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C00-1339-16

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A Study of Fission Product Levels and
Metabolism in Food Producing Animals
as Determined by Whole Body Counting

Progress Report

August 16, 1966 - August 15, 1967

Submitted by the
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Contract No. AT(11-1)-1339

Supported by: Division of Biology and Medicine
United States Atomic Energy Commission
Washington, D. C. 20545

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TABLE OF CONTENTS

	Page
1. SUMMARY: SCOPE OF INVESTIGATION AND SIGNIFICANT RESULTS OBTAINED	1
Kinetic Studies of Calcium Metabolism in Goats	1
Retention of Strontium in Pigs as a Function of Age, Dietary Calcium and Phosphorus Levels, and Breed.	5
Cesium-137 Metabolism in Cattle, Sheep and Swine.	10
2. LEVEL OF ACTIVITY	10
3. TECHNICAL REPORTS PREPARED FOR PUBLICATION.	11
4. REPRINTS OR PREPRINTS OF PUBLICATIONS	11
5. OTHER ACTIVITIES.	11

1. SUMMARY: SCOPE OF INVESTIGATION AND SIGNIFICANT RESULTS OBTAINED

Investigations conducted during this past year fall into two major areas: (1) Kinetic studies of calcium metabolism in goats; and (2) retention of strontium in pigs as a function of age, dietary calcium and phosphorus levels, and breed. Significant results obtained from the two areas are summarized below. Our studies of cesium metabolism have been concluded.

Kinetic Studies of Calcium Metabolism in Goats

Our studies of the kinetics of the "exchangeable Ca pool" (E) in dairy goats have been continued and expanded. One year ago five animals were included in the study. This summer 11 goats are being injected at two- to four-week intervals with Ca-47. The additional animals are being used to investigate the effects on Ca kinetics of dietary Ca: P ratios, thyro-parathyroidectomy, and growth of the young animal. Six mature goats have been selected for the Ca: P ratio study. Control data were obtained on these animals during the current project year and are reported in the results of this portion of the report. Ca pool kinetics in three immature goats are being measured to study the effects of growth and development. Two mature goats were thyro-parathyroidectomized, one in July and another in August. Their Ca pool kinetics are now being measured and will be compared with control measurements on the same animals prior to surgery.

Methods: The basic procedure is the same as that described in last year's progress report (COO-1339-3). However, a fundamental change has been made in the procedure for calculating and interpreting the blood specific activity data. A year ago we were interpreting our specific activity-time curves on the basis of the theory proposed by Bauer et al.

(1) and Heaney and Whedon (2), among others, that monoexponential removal of radiocalcium from the blood occurs during a time interval between 24 hours and 100 to 200 hours post-injection. Physiologically, the monoexponential removal phase was theorized as representing removal of radiocalcium from E via accretion and excretion; data obtained during this phase could be used to calculate E, $T_{1/2}^1$, and bone accretion rate (A).

Our more recently accumulated observations have led us to conclude that, in goats, the monoexponential removal phase is difficult to demonstrate in a reproducible manner. Even though as many as 14 plasma samples were taken between 24 and 140 hours after Ca-47 injection, the problem of selecting the "proper" portion of the specific activity (SA) curve seemed to require more imagination than precise measurement. Consequently, we decided to use a specific portion of the SA curve, from 20 to 60 hours post-injection for calculating E and $T_{1/2}^1$. A regimented blood sampling schedule has been adopted so that samples are obtained at regularly spaced time intervals from 20 to 60 hours post-injection, experiment after experiment. Two samples are also taken at approximately one and 15 hours after injection for use in describing the early portion of the SA curve. By using this procedure, we eliminate the guesswork of choosing "by eye" which points on a graph should be included in pool size calculations as well as variations caused by different blood sampling schedules from one experiment to another. The exact physiological meaning of our data might be questioned, but certainly no more than if we tried to find monoexponential functions where they do not always exist. By waiting until 20 hours post-injection, we can assume that the rapid mixing phase is complete and that our data do represent some function of the exchangeable Ca pool and its turnover. By using the same experimental procedure repeatedly on the same

animal during differing physiological and nutritional conditions, meaningful data will be obtained.

Results: Our measurements of E and $T_{\frac{1}{2}}$ for three of the five goats that were on experiment during the past year are shown in Figures 1-3. Results from the other two were similar, and depended on their physiological status.

Goat 1 (Fig. 1) has never been pregnant or lactating during the two years she has been on experiment. Her exchangeable Ca pool was small ($3-4\frac{1}{2}$ Gm) and was quite constant during the entire year. Except for July and August, the turnover of her pool was also rather constant and comparatively slow ($26-32\frac{1}{2}$ hr. $T_{\frac{1}{2}}$). Results from goat 4 (not shown), also nonpregnant and nonlactating, were like those from goat 1. Results from goat 14 (Fig. 2), when compared with those of goat 1, show the dramatic effects of pregnancy and lactation on calcium kinetics. She had been lactating $2\frac{1}{2}$ months as of July 1. Her pool size (26 Gm) and $T_{\frac{1}{2}}$ (18 hr) were much different than those of goat 1, presumably reflecting the lactational demand for Ca as well as previous fetal requirements and possible hormonal influences. Her pool size gradually decreased throughout lactation and the onset of her next pregnancy, leveling off at about 7 to 9 Gm when milk production ceased. Pool size did not change at the time of parturition, but rose markedly (to $24\frac{1}{2}$ Gm) during the next two months. Pool half-life fluctuated less than pool size, ranging between 14 and 18 hours except at the time of parturition (12 hr) and after three months of her second lactation when pool size was decreasing. Results from goat 7 (not shown), which also has gone through two pregnancies, were like those from goat 14.

Results from goat 6 (Fig. 3) are included to show an apparent decrease in pool half-life during pregnancy, from 30-35 hours pre-gestation to

19 $\frac{1}{2}$ hours at parturition. Pool size was fairly constant at 5 Gm until a small increase to 7 $\frac{1}{2}$ Gm was observed at parturition. It is interesting, as in goat 14, that pool size remained small at the time of parturition and that its turnover was increased. The cause of increased pool turnover during pregnancy could be a profitable subject for future study. Fetal Ca requirements should not impose a significant demand on maternal Ca stores until the third trimester of pregnancy, and yet turnover appeared to increase (decreased T $\frac{1}{2}$) throughout pregnancy. Endocrine secretions may be involved.

Figure 4 shows preliminary results from three young goats that were put on experiment beginning January, 1967. Each goat was approximately nine months old at that time. A similar pattern can be seen in all three animals. From 9 to 11 months of age their pool sizes were similar to those of adult goats or somewhat higher (7 $\frac{1}{2}$ -12 $\frac{1}{2}$ Gm). These results raise additional interesting questions as to future changes that will be observed as new data are obtained and the possible causes (season, age, endocrine status) of such changes. It may also be desirable to measure Ca kinetics earlier in the growth cycle to obtain a more complete picture.

Analysis of the plasma calcium concentration of the two thyroparathyroidectomized goats indicates that only one had total removal of its glands. For this goat, the plasma calcium decreased from about 10 mg per 100 ml before to 6.7 mg per 100 ml at 5 days after surgical ablation. Insufficient data have been processed to tell us what effect parathyroidectomy has on pool size and turnover. The two goats have been receiving daily injections of l-thyroxin (0.08 mg) in an attempt to maintain the euthyroid state.

Bibliography:

1. Bauer, G. C. H., A. Carlsson and B. Lindquist (1961). Metabolism and homeostatic function of bone. In: Mineral Metabolism, Vol. I B, C. L. Comar and F. Bronner, eds., pp. 609-676. Academic Press, New York.
2. Heaney, R. P. and G. D. Whedon (1958). Radiocalcium studies of bone formation rate in human metabolic disease. J. Clin. Endocrinol. Metab. 18:1246.

Retention of Strontium in Pigs as a Function of Age, Dietary Calcium and Phosphorus Levels, and Breed

During the past year a study to determine the retention of strontium-85 in standard-sized pigs was undertaken. From two litters consisting of a total of fourteen pigs, individual pigs were singly injected with the radionuclide at one of the following ages: at two or three days after birth, 2 months, 4 months or 6 months. Roughly half of each litter was raised on a commercial diet containing about 0.8% Ca and 0.6% P., whereas the other half was fed a prepared ration having 1.2% Ca and 0.8% P.

Methods: Whole body measurements were made on the pigs at weekly intervals to 200 days or longer after injection by the Illinois Animal Science Counter. In addition, urine was collected during the year from individually caged pigs at periodic intervals and was analyzed for its total hydroxyproline content by the method of Firschein and Shill.

Results: The data on fractional Sr-85 retention have not yet been fitted for the best-straight line by a regression equation. In addition, two pigs have not yet been counted for 200 days after injection. A summary table of the significant data obtained follows.

SUMMARY TABLE OF RETENTION DATA

<u>Age at Injection</u>	<u>Pig Number</u>	<u>Sex¹</u>	<u>Diet²</u>	<u>Fractional Retention</u>		<u>Weight in Pounds</u>	
				<u>Day 1</u>	<u>Day 200</u>	<u>Day 1</u>	<u>Day 200</u>
Birth ³	1		P	0.966	--- ⁴	3	---
	2	F	P	1.012	.200	3	160
	L1	F	A	.945	.360	2	158
	L2		P	.905	--- ⁵	2	---
			Ave.	.960	.280		
2 Months	3	F	P	.866	.120	10	250
	L8	M	A	.900	.170	10	224
	L9	F	P	.814	.060	12	252
			Ave.	.860	.120		
4 Months	8	F	P	.760	.180	53	370
	L5	M	P	.810	.135	49	320
	L7	M	A	.800	.220	68	290
			Ave.	.790	.180		
6 Months	5	F	A	.812	.250	168	395
	6	F	P	.780	.210	139	430
	L3	F	A	.920	.320 ⁶	121	300 ⁶
	L6	F	P	.870	.190 ⁶	105	350 ⁶
			Ave.	.845	.230 ⁷		

(See next page for footnotes.)

SUMMARY TABLE OF RETENTION DATA CONTINUED

- Notes:
1. M = Castrate Male (Barrow). F = Female (Gilt)
 2. P = Purina Pig Groweena 2101; 0.8% Ca and 0.6% P; 1.5:1 ratio.
 3. Littermate pigs 1 and 2 injected at 3 days; littermate pigs L1 and L2 injected at 2 days. None suckled on mother after 2 days of age.
 4. Pig 1 died at age 50 days.
 5. Pig L2 died at age 24 days.
 6. Retention and weights of pigs L3 and L6 determined at age 180 days.
 7. Average retention of pigs 5 and 6 only.

From the fractional retention data four points are evident:

(1) average retention values on day 1 decrease with age from birth to four months and then increase at six months of age; (2) average retention as measured 200 days after injection is maximal in pigs injected at birth and next in those dosed at six months; (3) without exception pigs raised on the Anderson ration with higher total calcium and phosphorus intakes and a higher Ca:P ratio than of the Purina diet retained more radio-strontium at 200 days than similar or littermate pigs injected at the same time; and (4) total weight or net weight change from day 1 to day 200 appear to have little or no relationship to retention. A statistical analysis of the data has not yet been started.

The results of the analysis of urinary hydroxyproline levels, although incomplete at this time, are presented in Figure 5. Several inferences may be made from the scatter plot of the urinary hydroxyproline levels in pigs as a function of age: first, individual variation between littermates on the same day or within the same animal on successive days is considerable; secondly, a pattern of low excretion levels (about 1 to 2 mg/24 hours) exists from soon after birth to roughly 110 days and is followed by gradually increasing levels (100-150 mg) to about 180 days or about 6 months of age; and thirdly, excretion levels in mature pigs over 6 months of age stayed at roughly these values (100-150 mg), but with a downward trend becoming apparent.

It is difficult from the data presented above to demonstrate a direct relationship between bone mineral turnover, as measured by strontium retention, and organic osteoid turnover, as measured by urinary hydroxyproline. The pigs injected at birth, which retained about 28 percent of the dose 200 days later, excreted very little hydroxyproline during the comparable period.

Two alternative explanations seem plausible: first, nearly all the hydroxyproline measured after resorption is reutilized in bone remodeling; or, secondly, very little degradation of bone tissue, both mineral and osteoid, occurred during this period which would have to be characterized as being almost entirely anabolic. The retention data seem to belie the second alternative, for approximately three-fourths of the injected Sr-85 was excreted from the growing pig. Exactly what mechanisms are operative in the growth of these pigs from birth to near maturity is certainly not apparent.

The retention data of the pigs dosed at two and four months of age show less avidity of the skeleton for strontium than in the first group. The two-month group demonstrates the highest bone turnover rate of all the groups during the 200-day post-injection period, whereas the four- and six-month pigs have approximately the same rates as estimated by the differences between the day 1 and day 200 retentions. The hydroxyproline values tend to correlate with the mineral retention data, since at around 110 days of age (50 days after injection of two-month-old pigs) the rate of bone turnover must be increased. Four-month and six-month pigs were injected beyond this point of change in hydroxyproline excretion. Whether the collagen synthetic mechanism increases or whether the need to reutilize hydroxyproline decreases at this time is open to question. A graphical analysis of the retention data may shed light on these questions.

Like dogs injected with Sr-85 at five or six months of age, the pigs administered the dose at six months retained more radiostrontium than the other groups at 200 days with the exception of the pig group injected at birth. In the pig puberty comes at or somewhat before six months. This pig age would be comparable to about 13 years for a human being.

Thus, if the results of the pig study can be extrapolated to man, it appears that both infancy and the teenage periods are potentially the most susceptible to harm that might result from the intake of large amounts of strontium-90 from fallout.

Two series of several young pigs each were used to develop a satisfactory method for hypophysectomizing pigs with implanted capsules containing radioactivity. Radon needles were used in the first series. Whereas the needles were quite easily placed directly in the pituitary following entry through the lateral skull wall by means of a trephine, the gamma rays of radon-222 were not efficient in destroying the pituitary. It has a physical half-life of 3.8 days and emits a gamma ray of 0.51 Mev. Yttrium-90, activated from yttrium-89 and with a half-life of 64 hours and a beta particle of 2.26 Mev, was somewhat damaging to surrounding brain tissue. The optimum yttrium-90 dose levels required to inactivate the pituitary of young pigs without causing undue brain damage still must be determined, but the high cost of the activation of stable yttrium has reduced the number of our experiments.

Cesium-137 Metabolism in Cattle, Sheep, and Swine

All phases of this study have been completed. Our progress report on this work consists of a draft copy of a paper soon to be submitted to Health Physics (COO-1339-14).

2. LEVEL OF ACTIVITY

The research objectives for 1966-1967 submitted with our revised proposed budget have been followed closely. No significant differences between actual level of activity proposed and that completed in the contract exist.

3. TECHNICAL REPORTS PREPARED FOR PUBLICATION

- a. COO-1339-12. "An inexpensive metabolic unit for baby pigs from birth to four weeks," by J. J. B. Anderson, L. D. Baker and A. R. Twardock. Accepted for publication in Laboratory Animal Care.
- b. COO-1339-14. "Cesium-137 retention in cattle, sheep and swine," by A. R. Twardock and W. C. Crackel. To be submitted for publication in Health Physics.
- c. COO-1339-15. "Note: Stepwise regression," by T. G. Lohman, P. M. Ribisyl and H. W. Norton. To be submitted for publication in Biometrics.

4. REPRINTS OF PUBLICATION

COO-1339-13. "Distribution of radiostrontium in the skeleton of the young pig with age" (Abstract), by J. J. B. Anderson and L. D. Baker, American Zoologist 7:204, 1967.

5. OTHER ACTIVITIES

- a. A. R. Twardock: "Whole-body counters for large animals"
T. G. Lohman, A. R. Twardock, H. W. Norton and W. J. Coffman:
"Factors affecting K^{40} measurements in the whole-body and body components."
Oral presentations at "Public Conference on Body Composition,"
University of Missouri, Columbia, May 4-6, 1967.
- b. J. J. B. Anderson: Presentation of Seminar on "Radiostrontium Retention as a function of age" at the University of Vermont, College of Medicine, July 24, 1967.

FIGURE 1

GOAT #1 --- NONPREGNANT, NONLACTATING

FIGURE 2

FIGURE 3

GOAT #6

FIGURE 4

Figure 5. Urinary Hydroxyproline Excretion as a Function of Age
(Points represent average of 2 or more pigs on a given day.)