# Dietary Strontium and Calcium and Deposition of $Sr^{89}$ and $Ca^{45}$ in the Bones of Rats.* (28110)

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Numerous papers demonstrate that the body "discriminates" against strontium in relation to calcium so that the ratio of radio-Sr to calcium in the bone is less than the ratio in the diet. This discrimination against strontium appears to occur mostly during absorption from the gut, although some may occur in the kidney and in the bone(1-3). Ordinarily, and in most of the studies which have been done, the diet contains very little strontium compared to the dietary calcium content. Thus it would appear that the mechanisms which provide for this discrimination are quite specific and able to distinguish traces of strontium in presence of large amounts of calcium. Several investigators have suggested that if strontium is relatively poorly absorbed and if the discrimination against strontium persists at higher levels of intake, addition of stable strontium to the diet, thus markedly decreasing its specific activity in the diet, should be effective in reducing the absorption of radio strontium and its retention in the body.

Efforts to test this hypothesis appear to have been uniformly negative. For example, Harrison *et al.*(4) gave rats as much as 0.12 g of stable strontium with $Sr^{89}$ and this carrier did not reduce the deposition of $Sr^{89}$ in the bone. They concluded that absorption of strontium was proportional to the dose. Kawin(5) also reported the non-effectiveness of carrier Sr in decreasing $Sr^{89}$ deposition, but this author, contrary to most others, found additional calcium was also ineffective. Mraz (6) fed chicks varying amounts of stable Sr up to 1.0% of the diet and found that these additions had no effect upon the bone retention of radio-Sr. On the other hand, injection of stable Sr shortly after injection of radio-Sr has been found to be partially effective in reducing the deposition of the radio isotope in the bones of mice(7).

Apparently all these studies have been done with administration of the radio isotope as a single dose followed by sacrifice of the animal shortly thereafter. Whether this technic influences the results obtained is problematical. In any event, the results reported here, where $Sr^{89}$ was fed over several weeks with varying amounts of carrier Sr, apparently demonstrate that the additional carrier is partially effective in decreasing $Sr^{89}$ deposition in the bones. It is not, however, as effective as might be expected and addition of stable strontium to the diet holds no promise as a practical means of decreasing the deposition of radio-Sr in the body.

*Methods.* Weanling female rats housed in groups of 4 received the various diets *ad libitum* for a 3 week period. They were then sacrificed and the left femur removed for analysis. The femurs were freed of most of the soft tissue and placed in boiling water for a few minutes. The remaining soft tissue was then removed and they were dried in a vacuum oven at 90°C. They were then extracted overnight with ether in a soxlet extractor, redried, placed in a tared dish and weighed. After ashing overnight at approximately 650°C, they were reweighed to obtain the weight of ash. The ash was dissolved in dilute HCl and made to volume.

Total calcium and strontium were determined by oxalate precipitation followed by permanganate titration and expressed as calcium. Total bone content was then calculated. Reference to the total bone calcium refers to bone Ca and Sr expressed as Ca. An aliquot containing 3 mg of calcium and strontium (expressed as Ca) was taken and precipitated as the oxalate which was plated for counting. Total activity (sum of $Ca^{45}$ and $Sr^{89}$) was recorded. The planchets were recounted after an aluminum disk (139 mg/sq cm) had been placed over the window of

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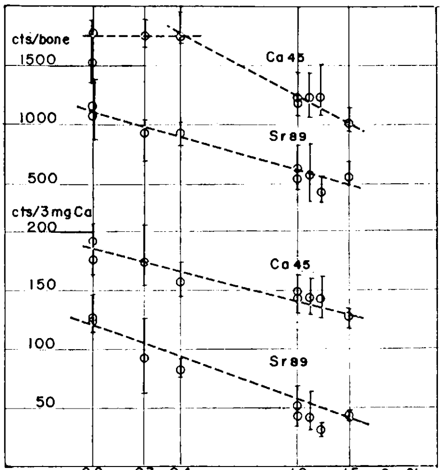
the detector tube. Repeated tests had shown that not more than 0.1% of the previously measured activity of $Ca^{45}$ was detected with the disk in place whereas 19.4% of $Sr^{89}$ ac-

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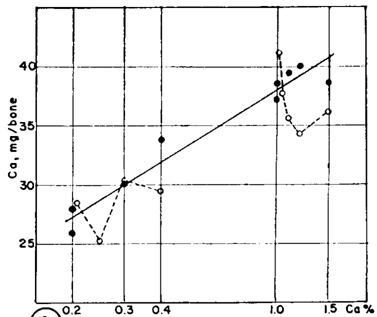


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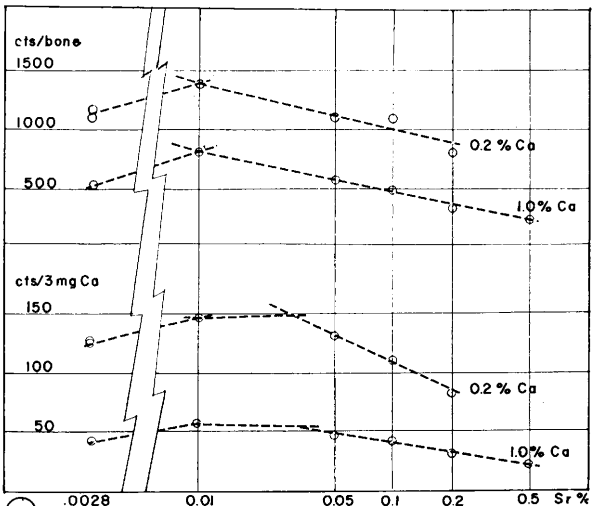


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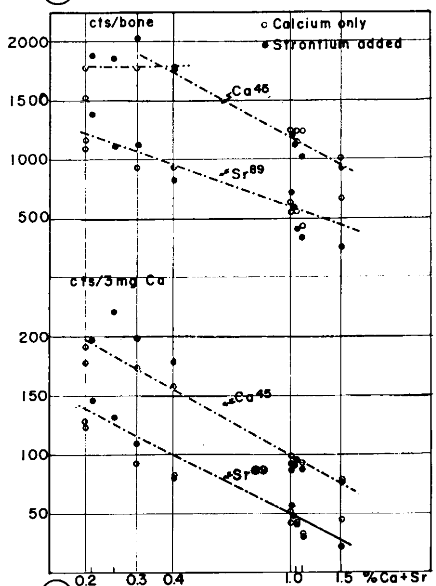


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FIG. 1. Uptake of $Ca^{45}$ and $Sr^{89}$ from diets containing different amounts of stable calcium. Bars represent range of values found in each group of animals.

FIG. 2. Effects of diets varying in calcium or calcium and strontium content upon total calcium and strontium in the femur. Diets containing only calcium are indicated by solid dots. Femur calcium was linearly related to dietary calcium. Several levels of strontium were added to the diet containing 0.2% calcium (open circles and dotted line at left) and to diets containing 1.0% calcium (open circles and dotted line at right). The sum of the calcium and strontium is indicated and the oxalate method for calcium determination also measures calcium and strontium in the femurs.

FIG. 3. Effects of addition of stable strontium to deposition of $Sr^{89}$ when added at various levels to either 0.2% or 1.0% calcium diets. The first value .0028% represents strontium content of a basal diet of similar composition and is included here to indicate the order of magnitude of the Sr content of basal diet.

FIG. 4. Comparative effects of the addition of either stable calcium or stable strontium upon uptake of $Ca^{45}$ and $Sr^{89}$. Strontium was added to the 0.2% calcium diet and the 1.0% calcium diet to produce the total indicated.

tivity was so detected. Total $Sr^{89}$ activity was then calculated and $Ca^{45}$ activity obtained by difference. Total bone $Ca^{45}$ and $Sr^{89}$ were then calculated.

The basal diet had the following percentage composition: 62.9, glucose; 18, purified casein; 9.5, hydrogenated cottonseed oil; 0.5, cod liver oil; 5, celluflower; 0.3, cystine; 0.5,† vitamin mix, and 2.5, low calcium salt mixture. This was the Jones and Foster(8) salt mix prepared without the $CaCO_3$. Various amounts of calcium carbonate and strontium carbonate were added to this diet to provide the levels described below. After the diets were prepared, a constant amount of $Ca^{45}$ and $Sr^{89}$ was added, approximately 1 $\mu$c per kilogram of diet.

The diets consisted of the following: 0.2, 0.3, 0.4, 1.0, 1.1, 1.2 and 1.5% calcium with no stable strontium added; diets containing 0.2% calcium to which were added 0.01, 0.05, 0.1 and 0.2% stable strontium and diets containing 1.0% calcium to which were added 0.01, 0.05, 0.1, 0.2 and 0.5% stable strontium.

The diet made with 4% regular Jones and Foster salt mix, which supplies 0.6% calcium was analyzed by activation analysis‡ and found to contain 2.8 ppm strontium. Although the strontium content may be partially dependent upon the amount of calcium carbonate in the diet, it is assumed that all diets without added Sr contained this amount. The absolute amount is not particularly important although the order of magnitude of the Sr content is of interest.

*Results.* The results obtained with diets of varying calcium content but with no stable strontium additions are shown in Fig. 1. As expected, the amounts of $Ca^{45}$ and of $Sr^{89}$ per mg of calcium fall with increasing dietary calcium. These curves in the lower part of the figure are roughly parallel, indicating approximately proportional effects of dietary calcium on deposition of both $Ca^{45}$ and $Sr^{89}$ although the slope of the $Sr^{89}$ line may be slightly greater than that for $Ca^{45}$. Approximately a 5-fold increase in dietary calcium was required to reduce the deposition of the isotopes by one-half. Interpretation of the results is complicated by the effects of the dietary calcium upon total bone calcium (Fig. 2). Total bone calcium increased with increasing dietary calcium although not proportionately. This presumably accounts for the shape of the upper curve in Fig. 1 which indicates that over 0.4% calcium was required before total $Ca^{45}$ activity per bone began to fall.

When increasing levels of stable Sr were added to the diets, the effects on total bone calcium (or ash) were less consistent (Fig. 2). With additions to the diet containing only 0.2% calcium there was a general tendency for the total bone salt to increase whereas addition of strontium to the high calcium diet, 1.0%, appears to have had an adverse effect. Again the groups were not of sufficient size to determine the significance of the variations encountered in the individual groups.

The effects of strontium additions in the 2 diets upon the deposition of $Ca^{45}$ and $Sr^{89}$ are shown in Fig. 3. As indicated previously, the basal diet is assumed to contain approximately 2.8 ppm Sr based upon one analysis of a similar diet by activation analysis. This is probably only an indication of the order of magnitude of the Sr content. In any event, it is of interest that addition of 0.01% of stable strontium to the diet, sufficient to decrease the specific activity of the dietary Sr to about one-fourth that in the basal diet, consistently resulted in a slight increase in the deposition of $Sr^{89}$ whether expressed per bone or per mg of bone calcium. Addition of larger amounts to either diet resulted in decreased deposition, the variation in the curves in the upper and lower parts of Fig. 3 presumably reflecting the effects of the additions upon total bone salt. Comparison of these results with those shown in Fig. 1 suggests that addition of stable strontium and calcium has approximately the same effect upon the deposition of $Sr^{89}$.

† Supplied 4 mg thiamin hydrochloride, 8 mg riboflavin, 4 mg pyridoxine HCl, 25 mg calcium pantothenate, 40 mg niacinamide, 1 mg folic acid, 0.1 mg menadione, and 0.2 mg biotin per kg of diet in glucose.

‡ Analyses performed by the Activation Analysis Service, General Atomic Division of General Dynamics Corporation, San Diego, California

A similar conclusion is derived from the data in Fig. 4, in which $Ca^{45}$ and $Sr^{89}$ deposition is plotted against the sum of the total calcium and strontium in the diets. Again it will be apparent that there was often a tendency for an increased deposition of the radio isotopes with addition of small amounts of stable strontium. Larger amounts of either strontium or calcium had generally similar effects.

*Discussion.* As stated above, it is known that the body is able to discriminate against radio strontium as compared to calcium and that this appears to be accomplished principally in the gut. If a similar degree of discrimination persisted at all levels of total strontium intake, one might then assume that addition of some stable strontium to the diet, thus decreasing its specific activity in the diet, might markedly decrease the amount of radio strontium deposited. Clearly this is not true. The data suggest, in fact, that small additions of stable strontium may actually increase the deposition of either $Ca^{45}$ or $Sr^{89}$. Larger amounts do have a diluting effect, but this is comparable to equal weights of dietary calcium. Since the atomic weight of strontium is approximately double that of calcium, it may be somewhat more effective than calcium on a molar basis in diluting the radio isotopes. The results of this type of experiment using growing rats over a relatively short period are difficult to interpret because of the effects of the dietary calcium and strontium upon bone growth. These complexities do not, however, affect the conclusion that additions of stable strontium to the diet appear to have no promise as a means of decreasing the deposition of radio-strontium.

Recent studies apparently show that calcium is actively absorbed by the intestine of the rat only in the upper portion of the duodenum(9) and absorbed by diffusion in the lower portion of the intestine(10). Both processes appear to be influenced by the Vit. D status of the animal and the active process at least by the calcium content of the diet. Strontium is apparently not actively transported(11). Questions remain as to which process is the most significant in terms of calcium nutriture, their relative roles at different levels of intake, the performance of strontium in the diffusion process, etc. It is probable(1) that the chemical dynamics of the bone mineral may also change in bone relatively high in strontium. The suggestion that relatively small amounts of strontium in the diet may somehow stimulate absorption or deposition of radio-calcium or strontium would appear to be worthy of investigation.

*Summary.* Diets containing various levels of stable strontium and calcium and containing low and constant levels of $Ca^{45}$ and $Sr^{89}$ were fed to young rats during a 3 week period. The amounts of the 2 isotopes in the bones were measured. Addition of small amounts of stable Sr, sufficient to reduce the specific activity of the dietary Sr to one-fourth, did not decrease the deposition of radio-Sr and may have caused a slight increase in the deposition of both $Ca^{45}$ and $Sr^{89}$. Larger amounts of stable Sr were no more effective than calcium in reducing the amounts of the isotopes deposited in the bones.

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