COMPARATIVE ENVIRONMENTAL PHYSIOLOGY OF MAMMALIAN LONGEVITY: METABOLIC AND THERMOREGULATORY EFFECTS OF IONIZING RADIATION

DISSERTATION

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By

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF TABLES (CONTINUED)

LIST OF FIGURES

LIST OF SYMBOLS AND NOMENCLATURE

α	Activity phase; active period $\tilde{=}$ 1800-0600 hours.
ρ	Resting phase; inactive period $\tilde{=}$ 0600-1800 hours.
ϕ	Acrophase; lag in a reference function used to estimate a rhythm.
τ	Period; tau, the time a rhythm repeats itself.
ω	Angular frequency; number of repetitions of a periodic process.
Δ	Change in ... a parameter measurement.
A, Amp	Amplitude; range of parameter response above mesor (mean) of a cosine function; or above baseline parameter response if a natural rhythm.
Act.	Spontaneous motor activity.
C	Thermal conductance in $cc O_2 gm^{-1} hr^{-1}/°C$.
C_0	See mesor
EM	See existence metabolism
LD	Light-Dark cycle; e.g., LD 12:12 = lights on 12 hours, lights off 12 hours.
MR	Metabolic rate; metabolism or oxygen consumption in $cc O_2 gm^{-1} hr^{-1}$.
Q_{10}	Change in oxygen consumption for every 10°C change in temperature.
R	Correlation coefficient.
R.Q.	Respiratory quotient; ratio of CO_2 formed and O_2 used during oxidative metabolism.
SDA	Specific dynamic affect; metabolism of foodstuff.

T_A	Ambient or environmental temperature (°C).
T_B	Deep body temperature (°C).
$\overline{T}_B$	Mean deep body temperature (°C).
T_{LC}	Lower critical ambient temperature of the zone of thermoneutrality.
TMR	Thermoneutral metabolic rate ($cc O_2 gm^{-1} hr^{-1}$); includes specific dynamic affect and activity.
$\dot{V}_{O_2}$	MR; volume of oxygen consumed in $cc O_2 gm^{-1} hr^{-1}$.
Case 1	Steady-state temperature environment; T_A = 27C.
Case 2	Temperature condition similar to the natural environment; T_A = 32C ρ, 15C α.
Case 3	12-hour T_A phase shift; T_A = 15C ρ, 32C α.
Diurnal	Daytime, or about a day, as a diurnal rhythm; e.g., 24-hr cycle.
Existence metabolism	Difference between maximum and minimum non-running MR.
Mesor	Mean parameter value (C_o) of a least squares cosine wave.
Nychthemeral	About a day and a night (≅ diurnal cycle).
Period	Time a rhythm repeats itself; 1/frequency of a circadian oscillation.
Rhythmometric	Graphic summary of periodic parameters associated with a least squares cosine fit of 24-hour data.

INTRODUCTION

The predictability of internal physiological stability in homeotherms has been shown to be in part a result of entrained oscillatory cycles in body temperature, oxidative metabolism and activity (Hart, 1950; Sealander, 1953; Aschoff and Pohl, 1970; for review see Whittow, 1971; Bünning, 1973). Maintenance of these homeostatic or steady-state cycles was found to be dependent upon yearly, seasonal, microclimatic and other environmental factors, and behaviorally controlled mechanisms (Yamamoto, 1965; Jones, 1973). Consistency in the regulation of these control mechanisms may be thought of as an adaptation to reduce the effects of the environment, such as ambient temperature changes (Pittendrigh and Caldarola, 1973; Coldwell, 1974).

Hart (1971) observed that metabolic rate and activity in small mammals was more closely related to ambient temperature variability than with seasonal changes in temperature or photoperiod. Body temperature has been shown not to be dependent upon environmental cues such as food and light (Gaertner, et al., 1973). However, ambient temperature changes during different periods of an animal's activity cycle may cause body temperature to become quite

labile (Hart, 1951). Ambient temperature, as well as solar radiation, convective heat loss, thermal insulation, etc., have the effect of modifying metabolic and thermoregulatory processes (Porter and Gates, 1969). The thermo-energetic state of an organism can therefore be directly influenced by the predictability and regularity of the environment in which it lives.

Variability in physiological maintenance has been suggested by Kohn (1971) to be an important mechanism in the aging process. The relative differences in longevity between species may display the same kind of physiological variability (Williams, 1957). Differences in longevity may also be due to a reduction in the accumulation of physiological errors. Accumulation of physiological errors rather than organ-system failures was proposed by Samis (1968) to be a primary mechanism in the aging process. If increases in longevity are in part based upon an animal's ability to minimize the effects of ambient conditions, then we should see decreases in the variability of physiological functions and increases in body temperature and metabolic performance. Increased performance in response to environmental change may therefore impart increased longevity to an animal.

Longevity can be thought of as an evolutionary consequence of changing response patterns to ecological inputs, such as partitioning time and energy to maximize fitness

(Emlen, 1970). Natural selection operates to minimize the effects of enviornmental pressures. Thus, selection towards lengthening life-span (Williams, 1957) may be a product of reduced physiological variability. The adaptive significance of reduced variability and increased performance must in the final analysis be viewed in terms of life history.

Physiological performance can be modified by exposing animals to ionizing radiation. Cassarett (1968) reported that irradiated animals surviving sub-lethal doses recover from the various syndromes associated with ionizing radiation effects, but remain susceptible to physiological alterations throughout the remainder of their lives. Examples include late effects in hemopoetic and enzyme repair, reduced immunological response, chromosomal damage, changes in nerve conductivity and morphological abnormalities (Van Cleave, 1968; O'Farrell, 1969).

Delayed alterations in biological functioning due to ionizing radiation, termed late effects, are difficult to study and the relationship between cause and response is not readily testable. What can be tested are the differences between the ways certain physiological processes are maintained in integrated performance, such as metabolism, body temperature and activity. Thus, the variability of these parameters to steady-state maintenance physiology can be

compared.

Ionizing radiation has been thought of in the past as an aging or longevity reducing perturbation (Upton, 1957; Neary, 1960). At the population level the effects of ionizing radiation appear to accelerate the senescence function of age specific mortality. Senescence function connotes aging in terms of biochronological entropy, not to be confused with mortality due to predation or disease. Recovery of steady-state performance minimizing the senescence function may thus be a selective adaptation characteristic of animals with differing longevities.

Cricetid rodents in general (e.g., Peromyscus spp.) may be more radioresistent than Murids (e.g., Mus musculus) (Golley, et al., 1965; Sacher and Staffeldt, 1971). However, evidence from acute gamma dose studies (O'Farrell, 1969) and for chronic gamma doses (Sacher and Staffeldt, 1971) indicates that rigid taxonomic characterization may be misleading. Reduced variability in oxygen consumption in response to varying levels of ionizing radiation was reported by Plyushchev, et al. (1973) to be a reliable index of radiosensitivity. Since resistence to ionizing radiation has been reported to be a criteria for general fitness (Roderick, 1963), reduced costs in metabolic performance may be directly associated with increases in longevity. Differences in maintenance performance may therefore provide

us with a new test of taxonomic radiosensitivity (Sacher and Staffeldt, 1971). For this study, sub-lethal acute ionizing radiation was used as a short-term perturbation, similar to temperature stress, but which had not been previously experienced in the genetic history of these mice.

The white-footed mouse, Peromyscus leucopus novebora-censis, has lived 2.1 to 2.5 times longer in the laboratory than the wild house mouse, Mus musculus (Duffy, per. comm.). Longevity records at Argonne National Laboratory, Biological and Biomedical Research Division, show that Peromyscus have lived to a maximum age of 96 months while Mus have lived to a maximum age of approximately 36 months. Peromyscus can be found in more diverse kinds of habitats including those with wider temperature extremes (King, 1968), but was of more recent evolutionary origin than Mus; however, the paleontological record is not entirely clear (Romer, 1966). The ecological plasticity apparent in Peromyscus could be a function of increased behavioral and physiological adaptability to Pleistocene environmental changes prevalent during its rapid distribution into North America (Hibbard, 1968). Mus's thermal evolution was perhaps less severe because of its close association with man during Pleistocene and Recent dispersal.

Morrison and Ryser (1959) observed that Peromyscus displayedwide thermoregulatory responses to ambient temperatures with a greater variance in temperature regulation

than mice from similar geographic regions. They suggested that this labile response in body temperature may be of ecological advantage by extending its range of temperature tolerances. Morhardt and Hudson (1966) reported that reduced temperature regulation in Peromyscus was not a consequence of faulty regulation but a behavioral and physiological mechanism facilitating hypothermia and daily torpor. No such mechanism has been reported for Mus, although a modified version of thermo-lability was mentioned by Morrison and Ryser (1959).

Two points are made concerning the relationship between metabolic and thermoregulatory performance and longevity. First, as previously noted, Peromyscus was shown to have a labile body temperature and depressed oxygen uptake during times of low ambient temperature or changes in food availability (Gaertner, et al., 1973). Hill (1975) observed that Peromyscus often experienced daily excursions of torpor during which time an energetic savings of up to 30% resulted. This species has adapted to diurnal environmental conditions by means of reducing the costs of maintaining high energetic expenditures during part of its daily physiological cycle.

The second point concerning steady-state performance and longevity relates to the total expenditure of energy and the requirements for thermoregulation. In terms of daily caloric output, it should cost an animal less to

maintain a high body temperature and a low metabolic output than for an animal that maintains a high metabolic output with a low body temperature. Therefore, as discussed by Sacher (1975), "... a decrease in body temperature at constant metabolic rate is associated with shorter life, and an increase of body temperature for constant metabolic rate is associated with longer life".

The objectives of this project were to study the effects of environmental temperature on oxygen consumption, body temperature and activity in two species of wild rodents with differing longevities, and on animals subjected to sublethal levels of ionizing radiation. The study addressed itself to the comparative aspects of interspecies performance to temporal changes in ambient temperature and focused upon the costs of maintaining steady-state physiology.

The view was taken that species longevity is in part a function of metabolic and thermoregulatory adaptation to varying environments. Animals with differing longevities, but of seemingly closely related phylogenetic origin, should respond differently to changes in ambient conditions. Accordingly, the following null hypothesis was tested: No differences exist in maintaining oxygen consumption, body temperature and activity in Mus musculus and Peromyscus leucopus during temporal phase changes in environmental temperature; and no difference in maintenance physiology was

also hypothesized for those animals which had recovered from sub-lethal acute levels of gamma ionizing radiation.

It must be emphasized that because of inherent morphological and behavioral changes due to laboratory rearing, this experiment did not purport to reflect direct quantitative comparisons between these mice and their free-living conspecifics. Only by using feral mice studied *in situ* can we be sure of a more reliable prediction of the physiological adaptive significance of the data that follow.

MATERIALS AND METHODS

Experimental procedures were designed to simultaneously record oxygen consumption, deep body temperature, and spontaneous motor activity in Peromyscus leucopus and Mus musculus. The mice were monitored under conditions which could be systematically repeated without loss of precision. Preliminary analytical techniques in protocol reduced the likelihood of experimental error (Braham, 1973).

Oxygen consumption, body temperature and activity are in direct association with the behavioral acclimation of an organism within the laboratory environment (Kavanau, 1967; Heusner, et al., 1971a). The principal difficulty encountered in metabolism studies in the laboratory involve inadequate space requirements at the time of measurement, altering normal behavior. Therefore, it was necessary to simulate conditions during the experiment which closely approximated those conditions under which the mice were reared.

My mice were housed singularly in plastic mouse boxes (28 by 18 by 13 cm) for four to five weeks before the experiment to acclimate them to the cage in which measurements would be taken. These breeder cages were approximately 2/3 the size of those cages in which the mice were raised throughout their lives. Behavioral observations made on

mice over several years at Argonne National Laboratory, Division of Biological and Biomedical Research where this research project was done, had not disclosed any apparent modification in mouse behavior using the breeder cages.

Mice were placed into a maintenance incubator (27°C, LD 12:12) two to three weeks before experimental analysis to acclimate them to controlled conditions. Prior to any measurements they were transferred to an identical experimental incubator and allowed to adjust for approximately 24 hours. This technique insured that the animal's behavior was not affected by handling during preoperational procedures. The experimental measurements proceeded by placing the breeder cage and animal into an airtight plexiglass module (30 by 19.5 by 19 cm) to which airflow and electronic recording devices were attached. Dual modules were connected into a Hotpack Refrigerated Incubator (Model 52720; temperature range 2-50°C) which acted as the controlled environmental chamber. Temperature timing and programming was installed into the incubator by the manufacturer. Light programming (Sears Time Switch, Model 5870) was installed by me. Once the modules were installed the animals were monitored continuously over the range of experimental conditions.

Animals

Twenty mice were used in the experiment, ten Peromyscus and ten Mus. To avoid differences in metabolic output as a

function of age and sex (Sacher and Duffy, unpublished data) only pubescent male mice were used. All Mus in the colony (17 animals) that were between the ages of three and five months, and Peromyscus between four and seven months old (15 animals) were assigned random numbers. From these age categories ten mice from each group were randomly drawn and concurrently randomized into two groups per species.

Mice from this colony were approximately 20 generations wild, founder parents were trapped on the grounds of Argonne National Laboratory in 1963 and 1964 (Staffeld, E., personal communication). They were routinely housed in the larger plastic mouse cages (44.5 by 20.5 by 13 cm) at 23-27C from birth on a light cycle of L 0600-1800, D 1800-0600 and given ad libitum food and water.

Oxygen consumption

Oxygen consumption (i.e., metabolic rate; $\dot{V}_{O2}$ or MR in $cc O_2 gm^{-1} hr^{-1}$), an expression of energy metabolism (Bartholomew, 1972), was measured by using a continuous flow open-circuit system. Briefly discussed, circulating air was dried prior to entering the animal chamber. Mixed gases leaving the module were again dried and CO_2 removed. The difference in oxygen content of the air entering and leaving the module was electronically detected and recorded using a Beckman G-2 Oxygen analyzer (Model G2-1AA-A5A) and Honeywell Electronik Model 15 recorder. Airflow through the system

was maintained at 250 cc min^{-1} and automatically corrected for standard temperature and pressure (s.t.p.) every hour. Barometric changes affecting the flow rate were in part compensated for by use of a Cartesian diver. A petri dish of Drierite (calcium sulfate) was placed in the module to help stabilize humidity at the different temperatures. Humidity was measured by means of a portable aspirated psychrometer (Bendix Fries Psychron, Model 566) and was recorded to vary from 39% (32C) to 46% (15C) during the various experimental temperature changes. Since humidity did not vary greatly between temperatures, and moisture was removed at several places within the system, it was not necessary to correct for water vapor interference in oxygen consumption calculations (Beaver, 1973). Corrections for temperature interference on s.t.p. were taken into consideration when calculating final oxygen consumption.

An accurate prediction of the volume of oxygen taken-up by an animal, expressed on a per gram animal basis, was a function of weight, flow rate of air through the system, temperature, barometric pressure, and, if present, recording chart errors. The expression used for the determination of oxygen consumption was:

$$\dot{V}_{O2} = \frac{T \times [(O-E)\ H] \times A \times CF}{W}$$

where: $\dot{V}_{O2}$ = volume of oxygen uptake
= cc O_2 $gram^{-1}$ $hour^{-1}$

T = time
= 60 min $hour^{-1}$

O = percent oxygen recorded
= 20.00 - 21.00% O_2

E = zero gas error term
= 0.005%

H = Correction for Hill (1972) transformation (condition 'B')

where: Y = 4.25 + 1.25 X
= corrected % O_2 from chart recording
X = uncorrected % O_2 from chart recording

A = airflow
= 250 cc min^{-1}

CF = s.t.p. correction factor (B.P. = 808 mm Hg)
15°C (285°K) = 1.008
27°C (297°K) = 0.967
32°C (302°K) = 0.951

W = weight in grams

Airflow System. Room air circulating in 4 mm (inside diameter) copper tubing was first passed through a Drierite trap ($CaSO_4$ - 8 mesh, 2000 cc or 4 lbs.) which dried the air. Air leaving this trap had an oxygen content of approximately 20.953 $\pm$ 0.002% which met the oxygen tension requirements for the Beckman G-2 analyzer. Air then passed into the module and circulated within the animal's immediate environment. Mixing of the air was determined to be uniform following an exponential response curve. Air leaving the module (for convenience termed mixed gases) flowed through dual solenoid switching systems which were turned on or off

depending upon which animal was being analyzed at the time. Gas analysis was thus recorded three times an hour for each animal by switching back and forth between modules every ten minutes. The automatic solenoid device was connected to a Singer timer so individual animals could be tested separately or sequentially. Mixed gases passing through the solenoids entered a tantallum airflow rotometer (Brooks Division, Emerson Electronics Co.) set at 250 cc min^{-1}. To reduce backpressure in the system an exhaust bypass at this point was installed to release gases not being analyzed. Mixed gases were then passed through a water trap (1N NaOH, 300 ml) and a second Drierite trap (250 cc) further drying the air and removing respirated CO_2. Circulated gases at this point passed through two more bypass solenoids which were activated during the calibration of zero gas (20.768%) and span gas (19.000%). Mixed gases were vented out of the system when standardization took place. Circulating gases then entered a Hastings mass flowmeter (Model ALL-500X), maintaining the flow rate at 250 cc min^{-1}, and finally entered the G-2 analyzer. A second recorder (Brown potentiometer, 0-10 millivolt) was installed to make a duplicate record of the air flow throughout each experimental run.

Removal of CO_2 before entering the analyzer conformed to condition 'B' specified by Hill (1972) and thus eliminated the computational error in CO_2 interference.

Deep Body Temperature

Deep body temperature (T_B) was continuously monitored by means of surgically implanted thermal transmitters. These thermistors ranged in size from 2.842 to 3.186 grams, were equipped with Mallory RM400R mercury batteries, and had a transmitting potential of 1.35 volts D.C. at a frequency of 500 KHz (recording 100-200 c.p.s.). The battery and transmitter were encapsulated in parafin and bee's wax insuring a life span of six to nine months over a temperature range of 20-40C.

While mice were under pentobarbital anaesthetization (10% slaine-Nembutal solution, 0.012 cc gram^{-1} animal) the thermistors were implanted within the peritoneal cavity (Barr, 1972). A minimum of five days was allowed for surgical recovery prior to an experimental run (Wang, 1972). Each thermistor was calibrated before implantation and after removal upon the completion of each experimental run. Since frequency of the transmitted signal is temperature dependent, quantitation of the coefficients according to the cubic function $Y = a_o + a_1x + a_2 x^2 + a_3 x^3$ represented the best fit of frequency to temperature conversion.

Each animal cage was wired for receiving the impulses from the thermistor. The antennae, wrapped around the cage, was plugged into a socket mounted in the side of the module which led to a preamplifier. The preamplifier was connected to an amplifier and pulse shaper which converted

and sent frequency impulses to a count rate meter and an analog potentiometric recorder (Brown Honeywell Electronik, Model 143X, 0-10 mV, 50-5000 c.p.s.). A continuous print-out was thus recorded at a chart speed of two inches per hour.

Activity

Spontaneous motor activity (Act.) was continuously monitored by means of electronic impulses using a crystal phonograph cartridge and stylus, serving as a transducer, similar to the ones described by Redetzki (1965) and Aschoff and von Saint Paul (1973). When activated by movement of the mouse the transducer gave off a reliable electronic signal which was transformed to a potentiometric tracing. The sensitivity of the device was such that it readily conformed to the conclusion reached by Bramante (1959) that slight changes in motor activity may correspond to changes in oxygen consumption.

Activity Apparatus. The mouse cage was placed on a plastic plate (28 by 17.5 by 0.5 cm) resting on four springs within the plexiglass module. The weight of the cage and contents (1300 grams) was such that the cage would vibrate on the springs with light movements of the animal. The cartridge was hinged to the floor of the module and was moved up or down by means of a bolt passing through the floor of the module precisely contacting the bottom of the plate. This modification insured the desired pressure of

the plate on the stylus. The bolt was passed through a rubber O-ring to maintain the module as an air-tight unit. Leading from the cartridge to an air-tight coupling mounted in the back of the module was a single conductor shielded wire. Inserted from the outside and into the coupling was another single conductor shielded wire which led to a two-channel operational amplifier. From the amplifier two single wires led to a two-channel Rustrak recorder (operating at one inch per hour chart speed), thus two animals could be recorded simultaneously. Gain and zero calibrations were conducted separately for each module at the amplifier.

The magnitude of the response in recorded activity between animals was standardized by adjustments in gain and amplitude according to pre-set behavioral patterns elicited by the animals. In this way changes in recording deflections among animals was a reflection of behavior (i.e., activity) rather than differences that may exist in the cages, springs, stylus pressure, etc. Sensitivity of the activity device was such that essentially no recording occurred (scored at a +1) when the mice were at rest, breathing hard, or during localized movements (e.g., rolling over, changing positions in the nest, etc.). More gross motor activities such as preaning, scratching, walking, face washing, feeding, nest building, etc. approximated moderate behavioral patterns and thus were scored as a +2. Running, jumping, climbing and tunneling were scored as a +3. From preoperational

standardization practices activities were recorded on the Rustrak recorder using the following approximations: 0 to 1/8 scale was scored as a +1, 1/8 to 1/2 scale scored as a +2, and 1/2 to full scale scored as a +3. Quantitation of recorded activity based upon qualitative behavioral patterns was a modification of the arbitrary scales reported by Bramante (1961) and Tucker (1966). These scores should not be construed as a means of predicting absolute behavioral comparisons between animals. They were, however, a useful tool in describing temporal activity patterns.

Environmental Temperature

Ambient temperature (T_A) within the incubator and the module was controlled by means of a timing programmer installed by the manufacturer as a standard component of the refrigerated-heating system. Because of the necessity to know the temperature stability within both the incubator and the module an additional dual thermocouple device was installed. The incubator temperature was monitored with a single wire thermocouple connected to a dual channel Rustrak recorder. A thermocouple leading from the module was connected to the second channel of the Rustrak recorder. Between temperatures of 10-40C within the incubator, T_A fluctuated no more than $\pm$ 1.0C under the experimental conditions. The T_A in the module remained essentially constant ($\pm$ 0.5C). Both temperature recording devices were calibrated and standardized for zero and span prior to and

at the completion of the experiment.

Ionizing Radiation

Five Peromyscus and five Mus were exposed to ionizing radiation using a Cobolt-60 source. A lethal dose was used which was low enough to reduce the likelihood of somatic death and yet large enough to simulate an acute level. Peromyscus was subjected to a $LD_{16(30)}$ (i.e., a lethal dose to 16% of the population in 30 days) of 900 r (r = Roentgens) and Mus to a $LD_{16(30)}$ of 565 r. These levels were chosen after extrapolation of dose response curves from data on $LD_{50(30)}$'s for Mus and Peromyscus reported in Golley, et al. (1965) and Dunaway, et al. (1969).

Experimental Design and Protocol

To ascertain how each animal physiologically adjusted to temporal changes in T_A, steady-state maintenance metabolism and thermoregulation measurements under laboratory conditions were a necessary recording prerequistite. These measurements included documentation of entrainment to periodic factors such as predictable light regimes, ambient temperatures within or approaching the thermal neutral zone, and ad libitum food and water.

Light is a very important environmental cue acting as a Zeitgeber (i.e., synchronizer) for many physiological processes (Aschoff, 1965; Bünning, 1973; Hillman, 1973; Smith, 1974). Hence, light was a controlled variable, first

regulated on a LD 12:12 cycle when measuring steady-state conditions and then held constant (DD) for the remainder of the experiment.

To compare the cost of endothermy between species and between irradiated and non-irradiated mice, T_A was changed between the upper and lower limits of the zone of thermoneutrality and below thermoneutrality in-phase and out-of-phase to ambient conditions. The series of environmental conditions encountered were protracted over four 24-hour continuous runs (Table 1). During the first 24-hour T_A regime, T_A was held constant at 27C in-phase to the laboratory light cycle (L 0600-1800 D 1800-0600). It was, therefore, a test for steady-state responses (termed Case 1). During the second 24-hour period (also Case 1) T_A was 27C but with the lights off (DD). Light illumination during the diurnal time (lights-on period) was measured at 10 lux and during the DD period 0.1 lux red light. Red light was used to enable the operator to view the behavior of the mice.

The third T_A regime (Case 2) consisted of raising and lowering T_A to coincide with the natural change in ambient fluctuations. The temperature was thus changed to 32C during the diurnal cycle, ρ (i.e., inactive time; $\sim$ 0600-1800) and 15C during the nocturnal cycle, α (i.e., active time; $\sim$ 1800-0600). The final experimental sequence (Case 3) introduced a 12-hour displacement of T_A to ambient conditions. During this 24-hour period each mouse experienced a 32C T_A

Table 1. Summary of the laboratory environmental conditions for time, temperature and light used in the comparative physiological study of Mus musculus and Peromyscus leucopus at Argonne National Laboratory, Biological and Biomedical Research Division, 1974-75.

Day	Time	Temperature (T_A)	Light	Hours
1	0600	27°C	L0600 D1800	24
2	0600	27	DD	24
3	0600-1800	32	DD	12
	1800-0600	15	DD	12
4	0600-1800	15	DD	12
	1800-0600	32	DD	12

during it's active period (α) and 15C during it's inactive period (ρ). The first and second 24-hour runs served as controls for the third and fourth 24-hour periods; the first for entrained conditions experienced in the laboratory history of the mice, and the second for a steady-state replicate of the first. The light and temperature conditions used during each of twenty 96-hour experiments are described in Figure 1.

Ambient temperatures were selected on the basis of these criteria:

1. 27C and 32C have been reported to be near the lower and upper critical temperatures in the zone of thermoneutrality for both species (Weidner, 1970; Pennyciuk, 1972); although 32C may be a mid-point for thermoneutrality in Peromyscus (Morrison and and Ryser, 1959).
2. Between 16C and 32C ionizing radiation effects have been reported to be independent of T_A in Peromyscus (Williams, et al., 1968).
3. 15C represented an approximate median summer's evening air temperature (< 30 cm above the surface) in an open field at Argonne National Laboratory (Moses, 1967).
4. 27C was near the upper ambient temperature at which these mice are raised.
5. Both P. leucopus and M. musculus have been shown to preferentially select approximately 32C in the

Figure 1. Light and temperature conditions for each of four 24 hour continuous periods during measurements of oxygen consumption, body temperature and activity in control and irradiated Mus musculus and Peromyscus leucopus. This experiment was carried out at the Division of Biological and Biomedical Research, Argonne National Laboratory from 1974 to 1975.

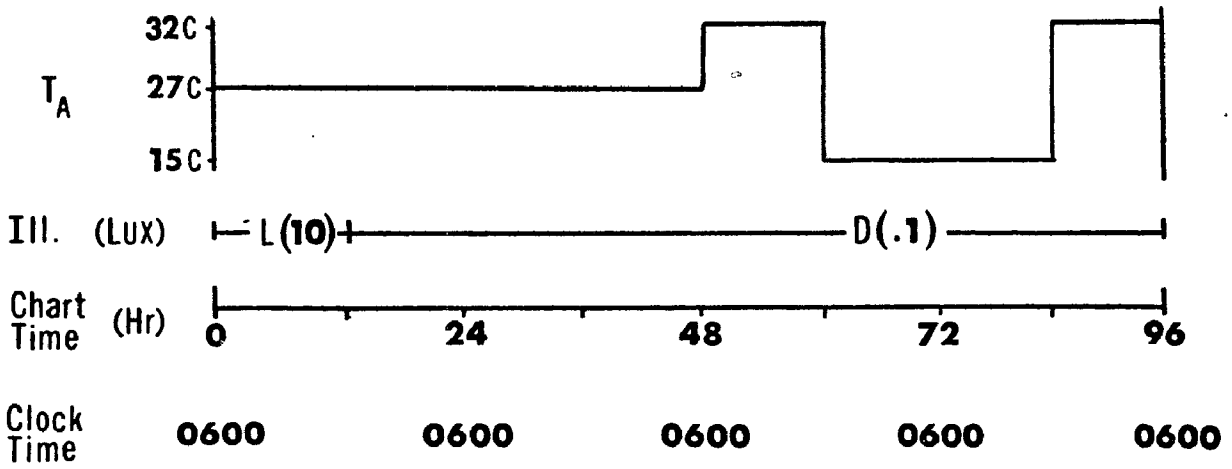
32C
27C
15C
T_A
Ill. (LUX)
L(10)
D(.1)
Chart Time (Hr)
0
24
48
72
96
Clock Time
0600
0600
0600
0600
0600

Figure 1

laboratory (Ogilvie and Stinson, 1966).

Statistical Analyses

All statistical analyses were performed using programs in Speakeasy (Cohen and Vincent, 1972) run at Argonne National Laboratory, and SAS, the Statistical Analysis System (Service, Barr and Goodnight, 1972) run at The Ohio State University Computer Center. The 95 percent confidence level was used throughout this study as a measure of statistical reliability.

Each of the three parameters (i.e., MR, T_B and Act.) were analyzed in terms of the variability they displayed in time, between species, between treatment groups, as a function of ambient temperature and for the interactive effects. Analysis of variance, regression, time series analysis and cross-correlation analysis were the major components of the statistical package.

An analysis of variance three-factor fixed model design (the factors were treatment, species and time) was used to determine how much of the variation within and between observation samples was due to variation in each factor, thus a comparative test for performance. The natural logarithm of MR was used in the factorial analysis of variance. This transformation was necessary to alleviate problems of heterogeneous variance and non-normality. Since within cell variance and sample size are positively correlated, and as data point sample sizes were large, the remaining heterogeneity should have had little deleterious effect (Scheffe,

1959). Slight deviation from normality is also not a serious violation of the assumptions in analysis of variance if data sample sizes are large (Li, 1968). Because of linearity between parameters, and large sampling events, it was not necessary to transform the data for additional statistical analyses (regression and correlation testing).

Regression analysis was used to measure the relationship among parameters as each changed in time. Changes in slope of the regression for metabolism-body temperature correlates were used to evaluate differences in thermoregulatory responses between animals. Correlation analysis, an expression of intensity or degree of accuracy (i.e., closeness of fit) of any two variables, was used to evaluate metabolic to thermoregulatory response interactions.

The degree to which each variate was related was a reflection of time series phenomena involving time, frequency of the parametric response, and the periodicity of the response. Cross-correlation analysis was used to measure the time varying processes of two different time series, therefore, it measured relationships between a present value of one function to a past value of another (Edwards and Yamamoto, 1965). This test was particularly important in predicting body temperature and oxygen consumption time-lags in animals.

To satisfactorily achieve an even time distribution in oxygen consumption versus body temperature it was necessary to standardize the data because of inherent physical time

lags in the oxygen detection system. Only the lag in oxygen flow from animal chamber to recorder (34 min.) was found to affect the accuracy of lag interpretation. An exponential time constant was therefore inserted into the time series statistical program to take into account the contribution from previous events which might bias the biological time lag between variables. Since the cross-correlates were equally time dependent, temperature (T_B) change was the function modified.

The modified temperature function was normalized to the decay rate of previous events contributed by lag in oxygen recording. The equation which described this function was:

$$TI_i = \sum_{i=0}^{t} T_i e^{-\alpha_t (t-i)\Delta}$$

where TI is the summation of decay constants from previous T events, T_i is body temperature at time i, α_t the animal chamber-oxygen detector exponential time constant (= 0.028988) and Δ the interval in sampling times (= 10 minutes).

Normalization of exponential time contributions from previous events in body temperature recordings should represent a reliable fit when equalizing previous event contributions of oxygen consumption. This method was used because differential integration of the data was not possible owing to potential human error when scoring analog strip-chart recordings.

Caloric Equivalents

A respiratory quotient (R.Q.) of 0.79 was estimated from the known gross energy yield in the mice's food (4.06 Kcal $gram^{-1}$). The percent yield was, carbohydrates 50.15%, protein 24.00% and fats 4.00%. The calculated caloric equivalent was 4.82 Kcal $cc O_2^{-1}$ for an average daily intake. Mus ate about 0.144 grams of food per gram animal and Peromyscus about 0.133 g g^{-1} animal (Peter Duffy, Argonne National Laboratory, Biological and Biomedical Research Division; pers. comm.).

RESULTS

No significant differences in body weights existed between control (19.7 ± 1.16 grams) and irradiated Mus (19.8 ± 0.97 grams, means ± S.D.), or between Peromyscus controls (25.8 ± 2.74 grams) and irradiated (29.1 ± 4.59 grams). Peromyscus was heavier than Mus ($P < 0.048$) using Wilcoxson's Rank Sum statistic (Hollander and Wolfe, 1973). No significant weight changes occurred in any group during the experiment.

Age differences between Mus treatment groups were not statistically significant (controls 128 ± 47.7 days; irradiated 120 ± 28.3 days) but irradiated Peromyscus were older (211 ± 11.3 days) than controls (193 ± 10.4 days) ($P < 0.048$). The difference of approximately 18 days was not considered biologically significant considering no difference in weights occurred between treatment groups of Peromyscus. Age and weight data for each mouse are summarized in Appendix A.

Results of metabolic recordings on several Peromyscus and Mus before thermistor implantation and after subjection to ionizing radiation are reported in Table 2. MR increased 12-17 percent between preoperation and post-irradiation periods (Table 2), however the differences were

Table 2. Lowest metabolic rates ($cc O_2 gm^{-1} hr^{-1}$) for several Mus musculus and Peromyscus leucopus before thermistor implantation and post-irradiation. The period for surgical recovery was a minimum of 10 days prior to irradiation, and oxygen consumption recordings took place two to 10 days after the radiation treatment. Values are means ± standard deviations.* This work was conducted at Argonne National Laboratory, 1974-75.

	Pre-operation	(n)	Post-irradiated	(n)
P. leucopus	1.74±0.36	(8)	2.11±0.67	(4)
M. musculus	2.33±0.80	(6)	2.63±0.25	(3)

*No significant difference after treatment nor among species groups using Wilcoxson's rank sum statistic (Hollander and Wolfe, 1973).

not significant.

Graphic plots and raw data for oxygen consumption, body temperature and activity on each mouse are reported in Appendix B and C.

From the data in Appendix B and C, general cyclic trends emerge in time (i.e., period, τ) and amplitude (i.e., range, A). Each animal displayed an approximate circadian rhythm in all three parameters, especially during days one and two. These nychthemeral patterns were related to periods of activity (α) and inactivity (ρ). Quantitation of these data revealed that differences existed in all three parameters depending upon time, treatment and species.

Analysis of Variance

Analysis of variance for metabolism and body temperature is reported in Table 3. If the F value for a main effect (e.g., treatment) was statistically significant but the interactive effect (e.g., treatment x species) was not, then differences in means between variables existed. If the interactive effect was significant then a component of one of the main effects explained the variability. F values for several ANOVA factors exceeded the probability of committing a type I error, using the Students t-statistic, warranting a rejection of H_o.

The larger F values for MR during day 1 by treatment (i.e., ionizing radiation versus controls) and by species

Table 3. Analysis of variance for a three-factor fixed model design (treatment, species, and time) recording the F-ratio (F) and probability (P) greater than F for control and irradiated *P. leucopus* and *M. musculus* during each of four 24 hour experimental periods measured at 10 minute intervals. MS = mean square. ANL, 1974-75.

FACTORS	d.f.		Day 1		Day 2		Day 3		Day 4	
			MR	T_B	MR	T_B	MR	T_B	MR	T_B
TREATMENT (T)	1	F	70,18	687,98	34,11	445,99	25,28	483,39	19,45	172,83
		P	,0001	,0001	,0001	,0001	,0001	,0001	,0001	,0001
SPECIES (S)	1	F	21,03	3,85	22,26	1,31	48,15	30,96	14,72	8,64
		P	,0001	,0470	,0001	,252	,0001	,0001	,0003	,0037
T X S	1	F	5,36	160,49	,329	185,96	1,01	20,85	17,28	119,91
		P	,0195	,0001	,574	,0001	,316	,0001	,0001	,0001
TIME (t)	143	F	9,12	9,22	7,09	7,94	40,80	2,32	11,13	5,78
		P	,0001	,0001	,0001	,0001	,0001	,0001	,0001	,0001
T X t	143	F	,240	,974	,382	,721	,418	1,38	1,03	,596
		P	1,00	,522	1,00	,994	1,00	,0026	,397	,999

Table 3. Continued.

FACTORS	d.f.	Day 1		Day 2		Day 3		Day 4	
		MR	T_B	MR	T_B	MR	T_B	MR	T_B
S X t	143 F	,992	1,41	,923	1.93	,579	2,08	,792	1,23
	P	,513	,0016	,731	,0001	,999	,0001	,965	,0359
T X S X t	143 F	,196	,475	,527	,707	,662	,575	,369	,340
		1,00	1,00	1,00	,996	,999	,999	1,00	1,00
RESIDUAL	2304 MS	,0506	,8942	,0579	1,034	,0776	1,311	,0758	1,348
TOTAL	2879 MS	,0687	1,517	,0731	1,635	,2277	1,707	,1122	1,752

(i.e., Mus versus Peromyscus) were greater than the interaction of treatment x species (Table 3). Differences in mean oxygen consumption were significant for both treatment groups and between species. A significant interaction suggested that separation of mean differences for these main effects cannot be made without consideration of species and treatment groups separately.

A somewhat different situation existed for T_B. The large F value for the treatment factor and the smaller F value for the species factor indicated that differences in body temperature were greatest between irradiated and control groups. The large F value for the interactive effect indicated that one of the two species was contributing the greatest to differences in T_B.

A better estimate of the biological significance of the analysis of variance can be made with the aid of data in Table 4. In Case 1 MR was higher for both irradiated species than controls. T_B for both species controls was higher than the irradiated groups (Table 4). The interactive effect for species x treatment was also significant for T_B (Table 3). The effects of ionizing radiation between species was significantly related.

Time displayed a significant effect on T_B as did the interaction of time x species (Table 3). The fact that time x treatment was not significant suggested that changes in time effecting T_B were not contributing to the separation

Table 4. Average daily body temperature (T_B), oxygen consumption (MR) and activity (Act.) (means ± standard deviations) for treatment and control groups of ten P. leucopus and ten M. musculus during each of four experimental days. N = 144, the number of events per parameter for each group per day. ANL, 1974-75.

Animal Groups		Days 1	2	3	4
P. leucopus	T_B	37.22±.959	37.10±1.09	37.77±1.29	37.12±1.27
(Controls)	MR	2.48±.701	2.46±.596	3.33±1.36	3.47±.918
	Act.	1.51±.699	1.53±.735	1.71±.808	1.36±.599
P. leucopus	T_B	36.74±1.19	36.82±1.28	37.02±1.41	37.04±1.59
(Irradiated)	MR	2.65±.871	2.68±1.02	3.22±1.40	3.46±.879
	Act.	1.73±.793	1.59±.708	1.71±.811	1.44±.676
M. musculus	T_B	37.60±.996	37.58±1.06	37.22±.891	37.51±.930
(Controls)	MR	2.47±.488	2.55±.628	3.31±1.76	3.84±1.25
	Act.	1.60±.798	1.73±.860	1.62±.826	1.61±.787
M. musculus	TB	36.22±1.30	36.26±1.30	36.59±1.19	36.46±1.20
(Irradiated)	MR	2.72±.600	2.70±.620	3.01±1.52	3.60±1.34
	Act.	1.64±.798	1.63±.811	1.56±.794	1.52±.733

of treatment groups. The main effect of treatment was more important. T_B was depressed in the irradiated groups by about 3 percent. This effect was especially evident in Mus (Table 4). Only slight differences in T_B by species was apparent from the data (less than 1 percent). MR was elevated in the treatment groups by about 7.7 percent and between species by about 4 percent; Mus was higher.

A significant effect appeared in T_B for species during days 3 and 4 (Tables 3 and 4). The first-order interactive effect for treatment x time and species x time during day 3 suggested that animals responded differently to changes in ambient temperature fluctuations. During day 4 the effect of time was more pronounced between species than treatment groups (Table 3). From these analyses, species differences in T_B during T_A changes were more important than treatment or time in separating animal groups (Table 3).

No second order interaction existed (T x S x t); thus the main effects of treatment and species and their interactions were used to explain differences in treatment groups of mice in response to temperature changes.

Metabolism

At 27C Peromyscus controls had a significantly lower MR ($P < 0.05$) than the irradiated group (Table 5). No statistical difference in MR was detected for Peromyscus

Table 5. Standard metabolic rate (MR;$cc O_2$ gm^{-1} hr^{-1}), body temperature (T_B) and thermal conductance (C ; $cc O_2$ gm^{-1} $hr^{-1}/^oC$) for control and irradiated P. leucopus and M. musculus at three temperature levels. Values are means ± standard deviations, N = 15 per cell. ANL, 1974-75.

	Mus musculus						Peromyscus leucopus					
	CONTROLS			IRRADIATED			CONTROLS			IRRADIATED		
$T_A(^oC)$	MR	T_B	C	MR	T_B	C	MR	T_B	C	MR	T_B	C
15	3.77± 0.30	36.6± 1.22	.176± .019	3.71± 1.30	35.2± 0.57	.185± .068	3.52± 0.39	36.7± 0.36	.162± .018	3.71± 0.59	36.1± 1.32	.177± .030
27	1.89± 0.18	36.8± 0.78	.195± .025	1.96± 0.18	34.9± 0.48	.248± .016	1.72± 0.26	36.4± 0.36	.183± .033	2.07± 0.09	35.6± 0.74	.241± .023
32	1.46± 0.21	37.4± 0.70	.294± .060	1.57± 0.16	35.8± 0.62	.418± .067	1.74± 0.45	37.2± 0.46	.330± .059	1.97± 0.20	35.8± 0.59	.524± .118

or Mus groups at 15C and 32C. Standard deviations were more than two times higher in control Peromyscus at 32C than control Mus (Table 5).

Comparing average daily metabolic rates by activity period, control Peromyscus had a lower MR than the irradiated group at both activity periods (ρ and α) during Case 1 ($P < 0.01$) (Table 6). No difference existed during Cases 2 and 3. In each case, however, control animals had a lower MR. Control Mus exhibited a lower MR than irradiated Mus during Case 1 ($P < 0.05$) and at ρ 15C ($P < 0.01$). Like Peromyscus, control Mus had a lower MR during the remaining activity periods. These differences were not statistically significant. Peromyscus controls had a lower MR than Mus controls during Case 1 (ρ, $P < 0.05$; α, $P < 0.01$) but not during Case 2 or 3. Irradiated Peromyscus had a lower MR than irradiated Mus only during ρ of Case 3 ($P < 0.01$).

In summary, the metabolic requirements for control groups were less, especially during steady-state, and during ambient temperature changes. That MR was generally not statistically different may be a reflection of the larger variances in data for irradiated groups when T_A fluctuated, or as a function of size differences among control animals.

Body Temperature

Body temperatures associated with standard metabolic

Table 6. Average metabolic rate, MR ($cc O_2 gm^{-1} hr^{-1}$), body temperature, T_B (^oC) and thermal conductance (C; $cc O_2 gm^{-1} hr^{-1} / ^oC$) for control and irradiated P. leucopus and M. musculus during each ambient temperature regime [Cases] corresponding to 12-hour activity periods, where ρ = inactive (0600-1800 hours) and α = active (1800-0600 hours). Values are means ± standard deviations; N = 360/cell. ANL, 1974-75.

	Mus musculus						Peromyscus leucopus					
	CONTROLS			IRRADIATED			CONTROLS			IRRADIATED		
TA(^oC)	MR	T_B	C	MR	T_B	C	MR	T_B	C	MR	T_B	C
Case 1												
ρ 27	2.16± 0.27	37.4± 0.74	.209± .024	2.33± 0.26	35.6± 0.72	.275± .023	1.94± 0.29	36.7± 0.35	.205± .027	2.44± 0.31	36.1± 0.94	.278± .028
α 27	2.88± 0.33	38.1± 0.58	.257± .023	3.09± 0.43	37.0± 0.86	.309± .034	2.41± 0.33	37.7± 0.68	.227± .028	3.44± 0.77	37.4± 0.77	.336± .064
Case 2												
ρ 32	1.86± 0.34	37.6± 0.72	.340± .074	2.00± 0.39	36.1± 0.78	.494± .065	1.98± 0.49	37.9± 1.11	.336± .121	2.07± 0.49	36.6± 1.61	.538± .069
α 15	4.31± 0.65	37.9± 0.82	.190± .022	4.46± 0.91	37.1± 0.97	.201± .020	4.16± 0.88	37.7± 1.10	.184± .019	4.17± 1.04	37.4± 0.97	.202± .030
Case 3												
ρ 15	4.33± 0.39	37.1± 0.86	.183± .022	5.12± 0.48	35.8± 0.92	.249± .023	4.10± 0.57	36.6± 1.19	.180± .021	4.13± 0.48	36.3± 1.74	.199± .034
α 32	2.82± 0.64	37.9± 0.67	.509± .098	3.07± 0.69	37.1± 0.93	.582± .095	2.82± 0.47	37.7± 0.99	.491± .102	3.23± 0.58	37.7± 1.04	.620± .139

rates (Table 5) revealed that in general irradiated mice had a significantly lower T_B than controls. This was true for Peromyscus at 27C ($P < 0.05$) and 32C ($P < 0.01$) but not at 15C. T_B in Mus controls was higher during 27C and 32C ($P < 0.01$) and at 15C ($P < 0.05$). No statistical differences in T_B existed among species controls or irradiated groups.

Within each Case (Cases 1-3) differences existed in T_B and MR between controls and irradiated animals. During Case 1 Mus and Peromyscus controls did not significantly increase T_B or MR when active (α at 27C). Irradiated groups did however (T_B, $P < 0.05$; MR, $P < 0.01$). In Cases 2 and 3 the irradiated groups significantly elevated T_B when active while controls did not. Both irradiated groups had higher metabolic rates than controls during Case 2. Controls reduced MR during α 32C in Case 3 more than the irradiated groups did. These data thus suggest that ionizing radiation adversely affected homeothermic mechanisms.

Activity

Peromyscus controls were less active (18 percent) than irradiated Peromyscus (X^2: $P < 0.0005$) (Table 7). Mus controls were more active (16 percent) than irradiated Mus (X^2: $P < 0.0028$).

Within-species activity levels were not significantly different when compared to average daily activity levels

Table 7. Chi-square contingency table for total activity at three activity levels in control (C) and irradiated (I) groups of Mus musculus (M) and Peromyscus leucopus (P) over 96 hours measured at 10 minute intervals. N = 2880 activity events on five animals in each group.*

	Total Activity							
	Mus musculus				Peromyscus leucopus			
Activity Levels	C	I	E†	χ^2	C	I	E	χ^2
+1	1665	1721	1693	0.92	1742	1583	1661	7.60
+2	574	621	598	1.84	739	809	774	3.16
+3	641	538	589	9.00	399	488	443	8.94
N =	2880	2880	2880	$\Sigma = 11.76^{\#}$	2880	2880	2880	$\Sigma = 19.70^{\#}$

*$\Sigma\chi^2 = \frac{(O-E)^2}{E}$ = Chi-square summation, d.f. = 2

†E = expected values

#$\chi^2_{0.99} = 9.21$

(Table 4). Among-species differences existed only for control groups; during day 4 Peromyscus was less active than Mus ($P < 0.05$). By pooling average activity for each group by day (Table 4) or when total activity levels are compared (Table 7) a bias resulted because activity levels differ between ρ and α. Therefore, frequency distributions and chi-square contingency tables for each activity period by individual treatment group and species were calculated using SAS (Service, et al., 1972).

Peromyscus leucopus. Both controls and irradiated mice were significantly less active than expected during ρ of Case 1 (X^2: $P < 0.0001$). Control animals were more active than expected during α from 2400 to 0600 hours, while the irradiated group was more active during α from 1800 to 2400 hours. The irradiated group was more active than controls throughout α. During Case 2 controls were less active at ρ 1200 to 1800 hours (32C) than the irradiated group, but no difference in activity between these groups occurred during α 1800 to 0600 hours (15C). Both were more active than expected during the period of α (X^2: $P < 0.0001$). During Case 3 control mice were less active than expected during α 1800 to 0600 hours (32C). Controls were active from 1800 to 2100 hours and irradiated mice from 0300 to 0600 hours. The overall effect was that during steady-state, differences in activity levels between groups were only apparent during α. With changes in

ambient temperatures control Peromyscus became less active over a longer period of time than the irradiated group and were active earlier in the evening.

Mus musculus. Both groups were significantly less active during ρ 0600 to 1800 hours than expected (X^2: $P < 0.0001$). The irradiated group deviated from this slightly between 1500 and 1800 hours. Control mice were no more active during α 1800 to 0600 hours than expected, but the irradiated mice were (X^2: $P < 0.0001$). In Case 2 (ρ 32C) controls were less active than irradiated mice over a longer time period (0600 to 1800 hours versus 0900 to 1800 hours). During α (15C) of Case 2 controls were more active than expected, but for a shorter time period, becoming less active than irradiated mice from 0300 to 0600 hours. Irradiated mice were significantly more active than controls throughout α and were more active than expected (X^2: $P < 0.0001$). During Case 3 controls were significantly less active than expected during ρ 0600 to 1800 hours (15C) except from 0600 to 0900 hours (X^2: $P < 0.0001$). Irradiated mice were more active than expected from 0900 to 1200 hours (15C) (X^2: $P < 0.0001$). Controls were more active than expected during α 1800 to 2400 hours (32C) but less than expected from 2400 to 0600 hours. Irradiated mice were significantly more active than expected throughout α except from 0300 to 0600 hours, as in Case 2.

In general, irradiated mice remain[illegible]ive longer

than controls during α by as much as six hours. Controls responded to lowered T_A (32C to 15C) by reducing their activity. This was not the case for the irradiated group. Irradiated mice also responded more slowly to the 15C 12-hour phase shift by about three hours and maintained a higher activity level during α 32C. Irradiated mice responded more slowly to T_A changes than control mice by delaying the initial phase of their activity cycle, and in general were more active than controls throughout ρ and α.

Peromyscus versus Mus. Peromyscus controls (P.C.) were significantly less active than Mus controls (M.C.) during Case 1 (X^2: $P < 0.0001$). During Case 2 M.C. were generally less active than P.C. During Case 3 P.C. were significantly less active than M.C. (X^2: $P < 0.0001$). In general M.C. were more consistent at maintaining inactivity during ρ regardless of T_A and were more active during the earlier stages of α (e.g., 1800 to 0300 hours versus 2100 to 0600 hours) than P.C. P.C. maintained a higher activity level when T_A was reduced (15C) during α, while M.C. reduced activity during the later stages of α (X^2: $P < 0.0001$). This may have been a reflection of a different activity rhythm between species, or reduced thermoregulatory capacity. M.C. significantly increased activity during the 12 hour phase change in T_A but P.C. did not (X^2: $P < 0.0001$).

No significant differences in activity levels were

observed for irradiated Peromyscus (P.I.) versus irradiated Mus (M.I.) during ρ of Case 1. P.I. in general, however, were less active than M.I. M.I. were more active than P.I. during ρ 32C Case 2 (X^2: $P < 0.0001$). Both groups were more active than expected during Case 2 α (15C). M.I. maintained a higher activity level than P.I. during Case 3 except from 0300 to 0600 hours (α 32C). Few overall differences existed between irradiated species except that M.I. were generally more active than P.I.

Thermal Conductance

Thermal conductance (C) was determined for each mouse for standard metabolic rates, and average metabolic rates by activity periods (Tables 5 and 6). Calculation of C followed that of Bakken and Gates (1974) and Schmidt-Nielsen (1975) where:

$$C = \frac{MR}{T_B - T_A}$$

Peromyscus controls had a significantly lower thermal conductance than the irradiated group at 27C and 32C ($P < 0.029$), but not at 15C. The same was true for treatment groups of Mus. For both species C was lower in controls at 15C. No statistical difference in C among species control groups was observed. Peromyscus controls, however, had a lower C at 15C and 27C but a higher C at 32C than Mus controls. Irradiated Peromyscus had a lower

C at 15C and 27C but a higher C at 32C than irradiated Mus.

Average daily thermal conductance (means ±S.D. for both activity periods, ρ + α) for Peromyscus controls was significantly lower than irradiated mice at 27C ($P < 0.01$) and 32C ($P < 0.05$), but not at 15C. C for controls was 0.216 ± 0.028 and irradiated 0.307 ± 0.055 at 27C, 0.182 ± 0.018 versus 0.199 ± 0.027 at 15C and 0.419 ± 0.124 versus 0.584 ± 0.109 at 32C. The greatest differences between treatment groups occurred at 27C α and 32C ρ where Peromyscus controls had a 32 percent lower C than the irradiated group.

Average daily C for Mus controls was also significantly lower than irradiated Mus at 27C ($P < 0.01$) and at 15C and 32C ($P < 0.05$). C for controls was 0.233 ± 0.034 and irradiated 0.292 ± 0.033 at 27C, 0.187 ± 0.021 versus 0.225 ± 0.032 at 15C, and 0.425 ± 0.121 versus 0.538 ± 0.090 at 32C. At all three temperatures the greatest significant differences in C occurred during ρ, but not during α at 15C and 32C. The major difference in thermal conductance between irradiated and control Mus, therefore, occurred when metabolism and activity were at their lowest, suggesting that elevated MR's were compensating for an increase in C (more heat lost to the environment). The greatest difference between Mus treatment groups occurred at 32C ρ and 15C ρ where controls had a 31 percent and 27 percent lower C respectively.

Among-species differences were generally not significant. At 27C α control Peromyscus had a significantly lower C than Mus controls ($P < 0.048$; Table 6). C for irradiated Peromyscus was significantly lower than C for irradiated Mus only during ρ 15C ($P < 0.048$; Table 6).

Thermal conductance and body temperatures associated with C were also calculated for each animal during its period of maximum spontaneous activity and period of lowest activity (Tables 8 and 9). Mean values for ρ were taken from Table 5.

During all three ambient temperatures C and T_B were significantly higher during α than ρ ($P < 0.05$) (Tables 8 and 9). Peromyscus controls had a 6 percent higher C than Mus controls during α 32C, but an 11 percent and 12 percent lower C at 27C and 15C (Table 8). Irradiated Peromyscus had a 13 percent and 14 percent higher C than irradiated Mus at 32C and 27C but a 12 percent lower C at 15C (Table 9). No differences existed between Peromyscus treatment groups and between Mus treatment groups at 15C. At 27C Mus controls had a 12 percent lower C and at 32C a 20 percent lower C than irradiated Mus. At 27C Peromyscus controls had a 33 percent lower C and at 32C a 26 percent lower C than irradiated Peromyscus.

The percent change in C from ρ to α at all three temperatures was lower in treatment groups of Peromyscus than in treatment groups of Mus except at 27C. Irradiated

Table 8. A comparison of thermal conductance (C in $cc O_2 gm^{-1} hr^{-1}/^oC$) and body temperature ($T_B$) in Mus musculus and Peromyscus leucopus controls during inactive (ρ ≙ 0600-1800 h) and active (α ≙ 1800-0600 h) periods using lowest (SMR) and highest metabolic rates (maximum recorded for calculating C at three ambient temperatures). ρ is considered to represent resting (Wunder, 1970). ANL, 1974-75.

	Ambient Temperature (°C)					
	15		27		32	
	Mus musculus					
	C	T_B	C	T_B	C	T_B
ρ	.176*† ±0.008	36.6 ±0.67	.195 ±0.01	36.8 ±0.43	.294 ±0.02	37.4 ±0.39
α	.261 ±0.02	38.2 ±0.59	.309 ±0.02	39.0 ±0.25	.479 ±0.05	38.5 ±0.41
% of resting; ΔT_B	148	1.6°	158	2.2°	163	1.1°
	Peromyscus leucopus					
	C	T_B	C	T_B	C	T_B
ρ	.162 ±0.01	36.7 ±0.19	.183 ±0.02	36.4 ±0.19	.330 ±0.04	37.2 ±0.25
α	.229 ±0.02	38.3 ±0.76	.277 ±0.02	38.9 ±0.63	.511 ±0.05	38.5 ±1.13
% of resting; ΔT_B	141	1.6°	151	2.5°	155	1.3°

*N = 15 per cell for ρ and 10 per cell for α in T_B; N=15 per cell for C in all data.

†Means ± 95% confidence interval (S.E. x $t_{0.05}$).

Table 9. A comparison of thermal conductance (C in $cc O_2 gm^{-1} hr^{-1}/^oC$) and body temperature ($T_B$) in Mus musculus and Peromyscus leucopus irradiated groups. See Table 8 for a full description. ANL, 1974-75.

	Ambient Temperature (^oC)					
	15		27		32	
	Mus musculus					
	C	T_B	C	T_B	C	T_B
ρ	.185 ±0.04	35.2 ±0.32	.248 ±0.009	34.9 ±0.27	.418 ±0.04	35.8 ±0.34
α	.261 ±0.02	38.0 ±0.07	.354 ±0.02	38.1 ±0.78	.601 ±0.03	37.3 ±0.54
% of resting; ΔT_B	141	2.8°	143	3.2°	144	1.5°
	Peromyscus leucopus					
	C	T_B	C	T_B	C	T_B
ρ	.177 ±0.02	36.1 ±0.73	.241 ±0.01	35.6 ±0.41	.524 ±0.07	35.8 ±0.33
α	.288 ±0.02	38.0 ±0.76	.414 ±0.04	37.7 ±0.63	.691 ±0.02	38.2 ±0.63
% of resting; ΔT_B	.29	1.9°	172	2.1°	132	2.4°

Peromyscus had a higher change in C than Mus (Table 9).

Existence Metabolism

The amount of energy that an animal expends for maintenance has an important ecological bearing upon its temporal activity patterns. This capacity for activity, termed metabolic scope, is the difference between highest and lowest daily metabolic output. Since my animals were not forced to run nor allowed to exercise on a revolving wheel, activity was spontaneous. Metabolism associated with activity was not maximum but considered existence metabolism (EM) as defined by Kendeigh (1969). Therefore, my results were not expected to be directly comparable to those definitions of capacity for activity given by Bartholomew (1972). However, as a comparative tool for laboratory conditions, existence metabolism should be a reliable estimate of metabolic differences.

Direct comparisons between metabolic scope and activity have not generally been possible owing to lack of efficiency measurements and controlled environmental conditions (Bartholomew, 1972). However since this experiment has minimized environmental variability, and efficiency of activity between species or treatment groups was expected to be a random event, an estimate of the differences in performance between mice was made by using existence metabolic differences. Activity and T_B translated from

metabolic scope have been shown to be reliable predictors of performance (Bartholomew, 1972).

Mus controls had a 26 percent higher EM during Case 1 than did Peromyscus controls ($P < 0.048$), which corresponded with the general activity levels of these animals (Table 10). Change in T_B was not significantly different. Control Peromyscus were able to increase T_B 27 percent more than the irradiated group at a 31 percent lower metabolic output. Mus irradiated during Case 1 had a significantly lower T_B associated with EM than control Mus and irradiated Peromyscus ($P < 0.048$). Irradiated Mus also had a greater ΔT_B (40 percent) than irradiated Peromyscus ($P < 0.075$). No difference in EM between Mus treatment groups occurred but the irradiated mice had a significantly higher ΔT_B (34 percent) than controls ($P < 0.028$).

During Case 2, Mus controls had a significantly higher EM (13 percent) than Peromyscus controls ($P < 0.048$) (Table 10). The ΔT_B was not different; however, Peromyscus controls had a 15 percent higher ΔT_B. Peromyscus controls had a higher T_B than the irradiated group but no difference in EM or ΔT_B occurred. Mus controls had a significantly higher T_B than the irradiated group ($P < 0.048$) but not EM. Irradiated Peromyscus had a lower EM than Mus irradiated ($P < 0.075$), however, not for ΔT_B (Table 10).

When the ambient temperature was 12-hours out-of-phase to the natural environment (Case 3) control groups (both

Table 10. Existence metabolism (EM - the difference between lowest and highest metabolic rates in $cc O_2 gm^{-1} hr^{-1}$) and percent increase in EM for treatment groups of P. leucopus and M. musculus during each experimental 24 hour period. Change in body temperature is associated with the difference in T_B during LMR and HMR used to calculate EM. Values are means ± S.D., Δ = change in, and N = 30 per cell for case 1 and 15 per cell for cases 2 and 3.

	EM	% Δ EM	Δ T_B (°C)
		Peromyscus leucopus	
Case 1			
Controls	1,34±,155	42,3	+ 2,61
Irradiated	2,01±1,09	49,3	+ 1,90
Case 2			
Controls	3,24±,723	62,0	+ 1,13
Irradiated	3,25±,682	64,0	+ 1,56
Case 3			
Controls	2,46±,173	51,1	+ 0,61
Irradiated	2,10±,346	44,0	- 0,82
		Mus musculus	
Case 1			
Controls	1,82±,447	48,9	+ 2,11
Irradiated	1,92±,333	49,4	+ 3,18
Case 2			
Controls	4,23±,932	72,9	+ 0,85
Irradiated	3,75±1,18	69,4	+ 1,72
Case 3			
Controls	3,68±,523	63,0	+ 0,60
Irradiated	3,13±,414	60,4	+ 0,25

species) had a 15 percent higher EM than the irradiated mice ($P < 0.048$) (Table 10). Irradiated Mus had a significantly greater ΔT_B (86 percent) than irradiated Peromyscus ($P < 0.008$) and a 33 percent higher EM ($P < 0.016$).

In general, EM was inversely correlated to ΔT_B for control mice (both species) and positively correlated in irradiated mice (Figure 2).

Except for Mus controls (MC) during Case 1 and 3 and irradiated Peromyscus (PI) during Case 3, correlation coefficients (R) for the regression analysis were significant ($P < 0.01$). A positive correlation for the irradiated groups suggested that the metabolism associated with capacity for activity was the principal means of achieving changes in T_B. The antithesis of this (a negative correlation) meant that ΔT_B was more likely to have been associated with metabolism without activity. Hence, thermoregulation in controls was probably associated with behavioral mechanisms and thermal conductance. The angle (ϕ) between regression slopes for treatment groups, except during Case 3, supported the consistency of this interpretation among species.

The greater the slope of regression as a positive correlate meant that more metabolism was required to elevate T_B. Since irradiated Mus were generally more active than irradiated Peromyscus during Case 1 (e.g.) the

Figure 2. Least squares power fit models ($Y = a X^{b}$) for existence metabolism (Y = ln DMS) versus change in body temperature (X = ln ΔT_B) for Mus and Peromyscus controls and irradiated covering three ambient temperature regimes. N = 30 per group in Case 1 and 15 per group in Cases 2 and 3. ANL, 1974-75.

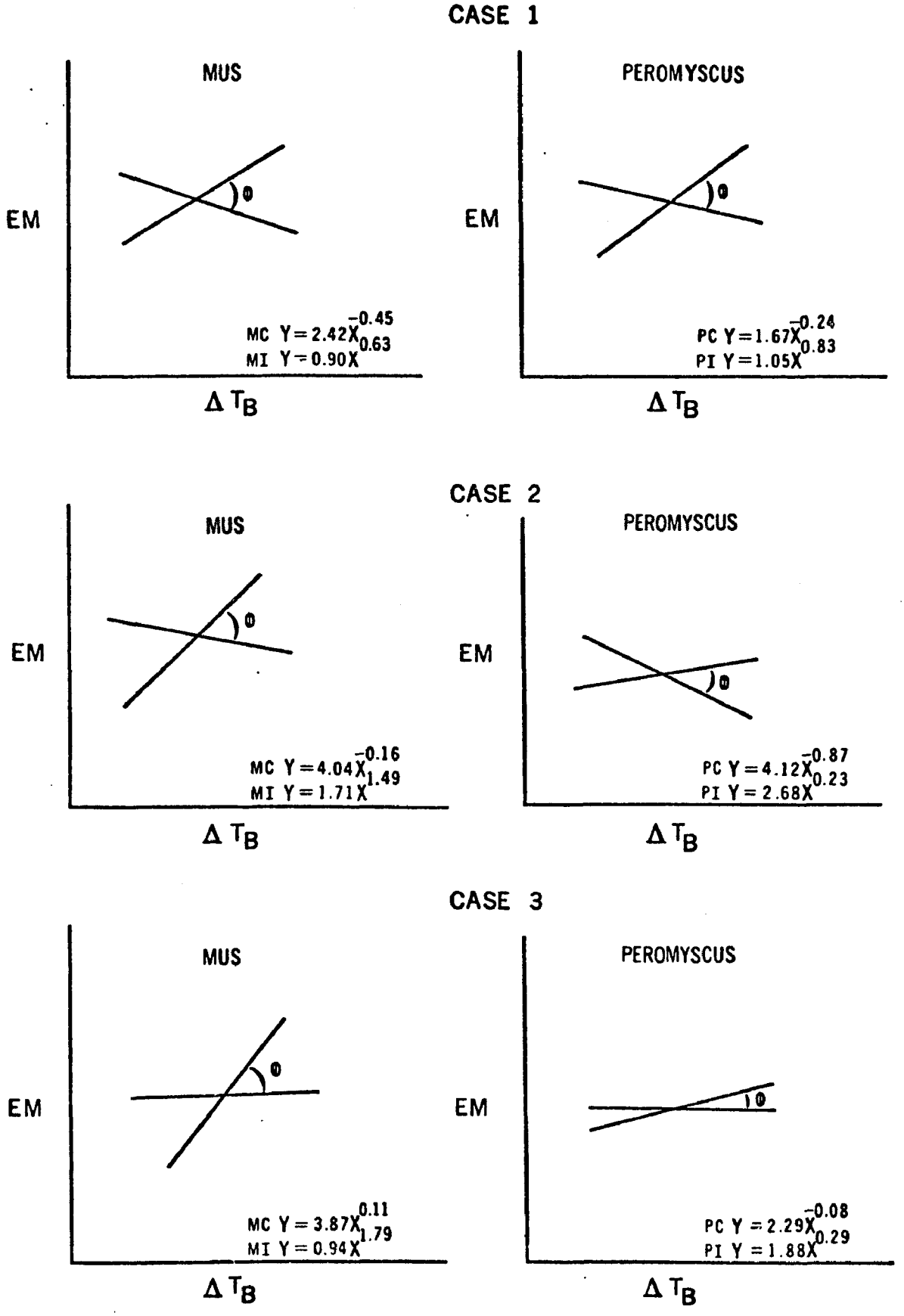
CASE 1
MUS
EM
MC Y = 2.42X^-0.45 0.63
MI Y = 0.90X
ΔTB
PEROMYSCUS
EM
PC Y = 1.67X^-0.24 0.83
PI Y = 1.05X
ΔTB
CASE 2
MUS
EM
MC Y = 4.04X^-0.16 1.49
MI Y = 1.71X
ΔTB
PEROMYSCUS
EM
PC Y = 4.12X^-0.87 0.23
PI Y = 2.68X
ΔTB
CASE 3
MUS
EM
MC Y = 3.87X^0.11 1.79
MI Y = 0.94X
ΔTB
PEROMYSCUS
EM
PC Y = 2.29X^-0.08 0.29
PI Y = 1.88X
ΔTB

Figure 2

greater elevation in EM required for T_B in Peromyscus suggested that both were achieving ΔT_B by slightly different means. It was evident that thermoregulation in Mus irradiated during Cases 2 and 3 (where $R = .987$, $P < 0.01$; $R = .895$, $P < 0.01$) was dependent upon metabolism, less than for irradiated Peromyscus (where $R = .925$, $P < 0.01$; $R = .969$, $P < 0.01$). Evidence for this can be seen in the smaller slopes for Peromyscus (Figure 2).

Regression slopes between control species were not different except during Case 2 where ΔT_B in Peromyscus was less dependent upon metabolic output associated with activity. Since no difference in activity levels was noted among species controls during Case 2, Mus expended more energy with less ΔT_B than Peromyscus for T_A simulating tne natural environment (Table 10). During Case 3 ΔT_B appeared to be independent of metabolic output for both species control groups; thus likely to be related to activity associated with T_A changes.

Temporal Associations Between MR, T_B and Act.

Linearity and correlation in MR vs. T_B, MR vs. Act. and T_B vs. Act. for individual animals were generally quite high yet when all data within a group were pooled, without consideration of individual contribution to the pooled mean, a bias resulted owing to differences in regression between individuals. Partitioning of the pooled data by animal

and time resulted in a reliable estimate of both correlation and regression. Since all members of a group had very similar within-parameter correlation coefficients, no loss of accuracy was expected (Ruland, per. comm.).

Representative correlation coefficients are reported in Table 11 for each treatment group by day for each association (MR vs. Act., etc.). In both control and irradiated mice, thermoregulation was more closely associated with MR than with activity during Case 1. This relationship appeared to be greater for Peromyscus than for Mus when comparing R values for MR vs. Act. or T_B vs. Act. (Table 11).

During Case 2 regulation of T_B was more closely related to activity in Mus but not in Peromyscus. This supported the earlier findings that Mus seemed to be slightly more dependent upon activity to ΔT_B than Peromyscus (Figure 2). During Case 3, metabolism became disassociated with the requirements for thermoregulation (Table 11). Behavioral thermoregulation was therefore an important mechanism to reduce energy expenditure. This seemed to be especially true during ambient conditions where T_A was 15C during ρ and 32C during α. Confirmation of this point appeared to come from the fact that no statistical relationship existed for MR vs. Act. for all groups during Case 3 (Table 11).

Table 11. Correlation coefficients (R) for associations of metabolism vs. body temperature (T_B-MR), metabolism vs. activity (MR-Act.) and activity vs. body temperature (Act.-T_B) during each of four 24-hour periods in control and irradiated Mus musculus and Peromyscus leucopus. All R values are significant ($P<0.05$) except MR-Act. day 4 for all groups of mice; N = 144 per cell. ANL, 1974-75.

		Mus musculus		Peromyscus leucopus	
		Controls	Irradiated	Controls	Irradiated
Day 1					
	T_B-MR	.8472	.8048	.7741	.7686
	MR-Act.	.7120	.7252	.5034	.4761
	Act.-T_B	.7416	.8018	.5343	.4549
Day 2					
	T_B-MR	.8850	.9021	.7322	.7163
	MR-Act.	.7645	.6780	.3776	.4046
	Act.-T_B	.7386	.7385	.5029	.4444
Day 3					
	T_B-MR	.3516	.4117	.6815	.6524
	MR-Act.	.5038	.5267	.4805	.6264
	Act.-T_B	.6678	.7405	.4885	.4000
Day 4					
	T_B-MR	-.3106	-.2813	-.5198	-.1602
	Mr-Act.	.0668	-.0839	.0341	-.0233
	Act.-T_B	.5880	.5445	.3825	.3507

Analysis of R by activity cycles indicated that T_B was more closely associated with MR during ρ, but that T_B was more closely associated with Act. during α (X^2: $P < 0.01$). While this trend generally held for all Cases it was especially pronounced in Case 1. At both activity levels (ρ and α) MR vs. Act. had an intermediate R value.

Linear Regression of MR and T_B

Differences between species metabolism and body temperature by activity period were calculated using linear regression analysis on Case 1 (Service, et al., 1972). Students t-test taken from the analysis of variance table revealed that both slope (b) and intercept (a) were significant (Table 12); thus H: $B \neq 0$ (a and b were not equal to zero). Correlation coefficients for the MR vs. T_B associations were also significant ($P < 0.001$) (Table 12).

A significant difference occurred between control and irradiated Peromyscus at ρ and α ($P < 0.05$) but not for Mus treatment groups. Control Peromyscus had a significantly lower slope than Mus controls during α ($P < 0.05$) but not ρ. No difference existed between the two irradiated groups (Table 12).

Detailed interpretation of regression analysis for Cases 2 and 3 were complicated by (1) a high degree of individual variation between animals presumably as a result of individual response to T_A changes; (2) non-significant

Table 12. Linear regression slopes (b) and intercepts (a) for oxygen consumption (Y) and body temperature (X) for each treatment group of Mus musculus and Peromyscus leucopus by activity periods (ρ and α) during Case 1 (days 1 and 2). The linear approximation Y = a +bX was used where N = 20 per cell. All slopes and intercepts are where H: B ≠ 0 and R for all slopes are P < 0.001. ANL, 1974-75.

Animal Groups	Inactive Period (ρ)		Active Period (α)	
	slope (b)	intercept (a)	slope (b)	intercept (a)
Peromyscus:				
Controls	.239	-6.15	.259	-7.60
Irradiated	.340	-2.12	.369	-11.60
Mus:				
Controls	.282	-9.30	.485	-15.61
Irradiated	.254	-6.68	.352	-9.94

regression and correlation between T_B and MR resulting in (3) a reduced confidence level and sample size from which to make meaningful conclusions. While specific data were not possible, some biological inferences can be made concerning data trends, and thus will be taken up in the discussion.

Time Series Analysis

Cross-correlation analysis was performed as a function of time on two continuous variables, body temperature and oxygen consumption. This analysis served to test regularity in an otherwise irregular record (time varying) or locate regular segments of two irregular, but assumed to be linearly related records. Reduced regularity was assumed to represent reduced performance. Time series analysis was not performed on activity because it was recorded as a descrete variable.

Peromyscus leucopus. In control mice MR lagged T_B (i.e., T_B versus MR) during ρ but generally no lag existed during α in Case 1 (Table 13 and Figure 3). The opposite appeared to be true for the irradiated group, however, a statistical difference existed only at 0600 to 1200 hours day 1 ($P < 0.05$). The amplitude of response between activity periods was higher for the controls. The most pronounced difference between treatment groups during Case 1 was found in the large variances between groups at each

Table 13. Summary of cross-correlation time lag data (in minutes) for control and irradiated *Peromyscus leucopus* by day (Cases 1-3) where positive values mean that MR is lagging behind T_B (T_B vs. MR) and negative values are where T_B lags MR (MR vs. T_B). Values are means ± st ndard error. Means ± S.D. are recorded for T_B and MR associated with each time period (0600-1800 = ρ; 1800-0600 = α); when N = 36 per cell. ANL, 1974-75.

Day	Time Period	Controls			Irradiated		
		Time-Lag	T_B	MR	Time-Lag	T_B	MR
1	0600-1200	10±3.1	36.9±.35	2.43±.71	-7.5±10.7	35.9±1.1	2.49±.62
	12-18	7±4.1	36.6±.34	2.06±.76	0±12.3	36.1±.70	2.04±.54
	18-24	-4±9.2	37.4±.69	2.46±.58	4±12.1	37.3±.88	2.90±.80
	24-6	6±6.7	38.0±.40	2.95±.57	6±12.1	37.4±.67	3.23±1.0
2	6-12	15±7.7	36.6±.64	2.50±.22	2±10.3	36.6±1.3	2.32±.62
	12-18	-4±9.2	36.5±.67	2.16±.42	0±11.4	35.9±1.2	2.09±.44
	18-24	0±7.0	37.3±.84	2.43±.43	6±12.5	37.3±.91	2.97±.93
	24-6	2±4.2	37.9±.55	2.85±.42	6±7.5	37.5±.88	3.34±1.4
3	6-12	8±5.6	38.2±1.4	2.71±.85	6±11.7	36.7±.69	2.42±.70
	12-18	2±3.6	37.6±.64	2.20±.87	8±14.6	36.5±.74	1.94±.43
	18-24	-46±12	37.5±.98	3.39±.86	-16±8.7	37.3±.79	3.57±.94
	24-6	6±5.9	37.9±.76	4.71±.45	14±6.8	37.7±.97	4.75±.89
4	6-12	-2±14.9	36.6±.80	4.14±.41	10±6.3	36.2±2.1	4.02±.79
	12-18	0±4.4	36.6±.87	4.04±.76	18±15	36.5±1.1	3.81±.68
	18-24	-40±15.9	37.3±.65	3.15±.55	-5±20.8	37.5±.98	3.34±.71
	24-6	-2±5.8	38.4±.40	2.69±.65	4±12.9	37.9±1.0	2.82±.83

Figure 3. Cross-correlation time series analysis on body temperature and metabolism for control (C) and irradiated (I) Peromyscus leucopus during inactive (ρ) and active (α) periods measured for steady-state (Case 1), an ambient temperature similar to the natural environment (Case 2) and a 12-hour T_A phase shift (Case 3). N = 36 at each recorded point (C or I). ANL, 1974-75.

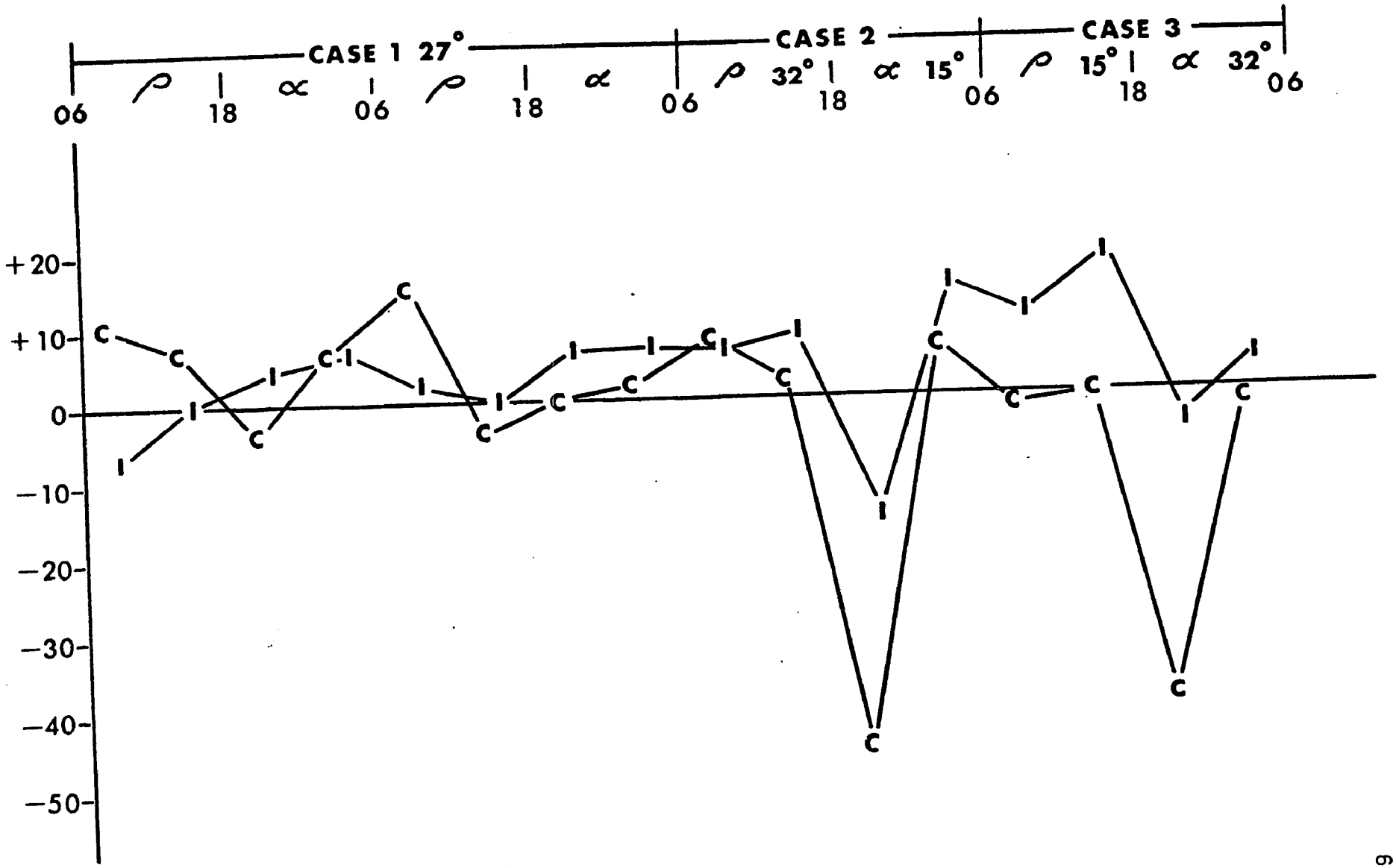
CASE 1 27°
CASE 2
CASE 3
32°
15°
15°
32°
06
18
06
18
06
18
06
+20
+10
0
−10
−20
−30
−40
−50

Figure 3

time interval (Table 13).

During day 3 separation in responses came only during the initial stages of α 15C (1800 to 2400 hours) where T_B lagged MR by about 45 minutes in controls and about 16 minutes in irradiated mice (Table 13, Figure 3). The difference between groups during this period was significant ($P < 0.05$). During day 4 (Case 3) both groups appeared to have responded to T_A out-of-phase in a similar fashion, however, controls showed little or no lag in T_B (Figure 3). The difference was not significant (Table 13).

Mus musculus. Cross-correlation data are summarized for Mus treatment groups in Table 14. T_B preceded MR throughout most of the four day experiment (Figure 4). A periodic trend in T_B versus MR was associated with activity cycles (Figure 4). The periods and amplitudes of these cycles appeared to be greater in the irradiated group.

There was less time lag between T_B and MR during ρ than α, supporting the earlier findings that T_B was more closely associated with Act. during α and with MR during ρ. When T_A changed from 32C to 15C the lag in T_B vs. MR was 40 to 50 minutes in both groups (Table 14). During day 4 irradiated mice had a greater lag in T_B vs. MR than controls during α at 32C ($P < 0.05$) (Figure 4). Variability in the data, especially in irradiated Mus, resulted in few statistical differences (Table 14).

Peromyscus versus Mus. Peromyscus and Mus responded

Table 14. Summary of cross-correlation time-lag data (in minutes) for control and irradiated Mus musculus. (See Table 13 for a complete description.)

Day	Time Period	Controls			Irradiated		
		Time-Lag	T_B	MR	Time-Lag	T_B	MR
1	0600-1200	10±10.9	37.0±.83	2.18±.20	-2±5.8	35.4±.40	2.42±.22
	12-18	2.5±4.2	37.1±.74	2.06±.22	0±10.5	35.5±.20	2.23±.16
	18-24	8±11.5	38.5±.39	2.90±.15	16±5.1	37.2±.66	3.24±.37
	24-6	6±5.0	37.8±.61	2.78±.17	20±4.5	36.8±1.0	3.00±.44
2	6-12	8±4.8	36.9±.83	2.17±.18	12±6.6	35.4±.19	2.51±.40
	12-18	8±16.4	37.2±.84	2.30±.54	4±4.0	35.5±.23	2.22±.24
	18-24	18±16.4	38.4±.25	3.02±.40	20±6.3	37.5±.79	3.31±.50
	24-6	8±14.8	37.8±.71	2.85±.53	16±5.1	36.6±.93	2.88±.39
3	6-12	20±13.4	37.6±.65	2.01±.25	10±5.2	36.2±.84	2.31±.43
	12-18	8±4.2	37.5±.67	1.67±.31	15±5.8	35.9±.55	1.70±.19
	18-24	54±9.2	38.5±.50	4.48±.66	46±12.9	37.6±.46	3.74±1.1
	24-6	2±3.7	37.4±.81	5.32±.94	15±17.3	36.7±1.2	4.52±1.8
4	6-12	18±5.8	37.1±.74	5.12±.66	2±7.3	35.8±.78	4.19±1.6
	12-18	4±11.6	37.1±.81	4.33±.64	10±3.7	35.8±.67	3.72±1.8
	18-24	15±5.7	38.0±.61	3.54±.65	63±7.6	37.2±.73	3.92±.76
	24-6	24±6.7	37.8±.66	2.43±.62	12±4.9	37.0±.90	2.59±.32

Figure 4. Cross-correlation time series analysis on body temperature and metabolism for control (C) and irradiated (I) Mus musculus during inactive (ρ) and active (α) periods measured for steady-state (Case 1), an ambient temperature similar to the natural environment (Case 2) and a 12-hour T_A phase shift (Case 3). N = 36 at each recorded point (C and I). ANL, 1974-75.

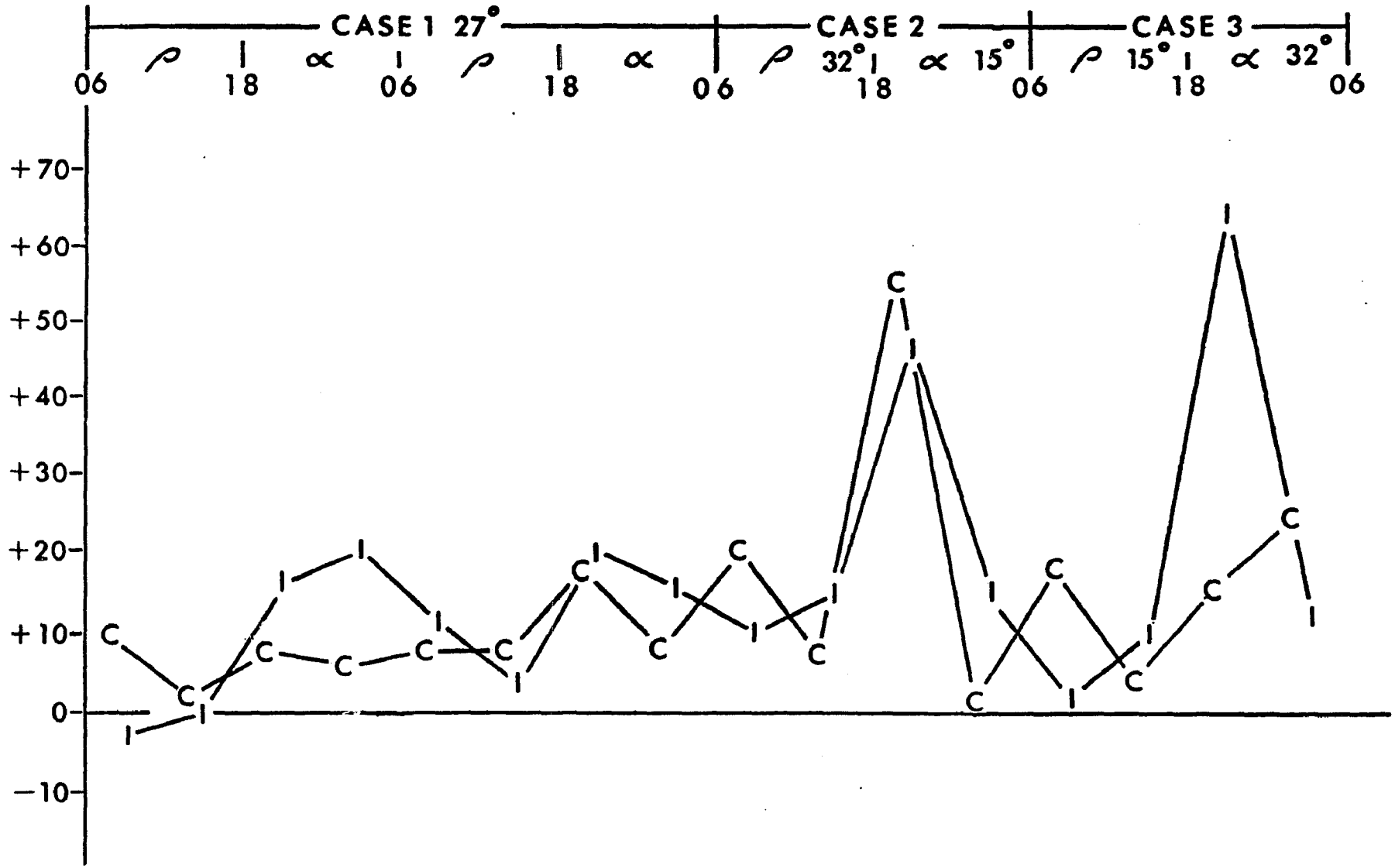
CASE 1 27°
CASE 2
CASE 3
ρ
α
06
18
06
18
06
32°
18
15°
06
15°
18
32°
06
+70
+60
+50
+40
+30
+20
+10
0
−10
C
I

Figure 4

differently to T_A changes during the initial stages of their active cycle (α). There seemed to be a species effect in the time lag intervals between T_B and MR, as Mus displayed a T_B vs. MR lag while Peromyscus displayed a MR vs. T_B lag (Figures 3 and 4).

During steady-state there was no difference in lag components; both species displayed a short lag of T_B vs. MR. MR followed T_B by about 20 minutes throughout Case 1, lower in Peromyscus than Mus during α but higher in Peromyscus than Mus during ρ (Figures 3 and 4). Initial response to α at 15C and 32C showed the greatest difference among species ($P < 0.01$). At ρ 32C and 15C no difference existed among species controls or irradiated groups.

Peromyscus was apparently metabolizing before elevation of T_B at 15C α, while Mus delayed metabolic output and allowed T_B to rise before equal increments of MR. This would seem to indicate that metabolism was the key thermoregulatory mechanism in Peromyscus when T_A changed but that behavioral thermoregulation was more critical in Mus. However, no significant elevation in T_B occurred for either group during this time (for control animals) nor was C different (Table 6).

During Case 3 Mus controls were more active than Peromyscus controls. Because the lag relationship was similar to Case 2 α, any interpretation seemed contradictory to the previous events (α 15C, Case 2). However, the

correlation between T_B and MR was a negative one. That is, T_B became disassociated with MR during Case 3 (Table 11). Therefore, T_B was lagging MR in the opposite direction for Peromyscus (MR was decreasing as T_B increased), and MR was lagging T_B in the opposite direction for Mus (Tables 13 and 14). The time lag in Mus during α 32C was not different than during steady-state but for Peromyscus the difference was significant ($P < 0.05$). Peromyscus responded to 32C during α by becoming less active and maintained a high T_B; Mus significantly increased activity during α 32C over α 15C.

Diurnal Rhythms of Metabolism and Body Temperature

Using methods of least squares cosine fit of 24-hour non-linear data (Halberg, et al., 1955, cited in Halberg, et al., 1971; Yunis, et al., 1974) diurnal rhythms were estimated for body temperature and oxygen consumption (Table 15). These estimates were diurnal rhythms (i.e., nychthermal) rather than true circadian rhythms because these animals were not entrained to free-running conditions long enough before measurements were taken (Pittendrigh and Daan, 1974).

Steady-state oxygen consumption (Figure 5) and 96-hour body temperatures (Figure 6) revealed that diurnal predictability in a circadian fashion resulted in differences in parameter means (termed mesor; Halberg and Lee,

Table 15. Mean±standard errors for 96-hour body temperatures and 48-hour metabolism in treatment groups of Peromyscus leucopus and Mus musculus fit to a 24-hour least squares cosine curve where T_B = body temperature, MR = metabolism, Mesor = mean values (in °C or $cc O_2 gm^{-1} hr^{-1}$), Amp = amplitude of the curve taken from mesor (in °C or $cc O_2 gm^{-1} hr^{-1}$) and Period = the time of the next repeating oscillation, diurnal rhythm (in hours). N = 2880 for T_B and 1440 for MR. See Table 16 for probability levels of significance.

	Peromyscus leucopus		Mus musculus	
	Controls	Irradiated	Controls	Irradiated
T_B Mesor	37.4±0.14	36.9±0.37	37.6±0.26	36.4±0.26
T_B Amp	0.80±0.05	0.87±0.14	0.61±0.06	1.07±0.14
T_B Period	24.7±0.40	24.2±0.18	23.9±0.22	23.9±0.27
MR Mesor	2.30±0.12	2.87±0.18	2.47±0.07	2.75±0.11
MR Amp	0.47±0.05	0.88±0.16	0.59±0.03	0.65±0.07
MR Period	24.6±0.14	23.8±0.30	23.5±0.43	23.5±0.39

Figure 5. Least squares fit of a 48-hour cosine function ($F_{(t)}$) to oxygen consumption in Mus musculus number 717; where the abscissa (X-axis) represents time in hours (beginning 0600 day 1 and ending 0600 day 2 = Case 1) and the ordinate (Y-axis) represents oxygen consumption (MR, $cc O_2 gm^{-1} hr^{-1}$). This computor printout plotted data for 48-hours and computed the 24-hour parameters of mesor (C_o, or mean value of MR), amplitude (A, in $cc O_2 gm^{-1} hr^{-1}$), angular frequency (ω) and period (τ) of the rhythm (in degrees and hours) and acrophase (ϕ, lag approximating the rhythm; in degrees or time) according to the equation $F_{(t)} = C_o + A \cos(\omega t + \phi)$ (Halberg, et al., 1971; Yunis, et al., 1974). P = predicted cosine rhythm; O = observed MR data. ANL, 1974-75.

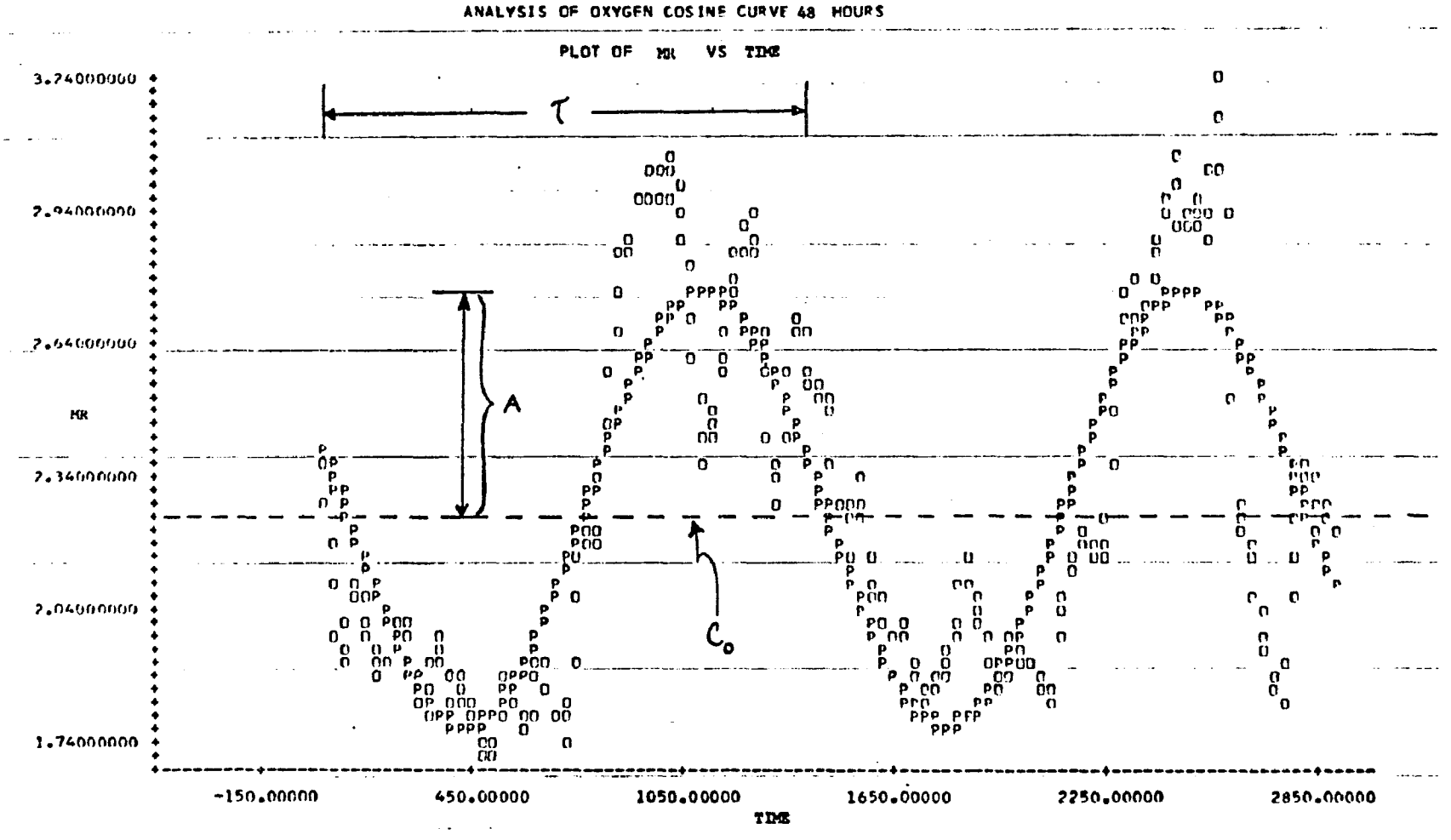
ANALYSIS OF OXYGEN COSINE CURVE 48 HOURS
PLOT OF MR VS TIME
3.24000000
2.94000000
2.64000000
MR
2.34000000
2.04000000
1.74000000
τ
A
C_o
-150.00000
450.00000
1050.00000
1650.00000
2250.00000
2850.00000
TIME

Figure 5

Figure 6. Least squares fit of a 96-hour cosine function to body temperature in Mus musculus number 730; where the abscissa (X-axis) represents time from 0600 day 1 ending 0600 day 4, and the ordinate (Y-axis) is body temperature °C. This cosine plot was averaged over 96-hours and 24-hour rhythm functions computed (see Figure 5). ANL, 1974-75.

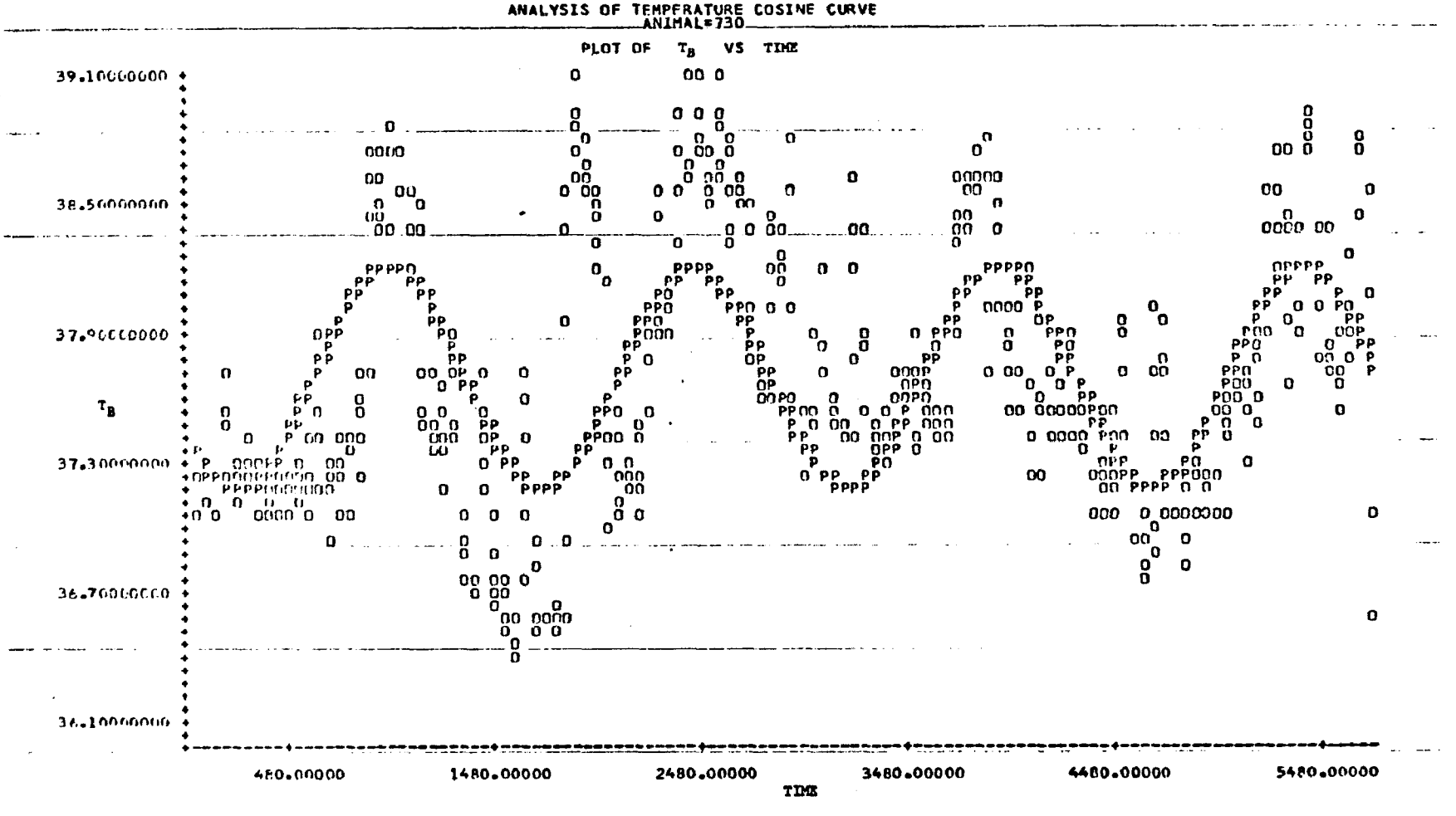
ANALYSIS OF TEMPERATURE COSINE CURVE
ANIMAL=730
PLOT OF T_B VS TIME
39.10000000
38.50000000
37.90000000
37.30000000
36.70000000
36.10000000
T_B
480.00000
1480.00000
2480.00000
3480.00000
4480.00000
5480.00000
TIME

Figure 6

1974) and amplitudes between species groups. Figure 7 summarizes these data from Table 15 using the rhythmometric technique employed by Halberg, et al. (1971) and Yunis et al. (1974).

Mus controls had a significantly lower T_B amplitude than Peromyscus controls ($P < 0.0283$) and a significantly higher MR amplitude ($P < 0.0472$) (Table 16). Irradiated Mus had a significantly lower T_B mean ($P < 0.0278$) and a significantly higher T_B amplitude than control Mus ($P < 0.009$) (Table 16). Irradiated Peromyscus had a significantly higher MR mean ($P < 0.0283$) and a higher MR amplitude ($P < 0.0090$). Irradiated Peromyscus also had a shorter MR period ($P < 0.0472$) than Peromyscus controls (Tables 15 and 16, Figure 7); characteristic of activity periods in aged mice (Pittendrigh and Daan, 1974). No significant difference in T_B mean, amplitude or period for treatment Peromyscus occurred, however, T_B mean in controls was higher and amplitude lower than in irradiated Peromyscus. No significant difference in MR mean and amplitude occurred between treatment groups of Mus, although mean and amplitude were higher in irradiated Mus. No difference existed among irradiated species (Mus versus Peromyscus) for either T_B or MR.

Figure 7. Rhythmometric summary of body temperature and oxygen consumption in control (C) and irradiated (I) Mus musculus and Peromyscus leucopus during steady-state ambient conditions (Case 1). Mesor = mean parameter value (± standard errors), amplitude = height of the parameter response from mesor, and period = the diurnal rhythm where the cosine wave begins to repeat itself. Five animals per group were used, N = 2880 for T_B and 1440 for MR per group. ANL, 1974-75.

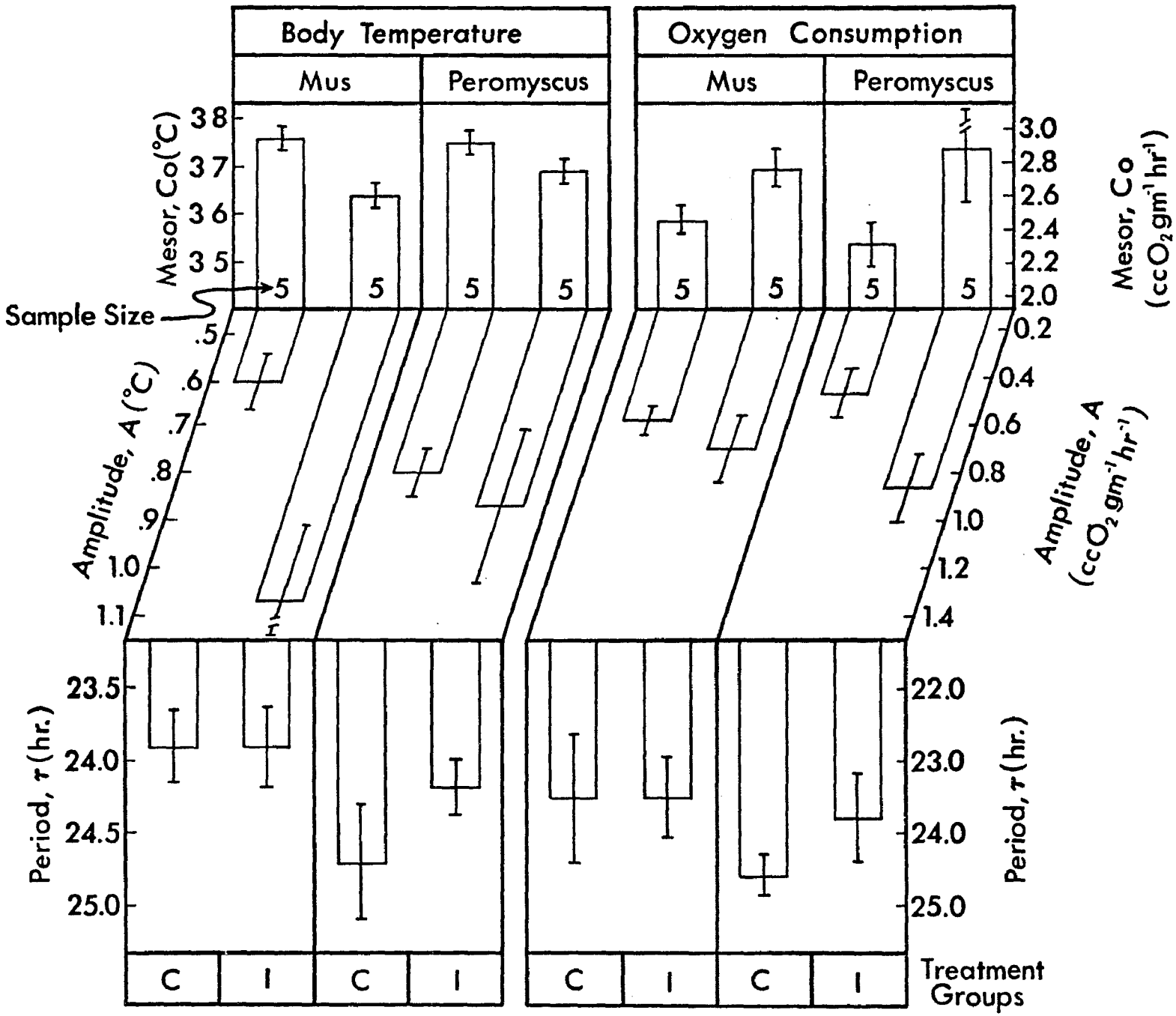
Body Temperature
Oxygen Consumption
Mus
Peromyscus
Mus
Peromyscus
Mesor, Co(°C)
Mesor, Co (ccO2gm-1hr-1)
Sample Size
Amplitude, A (°C)
Amplitude, A (ccO2gm-1hr-1)
Period, τ (hr.)
Period, τ (hr.)
C
I
C
I
C
I
C
I
Treatment Groups

Figure 7

Table 16. Probability levels of significance for body temperature and metabolism fit to a least squares cosine curve for comparisons between species and treatment groups of *Mus musculus* and *Peromyscus leucopus*. Mesor = mean parameter value, Amp = amplitude from mesor, Period = diurnal rhythm.*

	Body Temperature			Metabolism		
	Mesor	Amp	Period	Mesor	Amp	Period
Mus Controls vs. Irradiated	0.0278	0.0090	0.6015	0.1745	0.7540	0.7540
Peromyscus Controls vs. Irradiated	0.6004	0.2948	0.4020	0.0283	0.0090	0.0472
Mus Controls vs. *Peromyscus* Controls	0.7533	0.0283	0.0758	0.2948	0.0472	0.1425
Mus Irradiated vs. *Peromyscus* Irradiated	0.4647	0.2506	0.2948	0.6015	0.2506	0.7540

* Statistical analysis employing the Kruskal-Wallis test was used, modified for SAS by Gary White, Ohio Cooperative Wildlife Research Unit, Department of Zoology, The Ohio State University. Sample size for each group comparison was 10 (d.f. = 8).

DISCUSSION

On the basis of the weight specific allometric equation (Schmidt-Nielsen, 1975):

$$\dot{V}_{O2} / M_b = 3.8 \times M_b^{-0.25}$$

where $\dot{V}_{O2} / M_b$ is oxygen consumption (MR) in $cc O_2 gm^{-1} hr^{-1}$ and M_b is animal weight in grams; an estimate of standard metabolic rate (SMR) for each group of mice was made. The procedure followed the linear hypothesis model proposed by Brody (1945) and Kleiber (1932, 1961). The predicted standard metabolic rate for Mus (controls and irradiated) was 1.80 $cc O_2 gm^{-1} hr^{-1}$, 1.69 $cc O_2 gm^{-1} hr^{-1}$ for Peromyscus controls and 1.64 $cc O_2 gm^{-1} hr^{-1}$ for Peromyscus irradiated. These estimates assumed that the animals were not feeding, nor had fed for several hours previously, and were in thermal equilibrium with the environment.

At apparent thermoneutrality, using the linear approximation, my data underestimated MR for Mus (controls = 1.46 ± 0.21 $cc O_2 gm^{-1} hr^{-1}$) and overestimated MR for Peromyscus (controls = 1.72 ± 0.26 $cc O_2 gm^{-1} hr^{-1}$) (Table 5). The differences were not significant. The predicted SMR for Mus was 6% higher than Peromyscus while observed SMR for Mus was 15% lower. This paradox was in apparent violation of

the surface to volume law (Bartholomew, 1972) where smaller mammals lose more heat to the environment; hence, to maintain homeothermy, they have a higher MR (Schmidt-Nielsen, 1975).

However, at selected temperatures and during periods of activity or inactivity the differences in MR between species can change dramatically (Hart, 1950). Therefore, daily metabolic transition between similar sized animals cannot be made solely on the basis of weight and/or species but is also dependent upon level of activity and ambient temperature.

Animals seldom function under the strict limitations imposed by researchers who estimate SMR as representing an animal's metabolism (Bartholomew, 1972). One reason why I have detected a lower MR in Mus than expected, or from the literature (Hart, 1952; Pennyciuk, 1972), may have to do with the fact that SMR could be taken throughout a 12-hour period (ρ) when the mice were known to be at complete rest. My results were undoubtedly a reliable estimate of resting MR for both species since it might be expected that biases result in those cases where MR was taken shortly after an animal had been placed into a small container for metabolism testing (Heusner, et al., 1971b).

Another reliable estimate of metabolism can be gained from using the average daily MR or "thermoneutral metabolic rate", TMR (Webster, cited in Robershaw, 1974; Wunder,

1975) where the effects due to specific dynamic affect (SDA; i.e., oxidative metabolism of residual foodstuff) and activity are incorporated into the thermo-metabolic characteristics of a species (Tables 4 and 6). At 27C, where activity was independent of T_A, Mus had a 13.3 percent higher metabolic output (cal $gm^{-1}hr^{-1}$) than Peromyscus. At 32C Peromyscus had a 6 percent higher MR than Mus. Thus, the thermoneutral zone based on TMR, was apparently lower in Peromyscus than Mus. This was expected as Peromyscus was larger and had a lower critical temperature (T_{LC}). Also, Peromyscus has been reported to be a more cold acclimated species (Hart, 1953; Stinson and Fisher, 1953; Morrison and Ryser, 1959; Ogilvie and Stinson, 1966).

Wunder (1975) proposed a metabolic model which predicted the expected total metabolic character of a species based upon previously defined weight, temperature and activity related equations. Using predicted and observed values from my data, and approximating thermoneutrality, the model closely fits control animals (within 1 percent) but underestimated those values for irradiated mice. The equation Wunder used was:

$$MR = \alpha M_B + M_{TR} + M_A$$

where MR = total metabolism ($cc O_2 gm^{-1}hr^{-1}$) modified for activity, ambient temperature and animal posture; α = activity-postural coefficient, 1.0 = rest, 1.7 = active

(Taylor, et al., 1970); $M_B = 3.8W^{-0.25}$, standard metabolism (Kleiber, 1961); $M_{TA} = C\ (T_{LC} - T_A)$ or $(1.05W^{-0.50})$, $[(T_B - 4W^{+0.25}) - T_A]$, thermal conductance function (Morrison, 1960; Herreid and Kessel, 1967); and $M_A = (8.46W^{-0.40})v$, net cost of running (v = 0 for my study) (Taylor, et al., 1970).

At 27C and with a calculated T_{LC} of 28.6C for Mus and 28.0C for Peromyscus, the predicted MR for Mus controls was 2.18 $cc O_2 gm^{-1} hr^{-1}$ and observed 2.20 $cc O_2 gm^{-1} hr^{-1}$ (1 percent difference). Irradiated Mus had an observed value 8 percent higher than expected (2.35 versus 2.18 $cc O_2 gm^{-1} hr^{-1}$). The predicted value for control Peromyscus was 1.88 $cc O_2 gm^{-1} hr^{-1}$, the observed was 1.90 $cc O_2 gm^{-1} hr^{-1}$ (1 percent difference). Irradiated Peromyscus had a 20 percent higher MR than expected (2.30 versus 1.84 $cc O_2 gm^{-1} hr^{-1}$). These elevations in overall metabolism in irradiated mice correspond directly to changes in thermal conductance, suggesting that excess metabolic heat was generated because of a reduction in thermoregulatory efficiency.

Cost of Activity

Increase in oxygen consumption for activity above that needed for standard metabolism (EM) was about 4.2 times in Mus (6.12 $cc O_2 gm^{-1} hr^{-1}$ @ 15C α minus 1.46 $cc O_2 gm^{-1} hr^{-1}$ @ 32C ρ - thermoneutrality) and about 3.1 times in Peromyscus (5.31 $cc O_2 gm^{-1} hr^{-1}$ @ 15C α minus 1.72

$cc O_2 gm^{-1} hr^{-1}$ @ 27C ρ ~ thermoneutrality). These values were comparable to those in the literature for metabolic scope for Mus, 4.5 times (Hart, 1950; Jansky, 1959), but below those for Peromyscus, 5.7 times (Segrem and Hart, 1967). The difference, of course, lies in the fact that values from the literature represented animals at work using running velocity as an activity indicator, while my data represented spontaneous activity patterns analogous to existence metabolism (Kendeigh, 1969).

As discussed by Wunder (1970), the aerobic cost of activity may be either additive (i.e., if costs were independent of T_A, decreasing at increasing T_A).or partially substitutive (i.e., costs would decrease with decreasing T_A). In Wunder's data activity was partially substitutive for very active animals but additive at lower activity levels up to about 30C. From my data, activity in Peromyscus controls and both treatment groups of Mus was more than additive thus the costs for being active were less at higher temperatures than at lower temperatures (Figure 8). The cost for being active in irradiated Peromyscus was greater at 32C, hence partially substitutive. 32C was therefore a more critical event in irradiated Peromyscus than controls (Figure 8 and 10).

Irradiated Peromyscus appeared to have been more metabolically efficient during α at 15C than the other three groups of mice (Figure 8). They maintained their T_B close to that of control Peromyscus (Figures 9 and 10). A

Figure 8. Existence Metabolism (MR; $cc O_2 gm^{-1} hr^{-1}$) over maintenance, measured by subtracting maximum spontaneous non-running activity MR minus standard metabolism at three ambient temperatures (T_A, °C) in Mus musculus (M———M controls; M-----M irradiated) and Peromyscus leucopus (P———P controls; P-----P irradiated). N = 15 at each recorded point. ANL, 1974-75.

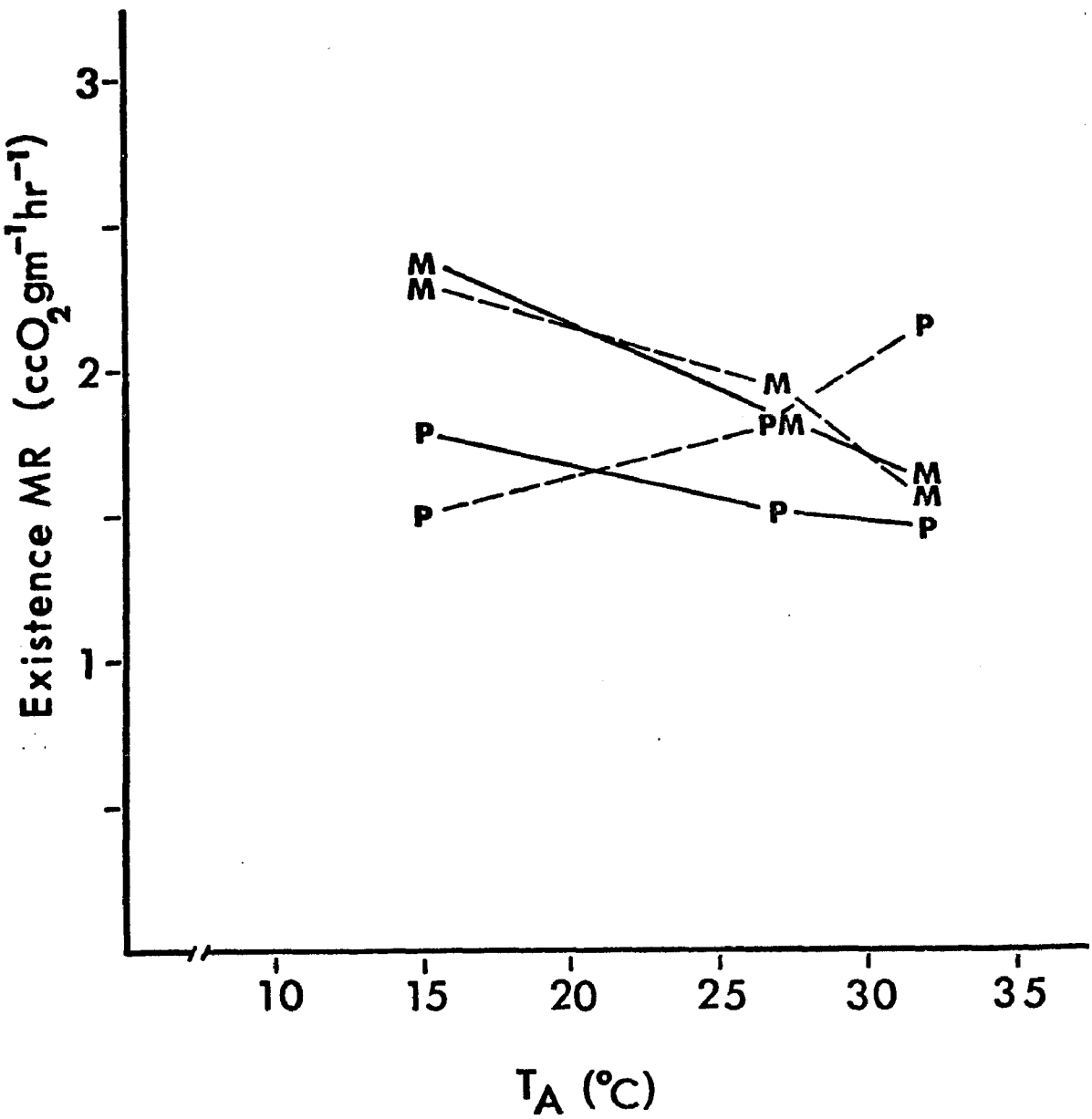

Figure 8

Figure 9. Thermal conductance (C; $cc O_2 gm^{-1} hr^{-1}/°C$) and body temperature ($T_B$; °C) at highest metabolic output during α and lowest metabolic output during ρ in control Mus musculus (ρ = M——M; α = M---M) and Peromyscus leucopus (ρ = P——P; α = P---P). N = 15 at each recorded point. ANL, 1974-75.

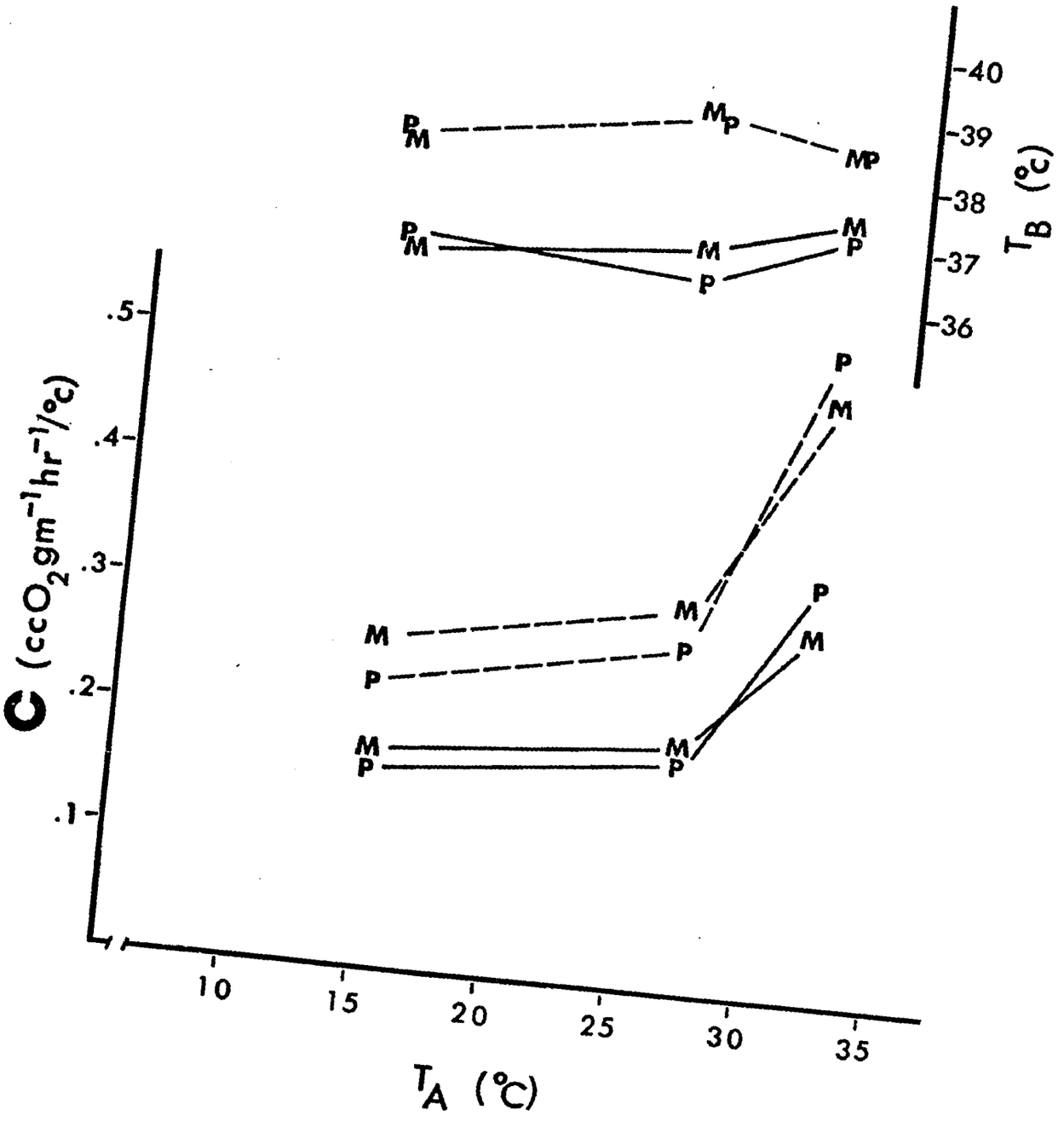

Figure 9

Figure 10. Thermal conductance (C; $cc O_2 gm^{-1} hr^{-1}/°C$) and body temperature ($T_B$; °C) at highest metabolic output during α and lowest metabolic output during ρ in irradiated Mus musculus (ρ = M——M; α = M---M) and Peromyscus leucopus (ρ = P——P; α = P---P). N = 15 at each recorded point. ANL, 1974-75.

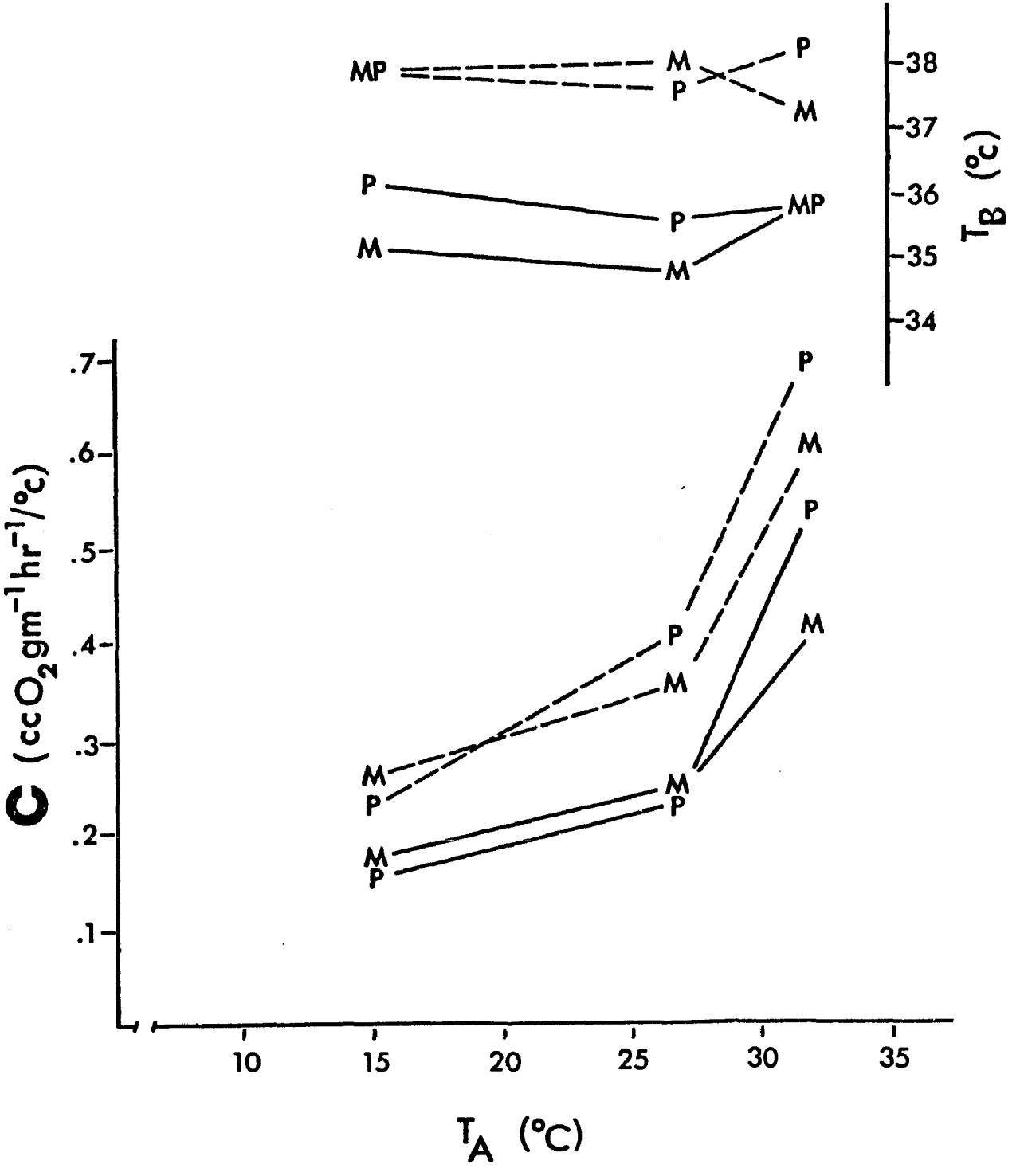
MP
M
P
P
M
P
P
MP
M
M
38
37
36
35
34
T_B (°C)
.7
.6
.5
.4
.3
.2
.1
C ($cc O_2 gm^{-1} hr^{-1}/°c$)
P
M
P
M
P
M
M
P
M
P
10
15
20
25
30
35
T_A (°C)

Figure 10

reduced metabolic output caused by ionizing radiation may have conferred an advantage at lower T_A's. However, at α 15C both controls and irradiated Peromyscus had the same metabolic-activity requirement (Figure 8); thus the effects due to ionizing radiation (the cost of performing) would be greatest at temperatures greater than T_{LC} in this species.

Thermal Conductance

The cost of activity at different ambient temperatures was evaluated by comparing change in heat loss to the environment (Tables 9 and 10). Thermal conductance followed a curvilinear relationship for spontaneous activity (this study) much the same for entrained activity reported for Merriam's chipmunk, Eutamias merriami (Wunder, 1970).

Mus lost more heat to the environment than Peromyscus at all three temperatures (α + ρ). Comparing average MR, the amount of heat lost through C was approximately 18.2 percent higher in Mus at 32C (2.04 cal $gm^{-1}hr^{-1}/°C$), 9.3 percent higher at 27C (1.12 cal $gm^{-1}hr^{-1}/°C$) and 4.3 percent higher at 15C (0.90 cal $gm^{-1}hr^{-1}/°C$). Heat lost to C by Peromyscus was 1.99 cal $gm^{-1}hr^{-1}/°C$ at 32C, 1.04 cal $gm^{-1}hr^{-1}/°C$ at 27C and 0.88 cal $gm^{-1}hr^{-1}/°C$ at 15C. These dat suggest that reductions in thermal conductance were a more effective mechanism to reduce heat loss in

Peromyscus than Mus. Size differences in mice could also help explain the differences.

Loss of heat to the environment in control mice was more dependent upon activity than T_A (Figures 9 and 10). The percent change in thermal conductance between 15C and 32C significantly increased in control mice between ρ and α ($P < 0.05$) (Table 8 and Figure 9). Between 15C and 32C, ρ to α, the percent change in C for irradiated Mus was similar, but rose during 27C for irradiated Peromyscus then dropped at 32C (Table 9 and Figure 10). This suggested that C was compensating for a high T_B in controls by equalizing heat gain and heat loss. At α 32C control animals reduced T_B presumably to avoid hyperthermia and thus had a higher C (Figure 9).

Irradiated animals had higher T_B increases from ρ to α than controls, but at the expense of a higher MR and C. Irradiated Mus apparently experienced hyperthermia at T_B approximating $\overline{T}_B$ for controls (37.8C) but effectuated a reduction in T_B through reduced activity and MR at 32C α, maintaining T_B near $\overline{T}_B$ (37.4C). Irradiated Peromyscus on the other hand, became hyperthermic at α 32C as a significant increase in C did not reduce T_B. This suggested that the upper critical temperature (T_{LC}) in irradiated Peromyscus had been shifted down (to the left). Irradiated Peromyscus may have been experiencing an excess heat load at 32C and thus become hyperthermic within a few fractions

of a degree above $\overline{T}_B$ for controls (37.8C). The fact that T_B in irradiated mice was maintained at 1-2C below controls with up to twice the metabolic costs associated with thermal conductance further supported the hypothesis that thermoregulatory performance was reduced by ionizing radiation. The differences in thermoregulation were expressed in higher metabolic rates and excess loss of heat through C, with an inability to maintain T_B consistent with controls.

The effects due to ionizing radiation did not appear to reduce the thermoregulatory capabilities of these mice at 15C, however, to achieve the same capability they were more active at both ρ and α. Irradiated mice became hypothermic at 27C and at 15C during ρ with a slight elevation in C. Apparently heat retention mechanisms were reduced by ionizing radiation at temperatures below the lower critical temperature ($\sim$ 27C) as heat production was higher in irradiated mice than controls. The effect was more pronounced in Mus than Peromyscus (Figure 8). Hypothermia would not seem to be a special ecological adaptation to ionizing radiation since mice and rats not hypothermic before being irradiated (T_B < 25C) were reported to be equally susceptible as controls (Storer and Hemplemann, 1952; Mraz and Praslicka, 1961).

Thermal conductance (C) can be predicted for temperatures below thermoneutrality for small placental mammals

using the following equation (Bartholomew, 1972):

$$C = 0.031W^{-0.51}$$

where C is in $cc O_2 gm^{-1} hr^{-1}/°C$ and weight (W) is measured in Kg. For a 20 gram mouse (Mus controls 19.7 gm and irradiated 19.8 gm) C predicted was 0.229 $cc O_2 gm^{-1} hr^{-1}/°C$. From the data (Table 5) C expected for Mus controls was intermediate between 27C and 32C but for irradiated Mus the equation nearly predicted C at 27C. The same was true for Peromyscus. At 27C (just below T_{LC}) control animals had a lower C than expected (Mus 15 percent, Peromyscus 9 percent), but irradiated mice had a higher C than expected (Mus 8 percent, Peromyscus 22 percent). Irradiated mice lost 25-30 percent more heat to the environment during metabolic-insulative activities than controls. At 15C, thermal conductance in Mus controls was 23 percent lower than predicted while for irradiated Mus C was 19 percent lower than predicted.

If one assumes that average thermal conductance at 15C ρ represented a reliable prediction of overall C during periods of inactivity (Table 6), then differences in C between treatment groups of Mus were more striking. At 15C ρ, observed C in controls was 20 percent lower than expected, but C was 8 percent higher than expected in the irradiated mice. The same relationship held true at 27C; and, at 15C and 27C for Peromyscus where C observed was 10

percent lower than expected in controls and 5 percent higher in irradiated Peromyscus (Table 6). Peromyscus can adjust to low ambient temperatures by going into torpot (Gaertner, et al., 1973) and thus reduce activity. Mus compensated for lower ambient temperatures by becoming more active than Peromyscus, hence increasing heat production. The thermoregulatory cost to Peromyscus at these temperatures was therefore less, even though none of my Peromyscus became torpid.

Higher energy requirements and excess heat loss to the environment in irradiated mice suggested that ionizing radiation reduced insulative capacity. Although not indicated from metabolic data using standard metabolic measurements (Tables 5 and 9, and Figure 10), the averaged effect (Table 6) demonstrated that the temporal aspect of C during an animal's normal diel cycle was a critical comparison when understanding adaptation to varying environments or effects due to perturbations such as ionizing radiation.

When temperature fluctuations change the energy balance in an animal, deliberate control actions take effect; the response would be characteristic of a negative feed-back system (Mitchell, 1974). Since thermoregulation is achieved by adjustments in heat transfer between core organs and peripheral surfaces (Tracy, 1972; Porter and Gates, 1969), perhaps feedback reception was altered in those mice with reduced thermoregulatory performance.

Whether temperature reference-reception or reference-comparison was altered, or reduced effectiveness in being able to respond to the disturbance occurred (Mitchell, 1974), cannot be analysed at this time. The mechanisms underlying investigations on temperature receptors await further testing by laboratory physiologists (Jansky, 1966; Rawson and Quick, 1970, 1972).

Body Temperature-Metabolism Time-Lags: Some Theoretical Considerations

The following set of considerations were used to interpret and evaluate the complex nature of the cross-correlation between MR and T_B. The assumptions made by me were in lieu of any known interpretations counter those given. No literature on this subject was found which directly related to the kind of analysis performed. Discussions with experts in the field of physiological ecology (e.g., Dr. George Bartholomew, UCLA; Dr. Robert Chew, USC; Dr. Franz Halberg, Univ. Minnesota; Alan French, UCLA; Dr. R. B. Lindberg, UCLA; Dr. Warren Porter, Univ. Wisconsin; and Dr. Michael Smith, Univ. Georgia) indicated that detailed temporal relationships, such as those I have performed, were not known to exist in the literature.

Because T_B preceded MR throughout most of steady-state (Tables 13 and 14), and the association(i.e., correlation) between these two parameters was positive, then several possible explanations exist. Each of these considerations

will be discussed in detail later.

1. T_B and MR were on different circadian rhythms (i.e., oscillatory frequencies).
2. Anaerobic metabolism may have taken place, elevating MR prior to any measurable detection in oxidative metabolism.
3. Specific dynamic affect, brown-fat metabolism and/or nonshivering thermogenesis may have resulted in only small increases in metabolism relative to T_B increases.
4. Decreased thermal conductivity (C) and behavioral thermoregulation prior to MR increases may have occurred reducing heat flow from the mice and elevating T_B at or above T_{LC}.
5. Activity and/or T_A may have phase-shifted or reset the clock mechanism(s) which regulated the periodicity of MR and T_B; uncoupling of the rhythm to ambient conditions may have occurred.
6. Metabolism may not represent the major contributing factor in T_B regulation, especially during certain periods of the diel cycle; these associations were thus complexed with activity.
7. No cause and effect relationship existed.

Anaerobic respiration during short periods of time are known to exist, however, when considering time lags approaching 30-40 minutes (Figures 3 and 4) it would not

appear as though this mechanism was a reasonable possibility.

Lindberg and French (pers. comm.) stated that 5 percent increases in T_A may cause a resetting of the T_B rhythm in Perognathus longimembris. If such a mechanism was in operation it would be more likely to have occurred in Peromyscus at T_A changed (as in Case 2, 32C to 15C) because T_B lagged MR during this time period, but not in Mus. Peromyscus is considered an incipient hibernator (Morrison and Ryser, 1959) somewhat characteristic of Perognathus (Chew, et al., 1965; 1967); thus phase-shifting of the T_B lag could be possible if the T_B rhythm was not independent of T_A. Such was not the case as evidenced from the T_A phase shift during Case 3 where T_B did not uncouple with T_A.

Activity was shown to have a higher correlation to T_B than to MR at various times during the diel cycle (Table 11); thus the contribution made by metabolism only partially explained ΔT_B. Mills (1973) stated that T_B can "...cause an increase in (MR) ...", but since metabolism apparently became uncoupled to T_B during Case 3 (or at least negatively associated with T_B), a lag in either parameter became complexed in the temporal displacement of the expected rhythm during T_A changes.

Specific dynamic affect (SDA), brown fat metabolism and/or non-shivering thermogenesis would not be likely to

produce heat toward T_B changes without increases in MR (Bartholomew, 1972). Brown adipose tissues were not found in any of the mice dissected. Brown fat, specific dynamic affect and non-shivering thermogenesis produce heat by oxidative metabolism therefore MR would tend to increase as T_B increased. Since MR lagged T_B during most of the steady-state diel cycle (and especially for Mus during Case 2) these mechanisms probably did not account for the lag. Although, in Peromyscus during Case 2 SDA may have contributed greatly to MR increases in advance of T_B. Mus and Peromyscus were not acclimated to 15C temperatures thus non-shivering thermogenesis was probably not in operation.

At this time it is impossible to adequately evaluate the possibility that MR and T_B were on separate rhythms. The experiment designed to test T_A out-of-phase phase shifts for T_B and MR (Case 3) suggested that T_B and MR did uncouple and that MR reversed its diel pattern. Body temperature rhythms independent of T_A are documented in the literature (Folk, et al., 1958; Aschoff and Pohl, 1970); and, it has been suggested that body temperature rhythm represents a different circadian oscillator than the one for metabolism (Aschoff and Pohl, 1970; Mills, 1973).

Decrease in thermal conductivity would appear to represent the most plausable explanation for MR lagging T_B. Rüdgier and Seyer (1965; cited by Hart in Whittow, 1971) suggested that behavioral control of temperature

regulation probably starts before the initiation of metabolic thermoregulation in the cold. Evidence from my data (at 27C) revealed that C appeared to be not only temperature dependent but activity dependent (Table 6); lower during ρ than during α. From Figures 3 and 4 a lag of MR following T_B occurred in a somewhat cyclic manner for Case 1 (steady-state) with a greater lag during ρ (in general) for controls than during α. This coincided with the differences in C during ρ and α, about 18 percent lower during ρ in Mus controls and about 10 percent lower in Peromyscus controls. This cyclic phenomenon was reversed in the irradiated group which further supported the hypothesis that the energetics and timing of thermoregulation were temporarily altered by ionizing radiation.

Aschoff and Pohl (1970; page 1550) stated "... that $\dot{V}_{O_2}$ (MR) follows activity rather than body temperature is less plausable but cannot be excluded a priori." My data confirmed this idea; that during normal diurnal patterns of activity T_B preceded MR in a cyclic manner between ρ and α (Figures 3 and 4).

In Peromyscus during Case 1 the amplitude of response in T_B versus MR time lags was higher in control than irradiated mice (Figure 3). If controls represented a model mouse then irradiated mice demonstrated reduced performance. The difference between lag response during Case 2 suggested that the relationship between metabolism

associated with thermoregulation was greater in controls than in irradiated Peromyscus, as no difference in activity existed for these groups at 15C α.

The overall costs in metabolism during Case 2 were greater for irradiated animals; thus the shorter lag between MR and T_B may have represented a reduction in response to T_A. Since T_B elevation significantly increased in the irradiated group ($P < 0.05$) but not in controls, it seemed reasonable to conclude that the shortened lag in T_B was in response to significant expenditures of energy as T_A changed from 32C to 15C. The fact that controls did not require a significant elevation in MR or T_B suggested that thermoregulatory performance was in part facilitated by a reduction in heat loss. If C was altered in the irradiated group a significant elevation in T_B associated with MR and/or activity would result in a shorter time period between metabolic output and T_B change. This apparently occurred; thus irradiated Peromyscus responded slower to changes in T_A. Therefore, controls were more efficient than irradiated mice at metabolic and thermoregulatory control from the 32C to 15C transition between inactive and active states.

During Case 3 metabolism was less associated with thermoregulation (Table 11). It is interesting to speculate on the idea that because MR and T_B were negatively correlated, perhaps MR became uncoupled to T_B and was thus

expressed as a different diurnal rhythm (Aschoff and Pohl, 1970).

In Mus during Case 1 the lag in T_B versus MR was similar to Peromyscus (Figure 4). The periods and amplitudes of these cycles were somewhat different in that amplitude was higher in irradiated animals than controls. Irradiated mice had a longer lag during α than controls and the reverse was true during ρ.

The long lag in MR following T_B during Case 2 for Mus (32C to 15C, ρ to α) can only be speculated on at this time. Presumably behavioral control mechanisms and changes in C might result in T_B preceding MR. The lag would be, of course, more pronounced at 32C than 15C, because below T_{LC} C was essentially constant. During Case 3 irradiated Mus had a significantly longer T_B versus MR lag than controls ($P < 0.05$). T_B and MR were negatively correlated during this time period (Table 11) suggesting that a greater increased heat load in irradiated Mus may have caused a greater time differential between the variables.

One explanation for the difference between species controls during Case 2 may be because Peromyscus was generally more active during α 15C than Mus. Metabolism should rise at a rate equal to or exceeding T_B elevation in Peromyscus (as was the case), and MR would probably be less likely to rise in response to T_A in Mus. Thus, T_B in Mus

could be regulated by behavioral mechanism without major changes in MR. If these two relationships were true, then the difference between MR versus T_B in Peromyscus and T_B versus MR in Mus can be explained.

Mus significantly increased activity during α 32C but not during α 15C. Presumably Mus was selecting a more preferred temperature (32C) and because of this, increased activity at no significant increase in metabolic output. Peromyscus, on the other hand, significantly reduced activity at α 32C and thus may have selected against 32C as an optimum temperature for activity. The differences in time-lags between species during α, therefore, could be a result of species differences in their thermal evolutions.

Linear Regression of MR and T_B

Part of the original hypothesis stated that as T_B and MR change in time in response to T_A, the energetics associated with thermoregulation and metabolism would be compared in terms of costs to the animal; a test of performance. Generally speaking, control and irradiated Mus had a lower slope than Peromyscus during the temperature phase change from 32C ρ to 15C α (Case 2). The intercepts (Y = MR) were positive for Mus with a shallow positive slope, but Y was negative in Peromyscus with a much steeper positive slope; indicating (in Peromyscus) a sharp rise in MR relative to T_B. This was expected considering the time-lag

differential witnessed in Figures 3 and 4. Unfortunately, the meaning of this drastic difference in metabolic and T_B response to T_A cannot be adequately evaluated until a better method is developed to analyze the time-dependent parameters associated with circadian periodicity and their phase relationships (Halberg, et al., 1971).

The only other unusual event relative to slope changes occurred at 32C α where the slope in Mus was generally positive while in Peromyscus the slope was negative. I suspect that this was directly related to the time-lag differences discussed earlier (Figures 3 and 4). During 32C α irradiated Peromyscus had a larger negative slope than controls and therefore was less capable of maintaining T_B as MR became disassociated with T_B. This may help explain why irradiated Peromyscus had a greater difference in metabolic output associated with activity than the other mice (Figure 8). Mus controls had a larger positive slope than irradiated Mus. They would thus be expected to have a closer association between metabolism and body temperature during 32C α since MR was decreasing as T_B increased. One would predict, therefore, that the lag between T_B versus MR would be less (Table 14 and Figure 4).

Four criteria were proposed which helped explain performance differences when comparing metabolism for thermoregulation. Performance was defined for this study as the ability to increase body temperature at reduced MR

expenditures. The hypothesis stated that the higher the T_B relative to MR, associated with a high statistical correlation, the greater the performance. This hypothesis was testable by comparing correlation coefficients (R) and the slope (b) of MR (dependent variable) versus T_B (independent variable) regression (Draper and Smith, 1966). The criteria chosen were:

1. Low association (R), low increment increase (b).
2. Low association, high increment increase.
3. High association, high increment increase.
4. High association, low increment increase.

Conditions (1) and (2) stated that performance reliability was low because an estimate of correlation between parameters was weak. Condition (3) resulted in low performance because little T_B was realized as MR increased. Performance was considered highest during condition (4) because less MR output resulted during T_B elevation. Under condition (4) less energy was expended for thermoregulation (ΔT_B, a Q_{10} effect); thus the costs of maintaining integrated performance were less.

The results of this study for steady-state (Case 1) revealed that on a performance criteria basis, Peromyscus controls were expending less energy to elevate body temperature than Mus controls (Table 12). When going from ρ to α, Peromyscus did not apparently change metabolic-thermoregulatory precision as no difference in slope nor inter-

cept resulted. Mus did display a significant difference in slopes (Table 12). Curiously, irradiated Mus had a lower slope than controls during α. This was not because of a reduction in metabolic enhancement but explained if one remembers that irradiated Mus had a significantly lower T_B than controls; thus any increase in MR might be expected to elevate T_B to a greater extent. The slope of MR versus T_B for Mus controls should be higher, which it was (Table 12), and T_B should be significantly higher from ρ to α in irradiated Mus, which it was (Table 6).

The general trend in angularity of scope between Mus and Peromyscus during temporal phase changes in T_A also revealed that the correlations between MR and T_B at different activity cycles were markedly different. Although specific data are not provided due to a lack of statistical reliability alluded to earlier, qualitative considerations reveal some interesting differences. As a general rule, correlations between MR and T_B were quite low during α 15C and α 32C in Mus treatment groups, but not Peromyscus. Since the variability was greater in Mus (standard errors were 50-100 percent higher) it is suggested that the mechanisms of metabolism associated with thermoregulation were operationally less defined in Mus than in Peromyscus, resulting in low associations. Increases in activity would also result in reduced T_B regulation.

Since performance criteria number (4) seemed to be a

more consistent property of Peromyscus controls than in Mus controls during both steady-state (Table 12) and possibly during T_A phase changes, it is concluded that Peromyscus was responding more favorably than Mus to those sets of environmental parameters imposed by this experiment. These findings may at first appear to be simply a reflection of weight-specific metabolism and thus not comparable. However, the fact that metabolic and activity differences did not exist within optimum thermal regimes (at least in controls), comparative differences were real and not biased by weight.

Diurnal Rhythms of Metabolism and Body Temperature

The comparisons made on control animals, a longevity related test, revealed that a significant reduction in amplitude for body temperature in Mus (Table 16) may be of some importance when considering aging between species (Yuris, et al., 1974). The reverse may be true for metabolism since increased amplitude during metabolic output could be energetically more expensive. My results (Figure 7) offered just such a suggestion as Peromyscus had a significantly lower MR amplitude than Mus (Table 16). There was also a difference in T_B periods among controls; Mus 23.9 hours, Peromyscus 24.7 hours (Table 15). No period differences for metabolism between control mice occurred (Table 16). The ratios of MR and T_B period lengths

corresponded closely to values reported in Aschoff (1965: page 88) for activity periods in these mice (Peromyscus had a slightly longer free-running rhythm).

Mus' T_B diurnal rhythm was 2 percent shorter than its MR rhythm; Peromyscus' T_B and MR diurnal periods were apparently synchronized to within 1 percent of each other. Phase synchronization of T_B and MR rhythms should be close in control animals since their free-running periods were undoubtedly influenced by previously entrained light cycles (Pittendrigh and Daan, 1974).

It would appear that my data, at least in part, supported the hypothesis that reduced amplitude may be a parameter of primary aging. However, first the disease of aging must be shown to have separate effects, or at least potentially distinguishable from those factors thought to be of primary aging (Yunis, et al., 1974). If a response to a perturbation commonly thought of as a disease of aging were to result in a similar reduction in amplitude then separation of these parameters of aging might be difficult. If, on the other hand, an opposite result occurred (e.g., amplitude were to increase) then separation of these two parameters may strengthen the concept of a parameter of primary aging. Ionizing radiation has been interpreted as an aging disease (Yunis, et al., 1974); thus I would expect T_B mean to be lower in irradiated mice, because of the aging effect, and T_B amplitude to be higher

as a consequence of the disease effect. Because of the metabolic enhancement effect of ionizing radiation, observed during the preliminary stages of this experiment (Table 2), I would also expect MR mean and amplitude to be higher than in controls.

The data appear to have supported the contention that the disease of aging, or an agent thereof, may be separated from the conditions proposed as parameters of primary aging. Amplitude of the T_B rhythm was lower in short-lived mice; perhaps a property of longevity differences. T_B amplitude, MR mesor and amplitude were higher in irradiated mice. Response to the disease of aging (e.g., ionizing radiation) in T_B amplitude was independent of the response due to animals with different longevities. Therefore, a decrease in T_B amplitude appeared to be an age associated parameter rather than a disease of aging (Yunis, et al., 1974).

The importance of these findings will be clarified upon completion of experiments using entirely free-running animals (Pittendrigh and Daan, 1974). Experiments which relate the maintenance of amplitude, mean and period homeostasis (Pittendrigh and Caldarola, 1973) when animals are exposed to perturbed ambient conditions, such as those performed in my experiment during day 4, may also reveal age versus disease associated differences.

The response of T_B to ambient phase changes in T_A

(Cases 2 and 3) were incorporated into a 96-hour cosine curve reported in Figure 6. The reason for the 96-hour inclusion was that T_B was independent of T_A, and at 32C α T_B became disassociated with MR. A predictable diurnal rhythm for T_B occurred independent of in- or out-of-phase temperature perturbations. This was not so for metabolism.

Least squares cosinor method applied to metabolic data during day 4 gave some rather unexpected results. Since no periodic estimators could be made for the cosine wave function, only mean and amplitude values showed any potential trend in the data. Ambient conditions, T_A out-of-phase, destroyed any periodicity inherent in MR; thus quantitative interpretation must give way to qualitative speculation. During day 4 control Mus had a higher MR mean than irradiated Mus and control Peromyscus. Control Peromyscus had a higher mesor value than irradiated Peromyscus; the difference was < 0.5 $cc O_2 gm^{-1} hr^{-1}$. As expected, amplitude was a negative component. A larger negative value should mean that in time the response in MR was less, assumed to represent an overall reduction in MR costs. In this case, controls had lower amplitudes (larger negative values) than irradiated mice; Mus having a lower amplitude than Peromyscus.

These data on out-of-phase T_A conditions tend to support the earlier findings concerning differences between aging effects versus disease effects. Ho , because of

large differences in individual response to T_A, confirmation of aging parameters associated with environmental perturbation await further testing.

Effects of Ionizing Radiation

The effects of ionizing radiation on MR were greatest during ρ than α (Table 6), suggesting that activity may be compensating for reduced performance in heat production. If thermal conductivity was altered by ionizing radiation, T_B should have been lower during ρ and C higher relative to controls. Data from Tables 6 and 9 and Figures 8 and 10 support this contention; thus ionizing radiation affected the thermoregulatory and metabolic capacity of irradiated mice when they were at rest more than when they were active. The only exception was Peromyscus at 27C. Except at 15C, irradiated Peromyscus had a higher increase in MR relative to controls than Mus. At 15C ionizing radiation would affect Mus more, as metabolic costs were higher and thermal insulation lower than in Peromyscus. Therefore, Mus foraging in the natural environment might be more susceptible to winter conditions if exposed to ionizing radiation. Behavioral regulation would, of course, change heat loss such as when the animal is in its nest. Two of five irradiated Mus did not build nests, whereas all five controls did. The sizes of the nests were not substantially different and thus this behavioral

trait may not have been altered.

Mills (1973) stated that motor and perceptual performance was closely related to T_B. Increased T_B resulted in increased performance, and increased T_B related physiological variability (i.e., variance from given T_B responses) led to decreased physiological performance (Wilkinson, et al., 1964). Therefore, reduced T_B in irradiated mice under varying environmental temperature regimes may have resulted in a reduction of temperature related physiological processes (e.g., Q_{10}, nerve conduction and enzyme activity responses). It may be that ionizing radiation shifted the response time to T_A by reducing the effectiveness of T_B as a temporal environmental T_A monitor. A reduction in T_A assessment would cause the energetics of these mice to rise more dramatically, with a greater lag in response than controls (Figures 3 and 4). An expected lowering of association between variables would also result (Table 11). Therefore, Act. and MR should rise accordingly to the delay in response caused by insufficient timing of the T_B rhythm. This appeared to have occurred; to a greater extent in Mus than in Peromyscus (Figure 2 and Table 7).

It has been suggested that the effects of ionizing radiation interact with the effects of environment to influence longevity (Carlson and Jackson, 1959). In terms of protracted total life-span metabolism (assuming a

constant average daily MR throughout life, which is admittedly a biased assumption) the difference between Mus treatment groups would be about 9 percent, and for Peromyscus treatment groups about 6 percent. This would result in an overall advantage of 3 percent in total life-span metabolism for the longer lived irradiated animals (data taken from Table 4 Case 1). The difference under natural conditions (Case 2) were about the same, 3 percent. However, during out-of-phase ambient T_A conditions the difference would be about 2-3 times steady-state. Taking this one step further, and assuming environmental conditions imposed during Cases 2 and 3, irradiated Mus would experience metabolic senescence (i.e., maximum obtainable age at a fixed metabolic regression) about 14 percent sooner than controls. Irradiated Peromyscus would experience the same consequence at approximately 6 percent short of maximum life-span.

SUMMARY AND CONCLUSIONS

1. Under conditions experienced in the thermal history of these mice, short excursions of increased amplitude in body temperature, oxygen consumption and activity during rest periods and excursions of reduced amplitude during active periods indicate that transient phenomena may be a more natural characteristic of physiological performance than single or double wave circadian rhythms. During inactive periods (ρ), body temperature had a higher association to oxygen consumption. However, body temperature was more closely associated with activity during α than oxygen consumption. During out-of-phase temperature regimes (Case 3) oxygen consumption became disassociated with activity and body temperature.

2. Mus expended more energy at 27C than Peromyscus, and Peromyscus more at 32C suggesting that optimal zones of thermo-equilibrium were closer to a T_{LC} of > 32C for Mus and a T_{LC} of < 32C for Peromyscus.

3. Peromyscus had a 25 percent higher capacity to elevate its body temperature than Mus at 23 percent less expenditure of energy during ambient temperatures similar to the natural environment. At the same potential elevation in body temperature Mus exhibited a 34 percent

increase in metabolism relative to Peromyscus when ambient temperatures were 12 hours out-of-phase. Therefore, efficiency of performance in terms of expenditure of energy was lower in Mus than Peromyscus.

4. The metabolic costs of activity in non-irradiated mice were less at 32C than at 15C, the reverse was true for irradiated Peromyscus.

5. Insulation was 3 to 12 percent more effective in Peromyscus than Mus as a mechanism in reducing loss of heat to the environment. As a consequence, less energy was expended during maintenance thermoregulation. Irradiated mice lost 25 to 30 percent more heat through thermal conductance than control animals and thus were less efficient at thermoregulation.

6. Peromyscus experiencing late effects of ionizing radiation became hyperthermic at ambient temperatures normally within the upper range of thermal equilibrium. Both species of mice became slightly hypothermic below thermoneutrality ($< T_{LC}$). It is suggested that blood circulation to peripheral tissues in irradiated mice may have been altered, reducing the efficiency of thermal insulation.

7. In irradiated Peromyscus, the period (τ) of the body temperature and metabolic diurnal rhythm was shortened. Body temperature and metabolism apparently became de-synchronized during entrainment to laboratory ambient

conditions. Metabolism had a shorter period (23.8 hours) than body temperature (24.2 hours). Desynchronization of T_B and MR did not occur in the irradiated group of Mus.

8. Mus responded to ambient temperature displacement by delaying its activity cycle. Once active Mus remained active longer than Peromyscus. The delay in initiation of activity corresponded to a delay in metabolic enhancement, which was preceded by an increase in body temperature. Body temperature was therefore likely to have been regulated by, or under the influence of, a different oscillator than that for metabolism; thus helping to explain time lags of MR relative to T_B in excess of 30 minutes. Uncoupling of T_B and MR during out-of-phase temperature regimes supported the lability aspect of oxygen consumption disassociated with the requirements for thermoregulation.

9. The slope of the metabolism versus body temperature regression was higher in Mus than in Peromyscus. This was characteristic of earlier findings on mice in advanced age (Braham, 1973). The results of that study were inconclusive but suggested that a greater metabolic output was required for thermoregulation in older animals. This may be an important factor in species longevity. Data from this study supported those findings.

10. A decrease in the diurnal periodicity of amplitude in body temperature and an increase in the amplitude of

metabolism, offers support to the hypothesis that comparative physiological differences exist between animals of different life-spans. These data suggest that differences in diurnal amplitude may be evidence of a parameter of primary aging. Ionizing radiation resulted in a heightened amplitude in MR and a lowering of mean (mesor) T_B, perhaps an example of the disease of aging. What these differences in mesor and amplitude levels mean to the total energetics of longevity among species remains to be tested.

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Appendix A

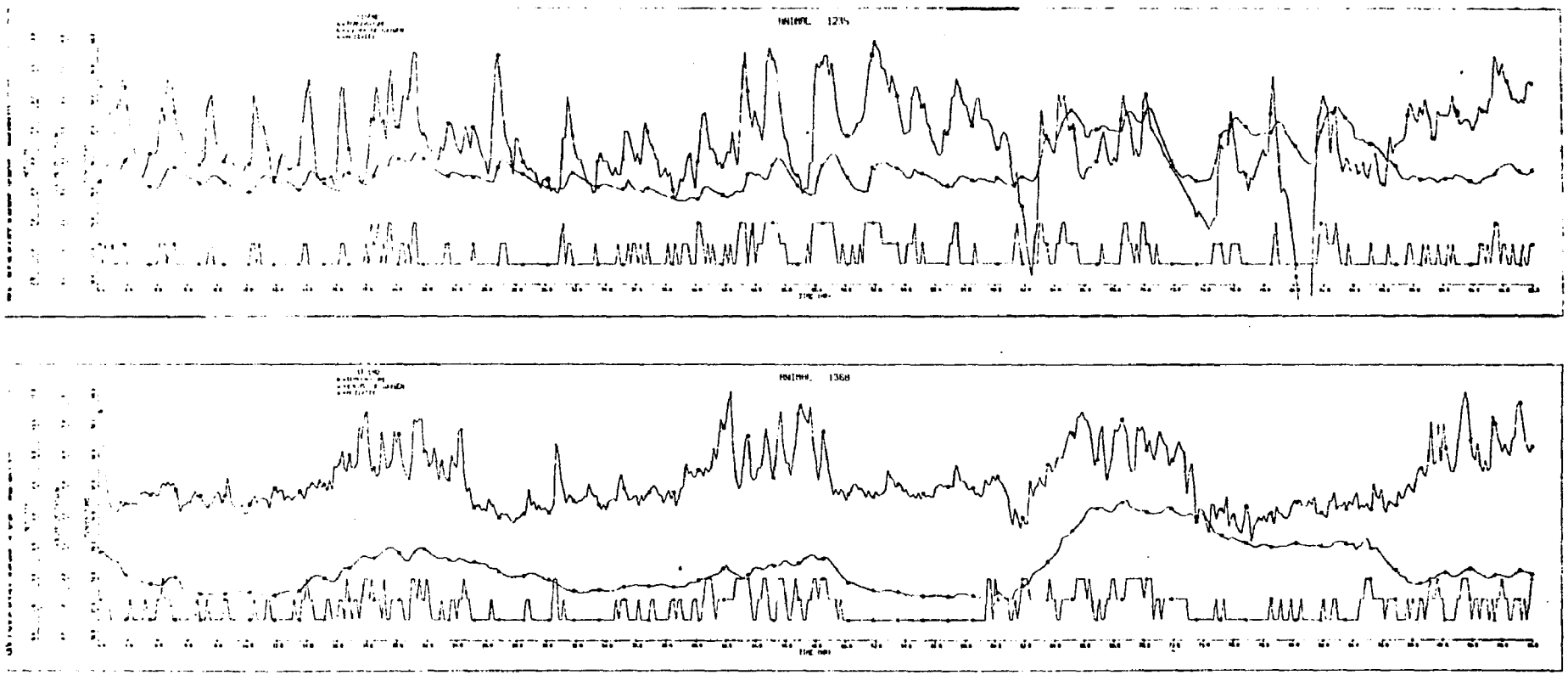
Age in days and weight in grams for each of 20 wild mice recorded at the time of metabolic measurement, Argonne National Laboratory, 1974-75. Means ± S. D.

Species Group Animal Number	Age	Weight
Peromyscus Controls	193.2±10.4	25.8±2.74
1235	182	24.5
1368	201	29.4
1374	203	27.7
1392	198	24.9
1412	182	22.4
Peromyscus Irradiated	211.4±11.3	29.1±4.59
1192	222	36.6
1213	211	30.0
1369	212	25.2
1370	219	27.6
1380	193	26.0
Mus Controls	127.8±47.7	19.7±1.16
700	110	20.1
717	127	19.6
730	109	18.0
766	94	21.2
772	99	19.5
Mus Irradiated	120.0±28.3	19.8±0.97
697	146	18.3
698	152	20.0
729	97	19.5
733	89	20.8
736	116	20.4

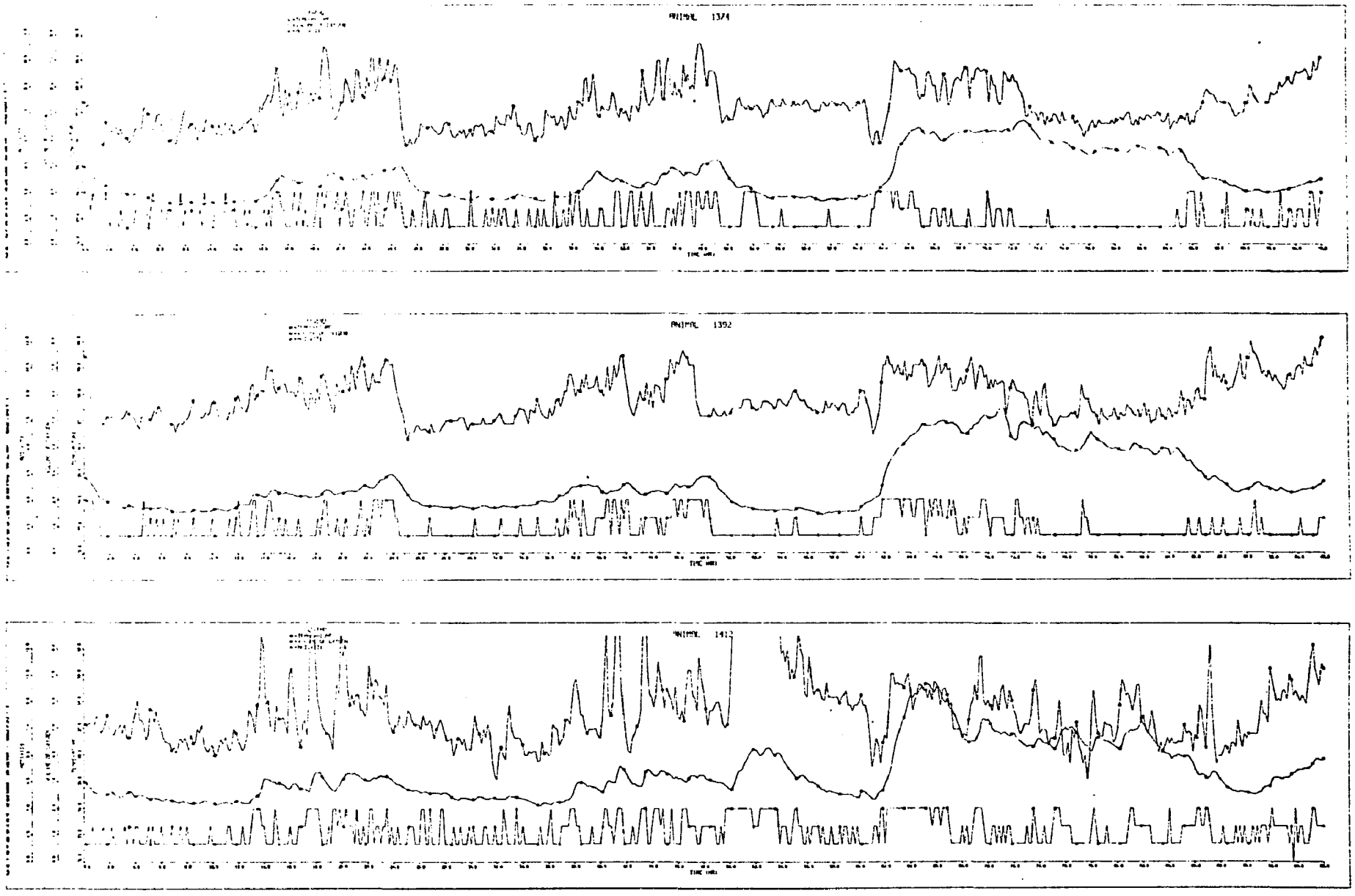
Appendix B

Plots of body temperature, oxygen consumption and activity over 96-hours for treatment groups of Peromyscus leucopus and Mus musculus recorded at Argonne National Laboratory, Division of Biological and Biomedical Research, Argonne, Illinois, 1974-75.

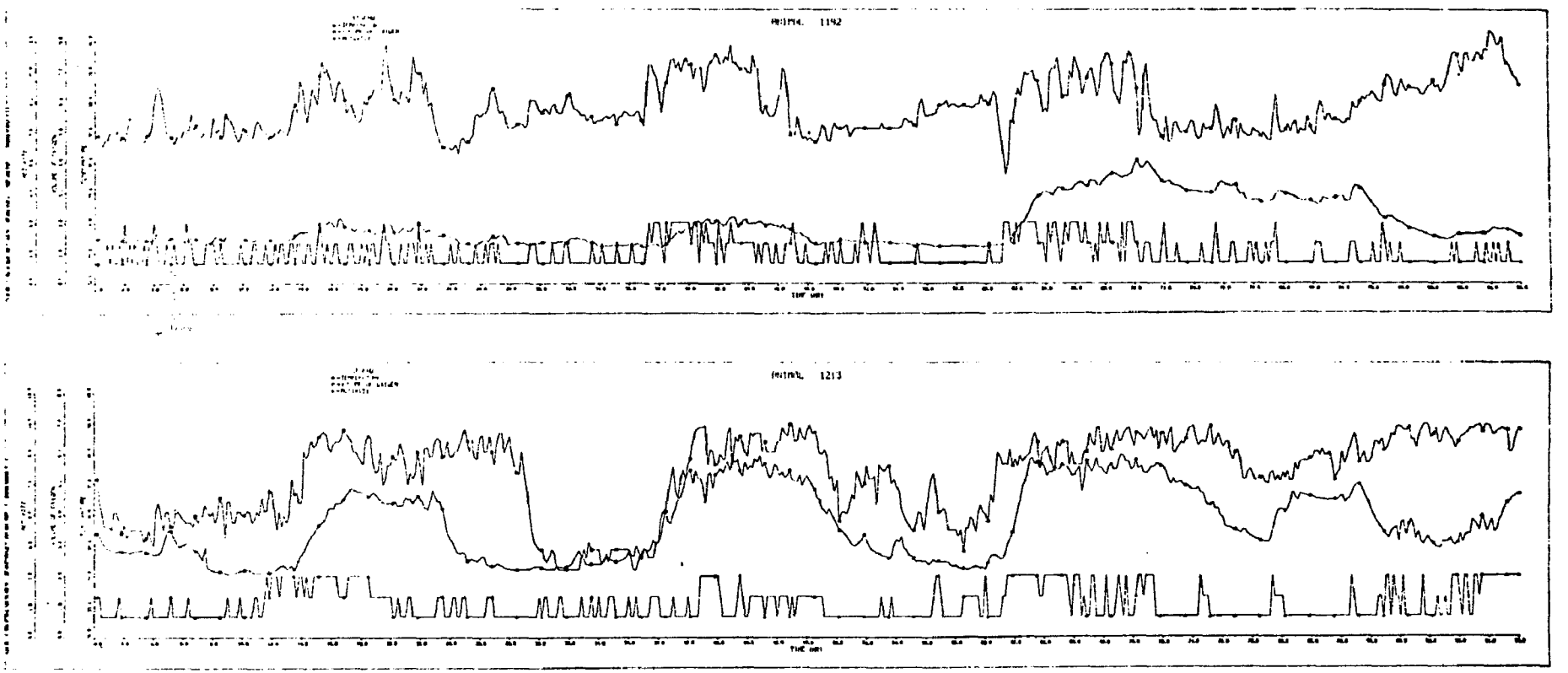
Abscissa represents time in hours from 0.0 (i.e., 0600 hours day 1) to 96.0 (i.e., 0600 hours ending day 4). Ordinate represents recorded values for body temperature ('TEMPERATURE'; 32° - 40°C), 'VOLUME OF OXYGEN' (0.0 to 8.0 $cc O_2 gm^{-1} hr^{-1}$) and 'ACTIVITY' (0.0 to 12 in arbitrary units).



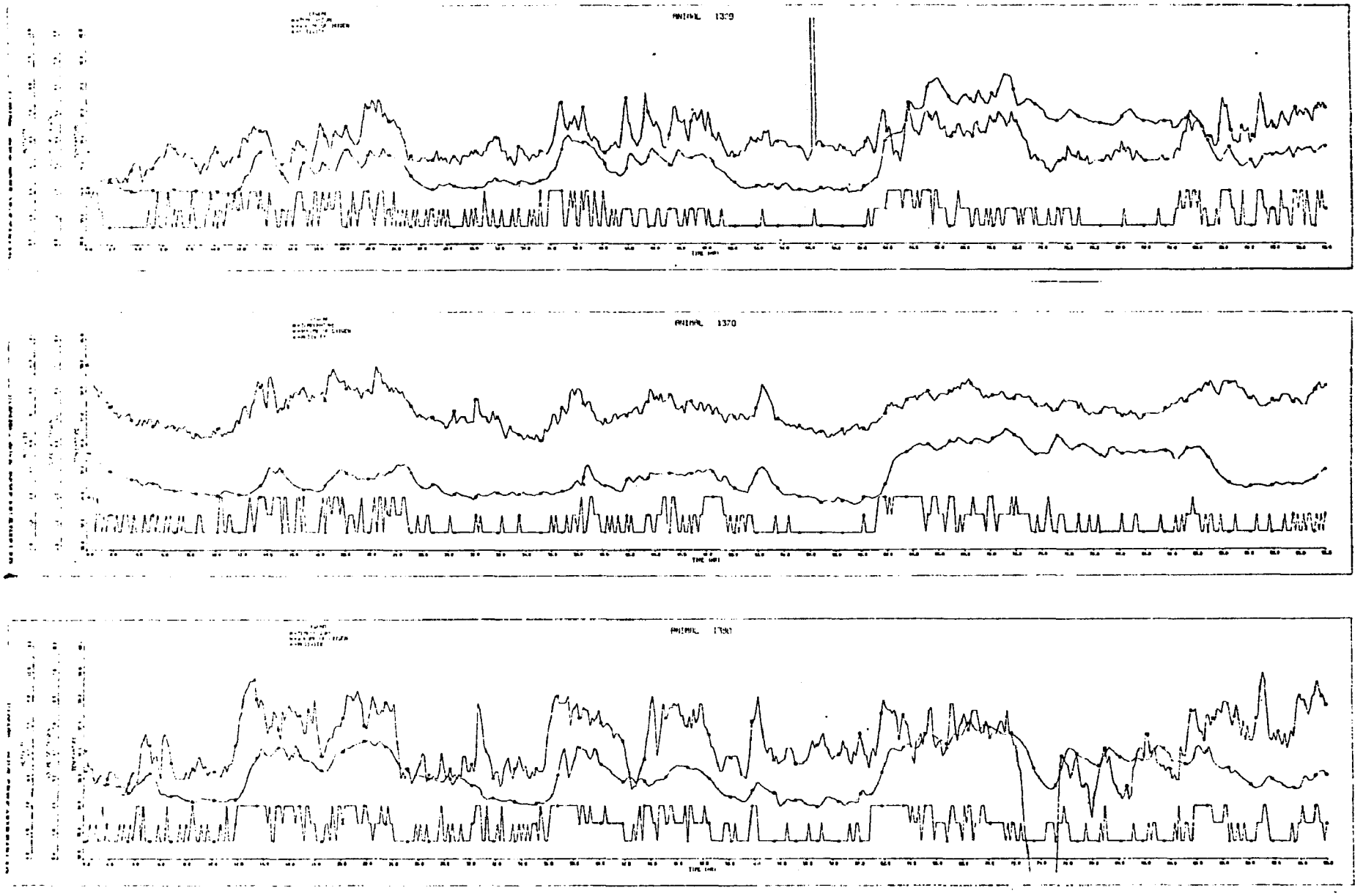
Peromyscus leucopus Control



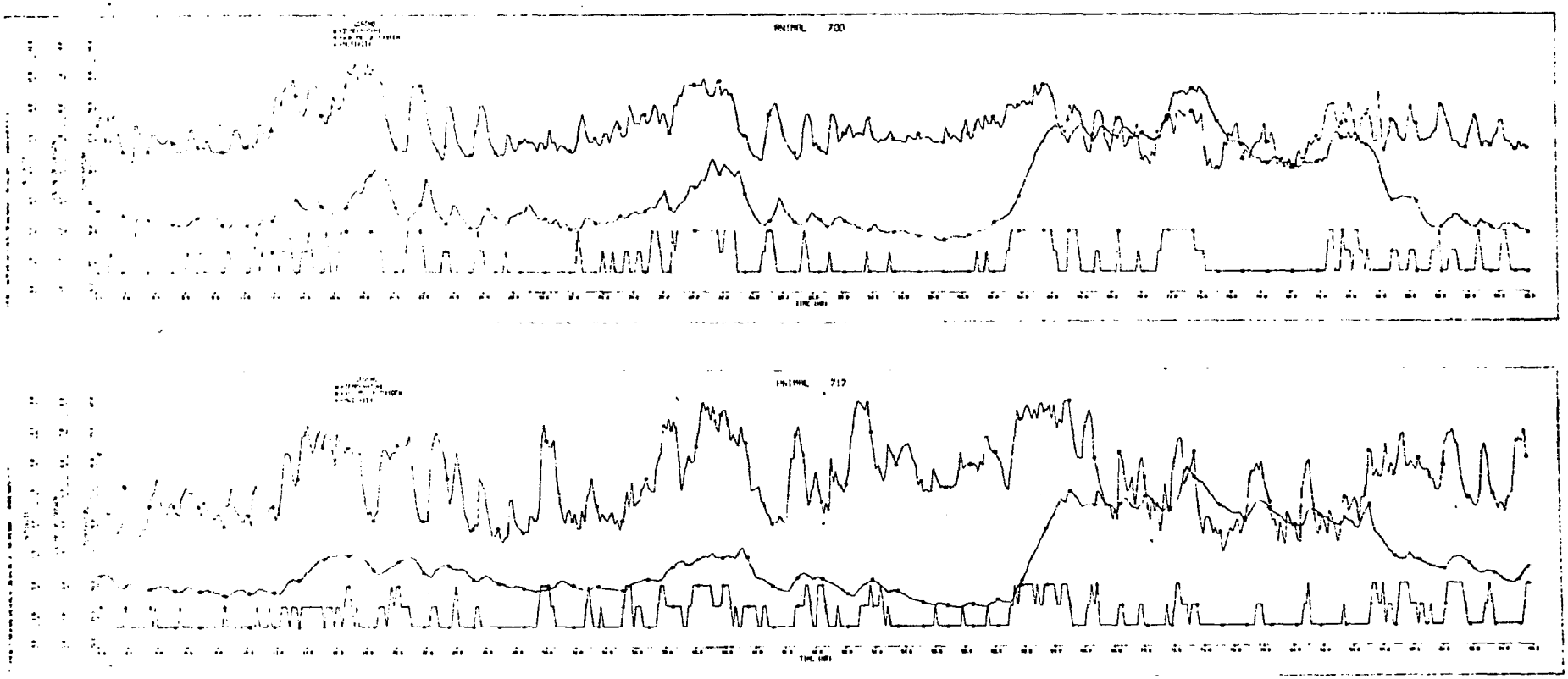
Peromyscus leucopus Controls



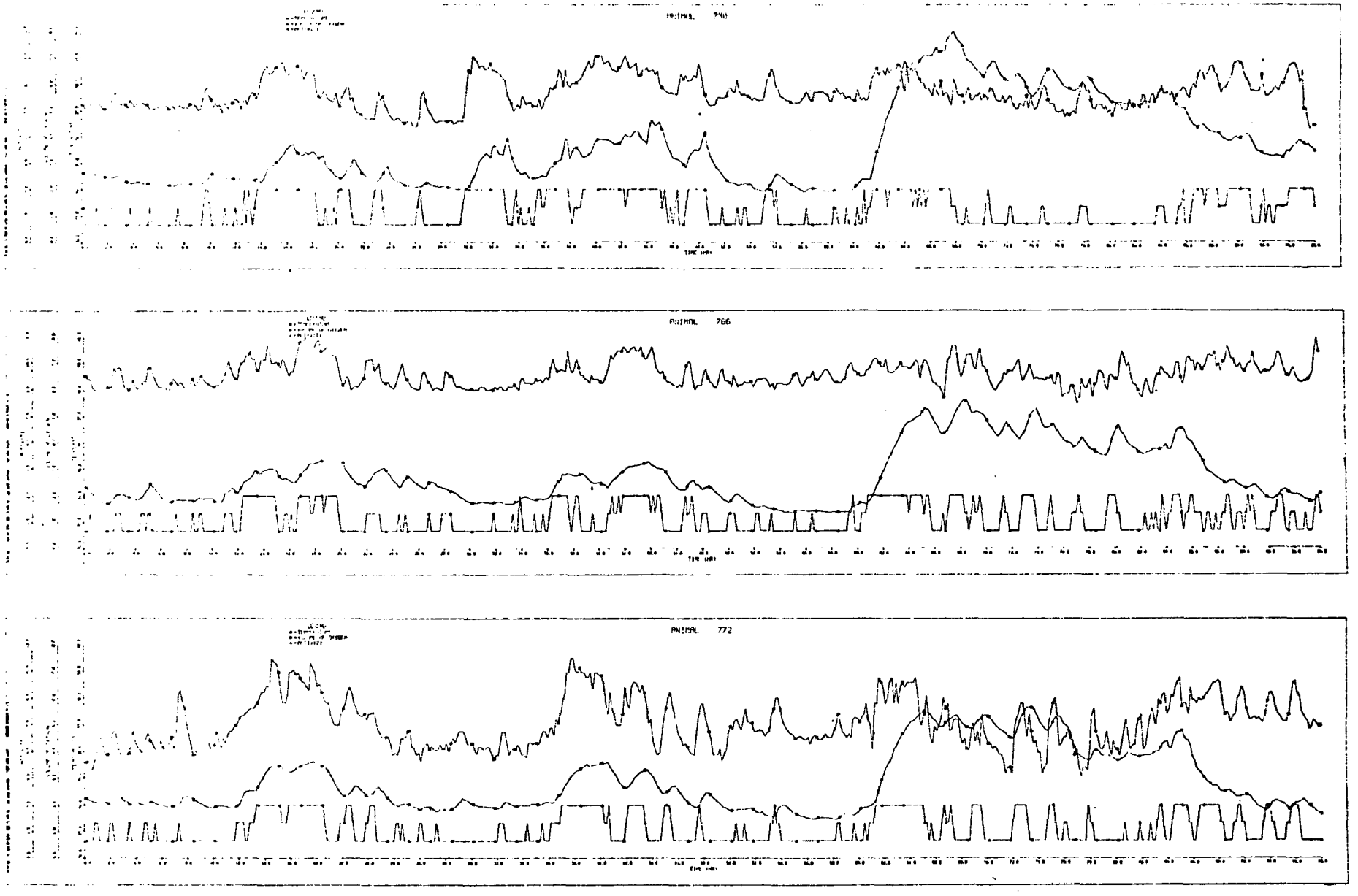
Peromyscus leucopus Irradiated



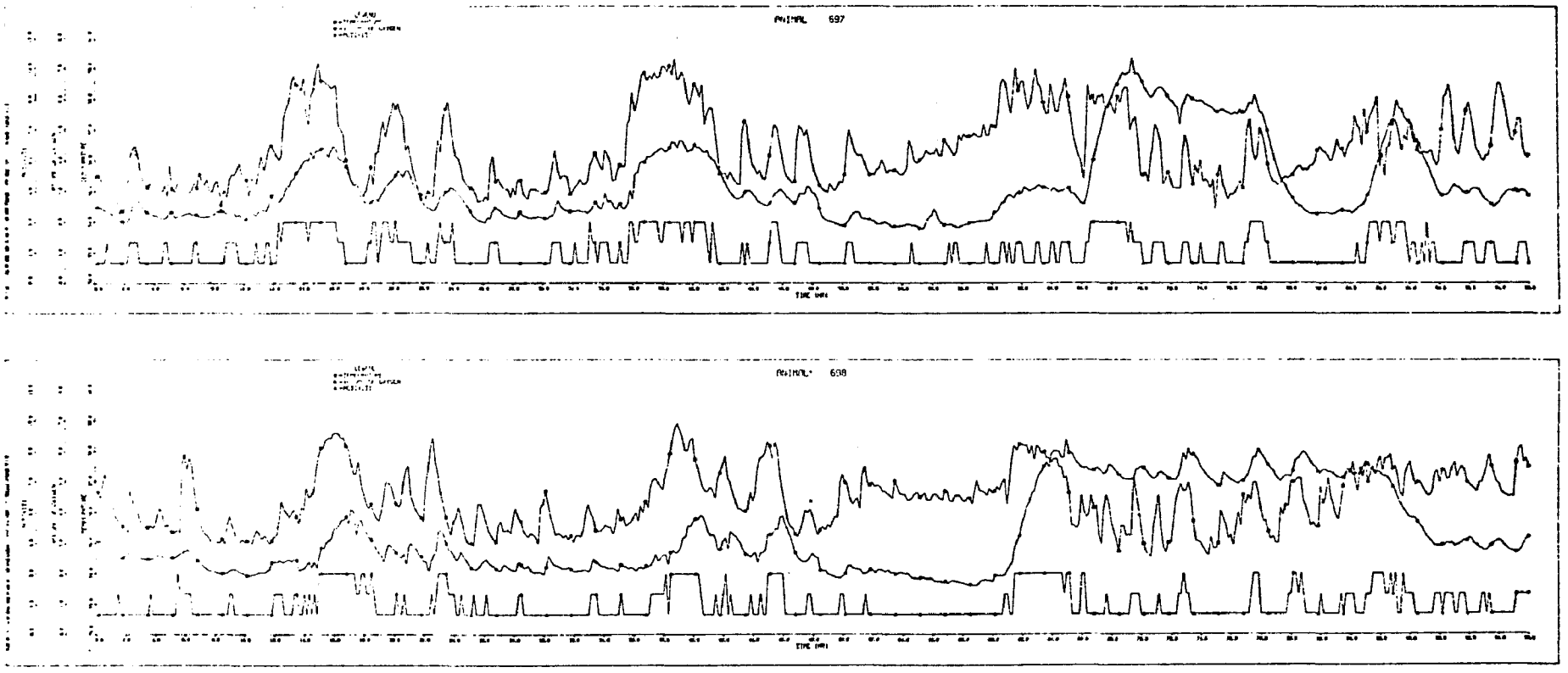
Peromyscus leucopus Irradiated



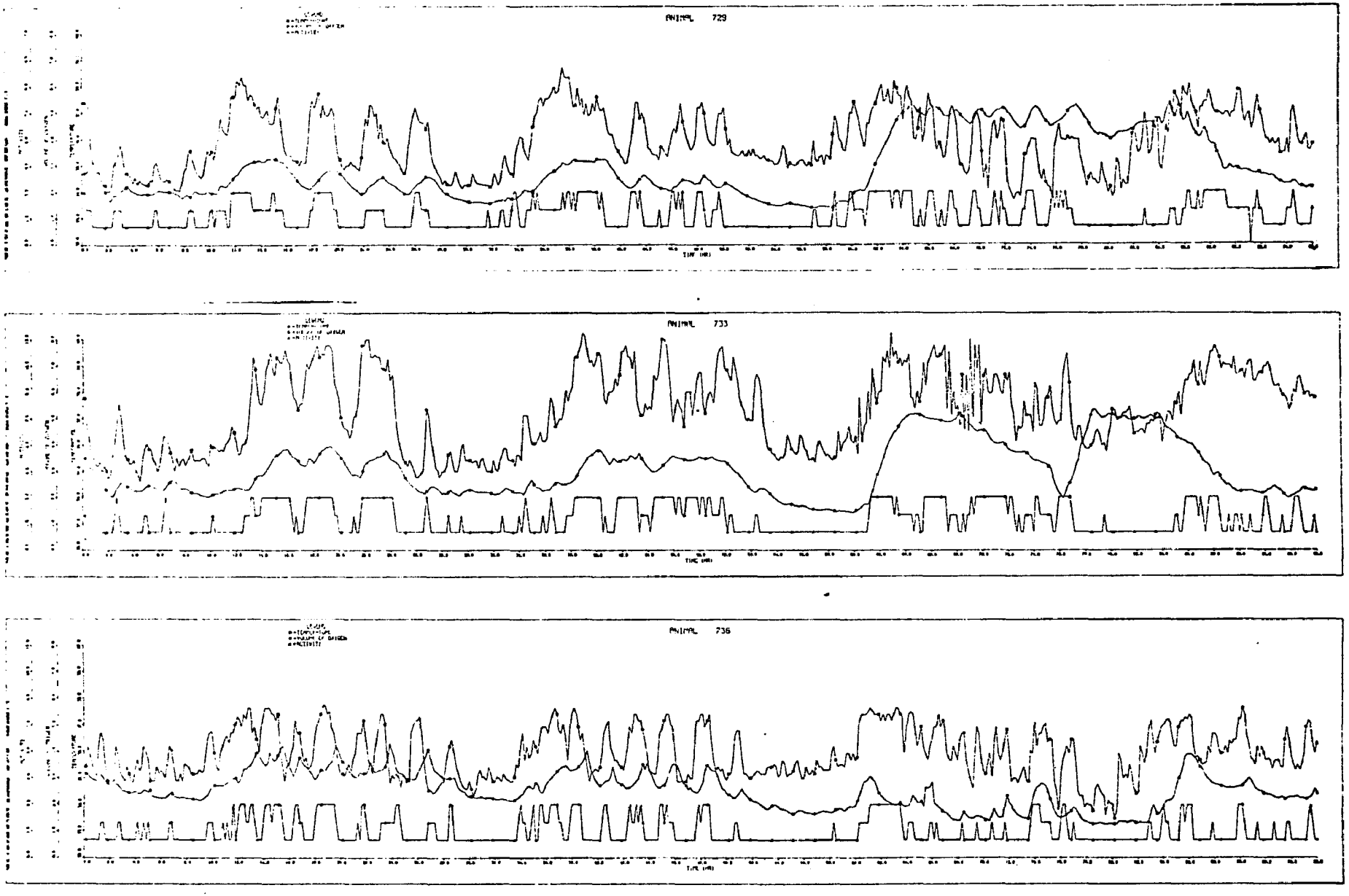
Mus musculus Controls



Mus musculus Controls



Mus musculus Irradiated



Mus musculus Irradiated

Appendix C

Summary data for 20 wild mice over four 24 hour periods recording body temperature (T_B, °C), oxygen consumption (MR, $cc O_2 gm^{-1} hr^{-1}$) and activity (Act.) (means ± standard deviation). N = number of measured events in time per parameter. Argonne National Laboratory, 1974-75.

Peromyscus leucopus Controls

Animal Number		Days (N=144) 1	2	3	4
1235	T_B	36.80±1.04	36.40±1.08	37.06±1.35	36.29±1.84
	MR	3.54±0.32	3.28±0.33	4.10±0.84	4.19±0.76
	Act.	1.29±0.54	1.44±0.68	1.62±0.78	1.33±0.57
1368	T_B	37.15±0.80	37.20±0.99	37.44±0.86	37.06±1.11
	MR	2.10±0.53	2.07±0.34	2.52±1.18	2.81±0.65
	Act.	1.48±0.64	1.56±0.73	1.69±0.77	1.50±0.63
1374	T_B	37.03±0.87	36.97±0.86	37.57±0.71	37.18±0.66
	MR	2.09±0.43	2.13±0.41	2.86±1.09	3.10±0.80
	Act.	1.75±0.76	1.52±0.75	1.54±0.76	1.24±0.57
1392	T_B	37.76±0.71	37.63±0.83	37.99±0.70	37.98±0.85
	MR	2.05±0.37	2.13±0.35	2.99±1.47	3.42±0.83
	Act.	1.49±0.75	1.53±0.74	1.63±0.79	1.19±0.46
1412	T_B	37.36±1.06	37.33±1.25	38.76±1.76	37.30±1.00
	MR	2.65±0.37	2.71±0.38	4.04±1.31	3.91±0.78
	Act.	1.55±0.72	1.63±0.78	2.08±0.84	1.60±0.69
Column Totals (N=720)	T_B	37.22±0.96	37.10±1.09	37.76±1.29	37.12±1.27
	MR	2.48±0.70	2.47±0.60	3.33±1.36	3.47±0.92
	Act.	1.51±0.70	1.53±0.74	1.71±0.80	1.36±0.60
Row Totals (N= 2880)	T_B	37.31±1.19			
	MR	2.93±1.05			
	Act.	1.53±0.72			

Appendix C. Continued.

Peromyscus leucopus Irradiated

Animal Number		Days (N= 144) 1	2	3	4
1192	T_B	37.52±0.78	37.98±0.86	37.83±0.84	37.94±0.97
	MR	1.60±0.19	1.55±0.30	2.11±1.00	2.43±0.62
	Act.	1.44±0.58	1.60±0.72	1.67±0.79	1.24±0.47
1213	T_B	36.80±1.13	36.83±1.75	37.36±1.10	38.21±0.56
	MR	3.27±0.94	3.60±1.39	3.99±1.38	3.98±0.66
	Act.	1.69±0.81	1.47±0.61	1.74±0.87	1.54±0.83
1369	T_B	35.56±0.76	35.90±0.62	36.23±2.30	35.88±0.58
	MR	2.55±0.47	2.73±0.29	3.28±1.59	4.14±0.59
	Act.	1.84±0.76	1.58±0.65	1.53±0.72	1.58±0.59
1370	T_B	37.44±0.76	37.13±0.49	37.29±0.60	37.61±0.38
	MR	2.56±0.38	2.46±0.36	2.97±0.94	3.32±0.62
	Act.	1.75±0.82	1.42±0.63	1.81±0.83	1.33±0.50
1380	T_B	36.41±1.17	36.26±1.08	36.41±0.69	35.66±2.21
	MR	3.21±0.78	2.97±0.56	3.27±1.13	3.45±0.52
	Act.	1.92±0.85	1.88±0.79	1.78±0.81	1.56±0.72
Column Totals (N= 720)	T_B	36.74±1.19	36.82±1.28	37.02±1.41	37.04±1.59
	MR	2.65±0.87	2.68±1.02	3.22±1.40	3.46±0.79
	Act.	1.73±0.79	1.59±0.71	1.71±0.81	1.44±0.68
Row Totals (N= 2880)	T_B	36.91±1.38			
	MR	3.00±1.11			
	Act.	1.62±0.76			

Appendix C. Continued.

Mus musculus controls

Animal Number		Days (N= 144) 1	2	3	4
700	T_B	37.51±0.88	37.32±0.73	37.31±0.56	37.06±0.56
	MR	2.65±0.45	2.68±0.56	3.32±1.50	3.91±1.43
	Act.	1.58±0.80	1.59±0.83	1.47±0.78	1.44±0.71
717	T_B	37.23±1.02	37.27±1.26	38.03±1.09	37.06±1.03
	MR	2.26±0.43	2.25±0.38	2.80±1.42	3.63±1.00
	Act.	1.39±0.57	1.60±0.75	1.54±0.76	1.50±0.74
730	T_B	37.48±0.55	37.83±0.82	37.73±0.41	37.61±0.58
	MR	2.60±0.39	3.07±0.74	4.12±2.22	4.91±1.03
	Act.	1.72±0.89	2.23±0.90	1.76±0.88	1.72±0.84
766	T_B	38.65±0.54	38.51±0.48	38.57±0.37	38.61±0.45
	MR	2.41±0.51	2.36±0.45	2.93±1.56	3.38±0.99
	Act.	1.66±0.82	1.65±0.81	1.74±0.85	1.72±0.77
772	T_B	37.11±1.03	36.96±1.04	36.98±0.80	37.19±0.86
	MR	2.48±0.54	2.41±0.56	3.12±1.58	3.51±1.16
	Act.	1.67±0.84	1.63±0.84	1.58±0.82	1.72±0.86
Column Totals (N= 720)	T_B	37.60±1.00	37.58±1.06	37.72±0.89	37.51±0.93
	MR	2.48±0.49	2.56±0.63	3.31±1.76	3.84±1.25
	Act.	1.60±0.80	1.74±0.86	1.62±0.83	1.61±0.79
Row Totals (N= 2880)	T_B	37.60±0.97			
	MR	3.04±1.28			
	Act.	1.64±0.82			

Appendix C. Continued.

Mus musculus Irradiated

Animal Number		Days (N= 144) 1	2	3	4
697	T_B	36.06±1.25	36.29±1.33	36.76±1.08	36.33±0.88
	MR	2.80±0.68	2.94±0.79	3.18±1.67	3.91±1.37
	Act.	1.69±0.80	1.65±0.81	1.46±0.69	1.50±0.72
698	T_B	36.27±1.01	36.26±1.01	36.47±0.87	36.61±0.85
	MR	2.61±0.48	2.58±0.51	3.39±1.70	4.53±1.13
	Act.	1.53±0.76	1.45±0.74	1.42±0.76	1.51±0.68
729	T_B	35.75±1.18	35.93±1.20	36.10±0.93	35.91±1.16
	MR	2.37±0.44	2.30±0.50	3.09±1.53	3.95±1.00
	Act.	1.62±0.74	1.69±0.80	1.69±0.83	1.61±0.77
733	T_B	37.04±1.66	36.98±1.60	37.56±1.42	37.68±1.04
	MR	2.83±0.60	2.78±0.57	3.20±1.37	3.49±1.14
	Act.	1.77±0.90	1.81±0.88	1.83±0.88	1.60±0.79
736	T_B	36.01±0.88	35.84±0.95	36.06±0.87	35.80±0.98
	MR	3.01±0.56	2.89±0.46	2.04±0.48	2.17±0.69
	Act.	1.60±0.78	1.56±0.78	1.40±0.71	1.38±0.68
Column Totals (N= 720)	T_B	36.23±1.30	36.26±1.30	36.59±1.19	36.46±1.20
	MR	2.72±0.60	2.70±0.62	3.01±1.52	3.60±1.34
	Act.	1.64±0.80	1.63±0.81	1.56±0.79	1.52±0.73
Row Totals (N= 2880)	T_B	36.38±1.25			
	MR	3.00±1.15			
	Act.	1.59±0.78			