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STUDIES ON MINERALIZED DENTAL TISSUES

IX. A microradiographic study of the mineralization of developing enamel.

by

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INTRODUCTION

The mineralization of enamel in the course of its development is considered by some investigators to be a single continuous process, the calcium salts being deposited gradually in the first enamel layers to be formed, at the same time as the building up of the new enamel matrix proceeds towards the outer enamel epithelium.

Such is the conclusion drawn by *von Ebner* (1906) who, by means of the polarizing microscope, observed in ground sections of human tooth germs that the optical properties of enamel change during its development. He distinguished four stages, the boundary between them following the incremental lines. His opinion was confirmed by *Harders-Steinhäuser* (1938) who used the same method, and by *Chase* (1935) who observed the staining properties of decalcified sections of tooth germs from man and various animals. *Nuckolls et al.* (1947) also maintain that the mineralization of the enamel matrix definitely progresses from the dentino-enamel junction towards the surface. This statement is based upon examination under the light microscope (*Saunders et al.* 1942, *Nuckolls et al.* 1943). *Hals* (1953) has reached similar conclusions in a recent study of the amelogenesis using polarized light.

There are, however, investigators who do not agree that the formation of the enamel matrix and its final mineralization occur concurrently. They see in the amelogenesis two distinct phases — the formation of the matrix and its subsequent mineralization, the latter not starting until the enamel has attained a definite thickness.

On molar tooth germs from pigs *von Beust* (1928) established that the enamel at first assumes a horny consistency and is slightly impregnated with inorganic matter. This horny matrix is subsequently calcified en masse. *May Mellanby* (1929), studying the influence of diet on the teeth of young dogs, found that the enamel calcifies in two well defined stages, which she termed first and secondary calcification. She made the same observations on human enamel (1934). The claim of a mass change following the completion of an organic matrix is supported by *Kitchin* (1933) in his studies of ground sections of the rat incisor in polarized light.

Diamond and *Weinmann* (1940), in a monograph on the human enamel, write: "The calcification process begins after the completion of the adult organic matrix at the highest points of the cusp eminence of the posterior tooth or the highest points of the incisal surfaces of anterior tooth, and proceeds from those points in the direction of the dentino-enamel junction, radiating laterally in all directions until the entire incisal or occlusal surfaces are fully calcified, and then continues along the peripheral surfaces in the direction of the cervical line, until the entire enamel is fully calcified. The direction of such calcification is in transverse relationship to the incremental lines". This opinion is further borne out by chemical analyses of enamel in various stages of development (*Weinmann et al.* 1942).

Studies of the *Mellanby* dog material by chemical analysis and the examination of ground sections by soft x-rays tend to confirm that the secondary calcification proceeds cervically from the tip of the tooth (*Glock et al.* 1942).

Applebaum (1943) found support for this theory in the results of a study with soft x-rays (1938) while *Marsland* (1952) came to the same conclusions in the light of his work on amelogenesis in the rat.

Most of the methods applied in the study of the transition of the enamel from a soft percursor state to the hardest tissue in the body allow only of indirect study of the mineral component. In the present investigation a direct method — microradiography — has been employed for determining the distribution of the calcium salts in the course of amelogenesis. The dental application of this method has been surveyed by *Bergman* and *Engfeldt* (1954).

MATERIAL AND METHOD

The material for this study comprised mandibles of full term infants. The mandibles were fixed in 10 per cent neutral buffered formalin (*Lillie,* 1948). Before examination radiographs were taken. One half of the jaw was left for examination by ordinary histologic methods. From the other half of the jaw ground sections were made. After dehydration and embedding in methyl methacrylate thin slabs were cut vertically (longitudinally or transverse), or horizontally. A rotating diamond saw was used. These "serial" slabs were then ground to 50—100 μ sections by the method evolved by *Hammarlund-Essler* (1955).

Microradiography with secondary magnification was performed using a Machlett OEG 50 x-ray tube energized with 20 kV fully rectified D.C. The radiation was filtered by a 0.2 millimetre beryllium window. The distance from the focus to the emulsion was 20 cm. The microradiograms were made on Eastman Kodak spectroscopic plates No. 649, which have a resolving power of approximately 1 μ.

FINDINGS

On the microradiograms the distribution of mineral salts and the degree of mineralization are immediately evident, the light parts being regions with a high x-ray absorption and hence a high content of calcium salts. In this paper the physiologic deposition of calcium salts in a tissue is called mineralization, and the term calcification should be reserved for pathologic conditions (*Westin* 1943).

The central incisors

The microradiograms of the central incisors show that all the incisal two thirds of the lingual enamel and one half of the labial enamel is highly and uniformly mineralized (Fig. 1). The cervical parts have a low mineral content except for a zone along the dentino-enamel junction. On the labial side, from the incisal edge to the middle of the crown, there is a band of low calcium salt content below the highly mineralized superficial enamel (Fig. 3).

The lateral incisors

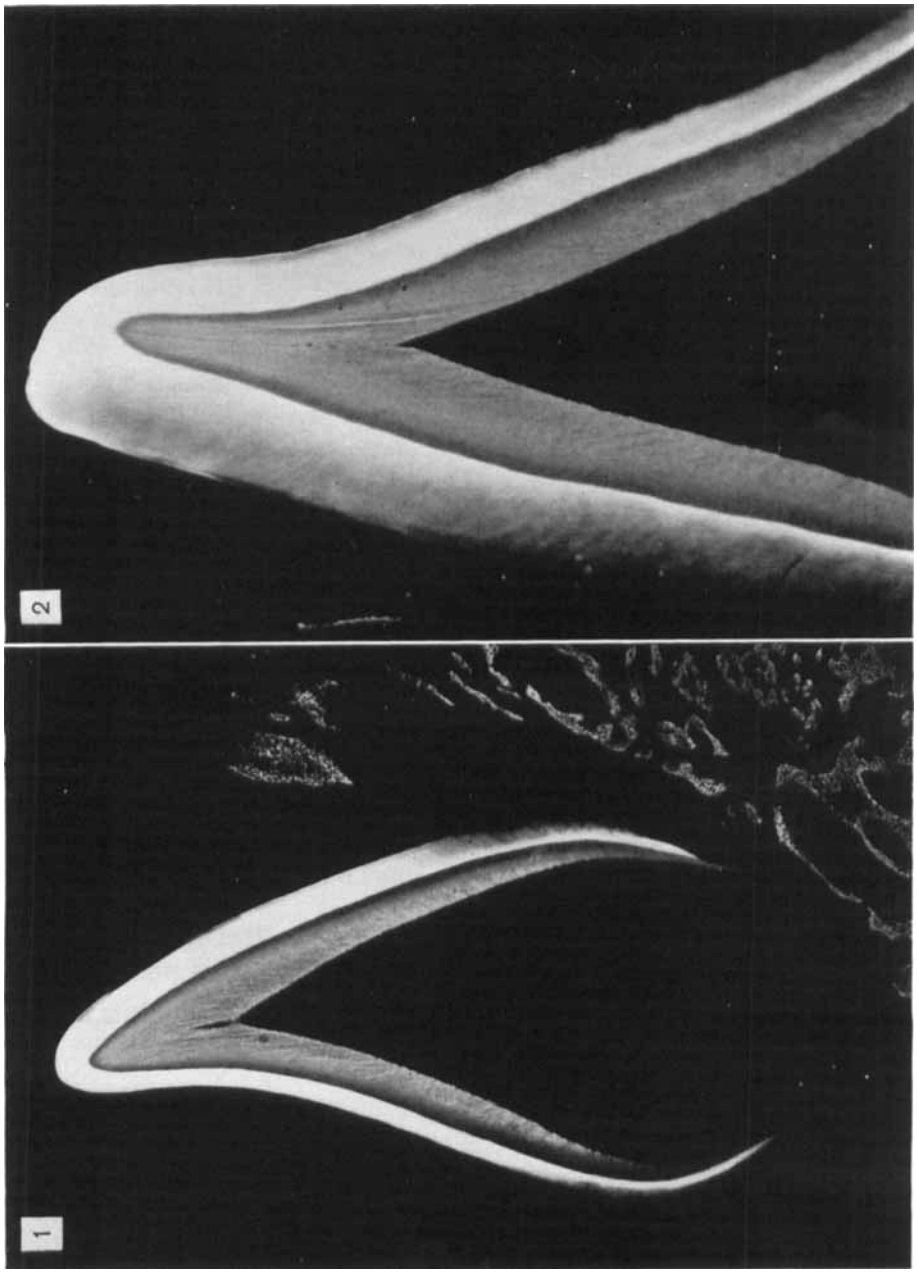
On the lateral incisors the incisal half of the lingual enamel is highly mineralized. Only in its extreme incisal part does the labial enamel evidence a high content of calcium salts (Fig. 2). With the exception of an innermost zone the rest of the labial enamel and the cervical half of the lingual enamel are seen to have a lower degree of mineralization. On the lingual side, starting near the incisal edge and continuing for one half the length of the crown there is a zone of low mineralization below the highly mineralized superficial enamel. There is a similar but shorter band on the labial side. The Hunter-Schreger bands are discernible in the labial enamel.

The canines

The highest degree of mineralization in the canines is in a zone nearest the dentino-enamel junction. Moreover, the mineralization is more advanced in the incisal than in the cervical enamel (Fig. 4). The higher mineralized incisal area extends a little further cervically on the lingual than on the labial side of the teeth.

Fig. 1. Microradiogram of 60 μ ground section through the central part of a central incisor. The mineralization of the lingual enamel is more advanced than that of the buccal. A thin zone with a low mineral content is visible inside the highly mineralized superficial buccal enamel, which is torn away in one place. On account of the curvature of the tooth the highly mineralized zone in the cervical part of the enamel appears wider than it actually is × 16.

Fig. 2. Microradiogram of a 60 μ ground section through the central part of a lateral incisor. One half of the lingual enamel shows a high mineralization except for a narrow zone below the highly mineralized superficial enamel. The mineralization of the buccal enamel is less advanced. The Hunter-Schreger bands are visible on the buccal side × 35.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The molars

The enamel of the cusps and marginal ridges of the first and second molars resembles that of the canines (Figs. 5—9). The occlusal regions of high mineralization are less extensive, however, in the second than in the first molar. In both the molars the regions of high mineralization are larger on the buccal than on the lingual cusps, and the mineralization is more advanced in the mesial than in the distal part.

General remarks

The pattern of mineralization described is visible in microradiograms of sections vertically through the central portion of the anterior teeth and of the cusps of the posterior teeth. Microradiograms of lateral and horizontal sections through various levels of the crowns show that the process of mineralization is less advanced in the lateral regions of the teeth (Figs. 4, 10).

In the enamel of all the teeth the highly mineralized areas pass gradually into areas of lower calcium salt content, with the exception of the highly mineralized zone along the dentino-enamel junction where the difference is more pronounced. In this innermost zone of high mineralization the enamel tufts are visible (Fig. 11).

DISCUSSION AND CONCLUSIONS

At birth the deciduous teeth are in various stages of development. The formation of the crowns of the incisors is practically complete. The incisal two-thirds of the canines and the occlusal

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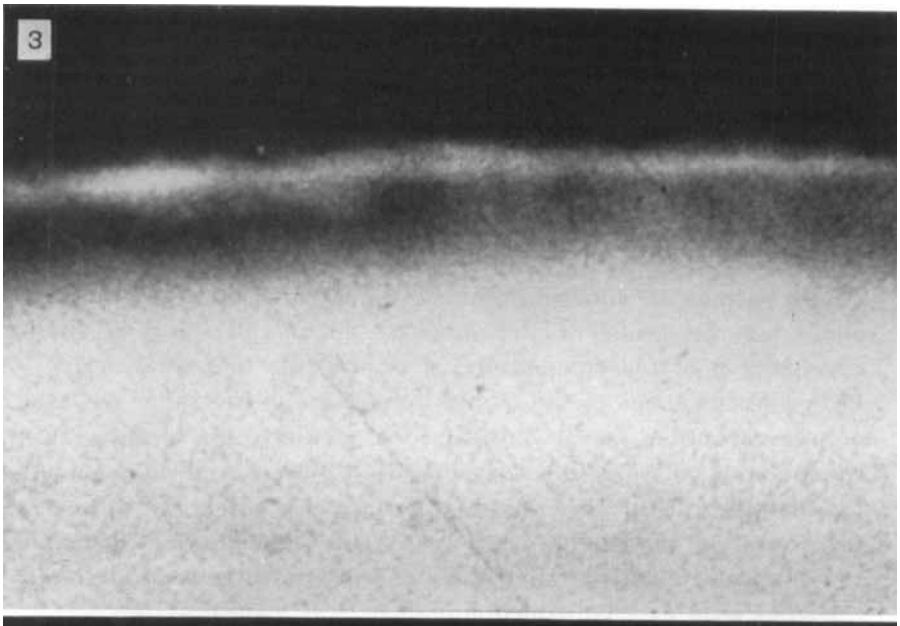
Fig. 3. Microradiogram, enlargement of detail of fig. 1. The zone of low mineralization below the highly mineralized superficial enamel. × 375.

Fig. 4. Microradiogram of a 50 μ ground section laterally through a lateral incisor and through the middle of the canine. Compared with fig. 2 the mineralization of this part of the lateral incisor is less advanced. The enamel of the canine, to the right, has a higly mineralized innermost zone and the advanced mineralization of the incisal part reaches a little further cervically on the lingual than on the buccal side. × 20.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parts of the first molars are built up, while the second molars are a little later in development. Specimens of jaws of newborn infants thus provide a good opportunity for studying the various developmental phases of the enamel. The material is suitable also for following the course of mineralization, in virtue of the rare disturbance of mineralization during foetal life, and the very regular formation of the prenatal enamel. Accentuated incremental lines — striae of Retzius — very seldom appear, *Schour* (1953).

The pattern of mineralization of the various teeth described above may be considered representing a series of stages in the development of the enamel, and it is probable that all anterior teeth and the cusps of the molars pass through the same course of mineralization. On account of their anatomy the picture for the molars as a whole must be somewhat more complicated than for the other teeth. Thus the microradiograms reveal the following course of the mineralization: — The enamel layer nearest the dentino-enamel junction assumes a high degree of mineralization as soon as it is formed, a feature first noticed by *Harders-Steinhäuser* (1938). The presence of a highly mineralized inner zone has been confirmed by *Glock et al.* (1942), *Saunders et al.* (1942), *Applebaum* (1943), and *Hals* (1953). This zone is, however, not uniformly mineralized, there being regions of little, if any, mineralization — the enamel tufts (Fig. 11).

In the enamel matrix formed outside the innermost zone, the calcium salt content increases much more slowly. The increase is first observable at the extreme incisal or occlusal parts of the dentino-enamel junction. It then extends to the incisal edge or the occlusal surface, being somewhat faster on the lingual side, at

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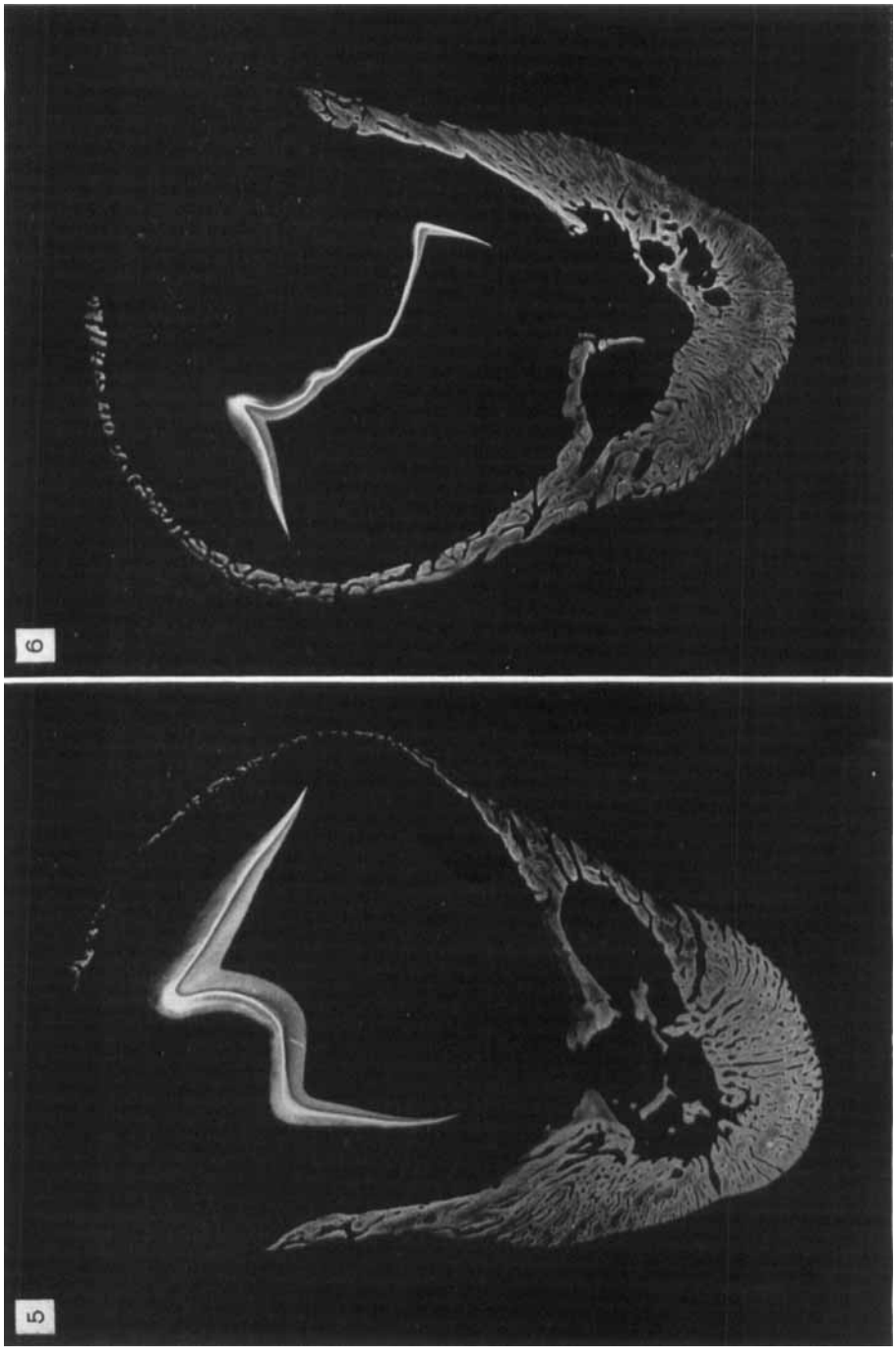
Fig. 5. Microradiogram of a 100 μ ground section through the mesiobuccal and mesiolingual cusps of the first molar. The pattern of mineralization is most evident in the mesiobuccal cusp of which the section is taken from the centre. The mesiolingual cusp is cut obliquely. Hence the structures are seen superimposed. × 10.

Fig. 6. Microradiogram of a 100 μ ground section through the distobuccal and distolingual cusps of a first molar. The advanced mineralization of the distobuccal cusp is visible. The section of the distolingual cusp is cut laterally, where only the innermost enamel is highly mineralized. × 10.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the same time as it spreads laterally. The lingual side is therefore leading in the process of mineralization and the lead is accentuated as the final mineralization continues towards the cervix of the tooth.

The course of the final mineralization is thus independent of the incremental lines. This is in contradiction to the opinion that the transformation from a soft to a hard mature enamel occurs parallel to these lines. However, the findings of this investigation support the view that the final mineralization starts at the dentino-enamel junction.

The course of mineralization revealed in the microradiograms differs also from the diagrammatic illustration of the "two phase" maturation process in the textbooks of *Orban* (1953) and *Schour* (1953), i.e. modifications of the original in the book by *Diamond* and *Weinmann* (1940). They state that the maturation process advances from the incisal edge or the cusps at right angles to the long axis of the tooth; they correlate the pattern of maturation to that of tooth eruption.

It is also evident from the microradiograms that the mineralization of the superficial enamel is in advance of that of the subjacent enamel. In erupted permanent teeth *Gustafson* (1955) often observed a hypomineralized zone below a highly mineralized enamel surface. Here, there is probably a persistence of this stage of mineralization.

The microradiograms show that there is no sharp boundary between the regions of high and low mineralization. This calls to mind the progressive transformation of the organic framework in the developing enamel described by *Sognnaes et al.* (1952).

In the second deciduous molar there is evidence of an advanced stage of mineralization before the full thickness of the enamel matrix is attained. According to *Schour* (1936), no part of the cusp enamel of the second deciduous molar has reached its full height at birth. Thus, the observations of the present study do not support the opinion that a definite thickness of the enamel matrix must be laid down before a further deposition of calcium salts can take place.

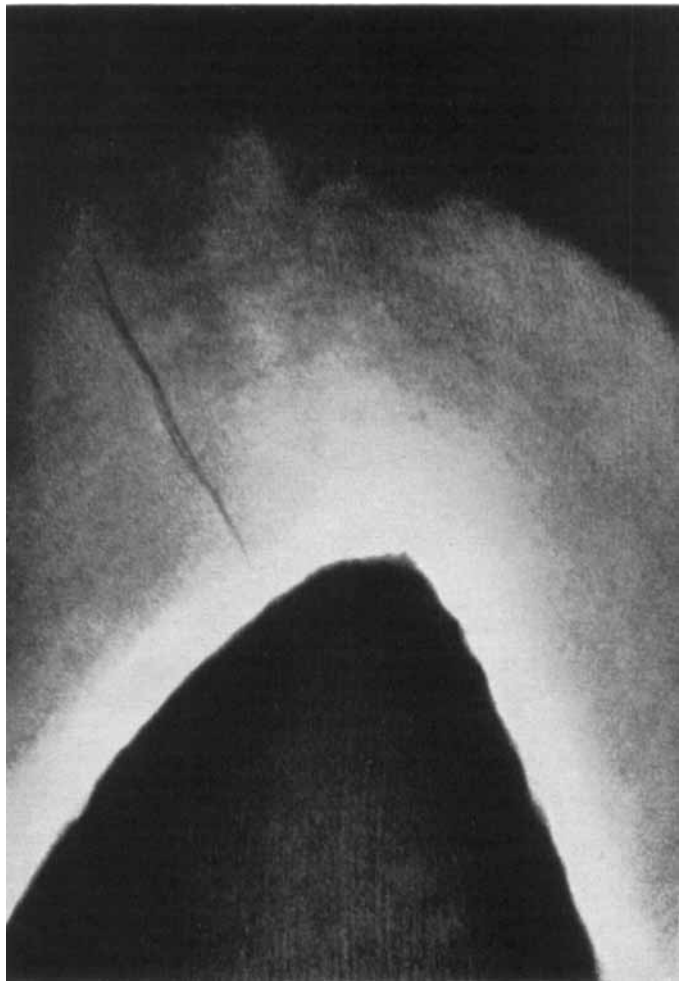
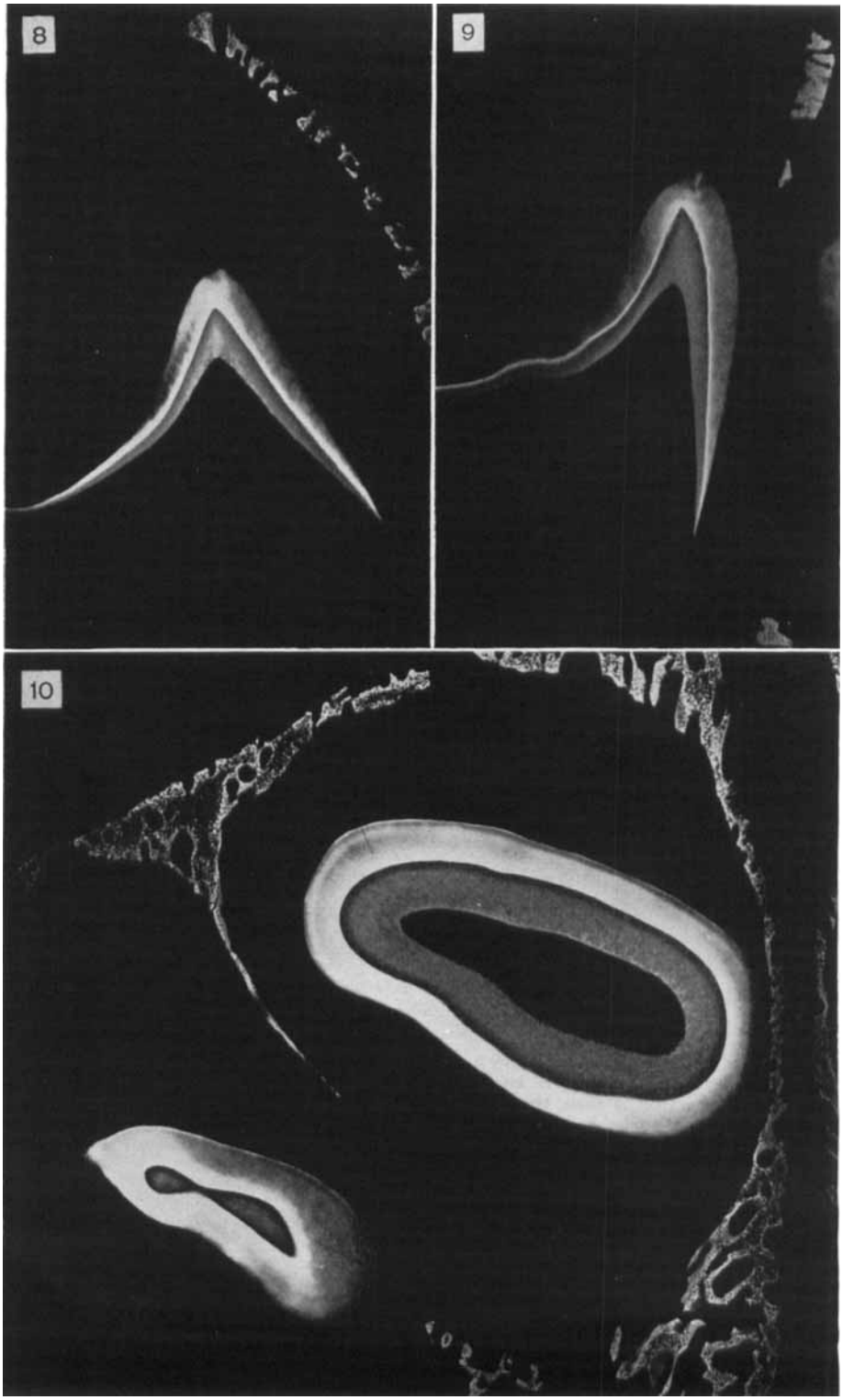


Fig. 7.

Fig. 7. Microradiogram of a 90 μ ground section through the distal marginal ridge of a first molar. A zone of high mineralization along the dentino-enamel junction is seen, this zone being widest in the occlusal part. Enamel prisms and dentinal tubules are visible. × 200.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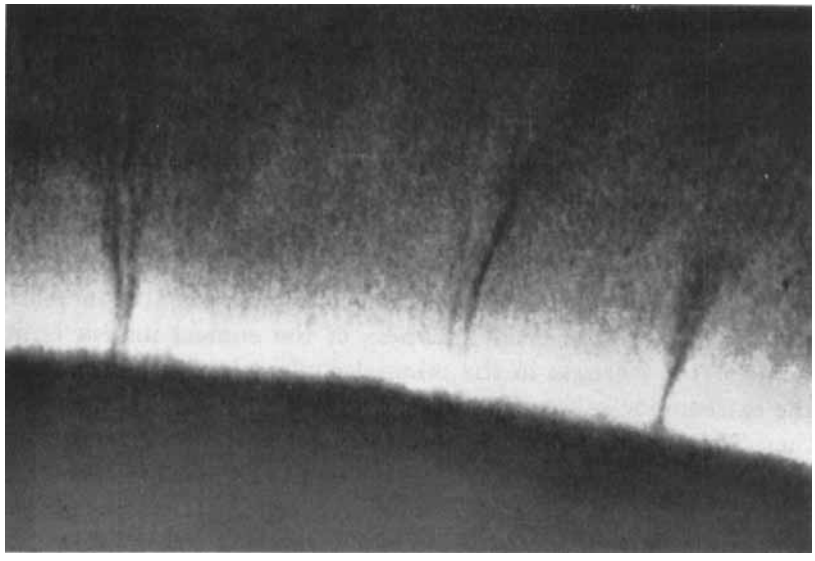

Fig. 11.

Fig. 8. Microradiogram of a 50 μ ground section through the centrobuccal cusp of a second molar. There is advanced mineralization of the enamel in the cusp tip. × 20.

Fig. 9. Microradiogram of a 55 μ ground section through the mesial marginal ridge of a second molar. Advanced mineralization of the occlusal enamel is evident. Incremental lines are visible in the mesial enamel. × 16

Fig. 10. Microradiogram of a 60 μ ground section cut horizontally through the middle of the crown of a central incisor and further incisally in the lateral incisor, which is cut obliquely. In both teeth the mineralization is more advanced in the lingual than in the buccal enamel. A narrow low mineralization zone is clearly visible below the superficial buccal enamel of the central incisor. × 24.

Fig. 11. Microradiogram of a 90 μ ground section horizontally through the cervical part of a lateral incisor. The enamel tufts are seen to pass through the highly mineralized inner zone of the enamel. × 475.

SUMMARY

Thin serial ground sections of deciduous tooth germs from the mandibles of new-born infants were examined by microradiography. Various stages of the development of the enamel were studied.

It was found that the enamel nearest the dentine rapidly attains a high mineral content. In the enamel that is subsequently formed the deposition of calcium salts occurs more slowly. The degree of mineralization in the inner enamel seems to increase, however, before the final thickness of the enamel matrix is attained. This increase in the mineral content is first observed at the extreme incisal or occlusal parts of the dentino-enamel junction. The mineralization continues faster on the lingual than on the buccal side, and consequently the course of the final mineralization of the enamel is neither parallel nor perpendicular to the incremental lines.

It was also found that the mineral content increases to a high level in the external enamel, before the underlying enamel has reached the same stage of mineralization.

RÉSUMÉ

ÉTUDES SUR LES TISSUS DENTAIRES MINÉRALISÉS

IX. Étude microradiographique de la minéralisation de l'émail en cours de développement

Une série de coupes minces par usure de germes temporaires provenant de mâchoires de nouveaux-nés ont été examinées à la microradiographie. Divers stades de développement de l'émail ont été étudiés.

Il est apparu que l'émail situé à proximité de la dentine acquiert rapidement un contenu minéral élevé. Dans l'émail formé par la suite, le dépôt de sels de calcium se produit plus lentement. Le degré de minéralisation de l'émail interne semble toutefois augmenter avant que l'épaisseur totale de l'émail soit atteinte. Cet accroissement du contenu minéral s'observe tout d'abord dans les parties insisales ou occlusales de la limite dentino-

adamantine. La minéralisation se produit plus rapidement du côté lingual que de côté buccal: en conséquence l'orientation générale de l'émail complètement minéralisé n'est ni parallèle ni perpendiculaire aux stries de Retzius. On a pu constater ègalement que le contenu minéral augmente considérablement à la surface de l'émail avant que l'émail sous-jacent ait atteint le meme degré de minéralisation.

ZUSAMMENFASSUNG

STUDIEN AN MINERALISIERTEN ZAHNGEWEBEN

IX. Eine mikroradiographische Untersuchung der Mineralisation während der Schmelzentwicklung

Dünne Serienschliffe von Milchzahnanlagen des Unterkiefers neugeborener Kinder wurden mit Hilfe der Mikroradiographie untersucht. Verschiedene Stadien der Schmelzentwicklung wurden untersucht, wobei der folgende Verlauf der endgültigen Mineralisierung des Schmelzes festgestellt werden konnte. Der Schmelz, der sich in unmittelbarer Nähe des Dentines bildet, erreicht schnell einen hohen Mineralgehalt. Die Einlagerung von Kalksalzen in den Schmelz, der danach gebildet wird, geschieht langsamer. Der Mineralisierungsgrad in den inneren Schmelzschichten scheint jedoch zuzunehmen, ehe die definitive Dicke des Schmelzes erreicht ist. Diese Zunahme des Mineralgehaltes kann zuerst über dem höchsten Punkt der Schmelz-Dentingrenze beobachtet werden. Die Mineralisierung schreitet danach schneller auf der lingualen Seite fort als auf der buccalen. Darauf folgt, dass die endgültige Mineralisierung des Schmelzes weder parallel mit den Retziusschen Streifen noch senkrecht zu diesen verläuft. Es konnte auch festgestellt werden, dass der Mineralgehalt in der Oberfläche des Schmelzes zu einem hohen Wert ansteigt, ehe der am nächsten darunterliegende Schmelz den gleichen Mineralisierungsgrad erreicht.

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