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# ELECTRON MICROSCOPY OF HERTWIG'S EPI-THELIAL SHEATH AND OF EARLY DENTIN AND CEMENTUM FORMATION IN THE MOUSE INCISOR

by

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### INTRODUCTION

Our knowledge of the formation and structure of dental hard tissues has been expanded greatly in recent years by the introduction of electron microscopy. Numerous investigations describing dentinogenesis have been published (4, 5, 6, 8, 15). These studies have been concerned mostly with the development of dentin along the labial curvature of rodent incisors. Less emphasis has been placed upon the development and ultrastructure of cementum. Only scattered information about the fine structure of this tissue in the mature state can be found (1, 2, 13, 14).

In the present investigation the epithelial sheath of Hertwig and its relation to the early formation of cementum and underlying dentin are described. The continuously growing mandibular incisors of the young mouse were selected for this study. In these teeth the various stages of cell proliferation and differentiation together with matrix deposition and calcification can be clearly distinguished. Also these teeth are well suited for electron microscopy since they can be quickly and easily dissected out and transferred to the fixative without damage to the delicate growing end.

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The mandibular incisors were dissected out from eight-day old albino mice. The teeth were fixed in a potassium dichromate-osmium tetroxide mixture, embedded in methacrylate, and sectioned with glass or diamond knives. Two-micron-thick sections were observed under phase contrast for general orientation and to facilitate final trimming of the embeddings so as to include the lingual portions of the tooth in the thin sections. Most teeth were sectioned at right angles to their long axes, starting from the growing end. When mineralized tissue was reached, some of the teeth were decalcified by immersing the methacrylate block in 0.1 N hydrochloric acid overnight before the sectioning was continued. Sections of unmineralized or demineralized material were placed on specimen grids covered with carbon substrates, and stained by floating the grids for 30 minutes on a 2 per cent solution of phosphotungstic acid, followed by 30 minutes on a saturated solution of uranium acetate in 50 per cent alcohol. Sections containing mineral were observed in the electron microscope without staining or after staining with uranium acetate only.

### RESULTS

The mouse incisor is covered on its labial curvature by enamel, while the lingual and approximal surfaces are covered by cementum. The apical end of this continuously growing tooth has the form of a cylinder with epithelium enclosing the dental papilla or primitive pulp. A sagittal section through the apical end of a mandibular mouse incisor is illustrated diagrammatically in Figure 1. On the labial curvature, which at a later stage of development will be covered by enamel, the epithelial tissue consists of the enamel organ. Laterally, the enamel organ adjoins Hertwig's epithelial sheath, which outlines the lingual and approximal surfaces of the developing tooth.

### Hertwig's epithelial sheath

The cellular arrangement near the posterior end of the epithelial sheath as seen in the phase contrast microscope is illustrated in Figure 2. In the electron microscope the epithelial sheath was found to be separated from the primitive pulp by a

delicate membrane (Figure 3). A similar membrane was found delimiting the epithelial sheath toward the dental sac or primitive periodontal membrane (Figure 4). At low magnification the separating membranes were observed to follow a wavy course (Figure 4). In longitudinal (Figure 2) and cross sections (Figure 6) the epithelial sheath near its apical end appeared to consist of two basal layers of cuboidal or columnar cells, separated by two or more intermediate layers of squamous cells.

The cell membranes at the basal ends of the epithelial cells were parallel to the separating membrane with an intervening space, 500 to 600 Å in width (Figures 3 and 4). The cells were connected by interlocking fingerlike projections between which relatively wide intercellular spaces were found (Figures 6, 7, and 8). Near their basal ends, however, the neighboring cells were in close contact over large areas so that the rather wide intercellular spaces did not extend basally to the separating membrane.

The cells of the epithelial sheath near its developing end contained oval nuclei surrounded by the characteristic double membranes. In any one section, ten to twenty mitochondria were usually seen in each cell. The mitochondria varied from 0.5 to over 3 microns in length. The cristae mitochondriales were arranged either at right angles to the long axis of the mitochondria (Figures 3 and 4), or in a more irregular pattern. The endoplasmic reticulum was sparse. The Golgi apparatus appeared as typical vacuoles and concentrations of parallel lamellae (Figure 7). Granules of varying sizes, up to 0.2 micron in diameter, were distributed throughout the cytoplasm.

The epithelial sheath was reduced in thickness at increasing distances from the apical end. The intermediate cell layers disappeared first. Gradually the length of the columnar cells lining the dental sac diminished (Figure 8), until they also disappeared, and only a single layer of epithelial cells remained. Finally, these cells assumed a cuboid or squamous shape (Figure 9). At this stage the separating membranes could no longer be seen.

With reduction in size of the epithelial cells the number of mitochondria present in each cell seemed to have diminished. Oval electron dense granules 0.3 to 0.5 micron in length, were now present in the cytoplasm. Their periphery was more electron

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dense than the central portion, and they could be distinguished from the cytoplasmic granules previously observed by their size (Figure 9). With the initiation of dentin calcification, the epithelial cells began to separate from each other and from the dentin surface, and collagen fibrils could be found in the spaces created by this cell migration (Figure 9). Occasionally two or more cells remained in close contact. In this case desmosomes were present and fibrils were not seen in the intercellular spaces (Figures 9 and 10). Eventually the epithelial cells became dislodged completely from the dentin surface and drifted out in the surrounding connective tissue (Figure 10).

### Dentin formation

The cells of the dental papilla near the apical end of the epithelial sheath were densely packed and of irregular shape (Figure 5). They contained large nuclei, and within the cytoplasm a few mitochondria and strands of endoplasmic reticulum. A space, at least one micron in width, was found between the separating membrane and the nearest cells. Collagen fibrils were located in the intercellular spaces. The separating membrane was lined on the pulp side by delicate 0.3 to 0.5 micron long nonstriated fibrils. These fibrils were oriented at right angles to the separating membrane, in a brushlike arrangement (Figure 3). Where the epithelial sheath had been reduced to a double layer of cells, the most peripheral cells of the dental papilla assumed a low columnar shape and formed an irregular layer along the separating membrane separated from the latter by the narrow space containing nonstriated fibrils (Figure 8). These cells were identified as odontoblasts. As the epithelial sheath became reduced in thickness from a double to a single layer of cells, the odontoblasts receded from the separating membrane and aggregates of collagen fibrils could be seen between the cells and the separating membrane. These fibrils had a diameter of about 250 Å and were characterized by the typical cross banding of collagen. Contrary to the nonstriated fibrils previously described, the collagen fibrils appeared very electron-dense after staining with phosphotungstic acid. They were frequently oriented more or less parallel to the separating membrane. Simultaneously with the appearance of fine collagen fibrils near the basal end of the odonto-

blasts, longer and thicker striated fibrils, up to 1,000 Å in diameter, were found between the odontoblasts (Figure 11). They extended intercellularly from the basal end in parallel arrangement. Gradually a definite layer of matrix which could be identified as predentin was formed between the separating membrane and the odontoblasts (Figure 12). At this time a cellular process could be seen extending from the receding odontoblasts into the matrix. When the predentin had reached a thickness of 3 to 5 microns, the fibrils nearest to the separating membrane became invested with an amorphous substance which did not contain inorganic crystals as substantiated by selected area electron diffraction (Figure 13). This layer of dentin matrix subsequently calcified as more predentin was formed. A similar separate intermediate phase between the fibrillar predentin and the mineralized dentin was not evident in the later stages of dentinogenesis. On decalcified sections it could be demonstrated, however, that the matrix of the mineralized areas always contained an amorphous substance in addition to the fibrillar component, indicating that crystal formation and ground substance deposition occurred simultaneously (Figures 14 and 15).

## **Cementum** formation

At the apical end of the epithelial sheath the dental sac contained several layers of long, slender fibroblasts arranged parallel to the sheath (Figure 2). Fine bundles of collagen fibrils were scattered between the fibroblasts and near the periodontal surface of the epithelial sheath (Figure 4). Nonstriated fibrils were found lining the separating membrane in an arrangement similar to that seen on the pulp side (Figure 4). However, they were shorter and fewer in number, and seemed to disappear as the epithelial sheath decreased in thickness. The fibroblasts of the dental sac contained, in addition to the other typical cytoplasmic structures, a considerable amount of endoplasmic reticulum, which frequently was arranged parallel to the long axis of the cell (Figures 16 and 17).

As deposition of dentin and simultaneous disintegration of the epithelial sheath proceeded, collagen fibrils located between the fibroblasts increased in number and formed thicker bundles

which were oriented either parallel to or at an angle to the epithelial sheath. These bundles could be conceived of as the first periodontal fibers (Figure 17).

With perforation of the epithelial sheath, collagen fibrils were observed between the separated epithelial cells and also between the cells and the dentin surface (Figure 9). The fibrils were of uniform diameter, 250 to 300 Å, and did not form bundles. As the epithelial cells drifted away from the dentin surface, fibroblasts were found close to the tooth (Figures 10, 18). These cells were now identifiable as cementoblasts.

In addition to the characteristic collagen fibrils between the cementoblasts, occasional bundles of fine nonstriated fibrils could be observed (Figure 18). Whether these fibrils were related to the nonstriated fibrils found near the separating membrane at earlier stages of tooth development, could not be determined.

The fibrils near the tooth surface gradually became more numerous and formed an irregular meshwork. An initial deposition of mineral crystals occurred around the widely spaced fibrils, and as the various foci of calcification coalesced, a thin layer of cementum was formed. In decalcified sections the fibrillar structure of the cementum matrix, as in dentin, was masked by an amorphous substance (Figures 18 and 19). The cementoblasts remained close to the tooth surface without a layer of cementoid present between the cells and the calcified tissue. Further increases in thickness appeared to result from the simultaneous deposition of crystals and amorphous substance in apposition to the first formed layer. As calcification advanced the collagen fibrils of the periodontal membrane, which were located near the tooth surface, became entrapped in the cementum (Figure 18). The maximum thickness attained near the incisal end of the tooth was in the order of a few microns, indicating a slow rate of growth. At this level the cementum matrix still contained only an irregular meshwork of collagen fibrils. No evidence was found for the presence of larger collagen bundles, which could have been identified as Sharpey's fibers.

The crystals in the newly formed cementum were 300 to 400 Å in length, and generally shorter and thinner than those in the dentin. They appeared densely packed and without preferred

orientation. In calcified sections the cementum was less electron dense than the dentin (Figure 20). A sharp cementodentinal junction, however, could not be identified.

#### DISCUSSION

In human teeth (3, 10) and in the rat molar (7) Hertwig's epithelial sheath consists of a double layer of cells, continuous with the outer and inner enamel epithelium. The present study demonstrated that in the mouse incisor, on the other hand, the epithelial sheath consisted of several cell layers near its posterior end. It gradually became reduced in thickness, however, and consisted of a double row of cells at the stage where differentiation of the odontoblasts began.

In agreement with the observations of Nylen & Scott (5), it was found that the initial elaboration of dentin matrix occurred by the deposition of collagen fibrils of two sizes. The first zone of predentin formed near the separating membrane consisted of thin, irregularly arranged fibrils. Concurrent with the appearance of this layer, bundles of thicker and longer fibrils, which presumably correspond to Korff's fibers, were found between the odontoblasts, arranged more or less at right angles to the separating membrane.

The present investigation also confirmed the earlier observation (5) that the formation of the initial layer of dentin matrix took place in two steps: first, a deposition of fibrils; and second, an incorporation of organic substance. Mineral crystals could not be detected in this initially deposited matrix. At later stages of dentin formation, however, the intermediate stage between fibril formation and calcification of the matrix did not exist as a separate phase, and the fibrillar matrix appeared to become calcified simultaneous with the incorporation of an amorphous substance.

The cementum of the young mouse incisor was exclusively of the acellular type. The cementoblasts, or the cells located close to the cementum surface, did not differ in cytoplasmic structure from the fibroblasts within the periodontal membrane. This does not preclude, however, that cementoblasts associated with more rapid cementum formation may represent a specialized type of

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cell as indicated by *Paynter & Pudy* (7). This is also supported by preliminary investigations of the formation of cellular cementum in the mouse molar, which indicate that the cementoblasts in areas where this tissue is being formed exhibit long cellular processes. In addition, they contain large amounts of roughsurfaced endoplasmic reticulum in their cytoplasm, thus resembling osteoblasts in their cytologic features (9).

It has been well established by optical microscopy that the formation of an initial layer of dentin, followed by disintegration of the epithelial sheath, is necessary before cementum formation is activated. At the optical level, Paynter & Pudy (7) observed that the inner cementum layer develops free of fibers. The present study indicated that in the mouse incisor this layer was not devoid of collagenous elements although evidence of a definite layer of cementoid was lacking. It appeared that sparsely distributed fibrils which originated in the periodontal membrane formed an irregular meshwork and that this network constituted the only fibrillar portion of the matrix. This is in contrast to the observations of Stern (11) who found large collagen bundles, Sharpey's fibers, penetrating deeply into the cementum. This difference may be explained on the basis of age correlated with functional activity. The mineral crystals were deposited in close association with the collagen fibrils in the same intimate relationship which has been found in bone (7) and dentin (12, 15)as pointed out more recently by Albright & Flanagan (1).

# SUMMARY AND CONCLUSIONS

An electron microscopic investigation of Hertwig's epithelial sheath in the continuously growing mandibular incisor of the 8day old mouse demonstrated that the epithelial sheath consisted of several layers of cells near its posterior end. When sections were made at greater distances from the developing end of the tooth, a gradual reduction in thickness of the epithelial sheath was observed. Differentiation of odontoblasts from cells of the dental papilla occurred when the epithelial sheath had reached a thickness of two cell layers. Elaboration of predentin was initiated as the sheath was reduced from a double to a single layer of cells. When the predentin layer was 3 to 5 microns thick an

amorphous investing substance obscured the fibrillar structure. As the dentin increased in thickness, a 3 to 5 microns wide zone of predentin was always found on the pulp side. Except for the initial stage of dentin formation, the deposition of mineral crystals and amorphous ground substance seemed to occur simultaneously.

When the first calcification occurred in the dentin, the cells of the epithelial sheath became separated, and scattered collagen fibrils from the periodontal membrane were found close to the peripheral dentin surface. The formation of cementum was initiated by the deposition of crystals around these fibrils simultaneously with the appearance of an amorphous ground substance. An intimate relationship between collagen and initial crystal deposition, similar to that found in bone and dentin, thus seems to exist in the cementum. The crystals of the cementum were 300 to 400 Å in length. They were generally smaller than those of the dentin. The cementum, which was entirely acellular, reached a thickness of a few microns near the incisal end of the tooth.

The periodontal support of the incisor in the 8-day old animal was characterized by loosely arranged collagen fibrils. Although these fibrils formed bundles in the periodontal membrane, no Sharpey's fibers were seen in the cementum.

# **RÉSUMÉ ET CONCLUSIONS**

# ÉTUDE AU MICROSCOPE ÉLECTRONIQUE DU MANCHON ÉPITHÉLIAL DE HERTWIG ET DU DÉBUT DE LA FORMATION DE LA DENTINE ET DU CÉMENT DANS L'INCISIVE DE LA SOURIS

Une étude au microscope électronique du manchon épithélial de Hertwig dans l'incisive à croissance continue de la souris de 8 jours a montré que le manchon épithélial se compose de plusieurs couches de cellules près de son extrémité postérieure. En faisant des coupes à de plus grandes distances de l'extrémité en voie de développement de la dent, on a constaté une réduction graduelle de l'épaisseur du manchon épithélial. La différenciation des odontoblastes à partir des cellules de la papille dentaire s'est produite lorsque le manchon épithélial avait atteint l'épaisseur de deux couches de cellules. L'élaboration de la prédentine débutait quand l'épaisseur du manchon passait d'une double couche à une couche unique de cellules. Lorsque la couche de prédentine avait une épaisseur de 3 à 5 microns, une substance amorphe d'inclusion obscurcissait la structure fibrillaire. Tandis que l'épaisseur de la dentine augmentait, on constatait toujours la présence d'une couche de prédentine de 3 à 5 microns de largeur du côté de la pulpe. Sauf au stade initial de la formation dentinaire, la formation de dépôts de cristaux minéraux et de substance fondamentale amorphe a semblé se produire simultanément.

Lorsque le début de la calcification se produisait dans la dentine, les cellules du manchon épithélial se séparaient, et des fibrilles collagènes du périodonte, dispersées, ont été trouvées près de la surface périphérique de la dentine. La formation du cément débutait par la précipitation de cristaux autour de ces fibrilles, en même temps qu'il apparaissait une substance fondamentale amorphe. Une relation intime entre le collagène et la précipitation initiale de cristaux, relation semblable à celle trouvée dans l'os et dans la dentine, semble donc exister dans le cément. Les cristaux du cément avaient une longueur de 300 à 400 Å. Ils étaient en général plus petits que ceux de la dentine. Le cément, entièrement acellulaire, atteignait l'épaisseur de quelques microns près de l'extrémité incisive de la dent.

Le support périodontal de l'incisive chez l'animal àgé de 8 jours était caractérisé par des fibrilles collagènes formant une structure lâche. Bien que ces fibrilles aient formé des faisceaux dans le périodonte, on n'a pu voir aucune fibre de Sharpey dans le cément.

## ZUSAMMENFASSUNG UND KONKLUSIONEN

### ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN VON DER HERT-WIGSCHEN EPITHELSCHEIDE UND VON DER FRÜHEN BILDUNG VON DENTIN UND ZEMENT IM FRONTZAHN DER MAUS

Eine elektronenmikroskopische Untersuchung von der Hertwigschen Epithelscheide des ununterbrocken wachsenden Unterkieferfrontzahns der acht Tage alten Maus zeigte, dass der posteriore Teil der Epithelscheide aus mehreren Schichten von Zellen besteht. Schnitte in steigenden Abständen von dem entwick-

lenden Teil des Zahnes zeigten eine allmähliche Abnahme der Stärke der Epithelscheide. Die Bildung von Odontoblasten aus Zellen der Zahnpapille entstand, als die Epithelscheide eine Stärke von zwei Zellschichten erreicht hatte. Als die Epithelscheide von zwei in eine Zellschicht reduziert wurde, fing die Bildung von Praedentin an. Wenn die Praedentinschicht eine Stärke von  $3-5 \mu$  hatte, verschleierte eine amorphe Substanz das Fasergefüge. Als die Dentinstärke zunahm, wurde immer eine  $3-5 \mu$ breite Zone aus Praedentin auf der Pulpaseite gefunden. Abgeschen von dem Anfangsstadium der Dentinbildung scheinen die Ablagerungen von Mineralkristallen und amorpher Grundsubstanz gleichzeitig einzutreten.

Als die erste Verkalkung in dem Dentin entstand, teilten sich die Zellen der Epithelscheide, und vereinzelte Kollagenfasern von der Wurzelhaut wurden in der Nähe der Oberfläche des peripheren Dentins gefunden. Die Bildung des Zementes wurde angefangen durch die Ablagerung von Kristallen um diese Fasern herum gleichzeitig mit der Erscheinung einer amorphen Grundsubstanz.

Ein enges Verhältnis zwischen Kollagen und beginnender Kristallablagerung, dem in Knochengewebe und Dentin gefundenen entsprechend, scheint also auch im Zement zu bestehen. Die Länge der Zementkristalle beträgt 300-400 Å. Sie sind im allgemeinen kleiner als die des Dentins. Das Zement, das völlig azellulär war, erreichte eine Stärke von einigen Mikra in der Nähe des inzisalen Teiles des Zahnes.

Die parodontale Stütze des Frontzahnes eines acht Tage alten Tieres wurde durch locker orientierte Kollagenfasern charakterisiert. Obwohl diese Fasern in der Wurzelhaut Bündel bildeten, wurden keine Sharpeyschen Fasern in dem Zement gefunden.

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Figure 1.

Diagrammatic representation of a sagittal section through the apical end of a mandibular mouse incisor. The box represents the area seen in Figure 2.

## Figure 2.

Longitudinal section of the posterior end of Hertwig's epithelial sheath. The epithelial sheath (E) consists of several layers of cells at its developing end (left). The cells of the dental papilla (P) are densely packed. The dental sac (S) contains long, slender cells arranged parallel to the epithelial sheath. Phase contrast. x 380.

# Figure 3.

Inner separating membrane. Non-striated fibrils (NF) line the separating membrane (SM) on the pulp side. Some collagen fibrils (F) in the primitive pulp have been cut in cross section. The cell membranes (CM) of the epithelial cells run parallel to the separating membrane. x 22,000.

# Figure 4.

Outer separating membrane. Fine bundles of collagen fibrils (F) are located in the dental sac. SM – separating membrane, NF – non-striated fibrils, CM – cell membranes of the epithelial cells, M – mitochondria. x 17,500.



# Figure 5.

Cells of the dental papilla. The cells are densely packed. They contain large nuclei (N), and a few mitochondria (M) can be identified in the cytoplasm. Collagen fibrils are present in the intercellular spaces. The separating membrane (SM) follows a wavy course. x 4,000.

### Figure 6.

Cross section of the epithelial sheath near its posterior end. The epithelial sheath is delimited toward the dental sac (top) and the dental papilla (bottom) by separating membranes (SM). Two basal layers of cuboidal epithelial cells, separated by several layers of squamous cells can be identified. x 3,400.



# Figure 7.

Cells of the epithelial sheath. The cells are connected by interlocking fingerlike projections (FP). Mitochondria (M), Golgi lamellae (L), and granules of varying sizes are located in the cytoplasm. N-nucleus, CM-cell membrane, SM-separating membrane, I-intercellular space. x 22,000.

# Figure 8.

Epithelial sheath consisting of a double layer of cells. The outer layer of basal epithelial cells (top) have diminished in size, while the cells facing the dental papilla (bottom) still form a low columnar arrangement. SM-separating membranes. x 3,400.



# Figure 9.

Disintegration of the epithelial sheath. Collagen fibrils (F) from the primitive periodontal membrane are observed near the lateral dentin surface. Some collagen fibrils are also located between the remaining epithelial cells (E) and the dentin (D). Electron dense granules are present in the cytoplasm of the epithelial cells. The arrows indicate desmosomes. FB-fibroblast. x 9,700.

## Figure 10.

The epithelial cells (E) have become separated from the dentin by a 2-micron wide space. Fibroblasts (FB) containing a considerable amount of endoplasmic reticulum and several mitochondria are now found near the tooth surface. Note the absence of collagen fibrils in the space between the two epithelial cells. x 12,000.



# Figure 11.

Collagen fibrils between the odontoblasts. These fibrils (F), approximately 1000 Å in diameter, are oriented parallel to the long axis of the cells. Finer collagen fibrils cut transversely are seen near the separating membrane (SM). x12,000.

### Figure 12.

Numerous collagen fibrils have accumulated near the separating membrane (SM) and between the receding odontoblasts (O). x 5,900.



### Figure 13.

The fibrillar structure of the predentin has been obscured by an amorphous investing substance. The epithelial sheath consists of a single layer of squamous cells. A few non-striated fibrils are located in the narrow space between the epithelial cells and the dentin matrix together with a fine line, which possibly represents remnants of the separating membrane (arrow). x12,000.

## Figure 14.

A 5 to 6 micron wide dentin layer with a 3 micron wide zone of predentin (PD) on the pulp side. Note the abrupt transition between the fibrillar predentin and the mineralized dentin. x 9,700.

## Figure 15.

Area similar to that in Figure 14 from adjacent section, which was decalcified on the grid with phosphotungstic acid. The presence of an investing substance in the dentin matrix is now evident. x 9,700.



# Figure 16.

Fibroblasts of the dental sac. The cells contain in their cytoplasm several mitochondria (M), Golgi lamellae (L), Golgi vacuoles (V), and endoplasmic reticulum (ER). Collagen fibrils are found in the intercellular spaces. N-nucleus. x12,000.

### Figure 17.

Periodontal membrane at the level of the first cementum formation. The collagen fibrils (F) have formed bundles which are oriented parallel with the fibroblasts. x 5,800.



# Figure 18.

The initial layer of cementum matrix (C), found in apposition to the dentin (D), contains a few collagen fibrils embedded in an amorphous ground substance. Collagen fibrils and a bundle of non-striated fibrils (arrow) are located between the cementoblasts (CB). Decalcified section. x 12,000.

# Figure 19.

Higher power of area similar to that of Figure 18, shows the association of collagen fibrils to the cementum matrix. The fibrils are of uniform diameter, 250 to 300 Å, and exhibit the characteristic cross banding of collagen. Decalcified section.  $\mathbf{x}$  60,000.



# Figure 20.

Near the incisal end of the tooth the cementum (C) has attained a width of 2 microns, while the underlying dentin (D) at this level has a thickness of 30 microns or more. The cementodentinal junction is not clearly delineated. CB- cementoblast. x 30,000.

