Electron probe microanalysis of secondary carious lesions associated with silver amalgam fillings

EINAR HALS & AGNAR HALSE

Department of Cariology and Research Laboratories, School of Dentistry, University of Bergen, Norway

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Secondary caries associated with silver amalgam fillings is characterized by outer lesions and cavity wall lesions. In this study the content of minerals and the penetration of elements from the amalgam into such lesions were analyzed. In sections of 11 teeth including cases of natural secondary caries and experimental *in vitro* and *in vivo* lesions around silver amalgam fillings the distribution of Ca, P, Mg, Zn, Sn, Cu, Ag and Hg was studied by means of two-dimensional X-ray images, linear scans and point analyses. Dentine wall lesions where microradiographs had shown increased radiopacity relative to intact tissue, exhibited considerably reduced Ca and P values. The outer portion of the radiopaque areas contained 5—8 % Zn and Sn, decreasing to < 0.1 % at a varying distance up to 130 μm from the cavity wall. Hg was not detected, nor was Ag, except in one specimen. The increased radiopacity of this zone as observed on microradiographs is, therefore, obviously caused by the presence of Zn and Sn. Zn and Sn had also penetrated into the moderately demineralized enamel lesions, but the concentrations were generally lower than those observed in the dentine.

Key-words: Dental caries, analysis; dental fillings; electron probe microanalysis.

Einar Hals, School of Dentistry, Årstadveien 17, N-5000 Bergen, Norway

The structure of secondary carious lesions associated with silver amalgam fillings has been dealt with in previous studies (*Hals & Nernaes*, 1969; 1971; *Hals & Leth Simonsen*, 1972; *Hals, Høyer Andreassen & Bie*, 1974). The lesions consisted of an outer lesion and a cavity wall lesion, the latter tending to encompass the whole filling, usually without penetrating deeply into the tissue (Fig. 1). Generally the microradiographs showed a subsurface demineralization in the dentine. However, some lesions had a surface layer of increased radiopacity, covering, or not covering, a layer of radiolucency. This observation could not be explained with the methods used in the previous studies, i.e. polarized light microscopy and microradiography. One could speculate whether the increased radiopacity could be due to a reprecipitation of reaction products from the underlying demineralized area. However, there was also a possibility that metal ions from the amalgam had been deposited in the dentine wall. Recently Sn and Zn have been demonstrated in carious dentine below amalgam fillings (*Kurosaki & Fusayama*, 1973; *van der*

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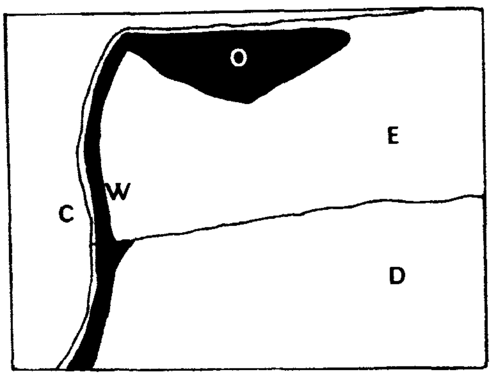

Fig. 1. Drawing of outer lesion (O) and cavity wall lesion (W) modified from microradiograph. C, cavity. D, dentine. E, enamel.

Linden & van Aken, 1973). The purpose of the present investigation was to subject representative sections from our studies to an electron probe microanalysis with special reference to the content of minerals in dentine and enamel wall lesions and the possible penetration of elements from the amalgam into these areas.

MATERIAL AND METHODS

The original material consisted of 11 teeth, including cases with natural secondary caries, experimental *in vitro* and *in vivo* lesions around amalgam fillings and one control without fillings or caries. A detailed description of this material has been given in our previous studies, mentioned above. Ground sections and microradiographs were available for the present study.

The free surface of the sections was covered by a thin layer of carbon and subsequently examined in an ARL electron microprobe operated at 15 kV and 0.1 μA. In one or more areas of each specimen elemental analyses were carried out using Ca Kα, P Kα Mg Kα Zn Kα, Cu Kα, Ag Lα. Sn Lα and Hg Mα X-ray emission. Characteristic X-ray images, showing the distribution of each of the elements, were displayed on an oscilloscope and photographed with a Polaroid® camera. Linear scans were made at right angles to the cavity wall. An estimation of the concentration of the elements was made by point analyses of the specimens and by comparison with mineral standards. Correlation with the micromorphology of the tissue was obtained by comparison with the images of sample current or secondary electrons and with ordinary incident light.

RESULTS

Natural secondary carious lesions, and experimental *in vivo* and *in vitro* lesions showed essentially the same pattern of elemental composition. They will therefore not be described as separate entities. However, differences in morphology and elemental distribution were observed between cavity wall lesions of the dentine and the enamel.

Cavity wall lesions in the dentine

A frequent type of dentine lesion consisted of a radiopaque surface zone covering a subsurface partially demineralized area (Fig. 2a). Elemental X-ray images showed that the radiopaque zone was relatively poor in P (Fig. 2b) and Ca (not illustrated). Corresponding to the low P zone, the contents of Sn (Fig. 2c) and Zn (Fig. 2d) were considerably higher than in the underlying dentine. Similar lesions, but without a subsurface demineralized zone, were also observed. In some specimens the surface layer of the cavity wall was radiolucent by microradiography. Even in these instances, the Ca and P contents of the surface layer were lower than normal, but the concentrations of Sn and Zn showed only a slight or no raise above the levels of unaltered dentine.

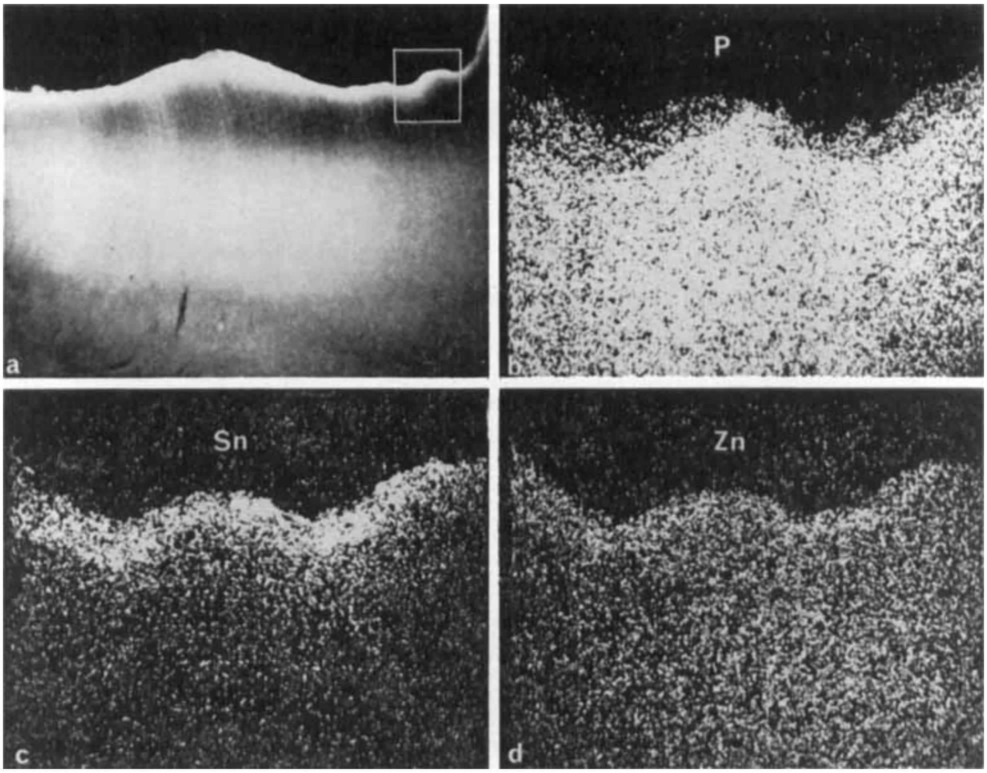

Fig. 2. Natural secondary caries. From bottom of cavity wall lesion in dentine.
a) Surface layer of dentine shows increased radiopacity relative to underlying dentine. Microradiograph. × 30.
b—d) Elemental X-ray images of enframed area in (a). × 225.
b) Loss of P in surface layer corresponding to area of increased radiopacity in (a).
c—d) Increased concentrations of Sn and Zn, respectively, corresponding to area in (b) showing loss of P.

The linear scans illustrated the demineralization pattern and the uptake of metals in greater detail. The width of the lesions as indicated by the Ca and P profiles was 15—70 μm and the degree of demineralization varied markedly (Figs. 3—5). In some specimens the profiles revealed a subsurface pattern, in which the surface of the dentine wall showed slightly reduced Ca and P values relative to intact tissue, while the subsurface area proper showed considerably lower values. The transition to the intact dentine was usually rather distinct (Fig. 3). In other instances, the Ca and P profiles corresponding to the lesions showed no subsurface pattern, but formed only uniformly lowered steps (Figs. 4, 5). Also the Mg values were lowered and showed a profile largely similar to those formed by Ca and P (Fig. 5).

In the demineralized area of the lesions an accumulation of Sn (Fig. 4a) and Zn (Fig. 4b) had taken place. With the allowance that must be made for analytical errors due to differences between the specimens and the standards, the maximum concentration of these elements was estimated to 5—8 %, decreasing to <0.1 % at a varying distance up to 130 μm from the cavity wall. This means that small amounts of Sn and Zn could also be

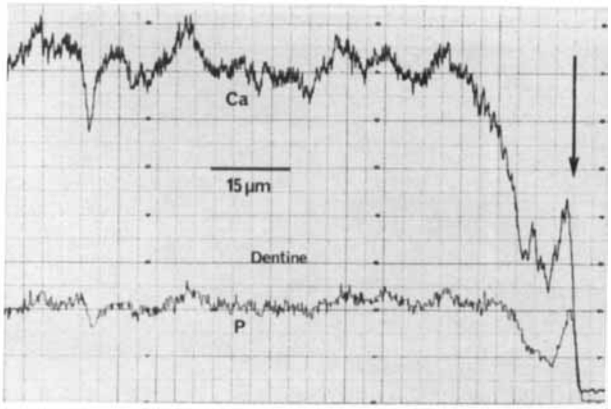

Fig. 3. Natural secondary caries. Concentration profile of Ca and P through dentine wall lesion. In this and all following reproductions of linear scans the surface of the cavity wall is indicated by a vertical arrow.

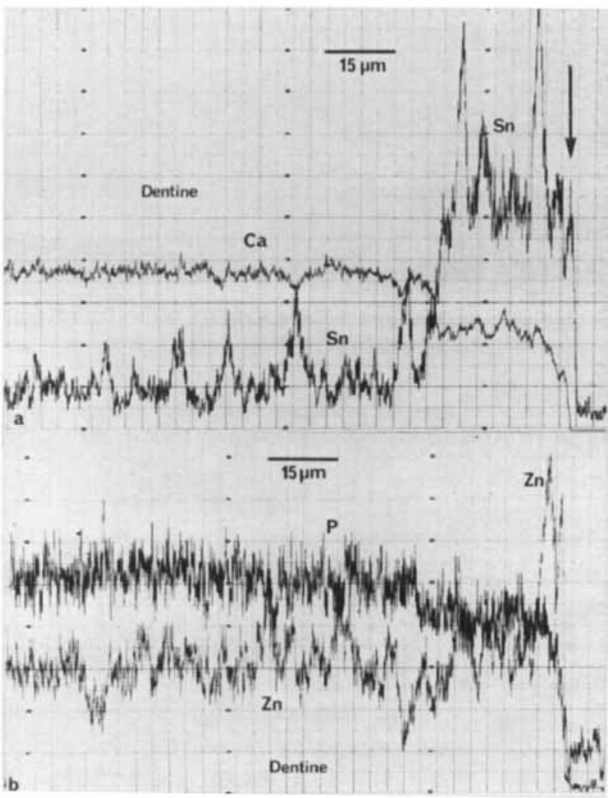

Fig. 4. Natural secondary caries. Concentration profiles of Ca and Sn (a), P and Zn (b) through cavity wall lesion in dentine.

observed at a depth where demineralization of the dentine could not be immediately recorded (Fig. 4). Sn was never observed without the presence of Zn or vice versa, but their profiles could differ to a certain degree (Fig. 4). Frequently small depressions in the Ca and P profiles corresponded to elevations of the Sn and Zn profiles (Fig. 4). In some instances Sn showed a high peak at the surface of the dentine wall and a penetration into the underlying tissue (Fig. 5). Corresponding to the peak

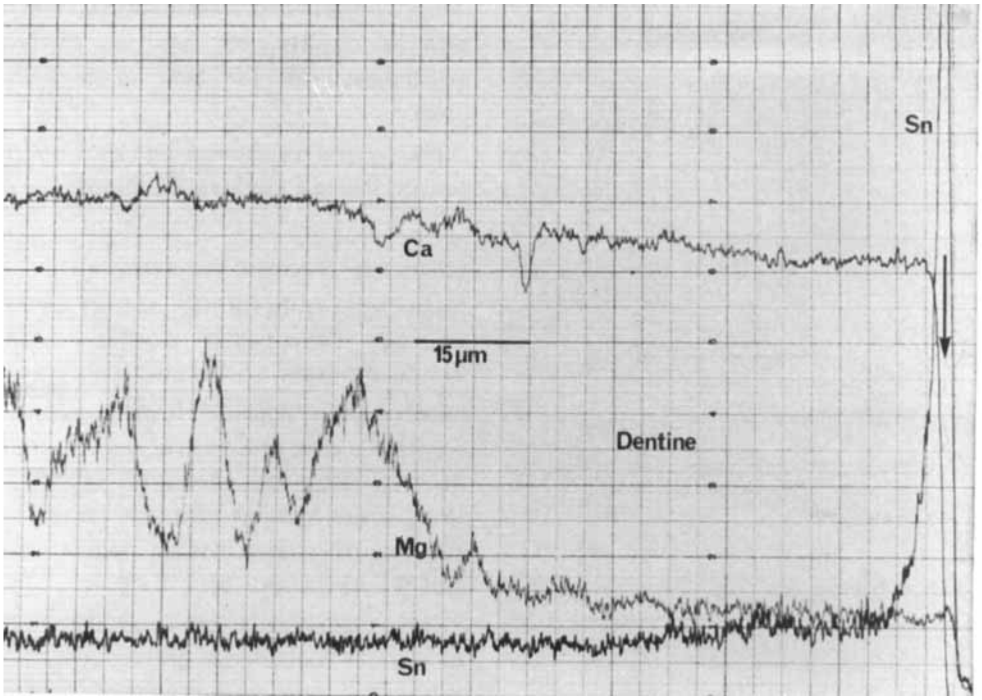

Fig. 5. Linear scan corresponding to »sc» in Fig. 7. Concentration profiles of Ca, Mg and Sn.

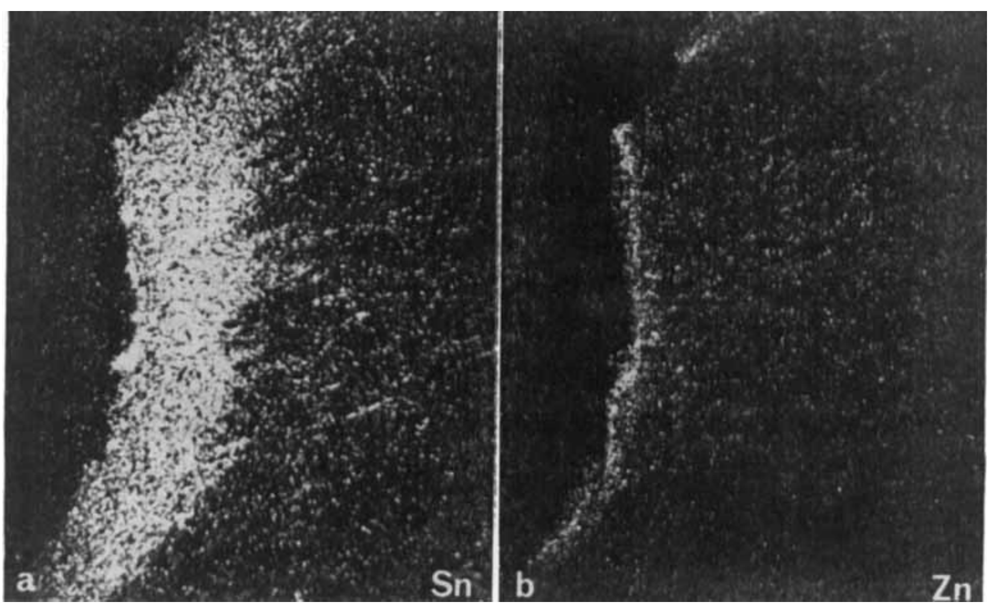

Fig. 6. Natural secondary caries. Dentine wall lesion. Occurrence of Sn (a) and Zn (b) in outer demineralized area. Strings of Sn and Zn, emanating from outer area follow direction of dentinal tubules. × 375.

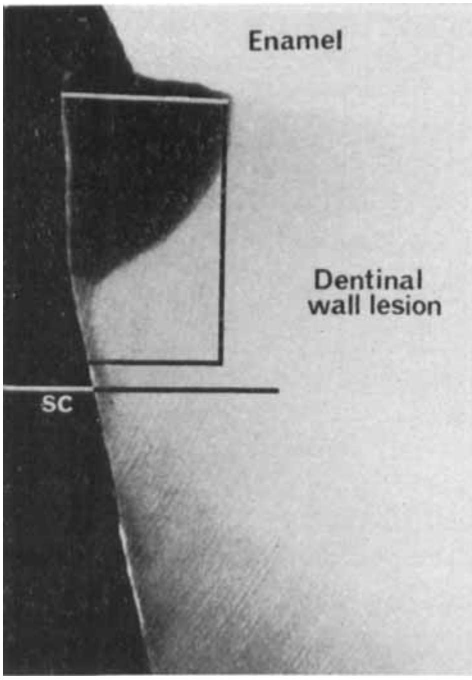

Fig. 7. Experimental *in vivo* »secondary carious» lesion. Cavity wall lesion in dentine. Sc, position of linear scan shown in Fig. 5. Microradiograph. × 85.

of Sn the microradiographs showed a radiopacity definitely higher than that of the underlying unaffected tissue (Fig. 7).

In one specimen with a 50 μm-wide wall lesion showing a considerable accumulation of Zn and Sn, strings of these elements had penetrated the underlying dentine to a depth of 130 μm from the surface (Fig. 6).

At the dentino-enamel junction, the microradiographic appearance of the lesions frequently differed from that of the other sites of the dentine cavity wall. Typically, the lesions were relatively wide and often formed a triangle with the baseline against the enamel (Fig. 7). The contents of Ca, P and Mg were low and there was a pronounced uptake of Sn and Zn (Fig. 8). The specimen illustrated (Figs. 7, 8) shows an outer zone of high radiopacity which corresponds to a zone of high Sn content, but with no obvious association with the area of high Zn uptake.

Hg, Ag and Cu showed only background emission in all sections examined.

Cavity wall lesions in the enamel

Microradiographs of the enamel lesions generally indicated a relatively slight demineralization. In some specimens a narrow outer zone of high radiopacity covering a zone of lower radiopacity could be seen (Fig. 9a). Elemental images fo these areas showed reduced content of Ca (Fig. 9b) and a distinct uptake of Sn and Zn (Figs. 9c, d).

The concentration profiles of Ca and P of enamel wall lesions showed the same subsurface pattern as primary carious lesions of the enamel. The width and depth of the lesions as judged by the Ca and P concentration profiles varied to a great extent (Figs. 9, 10). Also Mg showed lower values than in normal tissue. Corresponding to the areas of low concentration of Ca, P and Mg, there was consistently a relatively high content of Sn and Zn. The latter elements showed largely similar concentration profiles (Fig. 10b). In no instance was the subsurface radiolucency caused by reduced concentrations of Ca and P masked by the presence of Zn and Sn.

As in the dentine, Hg, Ag (Fig. 11) and Cu showed only background values in the enamel lesions, except for one specimen which contained some Ag (Fig. 12). In this case the concentration profiles revealed increased Ag values into a depth of approximately 40 μm from the enamel wall. Sn had reached the same depth while Zn had penetrated 90 μm into the dentine.

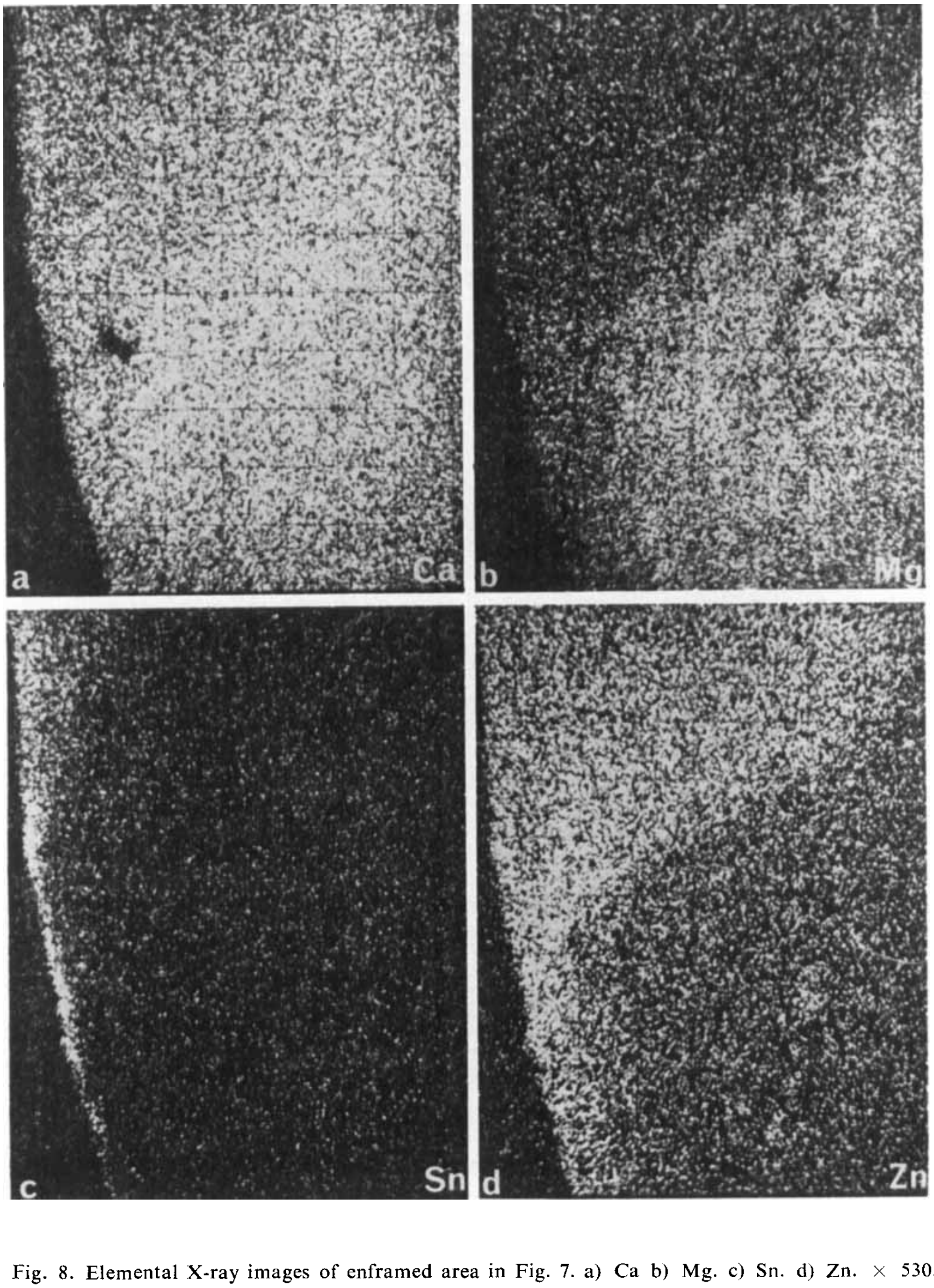

Fig. 8. Elemental X-ray images of enframed area in Fig. 7. a) Ca b) Mg. c) Sn. d) Zn. × 530.

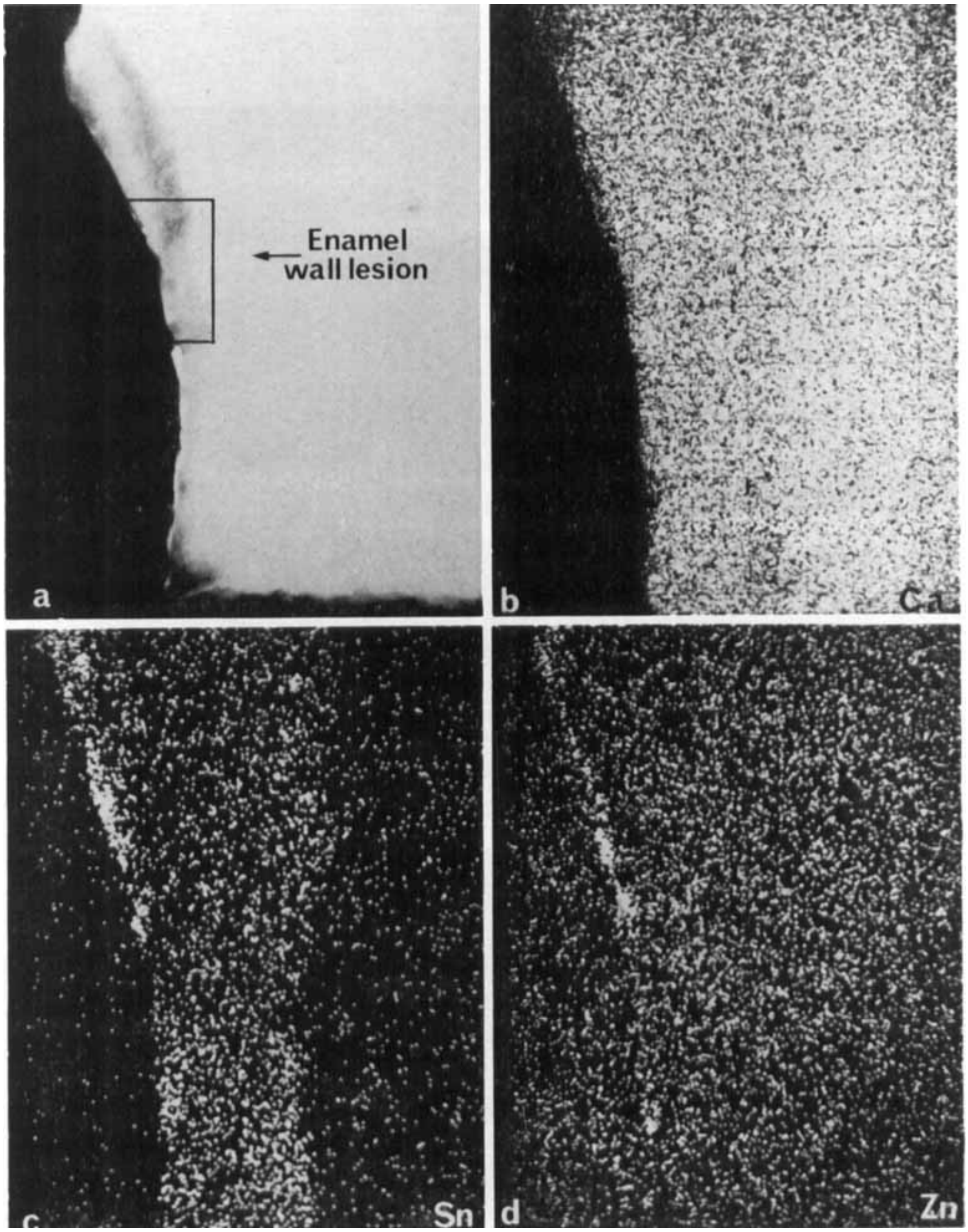

Fig. 9. Natural secondary caries. Enamel wall lesion.
a) Microradiograph. × 55.
b—d) Elemental X-ray images of enframed area in (a). × 450.
b) Loss of Ca in enamel lesion.
c) Accumulation of Sn in the corresponding area, especially in surface layer.
d) Slight accumulation of Zn in surface layer.

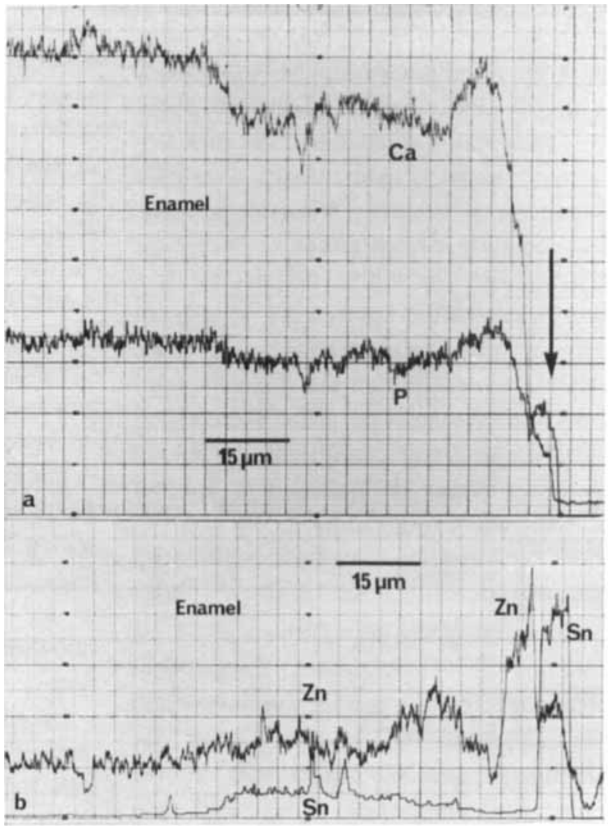

Fig. 10. The same enamel wall lesion as in Fig. 9. Linear scans through cavity wall lesion.
a) Ca and P
b) Zn and Sn

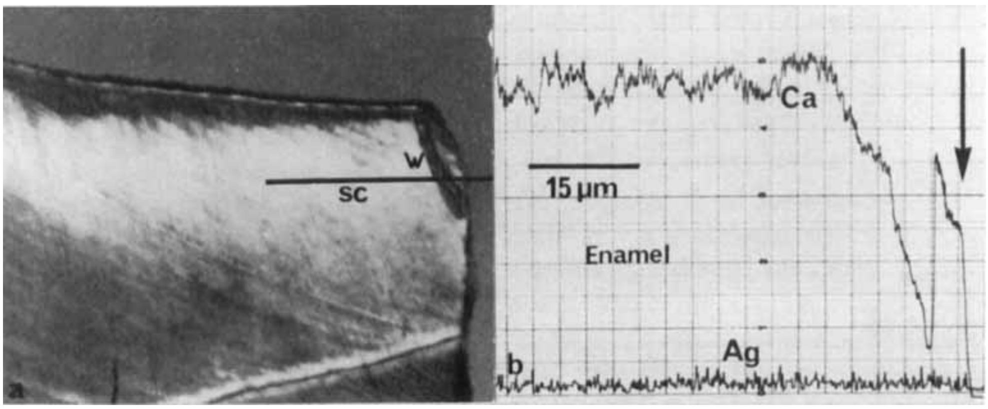

Fig. 11. Natural secondary caries.
a) Cavity wall lesion in enamel. Ground section imbibed in quinoline (n = 1.62). Polarized light. x 75. W, wall lesion; sc, position of linear scan shown in (b).
b) Linear scan through lesion seen in (a). Concentration profiles of Ca and Ag.

DISCUSSION

With the technique employed in this study it was possible to collect information about the chemical composition of incipient carious lesions around amalgam fillings. A zone with reduced Ca and P contents was regularly found, either in immediate contact with the filling material or as a subsurface lesion covered by a zone of relatively unaltered tissue. The space originally occupied by the dissolved minerals had obviously to some degree been filled up by metallic components released from the filling. The high atomic number of the penetrating elements, Zn and Sn, may explain the very high radiopacity observed in some areas (Figs. 7, 9a).

Considering the errors which might be expected from differences in physical characteristics between the specimens and the standards, we have preferred not to present detailed percentages of the elemental composition. Correlated electron microprobe and electron microscope studies have, however, demonstrated that the electron microprobe technique may provide informative data of the elemental composition, even when the mineral content is only a few percent (*Halse & Selvig*, 1974). Therefore, the principal findings of the investigation must be valid.

The phenomenon of fluorescence, i.e. excitation of characteristic X-rays at some distance from the impinging electron beam, may under certain circumstances represent a problem in analyses of volumes close to an area with a very high concentration of the elements in question, in the present study represented by the amalgam fillings. However, most fillings were lost during preparation of the ground sections, thus eliminating this possibility.

van der Linden & van Aken (1973) have shown that in clinical roentgenograms of some teeth an area with high X-ray absorption was visible in the dentine below silver amalgam fillings. Sometimes a radiolucent area was observed adjacent to the restoration. They concluded that in carious areas associated with amalgam fillings the reduced absorption of X-rays caused by decalcification had been compensated by the effect of Sn and Zn present in this soft material. The present study has demonstrated these phenomena at a much earlier stage of development, i.e. in narrow cavity wall lesions observable in microradiographs of ground sections. There can be no doubt that the increased radiopacity of the surface layers seen in Figs. 2a and 7 is due to concentrations of Sn and/or Zn overcompensating the radiolucency produced by the decalcification.

On the other hand, in the body of the dentine lesion (Fig. 7) and in the enamel wall lesions (Fig. 9a) the concentrations of Zn, and Zn and Sn, respectively, were not sufficiently high to obscure the radiolucency. *Hals & Nernaes* (1971) have produced subsurface wall lesions in dentine and enamel in association with fillings of nail varnish, a material that did not contain radiopaque elements in sufficient amounts to explain the radiopacity of the cavity wall. The subsurface pattern was, therefore, supposed to be due to the distribution of Ca and P. In the present study, Ca and P profiles, forming a subsurface pattern, were observed in some instances. Since Sn and Zn were present in all the actual areas, the microradiographic appearance must, apparently, be the result of the concentrations of these elements as well, as discussed above.

A zonation observed in some microradiographs could not in all cases be explained by the findings of the electron probe analyses. The reason for this appar-

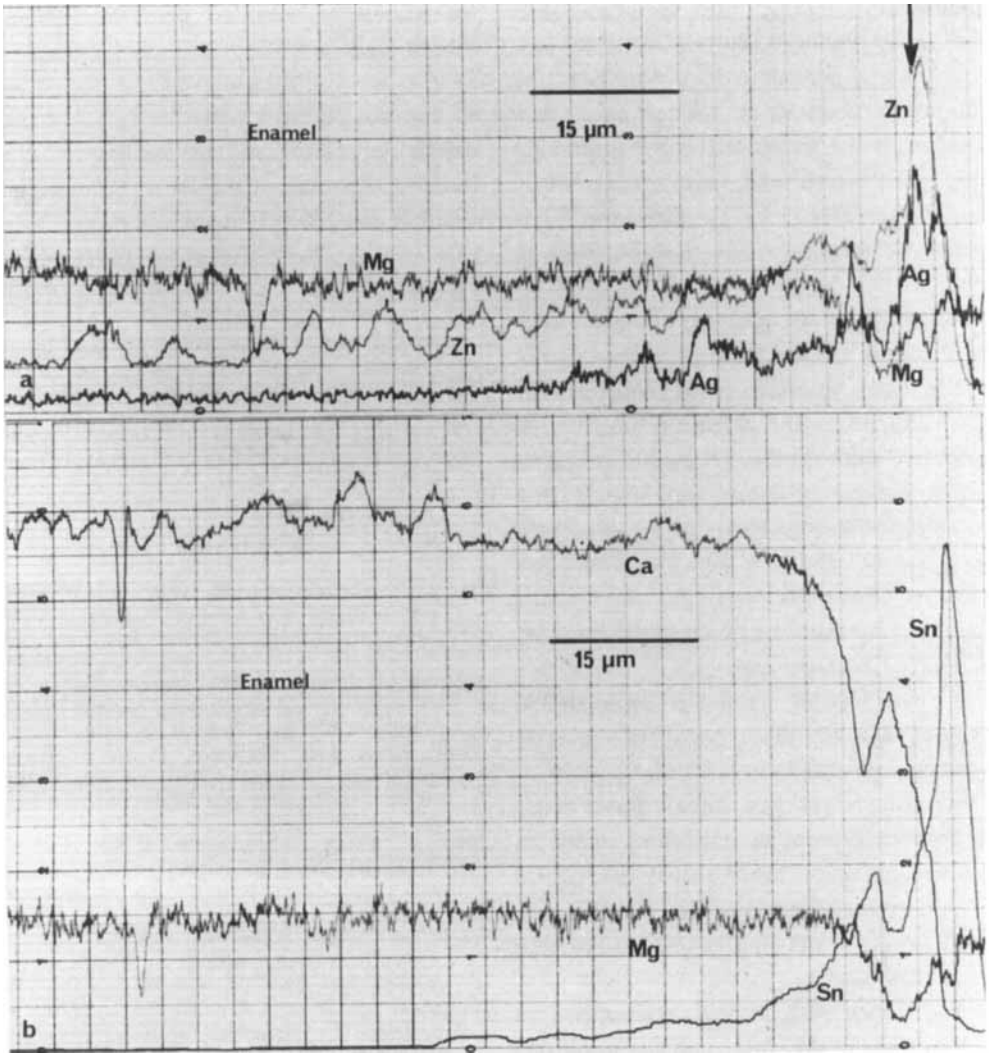

Fig. 12. Natural secondary caries. Linear scans through cavity wall lesion in enamel.
a) Concentration profiles of Mg, Zn and Ag.
b) Concentration profiles of Ca, Mg and Sn.

ent lack of correspondence is probably that the microradiograph offers an image based on the whole thickness of the section (60—120 μm) whereas the electron probe analyzed only the superficial 1—2 μm of the specimen surface.

Since Ag (except for one specimen), Hg and Cu showed only background values, the presence of the Sn and Zn in the wall lesions can be explained on the basis of corrosion of the amalgam material. Sn is considered to have dissolved from the γ_2 phase (Sn_7Hg) of the amalgam because of its high ionization tendency. The zinc products are the most soluble of the amalgam components with the highest ionization tendency (*Kurosaki & Fusayama*, 1973). Figs. 5 and 9a, c indicate that a layer of corrosion products containing Sn may cover the cavity walls in dentine and enamel and be continuous with Sn in the outer layers of these tissues.

Zn and Sn present in discoloured dentine subjacent to amalgam fillings have been located in the dentinal tubules indicating that penetration had taken place along this route (*Halse*, 1975). This would explain the strings of Zn and Sn seen in Fig. 6.

Although raised contents of metals have been found in filled teeth (*Söremark et al.*, 1968), the reported concentrations are generally below the detection limit of the electron microprobe. Previous electron probe analyses of dental hard tissues close to amalgam fillings have not demonstrated metals other than Sn and Zn. There are reasons, however, why the Ag profile in Fig. 12a should not be considered an artifact. Chemical analyses of the corrosion products of amalgam have shown that besides Sn these products regularly contain very small amounts of Ag and Cu. Obviously, these two metals have originally been present as impurities of the γ_2 phase (*Jørgensen*, 1967). In Fig. 12 Ag and Sn have penetrated to an equal depth into the enamel, a fact that is compatible with this theory.

The zones with altered radiopacity as demonstrated in our microradiographs represent initial stages of secondary caries. The phenomenon involves interesting clinical aspects. Even if the wall lesions described in this study would probably only exceptionally be detectable in clinical radiographs, *Hals et al.* (1974) have described wall lesions wide enough to be detected in this way. That elements from the amalgam can also penetrate deeply into a wide demineralized area is shown in Fig. 8d. The filling up of the porosities of the wall lesions with Sn and Zn can hardly be considered as healing of the lesions. However, it ought to be clarified whether the altered chemical composition will modify the progress of the carious process.

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