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A MORPHOLOGICAL STUDY OF THE SURFACE LAYER OF DOG TOOTH ENAMEL

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Dog tooth enamel has been studied by *Hals* (1945), *Schmidt & Keil* (1958), and *Gisela Hildebrandt* (1959). It appeared from the investigations in the last two publications that the superficial layer of enamel in this animal exhibited positive birefringence, whereas the main part of the enamel showed negative birefringence. Using polarization microscopy, the authors concluded that the surface of dog enamel was incompletely mineralized (*Schmidt & Keil* 1958), and that normal enamel could not easily be distinguished from carious enamel (*Hildebrandt* 1959).

The purpose of the present investigation was to make a further study of the morphology of normal dog enamel, especially with regard to the feasibility of using dog enamel in laboratory investigations of the carious lesion.

MATERIAL AND METHODS

Intact and fully erupted permanent teeth were obtained from six dogs of different age. After the jaws had been dissected out, the crowns of the teeth were separated from the roots by a cut below the cemento-enamel junction. One group of the teeth constituted the normal material. In the other group, the teeth were covered with wax except for a small area, and exposed to sugar-saliva mixtures at 37°C for 15 hours. Ground sections were made from different areas of the teeth of both groups. Both cross-sections and longitudinal sections were prepared. The sections were ground wet to a thickness varying from 60 to 80 microns, and both groups were examined in a Leitz polarization microscope after imbibition in distilled water. They were dehydrated in ascending alcohols to xylol and imbibed in Canada balsam as well as in Thoulet's solution with the same refractive index as Canada balsam (n=1.54). In addition, the sections were examined in polarized light in an air-dried or in a dehydrated state without imbibition. The same sections were also subjected to soft X-rays in a Philips microradiograph using a fine focus tube with Cu target. The exposures were made with kV 18—20, mA 20—30, exposure time 10—20 minutes. The distance from target to specimen was 20 cm. The images were recorded on Kodak Scientific Plates, Maximum Resolution.

EDTA treated teeth

The teeth were covered with Tenax wax except for a small area of the buccal surface which was exposed to Na_4 EDTA for 4 days and for 17 hours, respectively. The 4 days specimens were examined directly in a dissecting microscope. Ground sections were made from the teeth which had been exposed for 17 hours. The sections were subsequently viewed in the light microscope.

Replicas

Ground surfaces were made on intact teeth by cutting through the crown transversely with a carborundum disc. After polishing, the surfaces were treated with Na_4 EDTA for a few minutes. Replicas of the treated surfaces were produced by using the collodion strip method described by *Pantke* (1956). The replicas were mounted between slides in a dry state and examined in incident light.

Decalcified material

The material of this series was obtained from normal dog teeth, the crowns of which had been covered with acrylic splints. After the jaws had been fixed in 10 % neutral formalin and decalcified in 5.2 % nitric acid, they were cut in proper pieces and the acrylic splints dissolved by chloroform. The specimens were embedded in paraffin, and 5 microns thick serial sections were made. The sections were stained in hematoxylin and eosin.

OBSERVATIONS

1. *Ground sections of normal enamel*

A) *Polarization microscopy*

Sections imbibed in distilled water (n=1.33) showed an entirely negative birefringent surface layer. The prisms which could easily be followed in their curvy passage through the central part of the enamel, vanished towards the surface (Fig. 1). The enamel surface appeared more homogeneous than the adjacent layer of the enamel. When the same sections were imbibed in Thoulet's solution (n=1.54) without foregoing dehydration, no difference from the water imbibed sections could be observed with regard to the surface layer of the enamel (Fig. 2). On the other hand, it was apparent that the distinctness of the prisms of the middle part of the enamel was reduced to some extent. When the same sections were dehydrated through ascending alcohols before imbibition in Thoulet's solution, irregular areas of positive birefringence appeared in the subsurface layer (Fig. 3). These areas were interrupted by areas of negative birefringence or isotropy. A zone of isotropy regularly separated the positively birefringent area from the adjacent negatively double refractive enamel of the middle part. The course of the enamel prisms could be followed from the inner parts of the enamel to the surface. A similar picture was obtained when sections were dehydrated and imbibed in absolute alcohol (n=1.36). Sections that were dehydrated in alcohols and that went through xylol before imbibition in Canada balsam (Fig. 4), exhibited a picture which was identical to the one presented by the sections dehydrated and imbibed in Thoulet's solution.

B) *Microradiography*

The qualitative evaluation of the micro X-ray image revealed a fully homogeneous enamel (Fig. 5). No difference as to X-ray absorption could be discerned. Neither could any difference be observed in the absorptive quality of the prisms as compared with the interprismatic material.

Plate 1.

Figs. 1—4 are photomicrographs of a ground section of normal dog tooth enamel in polarized light after imbibition in the following media,

Fig. 1. Distilled water (n=1.33) without foregoing dehydration.

Fig. 2. Thoulet's solution (n=1.54) without foregoing dehydration.

Fig. 3. Thoulet's solution (n=1.54) after dehydration.

Fig. 4. Canada balsam (n=1.54).

Fig. 5. Microradiogram of the same sections as shown in Figs. 1—4.

Figs. 6—9 are polarization microscopic pictures of a ground section of dog tooth enamel exposed to sugar-saliva mixtures for 17 hours. The section was imbibed in the following media,

Fig. 6. Distilled water (n=1.33) without foregoing dehydration.

Fig. 7. Thoulet's solution (n=1.54) without foregoing dehydration.

Fig. 8. Thoulet's solution (n=1.54) after dehydration.

Fig. 9. Canada balsam (n=1.54).

Fig. 10. Microradiogram of the same section as shown in Figs. 6—9.

Fig. 11. Photomicrograph showing replica of ground surface of normal dog enamel. In the subsurface layer all prisms seem to concentrate in a direction perpendicular to the enamel surface (upper right corner).

Fig. 12. Enamel organa after decalcification. Hematoxylin and eosin. Organic network is basophilic. The amount of stained material and the basophilia increasing in the subsurface layer. A continuous membrane constitutes the peripheral surface.


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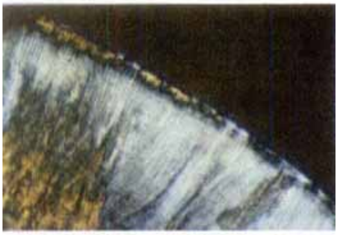
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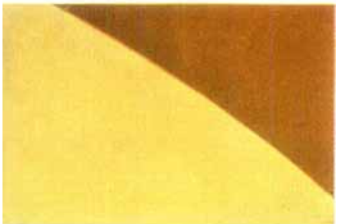
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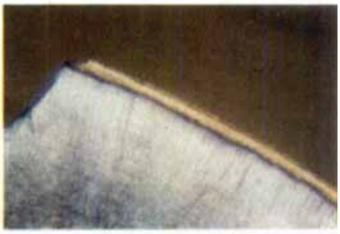
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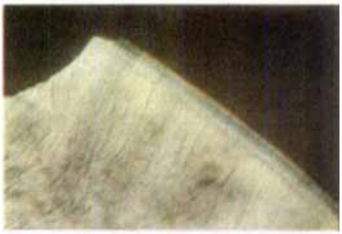
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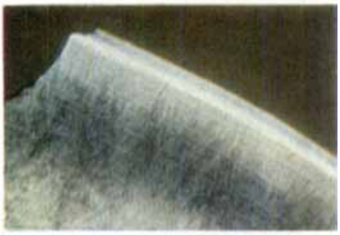
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2. *Ground sections of treated enamel*

A) *Polarization microscopy*

Sections which had been exposed to sugar-saliva mixtures for 17 hours, showed after imbibition in distilled water a positive birefringent zone located in the subsurface layer of the enamel (Fig. 6). In certain areas, this positive birefringence included the surface, although the main part of the enamel surface revealed negative birefringence. After imbibing the same sections in Thoulet's solution, the subsurface layer changed from positive birefringence to isotropy (Fig. 7). The negative birefringence of the narrow layer at the surface remained. This was, however, interrupted by smaller areas of isotropy.

Imbibition in Thoulet's solution subsequent to dehydration (Fig. 8) showed some reduction in the width of the isotropic subsurface layer when compared with sections that were not dehydrated. The superficial narrow layer exhibited an accentuated negative birefringence. In these sections the isotropic quality of the border against the normal enamel was changed to a zone of increased negative birefringence and the width of this zone was increased at the expense of both the positively and the adjacent negatively birefringent layers. In the sections imbibed in Canada balsam (Fig. 9), the zones described after dehydration and imbibition in Thoulet's solution were all over-accentuated. The surface showed a distinct negative birefringence. The subsurface layer was isotropic and the adjacent zone revealed an increased negative birefringence.

B) *Microradiography*

The microradiograms of the same sections showed a radio-opaque surface layer bordering on a radiolucent subsurface zone (Fig. 10). In the rest of the enamel — as far as this could be determined by a qualitative evaluation — no difference in the X-ray absorption could be discerned.

3. *Experimental cavities*

In the teeth which had been exposed to the tetrasodium salt of EDTA, a cavity to the dentino-enamel border was formed (Fig. 13). The cavity had regular right angle walls and corresponded entirely to the area not covered with wax. In particular, the mesial and distal walls showed a specific pattern. Bundles of prisms were observed in their wavy course from the dentino-enamel border towards the enamel surface. In the subsurface layer, these bundles coalesced in a homogeneous mass.

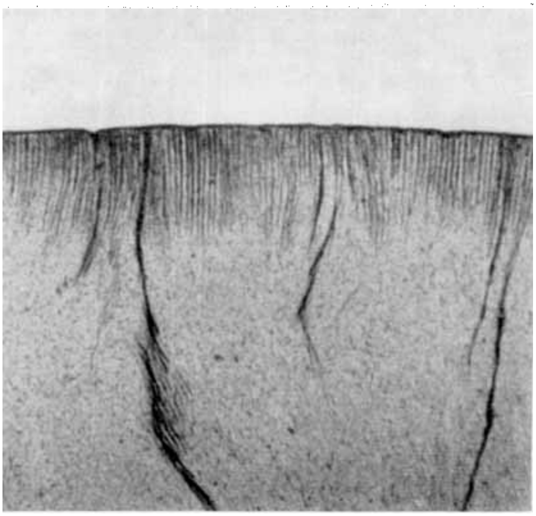

Fig. 13. Ordinary light microscopic picture of ground section of dog enamel treated with Na_4 EDTA, showing the direction of the prisms of the subsurface layer.

4. *Replicas*

The very same picture was found in the replicas of the ground surfaces (Fig. 11). Bundles of prisms could be followed from the central to the peripheral part of the enamel. In the subsurface layer, however, all prisms seemed to concentrate in a direction perpendicular to the enamel surface.

5. *Ground sections of Na_4 EDTA treated enamel*

In the light microscopic picture of sections of this series (Fig. 14), the direction of the prisms of the subsurface layer was made distinct by the darkness of the interprismatic material. The image of this layer left no doubt that the main direction of the prisms was perpendicular to the enamel surface.

6. *Decalcified material*

The remnants of the enamel after decalcification stained basophilically (Fig. 12). This basophilia increased from the subsurface layer on to the surface which appeared as a continuous membrane. The threads of the network showed a preferential

Fig. 14. Dog tooth exposed to Na_4 EDTA for 4 days. The mesial walls showing bundles of prisms in their wavy course. The subsurface layer appears more homogeneous.

arrangement fully in accordance with the picture already obtained of the direction of the enamel prisms, viz., a more or less wavy course through the main part of the enamel, and a concentration in the subsurface layer perpendicular to the enamel surface. The amount of stained material and the basophilia increased rather abruptly in the subsurface layer.

DISCUSSION

1. The degree of mineralization of the surface layer of enamel

According to *Schmidt & Keil* (1958), the subsurface layer of dog enamel is incompletely mineralized. This conclusion was recorded on the basis of the positive birefringence of this area when sections were viewed in the polarization microscope. The present series makes it clear that this positively birefringent zone does not show up after imbibition in distilled water (n=1.33) or in Thoulet's solution (n=1.54) without foregoing dehydration. On the other hand, when the same section was dehydrated and imbibed in absolute ethyl alcohol (n=1.36), the positive birefringent area appeared. The same picture was obtained after imbibition in Canada balsam (n=1.54). This goes to show that the appearance of a positive birefringence in the subsurface layer is entirely dependent on the treatment which has been given to sections before imbibition. A qualitative correlation existed between the polarization microscopic picture of water imbibed sections and the microradiographic image, viz., no difference as to the degree of mineralization of the subsurface layer as compared with the adjacent enamel. Microradiography is, probably, at present the most reliable method for the investigation of the mineral content of hard tissue (*Carlström* 1960). For this reason the conclusion may be drawn that the subsurface layer of dog enamel shows the same degree of mineralization as adjacent areas.

Enamel which was decalcified showed a greater amount of organic matter in the subsurface layer than the central enamel. In other words, the subsurface layer shows the same amount of mineral content, but a greater amount of organic material when compared with the adjacent enamel. This may explain the discrepancy between the polarization microscopic picture after dehydration and that obtained of other waterimbibed sections and the microradiograms.

2. The appearance of artificially produced carious lesions in the subsurface layer

The results obtained when artificially produced lesions were imbibed with different imbibition media, suggest that the subsurface layer is altered. In these cases, it was clear that this layer showed no difference when imbibed in a dehydrated or an undehydrated state. The microradiograms showed that a demineralization of the subsurface layer had taken place. The removal of the mineral salts from this area may explain why the subsurface layer could be imbibed after such treatment. The present series shows that even small differences between normal and demineralized subsurface layers of dog enamel can be registered. The lesions which were produced by exposing the teeth to sugar-saliva mixtures show essentially the same characteristics as does the human enamel under similar conditions.

3. The structural appearance of the subsurface layer

In the ordinary light microscopic picture and in the image of normal dog enamel in polarized light, each enamel prism can easily be traced in its wavy course from the dentino-enamel junction to the subsurface layer. Just below the enamel surface, however, the distinct borders between the prisms disappear and the subsurface layer takes a more homogeneous appearance. This was also confirmed by the appearance of the cavity walls after EDTA treatment. The pictures obtained of the replicas and of the ground sections of Na_4 EDTA treated enamel clearly demonstrate that the main direction of the prisms is not changed in the subsurface layer, but that the prisms assemble in a direction perpendicular to the enamel surface.

SUMMARY AND CONCLUSIONS

Normal and demineralized enamel of fully developed dog teeth were studied by ordinary microscopy, in polarized light, and by microradiography. It was shown that dehydration of the specimens causes the appearance of positive birefringence in the subsurface layer of the enamel. No difference in the degree of mineralization of the subsurface layer as compared with the adjacent enamel could be estimated from the microradiograms. The amount of organic material seemed to be greater in this layer than in the central part of the enamel. The difference between normal and demineralized enamel could easily be distinguished by means of polarized light and microradiography. In the subsurface layer the enamel prisms assemble in a direction perpendicular to the surface giving this layer a homogeneous appearance. It is, therefore, justified to conclude that dog enamel is well suited for experimental investigations.

RÉSUMÉ ET CONCLUSIONS

ÉTUDE MORPHOLOGIQUE DES COUCHES SUPERFICIELLES DE L'ÉMAIL DENTAIRE DU CHIEN

Il a été procédé a des examens d'émail normal et d'émail décalcifié de dents de chien entièrement évoluées, en microscopie ordinaire, en lumière polarisée et par microradiographies. Cette étude a montré que la déshydratation des specimens donnait une biréfringence positive dans la couche superficielle de l'émail. En se basant sur les microradiographies, il n'était pas possible d'évaluer de différence entre le degré de calcification de la couche superficielle et celui de l'émail adjacent. La quantité de matières organiques semble plus grande dans cette couche que dans la partie centrale de l'émail. La différence entre l'émail normal et l'émail décalcifié était facile à distinguer au moyen de la lumière polarisée et de microradiographies. Dans la couche superficielle, les prismes d'émail s'assemblent perpendiculairement à la surface, ce qui donne à cette couche un aspect homogène. On peut donc conclure que l'émail du chien convient bien pour les recherches expérimentales.

ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

EINE MORPHOLOGISCHE STUDIE ÜBER DIE OBERFLÄCHLICHEN SCHICHTEN DES SCHMELZES BEIM HUNDE

Normaler und entkalkter Schmelz von voll entwickelten Hundezähnen wurde lichtmikroskopisch, im Polarisationsmikroskop und mikroröntgenologisch untersucht. Es konnte gezeigt werden, dass Wasserentzug der Prüfkörper eine positive Doppelbrechung in den oberflächlichen Lagen des Schmelzes hervorruft. Es konnte durch mikroröntgenologische Untersuchungen kein Unterschied in dem Mineralisationsgrad der Oberfläche und der übrigen Schmelzschichten festgestellt werden. Die Menge an organischer Substanz scheint in dieser Lage grösser zu sein als in den tieferen Schichten. Der Unterschied zwischen normalem und entkalktem Schmelz konnte sowohl durch polarisiertes Licht als auch durch die mikroröntgenologische Untersuchung festgestellt werden. In der oberflächlichen Schicht des Schmelzes versammeln

sich die Prismen senkrecht zur Oberfläche. Hierdurch erhält diese Schicht ein besonders homogenes Aussehen. Es ist der Schmelz von Hundezähnen sehr gut für experimentelle Untersuchungen geeignet.

RESUMEN

ESTUDIO MORFOLÓGICO DE LA CAPA SUPERFICIAL DE ESMALTE DENTARIO DE PERRO

Se estudió el esmalte normal y descalcificado en dientes completamente desarrollados de perro, con el microscopio ordinario, con luz polarizada y con microradiografía. Se demostró que la deshidratación de las piezas confiere a la capa superficial del esmalte birefringencia positiva. Los microradiogramas no mostraron ninguna diferencia en el grado de mineralización de la capa superficial, comparándola con el esmalte subyacente. La cantidad de substancia orgánica parece ser mayor en esta capa que en la zona central del esmalte. La diferencia entre el esmalte normal y el desmineralizado puede apreciarse fácilmente por medio de la luz polarizada y la microradiografía. En la capa subsuperficial los prismas del esmalte se unen en una dirección perpendicular a la superficie dando a esta zona un aspecto homogéneo. Por estas razones se justifica la conclusión de que el esmalte de perro es favorable para ser utilizado en investigaciones experimentales.

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