Calc. Tiss. Res. 7, 133—138 (1971)

Synthetic Hydroxyapatite Crystals

IV. Magnesium Incorporation

W. F. Neuman and B. J. Mulryan
Department of Radiation Biology and Biophysics, School of Medicine and Dentistry,
University of Rochester, Rochester, New York

Received June 22, accepted October 31, 1970

Apatite crystals were synthesized under approximately physiological conditions and in the presence of magnesium ions. After ageing from 4 to 21 days, the crystals were analyzed and their exchange of incorporated magnesium studied with the use of ^{28}Mg.

Both the kinetics and the extent of the exchange showed that magnesium ions were strongly rejected by the apatite lattice. In aged crystals, nearly 90% of the magnesium was located in readily exchangeable (surface) positions. These findings support one concept that apatitic magnesium is essentially a surface-limited ion and help to explain the ready availability of skeletal magnesium in animals on magnesium deficient diets.

Key words: Hydroxyapatite — Magnesium — Crystals — Isotopes — Calcium.

Des cristaux d'apatite sont synthétisés dans des conditions physiological approximatives et en présence d'ions de magnésium. Après vieillissement de 4 à 21 jours, les cristaux sont analysés et l'échange du magnésium incorporé est étudié à l'aide de ^{28}Mg.

A la fois, l'étude cinétique et l'ampleur des échanges indiquent que les ions de magnésium sont nettement rejetés de la maille de l'apatite. Au niveau de vieux cristaux, près de 90% du magnésium est situé dans des positions (de surface) échangeables. Ces résultats confirment la théorie qui admet que le magnésium de l'apatite est surtout un ion de surface. Ils expliquent la grande labilité du magnésium squelettique chez des animaux soumis à des régimes pauvres en magnésium.

Apatitkristalle wurden unter annähernd physiologischen Bedingungen und in Anwesenheit von Magnesiumionen synthetisiert. Nach einer Ausreifungszeit von 4–21 Tagen wurden die Kristalle und der Austausch des eingebauten Magnesiums mittels ^{28}Mg untersucht.

Sowohl die Kinetik als auch das Ausmaß des Austausches zeigten, daß die Magnesiumionen vom Apatitgitter heftig abgestoßen werden. Bei ausgereiften Kristallen waren nahezu 90% des Magnesiums leicht austauschbar an der Oberfläche gelagert. Durch diese Befunde wird ein Konzept unterstützt, wonach das im Apatit vorkommende Magnesium hauptsächlich ein oberflächengebundenes Ion ist. Ferner läßt sich dadurch erklären, weshalb bei magnesiumarm ernährten Tieren so leicht Magnesium aus dem Skelet verfügbar ist.

Introduction

Although magnesium is an important constituent of bone, lack of a convenient isotope and problems in methodology have severely limited the study, and consequently our understanding, of the nature of bone magnesium and its possible functional significance.

For reprints: W. F. Neuman, Department of Radiation Biology and Biophysics, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14620, U.S.A.

This work was supported in part by the U. S. Public Health Service Training Grant No. 1 Tl DE-175 and in part by the U. S. Atomic Energy Commission Contract No. AT (30-1)-49 and has been assigned Report No. UR-49-1308.

Some years ago, a system was developed in our laboratory for synthesizing apatite crystals *in vitro* under conditions that approximate those occurring physiologically [8]. The system was successfully applied to the study of sodium [8], potassium [8], strontium [5], and carbonate [6] in relation to the formation of apatite crystals. It seemed of interest to reinvestigate the magnesium problem using this crystal-synthesizing system.

Methods

The apparatus employed for the synthesis of crystals, and much of the associated methodology, have been described previously [5, 6, 8]. Basically, 100 ml each of 0.1 M K_2HPO_4 (in 0.05 M $KHCO_3$) and of 0.16 M $CaCl_2$ (containing ^{27}Mg, 0.003 M) were simultaneously pumped into 500 ml of buffer over a period of 6 h. The buffer consisted of 0.14 M KCl in 0.025 M $KHCO_3$ which also contained Mg^{2+}, 0.001 M. Fifty milligrams of L-apatite [7] were added to "seed" the mixture. An automatic titrator maintained the pH at 7.4 by the addition of 2 M KOH throughout the precipitation process. During the period of crystal formation, the system was flushed with 95% O_2:5% CO_2. In the long-term experiments, the system was flushed with the gas mixture, sealed and flushed and resealed at periodic intervals. The crystals aged from 4 to 21 days.

In this system, the temperature and pH remained constant while ionic strength variations were less than 10% [8]. In the presence of the seed crystals, less accumulation of calcium, magnesium and phosphate occurred in solution. Concentrations were physiological, or less, throughout the last two-thirds of the precipitation process. At the end, approximately 98% of the calcium, magnesium, and phosphate pumped into the reaction flask was found in the solid phase.

For exchange studies, about 30 µC of ^{28}Mg (containing some ^{24}Mg) were added to the aged crystal slurry. The solids in aliquots of slurry, taken at various time periods from 3 min to 48 h after addition of isotope, were analyzed for H_2O, ^{28}Mg, ^{24}Mg, Ca, P, and CO_2 [5, 6, 8]. Ten milliliters of slurry were passed through a micro-millipore filtration apparatus and air pulled through for 3 min. The solution was saved for analysis. The solid, collected on the filter, was processed for analysis.

^{28}Mg (Brookhaven National Laboratories) is shipped as $MgCl_2$. However, it contains unknown organic impurities. Accordingly, the solution was evaporated in a platinum crucible and ashed at 600° for 30 min. The resulting ash was dissolved, with heat, in a minimum of dilute HCl and diluted to an appropriate volume (usually 5 ml) for aliquots. ^{24}Mg was analyzed by atomic absorption spectrophotometry (Perkin Elmer A.A.S. Model 303).

^{45}Ca (Oak Ridge) was precipitated with ammonium oxalate, isolated and washed by centrifugation. The washed precipitate was converted to $^{45}CaCO_3$ by placing in a muffle furnace for two hours at 600°. The $^{45}Ca_3$ was dissolved in a few drops of dilute HCl for dilution and subsequent use.

A few preliminary precipitations were conducted in the absence of CO_2:HCO_3^-. Crystals were taken at intervals from 15 min to 3 weeks, lyophilized, and examined by X-ray diffraction. The presence of magnesium slowed the precipitation and crystallization processes. Against the amorphous pattern of poorly crystallized apatite, several sharp aberrant diffraction peaks from an unidentified solid phase were observed. These same peaks were seen in the patterns given by solids precipitated in the presence of bicarbonate as well. In the unseeded preparations, then, varying and unpredictable mixtures were obtained; apatite, an unidentified solid and presumably some amorphous calcium phosphate [1, 4]. However, when the system was seeded with small amounts of preformed apatite crystals, the aberrant peaks were no longer observed. Moreover, the conversion to apatite seemed to progress promptly, suggesting an autocatalytic process. All solids reported in this paper exhibited diffraction patterns characteristic of hydroxyapatite only. Typical patterns are given in Fig. 1.

Results

Within this limited series of experiments, there were no trends in compositional change with varying age of the crystals. Therefore, averaged data from the

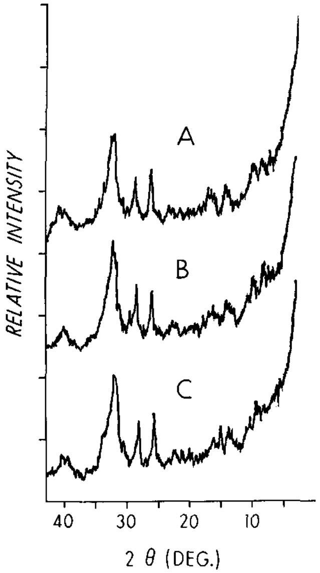

Fig. 1A–C. X-ray diffraction patterns of typical aged precipitates. A 4 days; B 2 weeks; C 3 weeks

Table. *Average composition of crystals and equilibrium fluid*[a]

	Solid % dry wt.	Solution mM
Water	79.6 ± 0.6[b]	—
Phosphate, P	15.7 ± 0.2	0.25 ± 0.06
Calcium	35.0 ± 0.8	0.44 ± 0.07
Magnesium	0.66 ± 0.04	0.61 ± 0.10
CO_2	6.03 ± 0.55	25[c]
Ratios (Molar)		
Ca/P	1.72 ± 0.02	1.75 ± 0.45
Ca/Mg	32.0 ± 2.0	0.72 ± 0.15

[a] Given as mean ± std. error, $n = 8$.
[b] Per cent of wet hydrated crystals.
[c] Set by experiment, not analyzed.

8 experiments are given in the Table. As with previous experience [6] the composition of the crystals closely approximated the composition of bone mineral: highly hydrated crystals of small size, containing 6% CO_2 and a Ca/P ratio, 1.72, near the theoretical value for apatite, 1.66. Like bone, when corrected for CO_2 content, the molar ratio, $(Ca + Mg)\text{-}CO_2/P$, was low, 1.50 [7]. The magnesium content, 0.66%, was also similar to that reported for bone [7]. However, it should be noted that the magnesium content of the crystals is fixed primarily by the design of the experimental conditions, i.e., the Ca/Mg ratio of the solutions pumped into the precipitating flask.

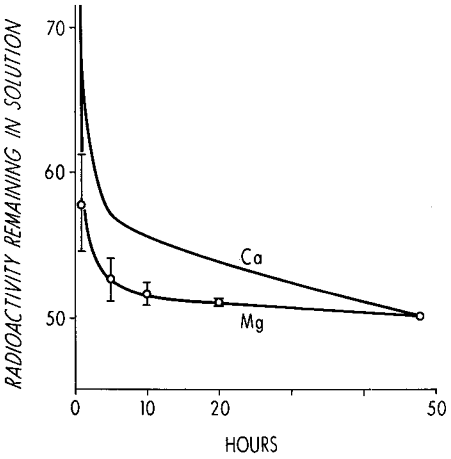

Fig. 2. A comparison of the kinetics of the exchange of ^{45}Ca and ^{28}Mg. The data from several experiments have been normalized to give coincidence at 48 h. Note the virtual cessation of further ^{28}Mg exchange beyond 10 h

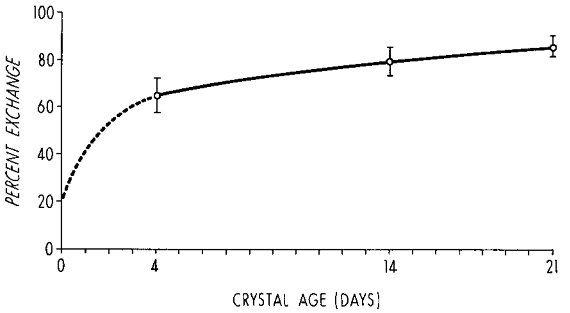

Fig. 3. The effects of crystal aging on the extent of ^{28}Mg exchange (48 h). The dotted line indicates the course of exchange if magnesium had been trapped randomly in the initial precipitation

The kinetic exchange data are presented in Fig. 2, where all of the experiments have been normalized to be coincident at 48 h. For comparison, ^{45}Ca exchange results are also given. Two points are worthy of emphasis. First, there is considerable Mg exchange occurring between 1 and 10 h after addition of the isotope. Second, between 10 and 48 h little further exchange occurs, in contrast to the case of ^{45}Ca.

The per cent of magnesium which exchanged in 48 h was dependent upon the age of the crystals. These data are given in Fig. 3, where it is seen that two-thirds of the magnesium resides in surface positions, even in crystals 4 days old. As the crystals age, more and more magnesium is extruded and becomes exchangeable, nearly 90% in 21-day-old crystals.

Discussion

Previous analyses of the kinetics of the exchange of apatite crystals [7, 9] have established that cations, residing in the hydration shell, equilibrate with the solution within minutes. Cations which are located in the lattice surfaces require hours of exchange, while interior positions take days, weeks, and even months to exchange. These separate phases are seen in the exchange of ^{45}Ca shown in Fig. 2. On the other hand, the kinetics of magnesium exchange show the first two phases clearly, but the slow, continuing exchange of interior lattice positions (from 10–50 h) is almost entirely lacking. The kinetic data, then, suggest strongly that magnesium, for the most part is, extruded from the lattice and resides in readily available positions in the surfaces and the hydration shells of the crystals.

The extent of the exchange confirms this conclusion. In aged crystals, not more than 20% of the calcium (which is distributed evenly through interior and surface positions) is readily exchanged (6; also Mulryan, unpublished results). Two-thirds of the magnesium was readily available in crystals four days old. By three weeks, 87% of the magnesium was found in the surfaces of the crystals.

The chemical data are also in accord with the concept that apatitic magnesium is essentially a surface-limited ion. The Ca/Mg ratio of the solid is 32 while the same ratio in the solution with which the crystals are in quasi-equilibrium averages less than unity, 0.72. Expressed as a discrimination ratio [3], the preference of the lattice for calcium is approximately 45.

These findings help to explain the ready availability of skeletal magnesium in animals on magnesium deficient diets. Blaxter (1956), who summarized a series of studies of magnesium deficiency, found that over half the skeletal magnesium could be transferred to soft tissue and the excreta of calves fed a deficient diet for a month. Although he reported reductions of skeletal magnesium of as much as 75%, more drastic deficiencies were terminated by tetany [2].

The state of magnesium in the skeleton unfortunately is rendered somewhat ambiguous by the presence of a non-apatitic, amorphous mineral phase [1, 4]. The present studies do not deal with the location or exchange properties of magnesium in an amorphous precipitate. Nor is it likely that such information will soon be forthcoming, since the amorphous phase is relatively unstable at 37° in aqueous suspension *in vitro* [1, 4]. One might expect that all ions in an amorphous phase would be relatively reactive and exchangeable. On the other hand, since an amorphous phase is by definition *structureless*, it would not be expected to show the discriminatory powers of a structured apatitic lattice.

References

1. Bachra, B. N., Trautz, O. R., Simon, S. L.: Precipitation of calcium carbonates and phosphates, III. Arch. oral. Biol. **10**, 731–738 (1965).
2. Blaxter, K. L.: The magnesium content of bone in hypomagnesaemic disorders of livestock. In: *Bone structure and metabolism* (G. E. W. Wolstenholme and C. M. O'Connor, eds.) p. 117–134. Boston: Little, Brown & Co. 1956.
3. Comar, C. L., Wasserman, R. H.: Strontium. In: Mineral metabolism (C. L. Comar and F. Bronner, eds.), vol. II, p. 523–572. New York and London: Academic Press 1964.
4. Eanes, E. D., Posner, A. S.: Intermediate phases in the basic solution preparation of alkaline earth phosphates. Calc. Tiss. Res. **2**, 38–48 (1968).

5. Neuman, W. F., Bjornerstedt, R., Mulryan, B. J.: Synthetic hydroxyapatite crystals. II. Aging and strontium incorporation. Arch. Biochem. Biophys. **101**, 215–224 (1963).
6. — Mulryan, B. J.: Synthetic hydroxypapatite crystals. III. The carbonate system. Calc. Tiss. Res. **1**, 94–104 (1967).
7. — Neuman, M. W.: Chemical dynamics of bone mineral. Chapt. III and IV. Chicago: Chicago University 1958.
8. — Toribara, T. Y., Mulryan, B. J.: Synthetic hydroxyapatite crystals. I. Sodium and potassium fixation. Arch. Biochem. Biophys. **98**, 384–389 (1962).
9. Pak, C. Y. C., Bartter, F. C.: Ionic interaction with bone mineral. I. Evidence for an isoionic exchange with hydroxyapatite. Biochim. biophys. Acta (Amst.) **141**, 401–409 (1967).