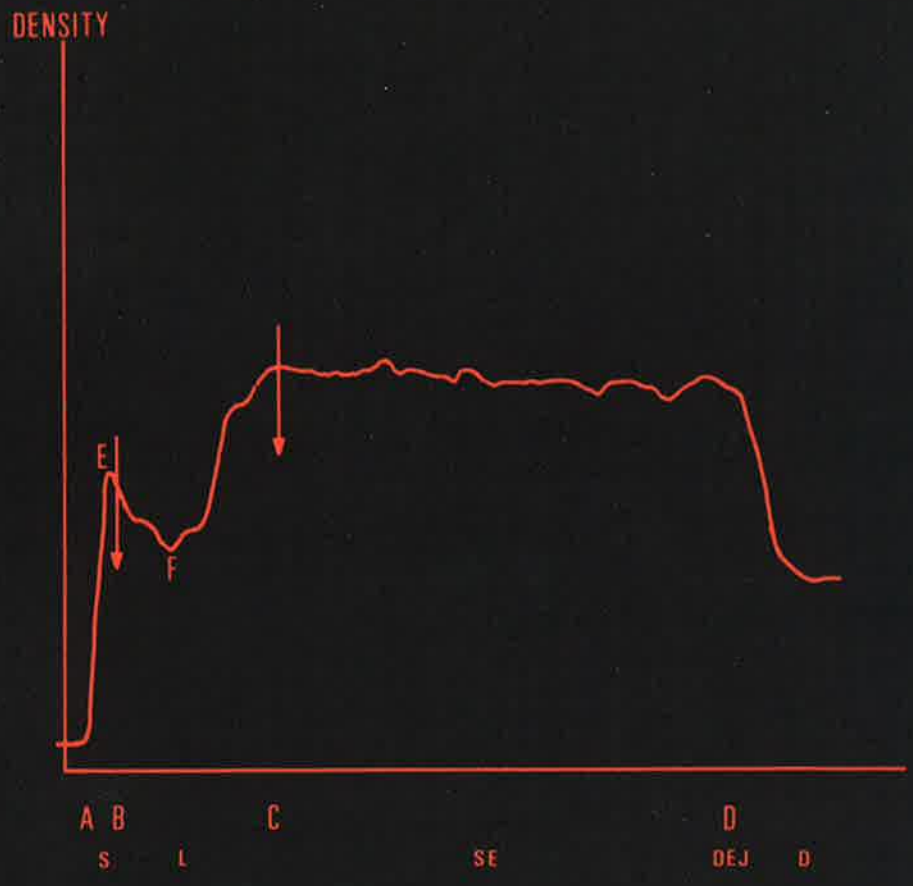
DENTAL CARIES

SOME ASPECTS OF ARTIFICIAL CARIES LESIONS EXAMINED BY CONTACT-MICRORADIOGRAPHY



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SOME ASPECTS OF ARTIFICIAL CARIES LESIONS EXAMINED BY CONTACT-MICRORADIOGRAPHY

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The author to his book:
I love you, hate you, love you,
hate you, love you, . . .
but I want you.

Shirley Bassey

Illustraties verzorgd door LUUK SCHOLTEN

Aan mijn moeder,
aan Annèt,
Agaath,
Arie,
Nico.

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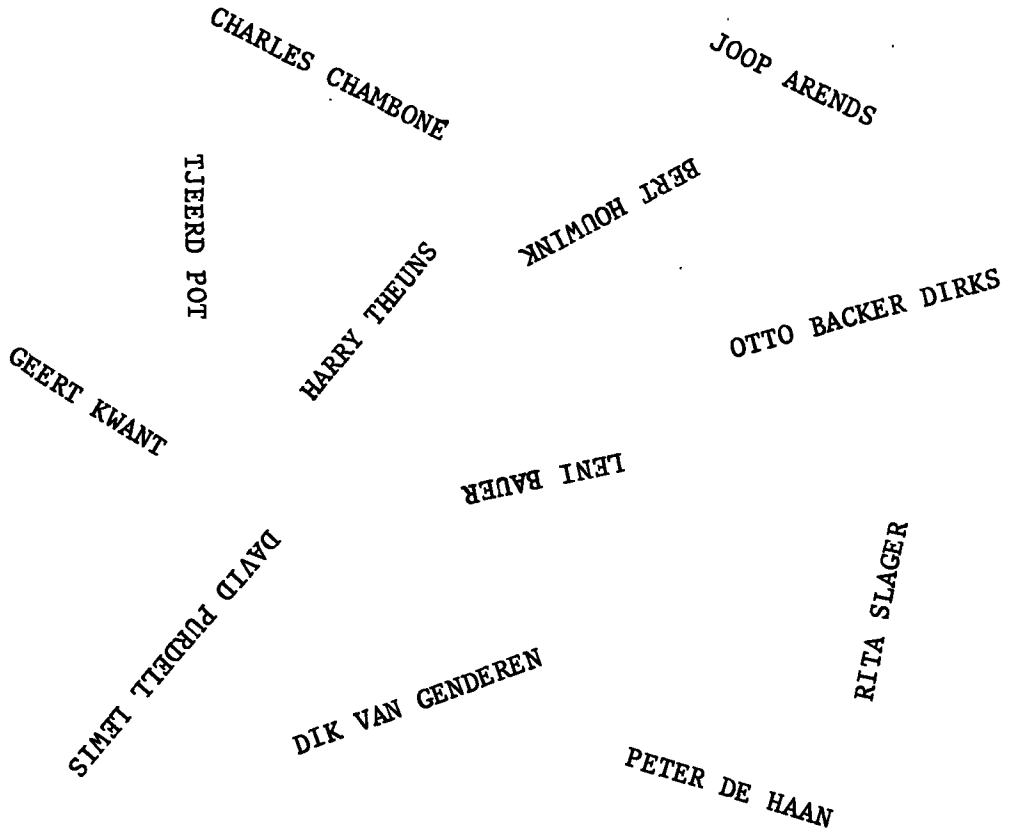
The author is greatly indebted to:



I would like to thank all those who have helped and encouraged me at the Laboratory of Microbiology in Utrecht and the Organization of Health Research T.N.O. in The Hague.

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CHAPTER I

INTRODUCTION

Dental caries may be defined as the progressive destruction of tooth substance, leading to cavity formation. The results of numerous epidemiological studies have shown that it is the most common disease in western society.

VAN ERP and MEYER-JANSEN (1966) examined a random group of 4½-year old children and found that only 15% had a caries-free decidious dentition. A recent study in a non-fluoridated area (Culemborg) in The Netherlands (KWANT et al., 1974) showed that there were *no* caries-free individuals in a representative group of 17- and 18-year old individuals. The mean number of decayed, extracted and filled teeth was 15.7 in the 17-year old group and 16.5 in the 18-year old group. Expressed as a precentage this means that 60% of the permanent teeth of every 17- or 18-year old individual were either decayed, extracted or filled. In another study of the Culemborg population POT et al. (1974) have shown that more than 30% have a full upper denture by the age of 34.

These figures demonstrate that social dentistry today faces the insoluble basic problem, that with neither the present nor the projected manpower will it be possible to restore the enormous number of diseased teeth in The Netherlands. It would therefore appear that the only way to create a dentally fit population is to prevent the formation of dental caries. Before any preventive measures can be contemplated a thorough knowledge of the etiology of dental caries is essential.

Dental caries is caused by the dissolution of tooth enamel. This complicated process is progessive and can be seen clinically in its initial stages as a 'chalky white spot' on the tooth surface. These 'white spot' lesions usually occur very soon after tooth eruption; 84% developing within 1½ years (see BACKER DIRKS, 1966). This dissolution may, depending on the oral environment, continue and lead to a partial breakdown of the enamel structure and subsequent cavity formation.

In a clinical study of the post-eruptive changes in enamel in a non-fluoridated area BACKER DIRKS (1966) showed that of 72 surfaces with white spot lesions found in a group of 8-year old children, 37 were diagnosed as sound, 26 as white spot lesions and 9 as cavities at the age of 15. This study illustrates that caries formation is a complex process and that only a small proportion of white spot lesions ultimately progress into cavities.

The suggestion that the dissolution of enamel is caused by acids, formed by the fermentation of food debris by bacteria, was first put forward by LEBER and ROTTENSTEIN in 1867 and later more thoroughly documented by MILLER in 1892. According to this theory, still considered valid, bacteria which are present in large numbers on the surface of all teeth, produce acids from dietary carbohydrates. Several kinds of organic acids are formed, but the main product of bacterial metabolism is lactic acid. Three main factors are essential for the development of dental caries (see fig. 1):

1. bacteria (microflora)
2. dietary carbohydrates (substrate)
3. dental enamel (host and teeth)

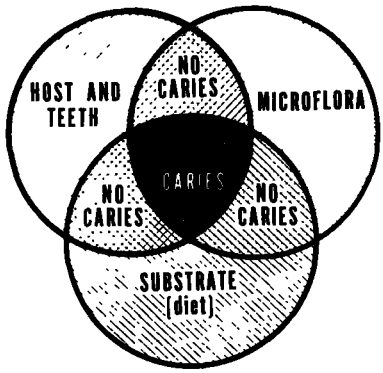


Fig. 1. The three factors involved in dental caries formation. If one of these is absent no caries will develop. (Paul H. Keyes: J.A.D.A. *79:* 1395 (1969).

No caries will develop if *one* of these three components is absent (KEYES, 1963). Research into the prevention of dental caries must therefore be directed at influencing one or more of these three factors. All researchers in the field of preventive dentistry in the past decennia have approached the problem of preventing dental caries by attempting to influence one or more of these components.

a. Bacteria and dietary carbohydrates

Bacteria are present in large numbers in the surface deposit on each tooth, the dental plaque; they account for approximately 70% of its weight. All the acid producing micro-organisms in this plaque, particularly the acidoduric fraction, are considered to be responsible for the dissolution of enamel. Two new important factors have been found in the past decade.

The first factor, the storage of iodophilic polysaccharides was discovered by GIBBONS and SOCRANSKY (1962). This synthesis, by many different kinds of bacteria, from sucrose, glucose, maltose, fructose, etc. is accompanied by increased acid production, since extra energy is required. The later degradation product of these polysaccharides is also acid which results in the pH of the plaque (VAN HOUTE, 1967) remaining at a lower level for a longer time.

The second factor is the synthesis of cell-bound extracellular polysaccharides by Streptococcus mutans from sucrose (WOOD and CRITCHLEY, 1966; GIBBONS and BANGHART, 1967; DE STOPPELAAR, 1967). These polysaccharides appear to be an important factor in plaque formation. They are sticky and enable the bacteria to adhere to the surfaces of the teeth. This is probably one of the main reasons why the plaque is not removed from tooth surfaces by the physiological clearing system of the mouth. Both factors have been shown to

operate in the human mouth (KRASSE, 1966; DE STOPPELAAR, 1967). Since these cell-bound polysaccharides are formed exclusively from sucrose, this sugar has been considered to be the most important dietary factor in the development of dental caries.

An important study by MARTHALER (1967) showed that very few of the children suffering from hereditary fructose intolerance developed caries although they consumed glucose, starch and other fructose free (and sucrose free) carbohydrate containing food products. It is tempting to deduce from these studies that diets with a much reduced sucrose content will lead to less dental caries. However, after plaque formation has been initiated by extracellular polysaccharide synthesis from sucrose by Strep. mutans, all the other sugars may contribute to the low pH at the enamel surface. VAN DER HOEVEN et al. (1974) have also demonstrated that there was a shift in the bacterial flora in animals fed on sucrose free, high glucose diets. This leads to a higher percentage of Actinomyces viscosus and the formation of extracellular polysaccharides, resulting in an increased caries score.

Although these results should not be directly applied to the situation in the human mouth, it can be concluded that a reduction in the *frequency* of the consumption of intermeal snacks, containing easily fermentable carbohydrates of *all* kinds, is necessary for caries prevention through dietary means. It is, however, extremely difficult to change the dietary habits of a whole population. Two studies in The Netherlands have shown that a dental health education programme does not result in a significant caries reduction after either 2 years (in Nijmegen, PLASSCHAERT, 1972) or after 3 years (in The Hague, PLASSCHAERT et al., 1974).

b. Dental enamel

The tooth enamel is the third main factor involved in the caries process. If, as it now appears, it is impossible, or at least very difficult, to prevent acid formation an alternative approach would be to make the tooth enamel more acid-resistant.

It must be noted here, that once a white spot lesion has progressed to a cavity the dissolution process is irreversable. The only way to stop the disease at this juncture is by restoring the cavity with the appropriate dental materials. Prevention of caries must therefore take place prior to, or at an early stage in, the formation of a white spot lesion. The results of many investigations have shown that the most effective method of increasing the acid-resistance of enamel is by fluoride treatment, waterfluoridation, fluoride tablets, mouth rinsing with fluoride containing solutions, or by topical fluoride application.

There are a number of indications that fluoride reduces the acid- and polysaccharide formation (WINKLER, 1946; VAN HOUTE, 1967) of the bacterial flora. The main action of fluoride is, however, thought to be its ability to increase the acid-resistance of enamel.

Clinical data on the effect of fluorides on dental caries

Many studies have shown the beneficial effect of artificial waterfluoridation. Table I shows the number of proximal lesions with dentine involvement (D-lesions) in Tiel (The Netherlands) after 16 years of waterfluoridation compared to the non-fluoridated town of Culemborg. It can be seen that, after the first two

Table I. Number of proximal D-lesions in molar region and maxillary anteriors per 100 children, 11 to 15 years of age; and the percentage of fewer lesions in Tiel.

Year of survey	1952	1953	1955	1957	1959	1961	1963	1965	1968
Culemborg	436	405	457	533	518	551	572	618	563
Tiel	409	407	450	389	339	270	213	170	140
% less D-lesions in Tiel				27%	34%	51%	63%	72%	75%

years, there was in Tiel a continuous reduction in caries until 1968 when this decrease reached a maximum value of 75%. Table I also clearly demonstrates that, in Culemborg the total number of proximal lesions has increased by nearly 30%. These results are depicted graphically in figure 2.

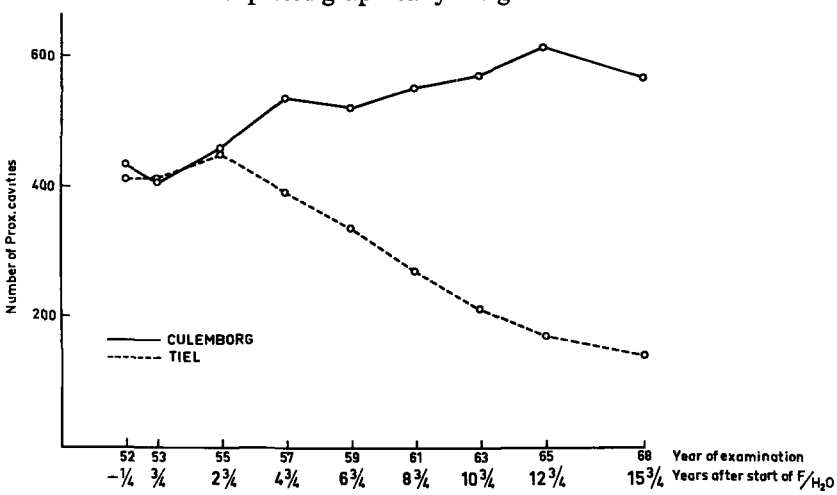


Fig. 2. The average number of proximal surfaces with fillings and cavities per 100 children aged 11 to 15. The year of examination, also expressed as years after the start of waterfluoridation in Tiel, is given on the horizontal axis. The non-fluoridated town of Culemborg served as the control.

Waterfluoridation has been found to be the most effective preventative measure against dental caries. The use of fluoride tablets, fluoridated salt or milk, fluoride

rinses and fluoride dentifrices is less effective and results in a caries reduction of between 15-40%.
There have been more successful results from clinical trials testing the effect of *topical* applications of fluoride. Stannous fluoride and acidulated sodium fluoride phosphate solutions appear to be the most effective. A clinical trial to study the effect of stannous fluoride has been completed in The Netherlands (HOUWINK, BACKER DIRKS and KWANT, 1974) on identical twins. It was found that topical application of a 1% F-solution (as SnF_2) twice a year resulted in a 55% reduction in the number of proximal lesions.

Some observations on the mechanisms of fluoride incorporation in enamel

The result of fluoride treatment can be divided into:
a. pre-eruptive effect
b. post-eruptive effect.

a. Pre-eruptive effect

Clinical studies have shown that waterfluoridation has a pre-eruptive effect.
The main component in mature enamel is hydroxyapatite often termed the 'mineral' content. Although the mechanism is not fully understood, F-ions probably influence the transition of the hydroxyapatite 'precursor' to hydroxyapatite. The precursor formed during the process of tooth formation is octacalcium phosphate or a similar material. In vitro experiments have shown that fluoride traces can accelerate the reaction:

$$2Ca_8H_2(PO_4)_6\,2H_2O \xrightarrow{\text{fluoride traces}} Ca_{10}(PO_4)_6(OH)_2 + 6Ca^{++} + 6HPO_4^{--}$$

octacalciumphosphate — hydroxyapatite

The formation of apatite crystals can probably only take place when there are small amounts of fluoride present in the surrounding fluids. The formation of uniformly *well mineralized* hydroxyapatite is, in all probability, an important caries preventative effect (NEWESELY, 1972). The pre-eruptive effect of fluoridated water is therefore presumably due to this formation of a well mineralized enamel.

b. Post-eruptive effect

The main component of enamel is hydroxyapatite (95% by weight). Although it is non-stochiometric, the simple formula $Ca_{10}(PO_4)_6(OH)_2$ will be used in this thesis.

Hydroxy-fluorapatite $Ca_{10}(PO_4)_6(OH)_{2-x}F_x$ or when x = 2, fluorapatite $CA_{10}(PO_4)_6F_2$ are only formed when hydroxyapatite is in contact with low concentrations of fluoride (approximately 1 ppm). Both compounds are less acid soluble than hydroxyapatite. The fluoride ions are not distributed homogeneously throughout the enamel. Their concentration depends upon the distance from the anatomical surface. The fluoride concentration at the surface may be several

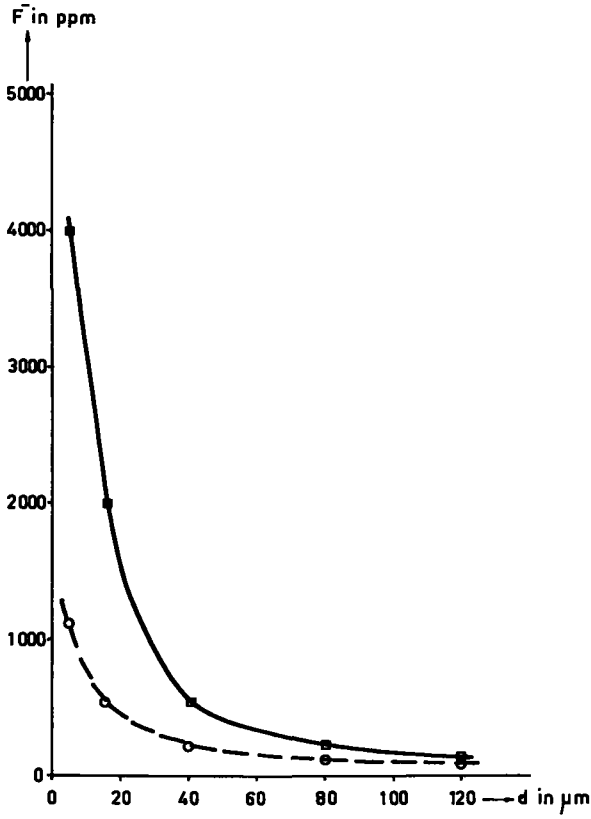


Fig. 3. The fluoride distribution in enamel as a function of the distance from the anatomical surface. The dashed line represents the average fluoride content after water fluoridation (buccal surface of premolars; age 15-18 y). The drawn line gives the average fluoride content immediately after a topical SnF_2 treatment (4% solution for 3 min.). (J. Schuthof and J. Arends private communication.)

orders of magnitude greater than that found at a depth of 250 µm. Figure 3 shows a typical example of the F^- content, given as a function of the distance from the surface, in sound enamel after waterfluoridation and topical application with a SnF_2 solution. The distribution of fluoride in a single enamel crystallite is probably also very inhomogeneous.

A second reaction occurs with higher concentrations (especially at pH = 4 or lower):

$$Ca_{10}(PO_4)_6(OH)_2 + 20\,F^- \longrightarrow 10CaF_2 + 6PO_4^{---} + 2\,OH^-$$

Depending on the pH, PO_4^{---} ions may form acid phosphate ions and the OH^- ions may form H_2O.

Calcium fluoride is more soluble than hydroxy-fluorapatite and can be lost by diffusion out of the tooth into the plaque and saliva (CHOW and BROWN, 1973). If, however, CaF_2 can be made to adhere to the surface by using aminefluoride or fluoridated sealants (ARENDS et al., 1974), it can act as a fluoride depot and fluorapatite will continue to be formed. This mechanism is certainly responsible for the more acid-resistant teeth which result from topical fluoride application.

The third important preventative effect is the *remineralization** property of fluoride solutions. BROWN (1973), ARENDS and DAVIDSON (1974) and MORENO and ZAHRADNIK (1974) have provided evidence which suggests that brushite, $CaHPO_4\,2H_2O$, or brushite-like materials, are formed in early caries (white spot) lesions (see Chapter VI) as a result of the dissolution of hydroxyapatite. Brushite reacts with fluoride at pH 4 to form fluorapatite. It is therefore probable that in a fluoridated area fluorapatite is formed in carious enamel. The observation by WEATHERELL (1971), that there is a high F^- concentration in carious enamel, is in line with this suggestion.

Fluorapatite is also formed after the local application of fluoride compounds. An important additional effect of heavy metal solutions (e.g. SnF_2) is that insoluble compounds other than fluorapatite e.g. $Sn_3F_3PO_4$ may be formed. These can refill the lesion and possibly make it more caries-resistant.

Caries lesion — microradiographic appearance

To be effective, caries must be stopped before the white spot lesion has progressed beyond the initial stages. The study of the white spot lesion is therefore of major importance. The early stages can be studied on ground sections. It can then be seen that the demineralization starts *underneath* a relatively intact surface layer.

Figure 4 shows a microradiogram of a section through a white spot lesion. 'S' denotes the intact surface layer which is approximately 40 μm thick. The radiolucent area at the right hand side of the intact layer indicates a zone of enamel demineralization which has already progressed through twothirds of the total thickness of the enamel layer.

HOLLANDER and SAPER (1935) and THEWLIS (1937) studied ground sections of caries lesions using soft X-rays. They suggested that the intact surface

* remineralization is used if 'mineral-like' materials are deposited in the lesion. It does not indicate that the original mineral is reformed.

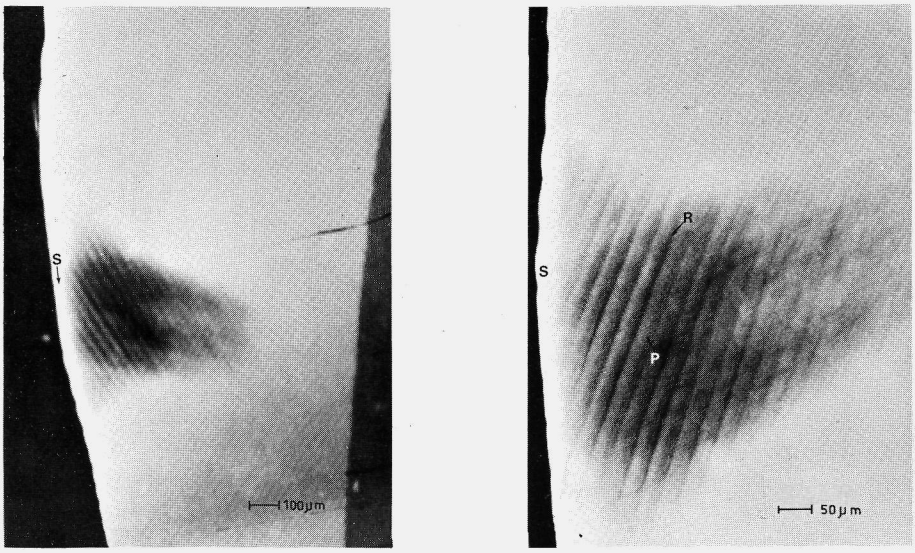


Fig. 4. Microradiogram of a longitudinal section through a white spot lesion. Some characteristic features can be seen: triangle shape, accentuation of Retzius lines, intact surface layer (S) ± 40/μ m thick, and sub-surface demineralization.

Fig. 5. Microradiogram of the section shown in fig. 4 at a higher magnification. 'R' indicates an accentuated Retzius line; 'P' = the prism border and 'S' = the intact surface layer.

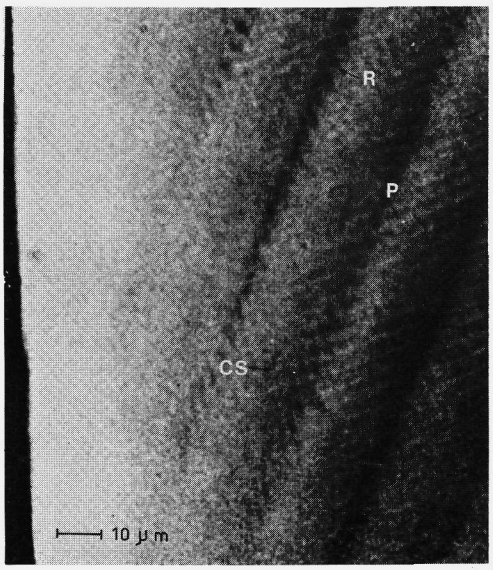


Fig. 6. Microradiogram of the section shown in fig. 4 at a much higher magnification. 'R' = Retzius line; 'P' = prism border and 'CS' = cross-striations, which are indicated by the small dark bands at right angles to the long axis of the prisms.

layer covering the sub-surface decalcification was caused by a photographic artifact (Mackie effect). APPLEBAUM (1940) provided the first evidence that this *'radiopaque line'* (S in fig. 4) was *not* an artifact, but a significant phenomenom of the initial caries lesion. Many authors have since confirmed these findings (BERGMAN and ENGFELDT, 1954; SONI and BRUDEVOLD, 1959; BERGMAN and LIND, 1966; CRABB and MORTIMER, 1967).

The following observations can be made about the microradiographic appearance of natural caries lesions from the results of these studies (figs. 4, 5 and 6):

a. The lowest mineral content is below the surface of the tooth in the body of the lesion (sub-surface lesion) indicated by the radiolucent area in figs. 4 and 5.
b. The relatively intact surface covering the sub-surface lesion is also demineralized, but to a much lesser extent (S in figs. 4 and 5).
c. Both the Retzius lines and the cross-striation of the prisms and the prism borders are accentuated (P and R in figs. 5 and 6).
d. The advancing front of the lesion is irregularly formed (fig. 4).
e. The overall shape of an initial caries lesion is that of a triangle with the apex facing the dentino-enamel junction (fig. 4).

Caries lesion — polarized light appearance

Many investigators have described the histological appearance of very small caries lesions when examined under the polarized light microscope (NISHIMURA, 1926; GUSTAVSON, 1957; DARLING, 1963; SILVERSTONE, 1966). The technique is, however, rather complicated and the interpretation difficult, because of problems such as form-birefringence, intrinsic birefringence and the influence of imbibition on the total birefringence (CARLSTRÖM, 1967). It is, however, necessary to describe this technique since it is widely used and many expressions such as 'translucent zone' and 'dark zone' are often used in describing the histological features of the caries lesion.

SILVERSTONE (1973) in a recent review on carious enamel, divided the caries lesion into *four* zones which are all clearly visible on ground sections mounted in canada balsem (fig. 7).

Zone 1: At the innerfront of the lesion there appears to be a translucent zone which has an average width of 40 μm. SILVERSTONE (1966) studied one hundred carious permanent teeth and found that only approximately 50 per cent of the lesions had this zone.

Zone 2: A dark area just superficial to the translucent zone. SILVERSTONE (1967) identified a dark zone in 90 per cent of the lesions in permanent teeth.

Both the translucent and dark zones are *irregularly* shaped, this usually results in a sawtooth effect at the edge of the lesion (DARLING, 1963).

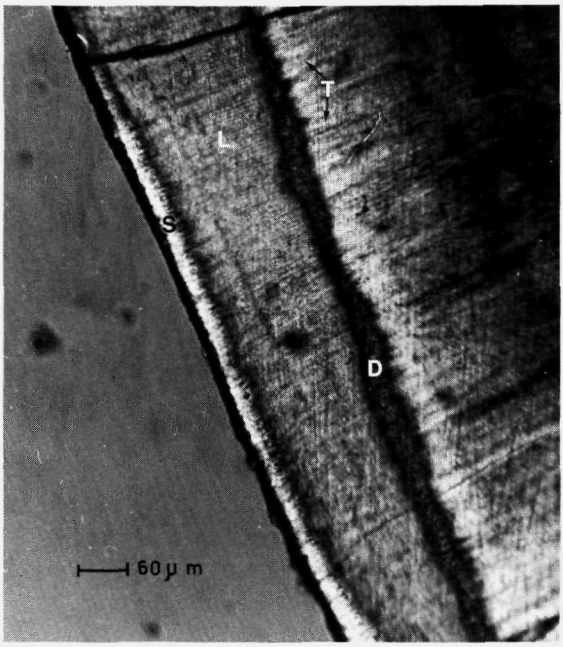


Fig. 7. Longitudinal section through a natural white spot lesion examined in canada balsam under polarized light. The four zones are clearly visible.
T = translucent zone
D = dark zone
L = sub-surface lesion
S = intact surface layer

Zone 3: The body of the lesion is considered to be the third zone. Demineralization is most pronounced in this area which is also the largest part of the lesion. Here, as in the microradiogram, Retzius lines and prism borders are accentuated.

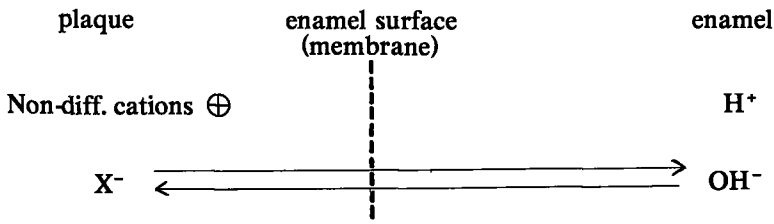
Zone 4: Microscopically as well as microradiographically the body of the lesion is covered by a relatively intact surface layer which is considered to be the fourth zone.

Caries lesions in vivo and in vitro

Many investigators have found that it is impossible to create caries-like lesions in *vitro* by exposing enamel samples to acid solutions alone. This suggests that plaque may play a role in caries lesion formation quite apart from acid production. Further research has revealed that the addition of suitable organic material (polymers) to lactic acid, results in an in vitro decalcifying solution which creates caries-like lesions in enamel.

VON BARTHELD (1961) suggested that demineralization takes place because

the pH *within* the enamel is lower than in the plaque on the surface. This might occur because of the Donnan-membrane effect in which it is presumed that the enamel surface acts as a semi-permeable membrane. In this hypothesis the plaque is considered to be a material which contains fixed non-diffusable cations. The Donnan-equilibrium situation is as follows:



The left compartment represents the situation in the plaque, which contains non-diffusable cations ⊕ and diffusable anions (X^-). The right hand side represents the situation inside the tooth. Enamel contains about 3-4% of water by weight. A very small amount of the *free* water will be dissociated in equal amounts of H^+ and OH^- ions.

The OH^- ions from within the enamel exchange with the X^- diffusable anions from the plaque to reach an equilibrium situation. It is, however, impossible for the H^+ ions to exchange with ⊕ cations from the plaque, since the latter ions are non-diffusable through the semi-permeable membrane. On the basis of a Donnan equilibrium it can be shown that under certain conditions the pH *in* the enamel *might* be lower than in the plaque. The magnitude of pH difference is uncertain, because too many unknown parameters are involved. At the present time no evidence has been produced which shows unequivocally that the Donnan equilibrium hypothesis explains caries lesion formation.

Gelatine, being a charged macromolecular substance, can act as a non-diffusable material containing cations and is therefore sometimes chosen to replace the plaque in in vitro models. MÜHLEMANN (1960), VON BARTHELD (1961) and SILVERSTONE (1966 and 1967) showed that this system creates caries-like lesions very similar to natural caries.

Figure 8 shows a caries-like lesion obtained by using this acidified gel technique. Both polarized light and microradiographic features show its similarity with natural caries. SILVERSTONE (1971) studied and compared the qualitative and quantitative behaviour of the histological zones in great detail and found *no* significant difference between the two types of lesions.

In contrast with the hypothesis of Von Bartheld, FRANCIS and GRAY (1963) showed that the addition of non-charged polymers to the decalcifying solution also results in a sub-surface decalcification. They explained caries lesion formation by diffusion phenomena and suggested that, at the enamel surface, there is a competition between a) the chemical reaction *at the surface* and b) reaction *in the enamel* caused by diffusion of the acid ions into enamel. If the reaction at the surface can be reduced, then the sub-surface reaction will become relatively more important thus resulting in sub-surface decalcification. The surface reac-

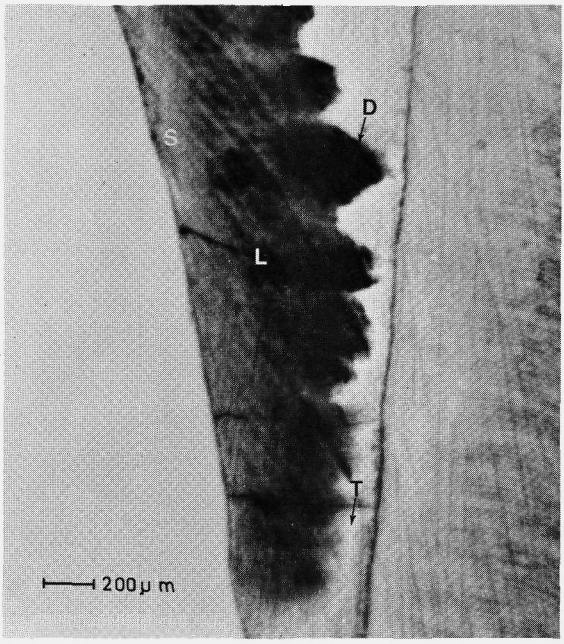


Fig. 8. Longitudinal section through an artificial caries lesion examined in water under polarized light. The lesion was produced in a 20% gelatine medium acidified with 30% lactic acid at pH 4. This lesion is very similar to a natural caries lesion. The accentuated Retzius lines in the body of the lesion, the sawtooth effect of the advancing front, the translucent zone, the dark zone and the intact surface layer are clearly visible.
T = translucent zone
D = dark zone
L = sub-surface lesion
S = intact surface layer
(Picture by courtesy of Dr. F. von Bartheld).

tion is reduced by the plaque in vivo and by adding organic material (polymers) to decalcifying solutions in vitro. A variety of water-soluble polymers were tested and hydroxyethyl cellulose (HEC) was found to be the most effective.

Figure 9 shows an artificial lesion formed by immersing a tooth in an HEC solution containing 0.1 M lactic acid. The four zones seen in a natural lesion are clearly visible when the section is examined under the polarizing light microscope. When a number of lesions is examined the translucent zone is usually absent and the dark zone is in some cases absent or irregularly formed. The importance of these features must not, however, be overestimated since both zones may also be absent or irregularly formed in natural lesions.

The microradiographical appearance of the lesions produced by acid HEC solutions is also slightly different from that of a natural caries lesion, although many details are quite similar. Figures 10 and 11 clearly demonstrate that the cross-striations, prism borders and Retzius lines are accentuated.

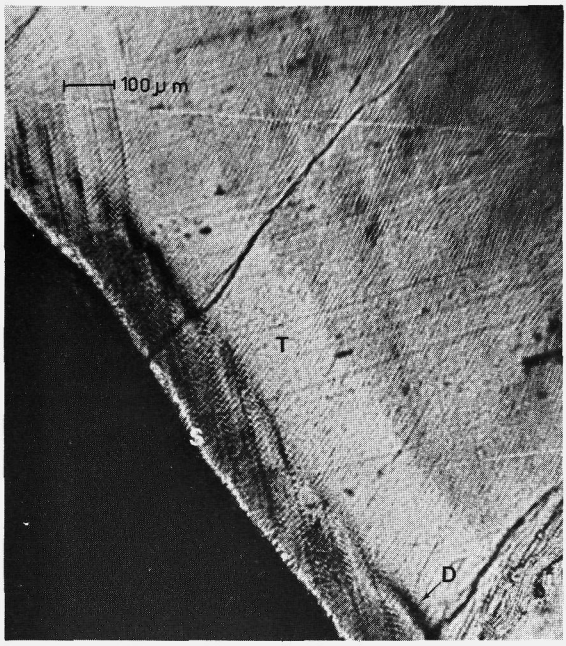


Fig. 9. Longitudinal section through an artificial caries lesion examined in canada balsam under polarized light. The lesion was produced in a 6% hydroxyethyl cellulose solution containing 0.1 M lactic acid adjusted to pH 4 for 96 hours. The inner front of the lesion is parallel to the enamel surface. Retzius lines and prism border are accentuated. A very broad translucent zone and a irregularly formed dark zone can also be seen on this section.
T = translucent zone
D = dark zone
S = intact surface layer.

In order to investigate the different factors responsible for lesion formation, it was necessary to create artificial caries lesions which were on one hand comparable to natural caries lesions and on the other relatively easy to study.

It became apparent during the course of this study that HEC produced lesions were:

1. regular in shape (no sawtooth effect at the edge of the lesion),
2. parallel to the anatomical surface (no triangle shape),
3. very homogeneous (see Chapter V),
4. highly reproducable (see Chapter VI).

Caries-like lesions produced in HEC solutions have therefore many advantages if compared with other systems.

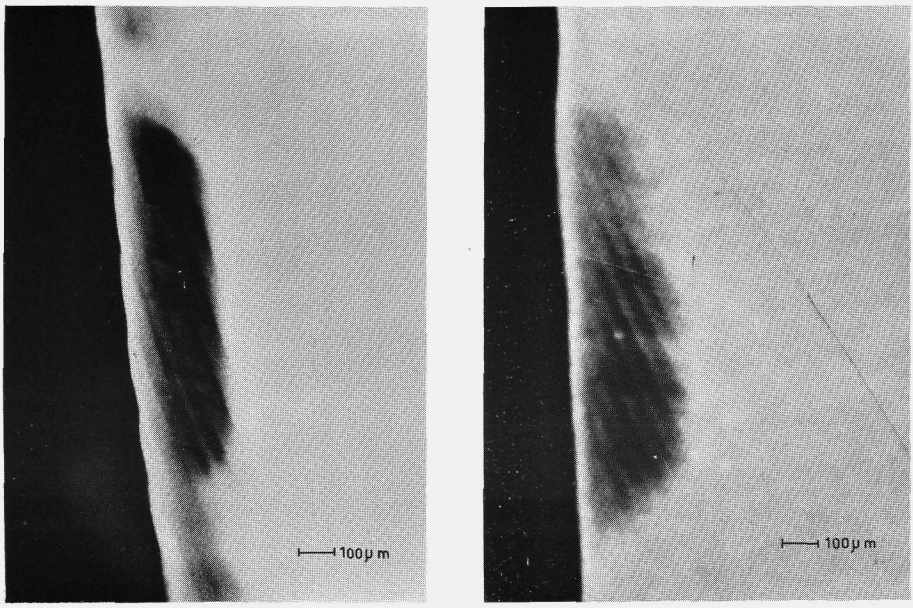


Fig. 10. Microradiogram of a longitudinal section through an artificial caries lesion produces in an HEC solution containing 0.1 M lactic acid adjusted to pH 4 for 96 hours. This figure shows the similarity between an HEC produced lesion and the natural lesion shown in fig. 11.

Fig. 11. Microradiogram of a longitudinal section through a natural caries lesion.

The aim of this investigation

The aim of this investigation was firstly to study the influence of three important parameters: the original mineral content, the pH of the demineralizing solution and the demineralization time on caries lesion formation and secondly to study the caries inhibiting and remineralizing effects of fluoride solutions.

Four lesion characteristics were usually investigated.

1. depth of the lesion
2. thickness of the intact surface layer
3. minimum mineral content in the sub-surface lesion
4. maximum mineral content in the intact surface layer.

A basic parameter in the caries process, which has received remarkably little attention until now, is the original mineral content of the enamel present before the caries lesion was formed. It might be expected that lesion formation is very different in enamel with a high or a low mineral content. The four above mentioned characteristics were therefore studied as a function of the original mineral content (Chapter IV).

The effect of demineralization time and pH are also important parameters,

which influence the four lesion characteristics. Special attention was paid to the correlation between the mineral content in the sub-surface lesion and the depth of the lesion, since the literature was contradictionary on this point (Chapter V).

A technique which enables microradiographic and microprobe measurements to be made along the same pathway was developed to obtain more detailed information about white spot enamel (Chapter VI).

The influence of fluorides on caries inhibition and remineralization is of direct practical importance. The inhibitory effect of two completely different types of solutions, an inorganic (SnF_2) and an organic (aminefluoride) on the four characteristics was therefore investigated (Chapter VII).

Stannous fluoride can also be employed as a remineralizing agent, this aspect was studied in more detail (Chapter VIII).

REFERENCES

APPLEBAUM, E.: The radiopaque surface layer of enamel and caries. J. dent. Res. **19**: 41-46 (1940).

ARENDS, J. and DAVIDSON, C.L.: The HPO_4^{--} content in sound and caries bovine enamel. Calc. Tiss. Res.: (1974) in press.

ARENDS, J.; JONGEBLOED, W.G. and SCHUTHOF, J.: Abstract I.A.D.R., Bruxelles (1974).

BACKER DIRKS, O.: Post eruptive changes in enamel. J. dent. Res. **45**: 503-511 (1966).

BACKER DIRKS, O.: The relation between the fluoridation of water and dental caries experience. Int. Dent. J. **17**: 582-605 (1967).

VON BARTHELD, F.: Membrane phenomena in carious dissolution of the teeth. Arch. oral Biol. **6**: 284-303 (1961).

BERGMAN, G. and ENGFELDT, B.: Studies on mineralized dental tissues II. Microradiography as a method for studying dental tissues and its application to the study of caries. Acta odont. Scand. **12**: 99-132 (1954).

BERGMAN, G. and LIND, P.O.: A quantative microradiography study of incipient enamel caries. J. dent. Res. **45**: 1477-1484 (1966).

BROWN, W.E.: Physicochemical mechanisms of dental caries. J. dent. Res. **51**: 53-57 (1972).

CARLSTRÖM, D.: Polarization microscopy of dental enamel with reference to incipient carious lesions. Advanc. Oral Biol., Vol. I: 255-296 (1964).

CHOW, L.C. and BROWN, W.E.: Reaction of dicalcium phosphate dihydrate with fluoride. J. dent. Res. **52**: 1220-1227 (1973).

CRABB, H.S.M. and MORTIMER, K.V.: Two dimensional microdensitometry. Brit. dent. J. **122**: 337-343 (1967).

DARLING, A.I.: Microstructural changes in early caries. In: Mechanisms of Hard Tissue Destruction (ed. Sognnaes, R.F.) p. 171-186, Amer. Ass. Advanc. Sci., Washington (1963).

VAN ERP, N.A.L.M. and MEYER-JANSEN, A.C.: Een experiment met kleutertandverzorging. Tijdschr. Soc. Geneesk. **44**: 458-467 (1966).

GIBBONS, R.J. and SOCRANSKY, S.S.: Intracellular polysaccharide storage by organisms in dental plaques. Arch. oral Biol. **7**: 73-80 (1962).

GIBBONS, R.J. and BANGHART, S.B.: Synthesis of extracellular dextran by cariogenic bacteria and its presence in human dental plaque. Arch. oral Biol. **12**: 11-24 (1967).

GRAY, J.A. and FRANCIS, M.D.: Physical chemistry of enamel dissolution. In: Mechanisms of Hard Tissue Destruction (ed. Sognnaes, R.F.), p. 213-269, Amer. Ass. Advanc. Sci., Washington (1963).

GUSTAFSON, G.: The histopathology of caries of human dental enamel with special reference to the division of the lesion into zones. Acta odont. Scand. **15**: 13-55 (1957).

VAN DER HOEVEN, J.G.; MIKX, F.H.M.; KÖNIG, K.G. and PLASSCHAERT, A.J.M.: Plaque formation and dental caries in gnotobiotic and SPF Osborne-Mendel rats associated with Actinomyces viscosus. Caries Res. **8**: 211-223 (1974).

HOLLANDER, F. and SAPER, E.: The apparent radiopaque surface layer of the enamel. Dental Cosmos **77**: 1187-1191 (1935).

VAN HOUTE, J.: Iodophilic polysaccharide in bacteria from the dental plaque. Thesis, University of Utrecht, The Netherlands (1967).

HOUWINK, B.; BACKER DIRKS, O. and KWANT, G.W.: A nine-years study of topical application with stannous fluoride in identical twins and the caries experience five years after ending the applications. Caries Res. **8**: 27-38 (1974).

KEYES, P.H. and JORDAN, H.V.: Factors influencing the initiation transmission and inhibition of dental caries. In: Mechanisms of Hard Tissue Destruction (ed. Sognnaes, R.F.), p. 261-283, Amer. Ass. Advanc. Sci., Washington (1963).

KRASSE, B.: Human streptococci and experimental caries in hamsters. Arch. oral Biol. **11**: 429-436 (1966).

KWANT, G.W.; POT, Tj.; GROENEVELD, A. and PURDELL-LEWIS, D.J.: Fluoride toevoeging aan het drinkwater V. Een vergelijking van de gebitstoestand van 17- en 18-jarigen in Culemborg en Tiel. Ned. Tijdschr. Tandheelk. **81**: 251-261 (1974).

LEBER, T. and ROTTENSTEIN, J.B.: Dental caries and its cause. An investigation into the influence of Fungi in the destruction of the teeth. Lindsay and Blakiston, Philadelphia (1867).

MARTHALER, T.M.: Epidemiological and clinical dental findings in relation to intake of carbohydrates. Caries Res. **1**: 222-238 (1967).

MILLER, W.D.: Die Mikroorganismen der Mundhöhle. Thieme Verlag (1892).

MORENO, E.C. and ZAHRADNIK, R.T.: Chemistry of enamel sub-surface demineralization in vitro. J. dent. Res. **53**: 226-235 (1974).

MÜHLEMANN, H.R.: Experimental modifications of the enamel surface. Helv. Odont. Acta **7**: 5-24 (1960).

NEWESELY, H.: Mechanisms and action of trace elements on the mineralisation of dental hard tissues. Zyma SA Nyon Switzerland (1972).

NISHIMURA, T.: Histologische Untersuchungen über die Anfänge der Zahnkaries speziell der Karies des Schmelzes. Schweiz. Mschr. Zahnheilk. **36**: 491-545 (1926).

PLASSCHAERT, A.J.M.: Preventieve maatregelen en gebitsgezondheid bij schoolkinderen van 7-9. Thesis, Kath. University Nijmegen, The Netherlands (1972).

PLASSCHAERT, A.J.M.; KÖNIG, K.G.; VOGELS, A.L.M. and BERGINK, A.H.: Tandcaries bij 5-, 7- en 9-jarige Haagse kinderen in 1969 en 1972. Ned. Tijdschr. Tandheelk. **81**: 129-143 (1974).

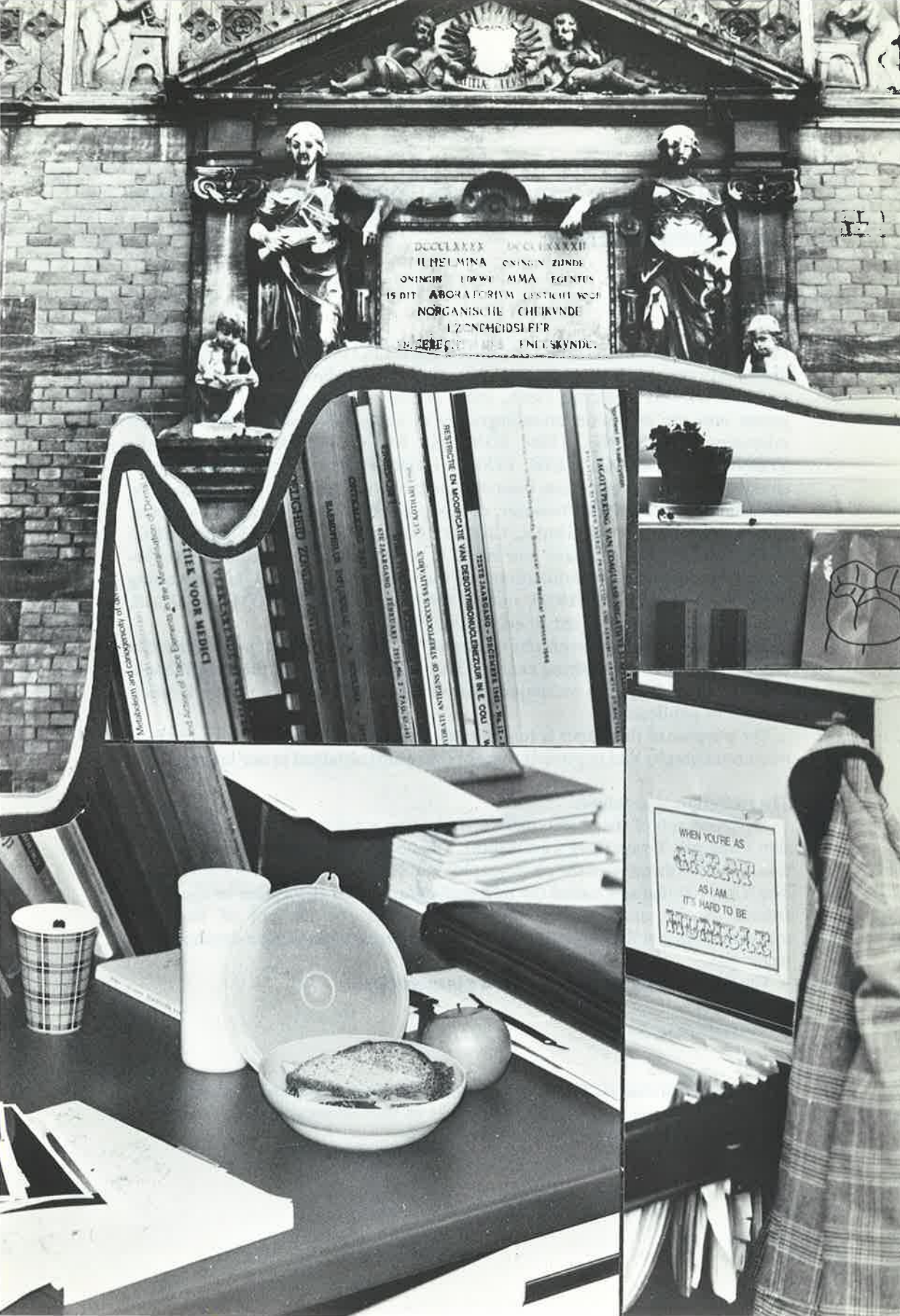
POT, Tj.; GROENEVELD, A. and PURDELL-LEWIS, D.J.: De invloed van 17 jaren drinkwaterfluoridering op het gebit van volwassenen. Een vergelijkend prothesedichtheidsonderzoek in Tiel en Culemborg. Ned. Tijdschr. Tandheelk. **81**: 5-12 (1974).

SILVERSTONE, L.M.: The primary translucent zone of enamel caries and of artificial caries-like lesions. Brit. dent. J. **120**: 461-471 (1966).

SILVERSTONE, L.M.: Observations on the dark zone in early enamel caries and in artificial caries-like lesions. Caries Res. **1**: 261-274 (1967).

SILVERSTONE, L.M. and JOHNSON, N.W.: The effect on sound human enamel of

exposure to calcifying fluids in vitro. Caries Res. 5: 323-342 (1971).
SILVERSTONE, L.M.: Structure of carious enamel including the early lesion. Oral Sciences Rev., Vol. III: 100-160 (1973).
SONI, N.N. and BRUDEVOLD, F.: Microradiographic and polarized light studies of initial carious lesions. J. dent. Res. 38: 1187-1194 (1959).
DE STOPPELAAR, J.D.: Streptococcus mutans, Streptococcus sanguis and dental caries. Thesis, University of Utrecht, The Netherlands (1971).
THEWLIS, J.: The calcification of enamel and dentine. Brit. Dent. J. 62: 303-307 (1937).
WEI, S.H.Y.: Scanning electron microscope study of stannous fluoride treated enamel surfaces. J. dent. Res. 53: 57-63 (1974).
WHEATHERELL, J.A.; ROBINSON, C. and HALLSWORTH, A.S.: Micro-analytical studies on single sections of enamel. In: 'Tooth Enamel' II (ed. Fearnhead, R.W. and Stack, M.V.), p. 31-38, Bristol: J. Wright (1971).
WINKLER, K.C.: Mottled enamel, fluor en tandcaries. Tijdschr. Tandheelk. 53: 1-12 (1946).
WOOD, J.M. and CRITCHLEY, P.: The extracellular polysaccharide produced from sucrose by a cariogenic streptococcus. Arch. oral Biol. 11: 1039 (1966).


WHEN YOU'RE AS
AS I AM,
IT'S HARD TO BE

CHAPTER II

SOME THEORETICAL AND TECHNICAL ASPECTS OF THE STUDY OF ENAMEL USING CONTACT-MICRORADIOGRAPHY AND MICRODENSITOMETRY*

Introduction

Many studies have been carried out since THEWLIS (1940) published his investigation into the application of soft X-rays to enamel studies. The results of SONI and BRUDEVOLD (1959a, 1959b, 1965) are very well known and most investigators now use contact-microradiography in conjunction with polarized light microscopy (DARLING, 1956; SONI and BRUDEVOLD, 1960; SILVERSTONE, 1967 and HOUWINK, 1970). A variety of data on the composition of enamel, dentine and bone have been obtained from studies using soft X-rays. The information obtained was, however, only expressed in a semi-quantitative form. The investigations of ANGMAR, CARLSTRÖM and GLAS (1963) and BERGMAN and LIND (1967) have now made it possible to use contact-microradiography for quantitative estimations of the inorganic material in developing (ANGMAR-MÅNNSON, 1971), sound (ANGMAR, CARLSTRÖM and GLAS, 1963) and carious enamel (BERGMAN and LIND, 1966).

Quantitative microradiography is one of the most sensitive non-destructive methods available for studying enamel. The rather complicated theoretical background and investigatory technique have, however, resulted in a relatively small number of publications.

The purpose of this paper is to give some background information on contact-microradiography and to present some of the results obtained in our laboratory.

The technique is as follows:

A photograph of the object under investigation is taken on fine grained emulsion using soft X-rays (long wavelength). The object, in this case an enamel section, is placed directly on the photographic emulsion (contact-microradiography). The X-ray photograph, called the microradiogram, is an absorption image of the enamel section and contains information about the amount of inorganic material. This can then be estimated in volume percentages using a densitometric technique.

The following points will be discussed in more detail:

1. soft X-rays,
2. absorption of X-rays,

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* already published as: 'Enkele theoretische en technische aspecten van glazuuronderzoek met behulp van contact microradiography en microdensitometry'. A. Groeneveld. Ned. Tijdschrift voor Tandheelkunde 80: 46-55, 1973.

3. densitometric technique,
4. quantitative estimation of inorganic material.

1. Soft X-rays

Radiation is produced by an X-ray tube.* Electrons, discharged when the cathode filament is heated, bombard the anode material which reacts by emitting X-rays. Lightwaves as well as X-rays are wave phenomena; the only difference between the two being that X-rays have a shorter wavelength (0.01-100 Å). Soft X-rays have a wavelength of between 1 and 10 Å. X-rays with a longer wavelength are designated as ultra-soft.

There are two kinds of radiation:
a. white radiation,
b. characteristic radiation.

a. White radiation

When electrons are suddenly stopped in the anode material, energy is released in the form of heat and X-rays with a continuous wavelength spectrum; this is termed white radiation. Each wavelength has its own intensity. This is shown in fig. 1 which gives the intensity and wavelength of the white radiation produced at different kilovoltages. It can be seen that increase in voltage results in a decrease

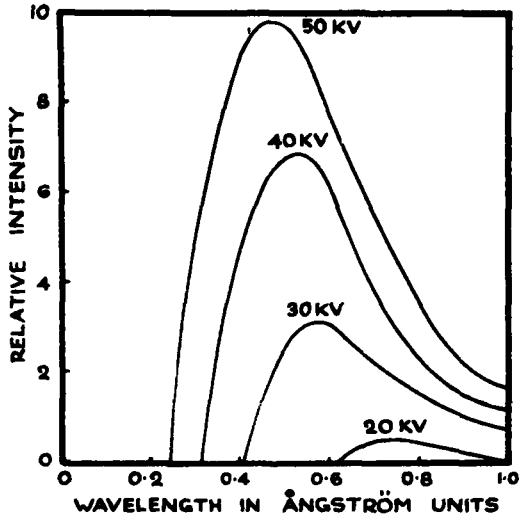


Fig. 1. White radiation: Increase in intensity and shift of the curve towards an area of shorter wavelenght with increasing voltage. (From: Modern Psysics.)

* ENRAF Diffractis 601.

in wavelength (harder radiation) and that the maximum intensity shifts to the shorter wavelengths. The intensity of the white radiation is proportional with the tube current and is thus directly related to the number of milliamperes used. An increase in the beam current increases the intensity without reducing the wavelength.

b. Characteristic radiation

When the speed of the electrons bombarding the anode is sufficiently high (this is adjusted by increasing the voltage), they are capable of removing one of the electrons in the inner shell of the atoms of the anode material. If one of the electrons in the K-shell is ejected, the vacant space will be occupied by an electron from either the L- or the M-shell. The energy difference, between the two energy levels, is emitted in the form of radiation. K_α radiation is emitted when the vacant space in the K-shell is filled by an electron from the L-shell and K_β radiation when the electron is from the M-shell. K_α (composed of K_{α_1} and K_{α_2}) and K_β radiation are called the characteristic radiation of the anode material.

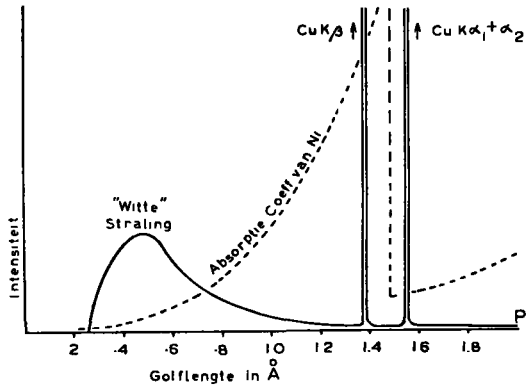
The energy needed to remove an electron from the K-shell increases with the atomic number of the anode material. This means that each anode has its own exciting voltage (table I). An increase in both the voltage and the tube current

Table I. Exciting voltage of some anode metals with the corresponding wavelength of K_α radiation. (Thewlis 1940)

Element	Atomic number	Wavelength (K_α) in Å	Exciting voltage in kV
Chromium	24	2.285	5.4
Manganese	25	2.097	5.9
Iron	26	1.932	6.4
Cobalt	27	1.785	6.9
Nickel	28	1.654	7.5
Copper	29	1.537	8.0
Molybdenum	42	0.708	17.5
Rhodium	45	0.612	20.0
Tungsten	74	0.208	59.0

increases the intensity of the characteristic radiation, but does not alter the wavelength. In quantitative microradiography it is preferable to use monochromatic radiation (one fixed wavelength), because of its constant absorption coefficient. Polychromatic radiation can be used for these determinations (ENGSTRÖM, 1957) by taking advantage of the absorption edge of the material under examination (see point 2), but this is a complicated technique. It is possible to

work with a source emitting only monochromatic radiation, but the use of a polychromatic source and a nickel filter is generally considered to result in an acceptable degree of monochromatic radiation. A nickel filter, 0.02 mm thick, absorps most of the K_β radiation so that the intensity ratio $K_\alpha : K_\beta$ is increased to 600 : 1 (fig. 2).



Spectrum van een buis met koper-anode, bedreven met 50 *kV*. Minimum golflengte volgens (7) = 0.23 Å. De toppen der karakteristieke straling *Kα* en *Kβ* vallen buiten de figuur; de intensiteit van *Kα* is ongeveer 6 × die van *Kβ*. De absorptiecoëfficient van *Kβ* in *Ni* is 6 × die van de *Kα*-straling; dit reduceert na passeren van 0,02 mm nikkel de intensiteitsverhouding van *Kα* en *Kβ* tot 600 : 1.

Fig. 2. X-ray spectrum of a tube with copper anode operated at 50 kV. Minimum wavelength = 0.23 Å. The end points of the curves of the characteristic radiation K_α and K_β are outside the figure. The intensity of K_α is 6 times that of K_β. The absorption coefficient of K_β in Ni is 6 times greater than that of K_α. After Ni-filtration the ratio of the intensities of K_α to K_β is 600 : 1. (From: Röntgenanalyse van Kristallen; J.M. Bijvoet, N.H. Kolkmeyer and C.M. MacGillavry).

2. Absorption of X-rays

The reduction in intensity, which occurs when a monochromatic beam of X-radiation passes through a material, can be calculated using the following formula:

$$X = X_0 e^{-/ut}$$

where X = the intensity of the transmitted X-radiation,
X_0 = the intensity of the incident radiation,
e = the base of the natural logarithm,
t = the thickness of the material,
μ = linear absorption coefficient of the material.

The linear absorption coefficient μ is a constant for a given element, but not for

substances formed by combinations of elements. In absorption calculations the mass absorption coefficient $\frac{\mu}{\varrho}$ (ϱ = specific density of the material) is normally used since it is a constant for any one substance and only varies with the wavelength of the X-radiation. The mass absorption coefficients of hydroxyapatite, organic material and water are expressed as a function of the wavelenght in fig. 3. In the

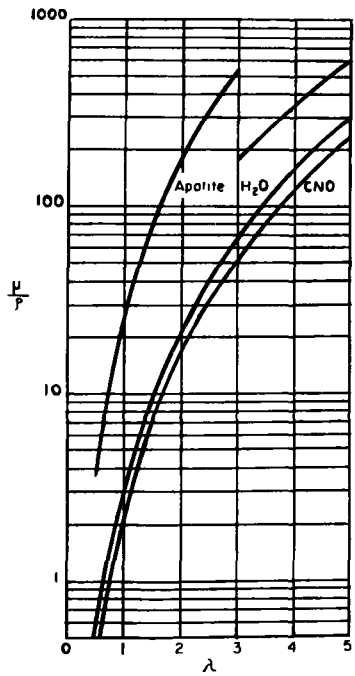


Fig. 3. Mass absorption coefficients of hydroxyapatite, organic material and water as a function of the wavelenght.

wavelength range between 0.5 and 3 Å the mass absorption coefficient of hydroxyapatite is approximately 10 times higher than that of organic material or water. An absorption edge (characteristic for hydroxyapatite) can be seen at a wavelength of 3.07 Å.

X-rays with a wavelength longer than 3.07 Å are less suitable for enamel studies, since the influence of the organic material and water on X-ray absorption is increased.

The anode material of the X-ray tube is usually copper; this has a characteristic K_α radiation of 1.54 Å. A nickel filter is also included in the apparatus to remove K_β radiation and thus provide a monochromatic source. The influence of this filter is shown in fig. 4. Other materials, such as titanium (K_α = 2.74 A) and scandium (K_α = 3.02 Å), were successfully used by GWINNETT (1967).

The photographic emulsion and the film-focus distance are important factors in determining the exposure time and tube voltage. The grain size of the emulsion should be very small, in order to obtain sufficiently high resolution of the micro-

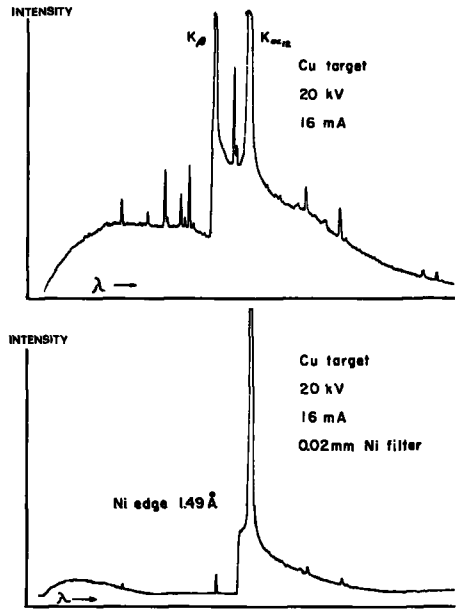


Fig. 4. The effect of Ni-filtration on polychromatic CuK$_\alpha$ radiation.

radiogram. It is also extremely important that the density of the emulsion is linearly correlated to the quantity of X-radiation striking the plate. The density curve of the Kodak High Resolution plates used throughout this study is shown in fig. 5.

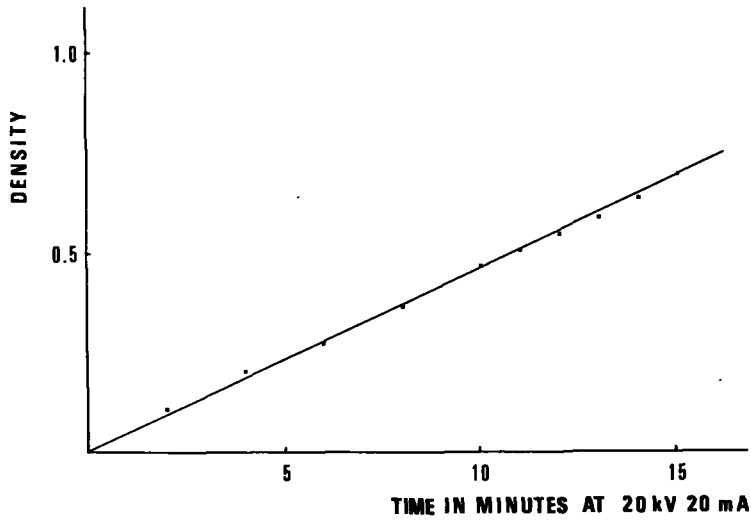
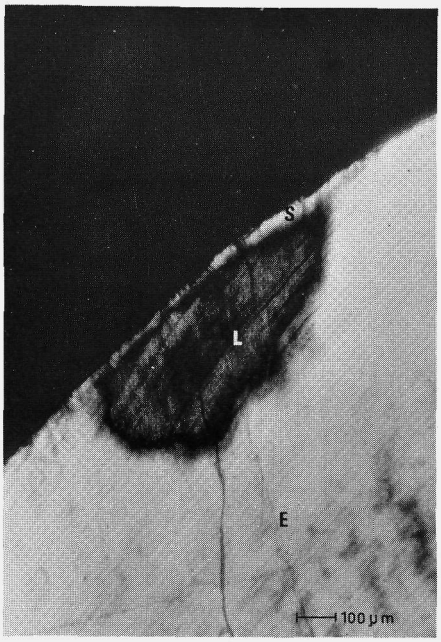


Fig. 5. Density as a function of exposure time. (ENRAF Diffractis 601 with Cuk$_\alpha$ radiation on Kodak High Resolution plates).

The X-ray apparatus was operated at 20 kV and 20 mA, since only a moderate exposure time is required at these settings. To ensure that the beam was relatively homogeneous a film-focus distance of 30 cms was used. HENKE, LUNDBERG and ENGSTRÖM (1957) calculated that the density of a microradiogram, suitable for densitometry, must be between 0.3 and 1.2 with an optimum of 0.7 (the optimum for visual inspection is 1.7). In order to remain within these values, the thickness of an enamel section should not be greater than 80-90 μm. It can be seen from fig. 5 that, under the above mentioned conditions, the optimum density also corresponds with an exposure time of 15 minutes. Figs. 6b and 7b show two microradiograms using these settings. The two photographs taken under polarized light shown in figs. 6a and 7a correspond to the microradiograms in figs. 6b and 7b. The amount of inorganic material present in the enamel specimen can be quantitatively estimated from the microradiogram with the aid of a densitometer.



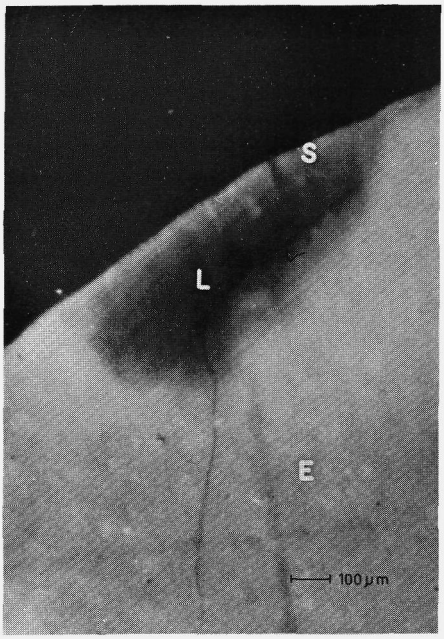


Fig. 6a Photomicrograph of a section through a natural caries lesion taken in polarized light (in water).

Fig. 6b. Contact-microradiogram of the section in fig. 6a.
S = intact surface layer
L = sub-surface lesion
E = sound enamel

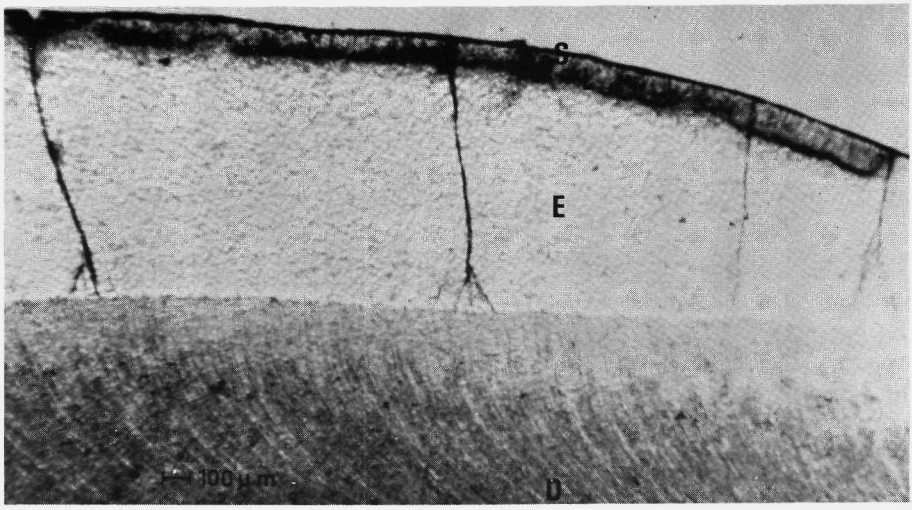


Fig. 7a Photomicrograph of a section with an artificial caries lesion taken in polarized light (in water).
S = intact surface layer
L = sub-surface lesion
E = sound enamel
D = dentine

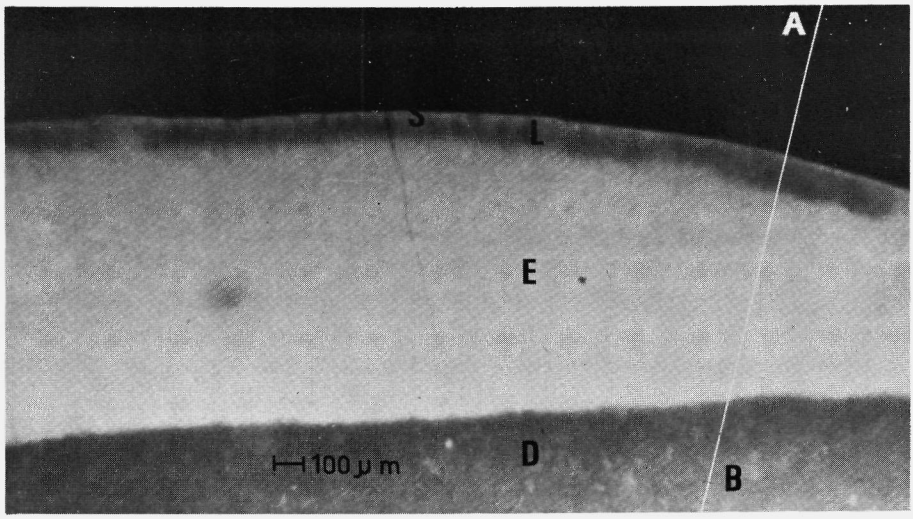


Fig. 7b. Contact-microradiogram of the section shown in fig. 7a.

3. The microdensitometer system

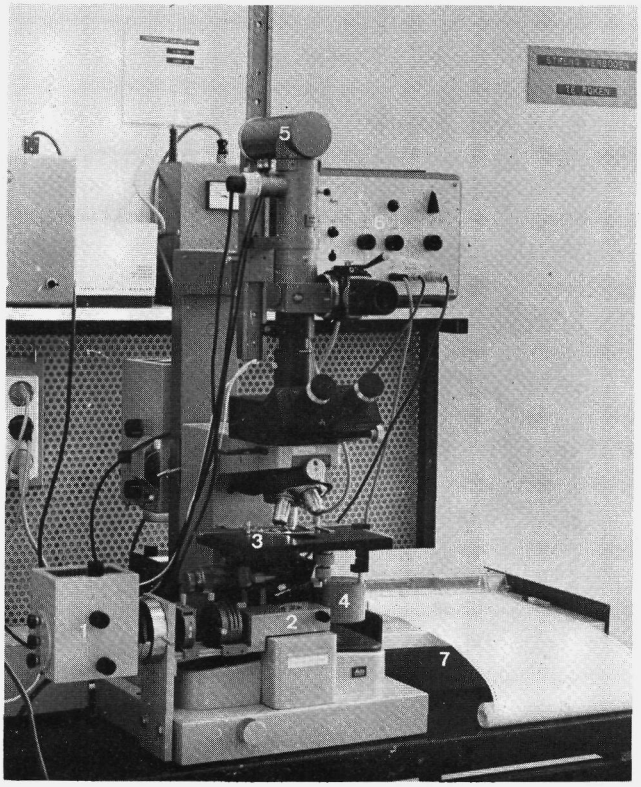


Fig. 8. Leitz MPV densitometer system. A stabilized light beam (from 1) is projected via a mirror (2) through the microradiogram on the object table (3) on to a photomultiplier (5), connected through an amplifier (6) to a recorder (7). A synchronized motor (4) moves the object table with a slow and fixed speed through the light beam.

Figure 8 shows a picture of the Leitz microscope-photometer MPV used in this study. A light beam passes through the microradiogram and falls on a photomultiplier which is connected to an amplifier and a recorder. The examination area is delineated by diaphragm, with an adjustable slit, placed between the microradiogram and the multiplier. The microradiogram is moved at a constant and relatively low speed (30 μm/min) through the light beam in order to obtain a representative density curve. Figure 9 shows a tracing through a caries lesion made along the line A-B of fig. 7b. Although a slit width of 1 by 1 micron is technically feasable, the influence of local differences in the microradiogram (i.e. grain size, emulsion) is then relatively large. A slit width of approximately 10 by 10 microns is therefore the practical minimum (see Chapt. III). LINDSTRÖM

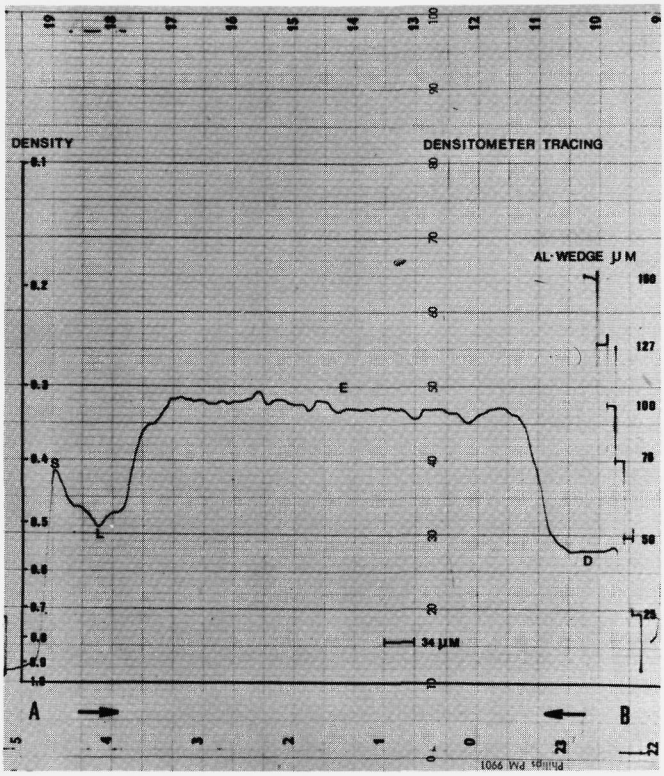


Fig. 9. Densitometric tracing made from points A to B in fig. 7b. The densities of the aluminium step-wedge reference system are on the right hand side of the tracing.
S = intact surface layer
L = sub-surface lesion
E = sound enamel
D = dentine

and PHILIPSON (1969) demonstrated that the error, in a similar densitometric system, was negligible when compared with the variations in the biological material being studied.

4. Quantitative estimation of inorganic material

THEWLIS (1940), WALLGREN (1957) and BERGMAN and LIND (1966) developed techniques for quantitative estimations using monochromatic X-radiation. The method of BERGMAN and LIND (1966) has been used in this study.

The mass absorption coefficient of the enamel specimen can only be approximated (see point 2 above) unless further measurements are made. The potential error is therefore too large for this figure to be used in calculations. To circum-

vent this problem a reference step-wedge system made of aluminium, 25-165 μm thick, each step being approximately 25 μm, is exposed to the X-rays at the same time and on the same photographic plate as the enamel section. Non-systematic errors, caused by the developing technique, are also eliminated using this procedure. The microradiograms are developed for 6 minutes at 20°C, rinsed in dust-free water and dried in a dust-free room.

It is now possible to estimate the density of any point on the microradiogram. The density (D) is the logarithm of the ratio between the incident (I_0) and the transmitted (I) light beam of the densitometer system,

$$D = \log. \frac{I_0}{I}$$

D is proportional to the transmitted X-radiation intensity (X),

$$D \sim X \qquad (1)$$

according to the *absorbtion law*

$$X = X_0 e^{-\mu t} \qquad (2)$$

Taking (1) and (2):

$$D \sim X_0 e^{-\mu t} \qquad (3)$$

or

$$\log. D \sim \log. X_0 - \mu t \log. e \qquad (4)$$

but because X_0 and e are constants:

$$\log. \frac{1}{D} \sim \mu t + constant \qquad (5)$$

it can also be said that for a given D value

$$\mu_a t_a = \mu_s t_s \qquad (6)$$

Here, t_a and t_s are the thickness of the aluminium stepwedge and of the sample respectively. Provided that the X-radiation is sufficiently monochromatic, μ_a will be a constant. If $\mu_a t_a$ is plotted against t_a (fig. 10), it is then possible, using

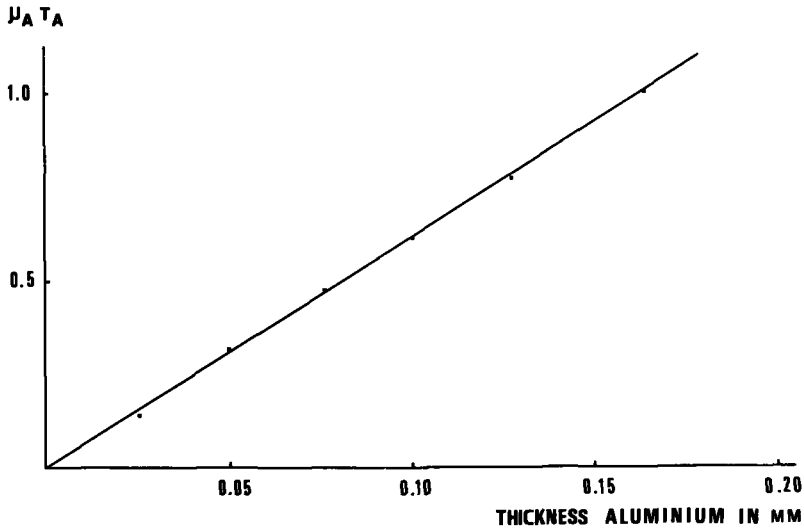


Fig. 10. Absorption of K_α radiation in an aluminium step-wedge, drawn as a function of the thickness of aluminium. The straight curve indicates that the copper K_α radiation used is sufficiently monochromatic.

this calibration line, to find the *corresponding* thickness of the aluminium step-wedge for every point on the microradiogram of the enamel section. ANGMAR (1963) used this thickness for quantitative estimation in volume percentages in the following formula:

$$V = \frac{100\,(131.5\,t_a - 11.3\,t_s)}{(260.5 - 11.3)\,t_s} \text{ in V \%;}$$

131.5 = linear absorption coefficient of aluminium
11.3 = linear absorption coefficient of organic material and water
260.5 = linear absorption coefficient of hydroxyapatite
t_a = effective thickness of aluminium in microns
t_s = thickness of the enamel section in microns

The formula can be simplified for practical reasons into:

$$V\% = \frac{50.48\,t_a}{t_s} \text{ (ANGMAR, 1963)}$$

without introducing any large errors.

A micrometer with an accuracy of 1 μm was used to measure section thickness in this study. The measuring error of a specimen, 70-80 microns thick, was approximately 1.3%. This error could be reduced if thicker specimens were used, but a thickness of more than 90 microns is unacceptable in enamel sections because of the minimum density requirement of 0.3. More accurate measuring systems using β-radiation and the interference lines of monochromatic light have been developed but, until now, have not been completely successful. BERGMAN and LIND measured the thickness under a light microscope by cutting the section perpendicular to the ground surfaces along the densitometric tracing. But, since the section is destroyed, the advantage of an otherwise non-destructive method is lost.

It is important that the ground surfaces of the specimen are planoparallel and as smooth as possible. The enamel sections used in this study were prepared by sectioning the tooth with a sawing machine developed by JANSEN (1950) and grinding and polishing on equipment described by FRIEND and SMITH (1965).

Accuracy of the method

The most important causes of non-systematic errors are:
1. errors in the photographic technique,
2. errors caused by difficulties in relocalizing the measured area,
3. errors in the determination of the thickness of the section (see Chapt. III),
4. errors caused by the densitometric system.

In order to estimate the influence of these errors, a microradiogram was taken, containing both a carious enamel section and a plate of pure gypsum crystal. A property of this gypsum plate is that the surfaces are planoparallel. The linear absorption coefficient of gypsum is 141 and the formula used is as follows:

$V = \frac{100\,(131.5\,t_a)}{141\,t_s}$ = volume percentage inorganic material (gypsum).

t_s = thickness of the gypsum plate.

Table II. Results in volume percentages of the mineral content in *10 microradiograms* containing both a section with an artificial caries lesion and a plate of pure gypsum crystal.

Number	Underlying sound enamel	Minimum mineral content in the lesion	Maximum mineral content in the surface layer	Sound enamel	Gypsum
1	81.3	19.1	56.6	82.2	100.7
2	81.9	19.1	55.5	82.8	99.4
3	85.8	19.6	60.0	86.8	101.9
4	81.9	17.9	60.0	82.8	98.2
5	81.3	19.1	54.4	82.2	99.4
6	84.7	19.1	58.9	85.6	100.7
7	83.6	19.1	60.0	84.5	100.7
8	83.0	19.6	58.9	82.8	100.7
9	81.3	20.2	59.5	82.2	101.9
10	84.1	18.5	62.2	85.1	101.9
Mean	82.9	19.1	58.6	83.7	100.6
Standard deviation	1.6	2.0	2.4	1.6	1.2

Table II shows the results, the mean of the estimations and the standard deviations.

Two densitometric tracings were made through each section, one through the caries lesion and one through the adjacent sound enamel. The same areas were compared in all the sections. A point close to the anatomical surface was chosen in the sound enamel tracing. Three areas were investigated in the caries lesion tracing: the sound underlying enamel, the lowest point in the sub-surface lesion and the highest point in the surface layer (see Chapt. V).

BERGMAN estimated that the standard error of 10 microradiograms of one section of sound enamel was 1.7. Table II, columns 1 and 4, give an indication of the errors in the present study. The mean value in column 4 is 83.7 with a standard deviation of 1.6. The 95% confidence limits of this mean value are 83.7 $\pm$ 1.0. When the estimation of the gypsum plate is considered the errors due to inaccuracies 2 and 3 mentioned above are eliminated. The standard deviation in column 5 is therefore smaller.

The influence of error 2, incorrect localization, is greatest at any point where there are large differences in the quantity of inorganic material in adjacent areas. This may be the case within both the sub-surface lesion and the surface layer of the lesion and is a possible explanation for the larger standard deviations in columns 2 and 3. In most experiments the sections are measured only once, errors due to difficulties in repositioning the microradiogram (point 2) do not therefore occur.

Table III. Results in volume percentages of 10 estimations of the mineral content of a section with an artificial caries lesion and a plate of pure gypsum crystal, *all made from the same microradiogram.*

Number	Underlying sound enamel	Minimum mineral content in the lesion	Maximum mineral content in the surface layer	Gypsum
1	77.5	17.4	58.3	99.4
2	78.5	17.4	59.5	100.7
3	78.5	18.5	57.7	98.2
4	77.5	17.9	59.5	98.2
5	78.5	17.9	60.0	98.2
6	79.1	18.5	57.7	98.2
7	80.2	19.1	59.5	99.4
8	80.8	19.1	59.5	98.2
9	79.1	18.5	57.7	99.4
10	79.1	19.1	57.7	100.7
Mean	78.9	18.3	58.7	99.1
Standard deviation	1.1	0.7	1.0	1.0

Table III shows the results of 10 estimations of the *same area* in the same microradiogram, errors due to points 1 and 3 are therefore eliminated. The standard deviation in column 1 is 1.1 with a mean value of 78.9. The 95% confidence limits of the mean value are, as might be expected, very high at 78.9 $\pm$ 0.7, since it is easier to locate the measured area in only one microradiogram. The standard deviations of columns 2 and 3 in table III are smaller than those in table II for this reason. The last column of table III shows the results of the estimation of the gypsum plate. Errors due to points 1, 2 and 3 are eliminated. It can be seen that the error due to the densitometric system alone (point 4) was found to be 1%.

Summary

Semi-quantitative microradiography is now being used in many studies but only recently has quantitative microradiography been possible through the work of, among other Angmar, Carlström, and Glas; Bergman and Lind.

The theoretical aspects of this method are discussed with special reference to three subjects: soft X-rays, absorption of X-rays and densitometry.

A diffractor (Diffractis 601 Enraf) equiped with a copper anode is used to generate X-radiation. The radiation is monochromated by placing an 0.02 mm thick nickel filter in the beam; CuK_α radiation with a wavelength of 1.54 Å is then obtained.

Microradiograms of enamel sections, 70-80 μm thickness, were taken on Kodak High Resolution plates by 15 minutes exposure at 20 kV and 20 mA, at a film-focus distance of 30 cms. The density of the microradiograms was between 0.3 and 0.7.

Next, quantitative determinations of the inorganic material were carried out by measuring densities in the microradiogram with a densitometer (Leitz MPV). The error of the densitometric system was found to be 1%.

REFERENCES

ANGMAR, B.; CARLSTRÖM, D., and GLAS, J.E.: The mineralization of normal human enamel. J. Ultrastruct. Res. **8**: 12-23 (1963).

ANGMAR-MÄNNSON, B.: A quantitative microradiographic study on the organic matrix of developing human enamel in relation to the mineral content. Arch. oral Biol. **16**: 135-145 (1971).

BERGMAN, G. and LIND, P.O.: A quantitative microradiographic study of incipient enamel caries. J. dent. Res. **45**: 1477-1484 (1966).

DARLING, A.I.: Studies of the early lesion of enamel caries with transmitted light, polarized light and radiography. Br. dent. J. **101**: 289-296 and 329-341 (1965).

ENGSTRÖM, A.; CREULICH, R.C.; HENKE, B.L., and LUNDBERG, B.: High-Resolution contact-microradiography with ultra-soft polychromatic X-rays. In: X-ray-microscopy and microradiography, Coolett e.a., Academic Press, New York, p. 218-233 (1957).

FRIEND, J.V. and SMITH, G.S.: Recent modifications to our technique of preparing thin, flat, polished tooth sections. J. med. Lab. Technol. **22**: 4-6 (1965).

GWINNETT, A.J.: The microradiographic image. J. dent. Res. **44**: 616 (1965).

GWINNETT, A.J.: The ultrastructure of the prismless enamel of permanent human teeth. Arch. oral Biol. **12**: 381-388 (1967).

GWINNETT, A.J.: Scandium as a target material in microradiography. J. dent. Res. **46**: 1479 (1967).

HENKE, B.L.; LUNDBERG, B. and ENGSTRÖM, A.: Proc. Symp. X-ray-Microscopy and Microradiography, Cambridge, England. 1956, p. 240 Academic Press, New York (1957).

HOUWINK, B.: Quantitative polarized-light microscopy of sound surface enamel. J. dent. Res. **49**: 370-377 (1970).

JANSEN, M.T.: An improved method for the preparation of 'serial' sections of undecalcified dental tissues. J. dent. Res. **29**: 401-406 (1950).
LINDSTRÖM, B. and PHILIPSON, B.: Microdensitometer system for microradiography. Histochemic. **17**: 194 (1969).
SILVERSTONE, L.M.: Observations on the dark zone in early enamel caries and artificial caries-like lesions. Caries Res. **1**: 260-274 (1967).
SONI, N.N. and BRUDEVOLD, F.: A microradiographic and polarizing microscopic study of sound enamel. J. dent. Res. **38**: 1181-1187 (1959).
SONI, N.N. and BRUDEVOLD, F.: Microradiographic and polarized light studies of initial carious lesions. J. dent. Res. **38**: 1187-1194 (1959).
SONI, N.N. and BRUDEVOLD, F.: Microradiographic and polarized light studies of artificially produced lesions. J. dent. Res. **39**: 233-240 (1960).
SONI, N.N.: Mineralization of dental tissues as measured by microradiography. Am. J. Med. Electronics **4**: 20-22 (1965).
THEWLIS, J.: The structure of teeth as shown by X-ray examination. Medical research council, Spec. Rep., No. 238 (1940).
WALLGREN, G.: Proc. Symp. X-ray-Microscopy and Microradiography, Cambridge, England, 1956. Academic Press, New York (1957).

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CHAPTER III

THE QUANTITATIVE MICRORADIOGRAPHIC/DENSITOMETRIC TECHNIQUE

Some systematic and non-systematic errors

Quantitative microradiography has, like every other measuring technique, specific limitations caused by the influence of systematic and non-systematic errors.

The most important sources of error in this technique, first classified by BERGMAN and ANNEROTH (1970) in an outstanding publication on the microradiography of oral tissues, will be discussed in this chapter.

A. The variation in intensity of the X-radiation field,
B. The influence of the thickness measurements of the sample (see Chapt. II), (non-systematic errors)
C. The use of the formula of ANGMAR (1963), (systematic error)
D. The errors of the densitometric system caused by the size of the slit, the speed at which the sample is transported through the light beam and the retardation of the photomultiplier/recorder system are also discussed in the chapter.

Note that some of the errors have already been evaluated and discussed in the preceding chapter.

A. The variation in intensity of the X-radiation field

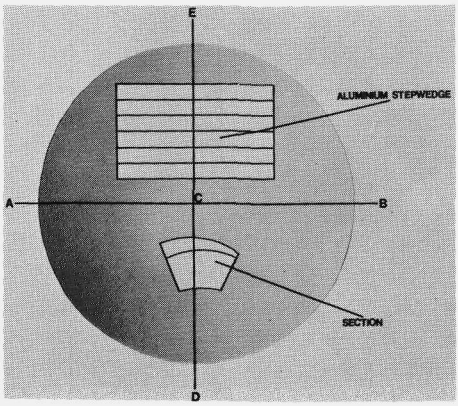


Fig. 1. Variation in intensity of radiation. The differences in darkening of the background circle indicate that the X-ray beam is inhomogeneous. The inhomogeneity is symmetrically distributed along the line D-E. When measuring along this line through the section and the step-wedge, the influence of the inhomogeneity is negligible (less than 0.01 in density difference).

Figure 1 shows a schematic drawing of the variation in intensity of the X-ray field produced by the apparatus (ENRAF Diffractus 601) used in this study. The differences in darkening of the background circle represent the inhomogeneity of the X-ray beam. The variation in intensity is symmetrically distributed along the line A-B, but decreases in intensity when either the film-focus distance or the diameter of the focal spot are increased. The focal spot should be as small as possible to minimize geometrical blurring (penumbra). It should not, however, be too small since this would result in an unreasonably long exposure time. The film-focus distance should also not be too long for the same reason. The best possible operating conditions were found to be a normal focus spot of 1×10 mm^2 and a film-focus distance of 30 cms. The exposure time was then approximately 15 minutes and the darkening of the emulsion was similar to that shown in fig. 1.

The darkening of the photographic emulsion is not homogeneous, although the differences are small. If the density at point C (fig. 1) is taken to be 0.7 then the density at point B is never less than 0.6 and the density at point A never greater than 0.8. The line D-E (and every line parallel to D-E) represents a line of equal density; the difference in density along the line D-C-E being approximately 0.01. The enamel section and the aluminium stepwedge are always placed on the emulsion as shown in fig. 1. The variation in density due to the inhomogeneity of the X-radiation field is almost entirely eliminated when the densitometric tracings are made parallel to the line D-E. It is therefore extremely important to standardize the position of the camera, section and reference wedge on the X-ray unit.

B. The thickness of the section

The thickness of the sections used in this study was measured using a micrometer with an accuracy of 1 μm. Several determinations in the same place on one section showed that the differences never exceeded 0.6 μm. For the estimation of the influence of this error on the quantitative data of the mineral content see Chapter II.

It is technically impossible to measure the thickness at exactly the same point as that analysed in the densitometric tracing. This disadvantage can be overcome by using plano-parallel sections and by taking the mean of a number of measurements. Only sections which differed 15 μm or less in thickness over a distance of 1500 μm were considered to be plano-parallel. The depth of the HEC produced lesions used in this study was approximately 150 μm. The difference in thickness over this distance was less than 1.5 μm. The error in the 70 μm sections used in these experiments is thus less than 2%.

Another important factor to be considered when dealing with section thickness is that, during the grinding and polishing procedure, selective removal of the lesion material (lesion material is softer) or rounding of the edge of the section (accumulation of slurry) may occur. To investigate this effect samples were prepared by cutting sections through the lesion perpendicular to the polished surfaces. Examination under the light microscope (400 ×) showed no evidence of

selective polishing of lesion material in 10 sections demineralized for 96 hours at pH 4. This indicates that the effect of selective removal was less than 0.5 μm.

The edges of the sections did, however, show a decrease in thickness of the outer 10 μm of between 2 and 3 μm. When three of four sections were polished at the same time, with the experimental surfaces adjacent to each other, this adverse grinding effect could no longer be observed.

C. The quantitative estimation of the mineral content

The formula $V = \frac{100\,(131.5\,t_a - 11.3\,t_s)}{(260.5 - 11.3)\,t_s}$ in V%; proposed by ANGMAR (1963) is often used in quantitative microradiographic work (see Chapter II).

It must be emphasized that, when using this formula for quantitative estimation, the numerical values obtained are based on the assumption that *all* the mineral present in the lesion or sound enamel is stoïchiometric hydroxyapatite with formula $Ca_{10}(PO_4)_6(OH)_2$. This is because the linear absorption coefficient of hydroxyapatite (260.5) is used in this formula. In sound enamel, where almost all mineral is hydroxyapatite, this assumption is correct. But in the sub-surface lesion and the surface layer of the lesion, other minerals can be formed and/or precipitated. From the investigations of BROWN (1973), MORENO and ZAHRADNIK (1974) and ARENDS and DAVIDSON (1974) it can be concluded that these are mainly phosphates, probably of the brushite type, $CaHPO_4\,2H_2O$. The linear absorption coefficient of brushite (141) is much smaller than that of hydroxyapatite (260.5). The total quantitative value of mineral is therefore underestimated in both the sub-surface lesion and the surface layer.

The numerical values of lesions, which are remineralized with heavy metal ions (SnF_2) (see Chapter VIII) are, in contrast, overestimated because of the very high linear absorption coefficients of heavy metals.

D. The densitometric tracing

The accuracy of the densitometric tracing or more correctly the density graph, depends on three factors: 1) the slit width, 2) the speed at which the microradiogram is moved through the light beam and 3) the retardation caused by the photomultiplier/recorder system.

The surface layer covering the sub-surface lesion, varies from 20 to 40 μm in thickness. The slit width, measured perpendicular to the anatomical surface, must not therefore exceed 20 μm, if information about the surface layer is to be obtained. Theoretically the slit width should be as small as possible to register every variation in density in the surface layer, but for practical reasons it is not feasible to use a slit width of less than 6 μm.

The slit width, measured parallel to the surface layer, should cover as large a part of this zone as possible to obtain a representative figure of the total surface

layer. The maximum width depends on the shape of the anatomical surface. The curvature of the surfaces of the human premolars selected for this study, allowed a slit width of 100-150 μm to be used. This is shown pictorially in fig. 2 where it can be seen that 10 μm inwards from the anatomical surface the slit is completely over the surface layer.

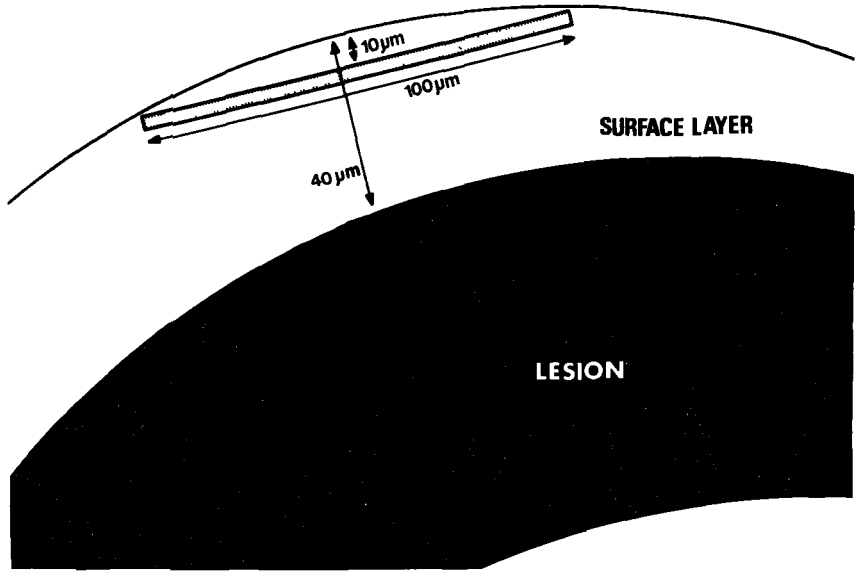


Fig. 2. Schematic drawing of the densitometer slit in a 40μm thick surface layer of an artificial lesion. After moving over a distance of 10μm the whole slit width of 100μm is positioned within the surface layer.

The Leitz MPV densitometer used in this study, is equiped with a drive mechanism for moving the samples at a speed of 30 μm/sec through the light beam. It was established that the shape of the densitometric curve is not influenced by this speed. The highest and lowest points of the densitometric tracing were found to be the same as those obtained by manual operation of the densitometer.

The right hand side of fig. 3 shows a normal densitometric tracing through a lesion. The vertical lines on the left hand side of this figure were obtained by moving the same microradiogram manually through the densitometer. Using the horizontal lines of the recording paper by the number and the paired numbers which indicate similar measuring positions on the two curves, it can be seen that the density values were unaffected by the mode of operation. It can therefore be concluded that it is extremely unlikely that the horizontal position of the curve is influenced by the relatively low speed of the sample drive mechanism.

The retardation of the photomultiplier/recorder system should be as small as possible. This means that, with a given sample speed and slit width, there should be no difference when the density at a point on the tooth, is estimated with the

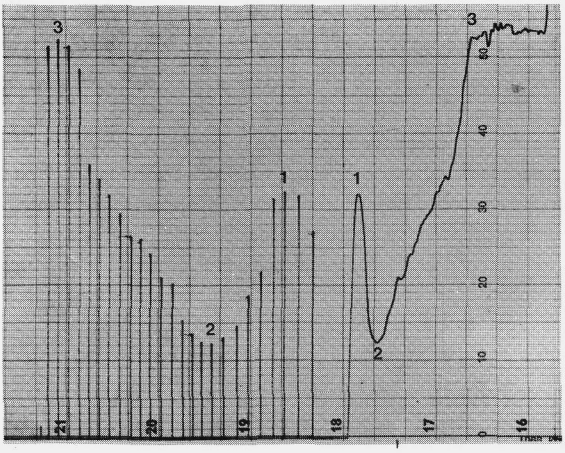


Fig. 3. Static (left side) and dynamic (right side) densitometric tracing. Points indicated by the numbers on the curves on the left and right side represent similar positions on the tracings. The vertical scale (= density) is the same in both sides of the figure. One division is equal to 34 μ m in the horizontal scale on the right hand side. The scale has been arbitrarily expanded in the left hand side.

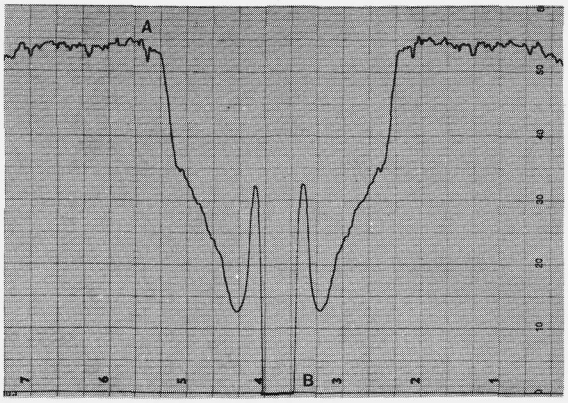


Fig. 4. Two densitometric tracings of the same section have been made here, one is the reverse of the other. The tracings are exactly the same, demonstrating that it makes no difference to the densitometric system whether the measuring procedure starts at A (point of low density) or at B (point of high density). The vertical scale represents the density.

densitometer scan coming from an area of either high or low density. Figure 4 shows that the density graph of a lesion, starting at a point of high density (B), made with the experimental set-up used in this study, is the same as the reversed graph starting at a point of low density (A). This result justifies the conclusion that the retardation of the photomultiplier/recorder system is sufficiently small.

REFERENCES

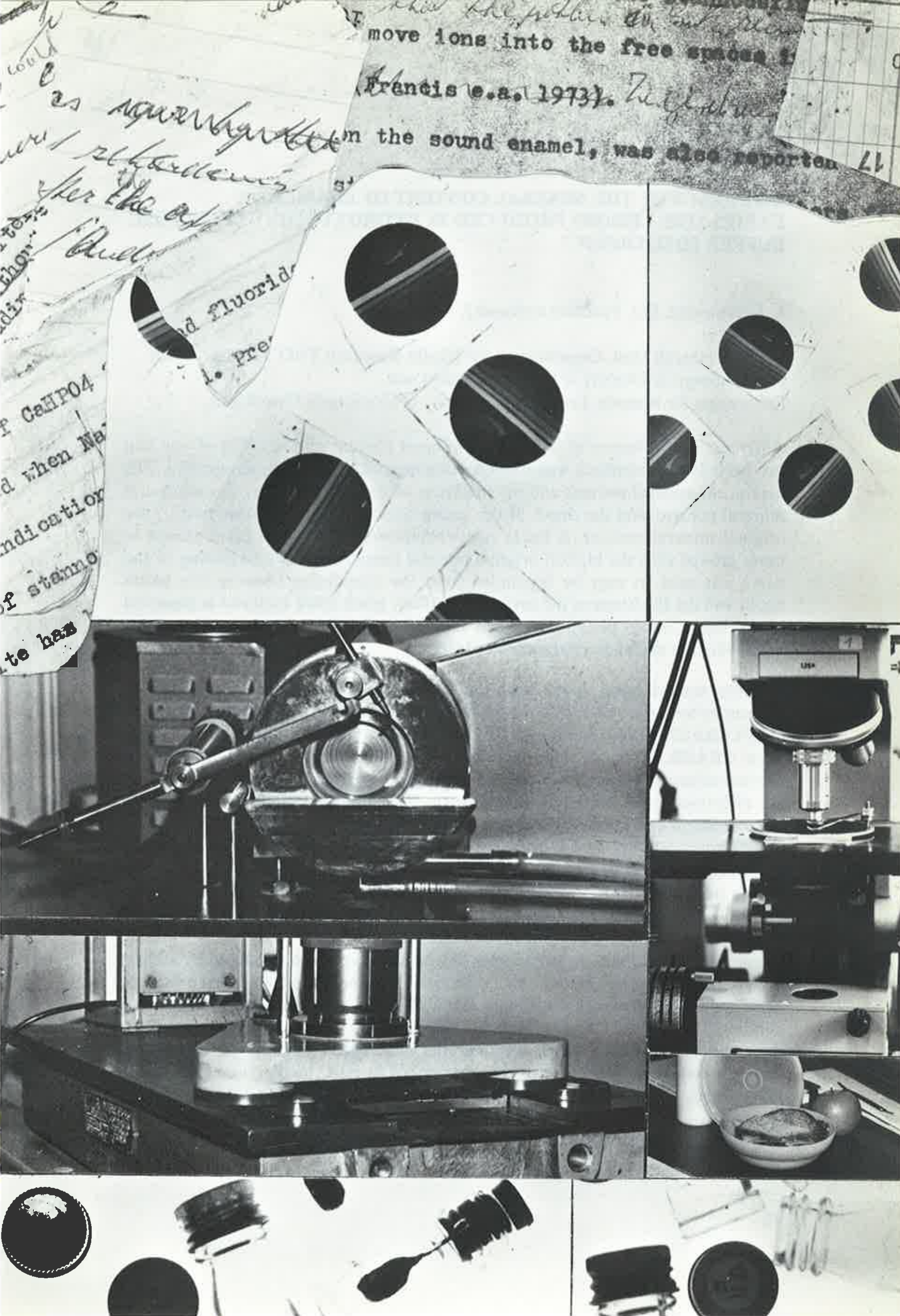
ANGMAR, B.; CARLSTRÖM, D., and GLAS, J.E.: The mineralization of normal human enamel. J. Ultrastruct. Res. 8: 12-23 (1963).

ARENDS, J. and DAVIDSON, C.L.: The HPO_4^{--} content in sound and caries bovine enamel. Calc. Tiss. Res.: in press (1974).

BERGMAN, G. and ANNEROTH, G.: Microradiograhy of oral tissues. In: Advances of Oral Biology, Vol. 4: 313-368 (Academic Press, New York, 1970).

BROWN, W.E.: Physicochemical mechanisms of dental caries. J. dent. Res. 53: 204-216 (1974).

MORENO, E.C. and ZAHRADNIK, R.T.: Chemistry of enamel sub-surface demineralization in vitro. J. dent. Res. 53: 226-235 (1974).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CHAPTER IV

INFLUENCE OF THE MINERAL CONTENT OF ENAMEL ON CARIES-LIKE LESIONS PRODUCED IN HYDROXYETHYL CELLULOSE BUFFER SOLUTIONS*

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Laboratory for Materia Technica, University of Groningen, Groningen

Abstract. The influence of the original mineral content on the effect of one and two periods of acid attack was studied using quantitative microradiography. The maximum mineral content and the thickness of the surface layer, the minimum mineral content and the depth of the lesion were depicted as a function of the original mineral content. A linear relationship was found in all cases except in those groups with the highest original mineral content, where a flattening of the curve was seen. It may be concluded from the correlation between the lesion depth and the thickness of the surface layer that, when more material is dissolved in the lesion, more precipitation can take place near the intact surface, thus increasing the thickness of the surface layer.

Many studies have shown that the caries-like lesions have a low mineral content in both the sub-surface lesion and in the surface layer covering the lesion (APPLEBAUM, 1940; SONI and BRUDEVOLD, 1960; BERGMAN and LIND, 1966; CRABB, 1968) as compared to the sound underlying enamel. The degree of demineralization depends not only on the severity of the acid attack, but also on the effectiveness of the defence mechanism, for example plaque formation and the structural and chemical characteristics of the enamel. BRUDEVOLD *et al.* (1968) suggested that a high degree of mineralization is one of the factors which results in a high caries resistance.

The purpose of this investigation was to study, by quantitative microradiography, the influence of the original degree of mineralization of the sound enamel on the effect of one and two periods of acid attack.

For the past 10 years, *in vitro* caries-like lesions have been produced by adding high molecular biopolymers to acid solutions. Two main types have been used in these studies: hydroxyethyl cellulose (HEC) (GRAY and FRANCIS, 1963) and gelatin (VON BARTHELD, 1961; SILVERSTONE, 1971). Gelatin media produce caries-like lesions which resemble the histological appearance of natural caries lesions more closely than those produced in HEC media. The HEC solutions have, however, the advantage of creating highly reproducable lesions

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(GROENEVELD and ARENDS, 1974) which are parallel to the anatomical surface.

Materials and methods

In this study, quantitative microradiographical data of 87 premolars were evaluated. In early experiments, the premolars were *all* treated in the following way. They were carefully cleaned with pumice and covered with blue inlay wax, with the exception of an 8 mm^2 window on the buccal surface. 40 of these teeth were then partly demineralized by immersion in individual 10-ml buffer solutions, at pH 4, containing 0.1 M lactic acid and 6% HEC for 24 h at 20°C. This group will hereafter be referred to as the 'white spot enamel group'. The remaining 40 untreated macroscopically sound teeth will be referred to as the 'sound enamel group', *irrespective of further treatment.*

Subsequently, *all* the teeth were treated with fresh buffer solutions, at pH 4.5, for 96 h at 20°C. Two groups were thus created, one group of 47 which was demineralized once and one group of 40 which was treated twice. Longitudinal sections of these lesions were ground planoparallel to a thickness of between 70 and 80 μm. Microradiograms were then taken and densitometric tracings made through the middle of the lesions, using a standardized technique (GROENEVELD, 1973).

Quantitative estimation of the minimum mineral content in the body of the lesion (V_L) and the maximum mineral content in the surface layer (V_{SL}) was carried out using the formula (ANGMAR *et al.*, 1963):

$$V\% = \frac{50.48\ t_a}{t_s} = \text{volume percentage mineral (hydroxyapatite)},$$

where t_a = effective thickness of aluminium and t_s = thickness of sections.

The thickness of the surface layer and the depth of the lesion were measured directly from the densitometric tracings (for details, see GROENEVELD and ARENDS, 1974).

Teeth from both the 'sound enamel group' and the 'white spot enamel group' were divided into five subgroups, each with a different mineral content in the underlying sound enamel (table I). The same two groups were later divided into five different subgroups according to the lesion depth (table II).

A separate experiment was carried out to study the homogeneity of lesions created using HEC buffer solutions. 24 sections were selected at random from the 'sound enamel group', and in all these sections three densitometric tracings were made instead of one.

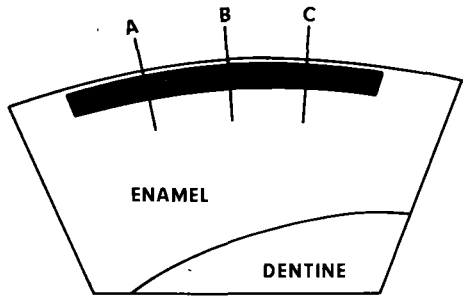
Table I. Number, ranges and mean values of the mineral content of the underlying sound enamel of five subgroups of both the 'sound enamel' and the 'white spot enamel' group; results after demineralization at pH 4.5 for 96 h at 20°C

Group number	Range of mineral content in sound underlying enamel, V%	Mean of V% in each group	Mean minimum content in the lesion V%	Mean maximum mineral content in surface layer, V%	Thickness of surface layer μm	Depth of the lesion μm	Number of teeth
Sound enamel							
1	> 89	92.8±0.9	67.4±2.8	75.0±3.7	38±4	114±5	10
2	87-89	88.0±0.2	66.2±1.8	71.3±2.0	32±4	118±4	8
3	84-87	85.6±0.3	60.7±2.6	70.1±2.0	32±2	123±5	11
4	81-84	82.4±0.3	57.4±2.4	66.2±1.4	37±3	124±4	9
5	< 81	78.3±1.1	51.6±2.4	60.0±2.2	31±4	121±7	9
White spot enamel							
1	> 89	91.8±0.7	35.1±3.7	46.0±2.9	31±3	105±10	7
2	85-89	86.6±0.5	43.5±2.6	50.9±1.8	35±4	107±10	9
3	82-85	84.1±0.3	36.1±3.3	42.7±3.6	30±3	109±9	7
4	80-82	81.1±0.2	34.4±4.1	42.3±3.6	28±3	109±8	8
5	< 80	77.4±0.8	28.1±3.3	39.2±2.5	27±2	113±7	9

Table II. Number, ranges and mean values of the lesion depths of five subgroups of both the 'sound enamel' and the 'white spot enamel' group; results of the mean thickness of the surface layer of each subgroup

Group number	Lesion depth, μm	Mean of lesion depth in each group	Mean thickness of surface layer	Number of teeth
Sound enamel				
1	>129	141±3	43±4	9
2	123-129	127±1	39±2.5	8
3	119-123	121±1	35±3	9
4	110-119	114±1	27±2	8
5	<110	100±4	29±1.5	11
White spot enamel				
1	>136	144±3	38±1.5	8
2	115-136	126±2	33±3	7
3	105-115	109±1	32±2.5	8
4	95-105	100±1	29±2.5	7
5	<95	84±2	22±1.5	10

Results



MEAN OF 24 sections	SURFACE LAYER	LESION	SOUND ENAMEL
A	69.0 ± 1.5	61.7 ± 2.0	85.3 ± 1.5
B	69.8 ± 2.0	62.6 ± 2.2	85.9 ± 1.3
C	66.4 ± 1.8	61.1 ± 2.2	84.7 ± 1.1

Fig. 1. The positions and results of three densitometric tracings A, B and C through a microradiogram. The data of the sound underlying enamel, the maximum mineral content of the surface layer and the minimum mineral content of the lesion are mean values of 24 different sections.

Figure 1 gives the results of the above-mentioned experiment and shows schematically the positions where the three tracings A, B and C were made through the lesions. The mineral content of the underlying sound enamel was calculated as was the maximum mineral content in the surface layer and the minimum content of the sub-surface lesion. The data shown are the mean of 24 observations on different teeth. The results indicate that lesions created in HEC buffer solutions are parallel to the anatomical surface and have a high degree of homogeneity (fig. 2). It is thus possible to obtain a representative densitometric tracing of a caries-like lesion taking one single midline reading.

Figure 3 is a scatter diagram of the vol% of the minimum mineral content in the lesion of the sound enamel groups expressed as a function of the mineral content of the sound underlying enamel. Figure 4 is a similar diagram of the data of the white spot enamel groups. The data giving the maximum mineral content in the surface layer of all groups are presented more simply in fig. 5 using only the mean values of each group.

The depth of the lesion and the thickness of the surface layer are also interesting parameters, if plotted as a function of the mineral content of the underlying enamel. The data in table I indicate that, as the mineral content values of the sound underlying enamel increase, the lesion depth tends to decrease. In contrast, the surface layer shows a tendency to increase with increasing mineral content of the sound underlying enamel. These data are however *not* statistically significant. When all the data are combined, it is possible to check whether there is a correlation between lesion depth and surface layer thickness. The thickness of the surface layer was plotted against the lesion depth in fig. 6. When drawing this graph, teeth were rearranged in five groups, according to the depth of the lesion.

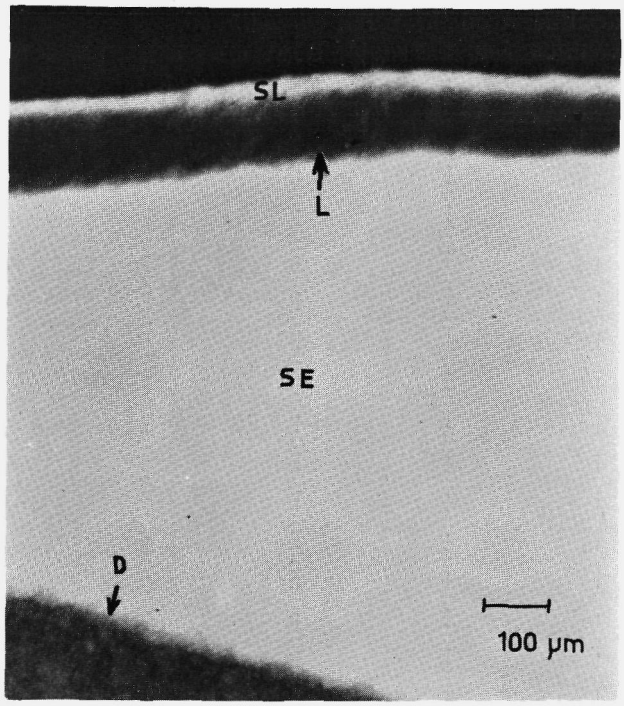


Fig. 2. Representative microradiogram of lesions created in hydroxyethyl cellulose buffer solutions. Note the homogeneity of the lesion and the parallelness to the anatomical surface. SL = Surface layer; L = sub-surface lesion; SE = sound enamel; D = dentine.

Discussion

It can be seen from fig. 1 that demineralization with HEC buffer solutions creates not only reproducible parallel sub-surface lesions, but also lesions which are very evenly decalcified. One densitometric tracing through the middle of the lesion gives a representative impression of the total lesion. For these reasons, HEC buffer solutions have many advantages over acidified gelatin solutions in physico-chemical experiments. Furthermore, if gelatin is used, for example in studies on the inhibiting effects of fluorides and phosphates, the results may be biased by the considerable amounts of these substances sometimes present in gelatin (WÖLTGENS, personal commun., 1974).

Several studies (SONI and BRUDEVOLD, 1959; ANGMAR *et al.*, 1963; GROENEVELD *et al.*, 1974) on sound enamel have shown that there is a slight increase in mineral content from the dentino-enamel junction outwards. Furthermore, BAUD and LOBJOIE (1966) have recently shown that, using microprobe and/or microradiography, there is no hypermineralized layer at the anatomical surface of the enamel as suggested in the past (THEWLIS, 1940).

The drop in mineral content over a distance of about 1500 µm from surface to

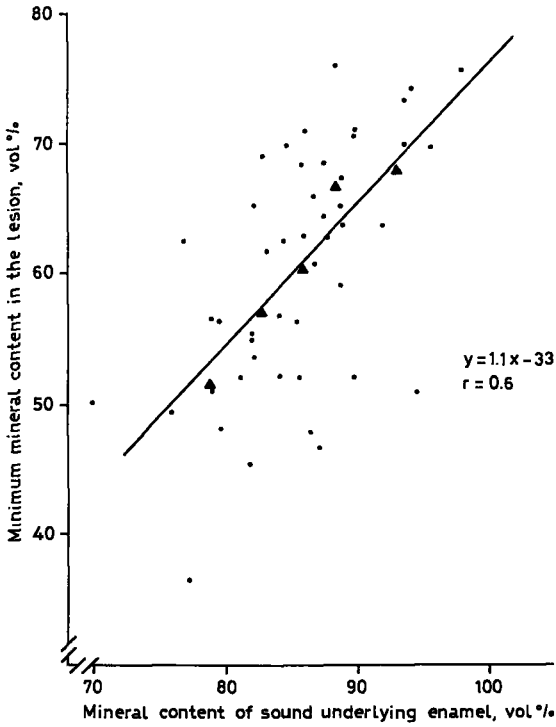


Fig. 3. Sound enamel group. Scatter diagram and regression line of the minimum mineral content of the lesion and the mineral content of the sound underlying enamel. Triangles indicate the mean values of the five subgroups of table I. The mineral content is given in volume percentages.

the dentino-enamel junction is about 4% by volume. This indicates that the mineral content at 200 μm below the anatomical surface (the distance of the most advanced lesion in this study) may be taken as approximating very closely with the mineral content of the surface.

Sound Enamel Group

The results as shown in fig. 3 and 5 indicate that after *one* period of demineralization the mineral content of the lesion and the surface layer appears to be linearly related to the amount of mineral *originally* present in the enamel.

If we denote the minimum mineral content in the lesion, the mineral content of the surface layer and the mineral content of the underlying sound enamel by $V_L\%$, $V_{sL}\%$ and $V_s\%$ respectively, the following equation can be written:

$$V_L\% = 1.1\ V_s\% - 33\%,$$

with r = 0.6, and

$$V_{sL}\% = 1.1\ V_s\% - 26\%.$$

with r = 0.6, where r is the correlation coefficient. The r values indicate that, when n = 47, the probability that r is not different from zero is less than 0.005.

The results demonstrate that the mineral content in the lesion and the surface layer can be directly correlated to the original mineral content of the enamel as suggested by BRUDEVOLD *et al.* (1968).

A theoretical linear relationship between $V_L\%$ or $V_{sL}\%$ and $V_s\%$ has been suggested by ARENDS and GROENEVELD (1974).

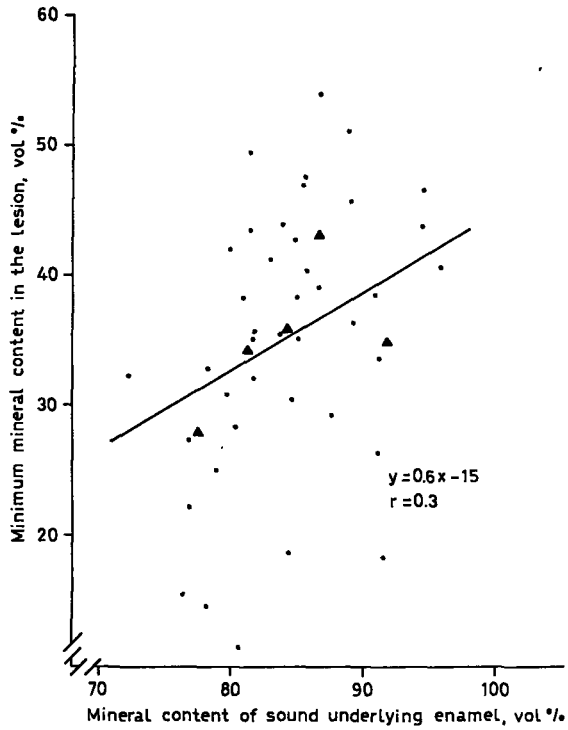


Fig. 4. White spot enamel group. Scatter diagram and regression line of the minimum mineral content of the lesion and the mineral content of the sound underlying enamel. Triangles indicate the mean values of the five subgroups of table I. The mineral content is given in volume percentages.

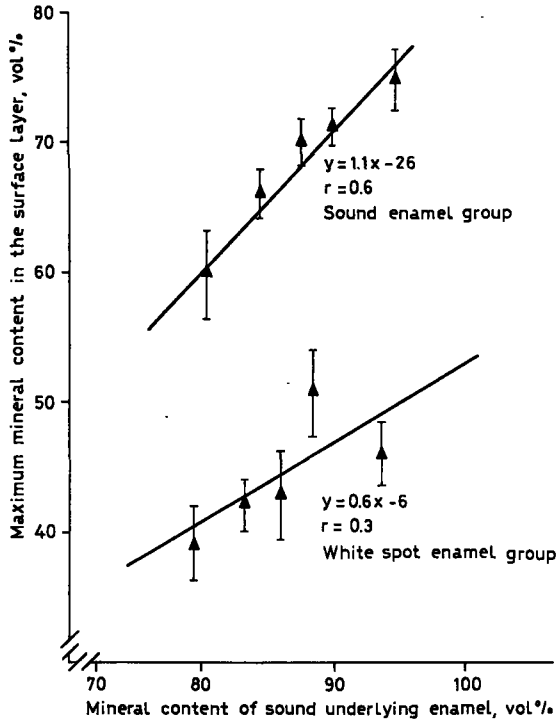


Fig. 5. The maximum mineral content of the surface layer as a function of the original mineral content of the enamel, for the sound and white spot enamel group. Triangles indicate the mean values of the five subgroups of table I. The mineral content is given in volume percentages.

White Spot Enamel Group

In vivo enamel is subject to numerous acid attacks. The white spot enamel group resembles the situation in the oral cavity more closely than the sound enamel group. The reason for the behaviour of the white spot group is not very clear, because this phenomenon cannot be due simply to a chemical or structural pattern in the enamel itself, but must also be influenced by precipitation of insoluble calcium phosphates from the first period of demineralization.

Two approaches are possible when considering the data of the white spot enamel group given in fig. 4 and 5. Firstly, if *all* the observations are taken into account:

$$V_L\% = 0.6\ V_s\% - 15\%,$$

with $r = 0.3$, and

$$V_{sL}\% = 0.6\ V_s\% - 6\%,$$

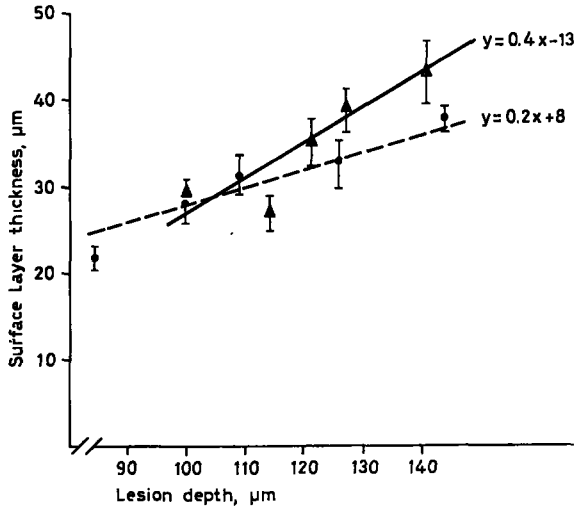


Fig. 6. Thickness of the surface layer plotted against the depth of the lesion. Dots and triangles indicate the mean values of the five subgroups of table II, for the white spot (---) and sound enamel (———) groups respectively.

with $r = 0.3$. Secondly, if only those observations, where the mineral content was smaller than 89 vol%, are considered:

$$V_L\% = 1.5\, V_S\% - 88\%,$$

with $r = 0.5$, and

$$V_{SL}\% = 1.1\, V_S\% - 47\%,$$

with $r = 0.5$.

When the results of these two approaches are compared, it can be seen that the linear dependence is statistically significant at the 5-percent level, if all data ($n = 40$) are considered. If, however, only those results ($n = 33$), where the V% mineral of the original sound enamel was less than 89%, are considered, the linear dependence is already statistically significant, for $p < 0.005$.

From the above equations and figs. 3-5 it can be seen that the second approach is most probably correct, although it is not possible to make a definite choice. An indication of this may be that, if only the lower 4 points on all the curves are considered, the slopes are nearly identical. This would indicate that with a very high mineral content in enamel (> 89 V%), demineralization is more pronounced than in average (87 V%) enamel. The same result, although less pronounced, can be observed in the sound enamel group. The reason may be that in lesion formation the structure of enamel is of particular importance when the original content is above 87 V%. Normal sound enamel (average mineral content of 87 V%) contains about 2% of organic material and 11% of water by volume (BRUDEVOLD and SÖREMARK, 1967). In enamel which has about 92% mineral by volume, the

organic material and/or water content must be considerably lower. Work, in progress, on the specimens used in this study has shown that highly mineralized enamel has a high water content.

Fig. 6 demonstrates that there is a positive correlation between lesion depth and thickness of the surface layer. It is possible to speculate that, in a caries lesion, part of the dissolved material precipitates in or near the intact surface covering the lesion as suggested previously by several workers. To a certain extent, it may be said that, when more material is dissolved in the lesion, more precipitation can take place, thus thickening the surface layer. Unfortunately, there appears to be little information on this interesting point.

Summarizing it can be said that (1) the original mineral content of the tooth directly determines the mineral content of the lesion and (2) the thickness of the surface layer increases with lesion depth.

Acknowledgements

The authors are indebted to Prof. Dr. P.G. de HAAN for his great interest and critical support, to Mr. C.A. CHAMBONÉ for the technical assistance, and to Miss L. BAUER for preparing the manuscript.

REFERENCES

ANGMAR, B.; CARLSTRÖM, D., and GLAS, J.E.: Studies on the ultrastructure of dental enamel. J. Ultrastruct. Res. **8**: 12-23 (1963).

APPLEBAUM, E.: The radiopaque surface layer of enamel and caries. J. dent. Res. **19**: 41-46 (1940).

ARENDS, J. and GROENEVELD, A.: The influence of the mineral content in enamel on dissolution behaviour. Calcif. Tiss. Res. (in press, 1974).

BARTHELD, F. VON: Membrane phenomena in dissolution of the teeth. Arch. oral Biol. **6**: 284-303 (1961).

BAUD, C.A. and LOBJOIE, D.P.: Biophysical investigations on the mineral phase in the superficial layers of human dental enamel. Helv. odont. Acta **10**: 40-46 (1966).

BERGMAN, G. and LIND, P.O.: A quantitative microradiographic study of incipient enamel caries. J. dent. Res. **45**: 1477-1484 (1966).

BRUDEVOLD, F.; McCANN, H.G., and GRON, P.: Caries resistant teeth. Ciba Symp., p. 121 (Churchill, London 1968).

BRUDEVOLD, F. and SÖREMARK, R.: Chemistry of the mineral phase of enamel; in MILES Structural and chemical organization of teeth, p. 248 (Academic Press, New York 1967).

CRABB, H.S.M.: Structural patterns in human dental enamel revealed by the use of microradiography in conjunction with two dimensional microdensitometry. Caries Res. **2**: 235-252 (1968).

GRAY, J.A. and FRANCIS, M.D.: In Sognnaes Mechanisms of hard tissue destruction, pp. 213-260 (Amer. Association for the Advancement of Science, Washington 1963).

GROENEVELD, A.: Enkele theoretische en technische aspecten van glazuuronderzoek met behulp van contact microradiografie en microdensitometrie. Ned. T. Tandh. **80**: 46-55 (1973).

GROENEVELD, A. and ARENDS, J.: The influence of pH and demineralization time on the mineral content, the thickness of the surface layer and the depth of artificial caries lesions. Caries Res. (in press, 1974).

GROENEVELD, A.; JONGEBLOED, W., and ARENDS, J.: The mineral content of decalcified surface enamel. A combined microprobe-quantitative microradiography study. Caries Res. **8**: 267-274 (1974).

SILVERSTONE, L.M.: The influence of acidified organic gels on the enamel surface *in vitro;* in STACK and FEARNHEAD Tooth enamel. II, pp. 197-202 (Wright, Bristol 1971).

SONI, N.N. and BRUDEVOLD, F.: A microradiographic and polarizing microscopic study of sound enamel, J. dent. Res. **38**: 1181-1187 (1959).

SONI, N.N. and BRUDEVOLD, F.: Microradiographic and polarized light studies of artificially produced lesions. J. dent. Res. **39**: 233-240 (1960).

THEWLIS, J.: The structure of teeth as shown by X-ray examination. Med. Res. Coun. spec. Rep. Ser., No. 238, pp. 1-82 (1940).

CHAPTER V

INFLUENCE OF pH AND DEMINERALIZATION TIME ON MINERAL CONTENT, THICKNESS OF SURFACE LAYER AND DEPTH OF ARTIFICIAL CARIES LESIONS*

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Abstract. Sound premolars were demineralized for 4, 6, 9, and 11 days in buffer solutions of either pH 4, 4.5, or 5. Lesions were examined by quantitative microradiography. The thickness of the surface layer, depth of the lesion and degree of demineralization were studied as a function of pH and demineralization time.

Previous studies have shown that it is possible to produce caries-like lesions. These are very similar to natural caries, having an area of sub-surface demineralization covered by a relatively unaffected surface (VON BARTHELD, 1961; SILVERSTONE, 1967, 1968; MANSON-HING *et al.*, 1972; DAVIDSON, 1973).

The objective of this paper was to study the behaviour of the surface layer and the sub-surface demineralization as a function of pH and demineralization time. The mineral content was measured by means of quantitative microradiography, in various enamel specimens, as a function of the distance from the anatomical surface. Special attention was given to changes in the thickness of the surface layer, the depth of lesion and the degree of demineralization of the surface layer and the sub-surface lesion.

Materials and methods

Fifteen sound premolars, extracted for orthodontic reasons, were divided into three groups and demineralized in buffer solutions at a pH of 4, 4.5, or 5, respectively. The demineralizing solutions consisted of 6% hydroxyethyl cellulose and 0.1 M lactic acid, developed by GRAY (1966).

After cleaning with pumice powder, the teeth were covered with blue inlay-wax, with the exception of four windows on the buccal surface from the cusp to the cervical region. Three of the windows were covered with wax during the exposure time; the first after 4 days, the second after 6 days and the third after 9 days. After 11 days the teeth were removed from the buffer solutions. Thus, the four windows

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were exposed to the demineralizing solutions for 4, 6, 9, and 11 days, respectively.

After demineralization, the teeth were sectioned (JANSEN, 1950) and ground planoparallel (FRIEND and SMITH, 1965) to a thickness of between 50 and 80 μm. Microradiograms were then taken on Kodak High Resolution plates, using an X-ray tube with a copper anode, beryllium window and nickel filter, at 20 kV and 20 mA. The film-focus distance was 30 cm and the exposure time was 15 min.

The density of the microradiogram was always kept between 0.3 and 0.8. An aluminium step-wedge of 25-165 μm thickness, with steps of approximately 25 μm, was used as a reference. One densitometric tracing was taken perpendicular to the anatomical surface, from the dentino-enamel junction, through the middle of each window, using a slit width of 6 × 100 μm. The thickness of the sections was measured using a micrometer with an accuracy of 1 μm.

The mineral content was calculated according to ANGMAR *et al.* (1963) in volume percentages using the formula:

$$V\% = \frac{50.48\ t_a}{t_s}, \qquad (1)$$

Here, t_a = effective thickness of aluminium, and t_s = thickness of sections.

The thickness of the surface layer and the depth of the lesion were measured directly from the densitometric tracings (fig. 2), being more accurate than measuring under the microscope.

The almost 90° change in direction at the left-hand side of the curve is taken to represent the beginning of the surface layer, point A. Those sections with an angled edge were discarded. Point B, the end of the surface layer and the beginning of the sub-surface lesion, is the position where the inner slope of the curve of the surface layer swings away from being a mirror image of the outer slope, thus indicating a radical change in density. Point C, the end of the lesion, is taken to be the point on the curve where it becomes horizontal.

A preliminary experiment showed that, when applying this technique to two windows placed in a 'horizontal' plane, the results were less reproducible than those obtained when the windows were placed 'vertically' (table I).

Results

Figure 1 shows a microradiogram of one of the sections demineralized at pH 4 for 4, 6, and 9 days. A representative densitometric tracing through the middle of the lesion, as shown in figure 1, is given in figure 2.

The mineral content at any point on the tracing may be calculated from the experimental data.

Two other parameters of interest are the thickness of the surface layer and the depth of the lesion. In figure 3, the experimental data concerning lesion depth and surface layer thickness are depicted as a function of the pH and demineralization time (t). Each point represents the average value of five observations on

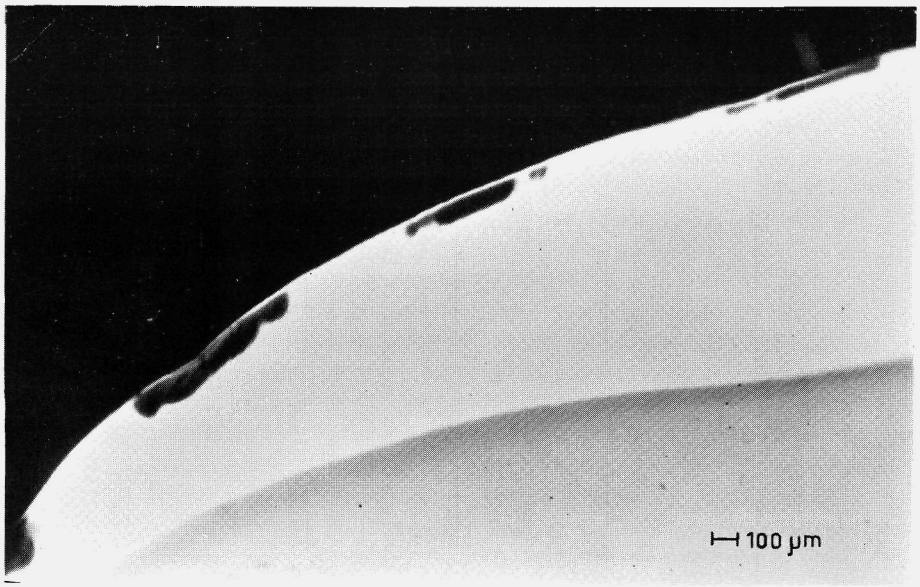


Fig. 1. Typical lesions formed with the artificial caries system. The microradiogram, from right to left, shows lesions after 4, 6, and 9 days, respectively, at pH 4.

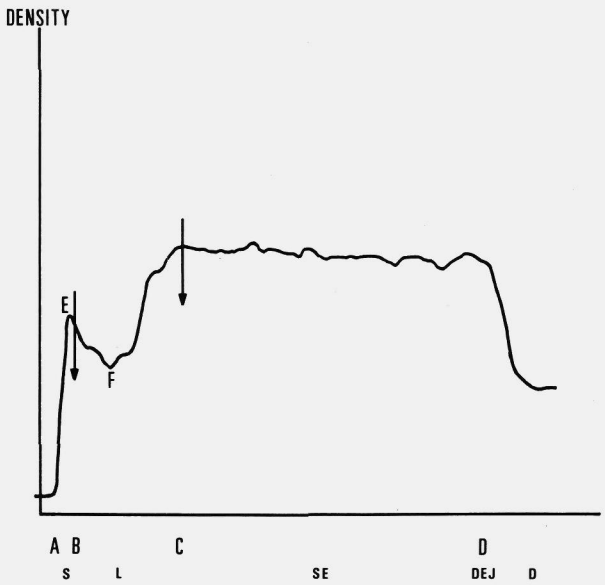
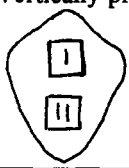


Fig. 2. A densitometric tracing obtained from microradiography. S = Surface layer; L = lesion; SE = sound enamel; DEJ = dentine enamel junction. The distance between the arrows AB was taken as the surface layer thickness. The distance between the arrows AC indicates the lesion depth.

Table I. Degree of reproducibility of 'horizontally' and 'vertically' placed windows

		Mineral content, V%		Means of differences	SD of differences
		I	II		
Horizontally placed windows (I and II)					
	Sound underlying enamel	84.9	83.1	5.4	1.6
	Minimum mineral content in lesion	50.1	46.5	5.1	1.6
	Maximum mineral content in surface layer	58.7	56.2	3.4	1.8
Vertically placed windows (I and II)					
	Sound underlying enamel	83.8	83.7	2.2	0.7
	Minimum mineral content in lesion	52.4	50.1	4.3	1.1
	Maximum mineral content in surface layer	56.9	55.4	3.7	1.0

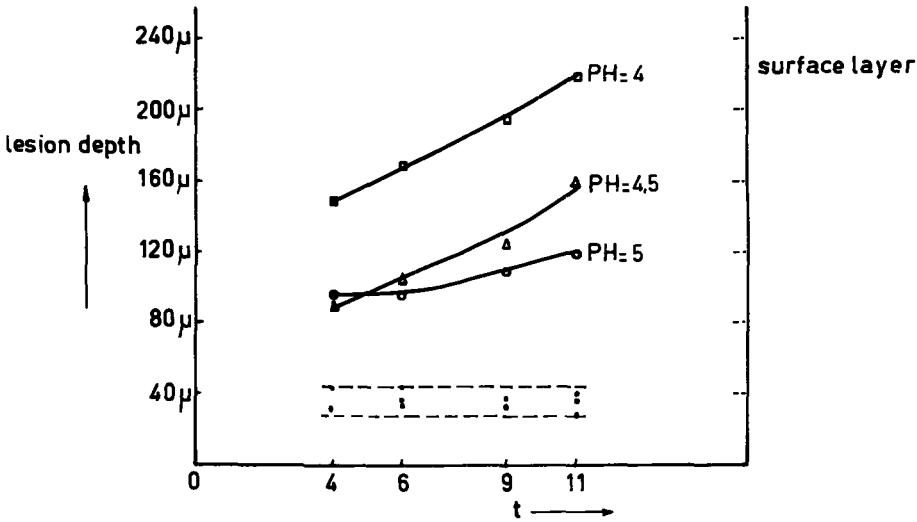


Fig. 3. The lesion depth is given as a function of demineralization time at various pH (continuous line). The broken lines show the scatter of the values observed for the thickness of the surface layer.

different teeth. The thickness of the surface layer is almost unaffected by the demineralization time and a lower pH reduces the surface layer only slightly. The depth of the lesion increases, as might be expected, with demineralization time.

The pH, however, is a more important parameter. A lower pH, at a fixed demineralization time, increases the lesion depth considerably. After 11 days of demineralization, the difference in lesion depth at pH 4 and 5 is about a factor 2. The mineral content was calculated from equation (1), as a function of demineralization time 't' and pH. The maximum mineral content in the surface layer and the minimum mineral content in the lesion have been compiled in table II.

Table II. Mineral content, thickness of surface layer and depth of lesion (mean of five measurements)

pH	Demineralization days	Mineral content, V%			Thickness of surface layer, μm	Depth of lesion, μm
		maximum in surface layer	minimum in lesion	underlying sound enamel		
4	4	61.2 (5.4)[1]	31.0 (4.7)	83.7 (1.8)	42 (7)	153 (13)
	6	56.6 (5.2)	26.8 (5.8)	83.3 (1.5)	44 (10)	170 (17)
	9	55.4 (6.3)	26.6 (4.3)	81.9 (2.9)	35 (9)	196 (19)
	11	47.7 (1.7)	19.1 (4.2)	76.8 (3.6)	39 (10)	219 (17)
4.5	4	67.6 (3.1)	52.8 (3.2)	88.0 (2.1)	38 (3)	93 (8)
	6	67.6 (2.2)	52.8 (3.5)	86.0 (1.2)	34 (6)	106 (10)
	9	64.8 (2.9)	49.6 (3.3)	86.4 (2.1)	38 (6)	126 (7)
	11	57.8 (4.2)	38.3 (3.4)	86.5 (2.3)	38 (4)	158 (14)
5	4	64.5 (2.4)	61.7 (3.2)	84.0 (0.8)	30 (2)	97 (13)
	6	65.9 (3.8)	59.8 (4.7)	84.0 (0.6)	33 (1)	97 (11)
	9	62.8 (2.9)	53.2 (5.0)	82.9 (0.6)	36 (3)	110 (9)
	11	57.6 (4.0)	49.0 (6.1)	81.1 (0.6)	27 (2)	120 (7)

[1] Figures in parentheses denote standard deviation.

The volume percentages of mineral content, as derived from the microradiographic experiments, are also shown in figure 4. They clearly indicate that the mineral content of the surface layer is only slightly affected by pH and demineralization time. The degree of demineralization of the sub-surface lesions tends, however, to increase with a lower pH and an increased demineralization time.

The influence of pH 4, 4.5, or 5, respectively, at a fixed demineralization time is shown in figure 5. The curves have been adjusted slightly in a vertical direction so that the sound enamel tracings are in one line, thus showing the differences in demineralization more clearly.

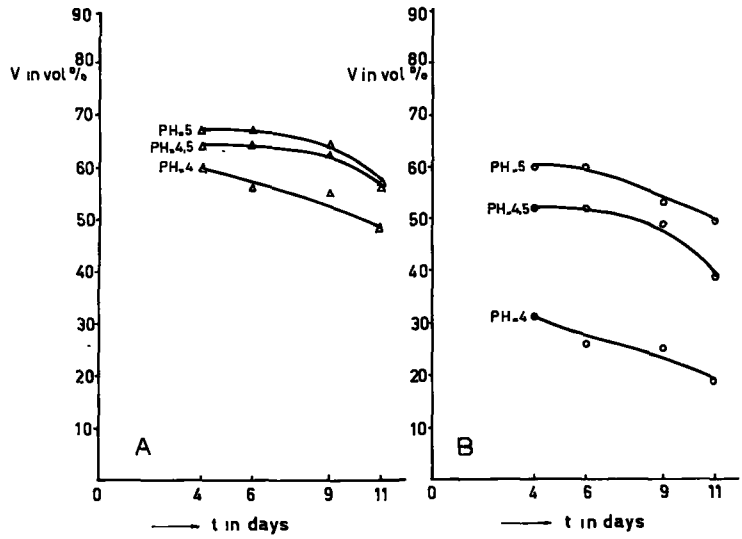


Fig. 4a. Volume percentage of mineral content in the surface layer as a function of demineralization time at pH 4, 4.5, and 5, respectively. *b* Minimum value of the mineral content *in* the lesion (average values).

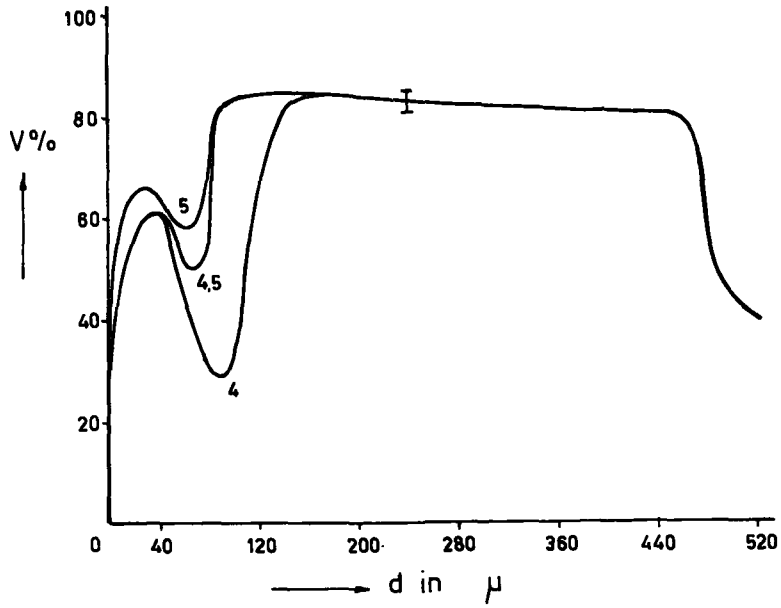


Fig. 5. The V% of mineral as a function of distance from the surface is given for pH 4, 4.5, and 5, respectively, after 4 days of demineralization. The maximum and minimum mineral contents are expressed as a percentage of the mineral content of the underlying enamel.

Discussion

The survival of the surface layer in the caries process is an important phenomenon. It is the existence of this intact layer that distinguishes the caries process in enamel from chemical etching of enamel. Furthermore, the surface layer is most probably an important factor in remineralization. It is therefore of interest to study this layer and the sub-surface lesion behind it, particularly with reference to the mineral content. Many investigations have already shown that the surface layer is only slightly demineralized in natural caries lesions (BERGMAN and LIND, 1966).

To the authors' knowledge no quantitative values, as a function of demineralization time and pH, have been published for artificial lesions.

The results in figure 3 indicate that the surface layer is of an almost constant thickness of between 25 and 40 μm within the pH range 4-5 and demineralization times of up to 11 days. This value is in agreement with the data on natural caries (BERGMAN and LIND, 1966) and artificial caries (MANSON-HING *et al.*, 1972). The volume percentage of mineral content in the surface layer is in the range of 50 and 65V% in the pH region 4-5 and is only slightly influenced by the demineralization time. This is in accordance with the data of HOUWINK (1972) who showed, by means of polarized light measurements, that no relationship between the degree of demineralization and the thickness of the surface layer could be observed.

From the available data it can be concluded that the surface layer created by the caries process *(in vitro* and *in vivo)* behaves like a porous layer of enamel. The original structure is more or less intact (DARLING, 1956; DAVIDSON, 1973) but permits transport of ions both in and out of the lesion. The relatively constant value of the degree of mineralization shows that once a certain degree of transport is possible (after the removal of about 20% mineral), the remaining material in this layer is removed very slowly. More research has therefore to be done on the very early stages of demineralization, when mineral loss at the surface is still less than 20%.

A second parameter, important in caries lesions, is the degree of demineralization as correlated to lesion depth. VON DER FEHR (1966), using *in vivo* produced lesions, showed a correlation between ^{32}P uptake and the lesion depth. HOUWINK (1972) on the other hand, using a similar technique, could not demonstrate a relationship between the degree of demineralization and lesion depth. The data in this study indicate that at pH 4 the lesion depth increases rapidly in the first few days of demineralization. The lesion depth in μm (d_1) for days 4-11 can, however, be described by:

$$d_1 \approx 150 + 10\,t, \qquad (2)$$

(where 't' is the demineralization time in days). At the same time, the vol% of mineral in the lesion can be approximated by:

$$\text{vol\%} \approx 31\text{-}1.7\,t \qquad (3)$$

Taking (2) and (3) together vol% $\approx$ 57-0.17 d_1 (in μm) is obtained. A similar expression, vol% $\approx$ 62-0.2 d_1 (in very good agreement), was obtained using the results given in table II. This indicates that, under the experimental conditions described, there is a direct relation between lesion depth and mineral content in vol%. It is possible that this result is partly due to the use of hydroxyethyl cellulose in the solution.

SILVERSTONE (1971) showed that by using a charged medium, acidified gelatin, it is possible to produce irregular areas of demineralization, very similar in shape to natural caries lesions. It is possible that such lesions will show different correlations.

REFERENCES

ANGMAR, B.; CARLSTRÖM, D., and GLAS, J.E.: Studies on the ultra-structure of dental enamel. J. Ultrastruct. Res. **8**: 12-23 (1963).

BARTHELD, F. VON: Membrane phenomena in carious dissolution of the teeth. Arch. oral Biol., suppl. 6, pp. 284-303 (1961).

BERGMAN, G. and LIND, P.O.: A quantitative microradiographic study of incipient enamel caries. J. dent. Res. **45**: 1477-1485 (1966).

DARLING, A.I.: Studies on the early lesion of enamel caries with transmitted light, polarized light and radiography. Brit. dent. J. **101**: 289-297 (1956).

DAVIDSON, C.L.: The softening of enamel; Ph.D. thesis Groningen (1973).

FEHR, F.R. VON DER: The caries-inhibiting effect of acid phosphate fluoride tested by the gold plate technique, Adv. Fluor. Res. **4**: 121-135 (1966).

FRIEND, J.V. and SMITH, G.S.: Recent modifications to our technique of preparing thin, flat, polished tooth sections. J. med. Lab. Technol. **22**: 4-6 (1965).

GRAY, J.A.: Kinetics of enamel dissolution during formation of incipient caries-like lesions. Arch. oral Biol. **11**: 397-421 (1966).

HOUWINK, B.: De ruimten in carieus oppervlakteglazuur. Ned. T. Tandheelk. **79**: 181-192 (1972).

JANSEN, M.T.: An improved method for the preparation of 'serial' sections of undecalcified dental tissues. J. dent. Res. **29**: 401-406 (1950).

MANSON-HING, L.R.; KELLER, S.E.; FEAGIN, F.F., and KOULOURIDES, T.: Microradiographic comparison of artificial caries systems. J. dent. Res. **51**: 923-929 (1972).

SILVERSTONE, L.M.: Observations on the dark zone in early enamel caries and artificial caries-like lesions. Caries Res. **1**: 261-274 (1967).

SILVERSTONE, L.M.: The surface zone in caries and in caries-like lesions produced *in vitro*. Brit. dent. J. **125**: 145-157 (1968).

SILVERSTONE, L.M.: The influence of acidified organic gels on the enamel surface *in vitro:* in FEARNHEAD and STACK Tooth enamel. II, pp. 197-202 (Wright, Bristol 1971).

CHAPTER VI

THE MINERAL CONTENT OF DECALCIFIED SURFACE ENAMEL*

A Combined Microprobe — Quantitative Microradiography Study

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Abstract. In a combined microprobe-microradiography experiment the calcium concentration and the mineral content has been measured in sound, decalcified and in decalcified SnF_2-treated enamel. The calcium content was estimated by the microprobe along the densitometric path of the radiography experiment. The results suggest the possibility that in the decalcified area a considerable amount (about 10-14 wt%) of the mineral is present either as precipitated orthophosphates or is adsorbed as Ca^{++} and HPO_4^{--} ions. Only a few wt% of calcium is lost from lesion and surface layer.

In enamel treated with stannous fluoride demineralization was less than in untreated specimens.

Recently many new analytical methods have been employed to study sound and carious enamel. The electron microprobe, in particular provides a very useful tool for a point-to-point analysis of microvolumes of enamel (MELLORS, 1964; FRAZIER, 1967; DAVIDSON *et al.*, 1973). FRAZIER (1967) stated that electron microprobe analyses, representing a micro-analysis of a surface layer of atoms about 3 μm thick, may be different from data obtained from quantitative microradiographic measurements. In microradiography the essential information is obtained from enamel sections of a defined thickness. 70-80 μm in this study.

The purpose of this study was to compare quantitatively the mineral content obtained from combined microradiograph and microprobe measurements on sound and decalcified human enamel. Using this technique information about the amount of hydroxyapatite transformed during demineralization can also be obtained from the demineralized enamel.

Both measurements were carried out in the same position in the enamel to avoid possible errors due to ultrastructural inhomogeneity or preparation.

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Material and methods

17 sound premolars which had been extracted for orthodontic reasons were carefully cleaned with pumice. They were completely covered with blue wax, except for two windows ($1 \times 2\ mm^2$) on the buccal smooth surfaces. The teeth were then immersed in a 10-ml buffer solution (pH 4) containing 0.1 M lactic acid and 6% hydroxyethyl cellulose (HEC) for 24 h. Demineralization thus occurred at the exposed windows of enamel. Subsequently, the enamel window nearest to the incisal edge was treated with 4% SnF_2 solution (pH 2.6) for 30 min and the second window was treated with tap water at 20°C for the same period of time.

To study the effect of the SnF_2 treatment the teeth were placed in fresh, pH 4, buffer solutions for 96 h. The teeth were then sectioned carefully, perpendicular to the buccal plane and ground planoparallel to a thickness of 70-80 μm.

Microradiograms were taken using an X-ray generator (ENRAF Diffractis 601) with a copper anode and nickel filter. The generator was operated at 20 kV, 20 mA for an exposure time of 15 min. The microradiograms were produced on Kodak High Resolution plates at a film-focus distance of 30 cm. The density of the microradiograms was kept between 0.3 and 0.8. An aluminium step-wedge of 25-165 μm was used as a reference system (THEWLIS, 1940). Densitometric tracings, perpendicular to the surface through the middle of the demineralized area, were made using a Leitz microscope-photometer type MPV with a slit width of $6 \times 100\ \mu m^2$.

Using a light microscope, a very thin hair was fixed to the surface of the enamel with cyanoacrylate adhesive to mark the line perpendicular to the enamel surface along which the density of the microradiograms had been measured. The

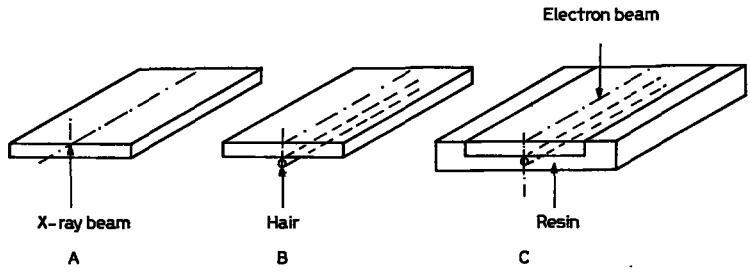

Fig. 1. Procedure for fixing the position of the microradiographic tracing for the microprobe analysis. *A* shows the section after the density measurement; *B* the marking of the position; *C* the specimen ready for microprobe analysis.

specimen was then embedded in epoxy-resin as indicated in figure 1, extreme care being taken to avoid surface damage and to obtain a planoparallel sample. When te resin had cured, the exact position of the hair was marked on the upper surface, again under the light microscope (fig. 1B). This elaborate procedure enabled us to fix the exact position of the microradiographic tracing, even after the layer

of carbon (about 200 Å) necessary for the microprobe analysis had been deposited.

The electron microprobe analyses were carried out with a JEOL.-SMS-U-3 scanning electron microscope and microprobe attachment. The beam current was 0.01 μA and the accelerating potential 15 kV. The Ca content was measured quantitatively along the densitometric path using the Ca K_α line. This can be carried out accurately provided that a fluorapatite single crystal is used for calibration (DAVIDSON *et al.*, 1973). During the continuous scan measurements the electron beam, causes characteristic X-rays to be emitted from the surface. The specimen moves at a speed of 20 μm/min from the enamel surface towards the dentino-enamel junction. The diameter of the electron beam at the surface was approximately 1 μm.

Results

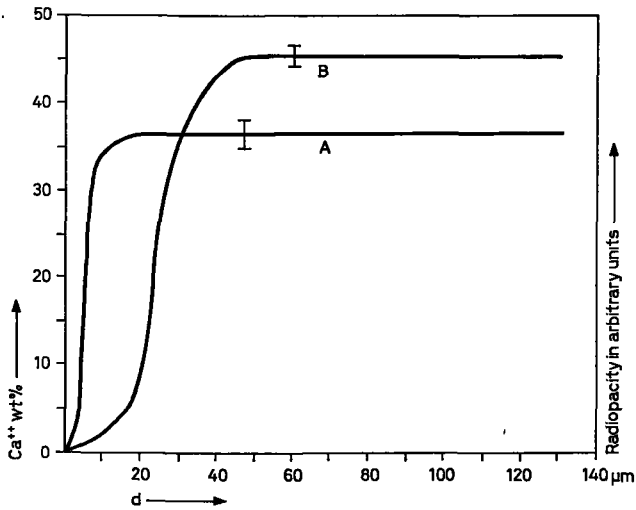

Fig. 2. Combined results of microprobe and microradiographic experiments on sound enamel. *A* is the microprobe tracing; *B* denotes the radiopacity. Differences between measurements on various samples are indicated by the bar.

Figure 2 shows the results for sound enamel. Curve A is the result of the densitometric tracing and curve B of the microprobe tracing in which the Ca concentration is expressed as a function of the distance from the enamel surface.

In figure 3 the results of both the microradiographic measurements and the microprobe tracing are given for demineralized SnF_2-treated enamel. The tracings of the treated and non-treated enamel show qualitatively an analogous behaviour. The most interesting quantitative parameters have been compiled in

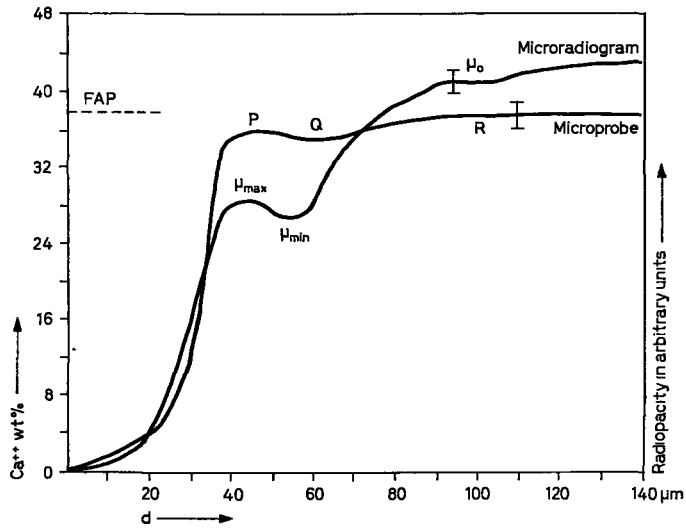

Fig. 3. Combined results of microprobe and microradiographic measurements on demineralized SnF_2-treated enamel. P, Q and R denote the surface layer, the lesion and sound enamel, respectively; μ_{max}, μ_{min} and μ_o indicate the radiopacity of the surface layer, the lesion and the sound material, respectively. Ranges between samples are indicated by the bar.

Table I. Summary of experimental results

	P_{Ca}^{++}	μ_{max}	Q_{Ca}^{++}	μ_{min}	R_{Ca}^{++}	μ_o
Demineralized enamel	34.0±1.2	100±2	31.6±1.2	88±2	37.0±1.2	216±2
Stannous fluoride-treated demineralized enamel	35.6±1.2	119±2	34.4±1.2	108±2	38.0±1.2	219±2

The Ca^{++} content in the surface layer (P), the lesion (Q) and in sound enamel (R) (in wt %). The linear absorption coefficient Ca K_α in the surface layer, the lesion and sound enamel is denoted by μ_{max}, μ_{min} and μ_o, respectively.

table I. P, Q and R denote surface layer, lesion and normal underlying enamel, μmax, μmin and μ_0 are the linear absorption coefficients of the surface layer, lesion and sound enamel.

From the Ca concentration the mineral content (hydroxyapatite — $Ca_{10}(PO_4)_6(OH)_2$) is also known. Table II shows the mineral content as calculated from the microprobe data, and the amount of mineral as calculated from the microradiographic measurements using the formula:

$$V\% = \frac{50.48\, t_a}{t_s}$$

t_a = effective thickness of aluminium, and t_s = thickness of the section (ANGMAR *et al.*, 1963).

Table II. Quantitative results from microradiography and microprobe

Material	Position	Microradiography		Microprobe
		mineral vol %	mineral wt %	mineral wt %
Untreated enamel	surface enamel	85±2	95±2	95±2.2
Demineralized enamel	surface layer (P)	39±2	66±2.5	85±2.2
	lesion (Q)	34±2	62±2.5	79±3.2
	sound (R)	83±2	94±2	93±2.2
SnF_2-treated demineralized enamel	surface layer (P)	45±2	72±2	89±2.2
	lesion (Q)	41±2	69±2	86±3.2
	sound (R)	84±2	94±2	95±2.2

The corresponding weight percentages of mineral have been calculated from the volume fractions, assuming the presence of the mineral hydroxyapatite (density 3.15) and water plus organic material (density 1.0).

The results of these microprobe experiments agree with recently published data (DAVIDSON *et al.*, 1973), showing a rather small Ca decrease in the lesion compared with the relatively high concentration of Ca remaining in the surface enamel. Figure 3 shows that the maximum Ca concentration in the surface layer, created by the demineralization process, coincides with the maximum mineral content as estimated by quantitative microradiography. On the other hand, the minimum concentration of Ca in the lesion does not coincide exactly with the minimum mineral content. The minimum mineral content is at 55 μm, and the minimum Ca concentration at 61 μm from the enamel surface. The depth of the lesion, taken as that point where the Ca content returns to normal again, is about 93 μm from the enamel surface for both measurements (fig. 3).

From this combined microprobe-microradiography experiment the mineral (hydroxyapatite) content, in weight percentages, can be estimated.

In the microprobe experiment the Ca concentration is measured in localized regions of about 40 μm^3 enamel. In the electron range employed, the analytical masses in which the Ca analysis is carried out are 71 and 117 ($\times 10^{-12}$) g for fluorapatite and enamel, respectively. Using these analytical masses and the fact that fluorapatite contains 39.7 wt% of calcium, the calcium concentration along the microprobe tracing can be calculated.

Discussion

The results obtained from both techniques on sound enamel are in good agreement with previously published data (ANGMAR *et al.*, 1963; BAUD and LOBJOIE, 1966). No hypermineralized layer could be observed in the originally intact enamel surface.

It can be seen from figure 3, that in the demineralized area the decrease in Ca concentration of the microprobe tracing was small compared with the relatively large decrease in mineral content measured by microradiography. It appears that few Ca ions diffuse out of the enamel during demineralization (table I). This is in excellent agreement with the mechanical properties of carious enamel as described by DAVIDSON *et al.* (1973).

In the demineralized area of the enamel section a surprising discrepancy (ranging from 10 to 14 wt%) between the mineral percentage calculated from the microradiographic and microprobe data can be noted (table II).

The difference is probably caused by two factors. Firstly, it is well known that lesion material has a lower density than sound enamel (LITTLE *et al.*, 1962; DAVIDSON *et al.*, 1973). This influences the microprobe results. If one takes into account the density differences in the electron ranges and analytical masses, this factor can explain a discrepancy of 3-4 wt%.

Secondly, in sound enamel hydroxyapatite is the main mineral constituent. Therefore, no differences greater than the experimental error were observed between the two techniques, but during decalcification of the lesion orthophosphates, $CaHPO_4$ or $CaHPO_4 2H_2O$ are formed.

The slow demineralization process causes a *partial* dissolution of the apatite crystallites. At the pH ≈ 4 the result of the dissolution will primarily be the formation of Ca^{++} and HPO_4^{--} ions. Through the porous surface layer, created by the acid, a small part of the dissolved material diffuses out of the enamel. From table I it is obvious that only 4-6 wt% of the Ca^{++} ions were lost.

As the dissolution process takes place both at the surface of the crystallites and in the centre of the crystallites (ARENDS, 1973), $CaHPO_4$ or $CaHPO_4 2H_2O$ formation will occur on internal and external crystallite surfaces. Furthermore, some of the ions may be adsorbed on the surfaces mentioned. The precipitated and adsorbed material has a lower density as well as a lower mass absorption coefficient for X-rays. Both parameters influence the formula of ANGMAR *et al.* (1963), which is correct if hydroxyapatite only is present; thus a lower mineral content is calculated.

Therefore, it is likely that this discrepancy in mineral content for carious enamel, is caused mainly by the presence of precipitated and/or adsorbed orthophosphates. More detailed calculations are in progress.

Another conclusion that may be drawn from the presented data is that in stannous fluoride-treated enamel the surface layer as well as the lesion are demineralized to a lesser extent than in untreated teeth. According to BERNDT (1970) CaF_2, $Sn_3(PO_4)_2$ and hydrated stannous phosphates are found in stannous fluoride-treated enamel. It is quite possible that the higher mineral content observed

in stannous fluoride-treated enamel is partially due to the presence of CaF_2 and Sn containing compounds (see Chapt. VIII). One of the favourable effects after a SnF_2 treatment, which may contribute to caries reduction, is a smaller calcium loss out of the enamel in both the lesion and the surface layer.

Acknowledgement

The authors are indebted to Mr. C.A. CHAMBONÉ for technical assistance, and to Dr. G. BOOM (Lab. for Physical Metallurgy, Groningen) for his cooperation in the microprobe measurements.

REFERENCES

ANGMAR, B.; CARLSTRÖM, D., and GLAS, J.E.: The mineralization of normal human enamel. J. Ultrastruct. Res. **8**: 12-23 (1963).

ARENDS, J.: Dislocations and dissolution of enamel. Caries Res. **7**: 261-268 (1973).

BAUD, C.A. and LOBJOIE, D.P.: Biophysical investigations on the mineral phase in the superficial layers of human dental enamel. Helv. odont. Acta **10**: 40-46 (1966).

BERNDT, A.F.: Reaction of stannous fluoride with hydroxyapatite. J. dent. Res. **49**: 638-642 (1970).

BROWN, W.E.: Physicochemical aspects of decay and decalcification in dental tissues and materials; in DRIESSENS Dental tissues and materials (1971).

DAVIDSON, C.L.; BOOM, G., and ARENDS, J.: Calcium distribution in human and bovine enamel. Caries Res. (in press, 1973a).

DAVIDSON, C.L.; HOEKSTRA, M., and ARENDS, J.: Microhardness and Ca content in decalcified enamel. Caries Res. (in press, 1973b).

FRAZIER, P.D.: Electron probe analysis of human teeth. Ca/P ratios in incipient carious lesions. Arch. oral Biol. **12**: 25-33 (1967).

GRAY, J.A. and FRANCIS, M.A.: Physical chemistry of enamel dissolution; in Mechanisms of hard tissue destruction, pp. 213-260. Publication No. 75 (Amer. Ass. for the Advancement of Science, Washington 1963).

LITTLE, M.F.; CUETO, E.S., and ROWLEY, J.: Physical and chemical properties of altered and sound enamel. Arch. oral Biol. **7**: 173-180 (1962).

MELLORS, R.C.: Electron probe microanalysis. 1. Calcium and phosphorus in normal cortical bone. Lab. Invest. **13**: 183-195 (1964).

THEWLIS, J.: The structure of teeth as shown by X-ray examination. Medical Research Council; Special Report Series No. 238 (1940).

CHAPTER VII

THE EFFECT OF SnF_2 AND CETYLAMINOHYDROFLUORIDE SOLUTIONS ON THE ACID DEMINERALIZATION OF ENAMEL*

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Abstract. The mineral content of enamel demineralized with and without preliminary F-application was measured using quantitative microradiography. The effect of an aminefluoride solution and SnF_2-containing solutions on the mineral content in the lesion and the surface layer was studied.

The results showed that local application of the aminefluoride solution to sound enamel was more effective than SnF_2; the demineralized area had a significantly higher mineral content in the lesion and a considerably thicker surface layer. There was no significant difference between the protective effect of both fluoride solutions on the mineral content of the lesion in the 'white spot' enamel groups. In the surface layer of the 'white spot' enamel groups, however, the SnF_2 treatment was significantly more effective.

The influence of many topically applied agents on caries reduction has been studied intensively. The effect of the inorganic fluorides NaF and SnF_2 in aqueous solutions and the aminefluoride solutions have been given particular attention (BRUDEVOLD *et al.*, 1967; MÜHLEMANN, 1967; LIM and HSIEH, 1971). In these studies, both the caries-inhibiting effect and the reduction in enamel solubility have been investigated.

As a result of the reduced solubility, following the application of fluoride, one might expect a higher mineral content after demineralization than in untreated enamel.

The purpose of this investigation was to study the mineral content in demineralized sound and carious enamel, with and without previous F-application, using quantitative microradiography. The effect of inorganic fluoride (SnF_2) and aminefluoride[1] solutions on the mineral content in the lesion, and in the surface layer covering the lesion, was also investigated and compared.

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1. Elmex 'fluid', Basel, Switserland; containing (1) component 297 bis-(hydroxy-ethyl-amino-propyl-N-hydroxyethyl-octadecyl-amine dihydrofluoride and (2) component 335 oleylamine-hydrofluoride.

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Materials and methods

100 microscopically sound premolars, extracted for orthodontic reasons, were selected for this study. They were carefully cleaned with pumice and covered with blue inlay wax with the exception of two windows (3 × 6 mm). They were then divided into two groups. One group of 50 premolars was demineralized by immersion in individual 10 ml buffer solutions at pH 4 containing 0.1 M lactic acid and 6% hydroxyethyl cellulose (HEC) for 24 h.

After this pre-treatment the teeth were divided into 4 groups of 25 premolars, two groups (I and II) with sound enamel and two with artificial white spot lesions (III and IV). Subsequently, one of the two windows of the sound enamel group I was treated with an inorganic fluoride solution (4% SnF_2, pH 2.6) and one window of group II with the aminefluoride solution (1% F^- pH 4.5) for 30 min. The control windows were treated with tap water for the same period. The two 'white spot' groups III and IV were treated with the two fluorides and tap water in exactly the same way. All teeth were demineralized in fresh buffer solutions at pH 4.5 for 96 h at 20°C. They were sectioned perpendicular to the buccal plane and ground planoparallel to a thickness of between 70 and 80 μm. Microradiograms were taken using an X-ray generator (ENRAF Diffractis 601) equiped with a copper anode and a nickel filter, operated at 20 kV and 20 mA, at a film-focus distance of 30 cm and an exposure time of 15 min. Densitometric tracings were then made through the middle of the lesions (for details see GROENEVELD, 1973; GROENEVELD *et al.*, 1973).

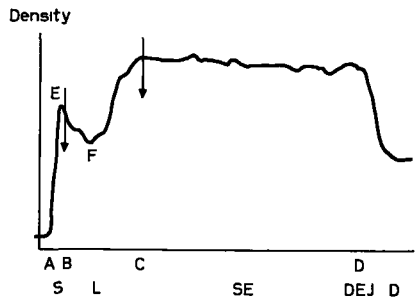

Fig. 1. A typical microradiogram of demineralized enamel. The optical density versus the distance from the enamel surface is given. 'E' and 'F' denote the maximum and minimum values for the surface layer and lesion. 'A-B' is the thickness of the surface layer, 'B-C' is the lesion depth.

Figure 1 shows a typical densitometric tracing through one of the lesions. 'E' denotes the maximum mineral content of the intact surface layer and 'F' the minimum mineral content in the body of the lesion. The distance 'A-B' and 'A-C' were taken to be the thickness of the surface layer and the depth of the lesion,

respectively, B and C indicating points of radical change in the direction of the curve.

The following formula was used for quantitative estimation of the mineral content:

$$V\% = \frac{50.48\ t_a}{t_s}$$

t_a = effective thickness of the aluminium step-wedge, and t_s = thickness of the section (ANGMAR *et al.*, 1963).

The difference in mineral content in one tooth with and without local application of a fluoride solution will be given as:

Δ V% = V% mineral (after F-application) — V% mineral (no F-application).

Results

The results given below in table I are the average values of 25 observations of the *minimum* mineral content in the lesion and the *maximum* mineral content in the surface layer covering the lesion in each of the four groups.

The data presented show that the average mineral content in the sound enamel was between 84 and 86 V%. This is in excellent agreement with previously published data (ANGMAR *et al.*, 1963; GROENEVELD, 1973).

Surface Layers

In all groups the maximum mineral content of the surface layer covering the lesion was 5-10 V% higher than the minimum mineral content of the underlying lesion. In the sound enamel groups Δ V% values of the surface layer for SnF_2 aminefluoride-treated teeth were 3.9 and 5.8%, respectively.

A completely different result was obtained in the 'white spot' groups. In the surface layer the Δ V% values for SnF_2 and aminefluoride-treated enamel were 6.4 and 0.4%, respectively.

The thickness of the surface layer in the two control groups was almost the same within the range of 29-35 μm (table II). The surface layers in the F-treated experimental groups were always slightly thicker than in the control groups, with the exception of the aminefluoride-treated group on sound enamel, where this layer was significantly thicker than in all other groups (46 μm).

Lesions

The Δ V% value of the lesions in teeth treated with aminefluoride was somewhat higher than in the SnF_2 group; the figures are 6.8 and 2.9% respectively. In the

Table I. Summary of the experimental results

Pre-treatment before application	Application with	Lesion average minimum mineral content V%	Surface layer average maximum mineral content V%	Underlying normal enamel average mineral content V%	Average decrease in mineral content		Differences due to F-treatment, V %	
					lesion	surface layer	lesion	surface layer
None	SnF_2	65.4 (2.3)	73.7 (1.8)	86.1 (1.3)	20.7	12.4	2.9(1.4)	3.9 (1.2)
None	control	61.7 (2.0)	69.0 (1.8)	85.3 (1.3)	23.6	16.3		
None	amine F^-	66.3 (2.0)	74.1 (1.3)	85.2 (1.0)	18.9	11.1	6.8(1.6)	5.8 (1.5)
None	control	59.7 (1.9)	68.4 (1.9)	85.4 (1.1)	25.7	17.0		
demineralized 24 h at pH 4. (white spot)	SnF_2	43.0 (2.0)	50.3 (2.0)	84.7 (1.1)	41.7	34.4	7.4(2.8)	6.4 (2.4)
demineralized 24 h at pH 4. (white spot)	control	35.1 (2.5)	43.3 (2.2)	84.1 (1.2)	49.0	40.8		
demineralized 24 h at pH 4. (white spot)	amine F^-	39.8 (2.5)	45.3 (2.3)	84.3 (0.8)	44.5	39.0	3.7(2.9)	0.4 (2.6)
demineralized 24 h at pH 4. (white spot)	control	35.4 (2.2)	44.3 (1.6)	83.7 (1.1)	48.3	39.4		

( ) = Standard deviation of the mean.

Table II. Summary of lesion depth and surface layer thickness

Pre-treatment before application	Application with	Thickness of surface layer, μm	Depth of the lesion
None	SnF_2	36 (2)	108 (4)
None	control	34 (2)	111 (4)
None	amine F^-	46 (3)	110 (4)
None	control	35 (3)	124 (3)
Demineralized 24 h at pH 4. (white spot)	SnF_2	32 (2)	92 (5)
Demineralized 24 h at pH 4. (white spot)	control	29 (2)	111 (5)
Demineralized 24 h at pH 4. (white spot)	amine F^-	31 (2)	84 (4)
Demineralized 24 h at pH 4. (white spot)	control	32 (3)	111 (5)

( ) = Standard deviation of the mean.

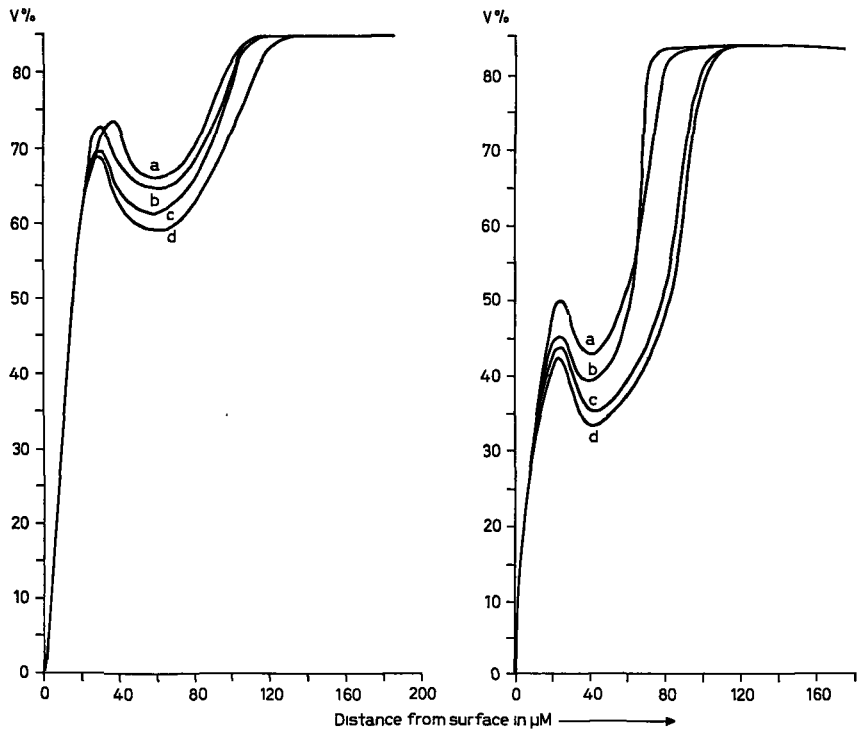

Fig. 2a. The V% of mineral is given as a function of the distance from the enamel surface. No pre-treatment before F-application, 'a, b, c and d' denote curve for aminefluoride, SnF_2 and the 2 control groups, respectively, *b* The V% of mineral is given as a function of the distance from the enamel surface. Demineralized before F-application, 'a, b, c and d' denote SnF_2 , aminefluoride and the 2 control groups, respectively.

'white spot' group this effect was reversed; 3.7 for aminefluoride and 7.4% for SnF_2 treated enamel.

The experiments show, however, that neither fluoride solutions have any great effect on the lesion depth. In figures 2a and b the results have been presented pictorially. Each line represents the average value of 25 densitometric tracings.

An interesting result can be observed if we compare the differences between the four control groups after one and two periods of demineralization, respectively. Firstly, it can be seen that a second demineralization (groups II and IV) has very little effect on the thickness of the surface layer. Secondly, tables I and II show the although demineralization of sound or 'white spot' enamel causes a similar lesion depth, there is a lower-V% of mineral in the 'white spot' groups. This indicates that once the lesion has been formed the main result of repeated demineralization is a reduction in the mineral content of the already formed lesion, rather than an extension of the lesion in the direction of the enamel-dentine junction.

Discussion

The experimental results show that, on sound enamel, local F-application with aminefluoride causes a considerably thicker surface layer than pre-treatment with a SnF_2 solution. The differences in mineral content (Δ V% values) are not, however, significantly different for the two types of F-treatment (Student t test). In 'white spot' enamel, the SnF_2-treatment gives a statistically significant increase in Δ V% value in the surface layer ($p < 0.05$).

When the lesion only is considered the application of the aminefluoride resulted in a statistically higher Δ V% value ($p < 0.05$; t test) as compared to SnF_2-treated sound enamel. The protection given by the two fluoride treatments is not significantly different in the lesions of the 'white spot' groups.

It is interesting to compare the microradiography results with previously published solubility reduction data. When doing so it should be remembered that the microradiographical method can distinguish between the surface layer and the sub-surface lesion, whereas solubility experiments measure bulk effects only.

MÜHLEMANN and SCHMID (1958) observed that *intact* enamel treated with SnF_2 or organic fluorides, showed a solubility reduction rate after 3 h of demineralization of about 77 and 88%, respectively. If one assumes that, in solubility experiments, the majority of calcium and phosphorus ions are removed from the enamel during demineralization there is a qualitative agreement with the microradiography data. In 'white spot' enamel, however, the solubility experiments showed that the most pronounced inhibition was found with SnF_2 solutions (MÜHLEMANN, 1960). From the microradiographic data it can be seen that this effect occurred mainly in the surface layer. This discrepancy might be caused by the use of different demineralization techniques. The demineralization technique of MÜHLEMANN (3 h treatment of phthalate buffer at pH 4) effects the enamel in a different way than an HEC solution.

The different behaviour of the amine- and inorganic fluorides used can be qualitatively explained as follows. In sound enamel the inorganic fluoride is attached to the outer surface. The aminefluoride, being a cationic detergent, penetrates over a considerable distance into sound enamel (ARENDS, unpubl. results). An indication of this is the significantly thicker surface layer covering the lesion in the aminefluoride-treated group and the relatively small mineral loss from the lesion. In 'white spot' enamel, however, adhesion and penetration properties are obviously not the most important ones. Most probably not only because of the already 'porous' structure, but also because of the presence of phosphates other than hydroxyapatite.

The insoluble precipitates (i.e. CaF_2, fluorapatite and Sn-containing precipitates), in the lesion and surface layer seem to be the most important protective factor. An indication of this may be that the lesion in both the fluoride-treated groups is shallower. Furthermore, in the SnF_2-treated group the precipitation also occurs in the surface layer.

Although the exact nature of the Sn-containing precipitates in enamel is unknown, it is probable that both $Sn_3F_3PO_4$ and $SnHPO_4$ will be present (JORDAN and WEI, 1971; NELSON and BAINBRIDGE, 1973). It is tempting to correlate the presence of the last mentioned compound with the known presence of $CaHPO_4$ 2aq. (DAVIDSON, 1973; GROENEVELD *et al.*, 1973). A detailed investigation of this point is in progress.

REFERENCES

ANGMAR, B.; CARLSTRÖM, D., and GLAS, J.E.: The mineralization of normal human enamel. J. Ultrastruct. Res. **8**: 12-23 (1963).

BRUDEVOLD, F.; McCANN, H.G.; NILSSON, R.; RICHARDSON, B., and COKLICA, V.: The chemistry of caries inhibition problems and challenges in topical treatments. J. dent. Res. **46**: 37-45 (1967).

DAVIDSON, C.L.: Decalcification of enamel; thesis Groningen (1973).

GROENEVELD, A.: Enkele theoretische en technische aspecten van glazuuronderzoek met behulp van contact-microradiografie en microdensitometrie. Ned. T. Tandhlk. **80**: 46-55 (1973).

GROENEVELD, A.; JONGEBLOED, W.G., and ARENDS, J.: The mineral content of surface enamel. Caries Res. (accepted, 1973).

JORDAN, T.H. and WEI, S.H.: $SN_3F_3(PO_4)$ the product of the reaction between SnF_2 and hydroxyapatite. Arch. oral. Biol. **16**: 241-246 (1971).

LIM, J.K. and HSIEH, J.B.: The solubility of surface enamel treated with precipitate-free aqueous solutions of SnF_2. J. dent. Res. **50**: 531-535 (1971).

MÜHLEMANN, H.R.: Protection of white spot enamel by different fluorides. Helv. odont. Acta **4**: 24-26 (1960).

MÜHLEMANN, H.R.: Die kariesprophylaktische Wirkung der Aminfluoride. Quintessenz **18**: 1-31 (1967).

MÜHLEMANN, H.R. and SCHMID, H.: Anti-caries dentifrices under laboratory conditions. J. dent. Belge **6**: 353-372 (1958).

NELSON, K.G. and BAINBRIDGE, C.A.: $SnHPO_4$ from the reaction of SnF_2 and hydroxyapatite at a low pH. J. dent. Res. **52**: 318-321 (1973).

CHAPTER VIII

REMINERALIZATION OF ARTIFICIAL CARIES LESIONS BY STANNOUSFLUORIDE*

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Abstract. The remineralizing property of SnF_2 was studied by treating artificial white spot enamel lesions for ½, 6 and 24 hours and one week in a 4% SnF_2 solution at 50°C.

Remineralization was observed in all treated samples and was studied using microradiography and microprobe analysis.

It appeared that remineralization started in the outer part of the intact surface layer covering the sub-surface lesion. With increasing time, the centre of the sub-surface lesion became remineralized. In a fully remineralized lesion it was no longer possible to distinguish between the surface layer and the sub-surface lesion. Some sections appeared to be fully remineralized after only 6 hours treatment with SnF_2.

Microprobe analysis showed that the variation in density was highly correlated with the Sn concentration. Some possible mechanisms and reactions that may be involved in remineralization are discussed.

Numerous studies have been carried out to investigate the remineralization of enamel. In vivo studies, as early as 1912, indicated that remineralization of enamel could take place after the application of a 'remineralization-powder' (ANDRESEN, 1921).

Remineralization also occurs in vivo without any pre-treatment, as shown by BACKER DIRKS (1966) in a longitudinal study. Several studies have since dealt with the remineralization properties of either calcifying solutions or saliva (LENZ and MÜHLEMANN, 1963; SILVERSTONE and POOLE, 1968; FEAGIN, KOULOURIDES and PIGMAN, 1969).

WEI and KAQUELER (1967) suggested that remineralization is not simply a matter of replacing the lost calcium and phosphate ions with similar ions from a calcifying solution, but is a chemical process which can involve a variety of ions

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e.g. fluoride and heavy metal ions. They tested calcifying, fluoride and heavy metal solutions and found that, based on microradiographic appearance, the stannousfluoride solution was the most effective in remineralizing etched enamel.

The purpose of this study was to examine the remineralization properties of stannousfluoride at 50°C, after ½, 6 and 24 hours and after one week using microradiography and microprobe analysis.

The temperature of 50°C was chosen firstly because MELLBERG and LOERTSCHER (1973) showed that heated fluoride solutions (up to 35°C) gave better results in reducing the solubility of enamel and secondly because 50°C is probably the maximum acceptable temperature in the mouth.

Materials and methods

Human premolars, extracted for orthodontic reasons, were used in this study. Ten were covered with blue inlay wax with the exception of four windows on their buccal surfaces. These teeth were then demineralized for 96 hours in a 6% hydroxyethyl cellulose (HEC) solution, containing 0.1 M lactic acid at a pH of 4.5. The teeth were subsequently cut into 4 pieces (each with 1 window). Thirty of these were put into individual bottles containing 5 ml of a freshly made stannous-fluoride solution (4% SnF_2) at 50°C. Ten pieces were removed after ½, 6 and 24 hours of treatment, respectively. The remaining group of ten which served as the control, was stored in tap water at room temperature. (Preliminary experiments indicated that storing at room temperature or 50°C produced no measurable differences.)

In a separate experiment another group of ten premolars (with 2 windows) was demineralized in a similar HEC solution at pH 4. These teeth were cut into 2 pieces each with 1 window. Ten pieces were remineralized for 1 week in a 4% SnF_2 solution in exactly the same way as described above. The other 10 pieces served as the control group. After this treatment, all the teeth were sectioned and polished plano-parallel to a thickness of 70-80 μm.

Microradiograms were taken on Kodak High Resolution plates using an X-ray generator (ENRAF Diffractis 601) fitted with a copper anode and nickel filter (0.02 mm Ni). The generator was operated at 20 kV, 20 mA and the exposure time was 15 minutes. The film-focus distance was 30 cm. Densitometric tracings were made using a Leitz microscope-photometer, type MPV with a slit width of $6 \times 100\ \mu m^2$.

The electron microprobe analyses were carried out with a JEOL-SMS-U-3 scanning electron microscope and microprobe attachment. The beam current was 0.01 μA and the accelerating potential 15 kV. The Ca, P and Sn content was measured along the densitometric tracings. During the continuous scan measurements the electron beam moved at a speed of 20 μm/min from the enamel surface inwards. The diameter of the electron beam at the surface was approximately 1 μm. Ca ($K\alpha_1$), Sn ($L\alpha_1$) and P ($K\alpha_1$) radiations were analysed with a pentaerythritol crystal.

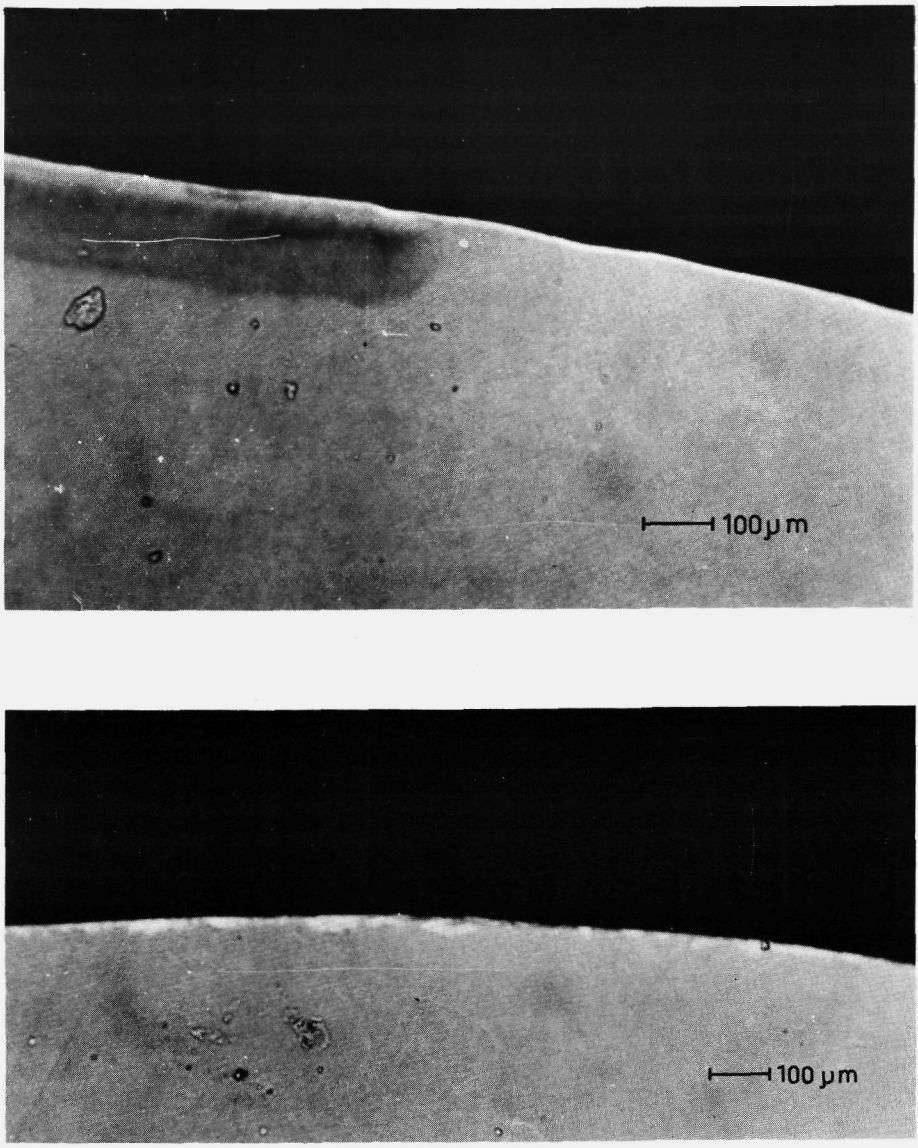


Fig. 1. Microradiograms of sections of enamel after ½ an hour of SnF_2-treatment at 50 C. *a.* a very thin radiodense layer can be seen in the outermost part (approx. 10 μm) of the sound enamel. This layer is continuous with that in the surface layer of the lesion seen in the left-hand side of this picture; *b.* a similar but irregularly formed radiodense layer was also seen on some sections.

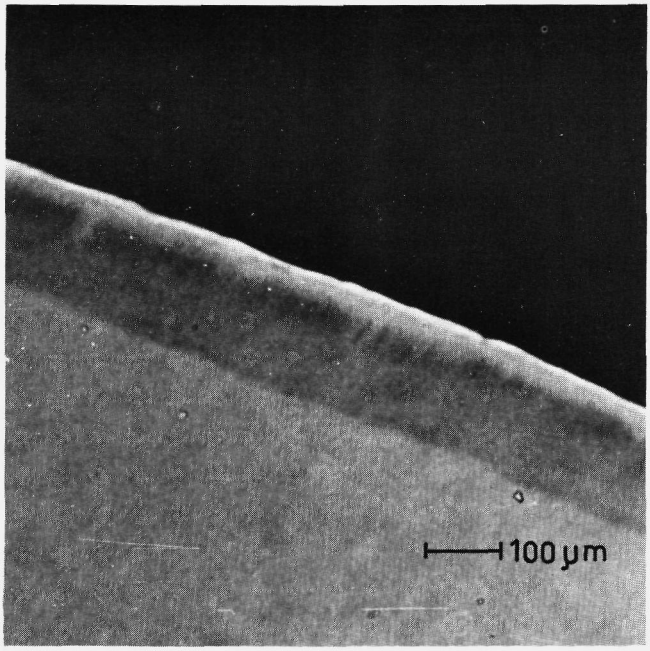


Fig. 2. Microradiogram of an artificial caries lesion after ½ an hour of SnF_2-treatment at 50°C. This very early stage of remineralization shows a completely formed homogeneous radiodense layer at the outermost part (approx. 10 µm) of the surface layer of the lesion.

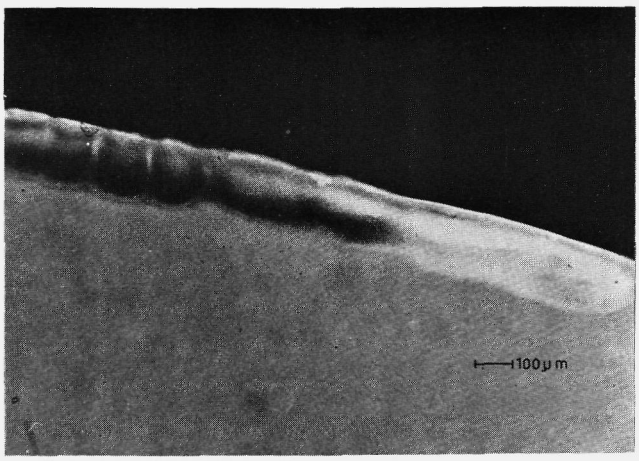


Fig. 3. Microradiogram of an artificial caries lesion after one week of SnF_2-treatment at 50°C. Selective remineralization probably occurs in more accessible areas in the body of the lesion. A very high degree of remineralization can be seen in the right-hand side, whilst heavily demineralized areas are still present.

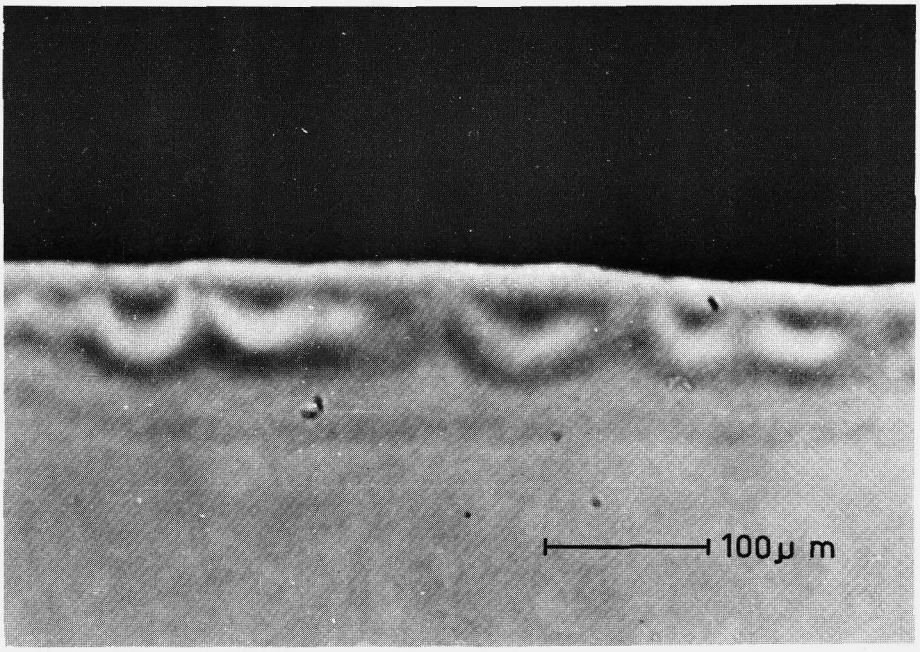


Fig. 4. Microradiogram of an artificial caries lesion after one week of SnF_2-treatment at 50°C. The surface zone and the body of the lesion have been remineralized. The radiodensity at the advancing front of the lesion is almost as high as that of the underlying sound enamel. In the body of the lesion the remineralization is at its highest point, surrounded by some less remineralized elongated areas.

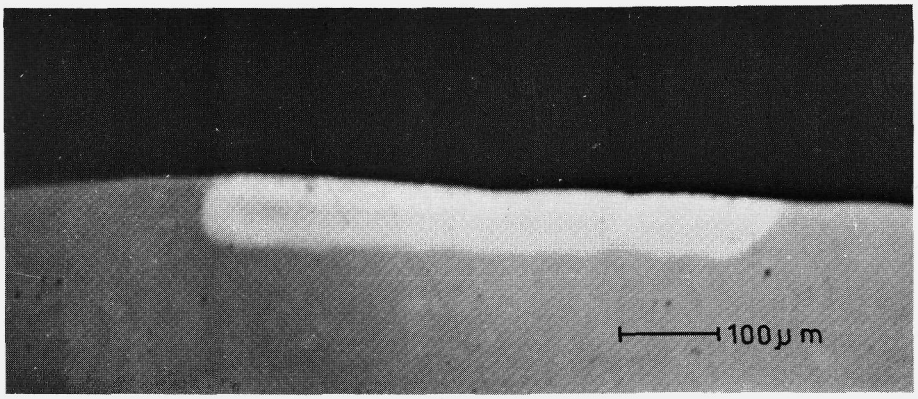


Fig. 5. Microradiogram of an almost fully remineralized artificial caries lesion after one week of SnF_2-treatment at 50°C. It can be observed that a distinction between surface layer and sub-surface lesion is no longer possible on a microradiogram.

Results

The results show that there was an increased X-ray density, as compared to the control groups, in all cases treated with SnF_2. An increase in radiodensity will be considered in this study as being caused by a remineralization process. A varying remineralization pattern was observed, not only in different sections, but also within the same lesions. In many sections a very thin (approx. 10 μm) layer of high density was seen just below the anatomical surface of the sound enamel. Figure 1a shows a regular homogeneous layer after only half an hour of SnF_2 treatment. This layer is often irregularly formed however, as shown in figure 1b.

Some different remineralization patterns found in *carious enamel* are given in figures 2, 3, 4 and 5. It can be seen in fig. 2 that, at an early stage, remineralization appears to be restricted to the outermost layer of the relatively intact surface layer covering the sub-surface lesion. This dense layer, very similar to that found on sound enamel, was always completely formed. It is also clearly visible in the microprobe tracings given in fig. 8.

A different pattern can be seen in fig. 3, here selective remineralization probably occurred in the more accessible areas in the body of the lesion. The most complete remineralization, in terms of X-ray density, is shown in figures 4 and 5. In fig. 4 the surface zone and the body of the lesion as a whole have been remineralized, with the exception of some elongated areas parallel to the inner front of the lesion. Figure 5 shows a lesion with a very high degree of remineralization. Radiographically, it is no longer possible to make a distinction between the surface layer and the sub-surface lesion after prolonged remineralization (figs. 4 and 5). The densitometric tracings in fig. 6 illustrate the changes in density which occurred with increasing remineralization. These 4 tracings show the results of 4 different treatments on sections which originated from one tooth and as might be expected, the degree of remineralization had increased with time. The pattern of the tracings in fig. 6 was generally followed by all teeth, although some showed maximum remineralization after only 6 hours, whilst others showed only a minor degree of remineralization after one week.

Figures 7 and 8 show the graphs of Sn, Ca and P content as determined by microprobe analysis as a function of the distance from the surface. The changes in density in the microdensitometric tracings made along the same track as the electronmicroprobe experiment are also given. Figure 7 is an example of a partly remineralized lesion and fig. 8 that of a more remineralized one. Both figures show a higher calcium and phosphorus content in the surface layer (about 40 μm thick) than in the sub-surface lesion. It can be seen from the graphs in figures 7 and 8 that the original surface layer is still indicated by the higher calcium and phosphorus content, even after extensive remineralization. Fig. 7 clearly indicates that Sn was present in relatively large amounts on the outermost part (about 10 μm) of the surface layer, but that the surface layer proper had a very low Sn content. Figure 8 shows that in the more remineralized lesion the surface layer also had a low Sn content. The Sn content in the sub-surface area was relatively constant in the more remineralized lesion but varied in the more poorly

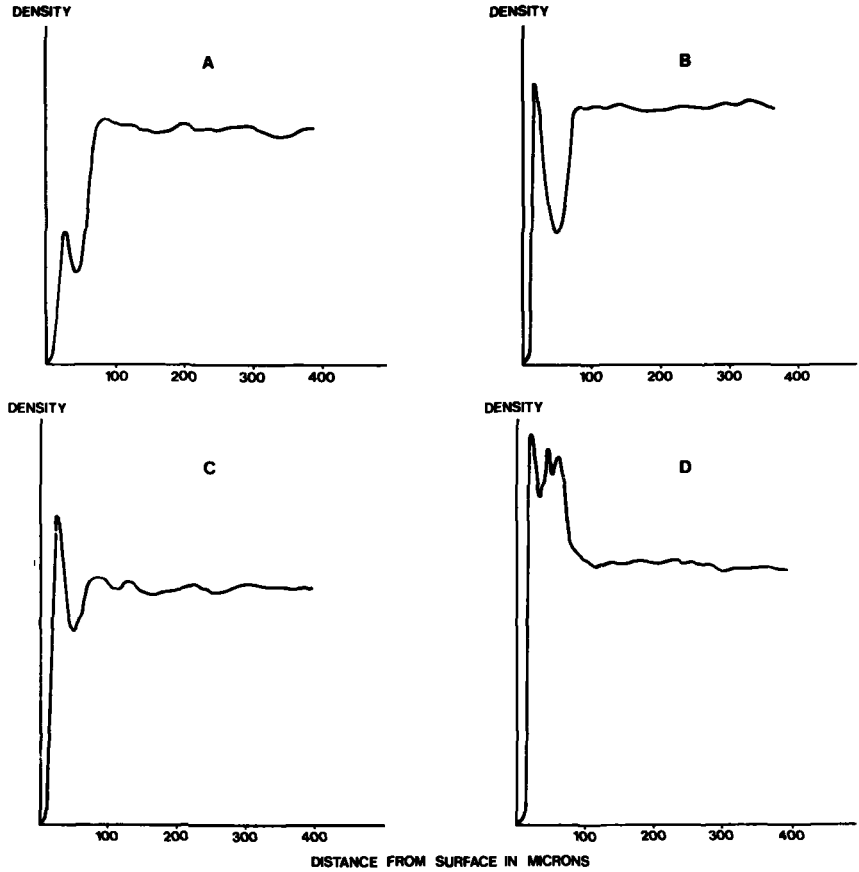


Fig. 6. Densitometric tracings of four treated sections which originally belonged to one tooth. *a. control group.* A typical densitometric tracing of an artificial caries lesion: the surface layer has a higher radiodensity than the underlying sub-surface lesion, but it is lower than that of the underlying sound enamel. *b. ½ an hour of SnF_2 treatment.* The density of the surface layer is already higher than that of the underlying sound enamel, whilst the density of the sub-surface lesion shows only a small increase. *c. 6 hours of SnF_2 treatment.* The density of the surface layer is still increasing and the sub-surface lesion shows a high density, almost the same as that of the underlying sound enamel. *d. 24 hours of SnF_2 treatment.* The density of the *surface layer and sub-surface lesion* is higher than that of the underlying sound enamel.

remineralized lesion illustrated in fig. 7. In both cases the Sn content drops to an undetectible level in the sound underlying enamel. The density varied greatly with the Sn content, as might be expected when the high X-ray absorption properties of Sn are considered.

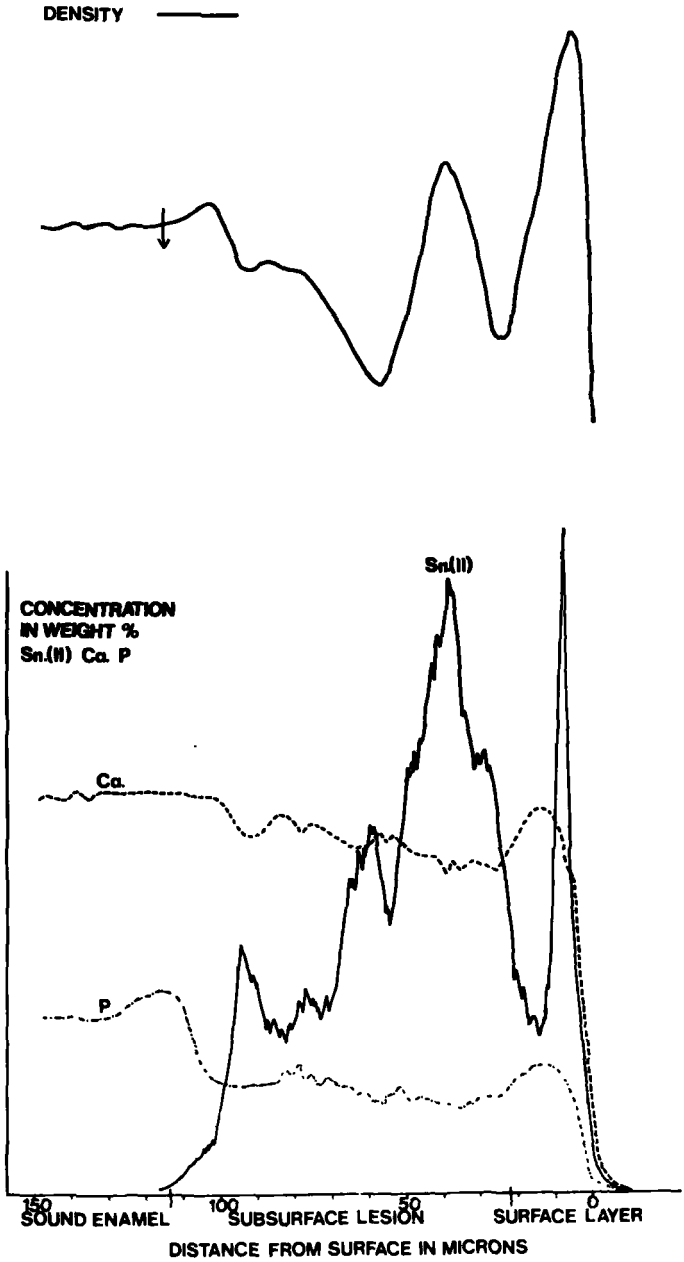


Fig. 7. Microprobe measurements of the section shown in figure 4. Ca, P and Sn concentrations are given as a function of the distance from the surface. The densitometric tracing made along the same path as the microprobe tracing has been drawn above the microprobe results.

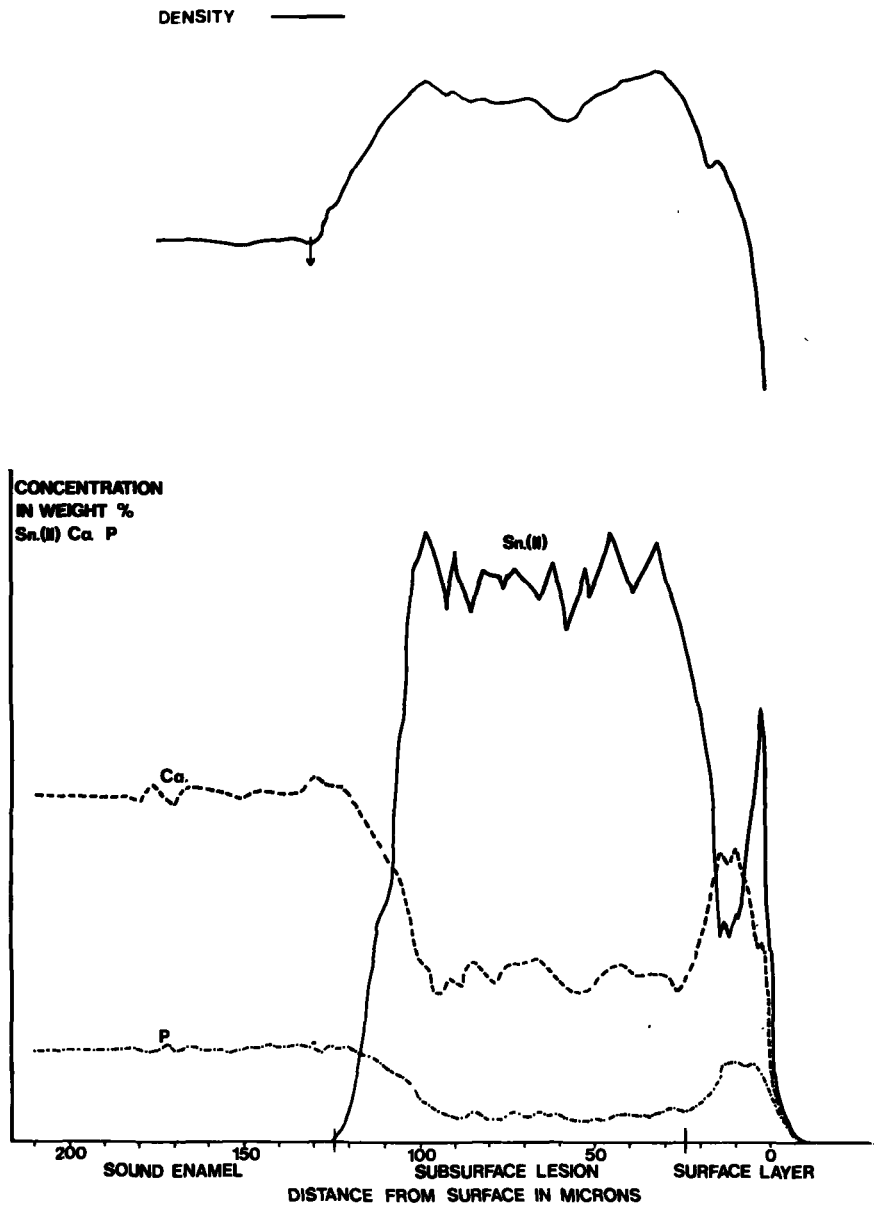


Fig. 8. The microprobe and microdensity results of the remineralized section of figure 5.

Discussion

The experimental results indicate that it is possible to remineralize caries lesions without calcium or phosphorus ions being present in the remineralizing fluid. As WEI (1967) suggested, it is probably better to differentiate between recalcification (Ca and PO_4) and remineralization (fluorides and heavy metal solutions).

Stannousfluoride has been used in several remineralization experiments (WEI and FORBES, 1968; FRANCIS, BRINER and GRAY, 1973). FRANCIS et al. (1973) showed that a 2% SnF_2 solution produced a degree of remineralization After 30 minutes application. Higher concentrations and longer application times caused, in contrast, a further dissolution of enamel. The temperature was not, however, reported by these authors.

FRANCIS et al. (1973) stated, that the problem in remineralization is: 'To move ions into the free spaces in the white spot without further damage.' The results of our investigation show that this problem might be overcome by the increased temperature of the stannousfluoride solution used. The irregular thin Sn-rich layer on the sound enamel, was also reported by WEI and FORBES (1974) using a 10% stannousfluoride solution. These authors found two Sn-rich zones, a phenomenon which was not observed in this investigation. These two zones were probably the effect of the higher concentration of the SnF_2 solution.

Although there is a variety of patterns, suggesting that the individual structure of enamel may be important, the results show that the overall remineralization process is similar to that depicted in fig. 6.

It can be observed from this figure that remineralization of the outer part of the surface layer covering the lesion, occurs after only half an hour of stannousfluoride treatment. This very thin Sn-rich layer has a thickness similar to that occurring on sound enamel, and is in some cases a direct continuation of this sound enamel layer.

The Sn-rich layer on the surface layer of the lesion is never irregular, and its X-ray density was higher than the density of the underlying normal enamel in 38 sections out of 40. The body of the lesion shows a very small increase in density at this early stage. The next step appears to be a remineralization of the central part of the sub-surface lesion, followed by remineralization of the inner part of the lesion. Afterwards the areas between the zones of higher density are filled in, beginning with the zone inside the surface layer. From that moment it is impossible to distinguish between surface layer and sub-surface lesion on microradiograms. This process appears to be a stepwise progression, almost a reversal of the stepwise pattern seen in caries lesion formation. This suggests that diffusion problems, similar to those of caries lesion formation, may be involved in the remineralization process.

The chemistry of this remineralization process is very complex. Stannousfluoride not only reacts with the hydroxyapatite of sound enamel, but also with the hydroxyapatite in the surface layer and the sub-surface lesion and the dissolution products, which are of the brushite-type, $CaHPO_4 2H_2O$ (ARENDS and DAVIDSON, 1974; BROWN, 1974; MORENO and ZAHRADNIK, 1974).

JORDAN et al. (1971); BERNDT (1972) and WEI (1974) gave convincing evidence that the reaction product of stannousfluoride and sound enamel is a crystalline material $Sn_3F_3PO_4$. These authors suggested that only small amounts of CaF_2 are formed whilst no evidence was found for the formation of fluorapatite.

The chemical reactions between carious enamel and stannousfluoride must be different because of the presence of $CaHPO_4$ $2H_2O$. WEI and FORBES (1968) showed that neither CaF_2 nor fluorapatite were formed in carious dentine after SnF_2-treatment at 37°C. This is in agreement with our infra-red experiments carried out 20°C and 37°C on SnF_2-treated brushite. On the other hand, fluorapatite appears to be present when brushite is treated at 50°C. X-ray diffraction and infra-red experiments were in agreement on this point.

Summarizing, it appears that at the surface $Sn_3F_3PO_4$ is formed; whilst in the body of the lesion a mixture of fluorapatite, $Sn_3F_3PO_4$ and other Sn-containing materials may be present.

REFERENCES

ANDRESEN, V.: Ueber Mineralisation und Remineralisation des Zahnschmelzes. Dtsch. Mschr. Zahnheilk. **39**: 97 (1921).

ARENDS, J. and DAVIDSON, C.L.: The HPO_4^{--} content in sound and caries bovine enamel. Calc. Tiss. Res. (1974) in press.

BACKER DIRKS, O.: Posteruptive changes in dental enamel. J. dent. Res. **45**: 503-511 (1966).

BERNDT, D.F.: Reaction of stannous fluoride with hydroxyapatite. The crystal structure of $Sn_3F_3PO_4$. J. dent. Res. **51**: 53-57 (1972).

BROW, W.E.: Physicochemical Mechanisms of Dental caries. J. dent. Res. **53**: 204-216 (1974).

FEAGIN, F.; KOULOURIDES, T., and PIGMAN, W.: The characterization of enamel surface demineralization, remineralization and associated hardness changes in human and bovine material. Arch. oral Biol. **14**: 1407-1417 (1969).

FRANCIS, M.D., BRINER, W.W. and GRAY, J.A.: Chemical agents in the control of calcification processes in biological systems. Ciba foundation symposium II: Hard Tissue growth, repair and remineralization, Amsterdam, Elsevier, p. 57-90 (1973).

JORDAN, T.H., WEI, S.H.Y., BROMBERGER, S.H. and KING, J.C.: $Sn_3F_3PO_4$: the product of the reaction between stannous fluoride and hydroxyapatite. Arch. oral Biol. **16**: 241-246 (1971).

LENZ, H. and MÜHLEMANN, H.R.: In vivo and in vitro effects of saliva on etched or mechanically marked enamel after certain periods of time. Helv. odont. Acta **7**: 47-49 (1963).

MELLBERG, J.R. and LOERTSCHER, K.L.: Fluoride acquisition in vitro by sound human tooth enamel from sodium fluoride and ammonium silicofluoride phosphate solutions. Arch. oral Biol. **17**: 1107-1116 (1972).

MORENO, E.C. and ZAHRADNIK, R.T.: Chemistry of enamel sub-surface demineralization in vitro. J. dent. Res. **53**: 226-235 (1974).

SILVERSTONE, L.M. and POOLE, D.F.G.: The effect of saliva and calcifying solutions upon the histological appearance of enamel caries. Caries Res. **2**: 87-96 (1968).

WEI, S.H.Y. and KAQUELER, J.C.: Remineralization of dentinal caries in vitro. I.A.D.R. 15th General Meeting, p. 79 (1967).

WEI, S.H.Y.: Remineralization of enamel and dentine. A review. J. dent. Child. **34**: 444-451 (1967).

WEI, S.H.Y. and FORBES, W.C.: X-ray diffraction analysis of carious dentine treated with stannous fluoride. Arch. oral Biol. **13**: 407-416 (1968).

WEI, S.H.Y. and FORBES, W.C.: Electron microprobe investigations of stannous fluoride reactions with enamel surfaces. J. dent. Res. **53**: 51-56 (1974).

WEI, S.H.Y.: Scanning electron microscope study of stannous fluoride treated enamel surfaces. J. dent. Res. **53**: 57-63 (1974).

SAMENVATTING

Tandbederf is de meest verbreide ziekte in de westerse samenleving. Bijna niemand is er vrij van, zoals uit een recent onderzoek in Culemborg blijkt, waar zelfs geen enkel individu met een gaaf gebit wordt aangetroffen in een groep van ongeveer 250 personen tussen 17 en 18 jaar. Op grond van dergelijke gegevens is het gemakkelijk te berekenen, dat het huidige aantal praktiserende tandartsen niet voldoende is om de bevolking door middel van *curatieve* hulp, tandheelkundig gezond te maken.

De enige mogelijkheid om dit toch te bereiken, is door *preventie* van tandbederf: die maatregelen treffen, die tandbederf voorkomen.

Tandbederf ontstaat wanneer het tandglazuur aangetast wordt door de inwerking van zuren, gevormd door bacteriën die zich in een dun laagje (de plaque) op het tandoppervlak bevinden. Deze zuur-vormende bacteriën vergisten bij voorkeur die suikers (saccharose = riet- of bietsuiker, en glucose = druivensuiker), die juist door de suikerverwerkende industrie in allerlei soorten versnaperingen worden verwerkt.

Tandbederf treedt alleen op wanneer bacterie, suiker en tand tegelijkertijd op elkaar kunnen inwerken. Dierproeven tonen aan, dat er na het geven van een suikervrij dieet geen tandbederf optreedt, maar ook is het zo, dat een suikerrijk dieet, gegeven aan kiemvrije dieren, geen aantasting van het gebit veroorzaakt. De bacterioloog en de voedingsdeskundige hebben dan ook waardevolle bijdragen geleverd op het gebied van de tandbederfbestrijding. In hoofdstuk I wordt hierop uitvoeriger ingegaan.

Het onderzoek naar de eigenschappen van het tandglazuur zelf biedt ook zeer goede aanknopingspunten tot het voorkomen van tandbederf. Met deze kant van de preventie houdt dit proefschrift zich bezig.

Het doel van deze studie is informatie te verzamelen over het ontkalkingsproces van het tandglazuur en middelen te beproeven die dit proces afremmen, tot staan brengen of zelfs in omgekeerde richting doen verlopen. Dit laatste is het geval bij de zogenaamde remineralisatie.

Het begin van het ontkalkingsproces is klinisch duidelijk waarneembaar als een krijtachtige, witte plek op het tandglazuur. Vrij snel na de doorbraak van de elementen zijn deze witte plekken al zichtbaar langs de randen van het tandvlees. Als het proces voortschrijdt, treedt een desintegratie van het glazuur op, wat aanleiding geeft tot de vorming van een caviteit. Het is duidelijk, dat preventieve maatregelen vóór die tijd genomen moeten worden.

Wanneer van deze krijtachtige, witte plek, 'de initiële carieslesie', een coupe wordt bestudeerd onder de microscoop, dan blijkt de eigenlijke ontkalking van het glazuur zich *onder* een intact gebleven oppervlaktelaag te bevinden. De verklaring voor dit verschijnsel is nog niet gevonden. Zeker is, dat inwerking van zuur alléén dit beeld niet te voorschijn kan roepen. Von Bartheld heeft als eerste de aandacht gevestigd op de rol die hoogmoleculaire stoffen in de plaque, naast de zuurvorming, bij het ontstaan van de carieslesie spelen. In hoofdstuk I wordt kort ingegaan op de achtergronden van Von Bartheld's hypothese, die gebaseerd is op

het ontstaan van een Donnan-membraan evenwicht, waarbij de oppervlaktelaag van de lesie als de semi-permeabele membraan wordt opgevat. Het blijkt in de praktijk mogelijk te zijn met behulp van aangezuurde hoogmoleculaire oplossingen in vitro carieslesies te maken, die morphologisch (bestudeerd met behulp van microradiographie en gepolariseerd licht) grote overeenkomst vertonen met natuurlijke carieslesies.

Door toepassing van hydroxyethylcellulose in een aangezuurde oplossing blijkt het mogelijk te zijn homogene, reproduceerbare carieslesies te creëren, die daardoor geschikt zijn om de invloed van verschillende factoren op het ontstaan van de lesie te bestuderen.

De in dit proefschrift gebruikte contact-microradiographische techniek wordt in hoofdstuk II en III beschreven. In hoofdstuk IV en V wordt de invloed van het mineraalgehalte van het glazuur, de zuurgraad en de ontkalkingstijd op de dikte van de oppervlaktelaag, de diepte van de lesie en het mineraalgehalte van de oppervlaktelaag en lesie onderzocht.

De volgende conclusies kunnen worden getrokken:

1. Er is een lineair verband tussen het *oorspronkelijke* mineraalgehalte van het glazuur en het mineraalgehalte van zowel de intacte oppervlaktelaag als de zich daaronder bevindende lesie. Een uitzondering is het glazuur met een hoog mineraalgehalte, boven 90 V%.
2. De invloed van het oorspronkelijke mineraalgehalte op de dikte van de oppervlaktelaag en de diepte van de lesie is gering, maar tendeert naar een positieve correlatie in het ene (oppervlaktelaag) en een negatieve in het andere (diepte van de lesie).
3. De dikte van de intacte oppervlaktelaag *neemt toe* met de diepte van de lesie, de dikte van deze laag beweegt zich tussen 20 en 40 micron.
4. De pH noch de ontkalkingstijd hebben een belangrijke invloed op de intacte oppervlaktelaag. Als deze, vrij snel, is gevormd wordt het mineraalgehalte in deze laag ongeveer 20 V% lager dan in het daaronder liggende gezonde glazuur, de dikte varieert van 25 tot 40 micron.
5. De pH heeft een grote invloed op de diepte en op het mineraalgehalte van de lesie. Deze invloed van de pH is aanzienlijk groter dan die van de ontkalkingstijd.

In hoofdstuk VI wordt, op grond van het feit dat de quantitatieve uitkomsten, verkregen met behulp van microradiographie, niet overeenstemmen met die van de electronmicropobe analyse, aannemelijk gemaakt dat brushiet of verbindingen van het brushiet type, na ontkalking, in de lesie zijn ontstaan. Dit is met name van belang omdat brushiet, vooral in een zuur milieu, omgezet wordt in fluorapatiet, wanneer fluoride-ionen aanwezig zijn. Tot nu toe blijkt dat de meest effectieve methode om glazuur minder oplosbaar te maken is: het toepassen van fluoriden, in verschillende vormen, op verschillende manieren.

Het fluoride-ion speelt daarnaast een grote rol in de pre-eruptieve fase, tijdens de tandvorming. Wanneer fluoride-ionen in het weefsel in een concentratie van 1 ppm aanwezig zijn, werken zij katalitisch bij de vorming van het hydroxylapatiet. Er zijn aanwijzingen dat door deze werking van het fluoride een goed gemineraliseerd glazuur ontstaat, dat alleen al daardoor een hogere weerstand

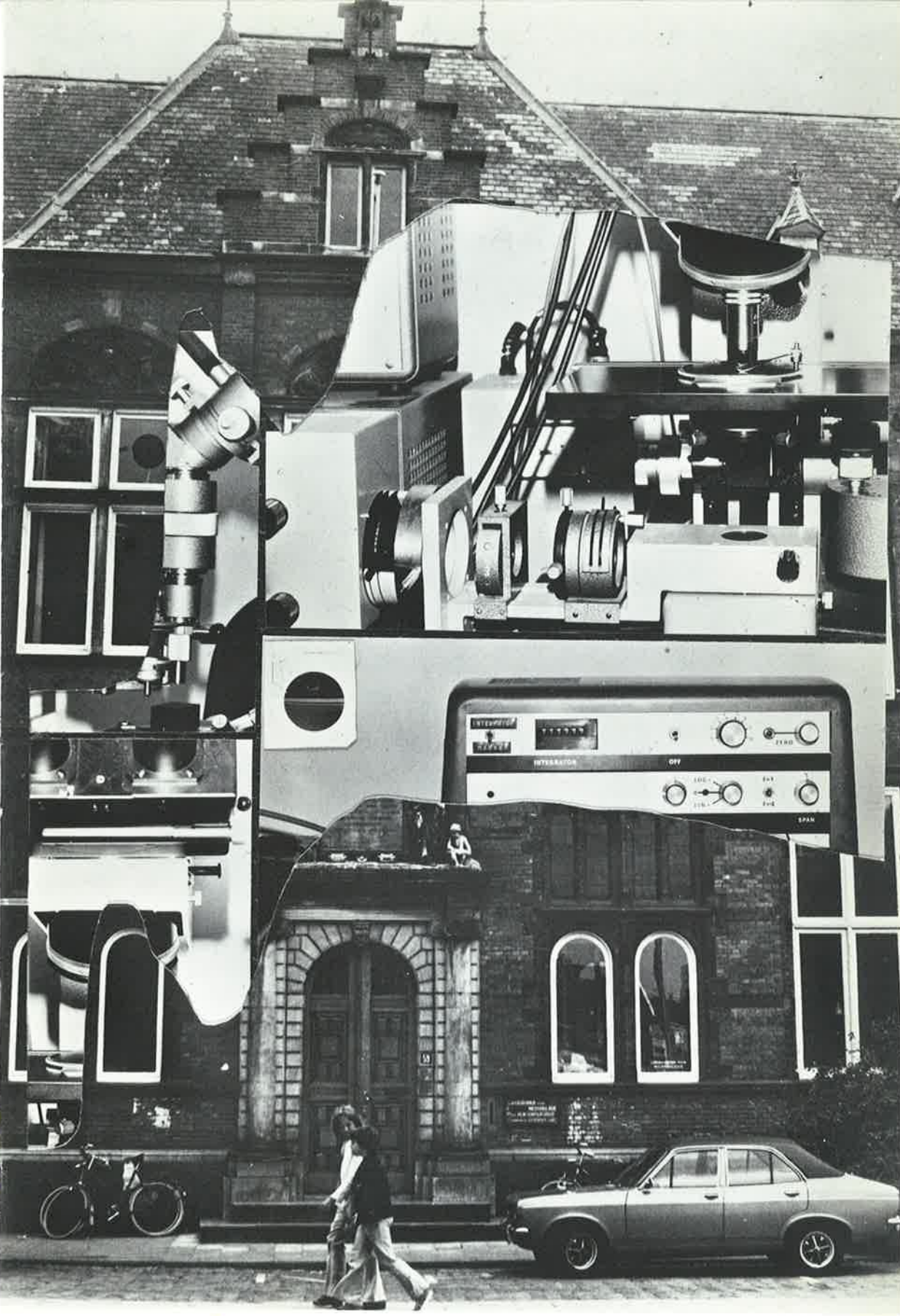
tegen zuurinwerking heeft. Dit effect kan belangrijker zijn dan het ontstaan van het minder oplosbare hydroxy-fluorapatiet.

Post-eruptief, na de doorbraak, kunnen fluoride-ionen direct inwerken op het glazuur, waarbij een gedeelte van het hydroxylapatiet omgezet wordt in het minder oplosbare fluorapatiet of hydroxy-fluorapatiet.

Een derde werkingsmechanisme van het fluoride-ion is, dat het een rol speelt bij de remineralisatie van reeds ontkalkt glazuur. In hoofdstuk VII wordt het effect bestudeerd van twee fluoride oplossingen op glazuur, een organische (cetylaminohydrofluoride — Elmex) en een anorganische (tinfluoride) verbinding. Het blijkt dat lokale applicatie van de organische fluoride verbinding op *gezond glazuur* een grotere remming van het ontkalkingsproces veroorzaakt dan de tinfluoride oplossing. Dit uit zich na de ontkalking met name in een significant hoger mineraalgehalte van de lesie en een duidelijk dikkere intacte oppervlaktelaag. In dat geval, dat de oplossingen geappliceerd worden op reeds *ontkalkt* glazuur blijkt het tinfluoride het meest effectief te zijn, vooral wat het mineraalgehalte van de oppervlaktelaag betreft.

Tenslotte wordt in hoofdstuk VIII de remineralisatie van ontkalkt glazuur door een tinfluoride oplossing bij 50°C onderzocht. Microradiogrammen tonen aan dat reeds na een half uur de buitenzijde (± 10 micron) van de intacte oppervlaktelaag geremineraliseerd is. Een enkele coupe vertoont een volledige geremineraliseerde lesie na een tinfluoride behandeling van 6 uur.

Hoewel op dit moment deze methode nog niet in de praktijk bruikbaar is door de lange applicatietijden, zijn de resultaten van dien aard, dat door modificaties een praktische toepasbaarheid in de toekomst tot de mogelijkheden kan worden gerekend.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