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## STUDIES ON MINERALIZED DENTAL TISSUES<sup>1</sup>

### VI. The distribution of mineral salts in the dentine with special reference to the dentinal tubules

*by*

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In dental caries there occurs a dissolution of mineral salts in the affected areas of enamel and dentine. This phenomenon can be studied by microradiography using soft x-rays and, with this technique, one is able to demonstrate a decreased x-ray absorption in the engaged areas. In some instances, however, one also finds an increased x-ray absorption due to an increased content of mineral salts in the adjacent parts of the dentine. In a recent investigation using microradiographic techniques it has been shown that areas with an increased content of mineral salts can be traced as far down as the dentine-predentinal border in very superficial carious lesions (*Bergman & Engfeldt 1954*). These areas of the dentine which show an increased x-ray absorption partly correspond to so-called transparent zones, but they sometimes also cover opaque areas. (We define a transparent zone of the dentine as an area of a ground section which, in transmitted light, shows up as brighter, more transparent, than the surrounding dentine. An opaque area is a part of the dentine which is dark in transmitted light). We have used longitudinal sections of teeth in our earlier investigation and the microradiograms so obtained have not permitted us to draw any definite conclusions as to the exact sites of the increased content of

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<sup>1</sup> Part I in this series is published in *Exp. Cell Research* 7: 381, 1954, parts II, III and V in *Acta odont. Scand.* 12: 99, 133, 193, 1954, and part IV in *Acta path. et microbiol. Scand.* 35: 537, 1954.

mineral salts in the dentine. The deposition of mineral salts could have occurred in the ground substance or in the dentinal tubules. By preparing thin ground sections perpendicular to the direction of the dentinal tubules and by obtaining high resolution microradiograms of these sections, it has become possible to study the distribution of mineral salts in the dentine more closely.

#### MATERIAL AND METHODS

Permanent teeth with superficial fissure caries have been extracted and immediately fixed in ethanol. Thin slices have been cut through the crowns perpendicular to the long axis of the teeth. These slices have been ground to a thickness of about 50  $\mu$ . The microradiograms published in this paper are all from sections situated so far from the carious lesions that no signs of demineralization whatever could be detected.

The microradiography has been performed according to the principle of secondary enlargement. A Machlett OEG x-ray tube with 0.2 mm Be window served as radiation source and the tube was operated with 24 kV fully rectified d.c. and 10 mA. The distance focus to emulsion was 25 cm which in our case gave a geometrical unsharpness of less than 0.1  $\mu$ . The microradiograms were registered on Eastman Kodak spectroscopic plates no. 649 which were developed in D.K. 50 for three minutes. The microradiograms were enlarged by photomicrography on Kodak 0 250 plates.

After the microradiography the sections were dehydrated, mounted in canadabalsam and photomicrographed in transmitted light.

#### RESULTS

The distribution of mineral salts in the dentine is demonstrated in Figures 1—3 which are arranged with the transmitted light photograph on the left and the corresponding microradiogram on the right. Light areas in the microradiograms correspond to areas with high x-ray absorption, that is, areas with a high content of mineral salts.

Figures 1 and 2 show two different areas from the same ground section of a third molar. The dentinal tubules have been cut

perpendicular to their longitudinal axis at two different levels. Thus some of the dentinal tubules situated in the immediate neighbourhood of the predentine zone and a group of tubules situated further away from the pulpal tissue are cross-cut. Figs. 1 a and b show cross-cut dentinal tubules close to the predentine zone. The dentinal tubules have here a diameter of about  $2 \mu$  (maximum  $2.5 \mu$ ). The microradiogram (Fig. 1 b) shows that the tubules have a sharp boundary towards the ground substance with its high x-ray absorption. A transitional zone between the dentinal tubules and the ground substance thus cannot be demonstrated in the microradiogram.

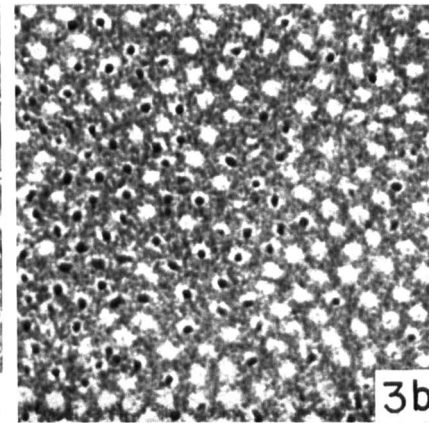
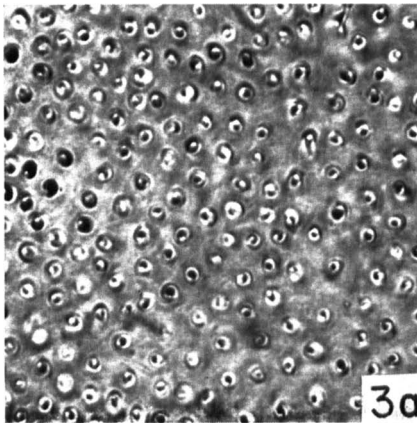
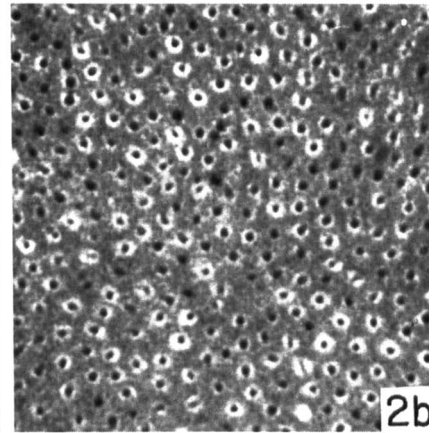
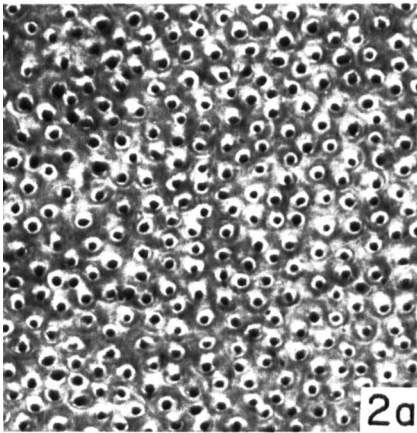
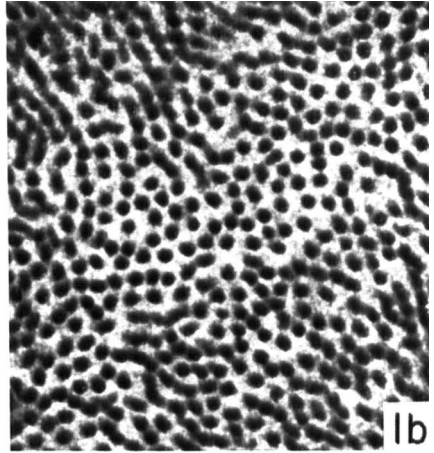
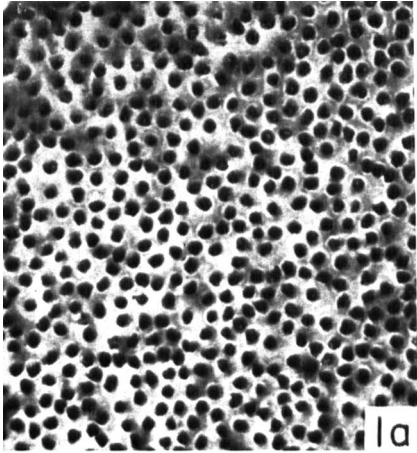
Figs. 2 a and b demonstrate the observations made in an area situated further away from the pulpal tissue. The diameters of the dentinal tubules are here decreased (maximum  $1.5 \mu$ ) and most of them are seen in the transmitted light photograph (Fig. 2 a) as surrounded by a light zone of varying thickness. The corresponding microradiogram (Fig. 2 b) demonstrates that most of the dentinal tubules are surrounded by areas of varying thickness having a higher x-ray absorption than the adjacent ground substance. A few tubules in this area, however, do not show such high x-ray absorption in their surroundings.

Figs. 3 a and b are taken from a ground section of another third molar. In these photographs one can observe the same details as in Figs. 2 a and b, and in addition the microradiogram (Fig. 3 b) shows that some of the dentinal tubules are completely filled with highly absorbing material.

#### DISCUSSION

The investigation of the distribution of mineral salts in the dentine from carious teeth, performed with microradiographic technique on ground sections cut perpendicular to the long axis of the dentinal tubules, has demonstrated different findings: 1) Dentinal tubules which are immediately surrounded by the ground substance, 2) dentinal tubules which are separated from the ground substance by a dense ring of mineral salts, and 3) tubules which are completely obliterated.

There are transitional forms of all degrees between the open dentinal tubules and the completely obliterated ones. This finding



seems to indicate that the tubules can be successively obliterated by a deposition of mineral salts, a process which starts in the periphery of the tubules. On the base of the material investigated to date, it is not possible to draw any definite conclusions concerning the nature of this process. There are of course different possibilities. The obliteration of the dentinal tubules might be a physiological process connected with the aging of the tissue or it might have something to do with caries. Because of the findings in our previous work on caries it appears very probable that the obliteration of the dentinal tubules by mineral salts is at least partly connected with caries and corresponds to such areas which in longitudinal ground sections of teeth appear as transparent or opaque zones when studied in transmitted light.

The resolution of the microradiograms is about  $0.5 \mu$  and thus it is not possible to decide with certainty if the tubules, which on the microradiograms appear to be obliterated, really are completely filled up by mineral salts or if they have a very thin lumen. A comparison with the corresponding photomicrographs taken in transmitted light indicates, however, that at least some of these tubules are void of lumen. *Miller* (1954) has stated that the dentinal tubules are always surrounded by a ring of highly absorbing material both in erupted and nonerupted teeth. However, this is in disagreement with our findings which show

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Fig. 1. *a*. Transmitted light photomicrograph of  $50 \mu$  thick unstained ground section of a third molar with superficial fissure caries. Cross-cut dentinal tubules near the pulp. *b*. Microradiogram from the same section. The dentinal tubules have a low x-ray absorption and appear dark. No evident transitional zone towards the highly absorbing ground substance.  $\times 600$ .

Fig. 2. *a*. Transmitted light photomicrograph of another area of the same section as shown in Fig. 1 *a*. Cross-cut dentinal tubules further away from the pulp. The tubules have smaller diameters than in Fig. 1 and are surrounded by light areas. *b*. Microradiogram of the same area as in Fig. 2 *a*. Some tubules have the same appearance as in Fig. 1 *b*, but have smaller diameters, while others are separated from the ground substance by rings of varying thickness showing high x-ray absorption.  $\times 600$ .

Fig. 3. *a*. Transmitted light photomicrograph of  $50 \mu$  thick unstained ground section. Cross-cut dentinal tubules beneath a superficial fissure caries lesion of a third molar. The tubules are surrounded by light zones of varying thickness. *b*. Microradiogram of the same section. Some tubules seem to be completely obliterated by mineral salts with high x-ray absorption, while others have lumina surrounded by highly absorbing rings.  $\times 600$ .

the absence of such highly absorbing areas around many of the dentinal tubules both in those parts of the dentine situated next to the predentine zone and further away.

#### SUMMARY

Ground sections from the crowns of molars with superficial fissure caries have been studied with microradiographic technique. The sections were taken perpendicular to the long axis of the teeth. The cross-cut dentinal tubules were sometimes obliterated with mineral salts, and the x-ray absorption of this structure was higher than that of the surrounding ground substance. In many cases the lumina of the dentinal tubules were surrounded by rings of varying thickness showing high x-ray absorption. Sometimes such areas were absent, and no transitional zones could be found between the tubules and the ground substance. Between these "open" tubules and the above-mentioned obliterated ones there was every transitional form. The question whether the process leading to an obliteration of the dentinal tubules is a normal or pathological one is discussed.

#### RÉSUMÉ

##### ÉTUDES SUR LES TISSUS DENTAIRES MINÉRALISÉS

##### VI. La distribution des sels minéraux dans la dentine, envisagée spécialement aux tubes dentaires

Des coupes pratiquées dans des couronnes de molaires superficiellement cariées ont été étudiées par une technique microradiographique. Les coupes ont été faites perpendiculairement à l'axe longitudinal des dents. Les tubes dentaires coupés transversalement étaient parfois obstrués par des sels minéraux d'une puissance d'absorption de rayons X plus grande que celle de la substance environnante. En certains cas les parois des tubes dentaires étaient recouvertes d'anneaux d'une épaisseur variée et d'une haute puissance d'absorption des rayons X. Entre les tubes dentaires sans anneaux et les tubes obstrués toutes les formes intermédiaires ont été trouvées.

On a discuté la question de savoir si la réaction, qui cause l'obstruction des tubes dentaires, est normale ou pathologique.

## ZUSAMMENFASSUNG

## STUDIEN AN MINERALISIERTEN ZAHNGEWEBEN

## VI. Die Verteilung der Mineralsalze im Dentin mit besonderer Berücksichtigung der Dentinkanälchen

Schliffe von Molarenkronen mit oberflächlicher Fissurenkaries wurden mit mikroradiographischen Methoden untersucht. Die Schliffrichtung war quer zu der Längsachse der Zähne. Die quergetroffenen Dentinkanälchen waren verschiedentlich durch Mineralsalze obliteriert, und die Röntgenstrahlenabsorption dieser Bezirke war grösser als die der umgebenden Grundsubstanz. In vielen Fällen war das Lumen der Dentinkanälchen von Ringen unterschiedlicher Dicke umgeben, die eine starke Röntgenstrahlenabsorption zeigten. Manchmal fehlten diese, wobei auch eine Uebergangszone zwischen den Dentinkanälchen und der Grundsubstanz nicht festzustellen war. Zwischen diesen "offenen" und den oben erwähnten obliterierten Dentinkanälchen konnten alle Uebergänge gefunden werden. Es wurde diskutiert, ob der zu einer Obliteration der Dentinkanälchen führende Prozess als normal oder pathologisch anzusehen ist.

## REFERENCES

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