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# THE MINERAL PHASE OF SOUND AND CARIOUS HUMAN DENTAL CEMENTUM STUDIED BY ELECTRON MICROSCOPY

*by*

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## INTRODUCTION

The mineral phase of dental cementum from human permanent teeth has been shown to undergo several changes in the carious process. These include demineralization and apparent remineralization as evidenced by the variation in radiodensity observed in microradiographs of carious cementum (*Furseth & Johansen,* 1968). In order to define these structural alterations more specifically and to determine the morphology and distribution of the crystals in the various zones of carious cementum, an electron microscopic study has been carried out. For control purposes sound cementum was also studied.

Only limited information is available on the morphology and distribution of the crystals from carious cementum. In a preliminary report, *Furseth* and *Johansen* (1965) described the presence of crystals of a larger size in the carious tissue as compared to the sound tissue. *Selvig* (1969) noted an irregular surface contour in some specimens of carious cementum and also the presence of closely packed microorganisms. Small mineral crystals and fragments of partially demineralized cementum were seen between the microorganisms.

A number of publications have dealt with the mineral phase of the noncarious tissue. Several investigators have described the mineral of sound

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cementum not exposed to the oral environment as consisting of aggregates of apatite crystals (*Dreyfuss & Frank*, 1964; *Glimcher, Friberg & Levine*, 1964; *Selvig*, 1965; *Furseth*, 1967). Some loss of crystals has been reported in unexposed cementum from teeth affected by chronic periodontitis (*Selvig*, 1966). Additional changes in the mineral phase have been described in cementum exposed to the oral environment. In some specimens, the surface layer of the tissue displayed a decrease in electron density, while in other specimens an increase in electron density was observed (*Herting*, 1967 a). The crystals of this surface layer were found to be larger than those of unexposed cementum (*Herting*, 1967 b). *Selvig* (1969) also found that the surface zone of exposed cementum frequently was highly mineralized and that the crystals were densely packed and thicker than the crystals of unexposed cementum.

## MATERIALS AND METHODS

All specimens used in this study were obtained from extracted human permanent teeth fixed in 4 % formaldehyde neutralized with calcium carbonate. For the first phase of the investigation, sections from 100 teeth with carious cementum and from 25 teeth with non-carious cementum were prepared for microradiographic studies. Details of the methods employed and the observations made have been published elsewhere (*Furseth & Johansen*, 1968).

The samples for the electron microscopic studies were chosen on the basis of the microradiographic findings and were from the cervical portion of the root. Selected areas from 10 sections each of non-carious and carious cementum were prepared for ultrastructural studies. Included in the material were sound tissues from unexposed cementum, 2 specimens of densely mineralized surface layers of non-carious exposed cementum, and carious tissues displaying a highly mineralized surface, a uniform subsurface demineralization and a subsurface demineralization characterized by alternating radially oriented areas of high and low mineral content.

The specimens were embedded in a mixture of 15 % methylmethacrylate and 85 % butylmethacrylate using 2 % benzoyl peroxide as catalyst. The methacrylate was polymerized for 48 hours at 60°C. Eight samples of calculus, partly from the same teeth as the ones with carious cementum, and partly from other teeth were also included in the study for comparative purposes. These were processed and embedded as described for cementum. All samples were sectioned on an LKB Ultrotome I using glass or diamond knives. The sections were deposited on carbon coated grids and examined in a Siemens Elmiskop I electron microscope operated at 60 kV.

## FINDINGS

### *Sound (non-carious) cementum*

Ultrathin sections of unexposed sound cementum from the cervical portion of the root displayed variation in electron density within limited areas (Fig. 1). Some sections also revealed alternating light and dark bands typical of mineralized collagen. The crystals of sound cementum were plate-like in shape as seen in Fig. 2. In edge views, they appeared as narrow, dense profiles resembling needles while in broad surface views they appeared less electron dense, displaying a variety of shapes. Some crystals were close to being rectangular, while others had a more circular, triangular or oval shape. Maximum crystal length was measured at 600 Å.

The densely mineralized surface layer of non-carious cementum that had been exposed to the oral fluids revealed some structural characteristics different from those of unexposed cementum. There was no indication of banding and individual crystals appeared elongated (Figs. 3 and 4). Crystal width seemed to be smaller than that observed in unexposed cementum, while the thickness and the length generally exceeded that of the plate-like crystals. In order to distinguish between these and the plate-like crystals, those of exposed cementum will be referred to as tablet-shaped.

### *Carious cementum*

Ultrastructural observations of the highly mineralized surface layer of carious cementum often revealed masses of bacteria attached to the surface. Microorganisms were also seen within lacuna-like spaces in the cementum surface (Fig. 5). The underlying tissue generally had a high mineral content but spaces devoid of crystals and also enlarged spaces between crystals were observed (Figs. 6 and 7). The crystals, as seen in Fig. 7, were similar to the tablet-shaped variety observed in the surface layer of exposed, non-carious cementum. The size of the tablet-shaped crystals varied but they generally appeared to be larger, more elongated and more regular in outline than those encountered in unexposed, sound cementum. Some crystals also seemed longer than those seen in unexposed, non-carious cementum. Lengths up to 900 Å and widths up to 200 Å were measured.

Carious cementum from the central portion of the tissue, which by microradiography showed a slight, uniform demineralization, sometimes revealed an uneven distribution of mineral when studied in thin sections (Fig. 8). Some areas displayed a high mineral content while other areas contained only a few scattered crystals (Figs. 8 and 9), indicating that localized demineralization might have occurred. Also, spaces devoid of mineral were

## PLATE I

Electron micrographs of sound, unexposed cementum from the cervical portion of the root.

Fig. 1. Ultrathin section illustrating regional variation in electron density within limited areas. × 36,000.

Fig. 2. Higher magnification of the crumbled edge of a section showing plate-like crystals. In edge views (E) the crystals appear as narrow electron dense profiles resembling needles; in broad surface views (B) they appear less electron dense and display various shapes. × 99,000.

## PLATE II

Electron micrographs of non-carious, exposed cervical cementum.

Fig. 3. Ultrathin section of the highly mineralized surface layer showing densely packed crystals in longitudinal, oblique and cross sectional views. × 74,000.

Fig. 4. Higher magnification of the edge of a section illustrating the elongated and uniform width of crystals. Because of these characteristics, the crystals have been referred to as tablet-shaped. × 99,000.

## PLATE III

Electron micrographs of densely mineralized surface layer of carious cervical cementum.

Fig. 5. Thin section showing bacterial plaque (BP) covering the highly mineralized surface zone (HZ). The microorganisms occupy lacuna-like spaces in the tissues. × 35,000.

Fig. 6. Thin section of surface layer showing spaces between crystals indicating porosity of the tissue. × 74,000.

Fig. 7. Higher magnification of the edge of a section illustrating tablet-shaped crystals of varying size. × 99,000.

## PLATE IV

Electron micrographs of carious cervical cementum from the central portion of a lesion, which by microradiography displayed a slight, uniform demineralization.

Fig. 8. Thin section exhibiting regional differences in mineral distribution as judged by the electron density. There is no evidence of bacterial penetration. Outlined area is shown in Fig. 9. at higher magnification. × 37,000.

Fig. 9. Higher magnification of area outlined in Fig. 8 showing the individual crystals which appear to be similar to the crystals of the sound tissue. × 84,000.

## PLATE V

Electron micrographs of carious cervical cementum from the central portion of a lesion, which by microradiography displayed uniform demineralization.

Fig. 10. Higher magnification of the crumbled edge of a section. These plate-like crystals resemble those of sound, non-exposed cementum. × 105,000.

Fig. 11. Thin section depicting several crystals with serrated edges (arrows). Note wide variation in size of crystals. × 74,000.

## PLATE VI

Electron micrograph of carious cervical cementum from a region which microradiographically showed alternating radiolucent and radiodense areas (brush-like appearance).

Fig. 12. Thin section illustrating densely mineralized cementum surrounding lacuna-like spaces containing bacteria (Ba). × 36,500.

## PLATE VII

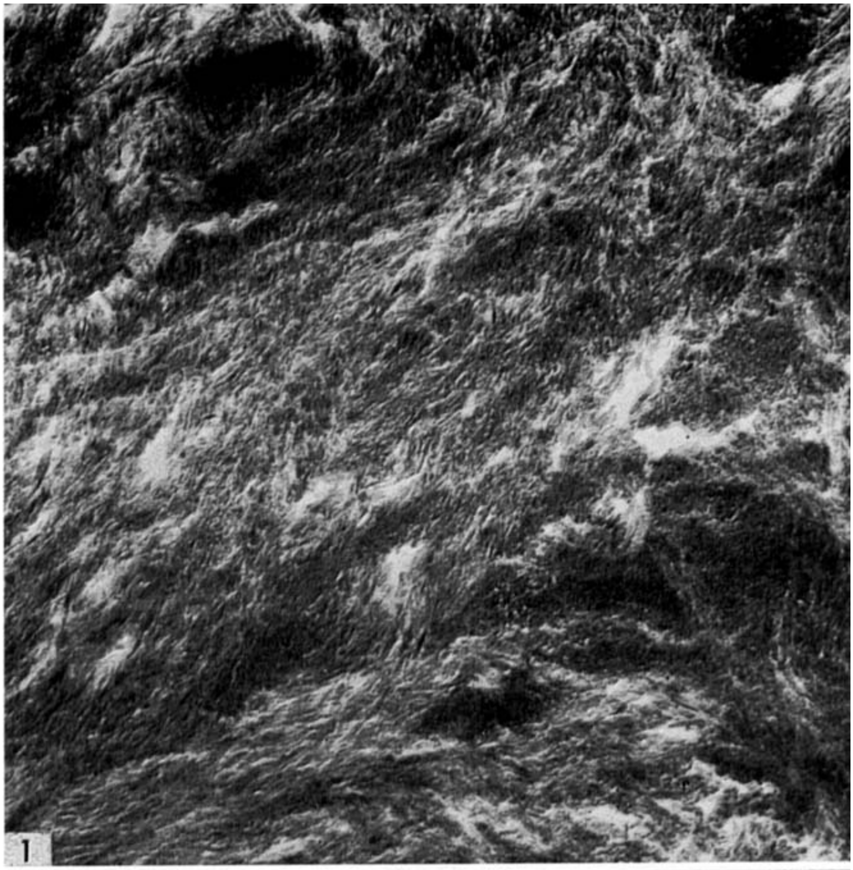
Electron micrographs of crystals from the central portion of carious cervical cementum and from calculus.

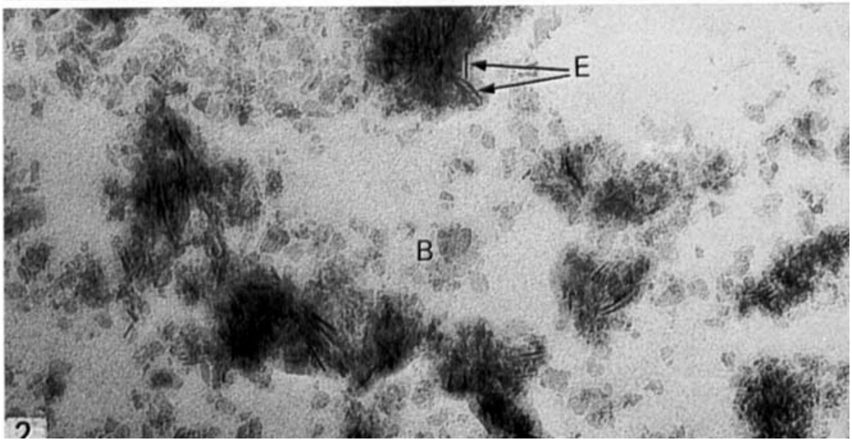
Fig. 13. Higher magnification of carious cementum as depicted in Fig. 12. Note variety of crystal morphology. Some appear as irregular plates while others are tablet-shaped. × 99,000.

Fig. 14. Thin section of calculus depicting small plate-like crystals located outside as well as within bacterial remnants (BR). × 99,000.

Fig. 15. Section of calculus demonstrating crystals of varying size and shape. These crystals were considerably larger than those of sound and carious cementum. × 99,000.

# PLATE I



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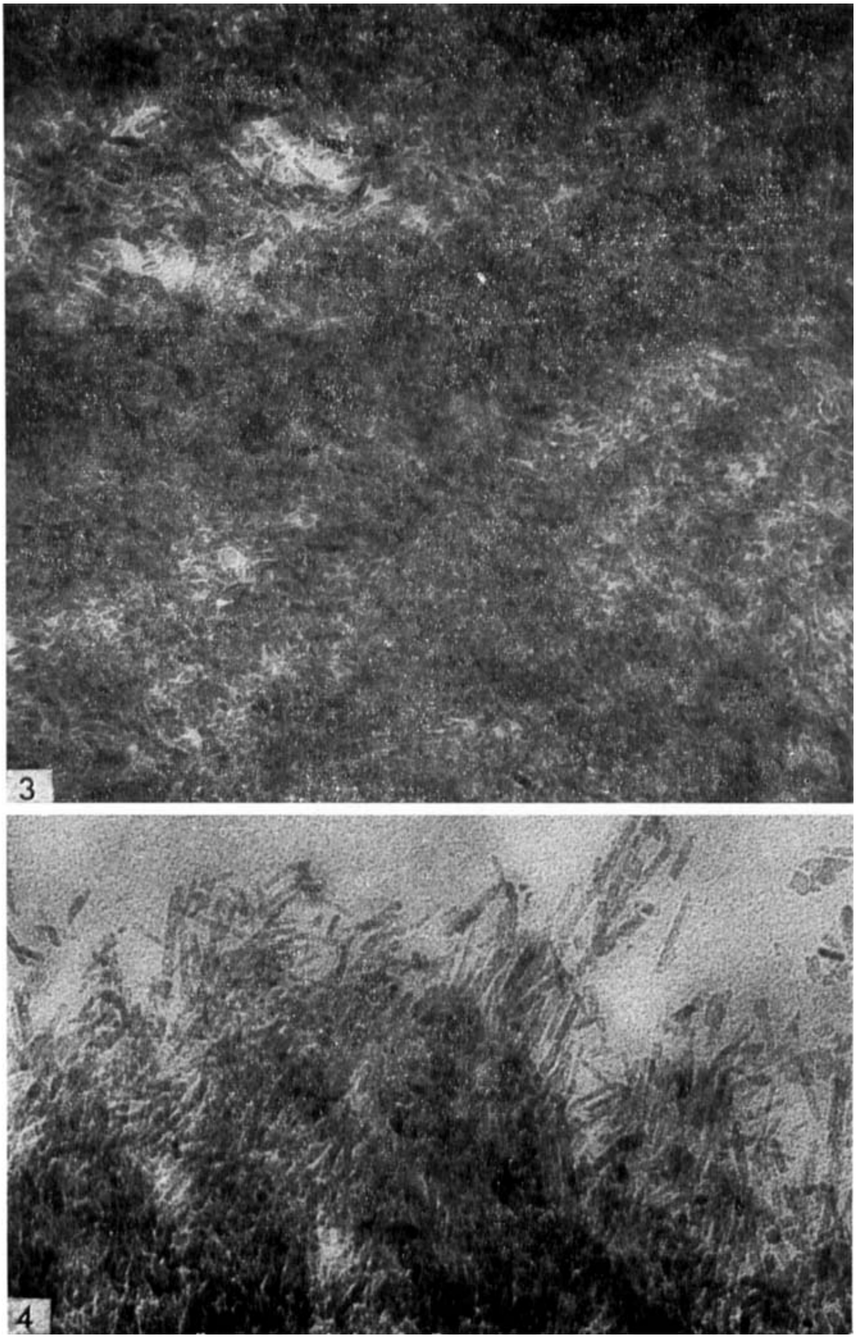

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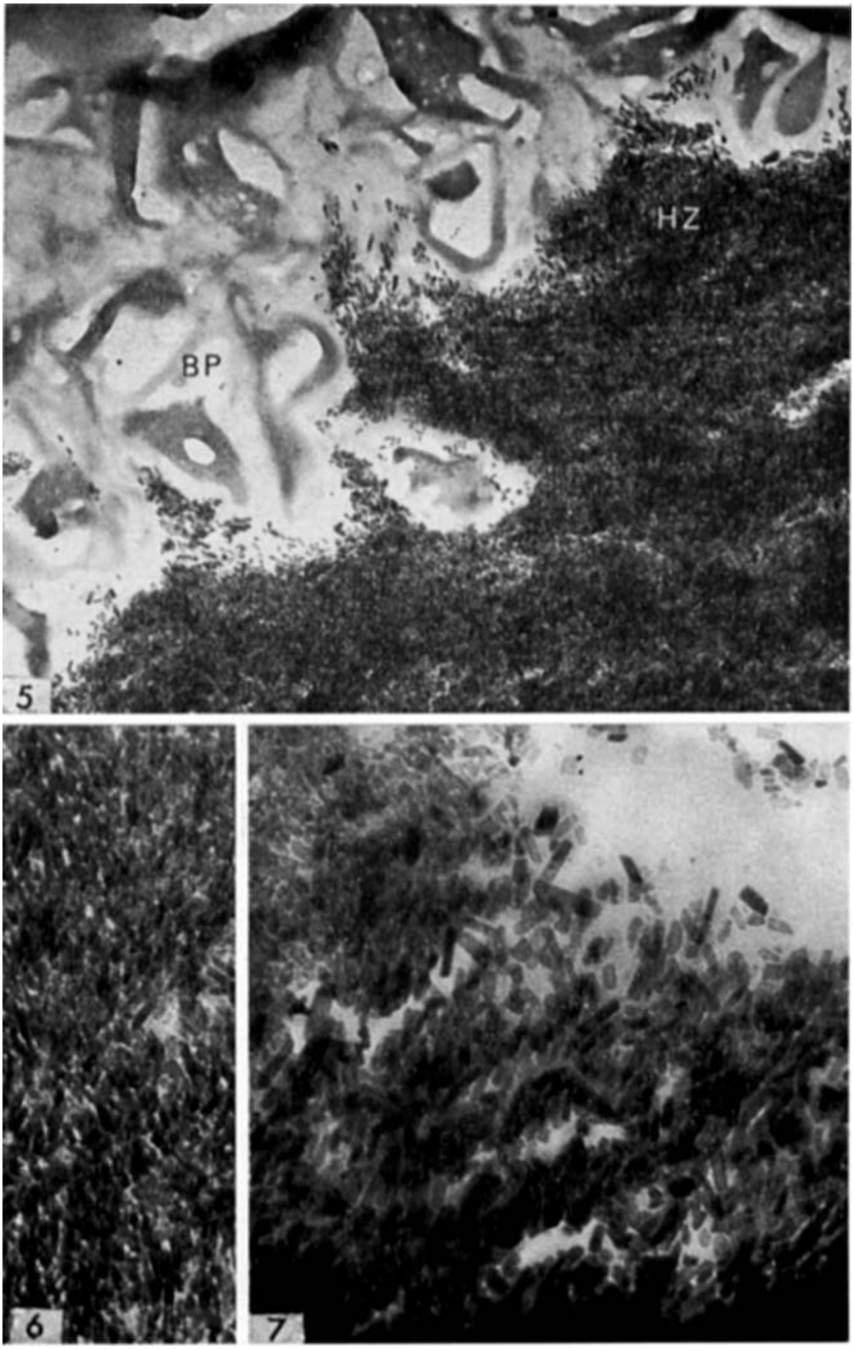

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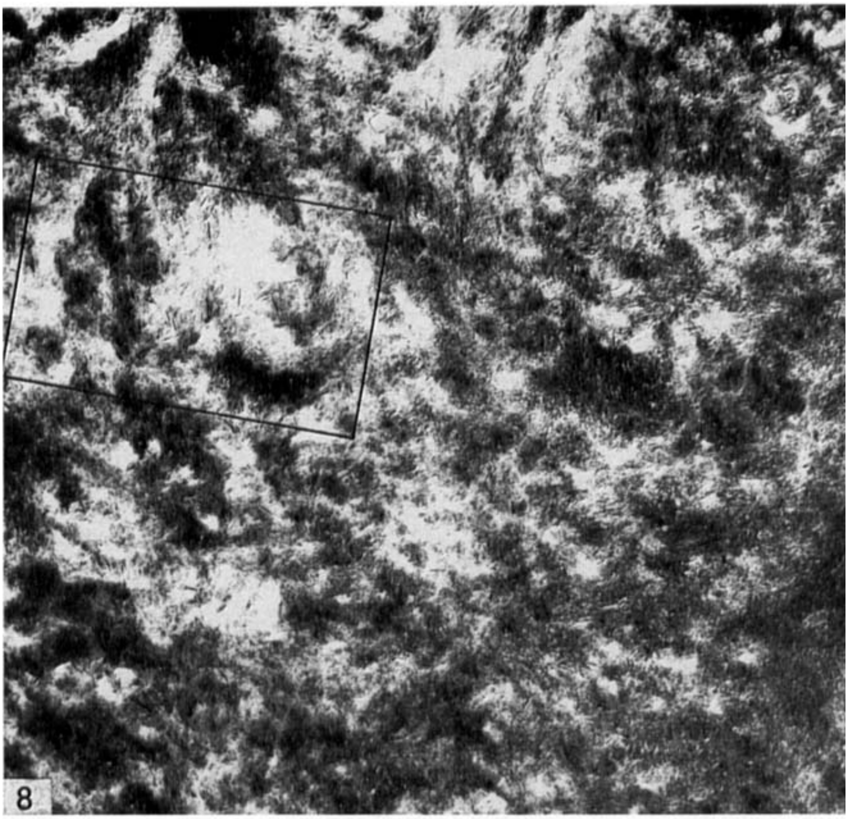


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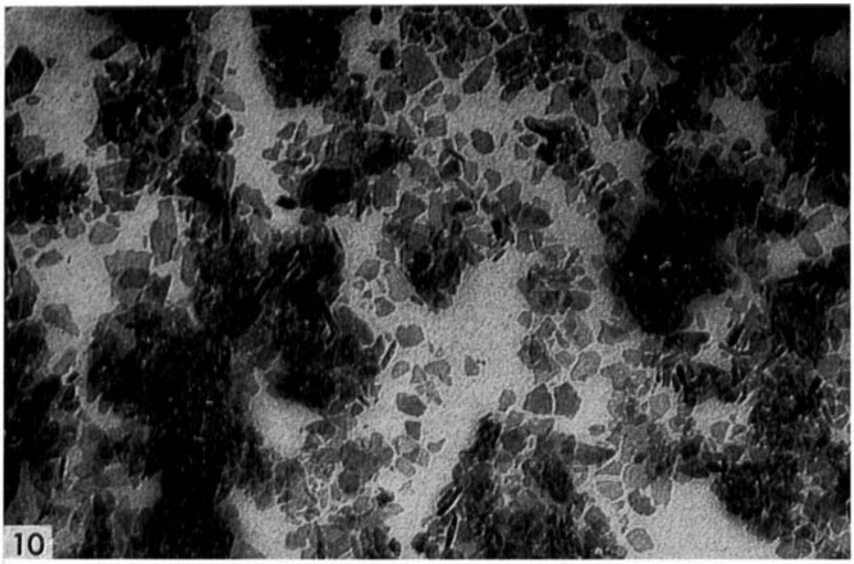

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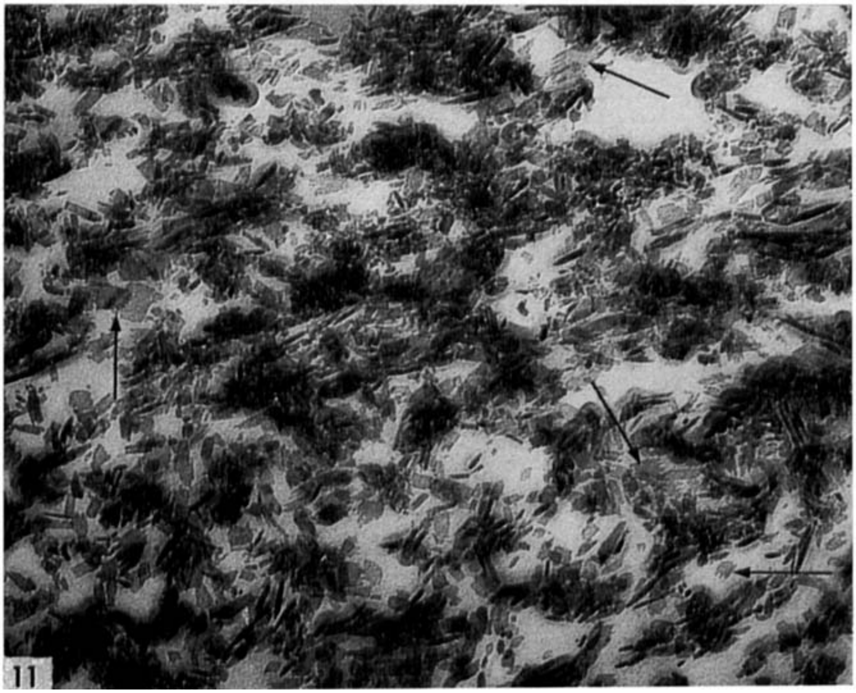

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PLATE VI

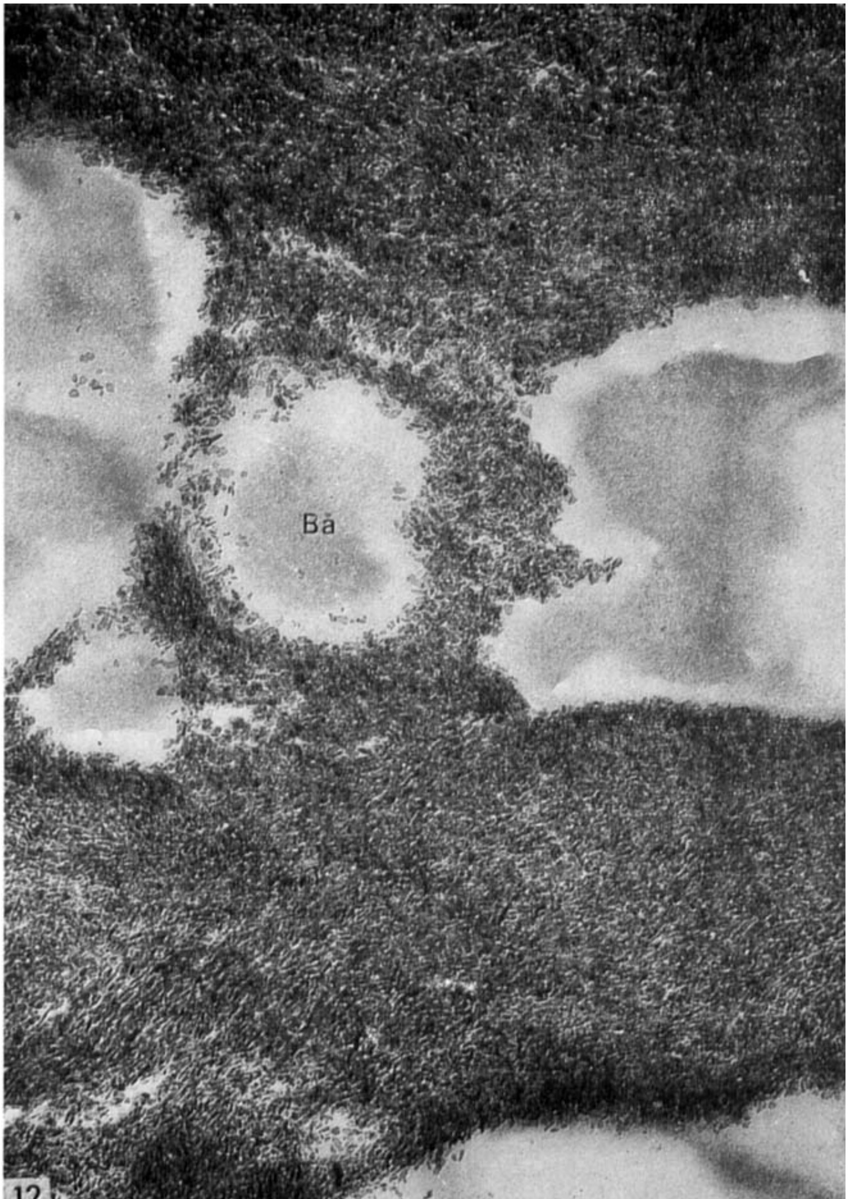
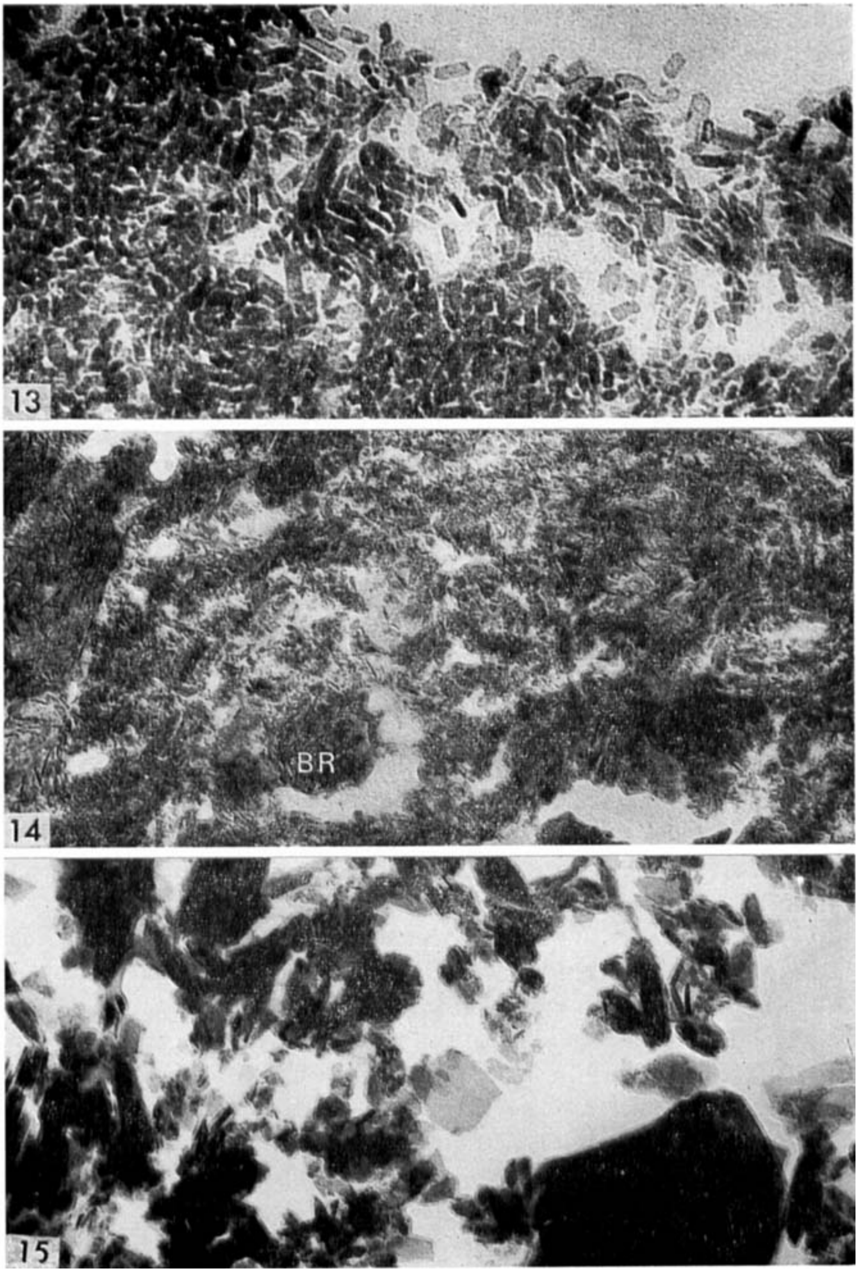


PLATE VII



interspersed throughout the tissue, but there was no evidence of bacterial penetration. It is possible that these voids represent microchannels through which movement of fluid from the surface to the underlying tissue may take place. Detailed study of the remaining mineral showed that the crystals were similar to those observed in sound, unexposed cementum. They were essentially plate-like in shape and of varying size (Figs. 9 and 10). In one specimen, several crystals with serrated edges were observed (Fig. 11).

Ultrathin sections of specimens, which by microradiography showed radiolucent and radiodense areas (brush-like appearance), revealed bacteria within lacuna-like spaces in the tissue (Fig. 12). The surrounding matrix was frequently densely mineralized and some crystals appeared as irregular plates, while others were tablet-shaped (Fig. 13).

Observations on the ultrastructure of the crystals of calculus were included in the study because of the occurrence of mineral deposits adjacent to carious cementum. In some specimens, plate-like crystals, approximately 200 Å in length were found both within and outside bacterial remnants (Fig. 14). More commonly, the crystals were of irregular shape and much larger than those of sound and carious cementum (Fig. 15).

## DISCUSSION

The microradiographic study upon which the present investigation was based (*Furseth & Johansen*, 1968) revealed a number of structural alterations in carious cementum as well as structural differences between exposed non-carious and unexposed (sound) cementum.

The ultrastructural findings on carious cementum confirm our previous conclusions based upon microradiography that demineralization and remineralization occur as part of the caries process. The most conspicuous evidence of mineral removal was the occurrence of lacuna-like spaces of varying size occupied by bacteria. Another indication of demineralization was the random occurrence of smaller spaces suggestive of microchannels. While it was not always possible to distinguish such spaces from artifacts resulting from tissue displacement during sectioning, their presence in tissue segments not disrupted in the sectioning procedures, support the idea that they were true spaces having resulted from crystal dissolution. Crystals with morphologic characteristics indicative of mineral deposition were also observed. Some tablet-shaped crystals were found to be larger than those observed in exposed non-carious cementum. The mineral for this crystal growth might have been derived from the salivary fluids and/or ions released from dentine and cementum in progressing carious lesions as previously postulated (*Furseth & Johansen,* 1968). Another indication of remineralization was the occurrence in one instance of large plate-like crystals with serrated edges. This configuration was observed only in the carious tissue, and because of the large size of these crystals, it seems most likely that they were the result of crystal growth rather than partial crystal dissolution.

The fact that non-carious exposed cementum revealed tablet-shaped crystals distinctly different from the plate-like crystals of the unexposed sound tissue, raises questions as to the mechanism involved in this transformation of the mineral phase. The most likely explanation is that recrystallization of the original mineral has taken place after exposure of the tissue to the fluid environment of the oral cavity. While unexposed (sound) cementum is maintained in the constant environment of the internal milieu of the body, the exposed cementum is subjected to the changing conditions of the oral cavity as mediated and modified by bacterial plaques. In addition to containing acids and other bacterial products, these plaques have the capability of concentrating phosphate and particularly calcium. The levels of concentration of phosphate have been found to be 3—4 times higher than that of saliva and calcium 19 times higher (*Dawes & Jenkins,* 1962) although saliva is supersaturated with regard to these ions (*Brudevold, Grön & McCann,* 1965). These high concentrations might be of relevance, since *in vitro* studies have shown that the relative amounts of calcium and phosphate in solutions may influence the size and shape of crystals formed. From a solution containing excess phosphate, lath-shaped crystals were formed, whereas crystals from solutions containing excess calcium ions were closer to being equilateral, with the length approximately 1 1/2 times the width (*Watson & Robinson,* 1953).

Another factor to be considered in relation to crystal transformation in non-carious exposed cementum and the persistence of these tablet-shaped crystals in the carious tissue, is the nature of the organic matrix. While unexposed (sound) cementum has a matrix largely composed of typical collagen fibers, the altered tissues have revealed modifications of the matrix. Loss of typical collagen structure adjacent to areas of inflammation has been reported (*Selvig,* 1966), and absence of collagen crossbanding has been observed in a narrow zone immediately beneath the surface of cementum exposed to the oral environment (*Selvig,* 1969). Partial or complete loss of crossbanding has also been demonstrated in the subsurface regions of carious cementum (*Awazawa,* 1961; *Johansen,* 1965). It is also likely that the surface layer may contain organic materials derived from oral fluids as well as products of bacterial metabolism. Since crystal morphology in the mineralized tissues of the tooth is clearly related to the nature of the matrix as exemplified at the dentino-enamel junction, the changes noted in the matrices of exposed non-carious cementum might well be sufficient to be co-factors in the formation of a new species of crystals under the conditions of the oral environment.

The observations on unexposed (sound) cementum were particularly valuable in the elucidation of environmental influence on crystal morphology and on organic matrix-crystal relationship. The fact that all samples revealed plate-like crystals similar to those of bone and dentine (*Johansen & Parks*, 1960) proves the stability of this crystal form in the environment of the tissue fluid. The alternating light and dark bands observed along the course of the fibers in some sections were similar to those seen in dentine (*Johansen & Parks*, 1962), and indicated a high degree of order in crystal arrangement. No evidence of such close matrix-crystal relationship was seen in the exposed non-carious and carious tissues.

The samples of calculus revealed crystals similar to those described by *Gonzales & Sognnaes* (1960) and *Schroeder* (1965). The plate-like crystals seen within and outside bacterial remnants were smaller than the plate-like crystals in carious cementum from the central portion of lesions, and the large irregularly shaped crystals observed in calculus were also different from the crystals of sound and carious cementum.

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## SUMMARY

The mineral phase of sound and carious cementum from human permanent teeth has been studied by electron microscopic techniques to characterize the ultrastructural changes occurring in dental caries. The specimens were selected from tissue preparations previously studied by microradiography. Based upon the microradiographic findings, representative samples of various types of non-carious and carious cementum were microdissected and processed for electron microscopy. Samples of dental calculus were also studied.

Sound unexposed cementum was found to display variation in density of mineral distribution. The crystals were plate-like in morphology. In the densely mineralized surface layer of non-carious cementum exposed to the oral environment greater uniformity in mineral distribution was observed. The crystals of this modified cementum were closely packed, elongated and of uniform width, and have been referred to as tablet-shaped.

Carious cementum showed the greatest variation in structural organization and crystal morphology. The densely mineralized surface layer was covered by bacterial plaque and microorganisms were found within lacuna-like spaces. The crystals in this region were tablet-shaped, but of varying size. Apparent loss of crystals was indicated by the occurrence of small spaces throughout this layer. The central areas of carious cementum which in microradiographs displayed a slight uniform demineralization was found to exhibit regional differences in mineral distribution. Areas of high as well as low mineral content were noted, giving the tissue a mottled appearance. Distinct spaces devoid of crystals were also seen; these resembled microchannels. While plate-like crystals similar to those of sound cementum predominated, some crystals with serrated edges were observed in one of the specimens. Carious cementum characterized by alternating radiolucent and radiodense areas (brush-like appearance) revealed a densely mineralized matrix containing large spaces filled with microorganisms. The observations on calculus revealed an ultrastructure different from that of cementum. The most conspicuous features of calculus were mineralized bacteria and a predominance of large irregularly shaped crystals.


## RÉSUMÉ

### ETUDE AU MICROSCOPE ÉLECTRONIQUE DE LA PHASE MINÉRALE DU CÉMENT DENTAIRE HUMAIN SAIN ET CARIEUX

La phase minérale du cément sain et carieux de dents permanentes humaines a été étudiée par des techniques de microscopie électronique pour déterminer les caractéristiques des modifications ultrastructurales se produisant au cours de la carie dentaire. Les échantillons ont été choisis parmi des préparations de tissus étudiées au préalable par microradiographie. Sur la base des résultats microradiographiques, des échantillons représentatifs de cément carieux et non carieux ont subi une microdissection et ont été préparés pour microscopie électronique. Des échantillons de tartre dentaire ont aussi été étudiés.

Il est apparu que le cément sain non dénudé présentait des variations dans la densité de la répartition minérale. Les cristaux étaient en forme de plaques. Dans la couche superficielle fortement minéralisée du cément non carieux dénudé et exposé au milieu buccal, on observait une plus grande uniformité de la répartition minérale. Les cristaux de ce cément modifié étaient tassés les uns contre les autres, allongés et de largeur uniforme; ils ont été décrits comme étant en forme de tablettes.

Le cément carieux présentait les variations les plus marquées dans l'organisation structurale et la morphologie des cristaux. La couche superficielle fortement minéralisée était couverte d'une plaque microbienne et on trouvait des micro-organismes dans des espaces ressemblant à des lacunes. Les cristaux de cette région étaient en forme de tablettes, mais de grandeur variée. La disparition apparente de cristaux était indiquée par le présence de petits interstices dans toute cette couche. Dans les zones centrales du cément carieux, qui, dans les microradiographies, présentaient uniformément une légère déminéralisation, on constatait des différences régionales de répartition minérale. On notait des zones à forte teneur minérale et des zones à faible teneur, ce qui donnait au tissu une apparence tachetée. On voyait aussi des interstices nets, ne contenant pas de cristaux; ces interstices ressemblaient à des microrainures. Alors que les cristaux en forme de plaques, semblables à ceux du cément sain, prédominaient, on a pu observer dans un des échantillons quelques cristaux à bords dentelés. Le cément carieux caractérisé par des alternances de zones radioclaires et radio-opaques (apparence en forme de brosse) présentait une matrice fortement minéralisée, contenant de grands interstices remplis de micro-organismes. Dans le tartre, on observait une ultrastructure différente de celle du cément. Les caractères les plus manifestes dans le tartre étaient la présence de bactéries minéralisées et la prédominance de cristaux de grande taille et de forme irrégulière.


## ZUSAMMENFASSUNG

### ELEKTRONENMIKROSKOPISCHE STUDIEN DER MINERALPHASE INTAKTEN UND KARIÖSEN ZEMENTES MENSCHLICHER ZÄHNE

Die Mineralphase in gesundem und in kariösem Zement bleibender menschlicher Zähne wurde elektronenmikroskopisch untersucht, um die ultrastrukturellen Veränderungen des Kariesprozesses zu charakterisieren. Die Präparate wurden zunächst mikroradiographisch untersucht. Auf die mikroradiographischen Resultate basiert, wurden repräsentative Präparate verschiedener Typen von gesundem und von kariösem Zement für das Elektronenmikroskop hergestellt. Zahnsteinpräparate wurden auch studiert.

Intakter nicht exponierter Zement zeigte unterschiedliche Dichte der Mineralverteilung. Morphologisch waren die Kristalle plattenähnlich. In der dicht mineralisierten Oberflächenschicht des nicht kariösen Zementes, der dem Mundhöhlenmilieu ausgesetzt worden war, wurde grössere Gleichförmigkeit in der Mineralverteilung beobachtet. Die Kristalle dieses modifi-

zierten Zementes waren dicht gepackt, langgestreckt und von gleicher Breite, und wurden als »tablet-shaped» bezeichnet.

Kariöser Zement zeigte die grösste Variation in der Organisationsstruktur und der Kristallmorphologie. Die dicht mineralisierte Oberflächenschicht war von Bakterienplaque bedeckt, und Mikroorganismen wurden in Lakunen gefunden. Die Kristalle in diesem Gebiet waren »tablet-shaped» aber von verschiedener Grösse. Scheinbarer Verlust von Kristallen wurde durch das Vorkommen kleiner Spalten durch diese Schicht angezeigt. Die zentralen Gebiete kariösen Zementes, welche mikroradiographisch eine leichte gleichmässige Demineralisierung darstellten, zeigten regionale Unterschiede in der Mineralverteilung. Es wurden Gebiete mit hohem und niedrigem Mineralgehalt beobachtet, welches dem Gewebe eine marmorierte Erscheinung gab. Gebiete ohne Kristalle waren auch deutlich zu sehen, welches an Mikrokanäle erinnerte. Plattenähnliche Kristalle, wie die im intakten Zement, beherrschten das Bild, jedoch waren einige Kristalle mit gezackten Kanten in einem der Preparate zu beobachten. Kariöser Zement, characterisiert durch abwechselnd röntgenhelle und röntgendichte Gebiete (Bürstenähnlich), zeigte eine dicht mineralisierte Matrix, die Mikroorganismen in grossen Gebiete enthielt.

Der Zahnstein zeigte eine Ultrastruktur, die von der des Zementes abwich. Am meisten auffallend waren mineralisierte Bakterien und ein übergewicht von grossen, ungleichmässig geformten Kristallen.

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