

ENAMEL ULTRASTRUCTURE OF FOSSIL MAMMALIA: EOCENE ARCHAEOCETI FROM KUTCH

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ABSTRACT

The application of the scanning electron microscope to the investigation of fossil mammalian enamel ultrastructure is very recent. Such studies provide a multidisciplinary approach to various aspects of the problem of biomineralization in a geologic perspective. The present study deals with the enamel ultrastructure of a Middle Eocene archaeocete whale (about 50 million years old) from south western Kutch. Prisms are circular in outline and conform to Boyde's Pattern One. Although, external dental morphology is far removed from that prevailing in Recent toothed whales, the enamel ultrastructure bears a close resemblance. Hydroxyapatite crystallites converted in minor amounts to fluorapatite, have undergone essentially no deformation during the process of fossilization.

INTRODUCTION

Scanning electron microscope investigations of primitive mammalian enamels provide significant information not only on the anatomical changes involved in the evolution of mammalian enamels but also on the ultrastructural responses of an animal's dentition to external stresses. Ultrastructural studies have recently shown the diversified nature of Cretaceous multituberculate enamels (Sahni, 1979) and their considerable morphological deviation from modern mammalian enamels (Boyde 1969). The present study on Middle Eocene archaeocete (Cetacea) enamels is part of a research programme which has the objective of studying fossil mammalian enamels of taxa which have just started diverging from an undifferentiated ancestral stock. Archaeocete cetaceans are ideally suited for the purpose as they (or their near relatives) are regarded as percussors to the modern toothed (odontocete) whales (Gregory 1915, Van Valen 1968) and yet retain, several primitive skeletal characteristics of their probable ancestors, the mesonychids (Van Valen 1966, Mishra 1976).

The archaeocete material on which this study is based, was collected from Middle Eocene limestones of south western Kutch, western India (Sahni and Mishra 1972, 1975). The ossiferous horizon which has produced an abundant archaeocete fauna is represented by a shallow-water, *Corbula*-rich chocolate limestone exposed along the Berwali stream section near the village of Harudi (68° 41' 15" E; 23° 30' 20" N) and has been described in some detail (Sahni and Mishra 1975). The Chocolate Limestone represents the Babia stage of the Berwali series and has been shown to be of Lutetian age on the basis of foraminiferal biostratigraphy (Tandon 1966, 1971, 1974). The general stratigraphy and geology of the area has been described by Biswas and Raju (1971) and Mohan and

Soodan (1970).

The Middle Eocene Kutch archaeocetes are fairly well diversified. They are represented by the family Protocetidae comprising of at least two genera, *Indocetus* and *Protocetus*, both known by dental material. Indian protocetids with short mandibular symphyses and "carnivore-like" dentition are similar to the Egyptian *Protocetus* described from the Fayum Basin (Qasr-el-Sagha Formation) and Lower Mokattam Formation near Cairo, by Fraas (1904), Stromer (1908) and Andrews (1906). Other Kutch primitive cetaceans with long, well fused mandibular symphyses are considered tentatively to belong to the odontocetes (Sahni and Mishra 1975). As these forms are known presently by secondarily altered gypsified teeth, they have been excluded from the present work.

In contrast to recent dental tissue which may have to freeze-dried or otherwise specially prepared for S. E. M. study, fossil enamels may be directly scanned after sputtering. Fossil enamels are in large part composed of crystalline calcium phosphate (hydroxyapatite) and differ from modern enamel tissue in the loss of organic matter and water content and the gradual replacement of (OH)⁻ ions by F⁻ ions in the apatite lattice, resulting in alteration to fluorapatite (Poole 1956). However, as the bulk of enamel is composed of crystalline mineral matter, little or no distortion of enamel prisms has been observed to occur during the process of fossilization. Differential acid-etch patterns in Recent and fossil enamels are essentially the same and in both cases, prism boundaries, which have a high organic-matter content, are preferentially etched.

Upper and lower molars of *Protocetus* were studied by scanning freshly fragmented enamel surfaces and by observing specifically oriented ground, polished and

etched surfaces, as described in detail earlier (Sahni 1979). All the dental material studied was taken from archaeocete skulls in the collection and not from isolated teeth. The ultrastructure of upper and lower teeth was found to be identical.

Studies of the ultrastructure of Recent mammalian enamel were initiated in a detailed manner by Boyde (1964, 1969). Prior to this, investigations were mostly confined to light microscope studies (Schmidt and Keil 1958) on human enamel as well as on rodent incisors (Korvenkontio 1934, Wahlert 1968). The first was motivated by direct application of results to modern dentistry and the second, as it provides a record of the continuous process of enamel secretion by ameloblasts. One of the earliest detailed studies by the light microscope to investigate the microstructure of various genera from a number of mammalian orders was that of Shobusawa (1952). The odontocete cetaceans sampled in that work include *Orcinus orca*, *Globicephalus melas* and *Delphinus delphis*. Later, Boyde (1969) in addition studied (but did not figure) the following cetacean genera: *Inia*, *Mesoplodon*, *Ziphius*, *Hyperoodon*, *Physeter*, *Delphinapterus* and *Tursiops*. In the present study, some thin sections of the Gangetic dolphin enamel have been used for comparative purposes.

RESULTS

This paper deals with the application of the S.E.M. to the study of a fossil mammal teeth dating back to about 50 million years. In surface etched enamels as well as in tangential and horizontal sections, prism outlines appear to be hexagonal in less etched samples (Plate I—3) or circular in better etched samples (Plate I—5). The slight differences in the outlines of prisms are mainly the result of the degree of etching of the prism sheaths. Prism sheaths in most fossil as well as Recent mammalian enamels have been found to be particularly susceptible to acid etching (Reif 1974). Prism sheaths, which envelop the prism cores, usually represent a higher concentration of organic matter, probably as a result of differing solubility influenced by the degree of permeability to the acid used for etching (Poole and Johnson 1968, Boyde 1970). In some instances, prisms appear to be horse-shoe shaped but this is perhaps related to the angle of scan as well as the orientation of the long axis of the prisms to the section under observation. When the same set of prisms is viewed normal to their long axis, their outlines appear to be circular. The enamel ultrastructure pattern of Eocene archaeocetes conforms to the Cetacean-Typen of Shobusawa (1952) representing Pattern one of Boyde (1969).

In view of the supposed derivation of the Eocene archaeocetes from Mesonychid condylarthra (Van Valen 1966, Sahni and Mishra 1975), an interesting comparison can be made of the enamel ultrastructure represented

by the series *Protungulatum* an arctyconid condylarthra from the Maestrichtian (Uppermost Cretaceous) of Montana (Sahni 1979), through terrestrial mesonychids of Middle Eocene age (Sahni, in preparation) to Eocene marine archaeocetes. *Protungulatum* is characterized by Pattern One arrangement of prisms with some sections showing Pattern two. The enamel ultrastructure of Lutetian mesonychids is still under study but preliminary investigation show the presence of hexagonal to circular prisms, similar to those found in Middle Eocene archaeocetes. Pattern one seems to be the dominant arrangement in this lineage, as it is for the majority of Cretaceous therians.

Slight etching of the enamel surface exposes the different orientations of the prisms (Plate I—2, 4). Several zones can be detected along which the prisms appear to be similarly inclined and differing in inclination from adjacent zones. However, a close observation of the junction between two adjacent zones reveals that there is no abrupt change in the direction of the prisms, rather, the change in inclination of prisms is continuous so that when medianly situated prisms in adjacent zones are observed, their inclinations diverge maximally whereas the degree of divergence decreases towards the junction (Plate I—4). This figure also illustrates the relatively more resistant nature of the prisms which stand out in relief as well as their sinuous courses.

In all sections normal to the outer surface, prisms follow an undulating course. In longitudinal sections (Plate I—6) there is a clear-cut differentiation of prisms into alternating parazonies and diazonies. Normally, zone formation is not a prominent feature of Pattern One prisms (Boyde 1969, p. 198). The parazone consists of a group of prisms with the long axis parallel to the section plane whereas in the diazonies, prisms are transversely sectioned. The parazonies may be oriented normal to the outer surface or may be slightly inclined. Parazone development starts very close to the enamel-dentine junction and extends to more than two-thirds the thickness of the enamel. In any one particular parazone, the prisms are essentially parallel to one another, though there may be minor divergence between adjacent prisms, leading to a marked resultant divergence between distantly separated prisms (Plate I—6). The course of individual prisms in a parazone can be traced for a considerable length. Generally, the inclination of prisms towards the top of a parazone is normal to the outer surface. The width of the parazone varies considerably depending upon the degree of bending ("sinuosity") of the prisms and the plane of sectioning. The width of the diazonies is also variable when measured along a horizontal plane, prism number varies between 7 and 14. In most instances, transversely sectioned prisms appear in definite rows which can be traced for short distances,

as seen towards the top of diazone at the left-centre of Plate I—6.

At the outer enamel surface, most of the prisms are cross-cut and the difference in the orientation of prisms in the diazones and parazonal zones becomes negligible. The outer most enamel surface comprising about $4\ \mu$ in thickness, represents the outer most prismless layer. This layer consists of crystallites oriented normal to the outer surface, constituting the interprismatic phase. It should be mentioned that towards the end of enamel secretion by ameloblasts, inter-prismatic material represents the dominant phase (Plate I—6).

The enamel ultrastructure of cetacean enamel raises some interesting problems regarding prism decussation which are represented also by the Hunter-Schreger bands (Boyde 1969). In Boyde pattern one enamel, there is no indication of true prism decussation involving a relative sliding movement of the ameloblasts past each. The apparent banded appearance in the archaeocete enamel is essentially a result of the sinuous course of the prisms from the enamel-dentine junction towards the surface. Plate II—2 shows the bands of longitudinally sectioned prisms alternating with transversely sectioned ones. Plate II—1 shows the parazonal-diazonal junction. Transversely sectioned prisms to the right of the picture appear to grade gradually into longitudinal sectioned prisms. There is no indication of an abrupt change in the direction of prisms as is illustrated by Boyde (1969 p. 182) in the case of dog deciduous molar enamel (fig. 38). Prism sheaths, which are considerably etched, are clearly visible.

Longitudinal sections show a prominent enamel-dentine junction (Pl. I—6). Pl. II—4 shows a natural fragmented longitudinal surface. Prisms towards the top of the enamel surface as well as to the enamel-dentine junction are oriented normally to both surfaces. In the central part of the enamel, there is some indication of the sinuous bending of prisms. Fracture planes are developed parallel to the long axis of prisms as well as normal to the this plane. The latter produces a distinct step-like fracture across transversely sectioned prisms.

Most of the longitudinal sections show a well differentiated outer dentinal layer which represents the Von Korff bundles. The Von Korff layer is a characteristic feature of Recent odontocetes (Boyde 1970) and it is interesting to note that even Lutetian (Middle Eocene) archaeocetes had a well differentiated Von Korff layer, suggesting a continuity of enamel ultrastructure from the Middle Eocene to the Recent. The phylogenetic implications of the Von Korff's layer were first mentioned by Poole (1971, p. 75). The von Korff's layer, discussed in some detail by Schmidt and Keil (1958), is the first differentiated tissue and precedes the formation of true enamel.

Histological studies have shown that during the

initiation of dentine formation, some collagen fibre bundles having their origin in the pulp get included in the dentinal matrix. These rather well differentiated coarse bundles are oriented nearly normal to the enamel-dentine junction and are rather distinct from the intertubular dentine. It is significant that in Eocene archaeocetes tubules are fairly common in the Von Korff's layer as well as in the intertubular dentine. Where preserved, the tubules have an orientation which is also normal to the enamel-dentine junction (Plate II—3). However, there appears to be marked density difference between tubules in the Von Korff's layer and those in the intertubular dentine, the latter being more common. The Von Korff's layer is of generally uniform thickness and is about $50\ \mu$ wide. Some spindle shaped projections occur at the junction of the enamel and dentine (Plate II—3) but the exact nature of these can not as yet be evaluated. These do not appear to be enamel spindles in the sense of (Boyde 1970, fig. 9) as these structures seem to be enamel in origin and are not projections of dentinal substance into the enamel. However, the association of enamel spindles with the Von Korff layer has been noted by Boyde (1970). The correspondence of dentinal and enamel tubules with the presence or absence of the Von Korff layer needs to be explained. Von Korff fibres are present in all but one genus of marsupials, which have tubular enamels (Boyde 1970). It is significant that in *Vombatus ursinus* (Marsupial), the enamel is not tubular. This condition is apparently associated with the presence of extensive Von Korff fibre bundles oriented parallel to the enamel-dentine boundary (Lester and Boyde 1968, Boyde and Lester 1967, p. 567). There is no clear indication that dentinal tubules extend across the enamel-dentine junction into the enamel. However, there are some tubule-like partings which are found in the enamel immediately adjacent to the dentinal boundary, though the precise nature of these cannot as yet be determined.

In transverse sections (Plate II—5), the undulatory nature of the prisms is clearly seen. Prisms follow a sinuous course from the enamel-dentine junction to the surface. Laterally as well, the prisms collectively occur in wave-form. In some places (right side of Plate II—5), the crest of this "wave" is near the central part of the enamel, with prisms essentially normal to the enamel-dentine junction. Towards the centre of the Plate II—5 lies the trough. Wavelength averages $300\ \mu$ (crest to crest) which is slightly more than the average enamel width. Towards the top of the enamel surface, incremental lines (lines of Retzius) are visible.

SUMMARY AND CONCLUSIONS

1. Enamel ultrastructure of Middle Eocene archaeocete cetaceans from Kutch conforms to Pattern One as in

the case of modern odontocetes.

2. Although in external morphology archaeocete molars are similar to their Mesonychid ancestors, the ultrastructural pattern is definitely cetacean. This implies that the internal ultrastructural response to selective pressure was greater than for external stresses.

3. The enamel ultrastructure of a primitive condylartha *Protungulatum* from the Cretaceous (Maestrichtian) from Montana has recently been studied by Sahni (1979). The ultrastructure of *Protocetus* is essentially the same, both being predominantly Pattern one.

4. The enamel ultrastructure of a Middle Eocene mesonychid condylartha *Ichthyolestes* has recently been investigated. Zone formation is a prominent feature. Preliminary studies have shown a close correspondence between the ultrastructure of Lutetian mesonychids and archaeocetes.

5. Prism decussation is absent in archaeocete enamels, although zone formation is a prominent feature. Diazone and parazone widths vary considerably. Prism decussation however is common in contemporary Lutetian rodents.

6. Von Korff's layer is a prominent coarsely differentiated layer of the dentine occurring internal to the enamel-dentine boundary. It is about 50 μ thick and contains definite tubules. The layer is also a prominent feature of Recent odontocetes.

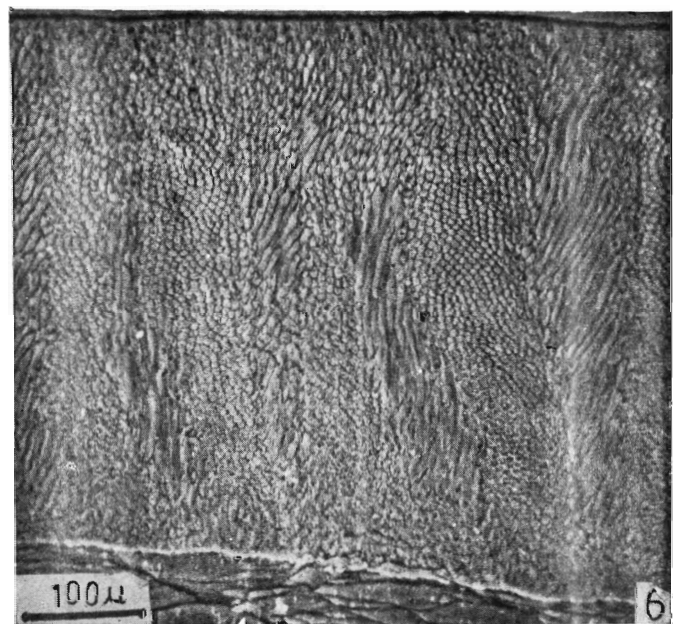
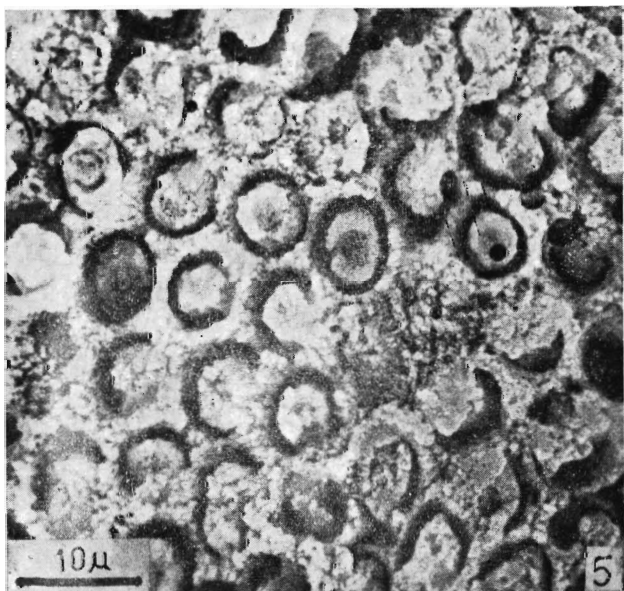
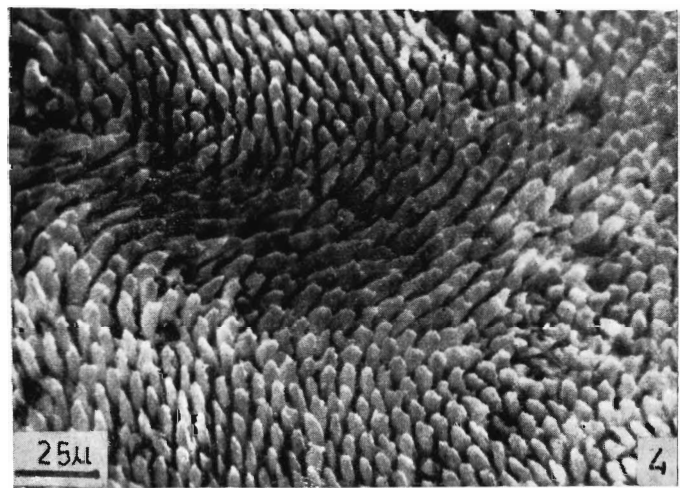
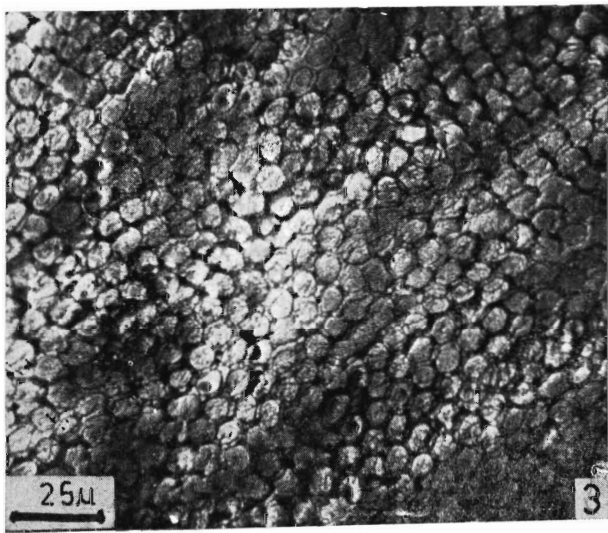
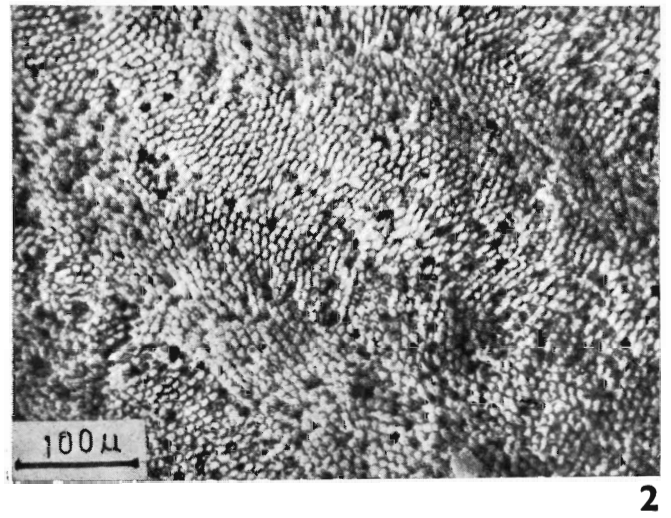
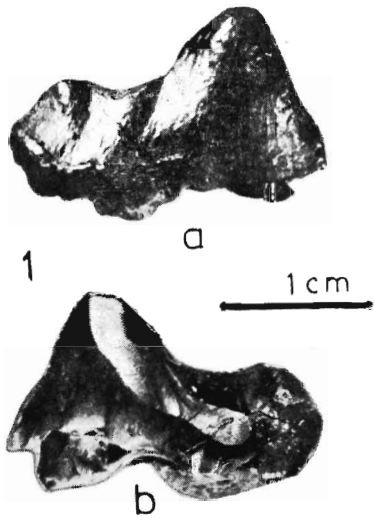
7. Tubules are common in the dentine. The orientation of tubules is normal to the enamel-dentine junction in longitudinal sections. There are some tubule-like structures in the enamel just across the enamel-dentine boundary.

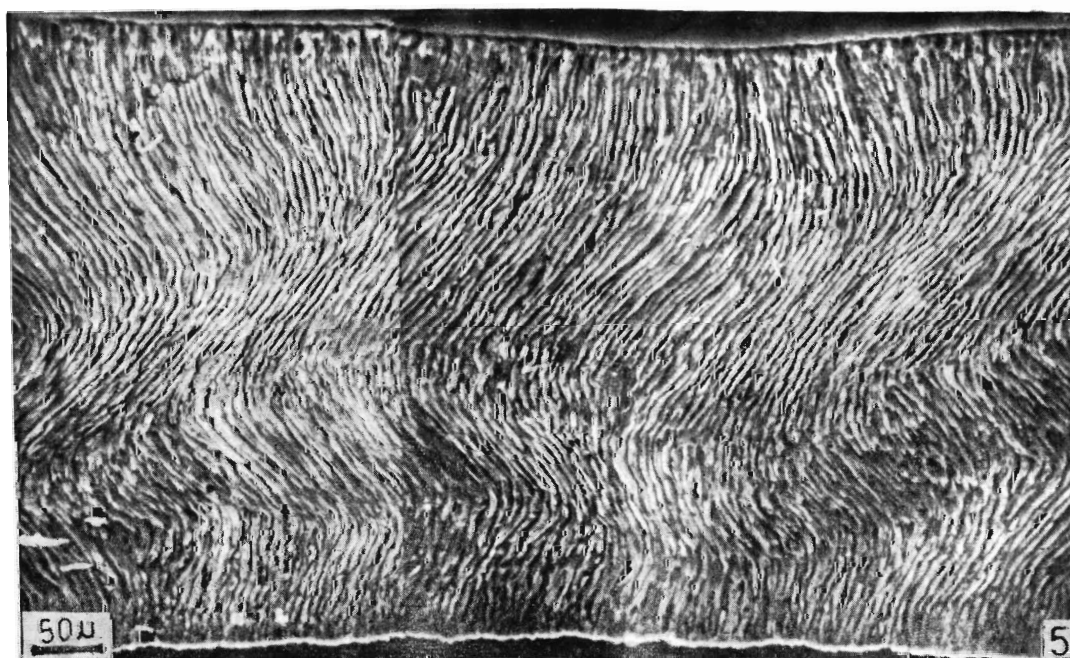
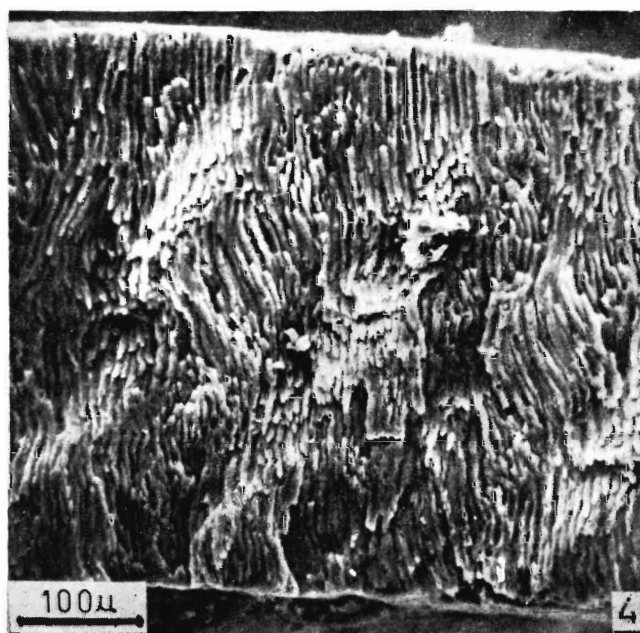
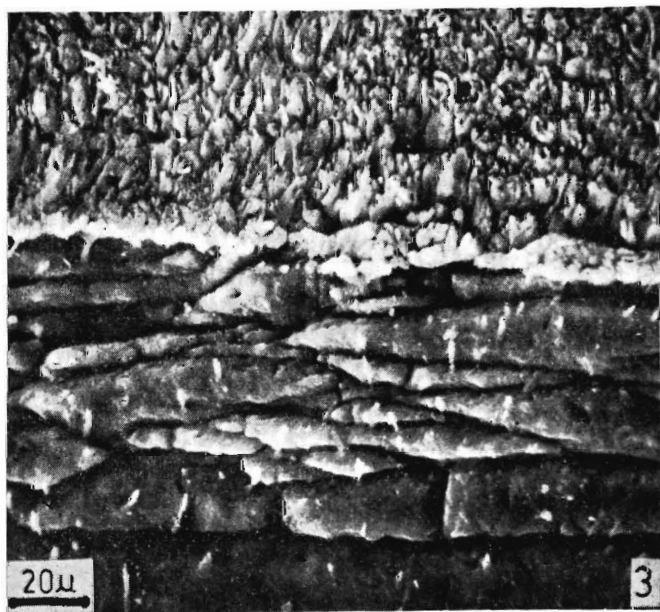
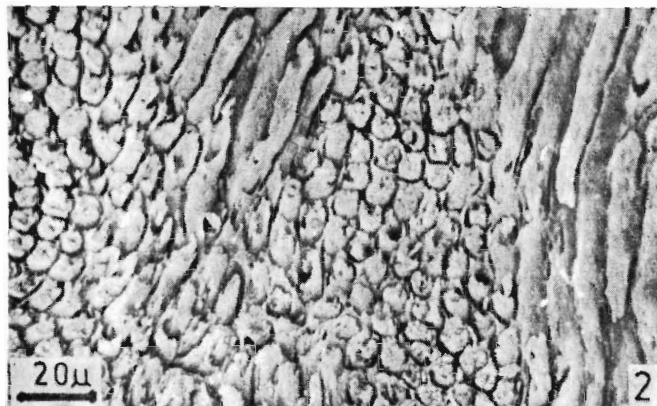
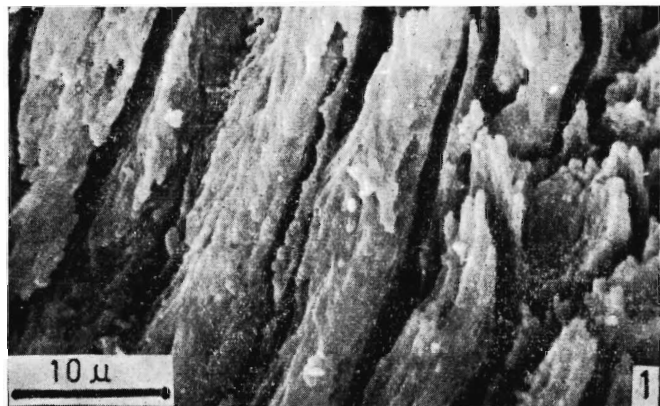
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EXPLANATION OF PLATES

PLATE I

1. Lower molar of *Protocetus*, a lingual view, b labial view.
2. Enamel surface showing transversely sectioned prisms.
3. Enamel surface, lightly etched with hexagonal to circular prisms.
4. Enamel surface, with divergent prism orientation, prism sheath considerably etched.
5. Enamel surface showing Pattern One prism arrangement, with hexagonal prism boundaries.
6. Longitudinal section of archaeocete enamel, with distribution of parazonal and diazonal zones.

PLATE II

1. Archaeocete molar, longitudinal section showing junction of parazonal with diazonal zone.
2. Longitudinal section, zone formation.
3. Archaeocete molar, longitudinal section showing enamel-dentine junction and Von Korff layer. Tubular dentine towards the bottom of the figure. Tubules also present in Von Korff layer.
4. Transverse section, natural fragmented surface. Fractures parallel to long axis of prisms and across prisms.
5. Transverse section, archaeocete molar showing sinuous bending of prisms. Wave-form distribution of prisms in horizontal plane.