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SOME OBSERVATIONS ON THE FINE STRUCTURE OF FLUOROSSED DENTAL ENAMEL

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INTRODUCTION

Relatively few investigations have been published describing the structure of enamel showing dental fluorosis. *Black* and *McKay* (1916) and *Williams* (1922) used lightmicroscopic methods and found that the changes produced in mottled enamel are usually limited to the outer third of the tissue. *Darling* and *Brooks* (1959) and *Newbrun* and *Brudevold* (1960) using microradiography showed that the surface layer of fluorosed enamel was well mineralized and that the subsurface area was hypomineralized. *Gustafson* (1961) found considerable variations in the degree of mineralization, prominent Retzius lines and that the prisms followed an irregular course in fluorosed enamel.

Darling and *Brooks* (1959) also examined decalcified sections and found an »apparent thickening of the organic matrix» in poorly mineralized areas and absence of organic matrix in areas of poorest mineralization.

The purpose of the present work was to study the fine structure of the organic matrix of enamel showing dental fluorosis. Since the authors were interested in the developmental effects of high fluoride intake, unerupted teeth were examined.

MATERIAL AND METHODS

The material consisted of four completely unerupted teeth (third molars with fully developed roots) which were removed surgically from a 26 year old woman with a clinically diagnosed dental fluorosis. The woman had always been living in Hettinger, North Dakota, where the fluoride content of the drinking water ranged from 4 to 4.8 ppm (*Vennes et al.*, 1962). The mottled areas in the enamel involved approximately 75 per cent of the buccal and lingual tooth surfaces and the degree of dental fluorosis was evaluated as *mild*, according to the system proposed by *Dean* (1938). Approximately 300 $m\mu$ thick sections from the mottled enamel were cut by means of »Gilling's Hamco Thin Sectioning Machine» under waterspray. When present small non-mottled patches were trimmed away. From these slabs ground sections varying in thickness between 15 and 30 microns were prepared according to the method described by *Silness* (1967).

Contact microradiographs were taken of all the ground sections with a Philips X-ray diffraction unit, type PW 1009, employing a copper target and nickel filter, at a target to film distance of approximately 26 cm. The machine was operated at 20 kV and 20 mA. To obtain firm contact between the section and the emulsion of the plate the evacuated cassette system proposed by *Scott, Nylen* and *Pugh* (1962) was used. Kodak Spectroscopic Plates 649-0 were used and developed in Kodak D-11 under constant agitation.

When adequate microradiographs had been obtained, the ground sections were decalcified according to a modified *Sundström* technique (*Sundström*, 1966, *Sundström & Zelandar*, 1968 a). The decalcifying fluid was prepared as an aqueous solution (0.5 per cent) of Chromic Sulphate Basic (approximately $Cr_2(SO_4)_3 \cdot 6H_2O$). The British Drug Houses Ltd., England. By adding alkali (NaOH) the chromium solution was brought to a pH slightly above 3 (3.1—3.3). The hard tissue sections were placed on the bottom of Petri dishes which were filled with the decalcifying fluid. The solution was changed daily for the time necessary to complete decalcification, 7—9 days. Assessment of the decalcification was made as explained by *Sundström* (1966). The demineralized, dehydrated matrices were placed in aluminium foil containers on the top of a polymerized layer of Araldite and immediately covered with Araldite syrup. After polymerization small, matrix containing resin blocks were placed on the bottom of gelatin capsules, which were subsequently filled with Araldite syrup. Within the capsules the specimens were oriented so as to permit sectioning in known relation to prism direction and to different tissue areas. Sectioning was done with glass knives on an LKB Ultratome. The sections were picked up on carbon reinforced, Formvar coated copper grids and examined in the Siemens Elmiskop I. The microscope was operated at 80 kV.

The average size values of the tissue components were derived by making the measurements at various photographic enlargements of plates with various magnifications.

RESULTS

Microradiographically all ground sections of mottled enamel showed three areas of differing densities. The surface area (area 1) was radiopaque, the subsurface area (area 2) showed radiolucency and area 3 on the dentinal side of the subsurface area was radiopaque (Fig. 1). The width of the radiopaque surface layer varied, and the subsurface radiolucency was more or less pronounced. A pattern of linear variations in radiodensity was confined to area 2 in which the alternating radiolucent and radiopaque lines were parallel to the prism direction. The radiolucent lines were narrower than the more dense ones, the width of which corresponded to that of a single prism.

The structure of the decalcified tissue as seen in the electron microscope, was of the same general character in all the specimens of mottled enamel.

In longitudinal sections from the surface area (area 1) the prism boundary could be recognized by the difference in the direction of the structural ele-

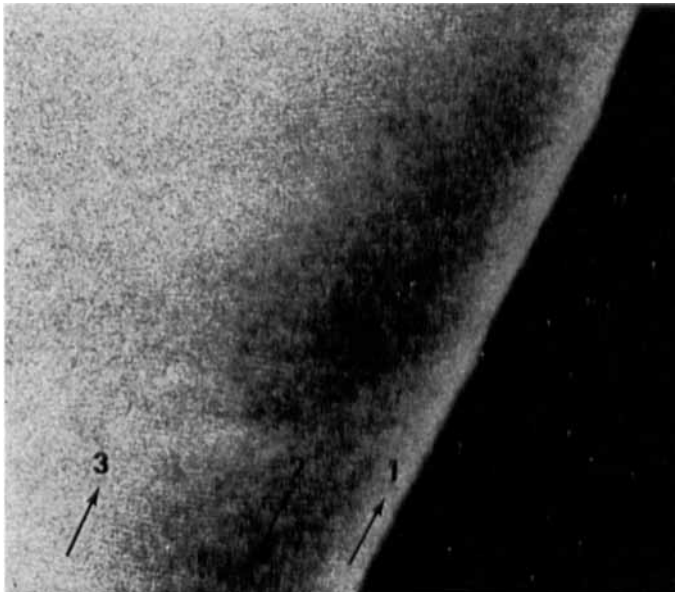


Fig. 1. Microradiograph of fluorosed enamel showing radiopaque surface layer (area 1), radiolucent subsurface layer (area 2), and radiopaque inner layer (area 3) (arrows). $\times 140$.

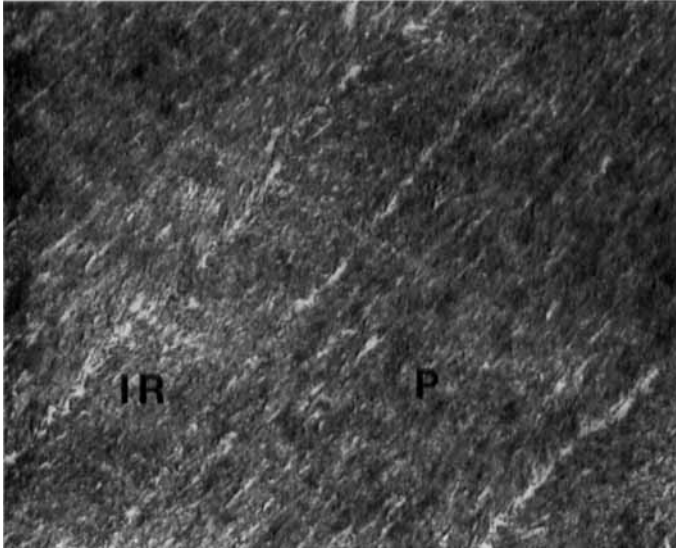


Fig. 2. Decalcified section showing prisms (P) and interprismatic regions (IR). Within the prism the contour lines are more or less parallel to the prism direction. The intraprismatic and the interprismatic lines are arranged at an angle to each other. Note that the tissues of the two entities are in close contact or separated by small electronlucent areas. $\times 10000$.

ments which looked like dense lines (Fig. 2). Within the prism these contour lines were more or less parallel to the long axis of the prism, and within the interprismatic tissue they were arranged at an angle to the axis. Occasionally, the prismatic and interprismatic structural elements impinged directly on each other, and from time to time the tissues of the two entities were separated by small localized electronlucent areas which made the prism boundaries look like light zones.

In the sections from the microcoradiographically radiolucent subsurface area (area 2) containing prisms cut crosswise and lengthwise, the organic matrix seemed uniformly dispersed throughout the prismatic and interprismatic tissue (Figs. 3 and 4). The prism sheath regions on the other hand, contained less chromium stainable matter than the rest of the tissues as evidenced by their lack of or reduced electron density. The outlines of the prisms were irregular and the width of the light boundary zones varied. The greatest widths (approximately 2000 Å) were measured on top of the incompletely encircled prisms.

In sections from area 3 the prism boundaries had different appearances. A frequent variant is shown in Fig. 5. The interprismatic and the prismatic

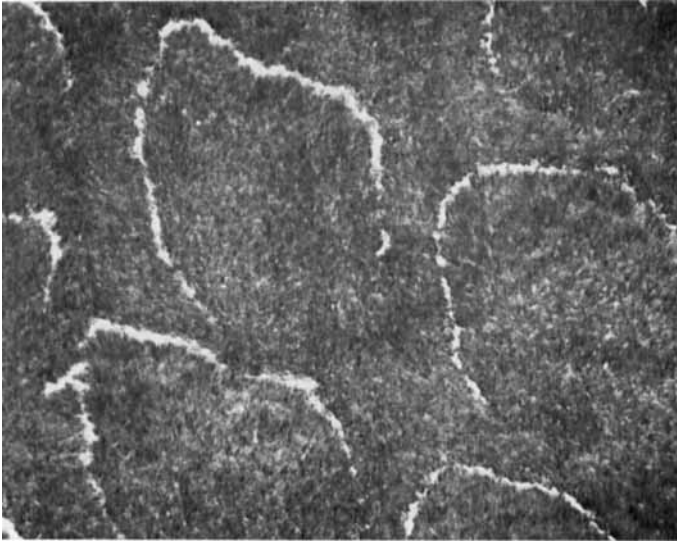


Fig. 3. Decalcified section showing prisms cut crosswise. Note the electronlucency of the prism boundaries. $\times 8400$.

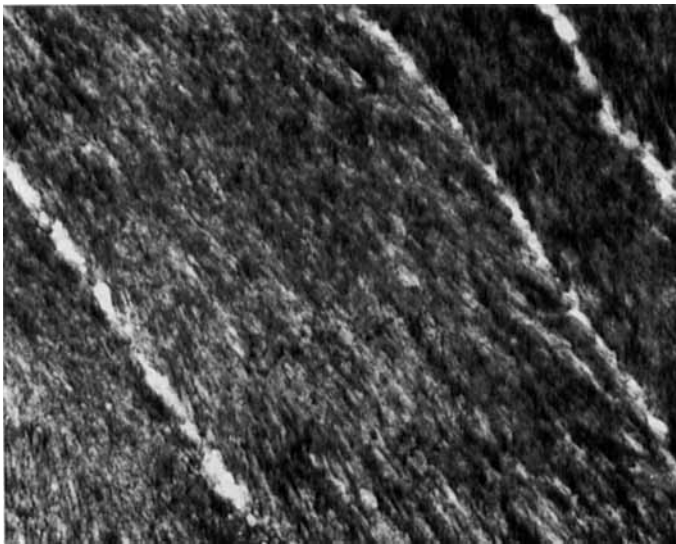


Fig. 4. Decalcified section showing prisms cut lengthwise. Note the electronlucency of the prism boundaries. $\times 13400$.

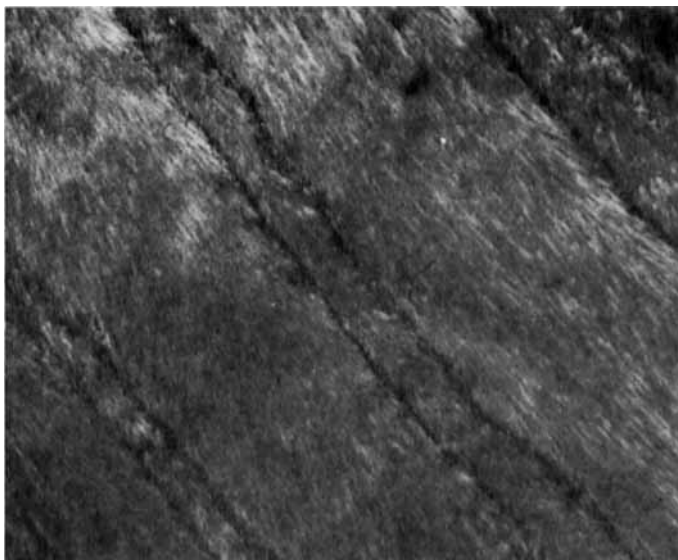


Fig. 5. Decalcified section showing prisms cut lengthwise. Note the electron density of the prism boundaries. $\times 6700$.

tissues abutted directly on to each other and there was a concentration of dense matter in the prism sheath region.

Observations on cross- and near cross-sections from areas 1 and 2 of the prisms and the interprismatic region revealed that a fraction of the recovered matrix was organized into compartments (Figs. 6 and 7). The thickness of the compartment walls was approximately 30—50 Å corresponding to that of the long dense contour lines seen in longitudinal sectioning planes from all three areas (Fig. 8). The shape of the compartments was that of polygons with hexagonal, pentagonal and tetragonal forms. In addition, a limited amount of less well-defined shapes was encountered in areas 1 and 2. In the transitional zone between area 2 and area 3 the appearance of the matrix seemed to have changed. Both well-defined and undefined forms as well as a range of intermediary forms could be seen (Fig. 9). In the depth of area 3 the compartment shapes were remarkably irregular on either side of the prism boundaries (Figs. 10 and 11). However, well-formed compartments could occasionally be observed in the prism sheath region (Fig. 11).

Most of the clearly defined compartments were characterized by having one dimension (width) greater than the other (thickness). However, equilateral or near equilateral forms did occur (Fig. 7). In all cases the compartments were randomly oriented in the plane of observation. Usually the compart-

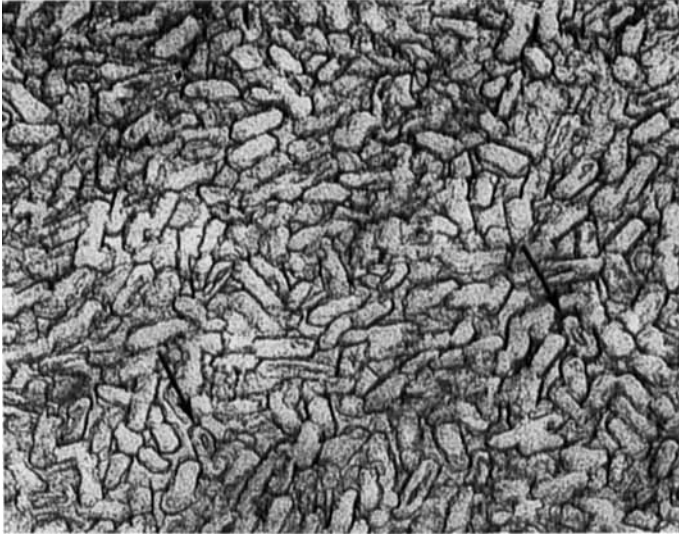


Fig. 6. Decalcified section showing mainly non-equilateral compartments. Note that few sub-compartments are visible (arrows). $\times 113800$.

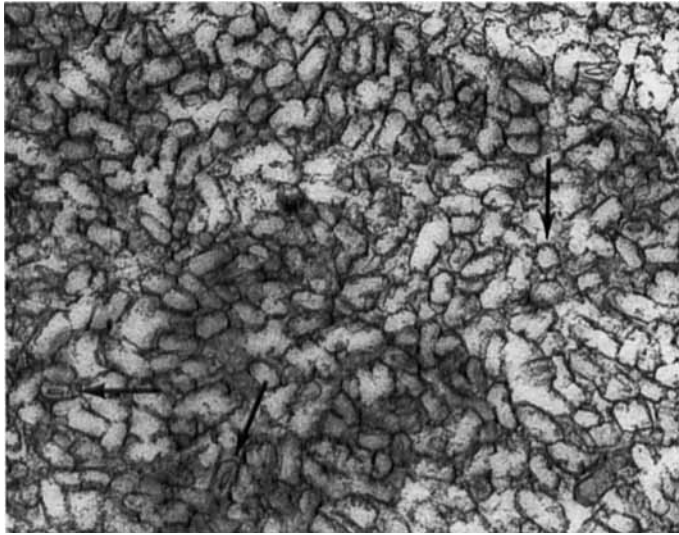


Fig. 7. Decalcified section showing variously shaped compartments. Note that equilateral (upper arrow) and near-equilateral forms occur. Also note that few subcompartments are visible (lower arrows). $\times 67000$.

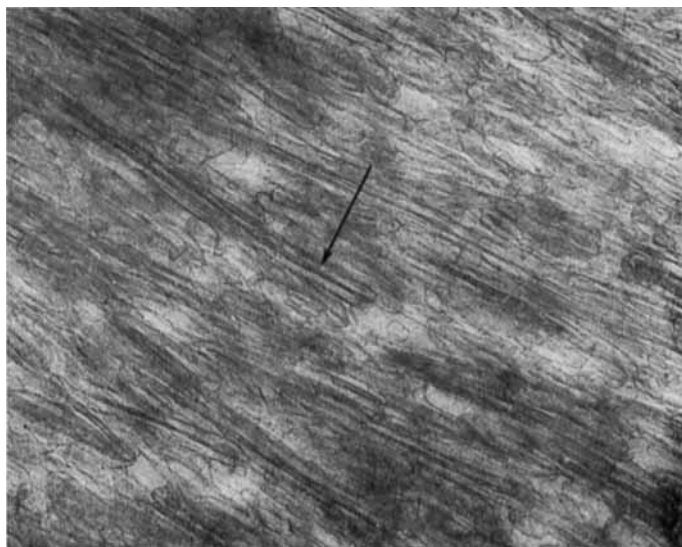


Fig. 8. Decalcified section showing longitudinally sectioned tissue. Note that paired, heavily stained lines are visible (arrows). $\times 50200$.



Fig. 9. Decalcified section showing well- and undefined compartment forms as well as intermediary forms. Note the high amount of sub-compartments which have an elliptical (right arrow) or irregular (left arrow) shape. Also note that the walls of the sub-compartments are heavily stained. $\times 27000$.



Fig. 10. Decalcified section. Typical micrograph characterizing the matrix in the depth of area 3. Note that it is almost impossible to discriminate between compartments and sub-compartments. $\times 134000$.

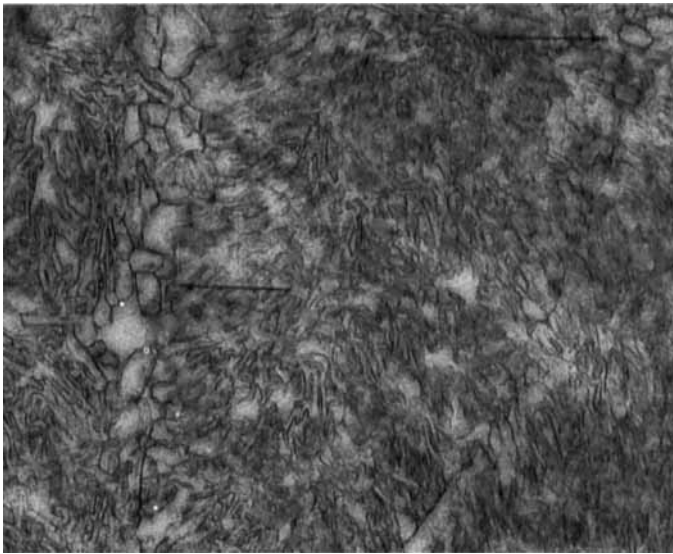


Fig. 11. Decalcified section showing prism boundaries with well-defined compartments (arrows). On either side of the boundaries identification of compartments and sub-compartments is barely possible. $\times 50200$.

ments were closely packed, although intercompartmental spaces did seem to occur.

Individual compartments contained few details or they showed slit-like sub-compartments which frequently had an elliptical shape (Figs. 6, 7 and 9). They ran centrally along the width dimension usually without coming in contact with the internal aspect of the compartment walls. The walls of the sub-compartments were structurally similar to those of the compartments. However, the former occasionally stained more heavily with the chromium salts (Fig. 9). In clearly defined compartments the sub-compartments also showed well-defined forms. When the compartments became irregular, it became evident that the sub-compartments also took on an irregular shape (Fig. 9). In sections from area 3 the irregularity of both structures was so pronounced that most frequently it was impossible to distinguish the one from the other (Figs. 10 and 11). Our observations indicated that the sub-compartments occurred less frequently in the outer layers as compared to the remainder of the tissue.

In longitudinally sectioned tissue it could be seen that some of the contour lines stained more heavily than others (Figs. 8 and 12). The strongly staining lines appeared in pairs and were separated by a narrow fissure which occasionally disappeared because the lines united. It was frequently observed that these lines touched or were continuous with the walls of the rounded or flattened end of the structural unit to which they probably belonged.

Measurements of the compartments were restricted to clearly defined structures i.e. to the compartments found in areas 1 and 2. The curve demonstrating the frequency of the values obtained for the width measurements showed a normal distribution around one maximum (Fig. 13). In contrast, the thickness values did not show a normal distribution. Two maxima were indicated, one at approximately 230 Å and one at approximately 450 Å, which may indicate that we are dealing with compartments of two size classes. Accordingly, mean values for the two classes were calculated separately.

Table I
Compartment Dimensions

| Type of compartment | No of compartments | Means and standard deviations | | | | Ratio W/T |
|---------------------|--------------------|-------------------------------|-------|----------------|-------|--------------|
| | | Width Å | | Thickness Å | | |
| | | \bar{x} | S. D. | \bar{x} | S. D. | |
| Non-equilateral | 110 | 664 | 107 | 238 | 26 | 2.8 |
| Near-equilateral | 110 | 701 | 143 | 498 | 76 | 1.4 |



Fig. 12. Decalcified section. Longitudinally sectioned tissue. Paired, heavily stained lines are indicated (right arrows). Left arrows indicate tubular ends. Note that the internal structures reach the ends of the tubes. Short arrows indicate width of the tube (approx. 600 Å). $\times 67000$.

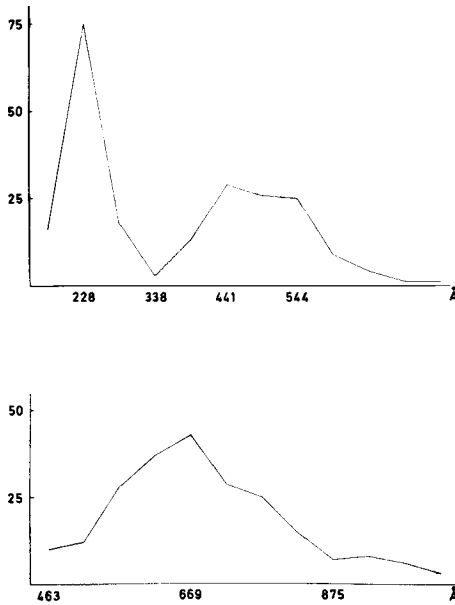


Fig. 13. Upper curve illustrates the distribution of the compartment thickness values. Note that two maxima are indicated. Lower curve illustrates the distribution of the compartment width values.

In the average non-equilateral compartment the width/thickness ratio was 2.8 and in the near-equilateral structure the ratio was 1.4 (Table I). Statistically the average thickness value of the near-equilateral compartment was significantly higher than that of the non-equilateral one ($p < 0.001$). As can be seen from the micrographs (Figs. 6 and 7) and the statistical data, the thickness was more uniform than the width in both groups. In longitudinal sections, when identification of the paired heavily stained lines was possible centrally between two parallel contour lines (Fig. 12), a maximum distance between the latter lines of 600 Å was indicated.

The results of the measurements of the sub-compartments are presented in Table II.

Table II
Sub-compartment Dimensions

| | No of sub-compartments | Means and standard deviations in Å | |
|-----------|---------------------------|---------------------------------------|-------|
| | | \bar{x} | S. D. |
| Width | 110 | 296 | 96 |
| Thickness | 110 | 59 | 10 |

As can be seen from Table II there was a considerable spread of the width measurements about the mean value. This may represent true differences, but probably also reflects the difficulty of determining true size due to oblique sectioning. The frequency distribution diagram which was prepared showed a somewhat skewed curve, with the longer tail on the right side of the maximum. In contrast, the thickness measurements indicated a little spread about the mean value. Measurements made on micrographs from longitudinally sectioned tissue indicated that the greatest length of the paired dense lines, which could be measured with a relative certainty, was approximately 9300 Å.

DISCUSSION

The present study has shown that thin sections prepared from mottled enamel of completely unerupted, fluorosed teeth may be decalcified to make a detailed study of the recovered organic matrix possible. In sections containing prisms cut more or less transversely the organic matrix was organized into compartments. In sections cutting the prisms lengthwise the matrix assumed the ap-

pearance of long contour lines which were parallel or near parallel to each other. This is essentially the same structural arrangement which has been reported for normal human enamel, bovine enamel and hedgehog enamel (Rönholm, 1962 c; Travis & Glimcher, 1964; Sundström & Zelandar, 1968b; Silness & Gustavsen, 1969; Gustavsen & Silness, 1969). The present study, therefore, indicates that the organization of the organic matrix of fluorosed enamel does not depart fundamentally from that of other dental enamels. The observations on the two main planes of sectioning together with the finding that the compartment walls and the lines had the same dimension, indicate that a fraction of the organic matrix was present as organic tubes. This interpretation is consistent with the opinions of earlier authors who studied normal human enamel, bovine enamel and hedgehog enamel (Rönholm, 1962 c; Travis & Glimcher, 1964; Sundström & Zelandar, 1968 b; Silness & Gustavsen, 1969; Gustavsen & Silness, 1969). Another fraction of the recovered matrix was observed within the tubular structures, and in a cross-sectional view it had the appearance of an elliptical, rectangular or more irregular sub-compartment. Frequently the sub-compartment linings stained more strongly with the chromium salts, and they could thus be identified in longitudinal sectioning planes with a relative certainty. This observation indicates that the intratubular structure has a length dimension arranged parallel to the long axis of the tube. The three-dimensional appearance of the former structure, therefore, probably is that of an elliptical or irregular cylinder. The presence of this cylindrical structure within the tubes can hardly be interpreted as a developmental disturbance related to the high concentrations of fluoride in the water (4—4.8 ppm) since similar structures were also observed in decalcified sound human enamel (Gustavsen & Silness, 1969). The observations on longitudinally sectioned tissue showed that the walls of the cylinder may reach the end of the tubes indicating that the internal structures go through the whole length of the tubes — at least occasionally.

The sections from the various tissue layers indicated a change in the appearance of the organic matrix in dentinal direction. Well-formed compartments which were found in the outer layers occurred gradually less frequently and, concomitantly, intermediary and irregular shapes became more frequent with increasing depth. In the depth of area 3 well-formed compartments were observed mainly along the prism boundary. Furthermore, the intratubular cylinders were seen less frequently in the outer layers compared to the deeper layers. The apparently increasing irregularity of the compartments together with the apparent increase in the prevalence of the internal structures in dentinal direction may be taken to indicate that the amount of recovered organic matter is increased in the same direction.

The cross-sectional shape and size of the tubes, their arrangement within the prism and within the interprismatic tissue as well as the random orientation of the compartments in the plane of observation resemble the morphology, size and arrangement of enamel crystals as shown repeatedly by various methods. It must be assumed, therefore, that the organic tubes have coated the inorganic crystals in the fluorosed enamel. That enamel crystals are surrounded by sheath-like organic material has been suggested by several earlier authors (Syrrist, 1949; Scott & Nylen, 1962; Rönnholm, 1962c; Travis & Glimcher, 1964; Sundström & Zeland, 1968; Silness & Gustavsen, 1969; Gustavsen & Silness, 1969). The discussion which follows will be based on the assumption that the tubes have accommodated the crystals in the fluorosed enamel.

The present study indicates that the crystals of fluorosed enamel may have several cross-sectional profiles. Since the crystals are closely or nearly closely packed only small changes, if any, can be expected as a result of further growth. The shape of the crystals in the surface and subsurface tissue seemed to be that of plate-like polygonal rods as previously indicated by Höhling (1961); Nylen, Eanes and Omnell (1963); Travis and Glimcher (1964), Silness and Gustavsen (1969) and others. However, in accordance with earlier works it was found that enamel crystals may also have an equilateral or near-equilateral cross-sectional shape (Frank, Sognæs & Kern, 1960; Johnsen, 1967; Silness & Gustavsen, 1969; Gustavsen & Silness, 1969). Deeper in the tissue there was a trend toward less well-defined shapes. In the depth of the tissue clearly defined forms were encountered mainly along the prism boundary, while the tissue on either side showed irregular and undefined shapes.

The determinations of the crystal size by means of the method used in this work are associated with errors. Even though it is likely that the compartments reflect the cross-sectional shape of the crystals, the tissue preparation procedures may have had a significant influence upon the measurements of the compartment dimensions. However, the most considerable error is probably introduced through oblique sectioning. This difficulty was encountered and discussed by several authors (e.g. Frank *et al.*, 1960; Rönnholm, 1962 b; Nylen *et al.*, 1963; Travis & Glimcher, 1964) so it is not specific to the method used in this work. None the less, size estimation has only a relative value. With the inherent restrictions it can be stated that the crystals in the surface and subsurface area had an average width of 660—700 Å. This value is somewhat higher than that reported for human enamel crystals in most electronmicroscopic works, in which the width was estimated to be approximately 400—600 Å (Robinson, 1952; Scott *et al.*, 1952; Little, 1955, 1959; Hall, 1958; Rönn-

holm, 1962 b). The crystal width in hamster, rat, bovine and hedgehog enamel also seems to be within a range of 400—600 Å (*Watson & Avery, 1954; Nysten et al., 1963; Travis & Glimcher, 1964; Silness, & Gustavsen, 1969*). The statistical data indicated that the well-defined crystals were of two size classes as regards thickness. In one the average thickness was approximately 240 Å, which is within the range of values reported in the afore-mentioned works. In the other the average thickness was approximately 500 Å. This value is higher than what has usually been found by electron microscopy, although *Frank et al., (1960)* reported that well-formed equilateral crystals vary from 500 to 1200 Å. On the other hand, recent works (*Johnson, 1967; Gustavsen & Silness, 1969*) indicate, that well-formed isodiametric or near isodiametric crystals of such sizes (1200 Å) in human enamel are characteristic of the prism borders. Although comparison with earlier works indicates that one set of the crystals of fluorosed enamel (width 700 Å, thickness 500 Å) is larger than usually reported for sound enamel, the restrictions of the methods and the heterogeneity of the values given in the literature do not permit any conclusion to that effect. Recent reports showed that the X-ray diffraction patterns of human (*Zipkin, Posner & Eanes, 1962*) and rat (*Menczel et al., 1962; Schraer et al., 1962*) bone apatites were better resolved as the fluoride content increased. It was also shown (*Posner et al., 1963*) that the fluoride, when incorporated in bone apatite, produced an improvement in crystallinity, where the more perfect and/or larger crystals are said to be more crystalline. By analogy, it would not be surprising that the same effects of fluoride might be produced in fluorosed enamel.

The crystals in the outer layers showed a high degree of morphological uniformity with a morphologically more perfect shape as compared to the less well-defined and undefined forms found in the deeper layers. In comparison *Johansen (1965)* reported that crystals from sound human enamel showed lack of morphological uniformity. Likewise, *Gustavsen and Silness (1969)* felt that the crystals on either side of the prism boundary were irregular and not well-defined in sound human enamel. However, it was not stated in the reports whether differences related to the various tissue layers might occur. It remains to be shown, therefore, whether the morphology of the crystals in the outer layers of fluorosed enamel is different from that of sound human enamel and thereby possibly an effect associated with the concentration of fluoride in the water supply.

The finding of elliptical and irregular cylinders suggests that organic matrix may be enclosed within enamel crystals as suggested by earlier authors (*Millard & Pautard, 1958; Frank et al., 1960; Rönholm, 1962 b; Johansen, 1965; Sundström & Zelandar, 1968 b; Silness & Gustavsen, 1969; Gustav-*

sen & Silness, 1969). On the basis of the presence of this ribbon-like organic cylindrical structure it may be said that interlayered or lamellar crystals may occur in dental enamel. It has been suggested by *Brown* (1965) that a core of octacalcium phosphate may occur in the mechanism of crystal growth. It was described as a ribbon, 10—30 Å thick and 200—300 Å wide. These dimensions are in harmony with the average values which resulted from the present measurements (thickness 59 Å, width 296 Å). Further, our observations seemed to show that the ribbon and the crystal may have the same length. The methods used do not permit any comments as to whether the core differs in lattice structure from the remainder of the crystal. However, our observations indicate that whatever the structure of the core may be, it is apparently coated by an organic layer.

Considering the data together, it is evident that the variations in crystal morphology, the variations in crystal size and the occasional subdivision of the single crystal into layers may have a decisive influence upon the solubility, the reactivity and the specific surface area of the apatite. The observations may, therefore, be important for a better understanding of the mineralization and demineralization processes of human enamel.

The microradiographs showed a well calcified surface enamel and a less mineralized subsurface layer. These findings confirm the observations by *Darling* and *Brooks* (1959) and *Newbrun* and *Brudevold* (1960) who found a hypocalcified area beneath a well mineralized surface layer in fluorosed enamel. These authors also observed the alternating radiolucent and radiopaque line pattern parallel to the direction of the prisms in the subsurface area and related the radiolucent lines to the interprismatic enamel. The correlated microradiographic and electronmicroscopic work by *Glas* and *Nylen* (1965) showed that in hypomineralized enamel (amelogenesis imperfecta) the radiolucent lines result from mineral-free prism boundaries rather than from less mineralized interprismatic enamel. This explanation was also suggested for the line pattern so frequently seen in hypomineralized human deciduous enamel (*Silness*, 1969). The present study has shown that the prism boundaries in the subsurface layer may be up to 2000 Å wide and usually free from compartments and contour lines, indicating that they were free from crystals in the ground section. It seems likely, therefore, that the radiolucent lines in the microradiographs should be attributed to the mineral-free prism boundaries and not to the interprismatic enamel. The presence of mineral-free slits separating the mineralized prisms in the subsurface area would essentially mean that this tissue has the properties of a diffraction grating. It is possible, therefore, that the mottling of the fluorosed enamel may be explained as a diffraction effect related to the structure of the enamel.

SUMMARY

Thin ground sections of mottled enamel from fluorosed teeth were examined by microradiography and subsequently decalcified. The recovered matrix was embedded in Araldite sectioned and its fine structure examined by means of electron microscopy.

Microradiographically the ground sections showed three areas of differing densities. The surface layer was radiopaque, the subsurface showed radiolucency and the enamel on the dentinal side of the subsurface area was radiopaque. Alternating radiolucent and radiopaque lines parallel to the prism direction characterized the subsurface layer.

As seen in the electron microscope one fraction of the decalcified tissue seemed to be built up by long organic tubes. Another fraction was occasionally contained within these tubes and had the appearance of an elliptical or irregular cylinder. It was assumed that the organic tubes had coated the enamel crystals. The finding of intracrystalline organic cylinders suggested that enamel crystals may occasionally be interlayered structures. On the assumption made the results also indicated that the crystals were morphologically more perfect in the outer layers of the mottled enamel as compared to the crystals in the deeper layers. It was discussed whether the mottling of fluorosed teeth may be a diffraction effect related to the structure of the subsurface enamel.

RÉSUMÉ

FINE STRUCTURE DE L'ÉMAIL DENTAIRE FLUOROTIQUE

Des coupes minces par usure provenant de l'émail tacheté de dents fluorotiques ont été soumises à un examen microradiographique, puis déminéralisées. La matrice ainsi obtenue a été incluse dans de l'Araldite, des coupes ont été réalisées et leur fine structure examinée au microscope électronique.

A l'examen microradiographique, les coupes par usure présentaient trois zones de densités différentes. La couche superficielle était radio-opaque, la couche située sous la surface présentait une certaine radiotransparence, et l'émail situé du côté de la dentine était radio-opaque. Des lignes alternées de radiotransparence et de radio-opacité, parallèles à la direction des prismes, caractérisaient la couche située sous la surface.

Au microscope électronique, une fraction du tissu déminéralisé semblait être constituée par de longs tubes de matière organique. Une autre fraction se trouvait parfois à l'intérieur de ces tubes, et avait l'aspect d'un cylindre elliptique ou irrégulier. Les auteurs ont supposé que les tubes organiques avaient revêtu les cristaux de l'émail. La mise en évidence de cylindres organiques intracrystallins semblait indiquer que les cristaux de l'émail

seraient parfois des structures à couches interposées. Suivant la supposition ci-dessus, les résultats indiquaient aussi que la morphologie des cristaux était plus parfaite dans les couches externes de l'émail fluorotique que dans les couches plus profondes. Dans une discussion, les auteurs considèrent la possibilité que l'aspect tacheté de l'émail fluorotique soit un effet de diffraction dû à la structure de l'émail de la couche située sous la surface.

ZUSAMMENFASSUNG

FEINSTRUKTUR DER ORGANISCHEN SCHMELZMATRIZE
VON FLUOROTISCHEN ZÄHNEN

Dünnschliffe von geflecktem Schmelz von fluorotischen Zähnen wurden mikroröntgenographisch untersucht und danach entkalkt um die Feinstruktur der organischen Matrize mit dem Elektronenmikroskop zu untersuchen.

Mikroröntgenographisch konnte festgestellt werden, dass drei Schichten vorhanden waren, welche die Strahlen in verschiedener Weise durchliessen. Eine Aussenschicht war opak, eine Unterschicht liess die Strahlen durch, und der Schmelz anderseits der Unterschicht war auch opak. Die abwechselnd hellen und dunklen mit den Prismen gleichlaufenden Linien waren für die Unterschicht des Schmelzes charakteristisch.

Elektronenmikroskopisch ergab es sich, dass ein Teil des entkalkten Gewebes von langen organischen Röhren bestehe. Ein zweiter Teil, der ab und zu innerhalb der Röhren befindlich war, sah wie ein elliptischer oder unregelmässiger Zylinder aus. Es wurde angenommen, dass die organischen Röhren die Kristalle umschliessen. Das Vorkommen von organischen Zylindern deutete an, dass Schmelzkristalle ab und zu lamellenartige Strukturen sein mögen. Die Auslegung der Resultate dürfte auch die Annahme rechtfertigen, dass die Kristalle der äusseren Schichte des fluorotischen Schmelzes morphologisch mehr vollkommen seien, als die der tieferen Schichte. Es wurde diskutiert, ob das gefleckte Aussehen des fluorotischen Schmelzes ein von der Struktur des Schmelzes bedingter Diffraktionseffekt sei.

REFERENCES

- Black, G. V. & F. S. McKay*, 1916: Mottled enamel. An endemic developmental imperfection of the teeth, heretofore unknown in the literature of dentistry. *Dent. Cosmos* 58:129—156.
- Brown, W. E.*, 1965: A mechanism for growth of apatitic crystals. In: *Tooth enamel*, pp. 11—14. Eds. M. V. Stack and R. W. Fearnhead. John Wright and Sons Ltd., Bristol.
- Darling, A. I. & A. W. Brooks*, 1959: Some observations on the mottled enamel of fluorosis. *J. dent. Res.* 38: 1226—1227.

- Dean, H. T., 1938: Chronic endemic dental fluorosis (mottled enamel). In: *Dental Science and Dental Art*, pp 387—414. Ed. S. M. Gordon, Henry Kempton, London.
- Frank, R. I., R. F. Sognnæs & R. Kern, 1960: Calcification of dental tissues with special reference to enamel ultrastructure. In: *Calcification in biological systems*, pp. 163—202. Ed. R. F. Sognnæs, Publ. No. 64. American Association for the Advancement of Science, Washington.
- Glas, J-E. & M. U. Nylen, 1965: A correlated electron microscopic and microradiographic study of human enamel. *Arch. oral. Biol.* 10: 893—908.
- Gustafson, A-G., 1961: The histology of fluorosed teeth. *Arch. oral. Biol.* 4: 67—69.
- Gustavsen, F. & J. Silness, 1969: Crystal shape in the prism sheath region of sound human enamel. *Acta odont. Scand.* 6: 617—629.
- Hall, D. M., 1958: Study of the submicroscopic structure of human dental enamel by electron microscopy. *J. dent. Res.* 37: 243—253.
- Höhling, H. J., 1961: Elektronenmikroskopische Untersuchungen am gesunden und kariösen Zahnschmelz unter besonderer Berücksichtigung der Ultramikrotomschnitt-Technik an nicht entmineralisierter Substanz. *Dtsch. zahnärztl. Z.* 16: 694—705.
- Johansen, E., 1965: Comparison of the ultrastructure and chemical composition of sound and carious enamel from human teeth. In: *Tooth enamel*, pp. 177—181. Eds. M. W. Stack and R. W. Fearnhead. John Wright and Sons Ltd., Bristol.
- Johnson, N. W., 1967: Some aspects of the ultrastructure of early enamel caries seen with the electron microscope. *Arch. oral. Biol.* 12: 1505—1521.
- Little, K., 1955: Electron microscope studies of teeth. *J. dent. Res.* 34: 778.
- Menczel, J., A. S. Posner, H. Schraer, G. Pakis & R. C. Likins, 1967: Comparative fixation of Sr⁴⁶ and Ca⁴⁵ by calcified tissues as related to fluoride induced changes in crystallinity. *Proc. Soc. exp. Biol.* 110: 609—613.
- Millard, A. & F. G. E. Pautard, 1958: Electron microscope observations on apatite crystallites in human dentine and enamel. In: *Intern. Conf. Electron Microscopy 4th Conf.* pp. 357—360.
- Newbrun, E. & F. Brudevold, 1960: Studies on the physical properties of fluorosed enamel. I. Microradiographic studies. *Arch. oral. Biol.* 2: 15—20.
- Nylen, M. U., E. D. Eanes & K-Å. Omnell, 1963: Crystal growth in rat enamel. *J. Cell. Biol.* 18: 109—123.
- Posner, A. S., E. D. Eanes, R. A. Harper & I. Zipkin, 1963: X-ray diffraction analysis of the effect of fluoride on human bone apatite. *Arch. oral. Biol.* 8: 549—570.
- Robinson, R. A., 1952: An electron microscopic study of the crystalline inorganic component of bone and its relationship to the organic matrix. *J. Bone Jt Surg.* 34A: 389—435.
- Rönholm, E., 1962 b: The amelogenesis of human teeth as revealed by electron microscopy. II. The development of the enamel crystallites. *J. Ultrastruct. Res.*, 6: 249—303.
- Rönholm, E., 1962 c: The structure of the organic stroma of human enamel during amelogenesis. *J. Ultrastruct. Res.*, 6: 368—389.
- Schraer, H., A. S. Posner, R. Schraer & I. Zipkin, 1962: Effect of fluoride on bone «crystallinity» in the growing rat. *Biochim. Biophys. Acta*, 64: 565—567.
- Scott, D. B., M. U. Nylen & M. Pugh, 1962: Some technical aspects of microradiography. *Norelco Reporter*, 9: 103—109.
- Scott, D. B. & M. U. Nylen, 1962: Organic-inorganic interrelationships in enamel and dentine — a possible key to the mechanism of caries. *Int. dent. J.*, 12: 417—432.
- Silness, J., 1967: Elements of the organic framework of dental enamel of the hedgehog (*Erinaceus europaeus* L.). Thesis. Universitetsforlaget, Oslo.

- Silness, J. & F. Gustavsen*, 1969: Structure of the organic matrix of dental enamel of the hedgehog (*Erinaceus europaeus* L.). *Odont. Revy*, 20: Suppl. 19.
- Silness, J.*, 1969: Some variations in the microradiographic appearance of human deciduous enamel. *Odont. Revy*, 20: 93—110.
- Sundström, B.*, 1966: A new technique for decalcifying ground specimens of adult human enamel. *Arch. oral. Biol.* 11: 1221—1231.
- Sundström, B. & T. Zelande*r, 1968 a: Routine decalcification of thin sawed sections of adult human enamel by means of a basic chromium (III) sulphate solution of stable pH. *Odont. Revy*, 3: 233—247.
- Sundström, B. & T. Zelande*r, 1968 b: On the morphological organisation of the organic matrix of adult human enamel after decalcification by means of a basic chromium (III) sulphate solution. *Odont. Revy*, 3: 249—263.
- Syrrist, A.*, 1949: En orientering i elektronmikroskopien med noen resultater fra histologiske undersøkelser av emalje og dentin. (An introduction in electron microscopy with some results from histological investigations of enamel and dentine. *Odont. T.*, 57: 79—105.
- Travis, D. F. & M. J. Glümcher*, 1964: The structure and organization of and the relationship between the organic matrix and the inorganic crystals of embryonic bovine enamel. *J. Cell. Biol.*, 23: 447—497.
- Vennes, J. W., J. E. Kwako, D. D. Swenson & J. K. Petterson*, 1862: Fluoride content of municipal water supplies in North Dakota. *J. — Lancet.*, 82: 388—391.
- Watson, M. L. & J. K. Avery*, 1954: The development of the hamster lower incisor as observed by electron microscopy. *Am. J. Anat.*, 95: 109—161.
- Williams, J. L.*, 1923: Mottled enamel and other studies of normal and pathological conditions of this tissue. *J. dent. Res.*, 5: 117—195.
- Zipkin, I., A. S. Posner & E. D. Eanes*, 1967: The effect of fluoride on the X-ray diffraction pattern of the apatite of human bone. *Biochim. Biophys. Acta.*, 59: 255—258.

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