THE EFFECT OF INJECTED RADIIUM ON THE ALKALINE PHOSPHATASE ACTIVITY OF BONE AND TISSUES

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Investigations have shown that the exposure of growing bones to x-radiation is followed by an inhibition of growth (1-4). Heller (5) confirmed this finding and, in addition, described a permanent stunting effect in young rats treated with radioactive Sr$^{89, 90}$. Wilkins and Regen (6), recognizing the importance of the enzyme phosphatase in bone growth from the work of Robison (7), performed experiments on the effects of x-rays on growth and phosphatase activity. They showed that retardation of growth and decreased alkaline phosphatase activity occur in bones of puppies exposed to 600 r.

Phosphatase is believed to be a product of the osteoblasts, the proliferating cartilage cells, and the cells of the inner layer of the periosteum (8). Robison and Soames (9, 10) have emphasized that alkaline phosphatase plays a specific role in the deposition and maintenance of mineral salts in bone and that phosphatase activity in bone is proportional to the rate of calcification and growth. They have also shown phosphatase to be present in high concentration in ossifying cartilage and essentially absent from those portions of bone that are not actively calcifying.

In a discussion of the histological changes produced by radium (at dosages of 0.5 to 1.0 γ per gm. of body weight) in the bones of rats and mice Heller (5) described a condition, seen 24 hours after injection, that included a marked reduction in the number of osteoblasts and osteocytes as well as diminished periosteal activity and dead cartilage cells. After 3 days, no normal osteoblasts remained and dead bone cells were numerous. Lower dosages of radium produced similar but less intense changes, and the time required to produce detectable changes was proportionately longer.

Studies of the metabolism of the alkaline earths (calcium, strontium, radium) (11, 12) have shown that these materials deposit preferentially in bone and especially in the areas of active calcification. In view of the results stated previously it appears that the alkaline earths are likewise deposited in the regions of greatest phosphatase activity. Therefore, it might be anticipated, in view of the results of Wilkins and Regen, that the

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administration of radioactive isotopes of the alkaline earths would also influence the alkaline phosphatase concentration of bone, perhaps to a greater extent than x-rays.

Radium was chosen for these studies because the high specific ionization of its α-rays will maximize the liberation of energy in the areas of actual radium deposition and therefore in the areas of active calcification and highest phosphatase activity.

**EXPERIMENTAL**

Solutions of radium chloride in isotonic saline, pH 3 to 4, were injected intraperitoneally into young (25 to 46 days old) Fischer rats, weighing from 35 to 65 gm. In one experiment adult, Sprague-Dawley rats, 110 days old and weighing 260 gm., were used. In any single run the experimental animals were litter mates and were selected to give maximum uniformity of weight. Four injected and four control animals were used in each experimental group. The animals were sacrificed at predetermined intervals after the injection of radium, along with controls that had been similarly injected with isotonic saline solutions at pH 3 to 4. In the various experiments the concentrations of the radium solutions were adjusted to give dose levels ranging from 0.001 to 0.75 γ of Ra per gm. of rat.

Blood samples were taken from the carotid artery immediately before sacrifice. Soft tissues and bones were removed immediately after sacrifice. The tissue samples were weighed and homogenized with 10 ml of water in a glass homogenizer similar to that described by Umbreit et al. (13). In the case of bone the homogenizer was constructed of stainless steel. During homogenization the apparatus was cooled by immersion in an ice-salt bath.

The homogenates were analyzed for alkaline phosphatase activity by the method of King and Armstrong as modified by the Paul-Lewis Laboratories (14). Values for alkaline phosphatase activity represent mg. of phenol liberated by the enzyme at 37.5° in 30 minutes from a substrate of disodium phenyl phosphate. The buffer used was diethyl barbiturate, which makes it possible to carry out the reaction at pH 9.0.

In additional experiments, the enzyme, both in serum and in bone homogenates, was irradiated in vitro, by using either x-rays or adding radium to the preparations to determine the rate of inactivation under these conditions. Alkaline phosphatase determinations were made as described above, along with determinations on identical non-irradiated control preparations.

**Results**

The intraperitoneal injection of radium chloride into either young or adult rats at dosages up to 0.75 γ of Ra per gm. of animal had no significant
effect on the alkaline phosphatase values of the duodenum, kidney, lung, spleen, and liver up to 4 days after injection. Serum values were somewhat depressed. Representative values for soft tissue, obtained with adult (260 gm.) Sprague-Dawley rats, are presented in Fig. 1.

Following such radium chloride injections, however, the alkaline phosphatase content of the bones (as represented by the femur, scapula, and tibia plus fibula) decreased markedly. The decrease in bone alkaline phosphatase at the lower dose levels was roughly proportional to the quantity of radium administered. However, at dosages above 0.1 γ per gm. the proportionality was no longer evident and levels of radium as high as 0.75 γ per gm. produced only slightly greater effects. The lowest observed phosphatase concentration at the higher dosages was 45 per cent of the control value. Inasmuch as the phosphatase content of bones in control rats was seen to vary considerably from experiment to experiment, presumably as a function of age of the animal and rate of growth, the results are expressed as the percentage of the control value in the particular experiment. The data are presented for all experiments in Fig. 2. The values for King-Armstrong phosphatase units per gm. of fresh bone in all the experiments ranged between 30 and 55 in young rats (30 to 45 days old)
and between 15 and 30 in adult rats (110 days old). In a single experiment in which litter mates were used, the values were reasonably constant. The spread of values encountered in a single typical run may be seen in Table I.

Fig. 2. The average alkaline phosphatase activity of the scapulae, femora, and tibiae-fibulae of rats 24 hours after the intraperitoneal injection of radium at various levels. The values are plotted for each experiment in per cent of the control value in that particular experiment. Except where indicated all animals are 25 to 40 days old.

TABLE I
Illustration of Spread of Experimental Values for Phosphatase in Bone Obtained in Single Typical Experiment

The values are given in King-Armstrong units per gm. of fresh bone. Age of rats, 33 days; weight, ~70 gm.; Ra dose, 0.75 $\gamma$ per gm.; sacrificed 3 days after injection.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Controls</th>
<th>Ra-injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat 1</td>
<td>Rat 2</td>
</tr>
<tr>
<td>Right tibia and fibula</td>
<td>38.5</td>
<td>36.8</td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right femur</td>
<td>35.7</td>
<td>32.8</td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right scapula</td>
<td>38.6</td>
<td>45.0</td>
</tr>
<tr>
<td>Left</td>
<td>34.5</td>
<td>49.8</td>
</tr>
</tbody>
</table>
Following the injection of 0.42 γ of Ra per gm. to 46 day-old Fischer rats, the alkaline phosphatase content of the bones decreased rapidly from the 1st hour to 6 hours and then rather slowly (Fig. 3) up to 24 hours. In other experiments, in which animals were sacrificed from 1 to 4 days following the administration of 0.75 γ of Ra per gm., there was only a slight further decrease in phosphatase during the interval. The combined results of these experiments make it apparent that, at these high dosages, the decrease in alkaline phosphatase in bone is restricted in time to the first few hours after radium injection.

When radium chloride was added to bone homogenates at a concentration of 0.8 γ per ml. and to serum at a concentration of 5 γ per ml. and allowed to stand for 24 hours, there was no decrease in alkaline phosphatase concentration nor did the control values change over this period. Inasmuch as no attempt was made to control the conditions with respect to radon in these experiments, it is difficult to determine the irradiation dosage accurately. Since the radium for the experiment was withdrawn directly from a radium chloride solution in a flask in which the radon was nearly in equilibrium with its parent, the contribution to irradiation level from radon and its daughters must have been appreciable. Assuming an average of 15 per cent Rn equilibrium over the interval, the irradiation dose was 348
rep (roentgen-equivalent-physical) in bone homogenates and 2180 rep in serum. The method of calculation of these values is shown later. The results are presented in Table II.

Similar bone homogenates were also exposed to 250 kev x-rays at a rate of 720 r. per minute until dosages up to 100,000 r. had been accumulated. The results (Fig. 4) showed no decrease in phosphatase concentration up to 10,000 r. Thereafter the phosphatase values decreased slowly to 58 per cent of the control at a total dose of 100,000 r.

**DISCUSSION**

The distribution of radium injected intraperitoneally or intravenously has been shown to proceed very rapidly (11, 12). In rats the bones attain their maximum concentration within 90 to 120 minutes, while the soft tissues, which initially (after 1 to 2 minutes) contain approximately 50 per cent of the injected dose distributed more or less uniformly, lose radium rapidly over a period of several days. Since bone phosphatase concentra-

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**Table II**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added Ra</th>
<th>Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \gamma \text{ per ml.} )</td>
<td>Control \text{ units per ml.}</td>
</tr>
<tr>
<td>Serum</td>
<td>5.0</td>
<td>0.54</td>
</tr>
<tr>
<td>Homogenized tibia</td>
<td>0.8</td>
<td>56.7</td>
</tr>
<tr>
<td>&quot; scapula</td>
<td>0.8</td>
<td>47.6</td>
</tr>
</tbody>
</table>

**Fig. 4.** Effect of x-irradiation on the phosphatase content (King-Armstrong units) of bone homogenates.
tions decreased most markedly during the first 3 hours after injection, and since the percentage excreted during this period is not very large, one may consider that the injected radium is approximately equally divided between bone and soft tissue. Since bone represents about 10 per cent of the body weight, the concentration in bone will be at least 10 times that in soft tissue. However, it is known from radioautographic studies (15, 16) that radium in bone is largely deposited in the epiphyseal areas. This localization within bone effects an additional concentration which may be estimated roughly by inspection of radioautographs to exceed that which would be encountered in a uniform distribution of radium by a factor of 10. Therefore, the radium concentration in epiphyseal bone must exceed that in soft tissue by a factor of approximately 100 during the first few hours after injection, which is the period when the greatest decrease in bone phosphatase activity is observed. Radium injected at levels over a range at least 100 times greater than the dose required to produce detectable decreases in bone phosphatase caused no decrease in soft tissue phosphatase. It is probable that the alkaline phosphatases of soft tissue are qualitatively not identical with those of bone. Such a concept has support from the work of Bodansky (17), who reported differences in the rates of hydrolysis of phosphoric acid esters by the phosphatases of different tissues under various conditions. Armstrong and Banting (18) reported that bone is the probable source of serum phosphatase, since it was not reduced by extirpation of the intestine, kidney, spleen, pancreas, liver, and testes. The results reported here are in agreement with those of Armstrong and Banting to the extent that the decreased phosphatase levels in bone are reflected by a small decrease in serum phosphatase. These data also indicate that bone phosphatase apparently does not contribute substantially to the phosphatase in the soft tissues investigated. No decrease in phosphatase concentration of these soft tissues could be demonstrated, even though the serum phosphatase concentration was diminished.

In view of the fact that the concentration of bone phosphatase, during the interval studied, was not further decreased by an increase in radium dosage from 0.1 to 0.75 \( \gamma \) per gm. and, further, since no marked decrease in the phosphatase concentration of bone occurred later than 3 hours after the injection of 0.42 \( \gamma \) per gm., it seems apparent that the capacity of radium to reduce phosphatase concentrations in bone is limited. Such results may reflect either (1) a partial coincidence of radium deposition on the distribution pattern of the phosphatase systems, thus rendering some phosphatase unavailable to the influence of radium, or (2) the presence of more than one alkaline phosphatase in bone, one or more of which is not highly susceptible to the effects of radium.

The radiation dosage in these radium-injected rats may be approximated
by use of the value of 15 per cent radon retention reported for rats by Evans et al. (19). The contribution of $\beta$- and $\gamma$-rays may be neglected since it represents a small fraction of the total energy dissipated by $\alpha$ particles. The radiation dose from 1 $\gamma$ of radium distributed uniformly in 1 gm. of tissue thus becomes

\[
\text{Dose in rep} = \frac{3.7 \times 10^4 \left[ E_{Ra} + 0.15 \left( E_{RaA} + E_{RaO(a)} \right) \right]_{86,400}}{35 \times 1.61 \times 10^{12}} - \frac{3.7 \times 10^4 \left[ 4.79 \times 10^4 + 0.15 \left( 5.49 \times 10^4 + 5.99 \times 10^4 + 7.68 \times 10^4 \right) \right]_{86,400}}{35 \times 1.61 \times 10^{12}} = 435 \text{ rep per day}
\]

where $E$ represents the energy in electron volts of the respective $\alpha$-rays. The remainder of the equation is formed by the appropriate constants as follows: disintegrations per second per microgram of Ra = $3.7 \times 10^4$, seconds per day = 86,400, electron volts per ion pair (approximate) = 35, ion pairs per gm. of tissue per rep = $1.61 \times 10^{12}$.

Studies of the metabolism of radium-injected rats have shown that the bones reach maximum radium concentration within 1 to 1.5 hours after injection. One femur contained about 2.5 per cent of the injected dose at this time and for 30 days thereafter (11). The average weight of a femur of the young rats used in the present work was 0.175 gm. If one uses the factor of 10 mentioned earlier to describe the concentration of radium in the calcifying areas in excess of that which would be attained by uniform distribution of the radium throughout the bone, the radiation dosage to the actively calcifying areas following the injection of 0.1 $\gamma$ of Ra per gm. to a 50 gm. rat (35 days old), may be estimated as follows

\[
\text{Ra dose} = 0.1 \times 50 = 5 \gamma
\]

\[
\text{"content of femur} = 5 \times 0.025 = 0.125 \gamma
\]

\[
0.125 \times 0.715 = 0.089 \gamma \text{ per gm. whole bone}
\]

\[
0.175 \times 10 = 1.75 \gamma \text{ per gm. actively calcifying bone}
\]

\[
\text{Radiation dose} = 7.15 \times 435 = 3110 \text{ rep per day or 129 rep per hr.}
\]

Because of the extreme difficulty in determining accurately the concentrations of radium in the fine structure of bone, the value presented above must be considered as only an effort at approximation of the radiation dosage associated with that quantity of radium required to produce maximum depression of alkaline phosphatase activity in bone. The estimate takes no account of the relative biological effectiveness of $\alpha$-rays in bone, although it is possible from Zirkle's results with microorganisms (20) that it is greater than 1. Doses of radium less by a factor of 100 than that used in the above calculations were observed to produce a definite effect, al-
though the time required for maximum depression of alkaline phosphatase activity at these dosage levels has not yet been ascertained.

Inasmuch as the irradiation in vitro of alkaline phosphatase in bone homogenate preparations by 250 kev x-rays and by the addition of radium to bone homogenates and to serum indicated the enzyme preparation to be rather resistant to radiation-induced inactivation, it is likely that the effect in vivo is not caused by irradiation of the enzyme per se. Rather it would seem to indicate damage to the phosphatase-producing systems of the parent cells or to the cells themselves.

Although it would appear that the causative agent in this phenomenon is the ionization derived from deposited radium, it is not possible at this time to exclude the purely chemical effect of radium itself. The experiments in vitro described earlier show that radium does not inactivate phosphatase preparations even in concentrations higher than those estimated to be present locally in the injected animals. However, this gives no clue as to its effect on the phosphatase-forming elements in vivo. Grier et al. (21) and Klemperer et al. (22) have shown that beryllium, in concentrations as low as $10^{-6}$ M, exerts an inhibitory action in vitro on preparations of alkaline phosphatase. Radium injected at a dosage of 0.1 $\gamma$ per gm. is sufficient to produce an average concentration in the entire rat of $4.4 \times 10^{-7}$ M, and local concentrations in bone may be 100 times this value. Further, since the toxicity of the alkaline earths is known to progress in the direction of the heavier elements, it is significant that the toxic effects of injected barium (the nearest non-radioactive analogue of radium) may be observed at dose levels as low as $10^{-7}$ $\gamma$ per gm.

It is apparent that this latter question may be settled by similar experiments with Ra$_{224}^{224}$ (radioactive half life = 3.6 days) in which the concentrations of Ra may be reduced by a factor of $10^5$ over those used here, the radiation dose being nearly identical.

**SUMMARY**

The influence of radium as an internal $\alpha$ emitter on the phosphatase activity of bone and tissue was studied in a number of rats injected with varying amounts of radium chloride.

The results indicate the following.

1. Radium deposited in bone causes significant reduction in the alkaline phosphatase content of bone.

2. The reduction of serum phosphatase concentrations following injections of Ra is compatible with earlier suggestions that it is derived from bone.

3. The phosphatase content of the soft tissues analyzed is largely independent of that of bone and is not affected by injection of radium.
4. Radiation dosages, from deposited radium, of the order of 10 to 100 rep per hour will produce significant reduction in the alkaline phosphatase content of bone within 24 hours.

5. Studies in vitro with x-rays and with radium added to serum and bone homogenates produced no change in phosphatase activity up to 10,000 r. Therefore, the effect of deposited radium as reflected by the reduction in alkaline phosphatase activity is probably on the phosphatase-forming elements of bone.

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