Surface Areas of Synthetic Calcium Phosphates and Bone Mineral[1] (34663)

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The surface or interface of bone mineral is an important parameter in bone metabolism. In particular, this surface must play a role in the interchange of ions between the body fluids and bone mineral. This is a report on a study of the specific surfaces obtained on a series of synthetic hydroxyapatites, synthetic amorphous calcium phosphates, and bone samples, treated in different ways. The surface measurements have been made with gas adsorption and small angle X-ray scattering techniques. A comparison of the results obtained by these contrasting methods adds to our knowledge of the interface between bone mineral and the remainder of bone which is essentially water and collagen. In addition, this study gives a comparison of surface measurements made by these fundamentally different methods.

Materials and Methods. 1. Synthetics. Large batches of a series of samples of poorly crystallized hydroxyapatite and amorphous calcium phosphate were prepared by the method described by Eanes *et al.* (1). Surface area measurments were obtained using low temperature adsorption of N_2 and the application of the BET method (2). Concomitant surface measurements by small angle X-ray scattering were performed.

It was ascertained by wide angle X-ray diffraction (3), that the hydroxyapatites were 100% crystalline (*i.e.*, contained no amorphous fraction) and the amorphous calcium phosphates were free of any crystalline apatite. This assay was necessary because the amorphous phase is a precursor in the chemical precipitation of hydroxyapatite (1) and one may contaminate the other.

The precipitated samples were separated from the solution by freeze-drying and then stored in stoppered vessels at room temperature. Prior to the adsorption measurements the samples were evacuated to 10^{-6} Torr at 300° for periods of 18–36 hr. Weight losses on these samples due to loss of water varied from 10–20% as determined by weighing the samples prior to and after evacuation. A standard volumetric BET apparatus was used in the adsorption measurement; for details see the work of Holmes, Beebe, and coworkers (4–6).

The small angle X-ray scattering patterns were recorded electronically on a commercially available apparatus of the Kratky (7) design. Aperture heights were chosen so that the corresponding scattering profile would approximate the shape of a curve produced by a beam of infinite height under the same conditions. Monochromatization of the X-ray beam was achieved by using Ni-filtered copper radiation and a proportional counter detector coupled with a pulse height analyzer. Fixed time, manual step-scanning was employed to collect the intensity data. The intensity at each step was recorded to within a probably error of 1.0% or less.

Specific surface values were calculated from the X-ray data in the region of scattering where the intensity fell as a function of h^{-3} (where, $h = 4 \sin \theta/\lambda$) according to the method of Porod (8). A polyethylene standard (courtesy, Professor O. Kratky) was employed to place the intensity measurements on an absolute scale. Simplified discussions of this method are found in two recent publications (9, 10). The method yielded a

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specific surface with a relative precision of ±5%.

2. Bone studies. Rabbit femur diaphysis was excised, frozen, and a portion was ground by hand to 200 mesh in an agate mortar (sample H) while a second portion was ground in a colloid mill to 300 mesh (sample M). The pools H and M were dried to constant weight at 110° and aliquots of each pool were taken for BET and small angle X-ray scattering surface area measurements. A series of samples of each pool were extracted in a Soxhlet apparatus using reagent grade ethylenediamine (followed by an absolute ethanol wash) for different periods of time to provide samples with decreasing amounts of bone collagen (See Table II for extraction times). BET surface area measurements were made as above except that samples of H and M with the least amount of collagen were subjected to a progressive heat-degassing procedure (with the same sample left in the apparatus after each temperature treatment) to ascertain the effect of this procedure on the surface area. Degassing times ranged from 20 to 100 hr, but in all cases the samples were degassed to a constant weight. Small angle X-ray scattering surfaces were measured on the ground untreated samples of H and M.

TABLE I. Surface Area (m^2/g) of a Series of Synthetic Hydroxyapatite (HA) and Amorphous Calcium Phosphate (ACP) Samples. Phase determined by wide angle X-ray diffraction.

Sample	X-Ray method	BET method	
		Based on original wt	Corrected for wt loss
1-ACP	75	51.4	61.0
2-ACP	57	52.1	62.5
3-HA	150	160.0	177.0
4-ACP	67	52.7	67.0
5-ACP	68	52.7	67.0
6-ACP	82	55.0	67.0
7-HA	202	139.0	156.0
8-HA	197	145.2	162.0
9-ACP	74	54.3	65.0
10-HA	221	172.0	195.0
11-ACP	66	47.0	62.0
12-HA	186	151.5	170.0

Results and Discussion. 1. Synthetic studies. The results on the surface areas of the synthetic samples by the two methods are given in Table I. It is noted that in most cases the area as determined by X-ray scattering is slightly larger than that determined by adsorption. Some of this difference may be due to change in surface area with degassing

TABLE II. Summary of Rabbit Femur Diaphysis Samples. Extracted with ethylene diamine, washed with ethyl alcohol.

Sample	Total extraction time (days)	Total % wt loss on extraction	Outgassing temp (°)	Surface area (m^2/g)	% Wt loss on degassing
H	0		RT[a]	1.94	4.08
	3	10.94	RT	44.90	1.31
	8	17.13	RT	91.45	2.74
	12	21.26	RT	112.84	
	15	24.00	RT	120.23	
			302	156.75	
			490	131.99	12.21
M	1	6.61	RT	17.01	1.09
	8	24.96	RT	91.06	0.96
	12	29.83	RT	115.13	2.34
	14	35.55	RT	138.63	
			295	160.02	7.70
	16	38.35	RT	122.54	
			296	152.99	
			496	125.06	10.96

[a] RT = room temperature.

temperature and/or to the difficulty of specifying the exact weight of the sample. We have reported surface areas by adsorption based on the original weight of sample and on the weight of sample after degassing at 300°. The latter figures are probably closer to the actual specific surface values. Considering the errors inherent in the two methods and the difficulties of preparing completely homogeneous samples in large batches, the agreement is remarkably good. Earlier workers have reported similar agreements between these two methods (9, 11).

It is of interest to speculate on the effect of the removal of water by evacuation at 300° on these samples. Holmes and Beebe (4) have made a more elaborate study of the effects of degassing temperature on the surface area and weight loss of these and other samples and also the relation of surface area to time of exposure to the preparation solution. It is possible that there is a microporous structure in some of these samples which might not admit the nitrogen molecules in adsorption process but which might still be observed by the X-ray techniques. This may account for the slightly higher values obtained by the small angle studies.

It is interesting to note that X-ray diffraction studies showed that no crystallization or phase change took place in the amorphous samples as a result of heating in vacuum at 300°. The amorphous material is quite unstable in water (1), dissolving and reprecipitating as hydroxyapatite in a short time. In addition this phase is unstable thermally in the atmosphere at 300°.

The surface area results on the amorphous phase are in excellent agreement with electron microscope observations on this material. Weber *et al.* (12) reported amorphous calcium phosphate prepared as above to be composed of spherical particles with diameters of about 300 A. A simple calculation (assuming a density of 3.0) shows that such particles have a specific surface of about 60 m^2/g, the same range as reported by both methods herein. The synthetic amorphous calcium phosphate particles appeared to be, in fact, hollow spheres in the electron microscope (12). This seems unlikely from these results since at least the X-ray scattering data should reveal such inner surfaces. At this point it would seem that the hollow nature of the particles is doubtful and may be due to some artifact of microscopy.

2. Bone studies. Table II gives the results on the bone measurements obtained by the BET method. The X-ray scattering studies showed the samples of whole bone to have the following specific surfaces: $H = 182$ m^2/g, $M = 200$ m^2/g.

The surface area in the ethylenediamine extracted bone (Table II) does not approach the value obtained by X-ray. This is no doubt due to the incomplete removal of collagen from the samples. The discoloration of bone samples apparent after heat treatment in the BET apparatus is further evidence of the presence of residual protein and protein breakdown products. If all of the protein is removed the bone powder is pure white even after heating. The usual water wash to remove low molecular weight cleavage products of collagen was not used to avoid altering the mineral phase. Finally, no change was seen in the wide angle diffraction pattern of either bone sample after ethylenediamine treatment supporting the view that this treatment did not alter the bone mineral.

It is clear from these results that hydrated collagen blocks the uptake of nitrogen on bone mineral surfaces. The X-ray surface measurement is independent of the presence of collagen and/or water and represents the interface between mineral and the rest of the bone. In the heat-degassing experiments (on the lowest collagen content bone samples) the surface begins to decrease when too much heat results in sintering of the mineral phase. No change was seen on the wide angle, X-ray diffraction pattern of either bone sample when heated, in vacuum, to about 300°.

One question arises from this study. If the hydrated collagen blocks the uptake of N_2 in the BET apparatus does collagen *in vivo* screen the surface of bone mineral from the body fluids? Rowland (13) has shown that a surprisingly small percentage (less than 0.2% of total bone calcium) of bone calcium is exchangeable, *in vivo,* with radiocalcium in

the body fluids. There is evidence for a chemical bonding between the collagen and the calcium phosphate of bone mineral (14, 15). Whether this intimate association results in preventing ion uptake on bone mineral remains to be proved. The surface measurement results reported herein lend support but do not conclusively prove such a viewpoint.

Finally, we feel that it would be useful in the study of bone and synthetic analogues of bone mineral to report surface area per unit weight along with other parameters such as percentage crystallinity, Ca/P ratio, crystal size, and others.

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