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OBSERVATIONS ON STRUCTURAL FEATURES AND CHARACTERISTICS OF BIOLOGICAL APATITE CRYSTALS
9. OBSERVATION ON DISSOLUTION OF CARIOUS ENAMEL CRYSTALS

BY

Takashi Ichijo, Yasuo Yamashita and Tatsuo Terashima*

Abstract

In a series of studies to investigate the basic structural featues and characteristics of the biological apatite crystals using a transmission electron microscope, we examined the ultrastructre of the human enamel, dentin, and bone crystals through the cross and longitudinal sections at near atomic resolution.

Subsequently, using the same approach, we have been able to directly examine the images of the lattice imperfections in the crystal lattices of the human tooth and bone crystals, and the images of the fusion of the crystals.

In this research, furthermore, using transmission and scanning electron microscopes, we examined the dissolution of the enamel crystals caused by the carious enamel from the same viewpoint. The material used for the observation of the dissolution of the enamel crystals was obtained from the region which corresponds to the middle layer of the enamel at the portion near the wall of a carious cavity caused by the fissure caries on the occlusal surface of the lower first molars.

Small cubes of the materials used for the observation by transmission electron electron microscope were fixed in glutaraldehyde and osmium tetroxide and embedded in epoxy resin using the routine methods. The ultrathin sections were cut with a diamond knife without decalcification. The sections were examined with the HITACHI H-800H type transmission electron microscope operated at 200 kV. Each crystal was observed at an initial magnification of 300,000 times and at a final magnification of 10,000,000 times and over.

The material used for the observation by the scanning electron microscope was the fractured surface obtained from the carious enamel. The fractured carious enamel surfaces were coated with carbon and gold and observed with the HITACHI HHS-2R type scanning electron microscope operated at 25 kV. The crystals were observed at a final magnification of 50,000 times.

As a result, we have confirmed that the dissolution of the enamel crystals caused by a caries occurs in the units of "hexagonal cell". We sincerely believe that the electron micrographs shown in this report are the first to show the images of the dissolution of the enamel crystals caused by a caries at near atomic resolution.

Key words: Apatite crystal, Hydroxyapatite, Enamel caries, Crystal dissolution, Crystal destruction.

Introduction

Recently, several investigators have reported their observations on the lattice imperfections in the biological and synthetic apatite crystals, such as dislocations or defect structures in the crystal lattice either by X-ray diffraction, electron microscope, or other methods [1–19].

Features or processes of the chemical

* 一條　尚，山下靖雄，寺島達夫：Department of Oral Anatomy (Chief: Prof. T. Ichijo), Faculty of Dentistry, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku)

dissolution of the crystals caused by acids or caries and those of the physical destruction caused by electron beam have also been observed [4, 20–24].

However, previous electron microscopic studies of the crystal dissolution or destruction have only been observed at the level of the striations originating from the crystal lattice, and there are many unknown details concerning the dissolution and the destruction of the crystals.

In the preceding study, we examined directly the ultrastructures of the human enamel, dentin, and bone crystals through the cross and longitudinal section at near atomic resolution by using a transmission electron microscope and showed the configuration of the hydroxyapatite structure where composed enamel, dentin, and bone crystals [25–29].

Thereafter, such methods have also been employed in the studies of the images of the lattice imperfection in the human enamel, dentin, and bone crystals, such as the point defect structure, line defect, and face defect [30, 31].

Subsequently, from these materials, the fusion between the adjacent crystals has been directly observed from the same viewpoint [32]. Thereafter, in a series of studies to investigate the basic features and characteristics of the biological apatite crystals such as tooth and bone crystals, detailed observations have been made on the dissolution of the enamel crystals caused by a caries at the same level. Furthermore, in order to comparatively analyze the chemical dissolution of the crystal by the caries and physical destruction of the enamel crystal by an electron beam irradiation, using a transmission electron microscope, ultrathin sections of the normal enamel were exposed to the electron beam and the process of the destruction of the enamel crystal was observed.

In this research, therefore, the dissolution of the carious enamel crystals was directly observed with the transmission and scanning electron microscopes using the same approach. As a result, we confirmed that the dissolution of the enamel crystals caused by a caries occurs in the units of "hexagonal cell", as well as the crystal destruction caused by the irradiation by the electron beam.

Material and Method

The material used for the observation of the dissolution in the enamel crystals caused by a caries was obtained from the slightly yellow-brownish region which corresponds to the middle layer of the enamel at the portion near the wall of the carious cavity caused by the fissure caries on the occlusal surface of the lower first molars. In all cases, the small cubes of the materials used for the observation by a transmission electron microscope, about 1 mm on each side, were cut from the carious region of the occlusal surfaces of the lower first molars using a dental diamond disk.

The small cubes of the material were fixed in 5% glutaraldehyde in sodium cacodylate at pH 7.4, postfixed in cacodylated-buffered osmium tetroxide and embedded in epoxy resin using the routine methods. Great care was taken to record the orientation of the small cubes.

The material used for the observation of the destruction of the enamel crystals caused by the electron beam was taken from the normal enamel of the lower first molar.

The ultrathin sections were cut without decalcification with a diamond knife attached to the Porter-Blum MT-2B type microtome. The sections were examined with the HITACHI H-800H type transmission electron microscope operated at an accelerating voltage of 200 kV. Each crystal was observed through the cross and longitudinal sections of the carious enamel crystals at an initial magnification of 300,000 times and at a final magnification of 10,000,000 times and over.

The ultrathin sections cut from the normal enamel for the observation of the destruction of the enamel crystals caused by the electron beams were irradiated a little longer with the electron beams under an electron microscope (about 10^3 coulomb/cm^2) and were then photographed. The ultrathin sections cut from the normal enamel for the observations of the crystal destruction by the electron beams were examined without additional treatment.

The material used for the observation by a scanning electron microscope consisted of fractured surfaces obtained from the carious enamel. The fractured carious enamel surfaces were coated with carbon and gold and observed with the HITACHI HHS-2R type scanning electron microscope operated at an accelerating voltage of 25 kV. The crystals were observed at an initial magnification of 20,000 times and at a final magnification of 50,000 times.

Results

Fig. 1 shows the representation of the hydroxyapatite structure drawn exactly on the same scale projected vertical to the

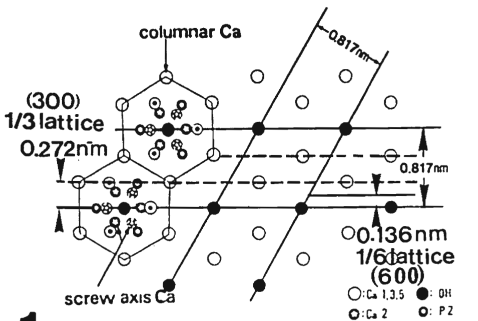

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Fig. 1 shows the representation of the hydroxyapatite structure drawn to the same scale projected vertical to the c-axis.

c-axis upon the basal plane, based on the description of the hydroxyapatite structure by Posner [33] and Kay et al. [34]. Furthermore, in the representation of the hydroxyapatite structure shown in Fig. 1, from our results of the observations on the ultrastructure of the enamel, dentin, and bone crystals [26–28], the 1/3 lattice images at 0.272-nm intervals and especially the 1/6 lattice images at 0.136-nm intervals among the lattice images of the (100) lattice planes spaced at 0.817-nm intervals of the hydroxyapatite structure frequently appearing in the cross and longitudinal sections were added.

In the drawing shown in Fig. 1, however, with the intention of avoiding the complexity, the PO_4 groups in the unit cell were drawn up excluding the sites of O and only the position of the Ca, P, and OH was described.

In the crystal in which the cross section cut vertical to the c-axis of the crystal was observed, the lattice image was observed as the parallel crystal lattices represented by the (100) lattice planes, three sets intersecting each other at a 120-degree angle. The crystal lattice images were spaced at 0.817-nm intervals.

In Fig. 1, the black dotted line represents its 1/3 lattice images. Two 1/3 lattice images appear at equal intervals of 0.272-nm among the lattice images spaced at 0.817-nm intervals. The black line represents the 1/6 lattice of the (600) lattice planes spaced at 0.136-nm intervals in the center of the 1/3 lattice dimension.

As seen in Fig. 1, when the hydroxyapatite structure is observed on the crystals sectioned transversely to the c-axis, the position of the Ca, PO_4, and OH, which constitute the unit cell in the hydroxyapatite structure of the crystal, is arranged in the order of the PO_4 group, screw axis Ca atom, and columnar Ca atom around the central OH group, forming the units of

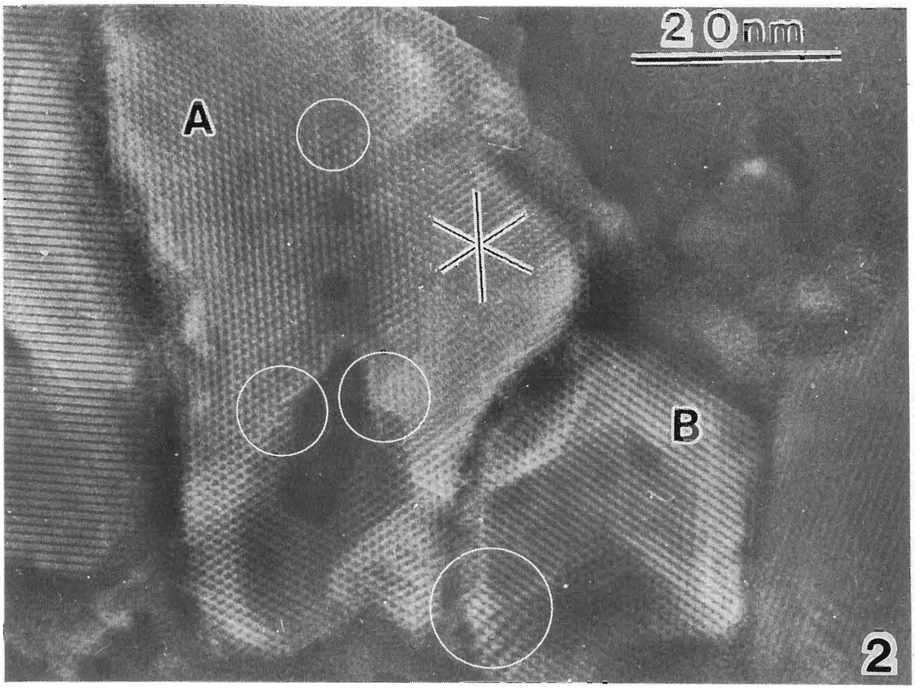

Fig. 2 shows the two crystals "A" and "B" observed in the cross section through the crystals in the carious enamel. In the part indicated by the circle, dislocations are observed.

the hexagons as a whole. These hexagon units are called "hexagonal cell" for the convenience of interpretation in this report.

Fig. 2 shows the two crystals ("A" and "B") observed in the cross section through the crystals in the carious enamel. In the two crystals respectively, there are three-directional crystal lattices of the (100) lattice planes with 0.817-nm intervals.

In the observations of the cross section of the crystals dissolved by the caries, the dissolution is found both on the interfaces and in the inner structure of the crystals. The dissolution occurring in the inner parts is more extensive and severer than that occurring on the interfaces.

As seen in Fig. 2, the transversal area of the dissolved region is largest at the surface of the section and becomes smaller toward the deeper part of the crystal. The dissolution progresses along the c-axis of the crystal, leaving a terraced cavity with an increasing volume.

Although the steps of the cavity have several different shapes, the edges of all steps of the cavity made by the carious dissolution process are expanded along the three-directional lattice images of the (100) lattice planes. No dissolution is observed which progresses along the (110) lattice planes, because the crystal dissolution occurs in the unit of hexagonal cells. In consequence, most of the dissolved portions are shaped like irregular polygons. The crystal dissolution on the surfaces of the cross-sectioned crystals does not always start in the neighborhood of the center of the crystal. Many cases are found in which the dissolution is most advanced at the parts near the edge of the crystals as in the case shown in Fig. 2.

Some such crystals are dissolved along their c-axis, leaving the terraced cavities with an increasing area, so that the cavities open on the side surfaces of the crystals. Fig. 2 includes an example of such a crystal (crystal "B").

Some observations suggest that as the internal dissolution proceeds preferentially at some points and the terraced cavity is enlarged, one dissolved part is connected with the neighboring one to form an irregular polygon.

The dissolution is also proceeding on the interface between crystals "A" and "B" in Fig. 2. A part of the crystals "A" and "B" in Fig. 2, where the dissolution is most advanced, is shown in Fig. 3 at a high magnification. In Fig. 2, in the part indicated by the circle, the dislocations are observed. In the edge region of the most advanced part, the lattice images are slightly shifted and tilted, a fault is seen, and the dislocation is observed. In Fig. 3, the slip planes are indicated with an arrow and letter S.

In the edge region of the part where the dissolution is most progressed, the dislocation is observed. The dislocations are also seen at the spot-like portions of the crystal "A" and a part of the interface of crystals "A" and "B" where the dissolution is preferentially proceeding.

Fig. 4 shows one cross-sectioned carious enamel crystal at a high magnification. In Fig. 4, the deepest region of the terraced cavity has only one hexagonal cell dissolved to leave a hollow area (arrow). Fig. 5 is an even higher magnification of that region in Fig. 4. The drawing of the hydroxyapatite structure shown in Fig. 1 was placed on the top of this electron micrograph.

From these observations, it was recognized that when the enamel crystals are dissolved by a caries, one hexagonal cell dissolves first preferentially at some por-

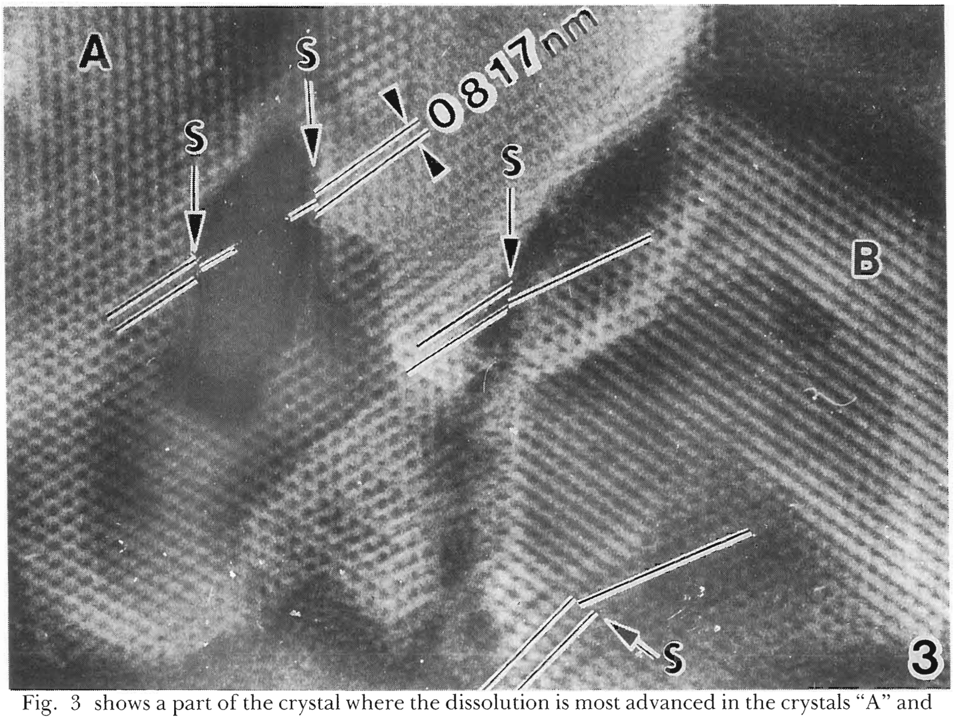

Fig. 3 shows a part of the crystal where the dissolution is most advanced in the crystals "A" and "B" shown in Fig. 2 at an even higher magnification. The area shown with the arrow S indicates the slip plane.

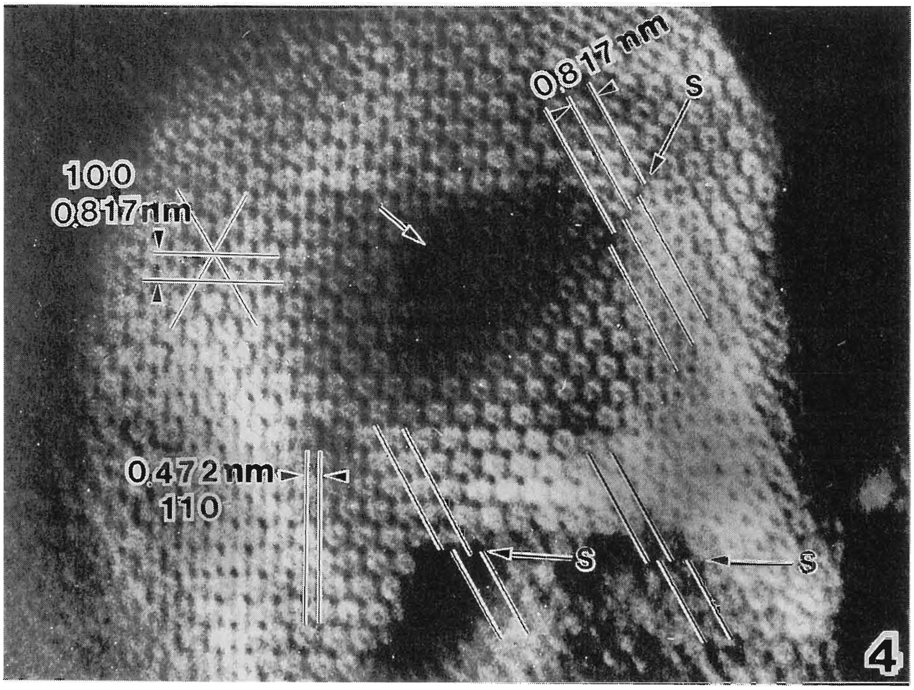

Fig. 4 shows one cross-sectioned carious enamel crystal at a high magnification. The deepest region of the terraced cavity caused by the dissolution of the caries has only one hexagonal cell dissolved to leave a hollow area (arrow). The arrow S indicates the slip plane.

tion in the crystals, after which they induce the dissolution of the adjacent hexagonal cells and the dissolution is expanded into the adjacent hexagonal cells, as well as the crystal destruction caused by the electron beam damage.

In the inner structure of a carious enamel crystal as shown in Fig. 4, the dislocations are also observed. The areas marked with an arrow and letter S indicate the slip planes. In the regions of the dislocation, the lattice images are slightly discontinuous and displaced, and the hexagonal cells are slightly disturbed. The dissolution of the crystal is progressing preferentially at these regions along the dislocations.

Figs. 6 and 7 show a scanning electron micrograph observed in the cross section (Fig. 6) and longitudinal section (Fig. 7) through the crystals in the fractured surface obtained from the carious enamel.

The observation of the cross section of the enamel crystals dissolved by the caries reveals many cavities formed as a result of the dissolution of the enamel crystals. In the cavities formed by the dissolution of the enamel crystals, there are also many crystals remaining only on the crystal surface of the cross section of the enamel crystals.

There are also the cavities opening onto the lateral surface of the enamel crystals. These cavities are formed as a result of the progression of the crystal dissolution from the surface of the cross-sectioned enamel crystals to the lateral surface. The carious enamel crystals showing dissolution only on the lateral surface of the enamel crystals can be seen.

When observed along the surface of the longitudinally sectioned crystals, some

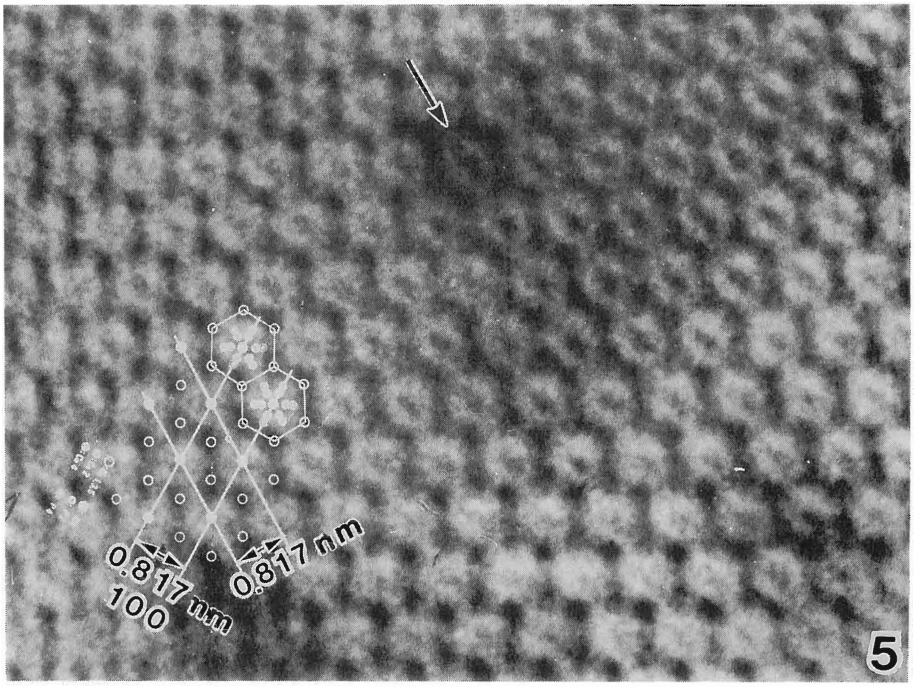

Fig. 5 is an even higher magnification of the region dissolved by the caries in Fig. 4. The drawing of the hydroxyapatite structure shown in Fig. 1 was placed on the top of this electron micrograph.

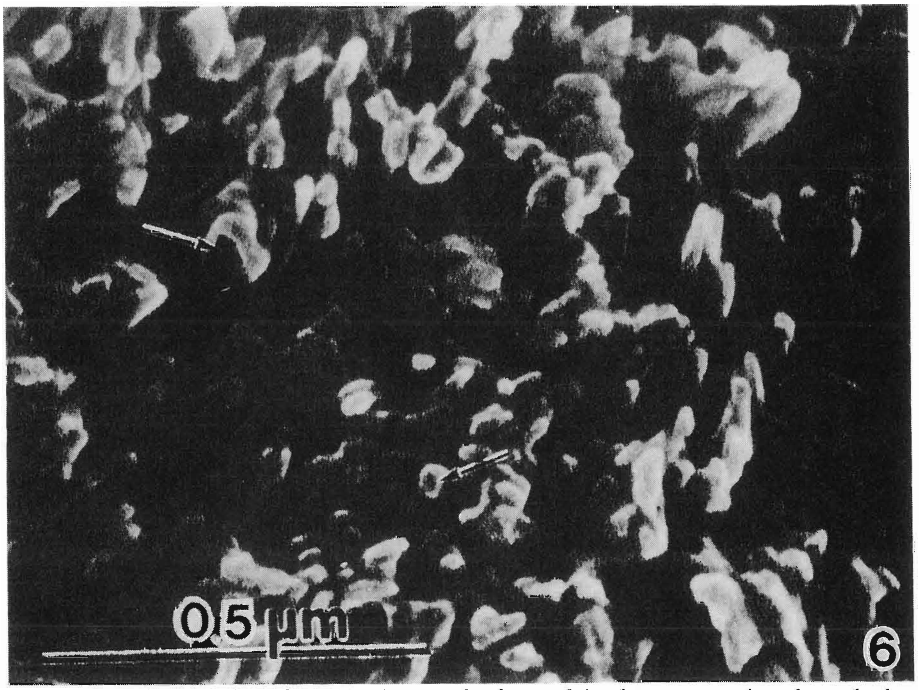

Fig. 6 shows the scanning electron micrograph observed in the cross section through the crystal in the carious enamel.

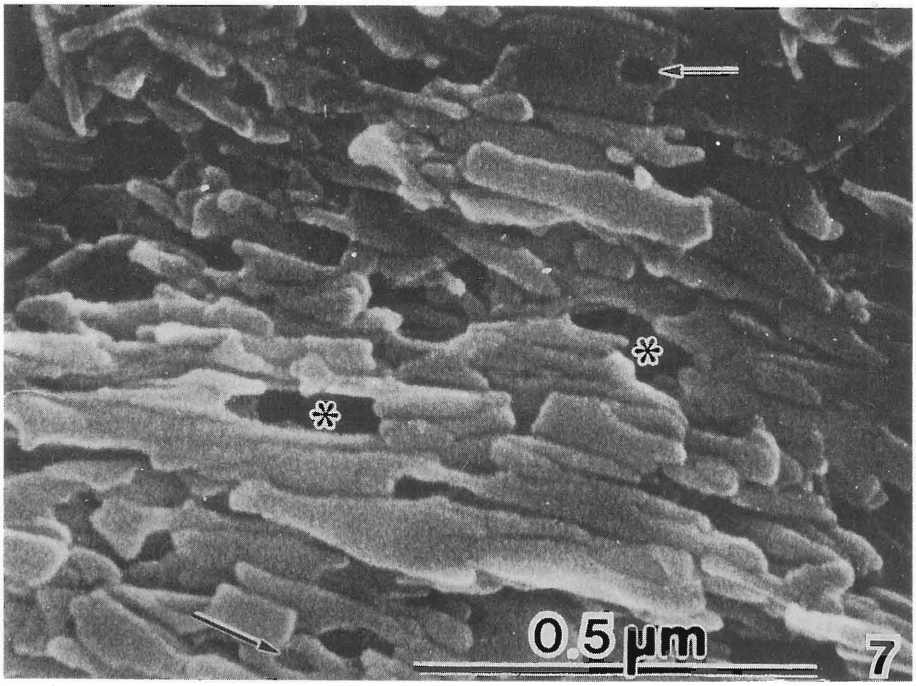

Fig. 7 shows the scanning electron micrograph observed on the longitudinal section of the carious enamel crystal.

crystals show progression dissolution along the c-axis of the crystal structure, while the other crystals show the dissolution of a part of the lateral surface of the enamel crystals. In a region where several crystals are in contact with each other, the adjacent crystals have dissolved at the site of contact in some specimens (marked with a "*").

In order to comparatively analyze the chemical dissolution of the enamel crystal caries and physical destruction of the enamel crystal by an electron beam, sections of the normal enamel crystal were exposed to the electron beam and the process of the destruction of the enamel crystal was observed.

In the crystal cross sections represented in Figs. 8 and 9, the lattice images are observed as parallel crystal lattices of the (100) lattice planes with 0.817-nm intervals, three sets intersecting each other at a 120-degree angle.

Beside there lattice images, the crystal lattices of the (110) planes with 0.472-nm intervals are observed.

At the part damaged by the irradiation of the electron beams, the crystals are distracted and have holes at some sites.

As seen in Figs. 8 and 9, when the crystal structure is destroyed by an electron beam, the changes in the crystal are expanded along the crystal lattice of the (100) lattice planes, three sets intersecting each other at a 120-degree angle, thus when viewed down the c-axis, damaged or changed regions are triangular, square, pentagonal, hexagonal, or irregular polygonal in shape. But as seen in Figs. 8 and 9, the changes in the crystals are scarcely detectable along the crystal lattices of the (110) lattice planes.

In the part marked with an "A" in Fig. 9, only a single hexagonal cell is destroyed. As above, when the crystal structures are

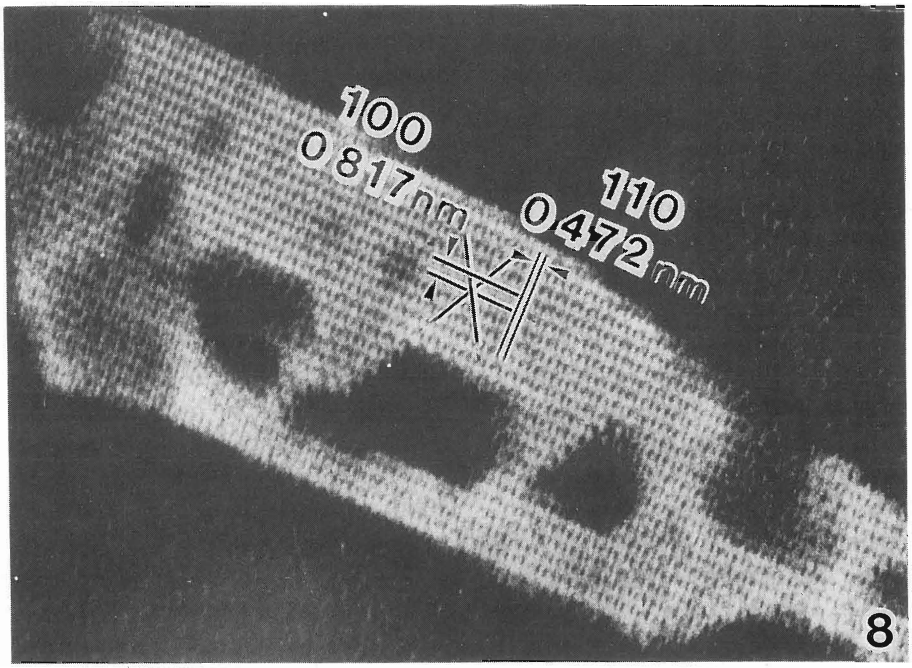

Fig. 8 shows the cross-sectioned enamel crystal damaged by the electron beam irradiation.

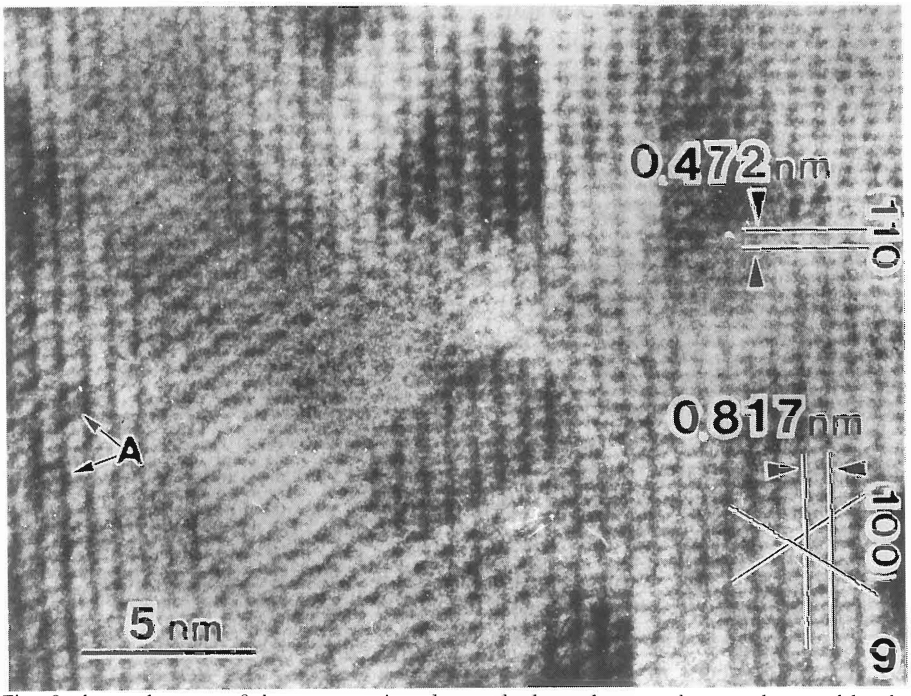

Fig. 9 shows the part of the cross-sectioned crystal where the crystals were damaged by the electron beam irradiation. In the part indicated by "A", only one hexagonal cell is damaged by the electron beam.

destroyed by the electron beam, the crystal destruction occurs in the unit of the hexagonal cells, as well as the crystal dissolution caused by the caries.

Discussion

It is well recognized that the acid-etched crystals and carious enamel crystals are dissolved from the center of each crystal to make the central holes.

Tyler [35] performed etching on the polished fluorapatite crystals with organic acids or EDTA and Jongebloed et al. [23, 36] treated the crystals of fluorapatite and hydroxyapatite with organic acids. Their observations of the etchpits using a scanning electron microscope revealed that the etchpits were perpendicular to the c-axis of the crystals. They also described that the dissolution proceeded parallel to the c-axis. They reported that the etchpits proceeded preferentially along the dislocation lines paralle to the c-axis of the crystals. They suggested that the dislocations and defects would work as the starting point for the dissolution process in the enamel caries.

Arends [37] also remarked on the same mechanism for the dissolution of the enamel crystals.

Many investigators have observed the crystals in the carious enamel and acid-treated crystals with a transmission electron microscope, and again, the dissolution from the central part and the formation of a central hole were observed. Furthermore, the preferential progression of the dissolution along the c-axis was recognized in the acid dissolution, too.

The electron beam was also found to make the small defects as small spots or voids, and the fact that the destruction originated by the defects in the crystals and that it tended to proceed along the c-axis were reported.

Based on the above observations, the dissolution of the crystals is considered to be initiated at the dislocations and defects. Though these former observations of the lattice imperfections and crystal destruction using a transmission electron microscope were aimed at the striations originating from the crystal lattice, there have been no published observations directly revealed at the level of near atomic resolution.

In the previous study, at first, we observed directly the ultrastructure of the normal crystals at near atomic resolution with a transmission electron microscope. Then, in recent studies, we observed the lattice imperfections in the normal enamel, dentin, and bone crystals [30, 31].

In this research, the observations were made on the dissolution of the crystals in the carious enamel and the destruction of the crystals by the electron beam, also to the level of near atomic resolution.

As a result, our observations found in the former research show that the defects and surfaces of the crystals were structurally unstable, and thus being the weak point against destruction, and that these parts are preferentially destructed by the electron beam and caries.

Frazier [21] and Swancar et al. [38] observed that acid-treated enamel crystals are dissolved in their center parts, and some of them have a C-, J-, or U-shaped dissolution.

We also observed many carious enamel crystals dissolved in such fingers as seen in Fig. 6. However, when the carious enamel crystals are examined with a higher magnification, all such crystals are found to be dissolved as the hexagonal cell units, just as in the case of the electron beam destruction. The dissolution of the crystals spreads along the three-directional lattice images of the (100) planes on the plane of the cross-sectioned crystals.

As in the electron-beam destruction, the dislocations in the carious enamel crystals are the weak points against dissolution. The dissolution of the crystals proceeds preferentially along the dislocations as seen in Figs. 2, 3, 4, and 5.

In Figs. 4 and 5, a single hexagonal cell at the position marked with an arrow is dissolved preferentially. Certain defect such as vacancy or substitution of the atom or group (Ca, OH, or PO_4) may have existed in the hexagonal cell.

From the above-mentioned results, the crystals are considered to have as the hexagonal cell units in the dissolution by the caries, as well as in the destruction by the electron beams. The lattice imperfections such as dislocations and defects are the initiation points of the crystal dissolution, being dissolved preferentially, and the dissolution of the hexagonal cell units successively spreads from that position.

As seen in Figs. 4 and 5, at the portion subjected to dislocation, the "hexagonal cells" are morphologically distorted, and at the dislocation boundary there occurs a change in the arrangement of the spot structures which compose the hexagonal cells. Also, the portion with the dislocationis apt to be damaged preferentially by the electron beams.

Furthermore, in a series of studies, we observed that the destruction of the enamel crystals caused by the electron beam irradiation is similar to that caused by the caries. When the crystal structures are destroyed by the electron beams or by the caries, the destruction occurs in the unit of the hexagonal cells, and consequently the changes in the crystal are expanded along the (100) lattice planes; thus, when viewed down the c-axis, the damaged or changed regions are triangular, hexagonal, or irregular polygonal in shape.

We sincerely believe that the electron micrographs shown in this report are the first to show the image of the dissolution of the enamel crystals caused by the caries and the destruction of the normal crystals caused by the electron beam irradiation at near atomic resolution.

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