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What is This?
Preliminary Report on the Mineralization of Human Dentin

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In spite of earlier extensive studies with the electron microscope, very little information has been gained concerning the inorganic phase of human dentin. A main reason for this lack of data is thought to be difficulty in specimen preparation. While more or less clear outlines of the crystalline structures in enamel have been brought out by replica technics,1 the methods have not been applied with equal success to the mineral components in dentin. Furthermore, it has not been possible to make useful thin sections of fully calcified dentin with the glass or steel microtome knives which have been available. Some of the difficulties have been circumvented through the use of softer developing dentin,2,3 but the full course of mineralization has not yet been thoroughly explored.

One of the important recent advances in ultra-sectioning technics has been the introduction of the diamond knife.4-7 Thin sections of undemineralized, fully calcified tissue, even enamel, can now be cut. This is making possible more extensive and systematic studies of mineralization. The present paper is a preliminary report on the mineral component of developing and mature human dentin.

MATERIALS AND METHODS

The majority of the specimens were partly mineralized deciduous tooth germs dissected from formalin-fixed human fetuses. The ages of the fetuses ranged from 3 to 4½ months. Some dentin from formalin-fixed, non-carious, fully calcified permanent molars was also used. Additional samples were obtained from unfixed, non-carious, fully calcified third molars rendered anorganic by ethylenediamine treatment.†

The samples were dehydrated and imbedded in an 8:2 mixture of butyl and methyl methacrylates, following the usual procedures. A microtome‡ equipped with a diamond knife was used for sectioning. Sections were picked up on specimen screens previously covered with carbon substrates and were subjected to electron microscopy and diffraction without removal of the imbedding medium. In some instances the sections were decalcified with 0.1 N HCl for a few seconds on the specimen screens.

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† Some of the anorganic dentin was provided by Dr. F. L. Lesee, National Naval Medical Center, Bethesda, Maryland (see Refs. 8–10).
‡ Porter-Blum.
RESULTS

Developing dentin.—In the organic matrix near the basal end of the developing tooth germ, various small areas can be seen which are infiltrated with dot- or needle-shaped particles (Fig. 1, A). It is interesting to note the occasional accumulation of particles at the periphery of the odontoblastic processes (Fig. 1, B), which thus become encircled by a narrow, dense layer.

The areas infiltrated by the dense particles increase in size and fuse (Fig. 1, C). Around the odontoblastic processes the layer is gradually widened, and its density increases because of the additional accumulation of particles (Fig. 1, D). The light round spots in Figure 1, B and D, are probably cross-cut matrix fibrils which have been outlined as the result of deposition of particles in the interfibrillar areas. Inside these cross-cut fibrils and scattered in various areas a few hazy granules can be seen.

The particles mentioned above are so small that accurate description of their dimensions is difficult. The needles, however, seem to reach 200–800 Å in length, and the granules are roughly 100 Å or less in diameter. When areas showing these particles are subjected to diffraction, the presence of apatite can be detected. Further, the particles disappear when the section is decalcified on the specimen screen, and diffraction patterns can no longer be obtained. It is thus evident that the particulate matter contains apatite, but it is not yet certain whether or not individual particles are single crystals.

Occlusal to the area from which the previous findings were made, dentinogenesis had progressed to a point where distinct layers of calcified dentin and predentin could be distinguished. The junction between the two is quite abrupt (Fig. 2, A). Further deposition of mineral seems to take place appositionally from this dentino-predental junction.

Under higher magnification it can be seen that the mineral particles at the junction are identical with those seen in Figure 1, A (Fig. 2, B). There are more or less definite indications that the needles accumulate into dense aggregates through side-by-side alignment. Between these aggregates, granular particles similar to those shown in Figure 1, D, can be seen.

One of the conspicuous findings in the dentin is the regular deposition of crystals on the matrix fibrils. Consequently, the fibrils become characterized by alternately arranged dark and light cross-bands (Fig. 2, C). Closer observation gives an impression that the dark bands are composed of rows of particles in the shape of platelets. The fibrils seem to be mineralized to a lesser degree than the interfibrillar regions.

In the dentin the areas immediately surrounding the odontoblastic processes (so-called peritubular areas) are easily distinguished from the rest of the intertubular substance by their more compact and homogeneous texture (Fig. 2, D).

Mature dentin.—The findings obtained from untreated mature dentin are quite similar in many respects to those from the developing dentin. There are, however, some significant differences between the two.

The peritubular area, especially, shows an extremely dense structure which is almost impossible to resolve (Fig. 3, A). The boundary between the peritubular and intertubular areas becomes very sharp, and the former appears as a ring-shaped structure. The bands on the matrix fibrils are seen in the intertubular area (Fig. 3, A and
FIG. 1.—Early stages of dentin mineralization near basal end of deciduous tooth germ. A: Cluster of needle- and dot-shaped particles. (Mag. ×45,000.) B: Accumulation of particles at periphery of tubule. (Mag. ×22,000.) C: Confluence of mineralized areas. (Mag. ×22,000.) D: Increase in peritubular mineralization. (Mag. ×45,000.)
Fig. 2.—Intermediate stage of mineralization in mid-region of developing deciduous tooth crown. 
A: Border between predentin (left) and dentin (right). (Mag. ×12,000.) B: Higher magnification of dentin close to predentin border. (Mag. ×45,000.) C: Mineral deposition on collagen fibrils. (Mag. ×22,000.) D: Persistence of density difference between peritubular (P) and intertubular (IM) areas. (Mag. ×45,000.)
In anorganic dentin the intertubular areas are filled with dense spindle-shaped or tabular objects (Fig. 4, A) which are considerably larger than the crystalline particles observed in developing dentin. Thus the spindle-shaped objects range from 330 to 2,200 A in length, and from 50 to 220 A in width, while the tabular objects vary in diameter from 110 to 300 A (Fig. 4, B). Although the spindle-shaped objects are very dense, there are indications that they may be composed of much smaller elongate units with fine cross-striations (Fig. 4 C). The impression is given by comparing Figure 4, B, with Figure 3, B, C, and D, that the above-mentioned tabular objects are related mainly to the matrix fibrils, and the spindle-shaped objects to the interfibrillar area.

The peritubular area appears as a highly mineralized region composed of closely packed, fine, round particles (Fig. 4, D), varying from 80 to 150 A in diameter.

**DISCUSSION**

During the present studies, great variation was noticed in the shapes and sizes of the mineral particles in dentin. The fine particles found in developing dentin may vary very well be single crystals, since their dimensions are the same as those obtained previously for crystals in dentin, bone, and cartilage by electron microscopy and X-ray diffraction. The larger mineral particles observed in mature dentin may result either from aggregation of the smaller crystals seen in developing dentin or from an increase in crystal size with advancing age, as pointed out for bone by Robinson and Watson. Furthermore, it is possible that the sizes and shapes of the inorganic components are related to the submicroscopic configuration of the organic matrix, as suggested by Nylen, Scott, and Mosley.

Besides the size of the mineral particles, other differences between developing and mature dentin were noticed. In the former the border between the highly mineralized peritubular area and the less mineralized intertubular area is usually transitional. In mature dentin, however, the border is quite abrupt, and the peritubular area appears as a well-defined ring-shaped structure of much higher density. The periodic structure on the matrix fibrils which can be seen in developing dentin is obscured in many places in mature dentin, probably because of additional mineral deposition.

On the basis of these findings, it could be said that calcification of dentin, at least in the early stages, proceeds through progressive deposition of mineral in the dentin proper, as well as through apposition at the dentino-predentinal junction. Such a continuous mineralization has also been suggested in recent radioautographic studies.

Opinions concerning the actual portion of the calcified tissues occupied by the inorganic components have been somewhat controversial, and the matter is still unsettled, even after electron-microscopic observations. Some researchers claim that the mineral in bone is localized primarily in the interfibrillar area and that the matrix fibrils are retained in an almost fully or completely unmineralized state. Contrary to this, other investigators are of the opinion that the collagen fibrils are themselves the main bearers of the bone crystals. Watson and Avery concluded that the initial mineralization in dentin seems to take place within the matrix fibrils and later extends to fill the interfibrillar region. It is evident from the present work that mineralization
Fig. 3.—Untreated, fully mineralized dentin from erupted, permanent tooth. A: Peritubular area (P) appearing extremely dense and sharply demarcated from surrounding intertubular matrix (IM). (Mag. ×24,000.) B: Enhanced cross-banding of matrix fibrils due to initial mineral deposition. (Mag. ×38,000.) C: Partial obliteration of striation pattern following additional mineral deposition. (Mag. ×38,000.)
Fig. 4.—Fully mineralized dentin from which the organic components were removed through ethylene diamine treatment. A: Difference in amount of mineral present in peritubular (P) and intertubular (IM) areas. (Mag. ×30,000.) B: Spindle-shaped and tabular objects in intertubular region. (Mag. ×45,000.) C: Higher magnification of area similar to B. Mineral objects appear to be composed of smaller units. (Mag. ×100,000.) D: Fine particles observed in peritubular area at high magnification. (Mag. ×100,000.)
can occur not only in the interfibrillar area but also within the matrix fibril itself. The location in which crystals first appear, however, was not determined.

As has been pointed out,11-16, 20, 24, 25 the fact that the minerals are deposited regularly on the collagen fibrils in a striated pattern would suggest that they are closely related to the original periodic structure in the matrix fibrils.

The existence and structure of the highly mineralized band (peritubular matrix) around the dentinal tubules have been described in previous replica studies.26 Although information on its development is still scanty, the present studies seem to indicate that this structure has its origin during the early stages of dentin mineralization. Further studies have to be done to determine whether or not the mineralization of the peritubular matrix is preceded by changes in the organic matrix, as reported by Bradford.27

**SUMMARY**

Undecalcified ultra-thin sections of the developing and fully calcified human dentin were observed under the electron microscope. Preliminary findings on the mineralization processes in the developing dentin were presented. Some differences between the mineralization in immature and mature dentin were also suggested.

**REFERENCES**