CALCIUM EXCHANGE; THE MECHANISM OF ADSORPTION
BY BONE OF Ca\textsuperscript{45}\textastisk signage

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Until recently, the difficulties of obtaining and of measuring the radio-
active isotopes of calcium have kept all but a few investigators from ex-
amining the distribution of calcium in the hard tissues of the body. Using
Ca\textsuperscript{45} as a tool, Campbell and Greenberg (1), Pecher (2), and Greenberg (3)
found between 15 and 28 per cent of a dose in the skeleton of mice or rats
within short periods of time. More recently, Armstrong and Barnum (4)
administered Ca\textsuperscript{45} and P\textsuperscript{32} simultaneously to rats and, after 5 days,
compared the percentages of each isotope in various tooth and bone tissues.
The total isotope content of the entire skeleton was not estimated, but
similar percentages of the Ca\textsuperscript{45} and P\textsuperscript{32} doses were reported in a number
of bone samples.

All of these workers agree that a substantial part of a Ca\textsuperscript{45} dose appears
promptly in the skeleton. Since the time periods of the studies \textit{in vivo}
were too short to permit growth and calcification to be major factors, the
experiments \textit{in vitro} reported below are believed to give grounds for a
hypothesis that can be applied directly to the rapid phase of Ca\textsuperscript{45} deposi-
tion in the living animal.

Previous work in this laboratory has shown (a) that P\textsuperscript{32} also appears
promptly in considerable proportions in the bone (5) and (b) that the
rapid phase of the radiophosphorus adsorption is adequately described as
a reversible exchange reaction (6). The incorporation of phosphorus into
bone glycol ash \textit{in vitro} over a period of 7 days reaches a steady state; at
this time about 20 per cent of the bone phosphorus has exchanged with
solution phosphorus. The magnitude of the exchangeable phosphate frac-
tion of the bone makes this exchange reaction important physiologically.

Since the principal mineral constituent of the bone has long been de-
scribed as a calcium phosphate, it is natural to inquire whether calcium

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behaves in an analogous manner; i.e., whether calcium of the bone takes part in a rapid reversible exchange in vivo with the calcium of extracellular fluid, and whether solution calcium would exchange in vitro with calcium of bone ash. The techniques which had been applied to the study of the mechanism of phosphate exchange in vitro have been adapted for the present work on calcium. It was observed that the adsorption\(^1\) of Ca\(^{46}\) by bone is an exchange reaction which follows a pattern in the rapid phase similar in most respects to that of the phosphate exchange as shown by P\(^{32}\).

**EXPERIMENTAL**

50 mg. samples of glycol-ashed rabbit bone, powdered and graded to size to an 80 to 100 mesh fraction, were exposed at 40° to 25 ml. volumes at CaCl\(_2\) solutions, pH 7.4, of three concentrations (viz., 1.25 \(\times\) 10\(^{-2}\) M, 1.25 \(\times\) 10\(^{-3}\) M, and 1.25 \(\times\) 10\(^{-4}\) M). Exposure times for different samples varied from \(\frac{1}{2}\) hour to 15 days, during which time the tubes containing the samples were rotated end to end continuously at 40 r.p.m. The 10\(^{-3}\) M calcium concentration was chosen because it approximated the concentration usually cited as that of “diffusible” calcium in blood serum. In order to observe the effects of changing the concentration upon the rate of exchange of calcium, 10\(^{-2}\) M and 10\(^{-4}\) M calcium solutions were used.

Each solution contained a known amount of radioactive calcium, Ca\(^{46}\), as determined by a mica window counter.\(^2\) Aliquots of the solution were counted directly if the activity of the samples exceeded 3 times the background count of 12 c.p.m.; samples of very low activity were counted after evaporation to dryness under an infra-red lamp. The counting efficiency was greatly enhanced (10 times) by counting the solid samples and, although the absolute standard error was higher in counts of solid samples, the percentage error was lower (Table I).

Radioactive calcium\(^3\) solutions were prepared as follows: 125 mg. of CaCO\(_3\) were dissolved in the calculated amount of 3 N HCl and heated to drive off the CO\(_2\). This material was diluted to about 800 ml. with dis-

\(^1\) The terms “adsorption” and “desorption” as used in this paper neither imply nor exclude a change in the total amount of calcium at the interface. Adsorption is used to describe the transfer of Ca\(^{46}\) from the solution to the solid; desorption is used to denote the reverse process.

\(^2\) The mica window had a thickness of 3 to 4 mg. per sq. cm. The counting tube was purchased from the Radiation Counter Laboratories, Inc., Chicago, Illinois.

\(^3\) Ca\(^{46}\), half life 180 days, was prepared by neutron bombardment in the pile at the Clinton Laboratories, Oak Ridge, Tennessee. One sample of Ca\(^{46}\) was obtained through the courtesy of Dr. Arthur K. Solomon of the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts. This Ca\(^{46}\) was prepared by transmutation from scandium and was essentially carrier-free.
tilled water and the pH was adjusted to 7.3 to 7.4 by the addition of several ml. of 0.001 M NH₄OH. As one of the reported radiocontaminants was argon (7), air was bubbled vigorously through the solution for 15 minutes before the solution was made up to 1 liter volume with distilled water.

The calcium content of the solutions and of the bone samples was determined by the method of Salomon, Gabrio, and Smith (8) before and after exposure of the bone ash to the solutions. Phosphorus was determined colorimetrically by the method of Fiske and Subbarow (9) in selected solutions after exposure to the bone ash, and occasionally in bone ash residues.

Results

In the adsorption experiments, 100 per cent of the Ca⁴⁶ was in the solution initially at each concentration, and 0 per cent was in the bone. As the time of exposure lengthened, larger and larger percentages of the Ca⁴⁶ appeared in the bone. Table II shows the percentages of Ca⁴⁶ in bone samples after their exposure to radioactive CaCl₂ solutions (10⁻², 10⁻³, and 10⁻⁴ M, respectively) for periods of 1/2 hour to 14 days. The figures are given as the means of several samples plus or minus the standard deviation. At each concentration there was an initial rapid increase followed by a prolonged horizontal curve which appeared to be a steady state and which was reached rapidly (4 hours) at 10⁻³ M, whereas at 10⁻² M and 10⁻⁴ M the plateau was reached more slowly (1 and 4 days, respectively). The percentages of Ca⁴⁶ on the bone at the three concentrations were in reverse order, as follows: 10⁻² M, 15 per cent; 10⁻³ M, 60 per cent; 10⁻⁴ M, 90 per cent.

### Table I

**Counting Efficiency in Counting Solid Samples**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>State*</th>
<th>No. of samples</th>
<th>No. of 5 min. counts</th>
<th>Average net count, c.p.m. per ml.</th>
<th>Standard deviation</th>
<th>Standard error</th>
<th>Per cent standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25 × 10⁻² M</td>
<td>Wet</td>
<td>20</td>
<td>40</td>
<td>150</td>
<td>0.6</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>10</td>
<td>40</td>
<td>1300</td>
<td>4.3</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>1.25 × 10⁻³ M</td>
<td>Wet</td>
<td>20</td>
<td>40</td>
<td>29</td>
<td>0.9</td>
<td>0.5</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>20</td>
<td>80</td>
<td>290</td>
<td>11.0</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>1.25 × 10⁻⁴ M</td>
<td>Wet</td>
<td></td>
<td></td>
<td>Too low to count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>20</td>
<td>80</td>
<td>40</td>
<td>1.4</td>
<td>0.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* "Wet" means in solution (2 ml. counting volume); "dry" means evaporated to dryness (2 ml. original volume).
**DISCUSSION**

_Exchange versus Deposition_—The adsorption of Ca$^{45}$ can be shown to be a reversible exchange reaction, as previously demonstrated for P$^{32}$ (6). Thus, the percentages of Ca$^{45}$ found in the bone increased with time at all three concentrations but equivalent increases in the calcium content of the bone sample could not be demonstrated. A change in the total calcium commensurate with the change in Ca$^{45}$ could have been easily detected by our analytical methods. For example, the bone sample absorbed approximately 60 per cent of the Ca$^{45}$ from a 25 ml. aliquot of the 1.25 × 10$^{-3}$ M solution. Since this solution contained 1.27 mg. of cal-

<table>
<thead>
<tr>
<th>Time</th>
<th>10$^{-4}$ M Ca (3)*</th>
<th>10$^{-4}$ M Ca (8)*</th>
<th>10$^{-4}$ M Ca (6)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>58 ± 0.2</td>
<td>31 ± 1.6</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>66 ± 0.6</td>
<td>37 ± 0.7</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>1.5</td>
<td>45 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>63 ± 0.8</td>
<td>62 ± 0.9</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>76 ± 1.2</td>
<td>65 ± 1.5</td>
<td>6 ± 0.7</td>
</tr>
<tr>
<td>8</td>
<td>87 ± 1.4</td>
<td>59 ± 1.6</td>
<td>6 ± 0.4</td>
</tr>
<tr>
<td>16</td>
<td>81 ± 1.0</td>
<td>59 ± 3.1</td>
<td>8 ± 0.8</td>
</tr>
<tr>
<td>days</td>
<td>83 ± 2.4</td>
<td>60 ± 2.8</td>
<td>12 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>85 ± 1.9</td>
<td>63 ± 1.9</td>
<td>12 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>92 ± 1.1</td>
<td>62 ± 1.2</td>
<td>14 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>91 ± 2.9</td>
<td>61 ± 2.5</td>
<td>14 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>99 ± 3.0</td>
<td>60 ± 2.0</td>
<td>13 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>64 ± 2.1</td>
<td>64 ± 2.1</td>
<td>14 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>61 ± 3.9</td>
<td>61 ± 3.9</td>
<td>13 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>92 ± 2.6</td>
<td>63 ± 1.7</td>
<td>15 ± 1.8</td>
</tr>
<tr>
<td>10</td>
<td>72 ± 1.3</td>
<td>72 ± 1.3</td>
<td>14 ± 1.6</td>
</tr>
<tr>
<td>11</td>
<td>62 ± 6.8</td>
<td>62 ± 6.8</td>
<td>14 ± 0.9</td>
</tr>
<tr>
<td>12</td>
<td>59 ± 5.3</td>
<td>59 ± 5.3</td>
<td>15 ± 0.9</td>
</tr>
<tr>
<td>13</td>
<td>67 ± 1.4</td>
<td>67 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>85 ± 3.6</td>
<td>60 ± 2.5</td>
<td>15 ± 0.9</td>
</tr>
</tbody>
</table>

* Each reading represents the mean of the number of samples indicated in parentheses. The per cent of standard error of all determinations = 1.9.
Calcium initially, the final content should have been $1.27 - 0.76$, or $0.51$ mg., if 60 per cent of the total calcium had been deposited on the bone. Actually, the average of several analyses (Table III) showed the final calcium content to be $1.48$ mg. Obviously, the net Ca$^{46}$ deposition in the bone had not been accompanied by a proportionate net deposition of the

**Table III**

*Comparison of Calcium Content of Solution Aliquots As Determined by Analysis with Calculated Contents from Ca$^{46}$ Data*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Initial Ca content of 25 ml. solution</th>
<th>Ca$^{46}$ loss from solution, 8 hrs.</th>
<th>Calculated Ca content</th>
<th>Analyzed Ca content</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.25 \times 10^{-2}$</td>
<td>12.7 mg.</td>
<td>6 per cent</td>
<td>12.0 mg.</td>
<td>13.0 mg.</td>
</tr>
<tr>
<td>$1.25 \times 10^{-3}$</td>
<td>1.27 mg.</td>
<td>59 per cent</td>
<td>0.52 mg.</td>
<td>1.48 mg.</td>
</tr>
<tr>
<td>$1.25 \times 10^{-4}$</td>
<td>0.125 mg.</td>
<td>78 per cent</td>
<td>0.028 mg.</td>
<td>0.125 mg.</td>
</tr>
</tbody>
</table>

Fig. 1. Desorption of Ca$^{46}$ from bone compared to adsorption from solution

non-radioactive calcium.$^4$ Similarly, at the other two calcium concentrations, if a net deposition of Ca$^{46}$ had occurred along with the adsorption of Ca$^{45}$, the calcium content of the final solutions should have decreased to the values shown in the 4th column of Table III. Instead, the final solutions by analysis gave the average values shown in the 5th column; there was a little gain in calcium content, if any change, but certainly not a loss at any concentration. The results are plausibly explained by as-

$^4$ This statement should not be interpreted to mean that only Ca$^{46}$ atoms are exchanging with Ca$^{46}$ atoms. The Ca$^{46}$ atoms serve to indicate the exchange of Ca$^{40}$ (the other isotopes of calcium) and Ca$^{48}$ ions between the solution and the solid phases.
suming that the Ca\(^{46}\) of the solution had exchanged with the Ca\(^{40}\) of the bone.

If the hypothesis is valid, then the reverse reaction should be demonstrable; Ca\(^{40}\) of the solution should exchange for Ca\(^{45}\) already on the bone. To test this hypothesis experimentally, a sample of powdered bone was allowed to absorb Ca\(^{46}\) from a 1.25 \(\times\) 10\(^{-3}\) M calcium solution for 9 days. The total Ca\(^{45}\) on the bone was arbitrarily set at 100 per cent and the losses when the bone was exposed to a non-radioactive, 1.25 \(\times\) 10\(^{-3}\) M, calcium solution were expressed on a percentage scale (Fig. 1) for increasing desorption times up to 9 days. The rapid initial exchange is strikingly reproduced; the desorption curve appears to be nearly a mirror image of the adsorption curve in this respect. After 24 hours exposure, the bone had about 75 per cent of its original Ca\(^{45}\) content, whereas the bone in the adsorption test had only 60 per cent of the total originally in the solution. If the controlling reaction in both cases were an exchange and if the same bone calcium atoms took part in both tests, it would seem logical to expect the ultimate distribution of Ca\(^{46}\) between bone and solution to be identical in the two experiments, regardless of where the Ca\(^{45}\) was initially. That a rough approximation of this identity was achieved is evident from Fig. 1; the curves for the ultimate distribution (i.e. 10 to 14 days) appear to be converging towards the same percentage. These data are in line with the behavior predicted if the reaction between solution and bone calcium is a reversible exchange.

**Exchangeable Fraction**—At what apparently is an equilibrium condition, the Ca\(^{46}\) is distributed between bone and solution in a system comprising all of the solution Ca\(^{40}\) and a fraction of the bone Ca\(^{40}\) (the bone ash contained on the average 37.5 per cent of calcium). The percentage of the bone calcium taking part in this exchange may be calculated at equilibrium by the following equation (10):

\[
\text{% exchangeable bone Ca} = \left(\frac{\text{mg. Ca}}{\text{c.p.m. Ca}^{46}}\right)_{\text{solution}} \times \left(\frac{\text{c.p.m. Ca}^{46}}{\text{mg. Ca}}\right)_{\text{bone}} \times 100
\]

After 14 days, at the two higher concentrations, nearly 25 per cent of the bone (glycol ash) calcium had exchanged with solution calcium. Insoluble as bone seems to be, this large fraction of exchangeable calcium gives bone extraordinary power as a mineral storehouse and regulator. The exchangeable calcium may be the source of the easily mobilized calcium (11) whose appearance was previously ascribed to a freely soluble bone salt. At the lowest concentration, the fraction of bone calcium exchanged was only 6 to 8 per cent; it is probable that equilibrium was not yet established. The exchangeable fraction of calcium in glycol-ashed bone is
strikingly similar in magnitude to the fraction of bone phosphate that exchanges with solution phosphate (6).

Armstrong and Barnum's measurements (4) of the Ca\(^{46}\) and P\(^{32}\) uptake by rat bones in vivo 5 days after injection showed unexpected similarities when the relative specific activities of calcium and phosphorus were compared. Their data recalculated on the basis of percentages of the doses of the two isotopes found in bone samples are given (Table IV) with the data in vitro from the current study for concentrations of calcium and phosphate often cited as characteristic of extracellular fluid (viz., of the order of \(10^{-3} \text{ M}\)). The absolute values of the percentages are not comparable but the ratios of Ca\(^{45}\) to P\(^{32}\) percentages are notably alike. It might be inferred that the similarity in the ratios is evidence of a similarity in the processes by which the amounts of Ca\(^{45}\) and P\(^{32}\) held by bone are determined. On the basis of the exchange hypothesis, the exchangeable fraction would be the limit in vivo or in vitro for the adsorption of either isotope. If the exchangeable fractions represent physical entities, the ratios should be constant whether the adsorption occurred in the femur of the intact rat or in rabbit bone ash in a test-tube.

In earlier work with P\(^{32}\), the exchangeable phosphate was shown to account for about 20 per cent of the total bone phosphate (12). From independent calculations based on surface area measurements, the apatite micro crystals were considered to have roughly 20 per cent of their phosphate groups in the crystal surfaces (6). These surface phosphate groups would constitute, therefore, an adequate source of exchangeable phosphate, and it was suggested that phosphate from the surfaces of the crystals was the only bone phosphate exchanging in the time periods studied. The nearly identical behavior of calcium, as shown by the Ca\(^{45}\) distribution, immediately leads to the hypothesis that surface calcium, like surface phosphate, is capable of rapid, reversible exchange with the solution ions. If a fifth of the calcium of the apatite were also in the surface layer of the

| Table IV Ca\(^{46}\) and P\(^{32}\): Percentages of Doses in Bones after 5 Days |
|-----------------|------|------|--------------|
| Experiment      | Sample                    | Ca\(^{46}\) | P\(^{32}\) | Per cent ratio, Ca\(^{46}\):P\(^{32}\) |
| Armstrong and Barnum, in vivo | Femur epiphyses          | 1.58       | 1.10       | 1.44         |
|                  | " diaphyses               | 5.0        | 3.7        | 1.35         |
| Present study, in vitro | Mixed glycol-ashed bone | 66.0       | 44.0       | 1.52         |
crystals and available for exchange, the magnitude of calcium exchange is reasonably well accounted for.

The calcium exchange after 14 days exceeded the phosphate exchange regularly by about 5 per cent. A similar comparison of calcium and phosphate exchanges at two other concentrations of each ion (unpublished data) demonstrated comparable differences; i.e., the calcium exchange was always somewhat greater than the phosphate. Such a difference might have been expected from the hypothetical picture Bale et al. (13) offered in which most of the calcium and phosphate of the bone is accounted for in the micro crystals of hydroxylapatite and the extra calcium, the carbonate, and other mineral constituents are "occluded, adsorbed or interstitially crystallized" (14). Presumably any interstitial or surface-attached calcium would be able to take part in an exchange with solution calcium at least as freely as the calcium atoms of the surface layer of the apatite crystals. If so, a slightly greater proportion of the total bone calcium than of the total bone phosphate would be exchanged. Additional

<table>
<thead>
<tr>
<th>Element</th>
<th>Per cent exchange in bone</th>
<th>Exchangeable fraction after ignition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycol ash</td>
<td>After ignition at 900°</td>
</tr>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>P</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>Ca</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

* Percentage of preignition value.

Table V gives the percentages exchanged after 48 hours exposure to $1 \times 10^{-3} \text{M}$ solutions of phosphate and calcium, respectively. The greater percentages of calcium exchanged in both types of ash are evidence of the greater availability of this element.

Many analyses of bone samples give Ca:P ratios of 2.2 and higher, whereas the theoretical ratio for hydroxylapatite is 2.14.

Some solution undoubtedly occurs. The calcium contents of the solutions in contact with glycol ash tend to increase slowly. The calcium content of 25 ml., at the concentration of $10^{-2} \text{M}$ at the initial time, is 12.7 mg., at the end of the rapid phase (4 hours) 14.4, and during the slow phase (10 days) 14.7; at the concentration of $10^{-3} \text{M}$, at the initial time the calcium content is 1.28 mg., at the end of the rapid phase (20 hours) 1.31, and during the slow phase (10 days) 2.30; at the concentration of $10^{-4} \text{M}$ at the initial time it is 0.125 mg., at the end of the rapid phase (8 hours) 0.131, and during the slow phase (10 days) 0.164.
Exchange Rates—In glycol ash, the calcium exchange follows an exponential course; the assumptions and equations derived by Merrell, Gellhorn, and Flexner (15) for the exchange of sodium between blood and extravascular fluid were modified and applied successfully to the calcium data. In Fig. 2, the logarithms of the differences (log Δ) between percentages of Ca\(^{45}\) on the bone at any time and at “equilibrium” are plotted as ordinates against the time; linear relations were obtained at each concentration. The rate constants of the equations have physical interpretations (15); however, the half time constants describe the over-all rates as follows: 10\(^{-2}\) M, 16 hours; 10\(^{-3}\) M, 2.2 hours; 10\(^{-4}\) M, 3.8 hours. Why the 10\(^{-2}\) M solution gave a slower reaction is not known. No suitable data.

Falkenheim, Underwood, and Hodge (16) described the rapid exchange of bone calcium with body fluid calcium in young rats.

Fig. 2. Rates of calcium exchange with glycol-ashed bone. The logarithms of the differences between percentages of Ca\(^{45}\) on the bone at any time and at “equilibrium” (log Δ) are plotted as ordinates against the time (Δ, 10\(^{-2}\) M; O, 10\(^{-3}\) M; □, 10\(^{-4}\) M).
in vivo on Ca\textsuperscript{45} distribution are available to permit a comparison with these rates in vitro.

Although the rates and magnitudes of exchange of Ca\textsuperscript{45} and P\textsuperscript{32} by glycol ash are strikingly similar, the processes must be different. When the log $\Delta$ values of the phosphate data are plotted (Fig. 3) against time, curves, not straight lines as for Ca\textsuperscript{45}, were obtained; i.e., the phosphate exchange is not a simple exponential process. This is true (Fig. 3) both in vitro (6) and in vivo (5) for the blood P\textsuperscript{32} and for the P\textsuperscript{32} of the femur ends of rats.

Further examination of the rates of P\textsuperscript{32} exchange revealed that these reactions follow hyperbolic courses both in vitro and in vivo. In vitro, at $10^{-3}$ M and $10^{-5}$ M, the half time constant for glycol ash is about 40 min-
utes. In vivo, the half time constant for the disappearance of P$^{32}$ from blood (following intraperitoneal administration) was 160 minutes; the process followed a hyperbolic course for at least 7 hours. In view of the prominent rôle ascribed the skeleton in the distribution of P$^{32}$ in the body, it is of considerable interest to find that the half time for the appearance of P$^{32}$ in the femur ends was also 160 minutes; this process also followed a hyperbolic course for more than 7 hours. A cause and effect relationship is suggested by the coincidence in rates; i.e., P$^{32}$ of blood exchanges via the extracellular fluid with the P$^{31}$ of the bone (and of soft tissues) so that the bone adsorption of P$^{32}$ is assumed to be the rate-controlling factor in the loss of P$^{32}$ from the blood.

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**Fig. 4.** Rates of calcium and phosphate exchange with fresh bone. Both phosphate (15) and calcium exhibit two straight lines relating the loss of P$^{32}$ or Ca$^{44}$ from the solution to the bone when the logarithm of the specific activities are plotted against time. Phosphate, Δ; calcium, O.
Exchange versus Recrystallization—Neuman and Mulryan (17) have shown that the P\textsuperscript{32} exchange in fresh bone follows a different pattern than that in glycol ash. The reaction is slower in fresh bone but does not fall off in rate in a matter of hours; in fact the P\textsuperscript{32} percentage is still increasing in fresh bone after a month’s exposure to the phosphate solution. When the logarithms of the specific activities of the phosphate solutions were plotted against the time (Fig. 4), two straight lines were obtained. Neuman and Mulryan interpreted the first “rapid” reaction as the surface exchange and the second, slower reaction as a recrystallization process.

Fresh rabbit femur shaft, ground to a powder, was shaken with calcium chloride solutions (approximately 1 × 10\textsuperscript{-2} M) containing Ca\textsuperscript{45} for periods up to 30 days to see whether the Ca\textsuperscript{45} exchange (6) would increase steadily like the P\textsuperscript{32} exchange. When the logarithms of the specific activities of the solutions in contact with the bone samples are plotted against time (Fig. 4), two straight lines were also found for Ca\textsuperscript{45}. If the interpretations of Neuman and Mulryan are applied to the calcium data, the first “rapid” reaction (half time, about 9 days) may be the exchange adsorption and the second slower reaction (half time, about 15 days) may be a recrystallization process. The rates of the two processes differ considerably for P\textsuperscript{32} (rapid, 10 days, slow, 58 days); there is obviously much less difference in the case of Ca\textsuperscript{45}. It is possible that the continuing increase in P\textsuperscript{32} and in Ca\textsuperscript{45} percentages in the bone is a continuing ionic exchange with non-surface P\textsuperscript{31} and Ca\textsuperscript{40}, respectively.

SUMMARY

1. Powdered bone ash adsorbed Ca\textsuperscript{45} from an aqueous solution of CaCl\textsubscript{2} without the simultaneous deposition of a proportionate fraction of the total calcium of the solution. This adsorption is adequately explained by assuming that bone calcium exchanged, rapidly and reversibly, with solution calcium.

2. About one-fifth of the calcium of the bone entered into the exchange reaction. Since the same fraction of the bone phosphorus is available for exchange, it is assumed that the exchangeable calcium and phosphorus, at least in the rapid exchange reaction, represent atoms from the surfaces of the microcrystals of hydroxylapatite.

3. The calcium exchange follows an exponential curve. Phosphate exchange is not exponential but is hyperbolic, both \textit{in vitro} and \textit{in vivo}.

4. Fresh bone takes up Ca\textsuperscript{45} in a slower but continuing process that may involve both exchange and recrystallization.

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