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THE NATURE OF BONE AND PHOSPHATE ROCK

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Carbonate has never been adequately explained as a constituent of bone, dentine, enamel, and the closely related phosphorites. Its failure to act quite like $CaCO_3$, upon heating for instance, has, since the time of Hoppe-Seyler[1] led to postulated calcium carbonate phosphate compounds as essential constituents of bone or enamel. Existence of optically homogenous apatites such as the minerals francolite[2] and collophane containing carbonate apparently supports this conclusion and has led to the acceptance of an apatite structure containing carbonate.

Preferential solution by acid of carbonate in bone has often been noted[3–5] and has been interpreted by some workers[4] as evidence for the presence of $CaCO_3$ as a separate phase. Crystalline $CaCO_3$, however, has not been observed in bone by the very sensitive test of its high birefringence. A Geiger-counter x-ray spectrometer, moreover, gives no evidence of its presence, while one-third as much calcite as would correspond to the analysis can be detected when mixed with hydroxylapatite, or naphtha-extracted bone. More incontrovertible evidence is afforded by the work of Logan and Taylor[5] which shows that an apatite phase will precipitate, from a solution having the inorganic composition of plasma, in the presence of carbonate and steadily withdraw CO_3^{--} from a solution unsaturated with respect to $CaCO_3$.

Bone, enamel, dentine and collophane, the last being the apparently isotropic component of phosphate rocks, give rather diffuse x-ray powder diffraction patterns identical, within their definition, with that of fluorapatite.[6] Francolite, the more highly birefringent material of phosphorites, can be obtained as optically clear single crystals containing more than 3% CO_2.[2] X-ray goniometer photographs of francolite crystals are identical with those of fluorapatite, even in very high orders of interference maxima. From the structural point of view it seemed necessary for a large number of CO_3 groups to be present in an apatite structure without changing the diffraction pattern, even in minor ways, which is very unlikely.

The average inorganic compositions of human bone, and enamel, based upon a great number of published analyses, can be represented by the following formulas[7] in which the number of positive ions is taken as 10.0:

Bone $[Ca_{9.50}Mg_{0.28}Na_{0.22}][(PO_4)_{5.66}(CO_3)_{1.38}][H_2O]_x$

Enamel $[Ca_{9.70}Mg_{0.19}Na_{0.11}][(PO_4)_{5.80}(CO_3)_{0.46}][OH]_{1.57}[H_2O]_x$

Three quite different suggestions for the structural nature of the compound present have been advanced in recent years. Trömel and Möller[8] and Bale and coworkers[6, 9] consider the material to be hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$, with "adsorbed" carbonate and possibly phosphate. Bale[6] states "Since the diffraction pattern of tooth and bone substance give no indication of a carbonate apatite being present, the carbonate ions are also probably most abundant in these regions of structural discontinuity that may be considered as marking the transition from one crystalline particle to another." Brasseur and Dallemagne in a series of papers published in Belgium during the war which were summarized in *Nature* in 1946[10] (also reference 4) concluded that bone is "tricalcium phosphate hydrate," $Ca_9(PO_4)_6(H_2O)_2$, with $CaCO_3$ present as a separate phase and that enamel is in part "carbonate hydroxylapatite." They state "We cannot admit, therefore, that the tertiary with a Ca/P ratio of 1.94 is hydroxylapatite with adsorbed PO_4 ions." Hendricks and Hill in 1942[7] reached essentially these same conclusions except that they considered the carbonate of bone to be present in the "tricalcium phosphate hydrate" lattice.

Properties of the several materials pertinent, without further discussion, to the question at issue are:

1. A single phase having the x-ray diffraction pattern of apatite and containing carbonate is present even though bone forms in a system unsaturated with respect to calcium carbonate.
2. In human bone this phase is shown by analysis to be a neutral substance. It is more basic, approaching hydroxylapatite, in parts of enamel.
3. A large portion of the carbonate in bone can be preferentially removed by solution in acid without destruction of the apatite lattice. Enamel and some "carbonate apatites" dissolve uniformly.
4. Citrate is present in bone as water insoluble form in amounts by volume approaching one-half that of carbonate.[11, 12]

The first and third of these properties indicate rather clearly that carbonate is on the surface of the bone material. Presence of citrate can also be explained in this way instead of being merely "adventitious." Bone also contains magnesium and sodium that might be expected to replace Ca^{++} in the apatite structure on the basis of ionic radii for Na^+ and Mg^{++}. These two are preferentially dissolved by acid,[5] however, indicating their location on the lattice surface. The difficulties of charge with

Na^+ replacing Ca^{++} and essential absence in solutions near neutrality of magnesium as Mg^{++}, rather being $Mg(OH)^+$, accordingly do not arise. There is still a size limitation for the surface, however, since Na^+ is taken up preferential to K^+.

The nature of "carbonate apatites" has been most confusing in its bearing on the type of compound present in bone. Lack of preferential solution in acid of carbonate in enamel was essentially the reason for Brasseur and Dallemagne considering the compound to be "carbonate hydroxylapatite." Another possibility, however, is that the carbonate groups of enamel and francolite and the citrate of enamel are also present on surfaces which are entrapped as crystallites grow and are surrounded by phosphate such that the final material has many occluded surfaces as schematically shown in figure 1. In the case of francolite, and to a lesser extent in enamel, the apatite lattice can be continuous throughout large crystals. The high density of francolite[2] indicates that voids cannot constitute more than 1% of the total volume.

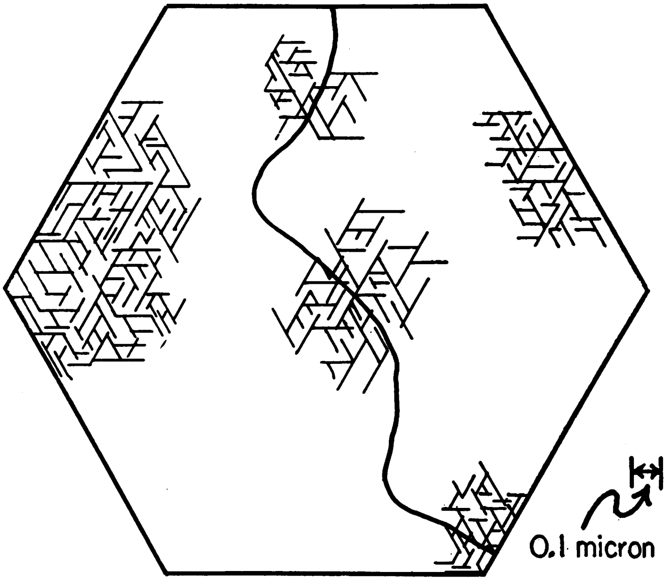

FIGURE 1

Entrapped surfaces in "carbonate apatites" as present in enamel and francolite, schematic.

Evidence in regard to entrapped surfaces for francolite and collophane comes from some peculiarities of their fluorine contents which have not previously been explained. These substances[2, 13] contain fluorine in excess of that required for an apatite structure, sometimes by a factor as great as

1.50, while fluorapatite has not been found to contain excess fluorine. Now if the surface occurs at primitive translations and all fluorine positions are filled then part of the surface fluorine atoms would be in excess of the unit cell requirements as shown in figure 2. Francolite is always observed to be secondary in formation to collophane and as it forms with a decrease in surface, excess fluorine present on the surfaces of primary collophane is exuded in limited amounts as secondary fluorite[14] in many phosphorites.

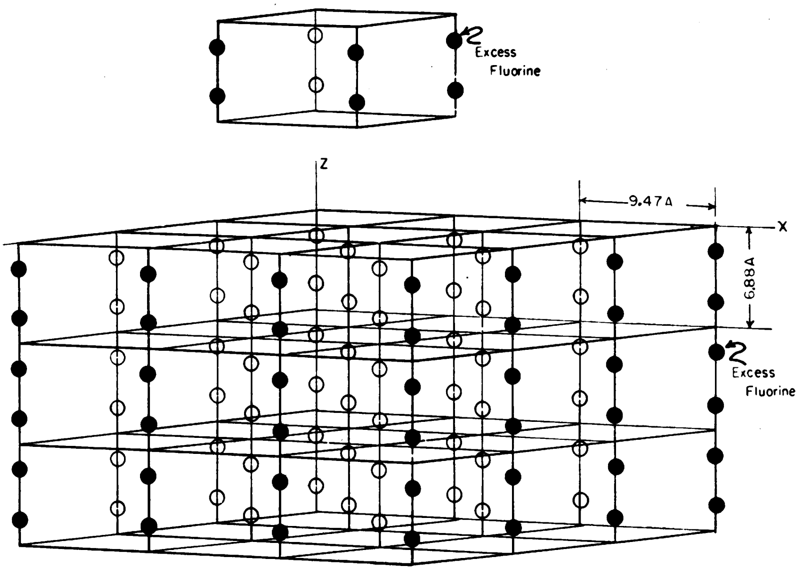

FIGURE 2

Excess fluorine in an apatite-type crystal due to lattice limitation.

TABLE 1

EFFECT OF FRAGMENT SIZE ON SURFACE AREA AND SOLUTION IN ACID OF SOME CARBONATE CONTAINING APATITE TYPE SUBSTANCES

SUBSTANCE	FINENESS SIEVE SIZE	SURFACE, $M.^2/G.$	PARTIAL SOLUBILITY IN ACID, PER CENT OF TOTAL[a]		
			CO_2	CaO	P_2O_5
Steamed bone	100–200	66.2	18.6	6.5	6.5
	16– 30	66.2	15.5	5.9	5.4
	4– 8	63.7	..	...	...
Francolite	Below 6 micron	7.3	26.1	7.2	2.9
	100–200	3.2	10.5	6.6	3.9
	20– 35	1.6	6.5	3.7	3.5
Enamel	Through 325	6.8			
	200–325	3.7			
	120–200	3.2			
	60–120	2.1			

[a] Analyses by J. H. Caro.

Other evidence for entrapped surfaces can be obtained from surface area measurements by nitrogen absorption (B.E.T.). These areas should depend markedly upon screen mesh size of material under test if entrapped surfaces are uncovered by breakage as indicated in figure 1. That this is true for enamel and francolite, but markedly less so for bone as required by the preferential solution of its carbonate in acid is shown by results in table 1. These surface areas were measured in the laboratory of Dr. V. R. Dietz of the National Bureau of Standards who, independently, had made the observations on enamel for Dr. R. C. Likens of the National Institutes of Health.

Attention is now turned to some detailed consideration of the surfaces. Bone, after removal of most of its organic matter, has a maximum surface of about 80–100 m.^{2}/g. (Reference 15 and extensive unpublished information of V. R. Dietz for bone ash) and contains about 7.5×10^{20} CO_3 groups/g.[7] The corresponding area per group, assuming a surface area of 100 m.^{2}/g., the larger value being taken in allowance for entrapped surfaces, is 13.3 A^2 which is about the value to be expected if the carbonate (and possibly citrate ions) forms a single surface layer containing cations and the maximum surface is realized.

Now consider the francolite sample analyzed by Gruner and McConnell[2] which contains 3.36% CO_2 and 1.08 times more fluorine than required for apatite. The excess surface fluorine is given by $2x + 1 = 0.08x^2$, where x is the number of primitive translations in a "crystallite" which is assumed to be equal in the x and y directions, figure 2. Thus x is about 25 corresponding to 234 A (25 × 9.35 A). The calculated "surface area"/g., made up chiefly of that entrapped, is about 62 m.^{2}/g. The "external surface" is surely less than 1 m.^{2}/g. A completely independent estimate of surface area is afforded by the carbonate content. For 3.36% CO_2 and an area of 13.3 A^2 per CO_3 group this is 61 m.^{2}/g. While the agreement with the other value is perfect the combination of the methods can only be trusted to about 25%, the greatest uncertainty being about the surface area per CO_3 group.

The nature of "tricalcium phosphate hydrate" having an apatite diffraction pattern, and serving as a prototype for bone, is now considered. This material can be formed by precipitating calcium phosphate in a system having a pH near that of plasma or by hydrolysis of $CaHPO_4$, i.e., under conditions where HPO_4^{--} is a predominant phosphate ion in solution. The composition of this material determined by independent groups is $Ca_9(PO_4)_6(H_2O)_2$[4, 7] or $Ca_9(PO_4)_6(H_2O)_{1.5}$,[16] the question of the exact water content of course being difficult to settle. A preparation made by slow hydrolysis of $CaHPO_4$ in a system more acid than pH 5.0 contained crystals measuring about 2 microns in length which were shown by electron micrographs to be well formed lath-like prisms with pyramidal faces. A material of this type gradually lost 4.70% H_2O (calculated for $Ca_9(PO_4)_6(H_2O)_2$ 3.73% H_2O) between 100° and 800°. Above 800° the substance is essentially anhydrous and gives the diffraction pattern of the beta modification of $Ca_3(PO_4)_2$ (stable below 1115°C. and possibly a stable phase in solution) which verifies the molal ratio. Hydroxylapatite behaves in a markedly different way in retaining its full content of 1.79% H_2O upon heating at 1000°. It is dehydrated fully only above 1300°.

Is this substance really what it appears to be or is it hydroxylapatite as was suggested many years ago by Trömel[8] (also references 6 and 9) with surface material modifying the composition? The "formula" on this basis would be

$$\begin{array}{ll} Ca_{10}(PO_4)_6(OH)_2\text{—Surface } (PO_4)_{0.67} \\ \qquad\qquad\qquad\qquad\text{Surface } (H^+)_{2.00} \end{array}$$

and would have to be approximated, on the basis of present evidence, over a pH range from about 4.6 to 7.3 in the precipitating system. The particular composition might vary somewhat and would be determined possibly by the surface and the pH. Surface areas of several materials made by precipitation varied from 26 to 64 $m.^2/g.$ With surfaces, separated by perhaps ten primitive translations and entrapped in part, covered with HPO_4^{--} ions, behavior upon heating might parallel that observed; namely, gradual loss of water in a manner very different from that of hydroxylapatite. In the case of bone, HPO_4^{--} would have to be present on the surface together with carbonate and citrate. Experimental results at hand cannot safely resolve the question, although the authors, as well as Brasseur and Dallemagne, have previously considered the evidence adequate for "tricalcium phosphate hydrate." The observation that the poorly developed x-ray powder diffraction pattern is closely the same as that of hydroxyl apatite should surely be given full weight,[6] but it alone is not conclusive.

This surface chemistry of bone and related materials markedly affects composition and is a result or probable cause of the large surface area. It can be generalized. An insoluble calcium compound can form surface compounds with other ions having insoluble or poorly ionized calcium salts (e.g., citrate). Thus $CaCO_3$ would equally be expected to form surface phosphates and citrates in much the same way as calcium phosphates form surface carbonates. Such reactions which are important for phosphate fertility of calcareous soils have been observed in tracer experiments by Dr. Sterling Olson of this Bureau.

The phenomenon discussed here, which can be studied in many ways, has broad implications in the formation and properties of bone and teeth. An explanation is afforded for the unusual relationship of fluorite, collophane and francolite in phosphate rock. Interpretation can be given to occurrence of uranium and other groups as surface material in phosphorites.[17] An understanding is afforded for the preferential use of certain phosphate rocks, particularly those from North Africa having high carbonate contents, for direct application to some crops.[18]

Modification of composition by small particle size has also been found for hydroxylapatite by P. W. Arnold of Cambridge University (personal communication). He observed that precipitates of hydroxylapatite contain $(OH)^-$ in excess of the formula requirements, such as to yield more than 2.0% water between 900° and 1400°C. (calculated 1.79% H_2O).

Summary.—The inorganic compounds of bone have a large surface on which carbonate, citrate, magnesium and sodium are located. Carbonate is present on entrapped surfaces and not as constituent within the lattice of the mineral francolite, the typical "carbonate apatite," that also by lattice limitation, contains fluorine in excess of the fluorapatite requirement. Enamel is similar to francolite in having entrapped surfaces. The over-all composition, including lattice and surface, of bone is that of a neutral calcium phosphate while enamel is more basic, approximating hydroxylapatite. The compound forming the lattice of bone remains to be established but it might well be hydroxylapatite with an excess of phosphate on the surface.

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