produced an adrenal response in intact animals not subjected to other exogenous stress as great as that in animals subjected to exogenous stress prior or subsequent to injection of virus.

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Kinetics of Strontium and Calcium Skeletal Metabolism in the Rat.* (32371)

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While the preferential absorption of Ca as compared to Sr across the gastro-intestinal tract and in renal excretion is well established(1), differential handling of Sr and Ca by the skeletal system, *in vivo,* has not been clearly demonstrated, although it is suspected that it exists, on the basis of the differing properties of these two elements. In a study employing peritoneal lavage in nephrectomized rats, the differential removal of ^{85}Sr and ^{45}Ca from the rat was shown to be due mainly to renal function and not to differential handling of the two elements by the skeleton(1a). In two studies employing compartmental analysis of data derived from double tracer studies in elderly human subjects, slight differences were found in a number of parameters of skeletal metabolism calculated from ^{85}Sr data when compared to those derived from ^{47}Ca data(1b,2). Because

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of the variability of the data in these elderly subjects, and also the statistical uncertainty of the individual measurements, there exists a question of the validity of the small differences observed between Sr and Ca kinetics. The statistical validity of the small differences observed in the turnover rate of bone-fixed ^{85}Sr and ^{47}Ca as measured with a whole-body counter over a 30-day period is also questionable. It is, therefore, of interest to investigate possible discrimination against the uptake of Sr by the skeletal system. The outcome of such a study can be used to evaluate the reliability of Sr as a tracer for Ca in metabolic studies of the skeleton.

In the present experiment, the skeletal uptake and turnover of Sr were compared with those of Ca, using the most precise tracer techniques available. A uniform population was obtained, and the data analyzed by means of a computerized program. For this purpose, the Berman SAAM-23 computer program was used to fit both ^{85}Sr and ^{47}Ca tracer kinetic data of adult rats to a compartmental model. In addition, the comparative turnover of skeletally-fixed ^{85}Sr and ^{47}Ca was measured in a specially designed rat whole-body counter. With these two techniques, it is possible to measure subtle differences in the kinetics of Sr and Ca with considerable precision and accuracy.

Method. Ten Sprague-Dawley male rats, 155 days of age (497 g) were divided into 2 groups and injected intraperitoneally with either 20 μc of $^{47}CaCl_2$ (>200 mc/g) or $^{85}SrCl_2$ (carrier-free). The rats were kept in individual metabolism cages for 72 hours for collection of the 24-hour urine and feces outputs. Ten serial plasma samples (125-350 λ) were taken from the tail of each rat over intervals from 0.5 to 72 hours. The rats were also counted at frequent intervals in a specially designed whole-body counter for 41 days following administration of the tracers. All counting was performed using NaI(Tl) detectors connected to a 400-channel pulse height analyzer. The details of the isotope technique employed and the counting instrumentation have been presented(3,4).

A previously formulated and tested compartmental model was used as the basis for this comparative study. The compartmental model delineated in Fig. 1 has been shown to fit equally well both Sr and Ca kinetic data from studies in man(2,3) and rats(4). The Berman SAAM program (modified for the CDC 6600 computer) was used to fit the above mathematical model to the data (5-7). The program, by an iterative system, obtains the best coefficients for a given set of differential equations describing the model. This set of coefficients is then used to determine the desired parameters of the system. These calculated parameter values with their computation errors provide a quantitative comparison of the fits of both the ^{47}Ca and the ^{85}Sr tracer data.

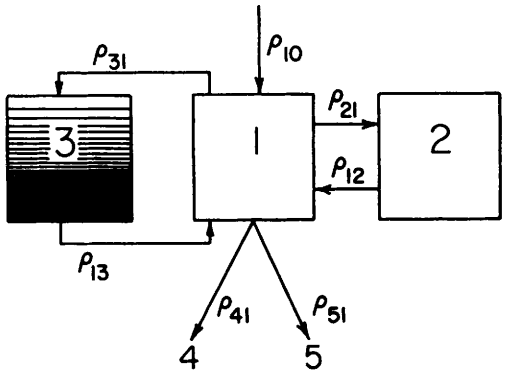

FIG. 1. Compartmental model of calcium kinetics. Compartments are designated as follows: 1, physiological pool of calcium in isotopic equilibrium within 1 hr (plasma-extracellular-intracellular); 2, physiological pool of calcium in isotopic equilibrium within 3 days (exchangeable bone); 3, calcium in "deep bone" or very slowly exchanging bone. The transfer constants, ρ, are designated as follows: ρ_{10} = calcium intake rate, ρ_{12} = Ca flow rate into compartment 1 from exchangeable bone, ρ_{21} = Ca flow rate into exchangeable bone from compartment 1, ρ_{13} = rate of resorption and slow exchange from bone, ρ_{31} = rate of accretion into bone, ρ_{41} = urinary calcium excretion rate, ρ_{51} = fecal calcium excretion rate.

Results. The parameters (compartment sizes and transfer rates) of skeletal metabolism as calculated from both the ^{47}Ca and ^{85}Sr data are presented in Table I. It can be seen that the mean values for all the parameters, for the ^{47}Ca data, except urinary excretion rate (ρ_{41}), do not differ grossly from those derived from the ^{85}Sr data. The urinary excretion (ρ_{41}) calculated from the ^{85}Sr data gave a value of 12.3 times higher than that calculated from the ^{47}Ca data.

The retention of ^{47}Ca and ^{85}Sr as measured by the whole-body counter over 41 days is presented in Fig. 2. The ratio of $^{85}Sr/^{47}Ca$ is also plotted in the lower curve. The slope and intercept of each curve was obtained (Table II), with a computer used to obtain a linear least fit to the straight line or final exponential component. The biological half time for ^{85}Sr is 113.7 days, or slightly less than that of ^{47}Ca, 128.3 days. The slope of $^{85}Sr/^{47}Ca$ from 13 to 41 days is described by a half-time of 354.9 days with a correlation coefficient of 0.603.

Discussion. On the basis of the compartmental analysis of the short-term kinetic data, most of the parameters calculated from the ^{47}Ca and ^{85}Sr data did not differ significantly. The major exception is the urinary excretion rate (ρ_{41}) while the size of the compartment 1 and the inter-compartmental flux ($\rho_{1,2}$ $\rho_{2,1}$) appears to be slightly smaller for the Ca data. The preferential renal excretion of Sr explains the difference in ρ_{41}, but the reason for the other differences is not apparent. The significance of the slightly larger size for compartment 1 when calculated from the Sr data is questionable. In past studies (1b,2), for example, the compartment sizes derived from ^{85}Sr data have generally been smaller than those from Ca data. There is no question that the most significant parameter of bone metabolism, the accretion rate (ρ_{31}) shows no significant difference whether calculated from Ca or from Sr data.

The potential errors in the previous studies in man were minimized in the present study by the use of one tracer per animal, larger concentrations of tracers used, and a uniform population. In addition, the objective handling of the kinetic data further acts to minimize any bias in the calculated parameters. The advantage of this digital computer analysis over previous analyses is that it affords a quantitative measure of the accuracy of the calculated parameters as well as a greater objectivity and reproducibility.

TABLE I. Parameters of Skeletal Metabolism Calculated from ^{47}Ca and ^{85}Sr Tracer Data.

Rat No.	Body wt (g)	Compartments (mg/kg) C_1	C_2	Transfer rates (mg/hr/kg) Urine ρ_{41}	Feces ρ_{51}	Accretion ρ_{31}	Flux $\rho_{12,21}$
^{47}Ca							
1.	500	41.02 ±2.74*	99.28 ±7.12	.200 ±.0088	1.01 ±.044	4.22 ±.046	8.74 ±.82
2.	449	43.51 ±2.81	104.94 ±7.58	.261 ±.0116	1.98 ±.085	3.75 ±.087	8.67 ±.78
3.	497	38.21 ±2.65	104.20 ±7.52	.179 ±.0129	1.77 ±1.13	3.28 ±.113	9.67 ±.84
4.	497	37.81 ±2.51	102.87 ±7.24	.205 ±.0092	1.23 ±.054	4.36 ±.056	8.56 ±.72
$\bar{X}$	486	40.14	102.82	.211	1.50	3.90	8.91
σ		±2.77†	±2.75	±.041	±.47	±.52	±.54
^{85}Sr							
1.	453	46.21 ±4.00	99.76 ±10.23	2.59 ±.14	1.37 ±.082	3.73 ±.17	6.76 ±.84
2.	513	47.09 ±4.21	107.62 ±11.21	2.38 ±.14	1.27 ±.078	3.98 ±.16	6.65 ±.76
3.	439	45.05 ±3.60	100.52 ±9.30	2.44 ±.13	1.14 ±.062	3.31 ±.14	6.84 ±.71
4.	467	46.52 ±3.92	113.03 ±10.14	3.15 ±.17	1.56 ±.090	3.89 ±.18	7.51 ±.83
5.	450	48.84 ±4.11	102.99 ±11.14	2.40 ±.13	1.15 ±.069	3.69 ±.15	6.15 ±.71
$\bar{X}$	464	46.74	104.78	2.59	1.30	3.72	6.78
σ		±1.63	±5.71	±.33	±.099	±.29	±.58

* Standard deviation of calculated value. † Standard deviation of group.

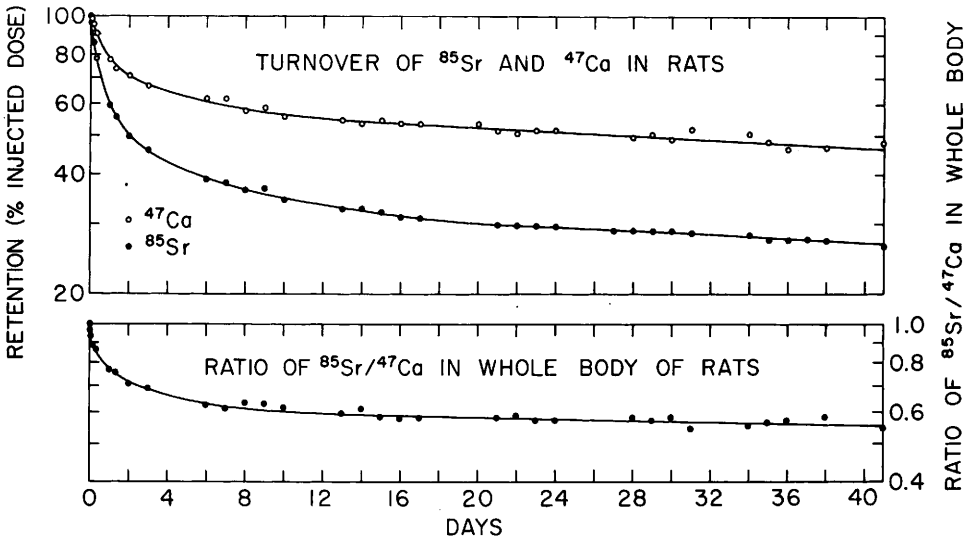

FIG. 2. Turnover of ^{85}Sr and ^{47}Ca in rats.

The average error of the calculated parameters, as indicated in Table I, is somewhat higher for Sr than for Ca, but this probably reflects only the larger error in the fit of the Sr urine data. By contrast, the standard deviation (σ) of most parameters, particularly the accretion rate values (ρ_{31}) for the group of Sr rats, is lower than those of the Ca data.

In a previous compartmental analysis, Bronner(8) concluded that only the fecal excretion rate and the bone accretion rate were the same for Sr and Ca. The authors stressed an important assumption inherent in the use of Sr as a tracer for Ca. Sr specific activity is generally expressed in terms of calcium or a circulating plasma volume, and not in terms of stable Sr as is the case for Ca tracer. Since the present calculated parameter values were based on the plasma volume (which is taken as a relatively absolute concentration of Ca), to obtain the absolute Sr concentration a different proportionality constant could be used to determine absolute levels of Sr based on the concentration of stable Sr in the plasma. However, since it has not been demonstrated that the amount of stable Sr in plasma is constant, it is more desirable to continue to express Sr in terms of plasma volume because of the homeostatically controlled constancy of the plasma Ca level.

In a previous study(2) using the quantitative methods employed here, the parameter values derived from Sr data for human subjects were, with few exceptions, lower than those derived from Ca data, although the correlation between the two was very high. The Sr values, however, were lower than the Ca values by about the same percent as the statistical uncertainty in the individual measurements. The variability in these pre-

TABLE II. Constants for Whole-Body Turnover of ^{85}Sr and ^{47}Ca.

Per cent retention $R = Ae^{-\lambda t}$

Isotope	Period of study (days)	No. of co-ordinates	A (intercept % injected dose)	λ days^{-1} (slope)	Biological half-time (days)	Correl. coefficient
^{47}Ca	13–41	19	58.40 ± .49*	.0054 ± .0003*	128.3 ± 7.2*	.828
^{85}Sr	17–41	16	34.22 ± .068	.0061 ± .0007	113.7 ± 1.2	.988
$^{85}Sr/^{47}Ca$	13–41	18	.609 ± .0027	.000195 ± .00016	354.9 ± 29.3	.603

* Standard error.

vious studies was due to the lower levels of tracer employed (of necessity), the error inherent in double tracer studies, geometry errors present in all whole-body counting of humans, and the variability present in an older pouplation.

The long-term (41-day) turnover data indicated that the biological half-time of Sr is only slightly shorter (5%) than that of Ca. Again, the potential errors in this technique were minimized here by the use of higher tracer concentrations administered, individual administration of tracers, and, of greatest importance, by the more reproducible counting geometry for the rats compared to that for the human subjects. In addition, the larger amount of data and the computer fitting gave a more objective and accurate evaluation of the turnover rate. Unfortunately, the only gamma emitting isotope of Ca, ^{47}Ca, has too short a half-life to permit these studies to be pursued longer than 41 days, without a significant loss of accuracy.

The $^{85}Sr/^{47}Ca$ ratio of the whole body as a function of time revealed essentially the same pattern as previously noted in man(2). As in previous studies, the rapid initial fall in the value of the ratio was followed by a relatively slowly changing ratio. In the past this ratio was considered to reach an asymptote after 2 weeks. In the present study, with its greater precision and accuracy, the ratio appears to develop a very low negative slope after 12 days. The very low rate of fall of the Sr/Ca ratio, ($\lambda = 0.00195$/d), in the face of the preferential renal excretion of Sr, does not in itself indicate whether Sr is leaving compartment 3 ($\rho_{1,3}$), at the same rate as Ca.

Conclusion. The present study demonstrates that the absolute values for the computer derived parameters of skeletal metabolism obtained with Sr tracer data do not differ significantly from those obtained with Ca. Further, the whole-body turnover of bone-fixed Sr is not significantly different from that of Ca as measured by whole-body counting. The evidence here supports the common assumption that Sr is a reliable tracer of calcium in skeletal metabolism, at least over a 40-day period.

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