Inhibitory effect of a fluoride-containing amalgam on development of cavity wall lesions *in vitro*

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This study aimed to compare caries-like lesions produced around fluoride-containing amalgam and conventional amalgam fillings *in vitro*. Class V cavities were prepared in 90 extracted premolars, a fluoride-containing amalgam was inserted in 45 cavities (experimental group) and a conventional amalgam in 45 cavities (control group). After three months in an acidified gelatin solution, a longitudinal ground section passing through the cavity was prepared from each tooth. The sections were imbibed in water, quinoline and some in air before examination by polarized light microscopy. When imbibed in Canada balsam the sections were photographed and areas of demineralization in the enamel measured morphometrically. Some sections were studied by microradiography and electron microprobe analysis.
Numerical scoring of the histological observations revealed significantly lower values for enamel and dentin cavity wall lesions in the experimental group, while the scores for the outer lesions were not statistically different between the two groups. The mean extension of enamel wall lesions was significantly lower in the experimental group, while no difference was observed in the outer lesions. A zone of increased mineralization in the enamel cavity walls was a frequent finding in the experimental group. This study has shown that fluoride-containing amalgam has an inhibitory effect on the development of experimental cavity wall lesions *in vitro*.

Key-words: Filling materials; caries

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Secondary carious lesions seem to be one of the most important factors leading to replacement of amalgam restorations (5, 32). The considerably lower incidence of secondary caries associated with silicate cement compared with amalgam restorations has been explained by the release of fluoride from the filling material (25, 31). In order to simulate the anticariogenic properties of silicate cement, fluoride has been incorporated into amalgam (2, 21, 29). It has been shown that addition of fluoride to silver amalgam reduces the enamel solubility of tooth structure adjacent to the restorations (22). Relevant literature, however, contains few studies of the possible cariostatic effect of this material.

Secondary caries consists of outer lesions and cavity wall lesions (14). Minoguchi et al. (29) observed a 25% lower incidence of secondary caries associated with a fluoride-containing amalgam compared with a conventional amalgam,

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after five years. Since this was a clinical study, only the outer lesions adjacent to the restorations could be registered.

Using acidified gelatin, von Bartheld (1) and Silverstone (33) have produced *in vitro* lesions simulating primary caries. Caries-like lesions adjacent to restorations can be produced by the same method (17,24), which was also used in the present study. This aimed to compare outer lesions and cavity wall lesions produced around fluoride-containing amalgam fillings with similar lesions around conventional amalgam fillings.

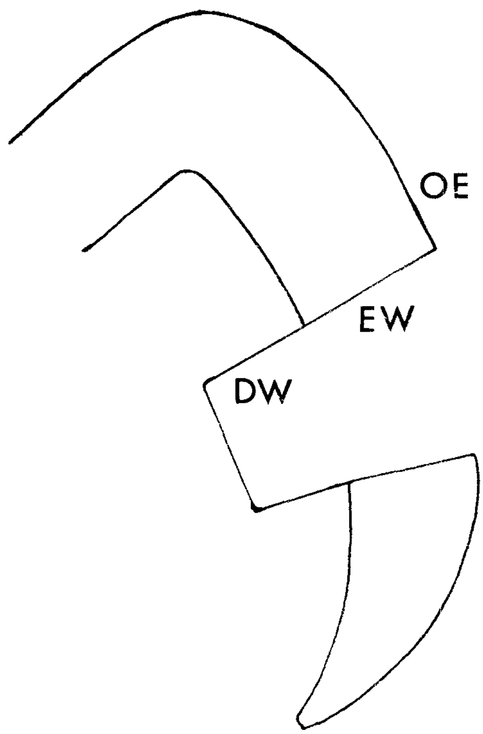


Fig. 1. Schematic drawing of a section through buccal cavity of a premolar. Outer enamel (OE) adjacent to the cavity, enamel and dentin cavity walls (EW, DW) each represents a caries risk. Each section represents an occlusal and a cervical set of risks.

MATERIALS AND METHODS

Ninety extracted human premolars which had been stored in 10% neutral formalin, were selected and randomly assigned to two groups. The teeth were not allowed to dry during any stage of the experiment. Class V cavities were prepared by an air rotor with adequate water cooling on clinically sound buccal or lingual surfaces in the middle or outer third of the dentin. The cavity walls were then treated with diamond (80,000 rpm) and finishing burs (15,000 rpm.)

After rinsing with water, the cavities were dried with compressed air. Fluoride-containing amalgam (Fluor Alloy®) was inserted in 45 cavities (experimental group) and a conventional amalgam (New True Dentalloy®) in 45 cavities (control group). The manufacturers' instructions were followed, and the material was condensed by hand instruments. The teeth were then covered by acid-resistant nail varnish, leaving an approximately 1 mm wide zone around the fillings uncoated. When the varnish had dried, the teeth were stored at 37°C in artificial saliva (28) to promote diffusion of the solution into the amalgam-tooth interspace. After 48 hours they were immersed in a 20% solution of gelatin, type A (J. T. Baker, Chemicals N.V., Denver, The Netherlands), adjusted to pH 4.2 by adding 30% lactic acid. Thymol was added to prevent bacterial growth. On the assumption that a possible inhibitory effect of the fluoride-containing amalgam would be small, the agent used to produce lesions should be sufficiently sensitive to discriminate a possible difference between the groups. The pH value 4.2 was selected on the basis of a pilot study. After 3 months the teeth were removed from the gelatin solution and a 60–120 µm thick, longitudinal ground section, passing in bucco-lingual direction through the cavity, was prepared from each tooth.

Histological examination

All sections were examined by polarized light microscopy as described by Hals &

Table 1. *Scoring system for numerical assessment of the degree of demineralization according to histological observations*

Tissue	Histological observations	Score	
Intact enamel	a) Negative birefringence in water	0	
		Narrow zone[1])	*Broad zone[2])*
Demineralized enamel	b) Zone with reduced negative birefringence in air, no change in water	0.5	1
	c) Translucent zone in quinoline no change in water	1.5	2
	d) Zone with positive birefringence in water,		
	subsurface	2.5	3
	surface	3.5	4
Hypermineralized enamel	e) Zone with increased negative birefringence in water	- 0.5[3])	
Intact dentin	Positive relative to dentinoenamel junction	0	
Demineralized dentin	Cavity wall characterized by von Ebner's phenol reaction,		
	subsurface	1	1.5
	surface	2	2.5

1) Narrow zone: < 20 μm wide
2) Broad zone: > 20 μm wide
3) To be added to score b-d, if present

Nernaes (17). The sections were imbibed in water (n = 1.33), quinoline (n = 1.62), some also in air (n = 1.0) prior to the microscopic examination. When sections are imbibed with phenols (9) or quinoline (17) the birefringence of intact dentin will be positive relative to the dentinoenamel junction, while the birefringence of demineralized dentin will be negative by the same orientation. This change, known as von Ebner's phenol reaction, was utilized to demonstrate demineralized areas of the dentin cavity walls.

In the present study, the cervical and occlusal outer enamel adjacent to the filling and the enamel and dentin cavity wall (Fig. 1) each represented a caries risk. The lesions in the outer enamel and in the enamel and dentin parts of the cavity wall were described separately. Changes occurring in the cavity floor were not included. After discarding some sections that were accidently damaged during the course of the experiment, the number of caries risks in the experimental and control group was 189 and 188 respectively.

In order to compare the degree of demineralization observed in the cavity walls and outer enamel in the two groups, a scoring system was used in the assessment of the results (Table 1). This is a modification and a further developement of a scoring system previously used by Grieve (12) and Jones et al. (23). The subsurface changes in the cavity walls were given lower scores than the surface changes because they were judged to represent a less advanced lesion (35). The score indicating the highest degree of demineralization within the single caries risk was adopted. Concerning the enamel, the scoring system is based on values from the imbibition studies of Darling (6,7,8), Silverstone (34,35) and Crabb (4). The situations described in b–d (Table 1) indicate an increasing degree of demineralization. On the other hand, the minus sign of the score is an expression of loss, not as in b – d an increase of positive form birefringence, judged as hypermineralization. This interpretation was supported by the microradiographic examination, which included microradiographs of six sections of enamel walls showing a translucent zone with increased negative birefringence when imbibed in water. Microradiographs were produced on spectroscopic plates (Kodak, Type 649–0) using nickel-filtered copper radiation.

Electron probe analysis

In order to elucidate which element could be responsible for the changes judged as increased mineralization (Table 1,e) three sections with such changes were analyzed by an Applied Research Laboratories electron microprobe. Analyses were carried out for Ca, P, Sn, Mg and Zn. The method of specimen preparation and the analytical procedure have been described previously (18,39).

Morphometric analysis

To compare the extent of the lesions in the two groups, all sections were photographed when imbibed in Canada balsam ($n = 1.53$) and the area of outer lesions and enamel wall lesions measured on the photomicrographs using a morphometric analyzer (Kontrom Messgeräte, GMBM, MOP-AMO3). Prior to the measurements the outline of the lesions was accentuated with a sharp pencil. For standardization purposes, the peripheral extension of the outer lesions was arbitrarily designated by a line drawn parallell to and at a distance of 0.6 mm from the cavity wall (Fig 2). Some sections in which the outline of the outer lesions was diffuse were excluded from measurement.

Statistical analysis

To test the difference between the numerical scores in the two groups, the normal approximation of the Wilcoxon rank-sum test was used (19). The normal approximation was also used to test the difference between the mean values of the demineralized areas in the two groups (19).

RESULTS

Histological observations

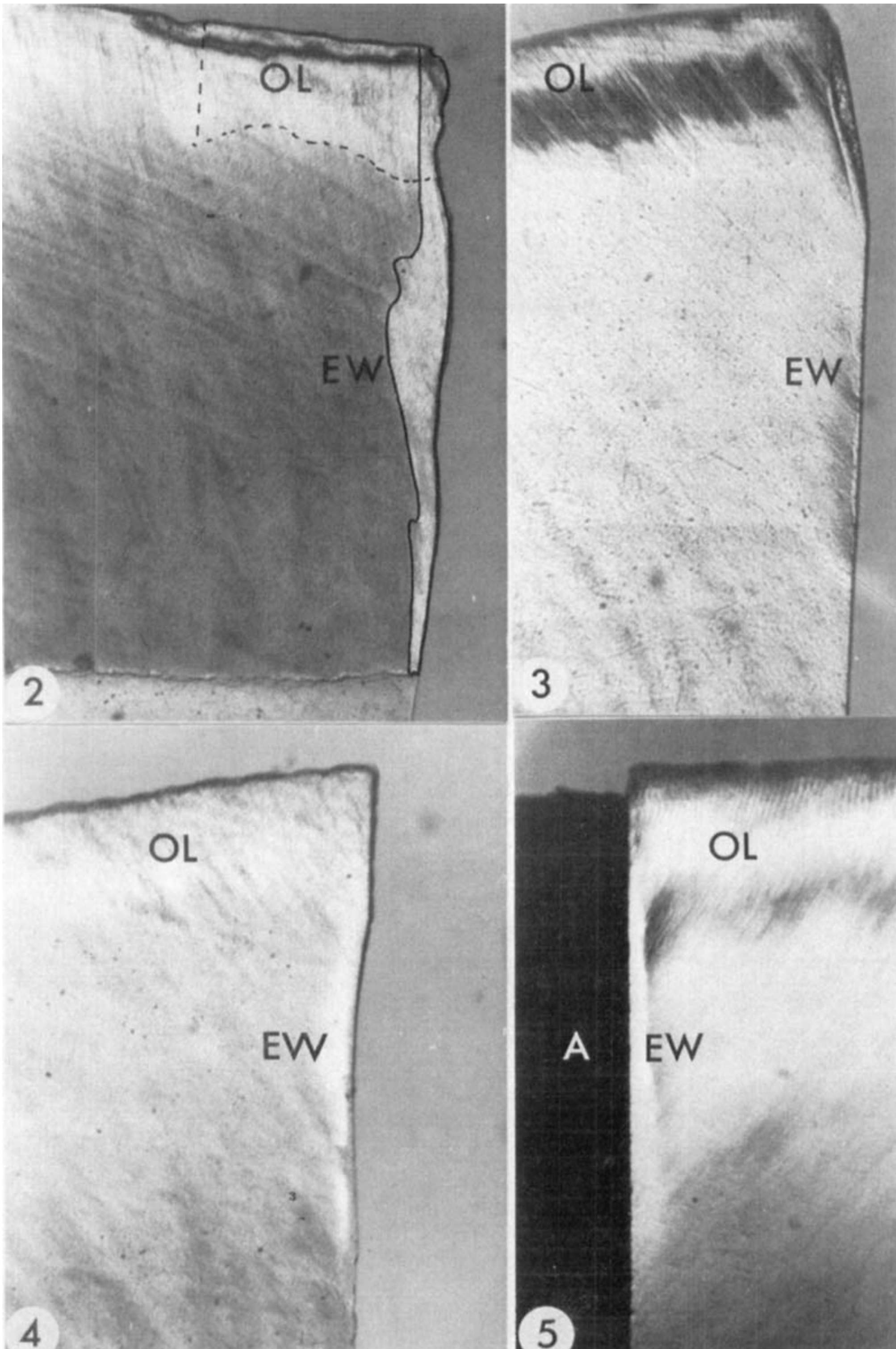
The results of the histological observations (Table 2) revealed markedly lower scores for the enamel and dentin cavity walls in the experimental group compared with the control group. The difference was highly significant. The scores for the outer lesions, however, were not statistically different in the two groups.

Fig. 2. Control section imbibed in Canada balsam. Polarized light. Outer enamel and enamel cavity wall showing translucent zones. Outline of outer lesion (OL) (broken line) and enamel cavity wall lesion (EW) (continuous line) defined with a pencil. In all outer lesions, the peripheral extension was arbitrarily designated by a line drawn parallel to the cavity wall at a distance of 0.6 mm. Original magnification 40X.

Fig. 3. Control section imbibed in distilled water. Polarized light. Outer lesion (OL) shows a broad subsurface zone of positive birefringence. Enamel wall lesion (EW) consists of a broad surface zone and a narrow subsurface zone both showing positive birefringence. Original magnification 60X.

Fig. 4. Experimental section imbibed in quinoline. Polarized light. Outer lesion (OL) shows broad translucent zone, enamel wall lesion (EW) a broad subsurface translucent zone of increased negative birefringence. Original magnification 40X.

Fig. 5. Experimental section imbibed in distilled water. Polarized light. In outer lesion (OL) a narrow surface and a broad subsurface zone of positive birefringence are present. Enamel cavity wall (EW) reveals a translucent surface zone, poor in structural details. The zone which displays increased negative birefringence covers a narrow, positively birefringent zone. Amalgam filling (A) in situ. Original magnification 60X.


OL
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2
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Experimental group

Outer lesions were observed in all sections. Imbibed in water they generally showed subsurface, broad zones of positive birefringence, similar to that in Fig. 3, thus indicating a demineralization claimed to contain more than five percent of spaces (6, 7, 8). In no instance was a break in the surface of the enamel observed.

In sections examined in quinoline, the lesions appeared translucent and with increased negative birefringence. In some cases the lesions included one or even two positively birefringent zones (Zone 2).

Enamel wall lesions. Fifteen percent of the walls showed no visible changes, while 10 % were given the score 2.5. The average score 1.53 indicates «a narrow translucent zone in quinoline, no change in water» (1.5). About 62 % of these zones were of the subsurface type (Fig. 4), while in the remaining cases the surface of the wall was affected as well. Eighteen percent of these translucent zones contained areas of positive birefringence.

When imbibed in water, 35 % of the cavity walls revealed a translucent surface zone very poor in structural details (Fig. 5). The zone displayed increased negative birefringence and in some instances covered a narrow zone of positive birefringence.

Dentin wall lesions. Approximately 72 % of the walls were found to be intact while 3 % of the observed lesions were given maximum score (2.5). When present, the lesions were usually narrow, subsurface demineralized areas.

Control group

Outer lesions were observed in all sections. Structurally they were very similar to those of the experimental group.

Enamel wall lesions. None of the walls appeared intact. Eight percent of the observed lesions were given the score 1.5;

Table 2. *Numerical scores of the degree of demineralization around cavities filled with a fluoride-containing or a conventional amalgam*

	Fluoride-containing amalgam				Conventional amalgam					
Caries risks	n	$\bar{x}$	x_{min}	x_{max}	n	$\bar{x}$	x_{min}	x_{max}	z	p
Outer enamel	63	3.81	2.00	4.00	65	3.92	2.00	4.00	1.29	N.S.
Enamel cavity wall	60	1.53	0.00	2.50	60	2.61	1.50	4.00	7.34	<0.001
Dentin cavity wall	66	0.37	0.00	2.50	63	1.72	0.00	2.50	6.61	<0.001

n = number of caries risks, $\bar{x}$ = mean score, x_{min} = minimum score, x_{max} = maximum score, z = calculated test-value.

N.S. = not significant

Table 3. *The area (mm²) of demineralization measured in Canada balsam-imbibed sections*

	Fluoride-containing amalgam					Conventional amalgam						
Caries risks	n	$\bar{x}$	s_x	x_{min}	x_{max}	n	$\bar{x}$	s_x	x_{min}	x_{max}	z	p
Outer enamel	48	0.241	0.066	0.161	0.480	44	0.230	0.077	0.141	0.452	0.72	N.S.
Enamel cavity wall	54	0.038	0.036	0.000	0.146	52	0.084	0.059	0.009	0.295	4.76	<0.001

n = number of caries risks, $\bar{x}$ = mean value, s_x = standard deviation, x_{min} = minimum value, x_{max} = maximum value, z = calculated test-value.

N.S. = not significant

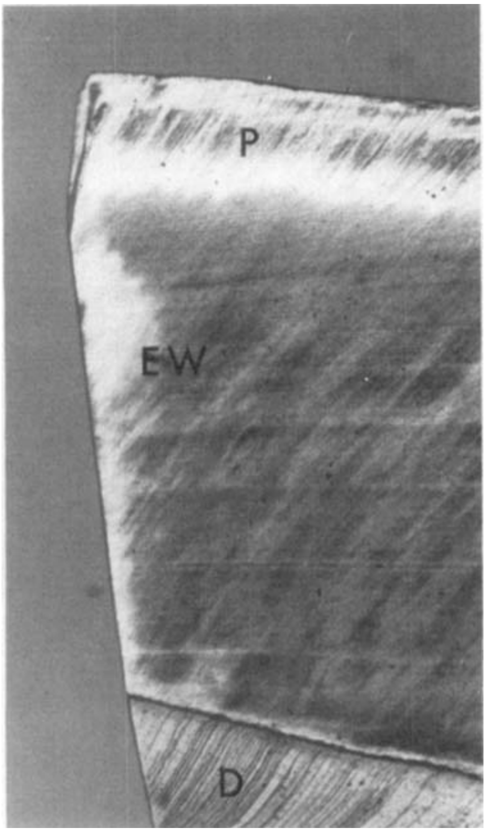
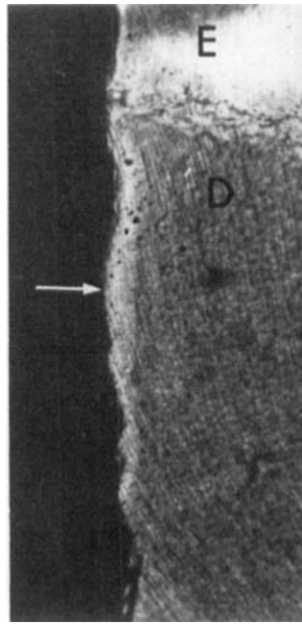


Fig. 6. Control section imbibed in quinoline. Polarized light. Outer lesion reveals a broad, positively birefringent zone (P) within a broad translucent zone of increased negative birefringence. Enamel wall lesion (EW) shows a broad, surface translucent zone of increased negative birefringence. D, dentin. Original magnification 40X.

11 % the maximum score 4. The average score, 2.59, comes close to a "narrow subsurface zone of positive birefringence in water" (2.5), (Fig. 3). The translucent zone seen in quinoline, however, also comprised the surface of the wall in most instances (Fig. 6), only 15 % appearing subsurface. Approximately 30 % of these translucent zones showed areas of positive birefringence.

In sections imbibed in water only 2 cavity walls (3.3 %) revealed translucent zones of increased negative birefringence of the type shown in Fig. 5. In both specimens, this zone was smaller and less dis-



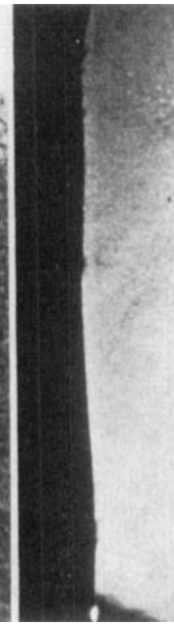


Fig. 7. Control section imbibed in quinoline. Polarized light. Narrow, surface dentin wall lesion (arrow) revealed by von Ebner's phenol reaction. E, enamel, D, dentin. Original magnification 100X.

Fig. 8. Experimental section. Narrow surface zone of enamel cavity wall shows increased radiopacity. Microradiograph. Original magnification 40X.

tinct than in the experimental group and covered an underlying narrow, positive zone.

Dentin wall lesions. Only 19 % of the dentin walls were found to be unchanged, while 41 % of the observed lesions were given the maximum score of 2.5. In most cases the lesions were of the surface type (Fig. 7), usually being broad, demineralized areas.

Morphometric analysis

The results of the morphometric analysis are summarized in Table 3. The mean values for the extent of enamel wall lesions in the experimental group were markedly lower than those for corresponding lesions in the control group, the statistical difference being highly significant. The dentin walls were not considered in these measurements. There was no statistically significant difference in the extent of outer lesions between the two groups.

Microradiography and electron microprobe analysis

A narrow surface zone of increased radiopacity was observed in three of the microradiographs (Fig. 8). Since a Mackie effect could be excluded – the microradiographs were continuously stirred during the development – the observations indicated an increased mineralization or uptake of elements with a high absorption-coefficient for the radiation used.

The electron microprobe analysis, however, showed no changes in Ca, P, Mg, Sn or Zn concentration profiles in these zones (Fig. 9 a, b). A detailed analysis of the distribution of fluoride will be published separately.

DISCUSSION

The results showed that, when compared with a conventional amalgam, the fluoride-containing amalgam had an inhibiting effect on the development of experimental cavity wall lesions *in vitro*, while the effect on outer lesions was insignificant.

A reduction of the development of *in vitro* lesions around amalgam fillings containing fluoride has also been reported by Heintze & Mörnstad (20). In their polarized light study, the sections were imbibed in quinoline only. By this method the extent, but not the degree, of demineralization can be assessed.

The demineralization in the enamel and dentin walls in the experimental group most often appeared as subsurface zones, which were judged less severe than surface lesions. This is also a characteristic of the wall lesions associated with silicate cement restorations (15,16). Mineral dissolved by a slowly progressing carious attack in the cavity wall would reprecipitate on its surface; the process being favored by fluoride released from the material. Since in the present study the demineralizing agent was the same for the two groups, the fluoride ions are most likely responsible for the great difference in the frequency of subsurface lesions. Sometimes reprecipitation may result in an increased mineralization compared with intact tissue. This will explain the surface zone of increased negative birefringence in water observed in enamel walls adjacent to silicate cement restorations (16) and to the fluoride-containing restorations in the present study. These translucent zones, poor in structural details, were in the present study considerably more frequent in the experimental group than in the control group. Only two enamel walls in the control group showed zones of this type, but smaller and less distinct than in the experimental group. In three of the microradiographs these zones were manifested as an increased radiopacity at the surface of the enamel wall, which again is a frequent finding in cavity wall lesions associated with silicate cement (16). The increased radiopacity indicates increased mineralization or uptake of heavy elements from the filling material. In this study, Ca, Zn and Sn seem to be the elements which may contribute significantly to the absorption of X-rays in the tissue. However, the electron microprobe analysis of this zone did not show any change in the normal concentration profiles of these elements. Therefore, it is supposed that the zone showing increased negative birefringence in water is hypermineralized, enhanced by the presence of fluoride, to a degree too low to be detected by the electron probe analysis. This may explain why this phenomenon is more frequently associated with fluoride-containing materials.

It has been claimed that normal en-

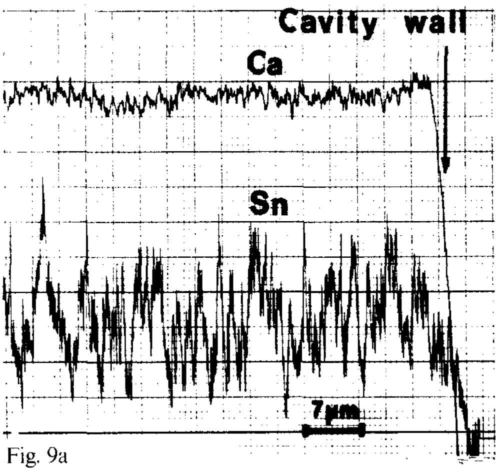


Fig. 9a

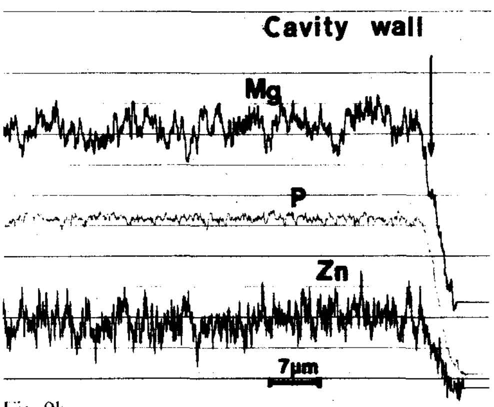


Fig. 9b

Fig. 9a, b. Electron probe microanalysis of experimental section showing a surface zone of increased negative birefringence in the enamel cavity wall, similar to that shown in Fig. 5. Linear scans of Ca, Sn (a) and P, Mg and Zn (b) traversing this zone in the enamel wall reveal no concentration changes.
Note: Each element has its own concentration scale.

amel contains approximately 0.2 % of spaces (13). Consequently, only a slight hypermineralization seems to be possible and is the reason why the zone judged as hypermineralization was given the low value, 0.5, in the scoring system.

It seems reasonable that the fluoride released from the filling would affect the cavity walls more than the outer enamel, since the cavity walls are in closer contact with the fluoride-releasing material. The greater inhibiting effect on the dentin walls than on the enamel walls could be explained by the greater fluoride uptake measured in the dentin from this material (27).

A positive zone in quinoline indicates a slow progression of the carious lesion (4) or a remineralization in the carious area (30, 36, 37). It was somewhat surprising that in this study the frequency of such a zone was higher in the control group than in the experimental group. However, only the number, not the extent of the zones was assessed.

The inhibiting effect on demineralization reported in this study may be due to fluoride present in the material and/or less marginal leakage around the fillings. The fluoride-containing amalgam in question releases fluoride (10, 38) and fluoride uptake into enamel and dentin from this material has been measured (11, 26, 27). The adverse effects of adding fluoride to amalgams have been discussed by several authors, but the general opinion seems to be that small amounts of fluoride added to amalgams will not essentially affect their physical and chemical properties (2, 29). There seems to be no difference in marginal leakage between conventional amalgams with and without stannous fluoride (3). Furthermore, the cavity wall lesions associated with the fluoride-containing amalgam resemble those which occur adjacent to silicate cement restorations. Consequently, the conclusion is that the inhibitory effect observed must be due to the fluoride in the material.

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