Texture and Composition of Bone

By combination of x-ray diffraction and microscopic methods Drew *et al.* (*1*) deduced that they found textural (microstructural) differences between bones of wild and domesticated animals. They did not claim that compositional distinctions exist and described the mineral constituent as "hydroxyapatite." This nomenclature itself is erroneous, but our comments are concerned primarily with the sampling methods used in conjunction with their powder patterns (their figure 2).

Three principal factors govern the relative intensities and the spacings of diffraction maxima: (i) orientation of the individual crystallites, (ii) their range of sizes, and (iii) their crystallochemical composition, including any defects. We shall discuss these matters in reverse order.

McConnell (*2*) and numerous later investigators found that changes in the relative intensities of the reflections (30.0) and (00.2) are related to compositional changes, but such changes probably are comparatively minor when bones of the same genus are considered. Thus, it is difficult to question their conclusions on this basis, although there may be very slight displacement toward greater or lesser angles of 2θ in their figure. The spacings (d values), as determined by the diffraction angles, particularly for prismatic reflections including (30.0), are quite sensitive with respect to compositional differences (*3*); consequently we deduce the compositional differences are probably minor.

The range in size of the crystallites, both the maximum and minimum, considerably alters the resolving power of the diffraction method. The most intense diffraction peak for bone, within the range 21° to 36° for 2θ, frequently represents a superposition of (12.1) and (11.2) both of which are intense diffraction maxima (*4*). We note that plot B (their figure 2) shows resolution of these two reflections—as indicated by a notched top—whereas the other two samples do not show such separation. Again we wonder whether this resolution, in one case but not in the other two, is related to size and orientation of the crystallites or does truly indicate compositional differences. That it is possible to obtain excellent resolution of diffraction maxima for bone samples is evident (Fig. 1) when the wavelength of the radiation is significantly reduced.

The relative intensities of ($hk.0$) versus (00.l) reflections can be altered in a most significant manner, however, depending upon textural characteristics of the sample. In a powder diffraction pattern of apatite crystals in the spinal column of a shark, for example, McConnell *et al.* (*5*) found (00.l) reflections entirely absent. We wish to demonstrate how this could occur, not in bone but in fetal dental enamel, through discussion of Fig. 2. One notes that even on a very small scale the fluxional arrangement appears to change direction about 90° within merely two dimensions. The three-dimensional orientation of crystallites within a sample may be very complex and could account for the differences found by Drew *et al.* (*1*) for their diffraction data. As a consequence, we surely agree that their samples show pronounced differences in orientation, but we seriously question the sampling method, and we cannot agree with their statement: "In these bones the intensity ratio is not reduced by layers of crystallites oriented radially." If "radially" refers to directions of elongation of the crystallites being perpendicular to the x-ray beam, the reflection (00.2) can have zero intensity.

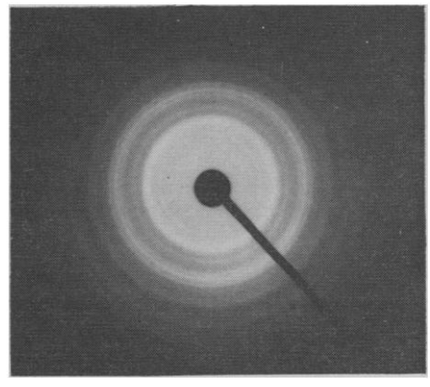

Fig. 1. Electron diffraction pattern of nondeproteinized bone, all maxima of which are attributable to dahllite (carbonate hydroxyapatite). The relative intensities have been altered by photomanipulation in order to enhance the weaker maxima at larger angles.

We also wonder whether their observations with the polarizing microscope comprise adequate sampling to justify their conclusions concerning differences in the textures of their samples. Again, a bone is a complex anatomical structure, and very small displacement in the position of a section might result in significant textural differences. We have shown (Fig. 2) that such differences exist on a very small scale for fetal dental enamel, and Drew *et al.* state that they obtained similar observations with the polarizing microscope, particularly when the gypsum plate was used to accentuate differences in interferences colors.

The birefringence of carbonate hydroxyapatite depends upon the carbonate content (*6*), but the total retardation (maximum interference) is always reduced because the individual

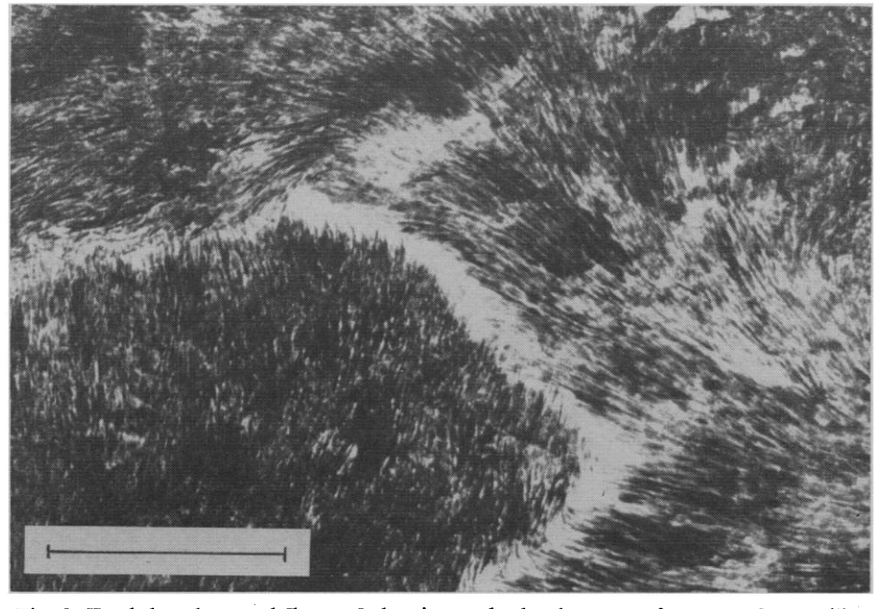

Fig. 2. Fetal dental enamel [human] showing early development of groups of crystallites in fluxional (subparallel) arrangement. There is no evidence of a so-called amorphous inorganic substance. The scale represents 1 μm.

crystallites are never perfectly aligned throughout the thickness of the section. Again, the optical effects are dependent upon both composition and texture (size and orientation).

Inasmuch as Drew *et al.* have employed two methods, both of which are related to both compositional and textural characteristics of bone, we cannot agree that their results are conclusive. Had chemical analyses shown the differences were not compositional, the textural differences could be accepted as valid provided the number of samples were adequate. However, when considering textures in which the orientation of crystallites can change within a distance of 1 or 2 μm (Fig. 2), the problem becomes a very knotty one.

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The purpose of our study was to present a new technique whereby archeologists and anthropologists could distinguish between the bones of domesticated animals and their wild counterparts. Detailed studies of the crystal chemistry of bone were not attempted. Therefore, we cannot agree that our characterization of bone mineral as "essentially hydroxyapatite" is erroneous. It is acceptable nomenclature mineralogically, it seeks to avoid current controversy as to the exact crystallo-chemical nature of bone, and it is familiar to our primary audience of archeologists, anthropologists, and zoologists through the fluorine dating method for bones, which depends on the change from hydroxy- to fluorapatite (*1*). McConnell appears to agree reluctantly with our original conclusion that the differences we observed are caused by differences in degree of orientation of crystallites.

Our research started out as a trace element study. We were unable to find any consistent chemical differences between the bones of wild and domesticated animals (*2*). Although major compositional differences seem unlikely, it would appear probable that nutritional deficiencies would be reflected in changes in trace element content. However, we have not yet discovered a satisfactory method for removing the calcareous minerals, which have thoroughly infiltrated most of the lacunae of the bones during 8000 years of burial, without altering the bone mineral.

The first series of x-ray powder patterns, represented by our figure 2A, were obtained in a second attempt to differentiate between wild and domestic animal bone. Tabulations of *d*-spacings and relative intensities for 50 random samples were remarkable only for their great similarity. The *d*-spacing for (300), which as McConnell points out is quite sensitive to variations in composition, varied only within experimental error, and no significant variations in the relative intensities of any of the reflections were noted, as stated in our report. We should like to caution against placing too much reliance on our figure 2. Intended only as an illustration and not as a research tool, this figure was mechanically reduced from x-ray diffractometer tracings chosen at random from among the tests made with the three groups of samples. The diffractometer settings were selected as giving the strongest reflections; however, the background noise was also increased so that the notched top McConnell observed in figure 2B may actually be a resolution of (211) and (112) or it may be merely "grass." Such notching appeared in about one-third of the patterns irrespective of whether they were powder samples or slices. When a slice of bone showing preferred orientation, as in our figure 2B or 2C, was pulverized and prepared as a powder specimen, we obtained an unoriented pattern, as in figure 2A.

McConnell seems to believe that faulty sampling techniques are responsible for our observations. We are confident of the reliability of our sampling. The 39 bones selected for thin sectioning and oriented x-ray diffractometer studies were chosen at random from much larger groups of bones which were (i) identifiable as to species, (ii) weight-bearing, and (iii) of known cultural status, that is, from wild or domestic animals. Every bone from all three species investigated showed the diagnostic criteria for the two groups as stated. In subsequent tests, bones of wild goats and domesticated sheep from a fourth archeological site (Ganj-Dareh in Iran) and a bone from a modern sheep, obtained from a local butcher, have yielded the same results. Since there appears to be no overlap in characteristics of the two groups representing wild and domestic animals, as measured by our techniques, it seems highly improbable that the consistent differences we reported could be produced by random sampling.

We believe that McConnell's chief difficulty with our work is one of scale. His figure 2 (fetal dental enamel) shows crystallites in subparallel arrangement in small randomly oriented groups which appear to average about 1 or 2 μm in size, or close to the optimum crystallite size for x-ray diffractometer powder samples. "If the crystallite size is small and the orientation highly random, a specimen of the proper dimensions is actually an excellent Debye-Scherrer 'powder sample' " (*3*). In other words, our techniques would provide unoriented diffractometer patterns of this fetal dental enamel, regardless of whether the sample were powdered or sliced.

The orientation effects we noted are on a much larger scale. The oriented layer of crystallites on the articulation surfaces of domestic animal bones averages 1 to 2 mm in thickness, or 1000 times larger than McConnell's oriented bundles of crystallites (*4*). The quotation from our report in McConnell's fifth paragraph refers again to the large-scale structure of the bone shafts. We found that in long bone shafts from domesticated animals the crystallites appear to be aligned radially [with respect to (002) planes] in the concentric Haversian lamellae, whereas the orientation in the interstitial lamellae is parallel to the length of the shaft. In contrast, in wild animal bones the orientation in the Haversian systems is more random.

What we have said earlier about the adequacy of our sampling holds equally for our optical work. Although we tried to section each type of bone in approximately the same plane for comparison purposes, difficulties encountered in producing petrographic slides from the friable bones caused unavoidable variations in the thin-section orientation. Since the submission of our original report, a humerus and several astragali have been sectioned along

planes perpendicular, and at various intermediate angles, to the original planes. Exactly the same results were obtained from these thin sections.

The primary purpose of a gypsum plate in optical mineralogy is not merely to increase the birefringence (the quarter-wave mica plate is more commonly used for that) but to indicate elongation. The apatite minerals are optically negative. Prismatic crystals are length-fast, tabular crystals are length-slow (*5*). The "blue rim" produced on the articulation surfaces of domestic animal bones when the section is placed so that the edge of the articulation surface is perpendicular to the slow ray of the gymsum plate suggests an alignment of tabular crystals oriented with the basal planes parallel to the surface of bone-to-bone contact. The strong enhancement of (002) reflections from the articulation surfaces corroborates the optical evidence.

We hypothesize that the alignment noted is a reaction to stress in the weight-bearing bones of domestic animals which, through poor nutrition or lack of exercise or both, possess insufficient bone matter when compared with the healthier wild animals. McConnell's figure 2 seems to support this hypothesis—the lack of large-scale orientation effects reflects the lack of stress in a fetal tooth. It might be worthwhile to compare the teeth of individuals of different cultural environments with our techniques, although since we have concentrated on the effects produced in the weight-bearing bones, and especially in the articulation surfaces of such bones, we do not know whether dental enamel would reflect similar stresses.

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The Thoreau-Reynolds Ridge, a Lost and Found Phenomenon

McCutchen's D-line (*1*), formed where relative motion exists between a surface film and the underlying liquid, was described as early as 1854 by Henry David Thoreau (*2*). The D-line is an abrupt change in surface curvature near the top of a small ridge raised by viscous shear stress at the edge of the film; it can be observed when a layer of oil spreads across a water surface, or where liquid flows under the edge of a raft of surface contaminants. It was discussed in the scientific literature first by Osborne Reynolds in 1881 (*3*), and later by other authors (*4–8*). So many times was it rediscovered that in 1936 *Nature* published a brief historical summary and commented, "When the rising generation of physicists see the Reynolds ridge, they should recognize it at once as an old friend" (*9*).

This expectation has been disappointed. The textbook (*5*) cited in the summary in *Nature* is now almost unobtainable, and modern texts do not mention the subject. Consequently the rediscoveries have continued (*1, 10*), the most recent one by McCutchen, who unwittingly ignored the earlier work.

It should not be ignored. Thoreau (*2*) understood, broadly, what happened at a D-line and had an inkling of its physical mechanism. His descriptions of the phenomenon as it occurs in nature remain some of the best available.

Reynolds' discussion (*3*) is longer, an unhurried essay that has a gentle Victorian charm. His efforts to understand the mechanism were only partly successful, because he needed a hydrodynamic concept that had not yet been thought of. Reynolds could not understand how the fluid at the surface could flow along at undiminished speed until, abruptly at the D-line, it almost stopped. He thought that viscosity ought to make the stopping occur more gradually. Missing was the idea of the boundary layer: that the direct effects of viscosity are confined to a thin layer of liquid immediately under the contaminant film, and extend only a minute distance upstream of the film's leading edge. Without the idea of the boundary layer Reynolds was forced into obscure speculations about surface tension to explain the narrowness of the D-line.

At least two of the later authors (*5, 7*) knew that the surface film dragged with it only a thin layer of the liquid beneath. But they seem to have learned about the boundary layer only from their experiments: they neither mentioned it by name, nor used the results of boundary layer theory. Like Reynolds, they needed to know that the viscous shear stress on the film tends to infinity at its leading edge. It is this stress peak that accounts for the sharpness of the D-line.

Perhaps the explanation is now complete enough so that the phenomenon will be remembered, and the earlier accounts will receive the attention they deserve as science and as good reading.

A different but closely related phenomenon has the appearance of an ascending Reynolds ridge (*4, 6, 11*): a contaminant film spreading over water will climb a vertical wetted surface, such as the wall of the container. The ascending edge produces what looks like a ripple, but interferometric measurements have shown this to be a round-cornered step rather than a true ridge. Except in contrived cases the water layer is very thin, both it and the film move very slowly, and the viscous forces are dominant everywhere. Reynolds' objection to an abrupt change in the speed of the surface now holds, and there is no D-line.

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