Histopathology of experimental *in vivo* caries around silicate fillings

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Caries was produced around 18 silicate fillings in permanent teeth in 12 patients, aged 14—60 years. The cavities were lined with 1 layer of Tubulitec® (Dental Therapeutics AB, Sweden), and insulated with a zinc phosphate base. The teeth were scheduled for extraction for orthodontic and prosthodontic reasons, and the experimental period ranged from 34—326 days with a mean at 131 days. Four teeth served as controls of the effect of the preparation procedure. Following extraction, ground sections were prepared, and examined mainly by polarized light microscopy and microradiography. Except for minor differences, the pathogenesis of the observed *in vivo* lesions was found to correspond closely to that of experimental *in vitro* caries around silicate fillings. Outer lesions occurred in 14 of the 32 »secondary caries risks» and in most cases revealed only slight demineralization. A cavity wall lesion was observed in the enamel in 24 out of 31 risks, and in the dentine in 3 out of 27 risks. Hypermineralization of enamel and dentinal walls ocurred in about one fifth of the cases. The low susceptibility to experimental caries associated with silicate fillings is explained through an action of fluoride released from the silicate material. The mechanism of this action is discussed.

Key-words: Dental caries; silicate cement; microscopy, polarization.

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The present study is part of a comprehensive investigation on the histopathology of secondary caries associated with various filling materials. In previous investigations the following aspects have been studied: Experimental (*in vitro* and *in vivo*) and natural secondary caries around silver amalgam fillings and experimental *in vitro* caries around silicate fillings (*Hals & Nernaes*, 1971; *Hals & Leth Simonsen*, 1972; *Hals*, 1971; *Hals, Höyer Andreassen & Bie*, (in press); *Hals*, (in press).

In all these studies the lesions displayed the same basic pattern: an outer lesion and a cavity wall lesion. However, when structural details are concerned, the lesions produced around silicate fillings differed typically from those produced around silver amalgam fillings.

The present study aims at describing the histopathology of experimental *in vivo* caries around silicate fillings for comparison with the corresponding *in vitro* study (*Hals*, (in press).

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MATERIAL AND METHODS

18 human permanent teeth in 12 patients, 14—60 years old, were used. Class V cavities, usually 2 x 2 mm wide and 2 mm deep, were prepared on clinically sound buccal surfaces. Great care was taken in the preoperative examination of the surfaces to exclude any that showed evidence of early carious attack. The preparation procedure was performed with an air-turbine with adequate water cooling. The cavity walls were finished in water spray with diamonds or finishing burs at 12000 rpm. After a thorough wash the cavities were dried with short blasts of hot air and lined with 1 layer of Tubulitec® that was not afterwards removed from the enamel walls. The floor of the cavities was further isolated with a zink phosphate cement base, and the silicate (Biotrey®, De Trey Frère S.A. Zürich) fillings inserted. In order to avoid cracking of the surface of the filling no strips were used (*Jörgensen*, 1971) but the surface was contoured with the instrument used for insertion and thereafter covered with vaseline. Before the patients left, the surface of the fillings were covered with varnish. Polishing was not undertaken until at least 24 hours later. Thereafter preformed orthodontic bands were attached to the teeth to ensure accumulation of plaque in order to produce secondary caries according to the method of *Hals* and *Leth Simonsen* (1972). The duration of experiments ranged from 34 to 326 days, with a mean at 131 days. All the experimental work was performed by one examiner (I-T. N.). Following extraction, the teeth were cleaned, the labial surface examined in a dissection microscope and the observations documented. Longitudinal sections 60—120 μm thick were prepared from the teeth with the fillings *in situ*. In addition to the experimental material four extracted teeth served as control of the preparation procedure and were not filled. Polarized and ordinary light microscopy and microradiography were used in the same way as described by *Hals* and *Nernaes* (1971).

For the examination and photography by polarized light all sections were imbibed in distilled water (n=1,33) and quinoline (n=1,62). Reexamination took place after mounting in Canada balsam (n=1,53). Since it was soon realized that this study would in particular be concerned with very early stages of enamel caries, many sections were also examined in air (n=1,0) and methanol (n=1,33) (*Silverstone*, 1966). Countour maps (*Darling*, 1958, *Crabb*, 1966) based on the appearance of isotropic lines occurring by imbibition in the series air (n=1,0), distilled water (n=1,33), Thoulets liquid (n=1,41) and Thoulets liquid (n=1,47) were prepared in a few cases.

RESULTS

Outer lesions

Table I shows that in 28 out of 32 cases of the secondary caries risks there was

Table I. *Experimental in vivo caries around silicate fillings. Frequency of outer lesions of different severity*

Severity of lesion	No.
No lesion	18
Only observable by polarized light (<5% spaces)	10
Lesions displaying radiolucency (>5% spaces)	4
Total number of secondary caries risks*	32

*) Every tooth represents 2 secondary caries risks

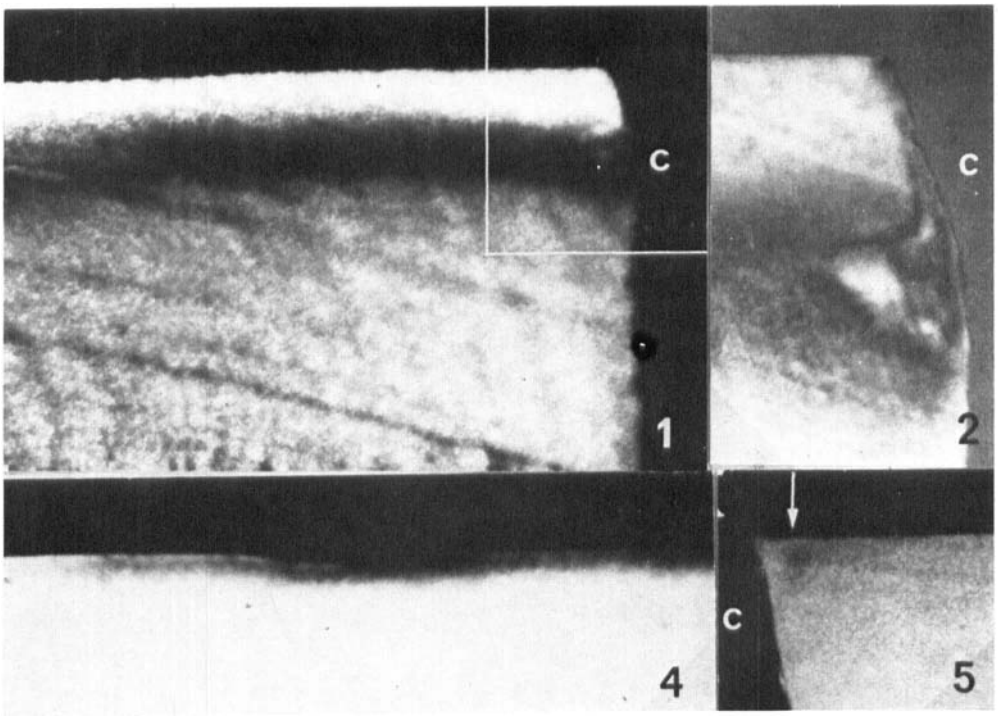

Fig. 1. Experiment 12. 101 days. Enamel incisally to the cavity C. Air-dried section. Polarized light. × 56.
Fig. 2. Enframed area seen in Fig. 1. × 140.
Fig. 4. Experiment 5. 326 days. Surface of enamel incisally to cavity. Microradiograph. × 140.
Fig. 5. Experiment 10. 78 days. C, cavity. Arrow, subsurface lesion in the cementum. Microradiograph. × 140.

either no attack at all or the demineralization was so slight that it could not be detected by microradiography. In 14 of the cases in which outer lesions were present, the proportion between the number of lesions only visible by polarized light (<5% spaces) and the lesions visible by microradiography (>5% spaces) was 2.5:1. All caries attacks were superficial without break of the enamel surface, and in 3 out of 4 lesions visible by microradiography, the degree of radiolucency was low. Nine out of 14 lesions including also the radiolucent lesions, which could be observed by polarized light, displayed a positive (dark) zone or, in a few cases, even two such zones. Figs. 1 and 3—5 are representative for outer lesions of the material. Figs. 1 and 3 correspond to a case in which the experimental time was 101 days. A section of the enamel occlusally to the cavity (Fig. 1) was airdried and shows a broad pseudoisotropic zone below a slightly narrower, positively birefringent, surface zone. No corresponding radiolucency was observed, and imbibition analysis indicated an outer lesion with an amount of spaces at the 1—5% level.

Fig. 3 a-e shows a section of the enamel cervically to the cavity. The microradiograph (a) reveals an opaque surface layer over a narrow, slightly radiolucent zone. Near to the cavity this slight radiolucency also includes the outer layer. Gradually fading, it extends deeper into the enamel. In (b) the section was imbibed in methanol

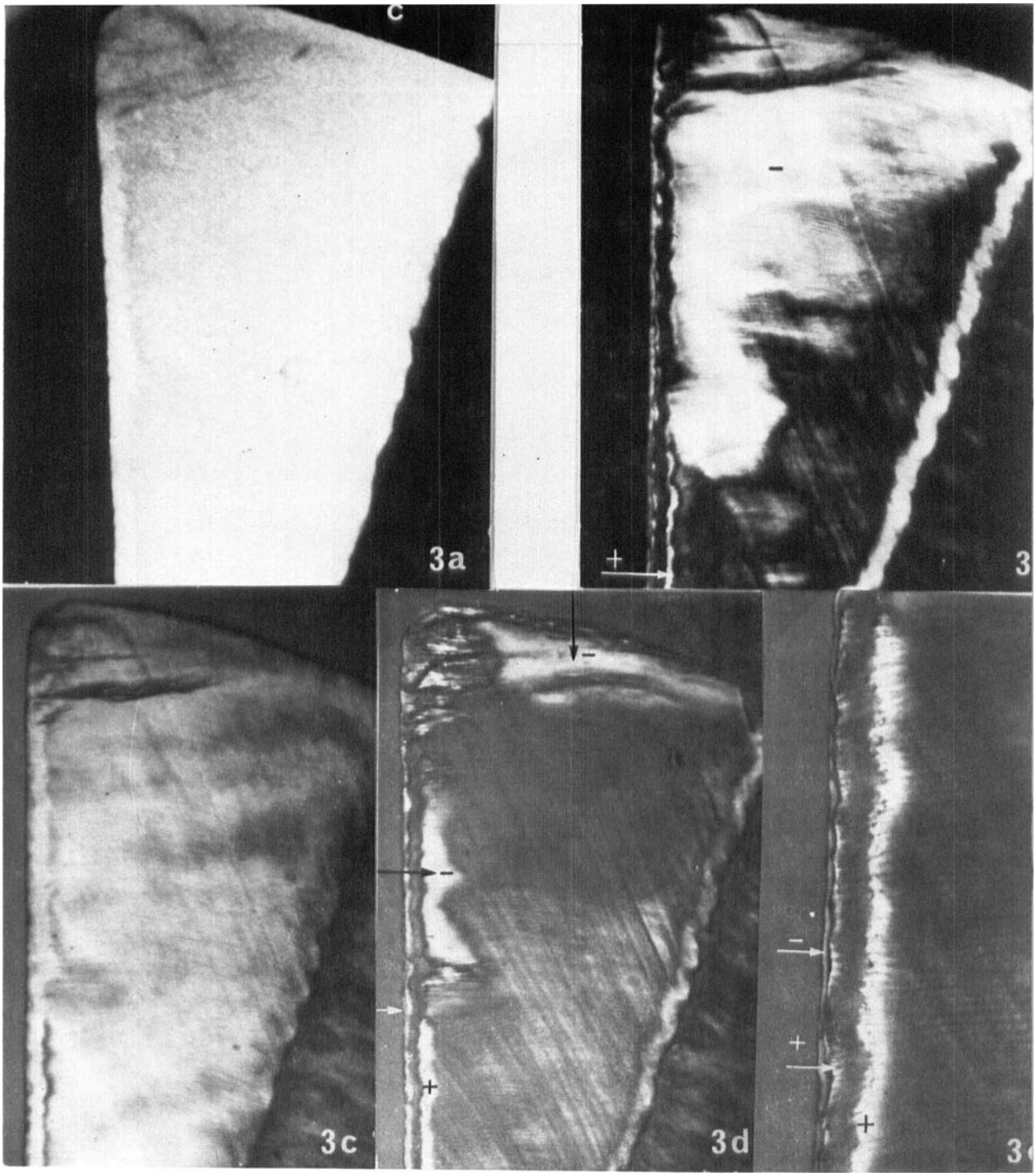

Fig. 3. Experiment 12. 101 days.
Enamel cervically to the cavity.
a) C, cavity. Microradiograph. × 140.
b) +, positive birefringence.
—, negative birefringence.
Methanol. Polarized light. x 140.
c) Distilled water. Polarized light.
Gypsum 1. order Red compensator.
× 140.
d) +, positive birefringence.
—, negative birefringence.
Quinoline. Polarized light.
Gypsum 1. order Red compensator.
× 140.
e) Outer layer of enamel cervically to white arrow in (d).
+, positive zone between pseudoisotropic borderlines.
—, negative birefringence.
Quinoline, Polarized light.
Gypsum 1. order Red compensator.
× 350.

for 24 hours. The large bright area, corresponding largely to the slightly radiolucent area in (a), displays strong negative birefringence whilst the narrow, subsurface bright zone, continuing in cervical direction, is strongly positively birefringent. The outer layer of enamel reveals a complicated system of zones, the nature of which is discussed in connection with (e).

In (c) the section was imbibed in distilled water. In the area of the cavity margin the negative birefringence of the enamel is slightly weaker than normally. A narrow subsurface zone shows positive birefringence and/or pseudoisotropy. Imbibition in quinoline (d) is included in this series because this method is generally used for the demonstration of the positive zone and the translucent zone in enamel caries lesions. In the present case a negatively birefringent and translucent zone is seen in two areas, and it is evident that the quinoline molecules have only penetrated part of the large, bright area seen in (b). The positive (dark) zone(s) has a rather superficial localization. The outer layer of the enamel cervically to the white arrow is seen by higher magnification in (e). Here, a narrow zone in the enamel surface displays strong negative birefringence. In the subsurface enamel a thin positive zone between pseudoisotropic lines is visible. Deep to this, another similar, but wider zone is observed, whilst the intermediate enamel exhibits week negative birefringence.

The microradiographic and imbibition analysis of this section indicates that the main area of the lesion only contains 1—5% spaces, whilst the area at the cavity margin and a narrow subsurface zone represent the 5—10% level.

The dominathing feature of the lesion is the extensive translucent zone. Generally, in enamel caries lesions, the translucent zone is confined to a narrow band at the advancing front of destruction.

Possibly, the area of the lesion bordering to the cavity wall may have developed as a wall lesion (compare the wide wall lesion seen in Fig. 8).

Fig. 4 — duration of experiment 326 days — shows a markedly radiolucent lesion beneath an extremely thin radiopaque surface layer. In one area also the surface is radiolucent. Examination by polarized light showed, however, that no break of the surface had taken place.

In one case, not included in Table II, the tooth surface at risk was cementum. Also in this case a subsurface lesion had formed (Fig. 5).

Wall lesions

Except for insignificant changes in two cavity walls the control sections appeared quite normal with the examination methods used. Frequency and features of wall lesions are seen from Table II. Since radiolucent lesions are also observable by polarized light the enamel walls

Table II. *Experimental in vivo caries around silicate fillings. Frequency of cavity wall lesions of different severity*

Severity of lesions	Enamel No.	Dentine No.
No lesion	7	24
Lesions only observable by polarized light	20	
Lesions observable due to radiolucency	4	3
Total number of secondary caries risks	31	27
Lesions displaying increased radiopacity of cavity wall surface	5	6

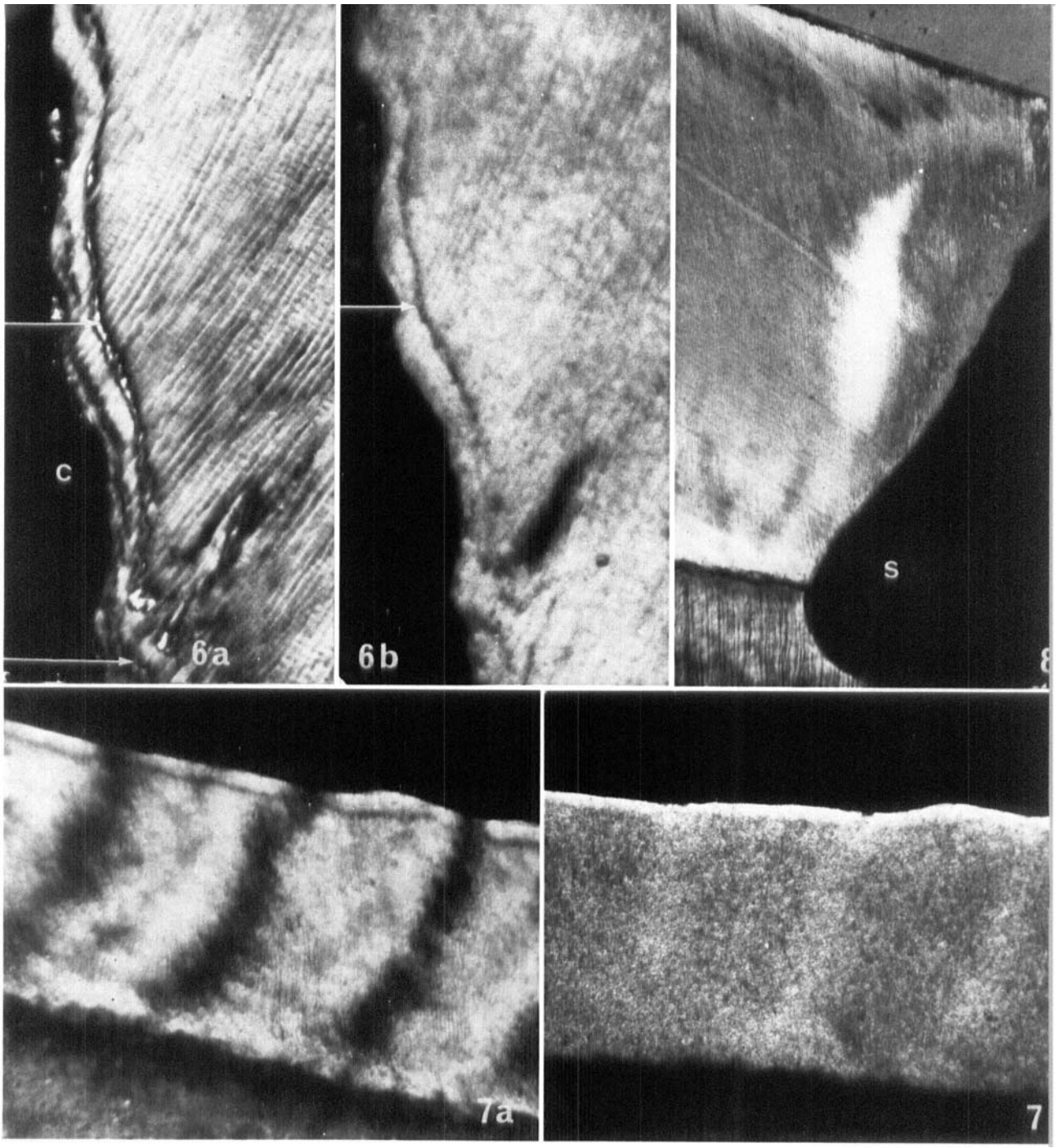

Fig. 6. Experiment 3. 171 days.
Enamel from cavity wall.
a) Arrows, narrow positive zones between pseudoisotopic lines.
Quinoline, polarized light. × 350.
b) Arrow, pseudoisotropic line.
Distilled water, polarized light.
× 350.

Fig. 7. Experiment 18. 78 days.
Enamel from bottom of a cavity.
a) distilled water, polarized light. × 84.
b) Microradiograph. × 84.

Fig. 8. Experiment 11. 101 days.
Enamel incisally to cavity.
S, silicate filling.
Quinoline. Polarized light.
Gypsum 1. order Red compensator.
× 140.

showed a total of 24 lesions that could be observed by the latter method. The lesions were similar to those occurring in the *in vitro* experiments. All of them displayed a positive zone between pseudoisotropic borderlines, one case even two such zones. The section shown in Fig. 6a was imbibed in quinoline and examined by polarized light. A rim in the surface of the enamel wall displays higher negative birefringence than the underlying intact enamel against which it is delimited by a narrow positive zone between pseudoisotropic lines, and which in the lower part of the photomicrograph extends deeper into the enamel. A corresponding zone is seen just below the surface of the enamel wall.

After imbibition of the section in distilled water (Fig. 6 b) the rim of enamel displayed a faintly visible increase of the normal negative birefringence, whilst the positive zones had changed to faint pseudoisotropic lines. Microradiographs of the section showed no definite changes corresponding to the rim. The findings would indicate a rim of enamel displaying a very slight increase of mineralization, not sufficient to be detected by microradiography. The positive/pseudoisotropic lines in Fig. 6a, b, represent very slight demineralization ($\leq 5\%$ spaces).

Fig. 7. shows the bottom of a cavity which in this case was cut entirely in the enamel. By polarized light, imbibition in distilled water (a) the wall lesion appears as a rim with a slight, but registrable increase of the normal negative birefringence of the enamel. The zone is delimited against the normal enamel by a thin pseudoisotropic line. In the corresponding microradiograph (b) the rim displays definitely increased radiopacity compared with the intact enamel. A narrow radiolucent line on the border to the latter is barely visible. This appearance could be expected on the base of the changes observed by polarized light. Actually, comparison of the findings strongly suggests that the radiopacity displayed in (b) is not formed as a Mackie effect.

Fig. 2 shows the enframed area in Fig. 1 by higher magnification. The triangular area, which must be regarded as a wall lesion, reveals partly pseudoisotropy, partly positive birefringence. As mentioned above the area is not radiolucent.

In Fig. 8, the wall lesion extends unusually deep into the enamel, from which it is delimitated by a very broad translucent zone displaying highly increased negative birefringence. A discontinuous positive zone, virtually consisting of patches of positive areas, is located in the body of the lesion, apparently without connection with the translucent zone. However, in sections imbibed in Canada balsam, the positive zone was broader and reached the translucent zone, at least in some areas. No changes could be detected in the enamel wall.

There is also an outer lesion. Just below the surface or in the very surface zone, there is a narrow positive zone, shown by higher magnification in Fig. 9. The delimitation of this zone against the underlying enamel is determined by the single prisms. Possibility exists that the border of the outer lesion is located deeper in the enamel, but this could not be assessed with certainty (Fig. 8). Neither the wall lesion nor the outer lesion were visible by microradiography. Analysis by the contour map method led to the conclusion that the lesions contain spaces at the 1—5% level.

In the dentine demineralized subsurface wall lesions were present in 3 cases, all covered by a surface zone of increased radiopacity. One of them was a »funnel» that was typical in the *in vitro* experiments

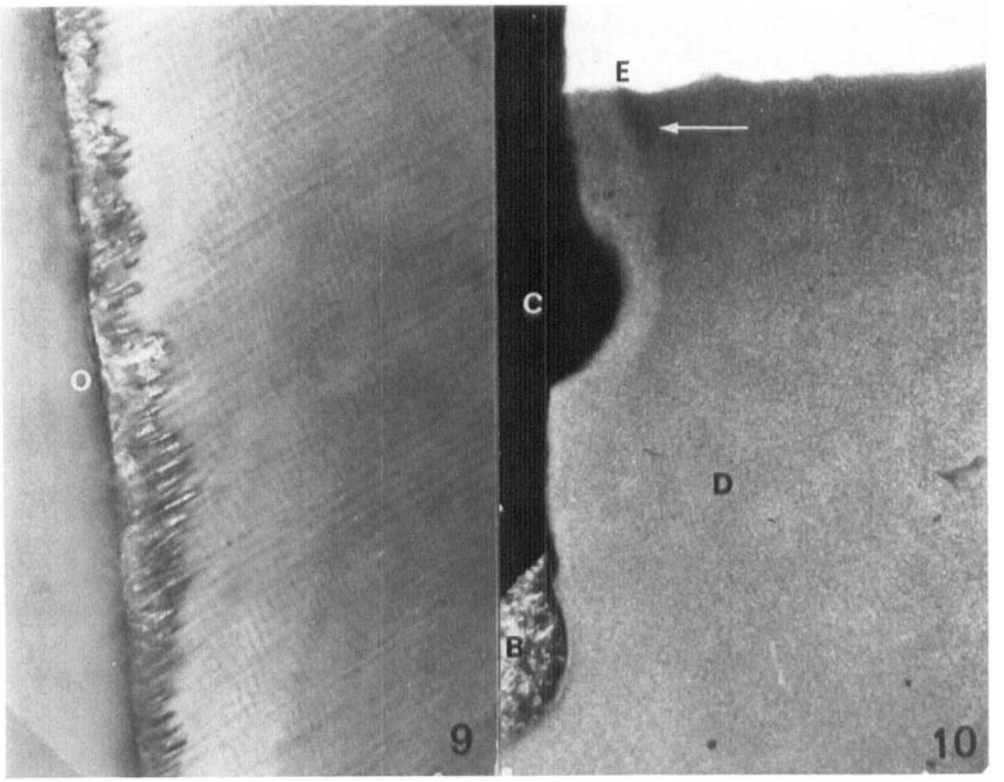

Fig. 9. Same section and technique as in Fig. 8. Outer layer of enamel incisally to cavity. O, outer surface of enamel. × 350.

Fig. 10. Experiment 3. 171 days.
D, dentine.
E, enamel.
C, cavity.
B, cement base.
Microradiograph. × 140.

(Fig. 10). In 3 other cases the dentinal wall was hypermineralized without an underlying demineralized zone.

DISCUSSION

It is well known that in the oral environment silicate cement disintegrates as a result of exposure to acids produced by the degradation of carbohydrates (*Skinner & Phillips*, 1968). The disintegration in the present *in vivo* experiments occurred after a remarkably short time of exposure and must be explained by the conspicuous accumulation of plaque that takes place with the method used.

The results showed that both the frequency and the severity of outer lesions around the silicate fillings were lower than in the case of outer lesions produced in the same way around silver amalgam fillings (*Hals & Leth Simonsen*, 1972). It would appear that the factor most likely to be responsible for this, is the fluoride contained in the silicate material. In the present experiments, however, the cariogenic effect of the plaque was active on the surface enamel several millimetres beyond the margin of the filling. It is not possible that enamel at such a distance from the point of contact with the filling could have received fluoride by ionic ex-

change directly from the silicate. The most likely explanation is that the marked caries resistance of the surface enamel is the result of a relatively high fluoride content of the plaque. This would result from the dissolution of the surface of the silicate filling, producing a constant release of fluoride into the plaque. According to *Dawes et al.* (1965) the concentration of fluoride in plaque from children in a low fluoride town was 25 ppm on a wet weight basis, whilst *Birkeland* (1970) arrived at somewhat lower values.

Some authors contend that the plaque fluoride is bound within the bacteria (*Jenkins et al.*, 1969), while others claim that fluoride bound in plaque is probably present in a calcium phosphate precipitate, from which it can be released when pH is lowered (*Birkeland*, 1973). Very little of the fluoride is present in ionized form.

In the present experiments it can be presumed that this high concentration of fluoride must have reduced the glycolysis in the plaque, which is known to be inhibited already at concentrations at the level 10 ppm F^- (*Jenkins*, 1970). Further it can be presumed that irrespective of the binding mechanism of fluoride, the plaque will very soon be supersaturated, resulting in considerable amounts of ionized fluoride that will spread in the plaque. Due to the experimental conditions the plaque could not be »diluted» with saliva to a degree that takes place in ordinary cases. Even if some fluoride is washed out by the saliva, much of it has apparently been taken up in the enamel or the cementum, rendering these tissues an increased resistance against dissolution. In addition, the F^- ions have favoured remineralization following possible demineralization. *Norman et al.* (1972) reported that plaque taken from the margins of silicate restorations revealed a distinctly higher carbohydrate-nitrogen ratio than plaques from the margins of other types of restorations. Concerning the presumed interference of the fluoride leached fom the silicate restorations with the metabolism of plaque carbohydrate, we are in agreement both with these authors and with *Volker*, *Belakis* and *Milillo* (1944).

Just as in the *in vitro* experiments the histopathology and the progression of the outer lesions seemed to be influenced by the increased fluoride uptake in the enamel. Besides the low degree of demineralization, the most characteristic feature was the usual absence of the classical triangle-shape of the carious lesions. A comparison of the lesions produced *in vitro* and *in vivo* reveals the following points of interest:

There is an almost complete coincidence when frequency of outer lesions is concerned. In the *in vivo* material the radiolucent lesions represented a higher percentage of the total number of lesions than in the *in vitro* material. The outer lesions produced *in vivo* showed in a few cases a very slight surface demineralization that could not be observed in the *in vitro* lesions. Even if frequency of the positive, dark zone was 3 times more frequent *in vivo* than *in vitro*, this zone was usually very thin, and the difference should hardly be ascribed any importance.

In the *in vitro* experiments formation of wall lesions was caused by lactic acid which had diffused from the gelatin covering the tooth surface into the microspace at the interface tooth-filling. This effect was superimposed on the initial effect caused by the phosphoric acid of the silicate material. It is an obvious conclusion that the cavity wall lesions *in vivo* have developed in the same way.

In the *in vitro* study, in which one half

of the cavities was unlined, whilst the other half was lined with Tubulitec®, the liner seemed to have reduced the number of wall lesions in the dentine, but not in the enamel. In the present study in which all the cavities had been lined, the frequency of cavity wall lesions in the enamel was approximately on the same level as *in vitro*, whilst the proportion of dentinal wall lesions *in vivo* versus *in vitro* was as 1:7. The present study therefore supports the impression gained from the *in vitro* study that, when silicate fillings are concerned, a lining with Tubulitec® will reduce the number of wall lesions in the dentine.

It should be noted that out of 54 enamel cavity wall lesions in the *in vitro* series none displayed radiolucency or radiopacity. In the *in vivo* material 4 lesions out of 24 combined radiolucency and increased radiopacity whilst 1 lesion showed increased radiopacity alone. The difference between the *in vitro* and *in vivo* series in this respect seemed to be too marked to be the result of chance. The hypermineralization of the enamel wall seemed to be related to the demineralization of the underlying enamel and it could be inferred that the phenomenon is unrelated to either of the factors duration of experiments and the use or non-use of Tubulitec®.

In conclusion, on comparing the »secondary caries» lesions produced *in vivo* and *in vitro* it seems justified to state that the former are slightly more advanced as long as the enamel are concerned. Obviously, the marked difference in the number of dentine lesions can be explained by the different use of liners in the two studies. In the present study the hypermineralization of the dentinal wall, which was frequent in both series, has been explained as reprecipitation of minerals following demineralization by phosphoric and lactic acids. It could perhaps be argued that it could be the result of a vital reaction of the odontoblasts in response to the acid irritaments. If so, the hypermineralized area would probably have stretched deeper into the dentine. Theoretically, increased radiopacity could also develope through emigration of ions from the silicate material. However, a preliminary electron probe microanalysis did not give any indication in this direction.

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