Influence of cementum on the demineralization and remineralization processes of root surface caries in vitro

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Dietz W, Kraft U, Hoyer I, Klingberg G. Influence of cementum on the demineralization and remineralization processes of root surface caries. Acta Odontol Scand 2002;60:241–247. Oslo. ISSN 0001-6357.

The influence of the nature of the root surface on the demineralization and remineralization processes within artificial fluoride-treated caries lesions was investigated using microscopic and X-ray microanalytical methods. Traces of fluoride were detected in the outer parts (about 25 μm) of the lesions after the application of fluorides, and a high mineral content was proved for the same region by means of microanalytical calcium estimation. The location of this mineral-rich band in relation to the root surface was deeper into the root depending on the existence and thickness of a cementum layer. However, within the dentine the location and intensity of the mineral content were unaffected by the cementum. Investigation of artificial caries lesions without fluoride treatment showed the following: The degree of mineralization was kept at a higher level near the root surface in the presence of cementum. Consequently, a cementum layer gives some initial caries resistance of the root surface. □ *Cementum; demineralization; fluoride; remineralization; root caries; X-ray microanalysis*

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Root caries is a major clinical problem in dentistry and attention is focused on the prevention and treatment of this disease. It is well established that fluoride has the ability to protect the orally exposed root surfaces from caries development. A variety of in vitro studies have shown that topical fluoride applications in the form of toothpaste or mouth-rinse retard lesion progression (1–3) and induce the formation of mineralized layers within the lesions (4–7).

The aim of this study was a qualitative and quantitative description of demineralization and possible remineralization processes, depending on the application of fluoride-containing toothpaste and the nature of the root surface, using microscopic and X-ray microanalytical investigations of artificial root caries lesions.

Material and methods

Specimens

Mesial or distal root surfaces of human premolars, extracted for orthodontic reasons from 9–12-year-old children, were investigated. Thirty-three natural (with or without cementum) and 8 polished root surfaces (cementum removed) were used.

In vitro demineralization

Experimental lesions were created within defined windows of covered root surfaces by means of 0.1 M Na-acetate buffer (pH 4.95) in 6% hydroxyethylcellulose with 1.5 mmol/l $CaCl_2$, 0.9 mmol/l KH_2PO_4, 150 mmol/l KCl at room temperature within 7 days.

Application of toothpastes

Toothpastes (AmF, 1400 ppm F^- or fluoride-free) were applied for 5 min immediately before and, once daily up to the 4th day, during the demineralization phase using a suspension of toothpaste and aqua bidest (1:2).

Investigated specimens

Test specimens: Demineralization, fluoride treatment
Negative control specimens: Demineralization, fluoride-free treatment
Baseline specimens: Untreated dentine beside the lesions (without demineralization and toothpaste application)

Section preparation

After the experimental period, one 250 μm and three 80–100 μm longitudinal ground sections through the lesion were prepared using a LEITZ low-speed saw microtome (Leica Instruments, Germany).

Polarized light microscopy (PLM)

Histological assessment and microphotographical documentation of the dentine lesions were performed with the polarized light microscope JENAPOL b (Zeiss Jena,

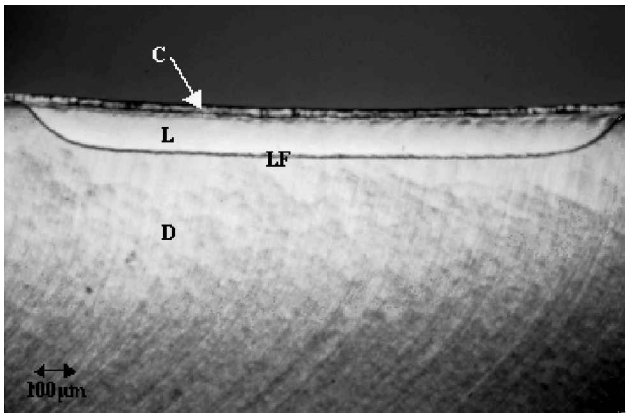

Fig. 1. Longitudinal ground section through a root caries lesion without fluoride treatment (negative control specimen), PLM. Cementum (C) covered dentine (D) with lesion (L) and lesion front (LF).

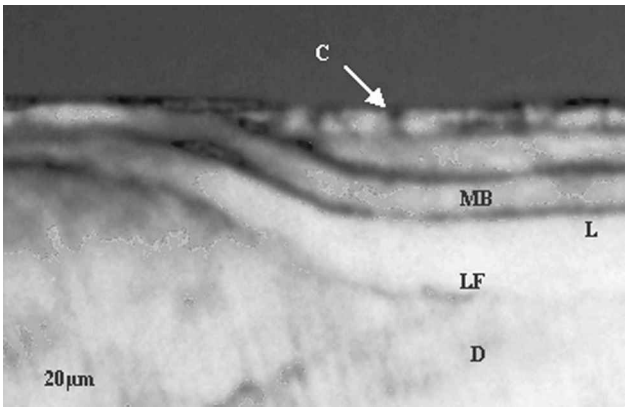

Fig. 2. Longitudinal ground section through a root caries lesion with fluoride treatment (test specimen), PLM. Cementum (C) covered dentine (D) with lesion (L), mineralized band (MB) and lesion front (LF).

Germany) after the inhibition of the 80–100 µm sections in water. Measurements were performed of the mean thickness of the lesion, the thickness of the cementum layer and the thickness of the optically distinguishable bands within the lesion using a video/computer-aided equipment.

X-ray microanalysis (XRMA) and scanning electron microscopy (SEM)

The 250 µm sections were prepared for XRMA (carbon-evaporation; Philips SEM 515; EDAX-DX4, ECON-window open, HV = 9 kV) and for SEM (gold sputtering). On the tooth sections, the X-ray profiles were taken at depths of 2.5–200 µm (steps according to Fig. 3) starting from the root surface and by line-scanning (20 µm scan-line) parallel to this surface. Semi-quantitative computing of weight percentages of the main constituents of the root hard tissue N, O, Ca, P and also F ($\Sigma = 100\%$) was performed. The quantity of both calcium and phosphorus is representative of the mineral amount of the root. In the present study, the degree of mineralization was estimated by the calcium content. Within each specimen, the calcium content measured in the untreated dentine under the lesion (200 µm below the surface) was fixed at 100%, giving the standard for the other calcium estimations of this specimen. Measurements beside the lesions (untreated dentine) according to the same steps of depth were used for the baseline specimens (6 specimens).

A total of 47 series of XRMA-spectra (19 in the baseline and negative control specimens and 28 of the fluoride-treated specimens) were investigated.

The influence of the nature of the root surface was characterized by dividing the spectra into 4 groups corresponding to the existence and thickness of any cementum layer: roots without cementum (polished), without cementum (natural), with a 2.5–7.5 µm and a 10–15 µm cementum layer.

Statistical analysis

The mineral distribution within the dentine lesions varied greatly from patient to patient, and from root to root, resulting in distinctly different amounts of calcium. However, the individual curves showing the calcium content as a function of the distance to the root surface presented a typical shape for every group. The mean values and the standard deviations were calculated for each group. Student's *t*-test was used for comparison of the discussed measuring points of the individual groups.

Results

Polarized light microscopy of the root caries lesions

The polarized light microscopic investigation of all specimens showed clearly marked dentine lesions. The median lesion depth of the negative control specimens with natural surfaces (Fig. 1) was estimated at 154.5 ± 8.9 µm and with polished surfaces at 178.7 ± 5.6 µm. In contrast, the lesions of the fluoride-treated specimens were distinctly smaller (107.8 ± 4.6 µm). In addition, a banding appeared within the lesions of these specimens (Fig. 2).

X-ray microanalysis of the mineral content depending on fluoride treatment

The XRMA allowed quantification of the calcium content within the lesions. Untreated cementum/dentine examined beside the lesions showed a nearly constant calcium content over the whole area investigated, beginning at the root surface up to a depth of 200 µm (Fig. 3, upper curve). In comparison to the standard amount (100%) fixed at the depth of 200 µm, the calcium content was determined between 99.6% and 100.3%. There were no differences caused by the existence and thickness of a cementum layer.

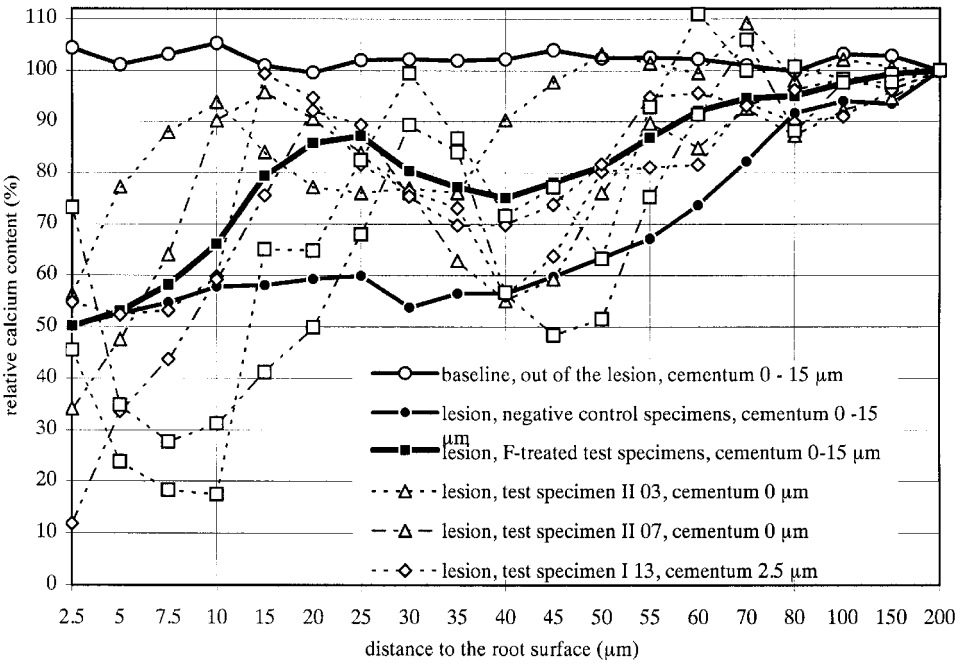

Fig. 3. Relative calcium content of caries lesions with fluoride treatment and controls as a function of the distance to the root surface. Bold continuous line = mean value of the fluoride-treated specimens; dashed lines = individual specimens; fine continuous lines = relative calcium content of the lesions without fluoride treatment (negative control) and out of the lesions (baseline).

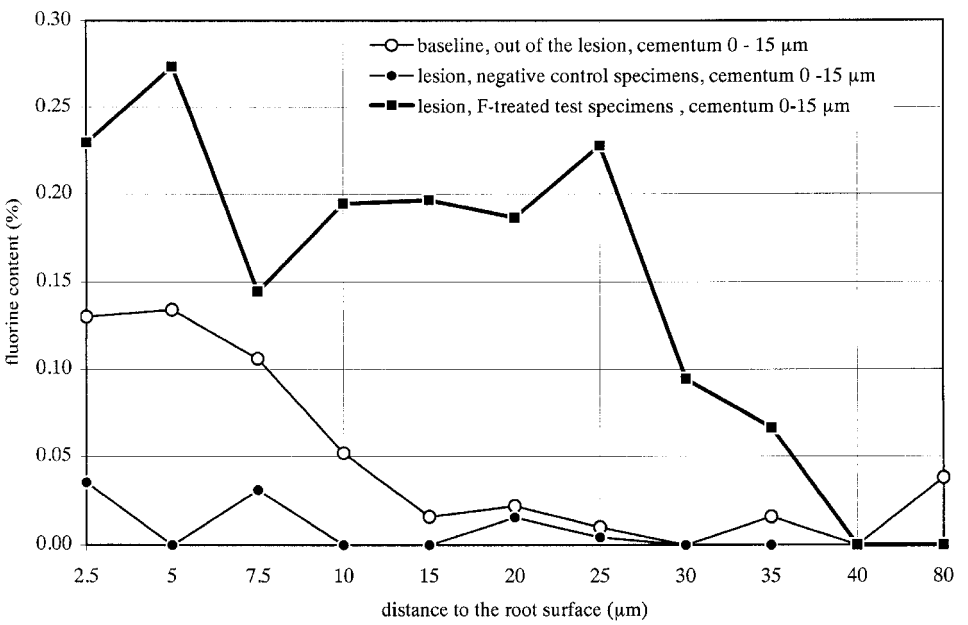

Fig. 4. Fluoride content of caries lesions (test and negative control specimens) and of the baseline as a function of the distance to the root surface.

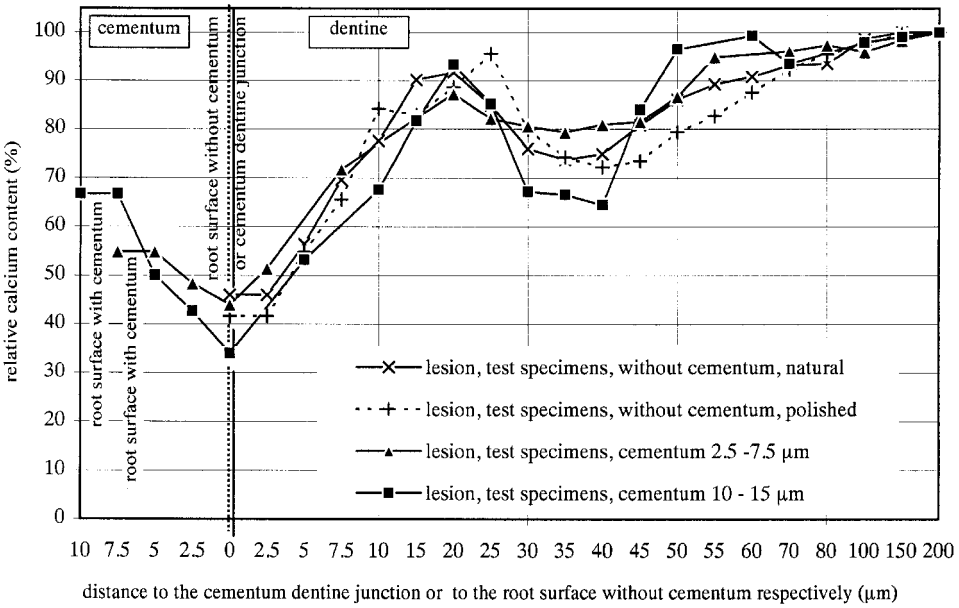

Fig. 5. Relative calcium content of caries lesions with fluoride treatment (test specimens) as a function of the distance to the cementum dentine junction and to the cementum-free root surface, respectively.

The lesions of the negative control specimens were characterized by a calcium loss of 40–50%, corresponding to the calcium content of 50–60% (Fig. 3, lower curve). Within the inner third of the lesion depth the calcium content had risen steeply and reached 94% of the standardized amount at the microscopically (SEM) visible front of the lesion at a depth of about 100 µm.

Contrary to the negative control specimens, the calcium loss within the lesions of the fluoridated test specimens was visibly smaller. The corresponding calcium content was determined between 50.0% and 87.2%, showing a distinct maximum at a depth of 25 µm and reaching 94.6% of the standardized amount already 70 µm under the surface (Fig. 3, bold curve).

The lesion depths of both the negative control and the test specimens were about one-third less when estimated by SEM and XRMA compared with PLM.

Fluoride amounted to about 0.2% within fluoride-treated lesions up to a depth of 25 µm (Fig. 4). No fluoride (<0.05%) was detectable within the negative control specimens. Control measurements beside the lesions showed a small amount (less than 0.15%) of fluoride near the surface (5 µm depth), but at 10 µm depth fluoride was not detectable (0.05%).

X-ray microanalysis of the mineral content depending on the nature of the root surface

The curves of the individual test specimens showing the calcium content as a function of the distance to the root surface presented nearly the identical shape; however, wide deviations were found with regard to the amount and location of the calcium minima and maxima (Fig. 3, dashed lines).

With respect to the nature of the root surface, the specimens were divided into 4 groups corresponding to the existence and thickness of the cementum layer. In addition, the curves of these groups were aligned by setting the cementum-dentine junction and the cementum-free surface, respectively, to zero (Fig. 5).

The medium calcium content at the polished and at the natural cementum-free surfaces amounted to 41.7% and 46.0%, respectively, at the roots with 2.5–7.5 µm cementum to 54.9% and with 10–15 µm cementum layers to 66.7%. The calcium content of the lesions reached the lowest values at the microscopically visible cementum-dentine junction and at the cementum-free surface, respectively (33.9–46.0%). The distinct calcium maxima (87.9–95.6%) of the individual groups were estimated at different depths under the root surface. However, these maxima were located at 20 µm depth within the dentine for all 4 groups investigated.

With respect to the cementum layer, the lesions of the negative control specimens showed different amounts of calcium loss near the root surface and approximately the same curve shape within the deeper part of the lesion (Fig. 6). For the group of thicker cementum layers (10–15 µm), the calcium content was estimated at 63.1–72.2% within the cementum and up to 10 µm under the cementum-dentine junction. The calcium content of thinner cementum layers (2.5–7.5 µm) and within the first 10 µm of the dentine amounted to 57.3–65.0%. The calcium content of the natural root surfaces without cementum was diminished to 44.1%. For the group of polished specimens, only 39.0% of calcium was estimated, dropping to 34.5% at

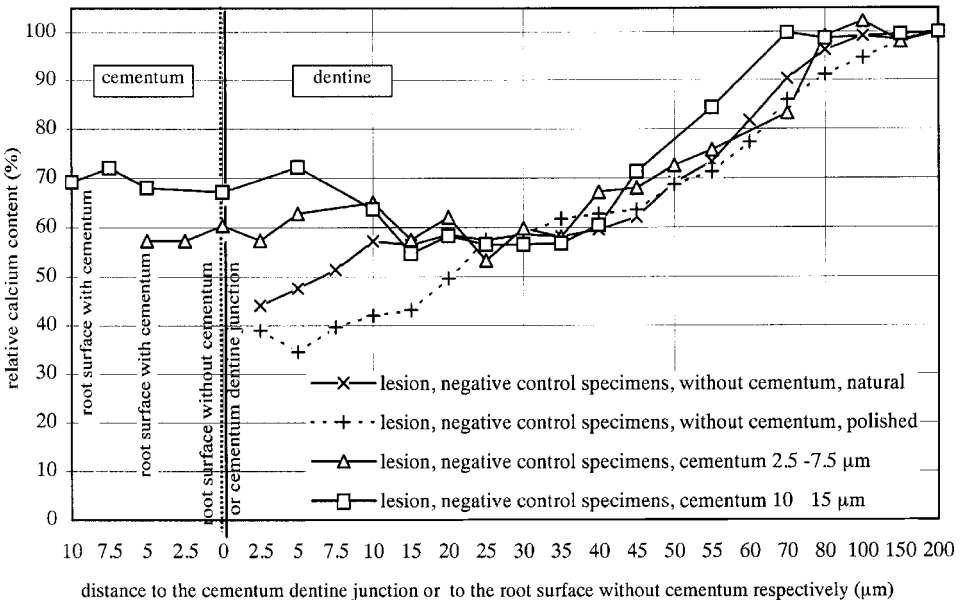

Fig. 6. Relative calcium content of caries lesions without fluoride treatment (negative control specimens) as a function of the distance to the cementum dentine junction and to the cementum-free root surface, respectively.

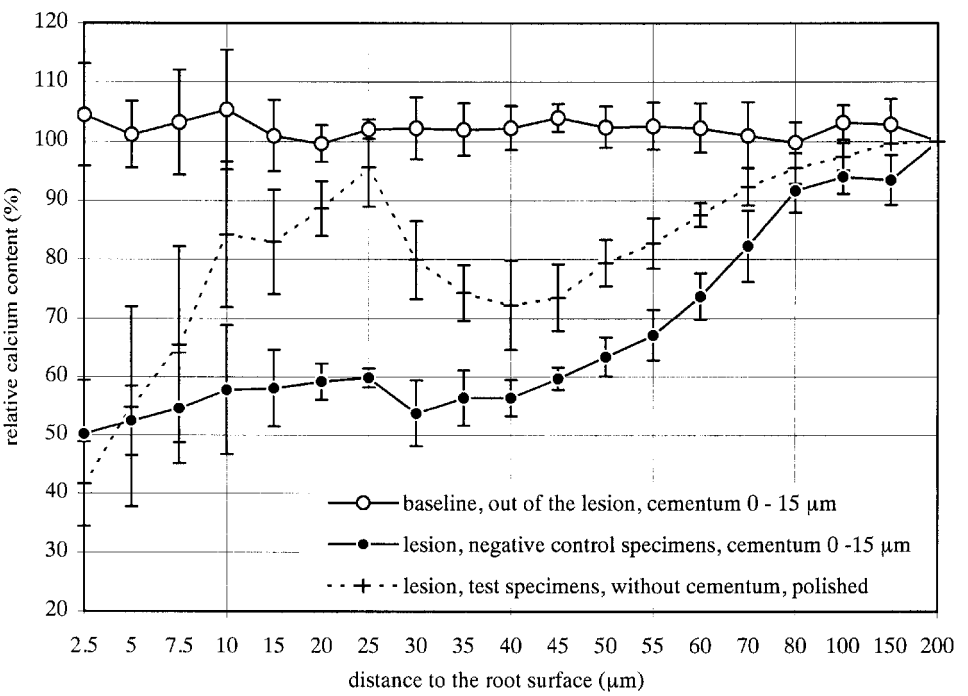

Fig. 7. Estimation of the standard deviation of the relative calcium content for the example of three individual groups.

5 μm under the surface. Independent of any cementum layer at a depth of about 25 μm within the dentine, all groups showed nearly the same calcium content (56.6–59.9%) continuously rising to 100% at the lesion front. In comparison to the fluoride-treated specimens, a greater lesion depth is indicated, especially in the polished specimens.

The highest standard deviation was estimated as 11% for the untreated dentine and control groups. The standard deviation for the individual groups of the fluoride-treated specimens reached 25% near the root surface (10 μm depth), about 15% for the middle part of the lesion, and was found less than 10% for the lesion front, as an example of the computed standard deviations (Fig. 7). The level of significance was set between $P = 0.02$ and $P = 0.13$ (Student's *t*-test) for comparison of the individual groups at prominent locations.

Discussion

Polarized light microscopy of the root caries lesions

In the present study, artificial root caries lesions, created without fluoride treatment, were characterized by polarized light microscopy as well-demarcated translucent areas.

The PLM allows the investigation of the lesions within aqueous media showing the original even surface and the real depth of the lesion. Drying the specimens during the SEM preparation and investigation results in a saucer-shaped surface and a smaller depth of the lesion. This shrinkage reaches to 30% of the lesion depth (8). The comparison of our PLM and SEM measurements of the lesion depth showed the same amount of shrinkage, which has to be taken into account when the results are applied in the clinical situation.

X-ray microanalysis of the mineral content depending on fluoride treatment

The X-ray microanalytical investigation of the artificial root caries lesions without fluoride treatment established a distinct mineral loss over the whole lesion depth (Fig 3, lower curve). On the contrary, the fluoride application during demineralization resulted in a smaller mineral loss within the whole lesion. The highest mineral content was shown at a depth of 10–30 μm by a microscopically visible banding and by the analytically determined calcium peak, which reached 90.6% of the standardized amount. This is in agreement with the results of microradiographic and other PLM investigations of fluoride-treated artificial root caries lesions (9–15). The determined high mineral content close to the surface supports the assumption that the fluoride-induced inhibition of demineralization is supplemented by a precipitation of mineral ions. These ions, dissolved at the lesion front, move outward and react with fluorides having diffused into the lesion (15). This process is discussed as a local remineralization (10).

In the present study, traces of fluoride (0.2%) were provable up to the depth of 25 μm. Although here the used EDX-system was working at its detection limit, this result corresponds to the XRMA estimation of the calcium content and thus to the location of the maximum mineralization. These findings are in good agreement with the more sensitive secondary ion mass spectrometry investigations (1) and proton probe analysis (16) showing distinct fluoride accumulation in the outer third of the lesions.

X-ray microanalysis of the mineral content depending on the nature of the root surface

The natural root surface is covered by a cementum layer of varying thickness. However, only a few data are available on the role of the cementum in the processes of root demineralization and remineralization. The cementum is usually removed by polishing the root surfaces to get a more standardized specimen (4) and to eliminate the influence of the higher fluoride content in the cementum (12).

Wefel et al. (11) created artificial root caries lesions with and without cementum but were unable to establish differences using polarized light microscopy. This agrees with our light microscopical findings of the fluoride-treated groups. However, deeper lesions were created in the polished specimens of the negative control specimens.

The microanalytical investigation of fluoride-treated artificial root caries lesions showed a distinct dependence on the cementum layer. As shown by the dotted curves in Fig. 3, the calcium content near the surface was changing within an extremely wide range and the calcium peaks of the individual specimens were localized at various depths under the root surface.

The influence of the cementum layer became obvious when dividing the specimens into groups according to the existence and thickness of the cementum layer and the alignment of the curves depending on the cementum dentine junction and the cementum-free surface, respectively (Fig. 5): The preservation of the mineral at the surface of naturally cementum-covered roots was significantly ($P < 0.03$) higher than that of cementum-free surfaces. The demineralization reached its highest degree at the cementum-dentine junction, achieving at least the same mineral loss there as at the surface of the cementum-free roots. No distinct differences were provable for the behaviour of the naturally cementum-free specimens and the polished ones. Thus, the highest mineral loss was always established at the beginning of the dentine without respect to the presence of a cementum layer. The calcium peaks pointing to a remineralization process were shifted deeper into the root according to the thickness of the cementum layer. However, interpreting Fig. 5, the location of this mineral-reach zone within the dentine was not affected by any cementum layer. Within that

highly mineralized band the outward diffusion of mineral ions is retarded. Thus a lack of mineral arises at the cementum-dentine junction, whereas the higher degree of mineral content is only kept right at the cementum surface. Consequently, the fluoride-induced caries inhibition is caused by an interactive mechanism of demineralization and remineralization processes. The mineral transport out of, within and into the lesion is influenced by the nature of the root surface.

Additional information was obtained by dividing the control specimens according to the existence and thickness of the cementum layer as above (Fig. 6). For the naturally cementum-covered roots, a distinctly higher mineral content ($P < 0.10$) of the upper part of the lesion, including the cementum layer and a few micrometres of dentine, was proved in comparison to the cementum-free surfaces. This indicates that the cementum layer works as a barrier against the diffusion of the mineral ions out of the lesion, resulting in precipitation within the upper dentine. This barrier effect was proposed in a recent paper (17) in which the calcium loss of cementum-covered lesions was studied using atomic absorption spectroscopy.

In conclusion, the fluoride-induced high mineral content localized within the experimentally created lesion supports the assumption that the inhibition of demineralization is even supplemented by remineralization. Traces of fluoride are detectable in the upper part of the lesion up to the region of highest mineralization. A cementum layer gives some initial caries resistance of the root surface. The thickness of the cementum layer influences the location of the highly-mineralized band in relation to the root surface. However, within the dentine the location and mineral content of this band are not affected by the existence or thickness of the cementum layer. The cementum layer must be taken into account when using natural dental roots for the studies of experimental caries processes. Polished roots are a suitable standard for in vitro investigations, even if they are more susceptible to demineralization compared to natural surfaces.

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Received for publication 21 November 2001
Accepted 28 March 2002