A Three-dimensional Reconstruction of the Rods in Rat Maxillary Incisor Enamel

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ABSTRACT

A three-dimensional reconstruction of the rod profiles seen in inner and outer rat incisor enamel was made from serial 1 µ cross sections of a decalcified upper incisor. The enamel rod was found to be an elongated structure which travelled incisally relative to its origin and ran continuously from the dentino-enamel junction to the enamel surface. The rod was divisible into an inner and outer enamel portion. The inner enamel portion began at the dentino-enamel junction and travelled incisally for about 20 µ with either a mesial or lateral tilt towards the outer enamel. The outer enamel portion of the rod was straight and ran incisally for about 60 µ as it gradually approached the enamel surface.

Inner enamel portions of rods were arranged in rows parallel to the cross sectional plane of the incisor. All the rods in each row were tilted either mesially or laterally such that individual rods of adjacent rows crossed each other at 90°. Outer enamel portions of rods were not arranged in rows but all passed incisally parallel to one another.

In the persistently erupting incisor of the rat two basic enamel patterns have been described, the inner enamel which is laid down first and forms most of the enamel thickness, and the outer enamel which is thinner and laid down last (see review in the preceding article; Warshawsky, '71).

Although several papers have presented morphological descriptions of the rat incisor enamel with the light and electron microscope (see Warshawsky, '71), the validity of speculations concerning the three-dimensional arrangement of enamel rods is limited in that these conclusions were always based on two-dimensional observations.

In this study a precise three-dimensional description of the nearly mature rat incisor enamel (Warshawsky, '71) was made by reconstructing serial sections which were thin enough to resolve individual rod profiles. Several difficulties, however, were encountered in such a reconstruction. First, a large number of thin (1 µ) serial sections were needed from which a consistent enamel field could be photographed with the light microscope. Second, a system of transcribing the information from the photographs was needed to allow three-dimensional visualization. The first difficulty was overcome by using serial, one micron thick sections of Epon embedded material and photographing the corresponding areas in each section with the light microscope. The second problem was solved by using clear methyl methacrylate plates ("plexiglass") upon which the rod profiles seen in the photographs were traced.

Because of the complex structure of the rat enamel, a model to represent the enamel rods in three dimensions was built. In the model, reconstructed enamel rods were superimposed onto the two-dimensional photographs, thus demonstrating, in three dimensions, the basic arrangement of these rods from their origin at the dentino-enamel junction, through the inner enamel layer, to their termination in the outer enamel layer.

MATERIALS AND METHODS

The methods employed to obtain adequate perfusion and decalcification for light and electron microscopy have been described in detail (Hommes, Warshawsky and Leblond, '66; Warshawsky and Moore, '67).
Orientation of the incisor segment was such as to yield cross sections of the enamel organ. One micron sections were cut with a Porter Blum ultramicrotome using glass knives.

The block to be sectioned was selected from the region of nearly mature enamel (Warshawsky, '71) of an upper left incisor from a 100 gm male rat (see fig. 2 of Warshawsky, '71, region indicated by the arrow). A portion of the central part of the enamel organ was trimmed to a small size and ribbons of 1 µ thick sections were cut. Each section was transferred with a hair to a glass slide. The sections were kept in their serial order on each slide using the method described by Merzel and Leblond ('66). By this method 125 1 µ serial sections were prepared and stained with toluidine blue.

A corresponding microscopic field was photographed in each of the 125 sections with a Zeiss Photomicroscope and each of the 125 negatives was printed to a final total magnification of X 800.

Analysis of the photographs was arbitrarily started in the middle of the series (e.g., photograph 62). A clear plexiglass plate was placed over the photograph and the ameloblast-enamel and dentino-enamel junctions, in combination with several widely scattered outer enamel rod profiles, were painted (using standard oil base paint) onto the transparent plate. These markings served as reference points. Then, several inner enamel rod profiles were selected arbitrarily and also painted onto the plexiglass plate. When dry, the painted plate was transferred to the next successive photograph, both incisally (from 62 to 125) and toward the growing end (from 62 toward 1), and the reference points (as indicated above) on the plate were aligned with the corresponding points on the new photograph. The periphery of the plate was outlined with a grease pencil and a fresh plexiglass plate was placed within the outlined area. Then, the reference points, as well as the rod profiles which were followed from the previous photograph, were painted onto the new plate. In this fashion the analysis continued until each of the 125 photographs had been examined. The plates could be stacked one on top of another in their proper order, and, by holding the stacked plates up to a light source, the three-dimensional arrangement of the enamel rods was visualized. By this method several hundred enamel rods were reconstructed.

RESULTS

A detailed description of the organization of the rat incisor (Warshawsky, '68) as well as the structure of the enamel (Warshawsky, '71), has been given elsewhere. After complete deposition of both inner and outer enamel, ameloblasts which synthesized this enamel differentiated into cells

**Abbreviations**

DEJ, dentino-enamel junction
IE, inner enamel
JIO, junction of inner and outer enamel
OE, outer enamel

Fig. 1 Light micrograph of nearly mature enamel in an upper incisor cut in cross section. 1 µ Epon section, Toluidine Blue. x1,100. This micrograph is typical of the serial sections used for the reconstruction. The inner enamel (IE) and outer enamel (OE) layers are made up of characteristically arranged rod profiles outlined by interrod material. The dentino-enamel junction (DEJ), as well as the junction between inner and outer enamel (JIO) are indicated.
which have been implicated in resorption (Reith, '63). These cells supposedly carried on the function of "maturing" the enamel, presumably by removing water and protein material. It was during this process of maturation that the gross structural units of the enamel, the rod profiles, became clearly visible with the light microscope.

**Two-dimensional structure of nearly mature enamel**

The term "nearly mature enamel matrix" referred to the matrix found in the maturation region just before the enamel was completely lost by decalcification (see definition in Warshawsky, '71). Cross sections of decalcified incisors cut in this region showed a clearly distinguishable pattern of inner and outer enamel (Warshawsky, '71 and fig. 1). Figure 1 shows this region and is representative of the type of photograph used for the serial reconstruction of the enamel rods.

**Inner enamel**

In 1 µ thick cross sections of the incisor, the inner enamel layer appeared as rows of oblong rod profiles (fig. 1). The rod profiles stained homogeneously and stood out prominently against a less intensely stained background. This background material was referred to as "interrod" material.

For descriptive purposes the inner enamel layer was divided into three regions by virtue of the clarity of these rod profiles. The first region was a narrow band adjacent to the initial layer of enamel at the dentino-enamel junction in which the rod profiles were small, variable in staining characteristics and irregular in outline. In some cases it was difficult to ascribe a rod profile to any particular row. The second region was a wide central region where the rod profiles were oblong, regular and tightly packed into obvious rows. The third region was thin and at the junction between the inner and outer enamel layers (fig. 1). In it the profiles became elongated and their periphery indistinct.

The oblong rod profiles of each row were aligned in single file and all profiles of that row were inclined or tilted in the same direction (either mesial or lateral). The rod profiles in the row above or below a particular row were inclined in an alternate direction at an angle of about 120°.

**Outer enamel**

In 1 µ thick cross sections of the incisor, the outer enamel layer was composed of a large number of thin "oval units" (interpreted as rods), the long axis of which was perpendicular to the dentino-enamel junction and to the outer surface of the enamel.

Again, for descriptive purposes, the outer enamel layer was divided into three regions. The first was near the junction between the inner and outer enamel layers where the oval rod profiles were large, widely spaced and not easily distinguished from the background material. The second was a short region where the oval rod profiles became very small (in long axis dimension) and less widely separated. Third, as the enamel surface was approached, the oval rod profiles became elongated, more regular in shape and closer together.

**Three-dimensional structure of individual enamel rods**

From the reconstruction it was evident that the oblong profiles seen in the inner enamel layer were sections cut through an elongated structure running more or less in the long axis of the incisor. It also became evident that the "oval units" of the outer enamel layer were indeed rod profiles which could be reconstructed as an elongated structure again lying in the long axis of the incisor. Furthermore, it was found that the rods of the inner enamel were continuous with the rods of the outer enamel. It was therefore concluded that a rod consisted of an inner and outer enamel portion.

The material between the rods resembled a homogeneous phase through which the rods penetrated. Since this material was between the rods, it was called "interrod material."

The course and shape of the individual enamel rods was illustrated in figures 2, 3 and 4. In these figures one rod from two adjacent rows was depicted.

The inner enamel portion of the rod was divided into three parts corresponding to the three regions seen in sections of inner enamel. The first part of the rod began at
the dentino-enamel junction, ran slightly incisally toward the enamel surface and turned either mesially or laterally. The central part was the largest and most regular portion of the rod and its course was a continuation of that described above. However, it was sloped more steeply toward the enamel surface. It is this portion of the rod which crossed the rods of adjacent rows (figs. 2, 3, 4).

At the junction between inner and outer enamel the rod was no longer bent mesially or laterally, but ran straight incisally for a short distance along which it decreased slightly in diameter. It then turned toward the enamel surface to become the outer enamel portion of the rod. This portion ran in the long axis of the incisor as it continued incisally, and gradually approached the enamel surface.

The inner enamel portion of the rod occupied about 20 $\mu$ along the length of the incisor, whereas the outer enamel portion occupied about 60 $\mu$. However, the total linear length of the inner enamel portion was greater than 20 $\mu$ because of its curvature and oblique path.

It should be stressed, moreover, that the rod showed small irregularities in diameter and course through the enamel layers (fig. 2) and, hence, it was not a straight, regular structure.

Three-dimensional organization of the enamel

The course of five arbitrarily chosen enamel rods over a distance of 78 $\mu$ was analyzed in a model constructed according to the description given in Appendix I (fig. 5).

The model demonstrated the following characteristics of the inner enamel portion of the rods: (1) they were arranged in rows which ran alternately in a mesial or lateral direction; (2) the member rods of each row crossed the rods of adjacent rows at a 90° angle; (3) the rods of all rows ran incisally relative to their origin at the dentino-enamel junction; and (4) all rods followed a steep course toward the outer enamel surface.

The outer enamel portion of the rods were not arranged in rows. Each rod travelled incisally in the long axis of the incisor and gradually sloped to the enamel surface.

DISCUSSION

John Tomes (1850) concluded from his studies that the inner enamel consisted of rods running obliquely to one another in alternating rows. Support for Tomes' interpretation was given by Watson and Avery ('54), Butcher and Taylor ('55) and Butcher ('56). Butcher presented illustrations which showed not only alternating rows of rods arranged at right angles to each other in the cross sectional plane of the incisor, but also that all the rods were bent 30° toward the growing end of the tooth.

The three-dimensional models presented here support Tomes' and Butcher's interpretations of the arrangement of the enamel rods within the inner enamel. The rods are arranged in alternating rows which cross adjacent rows at about 90°. The inner enamel portion of the rod travels incisally with either a mesial or lateral tilt and it is steeply sloped toward the enamel surface. The course of the rod can be described as an "S"-shaped curve.

The differing clarity of the rod profiles in sections, which were noted in the various regions of both the inner and outer enamel layers, was due to abrupt changes in the course and dimension of the enamel rods. In comparing the three-dimensional Figs. 2-4 Three-dimensional configuration of the enamel rod. Approximately x2,000.

Fig. 2 A schematic representation of two rods from adjacent rows. These rods are similar to those reconstructed from the serial sections and illustrate the average course taken by the rods.

Fig. 3 A drawing illustrating the relationship of the rods shown above to the anatomical boundaries of the incisor. The inner enamel portion of the rod begins at the dentino-enamel junction and proceeds incisally running steeply toward the enamel surface in either a mesial or lateral direction. The outer enamel portion of the rod proceeds incisally in the long axis of the tooth and follows a gentle slope to the enamel surface.

Fig. 4 A view of the rods looking from the dentino-enamel junction toward the enamel surface. The crossing of the inner enamel portions of the rods in adjacent rows is clearly seen. Note the difference in length of the incisor occupied by the inner and outer enamel portions of the rod. Note also that the amount of lateral or mesial displacement of the inner enamel portion of the rod from its origin at the dentino-enamel junction was usually 50 to 60 $\mu$. 
Figures 2-4
rod structure to the two-dimensional photographs, it is noted that: (1) the narrow region adjacent to the initial enamel layer at the dentino-enamel junction, in which the rod profiles were small and irregular, represented the initial part of the rods' course where it travelled incisally, medially or laterally, and with a slight bend toward the enamel surface; (2) the oblong, regular and obvious profiles in the wide central region of the inner enamel corresponded to the steady oblique course of the rod through this region; (3) at the junction between inner and outer enamel the elongated and indistinct rod profiles (region 3 of the inner enamel and region 1 of the outer enamel) were due to the abrupt change in direction, slope and diameter of the rods as they entered the outer enamel; (4) region 2 of the outer enamel depicted irregularities in the early portion of the rod in this layer; and (5) the elongated, regular and closely packed profiles of the rods in region 3 of the outer enamel represented the gentle, sloping path of the rods as they approached the enamel surface.

In summary, this reconstruction has demonstrated that rods, in the classical sense, are present in both the inner and outer enamel layers and that the rod runs continuously from the dentino-enamel junction, through the inner enamel into the outer enamel to end close to the surface of the enamel.

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LITERATURE CITED


APPENDIX I

CONSTRUCTION OF THE MODEL TO REPRESENT THE THREE-DIMENSIONAL ORGANIZATION OF THE ENAMEL (FIG. 5)

It became apparent that all rods had a similar three-dimensional pattern, but to represent all the rods in a given volume of enamel was too complex to permit construction and visualization by means of a painted plexiglass model. Therefore, five representative enamel rods were selected and recorded over a 78 µ sequence and painted on plexiglass sheets. From this the final model was constructed (fig. 5).

The 78 µ sequence was divided into five parts, these representing in forward pro-
Regression: 0 µ (start), 12 µ, 22 µ, 43 µ, 78 µ (finish). Mirror-image photographs were printed of each segment and were mounted back-to-back upon plexiglass sheets in such a way that a chosen point corresponded on each side of a sheet. Pieces of baseplate wax identical in thickness to the plexiglass were cut to the same shape as the rod profiles in each of the 78 photographs. These wax rod profiles were luted together and the five rods took the three-dimensional arrangement depicted in the painted plexiglass model.

Each rod was invested in yellow dental stone, the wax boiled out, and the impressed space filled with cold-cure acrylic. The formed acrylic rods were taken back to the mounted photographs and glued into their proper position.

Figure 5 shows the photographs depicted in the model. The five rods represented in the model have been drawn on these photographs, because photographing the original model proved difficult. However, the path of each of the five rods over the 78 µ distance can be visualized.