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Calcium Phosphates with Apatite Structure. I. Precipitation at Different Temperatures

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Precipitation of basic calcium phosphates from aqueous solutions by dropwise addition of ammoniacal calcium chloride and ammonium phosphate in stoichiometric ratio to ammonia buffer at pH ~ 9 and different temperatures from 25 to 98 °C has been studied. The products were investigated by chemical analysis and X-ray diffraction, and were identified as hydroxyapatite, $Ca_5(PO_4)_3OH.aq$, with properties similar to those of biological apatites.

The mineralized or hard tissue of teeth consists of an organic matrix embedding inorganic material. The former contains collagenous fibres and a structureless ground substance, and the latter includes crystallized and noncrystallized inorganic salts; it is often called the mineral. Strictly, a distinction must be made between the mineral phase as such and the inorganic fraction as a whole, since the latter includes any inorganic material associated with the organic or tissue fluid components. The various types of hard tissue, *viz.* enamel, dentine, cementum, and bone, differ slightly in their mineral content as shown in Table 1. The mineral phase of human teeth is essentially calcium phosphate with apatite structure, and the major ion constituents are Ca^{2+}, PO_4^{3-}, OH^-, and CO_3^{2-}.[1,3-6] Much research has been devoted to studies of structure, chemical and physicochemical properties of natural and synthetic basic calcium phosphates, and to comparison of these properties with those of biological apatites.[7-11]

In 1970 Jervøe [12] reported experimental evidence for changes in the crystallinity of mature human teeth irradiated both *in vitro* and *in situ*. The question whether the effect was primary or secondary, *i.e.* caused by products of radiolysis in the non-mineral phase, was let open. Since teeth consist of many different substances of both organic and inorganic nature, it is difficult to identify cause and effect of radiolysis in the crystalline structure. No method for removal of the organic constituents of enamel, dentine, and bone without structural modification of the mineral phase is known at present.[13-19] The literature concerning basic calcium phosphates is extensive. Most experiments described are often performed on purchased specimens with uncertain chemical composition, homogeneity, dispersity, polydispersity, and average crystal-

Table 1. Chemical contents of human enamel, dentine, cementum, and bone, after Orban.[1] Values are percentage dry weight.

Content	Enamel	Dentine	Cementum Bone
Water	2.3	13.2	32
Organic Matter	1.7	17.5	22
Ash	96.0	69.3	46
In 100 g of Ash:			
Calcium	36.1	35.3	35.5
Phosphorus	17.3	17.1	17.1
Carbon dioxide	3.0	4.0	4.4
Magnesium	0.5	1.2	0.9
Sodium	0.2	0.2	1.1
Potassium	0.3	0.07	0.1
Chloride	0.3	0.03	0.1
Fluoride[a]	0.016	0.017	0.015
Ca/P	2.08	2.07	2.28[b]
Ca/P (molar)	1.64	1.64	1.77[b]

[a] Depends on F^- content of drinking water. [b] Calculated from values given by Morse and Looney.[2]

linity. Even authors preparing their own specimens have not published data in such detail that we could use their prescriptions in our radiochemical experiments. For this reason we have synthesized pure calcium phosphate with structure, chemical, and physicochemical properties similar to those of biological apatites for later use in irradiation experiments. The method has been direct precipitation, according to the reaction

$$5Ca^{2+} + 3PO_4^{3-} + OH^- \rightarrow Ca_5(PO_4)_3OH$$

The calcium phosphates described in this paper can presumably be used with advantage in other than radiolytical experiments because they are well-characterized.

EXPERIMENTAL

Apparatus. Examinations of precipitates by X-ray powder diffractometry were carried out using both a Hägg-Guinie rcamera with Cu$K\alpha_1$-radiation and a General Electric diffractometer (type XRD-3) equipped with a scintillation counter, Speedomax recorder, and with Ni-filtered Cu$K\alpha$-radiation. The camera has been calibrated by means of recrystallized NaCl, a scale has been printed on the film before exposing, and an apparatus constant considering these properties has been calculated for the computer program.

Colorimetric determinations were carried out using a Beckman DU spectrophotometer.

Water content was determined using a Stanton automatic Thermo-recording balance, model HT-FS, in combination with a Univel-temperature programmer type TVP-2.

Materials. Calcium phosphate was prepared using C. P. grade chemicals and glass-distilled water. We shall not go into details but draw attention to the fact, that we have wasted a lot of time on unsuccessful experiments because we had not controlled the quality of the water. The samples were prepared by mixing equal volumes of solution A: 0.167 M $CaCl_2$, 0.40 M NH_3, and solution B: 0.10 M $(NH_4)_2HPO_4$, 0.20 M NH_3, with solution C: 0.10 M NH_4Cl, 0.10 M NH_3. The solutions A and B were added dropwise to solution C, which was heated to the desired temperature of precipitation. A pH of about 9 was calculated from the composition of the mixtures, and this value was considered favourable for the formation of hydroxyapatite. Variation of temperature enabled the preparation of fractions with different crystallinities comparable with those of human enamel and dentine. Ageing of the precipitate was allowed to proceed for 24 h. The solid phase was collected on a glass filter with a pore size of 10 – 20 μm, washed 6 times with 3 litre portions of boiled, deionized water, then once with acetone, and finally dried in air. A basic calcium phosphate, prepared by A. Tovborg Jensen, (ATJ), precipitated at 100 °C in 1936 as sample No. 8, allowed to age in a flask until 1942 when washed and dried in air, was used as a reference material in the X-ray diffraction investigation.

Analytical methods. Calcium was determined by complexometric titration,[20] phophorus colorimetrically using the molybdenum-blue method.[21] carbonate as described by Larsen,[22] chloride by potentiometric titration using silver nitrate, and ammonium with Nessler's reagent.

RESULTS

Table 2 shows the data from chemical analyses, expressed as weight %. The amount of calcium phosphate precipitated at 25 °C was too small to be analysed. Water content was calculated from weight loss at 900 °C after substraction of carbonate contribution to this figure. The percentages should add up to 100; however, $Ca_5(PO_4)_3OH$ is stable at 900 °C, which means that, if all phosphate present at this temperature is apatite, a correction of 0.5 mol water per mol apatite should be added. This has been done

Table 2. Results from chemical analysis on basic calcium phosphates with apatite structure precipitated at different temperatures.

Temp. °C	CaO %	P_2O_5 %	CO_2 %	H_2O %	NH_3 %	HCl %	Total %	Total corr. %
35	51.5	37.6	0.725	8.96	3.7×10^{-3}	0.02	98.8	100.5
45	50.6	37.8	0.602	7.51	4.1×10^{-3}	0.02	96.5	98.1
55	52.3	38.0	0.743	6.02	4.4×10^{-3}	0.02	97.0	98.7
65	53.0	39.8	0.387	5.41	4.1×10^{-3}	0.02	98.6	100.3
75	53.1	39.5	0.456	4.81	3.1×10^{-3}	0.01	97.9	99.6
88	54.0	39.5	0.456	3.07	3.4×10^{-3}	0.01	97.1	98.8
98	54.1	40.7	0.178	4.02	6.5×10^{-3}	0.03	99.0	100.7

Table 3. Calculated Ca/P molar ratio, CO_3^{2-} and OH^- contents, and molecular weight, from chemical analysis on basic calcium phosphates with apatite structure precipitated at different temperatures.

Temp. °C	Ca/P mol ratio	$CaCO_3$ mol	$Ca(OH)_2$ mol	H_2O mol	Molecular weight
35	1.73	0.093	0.097	2.72	568
45	1.69	0.077	0	2.35	552
55	1.74	0.094	0.118	1.75	552
65	1.68	0.047	0	1.61	536
75	1.70	0.056	0.040	1.40	536
88	1.73	0.056	0.127	0.79	532
98	1.68	0.021	0.011	1.16	526

in the last column of Table 2, and the total is seen to be correct within a standard deviation of 1 % which is completely satisfactory.

The second column in Table 3 gives the calcium to phosphorus molar ratio, which is $5/3 = 1.67$ in pure hydroxyapatite. All preparations contain a slight excess of calcium, which may be assumed to be present partly as carbonate, partly as hydroxide. In the third and fourth column of Table 3 these amounts are expressed as mol per mol apatite. The figures

Table 4. Unit cell dimensions of basic calcium phosphates with apatite structure precipitated at different temperatures obtained from least-squares refinement of a series of Guinier-film measured 2θ angles, and of human enamel and dentine obtained from least-squares refinement of a series of diffractometer-measured 2θ angles.

Temp. °C	*a*-axis Å	*c*-axis Å	Number of reflections
25	9.420(6)	6.891(4)	12
35	9.432(5)	6.888(3)	15
45	9.434(2)	6.892(2)	26
55	9.433(2)	6.892(2)	34
65	9.435(2)	6.890(2)	35
75	9.438(2)	6.893(1)	46
88	9.439(1)	6.896(1)	50
98	9.441(1)	6.894(1)	53
100 (ATJ)	9.443(2)	6.896(2)	43
Hum. Enamel [a]	9.437(3)	6.883(3)	28
Hum. Dentine [a]	9.434(7)	6.868(9)	18

Values within parenthesis refer to standard deviation in the least significant digits. [a] Values are the average for 23 samples.[23]

Table 5. *d*-Spacings, *hkl*-values from 98 °C precipitated calcium phosphate calculated from Hägg-Guinier photograph and relative intensities (I^x) calculated from diffractogram.[a]

d Å	*hkl*	I^x	*d* Å	*hkl*	I^x	*d* Å	*hkl*	I^x
8.166	100	12	1.945	222	29	1.408	413	6
5.268	101	4	1.891	312	13	1.349	512	8
4.084	200	6	1.872	320	4	1.317	431	4
3.891	111	8	1.842	213	33	1.308	520	6
3.447	002	46	1.808	321	15	1.281	423	6
3.172	102	12	1.782	410	12	1.267	602	4
3.089	210	17	1.755	402	12	1.257	215	6
2.819	211	100	1.722	004	15	1.245	610	4
2.780	112	67	1.645	322	8	1.238	414	6
2.725	300	62	1.612	313	4	1.222	522	6
2.634	202	27	1.574	330	4	1.177	315	6
2.530	301	6	1.543	420	4	1.159	433	4
2.298	212	6	1.531	331	4	1.149	006	4
2.267	310	21	1.504	214	6	1.137	523	4
2.153	311	4	1.476	502	8	1.116	442	4
2.063	113	5	1.454	304	8	1.110	325	4
2.039	400	5	1.453	323	8	1.104	206	4
2.000	203	4	1.434	511	6			

[a] The peak of maximum is arbitrarily assigned a value of 100 and the other peaks are expressed in terms of percentages of this value.

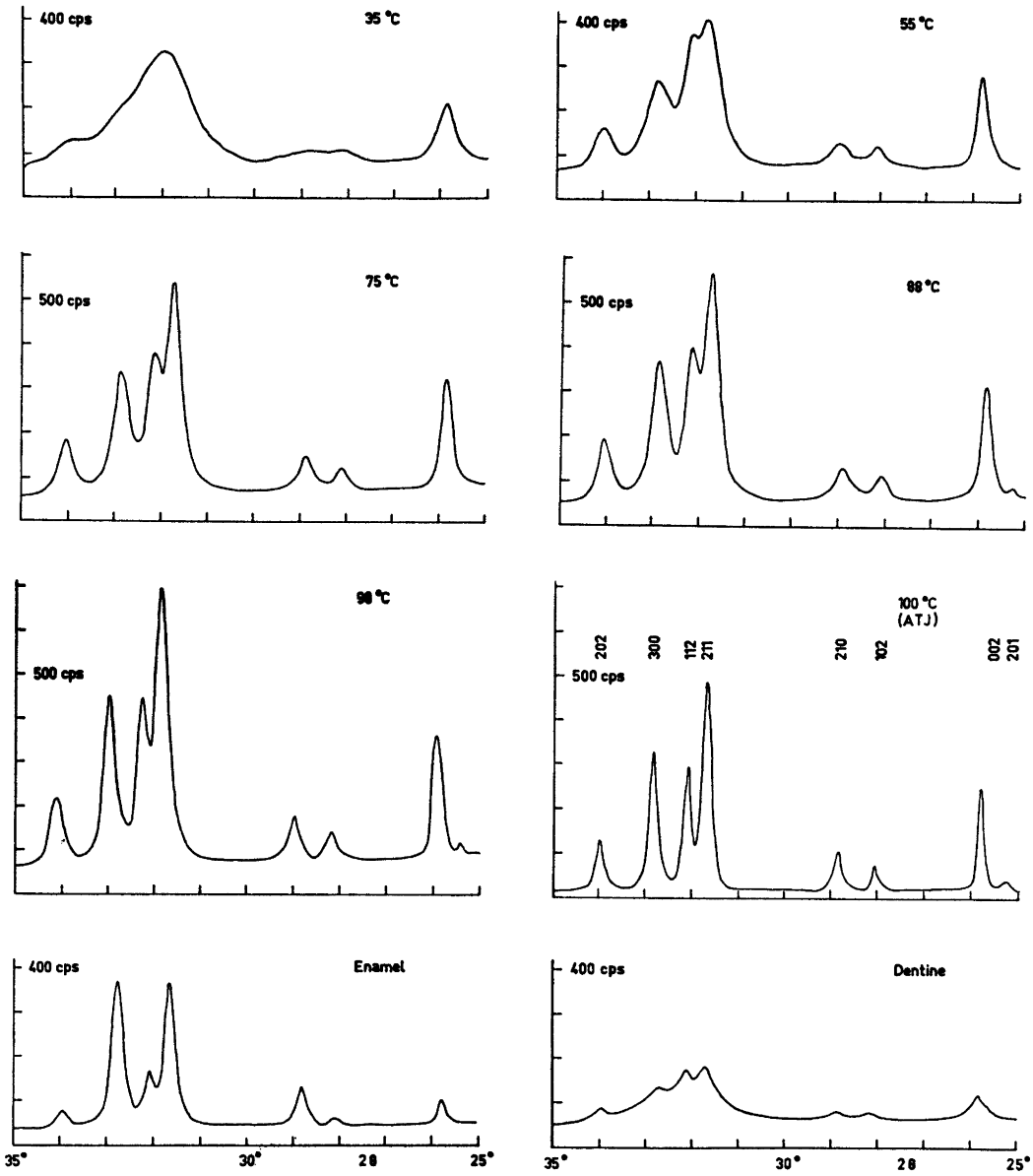

Fig. 1. Diffractograms of basic calcium phosphates with apatite structure precipitated at different temperatures, precipitated at 100 °C in 1936 (ATJ), and of human enamel and dentine.

in the fifth column express the total water content in the samples (water of crystallization or adsorbed water *etc.*) per mol apatite. Finally, the sixth column contains the "molecular weights", *i.e.* the amounts of substance in gram containing 1 mol $Ca_5(PO_4)_3OH$.

All X-ray reflections from both powder photographs and diffractograms were measured, *d*-spacings, *hkl*-values, and *a*- and *c*-axes of the unit cells were calculated. Spacings were compared with values obtained from X-ray powder patterns of the ATJ calcium phosphate, human

enamel, and human dentine.[23] The results are given in Tables 4 and 5. Diffractograms of calcium phosphates, and human enamel and dentine are shown in Fig. 1. in the 2θ range from 25 to 35°, which includes the most important reflections of hydroxyapatite. The diffractograms clearly reflect dependence of crystallinity of calcium phosphates on temperature of precipitation.

DISCUSSION AND CONCLUSION

The data of Tables 3, 4, and 5 as well as Fig. 1 establish the identity of all the prepared samples as hydroxyapatite with composition and crystallinity depending uniquely on the temperature of precipitation. The resemblance with the crystalline constituent of teeth is also obvious; the 55 °C fraction is very similar to dentine, the 75 °C to enamel.

The only important difference between our synthetic samples and biological apatites is the Ca/P ratio. Where the latter in general show a Ca deficiency of about 2 %,[1] see Table 1, the former contain an excess ranging from 0.6 to 4 %. This is of the same order of magnitude as the fraction of calcium ions, which are situated in the surface layer of the crystals. At a pH about 9 absorption of Ca^{2+} by the precipitate is very likely, much more so than in acid solution; presumably, OH^- acts as counterion (to assure electroneutrality), but is more or less exchanged for CO_3^{2-} during filtering, washing, and storage. Precipitation at a lower pH would probably lead to a lower Ca/P ratio, but then the preparation would require much more time, and it would be difficult to avoid contamination of the apatite by other calcium phosphates, in particular $Ca_4H(PO_4)_3$. Though metastable, this substance crystallizes readily from neutral solution and is transformed very slowly to hydroxyapatite, especially at low temperature.

As we would not expect the adsorbed layer to play any role in the behaviour of the synthetic apatites upon irradiation, we shall consider the results of experiments with these to be valid also for biological materials.

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