EFFECT OF AGE AND DIET ON EXCRETION OF STRONTIUM AND CALCIUM BY RATS*

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Abstract—Mature (8-month-old) and growing (26-day-old) rats, maintained on diets varying in calcium content from 0.03 to 2.0% were given a single injection of ^{90}Sr and ^{45}Ca and subsequent excretion of these radionuclides, in urine and feces, was measured over a period of 60 days. Similarities and differences in their patterns of excretion are discussed in relation to the probable mechanisms responsible for the behavior noted. Of particular interest was the decrease in ratio of urinary to fecal excretion of both ^{90}Sr and ^{45}Ca as a function of time following injection. This change was more evident for strontium than for calcium, was more evident in the adult than in the growing rat, and was most marked on a high calcium diet. To explain this variation it is hypothesized that strontium and calcium released from firm binding sites in bone may exist in the blood in a different form than strontium and calcium in equilibrium with freely exchangeable sites on bone surfaces.

PREVIOUS publications from our laboratory described the deposition and retention of ^{90}Sr and ^{45}Ca in the bone of mature [1] and growing [2] rats. These studies involved both single administration and continuous feeding of the radionuclides and were designed particularly to elucidate the effect of dietary calcium intake on the interrelated behavior of strontium and calcium. Whereas these earlier studies were concerned primarily with radionuclide retention, as measured in serially sacrificed groups of rats, we have attempted in the presently reported experiments to approach the problem by analysis of daily urinary and fecal excretion following single doses of ^{90}Sr and ^{45}Ca. Studies are reported in both mature and growing animals and at several levels of dietary calcium intake.

METHODS

Rats employed were females of the Sprague-Dawley strain. Studies were performed with mature animals, 7 months of age at start of experiment, and with weanling animals, 22 days of age at start of experiment. The mature animals were conditioned for 28 days to 0.03, 0.1, 0.5, and 2.0% calcium diets. All diets contained 0.5% phosphorus. The further composition of the diets is described in detail in an earlier publication. [1] Three animals were maintained on each of the diets. After the 28-day conditioning period, each rat received a single intraperitoneal injection of 30 μCi ^{45}Ca and 15 μCi ^{90}Sr. The rats were kept in individual metabolism cages and urine and feces were separately collected, daily, for 14 days following radionuclide injection. Thereafter, collections were made on each of 2 consecutive days out of each week, with the final collection made at 60 days postinjection.

The experiment with growing animals was similarly performed except that 5 animals were included in each dietary group, the conditioning period was reduced to 4 days, the radionuclide injection was reduced to 4 μCi ^{45}Ca and 2 μCi ^{90}Sr, the 0.03% calcium diet group was eliminated, and daily urine and feces collections were continued through only the first 9 days postinjection, with twice weekly collections thereafter. The animals within each dietary group were kept in a single metabolism cage and pooled excreta samples obtained.

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Table 1. *Summarized Excretion Data* (% *of injected dose*)

	Mature rats				Growing rats			
	^{90}Sr		^{45}Ca		^{90}Sr		^{45}Ca	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
0.03% *Ca Diet*								
Day 1	22	7.0	0.09	4.3				
Day 2	5.0	8.7	0.17	6.5				
Days 5–9 (Avg/d)	0.80	0.66	0.06	0.92				
Days 40–60 (Avg/d)	0.07	0.17	0.02	0.28				
Days 1–60 (Total)	42	33	1.8	35				
0.1% *Ca Diet*								
Day 1	30	3.6	0.90	2.6	4.9	4.3		0.45
Day 2	5.2	10.8	0.71	9.0	0.93	1.3	0.09	0.10
Days 5–9 (Avg/d)	0.82	0.74	0.18	1.3	0.60	0.54	0.01	0.03
Days 40–60 (Avg/d)	0.06	0.17	0.03	0.28	0.11	0.21		0.07
Days 1–60 (Total)	49	31	5.2	40	23	23	<1.0	3.1
0.5% *Ca Diet*								
Day 1	34	5.6	5.7	5.3	4.5	5.5		2.7
Day 2	6.2	8.4	1.4	10.	0.88	1.8		0.69
Days 5–9 (Avg/d)	0.80	0.62	0.30	1.5	0.50	0.54		0.16
Days 40–60 (Avg/d)	0.05	0.15	0.02	0.30	0.14	0.16	0.06	0.14
Days 1–60 (Total)	54	29	12	45	21	27	<5.0	15
2.0% *Ca Diet*								
Day 1	57	4.8	25	4.7	20	3.1	10	2.5
Day 2	5.5	8.1	4.2	9.6	1.4	2.8	0.66	2.3
Days 5–9 (Avg/d)	0.54	0.27	0.50	0.58	0.96	0.42	0.64	0.36
Days 40–60 (Avg/d)	0.02	0.08	0.02	0.14	0.17	0.10	0.17	0.12
Days 1–60 (Total)	72	20	38	29	45	20	31	19

Urine and feces samples were dry ashed at 600°C, the residue dissolved in nitric acid and aliquots assayed for ^{90}Sr and ^{45}Ca by a differential beta-particle absorption technique. Counting of samples was delayed for 2 weeks to ensure establishment of ^{90}Sr–^{90}Y equilibrium. All counting rate data were corrected for radioactive decay.

RESULTS

A summary of certain features of the excretion data, from both the mature- and growing-rat experiments, is given in Table 1. The average daily excretion of ^{90}Sr and ^{45}Ca in urine and feces is shown for the first and second days post-injection, for the period of 5–9 days, and for the period of 40–60 days. Cumulative figures are given for the total 60-day period. The first and second days' excretions of ^{90}Sr and ^{45}Ca were uniquely high, the second days' fecal excretion usually being higher than the first, due to holdup in the intestine. The 5–9 day period was one during which daily collections were made; it was beyond the early period of rapidly falling excretion rates; and it was a period of relatively stable urinary/fecal (U/F) excretion ratios. Data for the 40–60-day periods represent the average of six collections made during the final 3 weeks of the experiment. In several instances urinary ^{45}Ca data were unavailable from the growing animals, due to the smaller injection levels employed with these animals, and due to limitations of the differential beta particle absorption technique which make it difficult to quantitate small

amounts of ^{45}Ca in the presence of large amounts of ^{90}Sr.

There was a considerable scatter in day-to-day excretion values. This is illustrated in Fig. 1, where there are plotted daily average total ^{90}Sr excretion values for the growing animals on all three diets, and ^{45}Ca total excretion values for the growing animals on the 2.0% calcium diet.

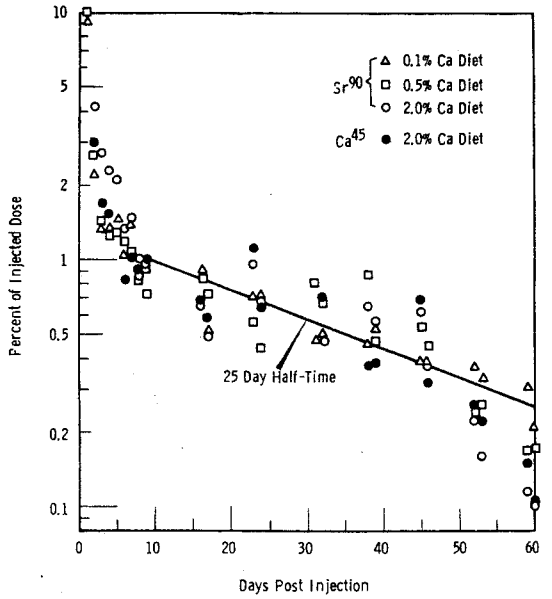

FIG. 1. Daily average total excretion of ^{90}Sr and ^{45}Ca injected intraperitoneally at age 26 days.

While fluctuations by as much as a factor of 2, from day to day, or even from week to week, were common, the general trend of change in excretion rate was clearly evident.

Strontium-90 and ^{45}Ca retention curves for the animals on each dietary regimen were constructed by subtracting each days total excretion from the initial injected dose. These curves are shown in Fig. 2 (mature rats) and Fig. 3 (growing rats). During the latter portion of the experiment, when excreta collections were not made daily, the daily excretion increment employed in the construction of the retention curves was read from an eye-fitted smooth curve drawn through the experimental excretion data. The line drawn in Fig. 1 is an example of such an interpolation curve.

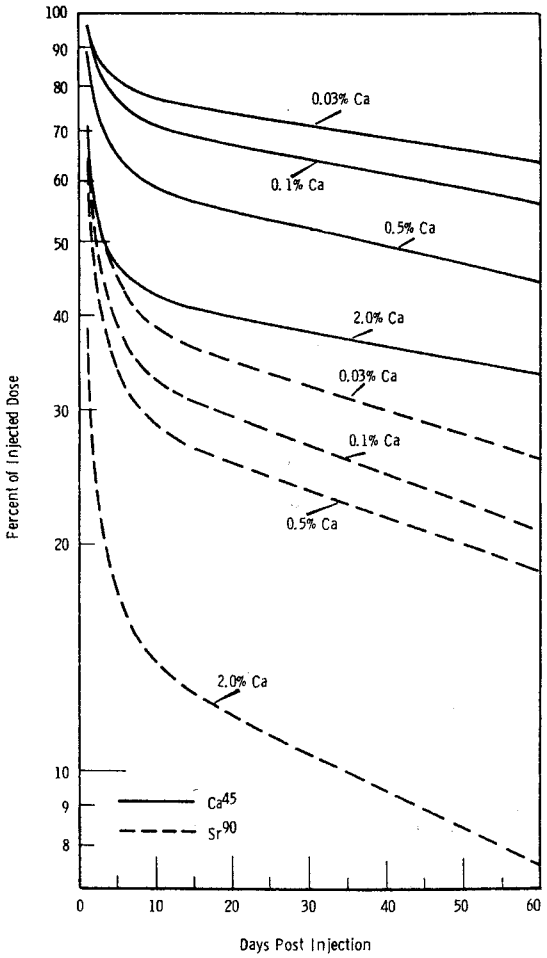

FIG. 2. Effect of dietary calcium level on the retention of ^{90}Sr and ^{45}Ca injected intraperitoneally at age 8 months.

An interesting feature of the experimental results was the variation in the U/F ratio of both ^{90}Sr and ^{45}Ca, between animals on different calcium intakes, and with time following radionuclide administration. This is shown in Fig. 4 (mature rats) and Fig. 5 (growing rats) where curves are drawn to indicate the change in U/F as a function of time postinjection. Because of the retention of feces in the intestine, U/F ratios, during the early period of daily collections, were calculated using a given day's urine value and the succeeding day's fecal value. The first day's urine value was compared with the sum of the

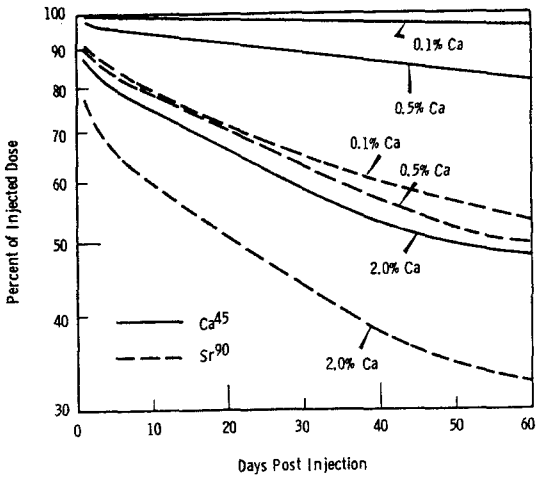

FIG. 3. Effect of dietary calcium level on the retention of ^{90}Sr and ^{45}Ca injected intraperitoneally at age 26 days.

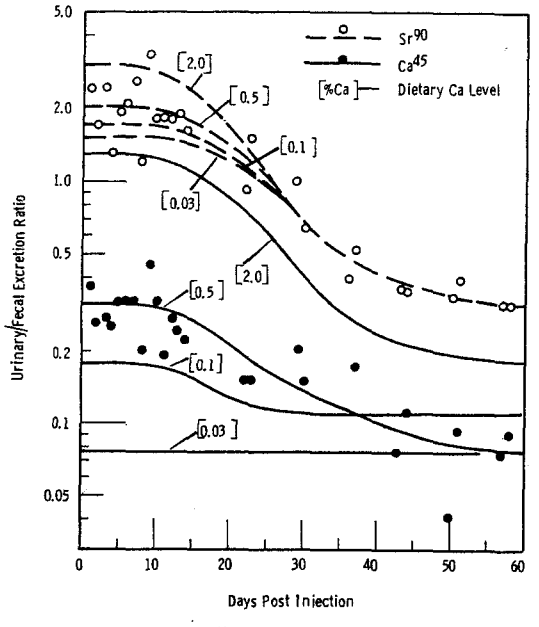

FIG. 4. Effect of dietary calcium level on the urinary/fecal excretion ratio of ^{90}Sr and ^{45}Ca injected intraperitoneally at age 8 months. Data points for daily average U/F ratios are shown for only the 0.5% calcium diet.

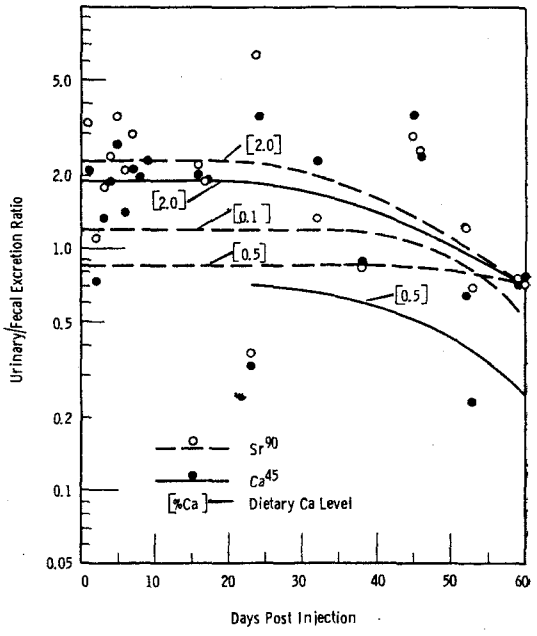

FIG. 5. Effect of dietary calcium level on the urinary/fecal excretion ratio of ^{90}Sr and ^{45}Ca injected intraperitoneally at age 26 days. Data points for daily average U/F ratios are shown for only the 2.0% calcium diet.

first and second day's fecal value. As one might anticipate, U/F ratios showed a considerable day-to-day variation. Individual data points (daily averages) are shown for two of the curves in Fig. 4 and Fig. 5 as an indication of the variability observed. The exact shape of the curves drawn are clearly not defined, and small differences between curves are of no significance. Certain of the curves, however, are clearly distinct from others.

DISCUSSION

The three diets employed markedly affected the general growth and skeletal development of the growing rats as indicated by the data obtained at sacrifice which is summarized in Table 2. More extensive data on the behavior of growing rats on these diets were obtained in earlier studies and are discussed in detail in a previous publication.[2] The 0.1% calcium diet was clearly inadequate for normal skeletal mineralization. The slight growth retardation on the 2.0% calcium diet was due to self-limited food

Table 2. Effect of Diet on Growth and Skeletal Development (Average values from five rats at sacrifice)*

	Calcium content of diet (%)		
	0.1	0.5	2.0
Animal wt. (g)	142	181	161
Femur, wet wt. (g)	0.33	0.51	0.44
Femur ash wt. (g)	0.094	0.22	0.19
Femur ash (% wet wt)	28	44	44

*Animals sacrificed at age 86 days, after 64 days on indicated diets.

intake. These discrepancies must be borne in mind in interpreting the ^{90}Sr and ^{45}Ca behavior in the growing rats.

Grossly, there was no indication of effect of diet on general appearance or weight maintenance of the mature rats. In earlier studies on the same diets, with somewhat younger animals (3 months old), there was a slight, but statistically significant increase in femur weight and ash content on the 2.0% calcium diet.[1] The possibility of significant radiation effects from these diets was discussed in connection with the previous studies,[1] and seems quite unlikely.

Retention in mature rats. The retention curves for ^{90}Sr and ^{45}Ca in the mature rats (Fig. 2) are qualitatively similar to those which have been described by many other investigators, working with a variety of mammalian species, including man.[1, 3-6] These retention curves were resolved into exponential components as detailed in Table 3. In all cases the retention subsequent to about 15 days postadministration was closely approximated by a single exponential expression involving a half-retention time of from 60 to 180 days. Subtraction of this long-lived component left a component, or components, with half-times of 2 days or less. It is important to note that there is no indication, in any diet group, of more than a single component of half-time longer than 2 days. Considering the manner in which these curves were constructed, and the relatively short duration of the experiment, the possibility of components of intermediate, or of very long half-time cannot be ruled out; however, it seems quite certain that

Table 3. Resolution of Retention Curves for Mature Rats

Diet group (% Ca)	^{90}Sr components		^{45}Ca components	
	Half-time (days)	% of injected dose	Half-time (days)	% of injected dose
0.03	90	40	180	80
	$\lesssim 2$	60	$\lesssim 2$	20
0.1	80	35	150	73
	$\lesssim 2$	65	$\lesssim 2$	27
0.5	80	30	120	61
	$\lesssim 2$	70	$\lesssim 2$	39
2.0	60	15	160	44
	$\lesssim 2$	85	$\lesssim 2$	56

any such components would constitute a small fraction of the total ^{90}Sr or ^{45}Ca. This conclusion is supported by the fact that the half-time for decrease in daily excretion rate, subsequent to about 15 days postadministration, was, in all groups, essentially the same as the retention half-time.

Within each diet group the retention of ^{90}Sr, as compared with the retention of ^{45}Ca, is characterized by the presence of a greater fraction of the total dose in the short-lived components, and by shorter long-lived components. In general, about twice as much ^{90}Sr is present in the short-lived components, and the half-time of the long-lived ^{90}Sr component is about half that of the long-lived ^{45}Ca component. The differences between adjacent diet groups are usually small and, in most cases, of questionable significance. There is an obvious trend, however, for larger short-lived components, and shorter long-lived components with increasing calcium intake.

Retention in growing rats. The retention curves for ^{90}Sr and ^{45}Ca in the growing rats (Fig. 3) are distinctly different from those obtained in the mature animals. As one might expect, ^{45}Ca is tenaciously retained on the 0.1% and 0.5% calcium diets. A single exponential component accounts for essentially all of the ^{45}Ca, with a biological half-time of approximately 1,000 days on the 0.1% calcium diet, and 200 days on the 0.5% calcium diet. A quite different pattern of retention is seen for ^{45}Ca in animals on the 2.0% calcium diet, and for ^{90}Sr on all diets. These are obviously multicomponent curves, consisting of a short-lived component (or components), an intermediate component (or components) which determines the greater portion of the available curve, and a long-lived component (or components) which is only suggested by the "tailing off" during the final 20 days of the experimental period. More positive evidence for the existence of a long-lived component may be deduced from the excretion curves. These are shown for ^{90}Sr on all diets and for ^{45}Ca on the 2.0% calcium diet, in Fig. 1. For the period from 10–60 days postinjection all of these data are reasonably fitted by an exponential function corresponding to a half-time of 25 days. The retention curves over this same time period, however, show a much slower rate of decrease, indicating that some substantial fraction of the ^{90}Sr and ^{45}Ca must be retained with a half-time much longer than 25 days.

Resolution of the retention curves for ^{90}Sr and ^{45}Ca in the growing rats is detailed in Table 4. The listed components must be considered quite approximate because of the very limited information which the retention curves provide concerning the long-lived components. The existence of an approximately 25-day half-time component for ^{90}Sr on all diets and for ^{45}Ca on the 2.0% calcium diet is established by the excretion data (Fig. 1) and these 25-day components were taken as a starting point in the graphic resolution of the retention curves. The

Table 4. Resolution of Retention Curves for Growing Rats

Diet	^{90}Sr components		^{45}Ca components	
Group (% Ca)	Half-time (days)	% of injected dose	Half-time (days)	% of injected dose
0.1	> > 25	50	1000	100
	25	40		
	< 2	10		
0.5	> > 25	45	200	97
	25	45		
	< 2	10		
2.0	> > 25	20	> > 25	35
	25	50	25	50
	< 2	30	< 2	15

long-lived components were assumed to have an infinite half-time. The curves were resolved into that combination of a 25-day half-time component and an infinite half-time component which best fit the retention data beyond about 7 days postinjection. Subtraction of these components left a short component of less than 2 days half-time. The long-lived component does not, of course, have an infinite half-time, but the data of this experiment provide no estimate of its true value, beyond the fact that it is evidently much longer than 25 days. Over the 60-day period of these retention curves, the choice between an infinite half-time or a 200-day half-time for the long-lived component would have little influence on the resolution of the remainder of the curve.

In comparing the behavior of calcium in growing and mature rats it seems clear that growing animals on an inadequate (0.1%) or marginally adequate (0.5%) calcium intake constitute a special case. The excretion of ^{45}Ca in both urine and feces of the growing rats on the 0.1% and 0.5% calcium diets was sharply reduced, as compared to the case of mature rats on the same diets (Table 1). These growing rats required essentially all of the calcium available and quite efficiently utilized their limited supply.

The behavior in growing rats of calcium supplied in excess (2.0% calcium diet), and of strontium under all dietary calcium conditions, are remarkably similar, and differ from the situation in mature rats in one major respect. The early rapid elimination observed in mature animals is greatly reduced in the growing animals, and the excess of retained calcium and strontium is lost gradually, with a half-time of about 25 days—such a 25-day half-time component is notably absent in the retention curves of the mature rats. Thus, on the 2.0% calcium diet, which provides perhaps a fourfold excess of calcium for the growing rat, total elimination of ^{45}Ca by urine and feces during the first 2 days postinjection amounts to about 15% of the injected dose—in mature animals on the same diet total elimination of ^{45}Ca during the same 2-day period amounted to nearly 45% of the injected dose (Table 1). Excretion during later time periods, however, was as high, or higher, in growing rats than in mature rats. This contrasting behavior of growing and mature rats might reasonably be explained by the much greater availability in growing rats of sites of new bone growth which tie up the injected ^{90}Sr and ^{45}Ca, preventing its rapid excretion. The processes of bone growth, however, involve continual resorption of old bone and deposition of new bone, so that the turnover of a substantial proportion of firmly deposited strontium and calcium occurs more rapidly in the growing rat than it does in the mature rat.

Mechanisms of strontium-calcium discrimination. In the mature rats the faster rate of ^{90}Sr excretion, as compared with ^{45}Ca excretion, is due principally to differences in urinary excretion (Table 1). The total fecal excretion of ^{45}Ca, on a percent of injected dose basis, is actually somewhat greater than that of ^{90}Sr. If allowance is made for the high urinary excretion of ^{90}Sr on the first day postinjection, the fecal excretion of ^{90}Sr and ^{45}Ca, on a retained dose basis, is very similar. This is true not only within a given diet group but is quite similar when comparisons are made between all dietary groups. This discrimination between strontium and calcium in renal clearance, and lack of discrimination in fecal excretion, has been noted in several studies in man. [7, 8, 9] As dietary calcium intake increases, the renal discrimination between strontium and calcium decreases, as is clearly evident in Table 1, and as has been indicated in studies with man reported by Samachson and Spencer-Laszlo. [10]

This effect of dietary calcium level on renal discrimination in the mature rat is also evident from a consideration of the urinary/fecal excretion ratios as presented in Fig. 4. On the 0.03% calcium diet, where retention of ^{45}Ca is at a maximum, the U/F ratio for ^{45}Ca is less than 0.1, excretion occurring predominantly via the intestinal route. With an increase in dietary calcium level, the initial U/F ratio increases to a maximum value of about 1.3 for ^{45}Ca on the 2.0% calcium diet. The initial U/F ratios for ^{90}Sr are all higher than those for ^{45}Ca, increasing as a function of calcium intake by only a factor of 2. In an earlier paper [1] we suggested that this type of behavior might be explained on "the assumption of a common threshold mechanism for the excretion of calcium and calcium-like elements. When the threshold

is not exceeded, excretion would be determined by the efficiency for each element of the reabsorption mechanisms operating. When the threshold is exceeded, there would be total and indiscriminate excretion of all calcium-like elements in excess of the threshold level. Thus, exceeding the threshold by only a small percentage could result in a markedly increased [percentagewise] excretion of the otherwise efficiently retained calcium, while having a quite negligible effect on the excretion of the always less efficiently retained strontium." Evidence indicating a maximum renal tubular transport of calcium has been presented by Copp, McPherson and McIntosh.[11] This simple hypothesis will not, however, explain the change in U/F ratios with time following radioisotope injection, which is a prominent feature of the data shown in Fig. 4, and which will be further considered in subsequent discussion.

In growing rats (Fig. 5) the initial U/F ratios for ^{45}Ca are much higher than was the case with mature rats, and very similar to those for ^{90}Sr on the same diet. These high U/F ratios are due to the very low excretion of ^{45}Ca, and to a lesser extent, ^{90}Sr, in the feces of growing rats. It would seem quite plausible that this lowered fecal excretion is due, not to a decreased secretion into the intestine, but to a more efficient reabsorption of the secreted ^{45}Ca and ^{90}Sr from the intestine. Intestinal absorption of calcium and strontium is known to be much more efficient in the young, than in the mature rat, and more responsive to dietary calcium intake.[12, 13] Direct comparisons of the numerical values of U/F ratios in growing and mature rats therefore have little meaning. Were it possible to correct the U/F values of the growing rats for enhanced reabsorption of intestinally secreted ^{90}Sr and ^{45}Ca, it is quite likely that they would fall below the U/F values for mature animals. What is more significant than the absolute numerical values is the close grouping of U/F values for both ^{90}Sr and ^{45}Ca on all diets. This is certainly indicative of a more similar treatment of strontium and calcium by the young rat. This fact was also evident in our earlier retention studies where young growing rats were observed to discriminate against strontium, relative to calcium, to a significantly lesser degree than the mature animals.[2] Studies in pigs,[14] dogs,[15] and humans[16, 17] have indicated a similarly lessened discrimination in the very young.

Evidence for differently excreted forms of ^{45}Ca and ^{90}Sr. The marked change in U/F ratios of both ^{45}Ca and ^{90}Sr, in mature rats, as a function of time following injection (Fig. 4) is an observation of particular interest. These animals (in any given group) were on a constant calcium intake dating from a month prior to ^{90}Sr and ^{45}Ca injection, and any change in the metabolic handling of these elements can hardly be attributed to a change in the physiological status of the animal. It seems necessary to postulate the existence of at least two different forms of calcium and strontium in the blood, which differ in their relative ease of excretion via renal and intestinal routes. The form preferentially excreted via the kidney is present in highest proportion immediately following injection, this proportion decreasing with time. The initial proportion of this form decreases with decreasing level of calcium intake.

The present experiment, with its lack of data on blood ^{90}Sr and ^{45}Ca, is of little help in further characterizing these forms. One may speculate that calcium or strontium released at long times following injection, from a site of "firm" deposition in bone, is present in some complexed form which is different from that released from "freely exchangeable" sites. It is tempting to equate the form of strontium and calcium exhibiting high U/F ratios with the short-lived component of the retention curves, and the form exhibiting low U/F ratios with the long-lived component. While qualitatively attractive, such a hypothesis does not appear to be quantitatively in accord with the data, since the high initial U/F ratios are maintained for longer periods than would be consistent with the 2 day or shorter half-lives of the rapidly lost components.

The relative constancy of U/F ratios in the growing rats might be taken to indicate a predominance, throughout the experimental period, of a single form of ^{90}Sr and ^{45}Ca, or, less plausibly, of a constant proportion of different forms. If the major proportion of the ^{90}Sr and ^{45}Ca injected into young growing rats is incorporated into the firmly-bound component of bone, as indicated by the small proportion lost

with a half-time of less than 25 days (Table 4), then nearly all of the ^{90}Sr and ^{45}Ca present in the blood and available for excretion must have been derived from stable bone, and be in the form which is characterized in mature rats by low U/F ratios. In other words, U/F ratios in growing rats do not decrease markedly with time because the ^{45}Ca and ^{90}Sr in the blood of growing rats is, essentially from the start, in the form which predominates in mature rats only after a period of several weeks.

Clearly, the further elucidation of this problem requires a more complete characterization of the physico-chemical state of calcium and strontium in the blood, in relation to excretion patterns, and as a function of the duration of retention of these elements in bone.

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