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ANIMAL METABOLIC STUDIES OF STRONTIUM AND CALCIUM
WITH SPECIFIC REFERENCE TO FALLOUT RADIONUCLIDES

Final Progress Report

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Calcium Kinetics During Pregnancy and Lactation in Jersey Cows

This experiment was designed to meet two objectives: (1) to characterize the pattern of changes in calcium kinetics during pregnancy and lactation in dairy cows, and (2) to determine the effects of optimal and excessive Ca:P dietary ratios on calcium kinetics in dairy cows over a prolonged time interval. The length of time a ration is fed can have a marked effect on whether or not observable changes in calcium metabolism occur. Stott (1965,1968) found that in a Jersey herd having a high incidence of milk fever, no further cases were observed after 189 days of feeding an optimal Ca:P ration, and that the disease did not reoccur for almost a year after removal from the optimal ration. He suggested that the predisposing factors for parturient paresis develop gradually, not suddenly. The fact that older cows are most often affected supports his suggestion.

Animals and Diets - Eight Jersey cows were assigned to one of two groups, equalizing the four animals in each group as much as possible according to age, pregnancy, and lactation status. Ages, body weights, parturition dates, and milk yields are shown in Table 1. Rations for the two groups were formulated to match the Ca:P ratios of two different hays: (1) timothy hay with a 2:1 ratio (control ration) and alfalfa hay with a 9:1 ratio (hi-Ca ration). Since the hay was fed ad libitum actual Ca and P intakes could not be measured, but the intake Ca:P ratio for each cow was constant because the ratio was almost identical in her hay and grain rations. Table 2 lists the compositions of the grain rations. The cows were fed only hay during the dry period; estimated daily consumption of 20 lbs/day (timothy) and 25 lbs/day (alfalfa) would provide approximately 36 gm Ca and 18 gm P, and 170 gm Ca and 21 gm P from the normal-Ca and high-Ca rations, respectively. Both hays provided similar daily P intakes in slight excess of NRC maintenance requirements during the dry period.

Grain was fed at a rate of about one pound per three pounds of milk produced. Feeding of the control and hi-Ca rations began on 5/23/71; prior to that time all cows consumed hay containing 0.63% Ca and 0.13% P (4.8:1 ratio) and, if lactating, grain containing 0.40% Ca and 0.46 P (0.88:1 ratio).

Readily Exchangeable Calcium Pool (RECaP) Measurements. RECaP size and turnover were measured in all cows at two- to four-week intervals using methods described previously (Anderson and Crackel; 1971). Twenty-five to thirty-five μCi calcium-47 (ORNL) was injected via the jugular vein and heparinated blood samples were withdrawn from the opposite jugular at approximately 1/2, 1, 3, 6, 11, 22, 34, 46, 52, 58, 70 and 76 hours post-injection. Stable calcium and radiocalcium were measured in blood plasma and the results were expressed as specific activity (percent injected dose/gram stable Ca). The specific activity-time curves for each cow and each injection period were fitted to a two-component exponential model by an iterative procedure utilizing a computer. The two-component solution yielded pool sizes in grams for small (E_1) and large (E_2) pools having shorter and longer half-lives, respectively. Calcium turnover in these pools was calculated as a transfer rate (TR), the product of E and the fractional turnover rate ($0.693/T_{1/2}$).

EDTA Infusion Studies. The cows were maintained on their respective rations and RECaP measurements were continued until November 3, 1971. Between November 9-18 each cow's calcium mobilizing ability was measured by ethylenediaminetetraacetic acid (EDTA) infusion according to methods employed by Muir et al. (1968). EDTA was infused at a rate of 98 mg/kg body weight for 240 minutes. Heparinated blood samples were taken immediately pre-infusion, at half-hour intervals during infusion through four hours post infusion, and at the following times from beginning of infusion: 9, 10, 11, 12, 13, 14, 24, 36, and 48 hours. Plasma Ca analyses were performed by a spectrophotometric

method (Malmstadt and Hadjiioannou; 1969) in which EDTA was used as the complexing agent; consequently only non-EDTA bound Ca was measured. Results were expressed in several ways: (1) half-recovery time = time in hours for plasma Ca to increase by half the difference between pre-infusion and terminal-infusion values, (2) total Ca reduction (Δ Ca) pre-infusion to terminal-infusion, (3) grams Ca mobilized per kg body weight, estimated from Ca chelated by EDTA infused plus Ca required to produce terminal-infusion Ca concentration based on total body plasma equal to 3.7% of body weight, (4) immediately available Ca reserves and Ca mobilization rate as calculated by Payne (1964), and (5) relative plasma Ca maintenance constant, as calculated by Muir et al.

Results and Discussion. Figure 1 shows E_2 values for both groups of cows from 40 days prepartum to 200 days postpartum. Since the point of reference (day 0) was parturition, the days pre- or post-parturition that injections took place varied among animals; each point in Figure 1 represents the midpoint of a range of days during which animals in each group were injected. For example, the points at 42 days prepartum include data obtained from injections made between 33-52 days prepartum, 42 days being the midpoint. Table 3 gives ranges and corresponding midpoints that were used to plot days pre- and post-partum. It is evident that the mean E_2 values were higher for the control group than the Hi-Ca group beginning about 30 days post-partum. The differences were not statistically significant, however, because of large standard deviations within groups. E_2 values were the same in both groups prepartum, which was before the cows were placed on the experimental diets; the times at which individual animals went on experimental diets ranged from day 0 to day 110 postpartum, all but one being prior to 66 days postpartum. The pattern shown by the E_2 values is similar to what we have seen before in goats (Twardock et al. 1970) and cows (Anderson and Crackel; 1971). RECaP

size increased dramatically following parturition and the onset of lactation, providing a larger reserve of readily available Ca to meet the stress of lactation.

The turnover of Ca in E_2 increased even more markedly than pool size, as shown by changes in $T_{1/2}$ and TR (Figures 2 and 3). Again, lower TR's were observed in the high Ca group but were not statistically different except at 110 days postpartum. Changes in E_1 size and turnover followed patterns similar to E_2 changes, but were not as marked. The radiocalcium kinetic data suggest but do not prove that there was a dietary effect on the RECaP. If such an effect existed, it supports the hypothesis that high-Ca rations might induce increased susceptibility to parturient paresis by reducing the size and turnover of the RECaP. These effects were not observed until late in this experiment because the animals had not been subjected to the experimental diets prepartum or for significant lengths of time immediately postpartum. If the cows had been continued on the same rations into the next parturition, induction of parturient paresis in the hi-Ca group might have been observed. Unfortunately it was not possible to continue the experiment.

Results of the EDTA infusion experiment are given in Table 5. Certain tendencies can be observed but the variation among animals within groups again precluded statistically significant differences. Preinfusion Ca concentrations were higher in the control group (9.7 mg/100 ml) than the high-Ca group (9.1 mg/100 ml), whereas at the end of infusion both groups had almost the same non-chelated Ca concentration (5.6 vs 5.5 mg/100 ml). The only difference worth noting was the more rapid Ca mobilization rate in the normal Ca group (0.51 vs 0.40 Gm/Hr). Figure 4 shows the average concentrations in both groups of nonchelated plasma Ca for the entire experiment. No remarkable differences

were observed between the two groups, except that the normal-Ca cows maintained a higher plasma Ca at both the beginning and end of the experiment. Our measurements of immediately available Ca reserves by Payne's technique gave results (6.8 Gm) similar to the range he observed in Ayrshires (5.8-7.6 Gm). His Ca mobilization rates (0.50-0.87 gm/hr) were generally higher than ours (0.28-0.81 gm/hr). We also plotted the reciprocals of nonchelated plasma Ca against infusion time for both groups (Figure 5) to compare our results with those of Muir et al. (1968). We found no difference between the slopes of the regression lines, indicating no differences in what Muir et al. call the "relative serum Ca maintenance constant" between the two groups. Equations for the regression lines were: plasma Ca reciprocal = $.104 + .019$ (hrs infusion) for controls and $.112 + .020$ (hrs infusion) for the hi-Ca group.

All in all, our results show no great difference in the calcium kinetics or mobilizing abilities of the two groups. The most likely explanation is that lactation has an overriding effect that obscures more subtle dietary effects. Muir et al. (1968) measured half-recovery times after EDTA infusion of 19.0 hours in vitamin D fed cows and 22.4 hours in control cows. Half-recovery times in our cows were much shorter, ranging from 1.8 to 4.1 hours (Table 4). This difference can be explained by the fact that our cows were lactating whereas those of Muir et al. were dry. Mayer et al. (1969) observed that at 1 day prepartum 11 hours were required for full recovery of plasma Ca following a one-hour EDTA infusion that chelated 4.82 gm; Ca recovery in the same cow after a second infusion 6 days postpartum required only 2.3 hours.

We believe that our results failed to clearly demonstrate the deleterious effects of the high Ca ration because those effects were obscured by high rates of Ca turnover caused by lactation. Had the experiment proceeded long enough to observe the cows during their dry periods, we believe more obvious differences between the two groups would have been observed.

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Table 1

47 Daily Milk Yields At Times of Ca-47 Injections (Pounds)								
Injection	Normal Ca Diet				High Ca Diet			
Date	<u>361</u>	<u>2306</u>	<u>2373</u>	<u>2513</u>	<u>364</u>	<u>2388*</u>	<u>2614</u>	<u>4575</u>
								Calved <u>2/2</u>
2/11/71								31.0
2/24/71		Calved <u>3/17</u>	Calved <u>3/23</u>					35.7
3/25/71		30.0	26.0					34.0
4/7/71		37.6	31.4			Calved <u>4/17</u>	Calved <u>4/10</u>	35.5
4/22/71	Calved <u>5/17</u>	33.8	29.5		Calved <u>5/18</u>	30.1	24.1	33.7
5/19/71	32.2	27.9	24.2		30.0	24.3	26.9	27.4
5/23/71	----- Normal and Hi-Ca Diets Begun -----							
6/2/71	40.0	27.8	26.7	Calved <u>6/4</u>	27.8	21.8	21.4	25.5
6/16/71	39.8	24.8	27.9	23.4	28.2	11.2	21.9	27.9
7/14/71	29.0	23.4	20.0	18.0	24.8	6.0	17.3	23.8
8/12/71	28.3	20.8	15.5	15.6	26.2	3.0	15.2	20.8
9/8/71	23.4	17.3	12.4	11.1	22.2	2.5	11.6	17.8
10/6/71	22.2	15.7	9.9	8.6	17.9	0.7	8.8	11.7
11/3/71	18.6	9.9	7.6	3.0	14.8	0.5	9.4	5.7
Average	29.2	24.4	21.0	13.3	24.0	11.1	17.4	25.4
Total	233.5	268.4	231.1	79.7	191.9	100.1	156.6	330.5
Age (Yr./Mo.) On 11/15/71	4/3	8/7	6/3	4/10	4/1	6/10	3/10	
Weight (Kg.) On 11/15/71	442.7	403.7	418.2	357.4	459.5	372.9	330.7	463.6

* Retained displaced abomasum, probably about 6/11; surgery 7/19.

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Table 2

Dairy Cow Concentrate Rations

<u>Ingredients</u>	<u>Control Ration</u>	<u>Hi-Ca Ration</u>
	(%)	(%)
Shelled Corn	82.4	73.45
Soybean Oil Meal	12.7	11.5
Dicalcium Phosphate	1.45	1.3
Trace Mineralized Salt	1.2	1.2
Vitamin A & D Supplement	0.05	0.05
Calcium Carbonate	2.2	12.5
<hr/>		
% Calcium	1.13	4.95
% Phosphorus	0.54	0.50
Calcium: Phosphorus	2.1:1	9.9:1

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Table 3

Pool Sizes and Injection Time Ranges and Medians

Injection Time		Pool Size (Grams)*					
Pre- and Post-							
Parturition (Days)		E ₁		E ₂		E ₁₊₂	
Median	Range	Control	Exptl.	Control	Exptl.	Control	Exptl.
-42	-(33 to 52)	5.79 $\pm$.98	6.22 $\pm$.75	21.9 $\pm$ 2.1	21.6 $\pm$ 5.4	27.7 $\pm$ 2.9	27.9 $\pm$ 5.8
-23	-(16 to 31)	7.38 $\pm$ 1.11	7.70 $\pm$ 1.20	20.9 $\pm$ 5.6	22.3 $\pm$ 9.4	28.3 $\pm$ 5.2	30.0 $\pm$ 9.8
10	5 to 16	6.61 $\pm$ 1.02	7.14 $\pm$ 1.18	26.8 $\pm$ 5.1	32.3 $\pm$ 4.1	33.4 $\pm$ 6.0	39.4 $\pm$ 4.8
30	22 to 39	7.78 $\pm$.56	7.22 $\pm$.95	40.6 $\pm$ 8.9	33.2 $\pm$ 9.7	47.8 $\pm$ 9.1	40.4 $\pm$ 9.9
57	51 to 63	6.38 $\pm$ 1.10	7.15 $\pm$ 1.49	34.8 $\pm$ 6.9	30.3 $\pm$ 8.8	41.2 $\pm$ 7.6	37.5 $\pm$ 10.0
116	108 to 124	6.61 $\pm$.44	5.92 $\pm$.36	30.9 $\pm$ 2.8	22.9 $\pm$ 4.9	37.5 $\pm$ 3.1	28.8 $\pm$ 5.2
142	134 to 151	6.37 $\pm$.91	6.85 $\pm$.67	28.03 $\pm$ 5.5	26.4 $\pm$ 3.9	34.4 $\pm$ 6.2	33.2 $\pm$ 4.2
170	162 to 179	8.15 $\pm$ 2.60	6.78 $\pm$.86	36.6 $\pm$ 20.5	25.2 $\pm$ 6.2	49.5 $\pm$ 22.8	32.0 $\pm$ 7.1
199	191 to 207	6.70 $\pm$.21	6.76 $\pm$.98	27.5 $\pm$ 3.1	25.2 $\pm$ 1.2	34.2 $\pm$ 3.3	32.0 $\pm$ 2.1

*Mean $\pm$ standard deviation

Table 4

Half-Lives, Transfer Rates, and Injection Time Ranges and Medians

Injection Time

Pre- and Post-

Pool One *

Pool Two *

Parturition (Days)Half-Life (Hr)Transfer Rate (Gm/Hr)Half-Life (Hr)Transfer Rate (Gm/Hr)MedianRangeControlExptlControlExptlControlExptlControlExptl

-42 -(33 to 52)

1.39 $\pm$.161.46 $\pm$.362.88 $\pm$.403.03 $\pm$.5621.4 $\pm$ 1.520.2 $\pm$ 2.10.71 $\pm$.110.76 $\pm$.27

-23 -(16 to 31)

1.79 $\pm$.081.70 $\pm$.302.86 $\pm$.483.15 $\pm$.1022.1 $\pm$ 2.119.9 $\pm$ 2.40.66 $\pm$.190.78 $\pm$.27

10 5 to 16

1.42 $\pm$.151.49 $\pm$.293.26 $\pm$.583.35 $\pm$.3613.0 $\pm$ 3.614.5 $\pm$ 1.41.42 $\pm$.151.49 $\pm$.29

30 22 to 39

1.64 $\pm$.071.45 $\pm$.203.05 $\pm$.393.45 $\pm$.6514.4 $\pm$ 1.313.3 $\pm$ 2.01.96 $\pm$.471.72 $\pm$.35

57 51 to 63

1.47 $\pm$.081.47 $\pm$.353.00 $\pm$.483.40 $\pm$.5214.3 $\pm$.7215.0 $\pm$ 5.81.70 $\pm$.381.53 $\pm$.57

116 108 to 124

1.56 $\pm$.141.63 $\pm$.382.95 $\pm$.282.60 $\pm$.5615.7 $\pm$ 1.5116.7 $\pm$ 5.81.37 $\pm$.221.03 $\pm$.40

142 134 to 151

1.57 $\pm$.091.60 $\pm$.152.83 $\pm$.522.98 $\pm$.5216.0 $\pm$ 1.9816.8 $\pm$ 3.11.44 $\pm$.441.13 $\pm$.32

170 162 to 179

2.15 $\pm$.891.63 $\pm$.132.71 $\pm$.472.88 $\pm$.1819.0 $\pm$ 2.016.4 $\pm$ 2.81.31 $\pm$.601.10 $\pm$.37

199 191 to 207

1.58 $\pm$.061.94 $\pm$.502.91 $\pm$.062.47 $\pm$.4518.3 $\pm$ 2.818.0 $\pm$ 4.61.07 $\pm$.281.00 $\pm$.19*Mean $\pm$ standard deviation

Table 5

EDTA Infusion Results

Ration	Cow No.	Body Wt. (kg)	EDTA Infused (Gm)	Plasma Ca (Mg%)			Grams Ca Mobilized/100 Kg	Half Recovery Time (Hrs)	Immediately Available Ca Reserves (Gm)	Calcium Mobilization Rate (Gm/Hr)
				<u>t₀</u>	<u>t₂₄₀</u>	<u>Δ</u>				
Normal Ca	361	442.7	41.78	9.30	4.94	4.36	1.17	1.85	5.41	.81
	2306	403.7	40.23	10.34	6.71	3.63	1.29	3.50	7.84	.39
	2372	418.2	40.81	9.92	5.80	4.12	1.23	4.10	4.74	.47
	2513	<u>357.4</u>	<u>34.36</u>	<u>9.24</u>	<u>4.99</u>	<u>4.25</u>	<u>1.19</u>	<u>3.80</u>	<u>9.23</u>	<u>.37</u>
Average		405.5	39.28	9.70	5.60	4.09	1.22	3.31	6.80	.51
High Ca	364	459.5	49.69	9.76	6.26	3.50	1.36	1.80	8.75	.58
	2388	372.9	38.52	8.53	4.75	3.78	1.26	3.50	6.31	.28
	2614	330.7	32.27	9.34	6.44	2.90	1.26	2.10	6.12	.33
	4575	<u>463.6</u>	<u>44.20</u>	<u>8.93</u>	<u>4.45</u>	<u>4.48</u>	<u>1.16</u>	<u>2.90</u>	<u>5.96</u>	<u>.41</u>
Average		404.4	41.15	9.14	5.48	3.66	1.26	2.58	6.78	.40

FIGURE 1

Figure 2

FIGURE 3

FIGURE 1

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FIGURE 5

Effect of time on retention of single injections of ^{85}Sr in standard and miniature swine from birth to six months of age

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INTRODUCTION

Long-term studies of the retention of a single injection of radiostrontium in experimental animals larger than a rat or rabbit, for the purpose of extrapolation of the animal model to man, have been relatively few. Two studies, among others, of strontium retention as a function of age in the dog resulted in comparable findings, namely, that retention at 200 days after injection was greatest when the animals were administered the radionuclide at 5 or 6 months of age. (1-5)

Still unresolved is the question of whether human infants from birth to two years or teenagers at the end of their growth spurt or any intermediate or even later age are most susceptible to the potentially deleterious effects of fallout ^{90}Sr . It is the purpose of this report to present new retention data following a single injection of ^{85}Sr in differently aged swine of both standard and miniature breeds. The findings indicate that, although neither canine nor porcine species serve as an ideal model for man, the dog is preferable to the pig because of the practical cessation of bone growth following sexual maturity, which is not the case in swine (6).

Animals. The 56 swine employed in these studies are listed according to standard or miniature breed in Table I. In addition, mean body weights at 200 days of age are included so that an assessment of growth according to body weight can be established. The values are mainly approximate interpolations from plotted growth curves of litters, since only a few animals were weighed at 200 days of age.

In experiments 5, 6, and 8 littermates were injected with ^{85}Sr at different ages whereas in the other experiments all littermates were injected at the same age. Sex of the animals was approximately evenly distributed within a given experiment.

Housing. Except during the period of suckling the swine were kept in indoor-outdoor combination pens, in which cinders served as the substratum. In experiments 10 and 11 all animals were maintained on concrete only.

Feeding. In experiments 5 through 9 the litters were split arbitrarily after weaning into two groups, one on a ration containing 1.2% Ca and 0.8% P by dry weight and the other 0.8% and 0.6%, respectively, the diet recommended by the National Academy of Sciences - National Research Council (7). Subsequently, all animals injected at a given age were pooled for retention calculations because of very similar findings in the two populations.

All other constituents of the two different rations were the same. However, the ration containing the higher percentage of calcium and phosphate was formulated at the University of Illinois feed stores whereas the lower ration, Pig Growena 2101, was made commercially (Ralston Purina, St. Louis, Missouri). Both rations contained 16% crude protein, which is more than recommended (7) when the pigs attain a body weight of about 125 pounds.

The animals were allowed to eat and drink ad libitum.

Whole-Body Counting and Retention Calculations. Each animal was injected intravenously with 0.25 to 0.5 μCi ^{85}Sr (pH 6.0, Nuclear Science and Engineering, Pittsburgh, Pennsylvania) on day 0 of an experiment. Whole-body measurements were made before and immediately after radionuclide administration in the Illinois

Animal Science Counter (ILLASCO) (8), a 4π liquid scintillation detector. The precount was used to determine the contribution of the naturally occurring ⁴⁰K radioactivity of the animal to the ⁸⁵Sr Compton-scatter band selected.

Retention of ⁸⁵Sr, R_t, at any time t, was calculated according to the relationship (1):

$$R_t = \frac{C_t (1-fw)S_o}{C_o (1-fw_o)S_t}$$

where C_o = pig radioactivity measurement in counts per minute (CPM) at time of injection, t=0

C_t = measured pig CPM at time t in days after injection.

S_o = measured CPM of standard dose at time of injection, t=0

S_t = measured CPM of standard at time t in days after injection

fw_o = fractional absorption of ⁸⁵Sr gamma photons in the body of pig of weight W_o in Kg at time of injection

fw = fractional absorption of ⁸⁵Sr photons in pig of weight w in Kg at any time after injection.

However, before retention could be calculated a correction was necessary for an animal's ⁴⁰K content at a given body weight up to 125 Kg. Beyond this weight (or mass) an increase in ⁴⁰K was not measureable under the conditions of the experiment. Because of animal variation and inherent systematic errors the counting accuracy beyond 150 days post-injection was decreased and, no doubt, accounts for some of the large variation observed in the 200-day retention values (See Table II).

All statistical comparisons of retentions as a function of age were made by Student's t-test.

Curve-Fitting. Attempts to fit the retention data by iterative computer techniques were made using single- and double-exponential and power functions. Best fits of the data, in general, were obtained with the double-exponential function. However, occasionally the second exponential portion of the curve was displaced downwardly and the computer-derived function did not appear to fit the data as well as a manually derived equation fit to the curve by eye.

RESULTS

Fractional Retention. Mean ^{85}Sr fractional retention at 1 and 200 days after intravenous injection in standard and miniature swine as a function of age at the time of injection is presented in Table II. In the standard breeds the progressively decreasing initial 1-day retentions indicate less uptake of the radionuclide with increasing age, a pattern also observed in dogs (2). However, in swine the initial uptake was considerably greater than for dogs beyond two months of age for both species. The mean fractional 200-day retention values for pigs injected at any time from birth to 6 months were quite similar, ranging between 0.23 and 0.31. The 2-month retention, 0.23, was less than the retention in swine injected shortly after birth, 0.28, and less than those injected at 4 or 6 months, 0.30. The swine injected at 1 and 2 months exhibited a slow rate of growth, which increased considerably just beyond 2 months in most of our animals before decreasing, but not leveling off, sometime after 9 months of age.

It is interesting to note that the one 13-month old pig used which continued to add weight (50 Kg. in 200 days) during its study, had the lowest initial uptake, 0.74, as might be expected, but by far the highest 200-day retention, 0.45,

which represents the greatest overall retention of the radionuclide of any of the swine studied. However, only one animal was employed at this age, which precludes any definitive comment.

The difference (Δ) between the day 1 and day 200 retentions, also included in Table II, show that the loss of ^{85}Sr in the groups injected between birth and 6 months is quite similar, except a hint is present that at the older ages more of the label is retained. This is certainly so in the 13-month old pig. The ratio of 200-day to 1-day retentions (Table II) also bears out this relation, namely, that more ^{85}Sr is kept by the animal the older it is when injected.

Retention was unaffected by sex, breed of standard-sized swine, relative growth rate of a given litter, or the two different dietary levels of calcium and phosphorus used in the animals sampled in these experiments.

In miniature swine a similar pattern of decreasing initial retention of ^{85}Sr was observed as in the standard breeds (Table II). However, the 200-day retentions in the animals injected at 1 and 2 months of age were considerably higher, 0.50 and 0.41 vs. 0.24 and 0.23, than in standard swine. These high 200-day retentions were also reflected in larger differences between day 1 and day 200 values and in larger ratios of the 200-day to 1-day retentions. Miniature swine which are administered the radionuclide within the first few months, may retain it more tenaciously than standard breeds because of their more flattened growth curves which begin to level off at around 250 days of age.

Curve-Fitting. The retention data of only 25 of the 55 swine studied through approximately 200 days post-injection could be described by a two-exponential function. Several other sets of data were fitted through 120 to 150 days post-injection, whereas six data sets could not be fit at all. The derived

functions for 28 standard and miniature swine injected at various ages are given in Table III. The derived functions for the standard swine illustrate trends for a slight decrease in the intercept (A_1) of the first function on the y-axis and for a slight increase in the intercept (A_2) of the second function. The negativity of the slopes of the first (b_1) and second (b_2) functions show no such relationship and are quite variable even within an age group, except for b_2 of the animals injected at 4 and 6 months.

For miniature swine (Table III) an opposite trend of increasing A_1 and A_2 values as a function of age at injection compared to standard swine was observed. Further more, a general trend of more negative slopes with increasing age of injection was also evident. These differences from standard breeds probably reflect the different growth curves of the standard and miniature swine as do the 200-day retention values.

Figures 1 to 7 are representative plots of retention data of different swine injected at various ages after birth, which have been drawn in along with the data points the best-fitted two-experimental function of the data. No examples are given for pigs in experiment 9, all injected at birth, because none of the fits could be achieved beyond 120-140 days post-injected. In Figure 1 are illustrated the pooled data of 5 Poland Cross littermates injected with ^{85}Sr shortly after birth. The computer-derived two-exponential equation for the data demonstrates the goodness-of-fit of this data. Other data from other experiments were generally not so well fit even though only the data of a single animal was used, particularly in some experiments in which an entire data set up to 200 days could not be included (Figures 2, 5 and 7). The fits

of two Duroc swine were quite good (Figures 3 and 4) as was one fit of a miniature swine injected at 1 month (Figure 6). Retention data of another miniature pig, injected at 6 months, could be fitted only to 126 days after administration of the ^{85}Sr (Figure 7). As noted above, the derived retention functions of the miniature swine were quite different from those of standard breeds.

DISCUSSION

These studies of standard and miniature swine clearly demonstrate that the two types of pigs retain a single intravenous injection of ^{85}Sr differently when measured 200 days after administration and that the differences in retention patterns are apparently related to the qualitatively dissimilar growth curves of the two types (Table II). Both types have s-shaped curves but, whereas the standard breeds have a rate of gain of about 0.75 Kg./day between 60 to 250 days of age, miniature swine gain only at a rate of approximately 0.25 Kg./day over a comparable period before the rates considerably slow down (Table I). Skeletal growth, although epiphyses remain open indefinitely (6), must decrease to a low rate at about 8 - 10 months to nearly a year of age after sexual maturity is attained, because the initial uptake of one pig injected at 13 months of age was much less than in younger swine (See Table II).

The retention patterns of the two types of swine, though different from each other, are even more distinct from the pattern obtained using experimental beagles (1,2). In Figure 8 are compared the 200-day fractional ^{85}Sr retentions of the two types of swine of this study and from dogs similarly studied (2). The 200-day values of standard swine injected at birth and at 4 and 6 months are greater than the values from swine injected at 1 and 2 months, but all of these values are much less than the 0.45 fraction of ^{85}Sr retained at 200 days

by one pig injected at 13 months of age. In the beagle, maximal retention at 200 days was obtained in dogs injected at 5 months of age, with lesser values observed for dogs injected at ages younger or older. In fact, a value of 0.2 was observed for dogs injected at slightly over a year of age (1), as against 0.35 to 0.4 at 5 to 6 months. In our limited series of miniature swine, retention at 200 days declined as a function of age at the time of injection to a value of just less than 0.3 at 6 months. Again, the retention pattern of the miniature swine studied is considerably different from that of the dog.

Because nearly all its epiphyses close within a short time after sexual maturity is attained at 8-10 months, the dog appears to be a better model than the pig for comparing the retention of a single administration of radiostrontium by a human subject, particularly during the teenage period of life. However, miniature swine, because of their slower growth rate and presumed lower skeletal turnover, may serve as a better model for radiostrontium retention by human infants only, particularly during the first year or so of life when skeletal turnover is high. The skeletal turnover of growing standard swine between 2 months and about a year of age is far too rapid for any valid comparison to be made between retention patterns of these swine and man. (Anderson, unpublished data). Moreover, the calcium (and phosphorus) intake of the standard breeds is estimated to be 5 to 10 times greater, or even more beyond 25 Kg. body weight (7), than that of a human of a comparable weight (9). For miniature swine compared to man the calcium intake ratio is closer to 2-4 to 1, even at older ages in swine whose mature body weight may be the same as that of an adult man. However, the dietary calcium (and phosphorus) ingested by a dog, including a growing puppy (10), is quite similar, if only slightly higher, to that of man and growing children.

The present study of age-related retention in swine following a single injection of radiostrontium, as opposed to continuous ingestion, provides information which, when coupled with dosimetric data at major sites of distribution of the radionuclide, such as the lining of bone marrow, can be predictive of potential radiation effects in the event of accidental ingestion or intake following a period of brief but intense fallout from nuclear weapons. The distribution patterns of radiostrontium have been reported to be nearly identical in swine following either a single injection(11) or continuous feeding (12). The continuous intake studies of swine (12, 13) and dogs (14, 15) more nearly simulate the long-term effects of constant fallout of radiostrontium and they have revealed many useful findings on the induction of blood dyscrasias and bone tumors in the experimental animals. The most appropriate model animal for the study of radiostrontium retention in man from birth through infancy and adolescence and into adulthood may not be any one animal. It is suggested that the dog extrapolates better to man during the teenage period, whereas miniature swine may be preferable as a model during human infancy. The artificial feeding, and handling of non-gnotobiotic swine can be initiated shortly after birth under proper management (16, 17) and such animals, particularly of a miniature variety, might prove upon extensive testing to be an excellent model for infants because of similar dietary intake and gastrointestinal physiology. A need for such a model exists (18, 19, 20).

TABLE I

SCHEDULE OF STANDARD AND MINIATURE SWINE USED IN STUDY

<u>EXPERIMENT NUMBER</u>	<u>BREED</u>	<u>NUMBER USED PER LITTER</u>	<u>APPROXIMATE MEAN BODY WEIGHT AT 200 DAYS OF AGE (Kg)</u>
5	Yorkshire-Hampshire Cross	5	75
	Yorkshire-Hapmshire Cross	7	67
6	Miniature ¹	7	45
7	Poland Cross	5	80
8	Duroc	11	110
9	Hampshire-Poland Cross	9	85
10	Yorkshire Cross	5	90
11A	Miniature	6	43
11B	Domestic Cross	1	50 ²
		<u>56</u>	

¹Obtained from Battelle-Northwest Laboratories, Richland, Washington

²Net weight gain between 400 and 600 days of age

TABLE II

MEAN FRACTIONAL ^{85}SR RETENTION (R_t) IN SWINE 1 AND 200 DAYS
AFTER INJECTION AS A FUNCTION OF AGE AT TIME OF INJECTION

AGE AT INJECTION	EXPERIMENT NUMBER	NUMBER OF ANIMALS	1-DAY FRACTIONAL RETENTION $\pm$ SD	200-DAY FRACTIONAL RETENTION $\pm$ SD	Δ R_1-R_{200}	RATIO R_{200}/R_1
A. Standard Swine						
Birth	5,7,9	16	$0.90 \pm .04^{2,3}$	$0.28 \pm .06$	0.62	0.31
1 Month	8,10	9*	$0.88 \pm .05$	$0.24 \pm .09$	0.64	0.27
2 Months	5	3	$0.86 \pm .04$	$0.23 \pm .05^{11}$	0.63	0.27
4 Months	5,8	6	$0.84 \pm .09^2$	$0.31 \pm .06^{1,11}$	0.53	0.37
6 Months	5,8	8	$0.85 \pm .07^3$	$0.30 \pm .06$	0.55	0.35
13 Months	11B	$\frac{1}{43}$	0.74	0.45	0.29	0.61
B. Miniature Swine						
1 Month	6	2	$0.95 \pm .02^3$	$0.50 \pm .02^{5,2}$	0.45	0.53
2 Months	6	1	0.85	0.42	0.44	0.48
4 Months	6	1	$0.86 \pm .06$	$0.26 \pm .04^2$	0.60	0.30
6 Months	6,11A	8 $\pm$	$0.80 \pm .06^3$	$0.26 \pm .02^5$	0.54	0.32

1, 11 Significantly different, p 0.10

2 Significantly different, p 0.05

3 Significantly different, p 0.025

4 Significantly different, p 0.005

5 Significantly different, p 0.001

*One animal died during the experiment.

$\pm$ Four animals died during the experiment.

TABLE III

COMPUTER-DERIVED TWO-EXPONENTIAL FITS OF EXPERIMENTAL RETENTION

DATA OF STANDARD MINIATURE SWINE INJECTED AT VARIOUS AGES

<u>AGE AT INJECTION</u>	<u>EXPERIMENT NUMBER</u>	<u>ANIMAL NUMBER</u>	<u>A₁ + SD</u>	<u>b₁ + SD</u>	<u>A₂ + SD</u>	<u>b₂ + SD</u>
A. Standard Swine						
Birth	5	2	0.593±.115	-0.0205±.0038	0.364±.119	-0.00303±.00156
	5	L1	0.383±.094	-0.2380±.0066	0.551±.099	-0.00225 .00099
	7	Pooled (N=5)	0.379±.021	-0.0730±.0115	0.552±.020	-0.00330±.00029
	9	1	0.462±.009	-0.0278±.0017	0.552± <u>±</u>	-0.00524±.00020
1 Month	8	1M	0.309±.048	-0.0663±.0204	0.570±.051	-0.00983±.00073
	8	2F	0.137±.021	-0.0630±.0210	0.877±.022	-0.00423±.00017
2 Months	5	3*	0.351±.0188	-0.3040±.0320	0.623±.010	-0.00899±.00022
	10	2	0.174±.025	-0.3790±.1080	0.811±.012	-0.00573±.00014
	10	4	0.359±.067	-0.0447±.0150	0.537±.071	-0.00541±.00086
	10	5	0.232±.026	-0.1340±.0390	0.720±.018	-0.00429±.00021
4 Months	8	2M	0.331±.037	-0.0389±.0081	0.608±.038	-0.00282±.00039
	8	3F	0.286±.004	-0.4530±.1220	0.678±.016	-0.00425±.00022
	8	5M	0.246±.024	-0.0647±.0159	0.761±.021	-0.00346±.00019
6 Months	5	L3	0.236±.024	-0.0511±.0121	0.680±.026	-0.00300±.00027
	5	L6	0.336±.049	-0.0341±.0085	0.556±.054	-0.00436±.00053
	8	OM	0.283±.025	-0.1340±.0300	0.659±.016	-0.00274±.00022
	8	4M	0.286±.033	-0.1910±.0510	0.679±.019	-0.00379±.00026
	8	7M	0.347±.032	-0.3760±.0650	0.616±.014	-0.00439±.00023

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B. Miniature Swine

1 Month	6	L2	0.135±.032	0.245±.1130	0.858±.0160	-0.00313±.00016
	6	R1	0.166±.021	0.203±.0500	0.808±.0113	-0.00263±.00012
2 Months	6	R2	0.276±.025	0.166±.0313	0.644±.0128	-0.00213±.00014
4 Months	6	L1	0.309±.028	0.440±.0795	0.659±.0108	-0.00451±.00019
	6	R3**	0.283±.016	0.529±.0610	0.723±.0060	-0.00398±.00013
6 Months	11A	M5***	0.245±.029	0.836±.1030	0.755± <u>±</u>	-0.00440± <u>±</u>

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* Only data through 142 days post-injection fit.

* Only data through 146 days post-injection fit.

*** Only data through 126 days post-injection fit.

± These parameters were held fixed during curve fitting.

LEGEND

1. Plot of pooled fractional ^{85}Sr retention data of 5 Poland Cross swine injected shortly after birth. A good fit of the data, obtained by an iterative computer technique, yielded the equation, $R_t = 0.379e^{-0.073t} + 0.552e^{-0.00029t}$.
2. Plot of ^{85}Sr retention data of a Yorkshire-Hampshire Cross injected at 2 months of age. A good fit of the data was obtained only to 142 days post-injection, at which time statistical variability increased because of the small amount of the retained radionuclide.
3. Plot of ^{85}Sr retention data of a Duroc pig injected slightly over 4 months of age. A fairly good fit of the data was derived by the computer up to 200 days post-injection.
4. Plot of ^{85}Sr retention data of a Duroc pig injected at 6 months of age. The computer fitted the data quite well with the two exponential function, $R_t = 0.283e^{-0.134t} + 0.659e^{-0.00274t}$.
5. Plot of ^{85}Sr retention data of a standard mixed-breed pig injected at 13 months of age. The data up to only 112 days post-injection could be fitted by a double exponential. A third exponential might be suggested to fit the remaining points, but the line had a slightly positive slope, which makes little sense in this experiment. The derived equation is $R_t = 0.635e^{-0.23t} + 0.380e^{-0.0031t}$.
6. Plot of ^{85}Sr retention data of a miniature pig injected at 1 month of age. The data are fitted quite well by a double-exponential equation, $R_t = 0.116e^{-0.203t} + 0.808e^{-0.00263t}$.

7. Plot of ^{85}Sr retention data of a miniature swine injected at 6 months of age. The computer program fit the data only to 126 days post-injection, at which time the values tailed off unexplainedly to yield an even more negative slope between 126 and 200 days. The equation best fitting the data is, $R_t = 0.245e^{-0.836} + 0.755e^{-0.0044t}$.
8. Comparison of the mean ($\pm$ SE) fractional ^{85}Sr retentions at 200 days post-injection of standard and miniature swine and of beagles administered the radionuclide at various ages. The dog data is replotted from Anderson and Comar (2). The different retention patterns as a function of age are apparently related to differences in growth, particularly skeletal growth.

Figure 1

Experiment 7

Pooled Data of 5 Poland Crosses,
3 males and 2 females

Injected at birth

FRACTIONAL RETENTION

Figure 2
Experiment 5
Yorkshire-Hampshire Cross No. 3, male
Injected at 2 months

Figure 3
Experiment 8
Duroc Pig No. 5, male
Injected at 4 months

Figure 4
Experiment 8
Duroc Pig No. 0, male
Injected at 6 months

Figure 5

Experiment 11B

Standard Mixed Breed, female

Injected at 13 months

Δ y-intercept of equations

Figure 6
Experiment 6
Miniature Pig RL, female
Injected at 1 month

FRACTIONAL RETENTION

Δ y-intercept of equations

DAYS POST-INJECTION

Figure 7

Experiment 11A

Miniature Pig M5, female

Injected at 6 months

Figure 8. Mean Fractional ^{85}Sr Retention ($\pm\text{SE}$) at 200 Days in Swine and Dogs as a Function of Age

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EFFECT OF TIME ON DISTRIBUTION OF INJECTED
RADIOSTRONTIUM IN THE SKELETON OF SIX-MONTH-OLD SWINE

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INTRODUCTION

A previous study (Anderson, 1968) showed that the distribution pattern of ^{85}Sr in the skeletons of two young growing pigs 25 and 50 days of age was not uniform. The largest percentages of ^{85}Sr per gram of calcium at 50 days of age were found in the tibia-fibula, the skull and mandible and the scapula, whereas radioactivity in the vertebrae was much less. When the per cent of radiostrontium retained following injection of $1\mu\text{Ci}$ at 24 days after birth was compared in the two pigs, the skull and mandible ranked first, followed by the long bones, and lastly by the vertebrae.

Blair et al (1963) found that retention of calcium in the skeleton as a whole was substantial in pigs from birth to 8 weeks of age. Bone growth, as a function of fresh wet weight, was determined in swine from birth to 28 weeks of age by McMeekan (1940, 1955) and Cuthbertson and Pomeroy (1962a, 1962b). Results from these studies pointed to increased rates of growth in the vertebral bones and less in the skull and mandible. The rate of growth of the long bones appeared to be scattered between those of the vertebrae and of the skull including

the mandible. Other studies (Anderson, 1968; Ragan et al., 1967) emphasized the accumulation of radiostrontium in the skull and mandible in swine dosed at an early age. Finally, Moulton (1923) demonstrated that the ash content of total body composition, though largely in bone, leveled off at a value of about 4% at 150-300 days of swine age, the time designated as chemical maturity. Somewhat similar values were found by Weniger and Funk (1954). Reproductive maturity also begins at about 150 days in swine.

To elucidate the relationship between bone growth as determined by stable calcium accretion and radiostrontium retention in individual bones of sexually mature swine, i.e., from ages six months to one year, this study was undertaken. In addition, data of other reports on bone growth from birth to six months of age were compared to growth beyond six months following a single intravenous injection of radiostrontium. A better understanding of the gross skeletal metabolism of radiostrontium as a function of age in swine, as a model for man, was the objective of this investigation.

METHODS

Each of 8 male Hampshire pigs was injected intravenously with 15 μCi of ^{85}Sr at 6 months of age. They were housed in a single outdoor pen with shelter and fed an identical ration (Ralston Purina Groweena, St. Louis) and offered water ad libitum. At 1, 10, 28, 60, 90, 120, 137, and 180 days after injection, a pig was killed. Thus, the last pig killed was one year of age. Selected bones (see Table 1) were cleaned and ashed in a muffle furnace at 400° C for 24 hours and then at 600° C for 48 hours. The ash was dissolved in a 1:1 $\text{HCl}:\text{H}_2\text{O}$ solution to final volumes varying from 100 to 2000 ml, depending upon the size of the bone. From these solutions, duplicate 15 ml aliquots were

removed for gamma spectrometric counting of ^{85}Sr and duplicate 1.0 ml samples were withdrawn for gravimetric determination of total calcium by oxalate precipitation. From the percent of the injected ^{85}Sr dose measured in each bone and the weight of stable calcium determined in each bone, the percent of injected dose of ^{85}Sr per gram of calcium (specific activity or SA; also called relative concentration or activity) was calculated. Standard computer regression programs were used for analysis of the specific activity data.

RESULTS

The percent injected dose of ^{85}Sr , quantity of calcium per bone, and calculated specific activity, including unweighted means, are presented in Tables 1, 2, and 3, respectively, for thirteen bones grouped according to type of formation and relative amounts of cortical and trabecular bone. Both Table 1 and Figure 1 show that there was a rapid loss of the radionuclide between days 1 and 10 post-injection and less so between days 10 and 28. It is noteworthy that, even with the variation and non-uniformity in the quantity of calcium and the percent of injected dose retained in each animal, the specific activity beginning on day 28 post-injection was relatively constant for a particular animal in all the bones examined (see Table 3 and Figure 1). This activity most likely represents ^{85}Sr that is buried in more slowly exchanging or remodeling bone.

In comparing means for pigs 2 and 8, the long tubular bones, i.e. Group II, had the largest percent increase in calcium per bone (8%) and the smallest percentage decrease of the originally injected dose of ^{85}Sr (24%) over the approximately six-month period (Table 4).

TABLE 4

Mean Percentage Changes in ^{85}Sr Retention, Calcium Accretion and Specific Activity in Three Groups of Bones of Swine Between Six Months and a Year of Age

Bone Group	Mean % Decrease in Retention of ^{85}Sr /Bone*	Mean % Increase in Calcium/Bone*	Mean % Decrease in SA/Bone*
I	44	60	69
II	24	87	59
III	50	75	71
All Bones	39	74	64

*Pig 8 (killed at 12 months of age) compared to Pig 2 (killed at 10 days beyond 6 months of age).

Individual bones of the largely trabecular vertebral group, i.e. Group III, exhibited remarkable homogeneity in the SA data, especially during the early times compared to the other two groups (Table 3). The highest mean SA during the early times post-injection was found in the bones of the head, i.e. Group I, (Table 3), which contain both cortical and trabecular tissue.

No statistically significant difference was found between any of the three group-means regressions of SA on time (Table 3 and Figure 1). Because only one animal was used per time, biological differences between animals contribute to the variations in the data of Tables 1, 2, and 3. The data of pig 1, killed 24 hours post-injection, were excluded from further analysis because time was probably too short for equilibration of the radionuclide. However, the semi-logarithmic plot of the SA versus time (Figure 1), which includes the data of pig 1, demonstrates a definite two exponential pattern.

Fitting a single exponential by a computer regression program to the SA data from day 10 to day 180 post-injection yielded the group mean equations plotted in Figure 1. The data obtained from the right fibula of pig 4 and from the mandible of pig 2 (Table 3) were excluded from the analysis on statistical grounds because of their extreme deviation from expected values. Because the standard deviations were sufficiently large, no statistically significant differences were found between the equations of any group by this analysis (Figure 1).

The time trend of SA of all the bones (pigs 1 to 8) was also approximated by a polynomial of the fourth degree. The three groups of bones did not differ in their respective values of the polynomial coefficients above the first degree. However, the linear coefficient, though not differing between Groups I and III, was clearly different ($p < 0.001$) for Group II, Group II values decreasing more slowly than those for Groups I and III.

Table 5 presents a comparison of data for ^{85}Sr retention, net calcium accretion and SA from a neonatally injected pig recalculated from Anderson(1968), and of similar data from the present study over a comparable 50-day time span, i.e., days 10 to 60 post-injection. The observed patterns of skeletal ^{85}Sr turnover of the differently aged swine was different. In the young pig, which grew quite rapidly, especially after the first two weeks, all bones of the skeleton laid down calcium at about the same rate for 50 days, whereas the smallest decrease in retained ^{85}Sr , and hence in SA, occurred in the bones of Group I. The very young pig had a net calcium accretion rate which was from about 10 to 20 times as great as in the six-month-old swine over a comparable time (Table 5). However, the rate of loss of ^{85}Sr by the neonate was only two or

three times greater than that from the same bones of the reproductively mature swine. The estimated mean decrease in SA of all the bones of the young pig was calculated to be only slightly greater than twice that of the older swine over a 50-day period of time (Table 5). Thus, gross skeletal turnover of the label in the young pig was greater than in the mature animals and no differences in the turnover between groups of any of the analyzed bones from young or mature swine are apparent regardless of type of formation. However, it should be emphasized that entire bones were assayed rather than trabecular or cortical parts whose rates of metabolism are known to differ. Furthermore, the mature animals certainly had not ceased growing since skeletal growth continues for at least 3 and 1/2 years in swine (Zietzschmann and Krolling, 1955).

DISCUSSION

In the present study values of SA of all bones were quite similar beginning with pig 3, killed at 28 days post-injection (Table 3 and Figure 1). Additionally, from this time on the progressive decrease in SA per bone group was remarkably constant. Similar observations were made for adult rabbits injected with ^{90}Sr by Kshirsagar et al. (1966), in which study the older animals had similar slopes to the older swine of this study (see Figure 1) but less steep slopes compared to curves of younger pigs (Anderson, 1968). Comparable SA data as a function of time are not available for swine continuously ingesting radiostrontium, but a report from Pacific Northwest Laboratory (Ragan et al., 1967) listed the highest retained ^{90}Sr to be in the bones of the head of mature swine killed at the end of a long-term continuous feeding study. In our acute study of swine the initial uptake was highest in the head bones.

Monoexponential regression analysis of the SA data of whole bones of six-month old swine between 10 and 180 days post-administration of ^{85}Sr revealed

qualitative (Table 3) but non-significant quantitative differences between mean values of bones, which were grouped generally according to type of formation (Figure 1). However, the long tubular bones of Group II retained more ^{85}Sr while during the same period their net mineralization of stable calcium was greater than either bones of the head of Group I or the vertebrae of Group III (Table 4). The SA data of day 1 post-injection were excluded from the analysis because the radioactive label undergoes considerable exchange within the first 24 hours, before reaching isotopic equilibrium, whereas probably by 10 and certainly by 28 days the ^{85}Sr that has not been eliminated almost entirely resides in deep slowly exchanging cortical bone which is also less susceptible to physiological remodeling and resorption compared to surface bone.

Nevertheless, the radioactivity data grossly suggest, at least, a two-component exponential model of the metabolism of ^{85}Sr in sexually mature but still growing swine, a rapid early removal over the first few days and a subsequent slow loss of the radionuclide with time. The initial uptake was by far the greatest in the bones of the head, which have much trabecular bone and which continue to turn over rapidly. Thus, even though the biological half-life of ^{85}Sr in the head bones, represented in the first exponential in the model, was somewhat similar to that of ^{85}Sr in the other bone groups, the high initial burden would represent a greater potential risk to those cells contiguous to the label. However, the long-term retained radioactive burden, represented by the second component of the model, would be highly similar in all bones because of its localization in the cortical tissue almost in its entirety. This fraction of the acute dose would have less likelihood of being deleterious

to the sparsely scattered cells in the mineralized matrix. Thus, young adult beagles have presented osteosarcomas after acute administration of ^{90}Sr (Mays et al., 1969), whereas uniformly labeled mature miniature swine and beagles have demonstrated the initial pathological changes to be in the marrow and blood rather than in bone tissue per se following the continuous ingestion of ^{90}Sr (Clarke et al., 1969; Bustad et al., 1969). In their review McClellan and Jones (1969) have suggested that the pathological effects of incorporated radiostrontium differ following a large, single dose compared to continuous ingestion of low levels because of the initially high radiation dose rate following a pulsed administration.

From our data in slowly growing sexually mature swine the bones of the head, particularly the mandible, would appear to be most susceptible to bone pathology because of the initial high uptake. If the two-component model obtained in swine following a single administration applies to man, the first component would likely contribute the greatest dose rate to the cells lining the marrow cavities of both the trabecular and cortical bone, as reported by Spiers (1969). It is of interest to note that although they appeared after myelolymphoproliferative disorders in the offspring of ^{90}Sr -fed swine, almost all the bone tumors were found in the skull and mandible (Clarke et al., 1969). Thus, it is possible that non-uniform skeletal uptake may lead to selective, rather than random, production and distribution of bone tumors following an acute dose even though subsequent retention of the radionuclide during the second component of the model would indicate rather uniform turnover rates throughout the skeleton.

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TABLE 1

Per cent of Injected Dose of ^{85}Sr in Selected Bones of Swine
at Several Times (Days) Post-Injection

		Pig Number							
		1	2	3	4	5	6	7	8
Days Post-Injection		1	10	28	60	90	120	137	180
Group I									
Skull		9.35	7.24	7.19	6.53	5.32	5.66	5.38	4.51
Mandible		<u>7.92</u>	<u>5.00*</u>	<u>3.00</u>	<u>3.16</u>	<u>3.02</u>	<u>2.54</u>	<u>2.57</u>	<u>2.35</u>
	Mean	<u>8.63</u>	<u>6.12</u>	<u>5.09</u>	<u>4.84</u>	<u>4.17</u>	<u>4.10</u>	<u>3.97</u>	<u>3.43</u>
Group II									
Right Scapula		1.86	1.20	1.22	0.91	1.18	1.01	1.04	0.89
Right Humerus		1.51	1.64	1.60	1.25	1.68	1.33	1.38	1.08
Right Radius-Ulna		2.11	1.25	1.10	0.94	1.24	1.10	1.02	1.02
Right Femur		1.95	1.86	1.76	1.25	1.17	1.42	1.50	1.43
Right Tibia		2.01	1.18	1.15	0.76	0.86	1.04	0.95	1.01
Right Fibula		0.22	0.21	0.16	0.11	0.13	0.11	0.13	0.16
7th Thoracic Rib		<u>0.39</u>	<u>0.31</u>	<u>0.32</u>	<u>0.22</u>	<u>0.26</u>	<u>0.17</u>	<u>0.18</u>	<u>0.19</u>
	Mean	<u>1.44</u>	<u>1.09</u>	<u>1.04</u>	<u>0.78</u>	<u>0.93</u>	<u>0.88</u>	<u>0.89</u>	<u>0.83</u>
Group III									
1st Cervical Vert.		0.81	0.50	0.43	0.31	0.41	0.36	0.34	0.26
2nd Cervical Vert.		0.59	0.40	0.32	0.17	0.30	0.40	0.20	0.14
7th Thoracic Vert.		0.63	0.28	0.30	0.20	0.23	0.17	0.16	0.17
4th Lumbar Vert.		<u>0.83</u>	<u>0.49</u>	<u>0.48</u>	<u>0.32</u>	<u>0.29</u>	<u>0.41</u>	<u>0.27</u>	<u>0.29</u>
	Mean	<u>0.72</u>	<u>0.42</u>	<u>0.38</u>	<u>0.25</u>	<u>0.31</u>	<u>0.33</u>	<u>0.24</u>	<u>0.21</u>

*Estimated Value

TABLE 2

Grams of Calcium Per Bone of Swine at Selected Times (Days)

Post-Injection

		Pig Number							
		1	2	3	4	5	6	7	8
Days Post-Injection		1	10	28	60	90	120	137	180
Group I									
Skull		81.26	113.20	126.20	119.10	130.30	154.20	193.70	166.70
Mandible		34.56	45.00	58.40	57.25	67.70	63.20	85.35	86.70
	Mean	57.91	79.10	92.30	88.17	99.00	108.70	139.52	126.70
Group II									
R. Scapula		18.13	15.58	22.63	18.84	27.95	28.73	33.43	33.10
R. Humerus		20.32	21.55	30.85	26.37	38.05	37.40	40.28	44.75
R. Radius-Ulna		14.78	18.33	22.78	21.37	29.15	29.15	32.80	34.98
R. Femur		13.00	28.83	32.55	27.83	27.85	40.38	41.58	46.53
R. Tibia		17.22	19.68	23.25	20.89	22.10	29.33	29.40	36.60
R. Fibula		2.65	2.98	3.33	6.04*	3.28	3.18	4.38	5.02
7th Thoracic Rib		3.35	4.73	5.18	5.09	6.59	4.86	7.10	7.40
	Mean	12.78	15.95	20.08	20.06	22.14	24.72	27.00	29.77
Group III									
1st Cervical Vert.		7.17	6.39	7.15	6.75	9.98	10.70	11.28	11.94
2nd Cervical Vert.		4.86	5.08	5.45	4.07	6.81	11.42	7.25	7.58
7th Thoracic Vert.		5.99	3.54	4.71	5.16	4.83	5.14	5.55	6.84
4th Lumbar Vert.		8.51	7.28	8.35	7.33	7.00	11.83	12.04	12.73
	Mean	6.63	5.57	6.41	5.83	7.15	9.77	9.03	9.77

Datum of R. Fibula excluded from calculated mean because of its unusually high value.

Caption

Figure 1.

Semilogarithmic plot of the specific activity (or relative concentration or activity), represented as percent of injected dose per gram of calcium, as a function of time in days of pigs killed after intravenous injection of strontium-85. Means of bone data of Groups I, II, and III and all bones (pooled) analyzed are shown for day 1 post-injection. Computer-derived monoexponential regression fits of specific activity of the three groups and all bones are given for days 10 to 180 post-injection.

The regression equations are as follows:

Group I	$y = 0.0672$	$e^{-0.00501 t}$
Group II	$y = 0.0677$	$e^{-0.00541 t}$
Group III	$y = 0.0768$	$e^{-0.00722 t}$
All Bones	$y = 0.0704$	$e^{-0.00590 t}$

Caption

Figure 1. Average OR body-diet values of six littermate dogs as a function of time after injection. The solid line represents values obtained while the animals were 3 months of age, whereas the dashed line represents the dogs at 5 months and ingesting 10% sodium alginate in their ration. If OR is assumed not to change over the two-month period, it can be said that the dietary alginate caused about a 40% reduction in ^{85}Sr absorption. ^{85}Sr and ^{48}Ca were administered together orally in a capsule and retained radioactivity of the animals was measured in a 2 pi total-body counter (Anderson and Comar, unpublished data).

WHOLE-BODY RETENTION OF SINGLE INJECTIONS OF ^{85}Sr IN SWINE AND DOGS AS A
FUNCTION OF AGE: A REVIEW

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Introduction. Comparative investigations of the metabolism of the poten-
tially deleterious radionuclides of strontium have been undertaken in ani-
mals in order to obtain information, unavailable directly in man, essen-
tial to a better understanding of the behavior and biological effects of
the nuclides in man. Many different experimental designs have been employed
but the emphasis of this review will be on studies of the total-body reten-
tion patterns of radiostrontium following single injections in dogs and
swine of various ages. A close examination of retention at times shortly
after birth and during the times at the attainment of sexual maturity will
be assessed because it is at these times in the skeletal development of
man that the ingestion of ^{90}Sr and ^{89}Sr would have its greatest potential
radiation effect.

Once the bone-seeking radionuclide enters the vascular compartment of
an animal, either following ingestion or intracorporeal administration of
a single dose, its metabolism is governed largely by skeletal dynamics
that are in turn age-related (Glad et al, 1960; Speckman and Norris, 1964;
Decker et al, 1964). Although renal function does change with age, the
magnitude of its change with respect to strontium reabsorption is much
smaller compared to that of skeletal uptake and turnover (Comar and Wasserman,
1964; Speckman and Norris, 1964; Decker et al, 1964). Thus, an analysis

of an animal's growth, in correlation with its strontium retention is essential for an assessment of the age-related potential for harm of the radionuclide. Too little attention has been accorded the variation of strontium metabolism with age.

A review of pertinent strontium retention data up to 200 days post-injection in dogs and swine, data on hydroxyproline excretion in swine and data on radial closure rate in swine leads to a major conclusion that there may be a need for a variety of animal models for subsequent extrapolation to man, largely because of differences in skeletal metabolism as a function of age.

Methods. Purebred beagle dogs from birth to 10 months of age were injected intramuscularly with 5 μCi ^{85}Sr and measured up to 200 days post-injection in a two-pi whole-body liquid scintillation counter (Anderson and Comar, 1968). The animals were reared on the dam, weaned and placed on dry feed. They remained healthy and were allowed unlimited exercise in large pens.

The swine employed, of several standard and miniature breeds, were injected intravenously with 0.25-0.5 μCi ^{85}Sr and measured up to 200 days post-injection in a four-pi total-body detector (Anderson and Crackel, unpublished). After weaning they were maintained throughout the experiment on a ration containing 16% crude protein.

^{85}Sr was used as a tracer for ^{90}Sr because of the relative simplicity of photon measurements. In each study whole-body fractional retention, R_t , at any time, t , was calculated according to the relationship first used by Decker et al (1964):

$$R_t = \frac{C_t (1-f_{wo}) S_o}{C_o (1-f_w) S_t}$$

where C_o = animal radioactively measured in counts per minute (CPM) at time of injection, $t=0$
 C_t = measured animal CPM at time t in days after injection
 S_o = measured CPM of standard dose at time of injection, $t=0$
 S_t = measured CPM of standard at time t in days after injection
 f_{wo} = fractional absorption of ^{85}Sr photons in animal of weight w_o in Kg at time of animal injection
 f_w = fractional absorption of ^{85}Sr photons in animal of weight w in Kg at any time after injection.

Additionally, in the study of standard swine, a correction for a contribution of the animal's naturally occurring ^{40}K to the measured activity was necessary. Statistical comparisons of retentions as a function of age were made by Duncan's multiple range test for the dogs and by Student's t -test for the swine.

The retention data for the dogs were fit quite well by a modified power function somewhat similar to that employed by Decker et al (1964). However, the swine data could not be fit at all well by a power function and it was found that two-exponential fits were much better. Iterative computer techniques were used for all curve-fitting.

In several litters of standard swine, urines were collected for hydroxyproline analysis (Firschein and Shill, 1966). In neonates, collection was made by a simple inexpensive metabolic unit (Anderson et al, 1967), whereas in older animals standard metabolic crates were used.

In two swine, one 2 months of age (20.7 Kg) and another 1 year (187 Kg), tetracycline (5-7 mg/Kg BW of oxytetracycline hydrochloride) was administered intraperitoneally. The animals were killed 14 days later and the radial closure rate of osteons in μ/day of bone sections removed from

the femur, tibia and rib was measured (Anderson and Simmons, unpublished data).

Results and Discussion. The retained fractional dose of ^{85}Sr as measured at 1 and 200 days post-injection was found to be almost identical in dogs injected at birth and two months of age, namely from about 0.90 at day 1 to almost 0.30 at day 200, whereas animals injected at 8 or 10 months retained from 0.62 to 0.69 at 1 day post-injection and 0.26 at 200 days. The five-months old dogs had only a 24-hr uptake of 0.75, but they retained 0.35 of the dose at 200 days, the highest burden of any of the age groups (Anderson and Comar, 1968). The log-log plots of these dog retention data are linear for about the first 50 days and then become curvilinear downwardly, but less so as the age at the time of injection increases.

In an independent study of beagles Decker et al (1964) reported very similar findings, except that they also injected additional dogs at about 3 years of age. The retention of these 3 year old dogs was linear on a log-log plot and the slope of this line, as determined by a best least-square regression fit of the data, was -0.41. The slopes of the other dogs injected between one month and a year declined from -0.85 to -0.34, whereas the slopes of the retention data of our dogs (Anderson and Comar, 1968) fitted by a similar regression program, yielded values ranging from -1.24 down to -0.32. The slope of the dogs injected at 5 months was -0.43. Thus, it can be seen that age has a significant effect on the retention pattern of ^{85}Sr during the first 200 days post-injection. However, beyond this time in the dog (Decker et al, 1964) the slope of the line of log-log retention plots does approach a constant value, -0.41, which indicates that physiological bone turnover and physicochemical exchange remain fairly constant.

The ^{85}Sr retentions in swine as a function of age were found to differ somewhat from those observed in dogs following a single injection (Anderson and Crackel, unpublished). In the standard breeds the 24-hour uptakes decrease as the age at the time of injection increases, a finding also noted in the beagles. However, the mean retentions at 200 days post-injection in pigs dosed from birth to 6 months of age varied between 0.21 and 0.27, somewhat lower than in the dogs, whereas the 1-day values were greater than in the canine species. Thus, in the standard swine, at least, bone turnover is considerably greater than in the dog over a similar period from birth to 6 months. This, of course, results from the almost linear rate of growth in standard swine beginning at 1-2 months and lasting to about 6-8 months of age, whereas the dog has a more nearly S-shaped growth curve with a spurt at about 5-6 months and then a gradual leveling off. Skeletal growth is nearly maximal at the age of puberty (Andersen and Floyd, 1963) and ceases almost completely by about a year in canine species because nearly all epiphyses close by then (Hare, 1961). However, in porcine animals growth continues well beyond sexual maturity as epiphyses do not seal off until an age of 3 years or so (Zietzschmann and Krolling, 1955). Thus, in swine, there is no final growth spurt following upon the onset of puberty as observed in dogs and man.

Compared to standard breeds, miniature swine have different retention patterns with age, and a different growth curve. Their growth curve is much less steep, or more flattened, than in standard swine and the mean 200-day retention of miniatures injected at 1 to 2 months of age ranged from 0.48

to 0.43, considerably greater than in standard animals and in dogs. The initial 1-day uptakes of the miniature breeds were similar to standard swine injected between birth and 6 months of age, ranging from about 0.80 to 0.95. At 4 and 6 months particularly, these initial retained values are somewhat greater than in the dog.

Table 1 summarizes the fractional ^{85}Sr retentions at 1 and 200 days post-injection of dogs and swine as a function of age at the time of administration of the radionuclide.

Retention (R_t) curves were best approximated in the dog by a modified power function of the form (Decker et al, 1964)

$$R_t = A(t+d)^b$$

where

- b = slope of the log-log transform
- d = constant having the unit of time in days
- A = constant independent of d .

However, in swine the curves could not be well fit by a power function, whereas they were adequately fit by a two-exponential function. It should be noted that a five-exponential function has been fit to the retention data of beagles singly injected with ^{90}Sr at the Utah Radiobiology Project (Dougherty, 1971). According to Marshall (1969) the power function model should adequately fit the retention data of all alkaline earths, particularly in the adult organism, because of the time-dependence of radial diffusion from canaliculi in bone.

In two standard-bred swine radial closure rates of osteons were measured by a tetracycline label and were found to be significantly different ($9 < 0.05$) for a given bone as a function of age, as illustrated in Table 2 (Anderson and Simmons, unpublished data).

Table 2
Radial Closure Rate of Osteons of Bone Sections Removed from
Femur, Tibia and Rib of Swine as a Function of Age
(Anderson and Simmons, unpublished data)

<u>Age</u>	<u>Bone</u>	<u>Number of Sections</u>	<u>Closure Rate μ/day</u>
2 months	Femur	51	2.56
	Tibia	50	2.21
	Rib	42	1.85
1 year	Femur	50	1.65
	Rib	49	1.28

These data serve to illustrate not only the reduction in closure rate with age but also the fact that in sexually mature swine the closure rate is still considerable.

Total urinary hydroxyproline excretion was measured in standard swine as a function of age in order to determine if a correlation exists between excretion of the imino acid and elimination of ⁸⁵Sr. Hydroxyproline output increased linearly from about 10 mg/24 hr shortly after birth to about 180 mg/24 hr by 5-6 months, at which time excretion leveled off. A broad range in excretion was observed. On a per Kg body-weight basis, excretion fell from a high of 5 in neonates to a minimum of 2.4 at about 3 months, rose to a second maximum of 3.3 between 4 and 6 months, and subsequently declined to 1.1 by about 18 months of age. These data (Anderson and Crackel, unpublished data) are presented in Table 3 and they demonstrate, in a qualitative way at least, that hydroxyproline elimination parallels the growth curve of standard breeds of swine from birth to six months of age, suggesting a direct relationship to bone collagen degradation requisite to growth remodelling. However, even at 1 1/2 years the animals are still putting out a large amount of hydroxyproline, although much reduced on a per Kg body-weight basis. The relatively high activity in the older animals is

also apparent in the ^{85}Sr metabolism of the older standard swine, a pattern not observed with beagles or the miniature swine. It has already been shown that these two indices of bone metabolism were in qualitative agreement when swine were stressed by exercise (Anderson et al, 1971).

In a study of simultaneous ^{85}Sr and ^{47}Ca retention by six immature littermate dogs the observed ratio between bone and diet ($\text{OR}_{\text{bone-diet}}$), a term designated by Comar et al (1956), was found to be decreased in dogs, ingesting 10% sodium alginate in their ration, up to 10 days following oral administration of the radionuclides (Anderson and Comar, unpublished data) (Figure 1). According to Della Rosa and Goldman (1961), OR values do not change, even though the retention of both radionuclides does decrease with age. Thus, it appears that the alginate had a true depressant action on the absorption of ^{85}Sr from the intestine (about 40%) and hence on the OR values of the immature dogs (5 months old) compared to the same animals at an earlier age (3months). These findings are in agreement with several reports of studies in a variety of species which will ingest such a high concentration of alginate.

Summary. The question of what all these data mean is raised. First, it has long been known that the retention of radiostrontium is age-dependent, but it has not always been obvious in reviews (e.g. Stara et al, 1971) that there may be significant differences with respect to age in the potential radiation dangers to man of this alkaline earth. Secondly, in data obtained from neonatal (Anderson, 1968) and from slowly growing sexually mature swine (Anderson et al, these proceedings) it would appear not only that the uptake of radiostrontium may be highly variable throughout the skeleton, being especially high in the bones of the head of the

mature swine, but also that the turnover of the retained label may be quite variable in the neonate and highly similar in the mature swine. Thirdly, it is re-emphasized on the basis of the data reviewed here that, because of differences in growth curves, no one animal model is adequate to represent the differential metabolism of the radionuclide as a function of age in man. Data of ^{85}Sr retention by dogs prior to and at the time of puberty appear to approximate quite well the human teenage period, whereas data from the more slowly growing immature breed of swine would appear to be as close to the neonatal and infant periods of man as possible of the models studied. However, it is recognized that neither of these models of possibly the most critical times for radiostrontium uptake and damage in the life of a human being is entirely satisfactory. Furthermore, it is recognized that pathological changes may represent a better end-point than retention data alone for comparative studies upon which extrapolation to man can be attempted. Nevertheless, full consideration of age-related aspects of skeletal metabolism is essential for better understanding of any model and hopefully of man.

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Table 1
Fractional ^{85}Sr Retention at 1 and 200 Days Post-Injection of
Beagles (Anderson and Comar, 1968) and Standard and Miniature Swine
(Anderson and Crackel, unpublished)

<u>Age at Injection</u>	<u>Number of Animals</u>	<u>Mean Day 1</u>	<u>Retention Day 200</u>	<u>Ratio: R200/R1</u>
BEAGLES				
Birth	9	0.90	0.29	0.32
2 Months	5	0.86	0.28	0.33
5 Months	4	0.75	0.35	0.47
8 Months	6	0.62	0.26	0.42
10 Months	4	0.69	0.26	0.38
STANDARD SWINE				
Birth	16	0.90	0.26	0.29
1 Month	4	0.89	0.22	0.25
2 Months	8	0.87	0.21	0.24
4 Months	6	0.86	0.27	0.31
6 Months	8	0.85	0.27	0.32
13 Months	1	0.74	0.45	0.61
MINIATURE SWINE				
1 Month	2	0.95	0.47	0.50
2 Months	1	0.85	0.42	0.41
4 Months	2	0.86	0.30	0.40
6 Months	8	0.80	0.28	0.35

Table 3
Urinary Hydroxyproline Excretion in Standard Swine as a
Function of Age
(Anderson and Crackel, unpublished)

<u>Age in Days During Collection (Mid-Time)</u>	<u>Number of Animals</u>	<u>Number of Samples</u>	<u>Hydroxyproline Excretion</u>		
			<u>mg/24 hr Mean</u>	<u>Range</u>	<u>mg/24 hr per Kg BW</u>
14-18 (16)	5	23	10.4	4.4-17.6	5.2
46-69 (58)	4	40	61.0	9.2-155.5	2.7
78-92 (85)	4	20	78.1	4.5-157.6	2.4
68-127 (98)	1	14	119.8	12.2-369.0	3.3
128-169 (148)	5	27	183.9	30.9-423.3	3.3
459-479	2	40	182.7	112.5-376.9	1.1

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