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Differential Removal of Strontium 85 and Calcium 45 from Rat Skeleton By Peritoneal Lavage. (23238)

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The need for understanding the behavior of strontium in various biological systems, especially in relation to calcium, is emphasized by the possibilities and implications of contamination of the biosphere with strontium 90 (1,2). In general, it has been shown that of the two elements, calcium is preferentially absorbed from the gastrointestinal tract, secreted in milk, and transported to the fetus; on the other hand, strontium is preferentially excreted in urine, in bile, and into the digestive tract(3-6). Attention has now been directed toward the comparative movement of calcium and strontium between body fluids and bone. The effects produced by physiological processes already mentioned make it difficult to observe in the intact animal any differential behavior directly related to bone. Lengemann(7), using tissue cultures of embryonic chick bones, showed that the Sr*/Ca* ratio was 1.08 in newly formed bone as compared with a value of 1 in the substrate, but that the ratio was 1.2 for the Sr*/Ca* in sub-

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strate being derived from labeled bone having a ratio of 1. Comar *et al.*(5), from daily ingestion studies of radiostrontium and radiocalcium in rats, showed that the Sr*/Ca* of bone was equal to or slightly higher than that of blood when plateau levels in each tissue had been reached.

In the present work, a direct comparison was made of removal of previously deposited calcium 45 and strontium 85 from normal and nephrectomized rats by peritoneal lavage.

Methods. Male rats (Carworth) weighing between 160 and 250 g were used. Normal rats were maintained on stock diet but were given no food the night before the experimental run. Nephrectomized rats were put on a salt deficient diet the night before removal of the kidneys and were maintained on this diet for duration of the experiment. Nephrectomy was done by ventral mid-line approach. Each animal received an intraperitoneal injection of a solution containing about 50 μc of Ca 45 and 5 μc of Sr 85, both essentially carrier-free. Calcium, phosphate, and Ca 45 analyses were done by standard methods(8) and the Sr 85 was counted in a scintillation well-counter. *Peritoneal Lavage.* The method is a modification of that reported by Talmage and Elliot(9); however, because of its simplicity and possible usefulness it is described in some detail. The catheter was made of glass tubing flattened perpendicularly on the end and containing 3 openings recessed behind the button-like end. About one-quarter inch behind the openings, the tube was ridged for holding the suture thread. The remainder of the glass tube was inserted into rubber tubing controlled by a screw clamp and placed into a graduated cylinder for collection of the peritoneal wash. The incision was made at the anterior end of the inguinal canal, after which the spermatic cord with its accompanying adipose tissue was stretched from the peritoneal cavity and tied off. The catheter was then slipped through the canal into the peritoneal cavity. A suture was stitched through the layers of the inguinal wall and tied around the ridge provided on the catheter thus preventing escape of fluid around the catheter and at the same time holding it far enough into the peritoneal cavity

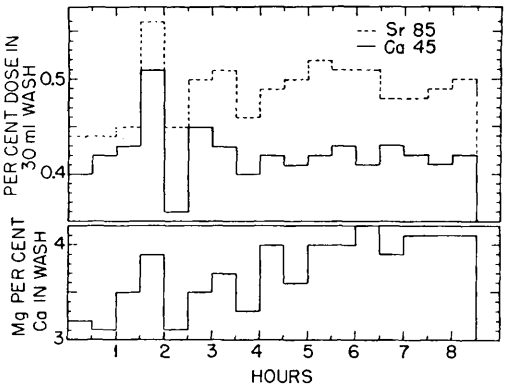

FIG. 1. Removal of Sr 85, Ca 45, and Ca from labeled rat skeleton in peritoneal lavage solutions collected every 30 min.

that the walls of the cavity would not close the openings. The button-like end prevented the intestines from blocking the openings. The testis was either cut off or sewed into the scrotum. The animal was maintained under nembutal anesthesia throughout. By this procedure the wash was normally collected merely by releasing the screw-clamp on the catheter; however, occasionally a slight pressure on the abdomen was needed to drain sufficient fluid. At the beginning of the experiment, 35 ml lavage fluid were placed in the peritoneal cavity either through the same catheter or through a polyethylene catheter placed in the abdomen under the liver. At the end of each equilibration period, 30 ml were removed and the same amount was replaced in the cavity. The lavage fluid consisted of 0.84% NaCl and 1% dextrose. Duration of equilibration periods and length of the experiment varied with experimental needs.

Results. Fig. 1 presents a typical histogram showing removal of strontium 85, calcium 45, and calcium in the lavage solutions collected every 30 minutes during an 8-hour period. This particular animal had been nephrectomized and had received the radioisotopes 24 hours before the start of the lavage procedure. The main features are (a) greater removal of Sr 85 than Ca 45 (as will be noted later these animals had the same amount of Sr 85 and Ca 45 in the bones at start of lavage); (b) general simultaneous changes of Sr 85, Ca 45, and Ca; and (c)

TABLE I. Removal of Ca 45, Sr 85, Ca and P from Rats by Peritoneal Lavage. (8-hr treatment with 30-min. equilibration periods.)

Exp. condition	Normal	Nephrect.	Nephrect.	Normal
Time between isotope dosage and lavage	24 hr	24 hr	48 hr	8 days
No. of animals	6	6	4	2
% of dose in skeleton*				
Ca 45	83 ± .5	87 ± 2	91 ± .5	75
Sr 85	76 ± 2	87 ± 2	89 ± 1	56, 57
% of dose removed*				
Ca 45	3.5 ± .2	5.9 ± .5	1.7 ± .1	.40, .38
Sr 85	3.7 ± .2	7.5 ± .6	2.5 ± .1	.33, .30
% of skeletal burden removed				
Ca 45	4.2	6.7	1.9	.51
Sr 85	4.8	8.7	2.8	.57
Electrolyte removed†				
Ca, mg/ml of wash	.035–.045	.030–.040	.025–.035	
P, mg/ml of wash	.015–.025	.025–.040	.035–.045	

* Mean ± stand. error of mean. † Range over 8-hr period of exp.

relatively uniform removal over the 8-hour period.

A study was made of the effect of time of peritoneal equilibration (between 15 minutes and 1 hour) upon rate of electrolyte removal from the body. Removal rate was maximum when 15-minute periods were employed; when 30-minute and 1-hour periods were used removal rates were about 70% and 45%, respectively, of the 15-minute values. For convenience, a 30-minute period was used for most studies.

Table I presents data first on amounts of Ca 45 and Sr 85 retained in the skeleton of normal and nephrectomized rats at 24 hrs, 48 hrs, or 8 days after intraperitoneal injection. The values are given as percentage of dose in the skeleton at start of lavage; they were obtained by analysis of the carcass at the end of experiment, to which values the amounts removed by lavage were added. Data are then given for the amounts of Ca 45 and Sr 85 removed during 8 hours of peritoneal lavage with 30-minute equilibration periods. The amounts removed are expressed both as percentage of dose and as percentage of skeletal burden at start of lavage. Values are also given to indicate amounts of calcium and phosphorus removed, expressed as range in mg/liter of wash during the 8-hour period.

Table II presents a definition of the term "Strontium-Calcium Observed Ratio (OR)" and gives calculated values for these ratios that permit more accurate estimation of the comparative removal of the 2 isotopes from bone(5). Values are presented for the discrimination in movement of the 2 elements between dose and bone, dose and lavage solution, and between bone and lavage solution.

Discussion. The data in Table I for percentage of dose in skeletons of rats at 24 hours after injection show that nephrectomy

TABLE II. Differential Removal of Ca 45 and Sr 85 from Rats by Peritoneal Lavage.

	Normal	Nephrect.	Nephrect.	Normal
	Time between isotope dosage and lavage			
	24 hr	24 hr	48 hr	8 days
	No. of animals			
	11	6	4	3
$OR_{bone\text{-}dose}$*	.92 ± .02	.99 ± .001	.99 ± .01	.77
$OR_{lavage\text{-}dose}$	1.03 ± .06	1.30 ± .05	1.50 ± .03	.79
$OR_{lavage\text{-}bone}$	1.12	1.31	1.52	1.03

* $OR_{bone\text{-}dose} = \frac{Sr^*/Ca^* \text{ in bone}}{Sr^*/Ca^* \text{ in dose}}$ (5).

had only slight if any effect on calcium retention, but did increase retention of strontium. Even at 48 hours after injection of nephrectomized rats, calcium and strontium retentions were almost equal. This supports earlier work(5,10) and demonstrates that renal discrimination is of major importance in the preferential removal of strontium once it is within the body. The greater retention of Ca 45 than Sr 85 at 8 days after injection into the normal animal reflects primarily the preferential urinary excretion of strontium during this period.

Let us now consider the removal of Ca 45 and Sr 85 by lavage; it can be seen from the 24-hour animals that nephrectomy increased the amounts of Ca 45 and especially of Sr 85 that were removed by lavage. This increase is probably due to loss in competition for electrolytes exerted by the kidney in the intact rat.

It is of interest to note the effect of length of time in the body on availability of radioisotopes for removal by lavage. For example, Sr 85 and Ca 45 in the body for 48 hours were only about one-third as effectively removed as that in the bone for 24 hours; after 8 days, the isotopes were only about one-eighth as available for removal. This behavior is a consequence of normal processes taking place in bone such as accretion, resorption, and redeposition in growth or remodeling, plus possible physical changes(6). It is pointed out that although these data demonstrate that such methods (artificial kidney) can in principle be used to remove radioactive calcium analogues from the body, they are inefficient and would require considerable improvement before being practical. The importance of treatment soon after exposure is also stressed.

The comparative retention in the skeleton as affected by urinary discrimination can also be expressed as an OR (Observed Ratio) value as shown in Table II. The $OR_{bone\text{-}dose}$ near unity for the nephrectomized groups, and the value of 0.77 for the 8-day normal animals again emphasizes the effect of preferential urinary excretion of strontium.

A main objective of this study was to estimate the comparative removal of the 2 isotopes from bone under conditions in which the kidney discrimination was not in effect. It was thought unlikely that significant physiological discrimination would be caused by any of the processes involved in the movement of calcium and strontium from the circulating fluids to the lavage solution. Accordingly, the discrimination that occurs between bone and peritoneal lavage solution in the nephrectomized animal is attributed to differential removal from bone. Interference resulting from the discrimination due to endogenous excretion into the gastrointestinal tract is probably not serious.

It is noted in Table II that the $OR_{lavage\text{-}bone}$ ratio in the nephrectomized animals was significantly higher than unity (1.3 to 1.5). This is interpreted to mean that Sr 85 was being preferentially released from bone as compared with Ca 45. The $OR_{lavage\text{-}bone}$ values in the normal animals have little meaning because of the masking effects of the urinary discrimination. The value of 1.3 for the 24-hour nephrectomized rats is in agreement with the value of 1.2 found by Lengemann(7) for the Sr/Ca released from bone in tissue culture. It seems, therefore, that the ratio of Sr/Ca released from bone is about 1.3 times the Sr/Ca in bone.

It was of interest to estimate the ratio of Sr/Ca that enters bone. This has been done with the use of the Sr/Ca ratio of 1.3 for the elements leaving bone, in conjunction with other data from the literature. The formula and calculation are shown in Table III. In principle, if one has (a) steady state values for Sr/Ca in bone and blood, (b) the bone accretion and resorption rate for calcium, and (c) the Sr/Ca ratio leaving bone, it is then possible to calculate the Sr/Ca ratio entering bone. Values for (a) have been reported for three groups of rats(5) and Bauer *et al.*(11) have estimated that in the whole tibias of young rats, the calcium accretion was 0.17 mg/hr and calcium loss by resorption was 0.13 mg/hr. When these values are used as shown in Table III, it is calculated that the Sr/Ca ratio entering bone was 1.5, 1.3, and 1.6 times the blood Sr/Ca ratio for the three groups of rats previously described.

Summary. 1) A simple technic has been described for removal of electrolytes from the

TABLE III. Calculation of Ratio of Strontium to Calcium Moving from Blood to Bone of Rats. (D = Sr/Ca ratio in diet.)

Group		1	2	3	Ref.
Sr/Ca in bone at steady state	(B)	.57 D	.45 D	.27 D	(5)
" " blood " " "	(P)	.46 D	.43 D	.21 D	(5)
Accretion rate of calcium	(A)	.17	.17	.17	(11)
Resorption " " "	(R)	.13	.13	.13	(11)
Sr/Ca leaving bone	(L)	1.3	1.3	1.3	
" entering "	(E)	1.5	1.3	1.6	

Note: Equation for E derived as follows:

$$\frac{\text{Sr in bone}}{\text{Ca in bone}} = \frac{\text{Rate of Sr entry} - \text{Rate of Sr leaving}}{\text{Rate of Ca entry} - \text{Rate of Ca leaving}} = \frac{(A)(E)(P) - (R)(L)(B)}{A - R} = B,$$

$$\text{and } E = \frac{B(A - R + RL)}{(A)(P)}.$$

rat by peritoneal lavage. From comparisons of strontium/calcium ratios in lavage solutions and bones of nephrectomized rats it is estimated that strontium was preferentially released from bone by a factor of 1.3 over calcium. 2) It was calculated that strontium preferentially entered bone from blood by a factor of 1.3 to 1.6. The differential removal of the 2 elements from the intact rat was shown to be due mainly to renal function. Eight hours of peritoneal lavage removed about 5% of the Sr* that had been deposited for 24 hours but only about 0.6% of that deposited for 8 days.

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Effects of Testosterone, Sesame Oil, and Castration on Tissue Respiration of Male Rats Exposed to Cold. (23239)

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Addition of testosterone *in vitro* to tissue slices is reported to have either an augmenting(1) or a depressing(2,3) effect on rate of oxygen uptake of tissue slices depending upon concentration. Smith, Kochakian and Fondal(4) report a decrease in rate of oxygen uptake of tissue slices and homogenates of liver and kidney from rabbits, rats and guinea

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