The ultrastructure of copper amalgam-covered dentin from human deciduous teeth

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The structure of dentin subjacent to Cu-amalgam restorations was studied. The restorations had been inserted 7–8 years before extraction of the teeth. No lining had been applied. The dentin subjacent to the restorations had a greenish-grayish discoloration, and in the pulpal end irregular secondary dentin had formed. Energy-dispersive X-ray microanalysis of areas showing discoloration confirmed the presence of Cu. Semithin and ultrathin undemineralized sections were studied. Vital pulp tissue and irregular secondary dentin were observed. Close to the predentin and halfway into the dentin most of the dentinal tubules were occluded. In the control material only an occasional occluded tubule was seen. Close to the Cu-amalgam restoration some tubules were occluded or partly occluded, and some were open. The odontoblasts had responded to the irritating agent by obturation of the tubules, thus reducing the permeability of the dentin. Furthermore, irregular secondary dentin had formed in another attempt to wall off the irritating agent. □ *Dental restoration; dentin permeability; dentin, secondary; odontoblasts*

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In a study of various aspects of the morphology of the dentin of primary teeth, a greenish-grayish discoloration of the dentin subjacent to copper amalgam restorations was noted. Irregular secondary dentin was also noted subjacent to cavity tubules. No information seemed to be available on the reaction pattern and long-term effect of Cu-amalgam restorations on the dentin, and a study was therefore carried out. This study is concerned with the basic reaction pattern of the dentin and is not meant to be part of the discussion about the pros and cons of the use of amalgam (and particularly Cu-amalgam) as a restorative material in dentistry. Previously, Cu-amalgam was frequently used in children's dentistry, but in later years it has seldom been used owing to its toxic effect on the pulp (1, 2).

Materials and methods

The material consisted of four second deciduous molars from patients aged 11 years. The teeth were extracted before normal shedding because of orthodontic treatment. Most of the roots had been resorbed at the time of extraction. Radiographic and clinical examination showed no pathologic processes.

Cu-amalgam restorations (Silibrin, containing 31% Cu, 67% Hg, and minor amounts of Ag, Cd, and Sn) had been inserted in the teeth by dentists in the School Dental Service in Oslo 7–8 years before extraction. (After 1986 Cu-amalgam was no longer used by the School Dental Service in Oslo.) No lining had been applied underneath the fillings. The cavity preparations reached approximately one-third into the dentin. Four deciduous second molars from patients aged 11 years with no restorations served as controls.

Immediately after extraction the teeth were divided in two with a pair of pliers (3). This made the pulp more easily accessible to the fixation fluid. The teeth were immediately immersed in a solution of 2.5% glutaraldehyde buffered to neutral pH with

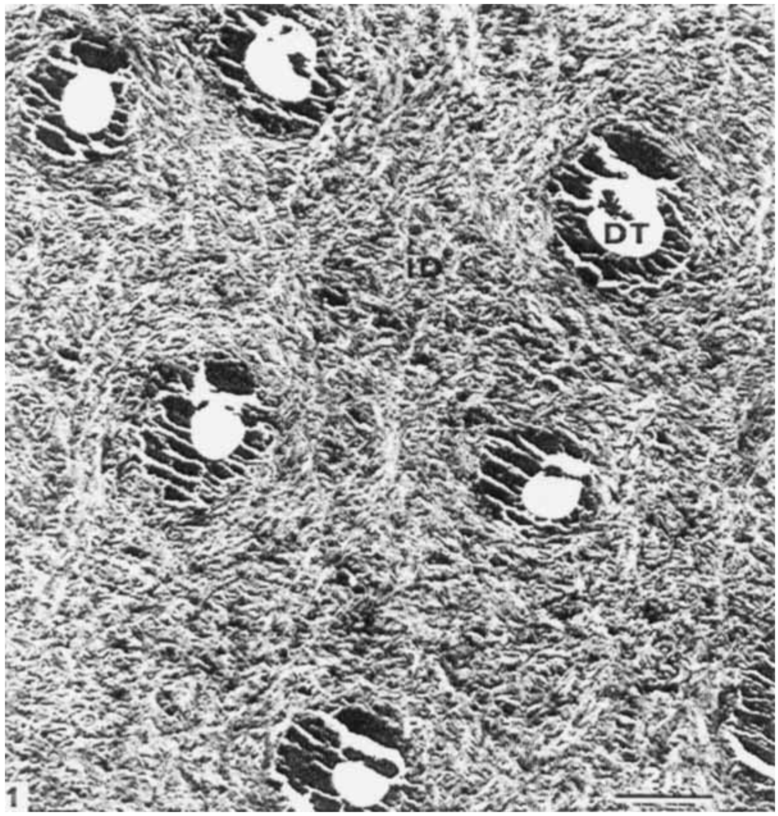


Fig. 1. Electron micrograph of undemineralized section showing cross-sectioned dentinal tubules (DT) from the outer part of the dentin in a control tooth. The dentinal tubules are surrounded by highly mineralized peritubular dentin. ID = intertubular dentin.

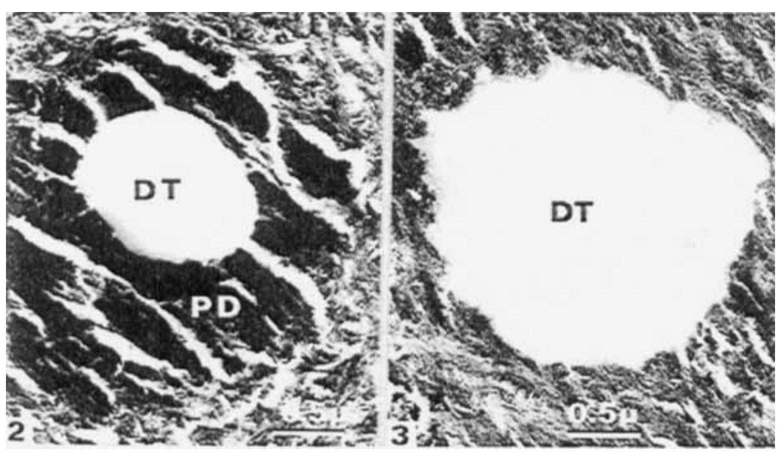
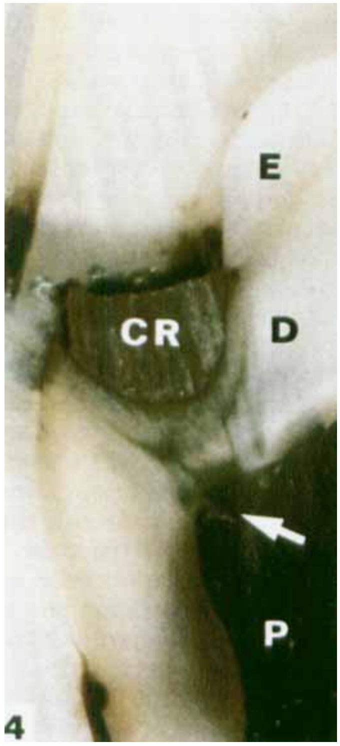
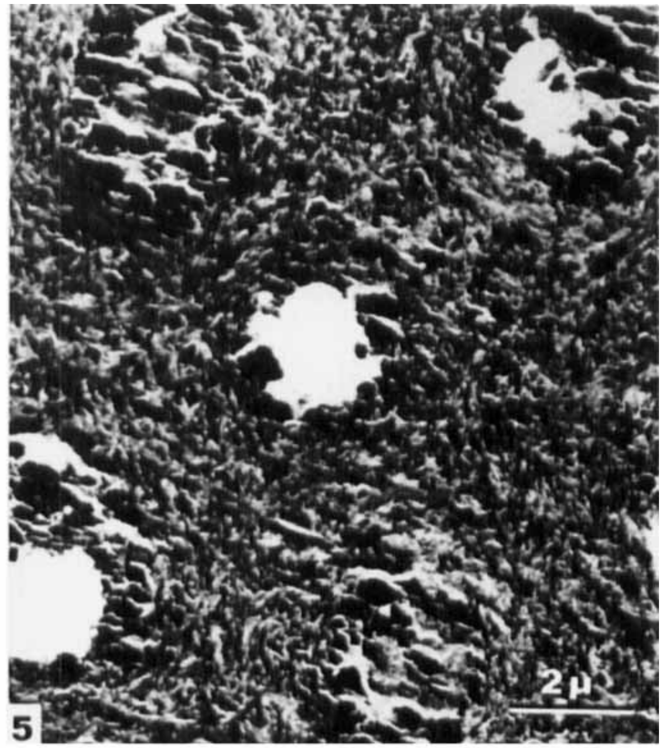


Fig. 3. High magnification of cross-sectioned dentinal tubule (DT) in the pulpal part of a control tooth. In this part of the dentin the peritubular dentin is absent.

Fig. 2. High magnification of a dentinal tubule (DT) in the outer third of a control tooth. Owing to the high mineral content, there are several cracks in the peritubular dentin (PD).





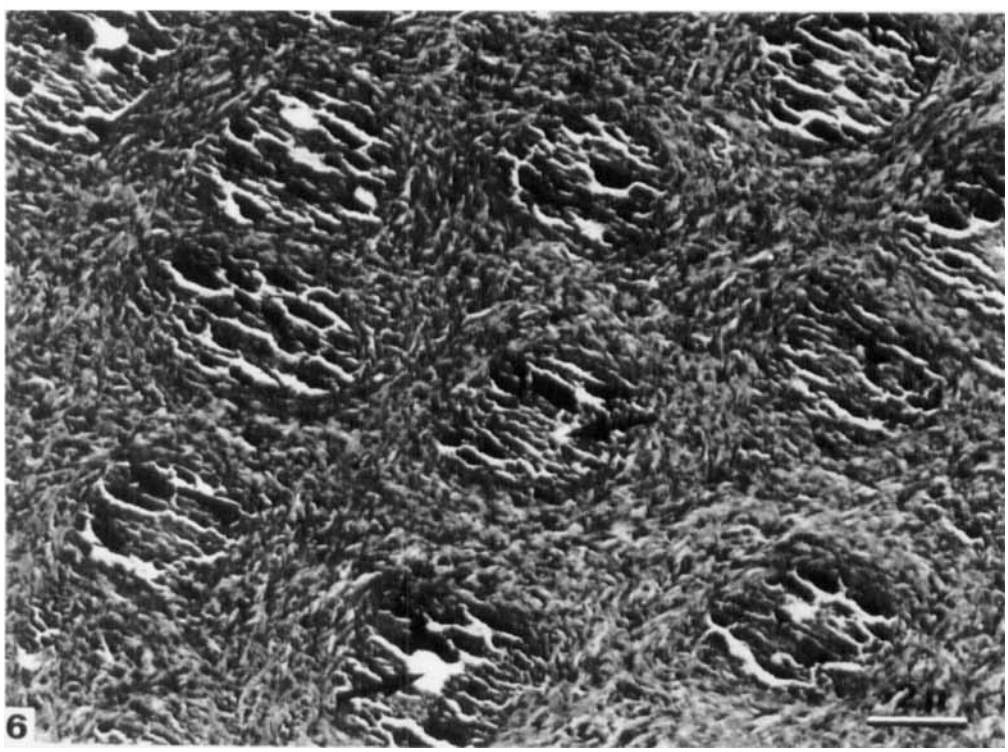


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Fig. 4. Ground section of deciduous molar showing a greenish-grayish discoloration of the dentin subjacent to the Cu-amalgam restoration (CR). Energy-dispersive X-ray microanalysis of discolored areas confirmed the presence of copper. E = enamel; D = dentin; arrow = irregular secondary dentin; P = pulp.

Fig. 5. Electron micrograph showing cross-sectioned dentinal tubules close to the Cu-amalgam restoration. Some of the tubules are partly or completely occluded, and some are open.

Fig. 6. Cross-sectioned dentinal tubules from the middle part of Cu-amalgam-covered dentin. Most of the tubules are occluded by an electron-dense material, but some tubules have a small, central open core (arrows).

cacodylate buffer. The teeth remained in the fixative for 24 h and were then kept on buffer for some days before postfixation in 1% cacodylate-buffered OsO_4 at pH 7.2.

The teeth were dehydrated in graded solutions of acetone and invested in Vestopal W. Sections approximately 500 µm thick were cut on a Gillings–Hamco thin sectioning machine, using a rotating diamond disc and ample water spray. The sections were examined in a dissection microscope, and photographs were made.

Cross-sectioned tubules subjacent to the amalgam restorations were studied at four different levels: close to the Cu-amalgam restorations, halfway into the dentin, close to the predentin, and at the predentin–dentin–pulp interface. Corresponding areas in the control material were also examined. In addition, odontoblasts subjacent to the restoration and odontoblasts from the control material were examined.

The desired areas were trimmed out, and semithin undemineralized sections were cut with glass knives and stained with 0.1% toluidine blue for light microscopy. On the basis of light microscopic findings, small areas were dissected out. Ultrathin sections were cut with a diamond knife on an LKB Ultrotome III and collected on Formvar- and carbon-coated grids. Most sections were examined unstained, but some sections were floated on a saturated solution of uranyl acetate in 30% alcohol for 25 min, followed by lead citrate for 5 min. The sections were examined in a Philips 400T electron microscope operated at 80 kV.

Energy-dispersive X-ray analysis of the 500-µm-thick ground sections was performed in a 515 Philips scanning electron microscope equipped with an EDAX ECON2 detector and a PV9900 analyzer. The microscope was operated at 20 kV. Spot analysis was performed of areas showing greenish discoloration as well as of unaffected areas and areas from the control material.

Results

Control material

Vital pulp tissue with regularly spaced odontoblasts were observed. The intertubular dentin showed an even mineralization in both the outer and the pulpal areas. Often the typical 640-Å cross-banding of the matrix collagen fibers was reflected in the mineral distribution (Fig. 1). The matrix fibers demonstrated the typical interlaced pattern of intertubular dentin (Fig. 1). Cross-sectioned dentinal tubules in the outer and middle part of the dentin had highly mineralized peritubular dentin (Figs. 1 and 2). Close to the predentin, however, highly mineralized peritubular dentin was absent (Fig. 3). Occasionally, a partly or completely occluded tubule was observed. The dentinal tubules in the predentin were evenly distributed and had a fairly regular outline.

Cu-amalgam-covered dentin: 500-µm-thick sections

Dentin underlying the Cu-amalgam restorations showed a greenish-grayish discoloration (Fig. 4). Energy-dispersive X-ray analysis of spots from discolored dentin confirmed the presence of Cu, whereas no Cu was detected in unaffected dentin or in the control teeth. Mercury was not detected.

Irregular secondary dentin subjacent to the cavity tubules was also observed (Fig. 4), and in one of the teeth the amount of secondary dentin was substantial.

Cu-amalgam-covered dentin: electron microscopic observations

Close to the Cu-amalgam some dentinal tubules were occluded or partially occluded, but quite a few were open (Fig. 5). The occluding material was slightly more electron-dense than the intertubular dentin (Fig. 5).

Halfway into the dentin most of the dentinal tubules were obturated by a material slightly more electron-dense than the intertubular dentin (Fig. 6). Some tubules, however, were only partly filled with electron-dense material. The areas devoid of electron-dense material were usually located centrically, but eccentrically located areas were also observed (Fig. 6).

Close to the predentin most of the dentinal

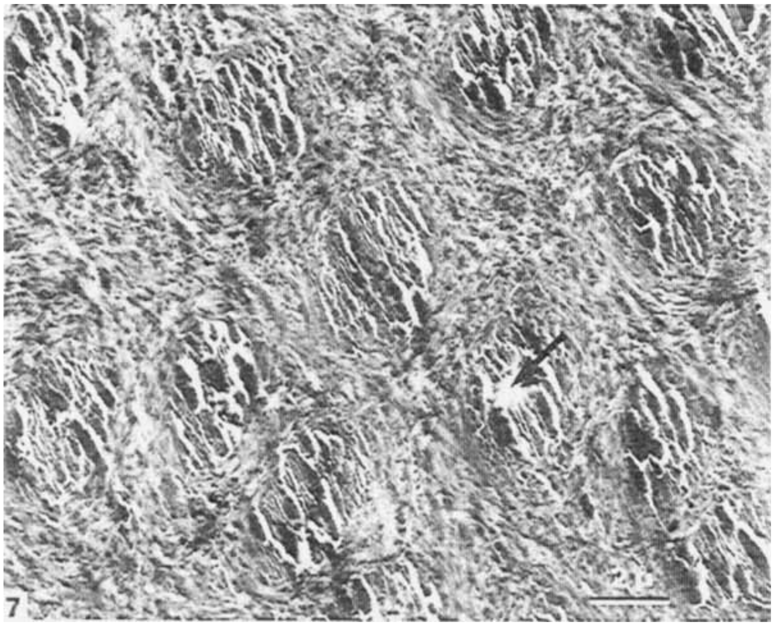

Fig. 7. Cross-sectioned, occluded tubules from the pulpal part of Cu-amalgam-covered dentin. As in the middle part of the dentin, some tubules have a central open core (arrow).

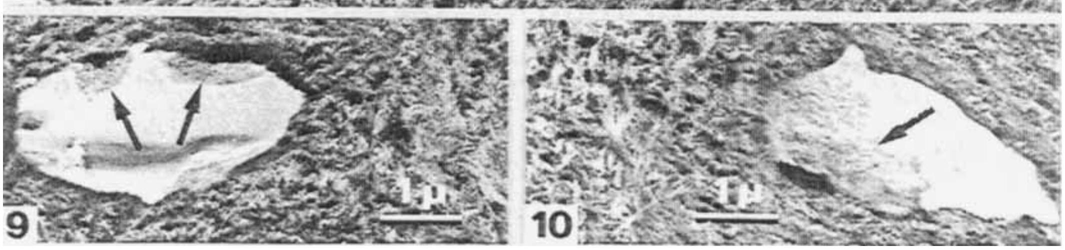

Fig. 8. Cross-sectioned tubules at the predentin-dentin interface of Cu-amalgam-covered dentin. The tubules have an irregular circumference, and deposition of mineral seems to be occurring in some of the tubules (arrows).

Figs. 9 and 10. Electron micrographs of cross-sectioned dentinal tubules close to the predentin. Tongues of mineralized tissue protrude into the tubular lumen (arrows).

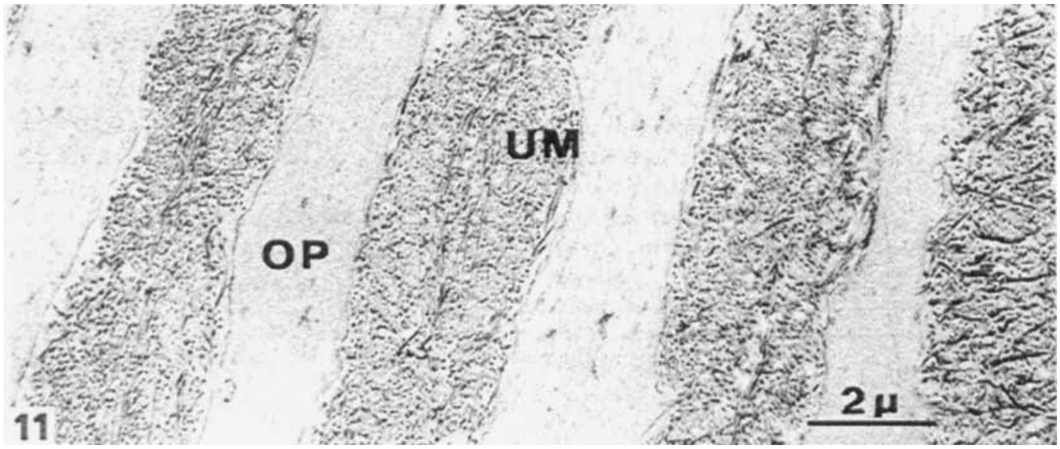

Fig. 11. Vital pulp tissue from Cu-amalgam-treated tooth. Longitudinally sectioned tubules from the predentin, showing unmineralized matrix (UM) and odontoblast processes (OP). (Lead citrate; uranyl acetate.)

tubules were also occluded (Fig. 7). As in the middle part of the dentin, tubules that were only partly filled with electron-dense material were sometimes observed (Fig. 7).

The dentinal tubules close to the predentin were evenly distributed and had an irregular outline (Fig. 8). Sometimes tongue-like, mineralized projections protruded into the tubular lumen (Figs. 9 and 10). Highly mineralized peritubular dentin was not observed. A wide zone of predentin with mineralized globules was seen. In the predentin a dense collagen matrix and odontoblast processes were noted (Fig. 11). The odontoblasts had a more irregular arrangement than what was observed in the control material.

Discussion

The effect of Cu-amalgam restorations and other restorative materials on the pulp of deciduous teeth of monkeys was studied by Mjör et al. (1). They found that high copper alloys caused more severe pulp damage than the other materials studied. Severe pulp reactions were observed in one-fourth to one-third of the teeth after observation periods of 3 weeks as well as after 3 months. In vitro studies indicate that Cu-release from the restoration is responsible for the cytotoxic effect of the Cu-amalgam (4, 5).

In the present study with an observation period of 7–8 years the pulp tissue was vital, even though the odontoblast layer was not as regular as in the control teeth. Irregular secondary dentin subjacent to the cavity tubules was also found. The state of the pulp immediately after the insertion of the Cu-amalgam restoration is not known. One might speculate that, despite the toxicity of the filling material, the release of toxic substances from the restoration has diminished through the years. Furthermore, the pulp had responded to the irritating agent by obturation of the tubules, thus reducing the permeability of the dentin. Irregular secondary dentin had also formed, in another attempt to wall off the irritating agent. In addition, Cu-amalgam has an antibacterial effect, and pulp reactions due to the presence of bacteria in the cavity are not likely.

The state of the cross-sectioned occluded dentinal tubules in the Cu-amalgam-covered dentin morphologically resembled the age changes observed in dentin in permanent teeth (6–8). However, in the control material only an occasional occluded tubule was found, indicating that age is not a likely explanation for the large number of occluded tubules seen in the Cu-amalgam-covered dentin. The similarity to age changes indicates that a gradual closure is brought about, probably under cellular control.

The normal growth of the peritubular dentin as an age change is a slow, gradual mineralization of the organic matrix, leading to complete or incomplete obturation of the tubules (6–8). Rapid ingrowth of mineralized matrix, primarily from one side of the tubule in corticosteroid-covered dentin (9, 10), has been reported. In the present study tongues of mineralized tissue protruding into the tubular lumen were observed, indicating that the obturation of the tubules in the Cu-amalgam-covered dentin is a more rapid change than obturation due to age.

The caries process that necessitated the restorations, together with the operative procedure and the restorative material, may have induced changes in the dentin. However, the extensive greenish discoloration of the dentin indicates that a substantial amount of the occlusion had occurred after the insertion of the Cu-amalgam, since the diffusion path for the Cu-amalgam is most likely the tubules (11). Halse (11) found Zn and Sn in high concentrations in dentin beneath amalgam fillings in human teeth. The metals were located primarily in relation to the dentinal tubules, and he therefore suggests that the pathway for the penetration of the elements is the tubules.

The present study gives no information about the binding site of the Cu. The color indicates that Cu is oxidized. The binding site could be organic or inorganic.

Mercury was not detected in the discolored dentin, and this agrees with findings by Halse (11), who did not detect Hg in discolored dentin subjacent to amalgam fillings in an electron microprobe examination. However, by means of autometallography, Hg has been found in dentin beneath amalgam fillings, and in a recent study of primary teeth with cold vapor atomic absorption, Hg was detected (13). This difference in results may be due to the variation in sensitivity of the analysis techniques used.

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