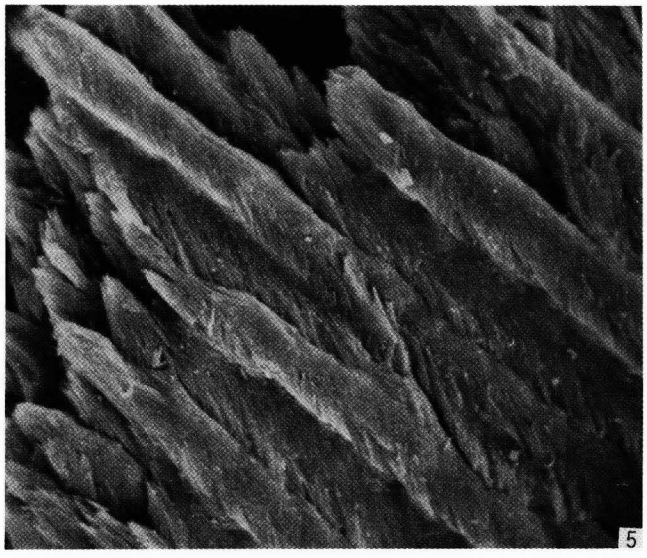
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Figure 1. Secretory ameloblasts on the developing enamel surface of the inner zone of a myomorph incisor. Fieldwidth 50 µm.
Figure 2. Dentine surface at the future enamel-dentine junction in a human third permanent molar. Fieldwidth 16 µm.
Figure 3. Developing enamel surface in the same human tooth, showing the morphology of development of pattern 3 prisms. Fieldwidth 36 µm.
Figure 4. Similar field at lower magnification showing different orientation of pits in different parts of the field. Fieldwidth 77 µm.
Figure 5. The same preparation fractured to reveal prism heads and tails with crystallites at a substantial angle to the long axis of the prisms. Fieldwidth 39 µm.
Figure 6. Adult human enamel etched to show the prisms. Fieldwidth 80 µm.

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Key terms and concepts

Enamel is secreted by **ameloblasts** (Fig. 1) on a mould of **dentine** (Fig. 2). The developing enamel surface is **pitted** (Figs. 3 & 4). The ensuing changes in orientation of the surface relate to the formation of repetitive bundles of crystallites called **prisms** (Figs. 5 & 6) delineated by boundaries or junctions where the crystal orientation changes abruptly. One **ameloblast** forms one pit and one prism junction (Fig. 3). Different arrangements of the surface pits give rise to three principal prism **Patterns**: Pattern 3 is shown in figures 3-6 and Pattern 2 in Figs. 7 & 8. Pattern 1, not figured, has complete boundaries, roughly circular in cross-section. A circadian, cyclical variation in the rate of elaboration of the enamel gives rise to features known as the **cross striations** or varicosities of the prisms (Figs. 9 & 10). A near weekly rate variation would explain the more regular class of the more widely spaced incremental growth lines known as the **striae of Retzius** in man (Fig. 11). Contrasting directions of cell movement during enamel secretion (Figs. 12-16) give rise in the mature, formed tissue to **Hunter-Schreger bands**, which are **zones** of prisms with contrasting, **decussating** orientations (Figs. 17-22). The local hardness and wear resistance of enamel depends upon both the crystallite and the prism orientation (Figs. 21 - 23). Enamel strength is critically dependent upon its being anchored dentine. The **enamel**-dentine junction **is appropriately complex** (Figs. 24-27). Cell process occupied spaces called tubules in dentine may be continuous with cell process generated tubules in enamel, best developed in the marsupials.

SEM methods for enamel

The structure of adult mammalian dental enamel can hardly be understood unless one studies its development. The developing enamel tissue contains a great deal of water and is very difficult to prepare without major artifacts. Two recent approaches have provided useful general solutions to the problem of visualizing the three-dimensional structure of the mineralizing front in developing mammalian dental enamel [12,16].

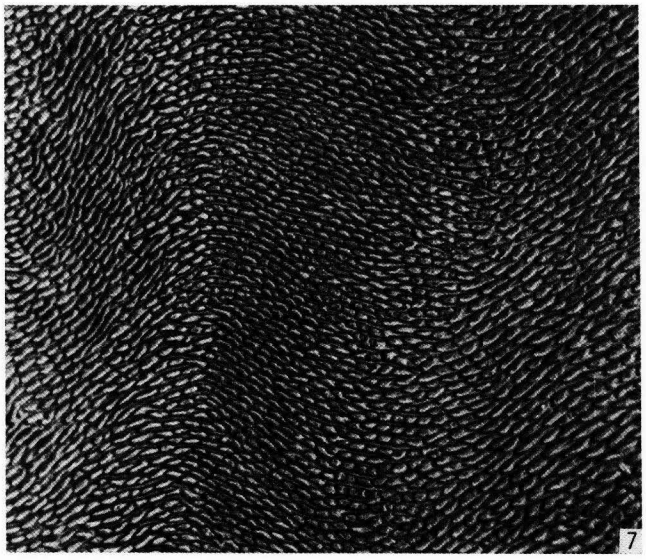
Developing enamel from unusual species which either may not be maintained as laboratory animals, or which may not be sacrificed specifically in order to obtain the tissue, is necessarily not obtained in a good condition. Such teeth usually arrive with the animal having been dead for a day or two so that it is not possible to have a good preparation of the formative cells. Fortunately, this may not be an essential requirement, since we can deduce many important facts about the circumstances during formation from the shape of the surface of the developing enamel which is made rigid and preserved to an extent by the development of the mineral component within the enamel. Furthermore, it is generally the case that one could not expect to obtain useful information from the morphology of the ameloblasts in respect of determining the structural features of developing enamel. However, in some instances this is not the case. For example, well preserved ameloblasts after osmium fixation demonstrate the relative positions of ameloblasts when they are not parallel to each other (Fig. 1).

Enamel is formed on the complex surface of a mould of dentine (Fig. 2). The relationships between the developing enamel surface (Figs. 3 & 4) and the crystallite orientation patterns which develop deep to it [2-10], can be confirmed by dissecting the developing enamel surface (Fig. 5). Deductions concerning the morphology of the adult enamel can be confirmed in etched (Figs. 6 & 7) or fractured (Figs. 8 & 9) preparations [1-3,6,8,9,12-14,16,18,21-24,26,28,32-34,36,37,43].

Most of our earlier preparations of developing enamel were made by gently washing the residual ameloblasts from the surface of the tooth, followed by air drying from a volatile non-polar solvent or freeze drying [2-6]. Later, we used critical point drying [9,10,16]. This technique has recently been improved by the Soxhlet refluxing procedure to ensure the complete removal of water and ethanol [19]. The best preparations are those made by oxygen plasma ashing methacrylate from PMMA embedded tissue, thus exposing the mineralizing front in the enamel (which is, for all practical purposes the same thing as the formative front in the case of enamel) and avoiding the need to subject the delicate tissue to surface tension forces during drying [12].

To examine the details of the surface of the enamel in a completed, mature tooth [7-9] only requires that it should be cleaned of bacterial dental plaque and calculus. The internal structure of adult enamel can be studied in the SEM in the simplest of specimen preparations. Teeth which are either accidentally or deliberately fractured [1,4,9,14,32] are cleaned of loose dust with a gas blast, coated and examined in the SEM. For more careful studies, deliberate fractures may be induced in chosen planes by grooving the unwanted part of the tooth with a diamond wheel. Details which can be studied in fracture planes include prism directions and cross sections, crystallite orientation, cross-striations and incremental lines and the relationship of these to the completed enamel surface morphology.

An alternative route to the examination of defined regions or oriented planes involves the preliminary step of grinding and polishing,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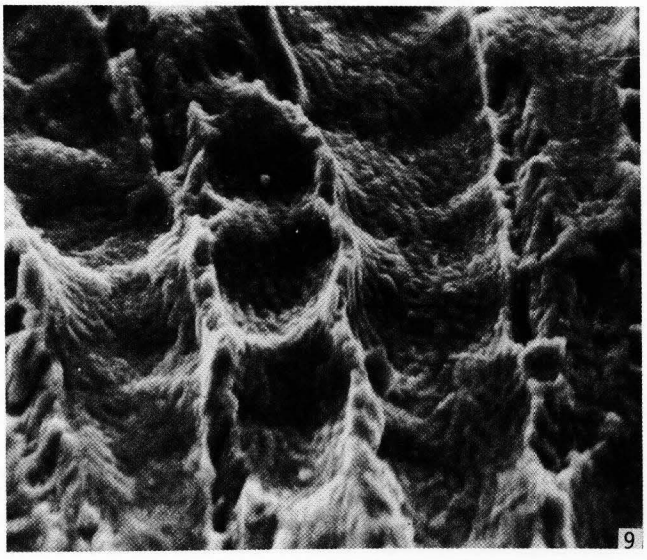
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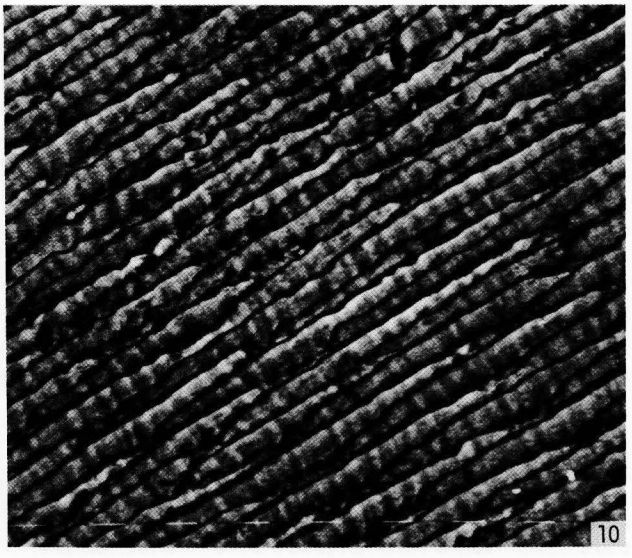
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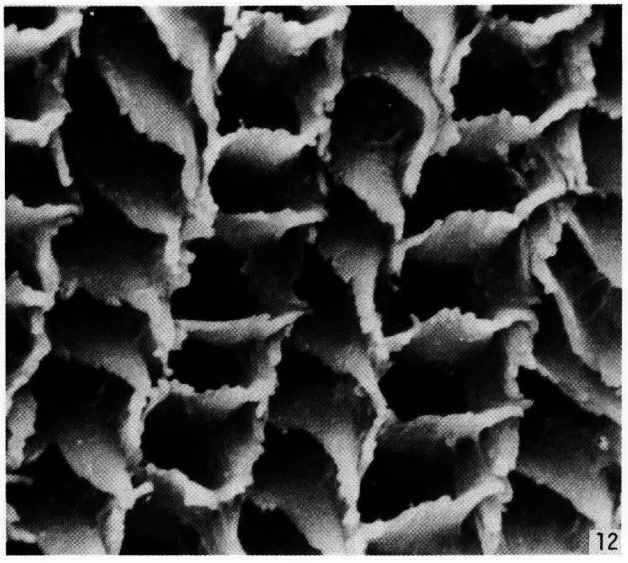
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Figure 7. Etched pattern 2 pig molar enamel. Cervical to right, occlusal to left. Fieldwidth 220 µm.
Figure 8. Fractured pattern 2 sheep molar enamel: rows of prisms have crystal orientation from WSW-ENE: the inter-row sheet crystal orientation appears to lie from WNW-ESE. The projection of both orientations is affected by the strong tilt angle of the surface in the SEM. Fieldwidth 25 µm.
Figure 9. Fractured elephant molar pattern 3 enamel showing varicosities, or cross-striations at prism boundaries. Fieldwidth 25 µm
Figure 10. Diamond micro-milled longitudinal section of human third permanent molar enamel imaged with symmetrical arrangement of back-scattered electron detectors in summing mode to produce high contrast from small differences in density in the field, (topography-free surface layer). Thus, showing the density dependence of the cross-striations. Fieldwidth 85 µm.
Figure 11. Fractured human enamel: fracture lines run parallel with superficial brown striae of Retzius. Fieldwidth 397 µm.
Figure 12. Developing enamel surface of inner layer of Jerboa (myomorph) incisor. Fieldwidth 22 µm.

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followed by etching with acids or chelating agents to reveal the prismatic structure [6,10,12,13,16, 21-24,33,43]. However, it is not necessary to have any other method of preparing an adult enamel sample than the production of an extremely flat surface without a smeared layer. Diamond micro-milling can produce ultra-flat surfaces of mature enamel and these are imaged using backscattered electrons in the SEM to show density-dependent features in the enamel [11,12,15]. Such images delineate both the enamel prism boundaries (Fig. 18) and the incremental features within the enamel (Fig. 10).

Wet etching has also frequently been used to remove the prism-free outer surface layers to reveal the underlying structure [23,43]. Acid etching is the procedure in most common use, but it may be over-done and present an excessively artefactual view of the real structure. As one example, etching may expand the space of the prism boundaries or junctions. As another, new calcium phosphate species may be deposited in the juxta-boundary regions; these more acid calcium phosphates may be left standing proud at a subsequent etching phase and interpreted as meaningful structures[13]. It should always be remembered that the morphology of an etched surface is mostly determined by what happened to what was removed, yet we try to interpret it in terms of what is left, which can hardly have yet been significant [18].

If acid etching is to be used with fresh or recent enamel, we recommend 0.5% phosphoric acid for up to 30 secs. In the case of fossilized enamel more rigorous acid etching regimes may be required [28,43]. Partly mature enamel will also require special treatment which may be designed around the need to solubilize some of the excess organic matrix component.

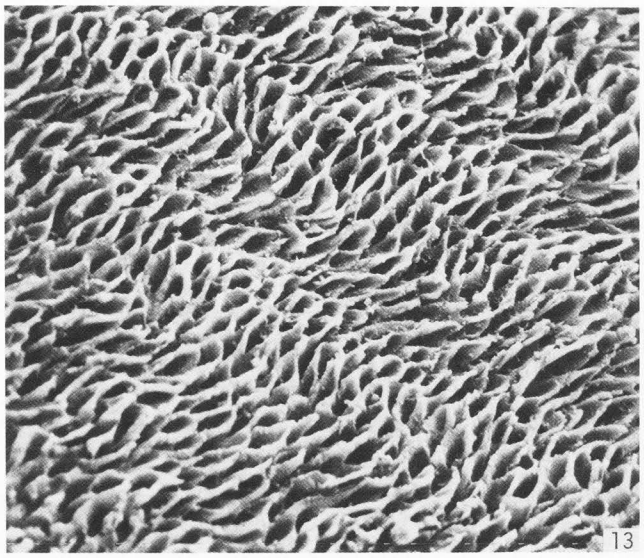
Because acid etching does damage enamel structure in depth, one has to know how much tissue has been removed and that that which remains has also been partly removed. Some of us, therefore, are testing physical etching techniques which do not significantly damage the tissue in depth. Two have been used extensively and with good effect. The first to be used was ion beam etching [18], in which, for example, 5 keV Argon ions (or neutral atoms of the same velocity) are projected at the polished surface of a specimen. The rates of removal of the enamel crystallites depend upon their crystallographic orientation: thus the prismatic structure is etched on the surface [18,2].

A more recent technique uses a high velocity jet of a soft abrasive propelled at the specimen surface (Figs. 21, 23 & 24): we have used the clinically available "cleaning" technique called Airpolishing™ [12,15]. The chances for the removal of tiny fragments of enamel are greatest next to structural discontinuities in the tissue. Thus, where the enamel prisms are etched head on, fragments tend to be removed closest to prism boundaries so that the centres of prisms remain proud (Fig. 23). If there are differences in the course of the enamel prisms, those which are parallel to the surface under attack are removed more rapidly than those which project perpendicularly (Fig. 21). The combination of these effects gives rise to very useful etch derived information about enamel structure without causing damage to the tissue in depth. (Figs. 22 & 23). The method can also be used to clean plaque and calculus deposits from the tooth surface, and dentine [15] or presoftened, anorganic dentine (Fig. 24) from enamel.

Since enamel shows interesting three-dimensional features, it is also useful to have in-depth etching procedures (Figs. 19 & 20). The best of these are based upon the use of chelating agents [6,13,26,36]. For example, EDTA will selectively remove enamel closest to the largest pore spaces in this tissue: these are the enamel prism boundaries which are expanded by treatment with this reagent. EDTA etched enamel can reveal considerable portions of the length of large crystallite groups (for example, prisms or inter-prismatic sheets) giving a good understanding of the courses of enamel prisms [6,10].

Examining a developing enamel surface shows the morphology of the pits (Figs. 3, 4, 12-16) caused and occupied by the single formative cells, the ameloblasts. These pits have different shapes which are important in the development of different enamel prism patterns. The simplest case to understand is developing Pattern 1 enamel (not illustrated here) in which pits have floors which are situated at a level below that of the general plane of the developing enamel surface. Thus a vertical discontinuity arises around each prism "body" separating it from an otherwise continuous interprismatic phase. The interprismatic phase is of interpit or shared ameloblastic origin [2-4,8,9]. The material formed in the pit floor which has been secreted at the distal pole of the secretory process of the ameloblast is spatially related to that one cell.

The other two main patterns arise where the floor of the pit is continuous with the interpit phase material at one side or one corner [2,8]. Pattern 3 enamel arises where the sloping floor of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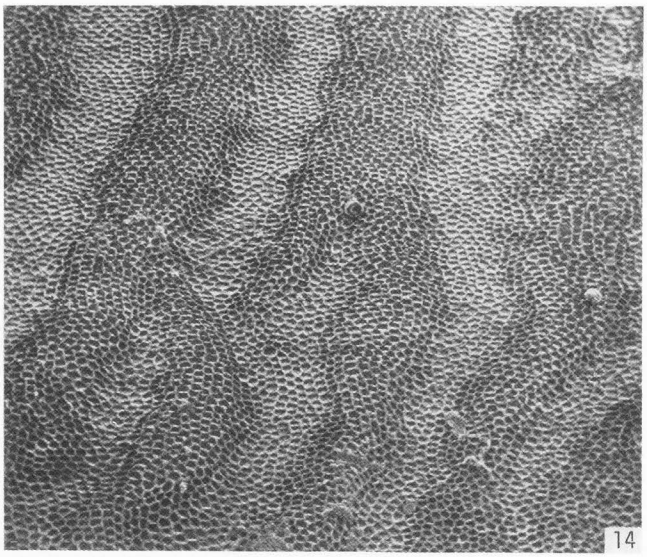
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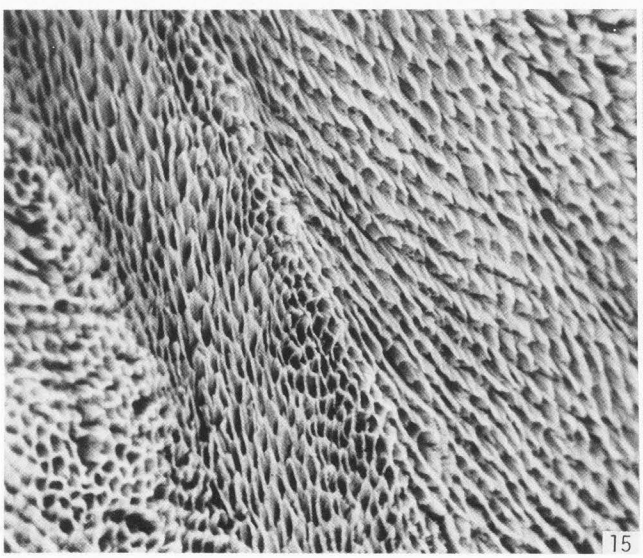
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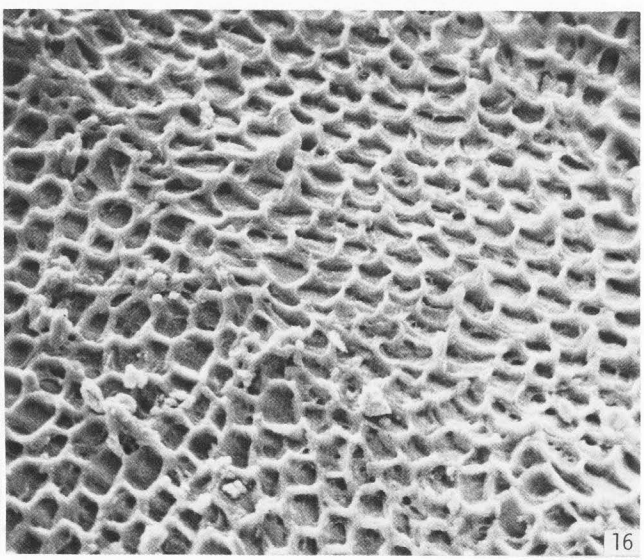
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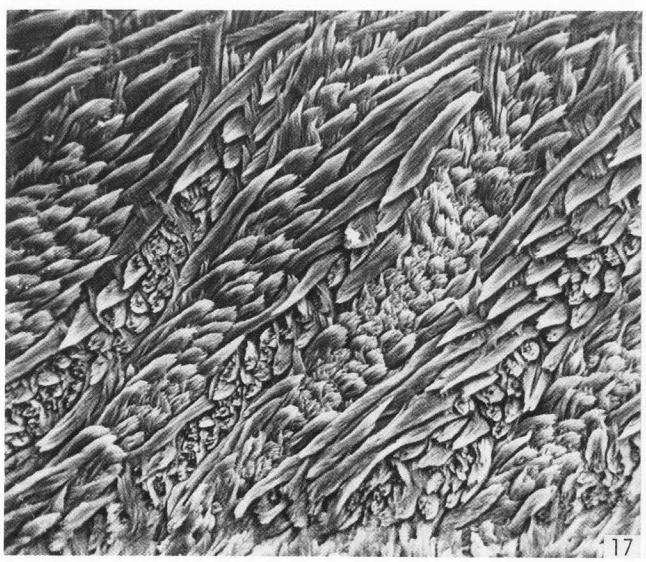
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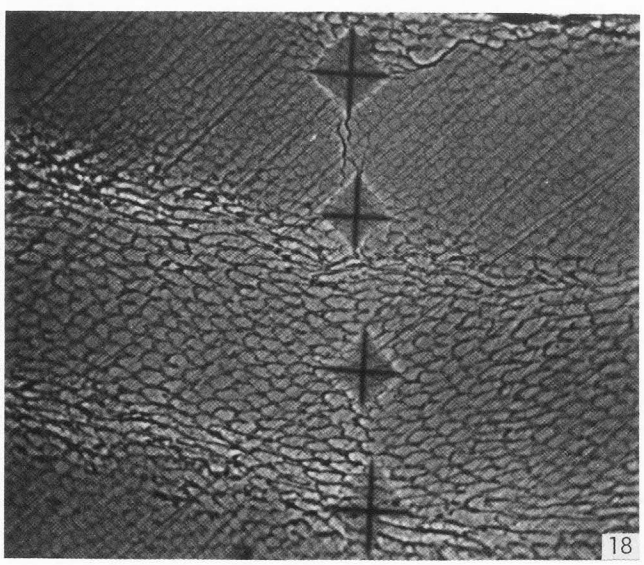
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Figure 13. Developing enamel surface of inner enamel layer of guinea pig (hystricomorph) incisor. Longitudinal direction from bottom left to top right. Fieldwidth 112 µm.
Figure 14. Developing enamel surface of cat canine enamel, longitudinal direction left to right, showing alternate bands of shading associated with different entry directions of the pits. Fieldwidth 361 µm.
Figure 15. Developing enamel surface of rhinoceros enamel, longitudinal direction NNW-SSE showing strong contrasting entry directions of pits in alternate zones. Fieldwidth 123 µm.
Figure 16. Developing enamel surface of African elephant molar enamel showing zones with different entry directions of pits. Fieldwidth 93 µm.
Figure 17. Etched longitudinal section of guinea pig (hystricomorph) enamel. Enamel dentine junction bottom. Outer enamel layer top, showing decussation of prism zones in inner enamel layer. Inter-pit phase crystallites are vertical in this image. Fieldwidth 112 µm.
Figure 18. Diamond polished section surface of rhinoceros molar enamel with contrasting orientation of enamel prisms in alternate zones. Field subject to diamond micro-hardness indentation study. Fieldwidth 220 µm.

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the pit runs up on to a wall between two adjacent pits (Figs. 3, 4, 14-16). Pattern 2 prisms (Figs. 7 & 8) arise where the sloping floor of the pit runs up on to the surface above the next pit in the same (usually longitudinal) row [2,6].

Owing to the very delicate nature of critical point dried developing enamel, it is possible to fragment the tissue with the prism boundary discontinuities ("prism sheaths") joining up to one another in the shortest possible directions (Fig. 5). It can then be directly demonstrated that the crystallite groups which form opposite the floors of the pits have a preferential orientation in the longitudinal direction of the bundle which we call the prism. Crystallite groups in the tail of Pattern 3 prisms form perpendicular to the inter-pit phase surface and, therefore, lie at a substantial angle to the main (longitudinal) axis of the enamel prism [2,4,8,9].

The different patterns of enamel prisms in modern mammals may correlate with different sizes of the formative cells [5]. The area covered by the ameloblast is measured in a standard location by examining the developing enamel closest to the surface of the dentine. Large numbers of pits are counted within a defined area to derive the average size of the secretory territories of the ameloblasts. There are, of course, considerable fluctuations about the mean value [24], but Pattern 1 enamel prisms most commonly fall in a size range between those of the smaller Pattern 2 and larger Pattern 3 [5]. There may also be a correlation between the enamel prism patterns and the rate of formation of the tissue [34]. Thus, whatever the main prism pattern, it is possible to find areas of Pattern 1 enamel formation at the enamel dentine junction (e.g some prism outlines in Fig. 24), where the process of secretion of the tissue may be slower because it has only just begun. It is also commonly the case that a layer of enamel contains Pattern 1 enamel prisms in the subsurface zone where the daily cross striation repeat interval is reduced, indicating a slower rate of formation [17]. Furthermore, within the bulk of enamel, areas of Patterns 1, 2, and 3 enamel may be mixed [2]. Nevertheless, within any one mammalian species, at any depth in the enamel and over vast areas of the enamel, one prism packing pattern usually predominates. The study of even a small fragment of enamel can, on occasions, conclusively exclude it belonging to certain taxons [17].

Incremental features

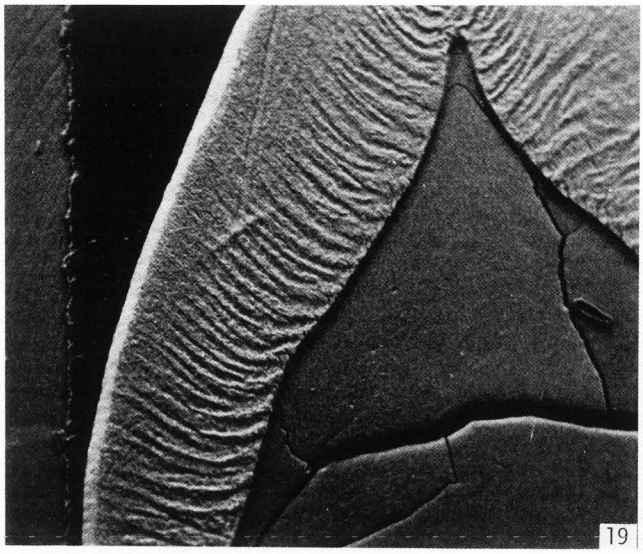
A hypothetical model explaining the development of the morphological aspects of the cross striations of the enamel prisms assumed that the rate of secretion of enamel varied over a 24 h period [25] and that the exact shape of the pits in the developing enamel surface was determined by a balance of dynamic factors [2,8]. It could be deduced that this balance would change as the rate of secretion changed. This hypothetical model correctly predicted the actual external shape of prisms at their junctional boundaries (Fig. 9) as they can often be seen in SEM images of fractured enamel [4].

Later, when good solid state backscattered electron detectors became available for the SEM, it was possible to demonstrate (using very flat polished or micromilled surface preparations, e.g. Fig. 10) that the cross striations also showed variations of density [11]. The change in density of the enamel in the cross striations is very small, but because of electronic contrast enhancement can be very simply demonstrated in the SEM. An extension of the earlier hypothesis suggested that this variation in density would be the natural consequence of the variation in the rate of production of the tissue [11]. Certain workers challenge the reality of cross-striations, believing that they are artefacts of methods of specimen preparation or observation [44].

The longer period incremental features known as the brown striae of Retzius in human enamel [38] also have explanations in both changes in density and crystallite orientation. They are planes of weakness in the tissue (Fig. 11) and are particularly prominent in the subsurface regions of lateral enamel [9]. In these locations, it is regularly possible to demonstrate a ratio of 7 or 8 cross-striations to one brown stria [2,20,25]. The latter crop out at the surface as the perikymata. This information may be used as a basis for determining the developmental age of a tooth or of dentitions [2,20].

Prism decussation

If one examines the surface of the developing enamel in many mammalian species, it is possible to see that the pits, which are caused by the ameloblasts' secretory processes, enter the surface in opposing directions (Figs. 4, 12-16). Turning this morphological information around into a developmental story, we can deduce that the ameloblasts (Fig. 1) producing the enamel (Fig. 12) have to move in contrasting directions. This phenomenon is most exaggerated in the development of the inner enamel of the incisor teeth of the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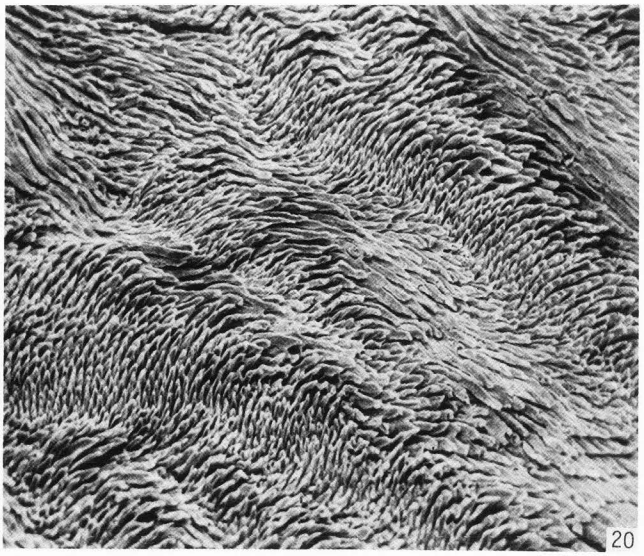
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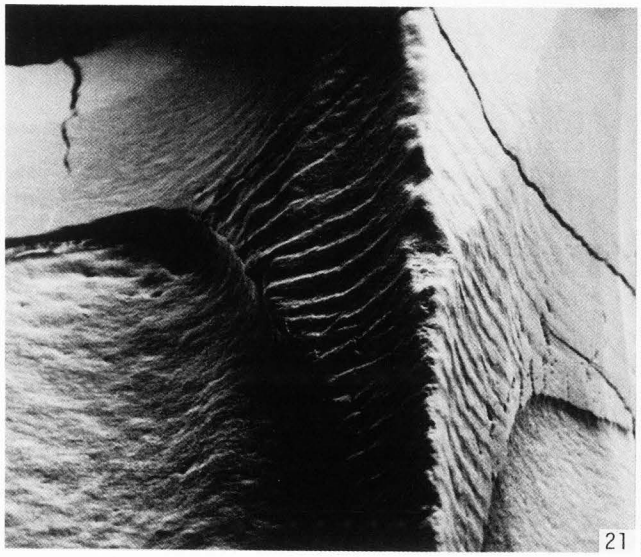
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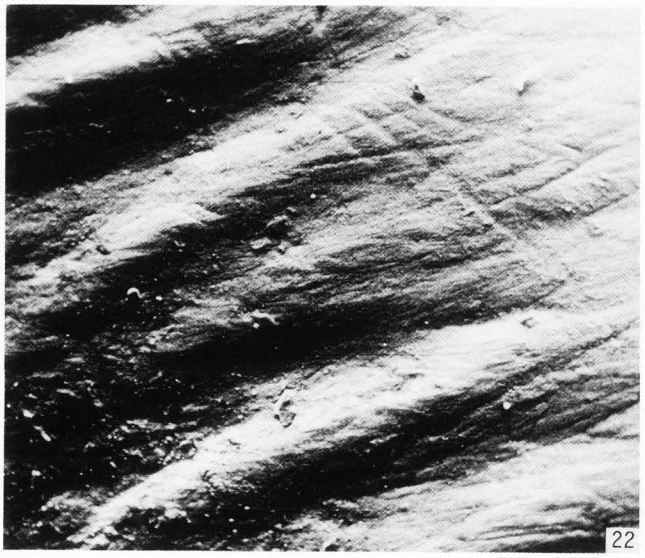
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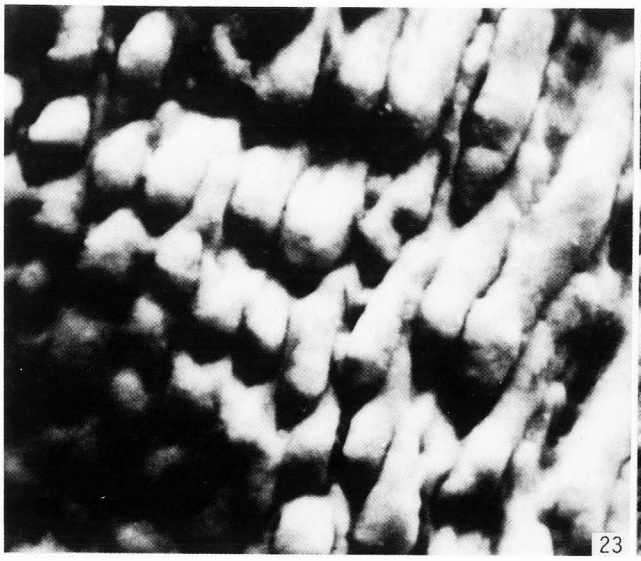
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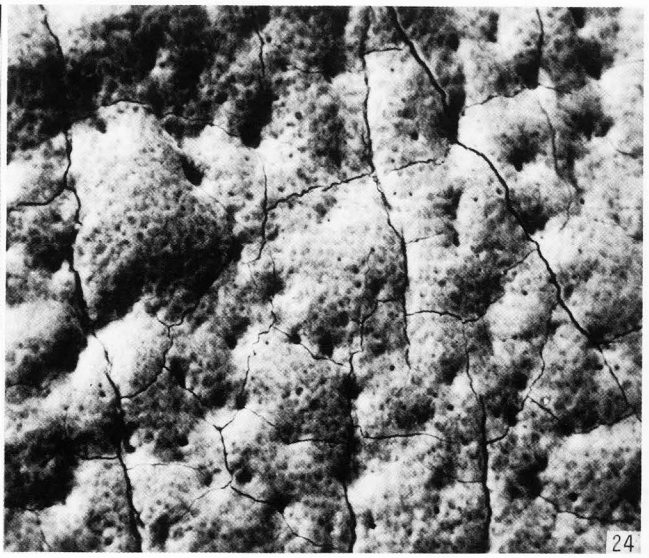
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Figure 19. Converted BSE image of EDTA etched longitudinal section of human tooth showing distribution of Hunter-Schreger bands prominent in the more internal layers of the enamel. Fieldwidth 4780 µm.

Figure 20. EDTA etched oblique section of human enamel showing changing directions of prisms in Hunter-Schreger bands. Fieldwidth 338 µm.

Figure 21. Sample of human permanent enamel prepared as a wedge in the inner enamel layer, then treated with Airpolishing™ to "etch" the Hunter-Schreger bands as a function of prism orientation at the cut surface. Higher rate of etching of dentine has undermined enamel. Fieldwidth 2000 µm.

Figure 22. Occlusal surface of inner enamel of rhinoceros molar showing serrated wear surface developed as a consequence of the vertical orientation of the Hunter-Schreger bands. Fieldwidth 965 µm.

Figure 23. Rhinoceros molar enamel, cut surface etched by Airpolishing™ to reveal the prism structure: rate of removal of enamel is dependent upon proximity to a prism boundary. Fieldwidth 50 µm.

Figure 24. Enamel-dentine junction in human molar prepared by softening the dentine by prolonged treatment with Na_2O_2 solution at 50°C, followed by removal of the anorganic dentine by Airpolishing™. Fieldwidth 222 µm.

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sciuriomorph and myomorph rodents (Figs 1 & 12). Examination of the internal structure of the enamel which develops in relationship to such a developing surface shows the contrasting orientation of the enamel prisms [10], further evidence of the fact that the enamel prism boundary is the fossilized path of movement of the secretory cell during the formation of this tissue.

In members of the hystricomorph rodents, bands can be identified, several pits wide, in which the pits exit in the same direction from the surface, that is, the cells in each band were moving in the same direction across the surface during the formation of the tissue (Fig. 13). Examination of the internal structure of this type of enamel shows that there are bands or zones of prisms having common orientation properties (Fig. 17). These are known as diazones and parazones, or the Hunter-Schreger bands [27].

In most mammals that demonstrate the phenomenon of prism decussation, the bands or zones are much wider, but they can nevertheless be traced at the developing enamel surface (Fig. 14). It is a remarkable fact that these bands or zones are usually parallel with the formative edge of the developing tooth, that is, the front at which preameloblasts are differentiating into ameloblasts following the formation of the local dentine platform upon which the enamel is to be secreted. There is an obvious correlation then between the direction of movement of the cells and the major anatomical axis of the tooth. In the case of the developing enamel of the very large plates of the molar teeth of elephants, this orientation cue for cell group movement is apparently lost, and in any one area of this surface, groups of pits can be found facing in any direction (Fig. 16): in other words, the pattern seems to have become randomized [6].

However, in one group of large extant mammals and in many now extinct mammalian orders [21,37] there is a well marked orientation of the Hunter-Schreger bands [21,37] and the associated cell movement during development [12] in a sense contrary to that found in the majority of mammals. This vertical decussation arises through groups, columns or zones of cells moving vertically either upwards or downwards across the developing enamel surface (Fig. 15), this process beginning only after some small amount of enamel has been produced on the surface of the dentine. It has many interesting consequences, not the least of which is that groups of cells with different differentiation ages enter the post-secretory maturation phase of enamel development at the same geographical level on the tooth. In terms of the functional requirements of the adult, completed, mature tooth, this arrangement gives rise to groups of prisms meeting worn occlusal facets in different orientations. Local parts of these worn facets which have prisms parallel to the surface are more worn than those which have prisms reaching the surface perpendicularly. Thus a micro-relief, or micro-serration is produced on parts of the occlusal surface as a function of the enamel structure (Fig. 22). Fortelius [22] has speculated that this may represent a functional advantage in mammals which had specialised in browsing. The pattern of attrition and abrasion in rhinos (Fig. 22) corresponds with that seen in experimental mechanical etching studies (reference 12 and Fig. 21).

In the context of examining local enamel wear rates as a function of enamel prism orientation, it is interesting to note that the strong prism decussation of the enamel of rodent incisors may in fact determine that they wear fast and in a predetermined direction, rather than that this arrangement of the tissue reduces the rate of wear.

The enamel-dentine junction

Enamel is strong because its prisms are interlocked in several senses (Figs. 2 & 24-27), but it is also very strong because it is anchored on to the surface of the dentine which has a high local-to-total surface area ratio. The surface of the dentine is composed of very fine collagen fibrils which project perpendicular to this surface (Fig. 2). Enamel matrix is secreted into the interstices of this web. Mineralization in the dentine and enamel is contiguous along this boundary. Sections perpendicular to the boundary examined by BSE SEM also demonstrate the incredible degree of interdigitation (Fig. 26). It is a well known fact that enamel attached to dentine is much more difficult to break than a fragment which has been cut completely away from this important support [9].

In marsupials, the continuity of tubules from dentine to enamel across the junction can be best visualized by exposing methacrylate casts of the tubules in embedded material [15,33]. Despite many suggestions otherwise, marsupial enamel tubules are ameloblast-related and necessarily reflect

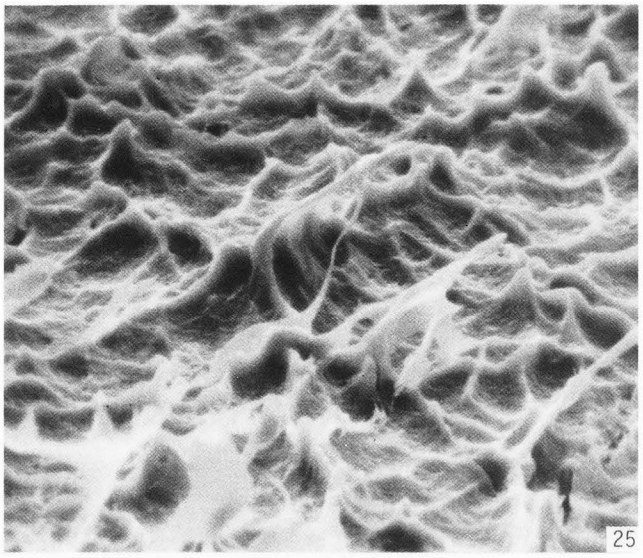

Figure 25. Decalcified enamel dentine junction in human tooth prepared by removing the overlapping enamel by acid demineralization. Fieldwidth 45µm.

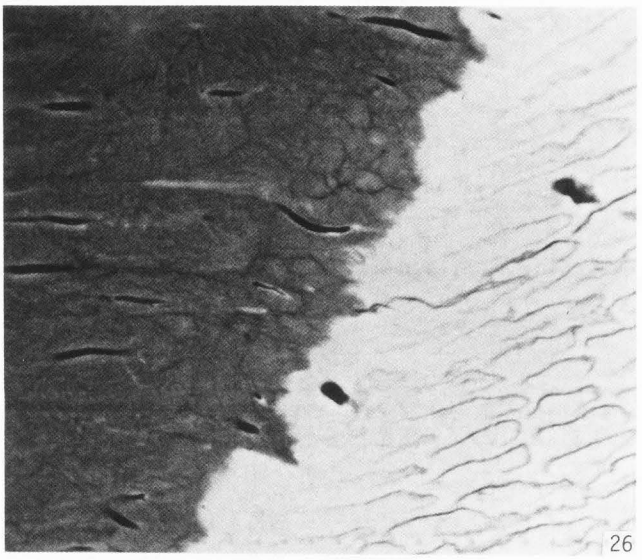

Figure 26. Longitudinal cut surface through enamel dentine junction in human permanent tooth, diamond polished BSE image showing prism boundaries, enamel spindle, dentine tubules, and details of variations in the degree of mineralization of dentine. Fieldwidth 101 µm.

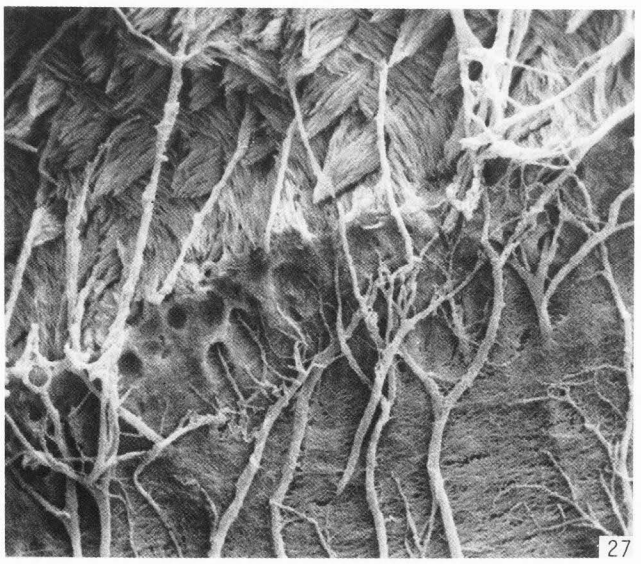

Figure 27. Longitudinal section through enamel dentine junction region of marsupial tooth which had been embedded in polymethylmethacrylate (PMMA) to make internal casts of the dentine tubules and the enamel tubules with which they are continuous. Fieldwidth 50 µm.

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ameloblast, and thus prism, course [4,30,31].

Fossil enamel

Enamel is really the only living fossil: there are no major structural changes we know of which happen to this tissue during the process of "storage" over millions of years [22,23,28,29,37,43]. It is therefore of great interest to understand possible correlations between development, structure, function and taxonomy. Only the future will tell how far we can go along these lines, but we would point out to other cell biologists that enamel may be the best in which to study several morphological aspects of cell function over the course of evolutionary time.

As regards the question of whether SEM is the best technique for making such studies, we should note that the SEM specimen only samples a very limited three-dimensional volume. A great deal of three dimensional information can be obtained from the examination of the developing enamel surface, but of course the developing enamel surface is not available for many extant species and, at least presently, for no extinct species in spite of efforts to utilize the depths of hypoplastic grooves in Miocene ape teeth [16], sites which had revealed preserved developing enamel surfaces in mature human teeth [7].

Although the resolution of optical microscopy cannot match that of SEM, its advantage in being able to examine the tissue in volume or in depth should not be underestimated. In respect of optical microscopy, it is very difficult to prepare thin sections of enamel, and particularly of fossil enamel, because it tends to fragment. We regard it as a major advantage, therefore, that a new microscope has recently been introduced which enables the internal structure of a bulk sample, like enamel on a tooth, to be examined without any need for the specimen to be prepared [17,12,33].

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References

[1] Beynon AD, Wood BA (1987) Patterns and rates of enamel growth in the molar teeth of early hominids. Nature. **326**: 493-496.

[2] Boyde A (1964) The structure and development of mammalian enamel. PhD thesis (available from Senate House Library), University of London.

[3] Boyde A (1965) The structure of developing mammalian dental enamel. Tooth Enamel (MV Stack RW Fearnhead eds) pp. 163-171, 192.

[4] Boyde A (1967) The development of enamel structure. Proc Roy Soc Med **60**: 1-6.

[5] Boyde A (1968) Correlation of ameloblast size with enamel prism pattern: use of scanning electron microscope to make surface area measurements. Z Zellforsch **93**: 583-593.

[6] Boyde A (1969) Electron microscopic observations relating to the nature and development of prism decussation in mammalian dental enamel. Bull Group Int Rech Sci Stomatol **12**: 151-207.

[7] Boyde A (1970) The surface of the enamel in human hypoplastic teeth. Archs Oral Biol **15**: 897-898.

[8] Boyde A (1976a) Amelogenesis and the structure of enamel. In: Scientific Foundations of Dentistry: B Cohen, IRH Kramer (eds). pp. 335-352, Heinemann, London.

[9] Boyde A (1976b) Enamel structure and cavity margins. Operative Dentistry **1**: 13-28.

[10] Boyde A (1978) Development of the structure of the enamel in the incisor teeth in the three classical subordinal groups in the Rodentia. In: Development, Function and Evolution of Teeth: PM Butler, KA Joysey, (eds). pp. 43-58, Academic Press, London.

[11] Boyde A (1979) Carbonate concentration, crystal centers, core dissolution, caries, cross striations, circadian rhythms, and compositional contrast in the SEM. J dent Res **58** Spec Suppl B: 981-983.

[12] Boyde A, Fortelius M (1986) Development, structure and function of rhinoceros enamel. Zool J Linnean Soc **87**: 181-214.

[13] Boyde A, Jones SJ, Reynolds PS (1978) Quantitative and qualitative studies of enamel etching with acid and EDTA. Scanning Electron Microsc. **1978; II**: 991-1002.

[14] Boyde A, Lester KS (1967) The structure and development of marsupial enamel tubules. Z Zellforsch **82**: 558-576.

[15] Boyde A, Lester KS (1984) Further SEM studies of Marsupial Enamel. Tooth Enamel IV. RW Fearnhead, S Suga, (eds). pp. 442-446. Amsterdam: Elsevier.

[16] Boyde A, Martin L (1982) Enamel microstructure determination in hominoid and cercopithecoid Primates. Anat Embryol. **165**: 193-212.

[17] Boyde A, Martin L (1984) A non-destructive survey of prism packing patterns in primate enamels. Tooth Enamel IV. RW Fearnhead, S Suga, (eds). pp. 417-421. Amsterdam: Elsevier.

[18] Boyde A, Stewart ADG (1962) A study of the etching of dental tissues with argon ion beams. J Ultrastructure Res **7**: 159-172.

[19] Boyde A, Tamarin A (1984) Improvement to critical point drying technique for SEM. Scanning **6**: 30-35.

[20] Bromage TG, Dean MC (1985) Re-evaluation of the age at death of Plio-pleistocene fossil hominids. Nature **317**: 525-528.

[21] Fortelius M (1984) Vertical decussation of enamel prisms in lophodont ungulates. Tooth Enamel IV. RW Fearnhead, S Suga, (eds) pp. 427-431. Amsterdam: Elsevier.

[22] Fortelius M (1985) Ungulate cheek teeth: developmental, functional, and evolutionary interrelations. Acta Zool. Fennica **180**: 1-76.

[23] Gantt DG, Pilbeam DR, Steward GP (1977) Hominoid enamel prism patterns. Science **198**: 1155-1157.

[24] Grine FE, Krause DW, Fosse G, Jungers WL (1987) Analysis of individual, intraspecific and interspecific variability in quantitative parameters of caprine tooth enamel structure. Acta Odontol Scand **45**: 1-23.

[25] Gysi A (1931) Metabolism in Adult Enamel. The Dental Digest pp. 661-668.

[26] Johnson NW, Poole DFG, Tyler JE~ (1971) Factors affecting the differential dissolution of human enamel in acid and EDTA. A scanning electron microscope study. Archs oral Biol **16**: 385-396.

[27] Kawai N (1955) Comparative anatomy of the bands of Schreger. Okajimas Folia Anat Japon **27**: 115-131.

[28] Koenigswald WV, Pfretzschner HU (1987) Hunter-Schreger-Bander im Zahnschmelz von Saugetieren (Mammalia). Zoomorphol **106**: 329-338.

[29] Korvenkontio A (1934-35) Mikroskopische Untersuchungen an Nagerincisiven unter Hinweis auf die Schmelzstruktur der Backenzahne. Histologisch-phyletische Studie. Annal Zool Soc Zool-Bot Fenn Vanamo (Helsinki) **2**: 1-274.

[30] Lester KS (1970) On the nature of "fibrils" and tubules in the developing enamel of the Opossum, Didelphis marsupialia. J Ultrastructure Res **30**: 64-77.

[31] Lester KS (1971) Fine Structure of "fibrils" and tubules in developing opossum enamel. Tooth Enamel II. RW Fearnhead, MV Stack, (eds). pp. 62-71. Bristol: Wright.

[32] Lester KS, Boyde A (1967) The structure and development of marsupial enamel tubules. Z Zellforsch **82**: 558-576.

[33] Lester KS, Boyde A, Gilkeson C, Archer M (1987) Marsupial and monotreme enamel structure. Scanning Microscopy **1**: 401-420.

[34] Martin L, Boyde A (1984) Rates of enamel formation in relation to enamel thickness in hominoid primates. Tooth Enamel IV. RW Fearnhead, S Suga, (eds). pp. 447-451. Amsterdam: Elsevier.

[35] Nasmyth A (1839) Researches on the development, structure and diseases of the teeth. Churchill, London pp. 165.

[36] Poole DFG, Johnson NW (1967) The effects of different demineralizing agents on human enamel surfaces studied by Scanning Electron Microscopy. Archs oral Biol **12**: 1621-1634.

[37] Rensberger JM, Koenigswald W (1982) Functional and phylogenetic interpretation of enamel microstructure in rhinoceroses. Palaeobiology **6**: 477-495.

[38] Retzius A (1837) Mikroskopiska undersokningar ofver tandernas sardeles tandbenets struktur. Kongl Vetenskaps Acad Handlinger Stockholm **Ar 1836**: 52-140.

[39] Shobusawa M (1952) Vergleichende Untersuchungen uber die Form der Schmelzprismen der Saugetiere. Okajimas Folia Anat Japonica **24**: 371-392.
[40] Smreker E (1905) Uber die Form der Schmelz Prismen menschlicher Zahne und die Kittsubstanz des Schmelzes. Arch f Mikr Anat **66**: 312-331.
[41] Tomes J (1849) On the structure of the dental tissues of marsupial mammals, and more especially of the enamel. Phil Trans Roy Soc Lond **139**: 403-412.
[42] Tomes J (1850) On the structure of the dental tissues of the order Rodentia. Phil Trans Roy Soc Lond **140**: 529-567.
[43] Vrba ES, Grine FE (1978) Australopithecine enamel prism patterns. Science **202**: 890-892.
[44] Warshawsky H, Bal P, Nanci A (1984) Lack of Evidence for Rhythmicity in Enamel Development. INSERM **125**: 241-256.