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FINE STRUCTURE OF THE INNER ENAMEL IN HUMAN PERMANENT TEETH

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Abstract

Using SEM after EDTA etching, the mid-coronal inner enamel of human permanent teeth was classified into three regions of the 1st, 2nd, and 3rd zones. The 1st zone showing a highly negative birefringence was the innermost 10 - 15 µm enamel. This zone consisted of arcade and circular initial prisms, and the succeeding arcade prisms only. These initial prisms arising perpendicularly to the dentine surface resembled pseudoprisms because these prisms showed a somewhat centripetal arrangement of crystallites and indistinct prism boundaries. The succeeding prisms were frequently bent following a faint slit within the prism. The 2nd zone adjacent to the 1st zone measured 20 - 40 µm in thickness. This zone was mainly composed of horseshoe-shaped prisms with EDTA-insoluble prism sheaths in the deep-etched prism boundaries, but the inner-half layer had dotted irregular prisms including circular, double marginal, and spiral shapes with the prism sheaths. Prismless structures were rarely seen in the 2nd zone. The 3rd zone was mainly occupied by horseshoe-shaped prisms without EDTA-insoluble prism sheaths in the deep-etched prism boundaries, although tuft prisms in the 3rd zone contained a large amount of EDTA-insoluble substances in the prisms, interprismatic regions, and the boundaries.

KEY WORDS. inner enamel, EDTA etching, scanning electron microscopy, prism arrangement, prism structure, initial prism, irregular prism, EDTA-insoluble substance, prism sheath, enamel tuft, prismless enamel.

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Introduction

In human permanent teeth, Gustafson and Gustafson [1967] reported that it was often impossible to distinguish individual prisms in the innermost enamel which had therefore a more or less homogeneous structure by polarized light.

By means of ground sections which were roughly tangential to the surface of a human tooth with serial focussing transmitted light, Osborn [1968, 1973] divided the mid-coronal inner enamel into three Zones: I, II, and III. Zone I was very close to the dentine-enamel junction (DEJ), and was a structureless layer of variable thickness up to about 5 µm from the DEJ; Zone II adjacent to Zone I, measuring about 20 µm in thickness, showed variable shapes of prisms such as U-shaped, circular, and spiral or double borders, that is one sheath inside another; and Zone III measuring up to 50 or 60 µm in thickness showed Pattern 2 prisms. In addition, Boyde [1965, 1976] classified enamel prisms into Patterns 1, 2, and 3 based on prism shapes and arrangements.

Osborn [1968, 1970, 1973] believed that there were no prism boundaries or poorly defined structures in Zone I. However, Hinrichsen and Engel [1966] showed prism boundaries in the innermost human enamel adjacent to the DEJ by transmission electron microscopy (TEM) of decalcified sections. Swancar et al. [1970] reported by using TEM on the replicas that irregular prism boundaries including circles, concentric circles, and spirals were most frequently observed in the area near the DEJ. Whittaker [1978] used scanning electron microscopy (SEM) after acid etching and observed enamel prisms to be closely associated with the dentine in human teeth, but there was a zone of altered enamel about 20 µm in width in contact with the DEJ. Similar images were reported by Kodaka [1978] using SEM after EDTA etching.

Recently, Boyde and Jones [1983], Boyde et al. [1988] and Boyde [1989] showed prism-free layers close to the dentine in human enamel using backscattered electrons of SEM. Fejerskov and Thylstrup [1986] reported that there was an aprismatic layer in the innermost enamel of a human tooth in SEM of the fractured surface. Nevertheless, Fejerskov and Thylstrup [1986] and Boyde [1989] also showed prism-like structures on the dentine surfaces in developing human teeth with SEM.

It is generally accepted that EDTA etching selectively dissolved prism boundaries [Hoffman et al., 1969; Johnson et al., 1971; Simmelink et al., 1974; Kodaka et al. 1989a, 1990b]. On the other hand, the inner enamel associated with enamel tufts were apt to be insoluble in EDTA [Weatherell et al., 1968; Weidemann and Eyre, 1971; Kodaka, 1978; Kodaka and Debari, 1982] or the tufts only [Amizuka and Ozawa, 1989; Robinson et al., 1989]. In the present study, the mid-coronal inner human enamel was investigated with SEM after EDTA etching.

Materials and Methods

Twenty-two human caries-free permanent premolars extracted from orthodontic patients aged 9 to 12 years were fixed in 10 % neutral formaldehyde for about one week and then were rinsed in running tap water. Three teeth were longitudinally sliced and 80 - 120 µm thick ground sections were prepared and observed under an Olympus polarized light microscope. Mid-coronal enamel of fourteen teeth was sliced about 1 mm thick at about 10 - 20^{o} angle to the transverse plane of a tooth with a diamond wheel, for the purpose of obtaining transverse prism planes in the inner enamel of a buccal side [Swancar et al., 1970]. The cuspal-side planes were polished with 0.3 µm alumina on polishing cloth and treated with ultrasonic cleaning in distilled water. Three specimens were observed with reflected light through a differential interference contrast (DIC) in a Zeiss photomicroscope III after coating with 10 - 15 nm thick platinum-palladium (Pt-Pd) in an Eiko IB-3 ion-sputtering apparatus in order to increase the reflected light values.

The ground enamel planes of the remaining eleven slices were etched with 2 % ethylene diamine tetraacetic acid (EDTA) at pH 7.2 for 15 minutes [Kodaka, 1978; Kodaka and Debari, 1982]. This was followed by rinsing in running tap water for one hour then dehydrating with ethanol. The mid-coronal enamel of the remaining five teeth were fractured transversely. They were ultrasonically cleaned in distilled water and dehydrated with ethanol. All specimens (in the inner enamel of a buccal side) were observed under a Hitachi S-430 scanning electron miucroscope (SEM) operated at 20 kV after critical point drying with CO_2 in a Hitachi HCP-2 critical point dryer and coating with a 10 - 15 nm thick Pt-Pd layer.

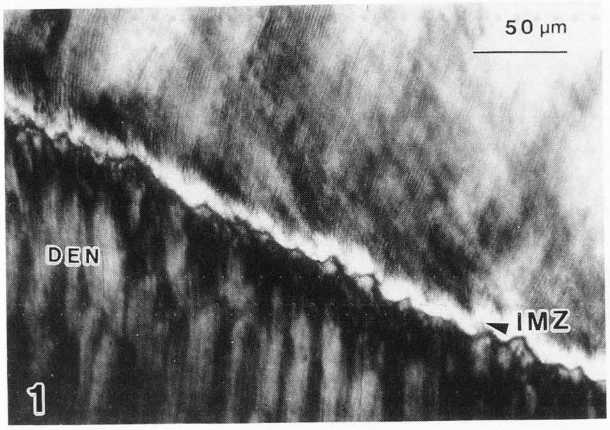
Results

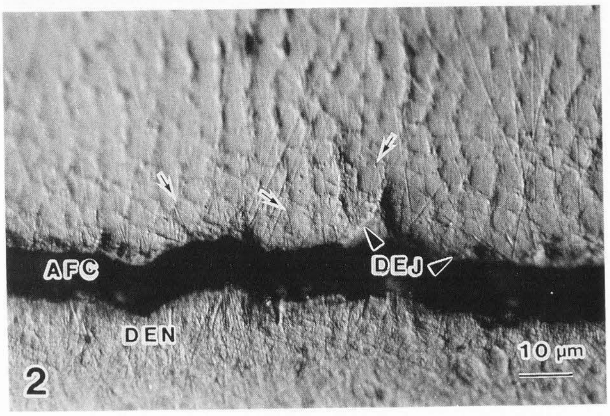
Using polarized-light microscopy with the longitudinal ground section of a human permanent tooth, a highly negatively birefringent layer measuring about 10 - 15 µm in thickness was observed in the innermost enamel covering the DEJ (Fig. 1). Prism structures were not found in the inner-half layer adjacent to the DEJ, but frequently seen in the outer-half layer. By reflected light through the DIC (Fig. 2), however, prism shapes were faintly observed in the inner-half layer of the transverse ground plane.

When transverse ground planes of the mid-coronal enamel were observed under the SEM following EDTA etching, the inner enamel layer (other than enamel tufts) was roughly classified into three regions of the 1st, 2nd, and 3rd zones (Fig. 3). The 3rd zone over about 30 - 50 µm distance from the DEJ was occupied by horseshoe-shaped prisms selectively dissolved in the peripheries with EDTA etching (Figs. 3 and 4). This zone basically showed Pattern 2 prisms.

The 1st zone was the innermost 10 - 15 µm enamel covering the DEJ and almost agreed with the highly negatively birefringent layer (Fig. 1). In the inner-half 5 - 10 µm layer, initial prisms were found (Figs. 5 and 6). The interprismatic regions showed a stronger resistance to EDTA than the prism bodies although these peripheral regions were selectively dissolved, so that the boundaries of the initial and the succeeding prisms indistinctly appeared in the 1st zone. These prisms basically showed an arcade shape (Figs. 5 and 13 - 15), although the prisms were more or less enclosed by the interprismatic regions. An indistinct circular shape, arising perpendicularly to the dentine surface, frequently appeared in the area closely surrounded by a smaller and deeper dome-shaped excavation of the DEJ (Fig. 6).

The 1st zone showed Pattern 3 prisms in the arcade prisms (Figs. 5, and 13 - 15) or Pattern 1 prisms in the circular prisms (Fig. 6). Crystallites in the initial prisms tended to show a





Figs. 1 and 2. Light-microscopic photographs of the inner enamel of human teeth.

Fig. 1: Polarized-light image in a longitudinal ground section. The innermost zone (IMZ) shows a highly negatively birefringent layer.

Fig. 2: DIC reflected-light image in a transverse ground plane (Fig 2). Arrows: prism-like structures, DEJ: dentine-enamel junction, DEN: dentine, AFC: artifactual crack.

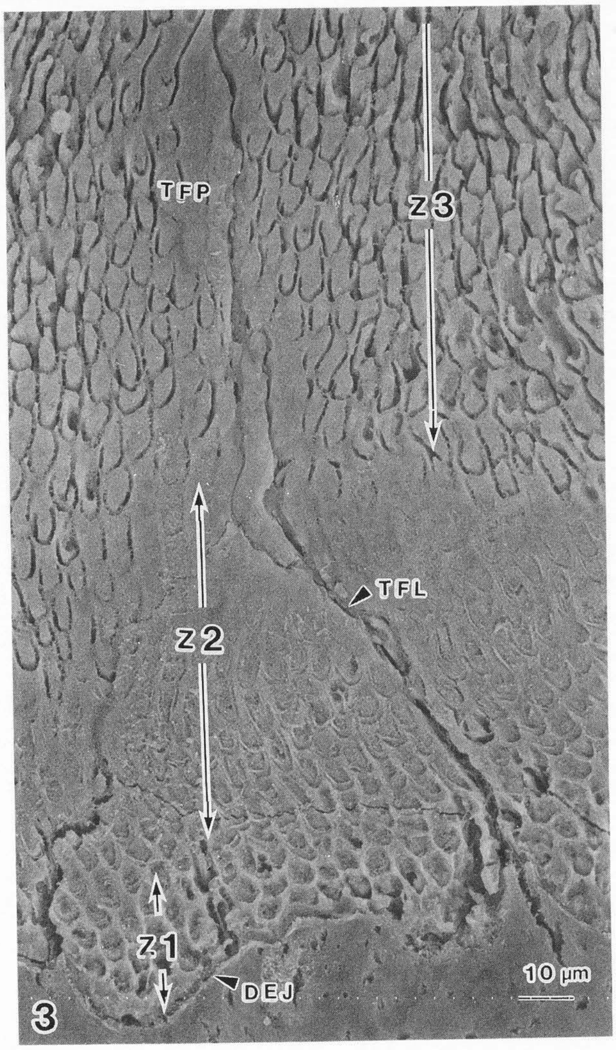
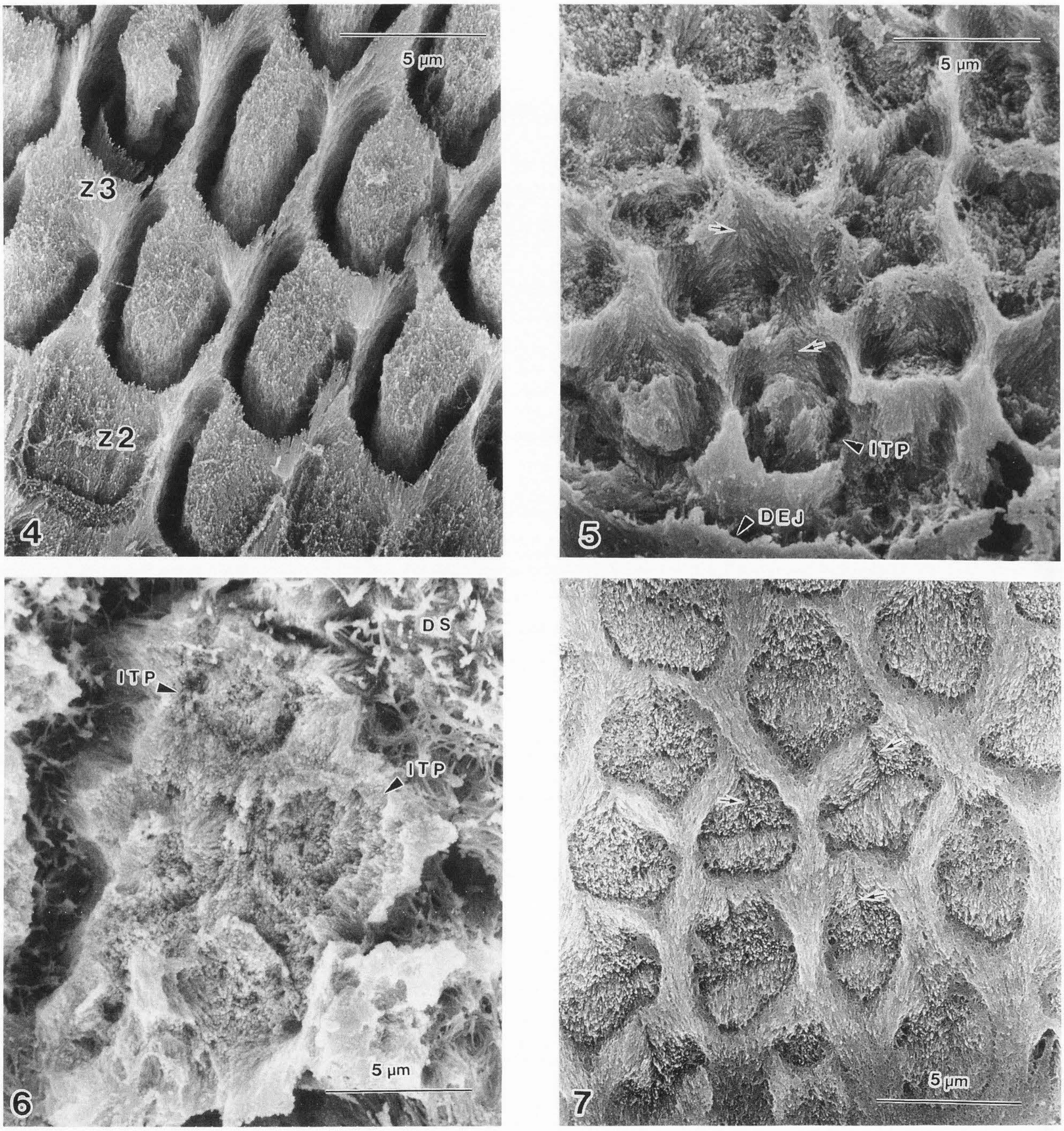


Fig. 3. A low magnification SEM micrograph of the inner enamel in a transverse ground plane etched with EDTA.

The 1st zone (Z1), the 2nd zone (Z2), and the 3rd zone (Z3) were roughly distinguished. DEJ: dentine-enamel junction, TFL: lamella-like structures of an enamel tuft, TFP: tuft prism.

centripetal arrangement (Fig. 5), especially in circular prism structures (Fig. 6). The c-axis crystallites in the prism central cores gradually changed towards the direction of the long axis of the prisms, and subsequently towards the prism peripheries. The succeeding arcade prism in the 1st zone had frequently an I (Fig. 5) or a T-shaped faint slit (Fig. 7) within the prism at about 5 - 10 µm from the DEJ.

The initial and the succeeding prisms in the 1st zone were also revealed by means of fractured methods (Fig. 8), although the prism shapes were not always clearly seen (Figs. 9 and 10). However, the prism structures enclosed by interprismatic regions, which probably include indistinct prism boundaries, were faintly distinguished by a fern-shaped crystallite arrangement in the prisms (Figs. 8 and 9). The initial prisms more or less arised perpendicularly to the dentine surfaces. Frequently, the prisms were abruptly bent at about 10 µm from the DEJ (Fig. 10).



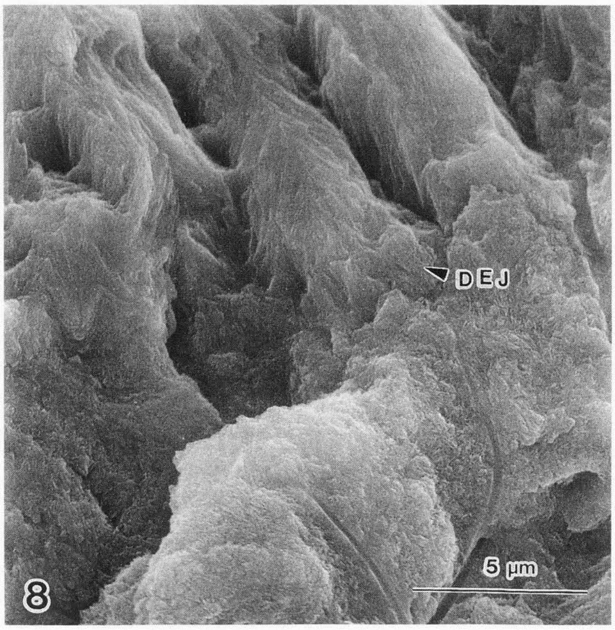
Figs. 4 - 7. SEM micrographs of the inner enamel in transverse ground planes etched with EDTA.

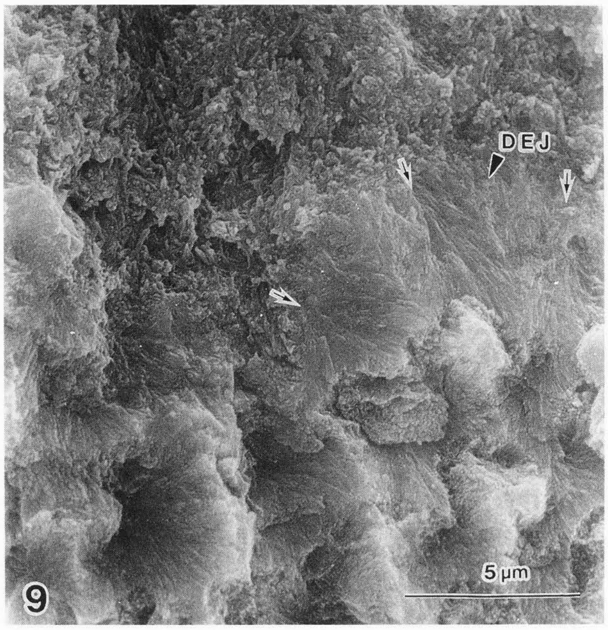
Fig. 4: The 2nd zone (Z2) adjacent to the 3rd zone (Z3).

Figs. 5 and 6: Initial prisms (ITP) basically show an arcade (Fig. 5) and a circular shape (Fig. 6) in the 1st zone.

Fig. 7: The 1st zone at 5 - 15 µm from the DEJ (below). Arrows in Figs. 5 and 7 show I and T-shaped faint slits. DEJ: dentine-enamel junction, DS: dentine surface.

The 2nd zone situated between the 1st and the 3rd zone was occupied by prisms possessing EDTA-insoluble prism sheaths in the deep-etched prism boundaries (Figs. 3, 11, and 13 - 15). This zone measured about 20 - 40 µm in thickness. The EDTA-insoluble prism sheaths were similar to prism sheaths of tuft prisms (Figs. 11 and 12), but the

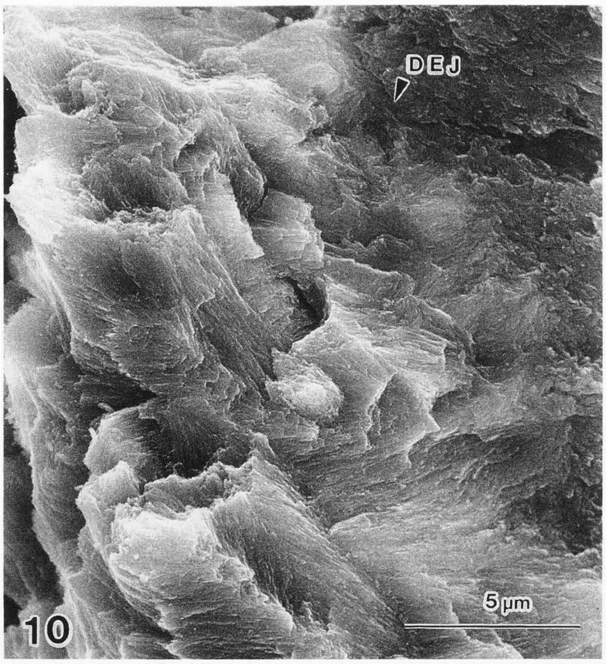




prisms and interprismatic regions in the 2nd zone were more or less strongly attacked by EDTA etching as well as those of the 1st zone, so that the prism sheaths lingered in the EDTA-etched plane (Fig. 11). On the other hand, in tuft prisms, large amounts of EDTA-insoluble substances were contained within the prisms and interprismatic regions as well as in the prism boundaries so that they existed in almost the same EDTA-etched plane (Fig. 12).

The 2nd zone was mainly occupied by horseshoe-shaped prisms, but circular, spiral, and double marginal prism structures were dotted here and there (Figs. 14 and 15). These irregular prisms existed in the inner-half 10 - 20 µm rather than in the outer-half 10 - 20 µm layer. The horseshoe-shaped prisms basically showed Pattern 2 prisms (Figs. 11 and 13), although the area containing variable shapes of irregular prisms generally showed Pattern 3 prisms (Figs. 14 and 15). The Pattern 3 prisms changed into Pattern 2 prisms towards the 3rd zone.

In two specimens, a 'prismless' enamel containing striations with a periodicity of about 5 µm was observed in an area enclosed by a wider and deeper dome-shaped excavation of the DEJ (Fig. 16). The 'prismless' areas showed a triangle shape and corresponded with the inner-half layer of the 2nd zone although the EDTA-insoluble prism sheaths were not visible. The size measured approximately 15 - 20 µm in thickness and width.

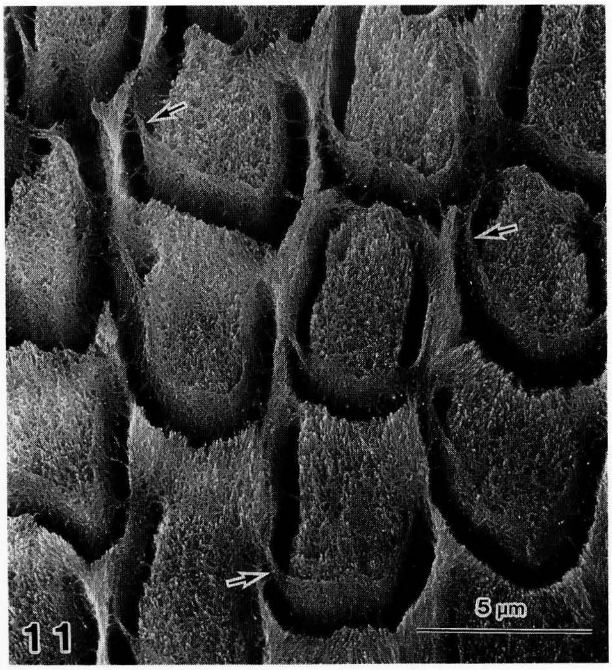


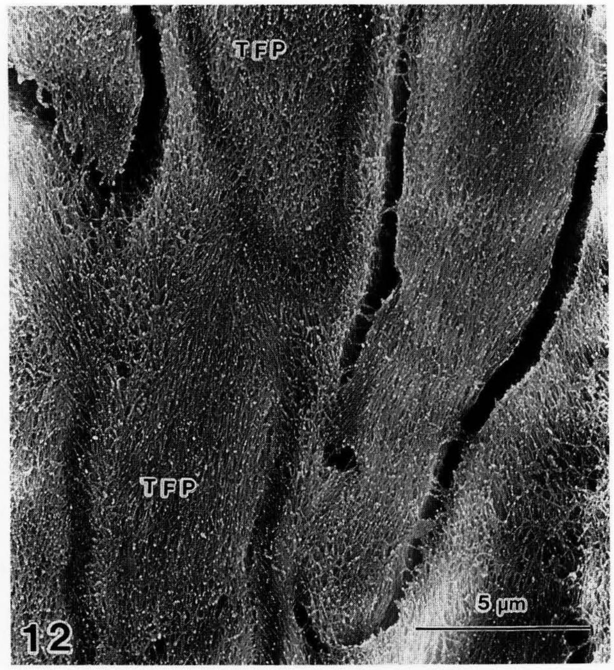
Figs. 8 - 10. SEM micrographs of the innermost enamel in fractured surfaces.

Fig. 8: Initial prisms adjacent to the DEJ.

Fig. 9: Indistinct prism boundaries (arrows) containing the interprismatic regions.

Fig. 10: Abrupt bend of prisms in the outer-half layer of the 1st zone. DEJ: dentine-enamel junction.





Figs. 11 and 12. SEM micrographs of the inner enamel containing EDTA-insoluble substances in transverse ground planes.

Fig. 11: Prisms in the 2nd zone at an about 25 - 35 µm from the DEJ. The arrows indicate EDTA-insoluble prism sheaths.

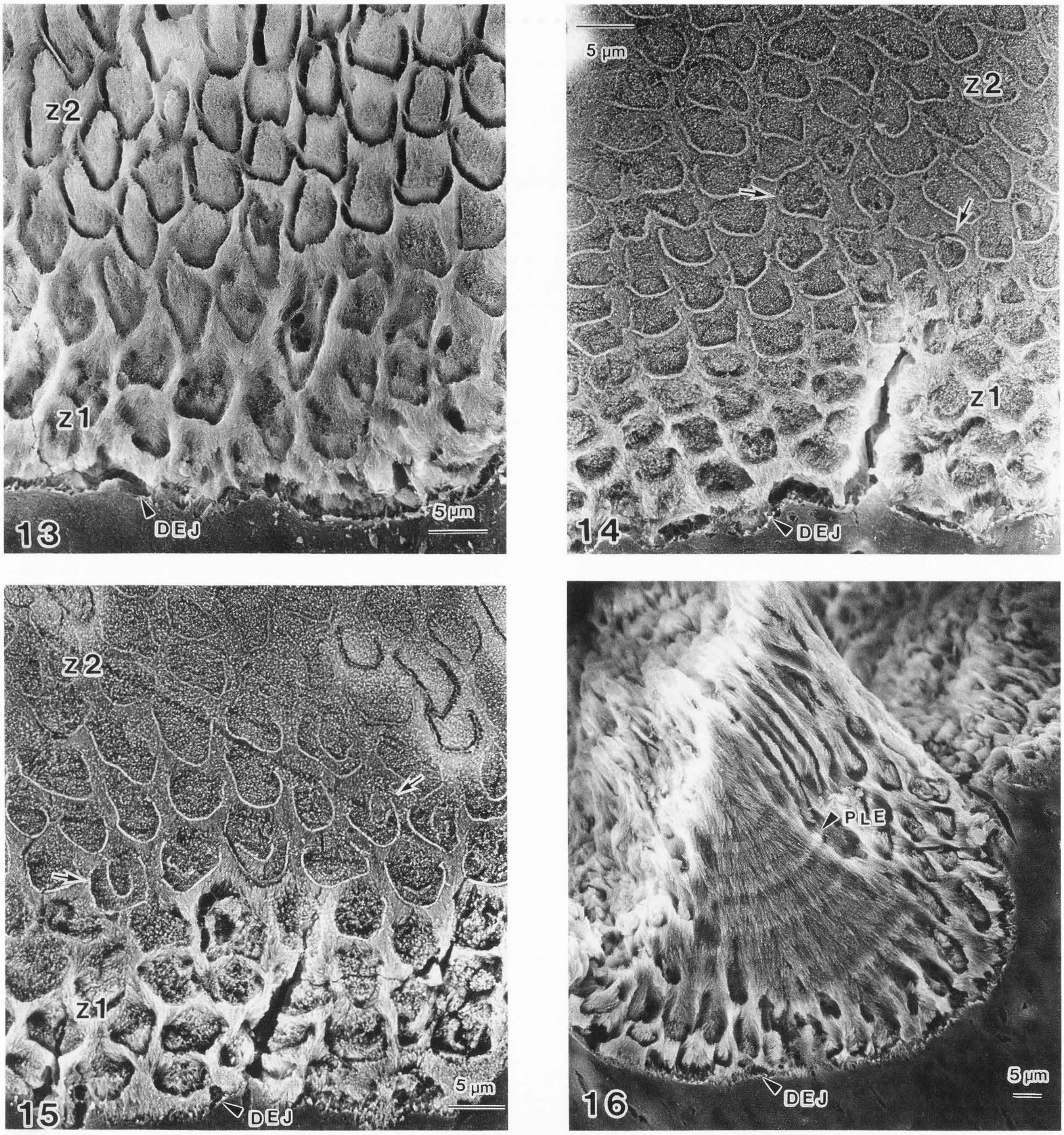
Fig. 12: Prisms in an enamel tuft and the surrounding enamel at about 60 - 70 µm from the DEJ. TFP: tuft prism.

Discussion

It has been reported that the innermost enamel close to the DEJ in human permanent teeth appears homogeneous under polarized-light microscopy [Gustafson and Gustafson, 1967], structureless by transmitted-light microscopy [Osborn, 1968, 1973], prism-free in ground longitudinal sections examined by backscattered SEM [Boyde and Jones, 1983; Boyde et al., 1988; Boyde, 1989], and also aprismatic in fractured specimens examined by SEM [Fejerskov and Thylstrup, 1986]. In addition, Warshawsky et al., [1981] initial enamel showed the innermost enamel of rat incisor aprismatic in TEM.

In this SEM study of human teeth, 'prismless' or aprismatic enamel [Gwinnett, 1967; Whittaker, 1982; Kodaka et al., 1989a, 1990b] was rarely seen in some areas of the inner enamel (Fig. 16), and could not be detected in the 1st zone adjacent to the DEJ. The SEM following EDTA etching data presented with the paper revealed prism structures in the 1st zone (Figs. 3, 5, and 13 - 15) similar to previous TEM [Hinrichsen and Engel, 1966; Palamara et al., 1989] and SEM studies [Kirino et al., 1972; Kodaka, 1978; Whittaker, 1978]; and moreover, the DIC reflected-light microscopy also showed prism-like structures in this zone (Fig. 2). These structures may be due to physical resistance to grinding between prism bodies and the interprismatic regions on the enamel ground plane.

In our previous study using energy dispersive electron probe microanalysis and the mid-coronal enamel of young human permanent teeth [Kodaka et al., 1990a], the innermost enamel at 5 - 7 µm from the DEJ (the 1st zone) contained significantly lower Ca concentration than that inner enamel layer situated at about 20 µm from the DEJ (the 2nd zone). Therefore, the highly negative birefringence in the 1st zone (Fig. 1) is not due to the presence of a 'prismless' enamel layer shown in the surface enamel of human deciduous and permanent teeth [Ripa et al., 1966; Gwinnett, 1966, 1967] and a hypermineralized layer [Losee et al., 1957; Gustafson and Gustafson, 1967;



Figs. 13 - 16. SEM micrographs of the inner enamel in transverse ground planes etched with EDTA.

Figs. 13 - 15: The 1st zone (Z1) and the 2nd zone (Z2). Irregular prisms (arrows) are visible in the 2nd zone (Figs. 14 and 15).

Fig. 16: Inner 'prismless' enamel (PLE) enclosed by a wider and deeper dome-shaped excavation of the DEJ. A fine incremental lamination is seen. DEJ: dentine-enamel junction.

Suga, 1987b], but may be due to the disorientations of crystallites [Osborn, 1973; Whittaker, 1978], so that this innermost zone should have low mineral content.

EDTA etching selectively dissolved the prism boundaries in the inner enamel over 30 - 50 μm from the DEJ (the 3rd zone) as observed in many previous studies, except for the prisms of enamel tufts shown in Figures 3 and 12 [Weatherell et al., 1968; Kodaka, 1978; Kodaka and Debari, 1982; Amizuka and Ozawa, 1989]. However, EDTA etching pattern of prisms in the inner 30 - 50 μm enamel (the 1st and 2nd zones) more or less differed from that of the 3rd zone.

In the 1st zone, EDTA etching dissolved more prism bodies than interprismatic regions (Figs. 5 and 6). Simmelink et al. [1974] described that the initial attack of EDTA to the enamel depended on the size of the spaces between crystallites and the tightly packed crystallites were not easily soluble. It is, therefore, suggested that EDTA etching particularly dissolves the low mineral content and disordered crystallites. In this SEM study, the crystallites of prism bodies showed a more or less centripetal arrangement in the 1st zone [Palamara et al., 1989], and the crystallite orientation in the boundary areas between the prisms and the interprismatic regions might be considerably disordered because the areas were selectively dissolved (Figs. 5 and 6).

When a 'prismless' enamel exists in the innermost layer of human teeth, its thickness may be less than 1 or 2 μm [Rønnholm, 1962; Fejerskov and Thylstrup, 1986; Boyde, 1989]. The thin 'prismless' structures might be formed during an initial formation of Tomes' process. The centripetal crystallite arrangement in the initial prisms showing an arcade (Fig. 5) or a circular shape (Fig. 6) will be produced by the cone-like shape of Tomes' process at the initial stage of prism formation [Osborn, 1970, 1973; Boyde, 1976; Osborn and Hillman, 1979; Wakita et al., 1981; Warshawsky et al., 1981; Lester, 1989; Lester and Koenigswald, 1989].

The 1st zone was a highly negatively birefringent layer under polarized-light microscopy in either transverse [Losee et al., 1957; Gustafson and Gustafson, 1967] or longitudinal ground section of the human tooth [Gustafson and Gustafson, 1967; Schmidt and Keil, 1971] as shown in Figure 1. These results probably indicate that the initial prisms more or less arise perpendicularly to the dentine surface (Figs. 6 and 8). Subsequently, the succeeding prisms will be abruptly bent (Fig. 10).

In the outer-half layer of the 1st zone, each I (Fig. 5) and T-shaped faint slit (Fig. 7) of an arcade prism resembled the "seam" termed by Lester and Boyde [1987], Lester and Hand [1987], Lester et al. [1988] and Lester [1989] or a convergence line [Lester and Koenigswald, 1989]. Lester and his co-workers showed the "seam" and convergence line in various SEM pictures of the enamel of many mammals and Procerberus, and suggested that these structures should indicate primitive forms of enamel prisms. The I and T-shaped faint slits might be such forms as well. On the other hand, we previously found Y-shaped slits similar to the I and T-shaped slits just before the regular striae of Retzius where prisms abruptly bent in the outer enamel layer of human permanent teeth [Kodaka, 1979a].

An appearance of the abruptly bent prisms in the outer-half layer of the 1st zone (Fig. 10) resembled that of the 'false prismless' enamel showing a highly negative birefringence but a clear prism structure in the outermost layer of human deciduous enamel [Hørsted et al., 1976; Kodaka et al., 1989a]. This was also seen in the outermost layer of human permanent enamel [Schmidt and Keil, 1971; Fejerskov and Thylstrup, 1986; Boyde, 1989]. These bent prisms in the innermost and the outermost enamel layer may be related to the beginning and ending of Tomes' process formation [Osborn, 1970, 1973; Nanci et al., 1987; Kodaka et al., 1989b].

In EDTA etching pattern, the 2nd zone showed basically an intermediate form between the 1st and the 3rd zone, beacuse the prism boundaries strongly attacked by EDTA etching more than the boundaries of the 1st zone, and the EDTA-insoluble prism sheaths did not exist in the 3rd zone (Figs. 3, 11, and 13 - 15). The prism sheaths were similar to those of tuft prisms (Figs. 3 and 12).

The EDTA-insoluble substances of tuft prisms and the prism sheaths in the 2nd zone may correspond to the ribbons of protein associated with fibers of enamel "floss" illustrated by Weatherell et al. [1968]. This protein was not amelogenin but enamelin [Amizuka and Ozawa, 1989], and tuft protein which represent a less specialized product of other epithelial tissues, perhaps related to keratins [Robinson et al., 1989].

The 2nd zone associated with the EDTA-insoluble lamella-like structures of enamel tufts and tuft prisms in the 3rd zone (Fig. 3) might be regions where the large amounts of EDTA-insoluble substances had been deposited during the secretory stage of enamel formation; and where the substances had been incompletely resorbed during the maturation stage [Reith and Coty, 1967; Robinson et al., 1978, 1989; Ozawa et al. 1983; Fejerskov and Thylstrup, 1986; Nanci et al., 1987; Suga, 1987a; Amizuka and Ozawa, 1989]. On the other hand, Suga [1960, 1987a] reported that the innermost enamel (the 1st zone) had less amount of organic matter already in the secretory stage of human permanent teeth.

Several factors probably related to ameloblast activity may be responsible for the appearance of irregular prisms in the inner-half layer of the 2nd zone. The surface 'prismless' enamel frequently contained irregular prisms [Whittaker, 1982; Kodaka et al., 1989a, 1990b]. Therefore the irregular prisms might be one of transitional forms to the 'prismless' enamel. The inner 'prismless' enamel (Fig. 16) might be produced by ameloblasts crowding with shoving and pushing within the area.

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Discussion with Reviewer

M. Goldberg: Have you compared the appearance of your inner enamel surfaces after various etching solutions?

Authors: We had observed the inner layers of human deciduous enamel etched with 0.5 % chromium sulfate at pH 3.5 for 2 hours [Kodaka, 1978] and permanent enamel etched with 0.01 mol lactic acid at pH 3.2 for 8 minutes [Kodaka, 1979b]. The sulfate and acid did not show organic prism sheaths and etching patterns as shown in the EDTA etching, although the 1st zone was divided by them as well as previous studies using HCl [Kirino et al., 1972; Whittaker, 1978].

M. Goldberg: You reported morphological and chemical variations in the inner enamel. Do you think that these phenomena are related to changes occurring in the secretory ameloblasts or modifications which occur in the forming enamel during matrix maturation and mineralization?

Authors: In this study, we did not used the developing enamel. The morpholgical variations should be induced by the secretory ameloblasts. On the other hand, the main chemical variations might depend on the modifications during the maturation stage, although the 1st zone showed already a high mineralization in the secretory stage [Suga, 1983, 1987a, b].

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