The Sciences 1 (4): 200-205
July - August, 2001

Organic Structure of Rat Enamel under Scanning Electron Microscopy

S. M. Taher and C. G. Rubler

The structure of the organic matrix of rat enamel was studied with the aid of scanning electron microscope (SEM) utilizing a replica technique. Samples of rat enamel were first deorganified with hydrazine and then impregnated with a low viscosity resin. These were then sectioned, longitudinally or transversely, polished and demineralized with either 0.1 or 0.5M EDTA (Ethylene diamine tetra acetic acid) for 12, 24 or 48 hours. Longitudinal sections were treated with 0.1M EDTA for short periods of time showed a non-fibrous layer, along the dentino-enamel junction, which was covered with small holes. When a 0.5M solution of EDTA was used, this non-fibrous sheet was completely etched away. The major portion of the organic could now be seen along the rod sheath. Demineralization of the sample left many small voids within the gel, where crystallites once were present, thus giving the organic matrix a fibrillous appearance. Transverse section, treated with 0.5M EDTA showed no evidence of any structures. When these sections were treated with 0.1M EDTA the organic material could be seen predominantly along the rod sheath. Large globules of organic, covered with small indentations, could be seen in areas which had no rods. Smaller globules, covered with holes, could be noticed along the tops of some of the arcades.

Key words: Organic matrix, EDTA, rat enamel, rodsheath, SEM

S. M. Taher
Department of Physics
Wichita State University,
Wichita, KS 67260-0032, USA

E-mail: syed.taher@wichita.edu

Department of Physics, Wichita State University, Wichita, KS 67260-0032, USA

Introduction

It is important to study the structure of the organic portion of enamel primarily, since it may aid in understanding the mechanism for caries. And it has an important role in affecting the material properties of enamel. It is known that hydroxapatite crystals alone would be too brittle, so that the organic matrix is necessary to stabilize this hardest of organic tissues. It may also plays an important role in nucleation and orientation of the crystals in enamel as collagen does in bone and dentin. Studies on the organic phase of enamel have been attempted as early as 1872 by C. Waldeyer and C. Wedl as reported in Sognnaes (1948). The first EMS studies provided evidence for existence of the organic matrix in enamel (Frank, 1950 and Scott, 1962) and more recently (Ducroc, 1972).

At present there is a insufficient knowledge about the structural aspects of organic matrix and its role in the decay processes of enamel. Two morphological entities of the matrix have been reported (Decker, 1973). One is the material located between the distal ends of secretory ameloblasts and mineralization front (Waston, 1960). The other morphological entity of the matrix has variously been described as fibrillar, lamellar (Helmcke, 1967) or as composed of compartments of tubules (Decker, 1973). It has also been leveled as artifacts (Fearnhead, 1968). The electron micrograph of decalcified sections show clearly the existence of an organic framework even in mature enamel with the same overall organization as the crystals (Warshawky, 1971).

From the early stages of enamel development to the beginning of the calcification, the organic phase turns from amorphous to fibrillous in appearance according to Scott *et al.* (1962 and 1971). Whereas according to Sundström and Zelander (1968) organic component appears to be a gel-like ground substance. It is still unclear where, among the rods, the majority of organic material is concentrated. According to Boyde (1967), the organic matrix was concentrated predominantly along the rod sheath. Soule's work (1970) confirms the existence of organic component throughout the rod and rod sheath. However he found that the organic matrix fibrillar in appearance with no indication of rod structure. He also noticed a series of pore-like openings having diameters about 0.2µm, which called microtubiculi, similar to tubules as observed by Jessen (1968).

In order to make any detailed study of specific surface features of enamel it is necessary to selectively remove the prescribed portions of enamel. The method used for demineralization was done with HCl by Sognnaes (1948). HCl was found to attack on the rod sheath first and afterwards spread to the interior of the rod (Nichol *et al.*, 1971). Hoffman *et al.* (1969) noticed that EDTA rapidly attacked areas with low concentrations of calcium(Ca) which corresponded to the rod sheath. Soule (1970) used 5% trichloracetic acid (CCl_3COOH) for demineralization. Samples showed no indication of any rod structure which was probably due to the fact that demineralizing solution was such a strong acid that it may have caused the rod structure to collapse. Sundström and Zelander (1968) used a metal salt, chromium (III) sulphate, ($Cr_2(SO_4)_3$ not only for fixing samples but also for decalcification. They tested other metal salts and found the $Cr_2(SO_4)_3$ to be the most successful.

Ethylene diamine ($H_2NCH= CHNH_2$) was one of the earlier methods used for deorganification. Termine and Posner (1967) found that $H_2NCH= CHNH_2$ caused recrystalization of apatite crystals. Their finding was later confirmed by Swedlow *et al.* (1972) who reported that in order for ($H_2N-CH= CH-NH_2$) to be effective it must be used as an aqueous solution at 118°C and in this state it causes recrystalization of apatite crystals. They noticed that ashing, another method for deorganifying, was poor since it did not completely penetrate the sample and also left the ash in place of the organic component and the best method for deorganifying is with hydrazine (H_2N-NH_2). This chemical causes the break down of peptide bonds on proteins thus ridding the sample of its organic material. Termine *et al.* (1973) found that after H_2N-NH_2 treatment the sample was almost completely free of organic material without any changes in the inorganic structure and very little change in the chemical composition.

The intent of this particular effort is to study the structure of organic matrix of rat enamel with the aid of the SEM, using a replica technique in which samples are deproteinated, impregnated with resin (which takes on the form of the organic matrix) and the demineralized.

Materials and Methods

Upper incisors from albino rats were used as specimens for this study for several reasons:

1. Rat incisors are more porous, i.e., they contain more organic material by volume than human teeth and therefore, it is easier to diffuse resin through them.
2. Incisors are constantly growing, therefore, it is possible to study both mature and developing enamel on the same sample.
3. Rat enamel has been found to contain twice as much organic material as human enamel (Stack, 1957).

Upper incisors were extracted from mature albino rats between six months and one year of age. After extraction, the teeth were placed in 100% ethanol (C_2H_5-OH) and stored at -5°C until further use.

Since the organic matrix of enamel dissolves in EDTA along with the mineral portion (Glimcher *et al.*, 1954), it was necessary to use a replica technique. The organic phase was replaced by impregnating they samples with epoxy resin which is resistant to EDTA. Deorganification and embedding the resin into the voids left by the organic component was done following the method described by Taher (2000).

The samples were then cut with the diamond saw into mesiodistal-longitudinal sections and transverse cross sections approximately 0.5mm in thickness. The dentin portion of the longitudinal section was removed by gently scraping it, if it had not yet fallen out by itself. Specimens were then glued with DUCO cement to aluminum specimen mounts with their dentino-enamel junction exposed. They were polished on emery papers from 240 grit up to 600 grit and then with 17.5 and 5µm aluminum oxide(Al_2O_3) powder. Samples were rinsed off thoroughly with mineral spirits between grades of Al_2O_3 powder to assure the removal of the coarser granules.

Polished sections were etched in 0.5M EDTA of pH 7.4, for 24 and 48 hr. at room temperature. In a second method, they were etched for 12, 24 and 48hr. and the results were compared to the original method. It was observed that when samples were allowed to stand in EDTA longer than 3 days, the entire enamel structure would disintegrate. To stop the etching process, it was necessary to rinse the samples thoroughly with distilled water and let them air dry. The remaining portion of the sample was now embedded with epoxy resin which had taken on the shape of organic matrix. Before placing the specimens in the microscope for observations, a sputtering device was used to assure an even and thorough coating of samples with gold. Enamel specimens were studied using the MAC 700 SEM operating with an accelerating potential of 20 KV and a working distance of 0.3 inch. Micrographs were taken of longitudinally cut enamel samples at the dentino-enamel junction.

Results and Discussion

The rats' teeth were cut into three sections. The first section, containing the oldest enamel, was closest to the incisal tip

Fig. 1: Longitudinal Section of Rat Enamel; 12 hours 0.1M EDTA etch of section 3 (400 X).

Fig. 2: Longitudinal Section of Rat Enamel; 24 hours 0.1M EDTA etch of section 3 (500 X).

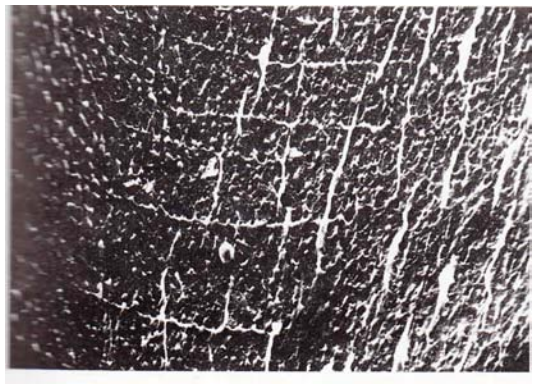

Fig. 3: Longitudinal Section of Rat Enamel; 48 hours 1.5M EDTA etch of section 3 (200 X).

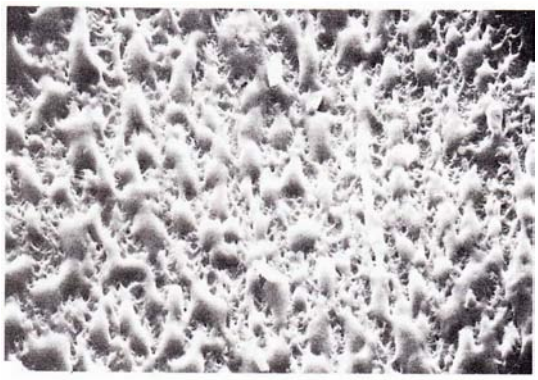

Fig. 4: Longitudinal Section of Rat Enamel; 48 hours 0.5M EDTA etch of section 2 (500 X).

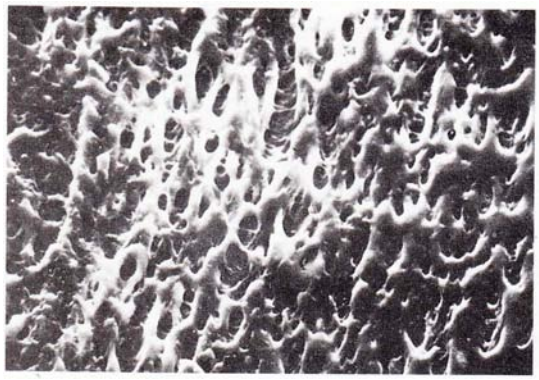

Fig. 5: Longitudinal Section of Rat Enamel; 48 hours 0.5M EDTA etch of section 2 (500 X).

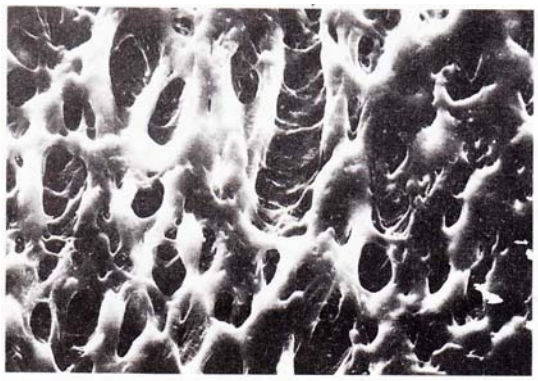

Fig. 6: Longitudinal Section of Rat Enamel; 48 hours 0.5M EDTA etch of section 2 (1,000 X).

while the third section was located within the mandible. The structures seen in the following sequence of micrographs are mostly composed of resin which through impregnation, has taken on the form of the organic matrix.

After 12hr. of 0.1M EDTA treatment, there appeared to be a homogeneous material which is forming a layer over the enamel along the dentino-enamel junction (Fig. 1). When observed more closely, small pore-like openings about 1-2μm in diameter could be noticed. When the sample was placed in 0.1M EDTA for 24hr. the pore-like openings became larger in size and exposing granular material (Fig. 2). Weber (1965) first noted the appearance of these holes. Warshawsky (1971) observed what he called "homogeneous electron dense material" which was near the dentino-enamel junction. And also noted the presence of "randomly distributed holes" within this homogeneous material which, from his micrographs, appear to be approximately 1-2μm in diameter. These holes were usually empty although, at times, they appeared to contain very small quantities of granular or filamentous materials. Washawsky (1971) also found that these holes, in developing enamel, contained the tips of Tomes Processes.

When a 0.5M EDTA was used as an etchant over a 48hr.

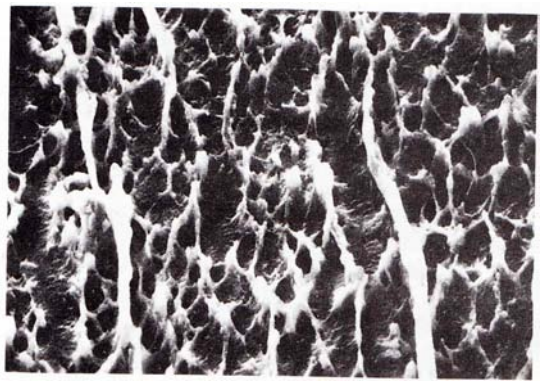

Fig. 7: Longitudinal Section of Rat Enamel; 48 hours 0.5M EDTA etch of section 2 (1,000 X).

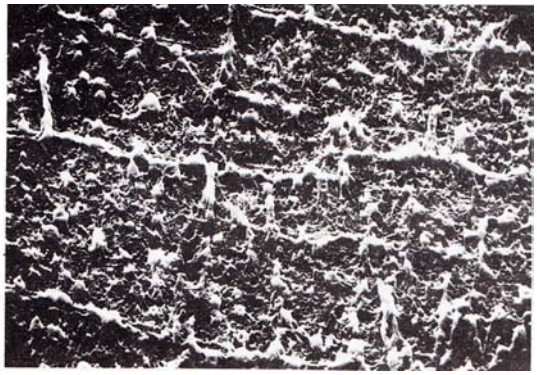

Fig. 8: Longitudinal Section of Rat Enamel; 24 hours 0.5M EDTA etch of section 1 (750 X).

Fig. 9: Longitudinal Section of Rat Enamel; 24 hours 0.5M EDTA etch of section 1 (2,000 X).

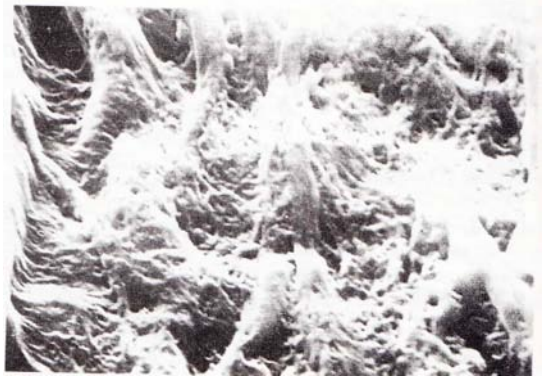

Fig. 10: Longitudinal Section of Rat Enamel; 24 hours 0.5M EDTA etch of section 1 (5,000 X).

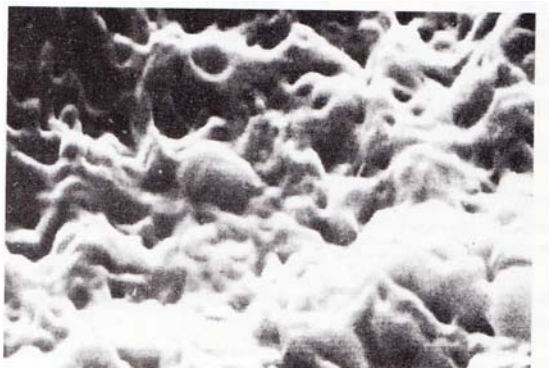

Fig. 11: Longitudinal Section of Rat Enamel; 24 hours 0.5M EDTA etch of section 1 (30,000 X).

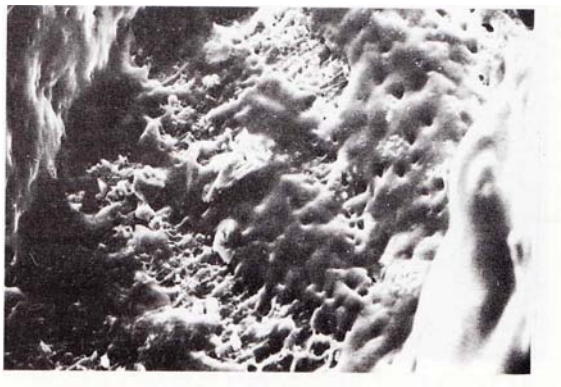

Fig. 12: Transverse Section of Rat Enamel; 12 hours 0.1M EDTA etch of section 2 (500 X).

period, there was no evidence of homogeneous material and rod sheath structure was clearly apparent (Fig. 3) which shows both transverse and longitudinal sections of the rod. Fig. 4 shows the large amorphous globs of organic material. As the sample was scanned, an area which had revealed further attack (Figs. 5 and 6). In Fig. 6, a partially exposed longitudinal rod can be seen and (Fig. 7) clearly shows both longitudinal and transverse rod sections along the enamel surface near the dentino-enamel junction (Tomes, 1850).

From these micrographs it appears that originally there is the presence of a non-fibrous sheet which covers the rods. Once this sheet has been etched away, the major portion of the organic component seems to be located along the rod sheath.

The sample shown (Figs. 8 and 9) had been treated with 0.5M EDTA for 24hr. The organic matrix was originally a gel with crystallites spread through it. Once these crystallites were removed through demineralization, the organic matrix had a fibrillous appearance. Also finger-like and globular protrusions of the organic matrix can be identified. These areas in which the organic material is denser may serve as a

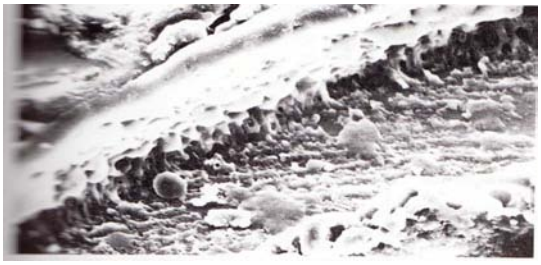

Fig 13: Transverse Section of Rat Enamel; 24 hours 0.1M EDTA etch of section 2 (500 X).

Fig 14: Transverse Section of Rat Enamel; 48 hours 0.1M EDTA etch of section 3 (500 X).

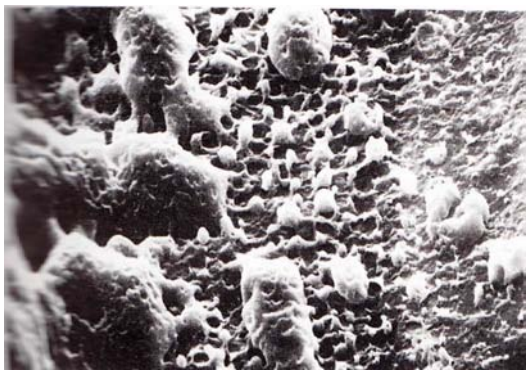

Fig 15: Transverse Section of Rat Enamel; 48 hours 0.1M EDTA etch of section 3 (1,000 X).

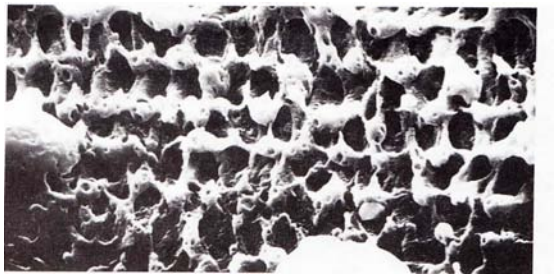

Fig 16: Transverse Section of Rat Enamel; 48 hours 0.1M EDTA etch of section 3 (2,000 X).

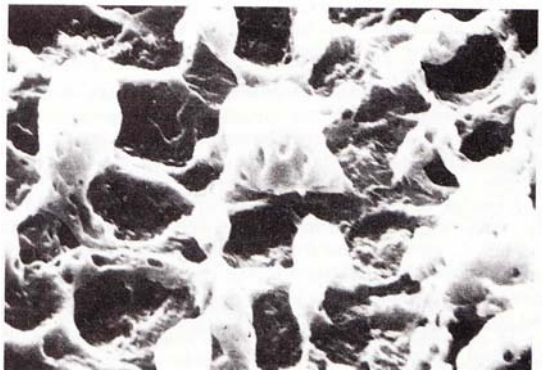

Fig 17: Transverse Section of Rat Enamel; 48 hours 0.1M EDTA etch of section 3 (5,000 X).

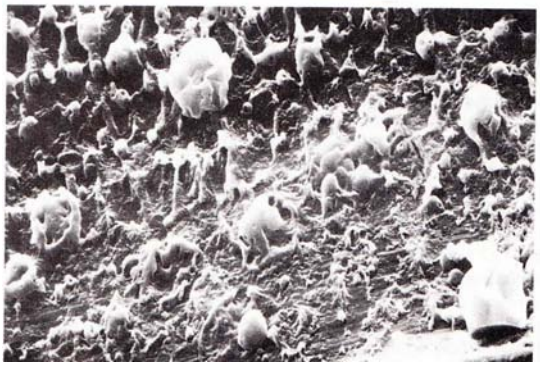

Fig 18: Transverse Section of Rat Enamel; 48 hours 0.1M EDTA etch of section 3 (2,000 X).

diffusion pathway for caries forming substances (Fig. 7). The fibrillous appearance of the organic matrix is much more apparent (Fig. 10). According to Scott *et al.* (1962) and Soule (1970) the organic matrix is amorphous when first laid down but once enamel matures it appears to be fibrillous.

At very high magnifications small holes could be seen throughout the organic matrix which measured about 0.1µm across (Fig. 11). Soule (1970) using foetal pig teeth, also noted small pore-like openings within the organic matrix with diameters of about 0.2µm. He called these holes microtubiculi and attributes the "porosity" of enamel to the size and hydroxy apatite crystals and it is just as likely that these pore-like openings contained such crystals before demineralization.

The next sequence of micrographs were taken of samples cut into transverse cross sections. In Fig. 12 the fibrillous organic matrix can be seen scattered between amorphous structure. The sample in Fig. 13 has been treated with EDTA for 24hr. Not much remains of the rod structure in this sample. If examined closely a few transverse sections of rods can be seen within the parentheses. Also rod structures, similar in form to those seen in Warshawsky (1971) micrographs, can be noticed along the lower portion of the wall (between the arrows). Large globules marked A and B are seen as though they had broken off from the wall area. Perhaps it is possible

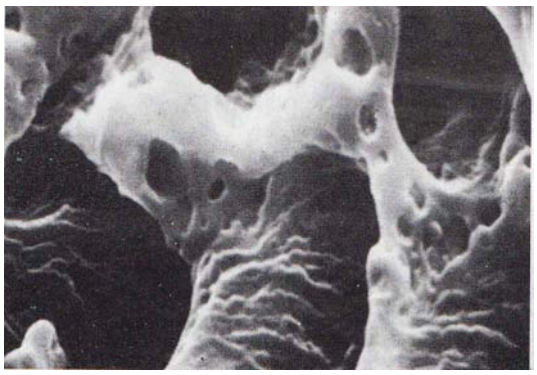

Fig 19: Transverse Section of Rat Enamel; 48 hours 0.1M EDTA etch of section 3 (10,000 X).

that the rod structure in this particular sample collapsed due to some trauma during rinsing and handling; thus there is very little evidence of any structure.

Fig. 14 showed the keyhole or in this case structure in the outer enamel region. There also appears to be large rodless areas where the organic matrix has taken on the shape of large amorphous globules which, when enlarged, are covered with pock marks (Fig. 15). The inner enamel in Fig. 14 showed virtually no structures, i.e., most of it has been etched away. The organic matrix appears to be in higher concentration along the rod sheaths. It seems to agree with the earlier observations (Scott *et al.*, 1971 and Boyde, 1967). In an enlarged micrograph (Fig. 16), globules of organic matter similar to those observed in Figs. 8 and 9 can be seen along the tops of some of the arcades. When these globules are examined more closely in Fig. 17 their surfaces appear to be randomly covered with pit holes between 0.4 and 0.6µm in a diameter. In another portion of the sample some organic matter can be seen along the inner enamel layer; however, there appears to be no structural organization, (Fig. 18). The organic matrix in this layer appears to be consisted of fibrils mixed with globular protrusions. It is interesting to note that the rods appear to run in opposite directions from the perpendicular plane of the picture in alternating rows; this may be better exemplified in Fig. 19.

Cross sectional specimens treated with 0.5M EDTA for periods of 12, 24 and 48hrs. showed no evidence of any structure. One possibility is that the crystallites in this particular orientation were more susceptible to etching and thus the structure that remained was so weak that it collapsed. Scott *et al.* (1971 and 1974) observed that the orientation of the crystallites with in the rod effects the etching pattern and rate. They found that crystals which were oriented such that their long axis was perpendicular to the direction of attack disappeared first while parallel crystals withstood the etch for a much longer time.

In conclusion, the following observations can be made: Longitudinal sections were etched with 0.1M EDTA for short periods of time showed a non-fibrous sheet covered with small "holes." Originally the organic matrix was a gel with crystallites spread throughout it. After demineralization with 0.5M EDTA the matrix appeared to be fibrillous. The transverse cross-sections treated with 0.5M EDTA showed no structures since the enamel was completely etched away. When a 0.1M EDTA solution was used, the organic material appeared to be concentrated along the rod sheath. Large amorphous globules, covered with pock marks, could be seen in rodless areas. Smaller organic globules, covered randomly with holes could be seen along the tops of some of the rcades. Transmission EMS investigation may reveal more detailed analysis of the ultra fine structure of organic matrix.

Acknowledgments

This research was supported in part at Wichita State University by Wichita State Research Committee and the St. Francis Foundation and in part at Rensselaer Polytechnic Institute by the National Institute of Dental Research under Grant 5T1 DE117-15.

References

Boyde, A., 1967. The Development of Enamel Structure. Proc. R. Soc. Med., 60: 923.

Ducroc, J. and J. P. Proust, 1972. Preservation of Enamel During Decalcification. J. Dent. Res., 51: 851.

Decker, J. D., 1973. Fixation Effects on the Fine Structure of Enamel Crystal-Matrix Relationship. J. Ultrastruct. Res., 44: 58.

Frank, R. M., 1950. Etudes sur l'Infracture Microscopique de l'email et sur la Surface Libre Coronaire de la Dent. Rev. Mens. Suisse d'Odontol., 60: 1109.

Fearnhead, R. W., 1968. Morphological Entity of Enamel Matrix. Archs. Oral Biol., (Special Suppl.) 8: 257.

Glimcher, M. J., U. A. Friberg and P. T. Levine, 1954. Etching of Organic and Inorganic Phases of Enamel Under EDTA. J. Biochem., 93: 202.

Helmcke, J. G., 1967. Ultrastructure of Enamel. Structural and Chemical Organization of Teeth, Vol. II, edited by A. E. W. Miles (Academic Press, New York), 135.

Hoffman, S., W. W. McEwan and C. M. Drew, 1969. Scanning Electron Microscope Studies of EDTA-Treated Enamel. J. Dent. Res., 48: 1234.

Jessen, H., 1968. Elliptical Tubules as Unit Structure of Forming Enamel Matrix in the Rat. Archs. Oral Biol., 13: 351.

Nichol, T., G. Judd and G. S. Ansell, 1971. Two Stage Model for Human Enamel Demineralization as Determined by Scanning Electron Microscope Anaylsis. J. Dent. Res., 52: 487.

Sognnaes, R. F., 1948. Organic Phase of Enamel. J. Dent. Res., 27: 609.

Stack, M. V., 1957. Minerals and Protein Levels in Enamel from Human, Monkey and Rat Molars. Odont. Revy Lund., 8: 89.

Scott, D. B., M. J. Ussing, R. F. Sognnaes and R. W. G. Wyckoff, 1962. Electron Microscopic Studies of Human Enamel. J. Dent. Res., 31: 74.

Scott, D. B. and M. U. Nylen, 1962. Enamel Development in Rat Teeth. J. Int. Dent., 12: 417.

Sundström, B. and T. Zelander, 1968. On the Microphological Organizationof the Organic Matrix of Adult Human Enamel After Decalcification by Means of a Basic Chromium (III) Sulphate Solution. Odont Revy., 19: 249.

Soule, J.D., 1970. The Organic Matrix in Developing Dental Enamel and Dentin Studied by Scanning Electron Microscopy. Bull. So. Calif. Acad. Sci., 69: 104.

Scott, D. B., J. W. Similink and V. Nygaard, 1971. Mineralization of Dental Enamel. Chemistry of Physiology of Enamel (University of Michigan, Ann Arbor, MI), 6.

Swedlow, D. B., R. A. Harper and J. L. Katz, 1972. Sample Preparation of Enamel Samples for Scanning Electron Microscopy. Proc. Worl. Bio. SEM (IIT Research Institute, Chicago, IL), 335.

Scott, D. B., T. W. Similink and V. Nygaard, 1974. Structured Aspects of Dental Caries. J. Dent. Res., 53: 165.

Tomes, J., 1850. On the Structure of the Dental Tissues of the Order Rodentia. Philos. Trans. Roy. Soc. London, 140: 539.

Termine, J. D. and A. S. Posner, 1967. Amorperus/Crystalline Interrelationship in Base Mineral. Calc. Tiss, Res., 1: 8.

Termine, J. D., E. D. Eanes, D. J. Greenfield, M. V. Nylen and R. A. Harper, 1973. Hydrazine-Deproteinated Base Mineral. Calc. Tiss. Res., 12, 73.

Taher, S. M., 2000, A Sample Preparation Technique to Study the Organic Phase of Tooth Enamel Under Scanning Electron Microscopy. J. Mat. Sci. Bull. Vol., 10: 35- 1725-1735.

Watson, M. L., 1960. The Extracellular Nature of Enamel in the Rat. J. Biophys. Biochem. Cylol., 7: 489.

Weber, D., 1965. Phase Microscopic Observations of Rat Incisor Enamel. Am. J. Anat., 117, 233.

Warshawsky, H., 1971. A Light and Electron Microscopic Study of the early Mature Enamel of Rat Incisors. Anat. Red., 169: 559.

MS received 15th May, 2001; Accepted 21st August, 2001