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THE DEVELOPMENT AND EVOLUTION OF MAMMALIAN ENAMEL: STRUCTURAL AND FUNCTIONAL ASPECTS

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ABSTRACT

Dental enamel is the most highly mineralized tissue of vertebrates and consists mainly of submicroscopic crystals of hydroxyapatite. Comparative analysis of enamel structure has revealed a marked structural diversity among vertebrates. In most cases, the enamel of amphibians and reptiles is aprismatic, since the crystallites are roughly parallel to each other and perpendicular to the enamel surface. The enamel of mammals is formed by prismatic structures, the diversity of which may be used to infer phylogenetic relationships and to identify mammalian taxa in higher orders. The complexity of enamel has been also related to feeding habits, since the patterns observed have usually evolved as functional adaptations in response to biomechanical stress imposed on teeth. In this article we review and discuss the modifications in enamel structure that occurred during mammalian evolution, as well as the functional and cellular aspects related to these changes.

Key words: Enamel, mammalian evolution, teeth

INTRODUCTION

The dentition of lower vertebrates (fish, amphibians and reptiles) generally has a simple structure, with no occlusion between the opposing jaws. The function of this dentition is usually limited to the capture and piercing of food, which is swallowed in large fragments. The formation of a dental occlusion in mammals allowed better processing of food and a consequent increase in the efficiency of nutrient intake by the digestive system. The development of an efficient masticatory system was, therefore, a key step that allowed mammals to cope with the increased demand for energy necessary to support high levels of activity and contributed to their diversification.

The advent of masticatory capability in mammals involved a precise adjustment of the temporomandibular articulation, periodontium, and teeth. The tooth is usually the most significant factor that controls occlusion since the adjustment between the maxilla and mandible during mastication is mainly regulated by occlusal contacts between the cusps, pits and fissures of the teeth in opposing jaws. During mammalian evolution, increased functional demands meant that the teeth were subjected to continuous abrasion and tensional forces, and had to remain functional for many years, since the polyphyodont dentition of reptiles was replaced by the diphyodont or monophyodont dentition found in most mammals. Changes in dietary habits, which shifted from soft insects to carnivorous and herbivorous diets [10], increased the likelihood of dental trauma. Additionally, the tendency to increase in body size, especially in the new mammalian species that appeared during the Paleocene and Eocene [1], led to an increase in life span and tooth size. Although these new features increased the dietary repertoire, they also increased the risk of dental trauma and wearing. This trade-off was compensated partly by the development of a more robust and complex tooth structure. Enamel is the tissue that receives the force of masticatory impact. The evolutionary changes in tooth structure were therefore determined mainly by an increasing complexity in enamel structure [20] and, ultimately determined by changes in ameloblast biology. In this article, we review the modifications in enamel structure that occurred during mammalian evolution, as well as the functional and cellular aspects related to these changes.

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THE ORIGIN OF ENAMEL PRISMS

Enamel is thought to have evolved from enameloid, a highly calcified tissue covering the

dentin of fish. Although enamel and enameloid have a similar composition, these tissues are formed by distinct developmental mechanisms. Enameloid is predominantly of mesenchymal origin and its matrix is composed mainly of collagen fibers, whereas enamel is of epithelial origin and its organic matrix does not contain collagen. True enamel probably appeared with the first amphibians that evolved from actinopterygian fishes during the Devonian period. This probably occurred by the development and expression of enamel-specific genes such as amelogenin, matrix metalloproteinase-20, kallikrein, enamelin and ameloblastin, which have not been identified in fish [12,22,].

The enamel of amphibians and most reptiles has a simple structure formed mainly by closely packed hydroxyapatite crystallites that are roughly parallel and oriented perpendicularly to the enamel surface (Figs. 1A and 2). Such enamel is termed aprismatic. The orientation of enamel crystallites is determined by the secretory surface of the ameloblast located at the distal end of the cell. The crystallites are oriented perpendicularly to the membrane of the secretory pole. This feature makes it possible to infer the morphology of the secretory end of ameloblasts by observing the orientation of the crystallites. Mammalian enamel is characterized by the presence of prisms, which are solid, rod-like structures that extend from the dentin-enamel junction to the enamel surface. This prismatic enamel is characterized by crystallite discontinuities in which crystallites are grouped in more or less parallel bundles that are limited by interprismatic crystallites oriented at a sharp angle to the crystallites in the prisms (Fig. 1C,D). The prismatic condition was considered to be a distinction between mammalian and reptilian enamel until Cooper and Poole [5] demonstrated the presence of prismatic structures in the agamid lizard *Uromastix*. Crystallite discontinuities were subsequently reported in the enamel of several recent and fossil reptiles [4,6,9,30]. Despite the presence of crystallite discontinuities, the enamel of most reptiles is not prismatic. Such enamel has been termed preprismatic or pseudoprismatic, and represents a transitional stage between prismatic and aprismatic enamel (Figs. 1B and 3). The transition from preprismatic to prismatic enamel is indicated by the presence of preprismatic enamel in the synapsids that gave rise to mammals [31]. In pseudoprismatic enamel, the crystallites usually form columnar structures without the formation of interprismatic substance (Fig. 1B).

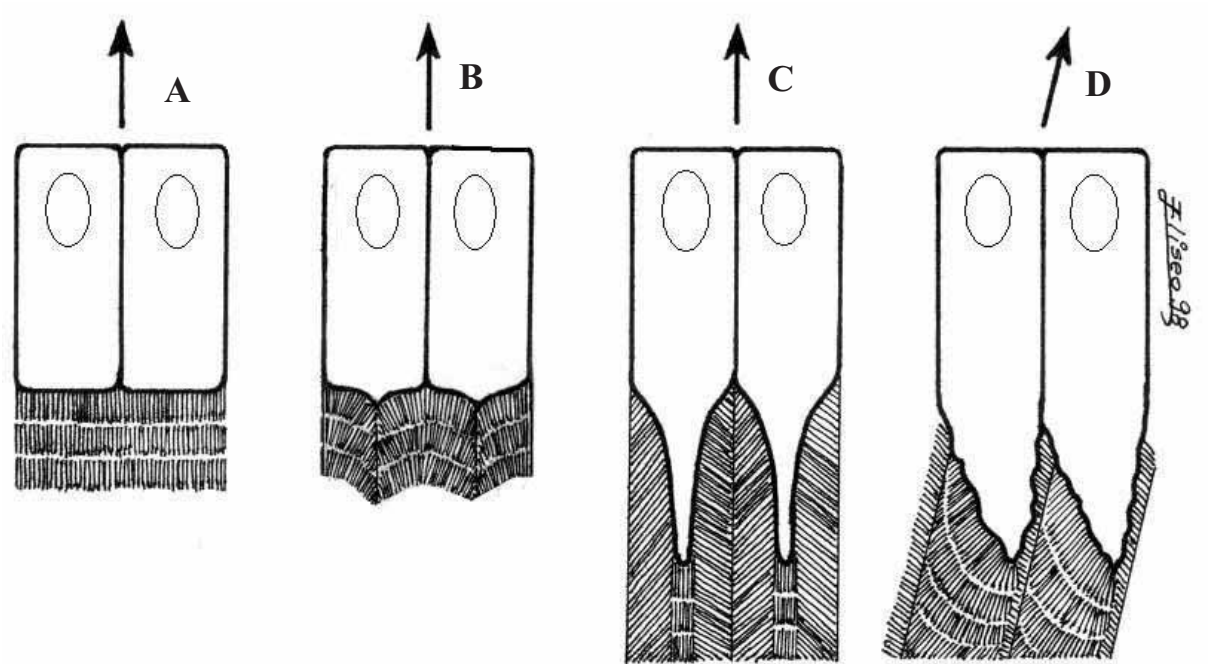

Figure 1. Evolution of ameloblast morphology and enamel structure. **A**) Aprismatic enamel produced by ameloblasts lacking a Tomes' process. **B**) Pseudoprismatic enamel produced by ameloblasts with a small Tomes' process. **C**) Radial enamel in which prisms follow a straight course from the dentin-enamel junction to the tooth surface. **D**) Prismatic enamel with Hunter-Schreger bands in which the ameloblasts migrate laterally in a wavy formation. The arrows indicate the direction of migration.

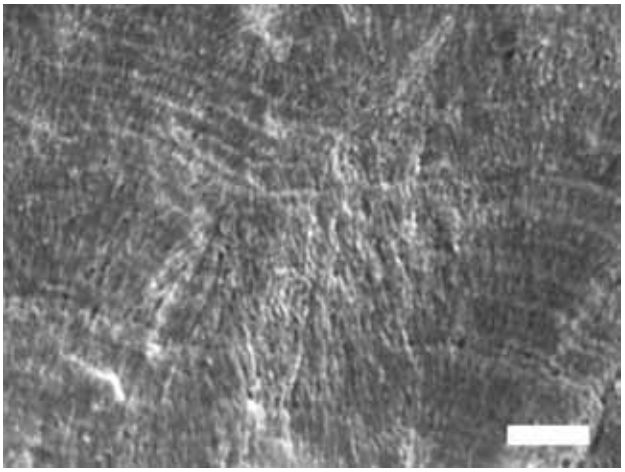

Figure 2. Scanning electron micrograph of a longitudinal aprismatic enamel from *Caiman crocodilus* (Reptilia, Crocodilia). Note that the crystallites are roughly vertical. The horizontal lines represent incremental growth marks. Bar = 9 μm.

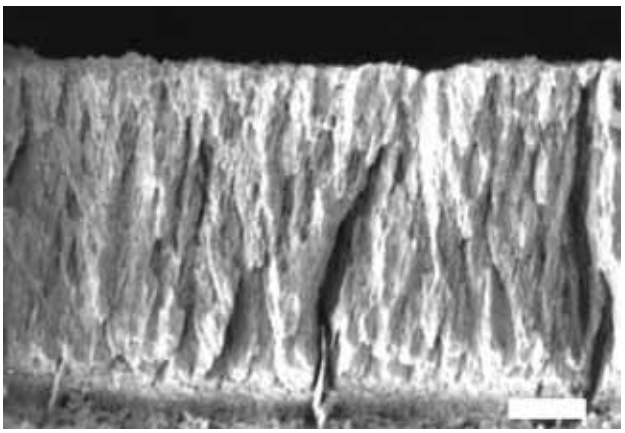

Figure 3. Scanning electron micrograph of pseudoprismatic enamel from a Canadian carnosaur (Reptilia, Theropoda) from the Cretaceous period. Note the columnar aspect of the enamel. Bar = 9 μm.

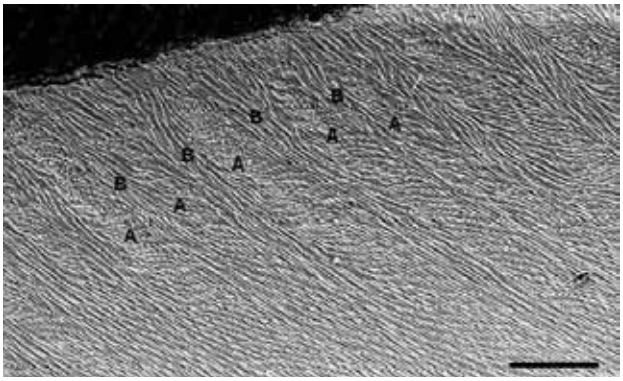

Figure 4. Scanning electron micrograph of human prismatic enamel. Note that some prisms are transversely cut (**A**), while other prisms run longitudinally (**A**). Groups of prisms with the same orientation form the Hunter-Schreger bands. Bar = 100 μm.

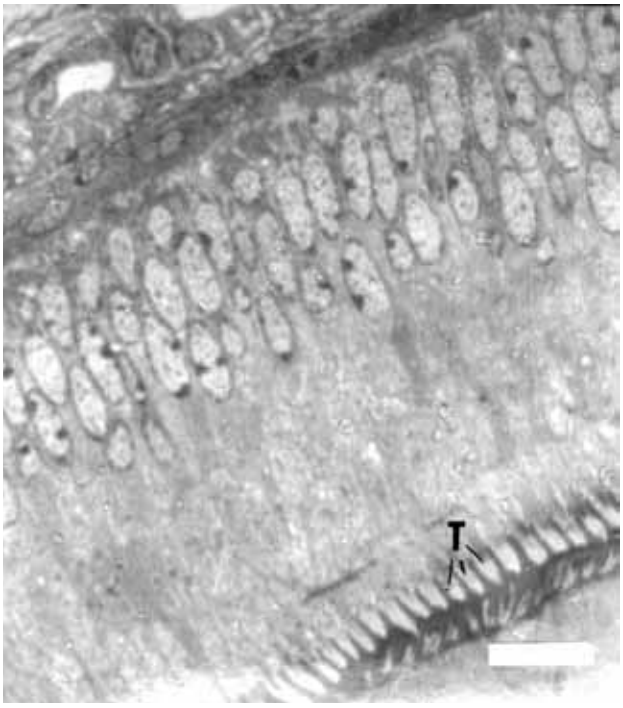

Figure 5. Transmission electron micrograph of rat ameloblasts. Note that the Tomes' processes (**T**) lie within enamel matrix. Bar = 20 μm.

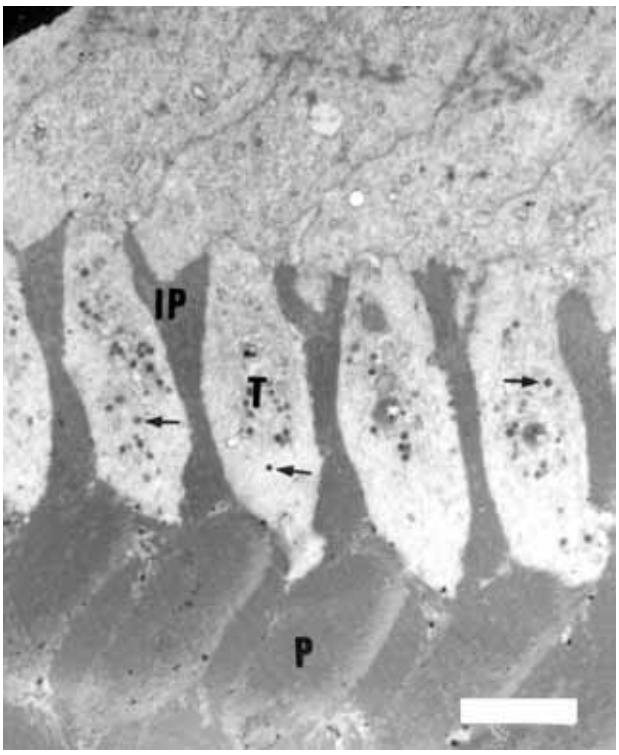

Figure 6. Transmission electron micrograph of the distal portion of rat ameloblast and enamel matrix. Note the large number of secretory vesicles (→) within Tomes' processes (**T**). Prismatic (**P**) and interprismatic (**IP**) enamel can also be seen. Bar = 4 μm.

The crystallite discontinuities in enamel probably developed to meet the functional demands imposed on teeth by mastication. Discontinuities in the crystallite orientation would help to dissipate the occlusal load, thereby reducing the risk of enamel fracture [33]. Variations in crystallite orientation would also contribute to optimal dental function through differential wear in functionally distinct regions of the teeth [21]. The selection of prismatic enamel was favored by the increasing biomechanical stress imposed on teeth by the advent of heterodonty and diphyodonty in advanced synapsids and primitive mammals [13]. The influence of functional demands on enamel structure is clearly illustrated by the degenerative history of enamel in a few mammalian species. The lack of or reduction in functional requirements is associated with rapid structural regression and a reduction in enamel thickness in odontocete whales, monotremes and bats. Prismatic enamel was present in middle Miocene monotremes, archaeocete whales and in their presumed ancestors (mesonychids), whereas pseudoprismatic and aprismatic enamel is the predominant type found in most living toothed cetaceans, *Ornithorhynchus* (platypus) and in the vampire bat *Desmodus* [11,17-19,25,29].

Enamel crystallites run roughly perpendicular to the secretory surface of ameloblasts [3,32]. Consequently, it is possible to infer that aprismatic enamel is formed by ameloblasts with a flat secretory surface (Fig. 1A). Likewise, the presence of crystallite discontinuities in the enamel of most mammals and some reptiles is consequence of morphological changes in the secretory surface of the ameloblast. Indeed, the distal end of ameloblasts that make prismatic enamel has a distal appendage known as Tomes' process (Figs. 1C, 5 and 6). Although the evolutionary appearance of the Tomes' process cannot be directly studied, its occurrence can be inferred by observing crystallite discontinuities in the enamel of fossils. This important step in mammalian evolution was accomplished by a rearrangement of the cytoskeletal components in the distal secretory portion of ameloblasts [16,25]. The importance of this event is exemplified by the presence of enamel prisms in nearly all extinct and extant mammalian taxa. The development of Tomes' process was a key innovation since it marked the crossing of an adaptive threshold that allowed the performance of a new function (crushing and grinding) by the first mammals during the Triassic. The development of Tomes' process is a rare example in evolution in which structural changes in a tissue were caused by modifications in cell morphology rather than by spatial cellular rearrangements.

EVOLUTION OF PRISM DECUSSATION

The extinction of the larger dinosaurs at the end of the Cretaceous, and the availability of new food resources caused by the radiation of the angiosperms during the early Paleocene, favored the diversification of eutherian mammals. This diversification was accompanied by an increase in body size that resulted in forms with bigger teeth and more robust masticatory muscles. In conjunction with increasing size, there was a shift in feeding habits from soft insects to carnivorous and herbivorous diets. These changes have greatly increased the stress imposed on teeth by the impact of mastication, and increased the risk of enamel fracture and abrasion. During the early Paleocene, the simple radial enamel, in which the prisms follow a straight course from the dentin-enamel junction to the tooth surface, gave rise to the typical arrangement of horizontal layers of prisms that formed the Hunter-Schreger bands (HSB, Fig. 4). The formation of HSB is believed to have improved the physical properties of enamel. Prism decussation can strengthen enamel and make it more resistant to tensional forces, thereby avoiding the propagation of vertical cracks. The importance of HSB is shown by the presence of these structures in the enamel of most placental mammals with a molar width larger than 4 mm [15].

There is considerable diversity in the structure and pattern of HSB among the various groups of mammals. These differences may be used to infer phylogenetic relationships and to identify mammalian taxa of higher orders [7,15,35]. The complexity of HSB has been related to feeding habits since the pattern of these bands usually evolves as a functional adaptation in response to the biomechanical stress imposed on teeth [2,15,27,35]. The enamel of arctiod carnivores consists of three types of HSB [34,35]: u*ndulating*, the simplest structure, in which the bands remain parallel throughout the enamel, *zigzag*, the most complex enamel type with increasingly greater amplitude in the waviness of the bands from the enamel-dentine junction towards the outer surface of the enamel, and *acute-angled*, which is structurally intermediate to the first two types of enamel. The observation that *zigzag* enamel is usually found in carnivorous species that crush bones regularly or in species that tend to supplement their diet with bones supports

the hypothesis that this structure developed to resist cracking under high tensile stress.

Although the majority of mammals have horizontally-oriented HSB, several classes of perissodactyls have developed vertical HSB. Vertical HSB, or intermediate forms between vertical and horizontal bands, have been found in some large, extinct Chalicotheriidae, Brontotheriidae and Tapiroidea [8,14], and is currently found only in the Rhinocerontidae [28]. The development of vertical HSB in these herbivores may have occurred as a functional adaptation since large mammals take longer to reach sexual maturity and enamel would have to remain functional for longer periods [15]. Vertical HSB wear at a slower rate than horizontal HSB [28], and this would enhance the functional durability of enamel, thereby extending the overall longevity of the teeth and the animal's life span.

The development of HSB was ultimately determined by changes in the pattern of ameloblast migration. Ameloblasts present in lower vertebrates, and in small, primitive mammals, have a nearly straight course of migration from the dentin-enamel junction to the enamel surface. In contrast, ameloblasts that form HSB would have a more complex interaction with their neighbours since they have to migrate upwards in the occlusal direction and also move laterally (Fig. 1D). The evolution of HSB may be inferred by observing fossil teeth and enamel ontogeny. The structure of enamel in most mammals that have HSB can be divided into three layers. The *innermost* enamel contains straight prisms, whereas the *inner* layer, which forms the bulk of the enamel, contains prisms that decussate to form HSB. The *outer* enamel contains prisms that run straight. Since each prism is made by a single ameloblast, the migration pattern of the ameloblast can be traced by following the path of the enamel prism.

Cytochemical and ultrastructural studies of rat and monkey amelogenesis have suggested a direct association between the course of ameloblasts migration and the arrangement of the distal terminal web (DTW) filaments and associated cell membrane adhesion molecules [23,24,26]. At the beginning of the formation of the innermost enamel, the Tomes' process is small and the distal terminal web filament bundles (consisting maily of F-actin) are arranged circularly near the cell membrane. At a later stage, the Tomes' process becomes ovoid and the distal portion of the cell changes to form elongated hexagons that are aligned in horizontal rows. This pattern appears to be determined by the orientation of the DTW filaments that form two distinct bundles at the cell periphery. The bundles of filaments are aligned with the straight rows of ameloblasts. The organization of the DTW with the formation of rows of ameloblasts apparently precedes the wavy movement of ameloblasts. This lateral movement would be possible through contraction of the web filaments in one side of the ameloblast row. Coordinated contractions of these filaments would cause neighbouring ameloblast rows to turn distinctly to the right or to the left, thereby forming decussating bands. Finally, during the formation of the outer enamel layer, the Tomes' process becomes small and round and the DTW filament bundles form a circular belt at the periphery of the cell membrane. These observations indicate that the development of HSB is determined by changes in the DTW filament bundles of ameloblasts. The evolution of HSB has apparently been a gradual process. HSB in early Paleocene mammals were limited to the central part of enamel and had a low angle of decussation, whereas in late Paleocene forms HSB extended throughout enamel and the angle between prisms of different layers reached nearly 90^{o} [15].

CONCLUDING REMARKS

The evolution of complex anatomical structures is a complicated process involving, mutations in genes associated with organogenesis and epigenetic changes. The expression and spectrum of action of these genes may be influenced by environmental factors. The evolution of enamel microstructure was determined by three major phenomena. The first phenomenon was the appearance of specific enamel proteins that allowed the formation of a true enamel in amphibians. The second phenomenon was the development of the small basal secretory appendage known as Tomes' process that is related to the formation of enamel prisms, and the third was the acquisition of a lateral wavy migration by ameloblasts in which migration in different directions gave rise to the HSB. The development of a secretory process and the acquisition of undulating migration by ameloblasts may be considered key innovations in mammalian evolution. These events conferred improved biomechanical properties to enamel, and allowed the adaptation to a crushing-grinding mode of chewing, thereby enhancing the evolution and diversification of mammals.

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