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EXTERNAL SHAPE OF ENAMEL CRYSTALS

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Abstract

Biological hydroxyapatite crystals are either small, as in bone and dentin, or large as in enamel. Enamel crystallites are unique since each is initiated and grows in length, thickness and width until the entire layer of enamel is secreted. In maturation, these extremely long crystallites grow only in thickness and width. Crystal growth in vitro follows physico-chemical principles, but lacks biological intelligence; in vivo this intelligence is contributed by protein templates. The location of the organic template in enamel is congruent with the crystallite, constituting the crystal ghost. Since crystals cannot accommodate proteins, the explanation is logically inconsistent. In sections, enamel crystallites, appear hexagonal, and this is interpreted as their cross-sectional shape. Since this hexagonal image also contains the crystal ghost, the notion that hexagons do not represent true cross-sections was explored with models of crystallite shape. Hexagonal rods were compared to rectangular or rhombohedral rods. Whereas segments of hexagonal rods in the section should project as octagons at the electron microscope imaging plane, and octagonal profiles are never found, rectangular or rhombohedral rod segments project as hexagons. Assuming the organic template covers the crystal exterior, the projected rhombohedral segment, appearing hexagonal, would seem to contain the protein, hence explaining the apparent presence of the crystal ghost.

Key Words: Enamel, crystals, crystallites, hydroxyapatite, parallelepiped-shaped, rhombooidal cut ends.

Introduction

My recent interest in amateur astronomy has led to reading about the search for extraterrestrial intelligence. Such intelligence is postulated to exist because of the probability that in our vast universe, the chances are overwhelming that we are not alone. Besides the comfort it gives us to believe in others beings elsewhere, there is the profit to be made in books, films and television. Also, vast sums of money are being committed to the SETI project (Search for Extraterrestrial Intelligence), a radioastronomy search for intelligent communication from the universe. While gazing at the stars in subzero weather, I also thought of enamel crystallites and an analogy came to mind. Is not the Medical Research Council of Canada funding us to look for intelligent life at the surface of enamel crystals? Now, what do I mean by intelligent life related to a crystal? It is well known that a supersaturated solution of calcium ions (calcium phosphate) will remain in solution until a seed is introduced to the system. This seed can be a single crystal of salt, or a scratch on the inside of the beaker resulting in glass fragments which act as seeds. The effect of this seed is the instantaneous precipitation of a slurry of crystals which, like a snowstorm, clouds the beaker and falls to the bottom. The creation of these crystals is governed by physico-chemical principles that are universal laws. These same principles apply to the precipitation of calcium/phosphate in mineralized tissues, but there is an overriding factor that controls the universal laws of chemical precipitation. There is a biological factor that imparts intelligence to the non-intelligent, physico-chemical reaction that occurs, without thought, in the beaker. This biological factor conveys constraints on the crystals and determines their size, shape, orientation and indeed whether they should exist at all.
The mechanism whereby intelligence is transmitted is by translation of the genetic code into protein molecules that are secreted from the cells and these proteins are endowed with information used to control various crystal parameters. These proteins are thus "templates" which direct the crystals of bone to be small, needle-like and oriented along the long axis of the collagen macromolecule, to be small and needle-like, but randomly oriented in calcified cartilage and to be present in bone, dentin and calcified cartilage, but not in normal connective tissue. Such a protein template also must direct the enamel crystallites to be long, plate or ribbon-like, and to be oriented in specific ways in rod and interrod enamel. The search for a template molecule in mineralized tissues is akin to the search for intelligent extraterrestrial life.

This review is divided into three sections; first, the structure of enamel will be discussed; second, what is known about templates will be reviewed. Also, the existence of proteins in the enamel, and their localization will be considered; and, third, a new conception of crystal shape, which accounts for the observations, will be described.

**Structure of enamel**

One-micrometer-thick Epon sections of rat incisor enamel in cross-sections of the tooth show enamel rods and the interrod enamel that separates the rods (Fig. 1). The distribution of interrod enamel into initial, inner, outer and final enamel layers is a consistent finding in rat incisors (Warshawsky, 1971) and in other teeth as well. A basic concept is that the "rod profiles" seen in histological or electron microscope sections are segments of elongated rod-like structures. Scanning electron microscopy of rat molar enamel shows the "rod-like" nature of rods and their continuity from inner to outer enamel layers (Fig. 2).

Reconstruction from serial sections produced models of enamel rods (Fig. 3) which confirm the sectional and SEM appearance (Warshawsky and Smith, 1971).

Transmission electron microscopy of sectioned inner and outer enamel and the sectional appearances of the ameloblasts responsible for the secretion of each layer of enamel confirms that the rod and interrod pattern is determined by the pattern and organization of Tomes' processes, the apical prolongation of the ameloblasts (Fig. 4; Warshawsky et al., 1981). Since these sections were cut from decalcified enamel, the material seen in the section is the matrix only.

Electron micrographs of undecalcified enamel show the crystallites in sections (Fig. 5). Within the inner enamel the rod and interrod profiles are seen by virtue of the differently oriented crystallites. At the secretion sites, the spaces occupied by Tomes' processes becomes filled with newly formed rod enamel (Fig. 5). Freeze-fracture replicas at the junction between rod and interrod enamel also show the distinction based on the different orientations of the crystallites. Rod and interrod enamel consist of bundles of crystallites running in different directions. These replicas clearly show the length of the crystallites, the difference in direction between rod and interrod enamel, their continuous nature, and the
Enamel Crystals

Figure 2. Scanning electron micrograph of a ground section of a rat molar etched to enhance the surface topography. The initial, inner, outer and final layers of the enamel are clearly evident and resemble the pattern seen in the light micrograph from the rat incisor (Fig. 1). The continuity of the inner and outer enamel rod portions is seen. The decussating pattern of the inner enamel, and the straight course of the outer enamel rod portions creates the distinction between the two layers of the enamel. (Courtesy of Dr. Steinar Risnes.)

Figure 3. Three-dimensional model of enamel rods reconstructed from serial sections of the upper incisor of the rat. The inner (IE) and outer (OE) enamel portions of the rods are seen, and the decussating relationship between the inner portions are evident.

shape of the crystallites at their fractured ends (Fig. 6). The actual shape of these ends is discussed in the Section "A New Concept of Crystal Shape" of this paper, but when seen in routine sections by transmission electron microscopy, the supposedly cross-cut profiles appear hexagonal in shape.

Examination of high resolution electron micrographs of crystallites cut in near cross-section (Fig. 7) show variation in density; some being translucent and others, completely opaque. The opaque, dark ones tend to be hexagonal, but elongated and with two peaks. Some have equal sided peaks and others have
Enamel Crystals

Figure 5. Section of inner enamel cut from an undecalcified incisor to show the organization of the crystallites into either rod (Rod or R) and interrod (IR) enamel. Cross-cut rods (labeled Rod) appear in rows separated by interrod enamel. Longitudinally cut rods (R) alternate with the cross-cut profiles. The spaces occupied by Tornes' processes (Tp) are progressively filled by the rod enamel until no spaces remain for the processes.

Figure 6. Freeze-fracture replica of glutaraldehyde fixed, glycerine-cryoprotected rat incisor enamel showing the groupings of crystallites into either rod or interrod enamel. The individual crystallites are long ribbon-shaped structures that can be viewed from their flat surfaces or from their narrow edges. When fractured, they tend to break at right angles to their long axes (arrows). Particles are present between the crystallites and represent the location of the amelogenin proteins. The parallel lines represent the average section thickness (100nm) and show how crystallites segments extend through the section. Thus, each crystallite image in section must represent a view of such a segment.

Figure 7. High resolution transmission electron micrograph of crystallites in near cross-section. The profiles vary considerably in electron density, and lattice fringes representing the unit cell structure of hydroxyapatite are seen on all profiles. The elongated hexagonal nature of these images and the fact that some profiles have peaks with equal sides (e) or unequal sides (u) is clearly illustrated. A three-dimensionalization of one of these hexagons by dashed lines, demonstrates an alternate interpretation of these hexagonal images.

Figure 8. Freeze-fracture replicas of enamel in the early part of the maturation zone (A), compared to enamel on the erupted portion of the incisor (B). In both cases the fracture plane passes between the rod and interrod enamel, thus crystallites cross each other at almost right angles. In the younger enamel the protein particles are present, but in the mature enamel the particles are gone. Although no evidence of imprinting of one orientation on the other is seen in the young enamel, the obvious grooves caused by the crossing crystallites is dramatic in the older and more mature enamel.

Figure 9. Isolated crystallites prepared from 4 M guanidine-extracted enamel of early maturation and plated on formvar-coated grids. The crystallites fall at random on the grid. The extreme length and uniform width of the ribbon-like crystallite are evident.

Figure 10. Unstained section of guanidine extracted enamel (A) compared to the adjacent serial section that was extracted and stained with phosphotungstic acid (B). The guanidine extracted enamel contains only the crystallites and the enamelin proteins. Thus, decalcification of this section in the acid medium removes the crystallites, but leaves an organic remnant that stains with phosphotungstate and resembles the crystallites, but with an empty central region. This organic material, supposedly the enamelines, represents the crystal ghost.

unequal sides at the peaks, a fact that will be important later. The fine lines on the crystal profiles are the lattice fringes given by electron diffraction from the repeat unit cells of the atomic structure in the crystal (Nylen and Omnell, 1962). As these crystals grow, the hexagonal profiles appear to become distorted. High resolution electron micrographs of older crystals (Selvig, 1970, 1972) show irregularly shaped profiles and a reduction of the space. In freeze-fracture replicas, we have found that this apparent irregularity of shape is caused by the crystals growing towards and partially around each other in a way which does not involve fusion (Warshawsky, 1985; Warshawsky et al., 1985). Freeze-fracture replicas at the junction of rod and interrod enamel in young enamel show the crystals crossing without leaving a trace on their surfaces, but in older enamel, the course of the crystals with one orientation is revealed by grooves left on the surface of the crystals that they cross (Fig. 8). In section, these perfectly regular variations of the ribbon-like crystals appear as irregularly shaped profiles.

Another way of looking at the crystals is to isolate and separate them from each other and plate them onto an electron microscope grid (Fig. 9). These isolated single crystallites lie randomly distributed on the grid, but with their long axes parallel to the grid surface. High resolution electron microscopy permits visualization of lattice fringes from these crystals of known orientation. Isolation is also useful in visualizing the extreme length of the crystallites, and overlapping of crystallites can be identified and the resulting moire patterns analysed.

Templates and Proteins in Enamel

Osteocalcin is a well categorized noncollagenous protein of bone (Hauschka, 1985). From its structure and the distribution of serines which can be phos-
phorylated, Hauschka has matched the phosphate positions in the protein with the spacing of calcium in the bone crystals. Thus, the molecule of osteocalcin may act as a template to seed and direct the growth of bone crystals. In the diagram published by Hauschka, the crystal being formed is depicted as hexagonal in shape and the unit cell is shown in relation to the hexagon.

A similar analysis of enamel hydroxyapatite showing the unit cell and the distribution of calcium, phosphate and hydroxyl groups, emphasizes the distances, 0.69 and 0.94 nm, of the unit cell (Traub et al., 1985). In the enamel protein, which is postulated to be the template (enamelin), the distribution of calcium binding sites in the protein can be found at distances that outline a unit cell with "amelodoms" of 0.69 and 0.94 nm (Traub et al., 1985). If this protein is indeed a template, it must be present in the enamel from the beginning of secretion until final maturation, when crystal growth stops. It must also be in close proximity to the crystallite surfaces.

In an attempt to look for this protein, sections of mineralized enamel, both unstained and stained, were examined. Little difference is apparent (Bai and Warshawsky, 1985); indeed, it was shown that staining sections of young enamel with aqueous solutions of uranyl acetate and lead citrate results in the complete removal of the crystal leaving behind a stained image which is identical to the original crystal (Bishop and Warshawsky, 1982; Nanci et al., 1983). High magnification of stained enamel reveals dots that may be stain bound to the protein, or defraction from the Epon, a question that could not be answered at that level. However, with freeze-fracture replicas, the proteins appear quite different from the crystals (Leblond and Warshawsky, 1979). Thus, we used this method to explore for proteins in the enamel (Bai and Warshawsky, 1985).

Freeze-fracture replicas of unfixed, glutaraldehyde only, and glutaraldehyde-acrolein fixed enamel, show the crystals and the presence of the protein as distinct particles between or on the surface of the crystals (Bai and Warshawsky, 1985). We used a routine biochemical method to remove the category of protein known as "enamelines" (Traub et al., 1985). Comparisons of control and extracted samples show the removal of all the particles by the extraction procedure. The other category of protein, the "enamelines", can only be removed by dissolving and destroying the crystallites in EDTA. Thus, the enamelines must still be present in preparations extracted by the 4 M guanidine.

Our concern about these particles is that given their size they really should be visible in routine sections. This prompted us to suspect that the particles may be a form of precipitation artifact caused by the freezing process. To prove this, we soaked the guanidine extracted enamel in protein solutions. We used albumin and ovalbumin and prepared the "protein infiltrated" enamel samples for freeze-fracture replicas. With both proteins, the freeze-fracture replicas are identical and in each case, the particles are restored. We concluded that the particles are indeed the result of precipitation caused by condensation and supersaturation due to withdrawal of the water during the freezing process (Bai and Warshawsky, 1985).

Having located the position of the amelogenins, we could now direct our attention to the location of the enamelines. First we looked at unextracted enamel. We compared unstained sections, sections that were extracted with formic acid and stained with uranyl acetate and lead citrate, and phosphotungstic acid extracted and stained sections. Unstained sections show no evidence of protein; extraction with formic acid removes the calcium and phosphate and staining with uranyl acetate and lead citrate restores images that are identical to the crystal images seen previously; phosphotungstic acid extracts the mineral and stains the remaining protein at the same time. These results suggest that a protein is present in very close association with the crystal. In order to determine what category of proteins are involved, the amelogenins were removed with guanidine and what remains was simultaneously either removed, or stained with phosphotungstic acid (Fig. 10). The images that remain resemble the crystals, but some are empty in the centre, and become expanding holes under continued beam irradiation. It is tempting to speculate that the crystals fit into these empty spaces. Measurements made by Bai and Warshawsky (1985) reveal that the outside of the crystals and the outside of the protein are identical in size, and that the empty space is statistically significantly smaller than the crystals. This makes us believers in the crystal-ghost hypothesis. This hypothesis was first proposed by Bonuccci (1969) and it proposes that crystals do not possess a background stroma of protein.

The results raise two possibilities: first, the protein is inside the crystal, as predicted by the crystal-ghost hypothesis, a situation prohibited chemically and crystallographically; or second, the protein has been incorrectly interpreted. We believe the latter and I will now explain our interpretation of the crystal shape.
A New Conception of Crystal Shape

The origin of the hexagonal shape theory begins with the electron micrograph published by Frank et al., (1960) which shows a single crystal as an equal-sided hexagon. The drawing published by Hohling (1961), depicts the crystals of enamel as elongated hexagons. The conception of crystals as hexagonal was firmly established by the electron micrographs of Nylen and Omnell (1962) and Nylen et al. (1963), where regular elongated hexagons were seen in rat incisor enamel.

Daculsi and Kerebel (1978) explained how crystals grow from thin rectangular plates to hexagons by the addition of crystalline planes (seen as lattice fringes) to both surfaces. Robinson et al. (1983) proposed that the protein particles seen in the freeze-fracture replicas regulate crystal growth and in their diagram show how a rectangular plate becomes a hexagonal rod.

The unit cell of hydroxyapatite predicts that the crystal should respect its symmetry. In fact all it dictates is that the crystal must respect the 60° angle of the 'hexagonal' unit cell.

The new conception of crystal shape begins with an analysis of enamel crystals in sections. In routine electron micrographs, longitudinal and nearly cross-sectioned crystal profiles are seen. In areas containing the supposedly cross-cut crystals there are two different images: translucent rectangular profiles and opaque hexagonal ones, neither of which represent true cross-sections (Fig. 7). Freeze-fracture replicas show how the crystals appear without sectioning. Two parallel lines drawn to scale representing the average thickness of a section predicts that segments of crystals extend through the entire section thickness (Fig. 6). We confirmed this by embedding and sectioning a crystal; thus obtaining the so-called "section-of-section" (Bai and Warshawsky, 1985). Goniometric tilting of a routine section shows that the rectangular and hexagonal profiles are one and the same (Warshawsky et al., 1987).

We examined two conceptions of what a crystal might look like, the widely accepted hexagonal rod, and the newly proposed rectangular rod model. With segments of the former shape one would expect to see octagonal profiles, and these are never seen. Segments of the latter would almost invariably present hexagonal profiles (Fig. 11). Whatever shaped crystal segment is present in the specimen, only a shadow of it would be seen at the film plane because of the nature of electron imaging. Since contrast is achieved by selective transmission of some electrons and reflection of others, the image is indeed a true shadow of the object present in the specimen. Thus, the shadows cast by a wooden block model of the hexagonal rod will be hexagonal only when the two cut surfaces are perfectly superimposed. In most instances the model would cast a shadow from an angle and such an image would project as an octagon (Warshawsky et al., 1987). On the other hand, shadows of the rectangular rod model project as hexagons (Fig. 12) except when the cut surfaces are superimposed.

A re-examination of supposedly cross-sectioned crystals (Fig. 7) illustrates the following: first, all images are hexagonal, and none are octagonal. Second, the hexagons have peaks with either equal or unequal sides. Third, the hexagons with equal sided peaks are narrower than hexagons with unequal sided peaks. Fourth, the long axes of the hexagons often do not have parallel sides. This distortion could result from projecting an image of a box-like structure that was tilted so that one end was closer to the film plane than the other end.

Re-interpretation of previously published images of crystals (Nylen et al., 1963) confirms that it is possible to extrapolate box-like images of the hexagons such that the central dark line appears as the edge of the box (Fig. 7). Indeed, the work of Nylen et al., (1963) and Frazier (1968) suggested that the central dark line behaves like an edge, giving Fresnel fringes at various levels of focus. When comparing sections of crystals with box-like extrapolations, to shadows of the model, it was seen that the orientation of the crystal profile could not be predicted from the shadow which it cast (Warshawsky et al., 1987).

There are two extreme extrapolations associated with the rectangular box, or parallelepiped model: these are first, that rectangular cut surfaces do not respect the hexagonal unit cell which has 60° angles; and, second, some of the angles measured from the shadows are closer to 90°. This objection can be eliminated by making the model a parallelepiped with both cut surfaces being rhomboidal and containing 60 and 120° angles (Fig. 12). Projected shadows from a model of a parallelepiped with rhomboidal ends show that equal sided and unequal sided peaks can be obtained from the same model (Fig. 12). Also, the angles are correct and hexagons with equal sided peaks are narrower than hexagons with unequal sided peaks (Fig. 12), a situation which is seen in actual electron micrographs (Fig. 7).

Finally, evidence for rhomboidal or rectangular cross-sectioned crystals was
obtained by freeze-fracture replicas with rotary shadowing (Warshawsky et al., 1987).

The most important corollary of accepting the parallelepiped model is that it explains how a thin, almost unmeasurable coat of protein acting as a template on the surface of a parallelepiped can produce a shadow which appears to be contained in the crystal. This is demonstrated by the cardboard box model constructed to conform to the shape of the wooden block. It is open at the cut upper and lower surfaces to mimic the protein coat at the surface of the crystallite segment. Projection of this empty model produces hexagonal shadows (Fig. 13), that are equal in shape and size to those cast by the solid model. Some of these show empty slits: images that resemble central dissolution of the crystallites such as supposedly occurs in incipient carious lesions (Simmelink and Nygaard, 1982). These empty slits are equivalent to the empty centers in the organic profiles seen after phosphotungstic acid extraction. In both cases the empty slits are smaller than the dense images that surround them, thus explaining the measurements published by Bai and Warshawsky (1985).

Summary

In order to place the matrix protein in the correct position to act as a template and regulator of crystal growth, that is, at the outer surface of the crystallite, and not within it, it is necessary to redefine the shape of enamel crystals. Instead of being hexagonal rods, the crystals seem to be elongated parallelepipeds with rhomboidal cut ends.

Figure 11. Schematic drawings to illustrate the sectioned surface (hatched) of the two models used to explain crystallite shape, and the three-dimensional shape of segments of each kind. The commonly accepted shape is of the elongated hexagon (A). A three-dimensional view of such a shape results in an octagonal figure. The rectangular shape shown in B is invariably seen as a hexagon.

Figure 12. A wooden block model of a parallelepiped-shaped segment with rhomboidal cut ends respects all the constraints of the hexagonal unit cell, and projects shadows that in all aspects resemble the dense hexagons seen in electron micrographs. Shadows with equal sided peaks (e, in A) and unequal sided peaks (u, in B) can be projected by the same model. Note that the shadow in B is wider than the one in A.

Figure 13. A cardboard box model made to represent a thin layer of protein on the surface of the parallelepiped-shaped wooden model projects a hexagonal shadow such that the "protein" appears inside the hexagonal shape and would be interpreted as the crystal ghost. Note that the shadow shows a central empty space resembling central dissolution of crystallites.

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Enamel Crystals

References


Discussion with Reviewers

D. R. Eisenmann: How does your theory fit with known crystallographic data on the lattice images of these crystals?

Author: The known crystallographic data show that when three sets of lattice fringes intersect at 60°, the "c" axis of the unit cell is parallel, to within 0.5°, to the direction of the electron beam. Since such intersecting fringes are seen on hexagonally shaped shadow projections of crystallite segments, it is assumed that the crystallites' long axes are also parallel to the beam and the crystallites are cut in cross-section. Thus, the hexagonal images are assumed to be cross-sections of hexagonal crystallites. What is factual is that the unit cell is oriented as predicted; what is assumed is that the orientation of the unit cell will absolutely predict the external shape of the crystal. Indeed, since the shape of the unit cell is rhombohedral, the crystals themselves ought to be rhombohedral in cross-section and not hexagonal. The use of the term "hexagonal" to describe the rhombohedral-shaped unit cell of hydroxyapatite is unfortunate in the light of the hexagons shown in fig. 13. Since the outside of these structures has "identical" dimensions with the crystals themselves, it is not clear how this fits with the presence of an organic coat on the outside of the crystal. The coat must have larger dimensions than the crystals.

Author: The question asked in this paper was, Why do the protein and the crystals themselves, and the atomic structure of the protein and the crystal be separate. This conclusion is inescapable although it is logically inconsistent. An alternate explanation for this observation was put forward and illustrated in this paper. In particular, the entire thrust of the argument is that the cardboard box model clearly demonstrates how the protein and the crystal images can appear to co-localize and yet be separate.

E. Kallenbach: Regarding the crystal ghost: .... The structures seen in fig. 10B are too regularly shaped to be explained convincingly by the mechanism shown in fig. 13. Since the outside of the crystallites is assumed to be octagonal, how can your crystals accommodate an organic layer?

Author: Because the measurements of crystal profiles and their ghosts are essentially identical; and a ghost would imply an organic stroma within the crystallite, a situation prohibited by the constraints of the atomic lattice, this alternate proposal of crystal shape becomes necessary. The apparent accommodation of the protein within the crystal is explained by the cardboard box model made to represent a thin layer of protein at the crystal surface (Fig. 13). Projection of this "protein sheath" results in a profile identical in size and shape to the crystals themselves and explains how proteins appear to be contained in the "crystal", which is a shadow of a crystallite segment contained in the section.

E. Kallenbach: The author states that in the 1987 paper, a given field was photographed at different settings of the goniometer, and rectangular profiles were actually observed. How does the rectangular cross section of the crystal change with the setting of the goniometer? This conclusion is inescapable although it is logically inconsistent. An alternate explanation for this observation was put forward and illustrated in this paper. In particular, the entire thrust of the argument is that the cardboard box model clearly demonstrates how the protein and the crystal images can appear to co-localize and yet be separate.

R.Z. LeGeros: Is your definition of 'structure' synonymous with 'morphology' when describing enamel apatite crystals?

Author: As an anatomist, I view the terms structure and morphology as identical. However, I was clearly discussing only the external shape of the crystallite and not its atomic 'structure'. Consequently, I am describing 'morphology' in the reviewer's context.
Enamel Crystals

R.Z. LeGeros: Several investigators have reported to observe preferential dissolution of the core of the enamel crystals and associated this with the observed central defects in these crystals. How do you reconcile this with your suggestions that the edges of the 'rectangular' crystals give rise to apparent central defects?

Author: The projection of the empty cardboard box model is a perfect explanation for central dissolution. I believe that the observation of central dissolution is an artifact seen only in cross sectioned crystallites in thin sections. Since these segments are cut so that the top and bottom of the segments are exposed and not protected by Epon they are most vulnerable to artifactual dissolution during sectioning (by the water in the knife boat) or during staining (by the aqueous and acid staining solutions) or during observation (due to electron beam sublimation). Since the two cut ends are nearly, but not quite superimposed, the resulting shadow would be an hexagonal image with a central empty slit, just as is seen in figure 13. I should also point out that central dissolution is not seen in crystallites lying within the plane of the section, that is, in longitudinal orientation and covered on both surfaces by Epon.

R.Z. LeGeros: Do you have an explanation for the preponderance of 'hexagonal shadow' from "rectangular" crystals? To have these shadows, the rectangular crystallites will have to be standing on edge, which is a very unstable position. Statistically is this observation reasonable?

Author: The preponderance of 'hexagonal shadows' is only seen in cross sections of enamel rods because in this situation the crystallites are more or less parallel and the rectangular crystal segments are "standing on edge". This is certainly not an "unstable position" since each segment is supported by the Epon, a solid plastic which surrounds and supports them in the section. Not only is this observation statistically reasonable, but is fully predicted and expected based on the "section-of-section" data illustrated in the article Warshawsky et al., 1987.

R.Z. LeGeros: Have you made some studies of known hexagonal crystals showing a preponderance of octahedral shadows? It would seem that these crystals would also be standing on edge, a likewise unstable position.

Author: If the crystals in enamel had no preferred orientation and were not embedded and sectioned, they would not stand on edge. On the other hand, known hexagonal crystals have never been plated or sectioned and no one has reported seeing octagonal images from any crystal-lite seen in biological material.