

# NATIONAL BUREAU OF STANDARDS REPORT

NBS PROJECT

NBS REPORT

0708-11-0707

December 31, 1957

5759

Progress Report

EFFECT OF CALCIUM TREATMENT ON SOLUBILITY AND CALCIUM UPTAKE  
OF SYNTHETIC HYDROXYAPATITE AND RAT MOLAR ENAMEL

by

R. C. Likins\*  
A. S. Posner\*\*  
A. C. Steere\*\*\*

- \* Dentist, National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.
- \*\* Research Associate, American Dental Association Research Division, National Bureau of Standards.
- \*\*\* Biologist, National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.

This work is a part of the dental research program conducted at the National Bureau of Standards in cooperation with the Council on Dental Research of the American Dental Association, the Army Dental Corps, the Air Force Dental Service, the Navy Dental Corps and the Veterans Administration.

## IMPORTANT NOTICE

NATIONAL BUREAU OF STANDARDS  
Intended for use within the Government. No additional evaluation and revision of this Report, either in the Office of the Director, National Bureau of Standards, or, however, by the Government agency to reproduce additional copies.

Approved for public release by the  
director of the National Institute of  
Standards and Technology (NIST)  
on October 9, 2015

Progress accounting documents  
initially published. It is subjected  
to reproduction, or open-literature  
use, is obtained in writing from  
the agency. Such permission is not needed,  
except if that agency wishes



U. S. DEPARTMENT OF COMMERCE  
NATIONAL BUREAU OF STANDARDS



EFFECT OF CALCIUM TREATMENT ON SOLUBILITY AND CALCIUM UPTAKE  
OF SYNTHETIC HYDROXYAPATITE AND RAT MOLAR ENAMEL\*

Abstract

A synthetic, calcium-deficient hydroxyapatite and specimens of intact, rat molar enamel were treated with calcium acetate solutions. This effected a reduction in both the acid solubility and radio-calcium uptake for both types of sample. The results may be interpreted to indicate that the calcium acetate treatment filled the empty calcium positions in the hydroxyapatite structure of the synthetic material by a process of recrystallization, thereby forming a more stable compound. This suggests that the major mineral phase of enamel is some form of cation-deficient hydroxyapatite, i. e. a so-called defect apatite.

-----

1. INTRODUCTION

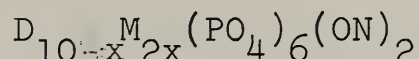
The existence of apatites deficient in divalent cations is supported by crystallographic evidence [1]. Certain chemically precipitated hydroxyapatites were shown to contain different amounts of calcium ions missing from structural positions, resulting in a family of defect apatites with low calcium to phosphorus ratios.

---

\* This investigation was supported in part by Research Grant D-572 to the American Dental Association from the National Institute for Dental Research, Public Health Service.

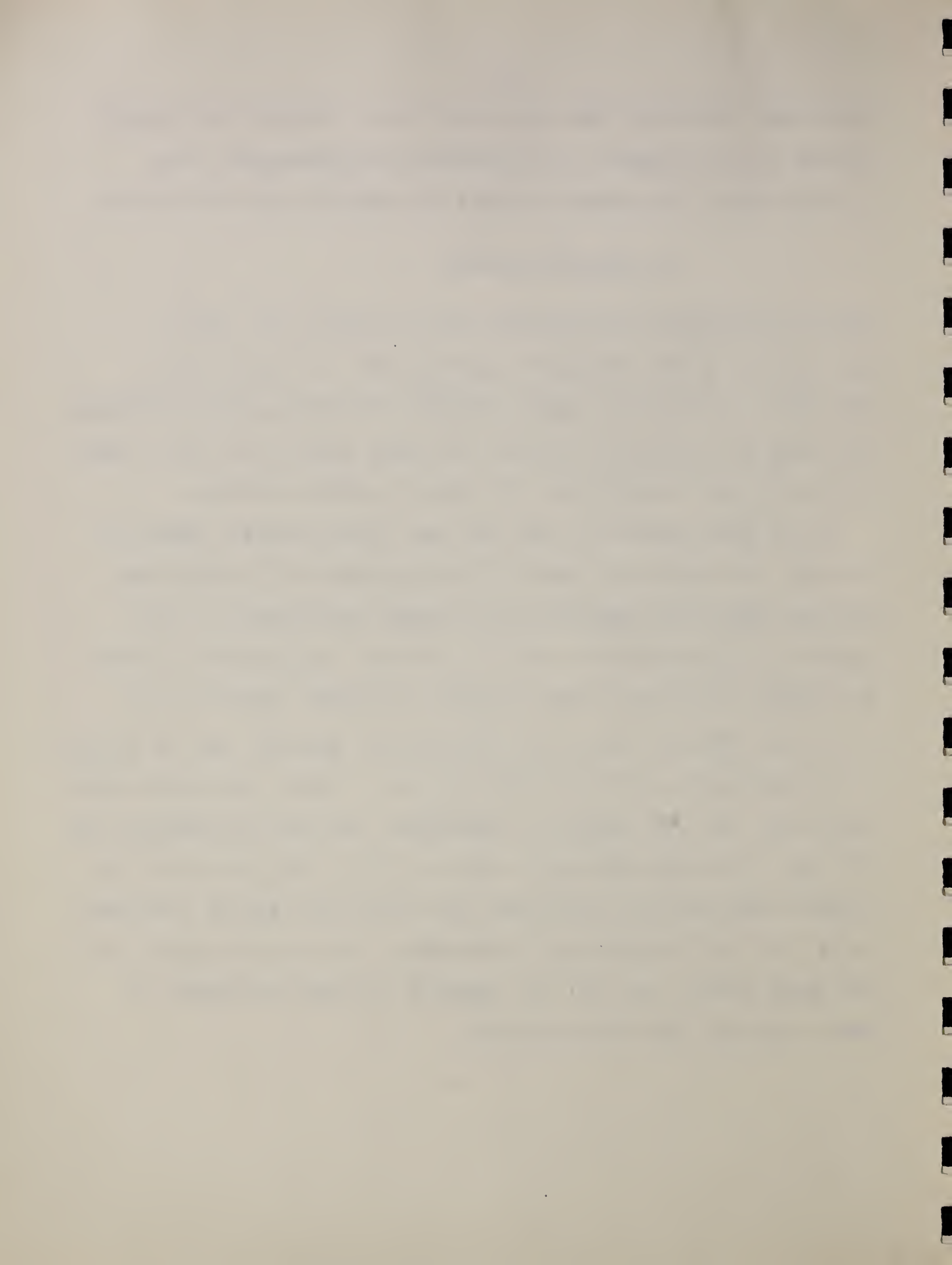


Electrical neutrality was postulated to be obtained by hydrogen bonding between oxygens of neighboring orthophosphate ions. In this light, the general formula for apatites may be written:



where D represents the divalent cations ( $Ca^{++}$ ,  $Pb^{++}$ ,  $Sr^{++}$ ,  $Mg^{++}$ , etc.), M the monovalent cations ( $Na^+$ ,  $N^+$ , etc.) and x the number of divalent cations missing from structural positions. The value of x has been reported to range from 0 to 2 [1]. When x is zero, the stoichiometry of perfect apatite obtains.

It is quite possible that bone and tooth mineral, which do not have the ideal Ca/P ratio of hydroxyapatite [2], may have calcium defect hydroxyapatite as a major constituent [3, 4]. Synthetic hydroxyapatites with low calcium to phosphorus ratios are thermally unstable when compared to perfect apatites [5]. It is also possible that the solubility of apatites may be related to the perfection of the calcium content. Other experiments have shown that the Ca/P ratios of synthetic, low-calcium apatites can increase in calcium hydroxide solution [6]. The purpose of the present study was to investigate the effect of calcium treatment on (a) the acid solubility of synthetic, low-calcium apatite and rat molar enamel, and (b) the capacity of these materials to remove calcium ions from solution.



## 2. EXPERIMENTAL PROCEDURE

### 2.1 Studies With Synthetic Hydroxyapatite

A synthetic, low Ca/P hydroxyapatite was prepared by chemical precipitation [1]. Eighteen samples, weighing 50.0 mg each, were transferred to 12 ml glass-stoppered centrifuge tubes. 10.0 ml aliquots of a 1 M calcium acetate solution (pH 7.2) were added to half of the tubes; 10.0 ml of distilled H<sub>2</sub>O was added to each of the remaining tubes. All were shaken for 48 hours at 20°C and centrifuged. The precipitates were washed three times by re-suspension and centrifugation in 10.0 ml of distilled water. After drying over calcium chloride, the samples were pooled to yield six composites, i.e., three control and three calcium treated. These were analyzed for calcium [7] and phosphorus [8].

All measurements of (a) radiocalcium uptake and (b) acid solubility reported below were based on the reaction at 20°C of 20.0 mg aliquots of the six sample pools with 10.0 ml of the test solution. Twelve milliliter capacity glass-stoppered centrifuge tubes served as containers.

(a) One aliquot from each sample pool was shaken for 55 minutes with  $8 \times 10^{-5}$  molar  $\text{Ca}^{40,45}\text{Cl}_2$  (1 uc/ml) adjusted to pH 6.5 with  $\text{NH}_4\text{OH}$ . The suspensions were centrifuged for five minutes, the supernates removed by aspiration, and the precipitates washed three times in 10.0 ml portions of distilled water. The samples were dried, weighed, dissolved in HCl, and analyzed for radio-calcium.



(b) One aliquot from each sample pool was shaken for 25 minutes with 0.001 N lactic acid, and centrifuged for five minutes. The supernates were removed by aspiration, filtered through sintered glass (nominal maximum pore size, 0.9 - 1.4 microns), and the filtrates analyzed for phosphorus.

## 2.2 Studies with Rat Molar Teeth

The molar crowns of 16 weanling male Sprague-Dawley rats were obtained as follows: At sacrifice the jaws were excised, freed of adherent tissue, and the clinical crowns cleaned with a soft brush and distilled water. Using a 7/8 in. circular steel saw in a dental handpiece, the teeth were sectioned transversely in situ approximately 0.1 mm above the gingival attachment.

After sectioning, the first and second molars of each rat were divided equally between two groups. The crowns were blotted and sealed, cut surface down, on paraffin-coated glass coverslips. Those of the control group were immersed for 60 hours in 50.0 ml of distilled water, while the crowns of the experimental group were immersed for the same time in 50.0 ml of the above 1 M calcium acetate solution.

Following treatment, the teeth were rinsed in eight 100 ml portions of distilled water, removed from the coverslips, and remounted.\* All were immersed for one hour in 50.0 ml of the radiocalcium solution and rinsed in distilled water as before. The crowns were pooled in groups of four, ground to 60 mesh, and the enamel separated from the

---

\* It was necessary to remount the crowns since the rinsing process occasionally dislodged teeth from the coverslips.



dentin [9]. The enamel samples were ignited for one hour at 550°C, weighed, dissolved in HCl and analyzed for radiocalcium.

To determine the effect of calcium treatment on solubility, the first and second molar teeth of eight weanling, Sprague-Dawley rats were sectioned, divided into two groups, and mounted on coverslips as before. After immersion for 18 hours in 50.0 ml of either distilled water or calcium acetate, the teeth were rinsed eight times in distilled water and removed from the coverslips. The first and second molars were mounted separately in groups of four and each group immersed in 25.0 ml of 0.005 N lactic acid at room temperature.\*\* After 30 minutes an aliquote of the solution was removed by aspiration, filtered, and the filtrate analyzed for phosphorus.

### 3. RESULTS AND DISCUSSION

The experimental results are summarized in Tables 1, 2, and 3. A standard error of the reported average is listed for most values in the tables. (See footnotes in each table). This standard error was calculated by pooling the variability among replicate determinations for all sets of replicates in the table-column. While this procedure ignores possible differences in precision for the different treatments, it is useful in assessing real differences in solubility and radio-calcium uptake. [Apparent differences in precision among the different treatments were not significant].

---

\*\* Stronger acid was needed to get measureable solubilities in the case of enamel than in the aforementioned synthetic apatite study.



The formula for pure hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , gives 39.8 percent calcium, 18.6 percent phosphorus, and a Ca/P weight ratio of 2.15. Comparing these values with those of the samples under test (Table 1), it is evident that the stoichiometry of the water-treated apatite specimens differs significantly from the theoretical for calcium, but not for phosphorus. These analytical data, which yield a Ca/P molar ratio of 9.2/6 instead of the theoretical 10/6, indicate that the preparation is a low-calcium (defect) hydroxyapatite.

Treatment of the apatite with calcium acetate resulted in a change in the Ca/P weight ratio from 1.99 to 2.11. This increase in Ca/P is apparently due to a relative decrease in the phosphorus content rather than an increase in the percent calcium. The reason for the lower phosphorus percentage of the calcium-acetate-treated synthetic apatite is not clear. Since less than 5 ug of the phosphorus were lost from the solid to the treatment-solutions, it is possible to rule out the surface replacement of phosphate by acetate.

Treatment of the synthetic apatite with calcium acetate resulted in a small but significant ( $p < 0.05$ ) reduction in the subsequent uptake of radiocalcium (Table 1). More striking are the findings with enamel (Table 2), where calcium treatment decreased ( $p < 0.01$ ) radiocalcium uptake by two-thirds. The extent of this decrease appeared to be somewhat greater in the case of the first molars. These results suggest that



some perfection of the calcium content of the apatite in the samples tested may have been achieved.

Treatment with calcium acetate markedly reduced the acid solubility (measured as phosphorus in solution) of both the synthetic apatite (Table 1) and the rat molar enamel (Table 2). In each instance the extent of this decrease was approximately 35 percent.

The study of the acid solubility of synthetic apatite was extended to include the calcium release as well as the phosphorous solubility, in order to obtain the  $[Ca^{++}][P_{total}]$  product. The methods employed were those previously described except that all reactions were carried out at room temperature. A sixty-four hour solubility determination was included for comparison with the thirty-minute value. In agreement with the findings of the previous experiment (Table 1), treatment of the apatite with calcium acetate resulted in a thirty-five percent decrease in the acid release of phosphorus (Table 3). While calcium release was also diminished, the extent of this decrease was only seven percent.

The excess of calcium over phosphorus in the acid filtrates of the calcium treated samples can most probably be explained by a release of adsorbed calcium. It seems unlikely, however, that this surface-held calcium is present as the acetate, since infrared absorption studies of calcium acetate-treated defect apatites have failed to reveal the presence of such a phase [10].



The results of a second acid leach of the 64-hour residues revealed no differences in solubility between the control and calcium acetate-treated apatite samples (Table 3). It appears, therefore, that the decrease in solubility following treatment with calcium acetate is the result of changes in the apatite crystal surface.

The findings of the present study, while not unequivocal, at least provide further basis for postulating the existence of defect apatites in enamel mineral. The presence of such a phase in enamel may be of importance in determining susceptibility to dental caries. Thus, the increased resistance to caries which accompanies advancing age may be due, in part, to a perfection of defect enamel apatite.

#### 4. SUMMARY

Treatment in calcium acetate reduced the acid solubility and radiocalcium uptake of both rat molar enamel and a synthetic, defect hydroxyapatite. The results suggest that the enamel mineral may contain a defect hydroxyapatite as a major phase.



BIBLIOGRAPHY

1. Posner, A. S., and Perloff, A. Apatites Deficient in Divalent Cations. J. Research NBS, 58:279 May 1957.
2. Neuman, W. F., and Neuman, M. W. The Nature of the Mineral Phase of Bone. Chem.Rev. 53:1 August 1953.
3. Posner, A. S., Fabry, C., and Dallemagne, M. J. Defect Apatite Series in Synthetic and Natural Calcium Phosphate. The Concept of Pseudoapatites. Biochim. et Biophys. Acta 15:304 October 1954.
4. Posner, A. S. A Discussion of a Defect Apatite Series. Proc. Int. Union of Pure and Applied Chem., Inorg. Section. Sept. 1954.
5. Sobel, A. E., Hanok, A., Kirschner, H. A., and Fankuchen, I. Calcification of Teeth. II. Comparison of Some Properties of Human and Rat Teeth. J. D. Res. 28:61 Feb. 1949.
6. Fabry, C. La Fixation de Chaux, en Milieu Aqueux, les Phosphates de Calcium Synthetiques de Structure Apatitique (Pseudoapatites). Biochim. et Biophys. Acta 16:377 March 1955.
7. Tisdall, F. F., and Kramer, B. J. Methods for the Direct Quantitative Determination of Sodium, Potassium, Calcium and Magnesium in Urine and Stool. J. Biol. Chem. 48:1 Sept. 1921.
8. Boltz, D. F., and Mellon, M. G. Determination of Phosphorus, Germanium, Silicon and Arsenic by the the Heteropoly Blue Method. Anal. Chem. 19:873 November 1947.
9. Manly, R. S., and Hodge, H. C. Density and Refractive Index Studies of Dental Hard Tissues. I. Methods for Separation and Determination of Purity. J. D. Res. 18:133 April 1939.
10. Posner, A. S. Unpublished data.



Table 1. Composition, solubility, and radiocalcium uptake of synthetic hydroxyapatite.<sup>1/</sup>

Treatment	Ca <sup>2+</sup> / %	P <sup>3+</sup> / %	Ca/P weight ratio	Total ug P dissolved in 0.001 N lactic acid <sup>4/</sup>	Radiocalcium <sup>5/</sup> uptake
Water	37.1	18.7	1.99	168	1.58
Calcium acetate	37.5	17.8	2.11	106	1.47

- <sup>1/</sup> All figures given in this table represent the average of three determinations.
- <sup>2/</sup> Standard error equals 0.33.
- <sup>3/</sup> Standard error equals 0.22.
- <sup>4/</sup> Standard error equals 7.2.
- <sup>5/</sup> Figures in this column represent the percent of total radiocalcium in treating solutions taken up per mg of enamel. Standard error of each figure equals 0.025.



Table 2. Solubility and radiocalcium uptake  
of rat molar enamel.

Treatment	Molar	Total ug P dissolved in 0.005 N lactic acid <u>/1</u>	Radiocalcium uptake <u>/2</u>
Water	1st	24.9	0.142(9)
	2nd	18.1	0.141(8)
Calcium acetate	1st	15.9	0.044(9)
	2nd	11.6	0.056(8)

/1 Figures in column are each the average of four determinations.  
Standard error of each equals 1.5.

/2 Figures in this column represent the percent of total radio-  
calcium in treating solution taken up per mg enamel. Each  
figure represents an average of the number of determinations  
shown in parentheses. Standard error of each figure equals  
0.006.



Table 3. Acid solubility of synthetic hydroxyapatite

No.	Sample	Hours in 0.001 N lactic acid	ug Ca per 20 ml <u>/1</u>	ug P per 20 ml <u>/2</u>	$[Ca^{++}][P_{total}]$	Ca/P
1	Calcium acetate treated	1/2	535	164	$1.78 \times 10^{-7}$	3.27
2	Calcium acetate treated	64	521	159	$1.67 \times 10^{-7}$	3.28
3	Water treated	1/2	565	259	$2.96 \times 10^{-7}$	2.18
4	Water treated	64	561	250	$2.82 \times 10^{-7}$	2.25
5	Residue of No. 2	1/2	592	272	$3.26 \times 10^{-7}$	2.18
6	Residue of No. 4	1/2	604	289	$3.51 \times 10^{-7}$	2.09

/1 Supernates of two 10 ml portions pooled for each analysis of Ca. Each value in this column represents an average of two determinations. Standard error for each figure equals 7.

/2 Figures in this column represent an average of four determinations, with a standard error for each figure shown equal to 4.

