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A study of enamel organization, from Reptiles to Mammals

D.N. Stern, A.W. Crompton

Samples of teeth from three extant reptiles, several non-mammalian cynodonts, and an early mammal were acid-etched and examined by scanning electron microscopy (SEM). This study had a three-fold purpose: 1) To extend existing information on enamel ultrastructure which might be used to establish taxonomic affinities within the reptile-to-mammal transition group. 2) To recognize whether enamel ultrastructure may be related to dental occlusion. 3) To present hypothetical models of the shape and secretory surfaces of ameloblasts in the forms studied and, based on these models, to propose how the transition from non-prismatic to prismatic enamel was achieved. None of the specimens examined had the features characteristic of the prismatic enamel of extant mammals, i.e. a prism (P) surrounded by a prism sheath (a space formed during development as a result of differing crystallite orientation, where organic material collects to be removed from the matrix as maturation proceeds) and interprismatic (IP) enamel. The extant reptiles had aprismatic enamel, except for *Uromastyx* which had an irregularly arranged prismatic appearance. In *Massetognathus*, round columns could be recognized within the enamel. The tritylodontid had shallow prism-like regions, but the structures were not very distinct, nor were they regular in distribution. *Pachygenelus* and *Megazostrodon* had a herringbone type of (pseudo)prism, and a seam in the center of each cellular domain. These domains were arranged hexagonally; we call these early prisms. Early prismatic enamel appears columnar (pseudoprismatic) when cut longitudinally, and it is difficult to discern P from IP enamel because the angle of crystallite orientation between the two is not yet great enough to form a prism sheath at that interface. We suggest that true prismatic enamel arose when the Tomes' process developed a non-secreting surface alongside a secreting one. From this study, the time of appearance of true prisms is not known. Based on results of this study, columnar enamel occurred in the mid-Triassic period. Early prismatic enamel that contains structures that have been called preprisms, pseudoprisms or herringbone prisms occurred in the late Triassic. Prisms with a distinct prism sheath and an abrupt change in the orientation of the crystallites of P and IP enamel probably arose in the late Cretaceous (see Crompton *et al.*, 1993 for description of *Groebertherium*).

Key words: Enamel evolution - fossil enamel - mastication - enamel prisms - reptile to mammal transition.

Introduction

Gross and microscopic dental characters of extant and extinct species are used extensively to elaborate phylogenetic relationships (for example, Tomes, 1849; Osborn, 1888; Carter, 1920; Korvenkontio, 1934; Crompton, 1972; Kemp, 1983; Sues, 1985; Carlson and Krause, 1985; Marshall and Kielan-Jaworowska, 1992; Wood, 1992). It, therefore, becomes increasingly important to extract as much information as possible from whole or fragmented fossilized teeth. The following study was undertaken, in part, in hopes of adding to the number of

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characters used to determine phyletic relationships among non-mammalian cynodonts and early mammals.

Not only has recent evidence supported a link between enamel ultrastructure and systematics (Boyde, 1978; Gantt, 1982; Fosse *et al.*, 1985; Carlson and Krause, 1985; Lester *et al.*, 1988; Koenigswald and Clemens, 1992), but also between the microanatomy of enamel and masticatory function (Rensberger and Koenigswald, 1980; Koenigswald, 1982; Fortelius, 1985; Boyde and Fortelius, 1986; Koenigswald *et al.*, 1987; Young *et al.*, 1987; Stern *et al.*, 1989; Maas, 1991; Rensberger and Pfretzschner, 1992; Crompton *et al.*, 1993). Complex postcanine tooth morphology, jaw movements during occlusion, and loss of alternate postcanine dental replacement occurred in traversodontids and tritylodontids (Hopson, 1971; Crompton, 1972; Grine *et al.*, 1979; Sues, 1985). We therefore undertook to compare the enamel ultrastructure of these forms with that of a trithelodontid (*Pachygenelus*), an early mammal (*Megazostrodon*), and three extant reptiles. Trithelodontids are considered to be the sister group of mammals (Hopson and Barghusen, 1986; Allin and Hopson, 1992; Crompton and Luo, 1993); this is based partially upon the assumption that both possess prismatic enamel (Shubin *et al.*, 1991). By comparing the species mentioned above it may be possible to relate changes in enamel crystallite arrangement to changes in occlusal relationships.

Speculations on the morphology of the ameloblasts in some of the species examined will be offered. Since the orientation of enamel crystallites is under cellular control (Greenberg *et al.*, 1983) then enamel can be viewed as the ameloblast's "footprint". Further support for a cellular role can be found in Boyde's (1964) discussion of crystallite orientation as being perpendicular to the mineralizing front and to the surface of the secreting membrane of the Tomes' process; Simmelink's (1982) description of a modified form of merocrine secretion (i.e. the production of a secretion which is not damaging to the cell itself) in rat enamel where crystallites are oriented parallel to the longitudinal membranous invaginations of the secreting portions of the Tomes' process; and Kallenbach's (1977), Sasaki's (1983), and Wakita and Kobayashi's (1983) descriptions of secreting and non-secreting surfaces of Tomes' processes. Results of our study will offer an opportunity to propose hypotheses of phylogenetic changes in cells involved in enamel matrix formation.

A review of the literature presents a lack of agreement as to when prismatic enamel arose in the fossil record. Those who have studied enamel ultrastructure in fossil species (for example, Poole, 1956; Moss, 1969; Schmidt and Keil, 1971; Osborn and Hillman, 1979; Sigogneau-Russell *et al.*, 1984) agree that the general reptilian condition is that of aprismatic or continuous enamel; the exceptions are the agamid lizard, *Uromastyx* (Cooper and Poole, 1973), some dinosaurs (Buffetaut *et al.*, 1986; Dauphin, 1988), and the gavial hatchling (Sahni, 1987). The situation is cloudier when non-mammalian cynodonts and early mammals have been examined, first because of the resolution limits of light and polarizing microscopy, and secondly because of the various definitions used to define a prism (which have yet to be agreed upon by those working on enamel ultrastructure).

Moss (1969) studied ground sections of several fossil and recent species (including therapsids, docodonts, multi-tuberculates, triconodonts, monotremes, pantotheres, and dryolestids) by transmitted light, polarization and phase optics. He concluded that "prismatic (discontinuous) enamel structure was acquired gradually only by therians, and that prismatic enamel appeared no later than the early Cretaceous".

Using the polarizing microscope in a novel way, Osborn and Hillman (1979) described the Triassic samples *Massetognathus*, *Diademodon*, and Liassic samples of *Morganucodon*. They claimed that in these forms crystals tilted toward the center of each column, and possibly had a prism sheath, but lacked interprismatic enamel. They considered this arrangement a reasonable link between the aprismatic enamel of most reptiles and true prismatic enamel of

therian mammals.

Grine and Vrba (1980) concluded that all mammals have prismatic enamel, but that it is not a unique feature of mammals. (We know now that certain Cetaceans do not have prismatic enamel [Ishiyama, 1987]). They (Grine and Vrba, 1980) identified prisms in the non-mammalian cynodont, *Pachygenelus*, and also in the early mammal, *Morganucodon* (*Eozostrodon*). Grine and Vrba concluded that "prisms may be a preadaptation for reduced dental replacement". Prisms allow, they claim, for the diffusion of masticatory forces which occur during complex forms of mammalian occlusion. This is in agreement with Poole's (1967) conclusion. They also suggest that prismatic enamel may have arisen before the separation of the trithelodontid and mammalian clades or it arose "independently and in parallel in the immediate ancestor of mammals and in at least one (and maybe more) cynodont lines" (Grine *et al.*, 1979).

Sigogneau-Russell *et al.* (1984) define preprismatic enamel as columns of crystals which deviate from the longitudinal axis of the column in a pinnate fashion, with no interprismatic enamel separating the columns (as opposed to pseudoprismatic enamel of Osborn and Hillman [1979]; in agreement with preprismatic enamel of Carlson, 1990). Preprismatic enamel was described in *Kuehneotherium* (Sigogneau-Russell *et al.*, 1984) and in *Morganucodon* (*Eozostrodon*) as well as an undetermined triconodont molar from the Rhaetic of France (Frank *et al.*, 1988). Frank *et al.* (1984) had also described these preprisms in the late Triassic Haramiyiids.

The problem of terminology in transitional forms of enamel, i.e. between aprismatic and true prismatic forms, has yet to be resolved. Until a resolution is reached, we realize that the situation is muddied by the use of new terms. At the same time, the situation is not clarified by the use of terms which are not adequately descriptive (see Carlson, 1990). Since the term pseudoprism connotes a "false" prism, or the enamel under the middle of one ameloblast (or the pointed portion of the Tomes' process) to the middle of another ameloblast (Lester and von Koenigswald, 1989), we prefer the term early prism (or preprism) which connotes a cellular domain. A clear cellular domain may be visible in etched enamel depending on the orientation of crystallites and the length of exposure to the etchant. The terms pseudoprism, preprism or primitive prism, however, all describe a similar type of ultrastructure. From an evolutionary perpective, it makes more sense to describe the product of a single ameloblast.

In this paper, we will define *early prismatic enamel* as possessing the following characteristics:

1) A prism domain (i.e. a unit of enamel related mainly to one ameloblast and whose crystallites are fairly parallel to each other or tilt toward a seam), regularly arranged.

2) Interprismatic enamel surrounding all, or part of, the prism domain.

3) An hexagonal packing pattern [as in Boyde's (1976) Pattern 1 and Pattern 3], or prisms arranged in regular rows stacked one prism upon another when seen in transverse section [as in Boyde's (1976) Pattern 2; see also Martin *et al.*, 1988].

True prismatic enamel also possesses these features but, in addition:

4) A prism sheath between the prismatic and interprismatic enamel on most of its boundary.

Materials and methods

Specimens examined

A. Reptiles

(1) *Caiman*

(2) *Crocodylus*

(3) *Uromastyx*

The caiman and the crocodile are extant reptiles which do not occlude their teeth and possess continuous tooth replacement as in most reptiles. *Uromastyx*, an extant Agamid lizard, is exceptional in that the teeth occlude and are not replaced (Cooper and Poole, 1973).

B. Therapsida

(1) Traversodontid - *Massetognathus*, a mid-Triassic herbivorous non-mammalian cynodont (uncatalogued specimen).

Complex occlusion developed within the gomphodont cynodonts, a group consisting of three families, one of which is the Traversodontidae (Crompton, 1972). Occlusion was analogous to that of mammals except that the mandible moved postero-dorsally during the power stroke when opposing crescents of upper and lower external cusps sheared by each other (Crompton, 1972). Heavy wear was evident on the postcanine teeth (Romer, 1967).

(2) Tritylodontid - a Late Triassic to early Jurassic non-mammalian cynodont.

The two rows of cusps on the lower molars fit between the three rows of cusps on the upper molars; the sides of the cusps sheared past each other as the mandible was pulled postero-dorsally (Crompton, 1972). The tritylodonts were the only "therapsids" (quotations are used to denote paraphyletic noncladistic terms, as in Allin and Hopson, 1992) with multiple-rooted teeth. Their dentition was highly specialized and dental replacement was limited (Sues, 1985; Hopson, 1987; Carroll, 1988). Traversodontids and tritylodontids (as well as *Uromastyx*) lose their teeth anteriorly and add teeth at the posterior end of the postcanine dentition; this mode of replacement has the advantage of not disturbing occlusal relationships.

(3) Trithelodontid - *Pachygenelus* cf. *monus*, from southern Africa, and recently discovered in Nova Scotia (Olsen *et al.*, 1987) (adult tooth with tooth bud = MCZ specimen no. 9150); early Jurassic.

This small carnivorous non-mammalian cynodont is considered to be the closest relative of mammals (Hopson, 1987; Rowe, 1988; Allin and Hopson, 1992; Crompton and Luo, 1993). Despite alternate replacement of the postcanines, the lingual surfaces of upper teeth sheared against buccal surfaces of lower teeth (Gow, 1980) to produce simple wear facets. As the replacing teeth erupted, they were worn when they contacted the matching tooth. Unlike the other non-mammalian cynodonts discussed above, and the Morganucodontids, complex wear facets were not formed (Gow, 1980).

C. Mammalia

Morganucodontid - *Megazostrodon*, a South African early Jurassic triconodont.

Morganucodontids are amongst the earliest known mammals. They retained the reptilian jaw articulation alongside a mammalian temporomandibular joint. During occlusion the buccal surface of the lower molars sheared against the lingual surface of the upper molars to form several well-defined wear facets (Crompton, 1974). The main cusp of the lower molar occluded between the posterior cusp (cusp C) of one of the uppers and the anterior cusp (cusp B) of the molar behind.

Preparation of specimens

All specimens are in the collections of the Museum of Comparative Zoology, Harvard University; all are uncatalogued, except for one of *P. monus*. All teeth were soaked in 2.5%

sodium hypochlorite for one to two hours in order to remove surface organic matter. After rinsing for 15 minutes in running tap water, the teeth were etched in 1N HCl for the following lengths of time (longer times - above 35 secs.- represent a cumulative series of shorter etches):

Caiman and crocodile - 35 secs.
Uromastyx - 20 secs.
Massetognathus - 40 to 60 secs.
Tritylodontid - 1 min. 30 secs.
Tritheledontid - 30 secs.
Megazostrodon - 10 secs. (0.5N HCl) plus 40 secs. (1N HCl)

Samples were etched until a definitive pattern or arrangement of domains were observed, and individual crystallites were clearly discerned. Samples were either filed and polished (with 400 and 600 grit sandpaper, then with levigated aluminum on frosted glass), or etched whole. One sample (*Pachygenelus*) was also embedded in Epon and then filed and etched. Specimens were mounted on double-sided tape on aluminum stubs, dabbed with conducting silver paint, checked uncoated in SEM for a suitable etch, and coated with palladium-gold (30 nm in a Technics Hummer II sputter-coater). All specimens were observed in an AMR-1000 scanning electron microscope, operating at an accelerating voltage of 20 kilovolts.

Results

Reptiles

Enamel from the tooth of a caiman (Fig. 1) displays the aprismatic structure typical of most reptiles. Aprismatic enamel, where the crystallites are aligned parallel to each other, was also observed in the crocodile (Figs. 2, 3). Large ovoid spaces, however, are concentrated

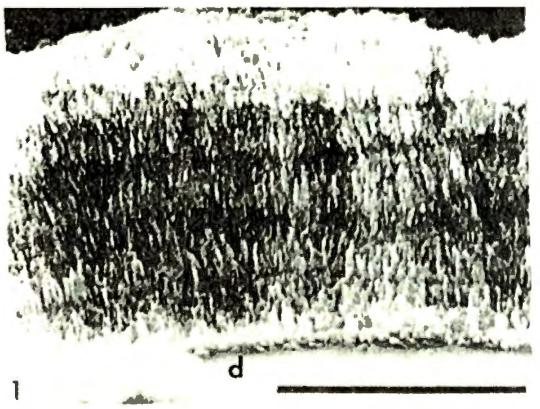


Figure 1. Transverse section through tooth of a caiman. Dentine is near the bottom of the micrograph. Crystallites are aligned parallel to each other, and the organization is typically aprismatic. Faint growth lines (arrows) are seen at various intervals. Bar = 10 µm. d = dentin

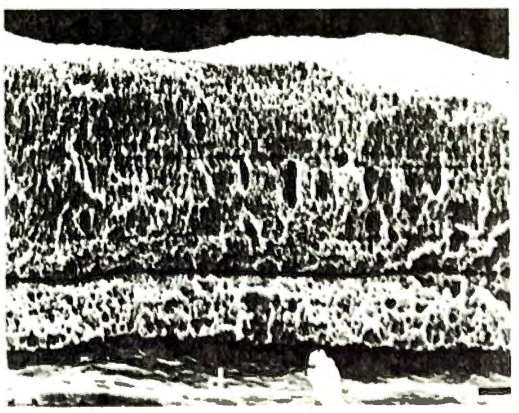


Figure 2. Longitudinal section through tooth of *Crocodylus*. Notice the large air-filled spaces. Pronounced line is due to environmental trauma. Bar = 10 µm.

in the middle layer of the crocodile enamel and occasional small round holes (arrows, Fig. 3) are observed in sagittal section.

Wear on the tips of the teeth of *Uromastyx* (Fig. 4) is seen in occlusal view. Round spaces are present in the enamel (Figs. 5, 6); these areas may have contained some mineralized material which etched more rapidly than the surrounding enamel, or contained organic material which was removed when the teeth were soaked in sodium hypochlorite. These deeply etched areas (about 2.5 μm in diameter) are not regularly arranged (Figs. 5, 6). A higher magnification

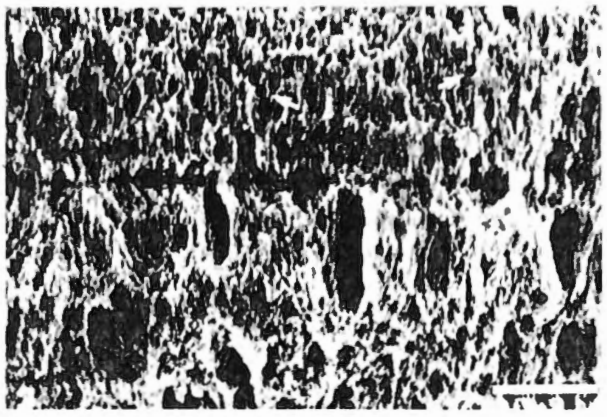


Figure 3. Higher magnification of large air-filled spaces in *Crocodylus*. Smaller tubular-like spaces are also visible (arrows). Except for the large spaces, arrangement of crystallites is aprismatic, typical of reptiles. Bar = 10 μm.

Figure 4. Occlusal view of hemi-mandible of *Uromastyx*. Notice the heterodont type of dentition not usually found in reptiles, and the wear on the occlusal surfaces. Bar = 1 μm.

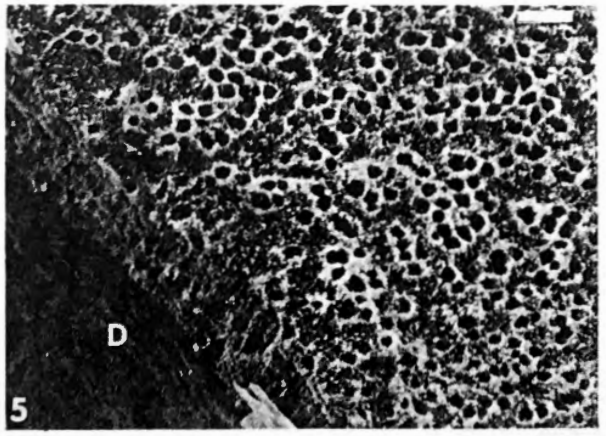


Figure 5. View of natural etched cervical surface of post-canine tooth of *Uromastyx*. Dentin is seen at the bottom left corner of the micrograph. Prism-like areas are seen, but are not regularly spaced. Holes are apparent where material (prisms) was preferentially removed, or which were filled with organic material. Bar = 10 μm.

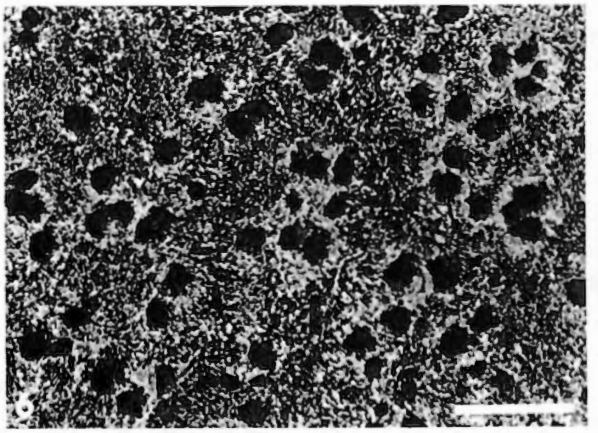


Figure 6. Buccal surface of *Uromastyx* enamel not far from the cusp tip. Notice holes of different sizes. The enamel does not appear to have a prism-like arrangement. Bar = 10 μm.

of the enamel (Fig. 7) shows a slight flaring of the crystallites. An enlarged area of crystallites (Fig. 8) between the spaces displays what appears to be an organic residue.

At the surface of the dentino-enamel junction (DEJ) (Fig. 9), the enamel layer after acid-

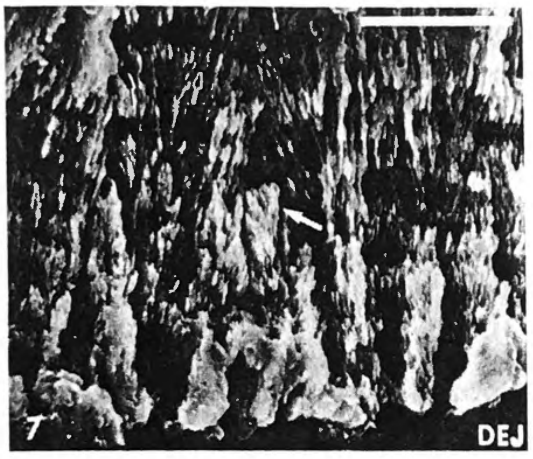


Figure 7. *Uromastyx* enamel as seen in occlusal view. Dentin is at the bottom of the micrograph. A "flaring-out" of crystallites is observed. Near the dentino-enamel junction (DEJ) some crystallites appear to be "bundled" into pipe-like arrangements (arrow). Bar = 10 µm.

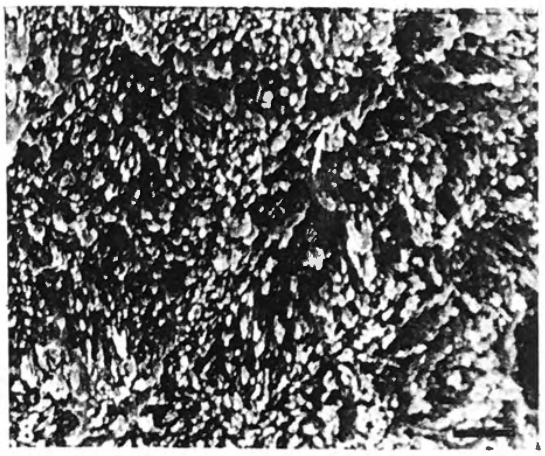


Figure 8. Higher magnification of area between holes seen in Figure 7. Arrow points to what appears to be organic material which remains after acid etching. Bar = 1 µm.

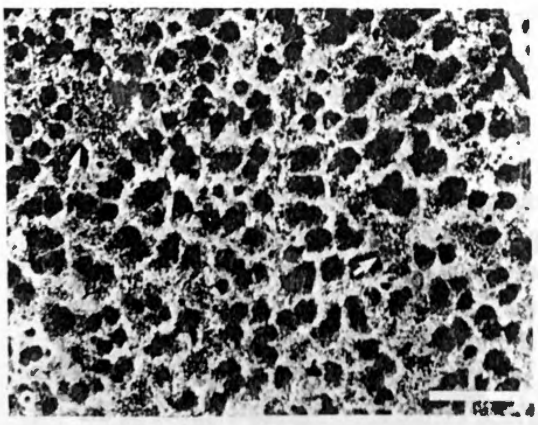


Figure 9. Enamel near the DEJ of *Uromastyx* after a strong acid-etch. Arrow points to some remaining organic material. Bar = 10 µm.

etching is reminiscent of interprismatic (IP) enamel. The spaces which contained prisms (Cooper and Poole, 1973; K.S. Lester, personal communication) are irregularly arranged.

A drawing of hypothetical secretory ameloblasts of *Caiman* and *Crocodylus* are shown in Figure 10. The ameloblasts of *Caiman* are shown with a relatively straight secreting surface, while those of *Crocodylus* are straight, but are leaving behind bits of cytoplasm due to a blebbing of the apical ends of the ameloblasts (may be a process similar to the formation of enamel tubules [Stern, 1989]). A drawing for *Uromastyx* is not shown; the enamel structures

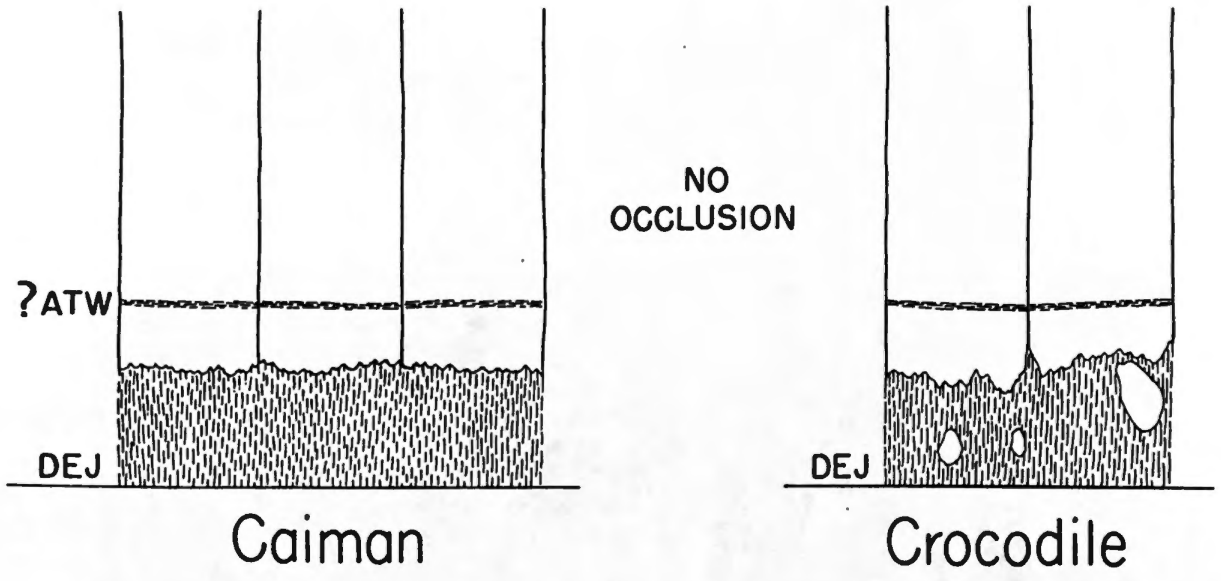


Figure 10. Diagram of reptilian ameloblasts secreting aprismatic enamel, and how ameloblasts of *Crocodylus* might look while secreting enamel with huge air-filled spaces. Theoretically, the apical end of the ameloblasts bleb, leaving large or small areas of cytoplasm in the matrix. The cytoplasm may eventually degenerate and leave spaces. Apical ends of ameloblasts are straight across, not tapered. No diagram is included for *Uromastyx* because it is difficult to recognize a cell's domain, and the pattern is not regular. The apical ends of the ameloblasts are almost certainly not tapered, however. ATW = apical terminal web.

cannot be precisely described because the territory covered by one ameloblast is unclear and there is a lack of developmental information in the literature to correlate to this.

Non-mammalian Cynodonts

The parasagittally fractured enamel of *Massetognathus*, a mid-Triassic traversodont, revealed a columnar structure; the columns (Fig. 11) appear to be directed towards the side of the tooth. In higher magnification (Fig. 12), columns of crystallites with periodic flaring are observed; the flarings line up at what might be incremental growth lines. Large spaces are observed between pinched portions of columns (arrow, Fig. 12); these spaces seem to be directed in a latero-apical direction. Seen from the outer natural (but etched) surface of the tooth, the tops of rows of columns (bottom of Fig. 13) appear similar to perikymata of mammalian teeth. Perikymata are structures on the outer surfaces of mammalian teeth where the lines of Retzius end (Boyde, 1964; Risnes, 1985); these features are considered to represent resting places of cohorts of cells (Warshawsky, 1985) during development. In *Massetognathus*, however, these are probably not perikymata because the growth lines are parallel to the DEJ (Fig. 12) and therefore do not intersect the outer surface of the tooth. Lines parallel to the DEJ are growth lines which probably represent 24-hour increments of enamel matrix (Asper, 1916; Boyde, 1964). These perikymata-like structures represent partially etched rows of columns with some intercolumnar enamel remaining between neighboring columns within a row; since acid etches preferentially along the c-axis, or long axis, of the crystallite (Johnson *et al.*, 1971), the crystallites which were originally perpendicular to the surface being etched (i.e. the intercolumnar or acolumnar enamel) will etch away more quickly and leave the round tops of the columns which are composed of more obliquely oriented crystallites (see ahead, Fig. 16). Some dislocations (Fig. 13) are observed which could represent a slight shift in packing of neighboring ameloblasts. The circular tops of columns are also seen in Figure

14, and a higher magnification of the column and intercolumnar areas is shown in Figure 15. Columns are randomly packed (Figs. 14, 15) so that no specific packing order can be recognized. A drawing of *Massetognathus* ameloblasts with a possible scenario of the arrangement of the cells secreting columnar enamel is shown in Figure 16. Two ameloblasts are drawn as if cooperating to form the pinched portion of the column by being closely juxtaposed at their apical ends at the right side of A_1 and the left side of A_2; the cells then modulate (i.e. shift back and forth from one arrangement to another) to form the flaring portions of the column by not being closely apposed at the sides of short, rounded Tomes' processes (that part of the cell below or apical to the apical terminal web [ATW, see Fig. 10]) When the cells are forming the pinched part of the column, there may be some blebbing (i.e. the cell may leave cytoplasmic remnants in the matrix) at the sides of the Tomes' processes

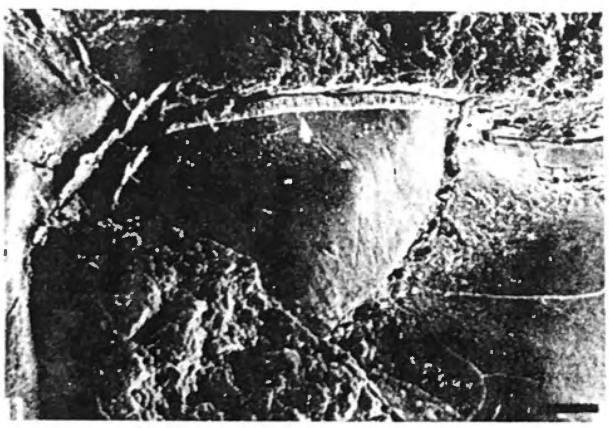


Figure 11. Cusp of *Massetognathus*, a Triassic traversodont reptile, which has occlusion and heavily wears its teeth. The tooth is embedded in matrix. Arrow points to longitudinal section of enamel which is exposed. The cusp is towards the upper left corner of the micrograph. Bar = 100 µm.

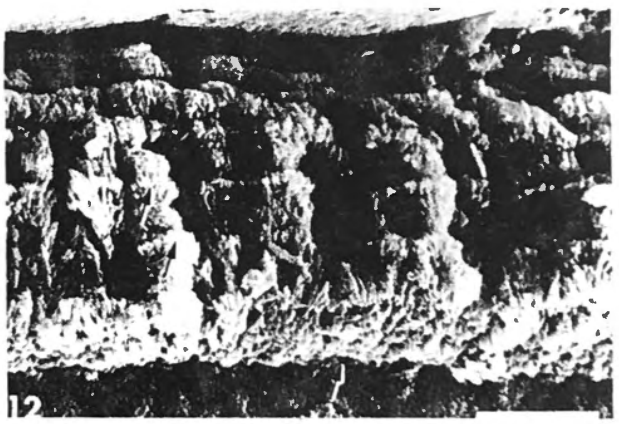


Figure 12. Enamel of *Massetognathus* in higher magnification. Columns are flared and pinched at repeating intervals. Flaring seems to coincide with growth lines. Bar = 10 µm.

Figure 13. View of back (e.g. mesial, buccal, lingual, or distal) surface. Upper left corner shows the columns as circular outlines. Linear arrangements are similar to perikymata in mammalian teeth. Bar = 10 µm.

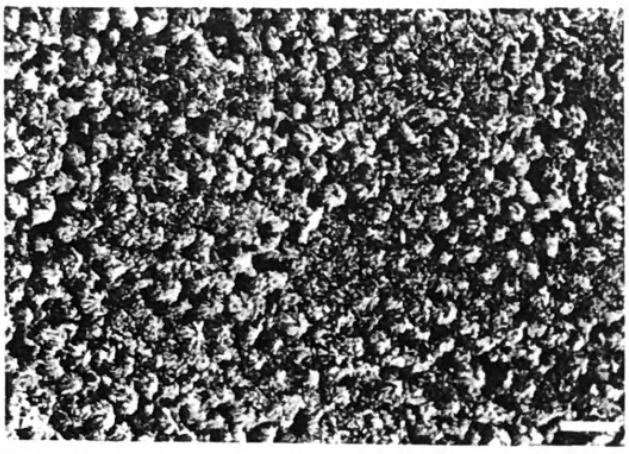


Figure 14. View of back surface of *Massetognathus*; circular outlines of columns are visible. This is not prismatic enamel because there is no clear evidence of interprismatic enamel. Bar = 10 μm.

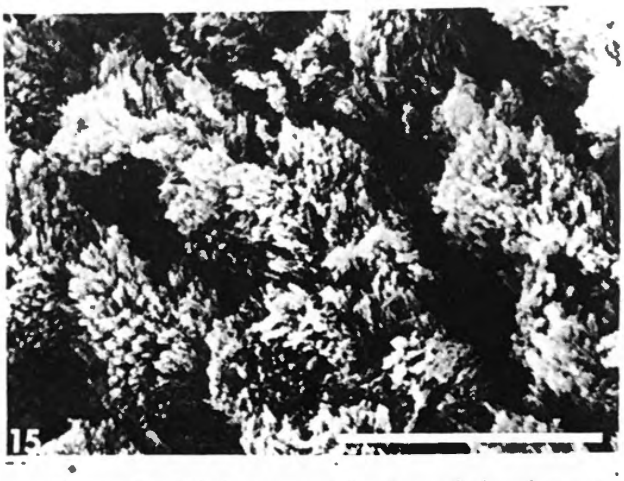


Figure 15. Higher magnification of circular outlines seen in Figure 12. Circular territories are about 8 - 10 mm in diameter. Bar = 10 μm.

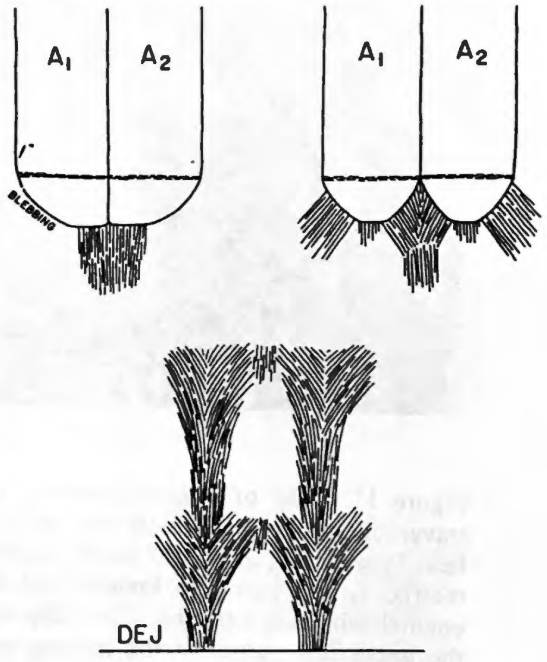


Figure 16. Drawing of columns of enamel found in *Massetognathus*, and a possible scenario as to how the ameloblasts secreted that enamel. Notice the changing shapes of the apical parts of the cells.

reminiscent of the formation of air-filled spaces in *Crocodylus* (Figs. 2, 3). Another possibility is that these spaces might have been filled with enamel which was more susceptible to the etchant. This model, and all subsequent ones in this paper, assume a diameter of about 4 - 6 μm for the ameloblast.

One of the anterior cusps of a tritylodontid lower molar (Fig. 17) was filed at an angle to the vertical axis of the tooth so that the dentine surrounded with very thick enamel was revealed. Enamel on the buccal side of this cusp (Fig. 18; arrow in Fig. 17) shows a layer of inner enamel (IE) composed of small tubular spaces, and a layer of outer enamel (OE) with an array of larger holes and structures which are sheet-like in appearance. The DEJ appears very straight with no apparent interdigitation between the two tissues. Stirrup-like patterns (Fig. 19) are obvious in a higher magnification of the area which is just above the enamel shown in Figure 18, or more towards the cusp apex due to the tilt of the filed surface. Near the DEJ, the stirrups are smaller (about 3-4 μm), and in the outer enamel they are about 12 to 20 μm wide. Often it appears as though 3 or 4 of the smaller stirrups (small arrows, Fig. 19)

unite to form a larger stirrup. One of the larger stirrups is shown in Figure 20; a very shallow prism appears to lie within boundaries of what we designate as interprismatic (IP) enamel. Crystallites of IP enamel are oriented perpendicular to the outer surface of the tooth, and oblique to the apex of the cusp (Fig. 20). Fractured and etched tritylodontid enamel (Figs. 21, 22) within a basin-like area between the cusps also reveals evidence of large and small stirrup-like areas (bold and thin arrows, respectively, Fig. 21). Incremental growth lines give a striped appearance to the enamel (Figs. 21 and 22). Prism-like areas can be seen on the outer surface of the tooth (Fig. 23; numbered 1 and 2 in Fig. 24); no clear prism sheath is

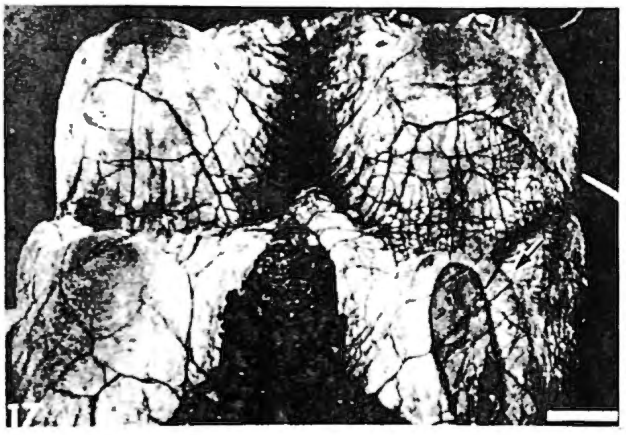


Figure 17. Lower left molar of a tritylodontid in antero-occlusal view. The anterior buccal cusp (lower right corner) has been filed tangentially. [During chewing, the two rows of the lower molar cusps fit between the three upper cusp rows and the mandible is pulled posteriorly.] There is a heavy shearing action on the sides of the lower cusp. Bar = 1 mm.

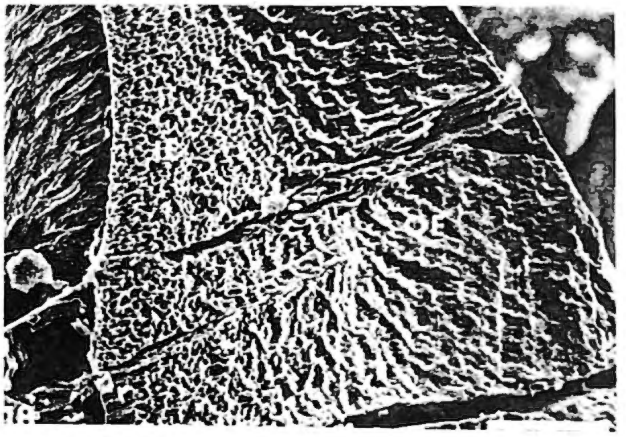


Figure 18. Near occlusal part of filed surface in Figure 14. Notice that an inner and outer enamel pattern can be seen. The inner enamel consists of smaller profiles. Bar = 100 μm.

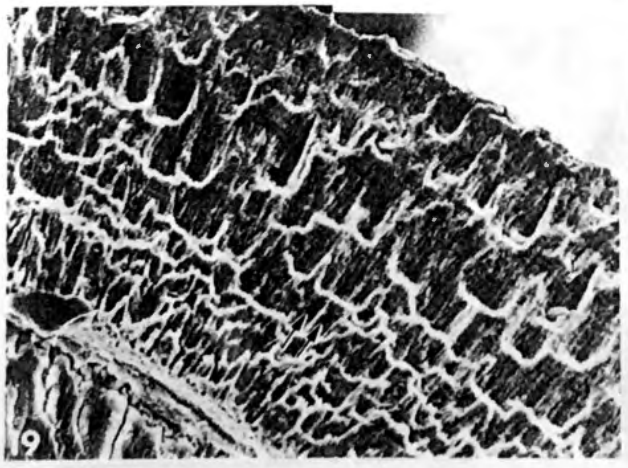


Figure 19. Higher magnification of inner and outer enamel near occlusal and postero-buccal side of filed cusp. Two (small arrows), and sometimes three, small early prisms appear to combine to form a large (12 μm wide) stirrup-shaped prism domain. Bar = 100 μm.

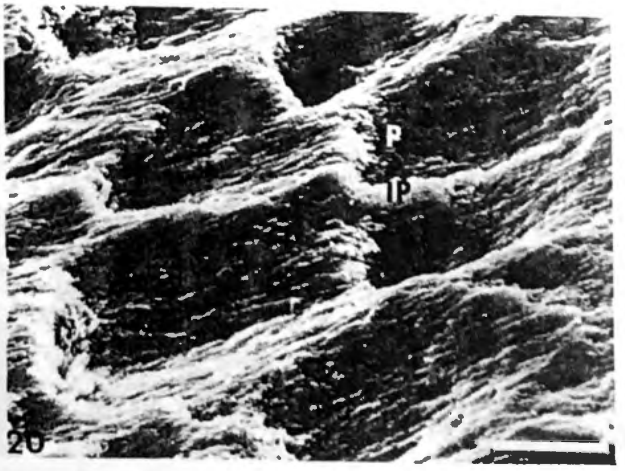


Figure 20. Higher magnification of one of the larger prism domains. Notice that there is no prism sheath and the angle between prismatic and interprismatic crystallites is small. Bar = 10 μm.

observed and the change in orientation of crystallites from prismatic to interprismatic areas is very subtle. Prism-like areas also do not appear to be identical to each other (Fig. 24) in orientation of prism crystallites or the appearance of IP enamel (i.e. which borders of IP enamel appear pronounced). The arrangements of these prism domains, and the orientation with respect to shearing forces during mastication is seen in Figure 25a. Interprismatic crystallites are shown perpendicular to the shearing surface and the prism crystallites are at a very slight angle, but almost perpendicular, to the shearing surface (Fig. 25a). A drawing of transverse sections of small prism domains blending to form larger prism domains is shown in Figure 25b; the IP enamel gradually fades away between some of the prisms. Below the diagram of the transverse sections (Fig. 25b) is a drawing of longitudinal prisms showing the conformations that the ameloblasts might have assumed while forming the small and large structures. The crystallites are drawn with a tilt toward the center of the prism only in the

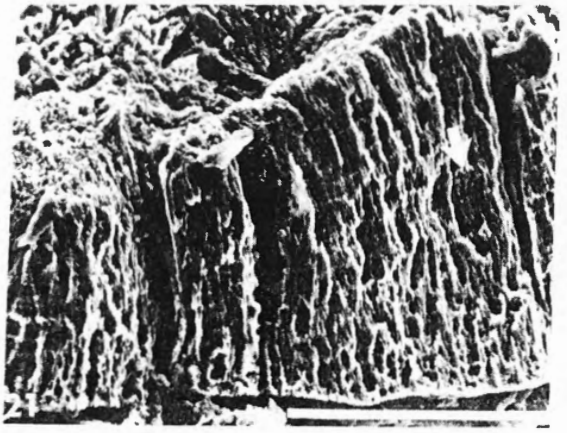


Figure 21. Fractured tritylodontid enamel in a basin-like area. Areas where about four prism domains cluster together as one, are obvious (arrow). Bar = 100 µm.

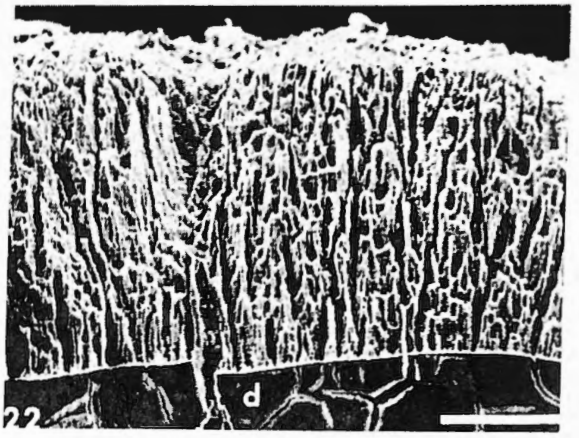


Figure 22. Enamel of the tritylodontid which is fractured more along the tops of prism domains. Bar = 100 µm.

Figure 23. High magnification of tritylodontid enamel on posterior surface, almost transverse to prism domains. Prism outlines are not very distinct, but are present. Again, prism domain seem to form groups. Different heights of enamel may be an effect of the fossilization layer. Bar = 10 µm.

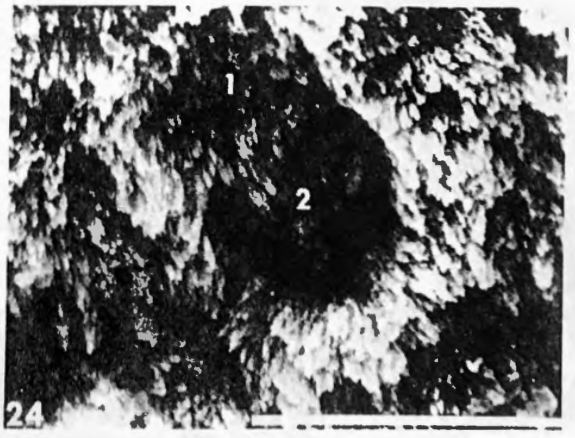
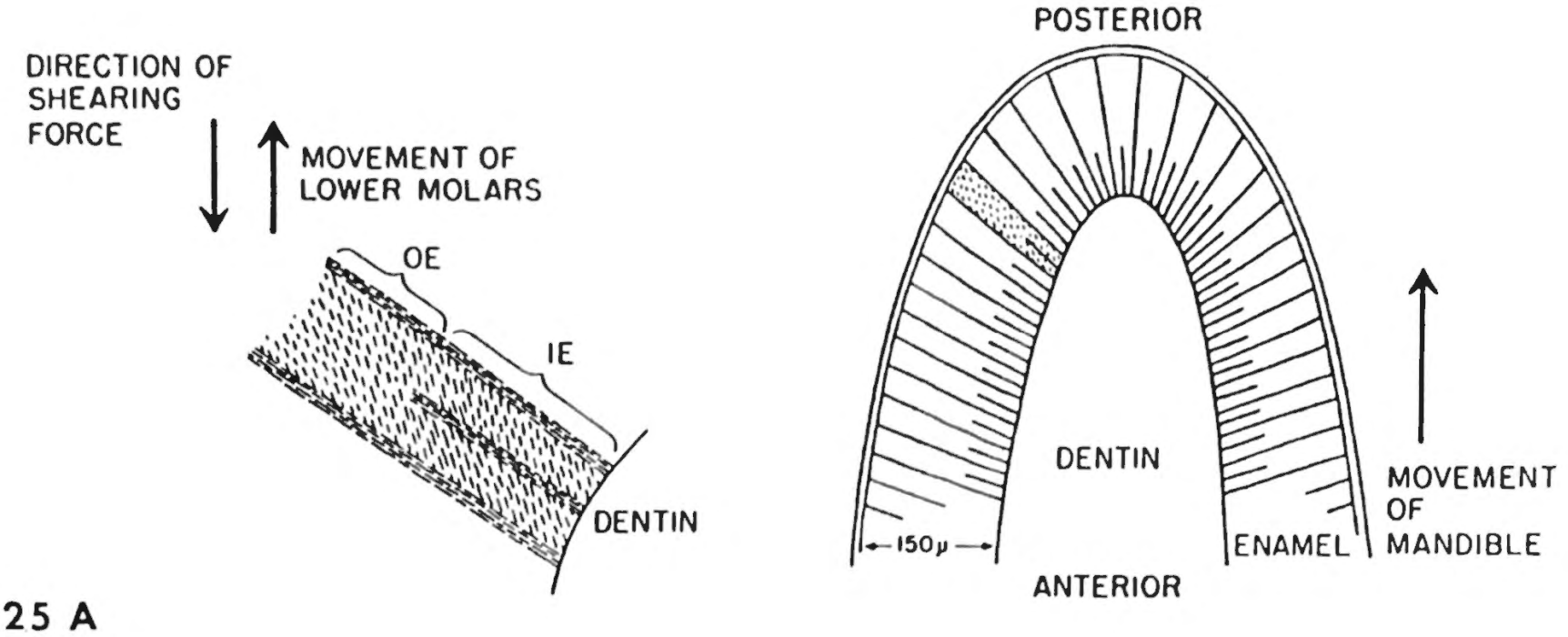
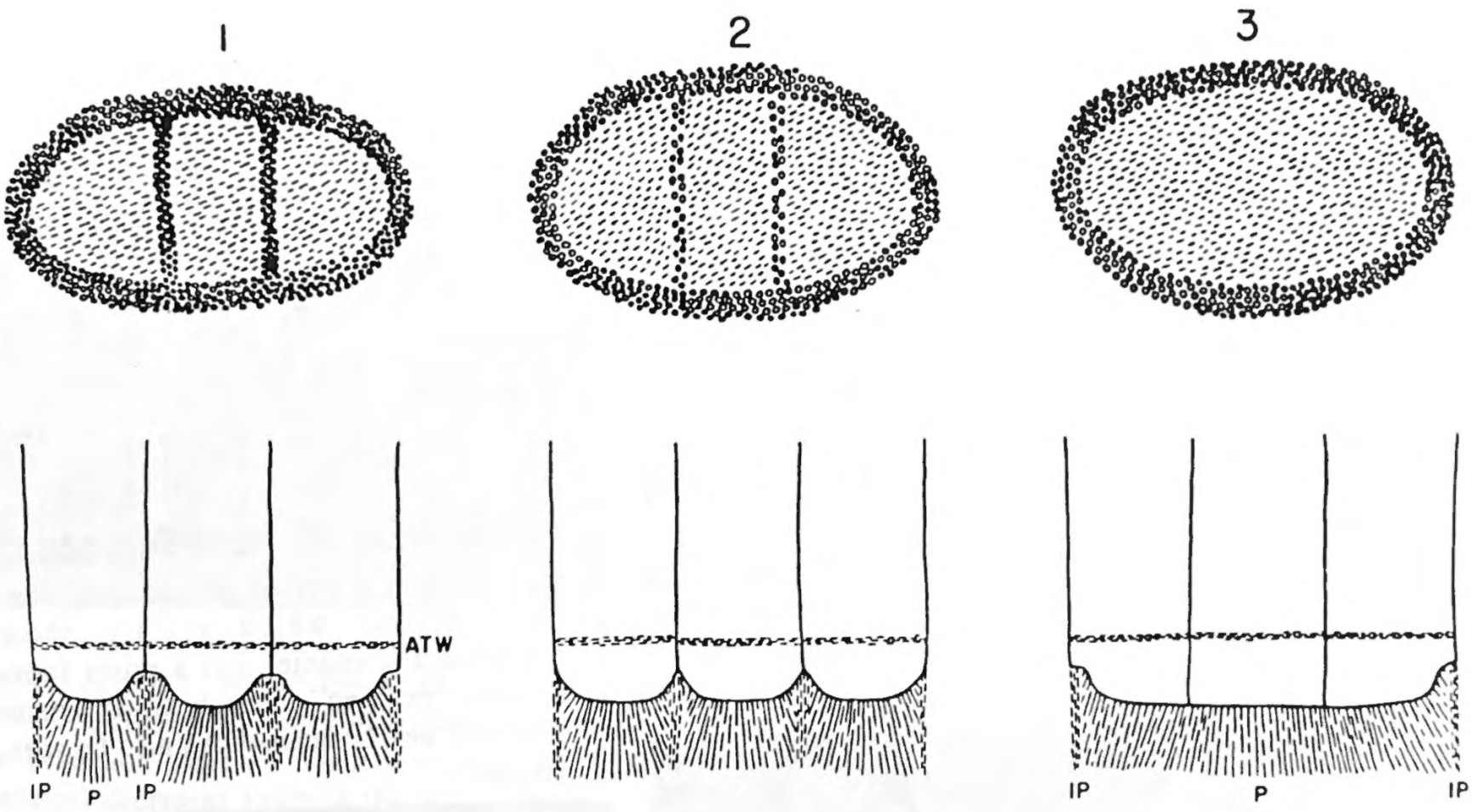


Figure 24. Higher magnification of area similar to that of Figure 23. Two prism domains next to each other (1 and 2) may have slightly different degrees of organization of crystallites. Bar = 10 µm.



25 A



25 B

Figure 25a. Drawings of orientation of crystallites in the tritylodontid. Upper sketch shows two prism domains blending together to form one prism domain. Shearing force is at an angle to prism direction. 25b. Diagram of prisms of the tritylodontid cut in transverse section showing several prism domains blending together to form a large prism domain. Notice how some of the IP enamel thins out. Ameloblasts are seen in longitudinal section in the positions in which they might have secreted inner and outer enamel. There may have been a slight tilt of crystallites towards the center of the prism domain which is not shown in the other views. IP = interprismatic enamel. P = prism.

longitudinal view.

A postcanine tooth of *Pachygenelus* (Figs. 26, 27) which was recently found in Nova Scotia (Olsen *et al.*, 1987), displays a very thin layer of enamel after acid-etching (Fig. 27). On the surface of the enamel, Type 1 and Type 2 etch patterns are observed. In Type 1 etch pattern (Silverstone *et al.*, 1975) the prism is etched more deeply than the IP enamel and in

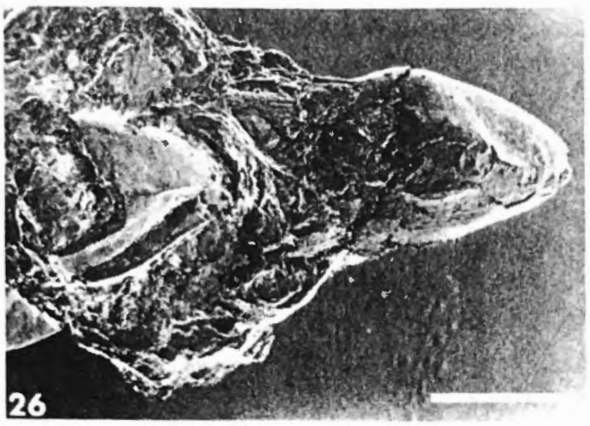


Figure 26. Mature and developing tooth of *Pachygenelus*, a North American Triassic ictidisaur. Sample is embedded in matrix. The teeth of *Pachygenelus* wear in direct occlusion, resulting in shear facets on the molars, especially the uppers (Gow, 1980). Bar = 1 mm.

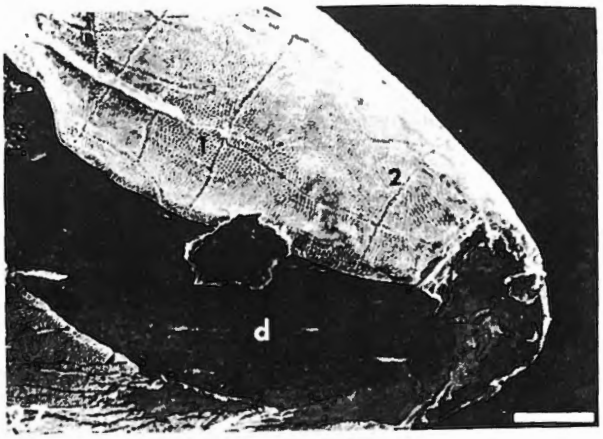


Figure 27. Higher magnification of the tip of the mature tooth of *Pachygenelus*. Pattern 1 and Pattern 2 etch patterns (Silverstone, 1975) are visible; see Results section. Bar = 100 μm.

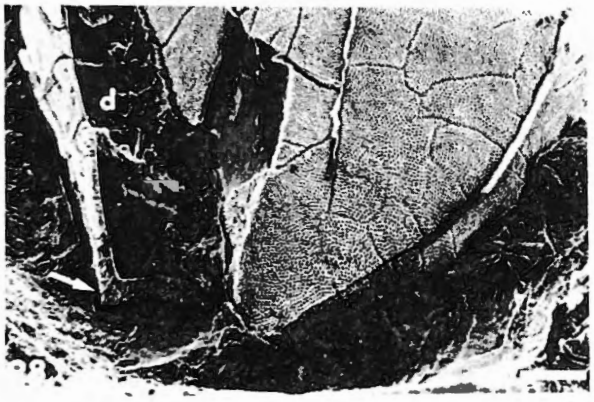


Figure 28. Developing tooth had been cracked during the process of fossilization. The entire enamel thickness can be seen at the fractured tip (arrow). Bar = 100 μm.

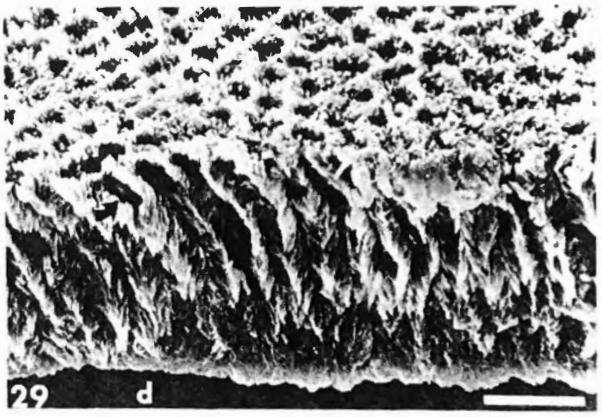


Figure 29. A fractured longitudinal view of the early prisms which mainly shows the interprismatic enamel, but a prism (arrow) can be seen "peeking" through the IP enamel. The tops of P and IP enamel can also be seen. Bar = 10 μm.

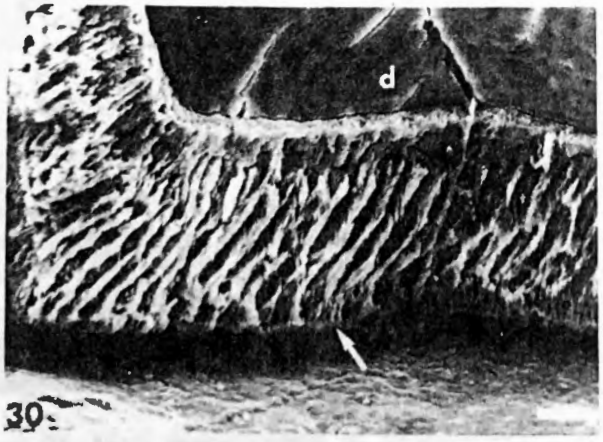


Figure 30. Higher magnification of the corner of the tooth seen in Figure 28. Notice the aprismatic layer (arrow) at the outer surface of the tooth, similar to that possessed by most mammalian prismatic enamel. Bar = 10 μm.

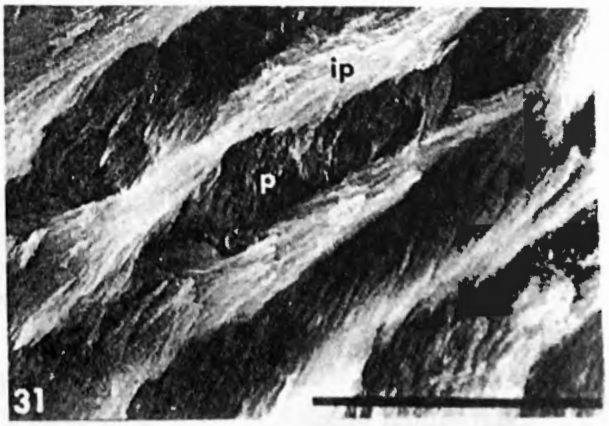


Figure 31. High magnification of what appear to be prisms, if prisms are defined by an abrupt shift in orientation of crystallites. Bar = 10 μm.

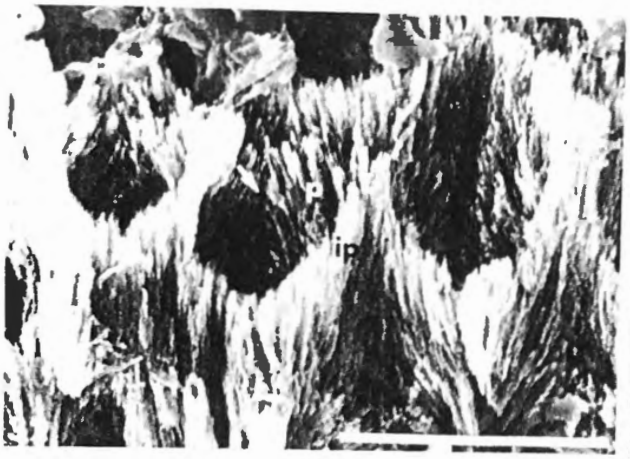


Figure 32. Slightly tangential view of early prisms seen in *Pachygenelus*. The early prism is pinnate-shaped with a seam (arrow) at the apex of the inverted "V". Interprismatic enamel surrounds the early prism, but there is no distinct prism sheath. Bar = 10 µm.

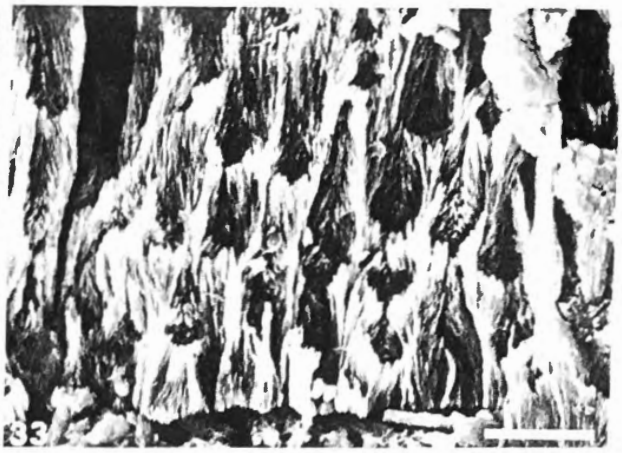


Figure 33. A lower magnification of Figure 31. Bar = 10 µm.

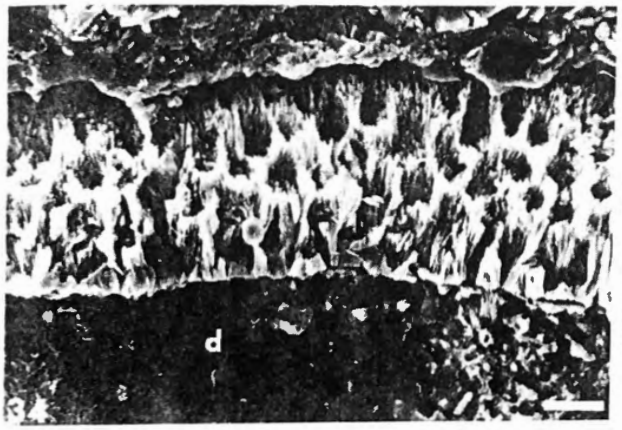


Figure 34. A tooth of *Pachygenelus*, filed from the occlusal direction. At the top of the micrograph is the plastic (Epon) in which the tooth was embedded. Prismatic areas are clearly visible. Thickness of enamel decreases towards the left. Dentine is at the bottom. Bar = 10 µm.

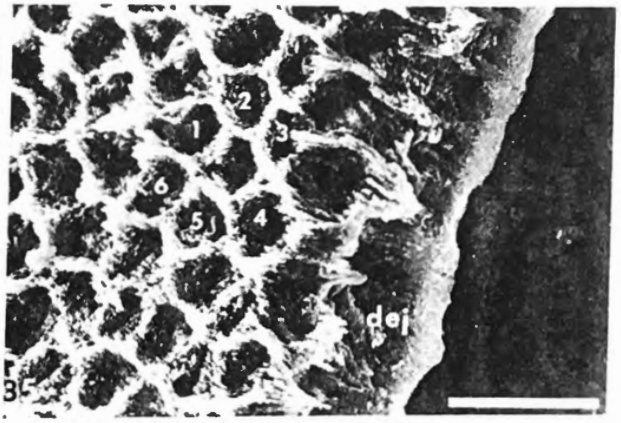


Figure 35. Pattern of etched enamel near the dentin-enamel junction (DEJ) in *Pachygenelus*. Some seams (arrow) are visible; they appear as lines crossing the early prisms in transverse view. Notice the hexagonal packing pattern (numbers). Bar = 10 µm.

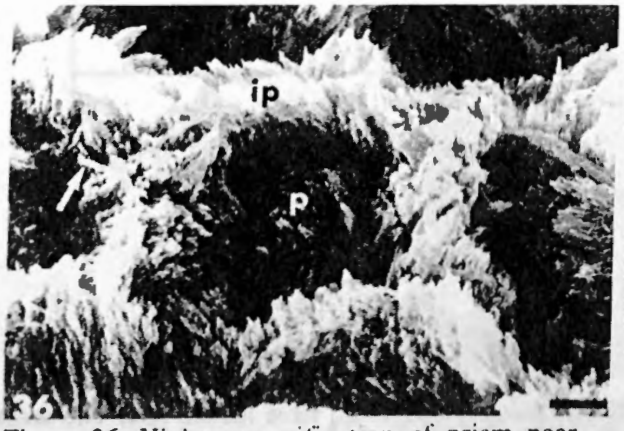


Figure 36. Higher magnification of prism near DEJ. Notice the enamel tubule protruding from the interprismatic enamel (arrow). Bar = 1 µm.

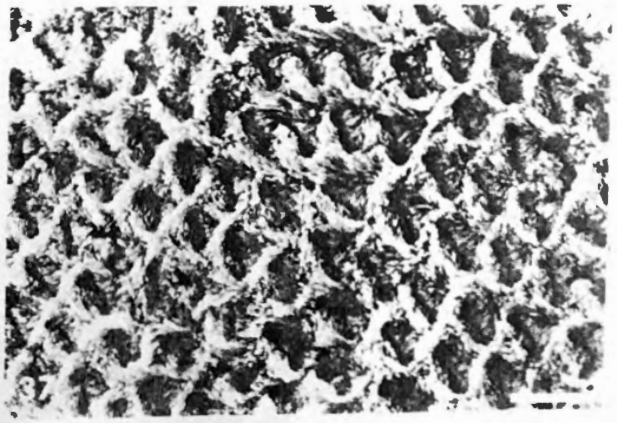


Figure 37. Occlusal view transverse to the long axis of the early prisms. Seams are clearly visible. Half of the early prism blends in with the IP enamel. Bar = 10 µm.

Type 2 etch pattern (Silverstone *et al.*, 1975) the IP enamel is etched more than the prism. The presence of these two patterns indicates a slight shift in orientation of the prisms to the surface exposed to the etchant.

Closer inspection of the unerupted tooth (Fig. 28) reveals an area near the tip of the cusp which is broken (arrow). The whole thickness of the enamel is present, essentially, and appears to have been protected from over-etching by the matrix which covers the outer surface of the tooth in certain locations. A fractured edge of the tooth (Fig. 29, complementary or appositional to area denoted by arrow in Fig. 28) displays a herring-bone columnar pattern, but the view from the outer surface (top of Fig. 29) reveals a regularly arranged prism-like pattern outlined by IP enamel. Very small prism-like structures (arrow) can be observed peeking through the thick IP enamel at the fractured side. A higher magnification (Fig. 30) of the area near the tip (arrow, Fig. 28) shows structures composed of thick IP enamel surrounding small early prisms; a further enlargement (Fig. 31) shows the change in orientation of crystallites between P and IP enamel. An early prism near the DEJ (Fig. 32) exhibits a herring-bone type of structure surrounded by IP enamel. A seam (see Lester and Boyde, 1987; Lester and Koenigswald, 1989) appears in the center of the early prism where the change in orientation is greatest (lower magnification in Fig. 33). A prism-like pattern is also seen in a transverse section of another *Pachygenelus* tooth (Fig. 34) which had been embedded in Epon. In an unembedded sample, the hexagonal arrangement of structures can be clearly seen (numbers in Fig. 35); this is a view of enamel near the DEJ such as that on the outer surface of the erupted tooth in Figure 27. At a higher magnification, a primitive prism near the DEJ (Fig. 36) clearly shows the difference between P and IP enamel. A view transverse to the prisms (Fig. 37) displays the seams found in the centers of the herring-bone pattern. A possible arrangement of ameloblasts secreting the type of early prisms found in *Pachygenelus* is depicted in Figure 38. The ameloblast is envisioned as having a V-shaped Tomes' process

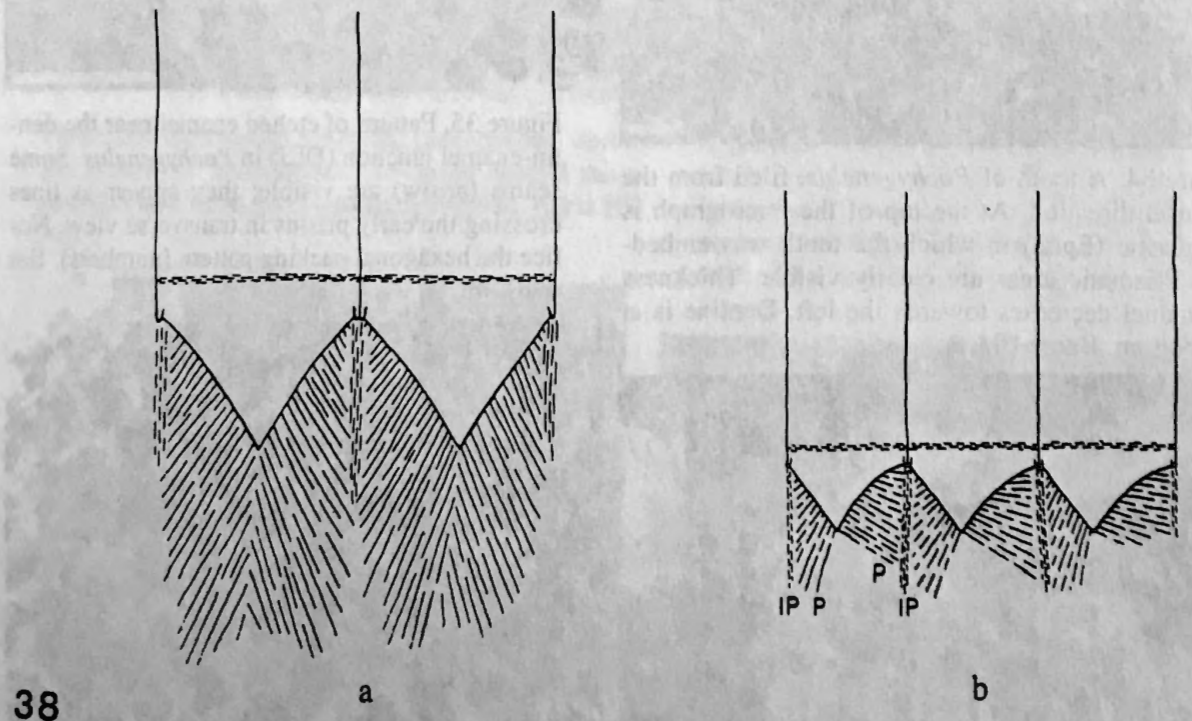


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Figure 38. Model representing what the ameloblasts of *Pachygenelus* could have looked like. The seam could be analogous to a prism sheath which results from an abrupt change in orientation of crystallites.

in Figure 38a. Processes at the proximal end of the Tomes' process are secreting enamel matrix in a direction parallel to (but in some areas more perpendicular or oblique to) the long axis of the ameloblast. The symmetry of the sides of the "V" is probably exaggerated in Figure 38a, since the two parts of the prism may not be always evenly divided (Fig. 37) or identical in crystallite orientation relative to the DEJ; a more asymmetric version of the Tomes' process is shown in Figure 38b.

Early mammal

A small right lower molar of the morganucodontid *Megazostrodon* (Fig. 39) displays wear facets (Fig. 40) formed by the shearing action against the upper molar cusps. A longitudinally fractured and etched section of enamel (Fig. 41) shows IP enamel extending from the dentin to the aprismatic layer of the outer surface. A line of Retzius is seen slightly oblique to the DEJ (Fig. 41) as is typical of mammalian enamel, and not parallel to it as in the reptilian enamel (Figs. 1, 2, 3, 5, 12). The herring-bone early prism, with a split in the middle, is seen with IP enamel surrounding the prisms (Figs. 42, 43) and also extending from

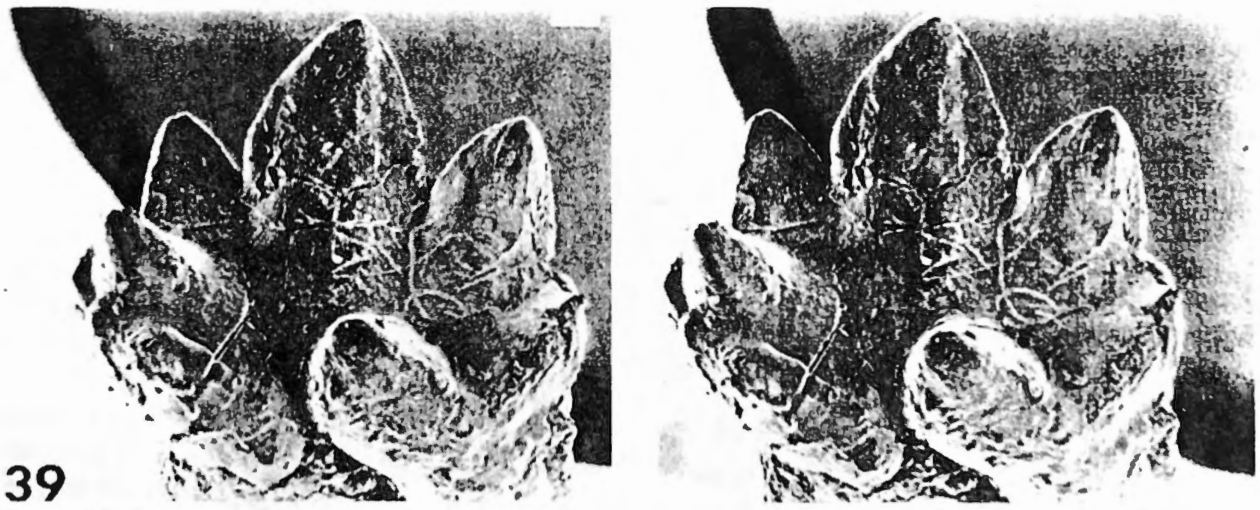

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Figure 39. Stereo pair of lower right molar of *Megazostrodon* seen from lingual view. Bar = 100 μm.

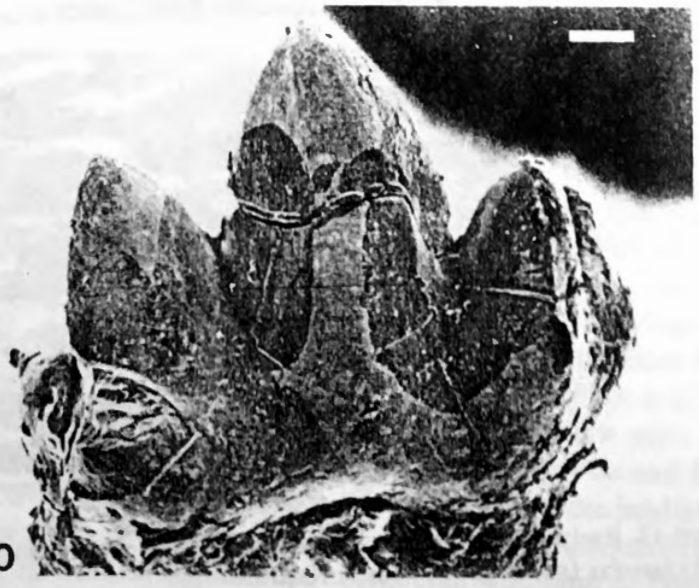

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Figure 40. Lower molar of *Megazostrodon* seen from the buccal side. Notice the worn areas where cusps of the upper molar sheared across the lower. The cusps have sharp carnassial-like edges. Bar = 100 μm.

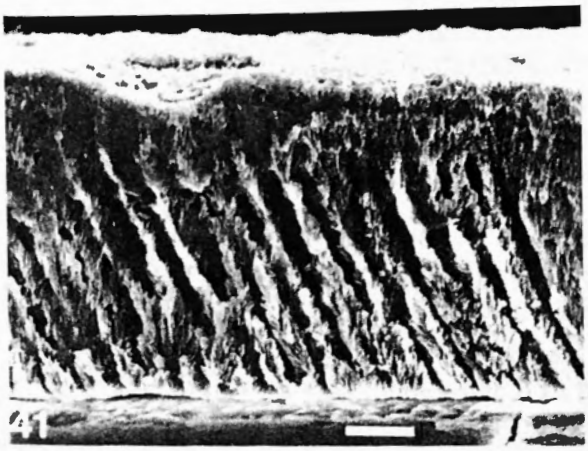
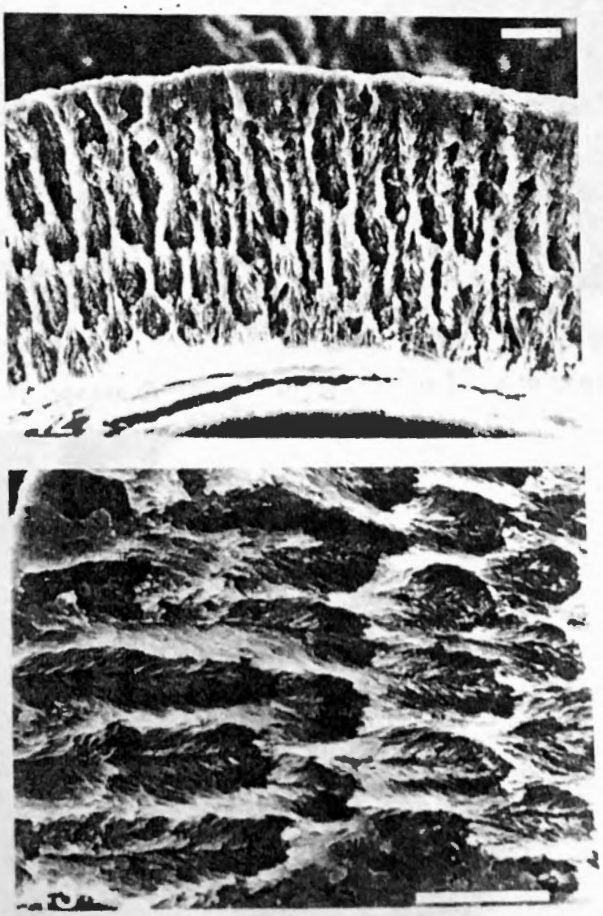


Figure 41. Longitudinal view of etched enamel. Only pinnate-shaped arrangement is obvious. Notice the lines of Retzius at an angle to the surface (arrow). Reptilian growth lines (see the tritylodontid) are parallel to the DEJ. Bar = 10 µm.



Figures 42 and 43. Early prism patterns in *Megazostrodon*. Arrangement of crystallites is very much like those of *Pachygenelus* (see Fig. 38). Bar = 10 µm.

the dentin to the outer surface of the tooth. The packing pattern appears to be hexagonal (Figs. 42, 43). From the micrographs in this study, it is difficult to tell if there are any major differences between the enamel of *Pachygenelus* and that of *Megazostrodon*. As shown in the drawing of ameloblasts for *Pachygenelus* (Fig. 38), essentially the same arrangement holds for *Megazostrodon*.

Where it is possible to observe orientation relative to masticatory function in *Pachygenelus* or *Megazostrodon*, the early prisms are either perpendicular or slightly oblique to shearing forces, i.e. the early prisms are directed laterally and cuspally (Figs. 34 and 42).

Discussion

Histochemical (Poole, 1956; Ferguson, 1981) and immunohistochemical data (Herold *et al.*, 1980) have shown that odontogenesis in reptiles and mammals are homologous processes. Furthermore, Herold *et al.* (1980) have demonstrated cross-reactivity between antisera to bovine enamel proteins and enamel of most vertebrate classes (see Slavkin *et al.*, 1984, for review). It is therefore of interest to record the transition from non-prismatic to early prismatic enamel in advanced mammal-like reptiles and the earliest mammals. Modern mammalian enamel is characterized by clearly defined prisms possessing interprismatic enamel and a prism sheath between the prism and IP enamel (Sahni, 1987). When these structures first appeared in the fossil record has not been clearly established.

The present study verified that reptilian enamel, as seen in the caiman (Fig. 1) and the crocodile (Figs. 2 and 3), is typically aprismatic, except that the crocodile enamel exhibits large spaces. These spaces were noticed earlier by Erler (1936) as being impenetrable by liquids, so it is possible that they were filled with some organic material prior to our treatment with sodium hypochlorite. The teeth of these two reptilian species do not occlude and movement is restricted to a vertical direction.

Uromastyx princeps enamel after a 20 second etch in 1N HCl displayed prism-like areas. Cooper and Poole (1973) claimed that prisms were present and etched more deeply than the IP enamel with 0.1N HCl, but micrographs of these samples were not shown. It may be that the etchants used in our experiment were too caustic for the *Uromastyx* enamel prisms to be observed clearly. The micrographs shown by Cooper and Poole (1973) were the results of one and two hour treatments with EDTA, pH 7.2. We observed a slight splaying of crystallites (Fig. 7) in addition to holes which apparently etched more deeply than the surrounding enamel (Figs. 5 and 6). These areas might have been filled with organic material, as stated above, or with a mineral which is more susceptible to the acid used in this experiment. Whatever these holes are filled with, however, they do not appear to occur at regular intervals; the amount of residual organic material (Fig. 8) after etching, make this enamel significantly different from most mammalian prismatic enamels (except for tubular enamels - see Stern *et al.*, 1989).

Massetognathus - No evidence of interprismatic enamel was seen between the columns of enamel in *Massetognathus* (Figs. 11, 14). Since the columns were about 8 μm in diameter, our scenario of the secreting ameloblasts includes the cooperative effects of ameloblast pairs. The idea of cells cooperating in the formation of a hard tissue is not new, since it is known that odontoblasts cooperate to form calcospherites of dentine (Boyde and Jones, 1972). The columns were directed towards the outer surface of the enamel at the medial and lateral sides (Fig. 11). If the crystallites (as opposed to columns or prisms) are almost perpendicular to the vertical axis of the tooth, this could explain the considerable amount of flattening at the cusp apices (upper left in Fig. 11). Crystallites which are parallel to abrasive forces wear more

quickly than those which are perpendicular, but discontinuities such as holes also cause enamel to wear more rapidly (see Boyde, 1984, for explanation of crystallite orientation as related to wear facets; also see Fortelius, 1985; Boyde and Fortelius, 1986 and Stern *et al.*, 1989).

The first evidence, in our study, of IP enamel occurred in the tritylodontid molar (Figs. 19, 20, 21, 22, 24). The stirrup shapes (about 20 μm wide) (Figs. 19, 20) can be seen as a combination of three or four smaller prism-like units. Here again (Fig. 25b) we see the result of a possible cooperation between ameloblasts. Within the interprismatic enamel (Fig. 20) lies a very shallow structure with no evidence of a prism sheath (Figs. 23, 24). This pattern may represent an early type of prism. However, since the tritylodontids may have arisen from traversodonts and only be distantly related to mammals (Crompton and Ellenberger, 1957; Allin and Hopson, 1992) the enamel structure might have evolved differently. These patterns may or may not represent an early step in the evolution of modern prisms. The inspection of more therapsid enamel will help clarify this point. But, as in recent mammalian enamel (Skobe and Stern, 1978), inner (closer to the DEJ) and outer enamel layers are observed (Fig. 18) when the enamel is examined in sagittal section. The DEJ is rather straight (Figs. 18, 21, 22) as we find in reptilian enamel (Poole, 1967; Piesco and Kallenbach, 1985), with a marked lack of interdigitation. Seen transverse to the long axis of the prisms (Figs. 23, 24), the pattern does not appear to be homogeneous across the enamel surface. The implication is that two adjacent ameloblasts may not be in exactly the same configuration. The prism-like structures appear to be shallow, which probably explains the sheet-like appearance after acid-etching (Fig. 18), and the domains within one row lack homogeneity. As shown in Figure 25a, the orientation of crystallites and prism-like structures is slightly oblique to the shear forces at the sides of the cusps; the IP crystallites are more perpendicular to the outer surface of the tooth and will, therefore, etch a bit more rapidly when the outer surface is exposed to the etchant (Johnson *et al.*, 1971).

In *Pachygenelus* (Figs. 29, 30), a herring-bone pattern with a seam in the middle of each domain (Fig. 37) is the predominant arrangement. Thick IP enamel surrounds each prism-like structure, and the change in orientation between the two is so slight that the point at which one grades into the other is difficult to distinguish. The greatest change in orientation between crystallites is in the middle of the herring-bone, at the locus of the seam (Fig. 32, 33, 38), and is likely to be the area of crystal-packing defects or an area of microporosity (Osborn, 1981) where the ends of groups of crystallites abut each other (Boyde, 1964; Osborn, 1981), and leave a small region for organic components to accumulate during maturation of the enamel matrix. Whether or not these seams are homologous to later prism sheaths which form between P and IP enamel, or to the seams found in bat enamel at the cervical end of the prism (Lester and Boyde, 1987; Lester *et al.*, 1988), is unclear, but this area would appear to be the likely site for breakdown products of the enamel proteins which are to be resorbed during maturation (see Glimcher *et al.*, 1964; 1965, for this phenomenon in bovine enamel).

Almost the identical pattern seen in *Pachygenelus* is found in *Megazostrodon* (especially Figs. 42, 43), so the model shown in Figure 38 would be equally applicable to *Megazostrodon*. Our results agree with those of Grine and Vrba (1980) and Grine *et al.* (1979), who also observed prism-like structures in *Pachygenelus* (they called them "prisms", however). We disagree slightly with Frank *et al.* (1984, 1988) and Sigogneau-Russell *et al.*, (1984) who describe herring-bone columns in Liassic mammalian enamel (*Kuehneotherium*, *Morganucodon*, and Haramyiids - see Introduction); we have shown that interprismatic enamel does exist in *Pachygenelus* and in *Megazostrodon*, and that the orientation of the IP crystallites differs slightly from that of prism crystallites by being more vertically oriented to the DEJ

than the crystallites of the herring-bone (Figs. 32, 36, 38, 43) making up the prism. We also do not agree with Sigogneau-Russell *et al.*, (1984) that the "V" points downward in the middle of one ameloblast's territory. We feel that the apex must point upward for the Tomes' process to assume a reasonable shape, and also to be compatible with images like Figures 32, 33, and 43.

No evidence of a prism sheath between the prism and IP enamel was found in any of the specimens examined. Results of this study, therefore, do not confirm the appearance of true prismatic enamel in the fossil record up to the Early Jurassic period. Based on the possible morphologies and secreting surfaces of ameloblasts that we have drawn (and these are by no means the only possibilities), we postulate that the cellular correlate of modern prismatic enamel would be an ameloblast with a Tomes' process that has developed a non-secreting, or sliding, surface. This phenomenon is discussed by Wakita and Kobayashi (1983) relative to dog and cat ameloblasts (also Kallenbach, 1977, and Sasaki, 1983). The fact that one surface slides and doesn't secrete, allows for a greater discrepancy in alignment of crystallites between

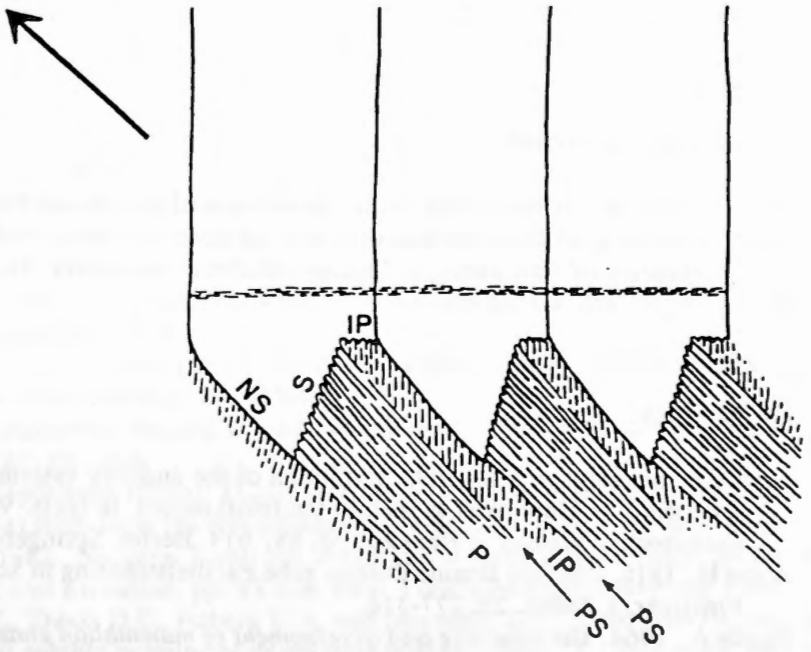


Figure 44. Diagram of ameloblasts with a non-secreting surface (NS) secreting Pattern 2 enamel as in *Didelphis virginiana*. IP in cell represents the proximal area of the Tomes' process which is secreting IP enamel. S = secreting surface of Tomes' process. Arrow = direction of movement of ameloblasts. IP = interprismatic enamel. P = prism. PS = prism sheath.

the prism and the interprismatic enamel and therefore a pronounced prism sheath. It is possible, therefore, that the evolutionary step from early prismatic enamel (or what has been called herring-bone, pseudoprismatic, or preprismatic enamel) to true prismatic enamel, is a change in the functional properties at discrete sites of the Tomes' process, and not due as much to a change in morphology. The model of ameloblasts secreting true prismatic enamel such as that found in the Pattern 2 enamel of *Didelphis virginiana* (Fig. 44) is included to demonstrate the concept. The condition of having a Tomes' process with all apical surfaces secreting may account for the confusion in the literature where advanced therapsid and early mammalian enamel ultrastructure have been described (hence columns, pseudoprisms, preprisms, etc.); the resultant structure is not clearly prismatic, and is especially confusing when cut obliquely or longitudinally.

Conclusions

The organization of enamel appears to be related to occlusion. Where crystallites are arranged in a pattern other than the typical reptilian-aligned-pattern, there is almost certain to be a correlation with an occluding dentition.

From this study we conclude that the ameloblast in the forms leading from reptiles to mammals could have varied in the following ways:

1) A conformational modulation back and forth of cooperating ameloblasts during the secretory phase, as in *Massetognathus* and a change in ameloblast conformation from inner to outer enamel as in the tritylodontid.

2) The development of a secreting portion at the proximal area of the Tomes' process which is responsible for IP enamel, as in *Pachygenelus* and *Megazostrodon*.

3) A change in Tomes' process shape (from rounded to pointed, for example), function (secreting to non-secreting), and length (length may be related to rate of enamel matrix secretion, as discussed by Grine and Vrba, 1980). All of these factors may contribute to a well-defined prism with a prism sheath and interprismatic enamel, as found in modern mammals.

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