February 20, 1970

To the Graduate Council:

I am submitting herewith a dissertation written by Charles Edwin Baker, entitled "Relationship of Cesium-137 and Iron-59 Elimination Rates to Metabolic Rates of Small Rodents." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Zoology.

James T. Tanner
Major Professor

We have read this dissertation
and recommend its acceptance:

J. C. Howell

Irden Eftmt

J. M. Giles

C. B. Hunaway

Stanley Hoover

Accepted for the Council:

Hatton A. Smith
Vice Chancellor for
Graduate Studies and Research

RELATIONSHIP OF CESIUM-137 AND IRON-59 ELIMINATION RATES TO METABOLIC RATES OF SMALL RODENTS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Charles Edwin Baker

March 1970

ACKNOWLEDGMENTS

This research was performed at Oak Ridge National Laboratory and was sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation. The program was carried out in the Radiation Ecology Section, Health Physics Division, while the author was under appointment as an Oak Ridge Graduate Fellow from the Oak Ridge Associated Universities. Thanks are extended to Drs. S. I. Auerbach (ORNL) and Louis A. Rayburn (ORAU) for administrative assistance and guidance.

Gratitude is expressed to Paul B. Dunaway, my research advisor at ORNL, for directing the program in a cordial and effective manner. James T. Tanner, my major professor, is also thanked for his helpful advice and guidance throughout my graduate career at the University of Tennessee, and for his criticisms of the manuscript. Other members of my committee, J. C. Howell, J. N. Liles, and G. E. Hunt, are acknowledged for their assistance in the final preparation of this paper.

Several members of the Ecology Section provided valuable help during the study, and I regret that all of these individuals and their contributions cannot be listed here. However, I would like to thank specifically the members of the Mammalogy Group, J. D. Story, J. T. Kitchings, J. B. Mathies, and especially L. E. Tucker, who gave much assistance and many helpful suggestions.

F. M. Rau and C. E. Ryan, Instrumentation and Controls Division, are thanked and commended for their excellent efforts toward the design and construction of auxiliary units for the carbon dioxide measuring

apparatus. Also, I thank M. B. Nathanson of Beckman Instruments Company for advice on the selection of proper equipment for making measurements necessary for this study.

A special acknowledgment is given my wife, Frankie, and my daughter, Dana, for enduring patiently the numerous hardships imposed on them during my graduate program. My wife also is thanked for typing all drafts and final copy of this paper, which she did cheerfully and cooperatively in the face of close deadlines and personal inconvenience.

Finally, I feel it is appropriate here to express my sincere gratitute to H. T. Shacklette, a friend and former professor, for being a constant source of advice and encouragement through the years. Through his words and example, Dr. Shacklette provided the incentive for me to enter graduate school and carry my program to completion.

ABSTRACT

Elimination of ^{137}Cs and ^{59}Fe by wild small rodents was measured in the laboratory and field to determine whether excretion rates of these nuclides are influenced directly by general metabolic rates. The object of this research was to establish a technique for measuring metabolism in the field for ultimate application in energy flow studies. A double-tagging method was employed, isotopes were administered intraperitoneally, and attention was focused on final-component elimination of each isotope. Cotton rats (Sigmodon hispidus), harvest mice (Reithrodontomys humulis), and white-footed mice (Peromyscus leucopus) were used in laboratory investigations, and cotton rats and white-footed mice were employed in field experiments.

Isotope elimination studies in the field were conducted in winter and spring, assuming that metabolic rates of rodents would be higher in winter due to lower temperatures. Also, metabolic rates in the field were expected to be higher than laboratory rates due to more physical activity of animals in nature. Average daily metabolic rates in the laboratory were determined by measuring carbon dioxide production with an infrared analyzer, and were examined in conjunction with ^{137}Cs and ^{59}Fe excretion rates. Animals were maintained under different experimental conditions, which were designed to create metabolic rate alterations.

Both species in field studies eliminated ^{59}Fe more rapidly than laboratory animals, but only cotton rats excreted the isotope faster

in winter than in spring. No differences between ^{137}Cs seasonal excretion rates of either species were found, and field values did not differ from those obtained in the laboratory. There was, however, a striking difference between species in rate of ^{137}Cs turnover, with Peromyscus excreting the isotope about twice as fast as Sigmodon.

Long-component biological half-times (T_b) of ^{59}Fe for Sigmodon were 108 days in winter, 144 days in spring, and 176 to 242 days in the laboratory; for Peromyscus, these values were 50 days in winter, 47 days in spring, and 289 days in the laboratory. Biological half-times of ^{59}Fe in Reithrodontomys ranged from 121 to 178 days at different laboratory treatment levels.

The T_b values for long-component ^{137}Cs elimination for Sigmodon were 7.5 days in winter, 7.9 days in spring, and 7.7 to 8.6 days at different treatment levels in the laboratory; in Peromyscus, values were 3.4 days in winter, 3.6 days in spring, and 3.5 days in the laboratory. Biological half-times of ^{137}Cs in laboratory Reithrodontomys ranged from 3.5 to 3.9 days.

Daily average CO_2 production rates (cc/hr/g; 29°C, 745 mmHg) for cotton rats ranged from 1.55 to 2.62; for harvest mice, 4.53 to 7.67; and averaged 3.73 for white-footed mice. Mean hourly CO_2 production rates were plotted to obtain daily metabolic cycles for individuals. These plots clearly indicated nocturnality in all three species, with Reithrodontomys being the most nocturnal. Regular peaks of increased CO_2 production were noted, and it was assumed that these peaks were concurrent with periods of accelerated physical activity.

Rate of long-component ^{137}Cs loss from rodents was not affected by metabolic rate changes at any treatment level; but, as in the field experiment, interspecific differences were noted in ^{137}Cs elimination rates, and also in metabolic rates. Long-component intercept values of ^{137}Cs, which indicated the level of incorporation or rate of initial-component excretion, exhibited a strong relationship with CO_2 production within a species. Based on this relationship, equations were derived to predict metabolism from Y-axis intercept values. These equations have potential use in estimating metabolism of rodents in the field for energy flow measurements.

Although ^{59}Fe elimination appeared to correlate with metabolic rates in the field, laboratory experiments failed to demonstrate a predictable relationship between excretion of this nuclide and general metabolism. Iron-59 was associated primarily with the blood, and the physiology of this tissue presumably controlled whole-body excretion of the isotope.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

I. INTRODUCTION

A. Statement of the Problem

More precise techniques for measuring animal energetics in the field are necessary for better understanding of energy transformations occurring in nature. A primary need is development of methods for determining metabolic rates of individual animals and populations in their natural environments. Without this metabolic information, the respiration component in energy flow can be only estimated from laboratory data (Golley, 1968).

"Metabolism" as used here is defined in the classical and ecological context, and may be regarded as the sum of energy processes in an animal which require oxygen and yield carbon dioxide (Kleiber, 1961; Odum, 1968; Wiegert, 1968). Volumes of these gases consumed or produced are convertible to caloric equivalents of respiratory heat loss and physiological maintenance. This component of energy flow must be considered in conjunction with estimates of biomass production, because most of the energy losses at all levels of an ecosystem are due to respiration (Odum, 1963).

A method for obtaining metabolic rates of animals in the field not only would provide vital information for energy flow studies, but also could be a useful tool for exploring modes of damage to populations by certain environmental forces. Effects of ionizing radiation, high population density, and severe climatic conditions could be

assessed from the metabolic rate aspect, and thereby generate a better understanding of stress factors.

The problem of measuring metabolism in the field has been approached with the theory that excretion of certain administered radionuclides is a function of metabolic rate, and that these excretion rates can be applied in the field to determine animal respiration. Such an application of radioisotopes in ecological research was alluded to by Auerbach (1958), formed into a hypothesis by Odum (1961), and later elaborated by Odum and Golley (1963) and Crossley (1963). With regard to the isotopic method, Odum and Golley (1963) stated that ". . . we can see no other way to measure metabolic rate in completely free-living populations."

B. Historical

Earlier experimental results which formed the basis for the hypothesis that metabolic rates of animals can be determined by radionuclide excretion were obtained from investigations on poikilothermous animals, chiefly arthropods (Odum, 1961). Most subsequent experiments conducted to validate and implement this technique also dealt with arthropods (Crossley, 1963; Reichle, 1967). Only recently have studies been conducted in an ecological context to extrapolate the method to homeotherms; a summary of these and other related experiments is presented here.

An investigation by Comar et al. (1962) indicated a lack of correlation between metabolic rate and ^{137}Cs retention in thiouracil-treated white rats. These workers were unable to detect any differences

in cesium retention rates between groups of animals with different metabolic and growth rates. Contrastingly, Furchner and Richmond (1963) demonstrated that lowered environmental temperature increased the rate of ^{137}Cs excretion in laboratory mice, indicating a possible connection between metabolism and isotope elimination. However, no metabolic rate measurements were made to confirm this apparent relationship. A statistically significant correlation between resting metabolism and elimination of ^{134}Cs in cotton rats was shown by Baker and Dunaway (1969).

Orr (1967) found no distinct differences between elimination rates of ^{65}Zn in animals kept in the laboratory and those released into field enclosures. There was a slight difference between laboratory and field groups during November-December, but not during the spring. Orr's assumptions were that field animals would require more energy due to increased activity and temperature stress, and that these factors would increase metabolic rates. If ^{65}Zn excretion is related to general metabolism, and if Orr's assumptions were correct, there should have been distinct differences between elimination rates of laboratory and field animals. Hemington (1967) and Pulliam et al. (1968) found the use of ^{65}Zn elimination rates to be promising as a technique for measuring metabolism of small mammals, but both of these investigations failed to disclose any predictable relationships with individual animals.

O'Farrell et al. (1966) found a relationship between whole-body retention of a halogenated pyrimidine, ^{131}iododeoxyuridine (IUDR), and

oxygen consumption in three species of rodents. The relationship was based on average values for each species, and not on individual metabolic rates. IUDR is incorporated into DNA of rapidly dividing cells, and elimination of the compound due to sloughing of old cells apparently is an index to cellular proliferation rates. These rates in turn may be related to general metabolism.

Experiments with ^{32}P excretion in cotton rats (Wagner, 1968) showed little promise that this nuclide could be used as a metabolic indicator because of wide variability in the data. Wagner also found that ^{32}P elimination was related to environmental temperature more than to metabolic rate.

Another method, somewhat different from the above cases, was investigated by Lifson et al. (1955), who demonstrated that total CO_2 production could be measured by means of doubly-labeled water ($D_2{}^{18}O$). This technique was validated by McClintock and Lifson (1958) and Lee and Lifson (1960), and was used later to measure energy metabolism of pigeons at rest and in flight (LeFebvre, 1964). Some authors have expressed confidence that this method will be most feasible for general application in field studies (Short and Golley, 1968; Wagner, 1968), but the technique is difficult and expensive to employ, requiring mass spectrometry.

C. Objectives

The primary objective of this research was to compare general metabolic rates of individual small rodents with long-component

elimination rates of ^{137}Cs and ^{59}Fe, determining whether these isotopes may be used as metabolic tags in nature. Secondary objectives of the study were (1) to provide experimental records of small-mammal metabolism as measured with newer techniques (as contrasted with the data of investigators such as Morrison, 1948a, 1948b; Morrison and Pearson, 1946; Pearson, 1947); (2) to add to the growing body of knowledge on element cycling in ecosystems by further elucidation of radionuclide movement in organisms (see Auerbach, 1965); and (3) to demonstrate the existence of possible alternative mechanisms for elimination of these isotopes, other than those related to general metabolic rate.

D. General Procedure

Three species of wild small mammals (Sigmodon hispidus komareki Gardner, Reithrodontomys humulis humulis Audubon and Bachman, and Peromyscus leucopus leucopus Rafinesque) were given ^{59}Fe and ^{137}Cs, both isotopes being administered simultaneously to each individual. This "double-tagging" technique allowed comparison of rates of elimination of both isotopes in the same animal to determine whether there would be concurrent changes in excretion patterns of the two nuclides during metabolic rate changes. As a secondary advantage, use of the method also avoided duplication of effort.

Elimination of these nuclides was investigated to determine whether excretion rates in the field would differ between winter and spring, and whether field elimination rates would differ from those in the laboratory. Laboratory animals were subjected to different

experimental treatments to produce metabolic changes, and to demonstrate whether isotope elimination rates were altered accordingly.

Metabolism was monitored by measuring rate of daily CO_2 production. Carbon dioxide production alone is assumed to be a valid index to the metabolic rates of small mammals kept under standardized conditions, but for total characterization of metabolism, measurements of oxygen consumption and protein catabolism also are desirable (Buckner, 1964).

E. Experimental Radionuclides and Animals

For these experiments, it seemed appropriate to select one isotope (element) which has little or no direct relationship to metabolic processes and another which is used actively in metabolism. Isotopes of cesium and iron were chosen because of such contrasting physiological roles. Cesium is metabolically similar to potassium (Davis, 1963; Relman, 1956; Ringer, 1882), which is important in maintaining ion balance and membrane potentials. Iron is related more to intermediary metabolism, being incorporated into the cytochromes and hemoglobin. The literature contains numerous reports of pertinent experiments which describe tracer techniques for both cesium (Hood and Comar, 1953; Johnson et al., 1968; Kitchings et al., 1969; Mraz and Patrick, 1956, 1957) and iron (Bonnet et al., 1960; Chappelle et al., 1955; Foreman et al., 1952).

Cotton rats and harvest mice were selected primarily because of their contrasting body weights. Body weight is related inversely

to metabolic rate (Kleiber, 1947), and using different sized species provided opportunity additional to experimental treatments to observe whether radionuclide elimination is affected by metabolic differences. Life histories, populational aspects, and habitats have been described both for cotton rats (Dunaway and Kaye, 1964; Meyer and Meyer, 1944) and harvest mice (Dunaway, 1968; Kaye, 1961).

Other species, such as short-tailed shrews and pine mice, were considered but rejected due to difficulties in laboratory housing or to the relative scarcity of the species. Also, the semifossorial habits of pine mice would have created problems in maintaining them in field enclosures. It was not planned originally to use white-footed mice, but this species was employed in the field study for reasons explained in Section IV.

II. GENERAL METHODS

Methods presented in this section were common to most phases of the project. Special methods for specific experiments will be discussed under the appropriate headings.

A. Acquisition and Maintenance of Animal Colony

Rodents for these experiments either were live-trapped on the Oak Ridge National Laboratory (ORNL) reservation or reared in the laboratory. It was assumed that a considerable period of acclimation of animals to the laboratory environment should be allowed before initiating metabolic rate experiments. Therefore, wild animals used in laboratory studies were trapped several months prior to the studies. All animals investigated were adults, except as indicated in one case.

Metal modified Sherman live-traps (Fig. 16, Appendix A), measuring 8 x 9 x 30 cm, were used for capturing animals. Each trap was placed inside a weather-proof metal container (Fig. 17, Appendix A), and set in an area well marked with animal runways. Trapping for both cotton rats and harvest mice was conducted in fields in early successional stages, with the former species always being more obtainable. Other details on trapping procedures and initial handling of captured animals in the laboratory were the same as given by O'Farrell *et al.* (1966).

Cotton rats were housed in polypropylene cages (35 x 22 x 13 cm) with steel grate tops; harvest mice were kept in clear plastic cages

(29 x 19 x 13 cm) with wire mesh tops; white-footed mice were held in the same type cages as harvest mice, but with steel grate tops. Types of cages used are illustrated in Fig. 18 (Appendix A). A contact-type absorbent bedding (Sterolit) was used. A metal can (8 x 8 x 11 cm), open at one end, was placed in each cage to provide a retreat, or "house." The can also was used for removing animals from their cages. White-footed mice and harvest mice were given cotton for nest-building, but this material was withheld from cotton rats because their larger size decreased the need for added insulation. Also, it previously had been noted in this laboratory that cotton rats were prone to chew and swallow large pieces of cotton, and sometimes died from gastric obstruction.

In addition to the standard diet of Purina Laboratory Chow, animals regularly were given sunflower seeds and lettuce. The temperature of the animal room averaged 22°C, and a photoperiod of twelve hours (0600-1800) was maintained. Rodents were kept under these conditions until termination of the experiments, except for the lower environmental temperature of one experimental treatment.

Shortly before the isotope excretion studies, cages of cotton rats were modified slightly to prevent self-contamination by animals excreting radioactive urine. The metal can described above was removed and replaced with a 12-oz beverage can which had been cut and trimmed to provide a "house" without a floor, and was wired to the top of the cage for stability. This procedure allowed radioactive urine to pass directly into the absorbent Sterolit on the bottom of the cage. Without

this modification, urine and feces would have remained for some time on the floor of the conventional can, and likely would have contaminated the hair and skin of the animal. The original-type can was reinserted in the opposite end of the cage during removal of a cotton rat for counting. The rat then was chased into the can and withdrawn. Changing the type of "house" was considered unnecessary for the other two species because they were provided with nesting material, and the cotton absorbed radioactive urine. Also, these smaller species were not expected to excrete as large a volume of urine as cotton rats. Cotton and Sterolit were changed frequently as a further measure for preventing self-contamination.

B. Tracer Techniques

Iron-59 was purchased from Nuclear Science Division of International Chemical and Nuclear Corporation, and ^{137}Cs was taken from stock obtained earlier from the Isotopes Division of ORNL. Both isotopes were in the chloride form. Injection solution was prepared by combining the two nuclides in distilled water so that approximately 0.8 μCi ^{59}Fe and 2.0 μCi ^{137}Cs was administered in 0.3 - 0.4 ml quantities. The mixed isotope solution was injected intraperitoneally using a 1-ml disposable hypodermic syringe (27-gage needle).

The injection mode of administration was selected because it was adjudged to be the most practical and convenient for eventual application in field studies. Although injection is an "unnatural" means of introducing radioisotopes into animals, there is little or no

evidence to suggest that mode of administration affects long-component excretion or tissue distribution of the nuclides used here (J. T. Kitchings, personal communication; Kitchings et al., 1969; Nelson et al., 1960).

During the injection procedure, each rodent was taken from its cage by means of the metal can and transferred to a plastic bag. The animal was directed into a crease of the bag, grasped by the nape, and held with its ventral surface up. The syringe needle then was inserted gently into the abdominal cavity, and isotope solution was released slowly. The animal was placed immediately into a ventilated paper food carton, and the top was secured by a band of wide masking tape. Pint-sized containers were used for cotton rats and white-footed mice, and half-pint cartons were used for harvest mice. Cartons were placed inside plastic bags to prevent leakage of radioactive urine into the counting apparatus. Animals were weighed while in the cartons, which were marked for identification, and taken immediately to the counting room for initial assay. Cartons were weighed to obtain tares after the rodents were returned to their cages. Only a few animals were handled at any one time to insure that they were not kept out of their cages any longer than necessary. Except for injection of isotopes, these same procedures were followed during subsequent counting.

Animals were counted for radioactivity four to six times after initial assay to establish long-component loss rates of isotopes. Previous work at this laboratory and the literature indicated that the final component of ^{59}Fe excretion in small rodents is reached after

about one week (Bonnet et al., 1960; Chappelle et al., 1955). The final component for ^{137}Cs elimination from harvest mice is reached after about three to four days, five to six days for white-footed mice, and about six days for cotton rats. Therefore, the first counts after initial assay took place at least seven days after injection. Counting was continued at appropriate intervals for about 30 days for ^{137}Cs and 45 days for ^{59}Fe.

Whole-body assay was accomplished with an Auto-gamma Spectrometer and Armac Liquid Scintillation Detector (Packard Instruments Company, Model 410 A/440; Fig. 19, Appendix A). These instruments were not designed primarily for mixed-isotope counting, but good resolution was obtained with the two nuclides used in these experiments. Radioactivity present was expressed for both nuclides as percent of initial by

$$\text{percent retention} = (100)(A_t/S_t)(S_o/A_o) \quad ,$$

where A is animal radioactivity, S is radioactivity of a standard, t is any given day after injection, and o is day of injection. Standards were prepared from polyethylene bottles containing a single-isotope solution and were counted in a geometry simulating that of the animals.

By transforming percent of initial radioactivity retained to natural logarithmic equivalents, a linear relationship between retention and time was attained, allowing slope and intercept values to be estimated by the method of least squares. Final-component excretion of both isotopes was then described by

$$Y = ae^{-kt} \quad ,$$

where Y is the percent of initial activity remaining at time t; a is the intercept of the regression line on the Y axis at time zero (not 100% in this case, because of more rapid components during early excretion); e is the base of the natural logarithms; and k is the regression coefficient, or rate constant. Biological half-time (T_b), or time required for the decay-corrected body burden to be reduced by one-half, was calculated by

$$T_b = \frac{0.693}{k} \quad ,$$

where 0.693 is $\log_e 2$, and k is as defined above.

Tissue samples were assayed for radioactivity with a dual-channel analyzer (Packard Tri-Carb Spectrometer, series 314E) and a 3-in Tl-activated NaI crystal detector. The instrument operated in conjunction with an automatic turntable, sample changer, and recorder. For preparation of samples, selected organs were removed from animals and placed in test tubes (25 x 150 mm). To obtain a constant counting geometry, 5% formalin was added to bring the level of all samples to the same height in the tubes. Formalin was used instead of water to preserve the organs in case it became necessary to retain them for further investigation.

C. Data Processing

Computer programs were used for most calculations and were run on an IBM 360/75-91 computer. Regression and correlation analyses were performed with program Regress (Van Dyne, 1965); analyses of variance

were calculated using Anova-Gr (Hull, 1967); and an unpublished program by R. I. Van Hook (Radiation Ecology Section, ORNL) was used for Duncan's Multiple Range Test. A program for reduction and analysis of metabolic data was written specifically for this project by Dan Foster of the Oak Ridge Gaseous Diffusion Plant's Computing Technology Center, and is discussed later in this paper.

III. PRELIMINARY INVESTIGATIONS

A. Dual-Isotope Technique

It was necessary to verify that combined ^{137}Cs and ^{59}Fe could be counted because the counting apparatus employed in this study was designed primarily for single-isotope analysis. Counting might have been performed with good resolution between isotopes on a multichannel analyzer, but this alternative was rejected because geometry problems in counting live animals with the detectors available would nullify any advantages of using such a system. Detectors used with multichannel analyzers in this laboratory consist of either one or two crystals (Tl-activated NaI), and slight movements of an animal being counted in these arrangements would introduce sizable error due to altered geometry. This problem would be particularly acute with the larger cotton rats, and could be avoided only by undue restraint of animals during counting. Geometry problems are not as great with the Armac because the liquid scintillation detector is essentially in a 4π configuration, and slight movements of animals during counting do not greatly affect detection efficiency.

Initially, relationships for resolving ^{59}Fe and ^{137}Cs in mixed samples were determined by counting standards of known radioactivities. The use of narrow counting modes ("windows") allowed virtual isolation of ^{59}Fe radioactivity in mixture with ^{137}Cs. However, a sizable fraction of radioactivity detected on ^{137}Cs settings was always attributable

to the presence of ^{59}Fe. Net ^{137}Cs therefore was determined by use of the following relationship.

$$\text{Net } ^{137}Cs = A - \left(\frac{B}{C} \times D\right) \quad ,$$

where A = sample counts on ^{137}Cs settings; B = ^{59}Fe standard counts on ^{137}Cs settings; C = ^{59}Fe standard counts on ^{59}Fe settings; and D = sample counts on ^{59}Fe settings.

For validation of the method, a small experiment was conducted with live animals to determine whether this relationship would hold at changing levels of radioactivity, that is, while the isotopes were being excreted by animals. Nine male cotton rats were selected on the basis of homogeneity of weight, and divided into groups of three. One group received ^{137}Cs only, one received ^{59}Fe only, and the other group received a mixture of the two isotopes. Excretion of the nuclides was followed for 39 days, and no differences were noted between the elimination rates of either single-isotope group and its counterpart in the mixed-isotope group. Therefore, it was concluded that the two isotopes could indeed be counted in combination, even in a dynamic situation.

The method was not without limitation, however, as the relative levels of radioactivity were an important factor in attaining good resolution. The quantity of ^{137}Cs had to be kept fairly low (<0.75 μCi), because above this amount the contribution of ^{137}Cs on the ^{59}Fe settings became apparent. However, this radioactivity due to ^{137}Cs was not considered significant as long as the level of ^{59}Fe radioactivity was kept high enough (>0.3 μCi). Because of rapid initial loss of injected ^{137}Cs,

more of this isotope was administered than was desirable, creating a small error in determining the initial amounts of both isotopes.

B. Oxygen Consumption Measurements

Two different environmental temperatures were to be used in the CO_2 production experiments, and it was desirable to know beforehand at what temperatures significant changes in metabolic rates could be expected. Also, it was important to characterize the metabolic profiles of the species to be used in the ensuing experiments, so that ranges of metabolic rates and relationships between species could be examined before further experiments were designed.

An apparatus for measuring CO_2 production rates was not available at that time, and a manometric respirometer (Minute Oxygen Uptake Spirometer, Aloe Scientific; Fig. 20, Appendix A) was used to determine oxygen consumption rates of rodents at several different temperatures. This instrument operated on a closed-system principle, whereby exhaled CO_2 was taken up by soda lime in the respiration chamber. The drop in chamber pressure resulting from CO_2 absorption caused oxygen to be drawn into the chamber from a reservoir, and the amount of O_2 entering was recorded. Time intervals also were recorded automatically. During the experiment, the respiration chamber was placed in a water bath (50-gallon aquarium), which contained heating and cooling elements to provide temperature stability.

A ventilated metal shelter was placed inside the chamber to provide a retreat for the animal during measurement, and presumably

aided the animal in arriving at a resting level more rapidly. It was necessary to measure animals at resting levels because the instrument used was capable of making only short-term measurements, and only at resting levels could mean values be used reliably to make comparisons between groups of animals.

Measurements were made on three adult cotton rats and three adult harvest mice. Values for oxygen consumption (corrected to STP) were plotted as a function of environmental temperature (Fig. 1), and were best described for both species by

$$\log Y = \log a - bx \quad ,$$

where Y is the rate of oxygen consumption (cc/hr/g) at temperature x, a is the intercept at $x = 0$, and b is the slope of the regression line. This semilogarithmic relationship for small rodents contrasts with the linear relationship described by Brody (1945), Kleiber (1961) and Prosser and Brown (1961), who are authorities for reference to metabolic data of this type. No zone of thermoneutrality was apparent in either species, and metabolism did not increase at the highest temperature used in these experiments. These relationships did not emerge until values were corrected to STP, and suggests for the range measured a Q_{10} of 1.4 for Sigmodon and 1.8 for Reithrodontomys.

The environmental chamber selected for use in the subsequent CO_2 production experiment maintained a lower temperature of 9°C. From these data it appeared that this temperature was sufficient to produce significant increases in metabolic rates of both species.

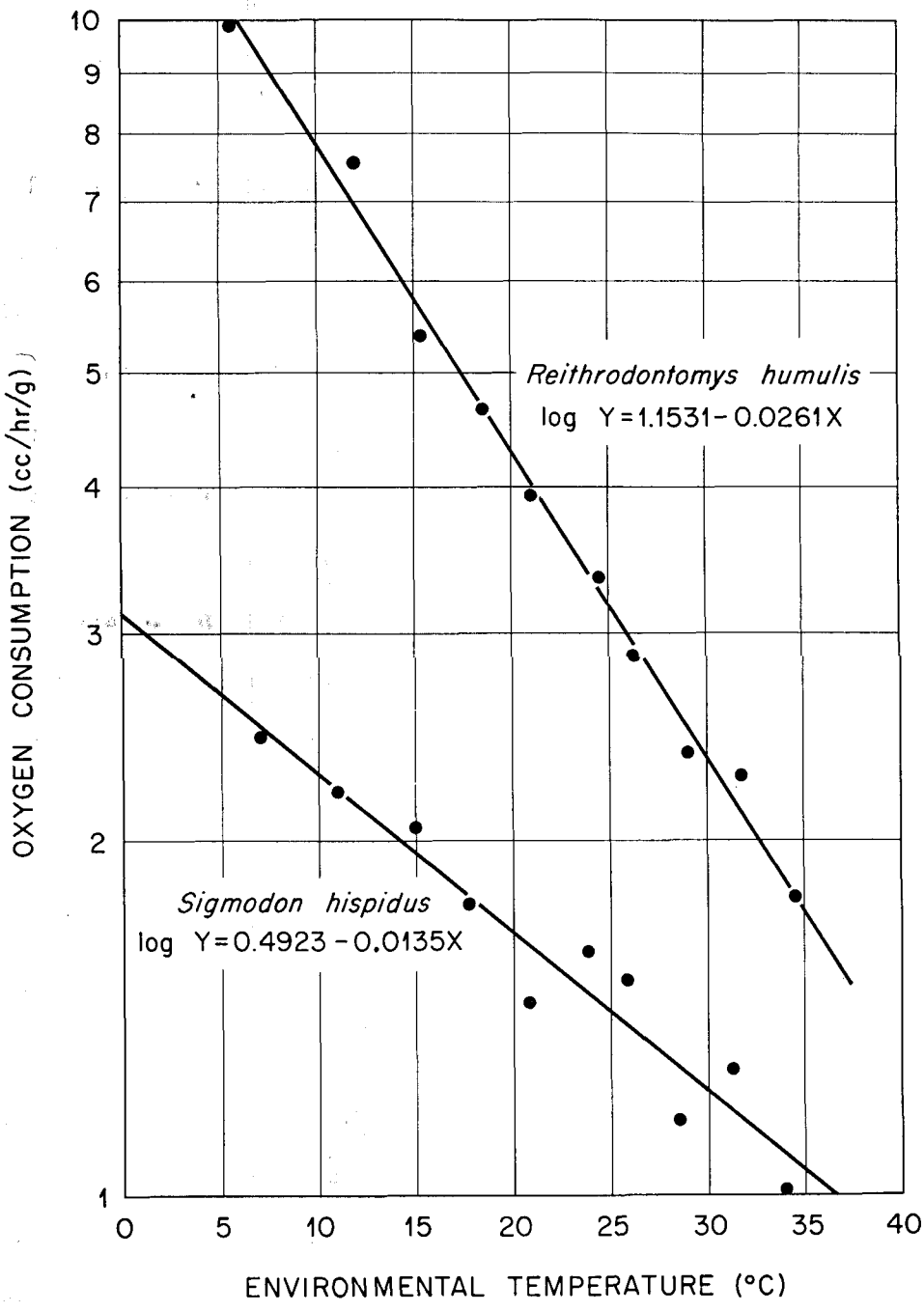

Figure 1. Oxygen consumption of two species of small rodents at resting levels as a function of environmental temperature.

IV. FIELD MEASUREMENT OF ISOTOPE ELIMINATION

A. Objective

The objective of this experiment was to examine the relationship of long-component elimination rates to general metabolism by measuring excretion of ^{59}Fe and ^{137}Cs by rodents under field conditions. Studies were conducted in winter and spring, and it was assumed that metabolic rates would be higher in winter than in spring due to lower environmental temperatures. Also, it was expected that field animals would have greater metabolic demands than those in the laboratory because of higher levels of physical activity in free-ranging animals. If these assumptions are correct and if elimination of these isotopes is related to metabolism, excretion rates should be more rapid in winter than in spring, and faster in the field than in the laboratory.

B. Methods

Experimental Animals

It was desirable to use for field studies the same species employed in laboratory experiments. However, harvest mice were extremely difficult to acquire when the field experiment was begun, and use of this species had to be abandoned. Field animals could not be trapped in advance and held in the laboratory because of the inadvisability of using rodents which were not acclimated to their experimental conditions. It was therefore necessary to trap all animals for each experiment within the span of three to four days. Because harvest

mice were unavailable, an arbitrary decision was made to use white-footed mice (Peromyscus leucopus) as a second species in the field. However, after introducing several of these mice into field enclosures at the start of the winter study, it was discovered that they were able to escape from the pens, which were designed primarily for cotton rats. Therefore, use of white-footed mice in the enclosures had to be discontinued, and a decision was made to use only cotton rats.

Some of the white-footed mice which escaped from the field pens remained in the study area. Since these mice already had been given isotopes, an attempt was made to follow excretion rates of these free-ranging animals and another group was released in spring. A number of traps were set periodically outside the pens for white-footed mice and some data were obtained. A total of four mice were captured regularly throughout the winter study, and five mice were followed in the spring experiment.

Description of the Area

The four 10- x 10-m field enclosures used in this experiment are located on ORNL Ecology Area 0911, and are constructed of 3-ft galvanized steel sheet. They are situated in an old-field habitat, adjacent to a semimarshy area (Figs. 21 and 22, Appendix A). The terrain is well-drained, sloping from the juncture of the four pens. Vegetation was predominately broomsedge and honeysuckle, with a clump of small sweetgum trees occupying a fairly large portion of one pen. A more complete summary of the flora of the plots is given in Appendix B,

which also contains a list of birds and other fauna observed or trapped on the area.

To discourage mammalian predation from outside the pens, an electrically charged wire was strung over the fences, and streamers were placed in trees and bushes about the area to frighten away avian predators. Small plastic barriers ("turn-ins") were placed over the top of each 90-degree fence juncture to prevent animals from climbing out the studded corners of the pens. A schematic diagram of the complete experimental arrangement is given in Fig. 2.

Weather Monitoring

Meteorological measurements were made to quantify microhabitat conditions during winter and spring. Due to the locations at which readings were taken, data from these measurements did not coincide with weather bureau information, but reflected the same trends. Relative humidity was measured 6 inches aboveground with a recording hygrothermograph. This instrument was located just outside the pens because the experimental rats might have mutilated it by chewing away taut hairs contained in the device. Maximum and minimum air temperatures were read from two thermometers placed 6 inches aboveground inside the pens. Soil temperature (2-in) was monitored in one location, and read only in the morning.

Mean maximum and minimum temperatures were obtained by averaging readings from the two max-min thermometers inside the pens with readings taken from the hygrothermograph. Mean daily temperature and mean daily relative humidity were obtained from hygrothermograph charts with the

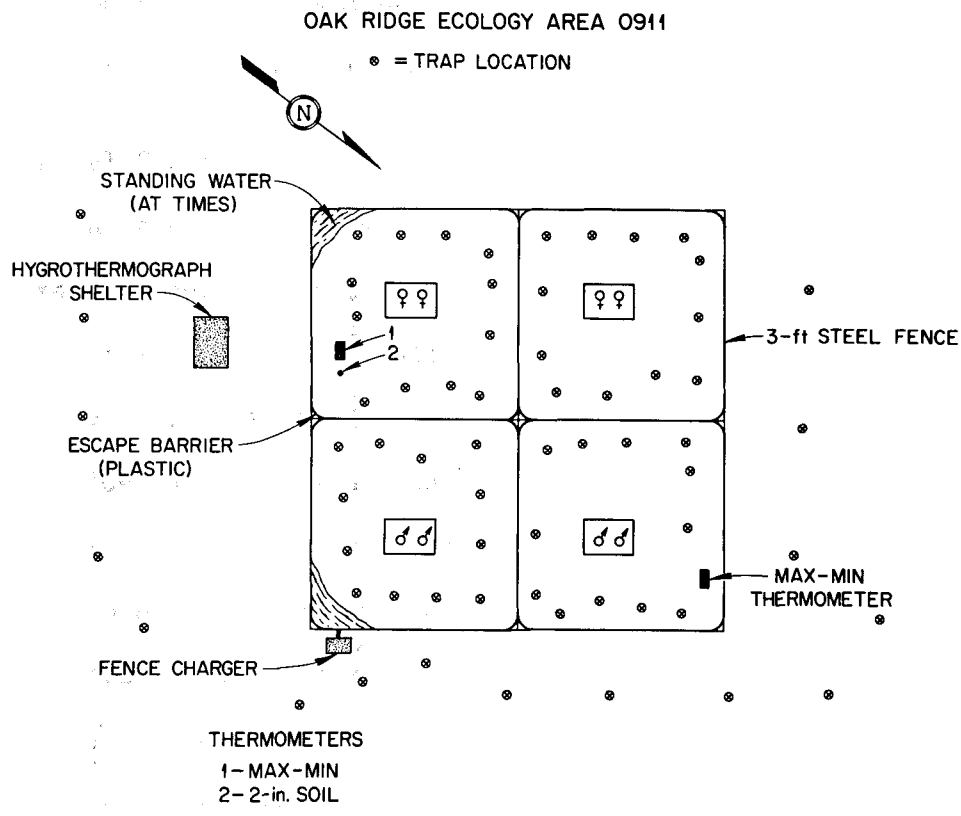

Figure 2. Schematic of field study area.

aid of a compensating planimeter. Hours per day of 100% relative humidity also were read from the hygrothermograph recording.

Experimental Procedure

Each seasonal study was handled as two replications, the second beginning two weeks after the first. This procedure obviated having to obtain a large number of animals at one time, and also allowed the study to continue over a longer period. The latter feature was important because ^{137}Cs was eliminated rapidly, and if the experiment had been designed without replications, excretion of this nuclide would not have been comparable with ^{59}Fe data in terms of elapsed time. Animals for the winter experiment were trapped January 28-31 and February 13-14, and for the spring study, April 3-8 and 22-25, 1969.

Animals obtained for the field study were brought to the laboratory, injected with isotope solution, and released immediately into the field enclosures. Thereafter, they were sampled weekly for radioactivity throughout the season. Captured animals were transported to the laboratory in the traps, counted for radioactivity, and returned immediately to the field while still in the cartons used for counting.

All four pens were used for each season (two for each sex), with an average of four animals per pen. At the beginning of the winter study the vegetation in the pens was sparse, and it was decided to supplement the natural food of the cotton rats with sunflower seeds. Therefore, four to five handfuls of these seeds were scattered over each pen twice a week. This procedure probably was not required during the spring, but was continued to keep the two seasonal studies as

similar as possible. However, spring animals likely consumed a somewhat different diet than the winter animals, due to the emergence and availability of green vegetation.

Very good sampling success was experienced during the entire study, and there was only one case of trap mortality. Except in a few instances all animals were trapped weekly, and only one important problem was encountered. A female in the spring experiment was undetectably pregnant at the time she was introduced into the pen, and about three weeks later gave birth to a litter. Four of these young were trapped subsequently, and kept in the laboratory to determine whether a significant amount of the mother's radioactivity had been imparted to the offspring. Enough radioactivity (^{59}Fe only) was present in each of the young rats to be counted for several weeks. It was of interest to follow the excretion of isotope from these young animals to determine whether the rate of turnover would differ from adults, as their metabolic rates were expected to be different because of smaller body size. In the data, these animals are referred to as "spring litter." It could not be determined from amount of radioactivity lost or weight change which female in the pen was the mother of the litter.

Four of the winter animals were brought to the laboratory after the field study was terminated, and elimination of ^{59}Fe was followed for several weeks (not enough ^{137}Cs was present for whole-body counting). The object of this procedure was to determine whether excretion rates would change after animals were taken from the field environment, where their metabolic demands were assumed to be greater due to lower

temperatures and physical activity. These animals are referred to in the data as "winter trailers." The remainder of the winter animals were sacrificed at the conclusion of the study for tissue distribution analyses.

For comparison with field data, a group of 12 cotton rats were injected with isotopes and maintained in the laboratory at 22°C. Elimination rates were obtained in a fashion similar to that of the field study. These laboratory "controls" also were used as controls in the CO_2 production experiments described in the next section.

C. Results

Average ^{137}Cs and ^{59}Fe elimination curves for both species are presented in Figs. 3 and 4, respectively. Based upon results from Duncan's 5% Multiple Range Test, no differences in long-component ^{137}Cs elimination rates (k) were found among winter, spring or laboratory cotton rats. Laboratory and spring intercept values (a) were not different from each other, but both differed significantly from winter intercepts. All three groups of cotton rats were significantly different in long-component elimination of ^{59}Fe, with the fastest rates being in winter and the slowest in the laboratory. Intercept values for ^{59}Fe were significantly lower in field animals, but no differences were present between winter and spring. These statistically significant differences in slopes and intercepts for both nuclides are summarized in Table I.

In Peromyscus, no differences were noted among the three treatment groups in long-component ^{137}Cs elimination rates, but this species

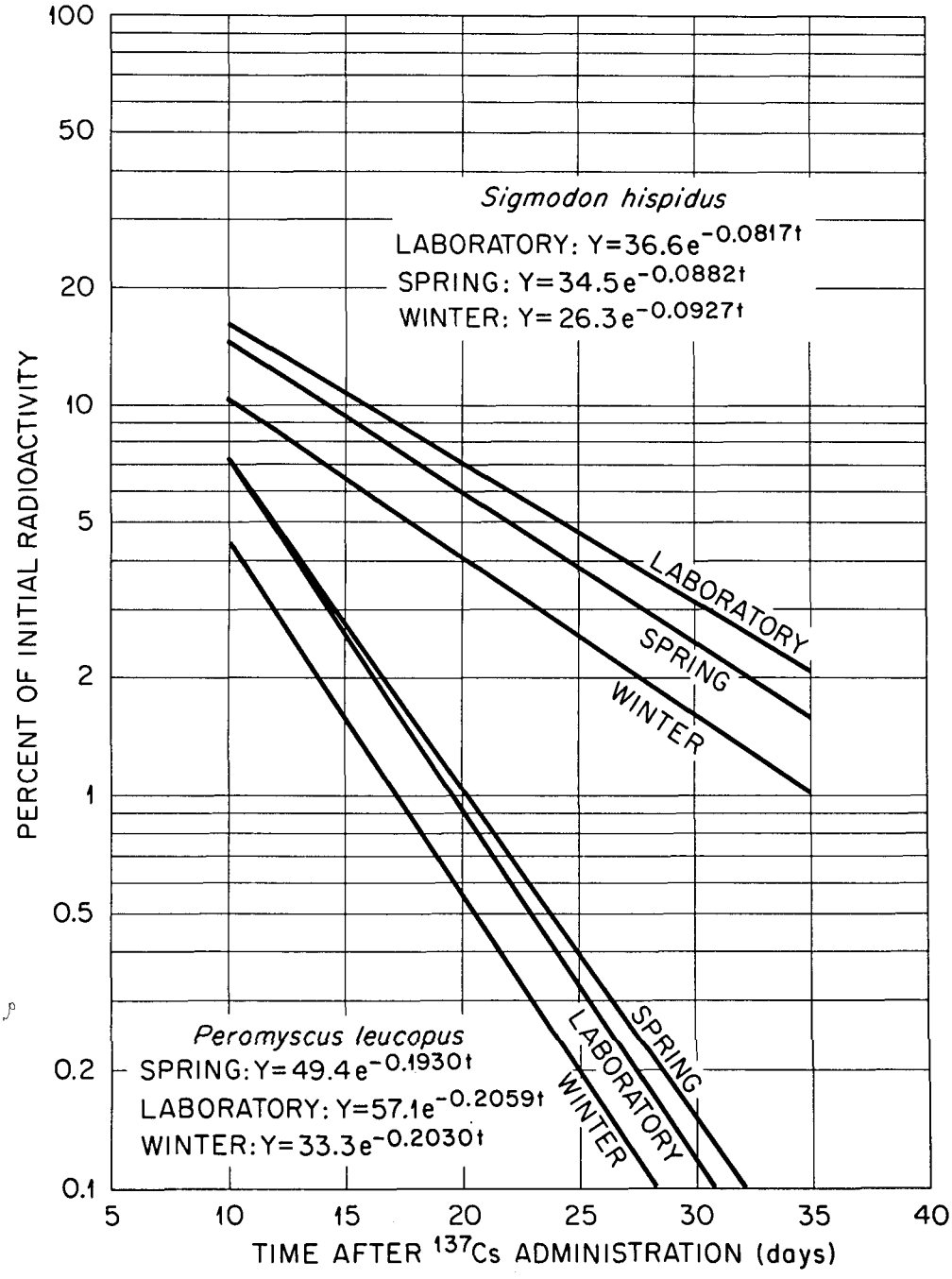

Figure 3. Elimination of ^{137}Cs by two species of rodents in laboratory and field.

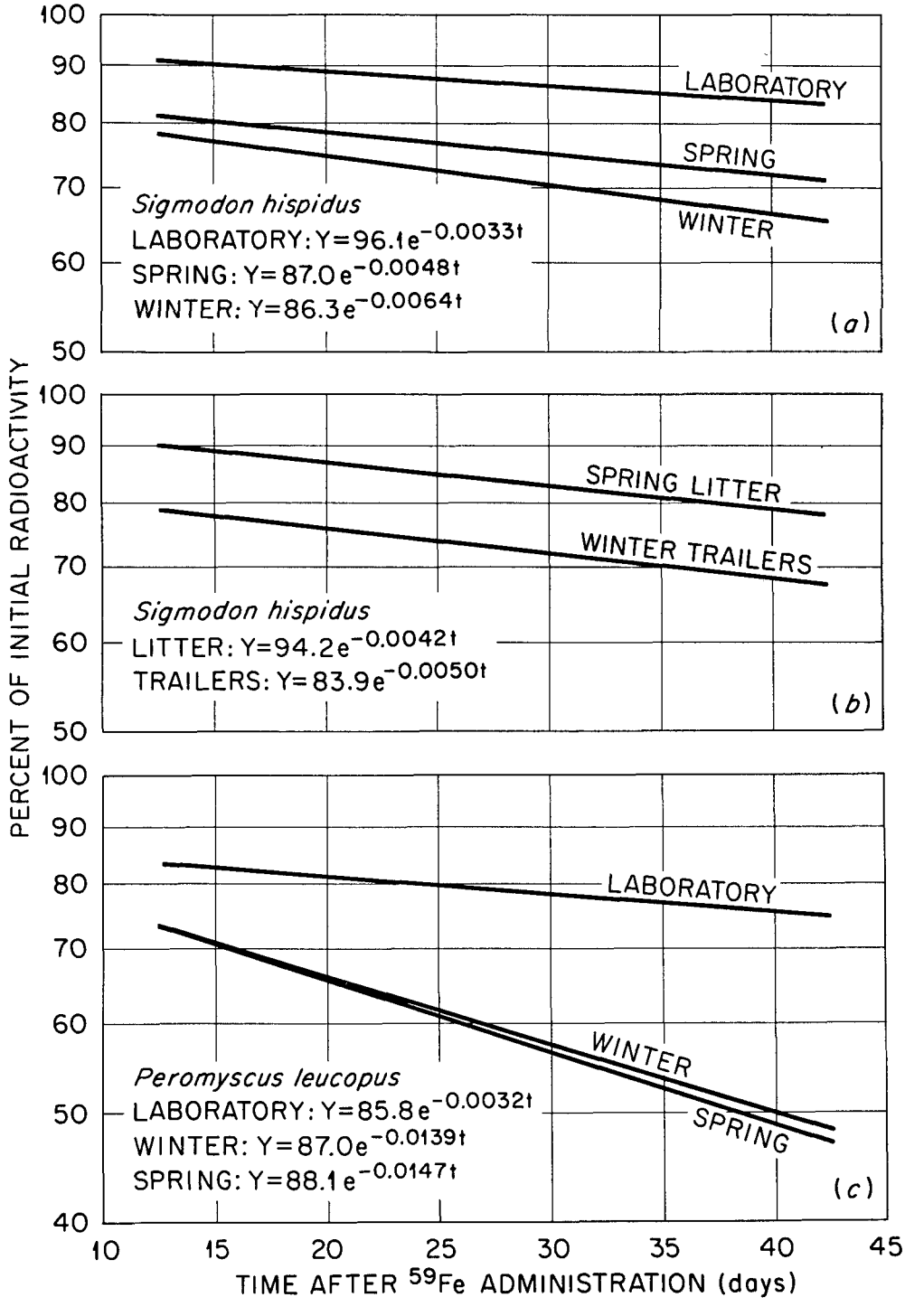

Figure 4. Elimination of ^{59}Fe by two species of rodents in laboratory and field.

TABLE I

RESULTS FROM DUNCAN'S 5% MULTIPLE RANGE TEST FOR DIFFERENCES IN SIGMODON ISOTOPE ELIMINATION VALUES BETWEEN LABORATORY AND FIELD*

Isotope Values	Winter	Spring	Laboratory
^{137}Cs Slope	-0.0927	-0.0882	-0.0817
^{137}Cs Intercept	26.3	34.5	36.6
^{59}Fe Slope	-0.0064	-0.0048	-0.0033
^{59}Fe Intercept	86.3	87.0	96.1

*Means underscored by same line are not significantly different.

excreted ^{137}Cs more rapidly than cotton rats (Fig. 3). Intercept values were different for all three groups, with winter intercepts lowest and laboratory intercepts the highest. White-footed mice in the field eliminated ^{59}Fe at markedly different rates than those in the laboratory (Fig. 4c), but winter excretion rates were not different from those of spring. No differences were seen in ^{59}Fe intercepts among the three groups of Peromyscus.

The "spring litter" rates (^{59}Fe) were indistinguishable from those of other cotton rats in the spring experiment. The "winter trailer" group, on an average basis, did not alter their ^{59}Fe elimination rates after being brought into the laboratory environment. The "winter trailer" curve of Fig. 4 can not be compared directly with the winter Sigmodon ^{59}Fe curve because the former represents only four animals of the latter; also, the time scale of Fig. 4b is not directly comparable with the other two graphs, because the days given are relative to the beginning of the experiment and not to time of isotope administration.

Weather data were averaged for the experimental periods and are summarized in Table II. The continua from which the averages were taken are depicted graphically in Fig. 5.

TABLE II

AVERAGE MICROMETEOROLOGICAL DATA TAKEN DURING ISOTOPE ELIMINATION STUDIES IN THE FIELD

Characteristic	Winter (1/22 - 4/10)	Spring (4/4 - 6/6)
Mean Daily Temperature	39.5°F (4.0°C)	60.5°F (16.0°C)
Mean Daily Rel. Humidity	79.0%	77.5%
Time/Day of 100% Humidity	8.5 hr	6.5 hr
Daily Maximum Temperature	58.0°F (14.5°C)	82.5°F (28.0°C)
Daily Minimum Temperature	26.5°F (-3.0°C)	43.5°F (6.5°C)
Soil Temperature (2-in)	39.0°F (4.0°C)	57.0°F (14.0°C)

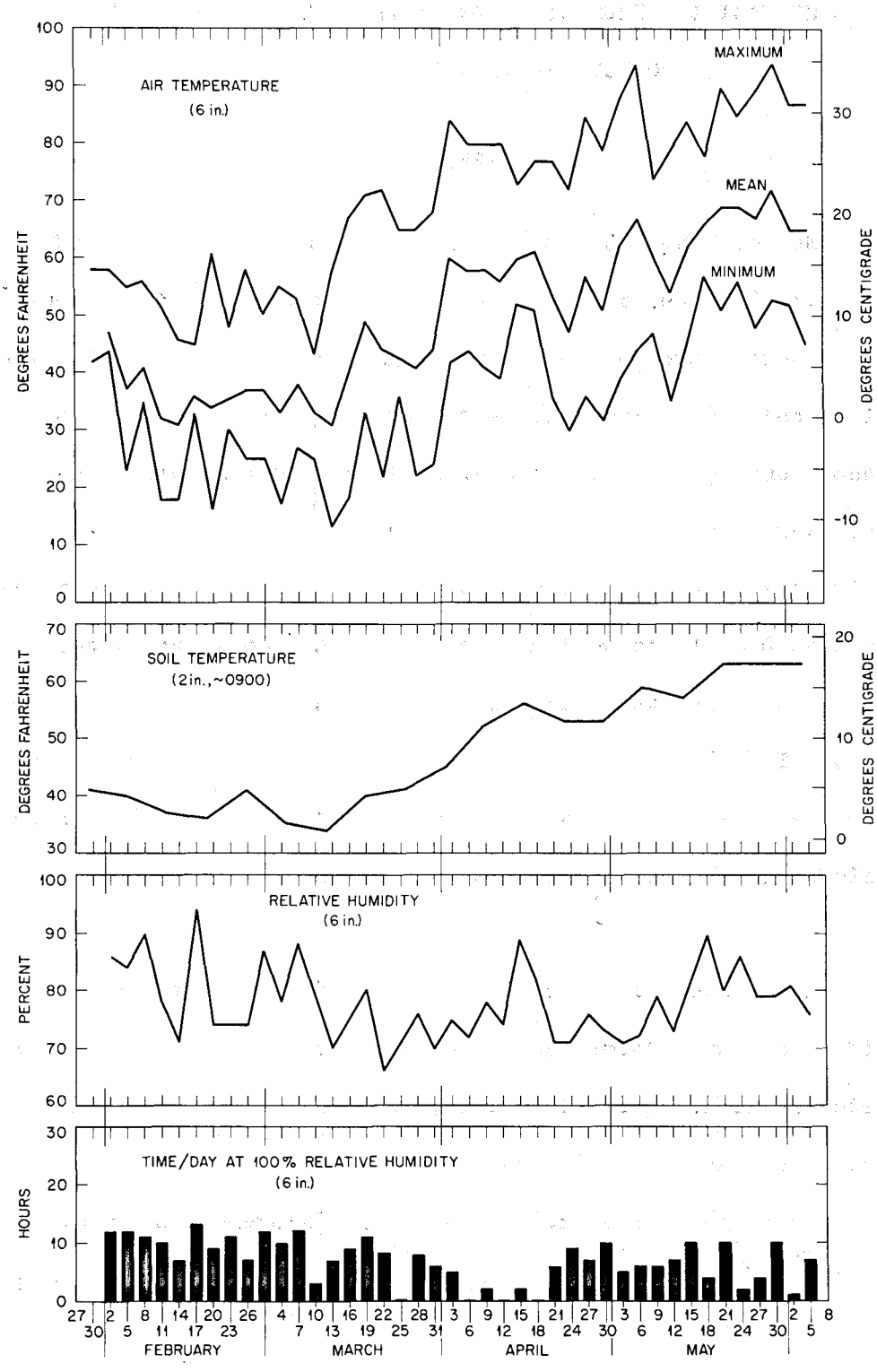

Figure 5. Weather data collected during study of isotope elimination in the field.

V. LABORATORY MEASUREMENT OF ISOTOPE ELIMINATION AND CARBON DIOXIDE PRODUCTION

A. Objectives

The laboratory phase of this study was designed to make comparisons between isotope elimination and metabolic rates of individual rodents. Besides the primary measurements of CO_2 production and radioisotope elimination, two other investigations were conducted to interpret factors which may be important in excretion of radionuclides by rodents. Food and water consumption rates of cotton rats were measured to determine whether these factors were related to isotope elimination. Some evidence indicates that "competition" from stable and analog forms of radioelements present in the diet may affect excretion of radioisotopes (Johnson et al., 1968; Mraz and Patrick, 1957). Tissue distribution studies were performed to define active sites of radionuclide incorporation, which presumably are the tissues controlling whole-body elimination rates.

An apparatus for making sustained metabolic measurements, incorporating the features desired for these experiments, was not available commercially as a unit. Therefore, one of the objectives of this experiment was to design and construct auxiliary components which could be combined with commercial equipment to form a working unit for measuring metabolic rates.

B. Methods

CO_2 Measurements

Metabolic rates of small mammals commonly are measured with manometric devices for determining oxygen consumption rates (Buckner, 1964; Pearson, 1947), and these instruments can be constructed so that measurements are feasible for extended periods. However, certain problems are inherent in the use of such instruments (Morrison, 1947), and a more satisfactory apparatus was needed. Therefore, an infrared gas analyzer (Beckman Instruments, Model IR-215A) was selected for monitoring metabolism of rodents over 24-hr periods. Analog data from the analyzer were converted to digital form by a teletype coupler (Beckman Instruments, Model 3108), and recorded on a teletype which provided both printed copy and computer punch tape.

For the system to be completely automatic, it was necessary to construct a special unit which would supply air to experimental animals and control the stream flow. The unit was required to switch the sample stream from one experimental cage to another, while still supplying air to the cages not being measured (three animals were measured during each sample period), and to control signal output to the recording instruments. This control unit was designed and constructed in collaboration with ORNL engineers (Instrumentation and Controls Division), and is shown schematically in Fig. 6. Figs. 7-9 show all of the components of the CO_2 measuring apparatus.

For measuring CO_2 production, the cages in which animals were housed were placed inside the respiration chambers (Fig. 9). This

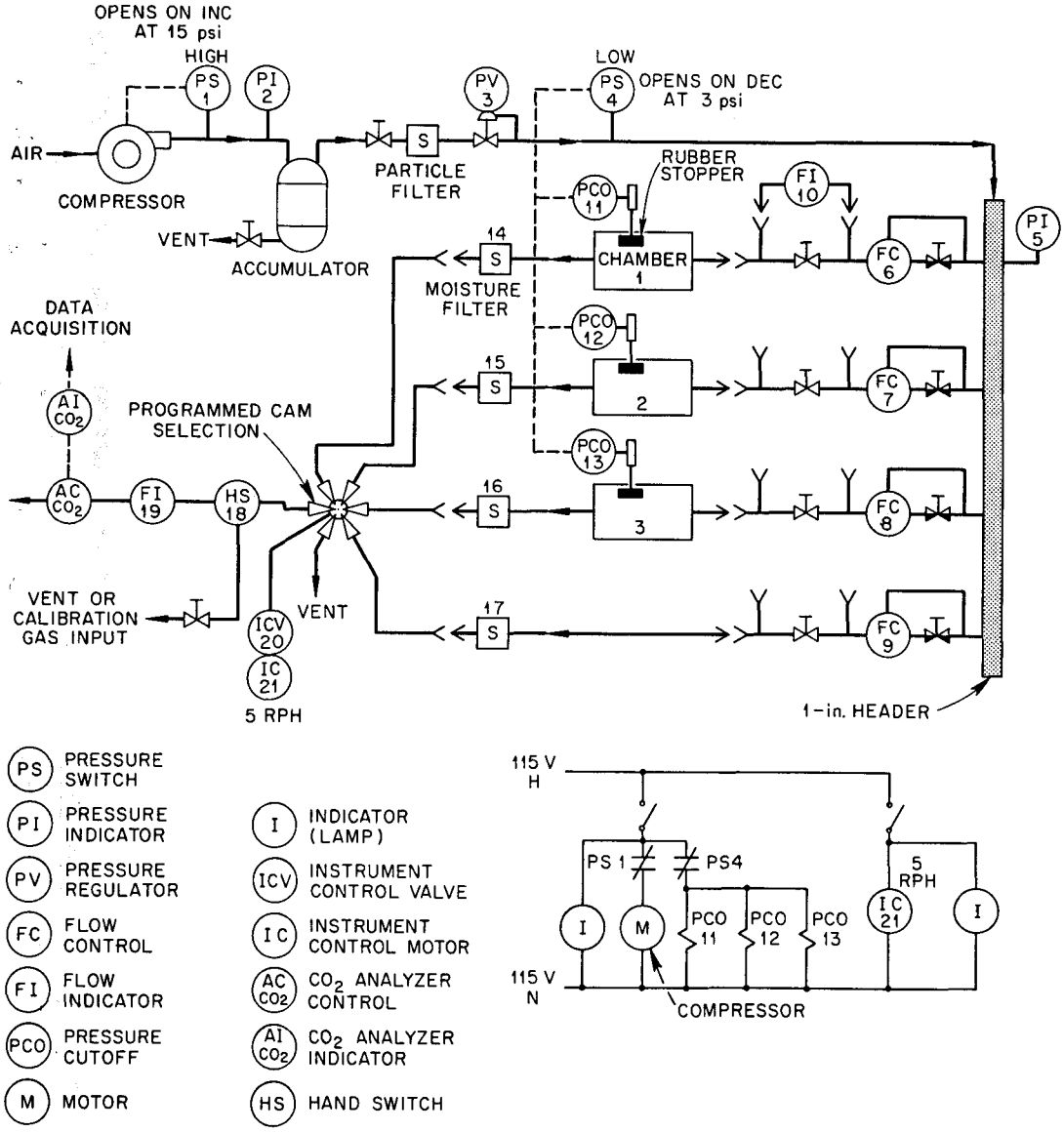

Figure 6. Schematic diagram of control unit specially constructed for use with infrared gas analyzer.

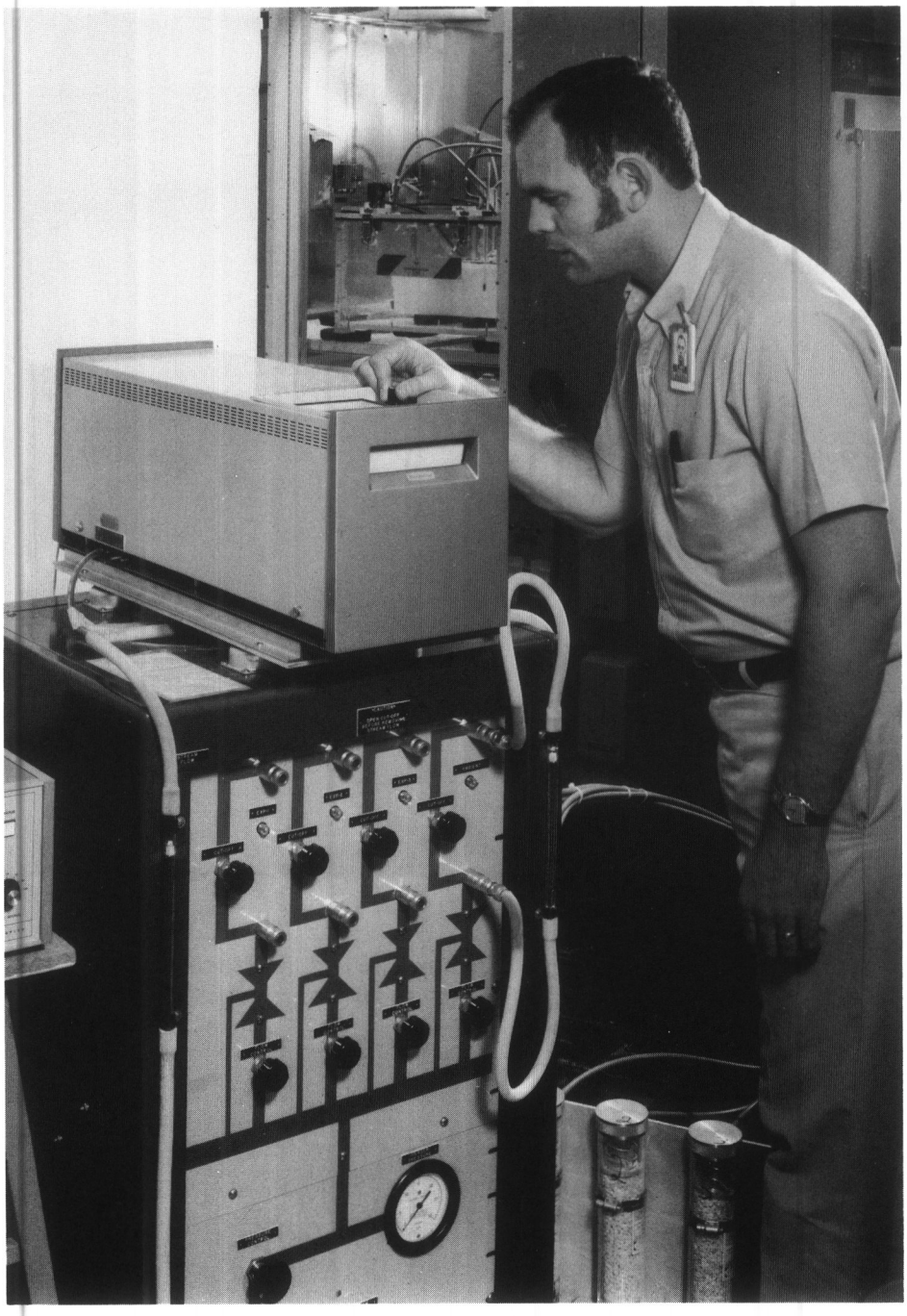

Figure 7. Beckman infrared gas analyzer resting atop specially constructed air supply and control unit, with measuring chambers in background.

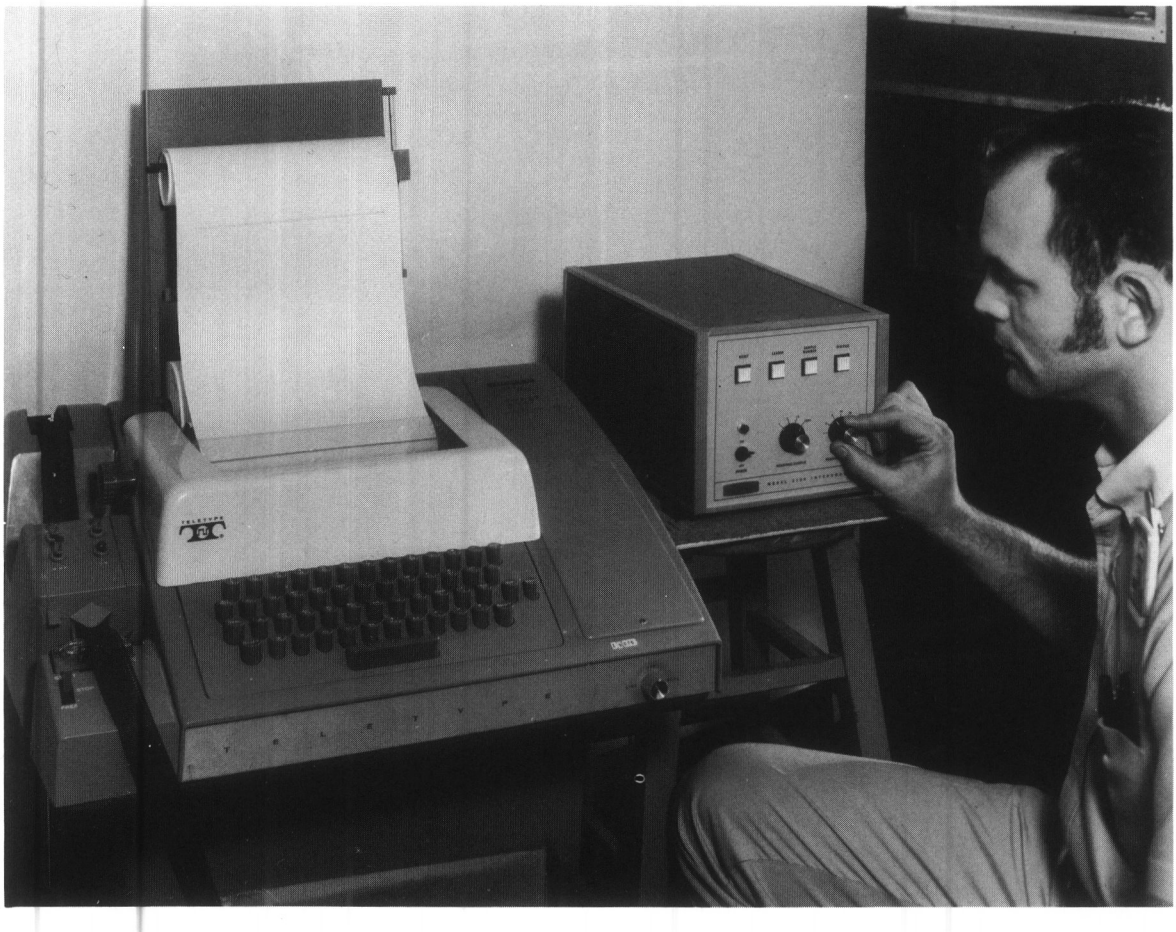

Figure 8. Beckman teletype coupler (right) and teletype (left) for recording CO_2 production data from infrared analyzer.

Figure 9. Specially constructed respiration chambers for holding rodents and cages in environment chamber during CO_2 measurements.

procedure reduced handling of the animals, and also allowed CO_2 production to be measured under practically the same conditions as isotope excretion. To minimize the contribution of CO_2 from bacterial respiration, clean cages were provided prior to measurement periods. This step was included even though tests failed to show that detectable levels of CO_2 evolved from extremely dirty cages. Temperature was monitored with a thermometer placed in each respiration chamber and a recording thermograph located inside the environment chamber. Temperature was stable throughout measurement periods, with fluctuations of less than 1°C except when the door of the environment chamber was opened. Actual measurement of CO_2 production was not begun until animals were allowed about a 1-hr period to equilibrate to the sample chamber and to recover from possible effects of being placed into a new environment.

Rodents were weighed immediately after removal from the chamber, and these weights were used for calculating CO_2 production on a per-gram basis. Carbon dioxide production rates were computed both as cc/hr and cc/hr/g for the 12-hr light period, for the 12-hr dark period, and as daily averages, yielding a total of six values with which to compare isotope elimination data. For these comparisons, the daily average value was considered of primary importance, because it probably was the best index to general metabolism.

All air passing through the respiration chambers was analyzed at 29 ± 1°C due to the warming effect of conduits leading from the chambers and due to heat from the analyzer. Therefore, assuming

negligible atmospheric pressure changes, no corrections to standard temperature and pressure were necessary for comparing one group of animals with another. However, to express CO_2 production at STP, all values reported need correction by a factor of 0.8502 which is appropriate for 29°C, 745 mmHg (Diem, 1962).

Each animal was measured twice (two separate 24-hr runs) during the period of isotope elimination. Except for a few instances when circumstances prevented it, these measurements were made within two weeks of each other. The two readings were averaged for each animal, and in most cases were very similar. This similarity indicated that the rodents had definite daily metabolic patterns (Appendix C), and that reproducible results were obtained from the measuring apparatus.

The air-supply and control unit is flexible and may be programmed to suit the design of the investigation. For these experiments, the equipment was arranged to measure three animals and ambient CO_2 concentration. The ambient value was subtracted from each experimental reading because animals breathed room air during measurement. In making multiple measurements with a single analyzer and recorder, it was necessary to measure one animal for a period of time, then switch to another, and so on. A total of 3 minutes per animal was allowed for any one reading period, the first minute of which was used for flushing the previous animal's air from the apparatus, and the remaining time was used for actual measurement. A recorded measurement every 5 seconds accounted for two lines of output on the teletype, with a 20-sec pause between lines. Only one line, 50 seconds, was devoted to the measurement

of ambient CO_2 (including flushing), after which the cycle returned to the first animal. This procedure yielded a total of seven lines for each 12-min cycle of measurement (Fig. 10).

Information provided as printed copy by the teletype was punched simultaneously on paper tape. The tape was used for data calculations which were done entirely by computer. Teletype print-out was used for monitoring purposes and for occasional editing of the punch tape. After conversion of the paper tape to magnetic tape, the following operations were required of the computer program; average the measurements represented by each two lines of output and subtract from each the average ambient reading for that 12-min period; correct for response characteristics of the analyzer; multiply relative data (all readings were recorded in percent of full scale) by factor appropriate for upscale calibration and air flow through the cages to obtain cc/hr; average five 12-min values of each animal to obtain hourly means; divide by weight of animal to obtain cc/hr/g; calculate dark-period, light-period, and daily CO_2 production; and plot hourly values of daily CO_2 cycle. Plotting was done on a CALCOMP cathode ray tube (CRT) plotter, which provided 35 mm photographic negatives of the graphs. Examples of these CRT plots are included in this report.

Experimental Treatments

The primary species investigated in laboratory experiments were cotton rats and harvest mice. Since white-footed mice were used in the field study, a group of five laboratory-reared animals of this species also were included.

```
01 0093 0094 0093 0094 0094 0095 0093 0094 0094 0094
01 0093 0094 0093 0093 0093 0092 0092 0091 0091 0091
02 0104 0103 0103 0103 0103 0102 0102 0102 0103 0102
02 0102 0102 0102 0102 0103 0102 0101 0100 0101 0100
03 0154 0152 0151 0153 0153 0155 0156 0156 0156 0154
03 0156 0156 0159 0161 0161 0162 0160 0162 0162 0163
04 0012 0014 0014 0013 0015 0014 0015 0015 0015 0015
```

Figure 10. Sample of teletype recording representing a 12-min CO_2 measurement on three animals.

First column is for identification, with number 04 the ambient CO_2 concentration. Numbers are read with one decimal place understood.

Rodents were selected to create heterogeneous treatment groups by making each group composed of both males and females, wild-caught and laboratory-born animals, and by having a wide range of body weights. The objective of this procedure was to create a group of animals which would exhibit metabolic differences, thus providing added opportunity to determine whether isotope elimination values would vary with metabolic rate. Whereas considerable constitutional differences existed within each group, an attempt was made to have experimental groups as similar to each other as possible, so that average values could be used. The primary objective in laboratory experiments was to make correlations on the individual level, and different treatments were provided only to induce metabolic changes in these individuals. Comparisons of group mean values is therefore only of secondary importance, but should reflect any significant correlations between isotope elimination and metabolic rates of animals within the groups.

Each treatment group except Peromyscus originally contained twelve animals. However, some mean values are based on less than twelve measurements per group due to mortality and also to occasional subjective discarding of invalid data (e.g., resulting from injection of isotopes into the gut or other organs, creating values which obviously were deviant from others in the group). In no case did the number in a treatment group fall below nine, and usually was eleven or twelve.

Treatment groups included a "control," which consisted of rodents kept at the usual laboratory colony temperature (22°C); one group held at a lower temperature (9°C); one which was irradiated at sublethal

doses and held at 22°C; and one which was kept at 22°C on a regimen of Tapazole (methimazole, Eli Lilly Company), which is a chemical metabolic inhibitor and goitrogen. Only cotton rats were treated with Tapazole, but the other three treatments were common to both principal species. Peromyscus were investigated only at the control level.

Animals held in cold temperature were kept in a chamber which normally maintained an environment of 9 ± 2°C. These rodents were placed in the cold temperature at least one month before experimentation to allow acclimation to the new environment. The chamber was the walk-in type, and during periods when the door was open the temperature not only rose, but required several hours to re-equilibrate. Therefore, it was important to minimize the frequency of entering the room to keep the experimental temperature constant. Temperature and humidity were monitored with a recording hygrothermograph, and the relative humidity averaged about 80%, considerably higher than the 40-50% of the colony room.

Irradiated animals were administered sublethal doses of gamma radiation from a Gammacell-200 ^{60}Co unit (Atomic Energy of Canada Limited), which delivered a dose of 7.0 rads/sec. Doses close to the LD_{50-30} probably are required to produce metabolic changes detectable by measuring CO_2 production rates. The LD_{50-30} values for cotton rats and harvest mice were estimated to be 958 rads and 947 rads, respectively (Dunaway et al., 1969). Therefore, half of each species received 900 rads and the other half received 700 rads. The lower dose was given because of the possibility of high mortality at 900 rads.

Irradiation took place two days before the first counting session after injection of isotopes, and was given in a single administration.

Tapazole-treated animals (cotton rats only) were supplied a 0.02% solution as drinking water 20 days prior to administration of isotopes. This concentration was chosen after reviewing experiments from the literature (Grosvenor, 1962), which used 0.0125% Tapazole solutions with laboratory animals. It was assumed that a slight increase in concentration would be appropriate for wild animals, since they may require higher levels to reduce thyroidal activity. The volume of drinking water consumed by these animals after being placed on Tapazole was measured, and it was found that they were consuming less (14.5 ml/day) than before being placed on the regimen. Assuming that the water had become unpalatable to the animals, and that unfavorable physiological effects might ensue, the concentration was reduced to 0.013% after 12 days on the previous concentration. Water consumption then increased to 15.6 ml/day, still somewhat below untreated water (app. 18.0 ml/day), but was left at this level. At the final concentration used, water consumption of these rats was equivalent to 2.03 mg Tapazole per day. The rats continued to receive Tapazole solution throughout the experiment.

Food and Water Consumption

Food consumption in cold-exposed and control cotton rats was measured to determine whether rate of intake was proportional to isotope elimination rates. Measurement was accomplished simply by weighing amounts of food added periodically to cage tops, and subtracting that

which remained at the end of the experiment. Water consumption also was measured on the same two groups of cotton rats by measuring the volume of water given, and subtracting the volume remaining at the end of the experiment. Relationships between rates of food and water consumption and isotope elimination values were tested by linear regression and correlation methods.

These methods for measuring food and water consumption are based on the assumption that disappearance of food and water from containers represents actual intake by the animals. Portions of food could be lost to the bedding material during gnawing, and water could be lost from the cannulate drinking bottles by an animal brushing against the aperture or "playing" with the drinking spout. Therefore, cages were examined for signs of these sources of error. No food was found on the bottoms of the cages, and no wet spots were observed in the area of the cage where dripping from the drinking tube might have occurred. Also, it was noted in the cages of animals which had high rates of water consumption that an area in the opposite end of the cage from the water bottle opening often became soggy with moisture. This indicated that relatively large quantities of water indeed were being consumed by these animals and deposited in another portion of the cage as urine. In view of these findings, confidence is placed in values obtained for food and water consumption.

Tissue Distribution Studies

Amount of radioactivity present in various organs of cotton rats was measured to determine which tissues accumulated the nuclides. The

fact that such studies had been performed previously (e.g., Nelson et al., 1960) was not a deterrent to performing this investigation because earlier studies did not involve the particular combination of isotopes and species used here. Also, previous investigations usually were conducted after isotopes had been present in animals for only a short time, whereas the present study was concerned with the distribution of the elements well into the final component of excretion. As mentioned previously, animals for these studies were remnants from the winter field study, and methods for tissue analyses are outlined in Section II.

C. Results

Measurements of primary interest were long-component elimination coefficients (k) of each isotope and daily average CO_2 production. Other elimination parameters determined from the data of each animal were biological half-times (T_b) and intercept values (a). In addition to daily averages, CO_2 production rates were calculated in cc/hr and cc/hr/g for both light and dark periods. Linear regression and correlation analyses were performed on all possible combinations of CO_2 production rates and isotope elimination values for both isotopes, using measurements from individual animals and also from treatment means. Correlation attempts were performed by treatment, by species, and on all treatments pooled. The results from all these tests cannot be reported, and in general it will be necessary to use group means as a somewhat qualitative way of reporting the findings. However, some of the more promising correlations based on individual animal data are discussed.

CO_2 Production and Isotope Excretion

Mean values of CO_2 production for all treatments are summarized in Table III. Mean hourly CO_2 production cycles for each treatment are shown in Figs. 11 and 12, and examples of individual daily CO_2 profiles from each treatment are included in Appendix C. Maximum-minimum and dark-light ratios of CO_2 production are given in Table IV.

Isotope elimination mean values are given in Tables V and VI. These values are presented graphically in Figs. 13 and 14 for the two principal species.

Tests for significant differences between treatment means, both for isotope values and CO_2 production, are given in Table VII. Results from other analyses of variance showed no differences in isotope or CO_2 values between males and females of any group. Tests also showed that treatment groups of the same species were not significantly different in mean body weights ($P = 0.01$).

For comparison and correlation purposes, all irradiated animals of the same species were considered as one treatment group. However, there were differences between 700- and 900-rad animals, and these differences followed the same trends in both species. In animals receiving 900 rads, CO_2 production was lower, ^{137}Cs elimination was less rapid, ^{137}Cs intercepts were higher, ^{59}Fe elimination was more rapid, but ^{59}Fe intercepts were almost identical to those of rodents receiving 700 rads. Although these differences were consistent and appeared to be predictable, only ^{137}Cs intercepts and ^{59}Fe slopes were significantly different at the two dose levels ($P = 0.01$). Only one case of mortality, a cotton rat, occurred among 24 irradiated animals.

TABLE III

MEAN CO_2 PRODUCTION VALUES FOR ALL EXPERIMENTAL GROUPS*

Treatment Group	Weight (g)	Light (cc/hr)	Light (cc/hr/g)	Dark (cc/hr)	Dark (cc/hr/g)	Daily (cc/hr)	Daily (cc/hr/g)
S.h. A	132.8	200.17	1.52	267.98	2.04	234.07	1.78
S.h. C	129.3	301.29	2.38	362.31	2.87	331.87	2.62
S.h. R	134.6	192.48	1.45	237.41	1.78	214.99	1.61
S.h. T	140.6	189.28	1.36	243.78	1.75	216.67	1.55
R.h. A	9.7	32.61	3.51	50.59	5.52	41.70	4.53
R.h. C	9.1	62.26	6.95	75.08	8.38	68.67	7.67
R.h. R	8.8	34.43	3.95	52.91	6.10	43.66	5.02
P.l. A	21.2	68.36	3.25	88.51	4.20	78.42	3.73

*S.h. - cotton rat, R.h. - harvest mouse, P.l. - white-footed mouse; A - ambient temperature (22°C), C - cold-exposed (9°C), R - irradiated, T - Tapazole-treated.

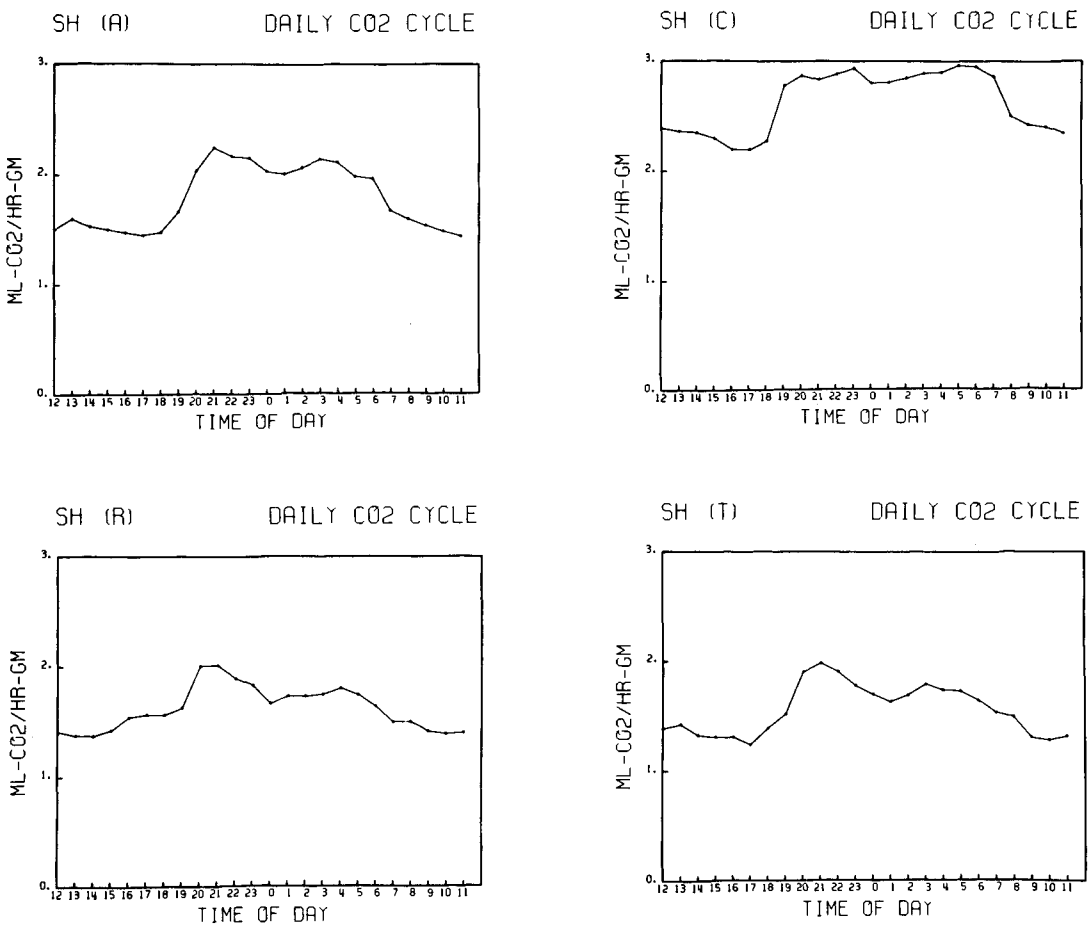

Figure 11. Average daily CO_2 production by cotton rats of different treatment groups.

A - 22°C; C - 9°C; R - irradiated; T - Tapazole-treated.

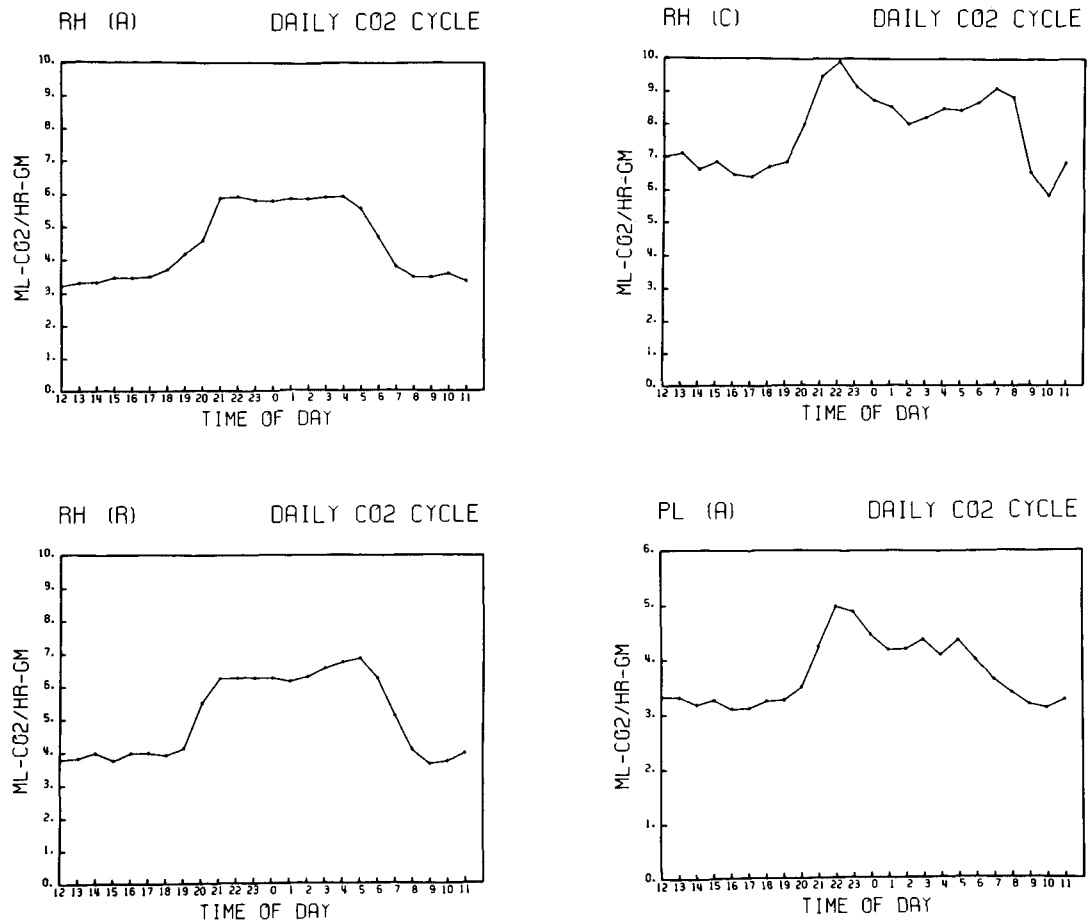

Figure 12. Average daily CO_2 production by harvest mice and white-footed mice of indicated treatment groups.

A - 22°C; C - 9°C; R - irradiated (Note different CO_2 scale for PL-A).

TABLE IV

MAXIMUM AND MINIMUM VALUES, DARK-PERIOD AND LIGHT-PERIOD AVERAGES OF CO_2 PRODUCTION*

Species and Treatment**	CO_2 (cc/hr/g)			CO_2 (cc/hr/g)		
	Maximum	Minimum	Ratio	Dark	Light	Ratio
S.h. A	2.46	1.24	2.01	2.04	1.52	1.37
S.h. C	3.32	2.09	1.59	2.87	2.38	1.25
S.h. R	2.19	1.23	1.79	1.78	1.45	1.23
S.h. T	2.07	1.15	1.84	1.75	1.36	1.29
R.h. A	6.72	3.01	2.22	5.52	3.51	1.57
R.h. C	10.39	5.05	2.05	8.38	6.95	1.20
R.h. R	7.46	3.36	2.21	6.10	3.95	1.53
P.l. A	5.12	2.80	1.85	4.20	3.25	1.30

*Max-min values based on highest and lowest hourly means; dark-light values based on 12-hr averages.

**S.h. - cotton rat, R.h. - harvest mouse, P.l. - white-footed mouse; A - ambient temperature (22°C), C - cold-exposed (9°C), R - irradiated, T - Tapazole-treated.

TABLE V

MEAN ^{137}Cs VALUES FOR ALL EXPERIMENTAL GROUPS*

Treatment** Group	K	T_b	a_{ln}	a_{arith}
S.h. A	-0.0817	8.6	3.6010	36.6
S.h. C	-0.0925	7.9	3.3266	27.8
S.h. R	-0.0900	7.9	3.9825	53.7
S.h. T	-0.0905	7.7	3.7178	41.2
R.h. A	-0.2041	3.7	4.4662	87.0
R.h. C	-0.1841	3.9	2.8326	17.0
R.h. R	-0.2142	3.5	4.1384	62.7
P.l. A	-0.2059	3.5	4.0453	57.1

*k = elimination coefficient.
T_b = biological half-time in days.
a_{ln} = intercept ($\log_e$).
a_{arith} = intercept (arithmetic).

T_b for each treatment obtained from

$$\frac{\Sigma(T_{b_i})}{N}$$, and not from $$\frac{0.693}{\overline{k}}$$

**S.h. - cotton rat, R.h. - harvest mouse, P.l. - white-footed mouse; A - ambient temperature (22°C), C - cold-exposed (9°C), R - irradiated, T - Tapazole-treated.

TABLE VI

MEAN ^{59}Fe VALUES FOR ALL EXPERIMENTAL GROUPS*

Treatment** Group	k	T_b	a_{ln}	a_{arith}
S.h. A	-0.0033	241.6	4.5659	96.1
S.h. C	-0.0035	225.7	4.4951	89.6
S.h. R	-0.0044	176.4	4.4822	88.4
S.h. T	-0.0045	192.1	4.4118	82.4
R.h. A	-0.0052	156.7	4.5511	94.7
R.h. C	-0.0046	177.8	4.4746	87.8
R.h. R	-0.0060	121.2	4.4308	84.0
P.l. A	-0.0032	289.0	4.4524	85.8

*k = elimination coefficient.
T_b = biological half-time in days.
a_{ln} = intercept ($\log_e$).
a_{arith} = intercept (arithmetic).

T_b for each treatment obtained from

$$\frac{\Sigma(T_{b_i})}{N}$$, and not from $$\frac{0.693}{\overline{k}}$$

**S.h. - cotton rat, R.h. - harvest mouse, P.l. - white-footed mouse; A - ambient temperature (22°C), C - cold-exposed (9°C), R - irradiated, T - Tapazole-treated.

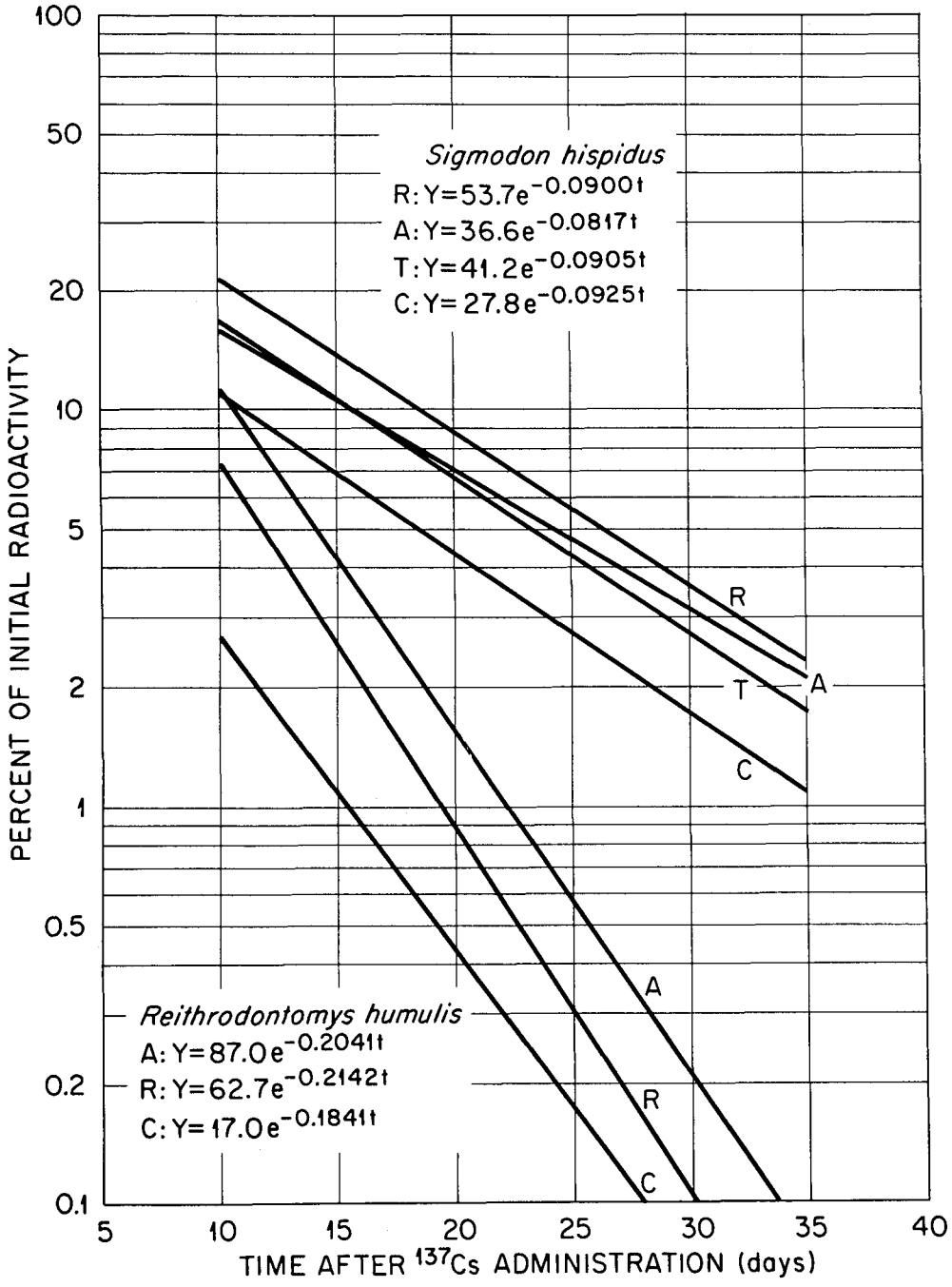

Figure 13. Elimination of ^{137}Cs by two species of rodents. A - 22°C; C - 9°C; R - irradiated; T - Tapazole-treated.

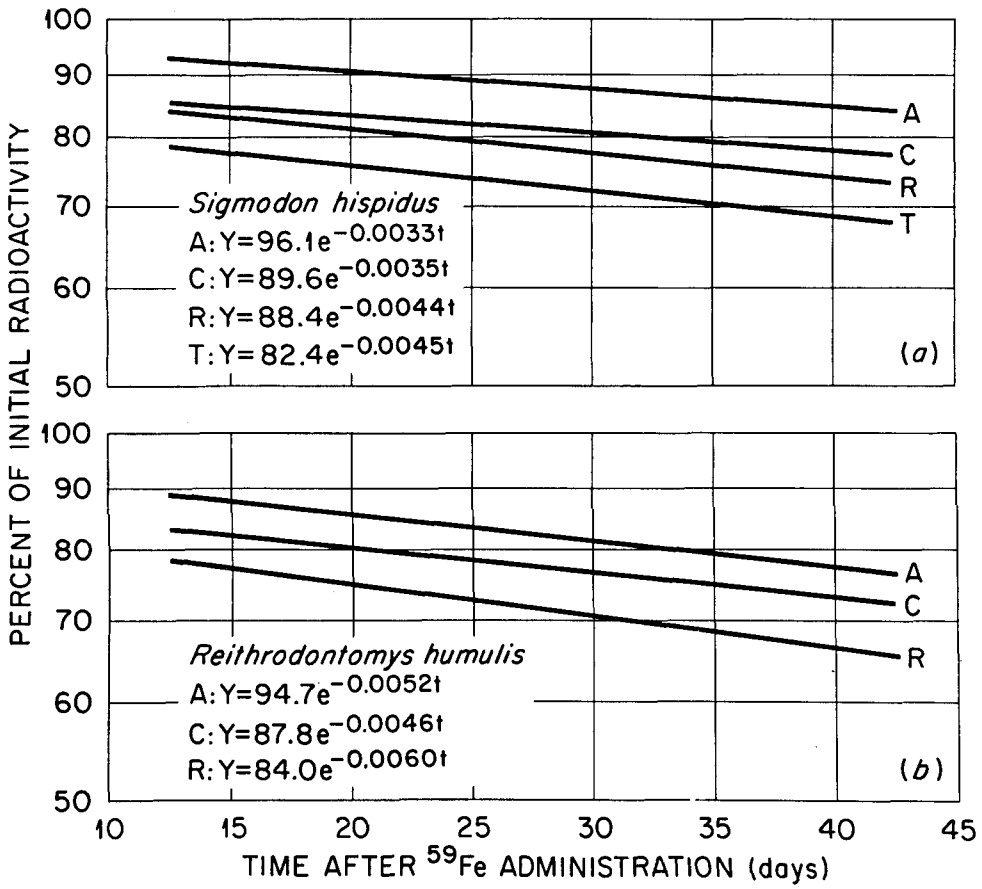

Figure 14. Elimination of ^{59}Fe by two species of rodents. A - 22°C; C - 9°C; R - irradiated; T - Tapazole-treated.

TABLE VII

RESULTS FROM DUNCAN'S 5% MULTIPLE RANGE TEST* FOR DIFFERENCES AMONG LABORATORY TREATMENT GROUPS**

Characteristic	C	A	R	T
		Sigmodon hispidus		
Daily CO_2 (cc/hr/g)	2.62	1.78	1.61	1.55
^{137}Cs Slope	-0.0925	-0.0817	-0.0900	-0.0905
^{137}Cs Intercept	27.8	36.6	53.7	41.2
^{59}Fe Slope	-0.0035	-0.0033	-0.0044	-0.0045
^{59}Fe Intercept	89.6	96.1	88.4	82.4
		Reithrodontomys humulis		
Daily CO_2 (cc/hr/g)	7.67	4.53	5.02	
^{137}Cs Slope	-0.1841	-0.2041	-0.2142	
^{137}Cs Intercept	17.0	87.0	62.7	
^{59}Fe Slope	-0.0046	-0.0052	-0.0060	
^{59}Fe Intercept	87.8	94.7	84.0	

*Means connected by same line not significantly different.

**A - 22°C; C - 9°C; R - irradiated; T - Tapazole-treated.

No significant correlations between general metabolism and excretion of ^{59}Fe were found. There were isolated cases of good within-treatment correlations both with slopes and intercepts, but for these to be valid the correlation should have been apparent among all treatments.

The only consistent correlation found with ^{137}Cs was between CO_2 production and Y-axis intercept values (a). Better correlations usually were found with logarithms of intercepts and were more predictable in harvest mice than in cotton rats. Equations for estimating metabolic rates from these relationships are, for Sigmodon

$$\text{Daily average } CO_2 \text{ production, cc/hr/g at 29°C, 745 mmHg} = 5.24 - 0.9172\ (\log_e a)$$

$$N = 39$$

$$r = -0.66\ (P < 0.01)$$

$$S_{y/x} = 0.36$$

and for Reithrodontomys,

$$\text{Daily average } CO_2 \text{ production, cc/hr/g at 29°C, 745 mmHg} = 12.51 - 1.80\ (\log_e a)$$

$$N = 31$$

$$r = -0.82\ (P < 0.01)$$

$$S_{y/x} = 1.02$$

These relationships are presented graphically in Fig. 15. Other equations describing statistically significant relationships for these two species, involving light- and dark-period metabolism, are given in Appendix D.

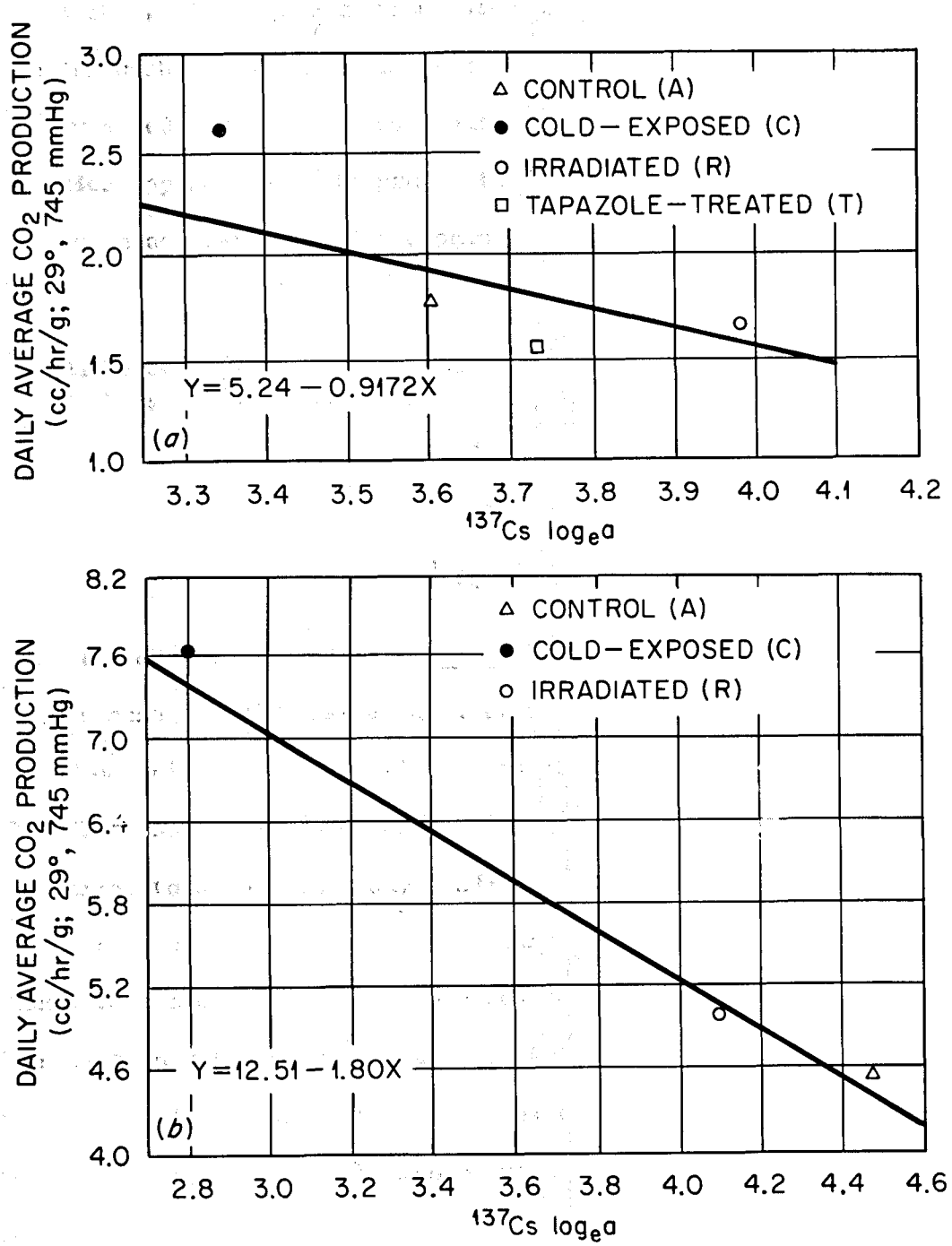

Figure 15. Index to metabolism in cotton rats (a) and harvest mice (b), using ^{137}Cs intercept values.

Line and equation derived from individual observations and not from treatment means as plotted. A - 22°C; C - 9°C; R - irradiated; T - Tapazole-treated.

Although ^{137}Cs elimination rates did not correlate well with metabolism within a species, there was a good interspecific relationship between CO_2 production and biological half-times (T_b). This correlation may be useful in predicting average metabolic rates in the field (assuming that the relationship between the two species is linear):

$$\text{Daily average } CO_2 \text{ production, cc/hr/g at 29°C, 745 mmHg} = 7.64 - 0.6773\ (T_b)$$

$$N = 86$$

$$r = -0.77\ (P < 0.01)$$

$$S_{y/x} = 1.42$$

No correlations were found with Peromyscus, probably due to the small number of animals and wide variability in the data.

Food and water consumption rates of cotton rats are given in Table VIII. Regression and correlation analyses on individual measurements failed to demonstrate any predictable relationship between rate of food or water consumption and elimination rates of either isotope. Instead, food and water intake appeared to be more closely linked with body size than with any other factor. Rate of food consumption differed significantly ($P = 0.01$) between cold-exposed and control group cotton rats, but no significant differences were found between these groups in rate of water consumption.

Tissue Distribution of Isotopes

Results from tissue distribution analysis for ^{59}Fe are given in Table IX. To determine the relative contribution of ^{59}Fe radioactivity

TABLE VIII

FOOD (PURINA LABORATORY CHOW) AND WATER CONSUMPTION OF COTTON RATS, WITH STANDARD ERRORS

Measurement		22°C	9°C
FOOD	g/day	10.4 (±0.42)	12.8 (±0.51)
	g/day/g*	0.078 (±0.0022)	0.090 (±0.0032)
WATER	ml/day	17.6 (±1.74)	19.4 (±1.98)
	ml/day/g*	0.135 (±0.0166)	0.145 (±0.0122)

*Body weight in grams.

TABLE IX

DISTRIBUTION OF ^{59}Fe IN COTTON RATS*

Component	Percent of Body Burden (±S.E.)	Percent/Gram of Tissue Weight
Kidneys	3.2 (±0.15)	3.72
Lungs	2.6 (±0.57)	5.53
Heart	1.6 (±0.27)	4.71
Liver	17.7 (±1.06)	5.01
Spleen	1.6 (±1.14)	14.55
G. I. Tract	11.1 (±1.03)	1.88
Skin-Hair	13.1 (±1.26)	0.70
Carcass (residual)	49.0 (±2.82)	0.76

*Data from 8 animals approximately 50 days after administration of isotope.

in the blood, samples were analyzed in 0.3 ml quantities. These samples averaged 3.2 ± 0.50 (S.E.) percent of the total body burden. Based on a relationship given by Diem (1962), the total blood volume of an average-sized cotton rat was calculated to be about 8 ml. If these calculations are correct, ^{59}Fe radioactivity in blood accounted for about 85% of the total body burden (3.2 [8/0.3]). Therefore, a major portion of radioactivity found in all tissues is attributed to the presence of blood. It is apparent from per-gram values of Table IX that hemopoietic and blood-rich organs accumulated ^{59}Fe. Tissues with low per-gram values probably reflected radioactivity solely from blood.

Approximately 80% of total body ^{137}Cs was found in the carcass and skin-hair compartments, with the remainder being uniformly distributed among other organs. Radiocesium has a high affinity for skeletal muscle (Nelson et al., 1960), and the distribution of the nuclide in the residual carcass could be explained on that basis. The skin also is endowed with muscle tissue, but a portion of the radioactivity in the skin-hair compartment possibly was due to external self-contamination.

VI. DISCUSSION AND CONCLUSIONS

A. Correlations Between Isotope Elimination and Metabolism

Iron-59

Elimination of ^{59}Fe by cotton rats in the field correlated well with expected metabolic rates during winter and spring. Biological half-times (T_b) were 108 days in winter and 144 days in the spring (Fig. 4a, page 28). Field groups of both Sigmodon and Peromyscus showed faster elimination rates for ^{59}Fe than laboratory animals, but when some of the winter cotton rats were transferred from the field to the laboratory, their elimination rates were not altered (Fig. 4b, page 28). Furthermore, no consistent relationship between ^{59}Fe elimination and CO_2 production could be demonstrated in the laboratory. On an average basis, however, ^{59}Fe intercept values of laboratory cold-exposed animals were lower than those of the control groups of both cotton rats and harvest mice. Intercepts of field animals were different from those of the laboratory, but no distinction was apparent between seasonal values (Table I, page 29).

Most of the ^{59}Fe was associated with blood, and it is obvious that changes occurring in this tissue would affect whole-body elimination rates. When red blood cells are destroyed and hemoglobin is degraded, mechanisms for recycling of iron must be functional, or a loss of iron will result. If the number of erythrocytes or amount of hemoglobin is altered, the total ^{59}Fe body burden could be affected (Bonnet et al., 1960; Stevens et al., 1953). Such hematological

impairments due to cold stress (Sealander, 1960, 1962) and irradiation (Ludewig and Chanutin, 1951; Okunewick and Kretchmar, 1967) have been reported, and these changes could affect ^{59}Fe loss rates. Moreover, it may even be possible to assess hemopoietic damage by measuring radio-iron elimination (Kitchings et al., 1968).

Hemoglobin and hematocrit values tend to increase during winter or prolonged cold exposure (Sealander, 1962). Apparently these changes precipitate rapid iron turnover, as evidenced by faster ^{59}Fe excretion by the winter field animals. However, environmental factors which caused ^{59}Fe to be eliminated more rapidly in the field apparently were not present in sufficient magnitude to affect ^{59}Fe elimination by laboratory cold-exposed animals. It is possible that low temperatures used in the laboratory were not sufficient to elicit responses similar to those of field animals, or perhaps differences in available iron between laboratory and field diets were important in iron retention. It also is possible that a long enough period of acclimation to the cold environment was not provided in the laboratory experiment. There appears to be a definite time requirement for such acclimation (Stullken and Hiestand, 1954), and perhaps the one-month period allowed was not adequate. One-month acclimation failed to produce total hematological adjustment in cold-exposed laboratory rats (Hannon and Young, 1959), but this same time allowance was adequate in another experiment (Deb and Hart, 1956).

Irradiated animals of both species incorporated less ^{59}Fe and eliminated it more rapidly than cold-exposed animals (Table VI, page 54).

A different mechanism is responsible for this rapid elimination because, unlike cold stress, ionizing radiation decreases erythrocyte numbers (Okunewick and Kretchmar, 1967). Despite this opposite effect on blood composition, both irradiation and cold increased the loss rate of radioiron.

No relationship between these hematological adaptations or damages (with subsequent faster turnover of ^{59}Fe) and general body metabolism was demonstrated in the laboratory. However, it is important to emphasize that laboratory isotope elimination rates of this nuclide must be interpreted with caution since they likely could be much different from elimination rates in animals undergoing long-term hematological adjustments in nature.

Cesium-137

Rate of final-component ^{137}Cs elimination within a species was not altered by any treatment used in laboratory or field (Figs. 3 and 13, pages 27 and 55), and therefore was assumed to be unrelated to general metabolic rate. However, there was a statistically significant interspecific relationship between metabolism and cesium turnover in cotton rats and harvest mice, which may have been due only to body size.

A better indicator for predicting individual metabolism within a species appeared to be ^{137}Cs intercept values, which indicate early levels of isotope incorporation or rates of initial excretion. It was assumed before the experiment that if radioisotope elimination and general metabolism were related, the relationship would be apparent

only during final-component excretion. This assumption was based on the thesis that initial excretion of injected isotopes represents extracellular flushing of unassimilated material, and would not be expected to relate to any particular metabolic processes. Therefore, it was somewhat unanticipated that intercept values would correlate so well with metabolic rates (Fig. 15, page 59). Such a correlation indicates that either the rate of initial assimilation or early excretion of injected ^{137}Cs is more related to metabolic rate than is the elimination of the nuclide once it is incorporated into body tissues. This conclusion is in qualitative agreement with Baker et al. (1968), whose data also suggested a correlation between metabolism and cesium intercept values, but is in contrast with findings by Furchner and Richmond (1963).

Using the predictive relationship derived from intercept values, cotton rats in the field had average metabolic rates of 2.24 cc/hr/g (29°C, 745 mmHg) in winter and 1.99 in the spring. Compared with values of laboratory controls and cold-exposed animals, 1.78 and 2.62, respectively, these estimated field metabolic rates were not as high as anticipated. Laboratory cold-exposed cotton rats were maintained at a higher temperature (9°) than the measured mean daily environmental temperature (4°) of winter animals, and still had higher metabolic rates than the winter animals. Moreover, laboratory animals were greatly restricted in physical activity. These estimations could indicate that the index to metabolism derived from intercepts is not valid, particularly in extrapolating to the field. They also might

suggest that free-ranging animals do not have unusually high average metabolic rates due to efficient behavioral mechanisms for heat and energy conservation. Animals in nature can reduce energy demands with well insulated nests and burrows, and by huddling in groups to conserve heat.

Cesium is excreted rapidly by rodents, and the duration of metabolic rate estimation from intercept values or initial-component excretion is therefore limited to about two to five days. Ideally, it would be desirable in energy flow studies to measure metabolism of free-ranging animals over a long period of time, but this does not appear to be feasible with ^{137}Cs. However, periodic sampling of a population at regular intervals may allow a series of short-term measurements to be integrated into a single long-term estimate of metabolism. It is conceivable that short-term measurements would be desirable in certain instances, such as during a short period of severe weather.

It was somewhat surprising that free-ranging animals did not eliminate ^{137}Cs more rapidly than animals caged in the laboratory (Fig. 3, page 27). Cesium apparently is closely linked with skeletal muscle functions (Nelson et al., 1960), and increased physical activity in the field theoretically should have affected the retention of cesium by these tissues. However, an investigation by Furchner et al. (1963) failed to demonstrate that enforced physical exercise effects the rate of ^{137}Cs excretion in mice.

The field data necessarily evoked conclusions based on indirect evidence and group mean values, but the laboratory phase of this project

sought correlations on the individual level. The latter was accomplished by making 24-hr metabolic measurements with a sophisticated apparatus, and comparing these values with isotope elimination parameters. Whereas the field experiment was considered essential, in that it served to test the method under "normal" conditions, the laboratory experiments must be considered more conclusive because of their more quantitative nature.

It is believed that if a relationship exists between CO_2 metabolism and long-component elimination of either of the isotopes employed, it would have been manifested during this experiment. The technology employed for making sustained metabolic measurements was highly reliable, and measurements were made with equal accuracy at all levels of activity. Other features of the technique which make the data worthy of confidence include (1) measuring CO_2 production of animals in their usual cages, (2) being able to measure several animals at a time, (3) having an open system, (4) providing extremely stable air stream flow through the measuring chambers, (5) obtaining digital output and handling data by computer, and (6) having a completely automatic system which minimized disturbance of experimental animals.

B. Carbon Dioxide Production

Excellent resolution between individuals was obtained in measurements of CO_2 production (Appendix C). In most cases, the two measurements made on each animal were similar, both in terms of average values and character of the daily cycle. Apart from correlations or

lack of correlations with isotope data, several observations were made concerning CO_2 production rates obtained for the different treatments.

As expected, cold-exposed animals produced more CO_2 than controls of both species (Table III, page 49). Light-period averages for cold animals of both species were higher than the dark-period averages of other treatment groups, indicating the important overriding influence of low environmental temperatures. All three species displayed definite nocturnality, as evidenced by the shape of daily CO_2 production curves (Figs. 11 and 12, pages 50 and 51) and dark-light ratios (Table VI, page 54).

Sigmodon displayed a subtle bimodal pattern during the dark, with a short period of "rest" occurring around midnight. This observation is in general agreement with physical activity patterns of cotton rats as measured by Calhoun (1945), and indicates that CO_2 production can be used effectively as an index to activity. The nighttime resting level never reached the low level of daytime metabolism, and the period of rest was less pronounced in the cold-exposed animals. Nocturnality was more apparent in the control group (A) than in any of the other three treatments.

Apparently the concentration of Tapazole used in this experiment was not high enough to produce distinct metabolic differences, although daily CO_2 rates of Tapazole-treated animals were significantly lower than controls. Tapazole animals had the lowest ^{59}Fe intercepts of all treatment groups, but ^{137}Cs intercepts were not different from the controls. A higher concentration of Tapazole probably would result in

rejection of drinking water. For future experiments Tapazole probably should be administered in food or by injection, allowing greater amounts to be delivered daily. Near-lethal irradiation produced no detectable metabolic rate changes in cotton rats.

In Reithrodontomys, all treatment groups showed definite nocturnality, with only the cold-exposed animals displaying biomodal activity during darkness. As in Sigmodon, the lowest hourly average CO_2 production rate during the dark was always greater than the highest hourly average rate during the light period. Irradiated animals had slightly higher daily average metabolic rates than controls, but showed about the same degree of nocturnality. Using dark-light ratios as an index to nocturnality (Pearson, 1960), cold-exposed harvest mice were less nocturnal than mice of the other two treatments.

Peromyscus was similar qualitatively to cotton rats in daily CO_2 patterns, but the secondary peak of production was less pronounced.

VII. SUMMARY

Elimination of ^{137}Cs and ^{59}Fe by wild small rodents was investigated in the field and laboratory. These investigations were conducted to test the hypothesis that elimination rates of radionuclides can be used in the field to determine animal respiration rates. A double-tagging technique was employed, and isotopes were administered intraperitoneally. Attention was focused on the final-component elimination phase of both isotopes, rather than on the complete characterization of excretion patterns.

For field testing, cotton rats (Sigmodon hispidus) and a lesser number of white-footed mice (Peromyscus leucopus) were live-trapped, brought to the laboratory, injected with isotopes, and released into field enclosures. Thereafter they were trapped weekly and counted for radioactivity to determine isotope elimination rates. This procedure was carried out in winter and in spring, assuming that metabolic rates would be higher in winter and would result in faster radionuclide turnover. Field elimination rates were compared with laboratory rates to determine whether greater metabolic demands in the field due to physical activity would increase isotope elimination rates.

Excretion of ^{137}Cs and ^{59}Fe by rodents in the laboratory was examined in conjunction with carbon dioxide production rates. Sigmodon hispidus and Reithrodontomys humulis were the primary species investigated, but some Peromyscus were used for comparison with field data. Metabolic rates were determined by measuring CO_2 production with an

infrared gas analyzer, which operated in connection with a specially constructed air supply and control unit. Continuous readings were taken automatically over 24-hr periods to establish daily average metabolic rates. These rates were compared with isotope elimination in animals maintained under different experimental conditions. Treatments included "controls" at 22°C, cold-exposure at 9°C, irradiation at near-lethal doses, and administration of a chemical metabolic inhibitor (Tapazole). These treatments were common to cotton rats and harvest mice, except only the former species received Tapazole. White-footed mice were investigated only at the control level.

Both species used in the field studies eliminated ^{59}Fe more rapidly than laboratory animals, but only cotton rats excreted the isotope faster in winter than in spring. However, when some of the winter cotton rats were transferred to the laboratory, they failed to alter their elimination patterns over a period of several weeks. No differences between seasonal values of either species were noted in ^{137}Cs excretion, and field values did not differ from those obtained in the laboratory. There was, however, a striking difference between the two species in rate of ^{137}Cs turnover, with Peromyscus excreting the isotope about twice as fast as Sigmodon.

Long-component biological half-times (T_b) of ^{59}Fe for Sigmodon were 108 days in winter, 144 days in spring, and 176 to 242 days in the laboratory; for Peromyscus, these values were 50 days in winter, 47 days in spring, and 289 days in the laboratory. Biological half-times for Reithrodontomys ranged from 121 to 178 days for the different laboratory treatment levels.

The T_b values for ^{137}Cs elimination for Sigmodon were 7.5 days in winter, 7.9 days in spring, and 7.7 to 8.6 days at different treatment levels in the laboratory; in Peromyscus, these values were 3.4 days in winter, 3.6 days in spring, and 3.5 days in the laboratory. Biological half-times of ^{137}Cs in laboratory Reithrodontomys ranged from 3.5 to 3.9 days.

Daily average CO_2 production rates (cc/hr/g; 29°C, 745 mmHg) for cotton rats ranged from 1.55 to 2.62; for harvest mice, 4.53 to 7.67; and averaged 3.73 for white-footed mice. Mean hourly CO_2 production rates were plotted to obtain daily metabolic cycles for individuals. These plots clearly indicated nocturnality in all three species, with Reithrodontomys being the most nocturnal. Regular peaks of increased CO_2 production were noted, and it was assumed that these peaks were concurrent with periods of accelerated physical activity.

Rate of long-component ^{137}Cs loss from rodents was not affected by metabolic rate changes at any treatment level; but, as in the field experiment, interspecific differences were noted in ^{137}Cs elimination rates, and also in metabolic rates. Long-component intercept values of ^{137}Cs, which indicated the level of incorporation or rate of initial-component excretion, exhibited a strong relationship with CO_2 production within a species. Based on this relationship, equations were derived to predict metabolism from Y-axis intercept values. These equations have potential use in estimating metabolism of rodents in the field for energy flow measurements.

Although ^{59}Fe elimination appeared to correlate with metabolic rates in the field, laboratory experiments failed to demonstrate a

predictable relationship between excretion of this nuclide and general metabolism. Iron-59 was associated primarily with the blood, and the physiology of this tissue presumably controlled whole-body excretion of the isotope.

LITERATURE CITED

LITERATURE CITED

Auerbach, S. I. 1958. The soil ecosystem and radioactive waste disposal to the ground. Ecology 29:522-529.

_____. 1965. Radionuclide cycling: current status and future needs. Health Physics 11:1355-1361.

Baker, C. E. and P. B. Dunaway. 1969. Retention of Cs-134 as an index to metabolism in the cotton rat (Sigmodon hispidus). Health Physics 16:227-230.

_____, _____, and S. I. Auerbach. 1968. Measurement of metabolism in cotton rats by retention of cesium-134. Oak Ridge National Laboratory, Report ORNL-TM-2069.

Bonnet, J. D., A. L. Orvis, A. B. Hagedorn, and C. A. Owen, Jr. 1960. Rate of loss of radioiron from mouse and man. Am. J. Physiol. 198: 784-786.

Brody, S. 1945. Bioenergetics and growth. Reinhold Pub. Corp., New York. 1023 p.

Buckner, C. H. 1964. Metabolism, food capacity, and feeding behavior in four species of shrews. Can. J. Zool. 42:259-279.

Calhoun, J. B. 1945. Diel activity rhythms of the rodents, Microtus ochrogaster and Sigmodon hispidus hispidus. Ecology 26:251-273.

Chappelle, E., B. W. Gabrio, A. R. Stevens, Jr., and C. A. Finch. 1955. Regulation of body iron content through excretion in the mouse. Am. J. Physiol. 182:390-392.

Comar, C. L., R. H. Wasserman, F. W. Lengemann, D. N. Tapper, A. R. Twardock, E. L. Gasteiger, J. C. Thompson, Jr., and B. W. Hess. 1962. Fission product metabolism and response in laboratory and domestic animals and planning study for evaluation of radioactive contamination of the food chain. U.S.A.E.C., Report TID-14780. p. 30-33.

Crossley, D. A. 1963. Use of radioactive tracers in the study of insect-plant relationships. In International Atomic Energy Agency. Radiation and radioisotopes applied to insects of agricultural importance. p. 43-54.

Davis, J. J. 1963. Cesium and its relationships to potassium in ecology. In Vincent Schultz and A. L. Klement, ed. Radioecology. Reinhold Pub. Corp., New York. p. 539-556.

Deb, C. and J. S. Hart. 1956. Hematological and body fluid adjustments during acclimation to a cold environment. Can. J. Biochem. Physiol. 34:959-966.

Diem, K., ed. 1962. Documenta Geigy: scientific tables. Geigy Pharmaceutical Co., Ardsley, New York. 778 p.

Dunaway, P. B. 1968. Life history and populational aspects of the eastern harvest mouse. Am. Midl. Nat. 79:48-67.

_____ and S. V. Kaye. 1964. Weights of cotton rats in relation to season, breeding, and environmental radioactive contamination. Am. Midl. Nat. 71:141-155.

_____, L. L. Lewis, J. D. Story, J. A. Payne, and J. M. Inglis. 1969. Radiation effects in the Soricidae, Cricetidae, and Muridae. In D. J. Nelson and F. C. Evans, ed. Proceedings of the second national symposium on radioecology. p. 173-184.

Foreman, H., R. L. Huff, J. M. Oda, and J. Garcia. 1952. Use of a chelating agent for accelerating excretion of radioiron. Proc. Soc. Exp. Biol. Med. 79:520-524.

Furchner, J. E. and C. R. Richmond. 1963. Effect of environmental temperatures on retention of cesium-137 by mice. J. Appl. Physiol. 18:786-788.

_____, _____, and G. A. Trafton. 1963. Lack of effect of exercise on the excretion of cesium-137 in mice. Los Alamos Scientific Laboratory, Report LAMS-3034. p. 34-40.

Golley, F. B. 1968. Secondary productivity in terrestrial communities. Am. Zool. 8:53-59.

Grosvenor, C. E. 1962. Methimazole: a goitrogen without apparent extrathyroidal effects in the rat. Endocrinology 70:934-936.

Hannon, J. P. and D. W. Young. 1959. Effect of prolonged cold exposure on the gross blood composition of the rat. Am. J. Physiol. 197: 1008-1012.

Hemington, J. G. 1967. Relationship between oxygen consumption and zinc-65 excretion in Perognathus longimembris. M. S. Thesis, University of Southern California.

Hood, S. L. and C. L. Comar. 1953. Metabolism of cesium-137 in rats and farm animals. Arch. Biochem. Biophysics 45:423-433.

Hull, N. C. 1967. Statpak. Oak Ridge National Laboratory, Report ORNL-TM-1863. p. 88-102.

Johnson, J. E., D. Garner, and G. M. Ward. 1968. Influence of dietary potassium, rubidium, or sodium on the retention time of radiocesium in rats. Proc. Soc. Exp. Biol. Med. 127:857-860.

Kaye, S. V. 1961. Laboratory life history of the eastern harvest mouse. Am. Midl. Nat. 66:439-451.

Kitchings, J. T., P. B. Dunaway, and J. D. Story. 1969. Uptake and excretion of cesium-134 from fallout simulant and vegetation by cotton rats. Health Physics 17:265-277.

_____, _____, _____, and L. E. Tucker. 1968. Use of radioiron (Fe-59) as an index to hemopoietic damage caused by ionizing radiation. J. Tenn. Acad. Sci. 43:85-87.

Kleiber, M. 1947. Body size and metabolic rate. Physiol. Rev. 27: 511-539.

_____. 1961. The fire of life. John Wiley and Sons, Inc. New York. 468 p.

Lee. J. S. and N. Lifson. 1960. Measurement of total energy and material balance in rats by means of doubly labeled water. Am. J. Physiol. 199:238-242.

LeFebvre, E. A. 1964. The use of $D_2{}^{18}O$ for measuring energy metabolism in Columba livia at rest and in flight. The Auk 81:403-416.

Lifson, N., G. B. Gordon, and R. McClintock. 1955. Measurement of total carbon dioxide production by means of $D_2{}^{18}O$. J. Appl. Physiol. 7:704-710.

Ludewig, S. and A. Chanutin. 1951. Effect of whole body x-irradiation on the storage of iron in liver, spleen and kidney of rats. Am. J. Physiol. 166:384-386.

McClintock, R. and N. Lifson. 1958. Determination of the total carbon dioxide outputs of rats by the $D_2{}^{18}O$ method. Am. J. Physiol. 192: 76-78.

Meyer, B. J. and R. K. Meyer. 1944. Growth and reproduction of the cotton rat, Sigmodon hispidus hispidus, under laboratory conditions. Mammalogy 25:107-129.

Morrison, P. R. 1947. An automatic apparatus for the determination of oxygen consumption. J. Biol. Chem. 169:667-679.

_____. 1948a. Oxygen consumption in several small wild mammals. J. Cell. Comp. Physiol. 31:69-96.

_____. 1948b. Oxygen consumption in several mammals under basal conditions. J. Cell. Comp. Physiol. 31:281-292.

_____ and O. P. Pearson. 1946. The metabolism of a very small mammal. Science 104:287-289.

Mraz, F. R. and H. Patrick. 1956. Organic factors controlling the excretory pattern of potassium-42 and cesium-134 in rats. J. Nutrition 61:535-546.

_____ and _____. 1957. Factors influencing excretory patterns of cesium-134, potassium-42, and rubidium-86 in rats. Proc. Soc. Exp. Biol. Med. 94:409-412.

Nelson, A., S. Ullberg, H. Kristoffersson, and C. Ronnback. 1960. Distribution of radiocesium in mice. Acta Radiologica 55:374-384.

O'Farrell, T. P., S. I. Auerbach, and P. B. Dunaway. 1966. Incorporation of a thymidine analog by indigenous rodents. Oak Ridge National Laboratory, Report ORNL-3907.

Odum, E. P. 1961. Excretion rate of radioisotopes as indices of metabolic rates in nature: biological half-life of zinc-65 in relation to temperature, food consumption, growth, and reproduction in arthropods (abstract). Biol. Bull. 121:371-372.

_____. 1963. Fundamentals of ecology. W. B. Saunders Co., Philadelphia. 558 p.

_____. 1968. Energy flow in ecosystems: a historical review. Am. Zool. 8:11-18.

_____ and F. B. Golley. 1963. Radioactive tracers as an aid to the measurement of energy flow at the population level in nature. In Vincent Schultz and A. L. Klement, ed. Radioecology. Reinhold Pub. Corp., New York. p. 403-410.

Okunewick, J. P. and A. L. Kretchmar. 1967. Mathematical model for post-irradiation hematopoietic recovery. The Rand Corporation, Memorandum RM-5272-PR.

Orr, H. 1967. Excretion of orally administered zinc-65 by the cotton rat in the laboratory and field. Health Physics 13:15-20.

Pearson, O. P. 1947. The rate of metabolism of some small mammals. Ecology 28:127-145.

_____. 1960. The oxygen consumption and bioenergetics of harvest mice. Physiol. Zool. 33:152-160.

Prosser, C. L. and F. A. Brown. 1961. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia. 675 p.

Pulliam, H. R., G. W. Barrett, and E. P. Odum. 1969. Bioelimination of tracer zinc-65 in relation to metabolic rate in mice. In D. J. Nelson and F. C. Evans, ed. Proceedings of the second national symposium on radioecology. p. 725-730.

Reichle, D. C. 1967. Radioisotope turnover and energy flow in terrestrial isopod populations. Ecology 48:351-366.

Relman, A. S. 1956. The physiological behavior of rubidium and cesium in relation to that of potassium. Yale J. Biol. Med. 9:248-262.

Ringer, S. 1882. An investigation regarding the action of rubidium and cesium salts compared with the action of potassium salts on the ventricle of the frog's heart. J. Physiol. 4:370-379.

Sealander, J. A. 1960. Hematological changes in deer mice acclimated at different ambient temperatures. Am. J. Physiol. 198:195-199.

_____. 1962. Seasonal changes in blood values of deer mice and other small mammals. Ecology 43:107-119.

Short, H. L. and F. B. Golley. 1968. Metabolism. In International Biological Program. Handbook 7: a practical guide to the study of the productivity of large hervivores. Blackwell Scientific Publications, Oxford and Edinburgh. p. 95-105.

Stevens, A. R., P. L. White, D. M. Hegsted, and C. A. Finch. 1953. Iron excretion in the mouse. J. Biol. Chem. 203:161-165.

Stullken, D. E. and W. A. Hiestand. 1954. Hematological changes influenced by short and long exposure to cold. Proc. Soc. Exp. Biol. Med. 86:253-255.

Van Dyne, G. M. 1965. Regress - a multiple regression and correlation program with graphic output for model evaluation. Oak Ridge National Laboratory, Report ORNL-TM-1288.

Wagner, C. K. 1968. Relationship between oxygen consumption, ambient temperature, and excretion of 32-phosphorus in laboratory and field populations of cotton rats. M. S. Thesis, University of Georgia.

Wiegert, R. G. 1968. Thermodynamic considerations in animal nutrition. Am. Zool. 8:71-81.

APPENDICES

APPENDIX A

This appendix contains photographs which illustrate experimental methods described in the text.

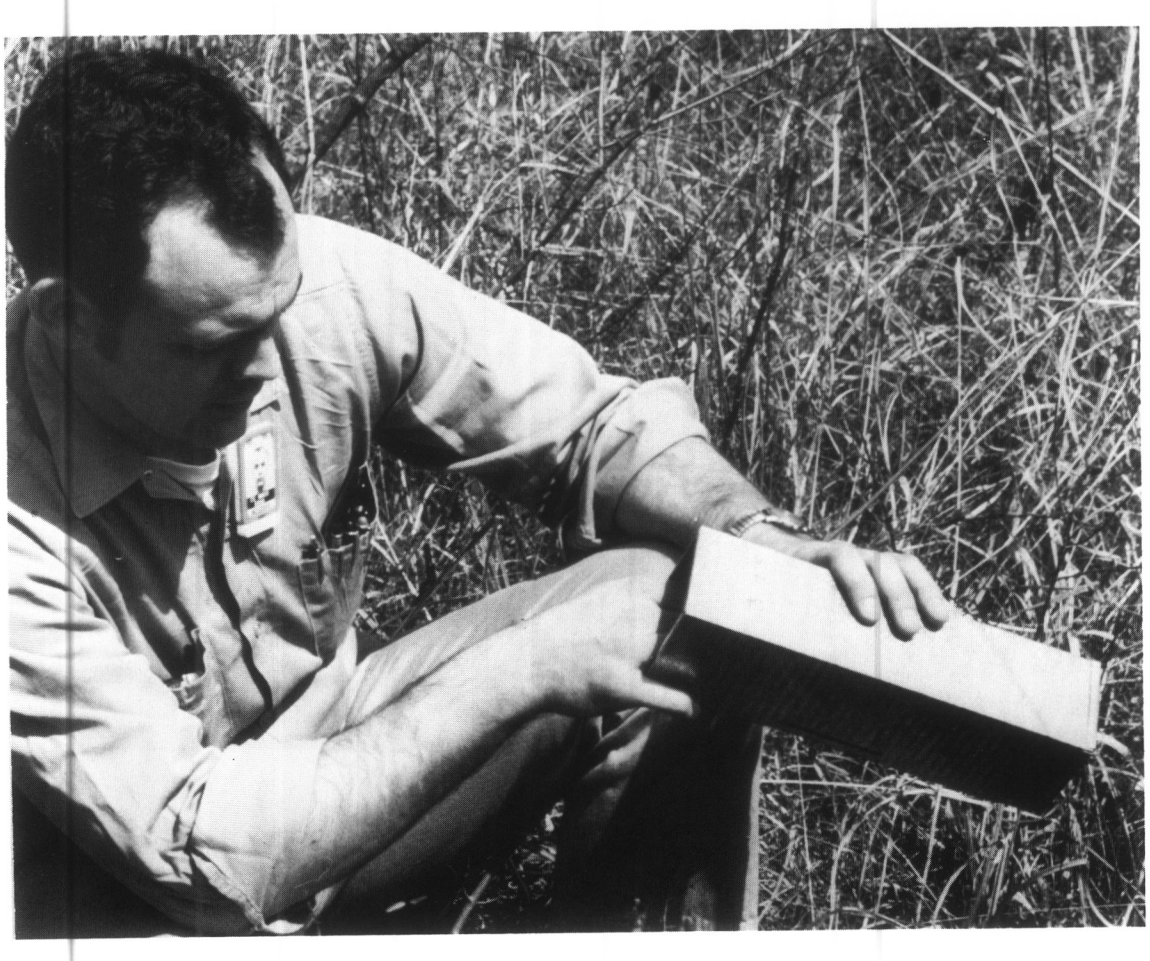

Figure 16. Sherman live-trap used for capturing small mammals.

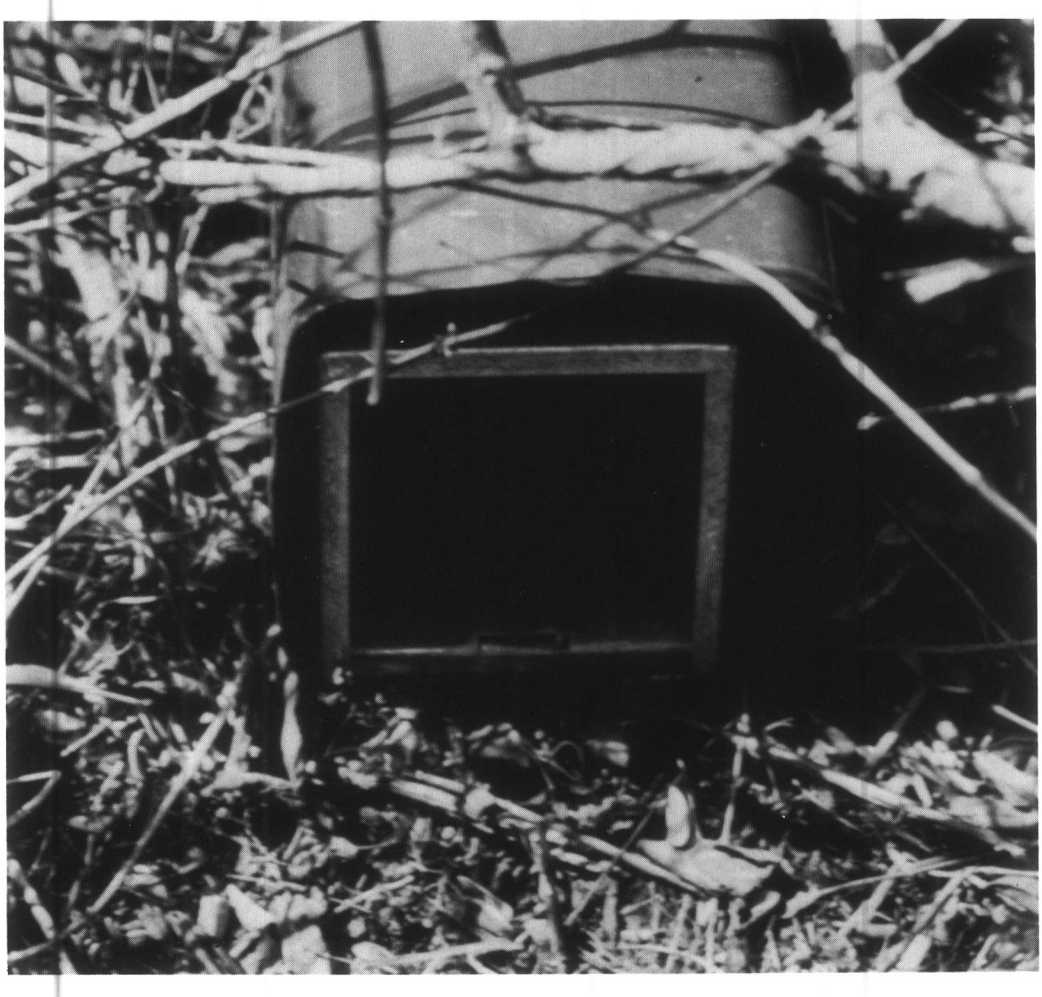

Figure 17. Mammal trap placed inside protective metal container and set for animal capture.

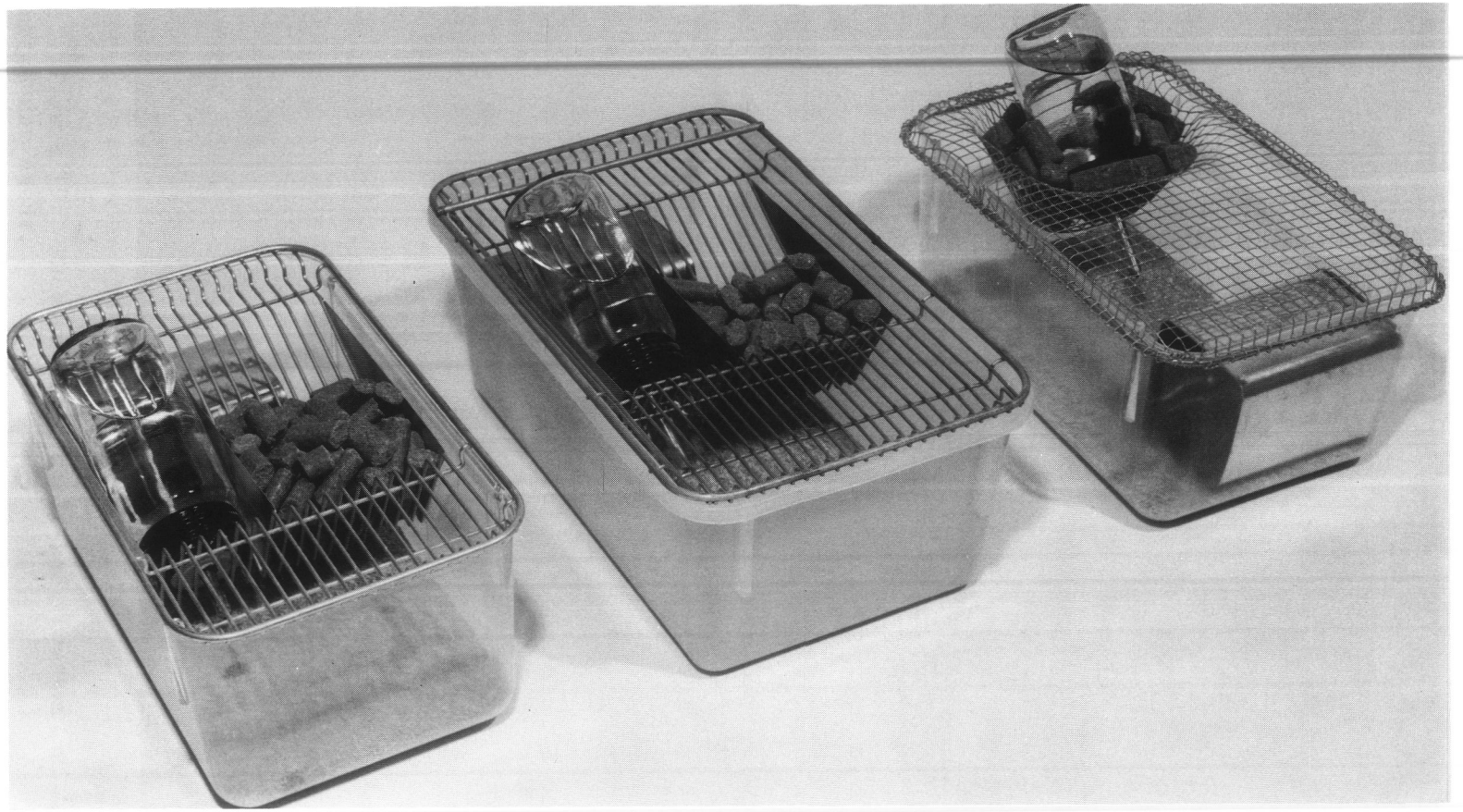

Figure 18. Laboratory cages used for (left to right) Peromyscus leucopus, Sigmodon hispidus, and Reithrodontomys humulis.

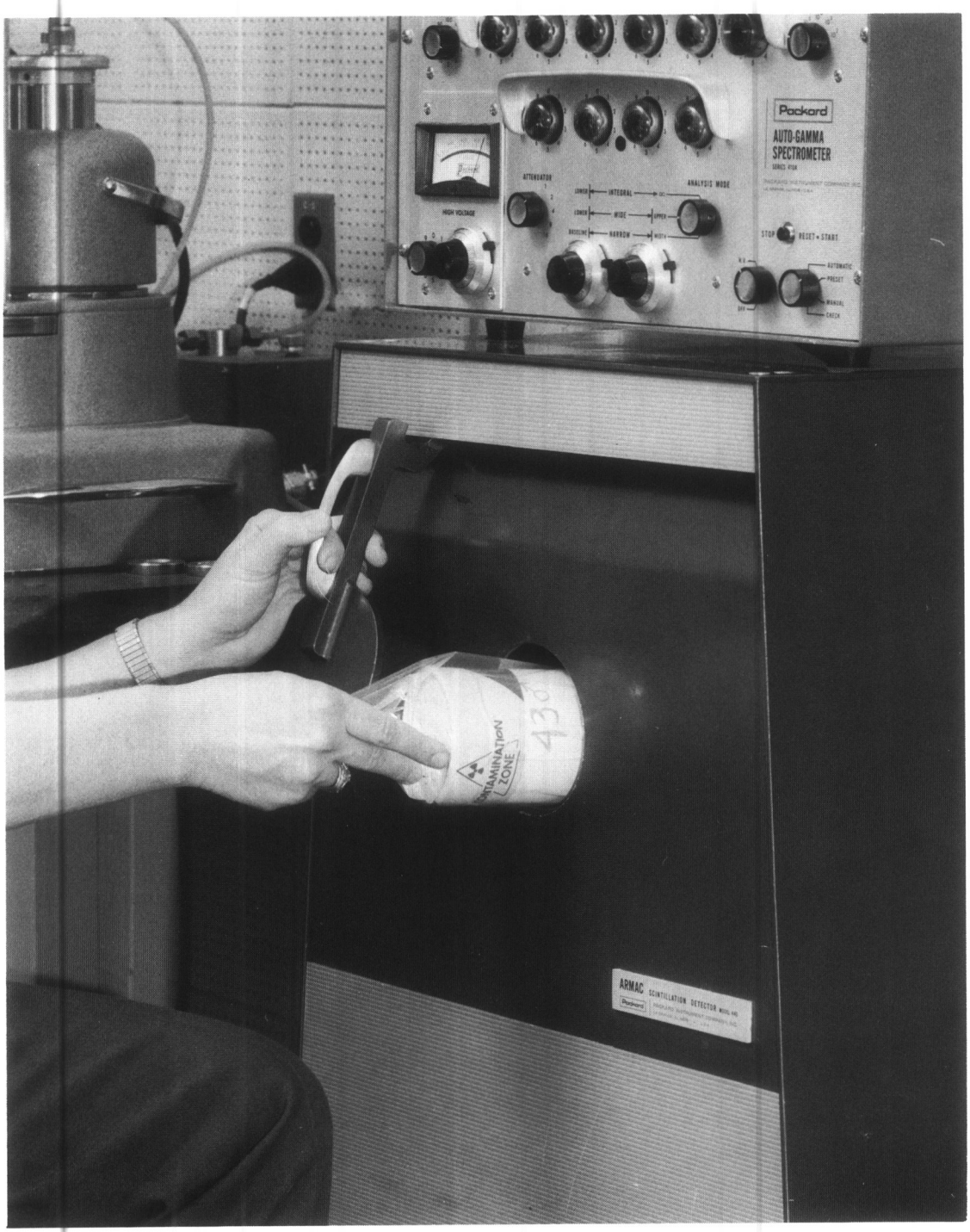

Figure 19. Packard Armac whole-body counter, with animal being inserted for assay.

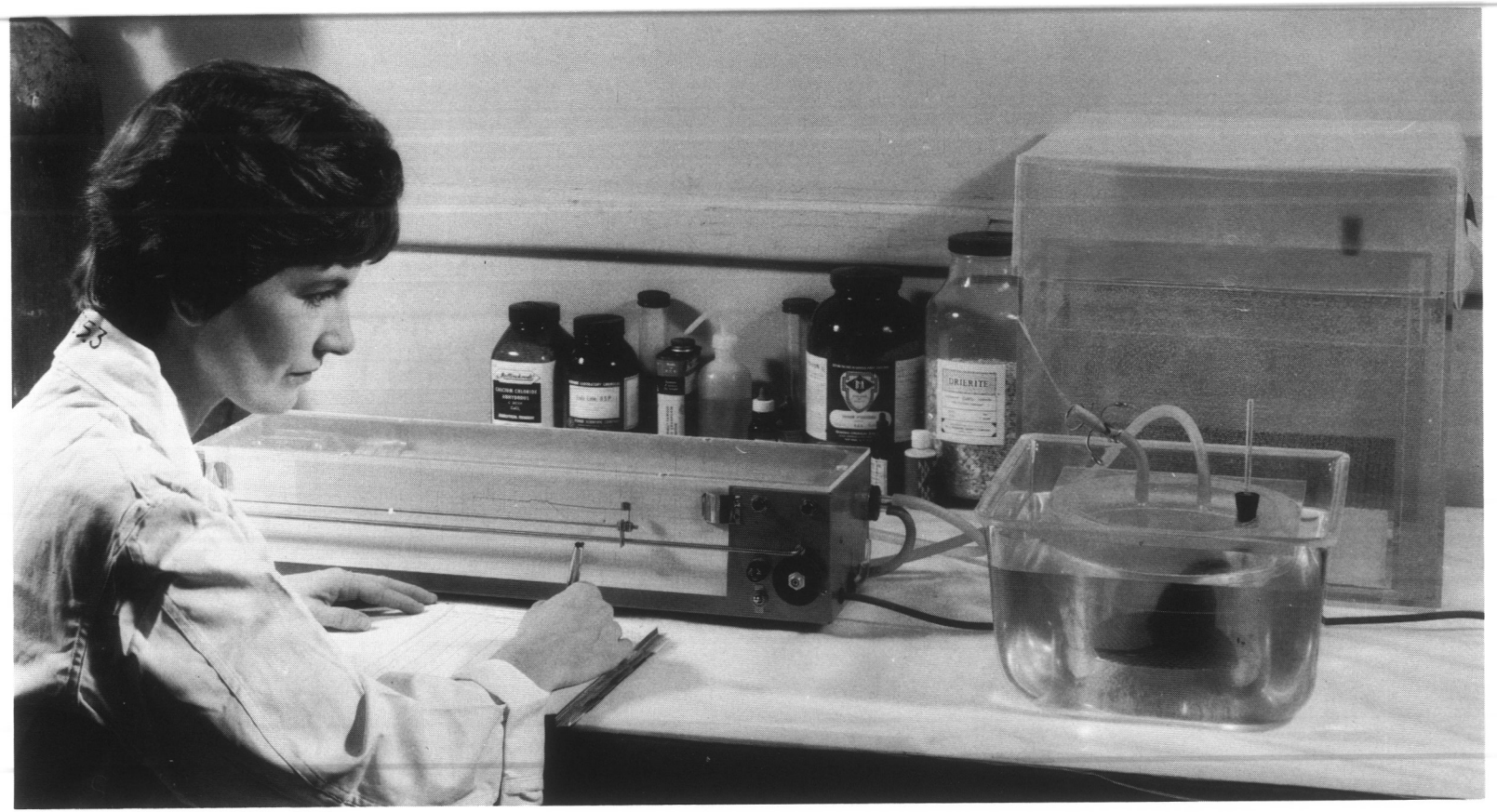

Figure 20. Aloe oxygen respirometer in operation; spirometer and recorder on left, animal chamber on right.

Figure 21. Field enclosures during winter experiments.

Nearest pen corresponds to lower-left enclosure of Figure 2, page 23.

Figure 22. Field study area as seen during spring experiments.

APPENDIX B

This appendix contains a list of identified biota in the field study area. No significance is attached to the order of any listings, which are provided primarily as a matter of record.

1. Flora Identified in Field Enclosures

   Blackberry (*Rubus* spp.)

   Sumac (*Rhus* sp.)

   Broomsedge (*Andropogon* spp.)

   Honeysuckle (*Lonicera japonica*)

   Goldenrod (*Solidago* spp.)

   Black-eyed Susan (*Rudbekia hirta*)

   Panic grass (*Panicum* sp.)

   Strawberry (*Fragaria virginiana*)

   Rush (*Juncus* sp.)

   Thistle (*Cirsium vulgare*)

   Small trees, few and scattered

   - Red maple (*Acer rubrum*)
   - Post oak (*Quercus stellata*)
   - Tulip poplar (*Liriodendron tulipifera*)
   - Dogwood (*Cornus florida*)
   - Blackgum (*Nyssa sylvatica*)
   - Sweetgum (*Liquidambar styraciflua*)
   - Black cherry (*Prunus serotina*)

Red cedar (Juniperus virginiana)

Virginia pine (Pinus virginiana)

2. Bird Species Identified on Field Study Area

White-throated Sparrow (Zonotrichia albicollis)

Field Sparrow (Spizella pusilla)

Cardinal (Richmondena cardinalis)

Phoebe (Sayornis phoebe)

Eastern Towhee (Pipilo erythrophthalmus)

Common Goldfinch (Spinus tristis)

Downy Woodpecker (Dendrocopos pubescens)

Blue Jay (Cyanocitta cristata)

Crow (Corvus brachyrhynchos)

Carolina Chickadee (Parus atricapillus)

Ruby-crowned Kinglet (Regulus calendula)

Blue-gray gnatcatcher (Polioptila caerulea)

Prairie Warbler (Dendroica discolor)

Yellow-throated Warbler (Geothlypis trichas)

Red-winged Blackbird (Agelaius phoeniceus)

Mourning Dove (Zenaidura macroura)

3. Other Fauna Trapped or Seen

Mammals

Rice Rat (Oryzomys palustris)

White-footed Mouse (Peromyscus leucopus)

Golden Mouse (Peromyscus nuttalli)

Pine Mouse (Microtus pinetorum)

Short-tail Shrew (Blarina brevicauda)

Eastern Cottontail (Sylvilagus floridanus)

Amphibians

Cricket Frog (Acris gryllus)

Chorus Frog (Pseudacris sp.)

Reptiles

Rat Snake (Elaphe obsoleta obsoleta)

Brown Snake (Storeria dekayi dekayi)

Garter Snake (Thamnophis sirtalis sirtalis)

APPENDIX C

This appendix contains examples of daily carbon dioxide measurements made on individual animals. Each figure shows results from three animals, two measurements on each, and identification of treatment and animal is given in the upper left region of each plot. Species names are abbreviated; SH - cotton rat, RH - harvest mouse, PL - white-footed mice. Treatment symbols are defined as follows: A - ambient temperature (22°C), C - cold-exposed (9°C), R - irradiated, T - Tapazole-treated.

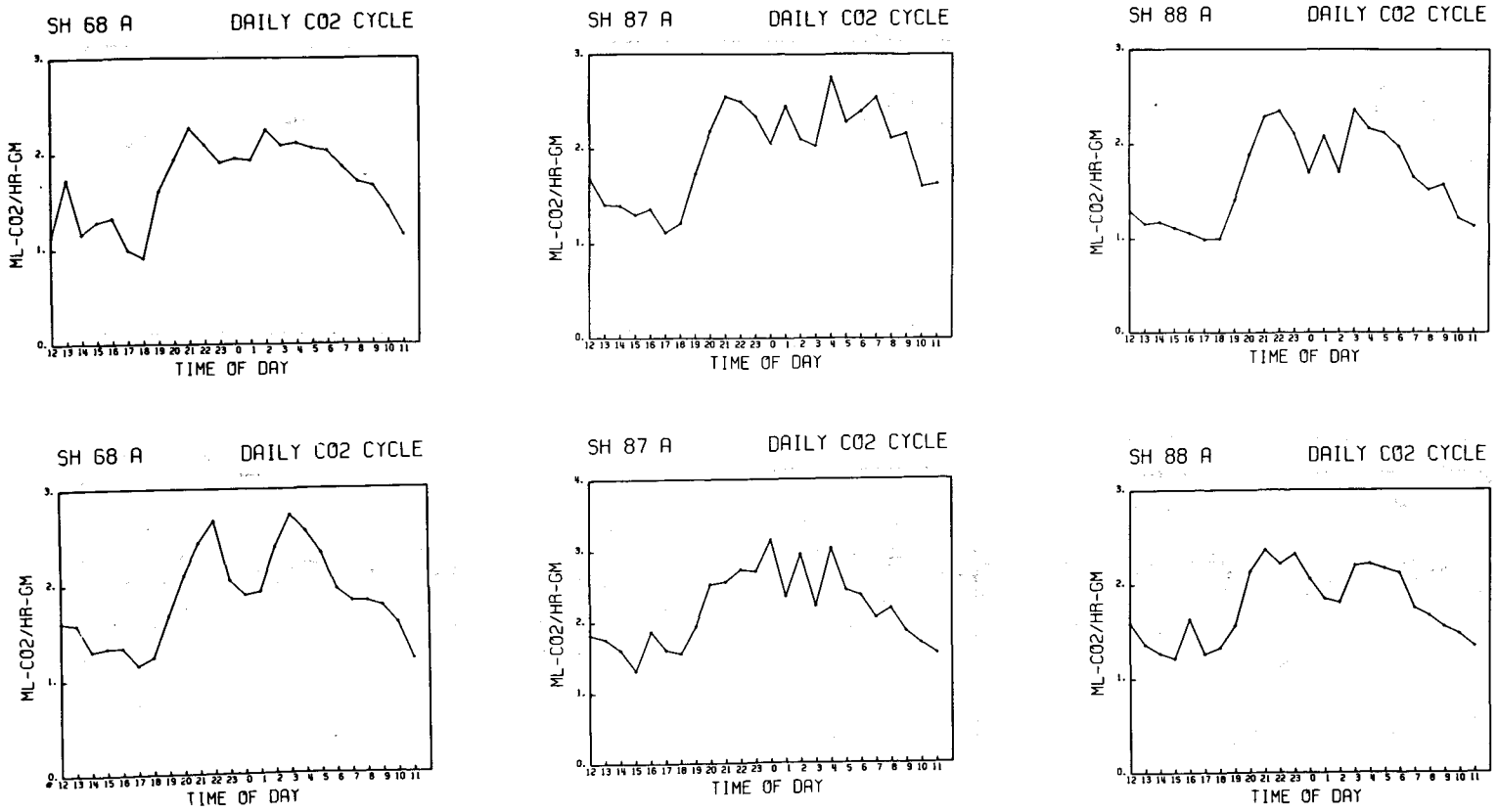

Figure 23. Sample of CO_2 measurements made on cotton rats at 22°C.

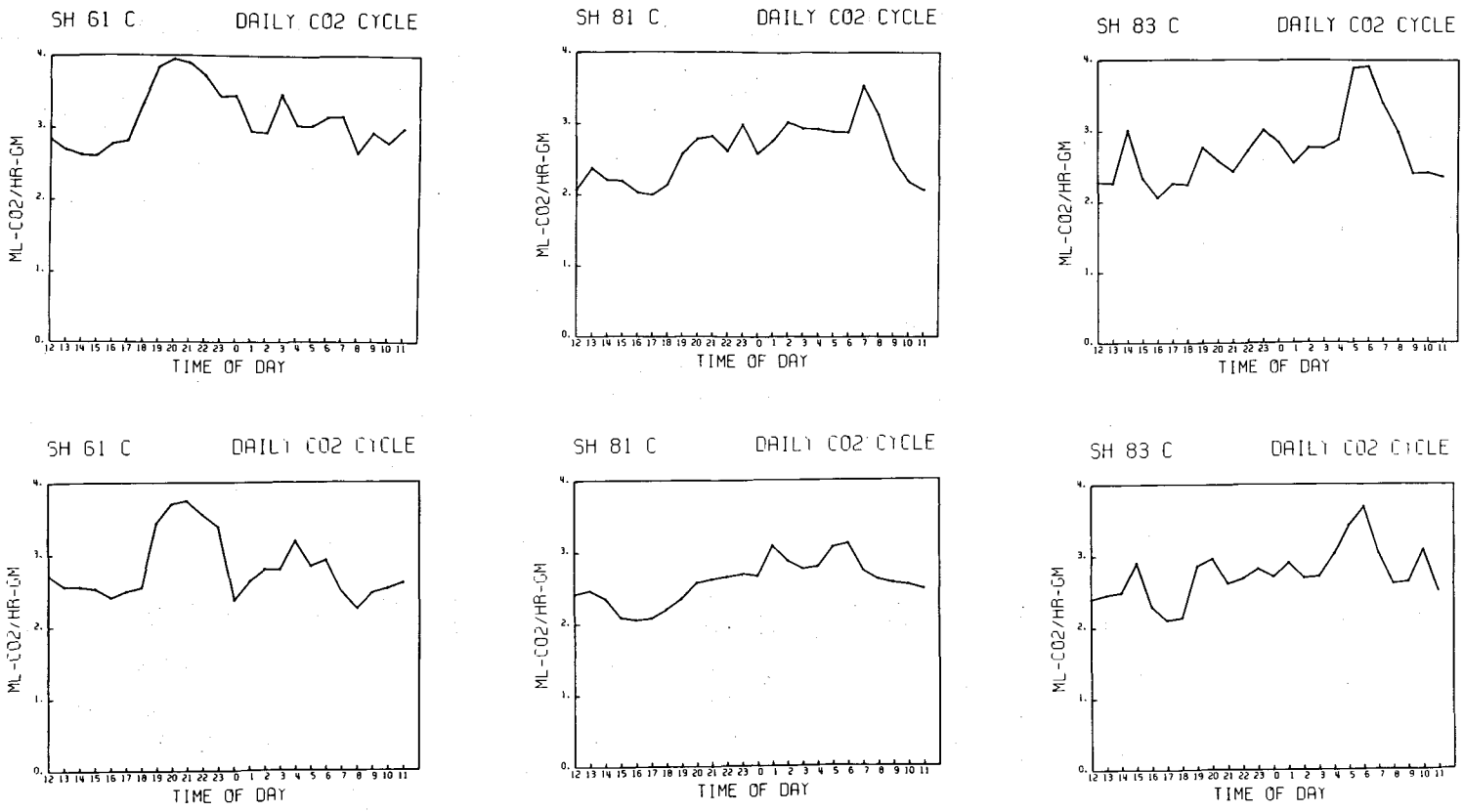

Figure 24. Sample of CO_2 measurements made on cotton rats at 9°C.

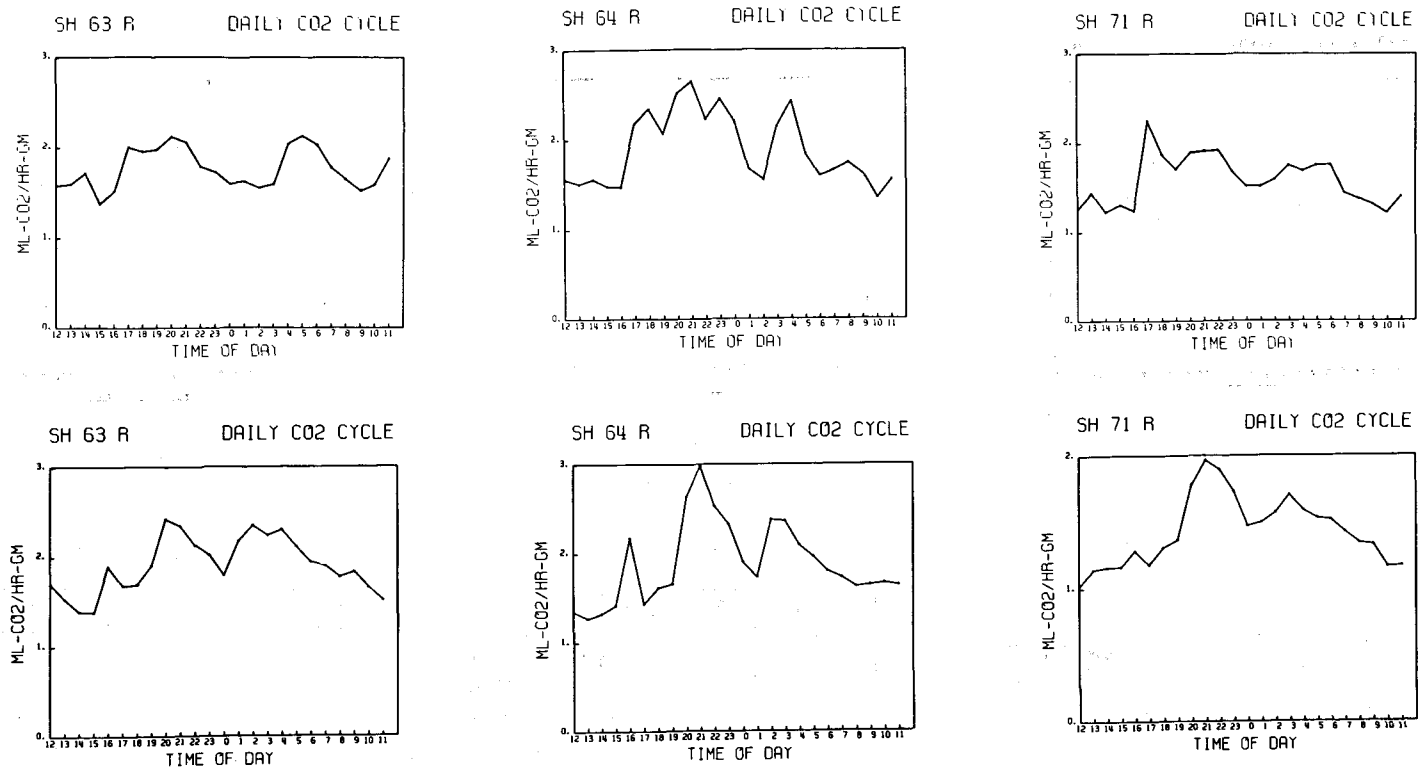

Figure 25. Sample of CO_2 measurements made on irradiated cotton rats.

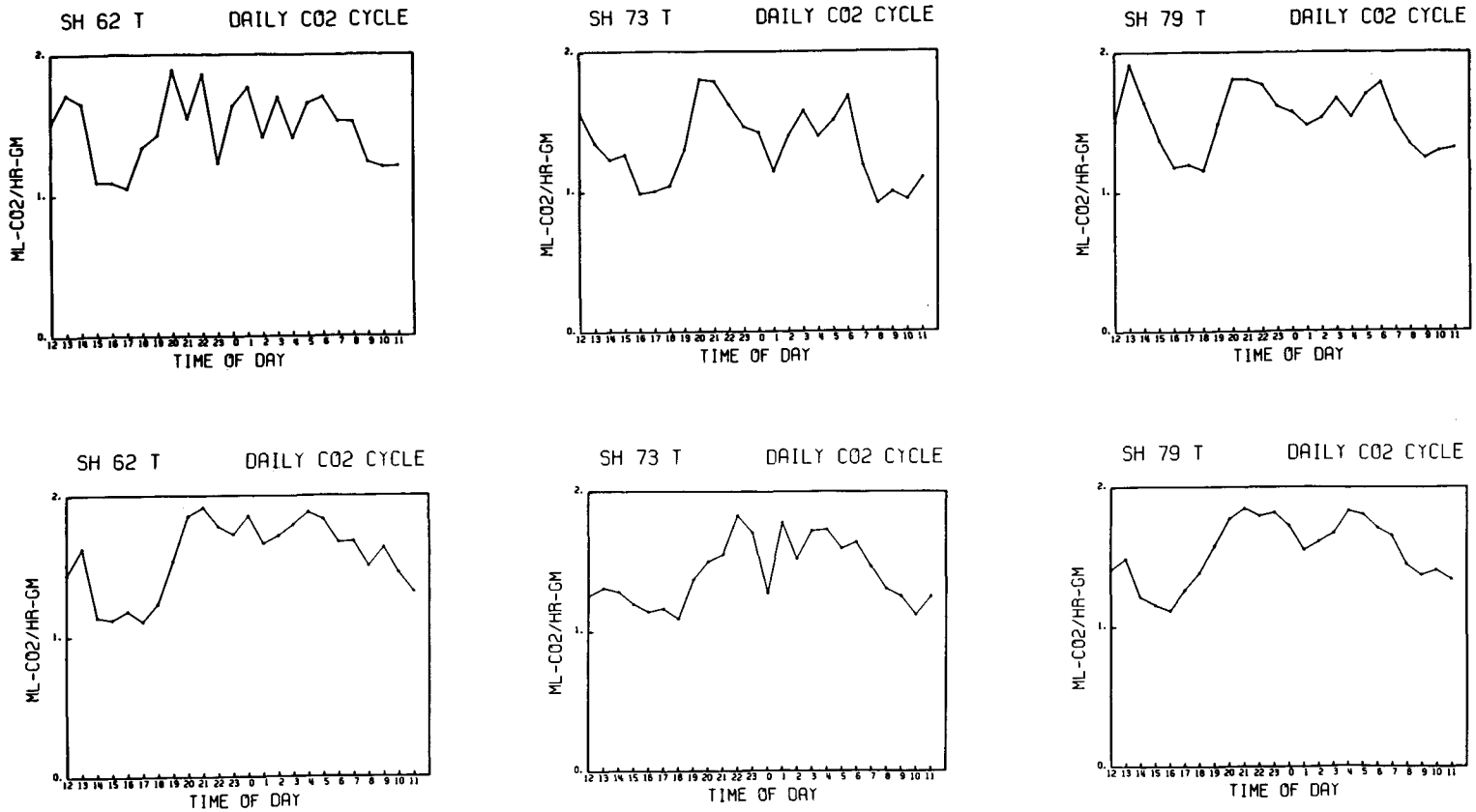

Figure 26. Sample of CO_2 measurements made on Tapazole-treated cotton rats.

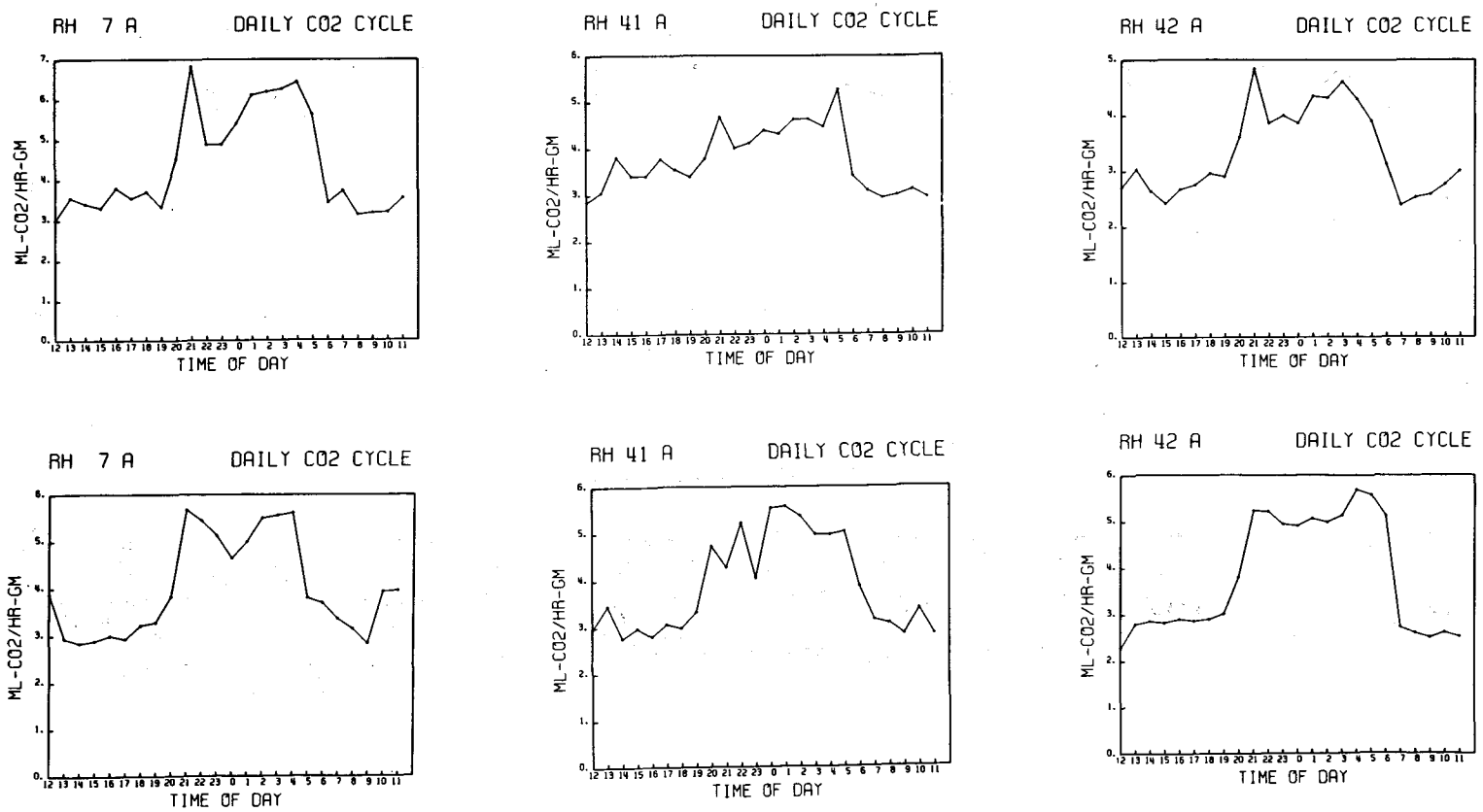

Figure 27. Sample of CO_2 measurements made on harvest mice at 22°C.

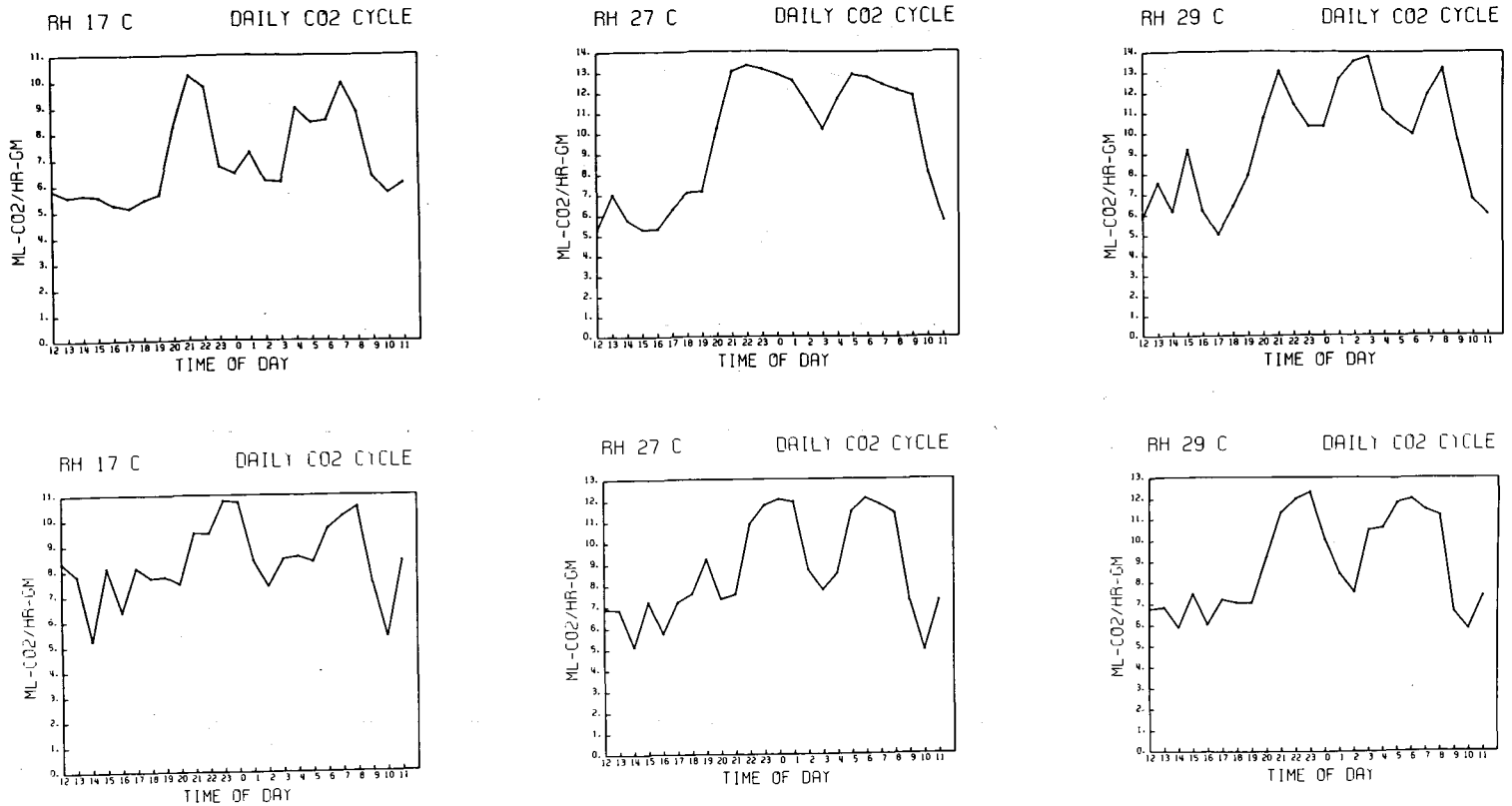

Figure 28. Sample of CO_2 measurements made on harvest mice at 9°C.

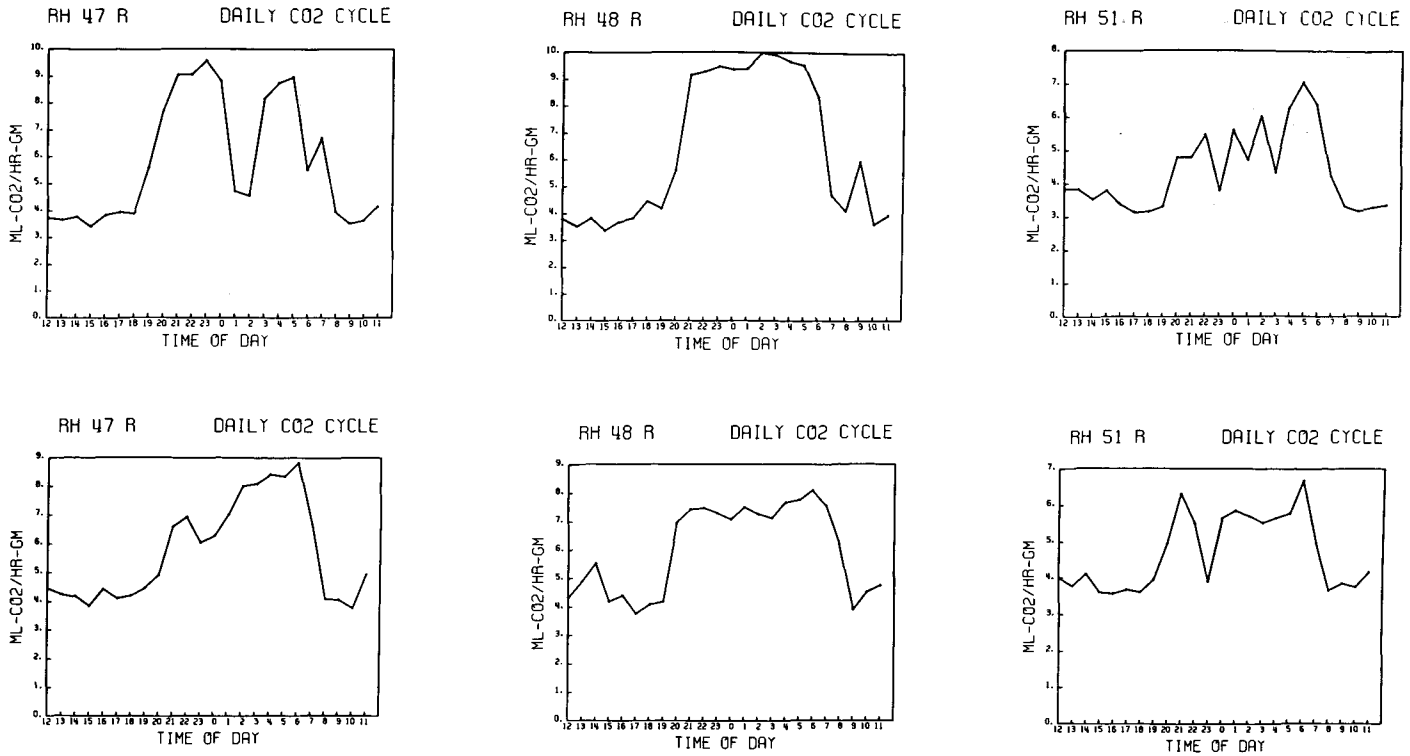

Figure 29. Sample of CO_2 measurements made on irradiated harvest mice.

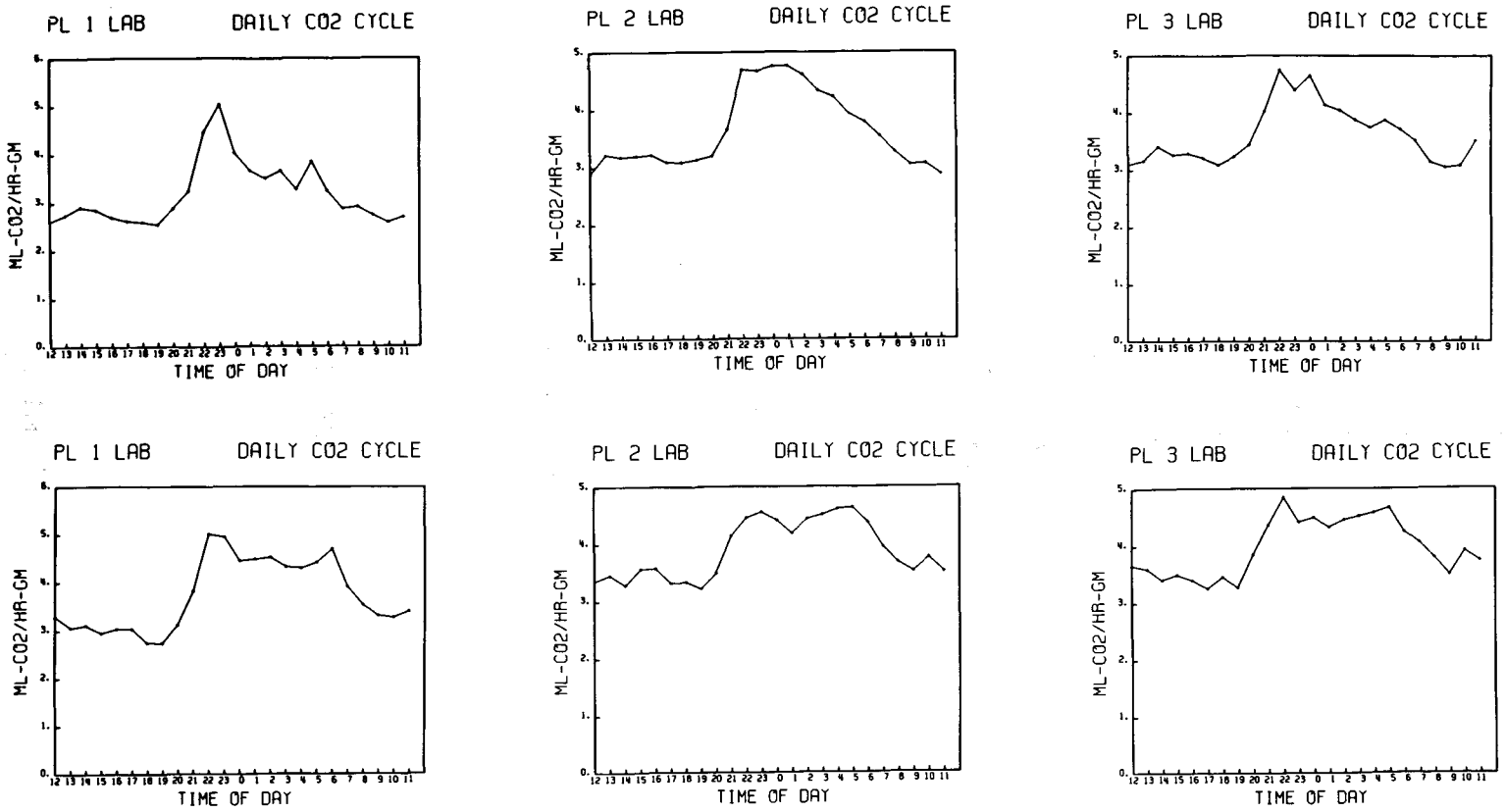

Figure 30. Sample of CO_2 measurements made on white-footed mice at 22°C.

APPENDIX D

This appendix contains additional equations derived from correlations of ^{137}Cs intercepts with metabolism in rodents. Unlike the primary relationships given in the text, some of these equations may be of potential value in predicting metabolic rates other than on a daily average basis. No attempt is made to elaborate on these relationships at present, and they are reported primarily because of their statistical validity ($P < 0.01$). All predictions are based on CO_2 at 29°C, 745 mmHg, and were derived from individual data rather than from treatment means.

For Sigmodon, potentially useful equations are:

$$\text{Light-period } CO_2 \text{ production (cc/hr/g)} = 4.60 - 0.8029\ (\log_e a)$$

$$r = -0.59$$

$$S_{y/x} = 0.38$$

$$\text{Dark-period } CO_2 \text{ production (cc/hr/g)} = 5.87 - 1.03\ (\log_e a)$$

$$r = -0.71$$

$$S_{y/x} = 0.36$$

Similar relationships for Reithrodontomys are:

$$\text{Light-period } CO_2 \text{ production (cc/hr)} = 106.26 - 16.65\ (\log_e a)$$

$$r = -0.88$$

$$S_{y/x} = 7.40$$

$$\text{Light-period } CO_2 \text{ production (cc/hr/g)} = 11.93 - 1.88\ (\log_e a)$$

$$r = -0.88$$

$$S_{y/x} = 0.83$$

$$\text{Dark-period } CO_2 \text{ production (cc/hr)} = 78.08 - 0.3367\ (a)$$

$$r = -0.71$$

$$S_{y/x} = 11.43$$

$$\text{Daily } CO_2 \text{ production (cc/hr)} = 111.55 - 15.96\ (\log_e a)$$

$$r = -0.84$$

$$S_{y/x} = 7.97$$

VITA

Charles Edwin Baker was born in Wheelwright, Kentucky, on December 18, 1939. He attended elementary and high schools in that city and was graduated in May, 1957. The following September, the author entered Georgetown College, Georgetown, Kentucky, and in May, 1961, received a Bachelor of Arts degree in Biology. After three years of public school teaching in Lexington, Kentucky, he accepted a teaching assistantship at the University of Tennessee and began study toward a Master's degree. He received his degree in Zoology in August, 1966, having performed his research at the Oak Ridge National Laboratory.

He continued graduate study toward the doctorate, remaining at the University of Tennessee, and again doing his thesis research at Oak Ridge. The Doctor of Philosophy degree, with a major in Zoology (Ecology), was conferred in March, 1970. The author holds membership in the following organizations and societies: Ecological Society of America, Human Ecological Society, American Society of Mammalogists, American Institute of Biological Sciences, Health Physics Society, Wilderness Society, Phi Sigma, and Sigma Xi.

He is married to the former Mary Frances Vargason of Owensboro, Kentucky, and the couple have a six-year-old daughter, Dana Leigh.