Surface Areas by Gas Adsorption on Amorphous Calcium Phosphate and Crystalline Hydroxyapatite

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Surface areas have been measured by the nitrogen gas adsorption method on a number of samples of precipitated calcium phosphates both in the amorphous form (ACP) and in the form of crystalline hydroxyapatite (HA). In all the samples studied the specific surface areas of the HA have from two to four times the values obtained for ACP, reflecting the smaller particle size of the crystalline form. In most cases, there is a marked decrease in surface area on storage over a period of months. This decrease is especially noticeable for the HA samples with high water content and is probably due to a slow growth and perfection of the crystals of HA.

A detailed study has been made of the surface area and weight loss of the calcium phosphates as functions of the degassing temperature. The amount of water retained on the HA after degassing at 20° but removable by degassing at 500° represents a close approximation to an absorbed monolayer. A similar analysis of the data for ACP would indicate over four monolayers on the basis of the postulate that all the water is held by adsorption forces. This postulate is rejected in favour of the alternative that most of the water is retained within the particles of the ACP rather than solely on the surface. These observations may have some importance in connection with the structure of bone, which contains both ACP and HA.

Key words: Apatite — Calcium phosphates — Gas Adsorption — Surface — Amorphous.

Des régions de surface ont été mesurées par la méthode d'adsorption gazeuse à l'azote sur un très grand nombre d'échantillons de phosphates de calcium précipités, à la fois, sous forme amorphe (ACP) et sous forme de cristaux d'hydroxyleapatite (HA). Au niveau de tous les échantillons étudiés, les régions de surface spécifique d'HA ont des valeurs deux à quatre fois supérieures à celles à l'ACP, traduisant une taille de particules plus étroite de la forme cristalline. Dans la plupart des cas, on note, au cours de ce travail, une diminution marquée en région de surface, en conservant les échantillons pendant quelques mois. Cette diminution est surtout nette pour les échantillons d'HA à contenu élevé en eau et semble liée à une croissance lente et à la perfection des cristaux d'HA.

Une étude détaillée de la région de surface et de la perte de poids des phosphates de calcium a été réalisée en fonction de la température de dégazage. Il apparait ainsi que la quantité d'eau, retenue sur HA après dégazage à 20°, constitue, en bonne approximation, une monocouche adsorbée: cette quantité d'eau peut être éliminée à 500°. Une analyse identique effectuée pour ACP indique quatre monocouches, en se basant sur le postulat que toute l'eau est maintenue par des forces d'adsorption. Ce postulat est rejeté en faveur d'une autre possibilité qui veut que la majorité de l'eau soit retenue dans les particules d'ACP, plutôt qu'au niveau de la surface seule. Ces observations peuvent présenter une certaine importance pour la structure osseuse qui contient à la fois de l'ACP et de l'HA.

Aus einer großen Anzahl Proben von gefälltem Calciumphosphat, die als amorphes (ACP) oder als kristallines (HA) Hydroxyapatit vorliegen, wurde die Größe der Oberfläche mittels der Stickstoffgasadsorptions-Methode bestimmt. Bei allen untersuchten Proben sind die spezifischen Oberflächenwerte des HA zwei bis viermal größer als jene des ACP, in Übereinstimmung mit den viel kleineren Partikeln der kristallinen Form. Es wird in dieser Arbeit gezeigt, daß die Oberfläche in den meisten Fällen deutlich abnimmt, wenn die Proben mehrere Monate aufbewahrt werden. Diese Abnahme wird speziell bei den HA-Proben mit hohem Wassergehalt beobachtet und wird wahrscheinlich durch ein langsames Wachstum und eine Vervollkommnung der HA-Kristalle hervorgerufen.

Die Größe der Oberfläche einerseits und der Gewichtsverlust von Calciumphosphat andererseits wurden als Funktionen der Entgasungstemperatur eingehend untersucht. Eine Auswertung der Resultate führt zur Annahme, daß die auf dem HA nach Entgasung bei 20° zurückgehaltene, jedoch bei 500° abgegebene Wassermenge mit dem Postulat einer monomolekularen Schicht vereinbar ist. Aufgrund der Annahme, daß alles Wasser durch Adsorptionskräfte haften bleibt, würde eine gleichartige Analyse der für ACP gefundenen Werte mehr als 4 monomolekulare Schichten ergeben. Dieses Postulat wird jedoch verworfen zugunsten der anderen Möglichkeit, daß nämlich das Wasser größtenteils eher innerhalb der ACP-Partikel als nur an deren Oberfläche zurückgehalten wird. Diese Beobachtungen können im Hinblick auf die Struktur der Knochen, welche sowohl ACP als HA enthalten, von einer gewissen Wichtigkeit sein.

Introduction

In a series of publications [3, 4, 6, 7] it was shown that an amorphous tricalcium phosphate (ACP) constitutes a precursor phase in the precipitation of hydroxyapatite, and further that under favorable conditions the ACP is transformed into the final crystalline hydroxyapatite (HA) after a few hours of exposure to the precipitation solution. The properties of the amorphous and crystalline forms and the possible significance of their interrelation in connection with bone formation is set forth succinctly in the introduction to a recent publication by Harper and Posner (1970). The authors point out that there is evidence to indicate that as much as 35–40% of the calcium phosphate in mature bone is present in the amorphous state, with the remainder to be found as finely-divided crystalline bone apatite, while in young bone there is an even higher fraction of ACP.

We have carried out a program of research using low temperature gas adsorption techniques to elucidate the surface chemistry of bone mineral [2, 8, 9]. In some of this work it has been shown that the adsorption characteristics of synthetic hydroxyapatite are closely similar to those of anorganic bone mineral extracted from bone [2]. In particular, this is true with respect to the order of magnitude of their specific surface areas and to the polarizing ability of the surfaces with respect to various adsorbates, especially water. Because of the potential importance of knowledge of the amorphous and crystalline phases to an understanding of the formation and function of the mineral in bone, we undertook gas adsorption studies on both ACP and HA. Although these synthetic materials represent a further step away from the situation *in vivo*, they are free from any effects of the rather drastic chemical treatment necessary to remove the organic constituents in the preparation of the anorganic bone mineral used in our earlier work.

Our first work on ACP and HA involved surface area measurements based on low temperature nitrogen adsorption on samples obtained from the laboratory

of the Cornell University Medical College. The results were compared with those obtained in the Cornell laboratory using a small angle X-ray scattering technique [10].

In the present paper we report the results of more extensive surface area measurements on ACP and HA prepared in the Carleton laboratory. Special attention has been given to the effect on surface area of such factors as time of exposure to the precipitation solution, storage time after lyophilizing, and temperature of outgassing the samples before nitrogen adsorption. In the interpretation of the results it turns out to be important to consider weight loss on degassing at successively higher temperatures in relation to the measured surface area of the sample, definable in terms of water held on the sample *per unit of area*. We find here a striking difference between ACP and HA which we believe may have some significance in relation to the way in which water is related to the mineral phases in bone.

Experimental Methods

Preparation of Precipitated Calcium Phosphates. To prepare large samples of the precipitated phosphates for our work and for comparison with previous samples sent to us from the Cornell laboratory, the scale of the preparation used by Eanes, *et al.* (1964) was increased but with no other modification of the procedure [3, 4]. A 3 l three-necked flask with ground joints and a Teflon stirrer was used as the reaction vessel. Into this flask 1 l of 0.25 M solution of $(NH_4)_2HPO_4$ was placed. One litre of 0.75 M solution of $Ca(NO_3)_2$ was then added quickly as possible from two separatory funnels with vigorous stirring. Freshly-distilled water and Fisher certified grade chemicals were used and both solutions were adjusted to pH 10.5 using concentrated aqueous ammonia. Attempts were made to exclude CO_2 by conducting these operations in a dry box flushed out by a continuous stream of N_2. Nitrogen was also passed through the reaction vessel during precipitation. Initial time was recorded from the first admission of the calcium nitrate solution, and samples of the slurry were removed by pipette at appropriate intervals. These samples were transferred to plastic centrifuge tubes cooled in ice and then placed in a refrigerated centrifuge at 8–10000 r.p.m. for 5–10min, sufficient to produce a clear supernatant liquid. Each sample was washed with shaking four times with cold ammonia water at pH 10.5 and recentrifuged as before.

After the final washing and centrifugation the precipitates were transferred to glass vessels and freeze-dried for 15–24 h. This lyophilization was expected to stop any further transformation from the amorphous to crystalline state. Care had to be taken at this stage to spread out the wet precipitate on the inner walls of the freeze-drier tubes. When this was not done portions became dry and hard on the outside while still wet on the inside. This permitted a continuation of the crystallization process within these portions after removal of the precipitate from solution. After the samples were dried they were crushed lightly in an agate mortar to break up small aggregates and were stored either in stoppered glass vials or in sealed ampoules evacuated to 10^{-5} torr at 300° for periods of 50–80 h depending on the size of the sample. All samples were finely divided and free flowing and appeared dry and non-hygroscopic to the eye. The samples prepared as described here had properties which were in good agreement with those of the smaller scale preparations obtained from the Cornell laboratory.

Surface Area Measurements. All surface area measurements used nitrogen gas at liquid nitrogen temperatures applying the B.E.T. method of Brunauer et al. (1938). Standard volumetric apparatus described in previous publications [8, 9] was used. In these measurements the degassing temperature was 300°, except for several series of measurements in which the effect of degassing temperature on surface area and on weight losses was being studied. A quartz spiral balance with a cathetometer to measure displacements (data of Fig. 3) as described in a previous paper [9] was also used. On most of our samples, weight loss measurements were made by sealing off the sample tube under vacuum after the
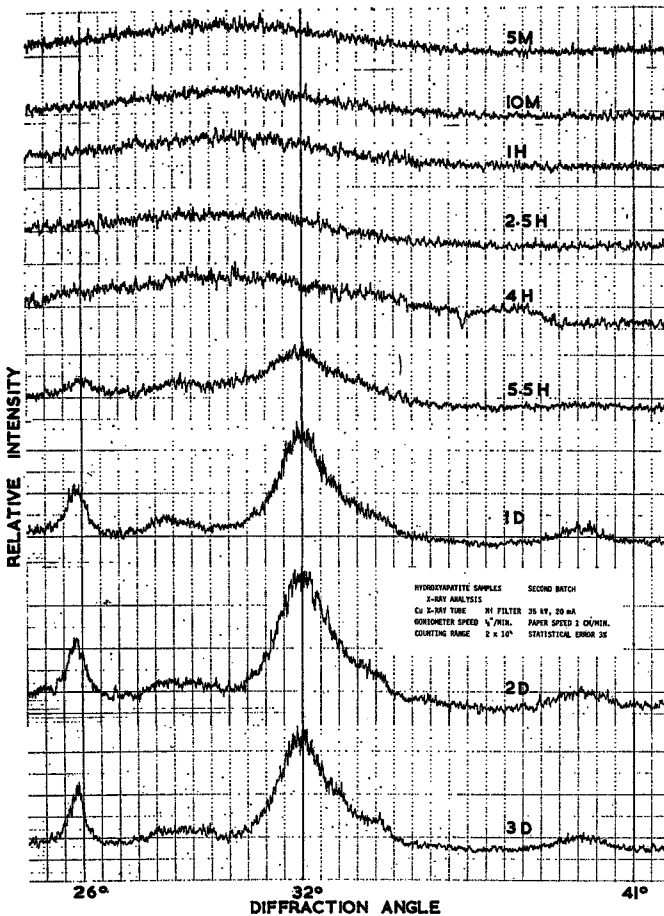
Fig. 1. X-ray diffraction patterns on one batch of precipitated calcium phosphate. Numbers on patterns represent time of removal from preparative solution. *M* minutes, *H* hours, *D* days

surface area measurement had been completed. After weighing, the tube was broken open with as small a scratch as possible, the glass was cleaned and the parts were reweighed. Agreement of duplicate measurements was better than 1%.

X-ray and Chemical Analyses. All samples prepared were examined[1] by X-ray diffraction using a copper X-ray tube with a nickel filter. Samples were examined at diffraction angles in the range 26° to 40° to check for crystallinity. Typical data for one of our preparations are reported in Fig. 1.

In the discussion of results, the contribution of CO_2 to the weight losses encountered on degassing the precipitated phosphates over a range of temperatures has to be considered. Analysis for carbon content was therefore important. Chemical analysis was performed on samples of both the ACP (2 g) and the HA (5 g) by Andrew S. McCreath and Son, Pittsburgh, Pa. Samples were taken from mixtures of large batches prepared with precautions to exclude CO_2. The percentage carbon reported was 0.120% for ACP and 0.142% for HA. Microanalyses (by Dr. Franz Pascher, Bonn, Germany) were used to monitor the separate

1 Kindly carried out by Dr. G. Chao of the Geology Department of Carleton University.

batches. In no case, for precipitates prepared with optimum precautions to exclude CO_2, did the analysis show greater than 0.25% carbon.

In working with large batches, it is difficult to exclude all CO_2 absorption during the preparation procedure. Although CO_2 could be excluded during the precipitation, a certain amount of exposure to atmospheric CO_2 could not be avoided during the centrifuging and washing stages.

Results and Discussion

1. X-ray Diffraction Patterns

The patterns in Fig. 1 represent a typical set of results obtained on the various batches made. Samples were run at periods of several months after preparation to check on the crystallinity. No change was detected in the crystallinity of our samples after the various periods of ageing at room temperature, or even after they had been evacuated at 500°. Although the data were not analysed for a quantitative estimate of the crystallinity factor as carried out by Eanes and Posner [6] it can be seen that we are in semi-quantitative agreement with these authors in the case of these larger samples. There was no clear evidence of crystallinity in the initially-precipitated amorphous material (5 min sample) and in most cases for samples removed from the slurry up to 5 or 6 h after initial precipitation. The characteristic pattern of hydroxyapatite became more sharply delineated with a longer period of contact between the precipitate and solution and crystallinity was nearly at a maximum after 6 h. We plan to concentrate most of our further work on samples removed at 5 minutes and 2 days, these being examples of the amorphous and crystalline precipitates respectively.

2. The Isotherms for Nitrogen Adsorption

In Fig. 2 isotherms are shown for the adsorption of nitrogen gas at −196° on samples of amorphous and crystalline forms of the precipitate. It is immediately apparent that the amount of adsorption increases greatly upon conversion of the amorphous to the crystalline form. A rough estimate of the volume of nitrogen adsorbed at the point where the first absorbed monolayer is complete can be made from the points B which have been located on the isotherms. A more precise value of the point of completion of the monolayer can be obtained by analyzing the isotherm plots and calculating the surface area by the B.E.T. method.. In each case straight lines were obtained at least up to a relative pressure of 0.25. In all these surface area measurements the adsorbent was degassed at 300° unless otherwise specified.

These curves are reminiscent of the adsorption isotherm for nitrogen on anorganic bone mineral reported in an earlier publication [9]. It should be noted that the isotherms on the synthetic preparations here exhibit the same hysteresis at relative pressures above 0.5 as are seen for the bone mineral. These hysteresis loops are indicative of a porous structure with pore radius 100 Å or greater. Attempts to determine a microporous structure on these samples by the use of "n" curves of Pierce (1968) or the "t" curves of Lippens and de Boer (1965) did not lead to conclusive results. We are of the opinion that the application of either of the above techniques will not suffice to determine the pore structure of our samples. It appears that while larger pores are present, present techniques do
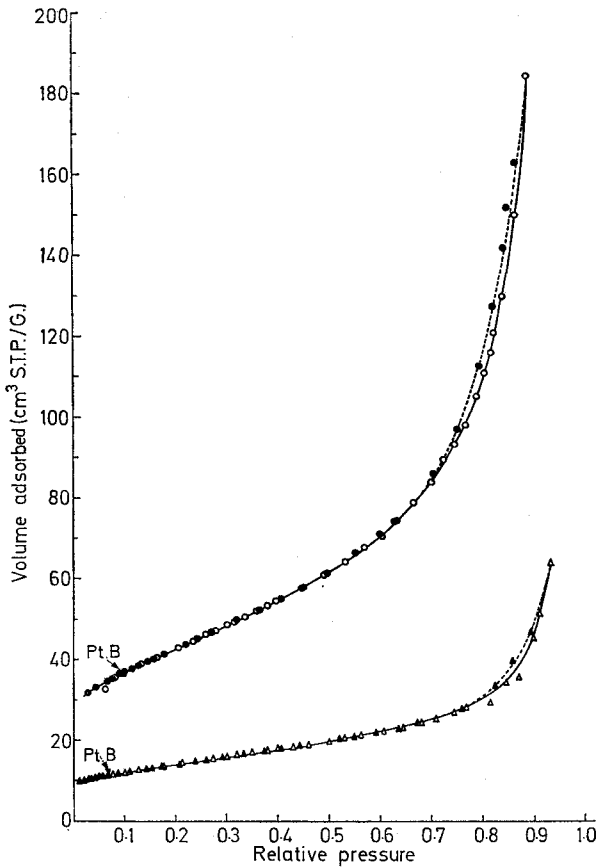
Fig. 2. Nitrogen adsorption isotherms on precipitated calcium phosphates at 78° K. Samples were degassed at 300° C prior to adsorption measurement. △, ▲ 5M sample (ACP) amorphous, ○ ● 2D sample (HA) crystalline. Open points adsorption, filled points desorption

not clearly indicate the presence of micropores (<20 Å) in our samples. The pore structure is definitely connected with the water content of the samples and more work will have to be done to completely elucidate the structure.

3. Surface Areas as a Function of Time of Exposure to the Preparative Solution

In Table 1 are shown some data of specific surface areas as determined by the nitrogen adsorption technique. Samples were withdrawn from the preparative solution at the times indicated and degassed at 300° to 10^{-5} torr pressure prior to the adsorption measurements.

In the literature it is recorded [10] that there is a large increase in surface area when the amorphous particles of ACP are transformed into HA, which consists of small crystals with a large surface area. This transformation normally occurs after about 6 h exposure to the solution. This large increase in area after

Table 1. *Surface areas of precipitated calcium phosphates as a function of time of withdrawal from preparative solution*

Exposure time[a]	Surface areas (m^2/g)							
	1	2	3	4	5	6	7	8
5 M	98	—	63	87	56	68	51	63
10 M	—	—	66	83	—	—	—	—
0.5 H	—	—	—	—	—	—	56	63
1 H	63	84	72	83	—	—	51	66
2 H	47	78	82	88	57	73	—	—
4 H	56	77	75	95	—	—	56	68
5 H	—	—	—	—	55	80	54	78
6 H	—	—	133	—	—	—	73	85
1 D	180	280	216	232	232	270	192	150
2 D	236	263	187	253	183	253	160	226
3 D	169	—	219	—	—	—	—	—

[a] This represents the time of withdrawal of the samples from the preparative solutions. M = minutes, H = hours, D = days.

some 6 h is apparent in the data of Table 1, and it has been consistently observed in all the samples we have examined.

There are smaller deviations in surface area among the samples of the ACP withdrawn after 4 h or less than among those of HA withdrawn after one day or more of exposure to the solution. These smaller deviations are possibly due to variations in stirring and mixing rates and temperature differences in different regions of the reaction system which may result in lack of homogeneity in particle size.

No effort has been made to determine particle size distribution for most of the samples studied. However, an average particle size can be estimated from the specific surface areas. When we do this we find diameters of the order of 300 Å for the ACP and crystal dimensions of the order of 100–150 Å for the HA. Electron microscopy on a few of the samples supports these estimates.

4. Surface Area Changes in Storage

We have observed changes in surface area on storage for both ACP and HA even in stoppered bottles or sealed vials at room temperature. After a period of one to 6 months, it was found that the surface areas had decreased, by as much as 25% in some cases. The data listed in Table 2 illustrate this effect. Samples listed in column A were transferred directly from the lyophylizing treatment to stoppered bottles. As seen from the Table, all these samples had a relatively high water content. The samples listed under B had been further (but not completely) dehydrated by evacuation at 300°; their decreased water content is shown in Table 2. It is apparent that there is a correlation between stability of surface area and water content, the samples of high water content being more labile.

Table 2. *Average surface areas of precipitated calcium phosphates as a function of ageing time at room temperature*

Ageing time, months[a]	Surface areas (m²/g)					
	5M[b] amorphous		1D[b] crystalline		2D[b] crystalline	
	A[a]	B[a]	A	B	A	B
0	60 (6)[c]	71 (6)[c]	196 (3)	265 (2)	201 (8)	239 (3)
1	54 (4)	76 (3)	—	—	—	240 (4)
2	53 (6)	—	153 (2)	—	152 (6)	—
6	52 (7)	74 (6)	145 (4)	241 (4)	155 (8)	231 (7)
Percent change in area after 6 months	−13.3%	+3.5%	−26%	−9%	−23%	−3.5%
Average weight loss on degassing at 300°	18.6% (6)	3.0% (6)	11.6% (3)	2.6% (3)	10.3% (8)	3.5% (5)

a Samples in A columns were transferred directly from lyophilizing to stoppered bottles and those in B columns were stored in sealed glass ampoules after partial vacuum dehydration at 300°.

b 5M, 1D and 2D refer to minutes or days of contact between the precipitated phosphates and the preparative solution before removal for freeze drying. The 5M samples are ACP while the 1D and 2D samples are HA.

c Numbers in parentheses represent the number of samples averaged to give the particular value recorded.

The observed decreases in area may be explained on the basis of a presumed reorganization of the sample resulting in an increase in particle size or perfection of the crystals. A notable exception to the observed decrease with time in the surface area is seen in column B for the 5 min samples. We have no ready explanation for this but it must be pointed out that these particular samples are amorphous and quite different in structure from the crystalline samples cited in the other B columns. Also it should be remembered that the method of surface area measurement is limited in accuracy.

The surface area changes shown in Table 2 were observed under conditions far removed from those of an *in vivo* situation. However, it is interesting to note that for the more strongly hydrated A samples, the percent loss in area after 6 months is almost twice as much for the HA samples as for the ACP samples in spite of the higher water content of the latter. Bone is known to contain both HA and ACP. It would seem likely, after studying the data of Table 2, that the crystalline form of HA is more susceptible to change (increase) in particle size than is the amorphous form ACP. This may also be true *in vivo*.

5. Weight Losses on Degassing

It is of interest to consider our data on weight losses on degassing in terms of the removal of adsorbed water from the surfaces of HA and ACP. Making use of our measured values for the surface areas we shall try to relate the weight
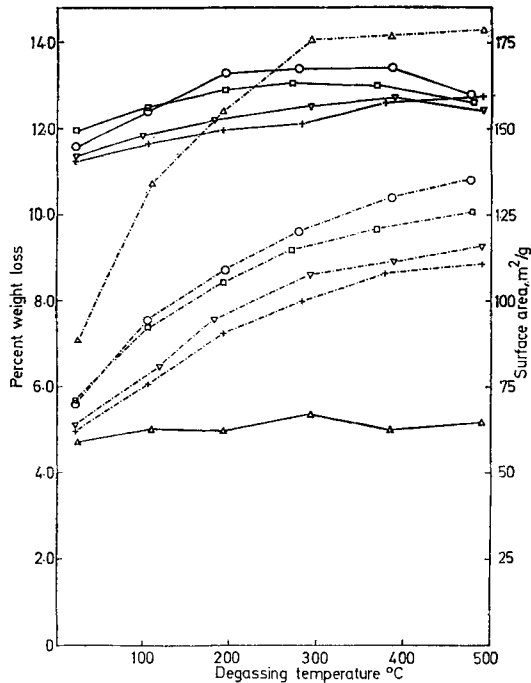
Fig. 3. Surface area and weight losses of precipitated calcium phosphates as a function of degassing temperature. Solid lines: surface area (right hand ordinate), Dotted lines: weight loss (left hand ordinate). △ 5 M sample (ACP), ○ 1 D sample (HA), □ 2 D sample (HA), + 4 D sample (HA), ▽ 8 D sample (HA). The numbers represent time of removal from preparative solution

losses to a number of adsorbed layers of water present on the surfaces considered. In the case of crystalline HA there is good evidence from our work and from earlier calorimetric work [2] that the *differential weight loss*, the amount of water retained after degassing at 20° but removable at 500°, represents less than an adsorbed monolayer.

Similar consideration of our data on ACP presents an interesting anomaly. The weight losses for HA and ACP are shown for successive degassing temperatures in Fig. 3 and in Table 3. The corresponding data for specific surface areas are also shown; it should be recognized that the initial weight losses upon degassing at 20° depend on the history of the sample with respect to exposure to an atmosphere of changing humidity after the freeze-drying of the samples. The 20° degassing step was used to establish a point of reference to consider any *further* weight losses in comparing samples. This further weight loss of a sample degassed initially at 20° and finally degassed at 500° is referred to as the differential weight loss. The values of this parameter can be read off the curves in Fig. 3. The differential weight loss is about 3% for the HA samples and about 7% for the ACP.

The weight of water necessary to form a close-packed absorbed monolayer can be calculated for any sample of known surface area on the basis of a postulated

Table 3. *Surface areas of precipitated phosphates as a function of degassing temperature*

Degassing temperature	Surface areas (m²/g)					
	Amorphous samples[a]			Crystalline samples[b]		
	1	2	3	4	2	3
0°	50	60	65	146	201	195
100°	48	—	63	157	214	210
200°	48	63	62	163	214	212
300°	47	63	61	159	207	203
400°	47	60	60	151	193	203
500°	42	57	56	135	155	157
Percent weight loss (total)[c]	22.2%	14.4%	15.2%	10.3%	10.3%	10.2%

[a] Samples of ACP removed from preparative solution at 5 minutes (5M).

[b] Samples of HA removed from preparative solution after 2 days (2D).

[c] This is the *total* weight loss of the samples after evacuation at the various temperatures up to 500°. These values depend on previous history of samples and are not as quantitatively significant as the differential weight losses referred to in discussion of Fig. 3.

value of 10.5 Å² [12] occupied by each adsorbed water molecule. From this result and from the values of the differential weight loss read from Fig. 3 it appears, for all the HA samples above, and for bone mineral examined earlier [9], that the weight loss up to 500° can be accounted for by the removal of some 70% of a monolayer of adsorbed water[2].

From the results of earlier work on adsorption calorimetry [2, 8] it is apparent that hydroxyapatite, whether of natural or synthetic origin, presents a highly polar surface after degassing at 500°. Upon exposure to the vapors of polar molecules such as methanol or water, a close-packed monolayer is first adsorbed with binding energies ranging from 22–14 kcal/mole. Any further adsorption results in a partially-filled second layer with heats of adsorption from 14 kcals down to the heat of vaporization (10.5 and 9.0 for water and methanol respectively). Any adsorbed molecules beyond the first tightly-held layer are desorbed upon degassing at room temperature. The first monolayer, however, is removed, under vacuum, only at much higher temperatures.

The ACP appears to be composed of spherical particles of about 300 Å corresponding to the observed specific area of about 60 m²/g [7]. We suggest that these spheres are amorphous agglomerates consisting of hydrated calcium and

2 We make the assumption that the weight losses are due entirely to the removal of water from the phosphate samples. This is not strictly true because of some contamination by the adsorption of carbon dioxide. The best values for the carbon content based on analyses of samples of ACP and HA from mixtures of the large batches are 0.120% and 0.142% respectively. If this carbon is given off as CO_2 on degassing at high temperature these values would correspond to 0.44% and 0.51% weight loss. While this amount is not negligible compared to the weight losses given here (from 3.0 to 7.0%), the argument is not seriously altered by the possibility of CO_2 evolution. For one thing, it is improbable that all the CO_2 would be given off between 20° and 500°. A more detailed study of the CO_2 evolution including an analysis of the effluent vapors on degassing is indicated. We plan to undertake a more quantitative study of the relation of water and CO_2 to the ACP and HA.

phosphate ions with little or no three dimensional order. Upon degassing under vacuum at successively higher temperatures this water is gradually removed. On the basis of the arguments above for the HA samples, we would expect that at room temperature any loosely-bound water with an energy of binding less than 14 kcal/mole would be removed by evacuation. It can be seen from Fig. 3 that the differential weight loss for the ACP is nearly double the value for the HA in spite of the fact that the ACP has less than 40% as much specific surface area. If the assumption is made that water associated with ACP after degassing at room temperature is in the adsorbed state it is necessary to postulate about 4 adsorbed layers to account for it. From analogy with the HA it seems improbable that more than one adsorbed layer is left after degassing at 20°.

It can be shown by calculation that a monolayer of adsorbed water on the ACP of specific surface area 60 m²/g would represent 1.7% by weight. This would mean no more than 1.7% of the 7% differential weight loss as due to adsorbed water can be accounted for. This suggests that 5.3% or more of the weight loss must be attributed to loss of water held within the ACP particles with an energy greater than 14 kcal/mole.

For a hydrate such as $Ca_3(PO_4)_2 \cdot xH_2O$, x would be close to 1 for a weight loss of approximately 6%. However, it seems improbable that any exact stoichiometric relationship exists. We suggest that non-integral values of x would be obtained which would depend on the history of the sample. Eanes (1970) suggests a value of x = 3 for his samples of ACP where weight losses of 15% on dehydration at high temperature were observed. Duplication of conditions in the freeze-drying procedure may well result in duplication of weight loss, as in samples 2 and 3 of ACP, and all three samples of HA shown in Table 3 above. In agreement with Eanes we calculate that values of x closely approximate to 3 for samples 2 and 3 of ACP. However, a value close to 4 would correspond to the 22% loss observed for sample 1. In other samples of ACP we have measured weight losses as high as 30%. This would result in an even higher calculated value of x. The 10% weight loss observed on the three HA samples is probably largely the result of multilayer adsorption on this material of high surface area. We conclude that a value of x based on weight loss is probably dependent on the freeze-drying technique and the history of the sample and that this value reflects the effect of both water of hydration and adsorbed water.

All the strongly-held water (heat of adsorption ≥14 kcal/mole) can be accommodated in an adsorbed monolayer on the HA and there is no evidence for water held inside the crystalline particles. In the case of the ACP however we are forced to conclude that only about 25% of the water can be in the adsorbed monolayer, the remaining 75% being held probably as water of hydration inside the amorphous particles. Since both HA and ACP constitute the mineral component in bone, these observations may have some significance toward an understanding of the microstructure of bone *in vivo*. A more detailed study involving the measurement of adsorption isotherms and ultimately heats of adsorption of water on HA and ACP is now in progress.

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