STUDIORUM PROGRESSUS

Hydroxyapatite: Preparation and Properties

The properties of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), both natural and synthetic, vary widely. The length of particles can be from 10^2 to 10^6 nm, the Ca/P mole ratio from 1.2 to 2.0 and solubility product constants may vary over a range of 10^{11}.

In order to interpret experiments involving hydroxyapatite it is necessary to have information on the behaviour of pure hydroxyapatites with well-defined properties. Slow progress in this field is taking place all the time and the purpose of this paper is to compare the properties of a hydroxyapatite prepared according to a method which has been used in this laboratory (LRS) for some years with a more recent method suggested by workers at the National Bureau of Standards (NBS) (Moreno et al.[1]). The methods and preparations will be designated LRS and NBS respectively.

Experimental. The LRS hydroxyapatite was prepared according to an adaptation of the method of Warington[2]. $(NH_4)_2HPO_4$ solution (42 g/l) was slowly added to a solution of $Ca(NO_3)_2 \cdot 4H_2O$ (19.3 g/l) at 70°C. The pH of the reactants was maintained in the range 9.0–9.5 by addition of concentrated NH_4OH. The precipitated hydroxyapatite was washed by decantation with glass-distilled water and then ethanol prior to air drying.

The NBS hydroxyapatite was prepared as described by Moreno et al.[1], in which $(NH_4)_2HPO_4$ (9.6 g/l) and $Ca(NO_3)_2 \cdot 4H_2O$ (31.4 g/l) solutions are reacted at 100°C in the presence of acetate ions. Precautions were taken against CO_2 contamination and the hydroxyapatite formed was washed with a solution of H_3PO_4 (pH 3) and then as in the LRS method.

The hydroxyapatites were characterized by the following methods: a) Chemical analysis after dissolution in a dilute solution of HCl; P by the colorimetric method of Fogg and Wilkinson[3], Ca by atomic absorption spectrophotometry. b) Degree of crystallinity by X-ray diffraction analysis. c) Specific surface areas by the continuous flow technique of Nelsen and Eggertsen[4]. d) Electron microscopy at magnifications up to 50,000. e) Solubility measurements over the pH range 6–7 by addition of H_3PO_4 to apatite suspensions and equilibration for 20 days before determining P and Ca in solution. f) Isoionic phosphate exchange (Neuman and Neuman[5]) by pre-equilibrating 0.25 and 0.5 g apatite in 700 ml glass-distilled water for 4 days prior to the addition of carrier-free $H_3{}^{32}PO_4$. g) Surface exchangeable P (Olsen[6]).

Results and discussion. Chemical analysis. Chemical analyses of the 2 preparations (Table I) show that the LRS preparation contained somewhat less Ca and P than the theoretical amount for pure hydroxyapatite, probably because of the inclusion of water and carbon dioxide in the lattice. The P content of the NBS preparation was almost identical to the theoretical P content and the Ca content was slightly higher than the theoretical value. The calculated Ca:P mole ratios are slightly higher than the theoretical value but they are both well below the upper limit of 2.0 for hydroxyapatite suggested by Arnold[7].

X-ray diffraction analysis. The X-ray diffraction patterns of the 2 preparations were both characteristic of hydroxyapatite and no extraneous peaks were observed. The pattern for the NBS preparation was considerably sharper than that for the LRS preparation (Figure 1), indicating a higher degree of crystallinity.

Surface area. The specific surface areas by the continuous flow method were found to be 32.0 and 11.9 m^2/g respectively for the LRS and NBS preparations. Moreno et al.[1] report a specific surface area of 6 m^2/g for their preparation but they used the BET method based on nitrogen as the adsorbate (Young and Crowell[8]).

Electron microscopy. The electron micrographs of the 2 preparations (Figure 2) show that the crystals obtained by the NBS method are more regularly cylindrical in shape and much larger than those from the LRS method. However, in both cases the crystals are rod-shaped.

The mean dimensions of the crystals were, respectively, LRS hydroxyapatite 240 × 34 nm, NBS hydroxyapatite

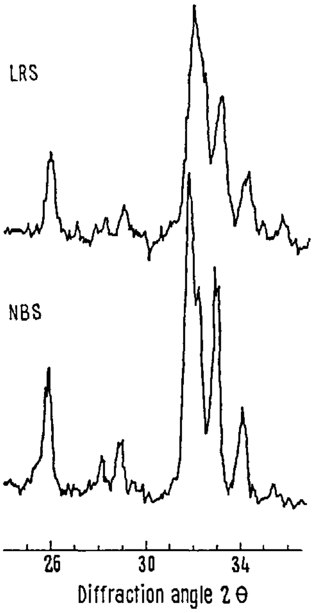

Fig. 1. X-ray diffraction patterns of the 2 hydroxyapatite preparations.

Table I. Chemical analysis and theoretical composition of hydroxyapatite

Preparation	Ca (%)	P (%)	Ca:P (mole ratio)
LRS	38.5	16.9	1.77
NBS	41.1	18.4	1.73
Theoretical value	39.8	18.5	1.67

[1] E. C. Moreno, T. M. Gregory and W. E. Brown, J. Res. natn. Bur. Stand. *72A*, 773 (1968).

[2] R. Warington, J. chem. Soc. *26*, 983 (1873).

[3] D. N. Fogg and N. T. Wilkinson, Analyst, Lond. *83*, 406 (1958).

[4] F. M. Nelsen and F. T. Eggertsen, Analyt. Chem. *30*, 1387 (1958).

[5] W. F. Neuman and M. W. Neuman, *The Chemical Dynamics of Bone Mineral* (University of Chicago Press, Chicago, Illinois 1958).

[6] S. R. Olsen, J. phys. Chem., Ithaca *56*, 630 (1952).

[7] P. W. Arnold, Trans. Faraday Soc. *46*, 1061 (1950).

[8] D. M. Young and A. D. Crowell, *Physical Adsorption of Gases* (Butterworth and Co. Publishers Ltd., London 1962).

1900 × 140 nm. The faster growth rate at the end of the NBS crystals is probably caused by less contamination with CO_2 in this preparation (LeGeros et al.[9]).

The specific surface areas calculated from the electronmicrographs are given in Table II. The calculation was based on the assumption that the crystals were cylindrical and that the specific gravity of hydroxyapatite is 3.2. Considering these approximations and the uncertainties associated with surface determinations the agreement between this method of determining the specific surface area and the continuous flow technique is excellent.

Solubility. The solubility product expressed as 10 pCa + 6 pPO_4 + 2 pOH (Table III) shows that the LRS hydroxyapatite was considerably more soluble than the NBS hydroxyapatite. Moreno et al.[1] found that the value for the solubility product of their preparation expressed as pK_{sp} of Ca_{10} $(PO_4)_6(OH)_2$ decreased from 116.2 to 111.2 when the pH was increased from 4.74 to 7.45. Our results indicated a similar relationship between pH and solubility product but the solubility of our NBS preparation was considerably less than that of Moreno et al. The reason for this may be found in the solid: solution ratio which was 0.36, 0.71 and 1.00 g/l in our experiment and 41.7 g/l in their experiment. It is well established that the solubility of hydroxyapatite can increase markedly with slurry density (Deitz et al.[10]).

The observed Ca:P mole ratios in the solution phase (Table III) are somewhat higher than the theoretical ratio of 1.67 for a solution saturated with hydroxyapatite. The excess calcium over phosphorus in solution is consistent with the slight excess of calcium in the solid as shown by chemical analyses (Table I). The much

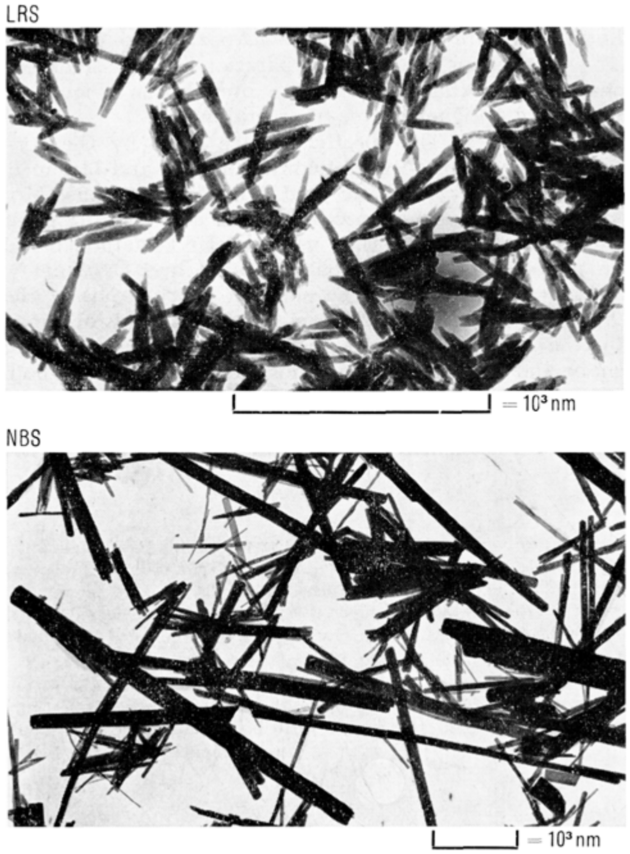

Fig. 2. Electron micrographs of the 2 hydroxyapatite preparations. (Please note different scales and units; 1 nanometre (nm) = 10^{-9} m.)

Table II. Surface area measurements of hydroxyapatite

Preparation	Method of measurement (area in m^2/g)		
	Continuous flow	Electron microscopy	^{32}P exchange
LRS	32.0	42.0	33.6
NBS	11.9	12.3	3.3

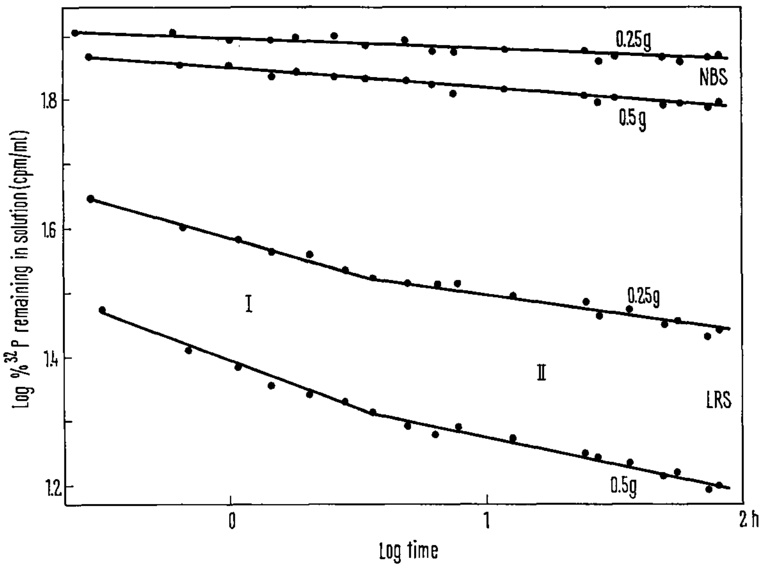

Fig. 3. The relationship between the logarithm ot percent ^{32}P remaining in solution and the logarithm of time.

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[9] R. Z. LeGeros, O. R. Trautz, E. Klein and J. P. LeGeros, Experientia *25*, 5 (1969).

[10] V. R. Deitz, H. M. Rootare and F. G. Carpenter, J. Colloid Sci. *19*, 87 (1964).

Table III. Solubility measurements

Suspension identification	Solid: solution ratio g/l	pH	Solubility (ppm solution) Ca	P	Ca:P mole ratio of dissolution	pK sp of hydroxy-apatite[a]
LRS	0.36	7.34	4.05	1.46	2.10	111.0
LRS	0.71	7.39	4.55	1.39	2.58	110.1
NBS	0.36	7.15	2.65	1.06	1.94	115.6
NBS	0.71	7.14	3.00	1.26	1.85	114.8
LRS + H_3PO_4	1.0	6.55	8.10	7.95		113.0
LRS + H_3PO_4	1.0	6.26	13.54	16.66		112.8
NBS + H_3PO_4	1.0	6.23	7.66	9.47		117.0
NBS + H_3PO_4	1.0	5.88	13.10	18.04		117.9

[a] Calculated according to CHAVERRI and BLACK[11].

greater Ca:P ratios in the solution than in the solid suggest that the excess calcium in the solid was greatest on or near the surface of the hydroxyapatite crystals[11].

Isoionic exchange. The results of the isoionic exchange determinations are given in Figure 3 in which the logarithm of percentage ^{32}P remaining in solution is plotted as a function of the logarithm of time.

In the case of NBS hydroxyapatite, only one linear relationship between log ^{32}P in solution and log time was observed during the experimental period of 90 h. The negative slope of this relationship was 0.027 and 0.019 respectively for 0.5 and 0.25 g hydroxyapatite per 700 ml of solution. In the case of the LRS hydroxyapatite, two linear relationships were observed in the same period. The negative slopes of these were 0.127 (I), 0.076 (II) and 0.108 (I), 0.053 (II) respectively for 0.5 and 0.25 g hydroxyapatite per 700 ml solution. Thus, only one reaction mechanism seems to be involved in the exchange of phosphate ions between the NBS hydroxyapatite and the bathing solution while two reaction mechanisms appear to be operating in the case of the LRS hydroxyapatite.

'Surface-exchangeable P' calculated according to OLSEN[6] was 8.25 and 8.40 mg P/g respectively for 0.5 and 0.25 g/700 ml suspensions of LRS hydroxyapatite and 0.80 and 0.83 mg P/g for similar suspensions of the NBS hydroxyapatite. The LRS hydroxyapatite appeared to have about 10 times more 'surface-exchangeable P' than the NBS hydroxyapatite, but the solid/solution ratio had little or no effect for either material.

OLSEN's conversion factor of 4.20 was used to transform surface-exchangeable P to specific surface area (Table II). Good agreement between the specific surface area measured by the continuous flow technique and the ^{32}P method was obtained for the LRS hydroxyapatite but not for the NBS material, suggesting that the factor differs between preparations.

Measurements of the surface area of finely divided solids are, however, notoriously unreliable and too much significance should not be attached to them. The relative surface areas, i.e. the specific surface area of the LRS hydroxyapatite divided by the specific surface area of the NBS hydroxyapatite could be more informative. This ratio is 2.7, 3.4 and 10.2 when determined respectively from the continuous gas flow technique, electron micrographs and the ^{32}P exchange method. The much higher ratio obtained by the last method suggests that the isotopic exchange process differed between the 2 preparations. It is probable that much more crystal growth would occur in the suspension of the less perfect LRS crystals than in the suspension of the near-perfect NBS crystals. Thus in the case of the LRS hydroxyapatite, loss of ^{32}P is likely to result from recrystallization as well as from surface exchange while in the NBS preparation only the second mechanism would be important.

Zusammenfassung. Es wurden sowohl ältere als auch neuere Verfahren zur Fällung von Kalziumhydroxyapatit aus wässriger Lösung vergleichend überprüft und die erhaltenen Präparate mittels chemischer und physikalischer Methoden charakterisiert, wobei sich ergab, dass das vom National Bureau of Standards entwickelte Verfahren die am besten definierten Kristalle liefert.

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15 October 1970.

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[11] J. G. CHAVERRI and C. A. BLACK, Iowa St. J. Sci. *41*, 77 (1966).

[12] Acknowledgment. The authors are indebted to the late Dr. R. V. COATES for the X-ray diffraction analyses and to Mr. R. D. WOODS, Department of Plant Pathology, Rothamsted Experimental Station for the electron micrographs.

PRO EXPERIMENTIS

Enzymatic Sulfation of 4-Methylumbelliferone

Enzymatic sulfation of aromatic hydroxy compounds is mostly investigated by using *p*-nitrophenol as the sulfate acceptor. This method, described by ROY[1], makes use of the solubility of the methyleneblue complex of the sulfuric acid ester. We developed a method for the demonstration of sulfating activity using one of the most fluorescent molecules known, 4-methylumbelliferone, 4-methyl-7-hydroxy-coumarin (MU). Various derivatives of MU are in use for the assay of hydrolases, but, so far as we know, no attempt has been made to use the parent