Adsorption of phosphates by calcified tissues may occur from the potassium hydroxide-ethylene glycol solutions used to extract the organic portion of tooth and bone substance (1, 2). Since it has recently been shown that dentin and enamel exchange phosphates with aqueous phosphate solutions (3), it is of interest to determine whether these systems are described by adsorption isotherms.

**Procedure**

Radioactive phosphate solutions were prepared from bombarded red phosphorus as has been described previously (1). Aliquots from these solutions were suitably diluted; the activities determined by the Geiger-Müller scale-of-four counter (4) were used as standards for calculating the experimental values.

The samples used were powdered (60 mesh) bone, dentin, and enamel. The bone and enamel samples had been glycol-ashed (2); the dentin was the unashed product of the centrifugal flotation separation process (5). The hydroxyapatite was Sample H1 (Ca:P ratio 2.10) described by Hodge, LeFevre, and Bale (6). It was not screened.

Suitable aliquots of the radioactive phosphate solution were made up to 50 cc. with solutions of disodium acid phosphate.* This work was supported in part by grants from the Carnegie Corporation of New York and from the Rockefeller Foundation.
Phosphate Adsorption

which varied in concentration from 0.2 to 0.00002 M by powers of 10. 25 cc. of the phosphate solutions containing radioactive phosphorus were stirred with 50 mg. samples of bone, dentin, enamel, or hydroxyapatite for 30 minutes at 40°. The solutions were decanted after centrifugation and the precipitates washed twice with distilled water. The precipitates were dissolved in about 3 cc. of normal hydrochloric acid. The counting procedure has been described previously (7) as well as the methods of calculation.

Data

In Table I are given the mg. of phosphorus picked up per gm. of bone, hydroxyapatite, dentin, and enamel at each of the final concentrations of phosphate found. There is a regular progression

<table>
<thead>
<tr>
<th>Equilibrium, M concentration of phosphate (approximate)</th>
<th>Average P picked up per gm. solid</th>
<th>Log X/M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone</td>
<td>Apatite</td>
</tr>
<tr>
<td>1.6 X 10^{-1}</td>
<td>55.2</td>
<td>45.7</td>
</tr>
<tr>
<td>1.6 X 10^{-2}</td>
<td>24.1</td>
<td>19.1</td>
</tr>
<tr>
<td>1.6 X 10^{-3}</td>
<td>4.4</td>
<td>1.8</td>
</tr>
<tr>
<td>1.5 X 10^{-4}</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>1.4 X 10^{-4}</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

for each substance; 20 to 50 mg. of phosphorus are picked up per gm. of solid exposed to the highest phosphate concentration and 0.04 to 0.2 mg. is picked up from the most dilute phosphate solution. Expressed as percentages of the total phosphate in the solution initially, the solids picked up approximately 1, 1, 4, 10, and 20 per cent from the solutions in order of increasing dilution.

When the logarithms of the mg. of phosphorus picked up per gm. of sample (log_{10} X/M) are plotted as ordinates against the logarithm of the molar concentration of phosphate remaining in the solution at equilibrium (log_{10} C), a straight line is obtained (Fig. 1) for each solid. These lines are not parallel; instead they diverge in the lower concentration ranges. However, since a
linear function is obtained for each calcium phosphate, this
criterion of adsorption is satisfied and each substance may be said
to adsorb phosphates under the conditions of the experiment.
The equation for each of these lines was calculated; the $n$ and $k$

\[
\log_{10} X/M = \log_{10} k + \frac{1}{n} \log_{10} C
\]

values for the Freundlich adsorption equation $\log_{10} X/M = \log_{10} k + \frac{1}{n} \log_{10} C$ are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Bone</th>
<th>Apatite</th>
<th>Dentin</th>
<th>Enamel</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>1.71</td>
<td>1.59</td>
<td>1.47</td>
<td>1.40</td>
</tr>
<tr>
<td>$k$</td>
<td>209</td>
<td>166</td>
<td>115</td>
<td>87</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The isotherms (Fig. 1) show that bone, dentin, and enamel adsorb phosphates from aqueous solutions at approximately body temperature. Furthermore, these calcified tissues adsorb phos-
Phosphates in a fashion mathematically comparable to the adsorption by a synthetic hydroxyapatite. This evidence supports the concept (6) that the principal inorganic molecule of tooth and bone substance is hydroxyapatite upon the minute crystal surfaces of which sufficient phosphates are adsorbed to account (at least in part) for the calcium to phosphorus ratio of 2.10 (theoretical Ca:P ratio for hydroxyapatite, 2.15).

In Fig. 1, the location of the isotherms indicates that in order of their ability to adsorb phosphates bone > dentin > enamel. Armstrong (3) has recently compared the ability of dentin and enamel to pick up phosphate from aqueous solutions at 37°. His data for dentin (log X/M = 1.1, log C = -1.2) fall almost exactly on our isotherm for dentin; however, his data for enamel (log X/M = +0.2, log C = -1.2) fall below our isotherm. Manly and Levy (1) have reported that, from ethylene glycol solution at 200° under conditions like those used in ashing calcified tissues, the calcified tissues adsorb phosphates in the order bone > dentin > enamel. Volker et al. (7) have reported the same order in the ability of these tissues to adsorb fluorides. The k value, characteristic of the adsorbent, expresses the adsorbing power quantitatively. When the k for enamel is set at unity, the k values for bone and dentin are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Bone</th>
<th>Dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate at 40°</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>&quot; 200° (1)</td>
<td>6.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Fluoride 40° (6)</td>
<td>2.3</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Undoubtedly this order arises from some fundamental difference in the crystals. One known physical difference is in the size of the ultimate crystals; these are much smaller in dentin and bone (10⁻⁵ to 10⁻⁶ cm.) than in enamel (10⁻⁴ cm.). The smaller crystals would have a larger effective adsorbing surface and should show a greater adsorbing power (k). The greater density of enamel (2.9) as compared to dentin (2.2) or bone (about 2.0) would limit the permeability of this tissue to aqueous solutions and thus tend also to give a lower rate of phosphate exchange.

When the data on phosphate adsorption at 40° are compared with those at 200°, two facts are obtained. First, there is (as
would be expected) less phosphate adsorbed at 200° than at 40°; e.g., for bone at 200°, \( k = 8.8 \); at 40°, \( k = 209 \). Second, the isotherms at 200° are approximately parallel to those at 40°; e.g., for bone at 200°, \( n = 1.6 \); at 40°, \( n = 1.7 \).

The delicateness of this radiophosphate method should be emphasized. The data for 50 mg. samples of bone in a 0.00002 M phosphate solution showed from eight analyses that an average of 9.6 γ of phosphorus were picked up. The eight analyses varied from this average by only 1 per cent (average deviation). To have established this fact chemically would have called for the difficult task of distinguishing between 4.25 and 4.26 mg. of phosphorus in each 50 mg. bone sample before and after exposure to the phosphate solution.

The concept of a mineral exchange in bone has been widely accepted. In bone (and probably in dentin) part of the phosphates is assumed to be in equilibrium with the blood phosphates (8). As the blood radiophosphorus level rises and falls, the labile bone \( P^{32} \) level also rises and falls. This rapid turnover may involve several equilibria in which adsorption plays a part. Exchange adsorption would increase the bone radiophosphorus level by exchanging \( P^{31} \) atoms of the surfaces of the minute crystals with \( P^{32} \) atoms of the blood. This process probably accounts in part for the rapid pick-up of \( P^{32} \) by various calcified tissues (9). Part of the normal mineral metabolism of labile bone may be a solution and reprecipitation of calcium phosphates. In these reactions, radiophosphorus would fit into the hydroxyapatite lattice in the place of \( P^{31} \) atoms. Furthermore, the new crystals would present surfaces upon which \( P^{32} \) atoms could be adsorbed in the ratio \( P^{32}:P^{31} \) currently in the blood.

In bone, the adsorption processes would be of some importance owing to the large fraction shown to be labile (one-sixth of epiphyseal bone in young rats) (8). However, in enamel, the slow interchange of fluids (by diffusion) would permit contact of radiophosphorus with a much smaller surface in a given time. Thus, a negligible turnover of radiophosphorus would be expected and has been described (10). On the other hand, if phosphate solutions (e.g. the saliva) with high \( P^{32}:P^{31} \) ratios were placed in contact with the teeth, even the relatively small surface exposed should adsorb sufficient \( P^{32} \) to give a discernible radioactivity. Volker
and Sognnaes (11) have observed higher P\textsuperscript{32} values for surface than for inner layers of enamel and suggest that adsorption is a factor. Calculations with the data of Table I have shown that the salivary levels (P\textsuperscript{32}:P\textsuperscript{31}) may be sufficient to account for as much P\textsuperscript{32} as has been found in surface enamel specimens. In these calculations it is assumed that area for area the phosphate is adsorbed in the proportions observed for powdered enamel samples. Thus, adsorption of phosphates may be in part the explanation for the finding of von Hevesy and Armstrong (12) that there are small but detectable activities in the enamel of the teeth of cats following the subcutaneous administration of highly radioactive phosphate solutions.

**SUMMARY**

1. Bone, dentin, and enamel adsorb phosphates at 40° in a manner comparable to the adsorption of phosphates by hydroxyapatite.

2. The adsorbing power of bone is greater than that of dentin which is greater than that of enamel. This order may depend on the fact that bone and dentin have smaller mineral crystals than are found in enamel and thus have larger adsorbing surfaces.

3. The importance of adsorption is indicated (a) in calcification phenomena and (b) in accounting for the presence of radioactive phosphorus in the surface of tooth enamel.

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THE ADSORPTION OF PHOSPHATES AT FORTY DEGREES BY ENAMEL, DENTIN, BONE, AND HYDROXYAPATITE AS SHOWN BY THE RADIOACTIVE ISOTOPE

Harold Carpenter Hodge, Grant Van Huysen, John F. Bonner and Stanley N. Van Voorhis


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