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STUDIES ON MINERALIZED DENTAL TISSUES

XII. MICRORADIOGRAPHIC STUDY OF CARIES IN DECIDUOUS TEETH

by

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Caries in the deciduous teeth has been considered to have special characteristics, owing to part of the crowns being formed after birth, this part being regarded of poorer quality. The borderline between prenatal and postnatally formed hard tissue is marked by the so-called neonatal line, first described by *Rushton* (1933, 1939) and later by *Schour* (1936). In ground sections, examined by transmitted light, it is seen in the enamel as a dark line, but is more difficult to identify in the dentine.

According to *Harndt* (1950), a carious lesion of the enamel may be temporarily arrested at this line, owing to "die bessere intrauterine Mineralisation". The same author states that circular caries in the deciduous front teeth can be explained by the cervical, postnatal enamel being more poorly mineralized than the incisal, prenatal enamel. He based this view on clinical observations and on histologic examination of ground sections.

In the present investigation, we focused particular attention on the microradiographic appearance of caries in the pre- and postnatal enamel, but we also studied the carious dentine, in which the incremental lines are amendable to microradiographic analysis. The potentialities of this technique in the study of caries have recently been illustrated by *Bergman & Engfeldt* (1954).

The stratified structure of the enamel and dentine — incremental stratification — has long been known. Thus, as early as 1836, brown, parallel streaks in the enamel were described by *Retzius* (lines of Retzius), and in 1840 corresponding lines in the dentine were described by *Owen* (Owen's contour lines). The mineralization pattern of the teeth became, however, more comprehensively elucidated by publication of the work of *Rushton* and of *Schour* and his co-workers.

Schour & Hoffman (1939) stated that the incremental lines normally have a very regular appearance in both enamel and dentine. Each incremental pair consists of a light and a dark line, each pair being 16 μ wide. In certain disturbances during development of the teeth, accentuation of the incremental lines may occur. The neonatal line can presumably be regarded as an accentuated line of Retzius.

In an extensive paper *Massler, Schour & Poncher* (1941) described how various developmental stages in the child are reflected in the calcification pattern of the enamel and dentine. During the infancy period, *i.e.*, from birth to about 10 months of age, slight disturbances in calcification are particularly apt to occur in the dental tissues, in the form of accentuation of the incremental lines, interglobular dentine and hypoplastic defects of the enamel.

PRESENT INVESTIGATION

Material and Methods

The material consisted of 80 deciduous teeth with carious lesions at different stages. Many of them had two or more such lesions. The teeth, which immediately after extraction had been fixed in neutral buffered formalin according to *Lillie* (1954), were dehydrated and embedded in plastic. One or two ground sections through the centre of the carious lesion, in the longitudinal direction of the tooth, were then prepared from each tooth. The sections were 80—100 μ thick.

Microradiographic examination was performed according to the method of *Engström & Wegstedt* (1951). Its application in dental studies was described by *Bergman & Engfeldt* (1954).

A Philips diffraction apparatus, type PW 1009, was used for microradiography. The tube had a copper anode and beryllium

window, the radiation being filtered through a nickel filter. The voltage was 20 kV and the current intensity 20 mA. The target-to-film distance was 25 cm, and the microradiograms were taken on no. 649 Eastman Kodak spectroscopic plates. They were enlarged by microphotography on Kodak 0 250 plates. On the microradiograms, the light areas represent high x-ray absorption (high mineral content) in the ground sections.

Results

Enamel

In most cases, the carious lesions had the same microradiographic appearance as that in permanent teeth. In cases of incipient caries, the surface of the enamel invariably had higher x-ray absorption (higher mineral content) than the underlying, partly demineralized enamel (Figs. 1b, 2b and 3b). Accentuation of the lines of Retzius and of the cross-striation of the prisms were also observed.

In a few cases of more advanced caries, distinct zones were visible in the enamel; they were reminiscent of those observed by *Gustafson* (1957) at polarization-microscopic examination (Fig. 5b).

Only two cases were encountered in which the carious lesions seemed to have been modified at the neonatal line (Figs. 3a, 3b and 4).

In ground sections, studied by transmitted light, the neonatal line was seen as a dark streak in the healthy enamel. In most cases, a dark line was also visible on the microradiogram, *i.e.*, the neonatal line had a lower mineral content than the enamel on either side of it. On visual evaluation, it was not possible to establish any definite difference between the mineral content of the prenatal and postnatal enamel.

In those cases in which the neonatal line could be traced into the carious lesion, a situation of the type illustrated in Figs. 6a and 6b was often observed. Thus, on the microradiogram of the carious enamel, a light line (higher mineral content) was seen to be bordered by two dark lines (Fig. 6b). The positional relation of the three lines to the neonatal line was hard to determine. This could, however, be done in one case. In this case, the light line lay on the dentinal side of the neonatal line (Fig. 4).

Dentine

On microradiograms of healthy dentine, the neonatal line appeared as a dark streak. Thus, as in the enamel, the mineral content of the neonatal line was lower than that of the adjacent parts. The incremental pattern of the dentine was particularly distinct on microradiograms of carious dentine, in which the incremental lines were often assembled in groups (Figs. 7b, 8b and 9b). In ground sections studied by transmitted light, the lines were, on the contrary, more difficult to identify (Figs. 7a, 8a and 9a). In none of the cases could the neonatal line be shown to have had any modifying effect on the carious lesion in the dentine.

It was evident from the present material, as from earlier investigations of permanent teeth, that a conventional microscopic study of ground sections does not give any reliable information about the mineral content in carious areas of the dentine. It is true that translucent areas often have a higher mineral content

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Fig. 1 a. Unstained ground section, 100 μ thick, showing approximal caries in upper first deciduous molar, seen by transmitted light. The surface is covered by a dental plaque. The tip of the carious lesion extends just beyond the neonatal line, which is vertical on the picture. ×100.

Fig. 1 b. Microradiogram of ground section in Fig. 1 a. The outer layer of the enamel has a high mineral content; the inner parts of the carious enamel are largely demineralized. The lesion seems to be unaffected at the neonatal line. ×100.

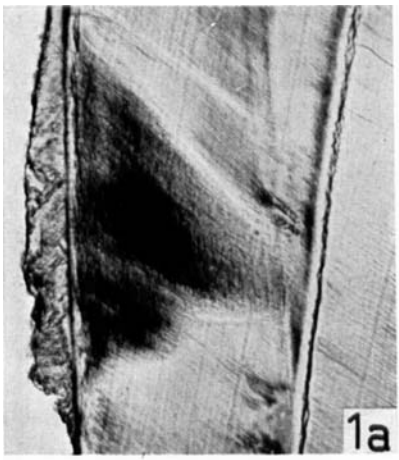
Fig. 2 a. Unstained ground section, 100 μ thick, showing approximal caries in lower second deciduous molar, seen by transmitted light. Below the surface, which is covered by a dental plaque, the carious lesion extends beyond the neonatal line. ×50.

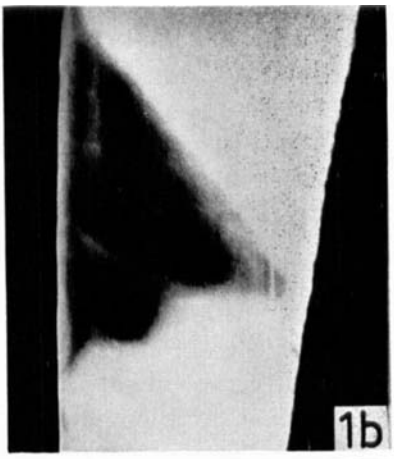
Fig. 2 b. Microradiogram of ground section in Fig. 2 a. Below the highly mineralized outer layer, a large proportion of the mineral salts have been dissolved from the deeper parts of the enamel. A group of lines with an alternately high and low mineral content is seen in the part of the carious enamel corresponding to the neonatal line. ×50.

Fig. 3 a. Unstained ground section, 80 μ thick, showing superficial caries in upper lateral incisor, seen by polarized light. The carious lesion is "flattened" at the neonatal line. ×25.

Fig. 3 b. Microradiogram of ground section in Fig. 3 a. The greatly demineralized area of the enamel is rather small. Close to the neonatal line is seen a slight decrease in x-ray absorption. ×25.

Fig. 4. Microradiogram of 90 μ thick ground section of upper second deciduous molar. Retrograde caries, extending to the neonatal line. The latter is visible as a dark band which, on the right, approaches the dentine. On the dentinal side of the dark line is seen a thin, light line (higher mineral content) in the carious enamel. ×25.


1a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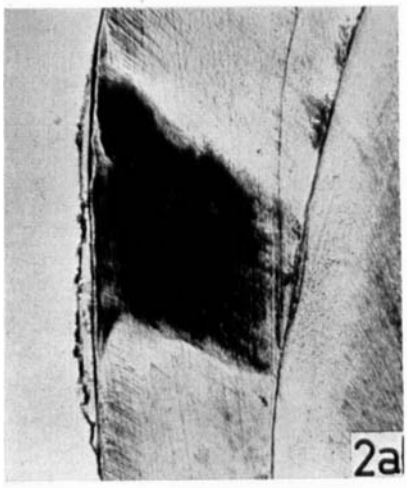
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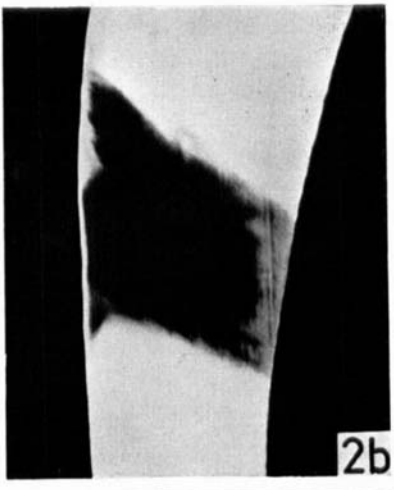
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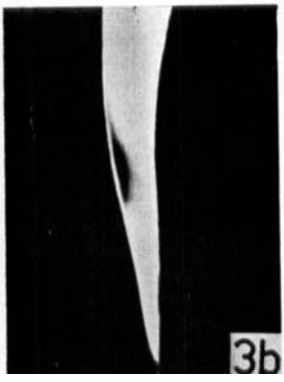
3a


3b


4

than the surroundings (Figs. 7a and b), but the reverse may also apply (Figs. 10a and b).

As a complement, healthy dentine from an area in the vicinity of the pulp is illustrated in Fig. 11. The dentinal tubules have a caliber of about 2 μ, which is in fairly good agreement with the corresponding caliber in young permanent teeth (*Bergman & Engfeldt* 1955).

DISCUSSION

It was mentioned in the introduction that *Harndt* (1950) had observed cases in which a carious lesion of the enamel became temporarily arrested at the neonatal line, owing to "better mineralization" of the prenatal enamel. Judging by the present material, it is questionable whether there is any difference between the mineral content of the prenatal and postnatal enamel, and the nature of the carious lesion is not usually affected at the neonatal line.

Fig. 5 a. Unstained ground section, 100 μ thick, showing an approximal carious lesion in a lower second deciduous molar, seen by transmitted light. The carious lesion involves both the prenatal and postnatal enamel, and extends into the dentine. The neonatal line is only indistinctly visible. ×30.

Fig. 5 b. Microradiogram of ground section in Fig. 5 a. The highly mineralized outer layer of the enamel is broken through, and the bottom of the cavity consists of enamel with low x-ray absorption (low mineral content). Beyond it, zones with alternating high and low absorption. The dark, straight line close to the upper part of the cavity is due to a crack in the ground section. ×30.

Fig. 6 a. Unstained ground section, 100 μ thick, showing retrograde caries in a lower first deciduous molar, seen by transmitted light. A light line runs diagonally across the picture, at the borderline between prenatal and postnatal enamel (the latter in upper right half of picture). Top left, healthy enamel; the rest of the picture shows carious enamel. ×240.

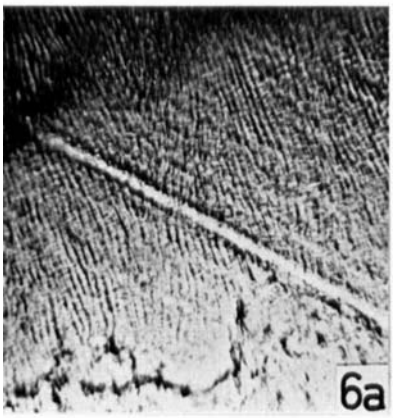
Fig. 6 b. Microradiogram of ground section in Fig. 6 a. The carious enamel is seen as a dark area with low x-ray absorption (low mineral content). A line with a higher mineral content than that of the adjacent carious enamel runs obliquely across the latter. The light line corresponds to the light line in Fig. 6 a, and passes without any sharp transition into the healthy enamel, which shows high x-ray absorption. ×240.

Fig. 7 a. Unstained ground section, 90 μ thick, seen by transmitted light. It shows the borderline zone between healthy and carious dentine in occlusal caries of lower second deciduous molar. The incremental lines are visible on the right in the carious dentine. ×110.

Fig. 7 b. Microradiogram of ground section in Fig. 7 a. The healthy dentine has high x-ray absorption, and is seen as a light area on the left. In the carious dentine, the incremental lines are visible as bands with a varying mineral content. ×110.

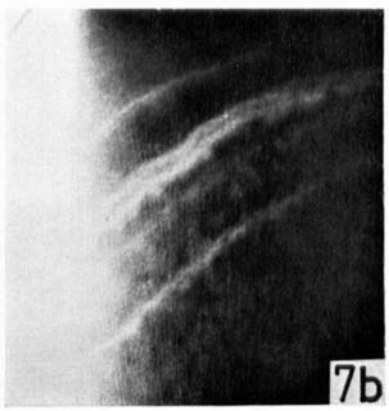

5a


5b


6a


6b


7a


7b

Of our two cases in which a modification of the carious lesion at the neonatal line appeared probable, one was "ordinary" caries (Fig. 3), and the other retrograde caries (Fig. 4). If differences in the degree of mineralization of the pre- and postnatal enamel were given as the explanation, the prenatal enamel would have had a higher mineral content in the former case, and the postnatal enamel in the latter. *A priori* this appears unlikely and the microradiograms gave no support to such a view. The possibility cannot, however, be ruled out that some property of the neonatal line itself or of its immediate surroundings may, in isolated cases, exert a modifying effect. It must nevertheless be borne in mind that no definite proof exists that an increase in the mineral content of the enamel is associated with any greater resistance to caries. Furthermore, the expression "better intrauterine mineralization" is misleading, in view of the fact that the prenatal enamel is mineralized to a great extent after birth.

The accentuated lines in the carious enamel in Figs. 6a and

Fig. 8 a. Unstained ground section, 90 μ thick, seen by transmitted light. It shows the borderline zone between healthy and carious dentine in deep approximal caries of upper first deciduous molar. At the top enamel with a distinct neonatal line. ×50.

Fig. 8 b. Microradiogram of ground section in Fig. 8 a. The healthy dentine is seen as a light area on the left. In the carious dentine (right), the incremental lines are visible either singly (top) or assembled in groups (further down). ×50.

Fig. 9 a. Unstained ground section, 90 μ thick, seen by transmitted light. It shows carious dentine in extensive occlusal caries of lower second deciduous molar. To the extreme left, a carious cavity. Right a small area of healthy dentine. ×30.

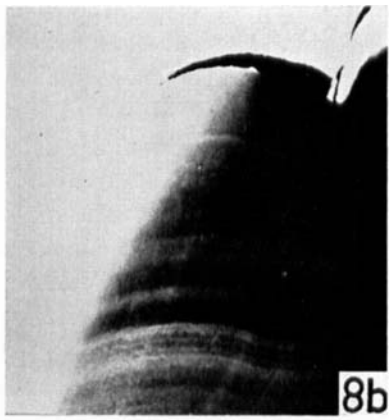
Fig. 9 b. Microradiogram of ground section in Fig. 9 a. Numerous, close incremental lines with a higher mineral content than the immediate surroundings are present in the carious dentine, which occupies the greater part of the picture. The cavity on the left is devoid of mineral salts, and causes a break in the incremental lines. ×30.

Fig. 10 a. Unstained ground section, 100 μ thick, seen by transmitted light. It shows advanced caries in the dentine of an upper second deciduous molar. Light and dark areas alternate in the dentine. No incremental lines can be identified. ×110.

Fig. 10 b. Microradiogram of ground section in Fig. 10 a. Greatly reduced x-ray absorption (dark areas) in large parts of the dentine, owing to dissolution of calcium salts by caries. In the vicinity of the cavity in the centre of the picture, softening of the dentine has caused bulging of the course of the dentinal tubules. No incremental lines are visible. ×110.

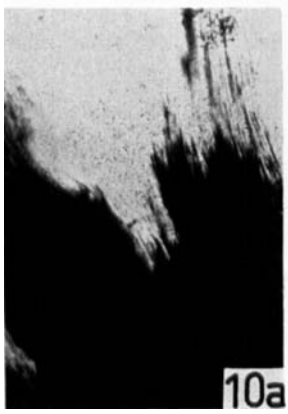
Fig. 11. Microradiogram of a 90 μ thick ground section, showing transversely cut dentinal tubules in healthy dentine close to the pulp in an upper canine. The caliber of the dentinal tubules is about 2 μ ×400.


8a

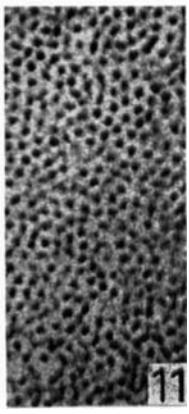

8b


9a


9b


10a


10b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6b are undoubtedly a kind of unmasking of the lines of Retzius. It is also apparent that they have an intimate relation to the neonatal line. We were not, however, able to determine with absolute certainty which of the three lines formed a direct continuation of the neonatal line which, in the healthy enamel, has a lower mineral content than the surroundings. The possibility cannot be completely excluded that, as *Hals* (1953) suggested, the neonatal line may consist of a group of parallel lines, and that only one of them is visible on microradiograms of healthy enamel, whereas the whole group of lines can be identified in carious enamel.

It is presumably unquestionable that the lines with an alternately high and low mineral content that are visible in the carious dentine are incremental lines, which reflect the rhythm in dentine formation (see microradiograms in Figs. 7b—9b).

In a careful study of the microradiograms under the microscope, it was possible to trace most of the incremental lines from the carious into the healthy dentine. This shows that the lines were present before development of the carious lesion, which had only caused them to become more prominent. The irregular distribution of the lines indicates that great variations exist in the developmental rhythm. Moreover, considerable individual differences were present. The lines occurred chiefly in the part of the dentine formed during the period from birth up to about 10 months of age. This lends support to the observations made by *Massler, Schour & Poncher* (1941), mentioned in the introduction to this paper. In ground sections, the incremental lines were barely discernible.

It is probably justified to regard the incremental-line pattern in the carious dentine as an accentuation of the less distinct pattern in the healthy dentine. The question then arises whether the sparse lines with a higher mineral content in Figs. 7—9b may be "normally" mineralized and the large intermediate areas "hypomineralized", or whether the latter areas are "normally" mineralized and the narrow, light lines "hypermineralized".

The lumina of the dentinal tubules were visible on the microradiograms at those sites where the tubules were crossed by the aforementioned bands with a higher mineral content. Consequently, the higher mineral content was not due to obliteration

of the tubules by calcium salts. An analysis of the significance of the incremental lines is, however, beyond the scope of the present paper. By way of conclusion, it may suffice to stress that the microradiographic technique appears to be a valuable tool in the study of the incremental pattern of the dentine. The method seems suitable for elimination of some of the sources of uncertainty inherent in the conventional methods.

SUMMARY

Ground sections 80—100 μ thick were prepared from 80 deciduous teeth with carious lesions. They were studied by transmitted light and with the microradiographic technique. On microradiograms of healthy enamel and healthy dentine, the neonatal line appears as a dark line, *i.e.*, it has a lower mineral content than the immediate surroundings. The appearance of the line in carious enamel nevertheless suggests that it may not be single, but that a group of lines is present in the borderline between the prenatal and postnatal enamel. On visual evaluation of the microradiograms it is not possible to establish any definite difference between prenatal and postnatal enamel with respect to their mineral content.

In most cases, the carious lesions are found to have the same microradiographic appearance as that in permanent teeth. Judging by the present material, it is highly uncommon for such a lesion to be altered at the neonatal line. In two cases, however — one of retrograde caries — the lesion seems to have been modified at this line. This observation is discussed against the background of the postulated better intrauterine mineralization.

In the areas of the microradiograms corresponding to carious dentine, an individually greatly varying pattern of "unmasked" incremental lines is present. Although these lines can be traced into the healthy dentine, they are more difficult to identify at this site. It is pointed out that it is hard to determine which parts of the pattern are to be denoted as "normally" mineralized initially. The possibility is suggested that "hypermineralized" areas may occur.

Finally, it is stressed that microradiography is a suitable method for a study of the incremental pattern of the dental tissues.

RÉSUMÉ

ÉTUDES SUR LES TISSUS DENTAIRES MINÉRALISÉS

XII. Étude microradiographique de la carie des dents temporaires

De 80 dents de lait cariées on a fait des coupes par usure d'une épaisseur de 80 à 100 μ. Ces coupes ont été examinées en lumière traversante et à l'aide de la technique microradiographique. Sur les microradiogrammes pris de l'émail sain et la dentine saine, la ligne néonatale paraissait comme une ligne sombre, c'est-à-dire que la ligne néonatale contenait moins de minéral que le voisinage le plus proche. Pourtant le caractère de cette ligne dans l'émail carié faisait croire à la possibilité qu'elle ne soit pas seule mais qu'il existe un groupe de lignes à la limite entre l'émail prénatal et l'émail postnatal. A l'examen visuel des microradiogrammes il n'était pas possible de constater de différence positive entre l'émail prénatal et l'émail postnatal en ce qui concerne la teneur de minéral.

Dans presque tous les cas les lésions de la carie avaient le même caractère microradiographique que dans les dents permanentes, et à en juger par les dents examinées ici, il est très rare que le caractère de la lésion de carie change à la ligne néonatale. Dans deux cas cependant il semblait que la carie se fût modifiée à la ligne néonatale. L'un de ces cas était un cas de carie retrograde. Cette découverte doit être discutée en tenant compte de la minéralisation intrautérine prétendue meilleure.

Dans les parties des microradiogrammes correspondant à la dentine cariée, il y avait un dessin de lignes de croissance "démasquées" qui était très varié d'un individu à l'autre. On pouvait bien suivre ces lignes dans la dentine saine mais elles y étaient plus difficiles à voir. On laisse entendre qu'il est difficile de décider quelles parties du dessin doivent être considérées comme normalement minéralisées dès le commencement. On suppose la possibilité qu'il existe des régions "hyperminéralisées". Pour finir, on accentue qu'on trouve dans la microradiographie une méthode propre à l'étude des "incremental pattern" des tissus dentaires.

ZUSAMMENFASSUNG

STUDIEN AN MINERALISIERTEN ZAHNGEWEBEN

XII. Eine mikroradiographische Untersuchung über die Karies der Milchzähne

Von 80 kariösen Milchzähnen wurden 80—100 μ dicke Schliffe hergestellt und diese im durchfallenden Licht und mit mikroradiographischer Technik untersucht. Auf dem Mikroradiogram von gesundem Schmelz und gesundem Dentin zeichneten sich die Geburtslinien als schwarze Linien ab, d.h. sie besassen einen geringeren Mineralgehalt als ihre nächste Umgebung. Das Aussehen der Linie im kariösen Schmelz deutete darauf hin, dass es sich möglicherweise nicht nur um eine Linie handelte, sondern dass sich an der Grenze zwischen prä- und postnatalem Schmelz eine Gruppe von Linien befand. Mit Hilfe der visuellen Beurteilung der Mikroradiogramme war es nicht möglich einen sicheren Unterschied zwischen prä- und postnatalem Schmelz hinsichtlich des Mineralgehaltes festzustellen.

Die Kariesangriffe hatten in den meisten Fällen das gleiche mikroradiographische Aussehen wie bei den permanenten Zähnen und nach dem vorliegenden Material zu urteilen, ist es äusserst ungewöhnlich, dass sich der Charakter des Kariesangriffes an den Geburtslinien ändert. In zwei Fällen schien es jedoch so, als ob die Karies an den Geburtslinien modifiziert worden wäre. Bei einem dieser Fälle handelte es sich um retrograde Karies. Dieser Befund wird in Hinsicht auf die, wie allgemein behauptet wird, bessere intrauterine Mineralisation diskutiert.

In den Gebieten der Mikroradiogramme die dem kariösen Dentin entsprachen fanden sich individuell stark variierende Muster von demaskierten Inkrementlinien. Diese konnten zwar bis in das gesunde Dentin verfolgt werden, waren dort jedoch schwer zu sehen. Die Schwierigkeiten zu bestimmen, welche Teile des Musters von Anfang an als normal mineralisiert bezeichnet werden sollen, werden diskutiert. Die Möglichkeit, dass hypermineralisierte Gebiete vorkommen können wird angedeutet. Zum Schluss wird darauf hingewiesen, dass die Mikroradiographie eine geeignete Methode zum Studium der Aufbaumuster der Zahngewebe ist.

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