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EXTENT OF STRONTIUM SUBSTITUTION FOR CALCIUM IN HYDROXYAPATITE

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SUMMARY

An X-ray-diffraction technique for the analysis of Ca and Sr in calcium–strontium hydroxyapatite solid solutions is described. Solid solutions representing the complete compositional range from calcium to strontium hydroxyapatite have been prepared by hydrothermal treatment of end member mixtures and by coprecipitation from solution. The influence of the cation : phosphorus ratio of precipitating solutions upon the discrimination against strontium in the precipitates was studied and the relationship to Sr adsorption by bone discussed.

INTRODUCTION

The danger to health caused by atomic-bomb fallout has produced a need for knowledge in the mechanisms of absorption and utilization of radioactive materials. As soon as Sr was shown to concentrate in bone, the substitution of Sr for Ca in the bone mineral, calcium apatite, was investigated from many different biologically oriented viewpoints. These studies demonstrated that the radioactive elements can be adsorbed onto previously formed CaHA-like crystals[1-4] and indicated that Sr might also form solid solutions[5] with apatites.

Aware of the special implications concerning the apatites, several workers[6,7] attempted to form the complete Ca–SrHA series by dry solid-state reaction, precipitation, and hydrothermal methods, but were unsuccessful. However, solid solution formation was still deemed likely to occur because: (1) both end members have analogous crystal structures[8]; (2) an incomplete lead–calcium fluoroapatite series had been described[9] (Pb atomic radius = 1.21 Å); hence, Ca and Sr, whose atomic radii are even closer (0.99 Å *versus* 1.13 Å), should form solid solutions; and (3) a continuous series had been reported for calcium–strontium fluoroapatites treated at 950° (ref. 10).

Complete solid solution between CaHA and SrHA was reported first by COLLIN[11]. Moreover, important to the public-health aspects of the problem, COLLIN showed that distinct discrimination against the substitution of Sr for Ca occurred. At high Sr : Ca ratios, he believed it necessary to have large excesses of cation in the precipi-

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Abbreviations: CaHA, calcium hydroxyapatite; SrHA, strontium hydroxyapatite; Ca–SrHA, calcium–strontium hydroxyapatite solid solutions.

tating solutions to prevent the Ca from being totally consumed before the reaction was complete and the P depleted. He determined by X-ray diffraction and fluorescence techniques that both the a_0 and c_0 lattice constants vary linearly as Sr is substituted. Since heat treatment at 950° improved the sharpness of diffraction patterns obtained from his precipitates, COLLIN concluded that the untreated precipitates were intimate mixtures of similar, but not homogeneous, solid solutions. In a second paper[12] he proved this point when, by keeping the cation ratios of the solutions constant during the precipitation process, he precipitated homogeneous solid solutions.

Thus, COLLIN has shown that Ca–SrHA can be produced readily by coprecipitation methods. However, the successful preparation of the entire Ca–SrHA series by ignition or hydrothermal means has not been reported. The purpose of the experiments carried on in this laboratory was: (1) to develop and test the precision of a X-ray-diffraction method for the determination of Sr substitution in apatite solid solutions; (2) to use this method to determine the composition of solid solutions formed by hydrothermal methods; and (3) to determine the influence of the cation:P ratio in solutions upon cation substitution in coprecipitates. This last point will be discussed in relation to the health aspects of the problem.

EXPERIMENTAL PROCEDURES

Materials

All chemicals, but the ethylenediamine, were of reagent grade. Demineralized water, boiled immediately prior to use, was used for the preparation and washing of precipitates. Stock solutions of 25 g% $Sr(NO_3)_2$ and $Ca(NO_3)_2 \cdot 4H_2O$ and 21 g% $(NH_4)_2HPO_4$ were prepared in demineralized water and diluted immediately before use to the proper concentration with freshly boiled water.

Preparation of solid solutions

Precipitation: The method of COLLIN[11] was used unless otherwise specified. This consists of adding a hot dilute $(NH_4)_2HPO_4$ solution dropwise to a rapidly stirred hot dilute $Ca(NO_3)_2$ and/or $Sr(NO_3)_2$ solution. Both solutions were made highly basic by the addition of 2.5 ml 85% commercial-grade ethylenediamine. The resulting precipitate was digested behind NaOH traps at 95–100° for 6 h. After digestion, the precipitate was cooled, decanted, and washed repeatedly until the wash water reached pH 7. The precipitate was filtered, washed, dried at 100°, and homogenized by grinding under alcohol.

This type of precipitate exhibited a relatively poor, but distinct apatite X-ray-diffraction pattern with no extraneous lines due to other phosphate phases. The pattern sharpness could be improved by heating the samples at 950° for 4 h or by treating them hydrothermally at 300°. Precipitates treated by the two methods gave equivalent results. The results given in this paper refer to dry heat-treated samples unless otherwise specified.

Hydrothermal reaction: Apatite end members, prepared by the method described above and mixed to give theoretical ratios of 10–90 atom% Sr, and intermediate unignited solid solutions, prepared by the coprecipitation method, were used for hydrothermal studies.

Approx. 0.05 g of sample was placed in a 2.5-mm dia. gold tube approx. 0.5 in long which had been previously sealed at one end. A small drop of water was added to the tube and the open end was sealed by welding. The seal was tested by heating the tube in excess of 100° and checking for weight loss. Sealed tubes were placed in a "test-tube"-type hydrothermal reactor described by ROY AND TUTTLE[13], filled with water, attached to the pressure system, and heated to the desired temperature. During the heating the system was kept closed and a pressure of several thousand lb/in^2 developed within the steam-filled reactor. Sample temperatures ($\pm$ 5°) were determined with a chromel–alumel thermocouple placed on the exterior of the bomb within $^3/_8$ in of the sealed tubes. After temperature equilibrium was reached (0.5 h for 300° and 1 h for 800°), the internal bomb pressure was raised to 15 000 lb/in^2 and maintained at that point ($\pm$ 300 lb/in^2) for the treatment duration. Samples treated at 300° were quenched to approx. 50° by immersing the bomb in cold water and were subsequently allowed to cool slowly to room temperature. Samples treated at 800° were air cooled to room temperature. Pressure was maintained at 15 000 lb/in^2 during the quench from 300°, but allowed to decrease naturally to room pressure as the reactors cooled from 800°. In hydrothermal experiments the gold tubes collapse to equilibrate the internal and external pressures.

X-ray-diffraction analysis

X-ray diffraction and fluorescence measurements were made with a General Electric instrument (Model XRD-3). For diffraction analysis Ni-filtered Cu radiation was used. The 211 reflection of all samples was scanned at $^1/_5$° 2θ per min and the peak center measured at one-half height. The angle 2θ was read to $\pm$ 0.005° and corrected for machine deviation by use of a quartz internal standard. Since the atom % of Sr and Ca was assumed to be a linear function of the angular shift of the 211 reflection between the two end members, a linear calibration curve was drawn between these two angles (CaHA = 31.820° 2θ and SrHA = 30.610° 2θ). The observed value of the angle 2θ was used to determine the atom % Sr to $\pm$ 0.5 %.

X-ray-fluorescence analysis

The X-ray unit was fitted with a LiF crystal for the fluorescence analyses. A scintillation counter was used in the Sr determinations, while a He flow counter was used for Ca analyses. Mixtures were made of gravimetrically analyzed[14,15] CaHA and SrHA end members (analyzed: CaHA:Ca, 40.35 %; P, 15.66%. SrHA:Sr, 59.67%; P, 9.33%; theoretical: CaHA:Ca, 39.88 %; P, 17.28 %. SrHA:Sr, 59.20 %; P, 11.58 %)* to contain 20, 50, and 80 atom% Ca. The ground samples were packed into holders and the intensities of the CaK$_\alpha$ and SrK$_\alpha$ lines were measured by counting for 20-sec periods. Calibration curves of the atom % of Ca and Sr *versus* measured counts were prepared from the results obtained with these standard samples.

This method eliminates the more tedious preparation of the samples in $Na_2B_4O_7$ pellets. To test adequacy of the packing method, borate pellets of the standards were prepared and the K$_\alpha$ intensities were measured by the same method. The curves were identical, but the intensities of the pellets were different from those of the powder. The easier packing method was used in all work reported here.

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* The cation:P ratios of these samples vary from the ideal. Standards were prepared to contain desired molar proportions of the two cations, but no correction was made for the low P content.

RESULTS AND DISCUSSION

Diffraction versus fluorescence

COLLIN[11] showed that in Ca–SrHA the a_0 and c_0 lattice constants for the hexagonal structure increase linearly as Sr is substituted for Ca. Because of this feature, the percentage of Sr within the lattice can be determined as accurately as the angle 2θ can be measured.

The samples listed in Table I were analyzed by both fluorescence and diffraction techniques (under the conditions used the fluorescence method has a precision of ± 1 %). Results from the samples, which represent almost the entire range from CaHA to SrHA, indirectly confirm COLLIN's finding that the a_0 and c_0 lattice constants increase linearly with Sr substitution and also demonstrate that the method provides answers that are within 2 % of the actual values.

TABLE I

ATOM % Sr IN HYDROXYAPATITE SOLID SOLUTIONS AS DETERMINED BY FLUORESCENCE AND DIFFRACTION METHODS

Samples were prepared by COLLIN's method from solutions with a stoichiometric cation:P molar ratio and were ignited at 950° for 4 h prior to analysis. The stoichiometric ratio was used to minimize the adsorption of excess cations.

Atom % Sr in precipitating solution	*Atom % Sr by X-ray analysis*	
	Fluorescence	*Diffraction*
98	99.0	99.5
85	85.0	84.5
80	80.0	79.5
60	61.0	63.0
50	50.5	51.5
40	40.0	42.0
20	22.5	21.5
10	10.5	11.5

The 211 reflection was chosen for this work because it is the most intense peak and, therefore, the least likely to be obscured by inhomogeneity of the samples. However, if a higher reflection (the less intense 213, for example) were measured in duplicate by this method, the cation contents within the apatite lattice could easily be determined to an accuracy of ± 0.2 %.

Presumably this same analysis could be used for other possible apatite solid solutions, such as Ca–Ba, Sr–Ba, Ca–Pb, and Ca–Cd, which might be studied in the future. This method is rapid, easily accurate to 2 %, and requires less than 0.05 g of material. Obviously, the method is not useful for the extremely low concentrations of the cations that are used in isotope studies. However, it is useful for studies *in vitro* where the special equipment required for isotope studies is not available or where the experimenter is interested in the grosser changes which occur in the presence of high levels of competing cations.

Hydrothermal treatment

Homogenization of coprecipitated solid solutions: COLLIN[11, 12] describes in detail his use of diffraction patterns to determine the crystallite size and homogeneity of solid solutions. Although not measured quantitatively in this work, changes in the sharpness of diffraction patterns were taken as indicative of changes in crystal size and/or homogeneity of solid solutions.

A moderate improvement in diffraction patterns was obtained by both hydrothermal treatment for 24 h at 300° under 15000 lb/in^2 pressure and by ignition at 950° for 72 h, whereas hydrothermal treatment for 4 h at 800° under 15000 lb/in^2 pressure produced sharper diffraction patterns than the other two methods. Extensive hydrothermal studies showed that temperature, rather than time or pressure, was the most important factor in pattern improvement; however, at a given temperature, hydrothermal treatment always yielded more improvement in pattern sharpness (and hence in crystallinity) than was obtained by ignition. No treatment used in these studies produced single crystals large enough to be identified or studied by petrographic methods.

TABLE II

ATOM% Sr IN HYDROXYAPATITE SOLID SOLUTIONS: SOLID-SOLUTION FORMATION BY SOLID STATE AND HYDROTHERMAL REACTION

*Symbol***	*Method of preparation*				*Atom % Sr*	
	Sample preparation	*Sample treatment*			*Treated mixture*	*Untreated mixture*
		Time (h)	*Temp. (°C)*	*H_2O pressure (lb/in^2)*		
PHA	Mixtures of end members containing the desired percentages of cations were ground together under alcohol	72	950		91	80
		24	300	15 000	93	80
		4	800	15 000	79	80
PHB		72	950		64	50
		24	300	15 000	8	50
		4	800	15 000	54	50
PHC		72	950		18	20
		24	300	15 000	4	20
		4	800	15 000	21	20
10CHA		4	800	15 000	98*	90
20CHA					84	80
30CHA					74	70
40CHA					67	60
50CHA					60	50
60CHA					50	40
70CHA					39	30
80CHA					27	20
90CHA					15	10
95CHA					7	5

* In this series the percentage of substitution was measured by scanning the diffraction pattern on fast speed and taking the average indicated substitution for 4 reflections. This method is less precise but more rapid.

** Samples are CaHA and SrHA mixtures: see column 7 for composition, columns 2–5 for treatment. Symbols are arbitrary.

Formation of solid solutions: The results of limited (PHA to C) and more complete (10 CHA to 95 CHA series are recorded in Table II. In both series, the samples were treated at 800° and the diffraction patterns of the treated mixtures indicated very homogeneous solid solutions which contained molar cation ratios almost identical to those of the untreated mixtures. This suggests that reaction was complete under these conditions. If very little or no reaction had taken place, the percentage of Sr determined by X-ray diffraction of the treated samples would be close to 100 for the high-Sr samples and close to zero for the low-Sr ones. Results which suggested incomplete reactions were obtained after hydrothermal treatment at 300° and ignition at 950°. The diffraction patterns of the products from these treatments were so diffuse that measurement of the extent of substitution was difficult. Judging from the patterns, ignition apparently did produce somewhat more complete reaction than was obtained with hydrothermal treatment at 300°.

HAYEK AND PETTER[16] described the hydrothermal preparation of large (3 mm) SrHA crystals* by the treatment of precipitates prepared in a manner similar to one used by COLLIN. The poorly crystallized material was treated in an autoclave for 72 h at 420° in 2 N NaOH. Later[6], they treated mixtures of CaHA and SrHA hydrothermally in NaOH, but were unable to prepare the complete series of solid solutions. Undoubtedly, their attempts at hydrothermal preparation were unsuccessful because they were unable to obtain favorable temperatures with their apparatus. Treatment of the mixtures at 300° in our apparatus did not produce solid solutions. Since high-temperature hydrothermal treatment, but not ignition of mixtures, produced homogeneous solid solutions, there is good evidence that water is necessary to accelerate this reaction.

The cation: P ratio

The series of experiments yielding the results given in Fig. 1 was prepared to show the influence of cation:P ratio upon the tendency of Sr to enter the lattice of the precipitates. At the stoichiometric ratio (1.67) there is no discrimination of the lattice against Sr. COLLIN[11] considered it necessary to retain a large excess of cation in the precipitating solutions in order to prepare the entire Ca–SrHA series. At low Ca concentrations he thought that the Ca in the system would be reacted well before the end of the precipitation. HAYEK AND PETTER[16] confirmed COLLIN's method, but were able to obtain solid solutions without the use of large cation excesses. They did not comment on the increased substitution of Sr found with precipitation from stoichiometric solutions. Our results demonstrate that, if COLLIN had used stoichiometric quantities of cation and P, he would have been able to prepare the solid solution series and have complete substitution of Sr for Ca. When the cation:P ratio is doubled to 3.2 the inclusion of Sr in precipitates is dramatically decreased. At a cation:P ratio of 6.0, approximately that used by COLLIN for high Ca concentrations, Sr substitution is further limited at the high-Sr levels. At ratios of 9.0 and 30.0 no Sr was found in the crystal lattice structure of precipitates formed from solutions which contained 5 % Sr. Thus, the Sr content of the product is shown to be directly related to the total cation available for precipitation as well as to the Ca:Sr ratio in the solution.

In a study which simulated the conditions of natural bone deposition, LENGEMANN[19] found that embryonic chicken bones grown *in vitro* discriminated against Sr when incubation was continued for more than 2 h. He also reported that discrimi-

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* Large crystals were not formed by the hydrothermal method used in this laboratory. As a result, only average indices of refraction could be determined for the crystals. Since the indices for CaHA and SrHA are so similar (1.643 and 1.649 for CaHA (ref. 17) *versus* 1.637 and 1.639 for SrHA (ref. 18)), it is not possible to use refractive-index differences to quantitatively determine cation substitution.

nation was related to the Ca available in the nutrient material. If the observations made by us or the *in vitro* studies mentioned above can be correlated with the body's tendency to incorporate Sr, the advantage of a high Ca intake is immediately apparent. However, many other factors enter the picture to complicate this direct comparison.

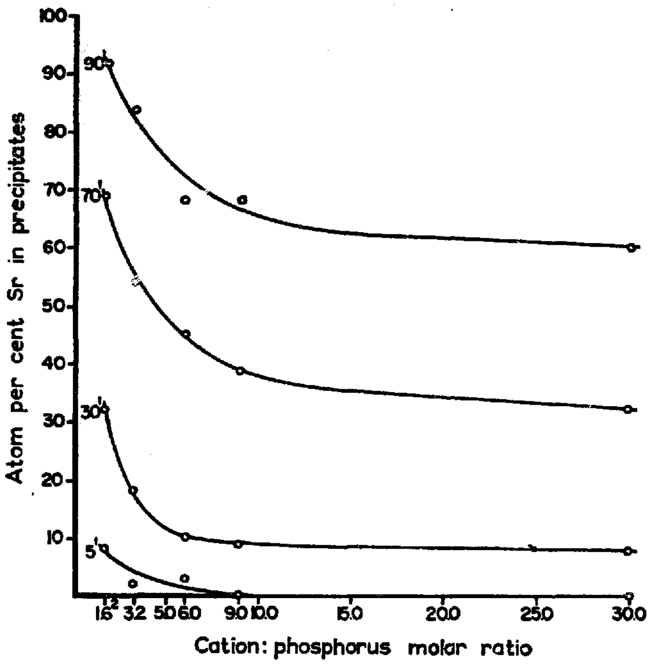

Fig. 1. Atom % Sr within solid solutions. Influence of cation excess in precipitating solutions upon the percentage of Sr substitution. ¹ Atom % Sr in original solution. ² Stochiometric ratio = 1.67.

Radioactive Sr adsorbed on bone crystals does as much damage as that which is located in the lattice. Although adsorbed Sr may be removed from the system more rapidly than Sr located in the lattice, both adsorbed and lattice Sr must be taken into consideration in studies of Sr poisoning. Accumulation of Sr in the system is limited by preferential absorption of Ca from the intestine and preferential excretion of Sr by the kidney[20]. At the same time concentrations of both cations in the blood are rigidly regulated to prevent excess accumulation.

The preferential uptake of Ca into apatites during precipitation has been repeatedly demonstrated in these experiments. This suggests that at a constant Sr level, the percentage of the available Sr which is actually incorporated into the bone mineral would be influenced by the intake of Ca. This phenomenon has been reported by many workers. The results of this study suggest that one of the mechanisms by which Sr substitution in bone is limited may be through preferential use of Ca during the deposition of bone.

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