From: The Biological Station of the University of Oslo, Dröbak, Norway.

THE TEETH OF ALLIGATOR MISSISSIPPIENSIS DAUD.

V. Morphology of the Enamel*

by

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The outer, very hard stratum on the crowns of the teeth of vertebrates is formed in two fundamentally different ways[3]. In fish and Amphibia the organic foundation is laid down by the dental papilla. The outer stratum of these teeth is of mesodermal origin and has, therefore, been termed *mesodermal enamel*. In reptiles is, for the first time in phylogenesis, formed an *ectodermal dental enamel*. The organic foundation for this enamel, as for that of mammals, is laid down by the innermost cell layer in the ectodermal part of the tooth germ, the so-called ameloblasts.

MATERIAL AND METHODS

For this investigation were used teeth from adult animals living in captivity. The teeth were shed in the alligator pool and collected from the mud at the bottom when the pool was cleaned. Most of the teeth had been kept dry for a varying length of time, but some were taken directly from the water when the work started. The teeth were fixed in 4 per cent neutral formaldehyd for two years or more.

Teeth large enough to be handled without embedding were cut in slices of approximately 1.5 mm thickness by moistened Joe Dandy discs in the hand piece of the dental engine. The slices were ground down to the desired thickness with carborundum on a glass slab, dehydrated, and mounted in Canada balsam.

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Smaller teeth were dehydrated in absolute alcohol and zylene and kept in Beetle Polyester Resin 4116* for a couple of days. Each tooth was then embedded in the resin to which was added the prescribed quantity of accelerator and catalyst. Small rectangular boxes of thin aluminum were used for this procedure. The specimens were left for two days at room temperature and the hardening of the blocks was finished in another two days in a thermostat at 50° C.

The polyester resin is easy to work with and air bubbles never occur. As the cured resin is very transparent the tooth embedded in it can clearly be seen, and the plane of the section can easily be determined. Thin slices were cut with a diamond impregnated disc and the further procedure was as described for unembedded teeth.

For the staining was used *Morgenstern's*[6] aqueous hematoxylin for ground sections, or the whole tooth was immersed in 2.5 per cent silver nitrate for a week before sectioning.

OBSERVATIONS

The enamel forms a very thin covering over that part of the tooth which projects from the gingiva. It is thickest at the tooth tip and gradually thins down to a sharp edge at the gingival border. The maximum thickness measured in the present material was 0.5 mm, but in most teeth the enamel layer was thinner.

In a longitudinal section of a tooth studied with low magnification light striae with darker intermediate layers are seen in the enamel (Fig. 1). At the tip of the tooth parallel striae surround the summit of the dentin in larger and larger curves, all of them beginning and ending at the dentino-enamel junction (Fig. 1 a). These curved striae finally reach the tooth tip.

Along the side of the tooth striae are likewise observed as parallel lines starting at the dentino-enamel junction. They run obliquely through the enamel meeting the enamel surface nearer the tooth tip (Fig. 1 c).

At higher magnification the impression of a coarse striation

* Manufactured by British Industrial Plastics Ltd., Tat Bank House, Oldbury, England.

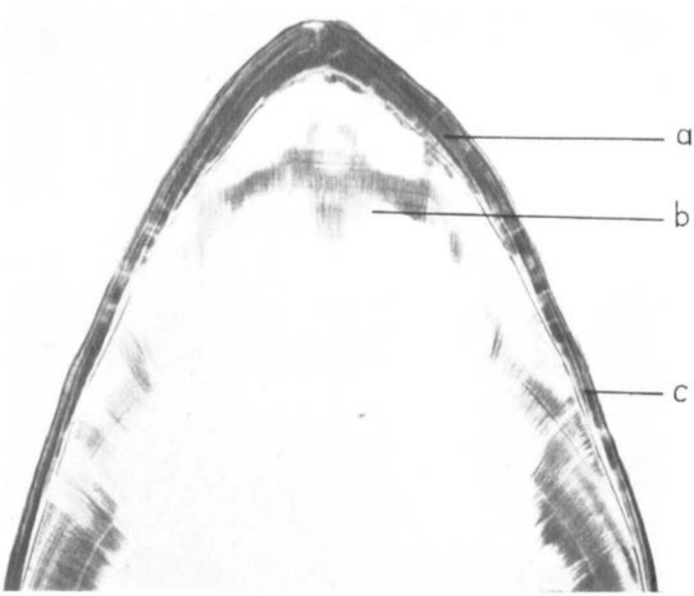

Fig. 1. Longitudinal ground section. X 10. a, incremental line in the enamel surrounding the tip of the dentin; b, dentin; c, incremental line in the enamel running obliquely from the dentino-enamel junction to the surface.

is conveyed by a much closer striation of fine, parallel lines going in the same direction (Fig. 2). The dark fields in Fig. 1 are groups of pronounced lines (Fig. 2 a) whereas in the light fields the lines are much less distinctive (Fig. 2 b). The individual lines are straight or faintly curved and can often be followed for quite a long distance without interruption.

In a transverse section of a tooth, concentrically arranged lines of varying distinctness are dividing the enamel into areas reminding of the dark and light fields seen in a longitudinal section (Fig. 3 a and b). The individual lines are straight or faintly undulated and can often be followed continually over a wide area.

Other lines in the enamel go perpendicularly from the dentino-enamel junction to the surface of the tooth. In a transverse section they cross the concentrical stripes at right angles (Fig. 3 d). They have the same general direction but are not absolutely parallel, small deviations from the straight course being often observed. In most cases the individual line is visible only for a short distance. Sometimes, however, one of them can be traced through the whole thickness of the enamel (Fig. 3 e). These lines are often more distinct than the ordinary ones, and sometimes

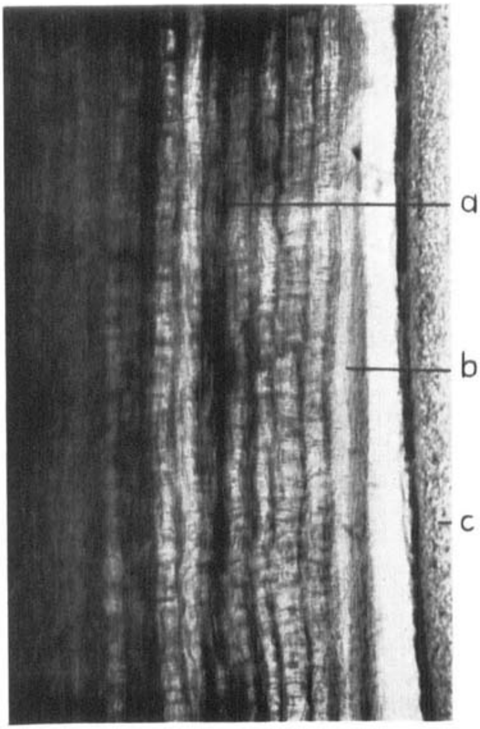

Fig. 2. Longitudinal ground section. X 360. a, dark field with pronounced incremental lines in the enamel; b, light field with faint incremental lines in the enamel; c, dentin.

two or more are united in bundles. In more extreme cases they remind of the lamellae found in mammalian enamel (Fig. 4 a). The lines usually end at the dentino-enamel junction. Some of the lamellae-like stripes, however, are traversing the junction and can be followed a short distance into the dentin (Fig. 5 a).

Lines going from the dentino-enamel junction to the tooth surface are also observed in longitudinal sections. Most of the stripes can be followed for a short distance only (Fig. 6 a), but some of them run uninterrupted through the whole enamel layer (Fig. 7 a). Also in longitudinal sections there are sometimes bundles of stripes reminding of lamellae (Fig. 8 a).

Staining of the enamel succeeded only to a small extent, but the structural details were in most cases satisfactorily discernible in unstained ground sections. Silver nitrate worked better than hematoxylin. After prolonged immersion in the staining solution the outer third of the enamel layer took a diffuse color (Fig. 9 a).

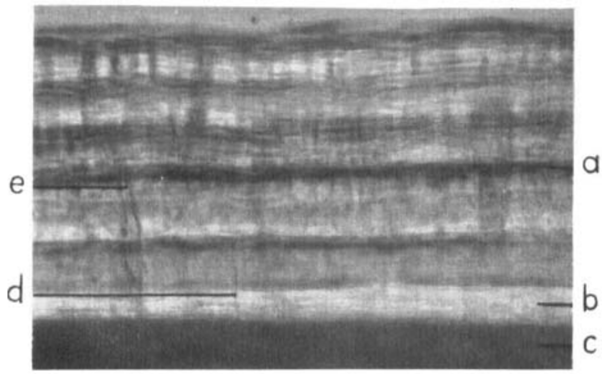

Fig. 3. Transverse ground section. X 360. a, dark field with pronounced incremental lines in the enamel; b, light field with faint incremental lines; c, dentin; d, perpendicular fiber in the enamel; e, perpendicular fiber traceable through the whole enamel layer.

The stain advanced along the lamellae-like stripes or followed cracks in the enamel to the dentino-enamel junction and spread out along the junction, often over a wide area, and mostly on the dentinal side of the junction (Fig. 9 b).

From the dentino-enamel junction the stain followed odd transversal stripes for a short distance toward the surface (Fig. 9 c). These partially stained stripes are often found some distance from the entrance of the stain to the dentino-enamel junction (Fig. 10 b), and in some places they seem to be continuous with terminal branches of dentinal fibers thus reminding of the dentinal fibers in mammalian enamel (Fig. 11 b).

DISCUSSION

The striae seen in longitudinal sections of the enamel surrounding the tip of the dentin and running obliquely through the enamel at the side of the tooth (Figs. 1 and 2) have much in common with the concentrical lines in the enamel in transverse sections of the tooth (Fig. 3). Compared with the outline of the enamel matrix at different developmental stages[4, 5], it seems obvious that each stria is a section through a layer of enamel which was deposited at the same time. The striae are,

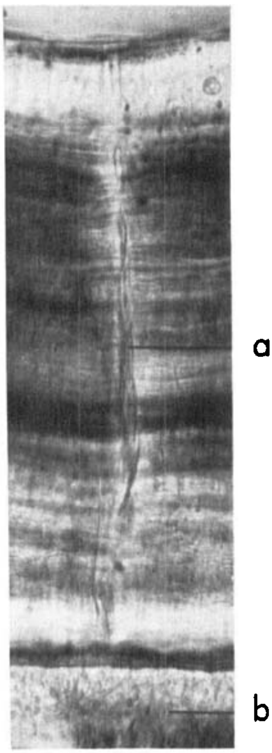

Fig. 4. Transverse ground section. X 360. a, lamella-like structure in the enamel; b, dentin.

therefore, incremental lines of the same nature as the lines of Retzius in mammalian enamel.

The incremental lines were not observed in the enamel matrix during the formation[4], they become visible only after decalcification. They most likely come into existence by variations in the degree of calcification. It was, however, impossible to get the less calcified incremental lines stained.

Poole[9] in *Agama atricollis* found the incremental lines already in the enamel matrix as "wide zones with the typical staining properties of the matrix separated by lines which do not stain so readily". In the matrix immediately before the final calcification the striae could be seen clearly.

Schulte[10] describes in crocodile both the concentrical lines in transverse sections and the longitudinal lines in longitudinal sections as fibers. *Erler*[2] found a "Wachstumsschichtung" in crocodile enamel both in transverse and in longitudinal sections of

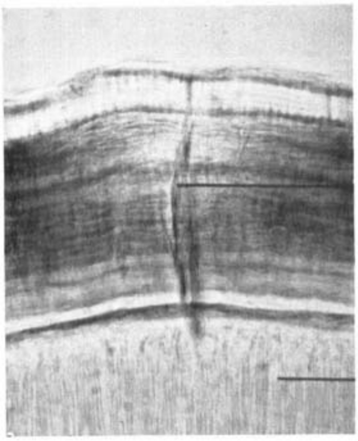

Fig. 5. Transverse ground section. X 180. a, lamella-like structure in the enamel traversing the dentino-enamel junction; b, dentin.

the tooth. She writes further: "Ausser der Wachstumsschichtung bieten Längsschliffe eine *Schrägstreifung* dar, die am Querschliff nicht wahrnehmbar ist" (p. 595). These two kinds of striation could not be demonstrated in alligator where the *incremental lines* in longitudinal sections had an oblique direction along the side of the tooth (Fig. 1 c). *Bradford*[1] in the enamel of *Alligator mississippiensis* found an incremental pattern only near to the cervix. In the present material the distinctness of the structures varied greatly, possibly due to some variation in calcification. The cervical part of the enamel is the last to be formed and, therefore, to be calcified, which may account for *Bradford's*[1] observation. In the enamel of some extinct mammal-like reptiles *Poole*[8] found very thin "layer lines" running parallel with the surface. They were probably incremental lines.

The lines running perpendicularly from the dentino-enamel junction to the surface in longitudinal as well as in transverse sections of the teeth (Figs. 3 d and 6 a) in most cases can be followed for a short distance only. A few of them, however, are visible through the whole enamel layer (Figs. 3 e and 7 a). This may indicate that the lines are fibers going from the dentino-enamel junction to the surface, but only in rare cases does the whole length of the fiber lie in the plane of the section.

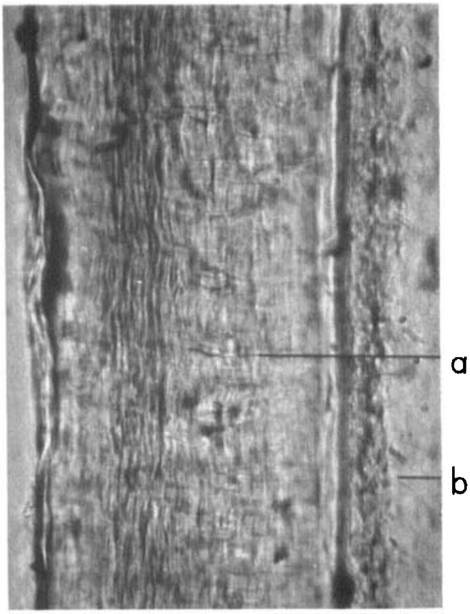

Fig. 6. Longitudinal ground section. X 360. a, perpendicular fiber; b, dentin.

During the development of the alligator enamel[4] fibers were demonstrated starting as tonofibrils in the ameloblasts and continuing as fibers in the enamel matrix running perpendicularly to the longitudinal direction of the tooth. The fibers in the enamel matrix very likely remain in the calcified enamel as the fibers demonstrated in this paper.

As mentioned it was impossible to get the enamel fibers stained, except for an occasional retrograde staining from the dentino-enamel junction (Fig. 9 c). This may indicate that the fibers are too strongly calcified to be penetrated by the staining solution. There is, however, also the possibility that the right staining method has not been found.

Erler[2] is of opinion that the striae perpendicular to the incremental lines are composed of very tiny, closed cavities containing air. The observation of fibers in the enamel matrix giving rise to these striae, however, makes the correctness of *Erler's*[2] view dubious.

Poole[8] examined in polarized light the enamel of extinct rep-

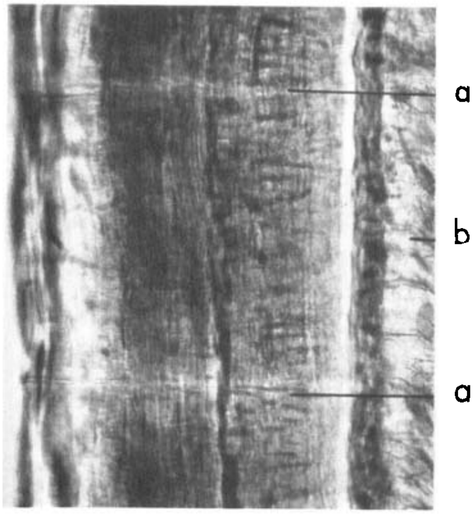

Fig. 7. Longitudinal ground section. X 360. a, perpendicular fiber traceable through the whole enamel layer; b, dentin.

tiles and found a pattern described as alternating black and white lines perpendicular to the enamel surface. According to his photomicrographs the dark lines may well be very coarse fibers of the same kind as those found in alligator. In the enamel matrix of *Agama atricollis Poole*[9] distinguishes between alternating light and dark stripes resulting from refraction and running vertically to the tooth surface, and fibers running in the same direction.

The lamellae-like structures often have a great resemblance to the lamellae of mammalian teeth (Fig. 4 a), but in contrast to the latter they appear as often in longitudinal as in transverse sections. They can, therefore, not have the same extent as mammalian lamellae which "extend in a longitudinal and radial direction of the tooth, from the tip of the crown toward the cervical region"[7] and are usually observed only in transverse sections of the tooth.

It is possible that the lamellae-like striae are bundles of coarse fibers that are less calcified than the rest of the enamel fibers since the stain passes along them so readily (Fig. 9). *Schulte*[10] describes the lamellae-like striae as cracks containing coarse

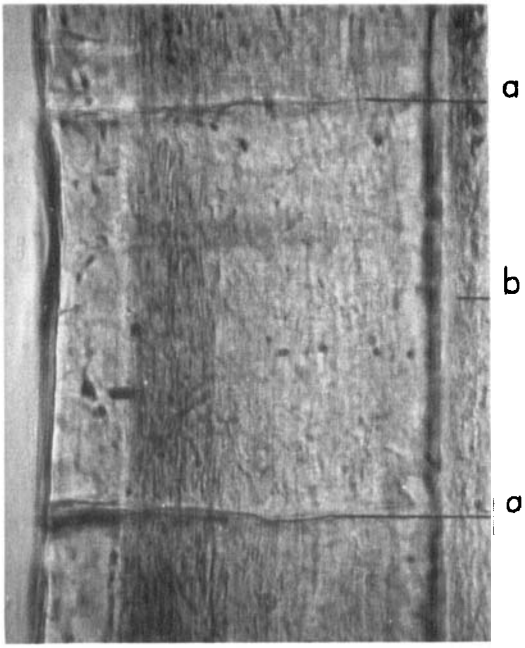

Fig. 8. Longitudinal ground section. X 360. a, lamella-like structure in the enamel; b, dentin.

fibers which he claims to have demonstrated after having dissolved the rest of the enamel in 5 per cent nitric acid. *Erler*[2] was of opinion that the lamellae-like striae were either artificial cracks or airfilled tubes forming an integral part of the enamel. The lamellae-like striae were not observed during enamel development in alligator[4] and they do not, therefore, form an integral part of the developmental pattern in the same way as *Bradford*[1] found in the enamel of *Crocodilus* and *Varanus*.

The lamellae-like striae were more numerous in sections from teeth which had been kept dry for a long time than from teeth which had been lying in water or fixation agent between shedding and sectioning. As dry enamel must be presumed to crack more easily than enamel soaked with moisture, this indicates that some of the lamellae-like striae are cracks formed after the shedding of the teeth. The lamellae-like structures which traverse the dentino-enamel junction and extend into the dentin (Fig. 5) are most probably cracks.

The teeth in the present material often lacked enamel at the

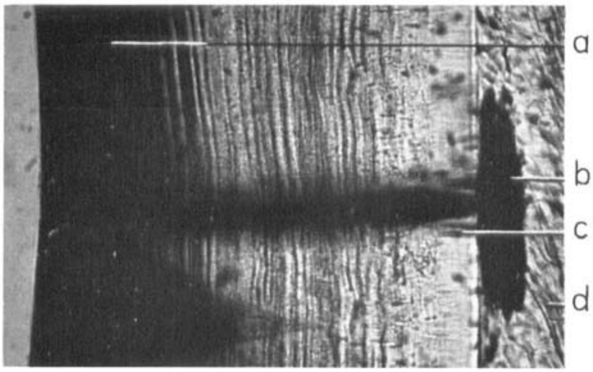

Fig. 9. Longitudinal ground section stained with silver nitrate. X 360. a, outer third of the enamel diffusely stained; b, stained lamella-like structure and spreading out of stain on the dentinal side of the dentino-enamel junction; c, retrograde staining of perpendicular fiber from dentino-enamel junction; d, dentin.

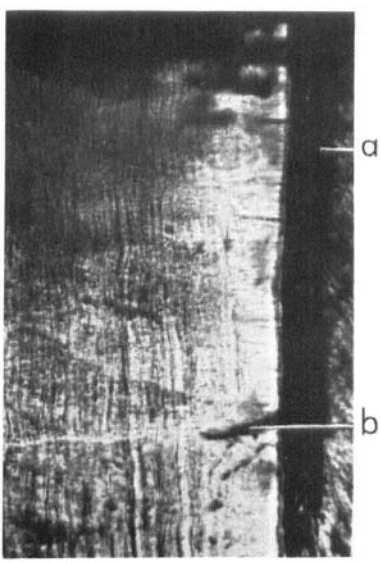

Fig. 10. Longitudinal ground section stained with silver nitrate. X 360. a, stain from lamella-like structure spreading out along the dentinal side of the dentino-enamel junction; b, retrograde staining of perpendicular enamel fiber.

tip which indicates a violent use of the functioning tooth. Such extreme force acting on a tooth may well produce cracks in the enamel which will show in the sections. The grinding of the specimens will also in most cases cause cracks even when carefully performed. There are thus so many possibilities for cracks

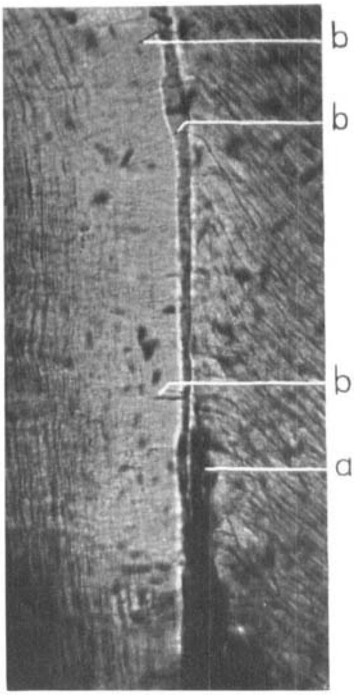

Fig. 11. Longitudinal ground section stained with silver nitrate. X 360. a, stain from lamella-like structure spreading out along both sides of the dentino-enamel junction; b, retrograde staining of perpendicular enamel fiber seemingly continuous with dentinal fibers.

in ground sections of alligator enamel that many of the lamellae-like striae must be presumed to be artifacts.

Schulte[10] is of opinion that dentinal fibers very frequently cross the dentino-enamel junction and end just inside the enamel. An apparent continuation of dentinal fibers into the enamel was often observed in the present material. Upon closer examination it always turned out to be terminal branches of dentinal fibers ending near the dentino-enamel junction which happened to be continuous with transversal fibers of the enamel retrogradely stained for a short distance (Fig. 11 b).

Schulte[10] claims to have demonstrated tufts (Schmelzfaserbüschel) in the innermost, frequently very transparent layer of the enamel. These structures are easily observed in polarized light, but can also be seen in ordinary light (Fig. 12 a). A close examination reveals that what *Schulte*[10] describes as tufts are the innermost part of the transverse fibers of the enamel which

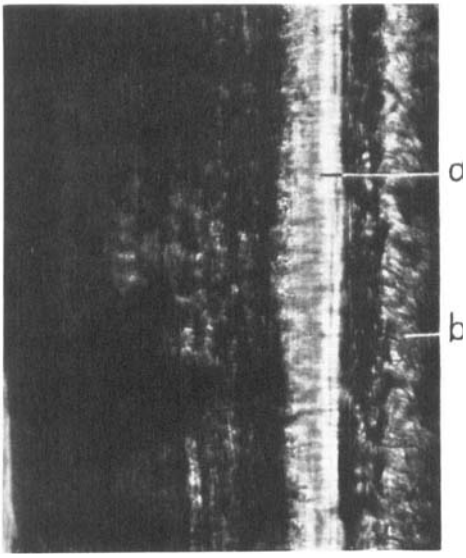

Fig. 12. Longitudinal ground section. X 360. a, transparent enamel close to dentino-enamel junction with faint perpendicular fibers; b, dentin.

are less distinct in the transparent inner layer, but which can be traced continually into the transverse fibers in the outer part of the enamel.

All earlier investigators agree that reptilian enamel is devoid of the prisms seen in mammalian enamel. The study of the present material corroborates this view.

ACKNOWLEDGEMENT

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SUMMARY

In the dental enamel of *Alligator mississippiensis* Daud. incremental lines surround the tip of the dentin, beginning and

ending at the dentino-enamel junction. Along the side of the tooth the incremental lines run obliquely from the dentino-enamel junction to the enamel surface nearer the tooth tip.

The enamel is traversed by fibers running perpendicularly from the dentino-enamel junction to the surface. In the same direction are seen lamellae-like striae.

RESUME

LES DENTS DE L'ALLIGATOR MISSISSIPPIENSIS DAUD. V. LA MORPHOLOGIE DE L'ÉMAIL

Dans l'émail dentaire de *l'Alligator mississippiensis* Daud., des lignes de croissance entourent l'extrémité de la dentine, commençant et se terminant à la jonction dentine-émail. Le long du côté de la dent, les lignes de croissance vont obliquement de la jonction dentine-émail à la surface de l'émail la plus proche de l'extrémité de la dent.

L'émail est traversé de fibres allant perpendiculairement de la jonction dentine-émail à la surface. Suivant la même direction on observe des stries ressemblant à des lamelles.

ZUSAMMENFASSUNG

DIE ZÄHNE VON ALLIGATOR MISSISSIPPIENSIS DAUD. V. DIE MORPHOLOGIE DES SCHMELZES

Den Schmelz auf dem Dentinhöcker der Zähne von *Alligator mississippiensis* Daud. durchziehen Wachstumslinien, die an der Schmelz-Dentingrenze beginnen und enden. Entlang der Kronenseite laufen die Wachstumslinien schräg von der Schmelz-Dentingrenze nach der freien Schmelzoberfläche näher die Zahnspitze.

Der Schmelz ist von Fasern durchzogen, die senkrecht von der Schmelz-Dentingrenze nach der Schmelzoberfläche gehen. In derselben Richtung verlaufen lamellenähnliche Streifen.

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