THE RELATIVE RETENTION OF STRONTIUM AND CALCIUM IN BONE TISSUE*

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The presence of strontium in the bone tissue of vertebrates has been reported by a number of investigators (1–6), although at least two workers found none in the samples analyzed (7, 8). The behavior of strontium in the animal body is of some interest, for, although its metabolic fate is very similar to that of calcium, it cannot supplant calcium in the diet without causing severe disturbances (9). Massive doses, however, accompanied by normal calcium intake, have been reported to be useful in clinical therapy of osteoporosis (10). Further stimulation of interest has followed the recognition of the potential hazards to human health from the radioactive isotopes Sr$^{89}$ and Sr$^{90}$ (11–13) produced in atomic fission. The purpose of the work reported here was to learn what relationship, if any, exists between the normal, natural calcium and strontium content of a diet of relatively constant composition and the calcium and strontium content of the bones of animals consuming that diet. Such information is valuable in estimating the radiostrontium burdens which would be attained in members of a human population by prolonged consumption of food supplies contaminated at some fixed level.

Materials and Methods

The primary specimens for study were the femurs of Carworth strain mice, Wistar strain rats, and albino guinea pigs, all descended from parents which had lived in the animal colony on the same diets as their offspring. The stock diets also were sampled and analyzed. Bones from a number of wild, Nevada desert kangaroo rats and rabbits and samples of typical flora upon which they feed were also added to the survey. Finally, although their dietary histories were unknown, bone specimens from domestic rabbits, a horse, and a cow were included.

Specimens were thermally ashed and analyzed for strontium and calcium. Strontium was determined in all samples by an emission spectro-

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graphic procedure. Calcium in the bones of the laboratory animals was determined by the conventional method of titration of the precipitated oxalate with standardized permanganate. The calcium content of the desert animal samples was determined by means of a flame photometer. The calcium content of the plant ash was determined by an emission spectrographic technique and verified by the oxalate-permanganate titration.

_Spectrographic Methods_—A spectrographic buffer-internal standard solution containing 1 mg. of CrO$_3$, 1 mg. of NaCl, and 1 mg. of Mg, added as the carbonate, per ml. of 6 N HCl was prepared for the analysis of plant ash. This buffer solution served to eliminate random variations in the strontium, calcium, and chromium line intensity ratios accompanying the wide variation in element composition associated with plant ash. All chemicals were reagent grade or better. The thermally ashed plant sample was diluted with the buffer solution to give a concentration of 1 or 3 mg. of ash per ml. of mixture. For samples containing less than 5 per cent calcium the 3 mg. per ml. concentration was used.

The buffer-internal standard solution used for the analysis of bone ash was the same as that described above, except that the NaCl and MgCO$_3$ were omitted. The bone ash was dissolved to give a concentration of 30 mg. per ml.

1 drop of the sample solution was evaporated on a collodion-treated, flat machined, spectroscopically pure, $\frac{1}{4}$ inch diameter graphite electrode. The dried sample was burned in a 5 ampere direct current arc and the spectrum was photographed on Eastman spectrum analysis No. 2 emulsion with a 2 meter, 24,400 lines per inch, grating spectrograph manufactured by the Applied Research Laboratories. Standard developing and den-
sitometric procedures were used to arrive at intensity ratios. The spectral emission line combinations used are presented in Table I.

### Table II
_Ashed Samples from Animals on Controlled Diets_

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Sr</th>
<th>Ca</th>
<th>Sr per 1000 Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.019</td>
<td>32.3</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.022</td>
<td>35.7</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>0.024</td>
<td>34.6</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.017</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.026</td>
<td>37.0</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.025</td>
<td>32.7</td>
<td>0.35</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Rats</td>
<td>35</td>
<td>0.018</td>
<td>34.0</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.016</td>
<td>34.1</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.015</td>
<td>35.4</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.020</td>
<td>36.2</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.023</td>
<td>36.7</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.019</td>
<td>37.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>70</td>
<td>0.16</td>
<td>35.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.19</td>
<td>34.4</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.17</td>
<td>36.4</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>0.17</td>
<td>35.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>2.2</td>
<td></td>
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</tbody>
</table>

### Table III
_Ashed Samples from Animals of Uncertain Dietary Histories_

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Sr</th>
<th>Ca</th>
<th>Sr per 1000 Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch rabbit</td>
<td>1400</td>
<td>0.12</td>
<td>37.9</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>1420</td>
<td>0.14</td>
<td>37.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Cow</td>
<td>0.081</td>
<td>37.4</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>0.091</td>
<td>37.6</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

Standards were prepared by the addition technique whereby strontium was added to a sample low in strontium and a plot of added strontium versus intensity ratio was extrapolated to zero intensity ratio to determine
the original concentration of strontium in the sample. Calcium values were also obtained by this method for plant ash and were verified by permanganate titration of the oxalate.

The method outlined permits the maintenance of a coefficient of variance of 0.1 for a single determination over several months of analysis. With particular attention to standardization a coefficient of variance of 0.05 can be maintained.

Results

The values obtained from the analyses are presented in Tables II to IV. All percentages were computed on the ash weight basis. Entries under the column heading Sr/1000 Ca express the ratio of Sr to Ca in terms of the number of Sr atoms present in the sample per 1000 atoms of Ca. For a given species, the weights of the animals are roughly indicative of their comparative ages.

DISCUSSION

First to be noted is the presence of strontium in all of the animal bone samples. This observation, together with the occurrence of small amounts of strontium in human bones reported by Hodges and coworkers (5), suggests the ubiquitous character of strontium in the food supply of vertebrates.

The constancy of the Sr:Ca ratio among the rat and mouse samples was striking (Table II). All these animals were fed the same stock diet that their parents had consumed (Rockland rat pellets). It is of interest
that reexamination of data reported previously (14) showed that the mean value of the per cent strontium in the ashed femurs of thirty-one normal mice ranging in age from 21 to 348 days was 0.024 per cent with a standard error of the mean of 0.001 per cent. The mean Sr/1000 Ca atom ratio was 0.32. These mice also were fed Rockland rat pellets.

The strontium burden per gm. of ash and the Sr:Ca ratios for the guinea pigs were very much higher than those for the mice and rats. Examination of the diet of these animals (Simplex rabbit pellets and lettuce) revealed a strontium content much higher than that of the rat pellets. However, even at this elevated strontium intake, the Sr:Ca ratio of the bones of these animals was constant.

The constancy of the strontium to calcium ratio, irrespective to age, requires that all animals reach a state of strontium equilibrium with their respective diets. However, it should be pointed out that, when massive doses of strontium are ingested, equilibrium may not be reached within the life span of the animal. In the case of mice drinking 0.1 and 0.05 M strontium lactate solutions, equilibrium was not reached until at least 250 days, although the animals had been started on the diet at weaning (14).

Not only did the Sr:Ca ratio in the bone ash indicate equilibrium with the diet, but in all cases it was significantly lower than the ratio in the diet. This situation implies a quantitative difference in the metabolic treatment of strontium with respect to calcium. To express this difference it is advantageous to introduce a term called the "bone retention factor," $F_B$, which may be defined as the Sr:Ca ratio of the bone divided by the Sr:Ca ratio in the diet. This term is most applicable when the diet has been of constant composition and the bones are from animals which have attained strontium equilibrium with the diet. This concept may be applied to any bone-seeking element. For example, the probable bone retention factor for Ca$^{45}$ is unity, since at equilibrium the Ca$^{45}$:Ca ratio of the bone is expected to equal the Ca$^{45}$:Ca ratio in the diet.

The magnitude of this factor appears to be somewhat species-dependent. In Fig. 1, a comparison may be made between laboratory animals and a large number of field animals. The individual points for the field animals indicate the mean Sr/1000 Ca ratio in the bone ash and the mean Sr/1000 Ca ratio of plant material selected as being representative of the diet for a particular collecting area. An estimate of the retention factor for each group of animals was gained by calculating the line of best fit which included the data and the origin. The slope of this line is the retention factor.

Although there appeared to be a definite difference in the retention factor among mice, rats, guinea pigs, and kangaroo rats, there seemed to
be no significant difference among guinea pigs, jack-rabbits, and cottontail rabbits. This implies a very similar metabolic behavior for strontium and calcium in rabbits and guinea pigs. However, whether or not these similarities and differences in retention factors prove to be real, the considerably greater retention of ingested calcium as compared with strontium was well demonstrated. This preference was noted in all of the animals studied. Furthermore, under conditions of fixed dietary composition, the strontium to calcium ratio of the bone ash of an animal may be expected to be between 0.15 and 0.40 that of its diet.

For the bone retention factor to be of much use, it must be independent of individual differences within a given species and relatively independent of the level of intake. If these conditions are met, it should be possible to predict the strontium burden of the bone from the retention factor for the animal and the strontium to calcium ratio of its diet. Sources of dietary calcium are inevitably sources of strontium. Even the purest grade of calcium carbonate which we have observed contained a significant amount of strontium. Thus, in all probability, this material establishes a lower limit for the Sr/1000 Ca ratio available to animals. The highest value

\[ \text{Sr/1000 Ca, PLANT MATERIAL} \]

This is a relationship of the Sr:Ca ratio of bone to that of the diet. The lines and their corresponding slopes (decimal values) represent the respective bone retention factors as follows: O, laboratory mice, Curve I; ●, laboratory rats, Curve II; ●, laboratory guinea pigs, and ●, wild cottontail rabbits, Curve III; ■, wild jack-rabbits, Curve IV; ▲, wild kangaroo rats, Curve V.

\[ \text{Sr/1000 Ca, BONE} \]

From Fig. 1 the retention factor for mice on a Rockland diet was 0.35. For eleven mice drinking strontium lactate solution for more than 250 days, the per cent Sr in the bone ash was very high, but the bone retention factor was still only 0.31 ± 0.02. The level of strontium intake for these mice was several hundred times that for the Rockland diet (14).
for the ratio in any normal diet was assumed to be that found in natural waters (15). Multiplying these ratios by the highest and lowest observed bone retention factors gave the range of Sr/1000 Ca for bone ash from which, in turn, were derived the extremes for per cent Sr shown in Table V.

All samples of normal bone ash which have been analyzed by the authors have fallen within the range of 0.015 to 0.19 per cent Sr, well within the predicted extremes of 0.0011 to 0.44 per cent Sr set forth in Table V. It is of interest to note that the mean per cent strontium in the bone ash of twenty-one salt water fish has been reported by Asari as 0.39 per cent (6). However, results have been reported which fall below the lower limit as stated in Table V. Forbes, Cooper, and Mitchell (8) reported that the level of strontium in the bone ash of one normal human was not detectable and therefore below 0.00001 per cent. Our analytical results suggest that there is some question as to the true limit of detection reported for the analytical methods applied by these investigators.

Comar and coworkers (16) have recently published data on Sr90 metabolism which are in rather gratifying agreement with the concepts presented herein. These workers raised rats, through two generations, on a diet in which the Sr90 activity per mg. of calcium was maintained at a fixed level.

The over-all utilization for bone growth was 3.6 in favor of calcium over Sr90 and the ratio was unaffected by the size of the rat. This "preferential utilization factor" is the conceptual inverse of what we have called the bone retention factor. Therefore, the reciprocal of Comar's factor for Sr90 should express his results in terms of $F_B$, i.e. 0.28, which is remarkably close to our value of 0.27 for stable strontium in rats. These results lend definite support to the concept that predictions of radiostrontium uptake by the human skeleton may be derived from knowledge of the bone retention factor, the strontium to calcium ratio of the diet, and the specific

<table>
<thead>
<tr>
<th>Source</th>
<th>Sr/1000 Ca</th>
<th>Maximal $F_B = 0.40$</th>
<th>Minimal $F_B = 0.15$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea water</td>
<td>14.5</td>
<td>0.44*</td>
<td>0.17</td>
</tr>
<tr>
<td>United States raw water</td>
<td>14.2 (Maximum)</td>
<td>0.44</td>
<td>0.16</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot;</td>
<td>0.45 (Minimum)</td>
<td>0.14</td>
<td>0.0052</td>
</tr>
<tr>
<td>Reagent grade CaCO3</td>
<td>0.097</td>
<td>0.0030</td>
<td>0.0011*</td>
</tr>
</tbody>
</table>

* Predicted extreme values.
activity of the radiostrontium in the diet. Data pertinent to such predictions are presently being sought.

SUMMARY

Bones of mice, rats, and guinea pigs raised in the laboratory, together with their respective diets, were analyzed for strontium and calcium by an emission spectrographic technique. Kangaroo rats and rabbits from the Nevada desert areas were also investigated, along with representative samples of their forage.

The number of atoms of Sr per 1000 atoms of Ca present in the bone samples ranged from 0.19 for rats to 2.5 for guinea pigs. This Sr/1000 Ca value was relatively constant for members of a given species, regardless of age.

The Sr/1000 Ca atom ratio of the bone tissue was always lower than that of the diet which suggested a quantitative difference in the metabolic treatment of strontium with respect to calcium, retention of the latter being favored.

The term “bone retention factor” was introduced to describe the ratio of the concentration of an element with respect to calcium for bone tissue divided by the ratio for the diet. The bone retention factor for strontium in mice was 0.35, in rats 0.27, and in guinea pigs 0.22. The retention factors for the Nevada desert animals were for jack-rabbits 0.20, cottontail rabbits 0.22, and kangaroo rats 0.16.

It was suggested that a prediction of the skeletal uptake of radiostrontium by humans, through continued consumption of contaminated food, might be made from a knowledge of this retention factor, the Sr:Ca ratio of the diet, and the level of radiostrontium contamination.

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