METABOLISM OF BONE SALT INVESTIGATED BY SIMULTANEOUS ADMINISTRATION OF $^{45}$Ca AND $^{32}$P TO RATS

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Within a short time after the parenteral administration of radiocalcium or radiophosphorus to animals the principal part of the dose is located in the skeleton. This uptake may be due to reversible processes (for instance, surface exchange) or to irreversible processes, or to both. Carlsson (1952) demonstrated that one day and onwards after the administration of radiocalcium to growing rats practically all of the activity recovered from the skeleton had been irreversibly deposited with the bone salt formed during the time of the experiment. Carlsson also showed how it is possible to determine the rate of formation of calcium salt in the incisor teeth from isotope data. Using Carlsson's technique, Bauer (1954a) determined the rate of formation of bone salt both in fractured and in intact bone shafts in adult rats.

The experiment to be described here was undertaken to investigate whether phosphorus is irreversibly incorporated in the skeleton fast enough to permit a calculation from isotope data of this rate. Radiocalcium and radiophosphorus were therefore administered simultaneously to rats, and the rate of bone salt deposition in various skeletal samples was calculated from the two sets of isotope data. It was found that there was no essential difference in the metabolism of the calcium and of the phosphorus of the bone salt.

EXPERIMENT

The series consisted of forty-three three-months old rats of both sexes from an inbred strain. Each rat received a subcutaneous injection of about 15 microC of $^{45}$Ca and 30 microC of $^{32}$P in an aqueous solution containing negligible quantities of calcium and phosphorus. The rats were killed by bleeding under ether anaesthesia in groups of three to five at intervals after injection ranging from one to 120 hours. The blood from each rat was collected and centrifuged. One millilitre of serum from each animal was mixed with 2 millilitres 15 wt per cent trichloroacetic acid. This mixture was centrifuged, and the clear residue was used for determinations of $^{32}$P activity, and of total P according to the colorimetric method of Youngburg and Youngburg (1930). The unprecipitated serum from each group was pooled and the serum Ca was determined for each group according to the method of Clark and Collip (1925). The $^{45}$Ca activity was determined on the solutions of Ca obtained in this way after they had been titrated with potassium permanganate.

In each animal the tibiae and the incisor teeth were dissected free from adherent soft tissues. By means of a circular saw the fibulae were removed from the tibiae, and the tibiae were divided in ends and shafts. In each animal the pooled right and left tibial ends formed one sample, the tibial shafts formed one sample, and the four incisor teeth formed one sample. Each sample was ashed in an electrical muffle at about 400 degrees centigrade, the ash was weighed, and the $^{45}$Ca and $^{32}$P activity determined on a nitric acid solution of the ash. The technique followed was that of Carlsson (1951 and 1952), except that a ratemeter was used instead of a scaler (Bauer 1945b).

The activity of each sample was determined with and without an aluminium absorber, which absorbed all the $^{45}$Ca radiation and 50 per cent of the $^{32}$P radiation. The counts per second (c.p.s.) emanating from $^{45}$Ca and $^{32}$P respectively were calculated from the data thus
obtained. Standard solutions were made in 100 millilitres of nitric acid containing $^{44}$Ca and $^{32}$P in the same proportion as in the injected dose. By reference to them the c.p.s. values of the various samples were expressed as per cent of the administered dose of $^{44}$Ca and of $^{32}$P. The activity values recorded hereafter are expressed in this way.

**RESULTS**

The $^{44}$Ca activity values of the tibial shafts and of the serum are recorded in Figure 1. The corresponding $^{32}$P values are recorded in Figure 2. From the values shown in Figure 1 the rate of deposition of calcium in the tibial shafts has been calculated according to the following formula (Carlsson 1952, Bauer 1954a): $U = \frac{C}{T \times S}$ where $U =$ calcium uptake in milligrams per hour; $C =$ the percentage of the dose of radiocalcium recovered from the calcified tissue in question, $T$ hours after the administration of the isotope; and $S =$ the average specific activity of the serum calcium expressed as a percentage of the dose per milligram of calcium during the interval of time in question ($O-T$ hours). (The expression $S \times T$ is represented by the area enclosed by the serum activity curve in Figure 1.)

![Graphs showing calcium and phosphorus activity values](image)

**FIG. 1** — Specific activity of the serum Ca and the $^{44}$Ca activity values of the tibial shafts at varying intervals after the simultaneous administration of $^{44}$Ca and $^{32}$P to young rats. Specific activity of the serum Ca is plotted according to scale to the left of the graph. The values of the pooled serum of each group have been indicated in the graph. $^{44}$Ca activity values of the tibial shafts are plotted according to scale to the right of the graph. The mean values are indicated $\pm$ the standard error. There were three animals in each of the 1, 2, 4, 8, 16 and 32 hour groups and five animals in each of the 24, 48, 72, 96 and 120 hour groups. Figure 2 — Specific activity of the serum P and the $^{32}$P activity values of the tibial shafts at varying intervals of time after the simultaneous administration of $^{44}$Ca and $^{32}$P to young rats. The specific activity of the serum P is plotted according to scale to the left of the graph. The individual values are indicated in the graph. $^{32}$P activity values of the tibial shafts are plotted according to scale to the right of the graph. The mean values are indicated $\pm$ the standard error. The number of animals used was the same as that recorded in the legend to Figure 1.

It is seen from Figure 3 that the tibial shafts show a constant value of $U$ from about twenty-four hours after the administration of the radiocalcium. This steady value represents a rate of deposition of calcium in the shafts of 0.03 milligrams of calcium per hour.

The $U$-values derived from the $^{32}$P activity values in Figure 2 are recorded in Figure 4. It is seen that the steady value represents a rate of deposition of phosphorus in the shafts of 0.015 milligrams an hour.

The ratio of the Ca/P deposition rates in the tibial shafts was thus two to one.
DISCUSSION

Recent investigations have demonstrated that rapid, reversible processes seem to be of minor importance compared to skeletal growth for the redistribution of calcium and inorganic phosphorus in the skeleton. Thus isotope, once deposited in the bone salt, is released to the circulation only when the bone salt, containing the isotope, is reached by a resorption process which removes (dissolves) the bone salt together with the organic matrix (Carlsson 1951, 1952, Bauer 1954a, b, c, d).

By means of the above "U-formula" the rate of deposition of calcium in various parts of the skeleton of rats has been calculated from isotope experiments. However, during the initial hours after the administration of the isotope the rate of deposition of calcium, arrived at by this method, is found to be higher than the subsequent steady rate (Fig. 3). The same is true for the phosphorus calculations (Fig. 4). The interval after the administration of the isotope during which this computation is influenced by reversible uptake processes is determined by the ratio of the exchangeable to the non-exchangeable fractions of the total activity uptake in a certain specimen of the skeleton. A more rapid fall in the serum specific activity respectively a lower ratio of the exchangeable fraction to the non-exchangeable fraction of the total activity uptake thus tend to decrease this initial time interval (Bauer 1954e). By comparison of the blood activity curves in Figures 1 and 2 it is seen that the calcium activity falls faster than does the phosphorus activity. Practically all the body calcium is located in the bone salt, whereas a considerable part of the body phosphorus is located outside the bone salt. The period after the administration of the isotopes during which the reversible processes might be expected to interfere with the calculation of the rate of the irreversible deposition process is thus longer in the $^{32}$P than in the $^{45}$Ca experiment. The present experiment confirms this view: Figure 3 shows that the rate of deposition of calcium in the tibial shafts can be calculated from the twenty-four hour activity values whereas the same calculation from the $^{32}$P activity values (Fig. 4) does not yield a reliable value until forty-eight or seventy-two hours after the administration of the isotope.

**Figure 3**—Calcium uptake in the tibial shafts as calculated from $^{45}$Ca activity data after the simultaneous administration of $^{45}$Ca and $^{32}$P to young rats. The calcium uptake has been calculated from the specific activity curve of the serum Ca and the tibial shaft $^{45}$Ca activity values shown in Figure 1 by means of the "U-formula" (page 659). For lack of space the 1 and 2 hour groups have been omitted. The individual values are recorded in the graph. Figure 4—Phosphorus uptake in the tibial shafts as calculated from $^{32}$P activity data after the simultaneous administration of $^{45}$Ca and $^{32}$P to young rats. Consult the note under Figure 3 for an explanation of the values recorded.

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**Figure 4**—Phosphorus uptake in the tibial shafts as calculated from $^{32}$P activity data after the simultaneous administration of $^{45}$Ca and $^{32}$P to young rats. Consult the note under Figure 3 for an explanation of the values recorded.
After the administration of radiocalcium, the activity ratio of two parts of the skeleton gives the ratio of their respective rates of deposition of calcium (Bauer 1954a), provided a time interval is chosen (after the administration of isotope) which is long enough to preclude the factor of "exchange" and short enough to exclude the importance of resorption of the newly built-in bone salt. The activity ratio between the tibial shafts and the incisor teeth should thus be the same whether $^{45}$Ca or $^{32}$P values are recorded. Table I shows that the two sets of data agree.

**TABLE 1**

**Activity Ratios Tibial Shafts/Incisors at Varying Intervals after the Simultaneous Administration of $^{45}$Ca and $^{32}$P to Young Rats**

<table>
<thead>
<tr>
<th>Interval after administration of the isotopes</th>
<th>$^{45}$Ca activity ratio tibial shaft/incisors</th>
<th>$^{32}$P activity ratio tibial shaft/incisors</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 hours</td>
<td>-40 (±040)</td>
<td>-43 (±010)</td>
<td>5</td>
</tr>
<tr>
<td>72 hours</td>
<td>-39 (±023)</td>
<td>-42 (±015)</td>
<td>5</td>
</tr>
<tr>
<td>96 hours</td>
<td>-38 (±046)</td>
<td>-39 (±031)</td>
<td>5</td>
</tr>
<tr>
<td>120 hours</td>
<td>-40 (±038)</td>
<td>-37 (±042)</td>
<td>5</td>
</tr>
</tbody>
</table>

Figures in brackets equal standard error of the mean.

The value of these ratios during the longer time intervals is about 0.4. Knowing that the deposition rate of calcium in the tibial shafts is 0.03 milligrams of calcium per hour (Fig. 3), the rate of deposition of calcium in the incisor teeth is thus 0.075 milligrams of calcium per hour. This figure is in agreement with earlier figures, in rats of the same strain and of about the same age, derived both from morphological methods and from isotope data (Bauer 1954a). From the above it is seen that the same holds true for the phosphorus values.

In an early isotope experiment (Armstrong and Barnum 1948) the conclusion was reached that there is a difference in the metabolism of the calcium and the metabolism of the phosphorus of the bone salt. It should be stressed, however, that the rate of deposition of bone salt can be determined only by referring skeletal activity to blood activity. This can be done either by direct reference ("U-values") or indirectly by recording the ratios of two skeletal activity values which meet the requirements pointed out above. If these conditions are not met with, misinterpretations might arise.

**SUMMARY**

After the simultaneous administration of radiocalcium and radiophosphorus to young rats the rate of deposition of calcium and of phosphorus in various skeletal parts was computed. Agreement was found between the two sets of data. No difference was thus found in the metabolism of the calcium and of the phosphorus of the bone salt.

**ADDENDUM**

Since this paper was submitted for publication (December 1954) further analysis of inter al. the data above has revealed that the formation rate (accretion) of bone salt can be computed even before the "U-value" has reached a plateau. This method permits also a computation of the magnitude of the exchangeable Ca fraction. (Bauer, G. C. H., Carlsson, A., and Lindqvist, B. (1955). Evaluation of Accretion, Resorption, and Exchange Reactions in the Skeleton. Kongliga Fysiografiska Sällskapets i Lund Förhandlingar, 25, Nr. 1.)

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REFERENCES


