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An Electron Microscope Study of Asiatic Elephant Tooth Enamel

By

Hisashi KAIBARA*

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Abstract

An electron microscopical observation of the replicas of polished and etched surface of asiatic elephant tooth enamel was carried out in order to examine the histological differences and varieties of enamel microstructure in relation to the position within an individual molar and the order of eruption as to five molars. Through the observation the histological differences were found to be almost negligible for the purpose of the correlation and identification of fossil proboscidea. Three different layers of the enamel were discriminated and one of them, intermediate layer, was recommended as the representative of the typical specific patterns.

Résumé

Une observation par le microscope électronique des répliques de la surface polie et corrodée de l'émail de dent des éléphants d'Asie a été exécutée afin d'examiner les différences histologiques et les variétés de la microstructure de l'émail dans leur rapports avec la position en deça d'une molaire individuelle et l'ordre d'éruption de cinq molaires.

Cette observation nous a amené à affirmer que les differences histologique étainet presque négligeable pour le but de la correlation et de l'identification de proboscides fossils.

Trois différentes couches de l'émail ont été discriminées et une d'eux: une couche intermédiaire a été recommandée comme la forme représentative de l'éspèce.

Introduction

Morphological evolutional trend of the mammalian teeth has been one of the most interesting subjects in the field of vertebrate paleontology, and notable investigations have been done both in details and perspectively. Then the writer is going to take up this problem from another view-point, the histological, and the teeth of fossil proboscidea are used here as the materials of investigation because of its relative abundance in the numbers of species and quantities in Japan.

* Department of Anatomy, School of Dentistry, Tokyo Medical and Dental University.

Furthermore he wishes that the investigation makes a good contribution to the problem of the proboscidien taxonomy and phylogeny which many investigators have dealt with hitherto.

As for the preservation of the fossils, dental enamel is the most useful material for study. Moreover through the histological study by optical microscopy, it is known that dental enamel is characterized by the bands of Schreger and the form of enamel prisms corresponding to the taxonomical positions such as order, family and species respectively (SHOBUSAWA 1952, KAWAI 1955, YAMAKAWA 1959). Accordingly, the writer intends to treat the dental enamel tissue of fossil proboscidien teeth. However, in advancing the study further in detail by electron microscopy, the writer expected to meet serious difficulties, such as the high mineral contents of the dental enamel which interfere with preparations of ultra-thin sections, the misunderstandings caused by the replacement of the materials during fossilization. Therefore for the first step of the study, the writer has made an electron microscopical observation on the dental enamel tissue of recent asiatic elephant by means of replication technique and has examined the histological differences or variation of the molars in relation to the order of eruption.

Materials and methods

According to SCHAUP (1948), the dental formula of asiatic elephant (*Elephas maximus*) is as follows:-

The milk dentition $\frac{1.\ \ 0.\ \ 3.}{0.\ \ 0.\ \ 3.}$

The permanent dentition $\frac{1.\ \ 0.\ \ 0.\ \ 3.}{0.\ \ 0.\ \ 0.\ \ 3.}$

The molars amount to six in all. The interpretation of the tooth position on these six molars has been a matter of controversy and yet disputable, but in the present study, it is set tentatively out of the question. Accordingly the six molars are described as I, II, III, ... VI. after MORRISON-SCOTT (1948). The discrimination of the molars was carried out on the lamellar number based upon the standard lamellar formula, that of FRADE's (1955) was adopted.

As for the materials treated here, the molars I,... V. were obtained from two asiatic elephants who died of the malady in zoo, but molar VI was not obtained. The I to III molars were got from a young female elephant aged about six months after birth. Their positions were recognized as lower jaw and right side. The molar I had completely erupted and attrition was observed on the whole occlusal surface. The molar II had partly erupted distal to the molar I and attrition was observed on the mesial three lamellae. The root was almost completed but cementum deposition was not recognized. The molar III had not erupted and only the upper halves of the lamellae were formed, without cementum deposition, two mesial of them were joined together but others remained separate. The three molars obtained from the left half of the same individual jaw have already been described in detail by TASUMI (1964), who kindly offered the materials of the opposite side to the present writer.

The molars IV and V were upper right and obtained from another individual whose age and sex were unknown. The molar IV had mostly erupted and mesial part of the root was slightly resorbed. The mesial eight lamellae were fairly worn, the root formation was apparently completed and the cementum deposition was not observed within the distal cervical part of the crown. The molar V was left incomplete and only two mesial lamellae had grown to unite together, the root was completely unformed.

For the replication the specimens were cut off from the mesial coronal part of each five molars with dental engine and polished. On the first stage of the polishing, a series of flat whet-stones, coarse, medium and fine grained, were employed, and the specimens were finally polished on a flat smooth glass plate using 3000-mesh emery powder used for the ordinary rock thin sections, moistened with ethylene glycole diluted with water. After the polishing, the surfaces of the specimens were cleaned with distilled water. The polished specimens were etched with 0.1 percent diluted hydrochloric acid for 10 to 15 seconds at about 20°C. Immediately after the etching treatment, the specimens were washed in a plentiful volume of distilled water and dried. Acethyl cellulose film was useful for replication, which is on the market as Bioden replica film. Three to five blank replicas were taken and the final one was washed in 0.1 percent diluted hydrochloric acid in order to remove the pseudo-replicas of apatite crystalls of enamel. Washing in a concentrated acid may interfere with the fidelity of reappearance of replica. The dilution to 0.1 percent and the relative short duration of the treatment, which was 30 seconds in this case, minimizes the artificial effect. After the washing and drying, the replica film was shadowed with evaporating Pt-Pd 8:2 alloy at an angle of 30 degrees under high vacuum. The preshadowed film was then coated with carbon evapolation under vacuum and placed on copper sheet meshes with the aid of Bioden Epocement, the binding agent for replica film. The replica film was dissolved away in methylacetate after the paraffin reinforcement. Then the Pt-Pd shadowed two-step carbon negative replica was ready to be observed under an electron microscope. Hitachi HS-7S electron microscope was used for the observation and photograph was taken at the magnification of 1000 to 5000.

Results

As regards the histological characteristic pattern, the dental enamels of most mammalians are generally divided into three layers: inner layer which contacts with dentine, outer layer contacting with coronal cementum or tooth surface, and intermediate layer which occupies most of the total thickness of enamel layer from dentino-enamel junction up to the surface or cemento-enamel junction (SHOBUSAWA 1955, KAWAI 1955, YAMAKAWA 1959 and TRAVIS et al. 1964). It is reported that the intermediate layer represents the best regularity of enamel prism arrangement and highly ordered histological features among the above cited three subdivisions.

To the observation the writer has paid much attention and has made efforts on avoiding the misunderstandings caused by the limited field of vision and the high magnification of electron microscope. Of course, the transversed or longitudinal form of individual prism and arrangement of prisms are considerably controlled by the angle of the surface plane of the polished specimen to the axis of the prisms, hence, for the purpose of comparing the prisms forms within five molars with each other, the strictly transversed and longitudinal sections of prisms were selected as carefully as possible under the electron microscope. The outlines of prisms in their various oblique sections and the bands of Schreger were also observed and photographed in order to study the histological differences which had been expected to be found out in different part of the individual tooth as well as among each of the five molars. As the observation was carried out on 30 to 40 photos of each of the five specimens as well as directly under the electron microscope, it can be said that the misunderstandings from arbitary selection of characteristics of enamel microstructures were able to be avoided for the most part.

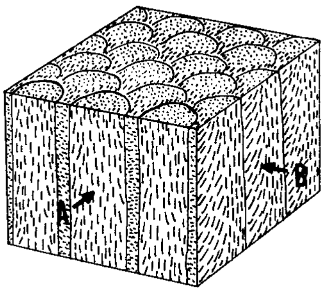
The results of the observation on the characteristics of the enamel microstructures are given as follows.

A) Histological variations in relation to the position in an individual tooth and the order of eruption.

One of the purposes of this study was to investigate the histological variations in relation to the positions within an individual molar, and to the orders of eruption which could be regarded as the differences of developmental stages. Regardless of the states of organization and arrangement of apatite crystals which were not successfully observed by the above-mentioned replication techniques, the histological features, especially the forms of enamel prisms, show none of significant differences within an enamel layer independent of their positions such as occlusal, lingual or buccal surface except for the neck of the tooth where the thickness of the enamel layer is reduced and wedged out. On the other hand, the differences between the three layers of the enamel are observed to be much more dominant. These facts can partly be attributed to the uniformity of the thickness of the enamel layer covering the whole coronal part of an elephant molar as in most other hervivorous mammalian molars. In the neck of the molars, however, the regularity of the arrangement of prisms is not observed and irregular and complicated features are common. Therefore intermediate layer of the coronal part of the equally thick enamel was examined for the comparison of histological features between each of the molars. Nevertheless, none of the structural differences of the intermediate layers could be observed corresponding to the tooth position and the order of eruption. Accordingly the descriptions of various characteristics of histological features, especially the forms of enamel prisms, were carried out independent of the order of eruption.

B) Intermediate layer of the enamel

Fundamental type in transverse sections of prisms is characterized by the well-ordered regular pattern of arch-formed outlines. The individual arch corresponds to prism sheath and shows a nearly semicircular outline without any notches, rather like the arrangement of roofing tiles. On the convex side of the arch, interprismatic area is observed. The arch of prism sheath caves in and forms an arch-shaped groove on the actual surface of the tooth specimen, so the etching treatment with acid had much more carving effect along the prism sheath than the other areas (Pl. 12, Fig. 1). An oblique section of the fundamental type shows distorted pattern. (Pl. 12, Fig. 2, 3).



Text-Figure 1. A schematic three-dimensional drawing of well-ordered enamel prisms of intermediate layer. A: a longitudinal section of prisms with alternative appearance of intra- and interprismatic substances. B: another longitudinal section without distinctive interprismatic substance.

The longitudinal section of the prism shows two different features according to the orientation, that of parallel to the enamel surface shows a clear alternation of prismatic and interprismatic areas (Text-Fig. 1A, Pl. 13, Fig. 1), and that of normal to the surface shows prism sheaths on only one side of the prism (Text-Fig. 1B, Pl. 12, Fig. 4) because the prisms are always arranged in the same direction facing the convex side of arch to dentino-enamel junction. Somewhat varied forms of the sheaths were observed, such as a slate-like (Pl. 13, Figs. 2 and 3) and a lemon-like type (Pl. 13, Fig. 4).

Absence of the interprismatic area was observed on some of the replica films which had been taken of the incompletely calcified and cementless ones, molars III and V. The prism sheaths of these specimens were inferred to be deeply hollowed on the surface of the specimen from the fact that high ridges of carbon replica film are observed along the prism sheaths. The apparent absence of the interprismatic area and the deeply etched prism sheath were rather frequently observed in the outer zone of intermediate layer (Pl. 14, Fig. 1).

Apatite microcrystals which are the main constituent of the enamel, called crystalloids or ribbons, were hardly recognized on the replica films of the transversed sections of prisms, but on the longitudinal sections the state of their arrangement was recognizable. The orientation of the crystals within a prism varies from nearly parallel to the long axis of the prism up to as much as forty degrees to the prism axis (Pl. 12, Fig. 4, Text-Fig. 1B). The crystals of interprismatic area were observed to have much more angles to the prism axes and some were almost perpendicular to the long axes of the neighbouring prisms (Pl. 13 Fig. 1, Text-Fig. 1A).

C) Outer layer of the enamel

It is reported that characteristic polygonal outlines of transversed prisms were observed in the outer layer of asiatic elephant tooth enamel (SHOBUSAWA 1952). Those forms of the prism outlines were, according to SHOBUSAWA, characterized by the irregular polygons just like natural stones for pavement works and were different from the characteristic regular alternation of polygons of carnivorous mammals. In the present study, however, none of those characteristic polygonal prism sections was noticed. In the outer layer of the enamel, the decrease of distinct prism sheath outlines and of amount of apparent prism number were observed, and the area occupied by the interprismatic substance increased toward the surface of the enamel and finally the superficial layer in contact with the inner surface of the coronal cementum was composed of entirely prismless enamel (Pl. 14, Fig. 2). The cemento-enamel boundary shows two different patterns: one is an almost straight line with little irregularity; the other is considerably uneven (Pl. 14, Fig. 3). The shape of the boundary would readily have been deformed by the exsiccation of the tooth specimen.

D) Inner layer of the enamel

TRAVIS et al. (1964) noticed the presence of the region near enamel-dentine junction which had irregular arrangement of prisms by electron microscopic observation of the ultra-thin sections of embryonic bovine enamel. Such irregularity in the prism arrangement in the inner layer of enamel is also observed in this replica study of asiatic elephant enamel. Enamel-dentine junction shows a nearly straight line and prismless zone of a few microns width is observed along the enamel zone of the boundary. However, in some of the photographs, the prisms contacting directly with enamel-dentine junction were noticed. Regardless of the presence of the prismless enamel, irregular arrangement of prisms along enamel-dentine boundary was found to exist in every specimen, and the dimension of the width of the irregular zone seemed to be less than that of bovine teeth reported by TRAVIS et al. (1964). The prisms near enamel-dentine boundary arise from the prismless zone with a smaller diameter and increase their dimensions toward the midway and on to cemento-enamel boundary (Pl. 14, Fig. 4).

Discussions

The varieties of enamel microstructure in relation to the positions within an individual molar and the order of eruption of the molars could not be found out despite the inferential expectation from developmental view-point. No reliable work on elephant or proboscidien concerning this point could be found. However, about some of rhodentia and lagomorpha, histological differences of the prism structure of enamel between incisors and molars have been proposed by KAWAI (1955). The incisors or tusks of young asiatic elephant are difficult to be obtained and much more the enamel crown of the tusk. None of the histological comparison of enamel structure between elephant incisors and molars could be found in the works made hitherto. In the present study, the structural changes of the enamel in the molar group were found to be less than those of positions within an individual molar. Histological characteristics of larger dimensions such as bands of Schreger, striations of Letzius and so on may show some variations in relation to the order of eruption. The fact that the changes of enamel structures on the molars of asiatic elephant is negligible may have an important possibility of application to the identification of fossil proboscidia. The correlation and comparative investigation of proboscidien fossil tooth can be convincingly made with a piece of their molar enamel which contains the whole thickness of the enamel layer regardless of the positions of them.

It has already been reported that histological differences are found in the inner, middle and outer layers of the enamel of various mammals. KAWAI (1955) has reported that the closed polygonal outlines of transversed enamel prisms were observed in the outer layer of asiatic elephant enamel. However, none of prism forms of such a kind was found in this study. This discrepancy can be ascribed to the difference of techniques between the observations of hematoxylene stained ground sections under the optical microscope and the electron microscopic observations of replica, or to the inevitable small visual field of the latter. Incidentally, the writer would like to remark that, as the typical characteristics of the enamel structure of asiatic elephant, that of the intermediate layer should be employed because this layer of the enamel represents the best regularity of histological appearances and is the dominant constituent of the whole thickness of enamel except for the occasion of molar I in which the intermedate layer is rather the minor.

Although the organization and the order of the apatite crystal arrangement in the prism and interprismatic areas cannot be discussed in detail on account of poor reliability of filmy replication, those which were observed in the longitudinal sections of prisms showed a good coincidance with hitherto reported results.

Resistivities of enamel constituents to the etching treatment with hydrochloric acid may have a proportional or inversely proportional relation—there have been two antagonistic hypotheses concerning this problem—to the degree of calcification. The surface collapse of etched enamel offers some information on the estimation of calcification degrees of enamel constituents such as prism sheath, interprismatic and intraprismatic substance. The prism sheaths apparent on the replica film are less resistant, and inter- and intraprismatic areas are more resistant to the etching. The prism sheath can be correlated with the approximately 425 A wide and crystal-free region in bovine embryonic enamel (TRAVIS et al. 1964). In order to make further investigation of the calcification degree by the replication method, much more perfect polishing and feiner etching treatment will be demanded. Quantitative measurement of the relief of replica film would be valuable.

Summary

1) Replica study of the asiatic elephant molar was made in order to examine the histological differences and varieties of enamel microstructure in relation to the position within an individual molar and the order of eruption.

2) The differences of the microstructure were found to be too slight and negligible for the purpose of correlation and identification of recent and fossil proboscidea.

3) Three different layers, inner, intermediate and outer, were discriminated in the elephant tooth enamel and the characteristics of each layer were described. The intermediate layer was recommended as the representative of the typical specific patterns.

4) Some instructive but insufficient information about the arrangement of the enamel apatite crystals and the degrees of calcification of enamel constituents was obtained.

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Explanation of Plate 12

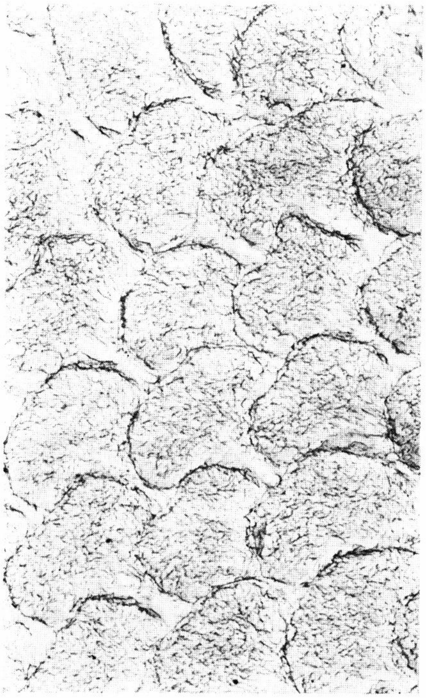
Electron micrographs of the etched surface replicas of asiatic elephant tooth enamel.

Fig. 1. Fundamental type of the transversed enamel prisms. Note the typical arch-form of the prism sheath. Intermediate layer of the molar V. ×3000.

Fig. 2. An oblique section of the fundamental type prisms. Intermediate layer of the molar V. ×3000.

Fig. 3. Another oblique section of the prisms of the same typical form. Intermediate layer of the molar I. ×3000.

Fig. 4. A longitudinal section of the prisms in the direction normal to the enamel surface (cf. Text-Fig. 1B). Prism sheaths are observed on one side of prisms. Note the state of arrangement of apatite microcrystals. Intermediate layer of the molar IV. ×3000.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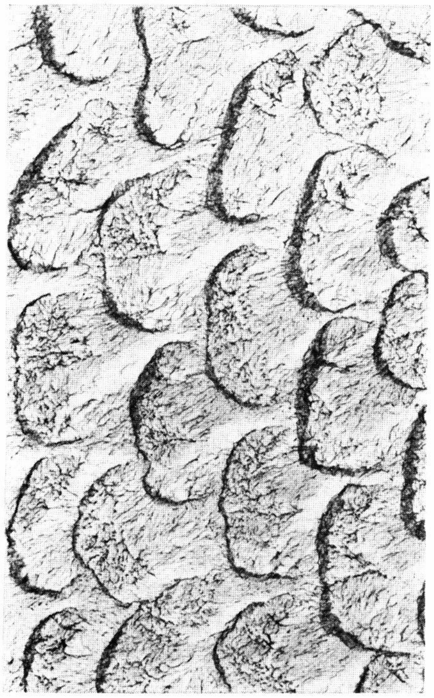
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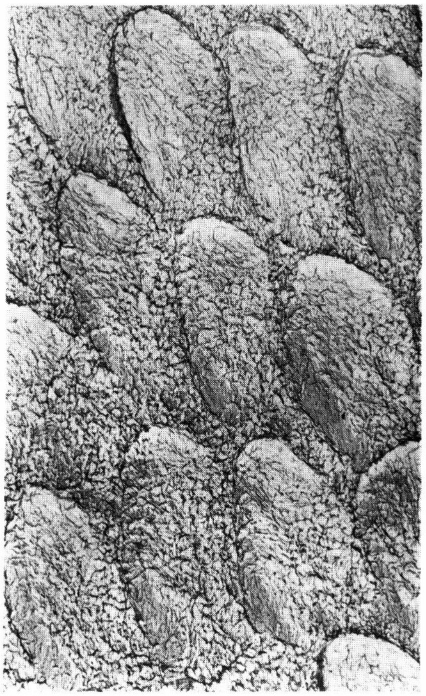
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KAIBARA: Asiatic Elephant Tooth Enamel

Explanation of Plate 13

Electron micrographs of the etched surface of asiatic elephant tooth enamel.

Fig. 1. Another longitudinal section of the prisms, parallel to the enamel surface (cf. Text-Fig. 1A). A clear alternation of prismatic and interprismatic areas. Intermediate layer of the molar V. $\times 3000$.

Fig. 2. A slate-like pattern of the transversed enamel prisms. Intermediate layer of the molar II. $\times 3000$.

Fig. 3. Another varied form of the prisms. Intermediate layer of the molar I. $\times 3000$.

Fig. 4. A lemon-like form of the transversed prisms. Intermediate to outer layer of the molar II. $\times 3000$.

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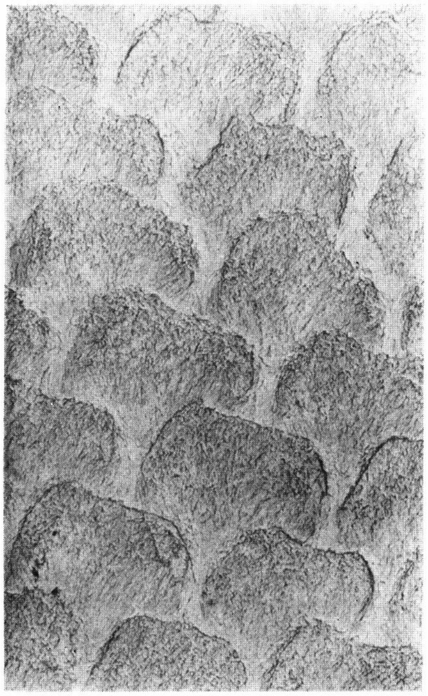

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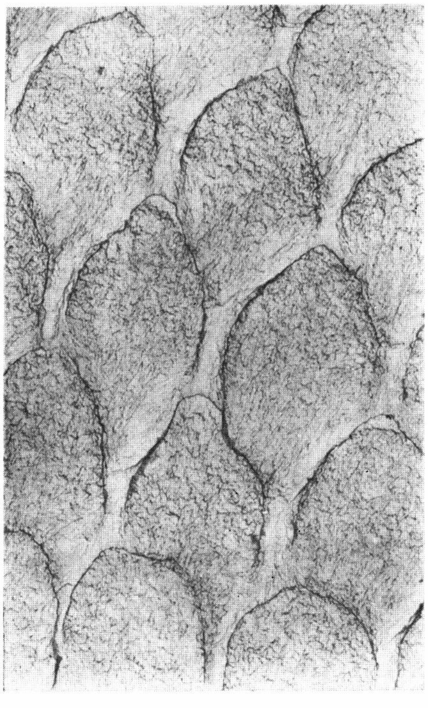

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KAIBARA: Asiatic Elephant Tooth Enamel

Explanation of Plate 14

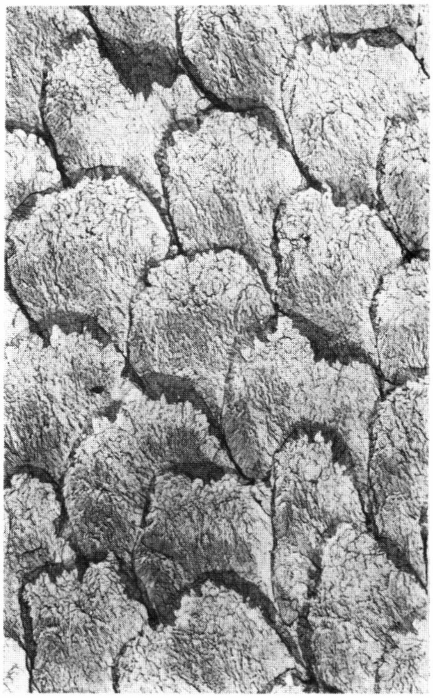
Electron micrographs of the etched surface replicas of asiatic elephant tooth enamel.

Fig. 1. Absence of the interprismatic areas and deeply etched prism sheaths. Outer zone of intermediate layer of incompletely calcified molar V. ×3000.

Fig. 2. Prismless enamel of the outer layer of enamel. Molar I. ×3000.

Fig. 3. Cemento-enamel boundary with a little irregularity. Cemento-enamel junction of the molar I. ×3000.

Fig. 4. Enamel-dentine boundary of the molar IV. Note the presence of prismless zone along the nearly straight enamel-dentine border line, and the irregularity of the prism arrangement of the inner layer of enamel. ×3000.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KAIBARA: Asiatic Elephant Tooth Enamel