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AN ULTRASTRUCTURAL STUDY OF CEMENTUM FORMATION

by

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INTRODUCTION

Classical histological investigations have demonstrated that the intercellular matrix of cementum, like that of bone and dentin, consists of collagen fibrils invested in an amorphous ground substance. The distribution of minerals within the matrix is, however, somewhat controversial, especially with regard to whether the Sharpey's fibers in cementum are uncalcified (5) or calcified (14). Otherwise, it is commonly held that the cementum consists of uncalcified collagen fibrils surrounded by a calcified ground substance (8, 11). It has been suggested, on the basis of histochemical staining reactions and histological appearance, that the inner layer of cementum develops free of collagen fibers, thus consisting of calcified ground substance alone (8).

Electron microscopic studies on the early development of dental root tissues in rodent incisors have been reported by the author (9). In continuation of these studies molar teeth of young and adult mice were selected as specimen material in order to

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examine an attachment apparatus more comparable to that of human teeth. The present report describes the results of this latter investigation.

MATERIALS AND METHODS

Mandibular molars with surrounding periodontal tissues were dissected out from 18-, 24-, and 78-day old albino mice. In the 18-day old animals only the first molars had erupted into the oral cavity. At 24 days the second molars had erupted, while the third molars were still covered occlusally by the oral mucous membrane. At 78 days all three molars had erupted. The specimens were fixed in a potassium dichromate-osmium tetroxide mixture and embedded in methacrylate with the long axes of the teeth oriented either horisontally or vertically in the gelatin capsules. The sectioning and staining techniques have been described previously (9). Thin sections were examined in the electron microscope[1].

RESULTS

The earliest stage in the development of cementum and periodontal membrane was seen in sections from the developing

[1] RCA EMU-2 or Siemens Elmiskop 1.

Figure 1. Longitudinal section near the apical end of a developing root. A 6-micron wide layer of dentin (D) has been deposited. Between the peripheral surface of the dentin matrix and the nearest cell of the periodontal membrane (upper left corner) a zone of irregularly arranged collagen fibrils is present. No appreciable amount of cementum matrix can be identified at this stage. PD — predentin, N — cell nucleus. Third molar from 18-day old mouse, decalcified longitudinal section. × 8,000.

Figure 2. A two to three microns wide layer of cementum (C) has been deposited. The first formed layer of cementum matrix contains irregularly arranged fibrils partly obscured by an amorphous investing substance. Single fibrils can be followed uninterruptedly from the periodontal membrane into the cementum. In the underlying dentin (D) the fibrils form broad sheets which run parallel to the root surface. CB — cementoblast. Twenty-four-day old animal, first molar. Decalcified longitudinal section. × 9,500.

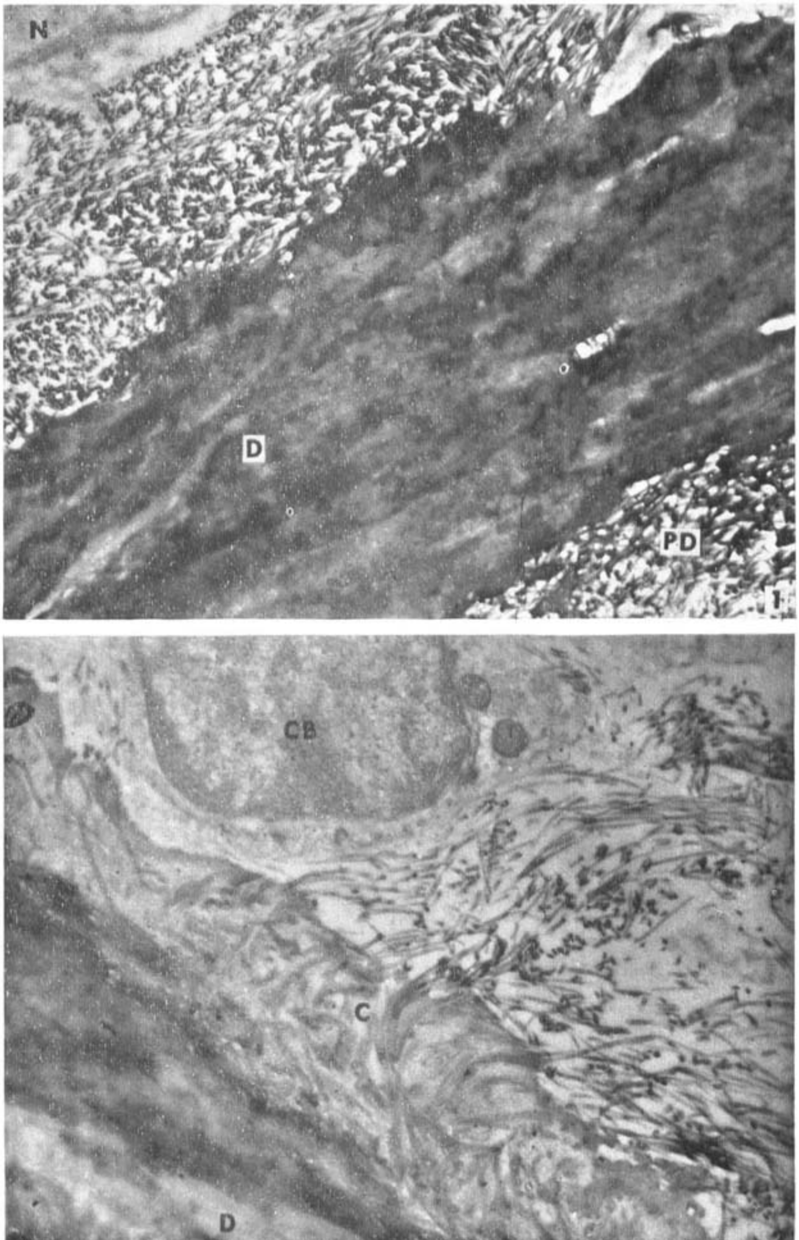

Fig. 1—2.

end of incompletely formed roots of molars from the 18- to 24-day old animals. Here, a zone of rather densely packed collagen fibrils was found adjacent to a thin layer of root dentin (Figure 1). These fibrils did not exhibit any preferred orientation. In the periodontal membrane, on the other hand, bundles of parallel fibrils were seen. The latter fibers were more or less parallel to the dentin surface and did not appear to enter the zone next to the dentin.

The formation of cementum, which was initiated before the teeth had erupted into the oral cavity, started by a simultaneous deposition of amorphous ground substance and of mineral crystals in close approximation to the root dentin. The first layer of cementum formed appeared in decalcified sections as a meshwork of irregularly arranged fibrils, partly obscured by an amorphous investing substance (Figure 2). Single fibrils or small bundles of parallel fibrils could be traced from the adjacent periodontal membrane into the cementum matrix. The cementum surface had an irregular outline, with protruding points corresponding to the points of insertion of each fibril or bundle of fibrils. Where a cell body was located in immediate contact with the cementum, the calcified tissue had a smoother surface (Figures 1, 2, and 3).

In the underlying dentin, the matrix fibrils generally were oriented parallel to the long axis of the root, and often formed broad bands. Thus, the inner layer of cementum could be distinguished from the dentin by a difference in fibrillar structure. In addition, the dentin matrix usually appeared more electron dense than the cementum matrix after staining of decalcified sections (Figures 2 and 4).

The cementum appeared to increase in thickness by simultaneous deposition of ground substance and mineral crystals along its surface. Sometimes islands of calcified tissue were found several microns away from the continuous part of the cementum. Occasionally, where cells of the periodontal membrane were located adjacent to the cementum, such islands were observed on the peripheral side of the cells (Figure 4). As the cementum increased in thickness, these cells eventually became enclosed in the calcified tissue.

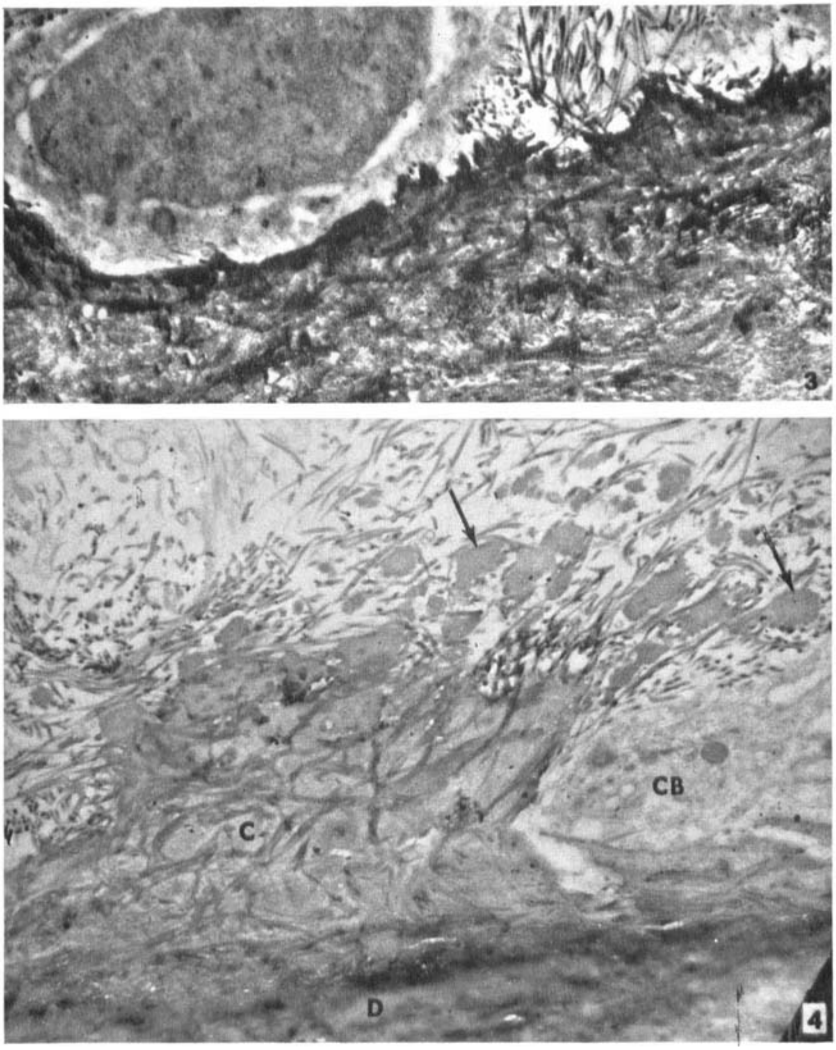

Fig. 3—4.

Figure 3. In areas where fibrils are attached to the cementum, the cementum surface protrudes. Adjacent to the cell body the surface is more smooth. Decalficied longitudinal section. × 8,000.

Figure 4. Formation of cellular cementum. Small foci of cementum (arrows) are located in the periodontal membrane several microns away from the continuous part of the cementum (C), partly entrapping the cell body of a cementoblast (CB). D — dentin. Twenty-four-day old mouse molar, decalcified, longitudinal section. × 7,500.

A more advanced stage in the development of the attachment apparatus than described above could be observed in the middle and cervical root portions of the same teeth. A greater amount of cementum was found in these areas than in the apical region, and thick bundles of collagen fibrils could now be traced from the cementum surface to the collagen fibers of the periodontal membrane (Figure 5).

Following eruption of the molars into the oral cavity, a change in orientation of the periodontal fibers became evident. While these fibers at early stages of root formation in general were oriented parallel to the root surface, they now were arranged more or less at right angles to the cementum surface. The fibers varied in diameter from two to five microns. Each fiber consisted of a great number of parallel collagen fibrils which could be followed within the fibers for seven to eight microns (Figure 6). However, due to the thinness of the sections, the fibrils often left the sectioning plane and their ultimate length could not be definitely ascertained.

In the 78-day old animals the periodontal membrane was even more densely packed with collagen fibrils. The zone closest to the root surface appeared structurally similar to the rest of the periodontal membrane (Figure 7). There was a marked increase in thickness of the cementum and two layers could now be identified. The inner layer, which was 1 to 3 microns in width, was characterized by an irregular arrangement of the matrix fibrils. In the outer layer the collagen fibrils formed thick bundles which were oriented more or less at right angles to the surface (Figure 7). The thickness of the cementum on molars of the 78-day old mice varied considerably in different areas, the thinnest layer being in the order of 5 microns.

The continuity of the fibrils in the cementum matrix with those of the periodontal membrane could be demonstrated in longitudinal as well as in cross sections of the teeth. Thus, bundles

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Figure 5. The root surface at some distance from the apex. Cross-sectioned bundles of collagen fibrils are seen near the cementum surface. Decalcified. × 6,000.

Figure 6. Periodontal fiber. The fiber consists of a great number of parallel collagen fibrils of indefinite length. × 8,000.

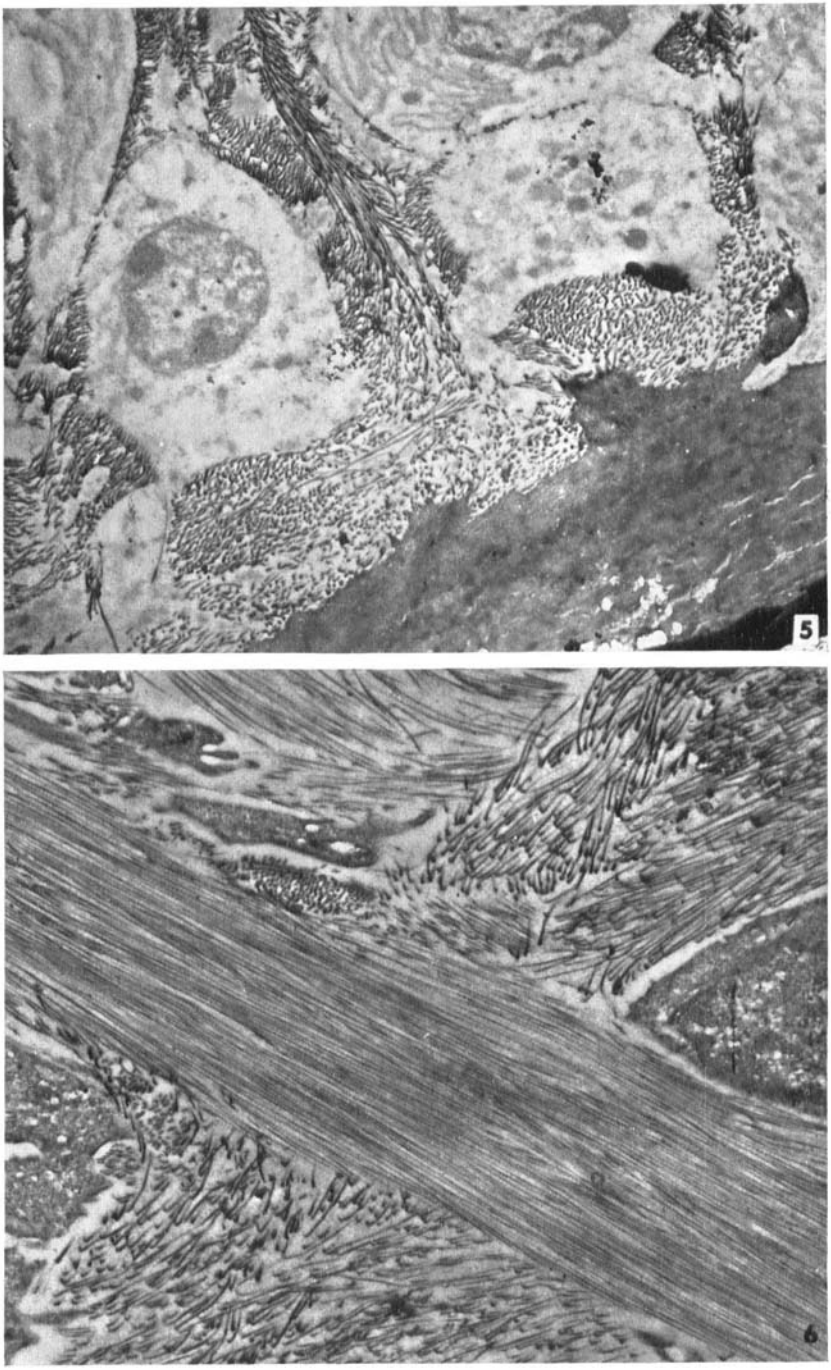

Fig. 5—6.

of parallel fibrils, 300 to 400 Å wide, could be traced from the periodontal membrane into the cementum, where they became obscured by the dense mineralized tissue. These bundles were identifiable as Sharpey's fibers. Where each fibril entered the cementum a small pyramid of calcified substance protruded, giving the surface a serrated appearance (Figure 8). At somewhat higher magnifications it could be observed that the pyramids consisted of small, threadlike mineral crystals which had been deposited along the collagen fibrils (Figures 8 and 9). These crystals were 300—400 Å in length, while their diameter was much smaller. They were oriented with their long axis parallel to the fibrils. Electron diffraction patterns indicated that this axis corresponded to the crystallographic c axis of hydroxyapatite.

In sections which were cut parallel to the root surface most fibrils of the periodontal membrane were seen in cross section (Figure 10). In areas where the sections touched the cementum surface the irregular character of this surface resulted in the appearance of small isolated islands of mineral in the section. The smallest islands of mineralized tissue contained one or a few crytals located on the surface of or within a single fibril. Others consisted of individual fibrils which were densely packed with mineral crystals. The mineralized areas apparently increased in size by apposition of more crystals on their surface, until the in-

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Figure 7. Cementum formation on a molar from a 78-day old animal. Bundles of densely packed fibrils in the periodontal membrane (PM) can be followed into the cementum. The inner layer of cementum (C) has an irregular structure. The cementum matrix does not contain unmineralized areas. The white lines are artefacts. D — dentin. Longitudinal section. x 9,500.

Figure 8. Higher magnification of an area from Figure 7. The fibrils are 300 to 400 Å wide and exhibit the typical crossbanding of collagen. The serrated appearance of the cementum surface is due to protruding pyramids of mineralized tissue. x 20,000.

Figure 9. Area similar to that in Figure 8. Some collagen fibrils have been sectioned longitudinally while others have been cut obliquely. The crossbanding of the collagen fibrils is almost completely obscured in the cementum. The fine threadlike mineral crystals are 300 to 400 Å in length. x 35,000.

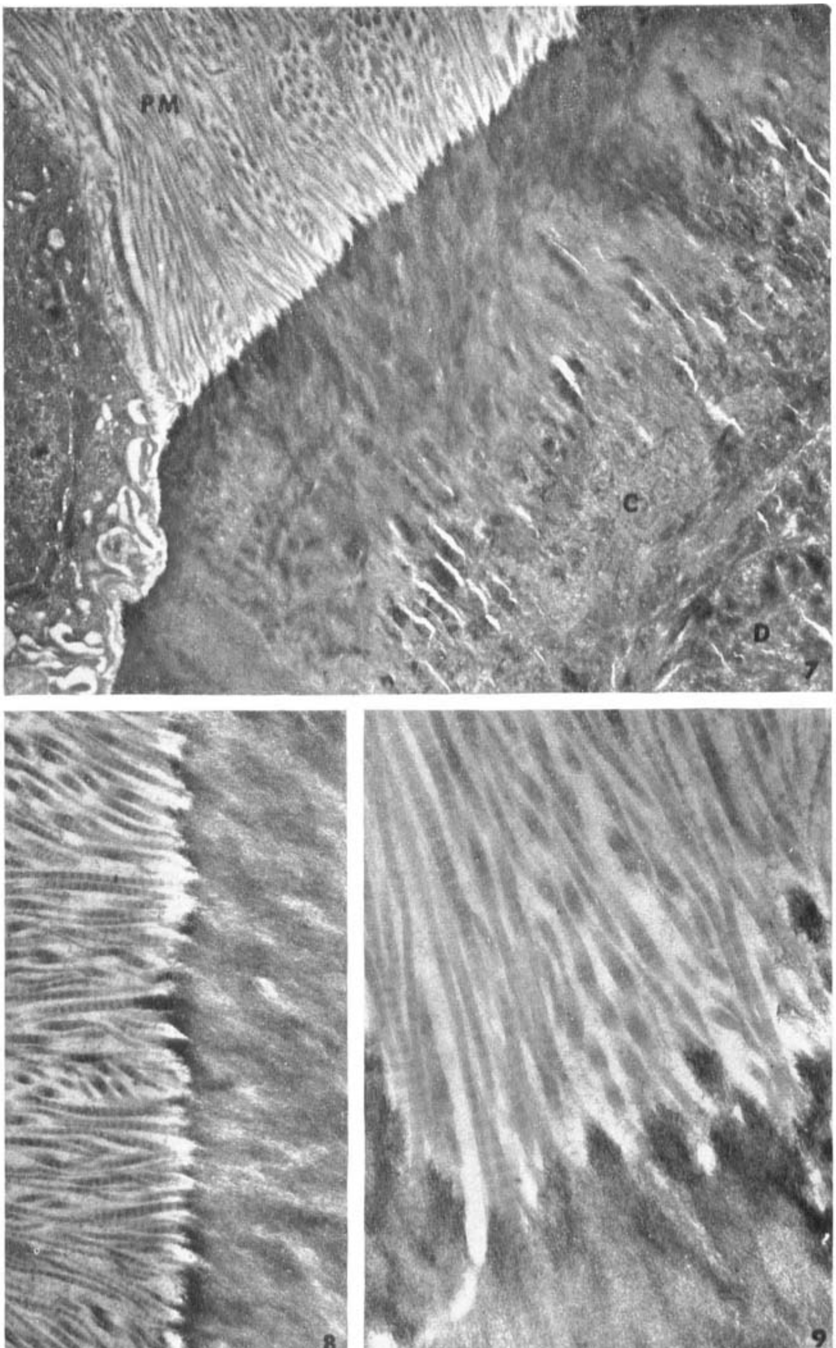

Fig. 7—9.

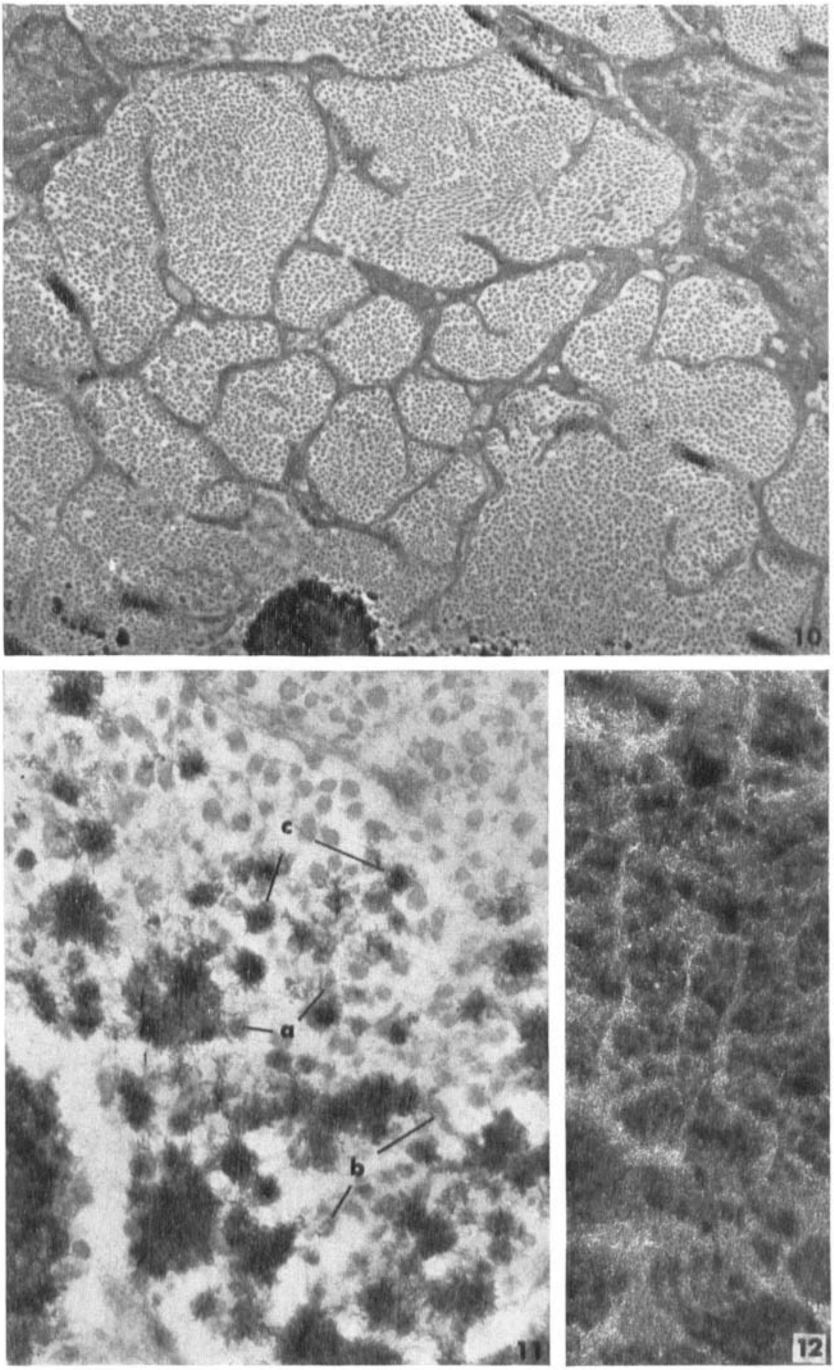

Fig. 10—12.

terfibrillar space was completely filled out (Figure 11). The crystals did not appear as points in the tangential sections, but as two-dimensional structures. Their greater dimension in this view was 100 to 200 Å, while the smaller dimension was less than 50 Å.

In sections cut parallel to the root surface through the cementum the mineral crystals were distributed homogeneously throughout the matrix (Figure 12). No areas of the cementum matrix were completely uncalcified.

DISCUSSION

In the mouse molars, as well as in the incisors (9), the formation of cementum was initiated immediately after the disintegration of Hertwig's epithelial sheath, at an earlier stage than was demonstrated previously (2).

When the observations on specimens from adult mice were compared with those from 18- and 24-day old animals, morphological differences were found in the periodontal membrane as well as in the cementum itself. In the periodontal membrane a gradually increasing density in the packing of the collagen fibrils was demonstrated. Near the developing end of the incompletely formed roots of the unerupted and erupting teeth bundles of parallel fibrils were found in the surrounding periodontal tissue, and these bundles were not attached to the root surface. This observation is in agreement with that of *Trott* (16), who

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Figure 10. Tangential section through the periodontal membrane near the cementum. The dense packing of the cross-sectioned fibrils is evident. Cellular processes from the cementoblasts separate the bundles of collagen fibrils. At the bottom edge the section includes some cementum. Seventy-eight-day old mouse molar. x 8,000.

Figure 11. Tangentially sectioned cementum surface. The collagen fibrils are seen in cross section. Mineral crystals have been deposited intrafibrillarly (a), and at the surface of some fibrils (b). Other fibrils are densely packed with crystals (c), while the larger islands of mineralized tissue apparently consist of several calcified fibrils connected by interfibrillar deposition of minerals. Seventy-eight-day old mouse. x 50,000.

Figure 12. Cementum sectioned parallel to the surface. The minerals are distributed almost homogeneously throughout the matrix. x 22,000.

found that these fibers were oriented in a crown-root direction and were not part of a ligament or band of fibers. Instead, a network of collagen fibrils was seen adjacent to the dentin. At some distance from the growing end in the direction of the crown, where some cementum formation had already occurred, fiber bundles could be traced uninterruptedly from the periodontal membrane into the cementum. Between the fiber bundles irregularly arranged single fibrils were seen. Finally, in the adult animals all the collagen fibrils of the cementum matrix appeared to be continuous with those of the periodontal membrane, and the zone adjacent to the cementum surface could no longer be distinguished from the rest of the periodontal membrane on the basis of morphological differences.

Thus, only at the early stages of root formation did the cementum on the mouse molars seem to form from a precursor tissue, or precementum. In the adult animal the cementum formation occurred by a simultaneous deposition of apatite crystals and ground substance in and around the collagen fibrils of the periodontal membrane. This process, by which even cells of the periodontal membrane become surrounded by the calcified tissue, can be described as a gradual mineralization of the periodontal membrane.

The difference in structure and orientation of the collagen fibrils of the tissue immediately surrounding the calcified root surface at different stages of cementum formation resulted in corresponding structural differences within the cementum of adult animals. Thus, the inner layer of cementum contained irregularly arranged matrix fibrils, while the outer layer consisted of fiber bundles which were oriented more or less horizontally. The inner layer, which did not contain Sharpey's fibers, presumably represents the amount of cementum formed before the teeth had been subjected to the forces of mastication. Similarly, in the cementum matrix of unerupted incisors a lack of preferred orientation of the collagen fibrils has also been noted (9) while, on the other hand, *Stern* (13) found that in adult rats Sharpey's fibers were present in incisor cementum as well.

The cementum appeared to increase in thickness by deposition of mineral crystals within and on the surface of the fibrils and, later, in the interfibrillar space. This is in agreement with the observations of *Albright & Flanagan* (1), who found that in mature cementum from human and hamster teeth the crystals were deposited on and within the fibrils rather than between them. These findings conform to the results from electron microscopic investigations of other calcifying tissues which contain collagen as the primary constituent of their organic matrices, such as bone (4, 10), dentin (15), and turkey leg tendon (7). The demonstration of crystals within the fibrils is in contrast to the general concept that the cementum consists of uncalcified fibrils surrounded by a calcified ground substance (8, 11).

Accurate size determinations of the mineral crystals were not attempted in the present investigation, but some information was obtained as to their orientation and shape. In general, the crystals were deposited with their long axes parallel to the collagen fibrils. When sectioned longitudinally the crystals appeared as needles. In cross sections, however, it was seen that they were two to four times wider than they were thick. Thus, in agreement with earlier observations (1), the crystals near the calcifying cementum surface were found to be thin, platelike structures.

The Sharpey's fibers in human and animal cementum have been thought to remain uncalcified in areas where the teeth are supported by intact periodontal tissues, and only become calcified in deeper layers of the tissue as a result of increasing age (5). Microradiographic investigations have demonstrated the presence of radially oriented uncalcified or semi-calcified structures in human cementum (12). On the other hand, observations on the penetration of dyes through cementum have indicated that Sharpey's fibers are as calcified as the rest of the matrix (14). In an electron microscopic investigation of human alveolar bone *Frank, Lindemann & Vedrine* (3) found that Sharpey's fibers in that tissue in general were uncalcified. In sections made parallel to the bone surface the Sharpey's fibers appeared circular, and were surrounded by an amorphous ground substance which gave the fibers a cloudy appearance. In the present investigation such structures were not observed. The intercellular matrix of the mouse molar cementum seemed to be nearly homogeneously calcified, and no areas were completely free from mineral. This study indicates, therefore, that as the cementum is formed minerals are deposited throughout the matrix in close relation to the collagen fibrils, including those which constitute the Sharpey's fibers.

SUMMARY

The matrix of the first formed cementum layer contained a meshwork of irregularly arranged collagen fibrils while, on the other hand, the cementum which was formed posteruptively contained bundles of densely packed fibrils which were oriented more or less at right angles to the surface. As the cementum increased in thickness the collagen fibrils of the periodontal membrane became incorporated in the cementum. Mineral crystals were, first, deposited within and on the surface of these fibrils and, later, between them. There was no difference in this respect between the bundles of parallel fibrils which made up Sharpey's fibers, and other collagen fibrils within the cementum. In the cementum of adult animals the intercellular matrix appeared homogeneously calcified. Thus, Sharpey's fibers appeared to be as calcified as the rest of the matrix, and cementum formation in the adult animal could be described as a slow mineralization of the periodontal membrane.

RÉSUMÉ

ÉTUDE DE LA STRUCTURE MICROSCOPIQUE DU CÉMENT EN VOIE DE FORMATION

La trame de la première couche de cément formée contenait un treillis de fibrilles collagènes irrégulièrement disposées, tandis que, d'autre part, le cément formé après l'éruption contenait des faisceaux de fibrilles bien serrées dont la direction était plus ou moins perpendiculaire à la surface. Au fur et à mesure que l'épaisseur du cément augmentait, les fibrilles collagènes du périodonte étaient incorporées dans le cément. Des cristaux minéraux se déposaient, d'abord à l'intérieur et à la surface de ces fibrilles, et ensuite entre elles. Il n'existait pas de différence à ce point de vue entre les faisceaux de fibrilles parallèles constituant les fibres de Sharpey et les autres fibrilles collagènes situées dans le cément. Chez les animaux adultes, la trame intercellulaire du

cément paraissait calcifiée de manière homogène. Ainsi, les fibres de Sharpey semblaient être aussi calcifiées que le reste de la trame, et la formation du cément chez l'animal adulte pouvait être décrite comme une lente minéralisation du périodonte.

ZUSAMMENFASSUNG

EINE ULTRASTRUKTURELLE UNTERSUCHUNG ÜBER DIE BILDUNG VON ZAHNWURZELZEMENT

Die Matrize der erst gebildeten Zementschicht enthielt netzartige, unregelmässig arrangierte Kollagenfibrillen, während anderseits das Zement das nach dem Durchbruch gebildet wurde, Bündel von dicht gepackten Fibrillen enthielt, die fast rechtwinkelig zu der Oberfläche ausgerichtet waren. Als das Zement in Dicke zunahm, wurden die Kollagenfibrillen der Wurzelhaut im Zement einverleibt. Mineralkristallen wurden erst innerhalb der Fibrillen und auf deren Oberfläche und, später, darunter deponiert. Es gab in dieser Beziehung kein Unterschied zwischen den Bündeln der parallellen Fibrillen, die die Sharpeyschen Fasern ausmachten, und den anderen Kollagenfibrillen des Zementes. Im Zement der erwachsenen Tiere erschien die Intercellularsubstanz homogen mineralisiert zu sein. In dieser Weise erschienen die Sharpeyschen Fasern gleichmässig mineralisiert zu sein wie das Rest der Matrize, und die Zementbildung des erwachsenen Tieres konnte als langsame Mineralisierung der Wurzelhaut beschrieben werden.

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