Electron Microscopic Observations on the Three-Dimensional Morphology of Apatite Crystallites of Human Dentine and Bone*, ‡

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ABSTRACT

In sections of human dentine (carious and sound) and bone examined with the electron microscope, apatite crystallites were seen to present long thin profiles somewhat suggestive of a cylindrical shape, broad profiles indicative of a plate-like shape, and profiles intermediate between these two extremes. With a special stereoscopic specimen holder allowing the specimen to be tilted through an angle of 30° it was possible to record images of two profiles of the same crystallite from different angles and thus gain information concerning the 3-dimensional morphology of crystallites showing a thin profile. In all fields so examined, the thin-profile crystallites that were properly oriented with respect to the axis of tilt exhibited a different width dimension in each of the two micrographs. From this it is concluded that the thin profiles actually represented edge views of plate-like crystallites.

INTRODUCTION

Two main concepts concerning the morphology of apatite crystallites of mineralized tissues have been proposed during the past several years. The more widely accepted view, namely that apatite crystallites are rod- or needle-shaped structures, is based on conclusions drawn from electron microscopic (1–3) and x-ray diffraction studies of bone (1, 4, 5). The opposing view, based on electron microscopic observations alone on bone (6, 7) and dentine (8), is that the crystallites are plate-like structures. A third point of view, concerning the mineral phase of bone, is that it takes the form of a continuous phase in which ellipsoidal masses of organic material are embedded (9, 10).

The observations presented here agree with the view that crystallites are plates. Both broad and thin crystallite profiles were seen in our sections, from which it was immediately obvious that many crystallites were plate-like structures. Further, it was seen with the aid of a stereoscopic specimen holder that thin profiles (superficially suggestive of a needle-like shape) were actually edge views of such plates.

Materials and Methods

Specimens of sound coronal dentine from non-carious erupted permanent teeth, of soft carious dentine from erupted permanent molars and premolars with occlusal lesions, and of alveolar bone of man were obtained from the Dental Clinic of Strong Memorial Hospital, Rochester, New York. They were fixed immediately after operative removal in cold (4°C.) 1 per cent osmic acid solution, containing sucrose (0.22 M) and buffered to pH 7.2–7.4 with 0.028 M veronal acetate buffer, in neutral 10 per cent formalin, or in 70 per cent alcohol. Some of the material was dehydrated in alcohol and embedded in butyl methacrylate catalyzed with 1 per cent benzoyl peroxide; the remainder was stored in 70 per cent alcohol and sectioned without further treatment. A few samples of fresh carious dentine were sectioned without preparative treatment.

Sections were cut with a Porter-Blum microtome equipped with a glass knife. Micrographs were taken with a Siemens Elmiskop Ia operated at 60 or 80 kv.

Evidence of the crystalline character of both the broad- and thin-profile objects that are interpreted

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here as being crystallites was obtained by making darkfield observations. This consisted in taking a micrograph of a given field, moving the objective aperture out of the optical axis of the microscope, and retaking the same field. With the objective aperture sufficiently displaced from the optical axis, only electrons scattered widely by an object can pass through the aperture and fall on the viewing screen. When crystals are present in the specimen, some may be so oriented that diffracted rays pass through the objective aperture. These crystals will appear much more strongly illuminated than non-crystalline objects or other crystals not so oriented, whose images are produced solely by randomly scattered electrons. Thus the ability to produce a strongly illuminated image in dark field is taken as evidence of crystalline character. Diffractograms were made to identify the apatite composition of the crystallites.

Information concerning the 3-dimensional morphology of crystallites was obtained by observing different aspects of the same crystallite. The stereoscopic specimen holder provided with the Siemens Elmiskop Ia allows a maximum tilt of the specimen through an angle of $\pm$ 5° from the horizontal. This amount of tilt was found to be inadequate to show an appreciable difference in crystallite appearance, so a special specimen holder was constructed in which the grid was held in a fixed plane, tilted 15° from the horizontal. A micrograph was taken of a given field, the grid then rotated through 180° around an axis perpendicular to, and passing through the center of, the grid, and a second micrograph made of the same field. Thus were obtained two aspects differing from each other by 30°.

OBSERVATIONS

Thin sections of uniform thickness were relatively easily obtainable from soft, carious dentine embedded in methacrylate. All fields observed of such sections contained a large number of both thin, dense and wider, less dense crystallite profiles (Fig. 3). Bone and normal dentine, being more heavily mineralized, did not section as easily or uniformly. Sections of these tissues were in most places of such a thickness that only the thin-dense crystallite profiles were visible as separate entities against the dense background; however, in the thinnest regions of these sections and especially at their edges, which tended to crumble, both types of crystallite profiles were easily distinguishable. It was generally true of all tissues studied that the thinner a section was, the more conspicuously abundant were the broad-profile crystallite images (Figs. 2, 4, and 6).

Both thin-profile and wide-profile objects were shown to be capable of forming brightly illuminated images in darkfield micrographs and were therefore interpreted as being crystalline (Fig. 7). Also, both gave apatite-like diffraction patterns.

The images of many thin-profile crystallites were not of uniform width throughout their length, and there was considerable variation in width among different thin-profile crystallites. It was therefore natural to suspect that the thin profiles were more probably edge views of plate-like structures than side views of rods or cylinders. This possibility was investigated with the aid of a stereoscopic specimen holder and it was found that thin-profile crystallites properly oriented with respect to the axis of tilt exhibited different dimensions when viewed from different aspects (Figs. 1, 3, and 5), thus revealing their plate-like structure.

In order to get some idea of the size of the crystallites studied here, the narrowest and the longest profile dimensions were measured in a number of micrographs taken from each of the three types of specimen used. These measurements indicated that the crystallites were 20 to 40 A thick with a maximum length of 700 A in carious dentine, 20 to 35 A thick and up to 1000 A long in normal dentine, and 25 to 40 A thick with a maximum length of 1000 A in bone.

DISCUSSION

If a thin plate-like object is viewed on edge, it presents a thin profile to the viewer. If the object is then tilted through 30° around an axis that is parallel to the long dimension of the thin profile, it will present a much wider profile. Since the long profile of a cylindrical object would show no change in thickness due to tilting, it follows that if a thin-profile crystallite lying with its long dimension parallel to the axis of tilt exhibits a different width before and after tilting, it must be of plate-like, and not cylindrical, shape.

The considerations stated above form the basis of our conclusion that the thin-profile, as well as the broad-profile, crystallites seen in our specimens of bone and dentine were plate-like rather than cylindrical. These observations considered alone suggest that all crystallites of bone and dentine may be of a plate-like shape, and thus accord with the views of Watson and coworkers (6–8). However, in view of the conflicting conclusion of numerous authors based on electron microscopy (1–3) and x-ray diffraction studies

(1, 4, 5) of bone from many classes and species of vertebrates, that crystallites are rod-like bodies, we should point out the possibility that the fields studied by us might not be representative of the totality of normal bone and dentinal matrix. It will be recalled that while in sections of carious dentine the obviously plate-like (broad-profile) crystallites were homogeneously distributed through the section, such crystallites were discernible only in certain preferred sites in sections of normal dentine and bone; namely, at very thin regions of sections or at the edges, where the material was often somewhat crumbled. It may be possible that these particular regions in normal dentine and bone, as well as the whole substance of carious dentinal matrix, owed their favorable sectioning characteristics in part to an unusual abundance of atypical (plate-like) crystallites, and/or the absence of rod-shaped crystallites. Fernández-Morán and Engström (1), who have seen small numbers of plate-like crystallites in their electron micrographs, suggest that such platelets may consist of lateral aggregations of rodlets, 30 to 60 A in diameter and about 200 A in length.

The possibility that fixation procedures might affect crystallite morphology should also be considered. This possibility seems unlikely, however, in view of the fact that crystallites in carious dentine that was sectioned without any previous treatment showed no morphological differences from those in the fixed and embedded material.

Regardless of what the ultimate significance of these observations may be, we believe it has been demonstrated that no valid conclusion can be drawn from a single micrograph concerning the 3-dimensional shape of a crystallite presenting a thin elongate profile. It is hoped that stereoscopic methods will be more widely used in future studies of crystallite morphology.

We can see no clear way at present of relating our observations on crystallite morphology to the view of Caglioti *et al.* (9), supported by Barbour and Cook (10), that the mineral substance of bone forms a continuous phase in which ellipsoidal masses of organic material are embedded.

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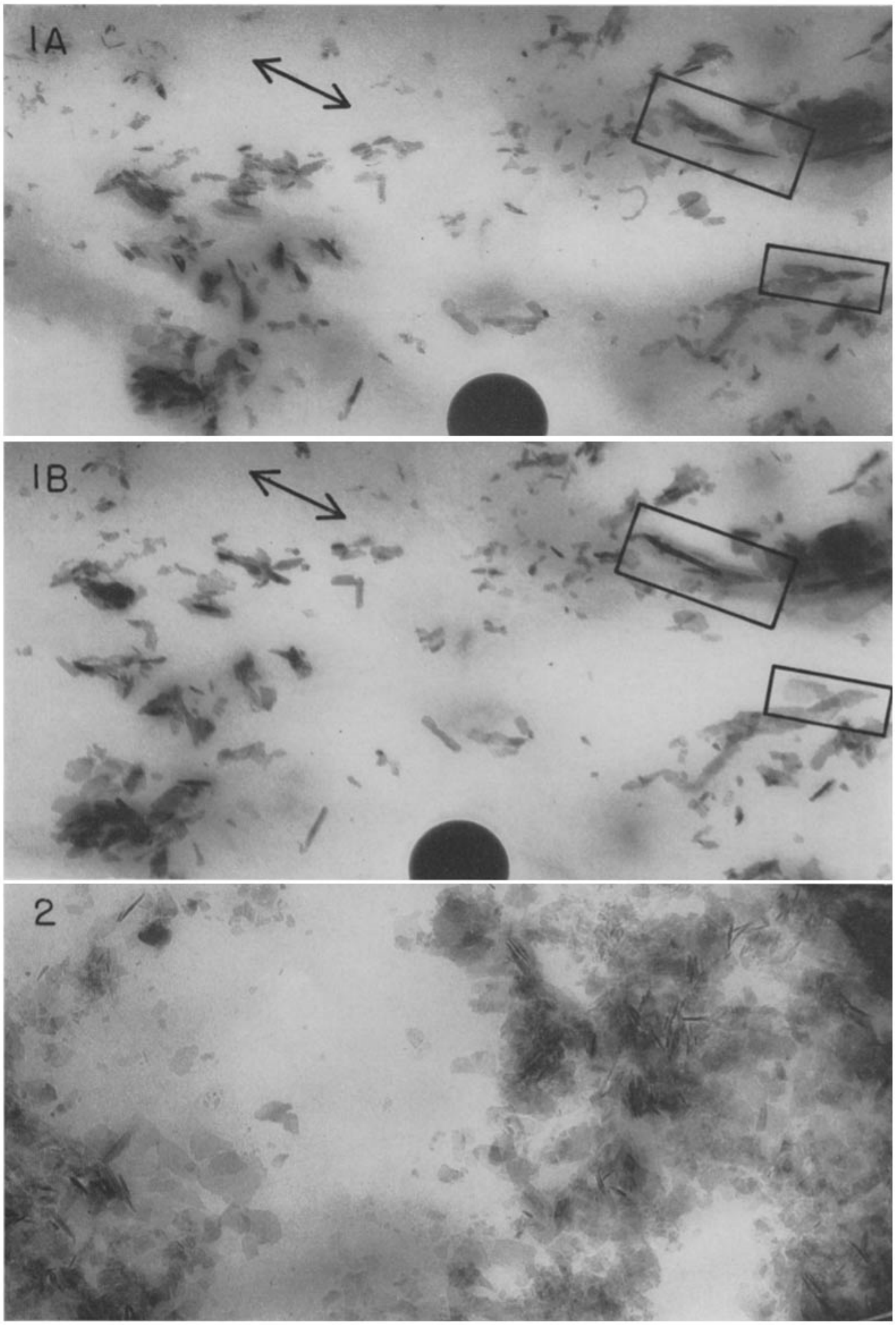
Explanation of Plates

Plate 377

Figs. 1 *A* and 1 *B*. Human alveolar bone crystallites. Two aspects of the same field were obtained by taking a micrograph before and after tilting the specimen through 30° (±15° from the horizontal). It can be seen that profiles of crystallites oriented with long axis approximately parallel to the axis of tilt (arrow) appear thin in one picture and relatively broad in the other, thus indicating the plate-like shape of the crystallites (note rectangles for specific examples). Magnification approximately 60,000.

Fig. 2. Human alveolar bone crystallites. This field shows that thin-profile crystallite images are conspicuous in thicker parts of a section and broad-profile images are more easily discernible in places where the section is thinner. Magnification approximately 96,000.

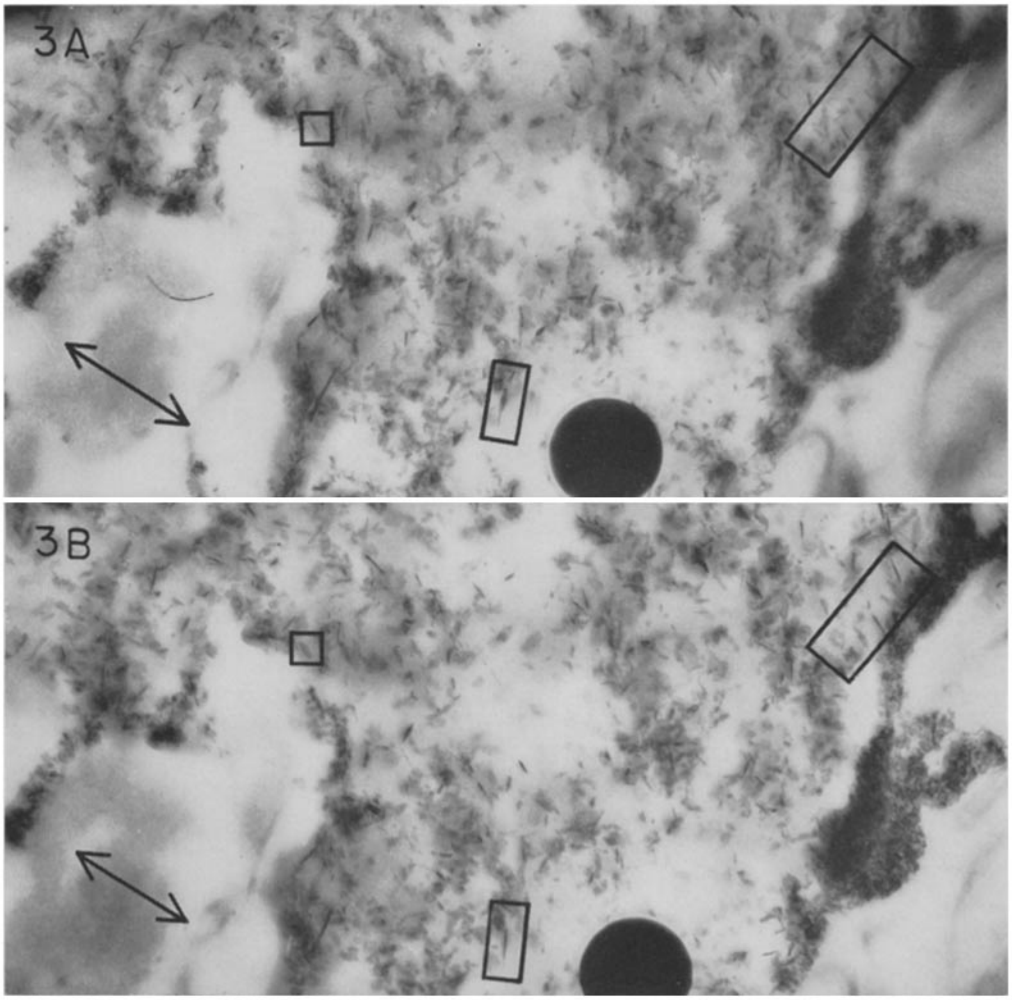


(Johansen and Parks: Three-dimensional morphology of apatite crystallites)

Plate 378

Figs. 3 *A* and 3 *B*. Carious human dentine. Two aspects of the same field were obtained by taking a micrograph before and after tilting the specimen through 30° (±15° from the horizontal). Many profiles, especially those of crystallites oriented with long dimension more or less parallel to axis of tilt (arrow), appear thin in one picture and broad in the other, thus indicating the plate-like shape of the crystallites (note rectangles for specific examples). Magnification approximately 60,000.

Fig. 4. Carious human dentine crystallites. Thin preparation was made by sectioning unembedded tissue. Where the material is spread thin or somewhat fragmented, as it is here, broad-profile crystallite images are considerably more abundant than thin-profile images. Magnification approximately 96,000.



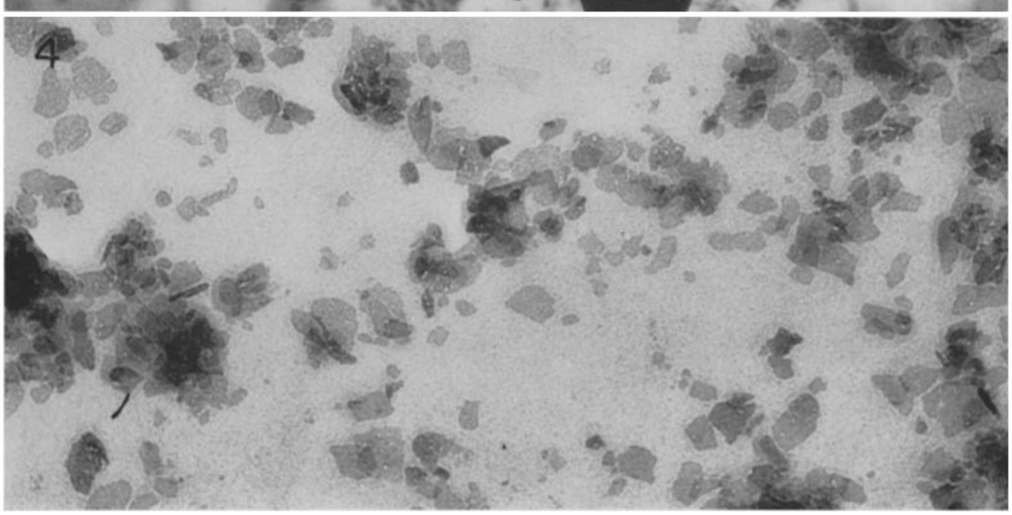


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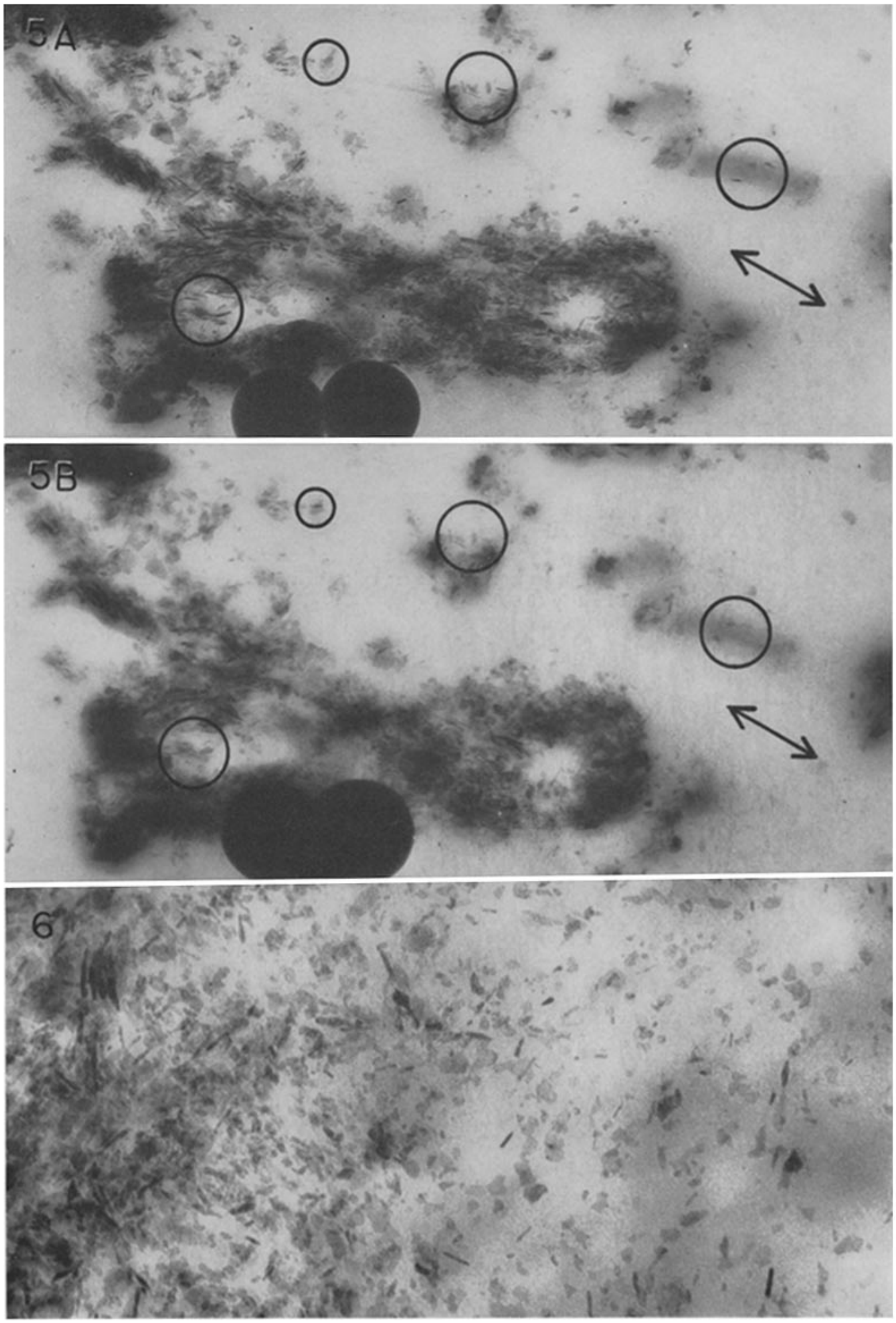
Plate 379

Figs. 5 *A* and 5 *B*. Normal human dentine. Two aspects of the same field were obtained by taking a micrograph before and after tilting the specimen through 30° (±15° from the horizontal). Profiles, of crystallites lying more or less parallel to the axis of tilt (arrow), that appear thin in one picture are appreciably broader in the other picture, thus indicating the plate-like shape of the crystallites (note circles for specific examples). Magnification approximately 60,000.

Fig. 6. Normal human dentine. This field, taken at the thin, and somewhat crumbled, edge of a section, shows that broad-profile crystallite images are conspicuously abundant in the thinnest parts of a section while only the dense, thin-profile images are readily discernible in thicker parts, as in left part of field. Magnification approximately 96,000.



(Johansen and Parks: Three-dimensional morphology of apatite crystallites)

PLATE 380

FIGS. 7 *A* and 7 *B*. Carious human dentine. These micrographs, showing darkfield and brightfield views of a single field, demonstrate that both thin-profile and broad-profile objects are capable of producing brightly illuminated images in a dark field (note rectangle for most obvious example). This characteristic is indicative of crystalline structure. Magnification approximately 60,000.

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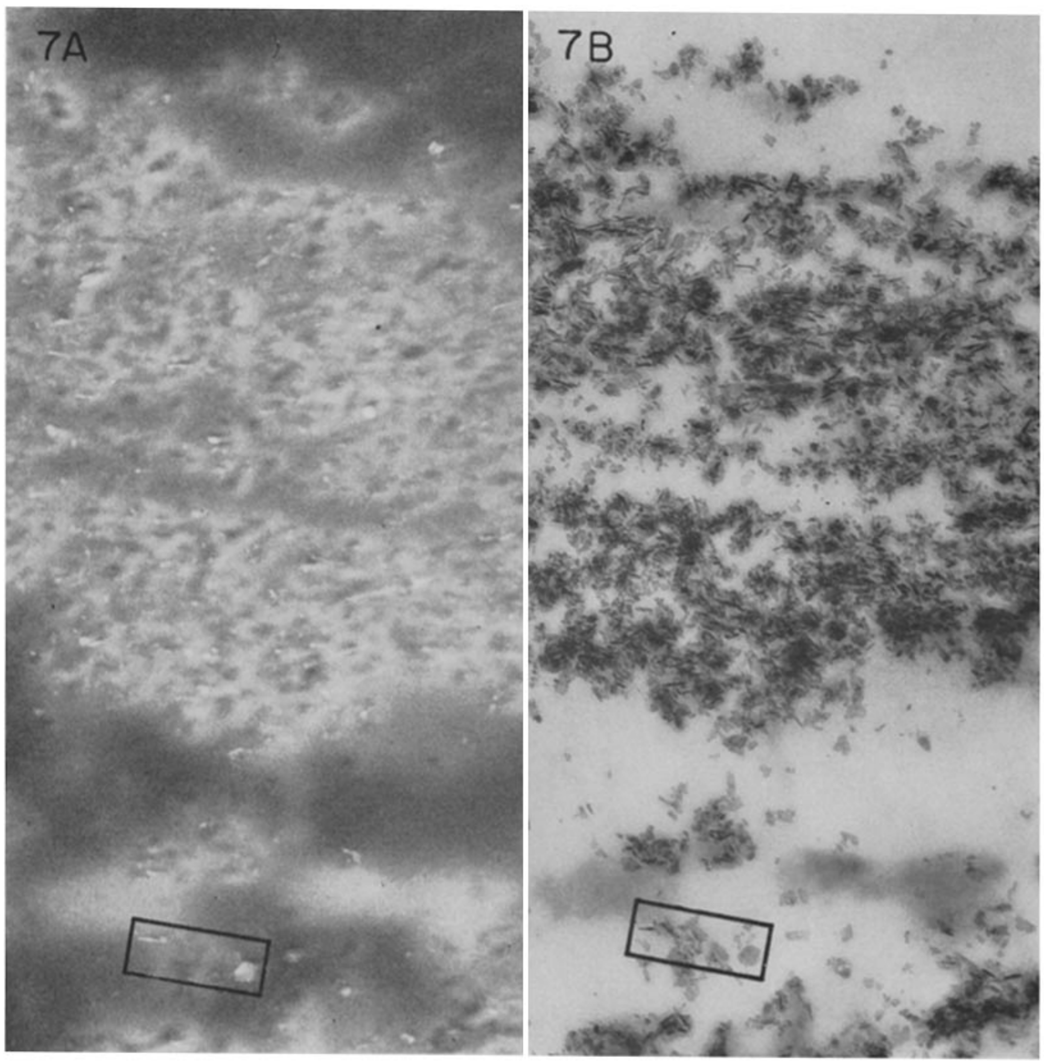

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