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MASTER

STRONTIUM-85 RETENTION IN SWINE AS A FUNCTION
OF AGE AND DIETARY CALCIUM AND PHOSPHORUS INTAKE^{1,2}

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ABSTRACT

Several litters of standard-size swine of different breeds were studied to determine the effects of age and dietary calcium and phosphorus intake at two different levels on their retention of administered Sr-85 up to 200 days post-injection (P.I.). Animals injected at birth and 6 months of age maximally retained Sr-85, 28%, as measured at day 200 P.I., whereas pigs injected any time between those ages retained less of the radionuclide. Retentions by swine on either a high Ca:P intake (1.2%:0.8%) or recommended intake (0.8%:0.6%) were not significantly different, although pigs on the higher intake did retain more Sr-85. However, this difference was removed when the groups on the two intakes were compared by dividing the amount of Sr-85 eliminated by net gain in weight over the 200-day experimental period. The retention data were fit better mathematically by a two-exponential function than by a modified power function. In absolute amounts swine injected near birth retained more Sr-85 than animals dosed at any other age. Comparison of retention behavior in swine and dogs as models for studying man is made.

Strontium-90 has been of considerable interest over the past two decades or so because of its relatively high yield upon fission, its long physical and biological half-life, and, thus, its potential radiation effects when ingested and metabolized in a similar way to calcium. Several species of mammals, including mice, rats, rabbits and dogs, have been employed in investigations designed to follow the metabolic pathways of radiostrontium, but swine have been used in such studies only relatively recently. The similarities between the pig and man, namely large body size, relatively long lifespan and similar gastrointestinal system, make the pig a good biological model for man. However, with respect to the anatomy of their skeleton, swine are less similar to man, because the long bones, in particular, continue to increase in length and width long after sexual maturity is attained at an age of about six months.

Swine grow, as measured by weight gain, at a rapid rate from birth to about 12 months and then growth levels off at about 24 months of age⁽¹⁾. Skeletal growth continues well beyond two years of age, as epiphyseal plates remain open⁽²⁾. The growth curve of pigs, like other mammals, follows a general sigmoidal pattern, but the birth to 12 months period is linear for all practical purposes⁽¹⁾.

During this rapid growth phase, it is of interest to know how much radiostrontium is retained as a consequence of the tremendous bone remodeling that occurs in young swine and to know at what specific age the radionuclide is maximally retained. To those ends, several different pig litters have been divided so that littermates were injected only one time with Sr-85 between birth and six months.

METHODS

Single injections of less than 1 μ Ci of Sr-85 were made intravenously, except for the newborn piglets which were injected intraperitoneally. Littermate pigs were injected in pairs whenever possible at the following ages: 2 to 3 days after birth, and one, two, four and six months. In the case of one litter all the piglets were injected shortly after birth, in order to test with greater numbers Sr-85 retention by newborns, which are considered by some investigators to be most susceptible to radiation damage. A total of 28 standard-sized swine were used in these experiments. The different swine breeds used are presented in Table I.

In addition, beginning at eight weeks of age about half of the pigs in each litter were fed a moderately high Ca and P ration containing 1.2% and 0.8% respectively, whereas the other half were given a ration consisting of lower amounts of Ca and P, 0.8% and 0.6%, as recommended by the NAS-NRC⁽³⁾. All other dietary constituents were held constant. The different rations with respect to Ca and P were used to test their relative effect on Sr-85 elimination, since McClellan and co-workers⁽⁴⁾ have shown that retention is reduced in swine continuously ingesting Sr-90 mixed in a high Ca and P ration. Pigs were fed the same high protein rations until the experiment was completed, which is not the customary practice in swine husbandry.

Due to insufficient housing facilities, the numbers of pigs injected at each age group in our study were small. A schedule of the litters is presented in Table I. Note the division of the litters according to injection age, the total number of pigs per age group, and a further breakdown of the swine according to high or low Ca intake.

Whole-body radioactivity in the swine was estimated by the Illinois Animal Science Counter, consisting of two large liquid scintillation tanks which allow nearly 4-pi detection geometry⁽⁵⁾. The tanks were housed in a steel vault made of 5-in. thick milled steel. Detection was at the Compton edge of the Sr-85 spectrum.

Fractional whole-body Sr-85 retention, R_t , was calculated according to the equation⁽⁶⁾:

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

Where C_o = measured pig count in CPM at time of injection, $\underline{t} = 0$.
 C_t = measured pig count in CPM at any time, $\underline{t}$, after injection.
 S_o = measured standard count in CPM at time of injection,
 $\underline{t} = 0$.
 S_t = measured standard count in CPM at any time, $\underline{t}$, after injection.
 f_{wo} = fractional absorption of Sr-85 gamma rays in the body of a pig of weight $\underline{wo}$ in kilograms at time of injection.
 f_{wt} = fractional absorption of Sr-85 gamma rays in the body of a pig of weight $\underline{wt}$ in kilograms at any time, $\underline{t}$, after injection.

In addition to the necessary correction for photon self-absorption as a function of the weight of each animal (Fig. 1), a second correction was required to eliminate the contribution of naturally occurring K-40 photons emitted by the swine in the Sr-85 energy channel (Fig. 2). Again this correction was made according to the weight of each animal. By about 200 days post-injection nearly all the radioactivity in the swine could be attributed to K-40.

Feed and water were offered all swine ad libitum and similarly aged animals were penned in small groups according to their designated diet. The health of the animals was good and their growth normal.

RESULTS

Table II shows the mean fractional retentions of all the swine at 200 days post-injection, according to age at injection and rate of gain of body weight. Typical semi-logarithmic plots of Sr-85 retention as a function of time post-injection in a litter of swine injected at various ages are presented in Figure 3. Note that:

- 1) retention in all pigs raised on either ration was about the same in all age groups except for those injected at two months;
- 2) no significant difference in retention existed between swine raised on the high or low Ca rations except at one month ($p < .05$), but in almost every age group retention was higher for swine ingesting the high Ca ration;
- 3) the rate of weight gain of the pigs was similar in each age group whether the pigs were ingesting a high or low Ca diet, but the rate of gain varied considerably between age groups; and
- 4) except for those injected at age one-month, pigs on the high Ca ration gained less over the 200-day period.

Another way of examining the same data of retention and net gain in weight is illustrated in Table III, in which the fractional quantity of Sr-85 lost from each pig, rather than the amount retained, was divided by the net weight gain in Kg. The values are almost identical for each age group whether the swine were on either a high or low Ca intake, with the exception of pigs injected at one month of age on the

high Ca ration, which had the highest rate of gain. These highly similar data indicate that the rate of removal of Sr-85 by normal processes is very closely related to the rate of gain, which in turn must be a fairly good index of bone remodeling activity in swine from birth to about one year of age.

Mean fractional retention $\pm$ 1 SD of five piglets injected at birth as a function of time post-injection is plotted in Figure 4. Note the large variability in the calculated values beginning at around 100 days, which was a common finding for all swine injected at any age. The data was fitted to a two exponential function by an iterative least-squares curve-fitting program (Los Alamos LA-2367). The fitted line is also shown in the figure. The two-exponential fit was better than the fit of either a power function, $R_t = At^{-b}$, or a modified power function, $R_t = A(t+d)^{-b}$, where b = slope of the log-log transform, d = time constant in days and A = constant independent of d , of Norris et al.⁽⁷⁾ to the data. The equation generated was $R_t = 0.3790 \exp - .0733t + 0.5519 \exp - .00330t$. Retentions of pigs injected at other ages have also been fitted by a two-exponential equation and the generated equations are given in Table IV. (These fractions do not add up to a total of 1 at $t = 0$ because of the variability in the data from around 100 days and on.) The half-lives of the two exponential phases are about 9.4 and 210 days.

The last table (Table V) lists the mean 24-hour post-injection retentions for all swine as a function of age at injection. Retentions are higher for piglets injected at birth or one month, but the values are not too different at any age.

DISCUSSION

A major finding of this study was that retention of a singly administered dose of Sr-85 as measured 200 days post-injection was quantitatively very similar in standard-sized swine of different breeds of a given age when injected at any time between birth and six months of age (Table II). Furthermore, these retention data were not significantly influenced by dietary calcium and phosphorus intake when the rations contained either 1.2% Ca and 0.8% P or 0.8% Ca and 0.6% P (Table II). However, swine fed the higher Ca and P ration (1.2% Ca: 0.8% P) than recommended by the National Academy of Sciences - National Research Council Panel⁽³⁾, in other words, 0.8% Ca and 0.6% P, did retain more Sr-85 as measured 200 days post-injection. Only in pigs ingesting the higher Ca and P diet and injected at one month was retention significantly greater ($p < .05$) than in swine on the lower Ca and P intake (Table II).

When net gain in weight was considered with respect to fractional removal of Sr-85 from the body (Table III), differences between age groups on either ration were eliminated. This finding indicates that the rate of gain in body weight was slightly better for swine on the lower intake of Ca and P which may be simply a reflection of qualitative differences, such as pelleting of the high Ca and P feed only, between the two rations. Differences as observed between age groups with respect to fractional Sr-85 loss divided by net gain can probably be attributed to differential growth rates, although small, as a function of age between birth and about one year, when swine injected at six months were last measured.

As previously demonstrated for dogs^(6,8), mean fractional Sr-85 retention at 24 hours post-injection declined as function of age at injection (Table V). After the period of injection from birth to one month, when the fraction of Sr-85 retained at one day approximated 0.9, retention leveled off at about 0.85. However, this strict ranking by age of retention 24 hours post-injection was eliminated in the subsequent 199 days when the readjusted ranking according to age and irregardless of Ca and P intake became: swine injected at birth, at 6 months, at 4 months, at 1 month and at 2 months (see Table V). These data demonstrate the significant differences between 200 day post-injection retentions of swine injected at different ages. A comparison of these data is plotted in Figure 5, in which, unlike dogs^(6,8), swine injected at birth or six months of age are clearly shown to retain Sr-85 maximally. Whether swine injected with Sr-85 at ages between six months and one year retain the radionuclide differently remains to be determined.

The pooled mean fractional Sr-85 retention data of swine of each breed at a given injection age were mathematically fit better by a two-exponential function than by a modified power function (Table IV). The reason for this observation is not readily apparent, but the substantial rate of gain and consequential bone remodeling of the swine between birth and one year may have an important bearing on the retention behavior of these rapidly growing animals. It should be noted that, unlike the fitting of beagle Sr-85 retention data^(6,8), the modified power function yields a "less good" fit to the experimentally derived retention findings. Such a difference between porcine and canine species can most probably be attributed to their patterns of growth.

A more ideal S-shaped growth curve than of pigs is characteristic of beagle dogs whose growth levels off between six months and a year in close relationship to the onset of puberty at about eight months of age⁽⁹⁾. The epiphyses of the long bones of the dog close within a few months after puberty^(10,11) most probably as a result of the surge in sex hormone secretion, whereas in swine they remain open to at least three years of age⁽²⁾, long after the onset of puberty around six to seven months of age⁽¹²⁾. Thus, long bones in swine have the potential to continue their growth in length about six times longer than the time elapsed from birth to puberty compared to dogs and human beings⁽¹³⁾, where the figure is only about three halves longer. From this perspective of skeletal growth, then, the dog may be a better model for the study of man than the pig. However, data presented in a companion paper on urinary hydroxyproline excretion by swine as a function of age indicate that swine serve as a good model for man⁽¹⁴⁾.

A comment is required on our finding that swine raised on a high intake of Ca and P retained more Sr-85 than comparably aged swine on a ration containing less Ca and P, a result which is contrary to that found by McClellan et al.⁽⁴⁾ in miniature swine continuously ingesting Sr-90. In our animals administered a total dose of 1 μ Ci or less of Sr-85 little chance existed for any bone damage due to incident radiation, whereas the radiation dose to the skeleton of the swine of McClellan and co-workers⁽⁴⁾ may have had an effect, though evidently not measurable. It would be assumed that the swine in our study on the higher Ca and P intake would have a reduction in bone resorption, aside from normal growth remodeling due specifically to less parathyroid hormone output resulting from the higher

gastrointestinal calcium absorption. Whether this assumption would also hold for the swine data of McClellan et al.⁽⁴⁾ is subject to the question of possible radiation effects to their animals. The fact that miniature swine were used by the Hanford group might help to explain the differences in findings.

As in a similar investigation with dogs⁽⁸⁾ on Sr-85 retention no effects of sex or of breed of swine were found in this study.

In conclusion, it can be stated that swine injected at birth retain more Sr-85 per Kg of body weight than swine injected at older ages up to six months, but in absolute terms they retain about the same fraction of the original dose as pigs injected at six months of age. The influence of dietary Ca and P at two different levels of intake on Sr-85 retention was not significant, but swine fed the high Ca and P diet did retain more of the radionuclide than did swine injected at the same age but ingesting the low Ca and P ration. Future experiments are planned to examine Sr-85 retention behavior in standard-size swine administered the dose between six months and a year of age and in miniature swine between birth and one year of age in order to determine with more certainty the age at which swine have the greatest retention of Sr-85. Extrapolation of the results obtained from swine to human beings will then be more feasible.

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TABLE I

Schedule of Swine Litters According to Injection
Age and Dietary Calcium and Phosphorus Intake

Number and Age at Injection					
<u>Breed</u>	<u>Birth</u>	<u>One</u>	<u>Two</u>	<u>Four</u>	<u>Six Months</u>
Mixed	2		3	3	4
Poland	5				
Duroc	—	<u>4</u>	—	<u>3</u>	<u>4</u>
Total	7	4	3	6	8
<u>Dietary Intake</u>					
High Ca and P	4	2	1	3	4
Low Ca and P	3	2	1	3	4

TABLE II

Mean 200-Day Post-Injection (P.I.) Fractional Retention of
Strontium-85 As a Function of Age and Dietary Calcium and
Phosphorus Intake and Mean Gain in Body Weight in Kilograms
Over the 200-Day Experimental Period

<u>Ration</u>	Mean 200-Day P.I. Retention $\pm$ S.D.				
	<u>Birth</u>	<u>One</u>	<u>Two</u>	<u>Four</u>	<u>Six Months</u>
High Ca and P	0.27 $\pm$.06	0.29 $\pm$.11	.17	.30 $\pm$.07	.32 $\pm$.06
Low Ca and P	0.28 $\pm$.08	0.14 $\pm$.09	.09 $\pm$.04	.22 $\pm$.11	.26 $\pm$.08

Mean 200-Day P.I. Gain (Kg)					
High Ca and P	75	150	98	122	94
Low Ca and P	81	137	105	128	111

No significance between age groups or between rations.

TABLE III

Mean Fraction of Strontium-85 Removed from Swine Divided
by Mean Gain in Body Weight in Kilograms Over the 200-day
Experimental Period as a Function of Age and Dietary
Calcium and Phosphorus Intake

<u>Ration</u>	<u>Birth</u>	<u>One</u>	<u>Two</u>	<u>Four</u>	<u>Six</u>	<u>Months</u>
High Ca and P	0.010	0.005	0.008	0.006	0.007	
Low Ca and P	0.009	0.007	0.009	0.006	0.007	

TABLE IV

Best-Fit Two-Exponential and Power Function Equations Generated by
Computer to Experimentally Derived Mean Fractional Strontium-85
Retention of Swine as a Function of Age

<u>Age at Injection</u>	<u>Two-Exponential Equations, $R_t = A \exp. -at + B \exp. -bt$:</u>		
	<u>Mixed Breed Swine</u>		
Birth	$R_t = .38 e^{-.024t} + .55 e^{-.0022t}$		
2 Months	$.34 e^{-.183t} + .59 e^{-.0056t}$		
4 Months	-- -- --		
6 Months	$.41 e^{-.024t} + .45 e^{-.0021t}$		
	<u>Poland Swine</u>		
Birth	$.38 e^{-.073t} + .55 e^{-.0033t}$		
	<u>Duroc Swine</u>		
1 Month	$.47 e^{-.047t} + .40 e^{-.0053t}$		
4 Months	$.30 e^{-.184t} + .67 e^{-.0034t}$		
6 Months	$.31 e^{-.234t} + .65 e^{-.0036t}$		
<u>Age at Injection</u>	<u>Modified Power Function, $R_t = A (t + d) \exp. -b$</u>		
	<u>Mixed Breed Swine</u>		
Birth	$R_t = 57.9 (t + 55.2) e^{-0.84}$		
2 Months	2.8	13.7	-0.45
4 Months	--	--	--
6 Months	17.6	46.7	-0.63
	<u>Poland Swine</u>		
Birth	2.0	8.4	-0.35
	<u>Duroc Swine</u>		
1 Month	744.3	96.8	-1.20
4 Months	6.1	36.4	-0.50
6 Months	1.5	5.1	-0.25

TABLE V

Mean 24-Hour Post-Injection Fractional Strontium-85 as
a Function of Age at Injection

<u>Age at Injection</u>	<u>Mean 24-Hour Fractional Retention</u>	<u>Mean 200-Day Fractional Retention</u>
Birth	0.91 $\pm$.05	[†] 0.28 $\pm$.06
1 month	0.90 $\pm$.07	0.17 $\pm$.16
2 months	0.86 $\pm$.04	[†] ,*0.12 $\pm$.06
4 months	0.84 $\pm$.09	0.26 $\pm$.10
6 months	0.85 $\pm$.07	*0.29 $\pm$.07

[†],* Means are significantly different at $P < .05$.

FIGURE LEGENDS

- Fig. 1 Plot of fw versus body weight in kilograms of swine.
- Fig. 2 Plot of potassium-40 (^{40}K) photon emission versus body weight in kilograms of swine.
- Fig. 3 Semi-logarithmic plots of ^{85}Sr retention as a function of time post-injection in a litter of mixed breed swine injected at various ages from birth to six months.
- Fig. 4 Plot of mean ^{85}Sr retention in a litter of swine injected at birth and the best "two-exponential function" fit of the data.
- Fig. 5 Comparison of the 200-day post-injection fractional retention of ^{85}Sr as a function of age at injection in months.

Fig. 2 ^{40}K Radioactivity As Measured In The Sr Channel of
"Cold" Swine As A Function of Weight in Kilograms

Fig. 4

Fractional
Retention
at 200
days P.I.

Fig. 5

0.5 -

○ High Ca & P Ration
□ Recommended Ca & P Ration

0.4 -

0.3 -

0.2 -

0.1 -

0.09 -

0.08 -

0

2

4

6

Age at Injection in Months