Calcif. Tiss. Res. 18, 13—26 (1975)

Precipitation of Calcium Phosphates from Electrolyte Solutions

III. Radiometric Studies of the Kinetics of Precipitation and Aging of Calcium Phosphates *

R. Despotović, N. Filipović-Vinceković **, and H. Füredi-Milhofer

Department of Physical Chemistry, "Ruđer Bošković" Institute, Zagreb,
Croatia, Yugoslavia

Received May 16, accepted September 30th 1974

Precipitation and precipitate transformation in the system sodium phosphate (pre-adjusted to pH 7.4)—calcium chloride (25°) was studied by means of radiometric analysis using ^{45}Ca and ^{32}P as tracers. Changes in the pH and the total concentrations of calcium and phosphate were followed during solid phase formation and the data were used to calculate composition changes of the precipitates and their supernatants. In all investigated systems two-step precipitation was observed, the precursor being more basic than the secondary precipitate. The composition of the latter was mostly within the range of the composition of octacalcium phosphate. The course of further chemical changes was dependent on the pH established during secondary precipitation. The heterogeneous exchange of the radionuclides between the solid phase and their supernatant solutions was also followed as a function of time. The results indicate that recrystallization through the mother liquid accompanied by composition changes is the dominant mechanism of equilibration of the solid phases.

Key words: Calcium — Phosphate — Precipitation — Aging — Kinetics

Introduction

Bones and teeth as living systems are in constant communication with the surrounding liquids and as such are subject to continuous changes. For this reason studies of the processes resulting in the formation and subsequent transformation of bone mineral and/or its main components, calcium phosphates have been drawing considerable attention [4, 9–16, 18–20, 25, 26, 28–31].

When calcium phosphate is precipitated from alkaline [9] and neutral [4, 30] solutions a noncrystalline, highly hydrated [18] precursor ("amorphous calcium phosphate", ACP) is frequently formed. Considerable attention has been

---

For reprints: Mgr. N. Filipović-Vinceković, Department of Physical Chemistry, "Ruđer Bošković" Institute, POB 1016, Zagreb, Croatia, Yugoslavia.

[*] Part of the results was presented at the International Colloquium on the Physical Chemistry and Crystallography of Apatites of Biological Interest, Paris, 10–15 Sept. 1975 and at the IV Meeting of the Yugoslav Society for Biophysics, Stubičke Toplice, Oct. 28–30 1973.

[**] Part of the M. Sc. Thesis of N. Filipović-Vinceković submitted to the Faculty of Science, University of Zagreb

given to the mode and mechanism of the conversion of ACP into crystalline apatite [4, 11, 14, 25]. It has been demonstrated that the crystalline precipitates may initially show characteristics of octacalcium phosphate [4, 14, 15] and/or calcium deficient apatites with low Ca/P ratios [15, 28] and that such precipitates are subject to subsequent changes in contact with the supernatant liquid [4, 27, 28]. The mechanisms of these aging processes have not yet been elucidated.

In previous papers from this laboratory [4, 14, 15] the results of solid phase analysis, showing morphological, compositional and structural changes of calcium phosphate precipitates in contact with the supernatant liquid were reported. In this work attention is primarily given to changes in solution composition during the formation and transformation of the precipitates. Some recently obtained X-ray data, concerning the conversion of ACP into crystalline apatite are also given. Besides, studies of the heterogeneous exchange of calcium and phosphate ions between the solid and liquid phase yield information on the mechanism of the transformation of the precipitates in contact with the supernatant solutions.

Materials and Methods

Chemicals

Analar grade chemicals (Merck p.a.), and twice distilled water were used. Samples were prepared by mixing calcium chloride solutions with equal volumes of sodium phosphate solutions ($H_3PO_4 + NaOH$ adjusted to pH 7.4) under constant stirring by a magnetic stirrer and were kept at all times in a water bath at 25°. The acidity of the systems was determined by a Radiometer M 4 pH-meter.

The distribution and heterogeneous exchange of calcium and phosphate ions between the supernatants and the respective precipitates were studied on double labelled systems using ^{45}Ca and ^{32}Pa as tracers. Radioisotopes were added to the reactant solutions before mixing (for radiometric analysis) or at time intervals t_A (t_A—aging time) after sample preparations (for heterogeneous exchange studies). At given time intervals the supernatant was separated from the solid phase by a T 20 Janetzki centrifuge (7000 g) and acidified to stop further precipitation. The radioactivity of the liquid phase was measured by a Nuclear-Chicago Liquid Scintillation Spectrometer Mo 720. The scintillator solutions were prepared by dissolution of 5 g of 1,4bis-2(4-methyl-5-phenyloxazolyl)benzene (POPOP) and 100 mg of 2,5 diphenyloxazol (PPO) in 1000 ml of toluene.

For X-ray diffraction analysis samples were prepared by mixing equimolar solutions of the precipitating components and were kept in contact with the mother liquid for 150 min. After that time the precipitates were collected on 220 nm Millipore filters and one part (*D*) was dried for 1 h at 100°. The other part (*W*) was used unaltered, i.e. wet with the residual mother liquid. X-ray powder patterns of dried and wetted samples of stoichiometric hydroxyapatite (prepared after Bett *et al.*, 1967) were recorded as controls. A Philips diffractometer with a scintillation counter and a single-channel pulse height analyser was used for X-ray analysis. The radiation was Ni-filtered Cu-Kα.

Treatment of Data

The results of the measurements of the distribution of radionuclides are expressed as changes of the molar concentrations of calcium and phosphate in the supernatant as shown in Eq. (1):

$$C_{(x)l} = C_{(x)_0}(A_t/A_0) \tag{1}$$

$C_{(x)l}$ and $C_{(x)_0}$ are the molar concentrations of calcium ($x = \mathrm{Ca}$) or phosphate ($x = \mathrm{P}$) in the supernatant at times t and t_0 respectively. A_t represents the radioactivity of the liquid phase at time intervals t, while A_0 is the radioactivity of the whole system which was measured as a standard at the same time intervals.

The sum of the molar concentrations of hydrogen containing species in the supernatant solution at time t ($C_{(\mathrm{H})l}$, in the following text referred to as "total hydrogen") was calculated from the experimental results according to the relation [13]:

$$C_{(\mathrm{H})l} = 3\,[\mathrm{H_3PO_4}] + 2\,[\mathrm{H_2PO_4^-}] + [\mathrm{HPO_4^{-2}}] + [\mathrm{CaHPO_4^0}] + 2\,[\mathrm{CaH_2PO_4^+}] + {} \\ + [\mathrm{H^+}] - [\mathrm{OH^-}] \tag{2}$$

or in the pH range considered in this paper:

$$C_{(\mathrm{H})l} = 2\,[\mathrm{H_2PO_4^-}] + [\mathrm{HPO_4^{-2}}] + \mathrm{CaHPO_4^0} + 2\,[\mathrm{CaH_2PO_4^+}] \tag{2a}$$

The molar concentrations of the ionic species listed on the right hand side of Eq. (2a) were obtained from the experimental data (pH and total molar concentrations of calcium and phosphate in solution) by means of a computer program using previously defined equations [16, 31]. For these calculations quasi-equilibrium in each point was assumed.

Ten subsequent determinations of the concentrations of calcium, phosphate and "total hydrogen" in the liquid phase of a precipitation system (3 mM in calcium and phosphate, aging time 150 min) gave the following results and standard deviations:

$C_{(\mathrm{Ca})l} = 1.80$ mM $\quad \sigma = 0.09$

$C_{(\mathrm{P})l} = 2.23$ mM $\quad \sigma = 0.08$

$C_{(\mathrm{H})l} = 2.97$ mM $\quad \sigma = 0.14$

The numbers of moles of calcium, phosphate and "total hydrogen" in the precipitates obtained from 1 l of sample at time t are:

$$N_{(x)_s} = C_{(x)_0} - C_{(x)l} \tag{3}$$

where $x = \mathrm{Ca}$, P or H

The zero time concentration of "total hydrogen", $C_{(\mathrm{H})0}$ is expressed [13] in terms of the molar concentrations of the reagents constituting the initially added sodium phosphate solution:

$$C_{(\mathrm{H})_0} = 3\,[\mathrm{H_3PO_4}] - [\mathrm{NaOH}]. \tag{4}$$

Values for $C_{(\mathrm{H})_l}$ and $N_{(\mathrm{H})_\mathrm{S}}$ are the resultant values, showing the balance of hydrogen and hydroxyl ions in the supernatant and the precipitate respectively. If $C_{(\mathrm{H})l} > C_{(\mathrm{H})0}$ hydroxyl ions are being incorporated into the precipitate (Fig. 2).

The results of heterogeneous exchange studies are expressed as the fraction exchange F for homogeneous distribution of radionuclides.

$$F = (A_0 - A_t)/(A_0 - A_\infty) \tag{5}$$

where:

$$A_\infty = A_0(1 + \alpha)^{-1} \tag{6}$$

and

$$\alpha = n_\mathrm{S}/n_\mathrm{L}. \tag{7}$$

The subscript letters correspond to exchange times t_E. α is the ratio of the amount of exchangeable ions in the solid (n_S) and liquid phase (n_L).

If the amount of the precipitate changes with time, as was the case in some of the investigated systems the corrections after Wolf [32] are introduced.

In the case of nonhomogeneous distribution of the radionuclides F becomes [8]:

$$1 < F < 1.3$$

Results

An earlier published [15], 24 h precipitation diagram of the system sodium phosphate (pH 7.4)—calcium chloride (25°) served as the orientational basis to select systems for examinations. The concentration products of the chosen sys-

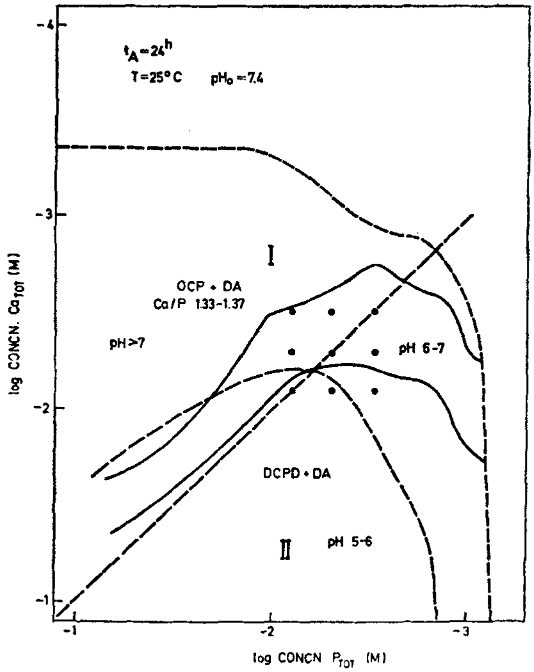


Fig. 1. Schematic presentation of the 24 h (25°) precipitation diagram of the system sodium phosphate (preadjusted to pH 7.4)—calcium chloride after ref. 15. Dots denote concentration products of systems studied in this work

tems (Fig. 1) lie mostly within the concentration region where octacalcium phosphate (OCP) and calcium deficient apatites (DA) with low Ca/P ratios were obtained 24 h after sample preparation. Besides, two systems were selected from the concentration region where mixtures of dicalcium phosphate dihydrate (DCPD) and DA were identified.

The results obtained may be conveniently divided into three parts. The first part comprises radiometric measurments and calculations of changes in solution concentrations (total reactant concentrations and concentrations of ionic species) resulting from the formation and transformation of calcium phosphate precipitates. In the second part some recently obtained X-ray diffractometry data concerning the transformation of ACP into crystalline apatite are shown. Finally, in the third part some results of heterogeneous exchange studies elucidating the mechanisms of further precipitate transformation in contact with the supernatant solution are described.

Part I

In all investigated systems changes in the pH and the concentrations of calcium and phosphate were followed as a function of time. Changes in the concentrations of free and complexed ionic species and of "total hydrogen" [Eq. (2a)] in the supernatant solutions were calculated from the experimental results. An example is shown in Fig. 2 (the concentrations of soluble calcium phosphate

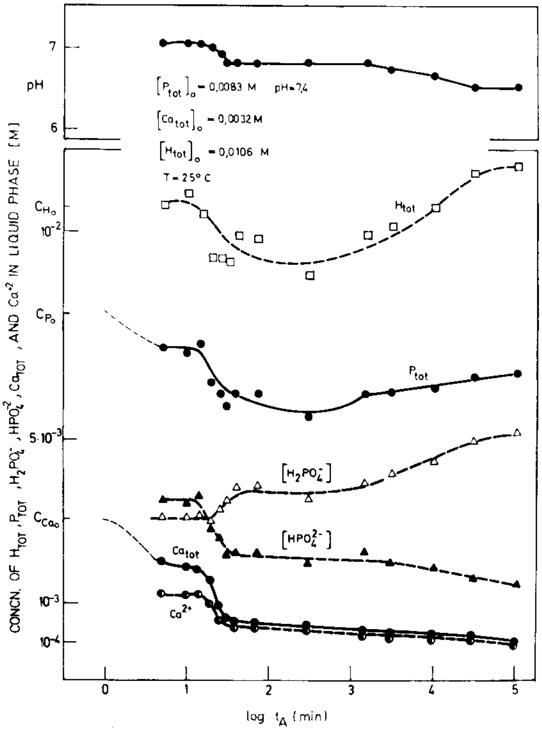


Fig. 2. Time dependent changes of the pH, the total molar concentrations of calcium (Ca_{tot}), phosphate (P_{tot}) and hydrogen containing species (H_{tot}) and of free $H_2PO_4^-$, HPO_4^{2-} and Ca^{2+} ions in the supernatant solution during precipitation. Initial reactant concentrations as indicated

complexes are not represented). In Fig. 3 time dependent changes of the molar Ca/P and H/P ratios of the precipitates formed in three systems with different initial reactant concentrations are shown. In the upper part of the diagram the respective pH curves are given for comparison.

In all investigated systems precipitation took place in two distinct steps, the initial formation of a metastable precipitate and secondary precipitation (Fig. 2). Precipitate formation, causing displacement of the phosphate containing species brought about significant changes of the solution pH [4, 15, 25]. In Fig. 2 the corresponding shift in ionic equilibria is represented.

In most systems the composition of the initial precipitate could not be determined by analysis of the supernatant since the expected changes in the total concentration of at least one of the components were within the experimental error. However, it was established that the first formed precipitates were always more basic than the secondary ones, discontinuous changes in composition occurring at the time of secondary precipitation (Fig. 3).

The stability of the initial and the composition of the secondary precipitates were dependent on the initial reactant concentrations. In most systems Ca/P

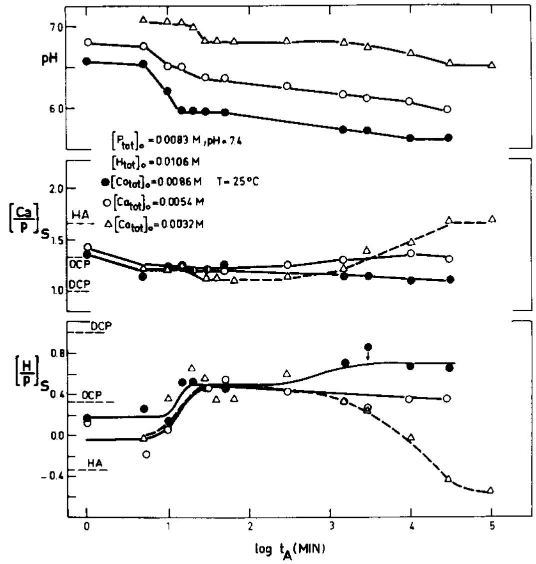


Fig. 3. Changes of the molar Ca/P and H/P ratios of the precipitates and the acidity (pH) of the precipitation systems as a function of time. Initial reactant concentrations as indicated

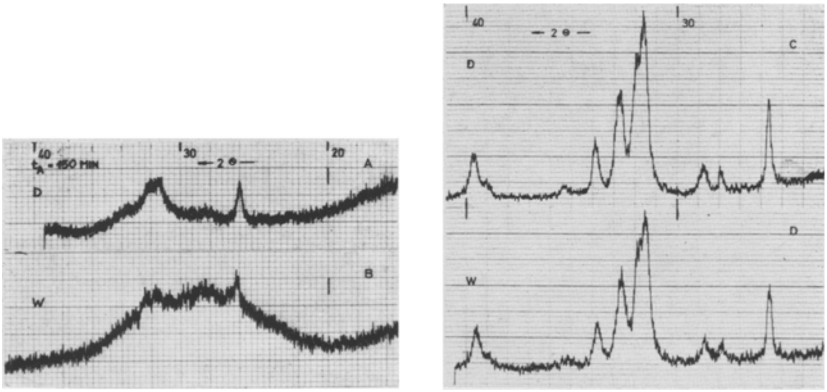
and H/P ratios of the secondary precipitate were in the region of those of OCP (Fig. 3). Only at initial reactant concentrations $Ca_{tot} = 8$ mM, $P_{tot} = 5$ mM H/P values of about 0.7, i.e. between those for OCP and DCPD were obtained. Further composition changes were dependent on the pH established during secondary precipitation. If this was within $5.8 < pH < 6.5$ composition changes were slow or negligible. At $pH > 6.5$, precipitates became increasingly basic and at $pH < 5.8$ increasingly acid with time (Fig. 3).

Part II

There are indications in the literature [24], that pretreatment of preparations, such as drying and other methods, might substantially alter X-ray diffractometry data obtained from calcium phosphate precipitates. For this reason some of our earlier results [4] were re-examined by a different technique as shown in Fig. 4. Curves A and B in Fig. 4a show X-ray diffraction powder patterns of a precipitate isolated shortly after secondary precipitation had taken place. Curve A which was taken from a dried precipitate shows a higher order of crystallinity than curve B which was taken while the precipitate was in contact with the supernatant solution. Apparently the structure of the precipitate significantly changed during drying. The controls, dry and wetted stoichiometric hydroxyapatite gave identical X-ray powder patterns (Curves C and D in Fig. 4b).

Part III

Figs. 5 and 6 and Table 1 show some results of measurements of the kinetics of heterogeneous exchange of calcium and phosphate ions (labelled with ^{45}Ca



a b

Fig. 4. Diffractograms of calcium phosphate precipitates taken 150 min after sample preparation (4a, Curves A, B) and of stoichiometric hydroxyapatite (4b, Curves C, D). Diffractograms A and C were obtained from dry (D) precipitates while B and D were obtained from precipitates which were in contact with the supernatant solution (*W*)

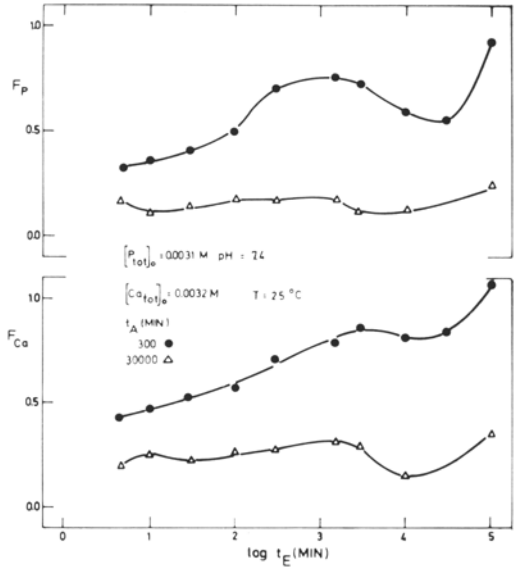


Fig. 5. The fraction exchange of ^{45}Ca (F_{Ca}) and ^{32}P (F_P) as a function of the exchange time t_E (log scale). Initial reactant concentrations and aging times indicated in the diagram

and ^{32}P) between calcium phosphate precipitates and their supernatant solutions. In Fig. 5 the exchange fractions F_{Ca} and F_P are plotted as functions of the exchange time t_E, for a relatively fresh ($t_A = 300$ min) crystalline precipitate and one that was aged for a considerable time ($t_A = 30000$ min) before labelling. In both cases characteristic maxima and minima are observed, the exchange capacity of the precipitates obviously decreasing with aging. When synthetic

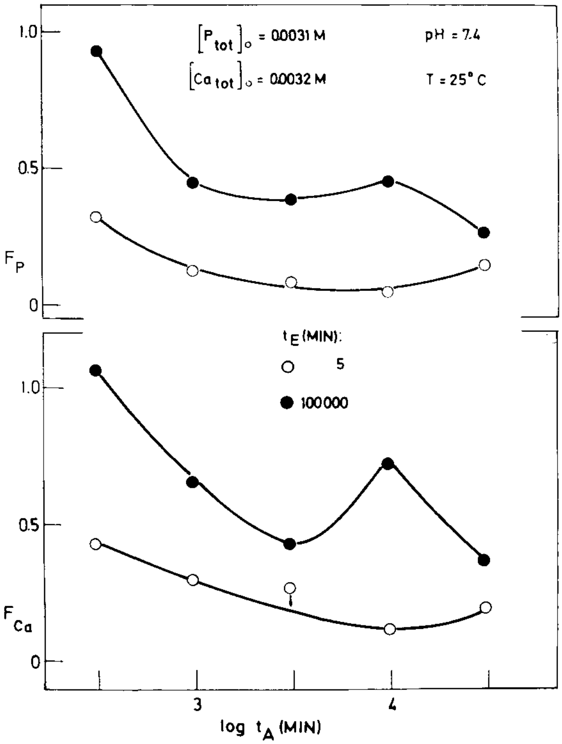


Fig. 6. The fraction exchange of $^{45}Ca(F_{Ca})$ and $^{32}P(F_P)$ as a function of the aging time t_A (log scale). Initial reactant concentration and exchange time as indicated in diagram

(prepared after Newesely, 1960) previously dried octacalcium phosphate was reequilibrated with near neutral solutions (pH 6–6.5) similarly shaped F_{Ca} and F_P v.s. t_E curves were obtained, only the maxima and minima were much less expressed.

In Fig. 6 the dependence of the same parameters on the aging time, t_A is shown. A complete set of data obtained from two characteristic experiments (system A: P_{tot} 3 mM, Ca_{tot} 3 mM, Figs. 5, 6; system B: P_{tot} 8 mM, Ca_{tot} 3 mM, Figs. 2, 3) is listed in Table 1. It is apparent from the Table that calcium ions are exchanging faster than phosphate ions.

Discussion

It has been shown (compare also ref. 13) that in unbuffered systems the pH can be used as an additional parameter to calculate composition changes of precipitates which incorporate hydrogen containing species and/or hydroxyl ions. Although the values of "total hydrogen" obtained in the kinetic experiments must be considered approximate and show only overall concentration changes, valuable additional information on the calcium phosphate system could nevertheless be obtained.

Table 1. The fraction exchange of ^{45}Ca (F_{Ca}) and ^{32}P (F_P) as a function of the exchange time t_E and the aging time t_A. Initial reactant concentrations: total calcium 0.0032 M, total phosphate 0.0031 M (system A) and total calcium 0.0032 M, total phosphate 0.0083 M (system B), pH of phosphate solution 7.4, $t = 25°$ C

t_A (min)	300			1000			3000			10000			30000		
t_E (min)	pH	F_{Ca}	F_P	pH	F_{Ca}	F_P	pH	F_{Ca}	F_P	pH	F_{Ca}	F_P	pH	F_{Ca}	F_P
System A															
0	6.37	—	—	6.27	—	—	6.17	—	—	6.10	—	—	5.87	—	—
5	6.37	0.43	0.32	6.27	0.30	0.11	6.17	—	—	6.10	0.12	0.05	5.87	0.20	0.15
10	6.37	0.47	0.35	6.24	0.34	0.12	6.17	0.21	0.19	6.10	0.16	0.10	5.87	0.25	0.09
30	6.37	0.52	0.42	6.27	0.32	0.12	6.17	0.25	0.11	6.10	0.37	0.31	5.87	0.24	0.11
100	6.34	0.57	0.49	6.26	0.42	0.17	6.15	0.29	0.11	6.09	0.30	0.24	5.87	0.28	0.17
300	6.33	0.71	0.70	6.24	0.32	0.17	6.15	0.21	0.07	6.04	0.17	0.13	5.80	0.28	0.18
1500	6.23	0.79	0.74	6.16	0.49	0.27	6.12	0.30	0.10	6.02	0.34	0.12	5.80	0.31	0.17
3000	6.15	0.86	0.72	6.10	0.37	0.45	6.11	0.26	0.09	6.03	0.35	0.18	5.80	0.27	0.10
10000	6.09	0.81	0.59	6.03	0.42	0.38	6.03	0.42	0.03	5.91	0.34	0.19	5.76	0.15	0.13
30000	5.87	0.84	0.55	5.80	0.69	0.37	5.80	0.39	0.09	5.76	0.31	0.12	5.76	—	—
100000	5.76	1.07	0.92	5.77	0.67	0.45	5.72	0.43	0.38	5.72	0.73	0.45	5.76	0.37	0.26
System B															
0	6.79	—	—	6.77	—	—	6.76	—	—	6.73	—	—	6.65	—	—
5	6.79	0.75	0.19	6.77	0.65	0.27	6.76	0.59	0.22	6.73	0.70	0.14	6.65	0.79	—
10	6.79	0.72	0.15	6.77	0.66	0.21	6.76	0.64	0.23	6.73	0.73	0.14	6.65	0.82	—
30	6.79	0.75	0.37	6.77	0.67	0.27	6.76	0.69	0.27	6.73	0.74	0.18	6.65	0.81	—
100	6.78	0.82	0.38	6.77	0.74	0.40	6.76	0.73	0.26	6.73	0.17	0.17	6.65	0.79	—
300	6.77	0.88	0.52	6.76	0.82	0.30	6.76	0.75	0.31	6.73	0.78	0.20	6.65	0.84	—
1500	6.76	—	—	6.76	0.84	0.44	6.75	0.94	0.53	6.72	0.99	0.73	6.64	0.86	—
3000	6.76	0.99	0.81	6.75	0.91	0.43	6.75	0.85	0.36	6.72	0.83	0.57	6.63	0.86	—
10000	6.73	1.02	0.81	6.72	0.97	0.78	6.72	0.93	0.59	6.65	—	—	6.60	0.94	—
30000	6.67	0.96	1.07	6.62	1.03	0.72	6.62	1.02	0.66	6.62	0.99	0.79	6.60	0.90	—
100000	6.57	—	—	6.55	0.98	—	6.55	0.98	—	6.62	0.99	—	6.60	0.94	—

The data pertaining to the initially formed precipitates (Figs. 2, 3) indicate that in most systems either equal amounts of HPO_4^{2-} and OH^- ions or no hydrogen containing species at all were incorporated.

Since earlier experiments [4] showed the presence of HPO_4^{2-} ions in amorphous calcium phosphate obtained under similar conditions, it follows that the composition of the precursor might be $Ca_9(HPO_4)_x(PO_4)_{6-x}(OH)_x$ ($x < 0.5$) as proposed by Heughebart and Montel (1973). We have found no evidence that dicalcium monohydrogen phosphate is a precursor [12] even to precipitates with Ca/P and H/P molar ratios between those of OCP and DCPD.

In accordance with earlier findings the composition of the secondary precipitates obtained in most of the experiments was in the range of the composition of OCP. It is thus confirmed, that in the neutral pH range this compound is likely to be a transient to apatite formation provided that the pH is allowed to change freely during the reaction. The range of the corresponding reactant concentrations has been previously given [15] and is also shown in Fig. 1. X-ray diffractometry data (Fig. 4, indicate, that the amorphous/crystalline conversion may not be completed during secondary precipitation. It is therefore feasible that calcium phosphates of different crystallinity and/or composition may persist for some time after secondary precipitation. The X-ray data seem to confirm the observations made by Pautard (1973) who suggested only recently that X-ray data obtained from pretreated bone mineral may lead to erroneous conclusions.

In order to obtain more information on the mechanisms of the equilibration processes, which take place after the conversion of ACP into crystalline apatite, heterogeneous exchange studies [7], were also performed. The heterogeneous exchange of constituent ions between solids and supernatant solutions may be controlled by different processes, such as recrystallization through the supernatant liquid in the sense of the Gibbs-Kelvin rule (Ostwald ripening and others), chemical transformation of the solid or liquid phase or both, chemical and physical surface reactions, self-diffusion into the crystal lattice and into the bulk of the solution etc. Plots of the fraction exchange, F, against the exchange time, t_E, indicate the dominant mechanisms of equilibration of precipitates with their supernatants.

In Fig. 7 a schematic F, t_E plot, indicating Ostwald ripening as the dominant mechanism in a system containing a large (L), a medium sized (M), and a small (S) crystal is shown. At the time of labelling, t_0, the fraction exchange, F, is zero. In the time interval from t_0 to t_1 crystals L and M grow incorporating radioactivity while the smallest crystal S will be completely dissolved. Thus the fraction exchange increased and reaches its first maximum. From time t_1 to t_3 the radioactive crystal layer of crystal M will dissolve while L grows, the net results being increasing solution radioactivity and decreasing fraction exchange. After a minimum has been reached (the radioactive layer of crystal M completely dissolves), only crystal L continues to grow incorporating radioactivity. The course of the exchange process then increases as a monotonous F, t_E function up to $F = 1$. In real systems F, t_E plots mostly show several minima and maxima, stemming from a wide size distribution and/or the contribution of other equilibration processes (structural and composition changes, etc.).

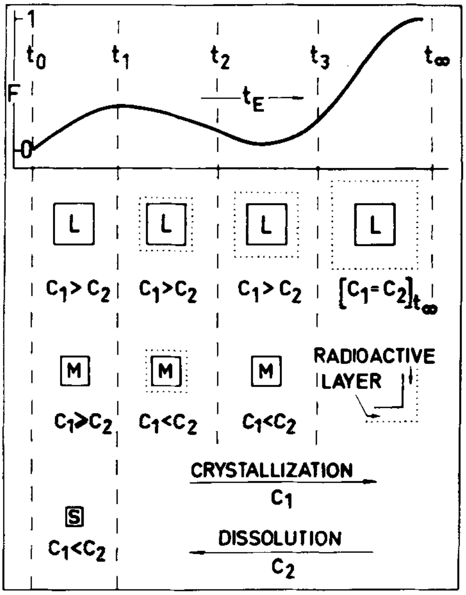


Fig. 7. Schematic presentation of the fraction exchange F versus the exchange time t_E function for the case when recrystallization in the sense of Ostwald ripening is the dominant mechanism determining the heterogeneous exchange process in the "solid-liquid" system

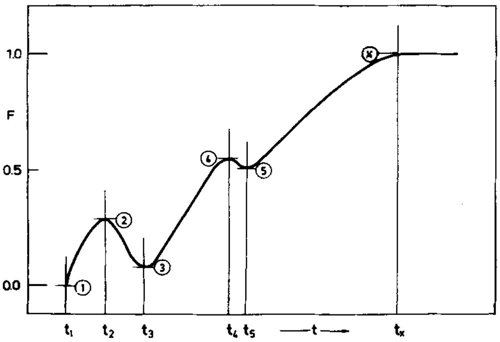


Fig. 8. Schematic presentation of the F vs. t_E function for real systems when recrystallization in the sense of Ostwald ripening is the dominant mechanism determining the heterogeneous exchange process

A schematic presentation is given in Fig. 8. If the system is labelled at t_1 (starting time), the exchange process is at the start, and $F = 0$ (point 1 in Fig. 8). Recrystallization takes place and after t_x (time of equilibration) the radionuclide is homogeneously distributed between the liquid and solid phase ($F = 1$, point x, in Fig. 8).

In systems labelled at times $t_2 \ldots t_x$ the course of the heterogeneous exchange processes is qualitatively equal as in systems labelled at t_1 but the maxima and minima are shifted to higher exchange times and total F values are different. Thus the equilibrium time t_x is reached after longer exchange times as the system is aged for longer periods (t_A) before labelling. In extremely aged precipitates in which particles are in morphological, crystallographic and dispersity equilibria, x points can occur at times corresponding to the rate of autodiffusion of the constituent ion used for systems labelling. In such cases the function F, t_E corresponds to the simple linear dependence in a form F, $\sqrt{t}$ [33].

Between extremely aged and fresh precipitates lie all real systems, which were investigated in this study. The curves F_P, t_E and F_{Ca}, t_e in Fig. 5 are an example showing that the dominant mechanism of equilibration of calcium phosphate precipitates under the given experimental conditions is recrystallization through the liquid phase. The driving force of this process is obviously the tendency of the system to minimize its energy by reducing the surface to volume ratio and/or by chemical transformation of the less stable phases. Plots of F_{Ca} and F_P vs. the aging time (Fig. 6) show curves of different shapes for calcium and phosphate ions indicating different exchange rates as is also apparent from Table 1. Thus chemical transformation may be considered a major event in this system as also follows from Figs. 2 and 3 and from the relevant literature [4, 27, 28]. The Gibbs-Kelvin effect is probably of minor importance as indicated by preliminary experiments on similar systems (J. M. Holmes, Lj. Brečević and H. Füredi-Milhofer, unpublished results), which show that the surface area of the precipitates might increase with aging time.

The large F_{Ca} values obtained in system B even for extremely aged precipitates (t_A up to 100000 min) and for very short exchange times (t_E 5–10 min; Table 1), are ascribed to the preferential adsorption of calcium ions in the hydration layer [19] and/or at the surface of the precipitate. Similarly Pak and Skinner (1969) have shown that calcium ions preferentially accumulate in the liquid film adjacent to synthetic apatites and octacalcium phosphate.

The importance of recrystallization in bone growth and transformation has been widely recognized (2, 5, 20, 25, 26], but the mechanisms of the processes involved are still subject to discussion. Interpretations range from the diffusion of ions mediated by lattice defects [20] to the Gibbs-Kelvin effect [2, 25, 26] and chemical changes [5, 26]. Clearly, the results of our experiments cannot be directly extrapolated to any biological system. However, some correlations might be relevant especially to phenomena considered in young bone formation and transformation.

Earlier investigations of heterogeneous exchange of radionuclides with calcium phosphates relate only to synthetic compounds and bone salts [5, 6, 20, 22, 23]. Investigations on the interaction of freshly-formed calcium phosphates in contact with supernatant solutions have not been reported previously.

Acknowledgements: The authors wish to thank Mgr. B. Purgarić for the computations and reading and discussing the manuscript, to Mgr. Lj. Brečević for helpful discussions, to Dr. S. Popović for the X-ray analysis and to Ing. V. Hlady for supplying stoichiometric hydroxyapatite. The financial support of the National Institutes of Health, Bethesda, Md. (PHS research grant No 02-002-1) and of the Republican Research Fund of Croatia is greatfully acknowledged.

References

1. Avnimelech, Y.: Analysis of ^{32}P and ^{45}Ca exchange between hydroxyapatite and its equilibrium solution. Israel J. Chem. **6**, 375–385 (1968)
2. Best, J. B.: Some theoretical considerations concerning crystals with relevance to the physical properties of bone. Biochim. biophys. Acta (Amst.) **32**, 194–202 (1959)
3. Bett, I. A. S., Christner, L. G., Hall, W. K.: Studies of the hydrogen held by solids. XII. Hydroxyapatite catalyst. J. Amer. chem. Soc. **89**, 5535–5541 (1967)
4. Brečević, Lj., Füredi-Milhofer, H.: Precipitation of calcium phosphates from electrolyte solutions. II. The formation and transformation of the precipitates. Calcif. Tiss. Res. **10**, 82–90 (1972)
5. Dallemagne, M. J., Bodson, P., Fabry, C.: Le calcium exchangeable de la substance minérale de l'os étudié à l'aide de ^{45}Ca. Biochim. biophys. Acta (Amst.) **18**, 394–406 (1955)
6. Dallemagne, M. J., Richelle, L.: Bone salts. Aeromed. Revs. 1–60 (1960)
7. Despotović, R.: Colloid stability in aqueous and non-aqueous media. Disc. Faraday Soc. No. 42, 175–209 (1966)
8. Despotović, R.: Kem. Ind. (Zagreb) **16**, 13–19 (1967)
9. Eanes, E. D., Gillessen, I. H., Posner, A. S.: Intermediate states in the precipitation of hydroxyapatite. Nature (Lond.) **208**, 365–367 (1965)
10. Eanes, E. D., Gillessen, I. H., Posner, A. S.: Mechanism of conversion of noncrystalline calcium phosphate to crystalline hydroxyapatite. Crystal Growth 373–376 (1967)
11. Eeanes, E. D., Posner, A. S.: Kinetics and mechanism of conversion of noncrystalline calcium phosphate to crystalline hydroxyapatite. Trans. N.Y. Acad. Sci., Ser. II **28**, 233–241 (1965)
12. Francis, M. D., Web, N. C.: Hydroxyapatite formation from a hydrated calcium monohydrogen phosphate precursor. Calcif. Tiss. Res. **6**, 335–342 (1971)
13. Füredi-Milhofer, H., Bilinski, H., Brečević, Ij., Despotović, R., Filipović-Vinceković, N., Oljica, E., Purgarić, B.: The formation of calcium phosphate precipitates. Metastable equilibria and kinetics. Proc. International Colloquium on Physical Chemistry and Crystallography of Apatites of Biological Interest, Paris 10–15 Sept. 1973 (in press)
14. Füredi-Milhofer, H., Brečević, Lj., Oljica, E., Purgarić, B., Gass, Z., Perović, G.: The influence of precipitation conditions on the formation and transformation of calcium phosphate precipitates, p. 109–120 in: A. L. Smith, ed., Particle growth in suspension. London-New York: Acad. Press 1973
15. Füredi-Milhofer, H., Purgarić, B., Brečević, Lj., Pavković, N.: Precipitation of calcium phosphates from electrolyte solutions I. A study of the precipitates in the physiological pH region. Calcif. Tiss. Res. 8, 142–153 (1971)
16. Füredi-Milhofer, H., Purgarić, B., Brečević, Lj., Pavković, N., Oljica, E.: Nucleation of calcium phosphate from solutions at physiological pH. Croat. Chem. Acta **41**, 37–42 (1969)
17. Heughebart, J. C., Montel, G.: Sur la transformation des phosphates amorphes en phosphates apatitiques, par reaction intracristalline. International Colloquium on Physical Chemistry and Crystallography of Apatites of Biological Interest. Paris. 10–15 Sept. 1973. Abstract book, p. 61
18. Holmes, J. M., Beebe, R. A.: Surface areas by gas adsorption of amorphous calcium phosphate and crystalline hydroxyapatite. Calcif. Tiss. Res. **7**, 163–174 (1971)
19. Neuman, W. F., Neuman, M. W.: The chemical dynamics of bone mineral. Chicago, Ill.: Chicago University Press 1958
20. Neuman, W. F., Weikel, J. H. Jr.: Recrystallization in bone mineral. Ann. N. Y. Acad. Sci. **60**, 685–695 (1955)
21. Newesely, H.: Darstellung von "Oktacalcium Phosphat" durch homogene Kristallisation. Mh. Chem. **91**, 1020–1023 (1960)
22. Pak, C. Y. C., Bartter, F. C.: Ionic interaction with bone mineral. I. Evidence for an isoionic calcium exchange with hydroxyapatite. Biochim. biophys. Acta (Amst.) **141**, 401–409 (1967)

23. Pak, C. Y. C., Skinner, C. W.: Ionic interaction with bone mineral. IV. Varying affinity of synthetic calcium phosphates for Ca^{2+}. Biochim. biophys. Acta (Amst.) **165**, 274–282 (1969)
24. Pautard, F. G. E.: The crystallinity of calcium phosphates in vertebrates and invertebrates. International Colloquium on Physical Chemistry and Crystallography of Apatites of Biological Interest. Paris 10–15 Sept. 1973. Abstract book, p. 21–22; Pautard, F. G. E., private communication
25. Smales, F. C.: The Kelvin effect and crystal growth of hydroxyapatite. Tooth Enamel II. (eds. Fearnhead, R. W., and Stack, M. V.). Bristol: John Wright 1971
26. Sobel, A. E., Laurence, P. A.: Crystal growth in mineralizing tissues. Biochim. biophys. Acta (Amst.) **41**, 1–8 (1960)
27. Strates, B. S., Neuman, W. F., Levinskas, G. J.: The solubility of bone mineral. II. Precipitation of nearneutral solutions of calcium and phosphates. J. Phys. Chem. **61**, 279–285 (1957)
28. Termine, J. D., Eanes, E. D.: Comparative chemistry of amorphous and apatite calcium phosphate preparations. Calcif. Tiss. Res. **10**, 171–197 (1972)
29. Termine, J. D., Peckauskas, R. A., Posner, A. S.: Calcium phosphate formation in vitro II. Effects of environment on amorphous crystalline transformation. Arch. Biochem. Biophys. **140**, 318–325 (1970)
30. Termine, J. D., Posner, A. S.: Calcium phosphate formation in vitro. I. Factors affecting initial phase separation. Arch. Biochem. Biophys. **140**, 307–317 (1970)
31. Walton, A. G., Bodin, W. J., Füredi, H., Schwartz, A.: Nucleation of calcium phosphate from solution. Canad. J. Chem. **45**, 2695–2701 (1967)
32. Wolf, R. H. H.: Heterogeneous exchange of precipitates, VII. Fraction exchange calculation when the composition of the system changes during the experiments. Kolloid-Z., Z. Polymere **205**, 120–121 (1965)
33. Ziemens, K. E.: Zur Kinetik heterogener Austauschreaktionen Arkiv Kemi, Mineral. Geol. *A* **20**, 18, 1–26 (1945)