

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND PHARMACOLOGY, SCHOOL OF MEDICINE AND DENTISTRY, THE UNIVERSITY OF ROCHESTER]

## Adsorption Studies on Enamel, Dentine and Bone<sup>1</sup>

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The adsorption of radioactive phosphorus by calcified tissues is possible both during the preparation of samples using the glycol-extraction procedure<sup>2</sup> and during calcification of these tissues in the animal body.

In previous work from this Laboratory using rats fed radioactive phosphorus, tooth and bone samples were freed from organic material by extraction with a 3% potassium hydroxide-ethylene glycol solution. If adsorption occurred during the extraction procedure, phosphate from the organic portions would be added to the inorganic material, thereby giving high results. Adsorption might be expected for tooth and bone apatites if their behavior is analogous to that of precipitated hydroxyl-apatite. Hodge, LeFevre and Bale<sup>3</sup> postulated that "commercial tertiary calcium phosphates are probably hydroxyl-apatite with more or less adsorbed phosphate ions to give empirical formulas approaching the theoretical."

Adsorption may also have a role in calcification *in vivo*, since the mineral salts have an apatite structure<sup>4</sup> yet contain greater than the theoretical amounts of phosphorus. A demonstration that phosphate ions in contact with the mineral phase of tooth or bone substance yield solutions which are described by the Freundlich adsorption isotherm would constitute an important confirmation of the adsorption hypothesis.

### Procedure

Radioactive sodium acid phosphate was prepared by dissolving radioactive red phosphorus in *aqua regia* and neutralizing with sodium carbonate to pH 6-7.

**Samples.**—The samples used were powdered, size-graded dentine, powdered enamel and bone, all of which had been extracted with 3% potassium hydroxide in ethylene glycol to remove the organic material.<sup>5</sup> The enamel and dentine had been separated according to the flotation method of Manly and Hodge.<sup>6</sup> Samples of approximately 100 mg. of the dried powders were placed in 50-cc. centrifuge tubes, 10 cc. of 3% potassium hydroxide

in ethylene glycol added and amounts of the radioactive sodium acid phosphate (not greater than 1 cc. volume) added to give various concentrations of phosphate. The mixtures were boiled for one and one-half hours, centrifuged and the glycol decanted according to the procedure already published.<sup>5</sup> The powder was then washed in boiling distilled water, centrifuged, and the water decanted and the powder washed with hot water into a porcelain filter set in a suction flask. After a second washing with hot distilled water, the powder was dissolved in hydrochloric acid, the solution evaporated down to 2 cc. and the activity determined with a Geiger-Müller counter.<sup>7</sup>

### Results

To ascertain the relation of the phosphate adsorbed to that remaining in the glycol, all the data have been plotted to test the agreement with the Freundlich adsorption equation.

$$\log_{10} \frac{x}{m} = \log_{10} k + \frac{1}{n} \log_{10} C$$

The curves for enamel, size-graded dentine and bone are found in Figs. 1 and 2. Regardless of the particle size or the amount of phosphate used for each tissue, when the logarithm of the amount of phosphate adsorbed per unit weight of adsorbent is plotted against the logarithm of the concentration remaining in the solution, a straight line is obtained.

### Discussion

Appreciable adsorption of phosphate takes place *in vitro* on enamel, dentine and bone. However, significant amounts of phosphate would be adsorbed during the preparation of samples for analysis only if the concentration of phosphate in the extraction medium is high. The calcified part of tooth tissues contains much larger amounts of marked phosphorus than the associated soft tissues, for example, the average percentage of the dose in incisor pulps is 0.007%, while that in the incisors from the same animals is 0.38%.<sup>8</sup> Therefore, even with a 95% adsorption of the phosphate from the pulp, the error would not exceed 2%, which is less than the error of the radioactivity measurement.<sup>7</sup>

According to Freundlich, the constant  $k$  is the

(1) The data in this paper were submitted by one of us (M. L. M.) in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Crowell, Hodge and Line, *J. Dent. Research*, **14**, 251 (1934).

(3) Hodge, LeFevre and Bale, *J. Ind. Eng. Chem.*, **10**, 156 (1938).

(4) Bale, LeFevre and Hodge, *Naturwissenschaften*, **40**, 636 (1936).

(5) LeFevre and Manly, *J. Am. Dent. Assoc. Dental Cosmos*, **25**, 233 (1938).

(6) Manly and Hodge, *J. Dent. Research*, **13**, 2 (1939).

(7) Bale, Haven and LeFevre, *Rev. Sci. Instruments*, **10**, 193 (1939).

(8) Manly and Bale, *J. Biol. Chem.*, **129**, 125 (1939).

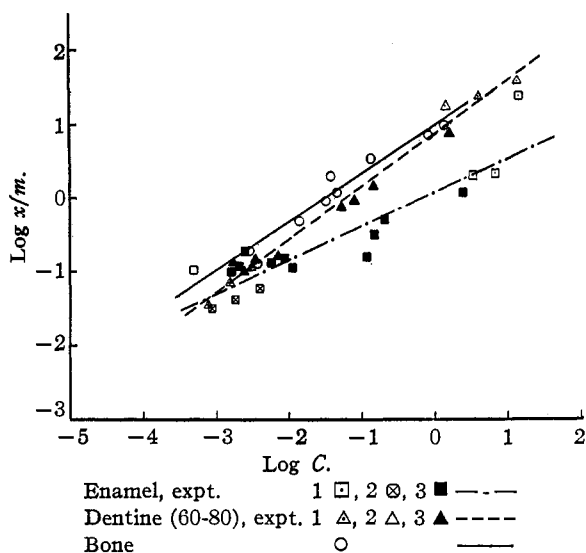


Fig. 1.—Showing the adsorption of radioactive phosphate by powdered enamel, dentine and bone. Note the adsorption in each case obeys the Freundlich equation.

adsorbent value; and, for any specified adsorbed substance, is characteristic of the adsorbent. The adsorption exponent  $1/n$  at a given temperature and for adsorbents of identical chemical composition is characteristic of the adsorbed substance. Although the mineral phase of dentine is known to have slightly more magnesium and carbonate than that of enamel, the principal constituents (calcium and phosphorus) are present in equal amounts in the two tissues<sup>9</sup> and the principal molecular lattices apparently are identical.<sup>10</sup> Therefore,  $1/n$  can be assumed to be independent of the adsorbent. From the curves for enamel, dentine and bone (Fig. 1) the calculated constants are as follows.

	Enamel	Dentine	Bone
Adsorption exponent ( $n$ )	2.3	1.4	1.6
Adsorbent value ( $k$ )	1.4	7.3	8.8

Since the adsorbed substance is the same for these experiments, the adsorption exponents should be the same. The values found for enamel, dentine and bone, 2.3, 1.4 and 1.6, respectively, are regarded as agreeing satisfactorily. However, the  $k$  values, which characterize the adsorbent, are not the same for the three tissues. Although the values for dentine and bone are nearly alike (7.3 and 8.8) the enamel value (1.4) is about one-sixth as large. This means a much greater adsorptive power for dentine and bone than for enamel. The greater adsorption is consistent with

(9) Armstrong and Brekhus, *J. Biol. Chem.*, **120**, 677 (1937).  
 (10) Bale and Hodge, *Naturwissenschaften*, **24**, 141 (1936).

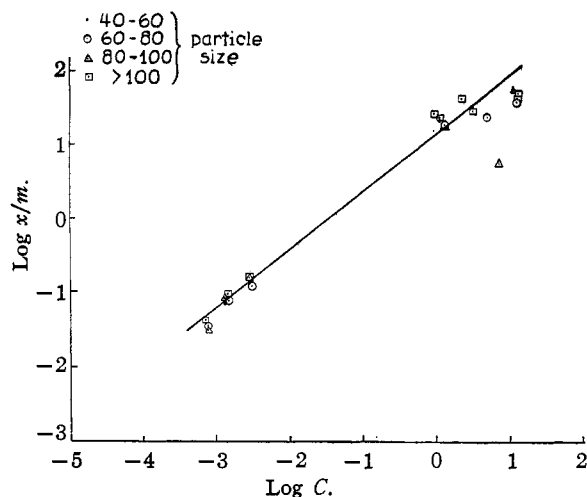


Fig. 2.—Showing the adsorption of radioactive phosphate by powdered size-graded dentine of different sizes. Note that there is no relation between adsorption and particle size.

the fact that the ultimate crystals of dentine and of bone are smaller than those of enamel (of the order of  $10^{-6}$ ,  $10^{-6}$  and  $10^{-5}$ , respectively).<sup>11</sup> The effect must be attributed to the ultimate crystal size, for differences in particle size do not affect the amount of phosphate adsorbed (Fig. 2).

Furthermore, the concept of Hodge<sup>12</sup> that tooth and bone crystals are hydroxyl-apatite with varying amounts of adsorbed phosphate is supported by these findings.

One point should be emphasized: these studies demonstrate true adsorption and not the establishment of a chemical equilibrium between the tooth or bone powder and the phosphate in the surrounding medium. At the beginning of the experiment, there is a certain ratio of marked to total phosphorus in the extraction medium. If there were a chemical exchange of phosphorus, a constant ratio between marked phosphorus in the tissue and in the extraction medium would be observed and there would be no change in the total phosphorus in either phase. The data presented here show no such linear relationship; they satisfy exactly the criteria for adsorption according to Freundlich's adsorption isotherm.

**Acknowledgments.**—The authors acknowledge the assistance of Dr. Harold C. Hodge and Dr. Richard S. Manly in supplying some of the samples and for aid in preparing the manuscript.

(11) Bale, Hodge and Warren, *Am. J. Roent. Rad. Therap.*, **33**, 369 (1934).  
 (12) French, Welch, Simmons, LeFevre and Hodge, *J. Dent. Research*, **17**, 401 (1938).

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### Summary

Quantitative measurements on the adsorption

of phosphate ion by powdered enamel, dentine and bone have been made. From varying concentrations of radioactive phosphorus as sodium acid phosphate in ethylene glycol, the tissues adsorb phosphate according to the Freundlich adsorption isotherm. The adsorptive power of dentine and bone are about the same and seven times greater than that of enamel.

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## A Redetermination of the Fluorine-Fluorine Distance in Potassium Bifluoride

BY L. HELMHOLZ AND M. T. ROGERS

### Introduction

In an electron diffraction investigation of gaseous hydrogen fluoride, Bauer, Beach and Simons<sup>1</sup> obtained the value  $2.55 \pm 0.03 \text{ \AA}$ . for the F-H-F distance and confirmed the existence of polymers  $(\text{HF})_n$ . The reported values for the F-H-F distance in crystals are:

$$\begin{aligned} \text{NH}_4\text{HF}_2 &= 2.37 \pm 0.1 \text{ \AA}.^2 \\ \text{NaHF}_2 &= 2.50 \pm 0.2 \text{ \AA}.^3 \\ \text{KHF}_2 &= 2.25 \pm 0.2 \text{ \AA}.^4 \end{aligned}$$

The assumption that the mean value  $2.25 \text{ \AA}$ . reported for potassium bifluoride is correct leads to the conclusion that the F-H-F bond is weakened and the distance correspondingly increased when additional hydrogen bonds are formed as in  $(\text{HF})_n$ . Also the distance is not accurately enough known to judge whether the two additional hydrogen bonds from fluorine to nitrogen in ammonium bifluoride cause a lengthening of the F-H-F distance over that in alkali bifluorides. In view of the large limits of error ascribed to the F-H-F distance, a redetermination of the parameter in potassium bifluoride has been carried out in order to test this conclusion.

In this work the value  $2.26 \pm 0.01 \text{ \AA}$ . has been found for the F-H-F distance in close agreement with the mean value reported by Bozorth.<sup>4</sup>

### Experimental

Potassium hydrogen fluoride crystallizes in the tetragonal system forming plates with  $\{001\}$  de-

veloped and sides parallel to  $\{110\}$ . The structure was determined by Bozorth,<sup>4</sup> who gives it the space group  $D_{4h}^{18}$ , four molecules in the unit cell, with the atoms in the following positions

$$4\text{H in } 0\frac{1}{2}0; \frac{1}{2}00; \frac{1}{2}0\frac{1}{2}; 0\frac{1}{2}\frac{1}{2}$$

$$4\text{K in } 00\frac{1}{2}; 00\frac{3}{4}; \frac{1}{2}\frac{1}{2}\frac{1}{2}; \frac{1}{2}\frac{1}{2}\frac{3}{4}$$

$$8\text{F in } u, \frac{1}{2} + u, 0; \bar{u}, \frac{1}{2} - u, 0; \frac{1}{2} - u, u, 0; \frac{1}{2} + u, \bar{u}, 0;$$

$$\frac{1}{2} + u, u, \frac{1}{2}; \frac{1}{2} - u, \bar{u}, \frac{1}{2}; u, \frac{1}{2} - u, \frac{1}{2}; \bar{u}, \frac{1}{2} + u, \frac{1}{2}$$

$$a = 5.67 \text{ \AA}. \quad c = 6.81 \text{ \AA}. \quad u = 0.14 + 0.01 \quad \text{F-H-F} = 2.25 \pm 0.02 \text{ \AA}.$$

Symmetric and asymmetric Laue photographs were taken with the beam normal to  $(001)$ . The parameter was narrowed down to a small range by the use of qualitative Laue data.

Theoretical curves for the dependence of intensity on the parameter  $u$  were calculated from the equation

$$I = C \left| S \right|^2 e^{-3.0(\sin \theta/\lambda)^2}$$

using  $f$  values from the Pauling-Sherman tables.

Comparing observed intensity ratios with the theoretical curves of Fig. 1, the upper and lower limits shown in Table I were placed on the value

TABLE I  
PARAMETER DETERMINATION FOR POTASSIUM BIFLUORIDE  
USING INTENSITIES OF LAUE REFLECTIONS

Reflecting planes	$\frac{\sin \theta}{\lambda}$	$\lambda$	Observed intensity ratio	Parameter
(802)	0.720	0.349	(802) < (732)	$u > 0.1393$
(732)	.687	.350		
(662)	.762	.333		
(752)	.773	.328	(752) > (662)	$u < .1413$
(914)	.851	.361		
(732)	.687	.367	(194) > (732)	$u > .1390$
(864)	.924	.406		
(934)	.884	.408	(864) > (934)	$u > .1381$

(1) S. H. Bauer, J. Y. Beach and J. H. Simons, *THIS JOURNAL*, **61**, 19 (1939).

(2) L. Pauling, *Z. Krist.*, **85**, 380 (1933).

(3) C. Anderson and O. Hassel, *Z. physik. Chem.*, **123**, 151 (1926).

(4) R. M. Bozorth, *THIS JOURNAL*, **45**, 2128 (1923).