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A STUDY OF FISSION PRODUCT LEVELS AND
METABOLISM IN FOOD PRODUCING
ANIMALS AS DETERMINED BY WHOLE BODY COUNTING
SUMMARY PROGRESS REPORT

December 1, 1963 - August 15, 1966

Submitted by the
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and the
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RELEASED FOR ANNOUNCEMENT
IN NUCLEAR SCIENCE ABSTRACTS

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Introduction

The original proposal for this project stated 3 primary objectives: (1) calibrating the Illinois Animal Science Counter (ILLASCO) for K-40 and Cs-137 detection efficiency in food producing animals and human beings, and studying the effects of fission product radiocontaminants on K-40 measurements as well as procedures for minimizing such effects, (2) studying Cs-137 metabolism in cattle using the existing Cs-137 content of hay as the source of intake, and (3) establishing a fission product monitoring program. At the suggestion of the Biology Division, the monitoring phase of the program was omitted and metabolic studies were emphasized.

Subsequent renewal proposals included continuations of the calibration work as the need for such information in different species arose, and extended the studies of Cs-137 metabolism to include measurements of oral and intravenous dose retentions in cattle and of intravenous dose retentions in sheep and swine. As a result of the most recent proposal, an investigation of Ca-47 kinetics was begun in dairy goats, with the objectives of studying Ca pool sizes and turnover rates during pregnancy and lactation.

It is expected that calibration work will continue in conjunction with body composition studies. When current Cs-137 retention measurements in swine are complete, the cesium metabolism phase of the program will be terminated. Future investigations are proposed on Ca and Sr metabolism in a number of species, including goats, cattle, pigs and dogs.

For this report, work that is described in detail in the current progress report (which should be available to the reviewer)

is referred to and a summary description is provided.

Research Accomplishments

Body Composition Studies and Effects of Fission Product Radiocontamination

Thereon

Preliminary studies of K-40 measurement in cattle revealed that the presence of fission products on the animal's hair coat and in its digestive tract must be taken into consideration, as well as the presence of Cs-137 in its tissues. Procedures for minimizing the effects of radiocontaminants and for whole-body counting K-40 in cattle are described in two papers recently accepted for publication by the Journal of Animal Science, copies of which are appended to this report. Summaries from these papers follow:

Symposium on Atomic Energy in Animal Science
Part I. The Illinois Animal Science Center;
Performance Characteristics and Animal Radio-
activity Measurement Procedures.

A large-volume liquid scintillation detector capable of measuring radioactivities in human beings and animals as large as cattle is described. The performance characteristics of the instrument are described, including short- and long-term electronic stability, energy resolution, detection efficiency calibration, and the use of instrument standards. Methods are discussed for preparing animals for K-40 measurements so as to reduce interference by radiocontaminants, and for loading and positioning animals in the detector so as to minimize errors caused by positioning and subject movement.

Part II. Estimation of Carcass Lean Muscle
Mass in Steers by K-40 Measurement.

Results are presented from a study of 42 steers relating whole-body and carcass K-40 contents, as measured by a large-volume, liquid scintillation counter, to carcass lean muscle mass as measured by chemical analysis. Variations in the amount of radioactivity of the gastrointestinal contents, in the counting efficiency of the whole-body counter, and in the positioning of the steers with respect to the scintillation detector, were found to be the main sources of error affecting the precision of estimated whole-body potassium and the precision with which carcass lean muscle mass could be predicted.

With procedures developed to account for or minimize the above sources of variation, whole-body count was repeatable from day to day to within 2 or 3% in 21 steers and predicted carcass lean with standard errors of estimate less than 3% within two narrow weight ranges. Carcass K-40 measurements predicted carcass lean with standard errors of estimate somewhat greater than the whole-body count (3.5%).

Body potassium can be measured precisely enough to suggest practical utility of the K-40 method for determining carcass lean muscle mass in live steers and in steer carcasses.

This project has supported body composition research activities through the participation of the ILLASCO operator, whose salary is paid by the project, through detection efficiency calibrations, and through studies such as those described in the above papers. Additional calibrations have been carried out using Cs-132 in 15 cattle, and K-42 in 25 pigs, 17 sheep, and 25 human beings.

Cesium-137 Metabolism in Cattle, Sheep, and Swine

Retention measurements of intravenously injected Cs-137 in 4 pigs are currently in progress. When complete, these studies will be published in conjunction with previously completed work on Cs-137 metabolism which is summarized in the following material and in the current (1965-1966) progress report.

(1) Cs-137 Intake and Body Burden in Cattle.

Materials and Methods. Two different cuttings of hay were selected, one to be relatively high and one relatively low in fallout Cs-137 content. The hays were pelleted and fed to two groups of Guernsey cows for 68 days, at which time the rations were reversed between the two groups and feeding continued for an additional 77 days. The animals were placed in the ILLASCO whole-body counter and their Cs-137 content measured at regular intervals during the feeding period. Non-lactating,

non-pregnant animals, 2 to 5 years old, were requested at the time of purchase, and were examined for pregnancy by local veterinarians prior to purchase. Nevertheless, 3 of the 6 cows calved before the end of the experimental period, a remarkable record for a group of "chronic non-breeders." Two of the three would have been only 30 days pregnant at the time of examination, which explains why pregnancy determinations were not made.

Three cattle were initially fed alfalfa pellets made from a second cutting hay (July, 1964) which received no rainfall during harvest. This hay contained 14.8% crude protein, 0.7% K, and approximately 0.63 nanocuries of Cs-137 per kilogram. Three other cattle were initially fed timothy pellets made from first cutting hay (June, 1963) which received 2 rainfalls during harvest. This hay contained 9.2% crude protein, 1.0% K, and approximately 2.16 nanocuries of Cs-137 per kilogram. Cesium-137 concentrations in hay cut by Ward et al. (2) during the summer of 1963 at Fort Collins, Colorado, ranged from 0.53 to 6.48 nanocuries per kilogram dry matter. Each animal was fed a maintenance ration, determined on a body weight basis according to the protein content of the hay and recommendations of the National Research Council. Mineral salt supplements were provided.

A 5" x 4" NaI(Tl) crystal detector and 400-channel analyzer were used to analyze feedstuffs for fission product and K-40 content. Known volumes and weights of feeds were placed in cardboard containers and counted. Standards were prepared having weights and volumes the same as the feed samples by mixing known quantities of the radionuclides to be analyzed with those feeds. For example, pelleted hay was counted in 800-gm aliquots. To another 800-gm aliquot of the same hay, a known

amount of Cs-137 was added and mixed. The spectrum of added Cs-137 was obtained by subtracting the count of the "unspiked" sample from that of the "spiked" sample. The net Cs-137 constituted the "library" spectrum for the known amount of Cs-137 in hay pellets. This method was used to prepare library spectra of Cs-137, K-40, Zr-Nb-95, Ru-Rh-106, and Mn-54 in pelleted hay, ground feed, oats, and chopped hay.

100-channel library spectra were punched on 8-channel paper tape with a "Tally" punch. The data were then transferred from paper tape to IBM cards in preparation for spectrum stripping, which was carried out on an IBM 7094 using a program provided by P. N. Dean of the Los Alamos Scientific Laboratory.

Results. Two animals refused to eat the hay pellets even though starved for a number of days, and were therefore useless in terms of the primary experimental objectives. One of these animals did not come back on feed and died suddenly 34 days after the initiation of pellet feeding. Autopsy revealed no obvious reason for death. The other animal that refused pellets was fed oats and was whole-body counted. Daily rations of the remaining animals were entirely consumed, eliminating the need for weighing back. By feeding pelleted hay, complete consumption of stems as well as leaves was obtained, and accurate intake records were kept.

Figures 1 through 4 show the Cs-137 concentrations of four animals during the time that the low Cs-137 and high Cs-137 content hays were fed. Because it was not possible to maintain constant body weights in all animals, results are expressed in terms of nanocuries per 100 pounds body weight. The Cs-137 content of the ration that was fed prior to the first pellet feeding period was not much higher than that of the

low Cs-137 hay. Body burdens of animals given low Cs-137 hay (1 and 2) declined slowly during the entire 68 days with no evidence of approaching steady state. Single exponential fits of these data gave half-lives of 294 days (1) and 264 days (2). When the animals were changed to high Cs-137 content hay a rapid increase in body burden was evident.

Animals 3 and 4 were initially fed high Cs-137 content hay and then, after 68 days, changed to low Cs-137 content hay. For these animals, both the rising and the falling portions of the body burden versus time curves were used to estimate biological half-lives of Cs-137. Figure 5 shows the body burden of animal 5 before the start of pelleted hay feeding, the declining body burden during the time that the animal refused to eat, a brief rise when oats and hay were fed followed by a steady decline when a constant intake of oats was established. The latter period of decline was curve fitted and described mathematically.

It is evident that the body burdens of all animals approached a condition of steady state after 60 to 80 days of eating a single ration. For purposes of graphic analysis, steady state values were assumed to have been reached even though other evidence may show that body burdens were still slowly changing. By subtracting non-steady state values (A) from steady state values (A_{eq}) for data from animals having increasing body burdens, it was found that the rate of change of body burden could be described with reasonable accuracy in terms of the sum of two exponential functions. A similar process was applied to the data from animals having decreasing body burdens, subtracting A from A_{eq} . Figure 6 shows typical data of ($A_{eq}-A$) from which half-lives were obtained. Table 1 summarizes the data in terms of the half-lives of short- and long-term components for all animals. The average half-lives were 2.2 days for

the short-term and 10.9 days for the long-term components.

The relationship between daily Cs-137 intake in pelleted hay and Cs-137 body burden is shown in table 2. The animals' body burdens as percentages of daily intake were quite different on the two rations, averaging 152% for timothy pellets and 454% for alfalfa pellets. Reasons for the differences are not readily apparent. The gradual decline in body burdens observed in animals initially fed low Cs-137 intakes might indicate that a much longer time is required for equilibration between intake and body burden than was employed in this experiment. Single-dose Cs-137 retention studies do not reveal the existence of a large compartment with the very long half-life needed to support this explanation, however. An alternative possibility would be a difference in the biological availability of Cs-137 in the two rations. Stewart *et al.* (1) reported greater Cs-137 secretion into milk by cows fed high-grain rations than in cows fed high-roughage rations. The crude fiber content of the ration was implicated as a determining factor in availability. We may have observed a similar effect; to obtain similar energy intakes, it was necessary to feed 1 1/2 times as much timothy as alfalfa pellets.

(2) Retention of Orally and Intravenously Administered Cs-137 in Cattle.

Procedures and results for these experiments are detailed in the current progress report (pp. 16-19). In summary: 3 cows and 2 calves were first given Cs-137 orally and whole-body counted for 60 days, and were then given Cs-137 intravenously and whole-body counted for an additional 60 days. The retention data were mathematically described as 3-component sums of exponentials having half-lives (in cows after oral dosing) of 1.3 days, 4.6 days, and 32 days, accounting for 0.21, 0.33,

and 0.43 of the administered dose, respectively. Calves excreted Cs-137 more rapidly than cows, primarily because greater fractions of the dose were lost by fast components 1 and 2. In cows, intravenous dosing appeared to accentuate the amount lost through the first component at the expense of the second, the size of the third remaining relatively constant. Pregnancy increased both the $T_{1/2}$ of compartment 3 and the fraction of the dose excreted through it. Biological half-times (B_T $1/2$; time required for excretion of half the dose) averaged 7 days for cows, 4.6 days for calves, and 16.5 days for 1 pregnant cow.

It is apparent that inconsistencies exist between the results of feeding fallout Cs-137 in hay and administering Cs-137 solutions either intravenously or by capsule. Retention patterns differed, depending on the form in which the radionuclide was given. In the case of hay feeding, a 2-component system with half-lives of 2 days and 11 days described most of the observed change in body burden, with some suggestion of a third component having a very long half-life. No evidence of the 4.6-day and 32-day components seen in single-dose experiments was present in the hay feeding results. No explanation for these observations is known to us at the present time.

(3) Retention of Intravenously Administered Cs-137 in Sheep.

Whole-body counting of 4 sheep for 60 days following intravenous dosing has recently been completed. A preliminary analysis and discussion of the data are contained in the current progress report (pp. 19-20). Again, 3-component exponential equations sufficed to describe the data. Half-lives were 1.2 days, 5.0 days, and 27 days, accounting for fractional excretions of 0.28, 0.33, and 0.39 of the dose, respectively. Excretion patterns in the cow and sheep were quite similar.

Calcium-47 Kinetics in Dairy Goats

For a complete discussion of methods and results, see current progress report (pp. 21-25). In summary: Methods for studying Ca-47 kinetics in dairy goats were investigated. From November 15, 1965, to August 15, 1966, fifteen intravenous injections of Ca-47 were made in each of 5 animals at 2- to 4-week intervals. Blood plasma specific activity-time curves and whole-body retentions were obtained, and exchangeable Ca pool sizes (E) and pool half-lives were calculated for various stages of pregnancy and lactation. Accretion rates remain to be calculated. Before pregnancy and during early pregnancy, E was rather constant for a given animal and ranged from 3 gm Ca to 14 gm Ca in 5 goats. E appeared to increase in one individual and to decrease in another at parturition. Improved experimental technique should provide more reliable data in future experiments at the time of parturition, when plasma Ca-47 concentrations drop very rapidly. Pool half-lives varied from 20 to 32 hours during early pregnancy, but dropped as low as 11 hours during late pregnancy and the early stages of lactation.

Present and Future Objectives

It is proposed that the research conducted under this project be directed to the field of calcium and strontium metabolism, with particular emphasis on retention and kinetic studies. Two closely related areas will be explored: (1) factors influencing strontium retention and excretion by mammals, and (2) factors influencing the kinetics of mobile Ca pools, with special reference to pregnancy and lactation.

The proposed study of strontium retention and excretion cannot be discussed in consideration of past results because it is an entirely new phase of the project. The scientific background, research objectives,

general plan of research, and present knowledge and comparable work in progress elsewhere which relate to this part of the project are described extensively in the renewal proposal accompanying this report. Briefly, the research objectives are:

- (1) to determine retention patterns of Sr-85 in the pig as a function of age of tracer administration, dietary Ca and P levels, and breed;
- (2) to measure urinary excretion patterns of hydroxyproline in the pig as a function of age, dietary Ca and P levels, and breed;
- (3) to relate radiostrontium and hydroxyproline excretion in the pig to comparable metabolic patterns in man;
- (4) to examine the effects of hypophysectomy, thyroidectomy, and parathyroidectomy (including hormone replacement therapy when possible) on radiostrontium retention as a function of age in the dog and pig; and
- (5) to investigate the influence of specific hormones on the age-related incorporation of radiostrontium into bone crystal in the pig and dog and to relate these findings to the human species.

Objectives 1, 2, and 3 might be half completed during the first year, with objectives 4 and 5 to be initiated during that time.

The past 9 months of research on Ca kinetics in goats have provided sufficient information and experience to support the feasibility of the proposed research and to suggest that important knowledge of mineral metabolism in milk-producing domestic animals can be obtained by the kinetic approach. Our opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine, and needed future investigations, is given in the renewal proposal accompanying this report (pp. 2-15). The objectives of this phase of the project are:

(1) to evaluate experimental procedures and mathematical approaches for studying Ca and Sr kinetics in domestic animals.

(2) to determine "normal" parameters of "exchangeable Ca" pool size and turnover rates in goats.

(3) to study the effects of various physiological and nutritional factors on pool size and turnover rates in goats, including:

(a) Ca content of diet

(b) Ca:P ratio of diet

(c) carbohydrate content of diet

(d) lactation

(e) pregnancy

(f) thyroid and parathyroid status

(4) to study other species, especially dairy cattle, when preliminary results warrant.

Experiments designed to provide information leading to objectives 1, 2, 3-d, and 3-e have been in progress for 9 months and will be continued. During the coming year, research bearing on objectives 3-a and 3-b will be initiated. Objectives 3-f and 4 would be pursued in the future.

Graduate Students

A number of graduate students have participated on a part-time basis in research conducted under this project. All have been half-time assistants paid by sources other than this project.

1. T. G. Lohman, Assistant in Animal Science. 10% of full-time to this project since December 1, 1963. Now in the final stages of preparing his Ph.D. thesis and should receive his degree in the fall, 1966. It is anticipated that he will become a half-time staff participant on this project. His special interests are in whole-body counting and body composition. Major chairman: H. W. Norton.

2. S. D. Farlin, Assistant in Animal Science. 10% of full-time to this project during 1965. Special interest was body composition in calves during growth. A new project has been selected because of excessive time requirements by the originally outlined study. Major chairman: U. S. Garrigus.
3. Y.-H. Kuo, Research Assistant in Veterinary Physiology and Pharmacology. 25% of full-time to this project during the past year. Completed M.S. in the fall, 1965; is now a Ph.D. candidate. He participated in kinetic studies of Ca metabolism in dairy goats during the past school year. He intends to write his Ph.D. thesis in this area of research. He will work full-time for a year beginning September 1, 1966, half of his time to be spent on this project. September 1, 1967, he plans to become a half-time graduate assistant again. Major chairman: A. R. Twardock.
4. R. H. Ball, Assistant in Animal Science. 10% of full-time to this project during the past school year. Special interest is body composition in swine during growth. Performed several K-42 calibrations in swine of various weights. Major chairman: B. C. Breidenstein.
5. P. M. Ribisl, Instructor in Physical Education for Men. Although not listed in our previous proposal, has devoted at least 10% of full-time to this project while organizing and carrying out K-42 calibration studies in men. Special interest is body composition in human beings and physical fitness. Major chairman: T. K. Cureton.

It should be mentioned that several veterinary medical students and undergraduates in animal science have been employed with funds from this project. One has entered graduate school and another is preparing to apply for an N.I.H. post-doctoral (post-D.V.M.) fellowship.

Publications Associated with This Project

1. Published In The Open Literature:
 - (a) Twardock, A. R., T. G. Lohman, B. C. Breidenstein and G. S. Smith. 1965. Whole-body counters: applications and potential in animal science and veterinary medical research. Ill. Vet. 8:3-6.
 - (b) Smith, G. S., T. G. Lohman, A. R. Twardock and B. C. Breidenstein. 1965. An animal's radioactivity is a measure of meatiness. Ill. Res. 7:6-7.

- (c) Lohman, T. G., O. B. Ross, B. C. Breidenstein, A. R. Twardock, A. L. Neumann and G. S. Smith. 1964. Estimation of fat-free lean tissue in live cattle, and carcasses by measurement of K-40. (abstract) J. Animal Sci. 23:862.
- (d) Ross, O. B., A. R. Twardock, T. G. Lohman, B. C. Breidenstein and G. S. Smith. 1964. Whole-body gamma-ray counting of cattle and sheep by the Illinois Animal Science Counter ("Illasco"). (abstract) J. Animal Sci. 23:863.
- (e) Breidenstein, B. C., T. G. Lohman, E. E. Hatfield, G. S. Smith and H. W. Norton. 1965. Comparison of K-40 measurement with other methods for determining lean muscle mass in sheep. (abstract) J. Animal Sci. 24:860.
- (f) Breidenstein, B. C., T. G. Lohman, G. S. Smith and H. W. Norton. 1965. K-40 and other indices of carcass lean muscle mass in pigs. (abstract) J. Animal Sci. 24:860.

2. Accepted For Publication In The Open Literature:

- (a) Twardock, A. R., T. G. Lohman, G. S. Smith and B. C. Breidenstein. Symposium on Atomic Energy in Animal Science. Part I. The Illinois Animal Science Counter: performance characteristics and animal radioactivity measurement procedures. J. Animal Sci.: accepted for publication.
- (b) Lohman, T. G., B. C. Breidenstein, A. R. Twardock and H. W. Norton. Symposium on Atomic Energy in Animal Science. Part II. Estimation of carcass lean muscle mass in steers by K-40 measurement. J. Animal Sci.: accepted for publication.

3. Monographs Prepared For Publicity Purposes:

- (a) Whole-body counters: potential and limitations in animal science research. Also abstracted as a text which was presented orally at the "Symposium On The Uses of Atomic Energy in Animal Science," August 13, 1964; Knoxville, Tennessee.
- (b) Illinois Animal Science Counter; ILLASCO; Whole-body radiation detector. A 4-page descriptive brochure given to visitors, and used to illustrate oral presentations.

Talks and Seminars

A. R. Twardock

- (a) Biophysics and Physiology Seminar (University of Illinois). Subject: Whole-body counters and their applications.
- (b) Nuclear Engineering Seminar (University of Illinois). Subject: Properties and uses of large-volume liquid scintillation detectors.

- (c) Radio program: "University Reports," WILL, Champaign-Urbana, Illinois. Subject: Measuring radioactivity in people and animals. (5 minutes).
- (d) Radio program for WIND, Chicago, Illinois. Subject: Radioactivity in animal products--fiction and facts. (10 minutes).
- (e) Brief presentation at 45th Annual Illinois Conference and Extension Short Course for Veterinarians. Subject: Whole-body counters and their applications. (10 minutes).

T. G. Lohman

Oral presentations of material published in abstract form, listed under publications (1,a) and (1,c), at the 56th and 57th Annual Meetings of the American Society of Animal Science (1964 and 1965).

B. C. Breidenstein

Oral presentation of material published in abstract form, listed under publication (1,d), at the 57th Annual Meeting of the American Society of Animal Science (1965).

G. S. Smith

- (a) Oral presentations of material published in abstract form, listed under publication (1,b), at the 56th Annual Meeting of the American Society of Animal Science (1964).
- (b) Whole-body counters: potential and limitations in animal science research. Paper presented at the Symposium on Uses of Atomic Energy in Animal Science, Knoxville, Tennessee (1964).

Present Division of Federal Research Support

A. R. Twardock

<u>Source</u>	<u>Amount For Current Year Including Indirect Costs</u>	<u>Percent of Full Time To Project</u>
This project		
A.E.C. AT(11-1)1339	\$ 34,005	30
N.I.H. HD-00727 (also under review for renewal)	26,784	30

J. J. B. Anderson

None other than this project. 50% of full time on project since June 15, 1966.

Bibliography

1. Stewart, H. F., G. M. Ward and J. E. Johnson. Availability of fallout Cs-137 to dairy cattle from different types of feeds. J. Dairy Sci. 48:709-713 (1965).
2. Ward, G. M., H. F. Stewart and J. E. Johnson. Effects of feeding practices on Cs-137 levels of milk. J. Dairy Sci. 48:38-43 (1965).

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Table 1

Biological Half-Lives of Cesium-137 in Cattle

<u>1. Increasing Body Burden</u>		<u>Half-Life (days)</u>	
<u>Cow</u>	<u>Short-Term Component</u>	<u>Long-Term Component</u>	
3	1.4	9.2	
1	2.6	12.3	
2	1.7	12.2	
4	-	9.2	
 <u>2. Decreasing Body Burden</u>			
5	-	11.7	
3	2.9	13.1	
4	-	8.8	
 <hr/>		<hr/>	
Average	2.2	10.9	

Table 2. Cs-137 Body Burdens in Cattle in Relation to Daily Intake.

Cow	Timothy Pellets			Alfalfa Pellets		
	Daily Intake (nc)	Body Burden at Steady State (nc/100 lb)	Body Burden as Percent Daily Intake	Daily Intake (nc)	Body Burden at Steady State (nc/100 lb)	Body Burden as Percent Daily Intake
1	11.72	22.2	190	1.94	9.7	500
2	15.32	18.5	121	2.79	8.5	304
3	11.49	18.0	156	2.24	10.5	470
4	<u>14.15</u>	<u>20.2</u>	<u>142</u>	<u>2.77</u>	<u>15.0</u>	<u>540</u>
average	13.17	19.7	152	2.44	10.9	454

Figure 1/ 1

Cow 1 Cs-137 Body Burden During
Consumption of Two Hays Containing
Different Cs-137 Concentrations

Figure 7 5

Cow 5 body Burden During Periods
Off Feed, Eating Hay and Grain,
and Eating Oats Only

Figure 6

Case 2 - Graphic Analysis of Data