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MARSUPIAL AND MONOTREME ENAMEL STRUCTURE

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Abstract

We present some recent developments in our understanding of two basic questions: the origin, extent, nature and course of marsupial enamel tubules; and the characterisation of monotreme enamel, more particularly, the prismatic nature of platypus enamel.

Methods used included SEM of methacrylate casts of marsupial enamel tubules, worn and cut surfaces of whole marsupial teeth, developing and erupted platypus teeth, and a well-developed molar of the newly discovered Miocene ornithorhynchid Obdurodon sp., and tandem scanning reflected light microscopy of intact marsupial teeth.

We conclude that there are significant species differences with respect to prism shape, row formation and tubule disposition in marsupials and, moreover, that these features change in a consistent way through the thickness of the enamel. Consideration of enamel prism course in incisor and molar enamel of Macropus eugenii, together with the tubule casts, enables us to conclude that there is a fundamental relationship of tubule to prism in the body of marsupial enamel. This and previously reported data put beyond dispute the essential relationship of the marsupial tubule to the formative ameloblast.

The enamel of Ornithorhynchus anatinus is shown to be prismatic only in part, with well-formed regular prisms not being a primary structural feature. The enamel of the fossil monotreme is prismatic and tubular and displays large areas of Pattern 2 prism packing. Monotreme enamel has been interpreted as representing a structural stage intermediate between that of known multituberculates and extant tribosphenid mammals.

Key Words: Enamel / Dentine / Structure / Development / Taxonomy / Phylogeny / Marsupials / Monotremes.

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Introduction

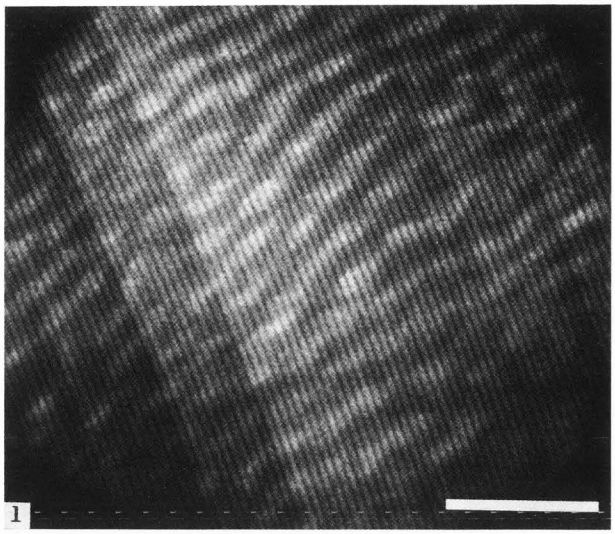
The aim of this paper is to present some recent developments in our understanding of two outstanding questions concerning metatherian and prototherian enamel structure, namely:

i) for marsupials other than the wombat, the origin, extent, nature and course of enamel tubules, and
ii) for monotremes, the prismatic nature or otherwise of platypus enamel.

Marsupial enamel

There is still, unfortunately, very little by way of a systematic study of marsupial enamel although many isolated, in-depth observations have been reported and the occasional review constructed to help organize the diverse findings. Reports on marsupial enamel revolve very largely around the subject of enamel tubules (known also as tubes, canals, fibres and fibrils). Light microscope studies on enamel tubules from Tomes (1849) through to Moss and Applebaum (1963) have been summarized in terms of the origins and positioning of tubules by Boyde (1964) and Boyde and Lester (1967). The major differences of opinion expressed in that time revolve around the question of tubule origin. The differences are explicable partly in terms of the variety of species and preparations examined and partly by the fact that the diameter of the enamel tubules, at 0.2 μm to 0.5 μm, may cross the useful limit of resolution of the light microscope. Further, the reality and detail of the extracellular nature of the secretion of enamel was not clearly demonstrated until 1960 (Fearnhead, 1960; Watson, 1960).

Problems concerning marsupial enamel structure are approached here through observations on plastic casts of tubules exposed against a clearly resolvable, three dimensional background of prismatic structure and through observations on prism course and packing pattern in a number of different species. The aim is to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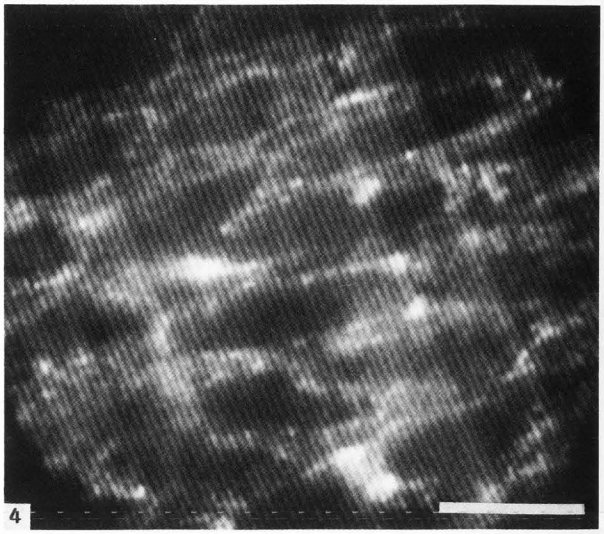
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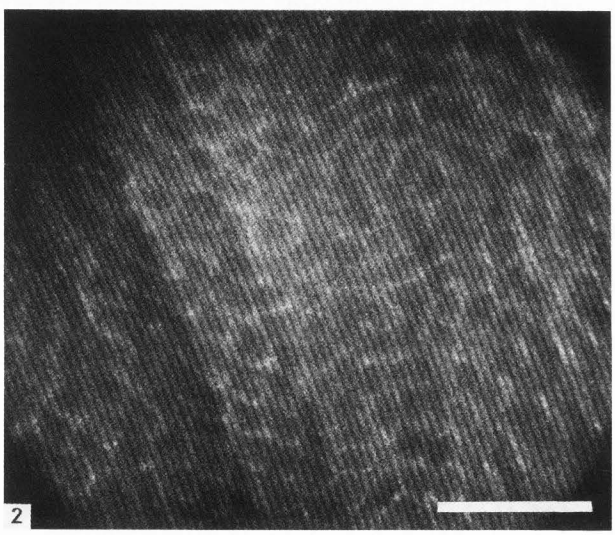
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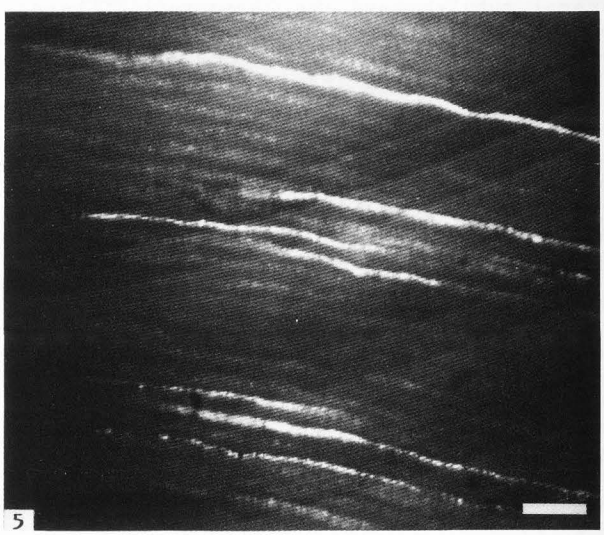
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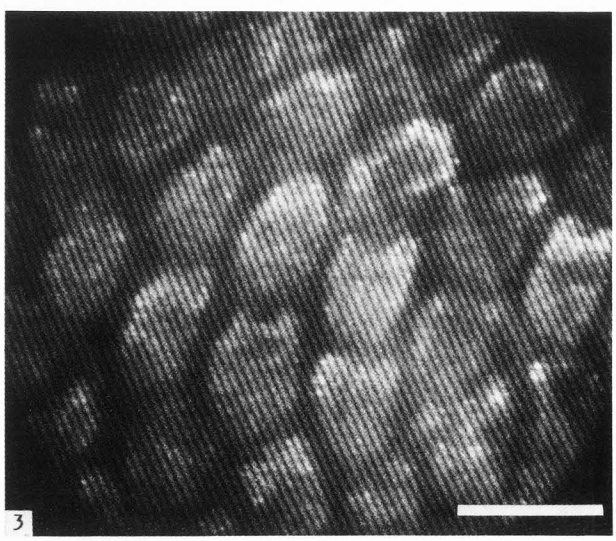
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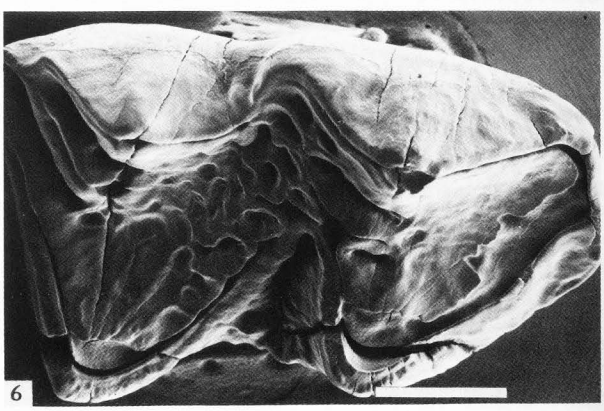
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Fig. 1 Intact labial enamel of a lower incisor of Phascolarctus cinereus by TSRLM. Small prisms occur in well-defined rows, the rows themselves in a gentle wave form. Bar = 25 μm

Fig. 2 Intact labial enamel of a lower incisor of Vombatus ursinus by TSRLM. The prisms are more ovoid and there is no dominant inter-row sheet. There is a slight tendency to row formation, again with some wave form. Bar = 25 μm

Fig. 3 Intact labial enamel of a lower incisor of the fossil labelled Nototherium mitchelli by TSRLM. The prisms are clear and appear in reverse contrast - Pattern 2 prism packing. Bar = 10 μm

Fig. 4 Intact labial enamel of a lower incisor of the fossil labelled Nototherium mitchelli by TSRLM. The prisms are in normal contrast and transversely sectioned. Enamel tubules appear as small bright areas. Bar = 10 μm

Fig. 5 Anorganic enamel of Macropus agilis by TSRLM showing highly contrasted longitudinally oriented tubules running parallel with the prisms. Bar = 25 μm

Fig. 6 Adult dried molar of Petauroides volans for occlusal morphology (SEM).Bar = 1 mm

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clarify the essential relationship of tubule to prism and thereby to formative ameloblast.

Monotreme enamel

There are two families of extant monotremes, the Tachyglossidae (echidnas) and the Ornithorhynchidae (platypus). The echidnas are toothless except for the temporary appearance of an egg tooth which Griffiths (1978) has examined and found to be similar to rudiments described by Hill and de Beer (1949) in Trichosurus vulpecula and Phascolarctos cinereus.

The platypus was presumed to be toothless until 1888 when Poulton announced to the Royal Society that teeth were formed. Subsequently Thomas, in 1889, reported that the teeth were functional for at least a short time in the juvenile (Poulton, 1888a; 1888b; Thomas, 1889). Present day zoologists regard as important the question of whether platypus enamel is prismatic or not (Griffiths, 1978; Grant, 1984); there being unfortunately some disagreement and vagueness in the literature on the subject (Poulton, 1888b; Tomes, 1904; Green, 1937; Hill and de Beer, 1949; Moss, 1969).

The question of the characterization of monotreme enamel is considered here largely by way of a review of recent work on platypus teeth (Lester and Boyde, 1986) and on a recently discovered Miocene monotreme molar (Lester and Archer, 1986).

Materials and Methods

Marsupial enamel

The species names used here are as given in "Complete Book of Australian Mammals" (Strahan, 1983).

1. Tandem Scanning Reflected Light Microscopy (TSRLM)

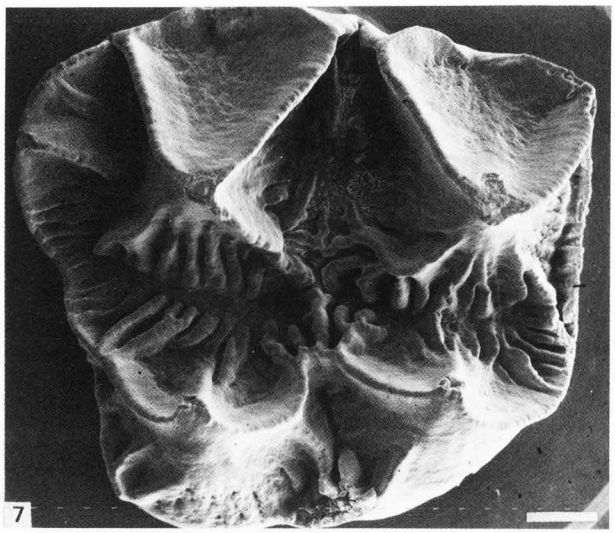
Whole mandibles of Phascolarctos cinereus, Vombatus ursinus, Trichosurus vulpecula and Sarcophilus harrisii were obtained on loan from the Zoology Department, University College London. Individual incisors (M3646 and M35958) and a half mandible (M43523) of the fossil labelled Nototherium mitchelli (almost certainly Zygomaturus trilobus) were obtained on loan from the Palaeontology Department of the British Museum (Natural History), South Kensington, London, U.K. (BM(NH)) - these were originally sent to London from Australia and described at macroscopic level by Richard Owen in 1872. The intact lower incisors of all specimens were examined at the labial face and at worn functional edges with oil immersion objectives by TSRLM.

Developing tooth germs of Macropus agilis also obtained from BM(NH) were refluxed in chloroform/methanol, critical-point dried, plasma-ashed to render them anorganic and photographed under oil by TSRLM.

2. Scanning Electron Microscopy (SEM)

Dried adult molars of Trichosurus vulpecula, Dasyurus maculatus, Wallabia bicolor, Macropus eugenii and Macropus rufus were refluxed in chloroform/methanol and embedded in polymethyl methacrylate. Sectioned surfaces were polished and etched (2% H_3PO_4 for 10 secs); or etched (either 0.5MHCl for 1 min or 0.1MHCl for 1 min) and made partially anorganic (5% NaOCl for 1-3 mins). The aim was to expose to different degrees the methacrylate casts created of the enamel and dentine tubules in their immediate relationship to resolvable detail of the prismatic structure of the enamel and the location of the enamel-dentine junction.

Grooves were cut with a 1 mm (diameter) plain tungsten carbide fissure bur in longitudinal and transverse planes of dried adult molars of Macropus rufus, Aepyrpymnus rufescens and Phascolarctos cinereus. The cuts were made to a depth of 1 mm across occlusal and axial surfaces. In order to reveal the prismatic and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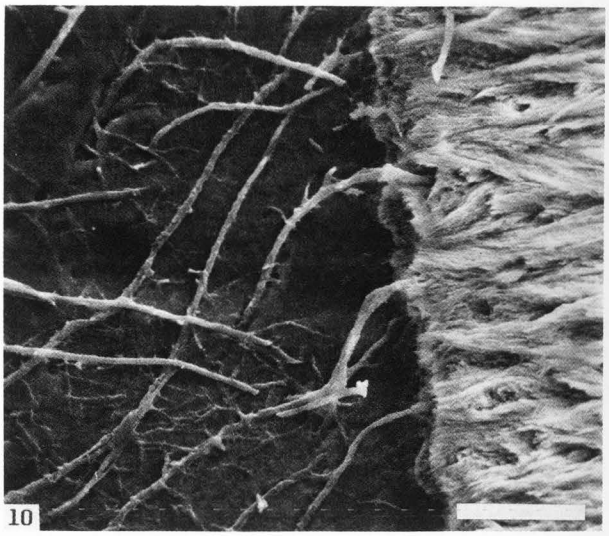
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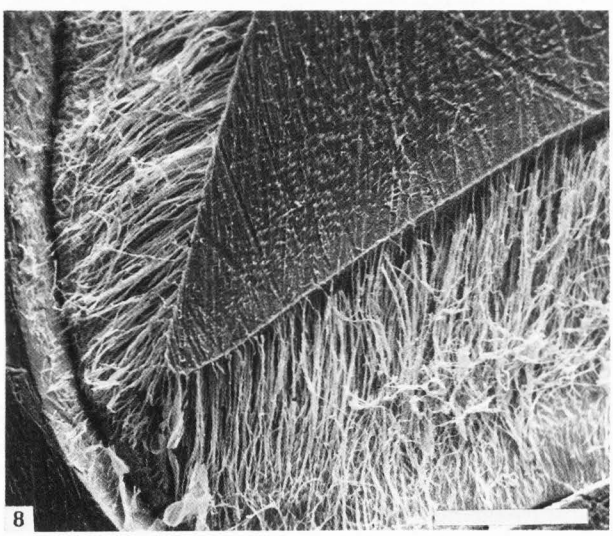
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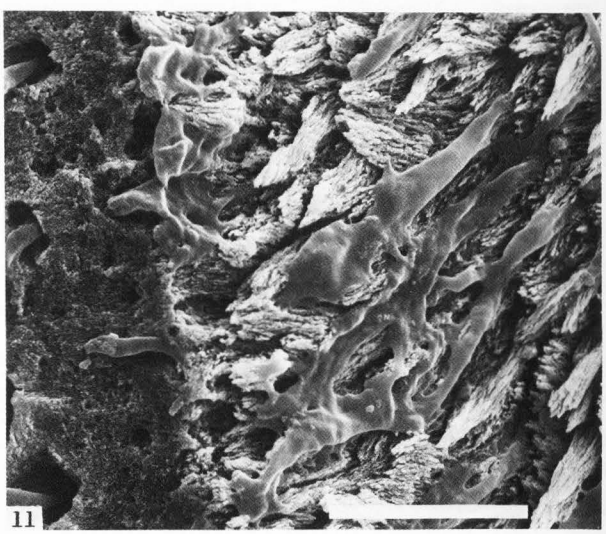
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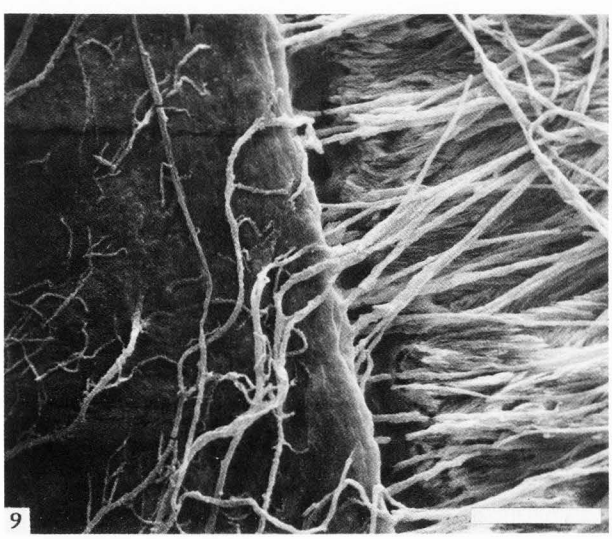
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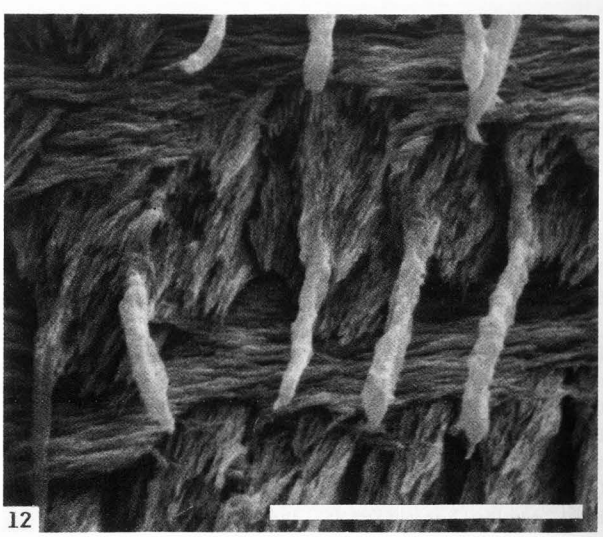
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Fig. 7 Adult dried molar of Phascolarctus cinereus for occlusal morphology. Bar = 1 mm

Fig. 8 Embedded, sectioned, polished, etched molar of Dasyurus maculatus. There is an extraordinary density of marsupial enamel tubules about this cusp - the enamel is completely etched from the specimen surface, the dentine remains. Bar = 100 μm

Fig. 9 Embedded, sectioned, polished, etched surface of molar of Trichosurus vulpecula showing enamel-dentine junction and the continuity of casts of dentinal and enamel tubules (dentine at left). Bar = 100 μm

Fig. 10 Embedded, sectioned, polished, etched Trichosurus molar showing bulb-like expansion of casts proceeding from dentine (at left) to enamel at the junction region. Enamel stands proud of the dentine in this specimen. Bar = 10 μm

Fig. 11 Embedded, sectioned, polished, etched specimen of Macropus rufus at enamel-dentine junction region (dentine to left) showing detail of branching and conjoining of tubule casts immediately on the enamel side of the junction. Bar = 10 μm

Fig. 12 Embedded, sectioned, polished, etched Dasyurus molar enamel showing ultimate relationship of tubule casts to individual prisms. Bar = 10 μm

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tubular structure, 2% H_3PO_4 and EDTA were used as solvent etchants and airpolishing™ (Boyde, 1984) as a physical etching technique. The functional edge of a lower incisor of Vombatus ursinus was airpolished™, etched and examined directly.

All specimens were sputter-coated with gold or carbon and examined by SEM.

Monotreme enamel

1. Ornithorhynchus anatinus:

Three alcohol-preserved specimens of nestling Ornithorhynchus anatinus were obtained on loan through the Australian Museum and the Museum of Victoria. Dissected tooth germs were rendered anorganic in 5% NaOCl (half strength stock solution) for 2 hrs, washed in distilled water and critical-point dried. Some were examined whole, others were fractured open and the remainder refluxed in chloroform/methanol and embedded in polymethyl methacrylate. Sectioned polished surfaces were variously plasma-ashed, sellotape-stripped to induce fracture, airpolished™, etched or cut with a sharp scalpel in efforts to expose internal structure. Resulting surfaces were examined in both secondary electron (SE) and back-scattered electron (BSE) imaging modes in the SEM.

Two air-dried platypus teeth were obtained on loan from BM(NH). These were embedded, progressively sectioned and polished, etched, airpolished™, plasma-ashed and examined in the SEM by both SE and BSE imaging modes after gold or carbon coating.

2. Obdurodon sp.:

The tooth, an upper molar of Obdurodon sp. cf. O. insignis, was originally collected from newly discovered middle Miocene limestone deposits in northwestern Queensland. It was prepared initially by immersion of the surrounding limestone in dilute acetic acid and subsequently washed in tap water. A 2 mm segment of the molar was obtained by longitudinal (axial) fracture. The fracture line included cusp tips, the root-crown junction and the pulp chamber. The fractured surface was etched with 2.5% H_3PO_4 for 18 secs and carefully washed with a stream of distilled water. The etched specimen was then dried, sputter-coated with gold and examined by SEM.

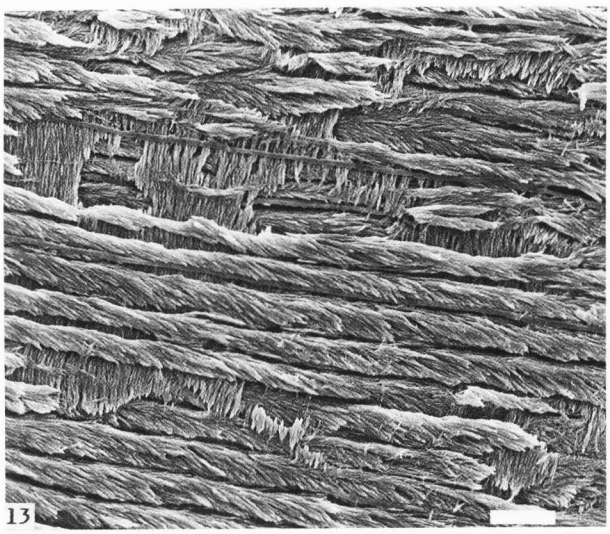
Observations

Marsupial enamel

1. Tandem Scanning Reflected Light Microscopy (TSRLM)

The TSRLM (Petran et al., 1968, 1985) allows calibrated focal sectioning from the outer enamel surface, through the enamel to the enamel-dentine junction in intact specimens (Boyde et al., 1983). Considerable variation was found in prism outline (completeness and shape) and packing as a result of through-focussing the enamel of the different species examined. Trichosurus consistently displayed well-spaced, open-ended ("horse-shoe") prisms. Phascolarctos had the greatest tendency to prism row formation and Vombatus the least. Tubules were observed to greatest advantage in Sarcophilus and Trichosurus.

Figure 1 shows the dominating inter-row sheets and narrow prisms of Phascolarctos cinereus as seen in the intact labial enamel of a lower incisor. The rows occur in the longitudinal axis of the incisor and display a continually changing shallow sinusoidal wave form on through-focussing. The lower incisor enamel



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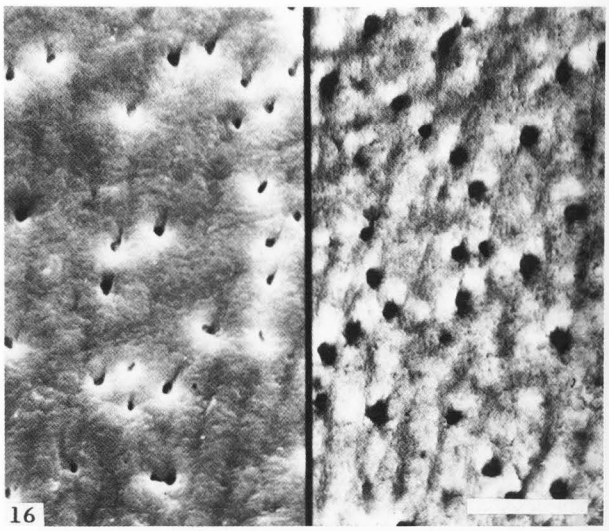

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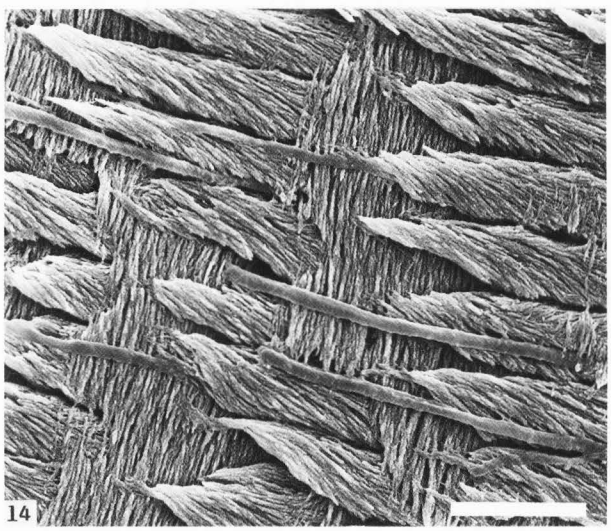

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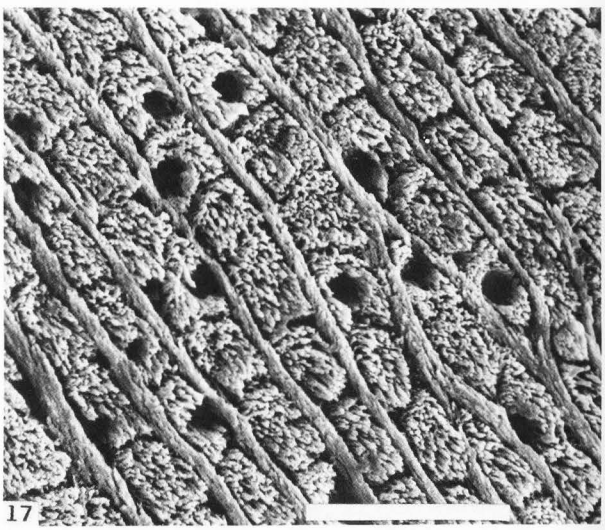

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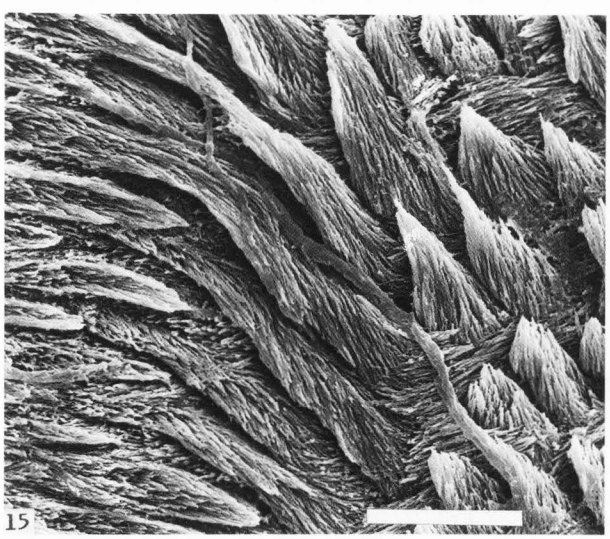

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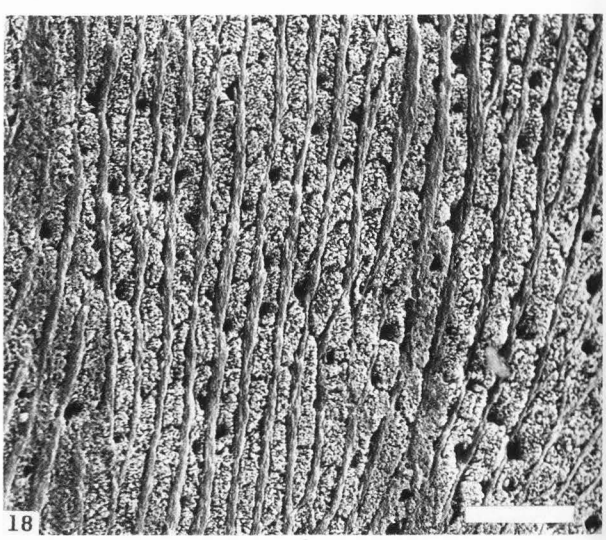

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Fig. 13 Embedded, sectioned, polished, etched surface of Wallabia bicolor molar showing long casts of enamel tubules in immediate association with and parallel to prisms. Crystallite groups in the inter-row sheet are oriented vertically. Bar = 10 μm

Fig. 14 Embedded, sectioned, polished, etched surface of Wallabia bicolor molar showing detail of enamel tubule casts and their intimate, consistent relationship with individual prisms (parallel prisms, parallel tubules). Bar = 10 μm

Fig. 15 Embedded, sectioned, polished, etched surface of Macropus eugenii incisor showing sudden change in prism course. A cast of an enamel tubule follows the change in course from prism outline (bottom right) to prism outline (top left). Bar = 10 μm

Fig. 16 A naturally occurring, worn occlusal surface of Macropus rufus molar treated only by airpolishing™. The surface is seen by SE imaging on left and BSE imaging on right, and shows tubules in what appear to be non-random arrays at the worn surface. Bar = 10 μm

Fig. 17 Airpolished™ etched, cut surface of Macropus rufus molar near the enamel-dentine junction showing numerous large tubules in association with the prisms. Bar = 10 μm

Fig. 18 Airpolished™, etched, cut surface of Aepyprymnus rufescens molar near the enamel-dentine junction showing dominance of inter-row sheet and irregular distribution of large tubules. Bar = 10 μm

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of Vombatus ursinus was quite different from the other marsupial enamels examined in that the prisms were more ovoid in cross-sectional shape, there was no dominant inter-row sheet, and on through-focussing the prisms appeared to move from row to adjacent row to effect a continually shifting spiral pattern (Fig. 2). It is instructive to examine the images obtained of Phascolarctos and Vombatus by TSRLM in conjunction with those obtained by SEM (cf. Figs 1, 2 with 23, 36).

Perhaps the most appropriate application of the instrument was to the fossil labelled Nototherium mitchelli. The incisor enamel exhibited a clear Pattern 2 prism packing (Boyde, 1964) with prisms in reverse contrast at different levels of the enamel (cf. Figs 3, 4). Transversely "sectioned" enamel tubules were also visible in this intact specimen (Fig. 4). Longitudinally oriented tubules were beautifully contrasted in a prepared whole specimen of anorganic developing enamel of Macropus agilis (Fig. 5).

2. Scanning Electron Microscopy (SEM)

It is important to appreciate the variety and complexity of crown morphology found within the marsupials as a group (e.g., Figs 6, 7). This raises an awareness of possible problems in sampling and in attempting to generalize on the histological detail of marsupial enamel.

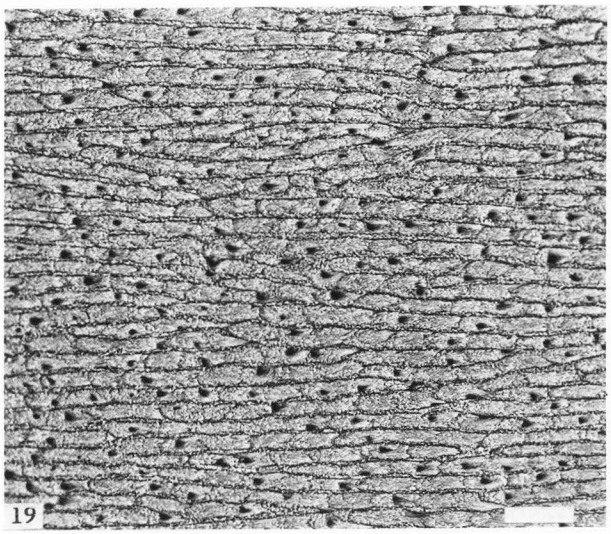
i) Casts of enamel tubules

The extraordinary density of marsupial enamel tubules which may occur is well illustrated over a dentinal cusp in Dasyurus maculatus (Fig. 8). The enamel in this specimen is completely dissolved from the section surface whereas the collagen content of the dentine has resulted in an entirely different response to the etchant. The terminal bending and arborization of the dentinal tubules is clear as is the continuity of tubules between enamel and dentine (Fig. 9). The crystallites of the enamel are evident in the subsurface of this specimen. Where the enamel is not as grossly affected by the etching process, the often described bulb-like expansions of the tubules may be observed on the enamel side of the enamel-dentine junction (Figs 10, 11). The cell surface contact relationship here in the formative stage between the developing Tomes' processes of the ameloblasts and the much-branched odontoblastic processes would be complex (see Lester, 1970). It is apparent, however, that very soon thereafter the enamel tubules begin to relate entirely to individual prisms (Figs 11, 12).

Where the prisms are straight, the tubules are straight (Fig. 13) and can be traced for considerable distances in relation to a single prism (over 140 μm in this specimen). This dependence on prism (and by inference ameloblasts) and independence of inter-row sheet is well shown in Figs 14 and 15. Similarly, a sudden change in prism direction ("diazone" to "parazone") is mirrored by the associated tubule (Fig. 15).

ii) Tubule disposition

As observed in worn and cut surfaces of whole teeth (see also Boyde and Lester, 1984), tubule size, location and frequency



Figs 19 and 20
Cut surfaces of Aepyprymnus rufescens molar, airpolished™ and etched showing at low (Fig. 19) and high (Fig. 20) magnification a regular, repetitive, discrete array of enamel tubules in association with the prisms. Bars = 10 μm

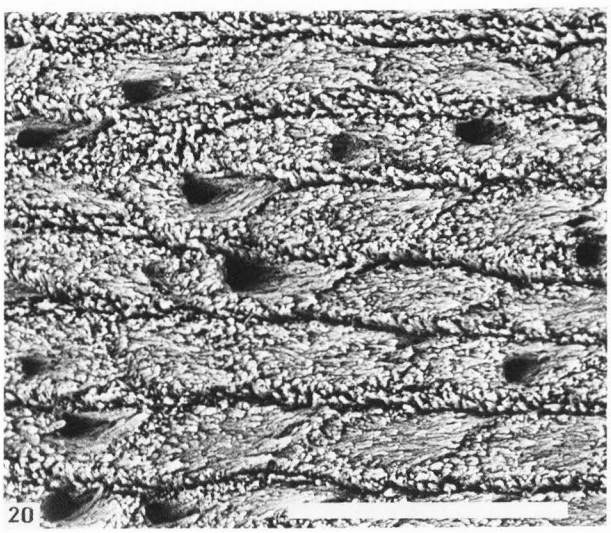
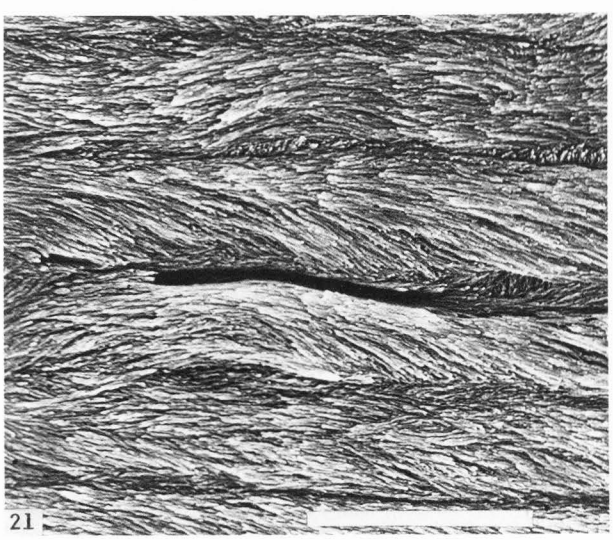


Fig. 21 A longitudinal section of enamel prisms of Aepyprymnus rufescens showing a longitudinally sectioned enamel tubule at the prism/inter-prism interface. Bar = 10 μm



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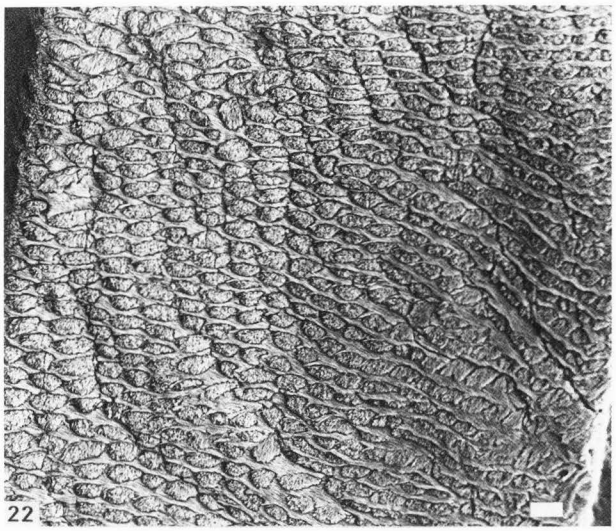
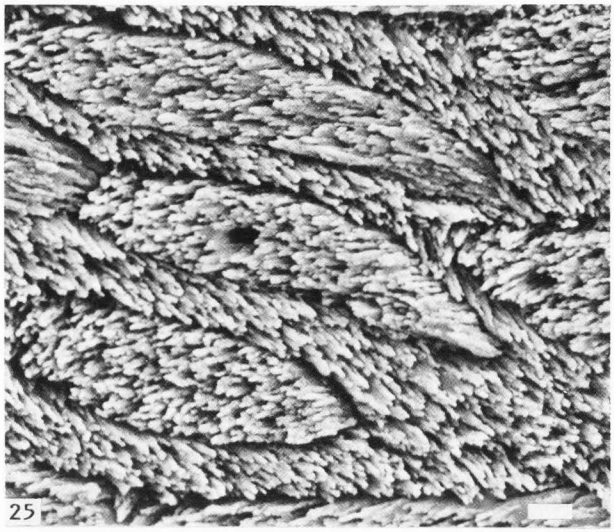
differ in different species, in different teeth of the same species and in different areas of the same tooth (Figs 1-32). Molar enamel of three species is presented here to illustrate this range with tubules both numerous and obvious in Macropus rufus (Figs 11, 17) to the even, more discrete distribution in Aepyprymnus rufescens (Figs 18-21) to Phascolarctos cinereus where the tubules are smallest and fewest (Figs 22-25).

Figure 16 is of a natural occlusal surface of Macropus rufus molar treated only by airpolishing™ and shows to great advantage the high density and the distribution of tubules. There was evidence to suggest that the tubules occur in non-random arrays. Etched prismatic detail of Macropus molar where the enamel has been purposefully exposed in the enamel-dentine junction region confirms the large diameter and high density of tubules (Fig. 17). Aepyprymnus rufescens displays a lesser density of tubules near the junction (Fig. 18), the enamel being dominated in this region by the inter-row sheet. In the middle third of the enamel, the inter-row sheet is less dominant and the tubule array more regular (Figs 19, 20). There is difficulty in confidently identifying a tubule in longitudinal section in an etched surface (Fig. 21) as the tubule may easily be confused with a fortuitously exaggerated boundary plane.

In Phascolarctos cinereus, tubules are difficult to identify (Figs 22, 23) elsewhere than at the enamel-dentine junction (Fig. 24). Where tubules do occur away from the junction, they are small (0.3 μm diameter) and within the substance of the usually quite narrow (2 μm diameter) prisms (Fig. 25).





Figs 22-25 are of a cut surface of Phascolarctos cinereus molar. Figs 23 and 24 are higher magnifications of Fig. 22.

Fig. 22 Whole enamel thickness - dentine bottom right. Pattern 2 packing is evident. The small prisms show tubules in association only near the enamel-dentine junction (see Fig. 24). Bar = 10 μm

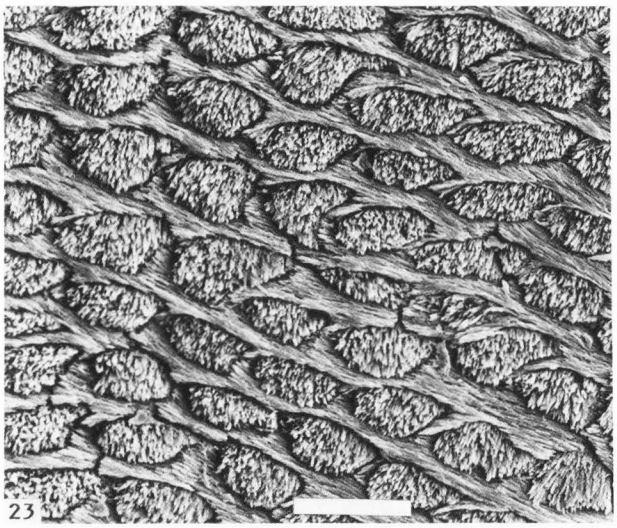
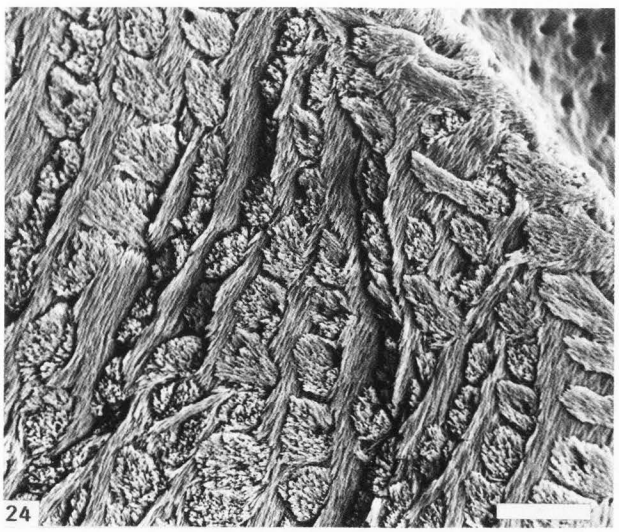


Fig. 23 An atubular area of outer enamel. Bar = 10 μm

Fig. 24 The enamel-dentine junction region showing dominant inter-row sheet and tubules in association with the enamel prisms (dentine top right). Bar = 10 μm

Fig. 25 Very narrow prisms and small and occasional enamel tubules found towards the outer enamel surface. Bar = 1 μm



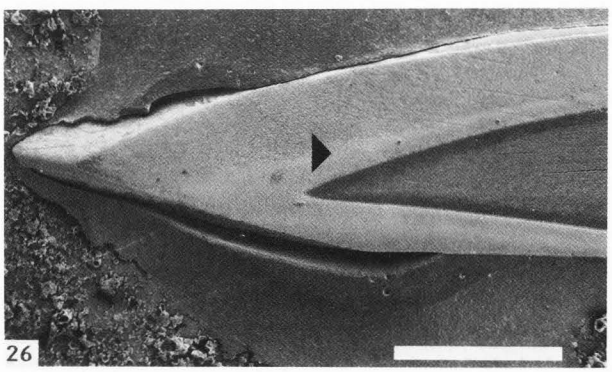
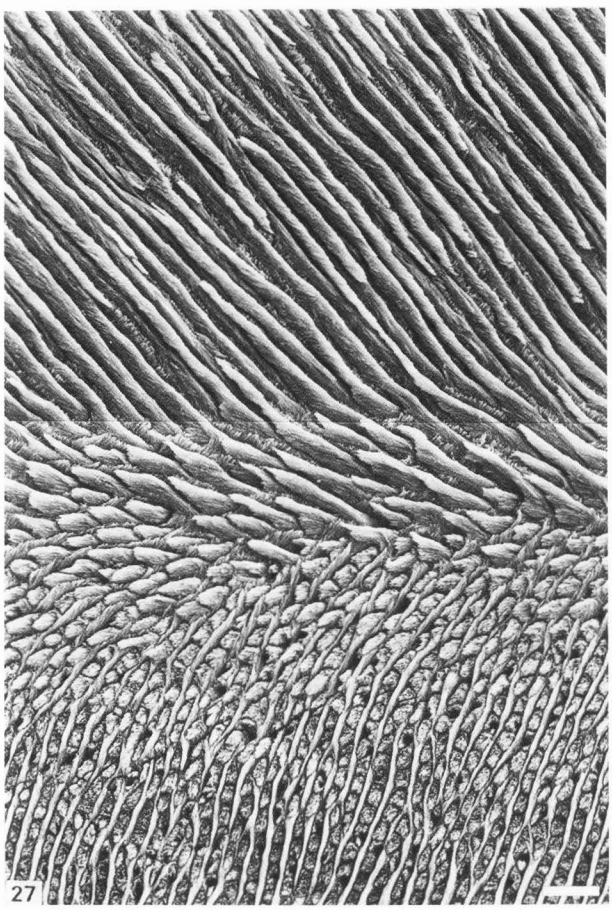
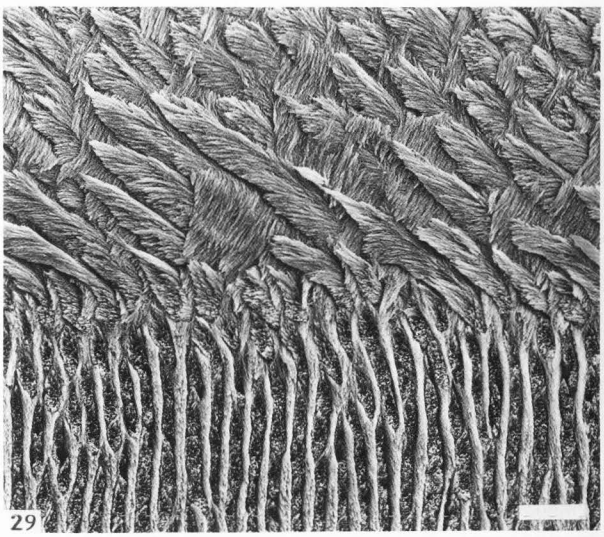
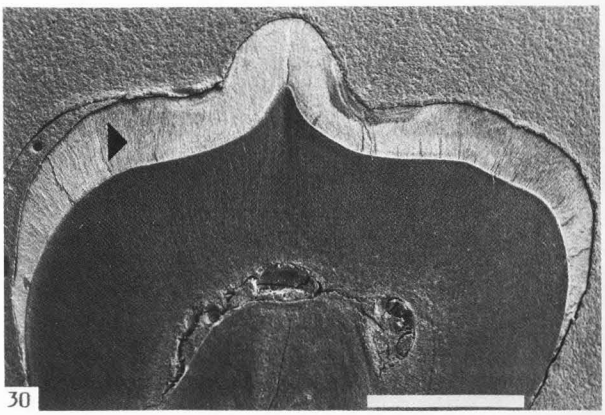
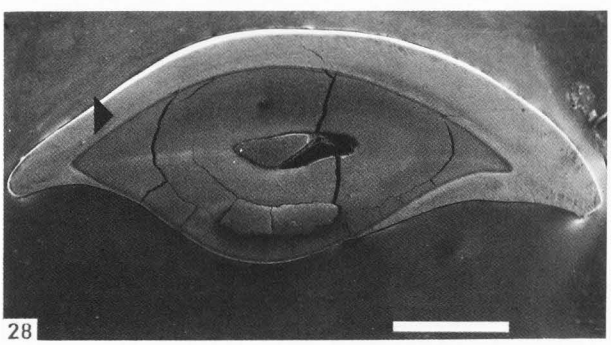


Fig. 26 A survey view of a longitudinal section of the incisor of Macropus eugenii. Arrow locates Fig. 27. Bar = 1 mm









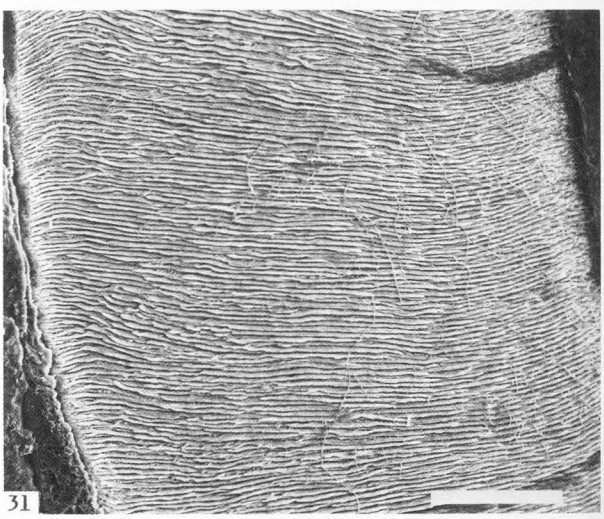


Fig. 27 A composite showing prism course in Macropus eugenii at the junction of inner and middle thirds in longitudinal section (refer Fig. 26). Note the sudden change in prism course. Bar = 10 µm

Fig. 28 A survey view of a transverse section of the incisor of Macropus eugenii. Arrow locates Fig. 29. Bar = 1 mm

Fig. 29 Junction of inner and middle thirds of enamel in transverse section (refer Fig. 28). Bar = 10 µm

Fig. 30 A survey view of a longitudinal section of a molar of Macropus eugenii. Arrow locates Fig. 31. Bar = 1 mm

Fig. 31 Higher magnification of enamel in Fig. 30 to show straight prism course in molar (refer Fig. 30). Bar = 100 µm

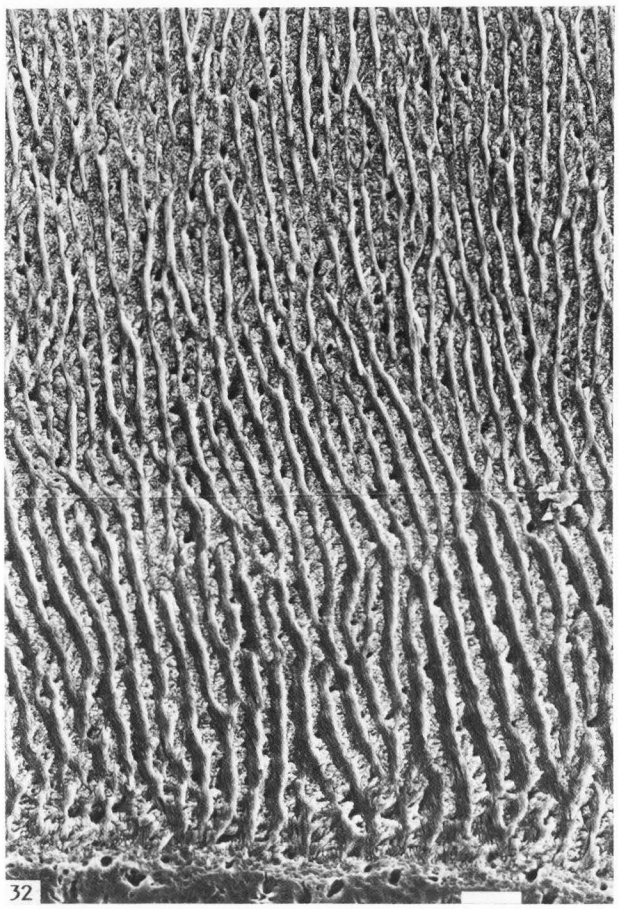


Fig. 32 Inner two thirds of enamel of transverse section of molar enamel of Macropus eugenii (dentine to bottom). Prisms are parallel (cf. Fig. 31) and the area dominated by inter-row sheet. Bar = 10 μm

iii) Prism course

Some details of prism course are described in teeth of two species:

a) in an incisor and a molar of Macropus eugenii as seen in longitudinal and transverse sections, and

b) in an incisor of the (somewhat neglected) atubular Vombatus ursinus as seen at a functional edge.

a) Macropus eugenii:

The incisor enamel exhibits a complete and sudden major change in prism direction at the junction of inner and middle thirds (Gilkeson, 1986) (Figs 26-29). Study of longitudinal sections (Figs 26, 27) and transverse sections (Figs 28, 29) shows that the prisms in the inner third enamel run parallel leaving the enamel-dentine junction at approximately 45° on the medial and lateral sides of the tooth and 90° in the mid-line. Prisms in the outer third are parallel with each other running to the outer enamel surface at approximately 45° in an incisal direction. At the medial and lateral edges of the incisor ("cusps" in transverse section), the line of change in prism direction expands to become an area of "gnarled enamel".

The molar enamel does not show the same sudden change in prism course about its circumference, the prisms passing straight from enamel-dentine junction to outer enamel surface (Figs 30, 31, 32). The exceptions are localized sites over dentinal cusps or under surface concavities or fossae where, again, the prism course becomes confused in the outer to middle third enamel.

b) Vombatus ursinus:

A functional edge of a wombat incisor is illustrated here as a partial but easily accessible window on prism course. The prisms in the mid-line of what is, in effect, a naturally occurring oblique transverse section show no decussation but are slightly wavy in their course (Fig. 33). Away from the mid-line, there are discrete "parazones" and "diazones" of 6-8 prisms in width (Fig. 34). Inter-row sheet is obvious where prisms are transversely sectioned (Fig. 35) as it is at the worn, exposed outer enamel surface (Fig. 36).

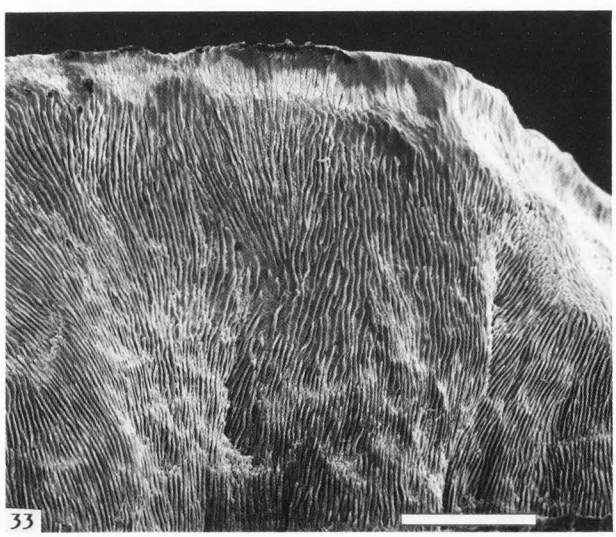
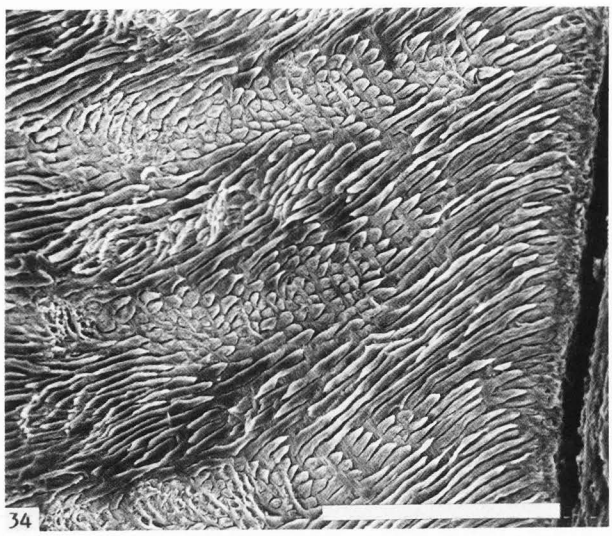


Fig. 33 Functional, etched edge of lower incisor of Vombatus ursinus showing relatively straight, slightly wavy course of prisms but little evidence of real decussation. Bar = 100 μm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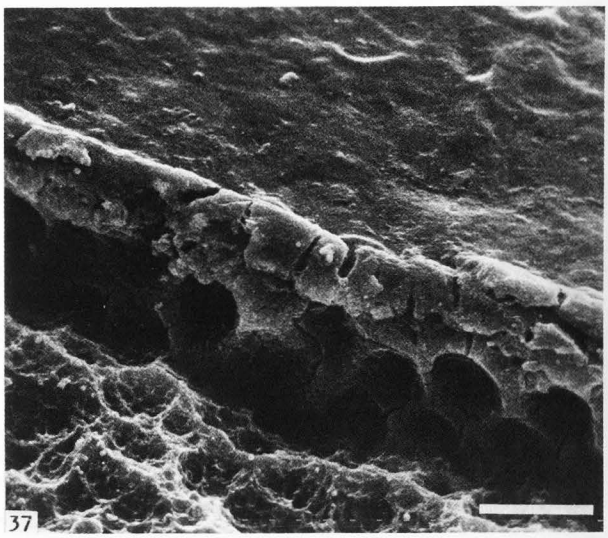
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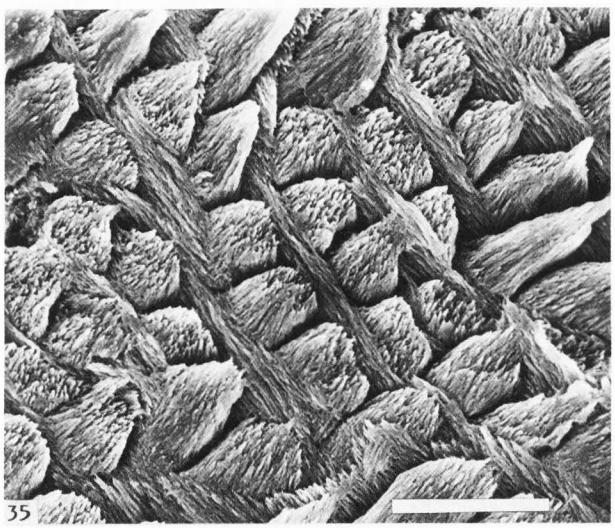
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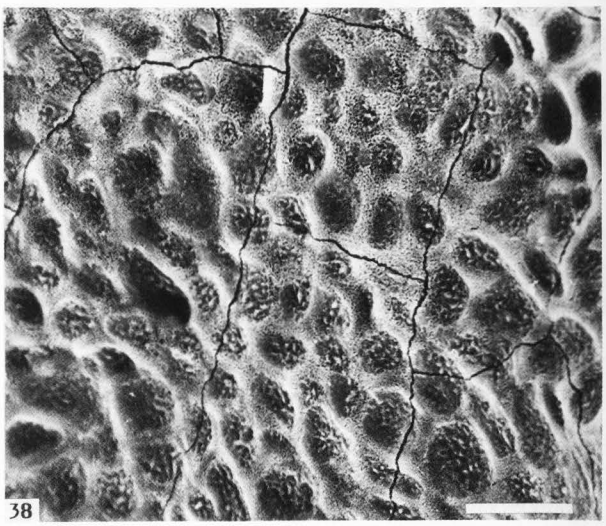
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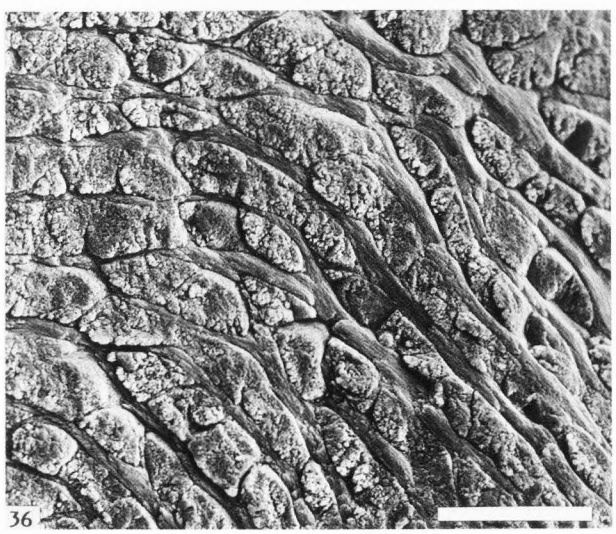
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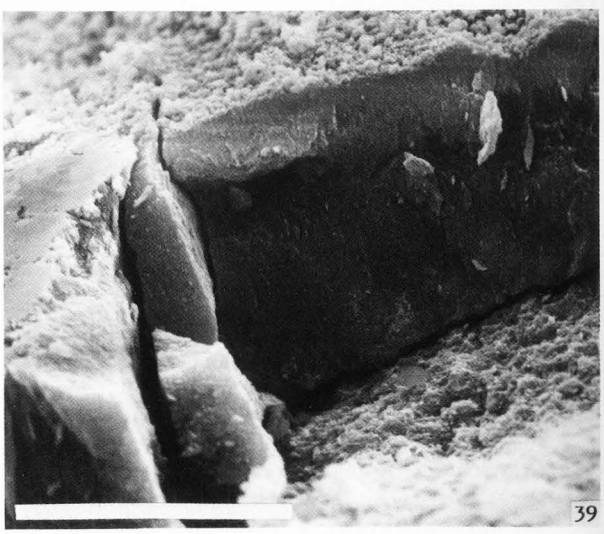
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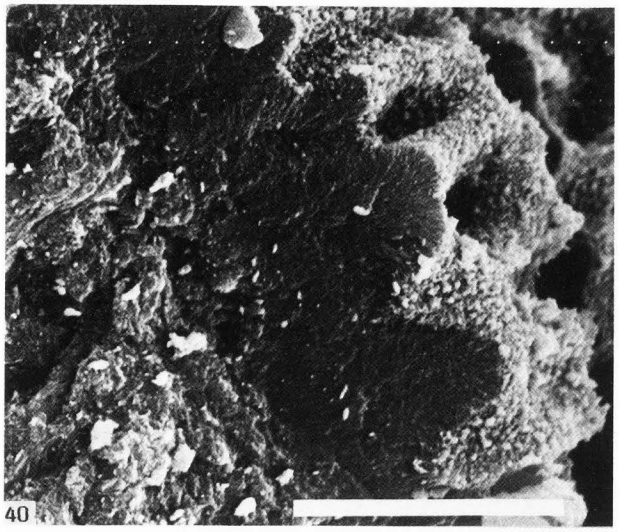


Fig. 34 Lateral edge of functional etched edge of lower incisor of Vombatus ursinus showing parazones and diazones, an indication of regular decussation. Bar = 100 μm

Fig. 35 Higher magnification of Fig. 34 showing definite prism stacking and occurrence of Pattern 2 prism packing within a diazone. Bar = 10 μm

Fig. 36 Larger area of transversely sectioned prisms at functional edge of lower incisor of Vombatus ursinus confirming Pattern 2 packing which is not generally as evident. Bar = 10 μm

Fig. 37 Plasma-ashed, embedded unerupted tooth of Ornithorhynchus anatinus showing a sectioned surface to the top right contiguous with that of a developing surface with Tomes' process indentations (methacrylate to bottom left). Bar = 10 μm

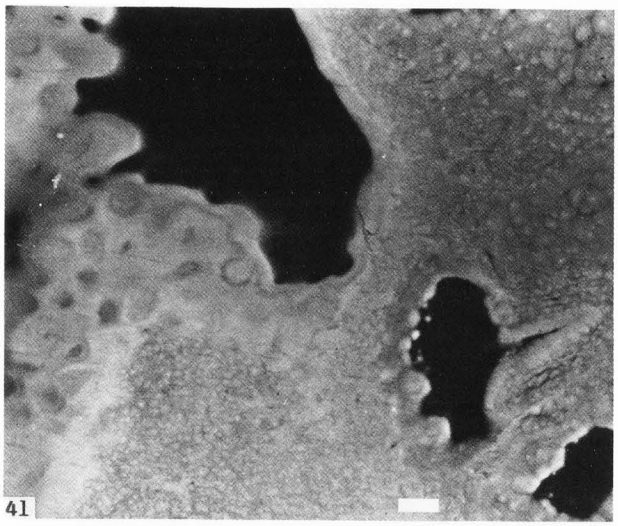


Fig. 38 An anorganic surface of an unerupted developing tooth of Ornithorhynchus anatinus showing the irregular and shallow nature of Tomes' process depressions in the developing front. Bar = 10 μm

Fig. 39 A fractured surface of developing enamel in an anorganic unerupted tooth of Ornithorhynchus anatinus showing the lack of repetitive structural detail (dentine to bottom right, outer enamel surface to top left). Bar = 10 μm

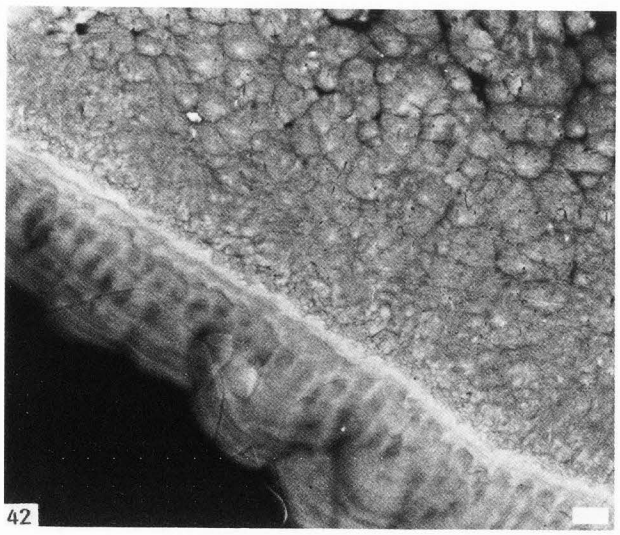
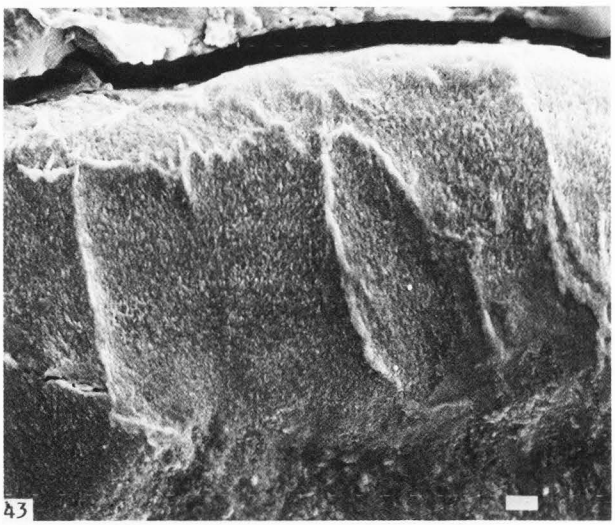


Fig. 40 Fractured, anorganic, developing Ornithorhynchus anatinus tooth germ (dentine to left, outer enamel surface to top right). Note the orientation of crystallite groups preferentially at right angles to the developing surface. Bar = 10 μm

Fig. 41 A BSE image of an embedded, sectioned, developing Ornithorhynchus anatinus tooth showing faint prismatic outlines (at left) in the thin enamel lining the dentine. Bar = 10 μm

Fig. 42 A BSE image of a polished, sectioned, embedded, anorganic developing tooth germ of Ornithorhynchus anatinus showing an indication of prism formation in the inner third of the enamel (bottom left). Note also the incremental lines and the radial features in the enamel. Bar = 10 μm



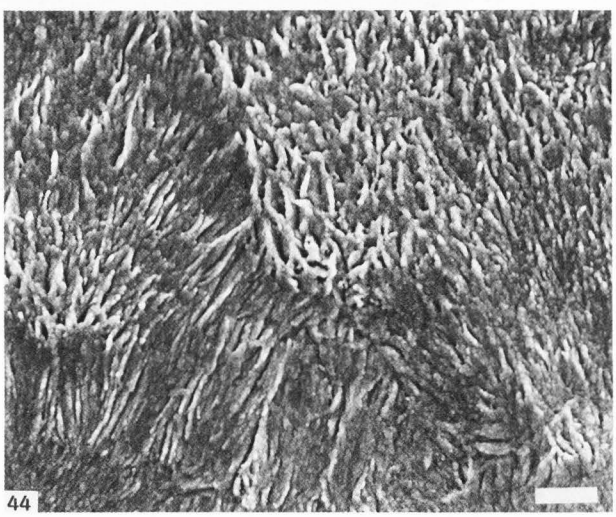
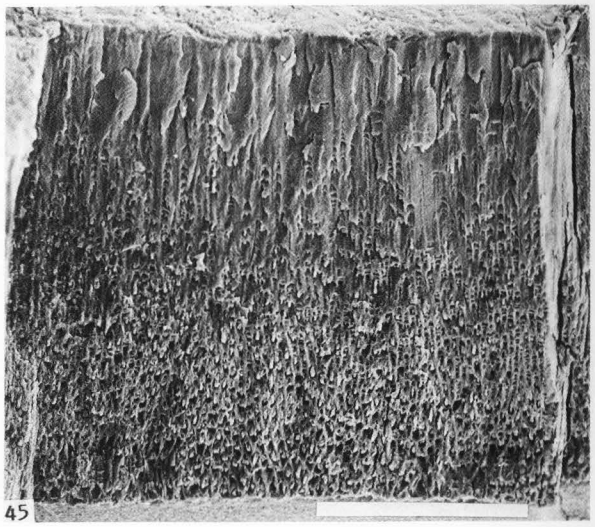
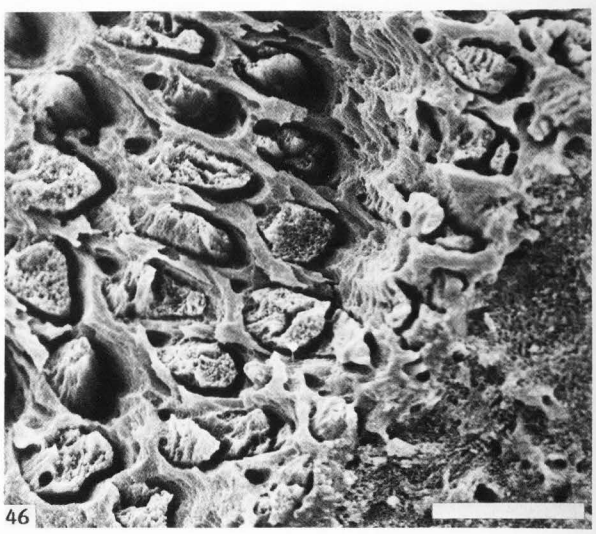
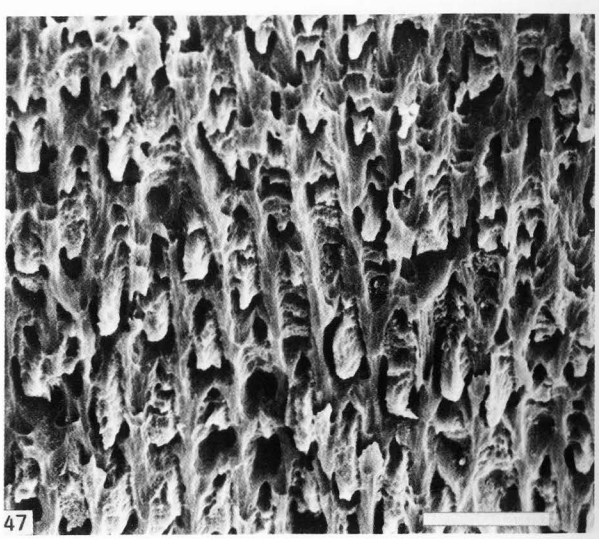


Fig. 43 Polished, etched surface of embedded, adult, Ornithornhychus anatinus tooth showing crystallite groups not oriented in prismatic array but with radial defects in the enamel (dentine bottom right). Bar = 1 μm

Fig. 44 Shows at higher magnification a clear boundary plane (running diagonally) between preferentially oriented enamel crystallite groups in adult embedded, etched, Ornithorhynchus anatinus enamel. Bar = 1 μm







Monotreme enamel

1. Ornithorhynchus anatinus:

Specimens of unerupted and formed platypus teeth are extremely rare. We have recently examined, by SEM, unerupted and formed teeth for the presence of typical mammalian features (Lester and Boyde, 1986).

Figs 45-47 are of fractured, airpolished^{TM} etched Obdurodon sp. molar enamel.

Fig.45 Full thickness fracture of enamel (outer surface to top). Enamel is prismatic and the prisms are parallel, curving slightly (transversely sectioned to longitudinally sectioned) through the enamel. Bar = 100 μm

Fig. 46 Enamel-dentine junction region (dentine bottom right) showing round-ovoid prisms, hexagonally packed in association with tubules. Bar = 10 μm

Fig. 47 Enamel in middle third. Prisms are stacked (Pattern 2) with prominent inter-row component. Bar = 10 μm

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The developing teeth are particularly small and fragile and difficult to examine in the normal way for morphological detail. Nevertheless, surfaces showing features typical for the developing fronts of mammalian enamel were identified in anorganic unerupted specimens (Figs 37, 38). Prepared fractured surfaces of unerupted anorganic specimens failed to develop any topographical relief which could be taken as evidence for a repetitive structural pattern (Fig. 39). Where resolvable however, crystallite groups were found to be preferentially oriented perpendicular to the developing front (Fig. 40). Although the basic prerequisites for prism formation for a Pattern 1 enamel were found, a widespread and regular prism pattern was not. Indications of a repetitive prism form appeared only in high contrast BSE images of sectioned, polished surfaces where the information is collected from the surface layers of the specimen in an additive way (Figs 41, 42). Even so, this pattern was clear only in the inner-third of the enamel of the developing teeth with well-marked incremental lines being the most prominent features in the more peripheral enamel. Etched, polished surfaces of the enamel of both developing and adult teeth displayed crystallite domains of variable size and outline, radial clefts, and fine tubules (Figs 43, 44).

2. Obdurodon sp:

A longitudinal fracture surface of an airpolished^{TM}, etched fossil molar has been examined recently by Lester and Archer (1986). It presents a type of enamel unique in its combination of the following features (Figs 45, 46, 47):

i) tubules in great number (a possibly plesiomorphic feature among mammals);
ii) prisms of small diameter (a possibly plesiomorphic feature within mammals);
iii) a substantial and coherent inter-prismatic and, in the outer enamel, inter-row structure (a possibly plesiomorphic feature within mammals);
iv) a simple, transversely sinous, mutually parallel, non-decussating prism course (a possibly plesiomorphic feature with mammals);
v) a predominantly Pattern 1 prism-packing in the inner enamel (together with (iii) a possibly plesiomorphic feature among mammals, but without (iii) a possibly derived feature and found in extant insectivorans, sirenians and some odontocetes);
vi) a distinct tendency to Pattern 2 prism-packing in the outer half of the enamel (a marsupial-like feature but one also found in the majority of living ungulates - although not rhinos or hippos - and to a much reduced degree in primates).

Discussion

Marsupial enamel

1. Prism shape and packing pattern

In a comparative study of prism shapes and packing patterns, Shobusawa (1952) examined Macropus and Dendrolagus enamel in haematoxylin stained, ground sections. He likened the enamel to that of ungulates, in having ovoid or oblong prisms (2-5 μm in diameter), arranged in lines or rows and with a wide "inter-prismatic" area between the rows, but noted that the enamel showed some peculiarities of its own. Boyde (1964) defined the now widely accepted cross-sectional patterns of enamel prisms and related these on a three dimensional basis to the contours of the developing enamel front and their intimate relationship with the Tomes' processes of the ameloblasts. Boyde also ascribed marsupial enamel to a Pattern 2 design: "horse-shoe" (cross-sectional shaped) prism boundaries, the prisms arranged in longitudinal rows with no regions definable as inter-prismatic between prisms in the same row. He further assigned marsupial enamel to one of two sub-groups (Pattern 2A) to differentiate it from primate (Pattern 2) and Myomorph and Scuiromorph incisor inner-enamels (Pattern 2B). Pattern 2A is as for

Pattern 2 but with a greater separation between the rows of prisms occasioned by inter-row sheets, this characteristic being shared with the ungulates and the lagomorphs.

Our observations show that prism shape, row formation, tubule disposition, and changes in these features through the enamel thickness are clearly resolvable by TSRLM in the intact labial enamel of extant (Figs 1, 2) and fossil (Figs 3, 4) marsupials. Thus, Trichosurus consistently displays well-spaced, open-ended (horse-shoe) prisms; Phascolarctos (Fig. 1) has the greatest tendency to prism row formation and Vombatus (Fig. 2) the least; and tubules are observed to greatest advantage in Sarcophilus and Trichosurus (see also Fosse and Holmbakken, 1971). Prism rows are arranged in the longitudinal axis of lower incisors in a sinusoidal fashion and with through-focussing, prisms and rows shift to create constantly changing spiral patterns. The degree of variation in prism outline (completeness), shape and pattern evident in the different species examined indicates some possible potential for species identification utilizing as indicators intact incisors.

2. Enamel tubules

Boyde and Lester (1967) summarized an earlier study of a number of species by a variety of microscopical techniques, including for the first time electron microscopy, as follows.

i) The undoubted continuity of some dentinal and enamel tubules could be traced in all species examined.
ii) The tubules leave more residue than the surrounding enamel when decalcified.
iii) The tubules are permeable to dyes in extracted teeth.
iv) The dyes methyl blue and trypan blue did not reach the enamel tubules from the pulp or blood-stream in in situ adult teeth of Metachirus nudicaudatus.
v) The tubular nature of the tubules is well demonstrated in scanning electron micrographs and replicas of fractured enamel and also in replicas of argon-ion beam eroded Macropus molar enamel surface.
vi) The tubules are situated within the enamel prisms.
vii) The tubules may be recognized in electron micrographs of developing enamel as regions in which crystallites do not develop.
viii) The study of enamel tubule development revealed no special features of the ameloblasts or of the nature of the first secreted enamel.

Boyde and Lester (1967) went on to state that enamel tubules were differentially diagnosable from spindles by the following features:

i) spindles are (or have) dentinal components (Frisbie, 1952; Schlack, 1940);
ii) spindles are thicker than tubules and prisms;
iii) spindles are shorter than tubules (reaching only some 50-100 μm from the dentinal surface); and
iv) spindles (or their origins) are apparent between ameloblasts before enamel development.

Lester (1970) further studied developing Didelphis marsupialis material by transmission electron microscopy and concluded that:

i) tubules contain cell processes which are bounded by plasma membrane initially and are extensions of ameloblast cytoplasm;
ii) there is an overlapping or interdigitation of odontoblast processes and the cytoplasm of presumptive ameloblasts immediately prior to enamel formation; and,
iii) this cytoplasmic relationship is maintained during subsequent enamel development and would account for the continuity of enamel and dentine tubules in the adult state.

Subsequently, Boyde and Lester (1984) applied some new approaches in specimen preparation and interpretation by SEM to this subject and concluded as follows.

i) Enamel tubules are open spaces which may be embedded with polymethyl methacrylate.
ii) Many enamel tubules are continuous with dentine tubules at the enamel-dentine junction and many are not, the details varying between species.
iii) The most frequent location of tubules is at prism border locations or in prism bodies.
iv) Although not every prism has a tubule some have more than one.
v) Tubule locations can be related to tubule deficiencies opening at the developing enamel surface (mineralising front).
vi) Enamel tubules can be seen arriving at mature outer enamel surfaces but are more readily identified at worn occlusal surfaces.

The body of ultrastructural evidence up until 1970 has subsequently been challenged by observations made on ground sections by light microscopy. Risnes and Fosse (1974) confirmed the earlier observations of Mummery (1914), Williams (1923) and Gustavsen (1972) on the

occasional departure of enamel tubule course from the apparent general prism direction. In the five species examined, Risnes and Fosse (1974) noted this deviation to be always in a cervical direction and usually at 45°, although more abrupt changes to 90° were also apparent. From this and other irregularities of tubule form, Risnes and Fosse (1974) argued against an ameloblastic origin of the tubules.

Osborn (1974), acknowledging that the optical contrast of prism sheaths in his ground sections was "generally poor", reported with the aid of helpful line drawings that some enamel tubules passed obliquely from "what appeared to be" the general prism direction. He too disputed the ameloblastic origin of enamel tubules.

Our more recent observations reported here on methacrylate casts of tubules exposed by a variety of etching procedures from sectioned, embedded specimens provide definitive evidence on the association of tubules with individual prisms in the body of the enamel (Figs 13, 14). The incisor enamel of Macropus eugenii is shown to exhibit a complete and sudden change in prism direction at the junction of the inner and middle thirds in longitudinal (sagittal) section (Figs 27, 28). Incisor and molar enamel demonstrate discrete and predictable areas of "gnarled enamel". We can discern that tubules follow the course of their parent prism through such areas (Fig. 15).

It is our contention that changes in individual prism and tubule direction would not always be resolvable in ground sections by light microscopy. We would like to suggest that our previously reported evidence (Boyde and Lester, 1967; Lester, 1970; Boyde and Lester, 1984) together with the present observations identify the fundamental relationship of tubule to prism in the body of enamel and, in so doing, put beyond dispute the essential relationship of the marsupial enamel tubule to the formative ameloblast. However, this conclusion must not be generalised to other recent or fossil mammalian enamels which also show tubules (see Carlson and Krause, 1985).

Monotreme enamel

Platypus enamel is prismatic only in part (Fig. 42): well-formed, regular prisms are not a primary structural feature (Lester and Boyde, 1986). Indeed, incremental lines and relatively poorly mineralized radial features are much more apparent. This can be related both to the variability inherent in the developing surface (Fig. 38) and to the thinness of the enamel layer (Fig. 41).

There are relatively few specific statements on the subject of the prismatic nature or otherwise of platypus enamel in the literature. Poulton (1888b) described and drew (his Pl.III, figs 1, 3) prisms in an undecalcified section of a developing tooth as finely striated, parallel to the surface, polyhedral in transverse section and varying considerably in size. Whilst not describing histological detail, Tomes (1904) and Green (1937) found the enamel "degenerate". Green is acknowledged in a footnote by Hill and de Beer (1949) as permitting them to state that the enamel "exhibits unquestionable prismatic structure"; and Moss (1969) thought the enamel of these "rudimentary fetal teeth" was non-prismatic. In neither of the last two cases was any pictorial evidence provided.

Our findings support the concept of platypus teeth as vestigial remnants but do not enable monotreme teeth to be as well characterized as for other groups of mammals. This was part of the reason for an examination of the well-developed molar of the newly discovered Miocene ornithorhynchid Obdurodon sp. It is possible to say now that monotreme enamel has been characterised (Figs 45-47) and, further, that the enamel is representative of a unique and relatively plesiomorphic mammalian group (Lester and Archer, 1986). Certainly, the enamel of Obdurodon sp. appears to represent a structural stage intermediate between that of known multituberculates (Sahni, 1979; Fosse et al., 1985; Carlson and Krause, 1985) and extant tribosphenid mammals. We acknowledge at the same time problems with polarities of many of the ultrastructural characters considered and their exact utility in phylogenetic reconstructions (Krause - personal communication). We also acknowledge the need for further quantification of features and for more extensive examination of variability.

Our findings based on enamel ultrastructure do not enable unambiguous placement of monotremes within a phylogenetic framework of mammals but they provide clear evidence for the notion that monotremes are at least similar to some multituberculates as well as to some tribosphenid therians (for current hypotheses, see Lillegraven et al., 1979; Kemp, 1983; Archer et al., 1985). Indeed, the highly distinctive inter-prismatic honeycomb (Fig. 47) is strikingly similar to the condition seen in some multituberculates. If multituberculates and monotremes are monophyletic, it would help to explain what has otherwise been a puzzling zoogeographic pattern in the distribution of each group: monotremes only being known from Australia while multituberculates were widespread in Nearctica but unknown in Australia.

Conclusions

Marsupial enamel

1. Tandem Scanning Reflected Light Microscopy
   i) Prism shape, row formation, tubule disposition, and changes in these structural features through the thickness of the enamel were clearly resolvable by TSRLM in the intact labial enamel of the different extant and fossil marsupial specimens examined.
   ii) Trichosurus consistently displayed well-spaced, open-ended (horse-shoe) prisms; Phascolarctos had the greatest tendency to prism row formation and Vombatus the least; and tubules were observed to greatest advantage in Sarcophilus and Trichosurus. Prisms in the fossil labelled Nototherium mitchelli are closed ovoids and packed in Pattern 2A.
   iii) Prism rows are arranged in the longitudinal axis of lower incisors in a sinusoidal fashion and with through-focussing, prisms and rows shift to create constantly changing spiral patterns.
   iv) Great variation in prism outline (completeness), shape and development of Pattern 2 prism packing was evident in the different species examined indicating some potential for possible species identification utilizing intact incisors.

2. Scanning Electron Microscopy
   i) Methacrylate casts of marsupial enamel tubules exposed by a variety of etching procedures from sectioned, embedded specimens provide definitive evidence of the association of the courses of the tubules with individual prisms in the body of the enamel.
   ii) Worn and cut surfaces of whole teeth show clearly that tubule size, location and frequency differ in different species in different teeth of the same species and in different areas of the same tooth. Of the three species examined, tubules were largest and most irregular in Macropus rufus, numerous but regularly arranged in Aepyprymnus rufescens and smallest and fewest in Phascolarctos.
   iii) The incisor enamel of Macropus eugenii exhibits a complete and sudden change in prism direction at the junction of the inner and middle thirds in longitudinal and transverse section. The incisor also demonstrates discrete and predictable areas of "gnarled" enamel.
   iv) The molar enamel of Macropus eugenii does not show the same sudden change in prism course about its circumference. However, predictable, localised sites display "gnarled" enamel and tubules follow the course of their parent prism through these areas.
   v) A functional edge of Vombatus incisor showed no prism decussation in the mid-line but discrete parazones and diazones in lateral areas. Inter-row sheet is obvious where the prisms are transversely sectioned. However, the inter-row sheet in Vombatus is not as well developed as in the other species examined.

Monotreme enamel

1. The enamel of Ornithorhynchus anatinus is prismatic only in part; "prisms" are not a primary structural feature and are best seen in BSE images in the SEM. Incremental lines and large, relatively poorly mineralized radial features are much more apparent.

2. Examination of a well developed molar of the newly discovered Miocene ornithorhynchid Obdurodon sp. has allowed monotreme enamel to be characterized. The enamel is definitely prismatic and tubular and displays large areas of Pattern 2 prism packing.

3. Monotreme enamel is representative of a unique and relatively plesiomorphic mammalian group, representing a structural stage intermediate between that of known multituberculates and extant tribosphenid mammals.

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Discussion with Reviewers

DFG Poole: Could the authors please give an opinion on the possible function of tubules, and on the significance of their presence in, or absence from, enamels in different mammals?
Authors: We are reluctant to speculate on the possible function of enamel tubules. However, we note that marsupial enamel develops rapidly, such that it is common to find thicker enamel than dentine, at least in a developing tooth. If a relatively thin shell of dentine were to persist in a functional tooth, it would be an advantage to have "an early warning system" for the pulp, so that odontoblasts could lay down secondary dentine before the close approach of the oral environment. This explanation presupposes the continuity of the enamel and dentine tubules.

L Moss-Salentijn: The application of various SEM techniques and TSRLM provide us with exciting new images of the enamel of marsupial and monotreme teeth. In this regard, the finding of the partial and imperfect prismatic nature of the enamel of Ornithorhyncus anatinus is of particular interest.

One of the problems that remains vexing, with respect to the structure of marsupial enamel, is the still unresolved nature of the organic constituents ("fibrils") enclosed in the enamel tubules. By now, there is adequate information on the morphology of the inorganic components and on the extent, density and origin of the tubules in marsupial enamel. The problem of preserving and imaging cell processes in hard tissues (such as dentine) has proved to be monumental. Careful TEM study of the development of enamel in staged pouch specimens must be the obvious next step to resolve this problem.
Authors: TEM studies (Kemp, 1983) have shown that enamel tubules do contain the cell process of an ameloblast during developmental stages. We agree that it would be extremely difficult to study the involution, decay, or degeneration of this process by TEM.

MM Smith: Is there a functional explanation for the difference in prism direction between incisors and molars? What are the implications of open tubules on the occlusal surfaces?
Authors: We presume that the differences in prism direction relate to different functional demands in terms of the stress applied to the enamel of the incisors and molars, respectively. A more detailed analysis of this problem is obviously required.

The implications of the open tubules on the occlusal surfaces are, given that enamel tubules are continuous with those in the dentine, that an early warning be given to the pulp of the impending approach of the external surface of the tooth as that is removed in functional wear.

G Fosse: In longitudinal sections of multituberculate enamel, Fosse et al 1973, (Calcified Tissue Res. 11, 133-150) and Fosse et al 1985, showed crossing of several prisms by individual enamel tubules in the same focal plane. How does this agree with an ameloblastic origin of the enamel tubules?
Authors: Our paper concerns the arrangement of marsupial enamel tubules and we would not wish to extrapolate from the structure we know well here to that of the multituberculates, which you have studied in detail. In the case of marsupials, we are confident that one enamel tubule was made by one ameloblast.