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INVESTIGATION OF PREFERENTIAL ETCH
PATTERNS CREATED IN HUMAN DENTAL
ENAMEL DURING ACID ATTACK

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The phenomenon of preferential etching in human dental enamel is discussed in light of contemporary knowledge of the structure of the enamel prisms and the results of five experiments performed by the author. The author presents evidence to discard the theory of crystallite orientation as the cause of acid etch patterns. The existence of a boundary diffusion barrier is proposed by the author to account for the etching effects created by acids at low pH and their similarity to etching effects created by ion bombardment. A small but measurable difference in the Ca/P ratio between the center and boundary region of an enamel prism was found by the author.

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I. INTRODUCTION

Dental caries is probably the most common of all human ailments, surpassing even the common cold in frequency of occurrence in the population at large. It is now thought by many to be an infectious disease, and a few think eventually a vaccine may be perfected to fight it. However, it is thought by many others that perfecting a vaccine to fight cavities may be as hard to accomplish as the task of curing the common cold. In any case, any miracle cure for caries is a long way off.

Meanwhile, much research is currently involved with the question of the mechanism of carious attack. In other words nobody really knows how people get cavities in their teeth. The mineralogy of the inorganic component of human dental enamel, hydroxyapatite, is still being debated. The question of why fluoride ions can inhibit carious attack remains a mystery, even though fluoride treatments have been used as a caries inhibiting device for many years. The structure of the enamel prisms, the biological structural units of dental enamel, is still being unraveled.

It is a curious fact that of the many researchers investigating carious attack, only a small proportion of them are materials scientists. The author finds this interesting, since although enamel is a living tissue, 98% of it is inorganic and made of various hydrated calcium phosphates, the properties of which are still

mostly unknown. The investigation of these properties should be the domain of people with materials backgrounds -- people who have the knowledge and training to deal with minerals.

In this work the results of several experiments dealing with the simulation of carious attack are presented. It is hoped that these results will add to the understanding of the mechanism of carious attack.

II. LITERATURE REVIEW

2.1 Anatomy of Tooth and Enamel

2.1.1 General Distribution of the Dental Tissues

Teeth--Human teeth are built up of three hard tissues, the enamel, dentin, and cementum (refer to Figure 1). Within the dentin is the pulp cavity, which contains the pulp tissue, a soft tissue.

A tooth consists of a crown and a root. The crown is that portion of the tooth that is covered by the enamel, and the root that which is covered by cementum. The term "neck" is sometimes applied to the area of slight constriction at the border between crown and root, or to the root near the cemento-enamel junction.

Many dentists and dental researchers use a terminology that describes the lines and planes which bound the different dental structures. The plane along which the dentin and enamel meet is the dentino-enamel or amelo-dentinal junction. The dividing line between enamel and cementum is the cemento-enamel or amelo-cemental junction. The plane of union of the dentin and cementum is the dentino-cemental or cemento-dentinal junction.

Figure 1. Longitudinal ground section through an upper central incisor of a young adult.

E, enamel; D, dentin; PC, primary cementum, SC, secondary cementum; DEJ, dentino-enamel junction; S, scalloped dentino-enamel junction; DCJ, dentino-cemental junction; CEJ, cemento-enamel junction; P, pulp chamber; RC, root canal; AF, apical foramen; GE, gnarled enamel; SR, Stripes of Retzius; IG, interglobular spaces; TL, Tomes' granular layer. 7X mag. After Kronfeld (11).

The shape of the crown is different in different types of teeth. In cuspids the enamel ends in one cusp. In bicuspid there are usually two cusps divided by a fissure. Molars have from three to five cusps which are divided by grooves and fissures. Fissures are narrow clefts between the cusps at the bottom of which the enamel is much thinner than on the rest of the occlusal surface.

The root is divided into the cervical portion and the apex. At the apex is the opening through which the nerves and the blood vessels of the pulp pass. This opening is known as the apical foramen.

The size of the pulp chamber and root canal alters as the individual grows older. During youth the pulp chamber is large, and the lumen of the root canal is wide. With advancing age the pulp horns are truncated, and the root canals become much smaller.

2.1.2 Enamel

The enamel is the hardest tissue of the body. It is very highly calcified, containing from 97 to 98% inorganic salts and only 2 to 3% water and organic material.

The enamel is composed of long, thin, calcified, fiber-like elements, the enamel prisms (sometimes called enamel rods). Figure 2 shows an end view of the modern

Figure 2. End view of the popular keyhole model of enamel prisms. The keyholes are said to be close packed, and each keyhole consists of a head and tail region. After Meckel, Griebstein, and Neal (9).

model of enamel prisms. The size of the average prism varies from 0.5-1.5mm in length and averages around 15 μ m diameter in the head region.

It is well agreed now that the enamel prisms radiate from the dentino-enamel junction toward the outer surface of the enamel, and that some of them do not extend all the way to it. This has given rise to ideas about a prismless layer, which is said to be approximately 25 μ m thick.

It is also agreed that the prisms have a keyhole-shaped cross section, and the component parts of the keyhole are referred to as the head and the tail. Throughout the enamel, most of the prisms are arranged so that their heads are directed towards the occlusal or incisal edge of the crown and their tails toward the cemento-enamel junction.

Each prism is composed of billions of small crystallites of hydroxyapatite. The crystallites are long and lathlike and cross sections have the form of elongated, irregular hexagons. The average size of the hydroxyapatite crystallites is now thought to be 321 ± 35 A long and 336 ± 17 A wide when they are measured using dark field electron microscopy, or $8,966 \pm 571$ A long and 378 ± 27 A wide when bright field microscopy is used (33). The course of these crystals in the central regions of

the heads is basically axial along the prisms. Toward the peripheries of the heads, the crystals diverge somewhat from the parallel course. In the tail regions they are distributed at a 35° to 40° angle to the axis of the prism (31).

All enamel is characterized by a number of consistent variations in structure and composition. These variations, the stripes of Retzius, Schreger's bands, and others, while they may be important in the overall picture of carious attack, will not be discussed here since only the role of prism solubility in enamel destruction was considered during this investigation.

2.1.3 Caries

Caries of the enamel is initiated through the action of a microbial plaque located either in a developmental groove or fissure or on a susceptible area of a smooth surface. It is thought that the acidic waste of the microorganisms present in the plaque somehow leads to the hypermineralization, demineralization, and eventual disintegration of the enamel. The mechanism of attack is not known, and many theories have been devised to account for the many observations on carious lesions that have been made (5, 6, 10, 12, 14, 16, 20, 21, 22, 25, 28, 29,

32, 34, 40, 41, 42, 43, 47, 64). Several of these will be discussed later.

2.2 Artificial Dissolution of Enamel

Many researchers have been concerned with the experimental dissolution of enamel. It has been hoped that observations of the changes occurring in the enamel during experimental dissolution will facilitate interpretation of the structural changes that have been observed in carious enamel. Inasmuch as some of these studies have provided insight into the fundamental properties of enamel, they can be a useful background for a subsequent discussion of the sequence of damage in caries.

Much of the early work in enamel dissolution was confined to the preparation of electron microscopy specimens (13,14). However, with the advent of scanning electron microscopy, the effects of artificial acid etching could be seen without replication or thin sectioning, and many papers have since been published on the subject of enamel dissolution (6, 9, 10, 17, 20, 21, 23, 24, 32, 34, 40, 43, 45, 46).

Although much has been argued and published about enamel dissolution, there are only two things that everyone agrees upon. The first is if a section of enamel

is polished to remove the prismless layer and the polished surface is approximately at right angles to the prism axes, then the polished surface after acid attack will look like Figure 3. The centers of the enamel prisms will be removed and a honeycombed structure derived from prism boundaries will remain. The second is that similar dissolution of enamel with the disodium salt of EDTA (ethylene diamine tetra-acetic acid) will produce a surface like that seen in Figure 4. The work of Tyler, Poole, and Johnson has been particularly concerned with this effect (21, 22, 25). This surface (Figure 4) has been attacked in a manner exactly opposite to the way the surface of Figure 3 was attacked. With EDTA, the prism boundaries have been removed, leaving the centers behind.

The disagreement among authors comes when theories are proposed to account for the two types of preferential dissolution. Poole and Johnson (21) concluded that the orientation of the enamel crystallites relative to the direction of attack, together with compositional variations within the enamel structure, contribute to the pattern of etching. No specific compositional variations were proposed, however. Sharpe (10) argued that the rates of dissolution of both acid and EDTA were governed by crystallite orientation and perhaps the existence of organic matter to be found in the boundary regions of

Figure 3. Enamel surface etched by immersion in
1/70N HCl adjusted to pH 2.3 for 10 hrs.
Au coated, 3,000X, SEM.

Figure 4. Enamel surface etched by immersion in 0.25M sodium versenate (disodium salt of EDTA) adjusted to pH 7.0. Au coated, 3,000X, SEM.

the prisms. No proof for the existence of such organic matter was given. Johnson, Poole, and Tyler (25) talk about the permeability of prism junctions being in part responsible for the preferential etching. Nichol, Judd, and Ansell (32) proposed a fantastic two stage model to account for preferential etching by hypothesizing that although attack starts in the prism boundaries in all cases, it is the direction of the spread of the attack that accounts for the differences in chemical etching.

The best argument for the difference in etching patterns that result from acid or EDTA attack so far has been that the EDTA calcium complex can only move in the less dense prism boundaries, and therefore the boundaries are preferentially attacked. It should be noted, however, that boundary attack occurs only when the pH of the EDTA solution has been adjusted to a high value, say pH 7 or so. EDTA attacks enamel like any other acid at its normal pH (pH 4).

Many maintain that the etching differences can be attributed to chemical differences within the mineral phase of the enamel, but no one has cited any evidence to back such claims. Perhaps this is because the regions involved are so small (15 μ m in diameter) that quantitative chemical analysis on rods and rod boundaries has been

impossible to carry out. It could also be because the mineralogy of the inorganic phase of the enamel has been a very complicated business. A brief description of the mineralogy of the inorganic phase of enamel will be included here.

2.3 The Mineralogy of the Enamel Crystallites

The principle inorganic phase of the dental enamel crystallites is the mineral hydroxyapatite, of the following proposed non-stoichiometric formulas (4):

1. $\text{Ca}_{10-x} \text{H}_{2x} (\text{PO}_4)_6 (\text{OH})_2$, $0 \leq x \leq 1$
2. $\text{Ca}_{10-x} \text{H}_x (\text{PO}_4)_6 (\text{OH})_{2-x}$, $0 \leq x \leq 2$
3. $\text{Ca}_{10-x} \text{H}_x (\text{PO}_4)_6 (\text{OH})_{2-x}$, $0 \leq x \leq 1$
4. $\text{Ca}_{10-x} \text{H}_x (\text{PO}_4)_6 (\text{OH})_{2-x} + (\text{H}_2\text{O})_x$, $0 \leq x \leq 1$
5. $\text{Ca}_{10-x-y} (\text{HPO}_4)_x (\text{PO}_4)_{6-x} (\text{OH})_{2-x-2y}$,
 $0 \leq x \leq 2$ and $y \leq 1 - \frac{x}{2}$
6. $\text{Ca}_{9-x} (\text{HPO}_4)_{1-2x} (\text{PO}_4)_{5-2x} (\text{OH})$ for Ca/P

mole ratio of 1.4-1.5

There are also additional phases thought to be present.

These include amorphous calcium phosphate and amorphous

calcium carbonate, and intercrystalline mixtures of octacalcium phosphate and hydroxyapatite. All of these mineral phases lend themselves to a wide range of non-stoichiometries and atomic substitutions, holes, and interstitials. Figure 5 illustrates some of the trace elements that have been found in healthy human dental enamel.

Stoichiometric hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is thought to belong to the hexagonal crystal system. A view of the hydroxyapatite unit cell is shown in Figure 6. An easy way to think of the rather complicated structure shown in Figure 6 is to imagine that the PO_4^{-3} tetrahedra that make up the basic building blocks of the system approximate spheres instead of tetrahedrons. These PO_4^{-3} spheres can then be packed in a standard hexagonal lattice. Looking perpendicular to the (0001) plane, one will then notice that a number of space channels pass through the sphere structure parallel to the hexagonal axis. 2/3 of these channels are occupied by Ca^{+2} (columnar calciums) ions which make up 2/5 of the total amount of calcium in the structure. The other 1/3 of the channels are filled by columns of OH^- ions packed head to tail OH OH OH OH, etc. The remaining 2/3 of Ca^{+2} ions partly line the channels occupied by the OH^- ions

<u>Element</u>	<u>Number of Samples</u>	<u>Mean Concentration $\pm$ S.D.</u>
Mg	8	0.28% $\pm$ 0.01
Na	8	0.7% $\pm$ 0.01
Cl	8	0.32% $\pm$ 0.01
Al	8	86.13ppm $\pm$ 4.54
Ca	8	37.03% $\pm$ 0.56
Cr	6	1.02 ppm $\pm$ 0.51
Ba	7	125.11 ppm $\pm$ 23.68
Sb	9	0.96 ppm $\pm$ 0.69
Ag	9	0.56 ppm $\pm$ 0.29
Zn	8	263.42 ppm $\pm$ 14.80
Co	10	0.13 ppm $\pm$ 0.13
Fe	9	118.27 ppm $\pm$ 71.65
Sr	7	111.19 ppm $\pm$ 9.86
Au	7	0.11 ppm $\pm$ 0.07
Br	8	33.79 ppm $\pm$ 5.71
Mn	4	0.59 ppm $\pm$ 0.04

Figure 5. The concentration of 16 elements in normal human enamel. From Retief, Cleaton-Jones, Turkstra, Delfef (1).

Figure 6. Hydroxyapatite structure projected onto the x,y plane. The numbers written in the symbols are the z parameters. Two of the O's in each PO_4^{3-} tetrahedron are superimposed and partially overlap the P in this projection. In some of the tetrahedra the two remaining P-O bonds are indicated by parallel lines, $=$. After Young and Elliott (53).

(see Figure 7). It should be noted that in Figure 7 the OH^- ions are not arranged in a head to tail fashion, due to OH^- substitution by F^- ions.

Pure stoichiometric hydroxyapatite has the monoclinic space group $\text{P2}_1/\text{b}$ rather than the hexagonal space group $\text{P6}_3/\text{m}$ usually found in apatites. This is thought to result from the ordered head to tail arrangement of the OH^- ions in pure hydroxyapatite (4, 7, 68). This is a recent discovery, since pure hydroxyapatite crystals have not been available for study until lately (4, 54, 61, 62). Apatites studied previously were never pure, and usually were combinations of fluorapatite, hydroxy-fluorapatite, chlorapatite, and others. The significance of Figure 7 is that it shows what the replacement of OH^- ions by F^- ions in the structure does to the ordered head to tail arrangement. If the head to tail arrangement is disrupted, the monoclinic space group detected for pure hydroxyapatite disappears, and the system appears to be completely hexagonal. Unfortunately, the hydroxyapatite crystallites found in dental enamel are not pure or stoichiometric or always hexagonal, or all hydroxyapatite, or large enough for some types of x-ray studies.

One of the interesting things about apatite is the large family of closely associated calcium phosphates from

Figure 7. Those calciums that line OH^- channels in the apatite structure are thought to form triangles about the OH^- ions. Here, the normal head to tail arrangement of OH^- ions is interrupted by the presence of an F^- substitution.

which it comes. When precipitating calcium phosphates from solution, for example, one simply has to change the Ca/P ratio of the reagents involved, and the result can be any number of calcium phosphates created, hydroxyapatite included. Figure 8 illustrates this. Also, a wide range of the Ca/P ratio can be tolerated within the hydroxyapatite structure itself. A relation exists between the Ca/P ratio and the lattice parameters of the unit cell of hydroxyapatite, as shown in Figure 9. This relation was used in the course of this study.

Figure 8. Forms of calcium phosphates. The weight ratio of Ca/P is 1.29 times the molar ratio. After Trautz (51).

Figure 9. Length of apatite axes of calcium phosphate precipitates versus Ca/P ratio. The values for the a axes are plotted in the upper half, those for the C axes in the lower half of the graph. After Trautz (51).

III. THE WORK DONE IN THIS INVESTIGATION

3.1 Ca/P Ratio vs. Interplanar d-spacings

Since one of the crucial experiments in this investigation was the x-ray analysis of enamel residues and a comparison of d-spacings to see if Ca/P ratios were different in different residues, an initial attempt was made to see if Ca/P ratio differences in apatite samples could be detected with an ordinary DeBye-Scherrer camera.

3.1.1 Procedure

Three precipitated apatites were prepared using the method of Eanes, Gillessen, and Posner (54). Since their paper stated that after initial mixing of the reactants, no detectable phosphorus could be found, while calcium was detected in the reacting medium until it was finally situated in the phosphate matrix (about a week of stirring), a simple variation was tried. The Ca/P ratios of the reactants were different in all three cases: 1.71, 1.60, and 1.50. It was thought that stoichiometric apatite (Ca/P = 1.67 mole ratio) would result from the solution in which the Ca/P ratio of the reactants was 1.71. Slightly calcium deficient apatite would result from the

solution in which the Ca/P ratio of the reactants was 1.60, and grossly calcium deficient apatite would result from the solution in which the Ca/P ratio of the reactants was 1.50.

The three solutions were stirred for two weeks at room temperature. Then the precipitates were vacuum filtered and washed three times with ammoniated wash water at pH 10.5. The precipitates were then air dried and powder patterns of each were made with a Debye-Scherrer camera. The radiation used was $\text{Ca}_{K\alpha}$, with a Ni filter. Film exposures were 10 hours for each sample. Line broadening and calcium fluorescence were problems that made line measurements difficult, but eventually the d-spacings for the (002), (222), (213), and (004) or (411) lines were calculated from five measurements made for each line for each sample. The five values were averaged, and the results were shown in Figure 10.

<u>Ca/P ratio of reactants</u>	<u>Interplanar d-spacings</u>			
	<u>002</u>	<u>222</u>	<u>213</u>	<u>004,411</u>
1.71	3.390	1.943	1.831	1.710
1.60	3.410	1.938	1.835	1.713
1.50	3.411	1.938	1.835	1.714

Figure 10. Values of interplanar d-spacings for four lines measured for apatites precipitated from solution. The Ca/P ratios shown are those of the reactants from which the apatites were formed.

3.1.2 Results

All three patterns were fuzzy because of the small particle size of each apatite precipitate and calcium fluorescent radiation which caused film blackening. However, the patterns were definitely those of apatites and there was a measurable, though small, relation between d-spacings and Ca/P ratios. It should be noted that the trend for the (002) and (004) lines is the reverse of that shown in Figure 9, if one uses the relation:

$$d = \left(\frac{1}{\frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{1}{c^2}} \right)^{\frac{1}{2}}$$

It is hard to see what direction the trend for the C-axis is between 1.7 and 1.5 in Figure 9, however.

3.1.3 Conclusions

If one measures d-spacings from powder patterns taken from several apatites, a relation will be found between d-spacings and the Ca/P ratios of the apatites. Generally, the more calcium deficient the apatite, the larger the interplaner d-spacings will be, at least for the 002, 222, 213, and 004 or 411 lines.

3.2 The X-ray Analysis of Enamel Surface Residue From HCl and EDTA Etched Enamel

3.2.1 Procedure

Of a jar of extracted permanent teeth obtained from a local dentist, William K. Cooper, D.D.S. (all teeth stored in absolute ethyl alcohol), a large upper incisor was selected and split longitudinally so that two relatively flat surfaces of enamel could be prepared. Each enamel chip was mounted on the end of a 5/8" aluminum rod 6" in length with Lakeside 70, a thermoplastic resin. Each enamel surface was then ground flat with 600 mesh aluminum oxide paper and polished with levigated alumina on a felt lap until the polished surfaces were microscopically free of scratches or pits.

The two chips were then immersed for 10 hours in an etching agent. One chip was immersed in $\frac{1}{70}$ N HCl adjusted to pH 2.3 with 0.25M NaOH solution. The other was immersed in 0.25M sodium versenate solution (sodium versenate is the disodium salt of EDTA) adjusted to pH 7.0 with 0.25M NaOH solution. After 10 hours, the two chips were removed and washed three times with deionized water, and then allowed to dry in a desiccator overnight.

The surface of each enamel chip was then carefully scraped with a razor blade and the powder that was removed

during the scraping was collected on a glass slide, one slide for each chip. The two powder residues were then used to make powder patterns in the DeBye-Scherrer camera. $\text{Cu}_{K\alpha}$ radiation with a Ni filter was used, and the exposure time for both samples was 10 hours. Four lines were measured five times each for each sample. The five measurements for each line were averaged.

3.2.2 Results

The results were as shown in Figure 11.

<u>Residue</u>	<u>Interplanar d-spacings</u>			
	<u>002</u>	<u>222</u>	<u>213</u>	<u>004,411</u>
HCl etched	3.430	1.949	1.843	1.719
EDTA etched	3.433	1.949	1.843	1.721

Figure 11. Interplanar d-spacings for four lines from the surface residues of two pieces of enamel taken from the same tooth and etched for 10 hours in two different mediums.

3.2.3 Conclusions

There appears to be no significant difference in the interplanar d-spacings of the two residues. Both are definitely apatetic in nature. There is no way to tell

whether they are nonstoichiometric and in what way they are nonstoichiometric with x-ray powder diffraction methods.

3.3 The Etching of Enamel With Argon Ions

3.3.1 Procedure

A $\frac{1}{2}$ " length of 1" diameter aluminum stock was cut and faced. A $\frac{1}{4}$ " diameter hole was drilled down the axis of the resulting disc, and four other $\frac{1}{4}$ " holes were drilled through the disc, surrounding the center hole. In two of the surrounding holes and in the center hole, a chip of enamel was embedded in epoxy cement. In the other two holes, small single crystals of fluor and hydroxy-fluorapatite were embedded in epoxy cement. After the cement was allowed to cure, the sample face of the disc was ground and polished with levigated alumina, until no microscopic scratches or pits were detected in the sample faces.

The disc was then mounted on a tantalum mask (the mask was actually half of the standard IMMI III specimen holder) and placed in an IMMI III (Ion Micro-Milling Instrument, model III, Commonwealth Scientific Corp.) for ion etching. The mask made it possible to limit the sample etching to a 2 mm circle on the enamel surface

in the center hole. The sample was bombarded with argon ions accelerated across a potential difference of 5KV.

The incident angle of the ion beam was 25 degrees, and the beam current was 25 μ A. The sample was constantly rotated about the axis of the disc during the milling operation.

The milling operation lasted 1 hour.

The sample disc was then carbon coated and the etched region of the enamel was observed with a scanning electron microscope (JEOL JSM-U3). The backscattered electron mode was used for sample observation with the SEM.

3.3.2 Results

The photograph in Figure 12 shows the argon ion etched surface of the enamel sample. There was not enough surface relief to use secondary electrons for the picture, so the backscattered electron mode had to be used. Even with the focusing help of a wave form monitor hooked to the scanning electron microscope, the picture still did not appear to be in focus after the exposures were made. So the best of 15 exposures was chosen, but it still appears to be poor quality. But it does show the centers of the enamel prism heads were removed at a rate greater than the prism boundaries, leaving a relief map of the prisms in cross-section.

Figure 12. Enamel surface etched with 5KV argon ions.
Carbon coated, 3,000X, SEM. Backscatter
mode.

3.3.3 Conclusions

If a sample of human dental enamel is prepared such that a flat polished surface is cut perpendicularly to the prism axes and bombarded with Ar^+ ions, the centers of the prism heads will be etched away at a rate greater than that of the prism boundaries. Structural differences within the enamel prisms are probably responsible for this effect.

3.4 The Ca/P Ratio of Prism Centers and Boundaries

3.4.1 Procedure

The argon ion etched enamel was placed in a JEOL JSM-50A electron probe for analysis. Under electron bombardment at 20KV and around 10^{-8} A absorbed current, the prism map could be seen when the backscatter mode was used. The combination of electron microscope and probe that makes up the JEOL JSM-50A probe made it extremely easy to place the electron beam on various parts of the prisms. One merely had to place the beam in the appropriate area on the picture screen, using the spot mode, to analyze whatever parts of the prisms (shown at 3,000X) one wished. Since the prisms had been etched with argon ions, no chemical change in the enamel

could have occurred during etching. The Ca/P ratios found were thus the actual Ca/P ratios that existed in the boundaries and centers of the enamel prisms. The disadvantage of this particular probe was that it had no device with which to monitor the actual beam current, so only count ratios could be compared, and no absolute Ca/P ratios could be calculated using the apatite single crystals that accompanied the enamel in the disc.

The relative intensities of the $P_{K\alpha_1}$ and $Ca_{K\alpha_1}$ peaks (minus background) were measured using 10 40 second counts for each peak for the fluorapatite standard, and the center and edge of a prism in the etched enamel. The 10 counts were then averaged for each and the Ca/P count ratio calculated for each. Although the beam current was not monitored, a crude attempt at calculating the Ca/P wt. ratios of the edge and center was made using the following formulas and an assumption that the fluorapatite standard was stoichiometric (Ca/P wt. ratio = 2.154):

$$\left(\frac{Ca}{P}\right)_{\text{center}} = \left(\frac{Ca}{P}\right)_{\text{std.}} \left(\frac{P \text{ counts std.}}{P \text{ counts center}}\right) \left(\frac{Ca \text{ counts center}}{Ca \text{ counts std.}}\right)$$

$$\left(\frac{Ca}{P}\right)_{\text{edge}} = \left(\frac{Ca}{P}\right)_{\text{std.}} \left(\frac{P \text{ counts std.}}{P \text{ counts edge}}\right) \left(\frac{Ca \text{ counts edge}}{Ca \text{ counts std.}}\right)$$

3.4.2 Results

The Ca/P wt. ratio for the center of the prism was 2.40 and the Ca/P wt. ratio for the edge of the prism was 2.58. Both these values are high relative to the Ca/P wt. ratio of the standard, especially in light of a recent publication by Wirsing, Judd, and Ansell (63). They claim that the Ca/P wt. ratio of the edge of the average prism is higher than the Ca/P wt. ratio of the center, but that the absolute ratio at the edge is stoichiometric and that of the center is slightly calcium deficient.

3.4.3 Discussion and Conclusions

The difference between the Ca/P ratio in the center and boundary region in one enamel prism was 7.04%. The difference in ratios for 26 enamel prisms, as measured by Judd, Ansell and Wirsing was 4.2%. One is pushing the limits of any machine to find a difference of 4.2%, and a difference of 7% did not set any noticeable trends in the numbers this author was getting with 40 second counts. It was only after an average of 10 counts was calculated that a difference in Ca/P counts between the center and edge of the prism could be seen.

The published value of the average Ca/P ratio of the centers of the prisms determined by Judd, Ansell,

and Wirsing was $2.07 \pm$ a 95% confidence limit of 0.06. That for the boundary regions was 2.16 ± 0.07 . According to J. B. Woodhouse, the resident microprobe analyst at the Materials Research Laboratory of the University of Illinois, such confidence limits should make it possible to see a trend forming at counts of around 10,000. This author did not see any numerical clue to show that a Ca/P ratio difference of even 7.04% between the center and boundary region of the prism was imminent at counts as high as 90,000 for phosphorous and 60,000 for calcium, not to mention counts around 10,000. This author simply did not get counts that agreed with the confidence limits set by Judd, Ansell, and Wirsing.

However, it should be emphasized that although the difference in Ca/P ratio between the centers and boundary regions of the enamel prisms is small and hard to measure, it definitely exists. Figure 13 illustrates this point. It shows the Ca/P count ratios actually found for ten measurements taken during the investigation. During the measuring, it is suspected that the beam current decreased slowly with time. The actual counts measured decreased as the beam current decreased, but it can be seen that the Ca/P count ratios were relatively unaffected. And more importantly, the % difference observed between

Figure 13. Ca/P count ratios for the center and boundary region of a normal enamel prism, taken from a healthy adult upper incisor, history unknown. Each measurement was a 40 second count of the $\text{CaK}_{\alpha 1}$ and $\text{PK}_{\alpha 1}$ peaks. It is suspected that during the investigation (measurement number increased with time) the beam current steadily but slowly decreased, lowering the $\text{CaK}_{\alpha 1}$ peak count for the center, for example, from 55,131 at the beginning of the experiment to 54,654 at the end. However, the Ca/P count ratios for the center and boundary region did not change in any manner that seemed to be dependent on time and thus the beam current. Even more important is the fact that the fluctuations in the % difference in the ratios appear not to be beam dependent, and cluster closely about the average difference of 7.04%, with a standard deviation for the ten % differences of 0.81%.

the ratios of the center and boundary region appeared not to depend on the beam current, staying tightly clustered around an average of 7.04%, with a standard deviation of 0.81%.

Many measurements on many prisms must be taken in order to determine an average Ca/P ratio for the center and boundary region of the average enamel prism. Also, the author believes the absolute concentrations of calcium and phosphorous must be determined for the center and boundary region of the average enamel prism before any speculation on the role of nonstoichiometry in enamel solubility can be made.

3.5 HCl Etch of Enamel in Ultrasonic Cleaner

3.5.1 Procedure

Two polished enamel surfaces were etched as before for the powder diffraction experiment in $\frac{1}{70}$ N HCl solution adjusted to pH 2.3 for 10 hours. This time, though, the etching was carried out in a plastic beaker in an ultrasonic cleaner. The two samples were then rinsed, dried and mounted on a 1" diameter aluminum disc and gold coated for observation with a scanning electron microscope.

Figure 14. Enamel surface etched with 1/70N HCl at pH 2.3 for 10 hrs. in an ultrasonic cleaner. Au coated, 3,000X, SEM.

3.5.2 Results

Figure 14 shows the appearance of the etched surface of the enamel at 3,000X. It appears the etching rate of the prism boundaries has greatly increased, as compared to the boundaries of Figure 3.

3.5.3 Conclusions

It appears that with constant agitation during etching, the etching rate with acid of the prism boundaries approaches that of the centers. This effect indicates two things. First, it appears that during acid etching under "normal" conditions (i.e., no agitation of the solution is involved), something is preventing the dissolution of the prism boundaries which would otherwise occur. Perhaps, after initial attack at the boundaries, a reaction product protects them somehow from further dissolution. Second, the claim that crystallite orientation governs the etching patterns of enamel prisms cannot be accepted.

3.6 Ion Probe Analysis of HCl Etched Enamel Residue

One way of chemically analyzing a material results from the bombardment of the material with ions (ion micro-probe). The secondary ions that are ejected from the sample surface during bombardment are characteristic of the sample composition. These secondary ions can be fed into a mass

spectrometer for a plot of mass-frequency relationships. The ideal ion microprobe can focus the bombarding ions into a submicrometer sized beam to bombard a very small region of the sample, thus making it possible to chemically analyze very small areas of the sample. Also, there are two advantages such an ion probe has over the standard electron probe. First, the ion probe can scan at once for every element on the periodic chart, while the electron probe is generally limited to analysis of elements with atomic numbers greater than that of boron, and the number of elements that can be looked for simultaneously is limited to the number of spectrometers on the electron probe. Second, the ion beam gradually drills into the material it is bombarding, enabling depth profiles of the material to be made, in addition to the standard x-y profiles that can be made with the electron probe. Theoretically, the ion probe can be used to make a quantitative 3-dimensional analysis of any material.

An experimental ion probe is currently being worked on at the Materials Research Laboratory of the University of Illinois--Urbana. Problems with the ion beam have kept the probe researchers from doing much of what they had originally intended to do, but it was possible to try once to see what the results of ion bombardment of etched enamel would result in.

A piece of normal human enamel was etched with HCl as in Figure 3. After gold plating, it was mounted in the ion probe and bombarded with a very wide beam of negatively charged oxygen ions. The enamel residue secondary ions were fed into a mass spectrometer tracked to the probe.

A look at the mass peaks on the mass-frequency chart that resulted from the experiment indicated mostly calcium and phosphorous and oxygen were present, but there were several peaks that simply could not be attributed to anything. Much work should be done on etched, normal, and carious enamel someday with an ion probe that has been perfected. This author believes the technique of ion probe analysis may someday contribute much to the understanding of the properties of enamel, especially in the piecing together of a 3-dimensional map of all the elemental concentrations in the enamel prisms.

IV. GENERAL DISCUSSION AND CONCLUSIONS

It was thought during the course of this investigation that the results of the five previous experiments could be tied together to form a general picture of how preferential enamel dissolution comes about, or, more precisely, how it can be explained in the light of existing knowledge about the enamel prisms.

As the agitation experiment shows, the honeycomb etch pattern so familiar to those who have done acid etching experiments is probably not the result of crystallite orientation with respect to the axes of the enamel prisms. Instead, it is thought by this author to be the result of some sort of reaction product diffusion barrier built up in the prism boundaries after initial boundary attack by the acid.

What, then, is responsible for this diffusion barrier? It must certainly be due to a chemical difference between the boundaries and centers of the prisms, but it is not clear just what it would be. The Ca/P ratio differences certainly cannot account for it alone, if lattice vacancies are all that result from the variable Ca/P ratios. Lattice substitution for Ca^{+2} or PO_4^{-3} ions is commonplace, but as Figure 5 shows, there are many elements to consider

before one can attribute the effects of preferential etching in dental enamel to one or more of them.

The experiment with the argon ion etching of enamel lends support to the theory of a boundary diffusion barrier. In experiments with polycrystalline materials, it has been found (65, 66) that ionic bombardment usually results in grain etching in a manner exactly opposite that of a chemical etch. During chemical attack, that crystal face which presents the least number of atoms packed per square unit to the etchant will be attacked at the greatest rate. That face which presents the most number of atoms packed per square unit will be attacked at the slowest rate. In ionic bombardment experiments the reverse has been true. The crystal face which presents the least number of atoms packed per square unit to the bombarding beam will be attacked at the slowest rate. The crystal face which contains the most number of atoms packed per square unit will be attacked at the greatest rate.

Of the crystallites present in the enamel prism in cross section, those presenting the (0001) face to the ion beam should be etched at the greatest rate. As it happens, these are the crystallites that are present in the prism head, and the heads were the regions attacked

at the greatest rate during ion bombardment. The other regions (tails and boundaries) contain crystallites that generally do not present the (0001) face to the bombarding beam, and should not be attacked at quite the rate of the head region. This is also what happened.

However, if acids generally are supposed to attack polycrystalline materials in a manner exactly opposite that of bombarding ions, why doesn't HCl attack leave prism centers and take away the boundaries? And why is the only etchant that actually acts in a manner reverse that of the ion beam EDTA at a pH sufficiently high to be called neutral? This author believes the answer may lie in a boundary diffusion barrier.

If a barrier exists, though, its existence may be hard to prove. The x-ray analysis of the enamel surface residues after etching indicates that the boundary residue left during HCl attack definitely shows nothing but apatite in the residue, and this residue appears to be identical to that left after EDTA etching. Such a barrier could be helpful in inhibiting acid attack, however, if it were found and its inhibiting mechanism analyzed. Perhaps then chemical methods could be invented to make all enamel produce such a shield during acid attack, and thus inhibit carious attack.

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