INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

FINAL REPORT

Edited by

R. G. Lindberg
Principal Investigator

February 1968

Prepared under Contract No. NASw-812 by
NORTHROP CORPORATE LABORATORIES
Hawthorne, California

for

Biosciences Program Division
Office Space Science and Applications
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
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FOREWORD

This report presents the results of a thirty-eight month effort by Northrop Corporation to elucidate the basic biology and physiology of pocket mice with the intent of defining space biology experiments utilizing their unusual physiology. The effort followed related studies initiated during a previous contract between Northrop and NASA (Contract No. NASr-91) following several months of preliminary studies supported wholly by Northrop Corporation. The results of these studies were used extensively to derive design parameters for space biology experiment hardware in support of other contracts, ("A Prototype Space Flight Experiment Package to Study Circadian Periodicity in Pocket Mice," NASw-1191, and "Feasibility Study for Conducting Biological Experiments Aboard a Pioneer Spacecraft," NAS2-4491). The prototype experiment hardware developed under Contract NASw-1191 was in turn used as a laboratory tool to study the stability of the circadian period of body temperature under Contract NASw-812.

A high level of interdisciplinary cooperation was required to fulfill the goals of this research. Physiologists, Geneticists, Radiobiologists and Ecologists and Engineers worked together closely to reinforce each other's studies and to make this report a meaningful composite. Particular recognition is due Dr. R. M. Chew of the University of Southern California, Dr. J. J. Gambino, and Mr. P. Hayden of Northrop Corporate Laboratories for their support and participation. We are also indebted to Dr. Colin S. Pittendrigh of Princeton University for his counsel and support in the area of biological rhythmicity. The full support and cooperation of managerial, technical and clerical personnel contributed materially to the successful completion of this task.

Many of the individual sections of this report were prepared and submitted for separate publication in scientific journals. It is hoped that the lack of consistent format and the redundancy that might be noticed as a result of this dual purpose will not detract from the value of this report.

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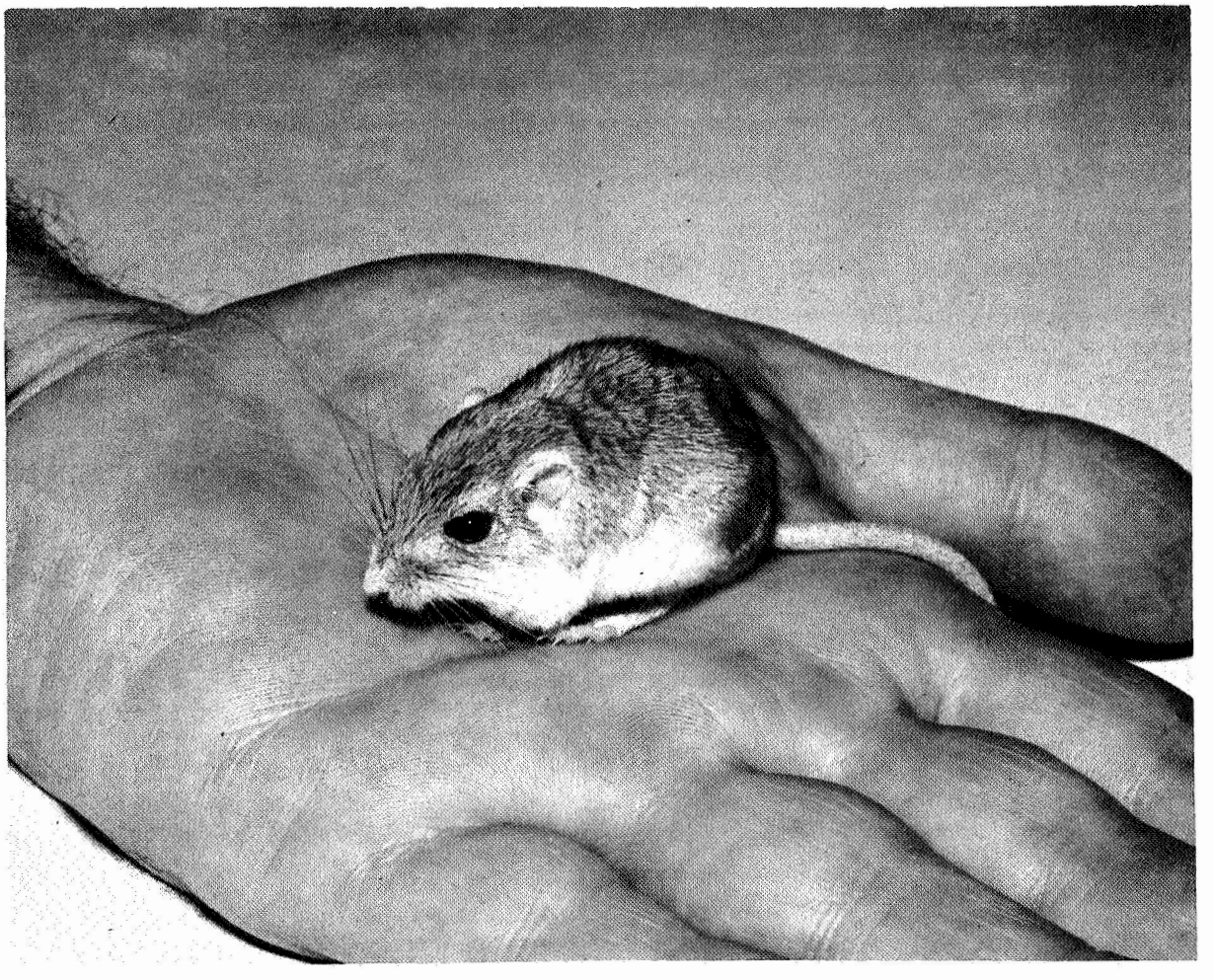

The Little Pocket Mouse

(Perognathus longimembris)

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

R. G. Lindberg

SUMMARY

Pocket mice (Genus: Perognathus) are proposed as particularly suitable subject matter for space biology research by virtue of their unusual physiology which permits significant simplification of the life support systems in biosatellites. The resultant savings in payload, coupled with the small size of the animals, permits the formulation of experimental designs involving large numbers of mammals, and a significantly higher probability of definitive results from this experimentation.

This report presents results of work in several broad biological areas. Specific data are presented on the following: breeding, growth and development; thermoregulation; hibernation; circadian periodicity; and radiobiology. From these data are derived: (1) metabolic baselines delimiting the nature and complexity of a life support system for pocket mice in space vehicles; (2) baselines of selected physiological trailts of pocket mice that are potentially useful for assessing the biological effects of extra-terrestrial residence; (3) specific design for a space biology experiment utilizing pocket mice to study the stability of circadian systems in space.

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

INTRODUCTION

Objectives

This contract was undertaken to establish the feasibility and practicality of using pocket mice (Heteromyidae: Perognathus) as subjects for biological experiments in space; to identify specific experiments in which their particular physiological attributes could be used to study biological phenomenon; and to determine those metabolic baselines that would define the nature and complexity of space experiment hardware.

Need

The inherent variability of biological material, and the nature of latent responses of organisms to chronic stresses suggest that the consequence of extraterrestrial habitation will not be readily resolved through experiences involving human subjects. Therefore, irrespective of the progress of manned space flight, sophisticated research of lower organisms must be pursued in space, concurrent with manned missions, throughout the foreseeable future.

The cost per unit weight, coupled with the necessary complexity of packages containing living organisms, places a major limitation on the design of biological experiments to be conducted in space vehicles. Because of this limitation, it is not feasible at the present time to pursue programs which include sufficiently large numbers of mammals to insure statistical validity of the data obtained in single flights. The inclusion of a large number of animals in any single flight is desirable in order to offset inherent biological variability and experimental error, and to permit a greater flexibility in the design of experiments and a more efficient and rapid pursuit of research programs.

An obvious means of facilitating this development in space biology experimentation is to find a small mammal which demands a minimum of life

support equipment and which is adaptable to the requirements of foreseeable research programs. Several species of pocket mice appear to fulfill these requirements.

Characteristics which suggest the potential use of pocket mice for space biological research are: small adult body size, a normal and complete absence of a requirement for intake of water, minimal excreta, subsistence ad libitum from air-dried seeds, and a hibernation and estivation behavior pattern controlled by ambient temperature and available food supply. These unusual traits do not disqualify pocket mice as representative mammals from which much experimental data applicable to man may be extrapolated. Unfortunately, however, insufficient information is available concerning the basic biology of these animals. One objective of the investigation reported herein was to obtain basic biological information as a step in the development of the pocket mouse into a unique research subject.

Scope

The contract effort focused upon comparative physiology within the genus Perognathus. The genus consists of a complex of species indigenous to the western United States and extending from Minnesota into northwestern Mexico. The species of interest in this investigation are found in the deserts of southern California and Nevada, and in Arizona. They differ in local habitat, behavior and adult size.

Since body weight is a primary factor in selection of a species for space research, P. longimembris with a body weight of $\sim$ 10 grams and P. flavus with a body weight of $\sim$ 6 grams appear to be the most promising. They are among the smallest of North American rodents, and are probably the smallest mammals potentially suited to the purpose. P. longimembris received the most attention in this study because of its availability.

Small body size is, however, associated with a more critical temperature regulation mechanism. Since the control of activity in small animals may be temperature dependent, some of the larger species may prove to have

characteristics which would result in their selection in spite of their greater weight (as much as 30 grams). Availability, breeding behavior, radiation sensitivity characteristics, or chromosomology of the larger species might also prove favorable to their selection.

The research reported generally falls into the catagories of breeding, growth and development; thermoregulation; hibernation; circadian periodicity; radiobiology; and experiment definition for space studies. The research has resulted in seven publications in the open literature (included in this report in the interest of continuity) and provided definitive engineering requirements in support of contracts NASw-1191 and NAS 2-4491.

The findings of this study, coupled with the data from contract NASr-91 which presented cytogenetic and hematological data, support the contention that pocket mice can now be used with confidence in many experiments in which conventional laboratory mice, rats, guinea pigs, and hamsters have been proposed to study the effect of space conditions on mammals.

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LABORATORY BREEDING OF THE LITTLE POCKET MOUSE, *PEROGNATHUS LONGIMEMBRIS*

BY P. HAYDEN, J. J. GAMBINO AND R. G. LINDBERG

ABSTRACT: A group of 160 female and 40 male captive pocket mice (*Perognathus longimembris*) were examined routinely for reproductive activity during January–June 1965. Limited observations were made throughout the summer and fall. Fifty-eight per cent of the females showed one or more estrous cycles during the routine inspection. Most exhibited polyestrus cycles of approximately 10 days duration. The incidence of first estrus was highest in March. Breeding was accomplished by pairing animals judged by external signs to be receptive. A total of 217 matings were attempted and 57 litters were produced. Five mice produced two litters each. Litters were born from late March through September. Males made sexually active with human chorionic gonadotropin sired several litters. Information on hormone treatment, estrus, estrous cycles, postpartum and juvenile estrus, mating, gestation, maternal care and nest building is presented.

INTRODUCTION

The only species of *Perognathus* cited in the literature as having been bred in captivity are *P. californicus* and *P. flavus* (Eisenberg and Isaac, 1963). Because of the aggressiveness of *Perognathus*, these matings were accomplished only by careful pairing methods and control of environmental conditions. Prediction of female receptivity, size of the mating pens and duration of pairing were extremely important.

There has been marginal success in breeding other captive heteromyid rodents, *Dipodomys* spp. and *Liomys pictus* (Butterworth, 1961a and b; Chew, 1958; Day et al., 1956; Eisenberg and Isaac, 1963). *Dipodomys* appears somewhat easier to breed than *Perognathus*, although both genera have solitary habits and strong intraspecific aggression. These behavior patterns present the major obstacles to laboratory matings, and successful breeding depends upon modification of the usual techniques employed for breeding most laboratory animals (Day et al., 1956).

Efforts to breed *Perognathus* in this laboratory have emphasized routine observations of natural estrous cycles in a large number of captive animals as a basis for determining most favorable mating time, and induction of sexual activity in males by hormone treatment.

MATERIALS AND METHODS

Holding facilities and animal husbandry.—A group of 160 female and 40 male pocket mice were trapped during the spring of 1964 in the vicinity of Whitewater Canyon, approximately 10 miles E of Palm Springs, California. The mice were kept individually in gallon jars containing 2 inches of sand.

A diet of equal parts hulled sunflower seeds, parakeet seed mix, rye, wheat and rye grass seed maintained the mice in good health, as judged by sleek coats, bright eyes and normal activity. However, there was a question whether this diet lacked a factor necessary for breeding. In the field such a factor may be present in the succulent plant materials that are present during the natural breeding season. Therefore, rye grass seed soaked in water soluble vitamins (Avitron, pet vitamins) was added to the diet. In addition, freshly cut vegetation, consisting of about equal parts unidentified grasses and weeds were given every other morning. The grass seed and fresh vegetation were avidly consumed.

The temperature was maintained between 20–23° C with relative humidity between 50–70%. Overhead fluorescent lights were on from 0600 to 1800 HR. During the dark portion of the light regime, a 7-watt light provided minimum illumination.

Observation routine.—The 160 females were separated into two equal groups for convenience of handling and data acquisition. One group was periodically weighed during the first portion of the study; otherwise, the groups were handled and treated similarly.

Routine observation of genitalia was begun in January 1965. At first, all females were examined every third day; beginning in February, sexually active females were examined every day. The entire group was examined every 3–7 days, and sexually active mice were added to the daily inspection group.

Beginning in late June, the inspection routine was limited to only those females that had produced litters. Because of somewhat intermittent observations, the data for this period are not included in the graphic presentation of estrous cycles.

Breeding chambers.—Three kinds of breeding chambers were used: 15-gallon glass aquaria; rectangular boxes ($8 \times 1.5 \times 1.5$ ft); and square, compartmented boxes ($4 \times 4 \times 1.5$ ft). The square boxes each had six peripheral compartments, opening into a central area. This allowed animals to establish home territories in the six outer chambers and use the central area for breeding. The long boxes had no dividers. All chambers contained 3–4 inches of desert sand and rocks. The boxes were provided with lengths of metal and plastic tubing of various diameters to afford many hiding places for the animals.

Pairing methods.—The method of selecting animals for pairing was direct. Females judged to be receptive by external signs were placed with males with large palpable, but not obviously scrotal, testes. The pairs were observed for 15–20 minutes to determine if they were aggressive. Sometimes animals could be switched around to match "personalities," but this was seldom successful. If the male was pugnacious or reacted violently to the female, he was removed immediately. If the paired mice seemed compatible, they were left together 3–4 hr, overnight, or sometimes longer. The female was then placed in an isolation cage for periodic observation.

Hormone injection.—The hormone human chorionic gonadotropin was used to stimulate both males and females. The lyophilized CGH (Calbiochem, 3625 Medford St., Los Angeles, California 90063) was dissolved and diluted with physiological saline to a concentration of 2,000 IU/ml and administered subdermally with a micrometer syringe. Doses of 100 units per day for 10 days were given to a sexually inactive group of 10 females and 10 males in June 1965. This group was observed for changes in external genitalia. A group of eight females were injected for 7 days, and ovaries were later taken for histological examination. Several other groups of females were treated with CGH and paired with untreated sexually active males.

RESULTS

Estrous cycle.—In our captive *P. longimembris*, estrous cycles started in mid-January and continued in some animals through December (time of this writing). An estrous cycle is completed in about 10 days.

The times of the first observed estrous periods, total number of estrous cycles and number of litters are presented in Table 1. The incidence of first estrous periods increased from late January to a maximum in March and then gradually declined to June. The sexually active females were observed

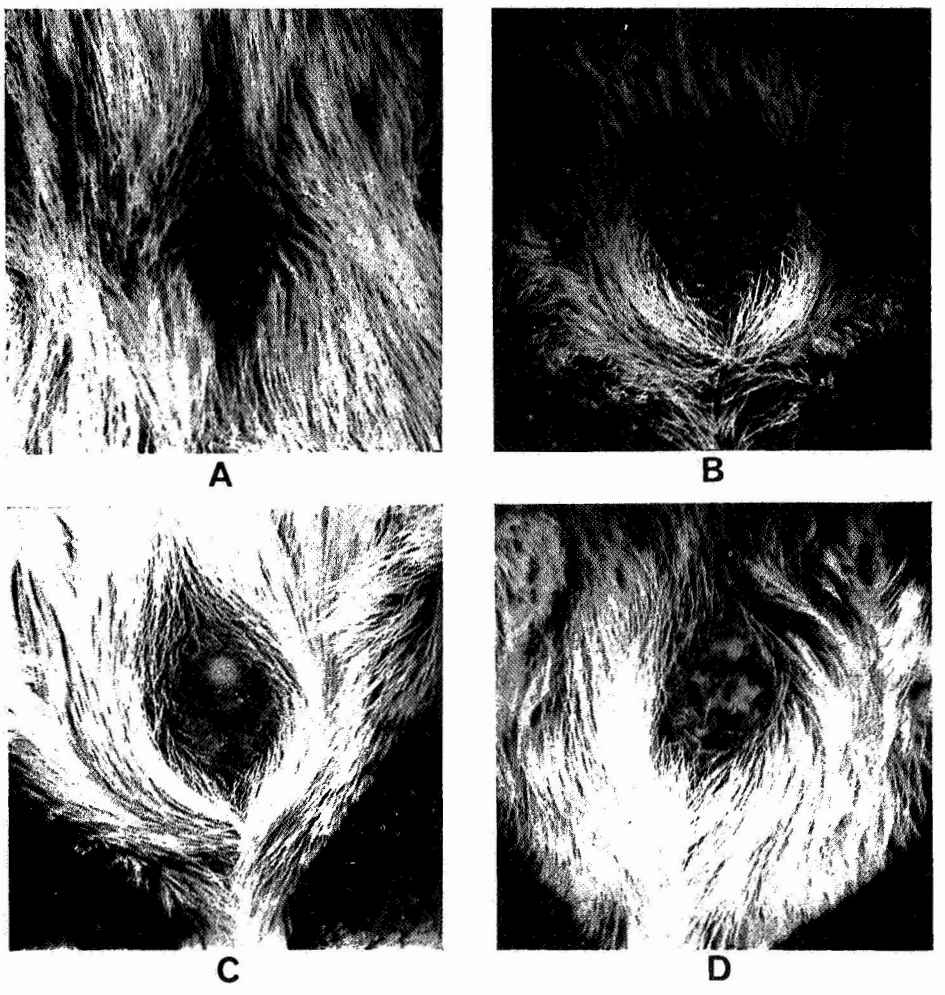

FIG. 1.—External genitalia of female *Perognathus longimembris* during estrous cycle. A. Anestrus—not swollen, edges of vagina closely oppressed or sealed. B. Proestrus—swollen but still sealed, future vaginal orifice well defined. C. Estrus—swollen, vaginal orifice open, edges evaginated (not well shown in picture). D. Metestrus—sloughed lining and mucous plug consolidated, swelling diminished.

One female born in the laboratory was noted to have swollen external genitalia, but a sealed vaginal opening at 41 days after birth. The next day a well-formed vaginal plug was observed, indicating that an estrous cycle had been completed. This suggests that sexual maturity may be reached in *P. longimembris* females as early as 41 days. This individual was again in estrus 30 days later and was paired with a male, but no litter was produced.

A gravid juvenile *P. formosus* was field-captured (22 June 1965) and littered in the laboratory but failed to care for the neonates. This animal was judged to be juvenile by the gray color of the coat and was evidently born early in the spring.

These data indicate that the early young-of-the-year are capable of breeding the same year they were born.

TABLE 1.—*Summary of observed estrous periods and litters of 160* Perognathus longimembris

Time period	No. first estrus		% of inactive females becoming active	Total no. estrous periods	No. litters
1–15 Jan.	0	6	3.7	0	0
16–31 Jan.	6			6	0
1–14 Feb.	5	18	11.7	13	0
15–28 Feb.	13			21	0
1–15 Mar.	14	32	23.5	37	0
16–31 Mar.	18			42	2
1–15 Apr.	5	23	22.0	43	0
16–30 Apr.	18			53	2
1–15 May	8	11	13.6	62	8
16–31 May	3			24	9
1–15 June	3	3	4.3	33	14
16–25 June	0			21	13
26 June–26 July	No obs.		No obs.	No obs.	9
Total	93			355	57

to have an average of 3.8 estruses, with a range of 1 to 11. At least one full estrous cycle was observed in 58% of the group, with 42% remaining inactive during the 5-month daily observation period.

During the anestrus portion of the year, the vaginal orifice is completely regressed and sealed with epithelium (Fig. 1, A). During the polyestrus portion, the orifice is alternately open and closed, with vaginal walls oppressed or sealed with epithelium. The vulva swells during proestrus, 1–2 days before estrus (Fig. 1, B). The vaginal orifice remains sealed during proestrus, but a characteristic transverse line is evident in the vulva. During estrus, the vaginal orifice is open, with the edges much enlarged and evaginated to various degrees (Fig. 1, C). This condition lasts from a few hours to a day. During metestrus, the external genitalia regress and the vaginal lining sloughs off (Fig. 1, D). The sloughed material and mucous form a plug which is retained in the vagina from 1 to 5 days. One of these plugs was removed intact from an animal. The consolidated material was a cast of the vaginal cavity complete with a bifurcated tip, which indicated that sloughing of the lining involved at least a portion of the uterine horns. These plugs are apparently the "secretory plugs" described in *Dipodomys deserti* and differ in several respects from "copulation plugs" (Butterworth, 1961a).

In two instances females ate their offspring shortly after birth and were returned immediately to routine observation. Estrus was observed at 2 days postparturition in one of these animals and at 4 days in the other. Both females were paired again, and the former bore a second litter. The timing suggests that true postpartum estrus occurred; however, it is difficult to distinguish this form a normal return to estrus after loss of a litter.

Reproductively active males.—Although males with conspicuously descended testes have been observed in the field, none was observed during this laboratory investigation. Some of the captive animals and the hormone-treated males had enlarged testes that could be forced down into the scrotum and retained for several seconds. The abdominal position of the testes does not prevent production of viable sperm, as evidenced by successful matings.

Along with testicular enlargement there is an increase in the amount of vascular tissue surrounding the baculum, giving a rugose texture to the penis. In the inactive male, the penis is characteristically thin and pale with little vascularization.

Induction of sexual activity.—In an early attempt at induction of sexual activity, estradiol and progesterone were used without success. However, CGH was found to give an externally visible effect in females after about a week and in males after 10–12 days.

Of the 10 anestrus females that were injected with CGH, six had open vaginal orifices before 7 days and remained so for 2–5 days; two opened after 7 days, and two were still sealed after 16 days. In these hormone-induced openings of the vagina, swelling and evagination of the vulva either did not occur or was much less than in normal estruses. Several other groups of females were given 100 units/day for 7 days and paired with both normal and hormone-induced males, but none of the females was receptive.

After seven injections of 100 units CGH/dose, several females who exhibited opening of the vagina were sacrificed for autopsy and ovarian tissue. In the uninjected control animals, the ovaries were small and contained no obvious developing ova; the uteri were transparent and flaccid. The ovaries of the injected animals were much enlarged with many developing ova, and uteri were well developed.

The testes of all injected males became enlarged and could be palpated after about 12 days, but the testes did not descend into the scrotum. Viable sperm developed in hormone-induced males, since they sired several litters with normal females.

Breeding.—Observations on frequencies of estrus, mating attempts and litter production are summarized in Figs. 2 and 3. These represent two randomly chosen groups of 80 females each; mice in Fig. 2 were weighed biweekly during the first 6 weeks, while those of Fig. 3 were not weighed. Estrus began earlier in the weighed group (January), than in the unweighed group (February). More weighed mice showed estrus (50 of 80) than the unweighed mice (43 of 80). Pairing of females in estrus with males was not attempted until 1 March.

Daily inspections of reproductively active females disclosed 355 estrous cycles in progress; 217 matings were made, which is a utilization of 61% of the potential receptive periods. A total of 57 litters resulted from the 217 pairings, which is a success of 26%. This is considered a high level of achievement, since sexual receptivity occurs only during the first half of estrus.

Litter size averaged four and ranged from one to six. The sex ratio of those young weaned by 30 June was 30 ♀♀ to 34 ♂♂ (47 : 53). In 11 instances, the first observed estrus and pairing resulted in the production of a litter. Five females have produced two litters each. The 52 females that bore litters were kept under observation biweekly after 25 June. They continued to undergo estrous periods to the time of this writing, 1 December 1965. Three more litters were produced, the last on 29 September 1965.

A total of 26 males sired the 57 litters; however, five males sired 52% of the litters (30 of 57). After several matings these males had short and severely scarred tails, indicative of the aggressive behavior of the females even at a "compatible time."

Fatalities occurred in eight matings (4 ♀♀ and 4 ♂♂). In three cases the animals were partially eaten. Subordinate animals were in a state of

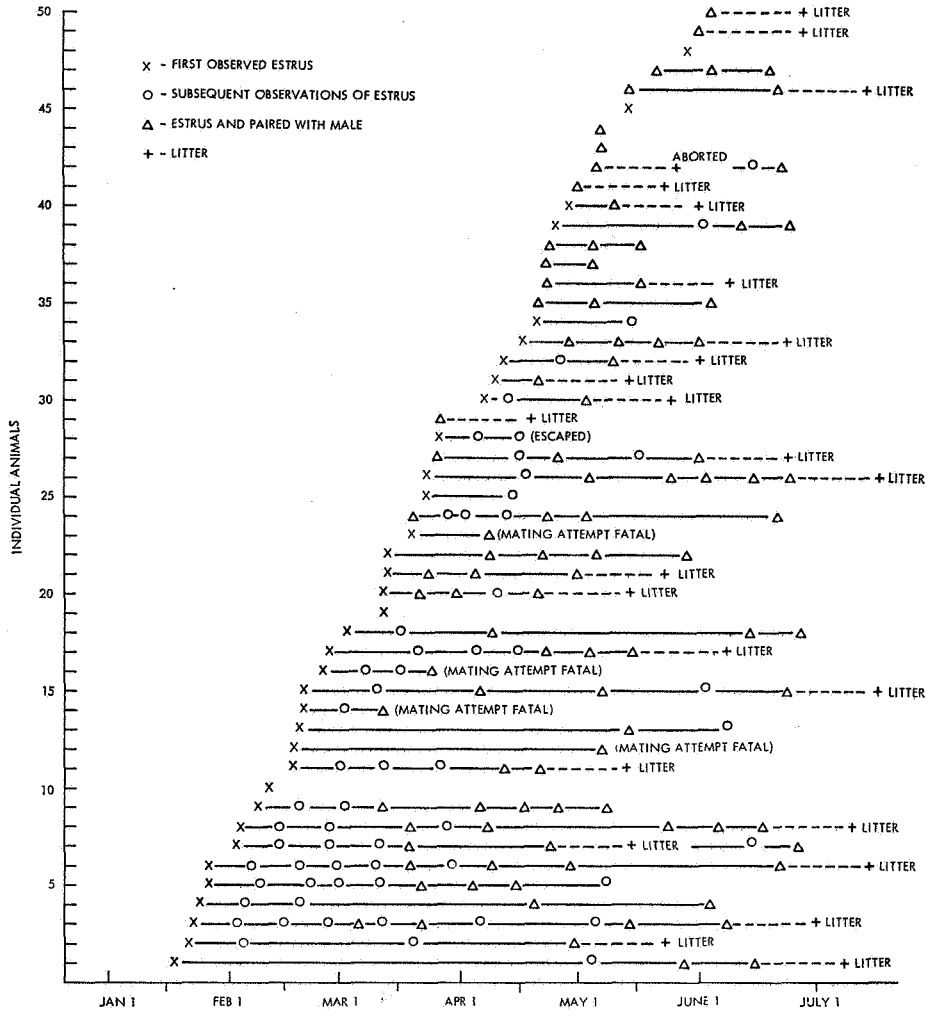

FIG. 2.—Estrus periodicities, breeding attempts and litter production in reproductively active females from a group of 80 *P. longimembris*. Animals weighed routinely.

torpor, although they seemed only superficially wounded by bites on back and tail.

Copulation.—The copulatory behavior of *P. longimembris* is sufficiently different from other species of *Perognathus* (Eisenberg, 1963) to warrant description. In one typical observed mating, the two animals approached one another directly after being placed in the breeding cage. After one nose-to-nose contact and a simultaneous leap, copulation ensued. The male mounted from the rear and both animals fell on their sides. Copulation was accomplished in this position. The male did not bite or grasp with his front feet, but grasped the female's tail with one of his hind feet and thrust rapidly. Mounts lasted no longer than 4–5 seconds and appeared to be terminated by the female. In a 10-minute period, there were 20–25 mounts with an undetermined number of intromissions. During the final encounter, the female bit the male on the head, which precipitated a brief kicking and scratching battle. The male ran to a corner, lay down and remained motionless. The female dug in the sand and preened herself. Twice the female approached the male and attempted to mount him, but he did not move and responded only by squeaking.

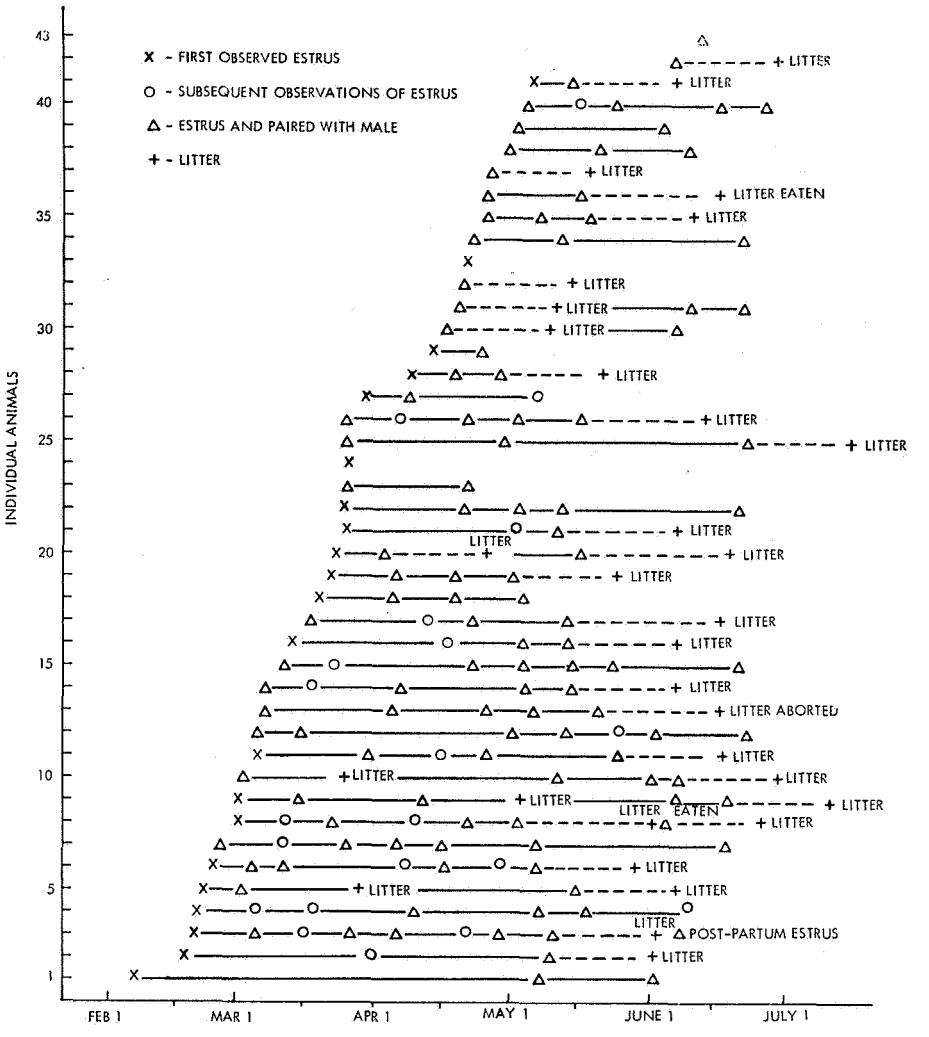

FIG. 3.—Estrus periodicities, breeding attempts and litter production in reproductively active females from a group of 80 *P. longimembris*. Animals not weighed.

The female was examined immediately after copulation. Her external genitalia were no longer swollen and evaginated as before copulation, and the vaginal orifice was sealed with a crust. Egoscue (pers. comm., 1962) noted this immediate disappearance of the vulval swelling after breeding in *Dipodomys* spp. This may indicate that superfecundation is not possible in heteromyid rodents.

Several other females were observed to assume a copulatory position (lying on one side with pelvis rotated 90° and one hind leg extended nearly vertically to substrate) when a male approached. This behavior was also noted when a male was no longer close to the female, but after initial contact had been made.

Gestation and maternal care.—Gestation periods were definitely established in 31 of the pregnancies. Gestation lasted 22 to 23 days in 74% of these cases; extremes of 21 days and 31 days were recorded. The one animal that littered at 31 days ate the litter shortly after parturition.

Abortions and subsequent eating of the young occurred in five of the 57 litters. Several isolated deaths of newborn mice were observed, but they could not be attributed to maternal neglect.

Maternal care often involved much apparently aimless carrying and shifting of neonates. This continued as late as the third week, when offspring were almost as large as the dam. Even at this age, the young did not struggle when picked up by the dam; rather, they facilitated the action by raising their legs. Litters were weaned at about 30 days.

Nest building.—Pocket mice generally build nests when material is available. Pregnant mice in our colony were provided with dry grass for nest building, and casual observations were made on nest building behavior and nest construction.

Size of nest seemed to vary with the amount of grass available. Fine grass was cut in ½-inch lengths and used for the bulk of the nest, while coarse grass was shredded and used as bedding. The bed area was about 1½–2 inches in diameter. If sufficient grass was available, the nest was covered.

Field samples of P. longimembris.—Animals that were trapped in the Whitewater Canyon area and returned to the laboratory during the period 5 March–28 June 1965 are listed in Table 2. The first gravid female was trapped in mid-April and juveniles began to appear about 7 weeks later. By mid-June, juveniles constituted 84% of the catch.

The mean weight of female animals when weighed several days after capture was 8.6 g (range 6.4–12.2 g). The same animals 9 months later weighed 9.4 g (range 7.4–11.0 g), indicating good adjustment to our laboratory conditions.

Breeding of other Perognathus.—Limited observations for incidence of estrous cycles and mating attempts as described for *P. longimembris* were made on three other species of pocket mice: *P. californicus*, *P. fallax* and

TABLE 2.—*Field collected* P. longimembris *received in 1965*

Date	NO. COLLECTED		% Juveniles	% Gravid ♀♀
	♂♂	♀♀		
5 Mar. 65	3	4	0	0
10 Mar. 65	18	6	0	0
17 Mar. 65	3	3	0	0
25 Mar. 65	24	16	0	0
19 Apr. 65	18	5	0	20%
6 May 65	9	8	0	50%
17 May 65	13	7	0	28%
2 June 65	21	17	18.4%	25%
8 June 65	25	25	52.0%	4%
14 June 65	24	28	84.6%	7%
22 June 65	49	59	60.2%	0
28 June 65	29	29	51.7%	0

P. formosus. Of the three species only *P. fallax* was successfully bred. Four litters were produced by *P. fallax*, with one individual producing two litters. Three of the litters had four each, and one had one only. The single neonate was dead and may have represented the remains of a cannibalized group. Gestation time varied from 24 to 26 days. Sex ratio was 42% males and 58% females. The first female to produce a litter (15 May 1965) was again mated 18 June 1965, 10 days after weaning the first litter. Sexual maturity in a female was first noted in early November (166 days old) but may have occurred earlier, as observations were somewhat limited. Although adult pelage was attained in about 2.5 months, one female retained juvenile pelage for 5 months. This suggests that age estimates based on pelage color alone could result in considerable error.

DISCUSSION

There is little doubt that the seasonal reproductive cycle of *Perognathus* in nature is correlated with annual environmental cycles, but is subject to perturbations by local climatic conditions. Chew and Butterworth (1964) made observations of *Perognathus longimembris* and *Dipodomys merriami* in their ecological study of rodents at Joshua Tree National Monument, California. In this study, they noted pregnant mice, males with scrotal testes and very young animals in February, March and April. French (1964) notes that juvenile pocket mice (*P. longimembris* and *P. formosus*) appear in numbers in June at the Nevada Test Site, suggesting that they are probably born in April or May. Hall (1946) indicates that nearly all pregnancies occurred in May. The peak of reproductive activity in these desert rodents has been attributed to seasonal variation in rainfall, plant growth and other ecological factors.

It is evident from our observations that initiation of reproductive cycles in laboratory-maintained pocket mice coincides with the natural breeding season, as judged by field collection data. For example, a high incidence of juveniles occurred in field samples taken in June 1965. This suggests that conception occurred in field animals at approximately the same time captive animals in this study had a high incidence of estrous cycles.

In the present study, captive pocket mice previously maintained in a stable laboratory environment for at least 8 months produced litters from March through September. This may indicate that preparative processes for breeding are associated with an annual endogenous cycle, but initiation and maintenance of the breeding condition are dependent upon diet.

The earlier onset of estrus in the weighed group in this study (Fig. 2) cannot be explained. Ostensibly, both groups were otherwise treated similarly; however, the difference could have resulted from some subtle differences in housing or handling of the animals, or it may represent random variation.

Laboratory breeding of *Perognathus longimembris* can be accomplished by proper selection of mating pairs. The key to obtaining sufficient compatible pairs is routine observation of large numbers of both sexes to ascertain their state of reproductive activity. Twenty-six per cent of the time females in full estrus (i.e., with open vaginal orifice) produced litters when placed with males with enlarged testes.

The use of hormones to induce sexual activity has been marginally successful. Pfeiffer (1960) used urine from pregnant women and human chorionic gonadotropin to produce a vulval condition resembling estrus in anestrus kangaroo rats. Although a similar condition was achieved in pocket mice, breeding attempts with hormone-treated females produced no litters.

Obviously, routine observation and selective pairing of large numbers of pocket mice are time consuming and expensive. Establishment of a breeding colony may depend upon whether animals conceived and reared in the laboratory will exhibit modified physiological and behavioral patterns, i.e., continuous estrous cycles and greater compatibility.

ACKNOWLEDGMENTS

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GROWTH AND DEVELOPMENT OF THE LITTLE POCKET MOUSE, *PEROGNATHUS LONGIMEMBRIS*

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Growth and development data are presented on 8 litters of *Perognathus longimembris*. This is the first recorded laboratory breeding of this species. Body weights and measurements of ear, hind foot, tail and total body length were taken on 26 individuals. At birth, body weight is about 1.3 g; the skin is nearly transparent and naked, with eyes and ears sealed. There are no cheek pouches at birth. By day 5 the pinnae are unfolded, but the meatus remains sealed. Sparse hair is evident at day 8; by day 14 a full juvenile coat is attained, and the eyes are open. Cheek pouches start to invaginate at about day 3 and are functional by day 14. About 65% of adult weight (8-10 gms.) is attained in 21 days, but the hind foot reaches near adult-size during this period. Semilogarithmic plots of growth measurements showed polyphasic growth with initial instantaneous growth rates which varied from 4.4% for total length to 14.8% for ear.

INTRODUCTION

Growth and development data for the Heteromyid rodents are very limited. This group contains kangaroo rats, pocket mice and kangaroo mice, nearly all of which are noted for their adaptation to an arid environment. These animals are solitary in nature and exhibit strong intraspecific aggression. Most attempts to breed them in captivity have been unsuccessful. However, several species of the genus *Dipodomys* have been bred in the laboratory, and various details of mating behavior, growth and development are available (Day *et al*, 1956; Chew, 1958; Chew and Butterworth, 1959; Butterworth, 1961a,b).

More recently, several species of pocket mice (genus *Perognathus*) have been mated under laboratory conditions (Eisenberg and Isaac, 1963). However, only limited growth data is presented on 3 litters of *P. californicus*, one litter of *P. penicillatus* and one litter of *P. flavus*. Successful breeding of *P. longimembris* and preliminary data on growth and development were reported earlier from this laboratory (Hayden *et al*, 1965). The current report presents representative data from 61 litters of *P. longimembris* that have been bred in our laboratory between April and September, 1965.

METHODS AND MATERIALS

Observations of growth and development were made on eight litters with a total of 26 individuals. One of the litters was from a pregnant female trapped in the field, while the rest were laboratory-bred. All of the females were from the Sonoran Desert of California (Whitewater Canyon area, about 10 miles east of Palm Springs). The observation group was reduced to 24 animals at 14 days and to 22 at 32 days because of death and escape of individuals.

Females and their litters were housed in galvanized boxes, 8″ × 11″ × 6.5″ with screen wire tops. Sand approximately 1-2 inches deep was provided in the cage for digging and grooming. A container (pint can, milk carton, etc.) was provided for nesting. Dry grass (timothy and rye or bermuda) was placed in the container for nest material. Torn paper towels were used in addition to the grass in several cases. Gravid females were placed in the maternity cages 2-8 days prior to expected parturition date to allow them to acclimate to the new environment. If the female appeared nervous or easily agitated, the screen top was covered with a towel, and her cage placed in a more isolated position in the animal room.

The diet was provided ad libitum and consisted of a mixture of about equal parts: hulled sunflower seed, rye grain, oat groats, hulled millet, parakeet mix, rye grass seed and rye grass seed enriched with water soluble vitamins (Avitron, trade name). Pieces of raw carrots were provided every other day.

Litters were handled only after the hands were washed thoroughly with soap and water, rinsed in ethyl alcohol and dried. The first few litters were handled hesitantly, but it was found that newborn mice could be manipulated from the first day of life with no apparent adverse effects.

Measurements were taken routinely on specific days of the week. However, since litters were added to the sample group as they were born, animals in the various litters were not all measured at precisely the same age. For this reason, plotted values do not represent the same number of individuals. This fact was taken into consideration by weighting the points when the curves were visually fitted. The following measurements were made on all animals: body weight, total body length, tail length, hind foot length and ear length. Tail and body measurements

were taken with a flexible plastic millimeter ruler, while hind foot and ear were taken with vernier calipers. Linear measurements were read to 0.5 mm. (except ear, to 0.1 mm.), weights to 0.01 gm.

Measurements of total body length were taken on active juveniles while the animal, held by the tail, extended itself in an escape attempt. Tail measurements were taken from the same position, using slight pressure of the ruler against the base of the tail. Such measurements on live animals are subject to inherent errors and do not represent absolute values.

Measurements were analyzed as in Brody (1945). Measurement values were plotted on a logarithmic scale versus age on an arithmetic scale. Linear segments of such a plot indicate periods when growth increments were a constant percentage of previous size. From these linear sections, instantaneous growth rates were calculated as:

$$K = \frac{\ln m_2 - \ln m_1}{t_2 - t_1}$$

The value *k* is the instantaneous percentage rate of growth for the unit of time in which t_2 and t_1 are expressed; $\ln m_2$ and $\ln m_1$ are natural logarithms of the measurements made at t_1 and t_2.

Observations and Discussion

General Development

At birth, the skin is hairless, pink, wrinkled and nearly transparent. The internal organs can be clearly seen through the ventral surface, as can blood vessels, brain and sutures in the skull. Vibrissae are present on the snout at birth and are about 1-1.5 mm. long.

Dark pigmentation starts to appear on the head and back at 4-6 days and eventually covers the body down to the lateral line area of the adult. Sparse dark gray hair appears on the pigmented area of the dorsum at 7-9 days with rather coarse white hair on the flanks and legs. By day 13-15 a full coat of juvenile pelage is present, gray on the back, whitish underside and with a buff cast in the head region. The adult colored pelage of pinkish or ochraceous-buff overlaid with blackish hairs on the dorsal surface and pale tawny to buffy white on the ventral surface starts to appear between 29-40 days. Color changes appear first on the back of the head or under the eyes.

The ears, sealed at birth, appear as protuberances. At 3-4 days, a groove deepens on the anterior surface of the auditory protuberance and forms the pinnae. By day 5, all pinnae are unfolded and are about 1 mm. long. The meatus is still closed at 13 days, and the exact time of its opening was not determined.

The eyes are sealed at birth and appear as large, heavily pigmented areas behind the thin integument. Eyelids start to develop at 6-7 days and are well formed by day 14. In most of the litters, the eyes were open by day 14-15, but in one litter they did not open until day 18. These data agree with those presented for *P. californicus, penicillatus* and *flavus* (Eisenberg and Isaac, 1963). See Figure 1 for features of general development.

The toenails are not evident at birth but are quite distinct by day 3. The incisors penetrate the gums as early as day 5 and are evident in all young by day 11. Cheek pouches are not present in the newborn. By day 3, creases lateral to the mouth are present. These creases gradually invaginate and by day 10 are approximately 1/8″ deep. The pouches increase in depth and are lightly haired by the time the juvenile pelage is acquired.

The external genitalia are very similar in both sexes but can be fairly well differentiated by 14 days. One female became sexually active at 60 days (vulva open and swollen). No males had descended testes up to 150 days (April-September).

Behavior

All neonates exhibited the righting reflex and were able to crawl, although very laboriously. Ability to move increased daily and by day 6, one animal was noted to perform digging motions involving coordinated movement of fore and hind legs. This occurred when the animal was placed upon a metal pan in preparation for weighing and was obviously disturbed by the cold metal. By day 8, the young moved about the cage freely and by day 12 were gamboling about, even though their eyes were still sealed.

Young mice were noted eating raw carrot at 10 days, and seeds were found in pouches by day 14; they appear to become self-sufficient at about 18 days. One litter survived when the mother died at 14 days.

Siblings were normally separated and placed in individual containers

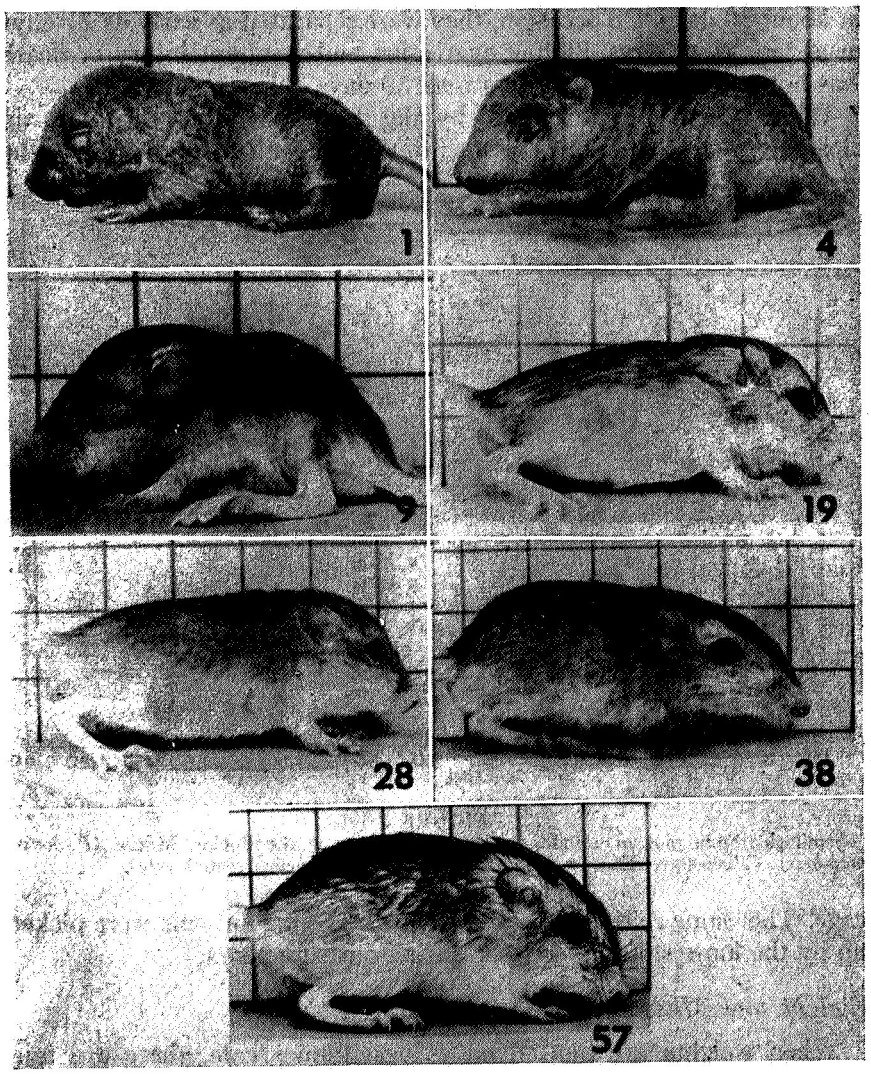

FIGURE 1

Development of the Little Pocket Mouse (*Perognathus longimembris*) from neonate to adult. Age in days is indicated in lower right corner in each sequence. The background grid is 1 cm^2. Note unfolding of auditory pinna between day 1 and 4, and rapid total development of animal between 4 and 9 days.

at about 30 days. On occasion, they were separated as early as 21 days or as late as 42 days. Sibling aggression and resultant injury or death was noted in several litters less than 30 days old.

When neonates were carried by the dam, they drew their legs up close to their body, thereby facilitating her movement around the

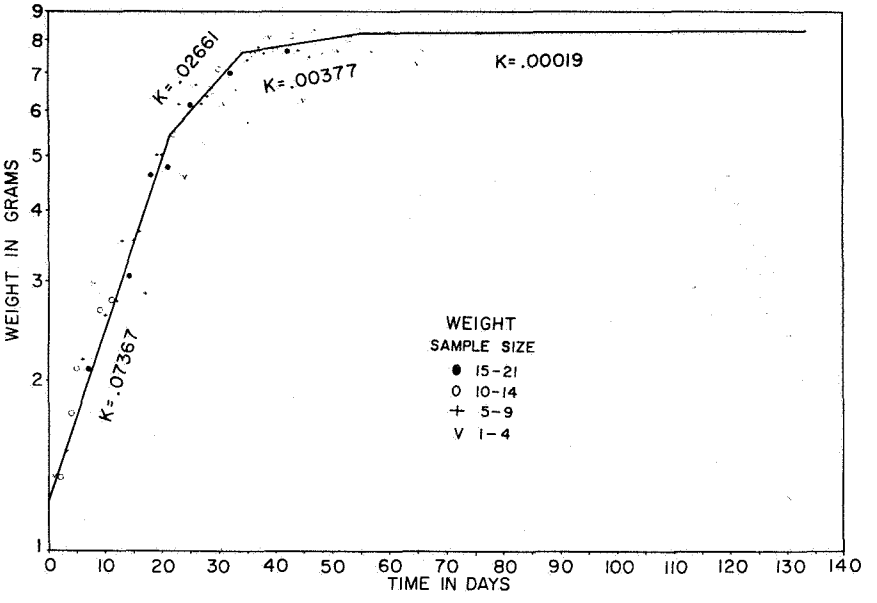

FIGURE 2
Semilogarithmic plot of weight increase with time of the Pocket Mouse (*P. longimembris*). Values above linear segments of plot are instantaneous growth rates.

cage. This same reflex was noted when the young animals were picked up by the loose skin of the back during measurements.

Weight and Measurement

When weights are plotted on a logarithmic scale, there are four distinct phases of growth during which the percentage of increase per day is constant (see Figure 2). The first phase of almost 7.4% size increase per day brings the individual to 65% of adult weight in 21 days. *P. californicus* attains only 39% of adult weight during the same

time (Eisenberg and Isaac, 1963). Unfortunately, comparative data for *P. flavus,* which is similar in size to *P. longimembris,* are not available, although this species has been bred in captivity (one litter, Eisenberg and Isaac, 1963). The initial growth rates of *Dipodomys* spp. (Chew and Butterworth, 1959; Butterworth, 1961) are greater, but are not sustained as long as in *P. longimembris* (11 days at 13.0% vs. 21 days at 7.3%). Kangaroo rats attain about 30-50% of maximum weight during this first 20-day period.

Growth rates for *P. longimembris* level off by about 50-60 days, at which time near maximum weight has been attained. After that time, body weight increases at a rate of about 0.02% per day. Maximum body weight probably is not a meaningful value, because body weight fluctuates seasonally as well as daily.

Inflections in weight increase are suggested to be associated with milestones in the development of wild rodents. Chew and Butterworth (1963) noted that the first inflection of the curve for the kangaroo rat, *D. merriami,* coincided with opening of the eyes and ears. No change was observed in *P. longimembris* at 14-15 days, the time of eyes opening. The first change of growth constants (7.4%-2.3%) did not occur until 21 days, which approximates the time of spontaneous weaning or self-sufficiency.

Increases of other dimensions commonly used as indices of growth are given in Figures 3-4. Like total body weight, total length, tail, ear and hind foot all had a four-part growth. Total length and tail length show their first growth inflections to lower values at about the 15th day (eye-opening) and the second inflection at the 26-27th. These data agree with those presented for the kangaroo rat, *D. merriami* (Chew and Butterworth, 1963).

The hind foot attained a near adult-size at about 20 days. This means that when the animal attained about 70% of its total length and about 60% of its weight, it had an adult-size foot. Inflection of growth rate occurred at 10 and 20 days. Although this species is not as dependent upon saltatorial locomotion as the genus *Dipodomys,* this mode of travel is used during escape attempts and at other times when maximum speed is valuable. The adaptive value of a fast-developing foot is self-evident.

The ear had the highest rate of growth, with a value of about 15%

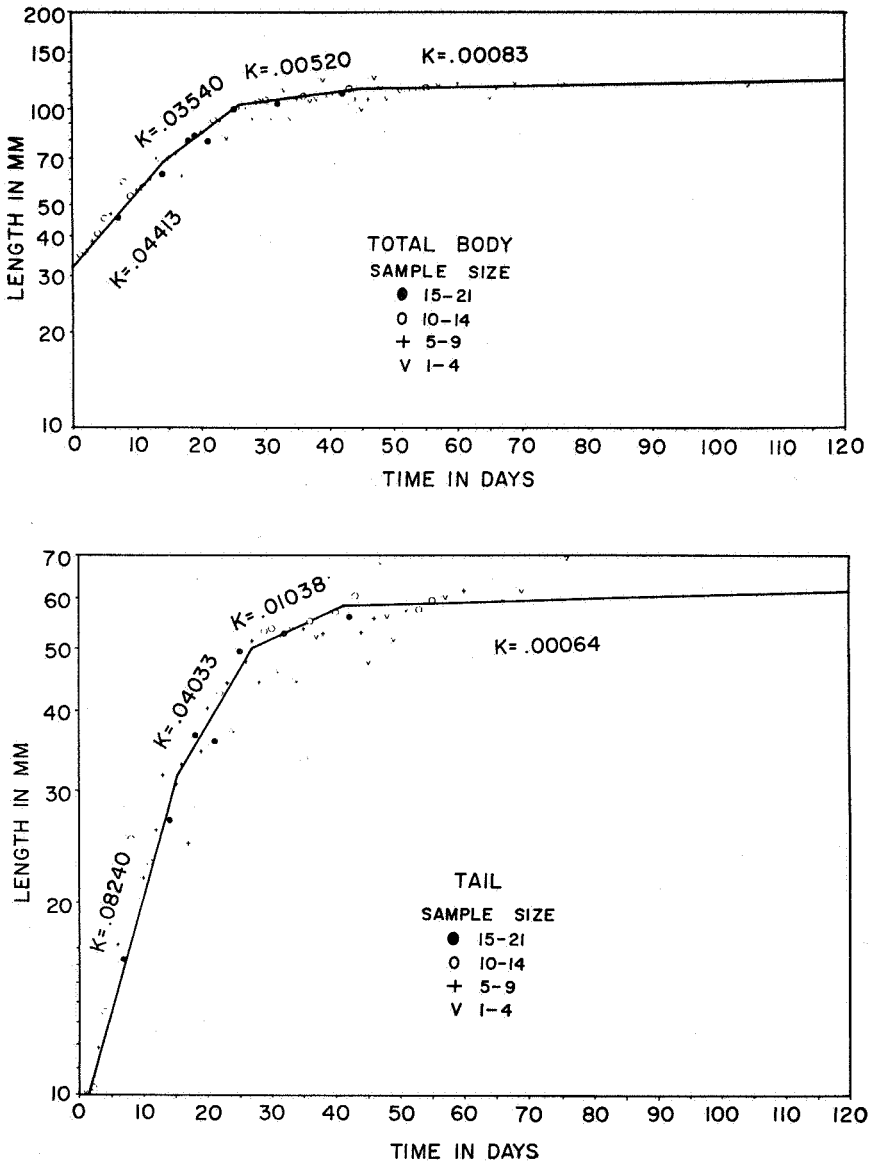

FIGURE 3
Semilogarithmic plots of total body and tail length of *P. longimembris*.

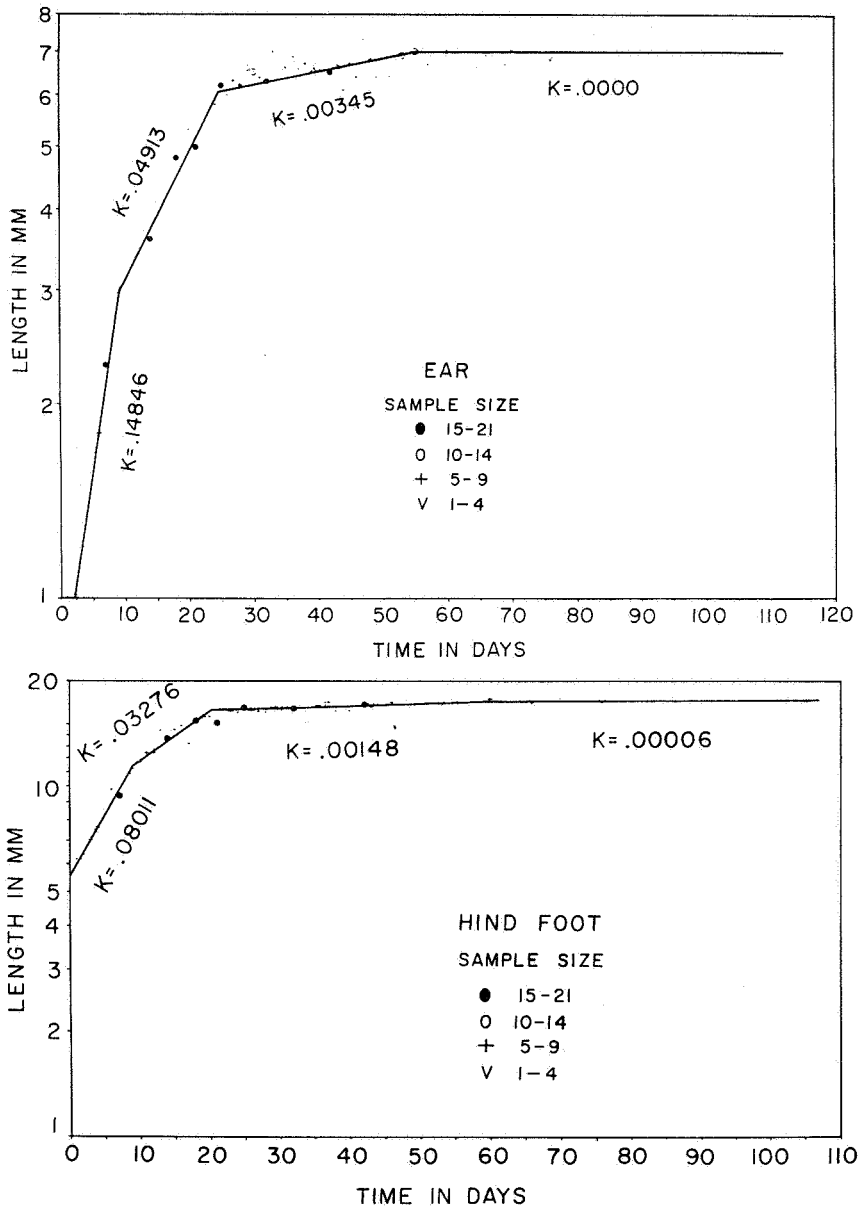

FIGURE 4
Semilogarithmic plots of hind foot and ear length of *P. longimembris*.

during the first 9 days. The inflections of growth rate are similar to those documented in the hind foot. Nearly 86% of adult size was reached by 25 days, with adult size being attained at about 55 days.

Summary and Conclusions

Data are presented on the growth and development of 26 individuals from 8 litters, 22 of which were the result of the first recorded matings of *Perognathus longimembris* in captivity. Newborn of this species of pocket mouse are naked and have a nearly transparent integument; pigmentation gradually fills the area above the future lateral line and is complete with sparse hair by day 9. Eyes and ears are sealed at birth with the pinnae developing at about day 4. The external meatus opens after day 13. Eyes usually open between day 14-15. Cheek pouches are not present at birth, but start to develop on day 3 and are functional by day 14.

Semilogarithmic plots of body weight, total length, and lengths of tail, hind foot and ear show polyphasic growth. All show a four-part pattern with initial instantaneous percentage growth rates which vary from 4.4% for total length to 14.8% for ear. Hind foot reaches near adult size by 20 days.

Acknowledgments

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SURVIVAL OF LABORATORY REARED POCKET MICE
(Perognathus longimembris)

Page Hayden

During the spring and summer of 1965 an intensive breeding program produced a cohort of 216 P. longimembris. The breeding techniques and growth characteristics of this species have been published elsewhere (1,2).

From this cohort several fatalities occurred during later breeding attempts; otherwise it has remained intact and unused for experimental purposes. These animals have been exposed to a physical environment that should be conducive to maximum survival, (moderate environment, food ad libitum, no predators). The effects of food reduction and "freedom" on longevity were obviously not evaluated.

The age at time of death and age of survivors is plotted in Figure 1. The initial high mortality rate during the first 60 days may be in part the result of the breakup of the litters into individual chambers at about 30 days. A mistake in the correct timing (too early or too late) of litter separation resulted in a stressful situation.

The mortality curve up to about the age of two years is characterized by a 0.8% death rate per month. After this 2-year period, however, there appears to be a 4 fold increase in death to about a 3.6% death rate. If this rate is extrapolated to time zero, it appears that this cohort should be dead in about 4¼ years. The curve probably will become more asymptotic with time as a few individuals will live beyond their expected demise.

Mortality for a cohort of this species under natural conditions has been estimated to be 85% during a yearly cycle. However, marked individuals have survived in natural conditions from 3 to 5 years (3). The maximum recorded life span for an individual of this species is 8 years (4).

As of this date, (February 1968) there are 3 individuals almost 3 years old, with remaining survivors (102) between 2-1/3 and 3 years old.

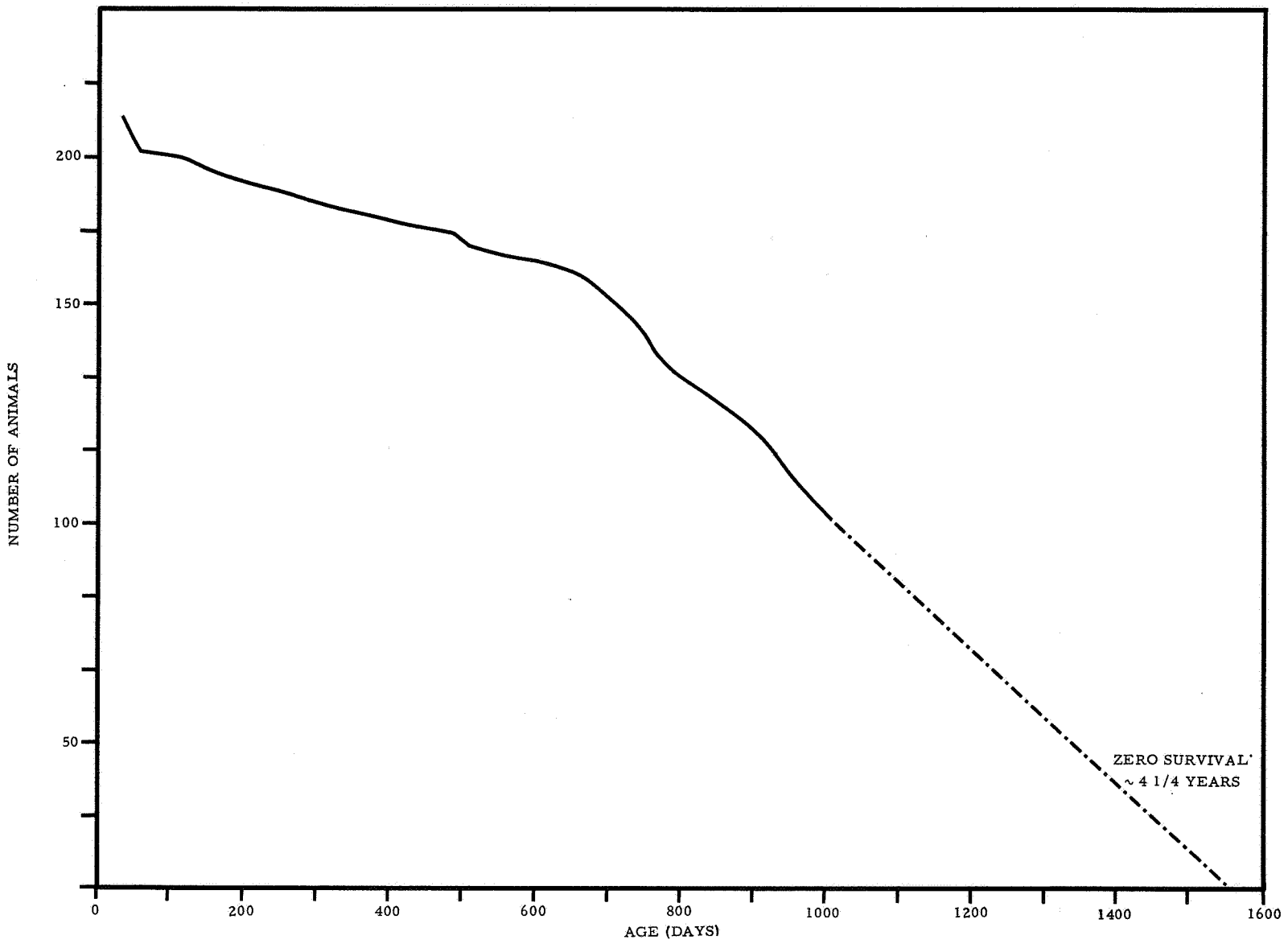

Figure 1 Age at the time of death in a cohort of 210 Perognathus longimembris born in captivity spring and summer 1965.

Most general information on small mammals gives a maximum life span of from 4-10 years, with the majority about 3-6 years (e.g., golden hamster 1-3/4 year, house mouse 3 years, albino rat 3½ years, hedgehog 4 years, guinea pig 6 years, eastern chipmunk 7½ years). It would appear that the life span of the little pocket mouse lies near the upper limits for mammals of much greater size. The occurence of periodic torpor may be involved in this prolongation of life.

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TEMPERATURE REGULATION IN THE LITTLE POCKET MOUSE, *PEROGNATHUS LONGIMEMBRIS**

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Abstract—1. Mice acclimated to ambient temperature (T_A) 22–24°C are good thermoregulators, when not torpid, at T_A 2–34°C.

2. The core-to-subcutaneous temperature gradient varied with T_A in a definite pattern that suggests four zones of temperature regulation.

3. *Perognathus longimembris* simulated conformation to Newton's Law of Cooling when metabolizing at a minimum maintenance level, but active mice had a variable conductance which decreased with T_A.

4. Mice entering and arousing from torpor went through several phases of temperature change. During cooling, conductance is greater than normal, and during warming it is less.

INTRODUCTION

THE little pocket mouse is a particularly interesting subject for study of temperature regulation because of its small adult size (8–11 g) and its facility for daily torpor.

Because of the relationship between heat flux and body size in homeotherms, one expects an increase in lability of body temperature (T_B) with decrease in body size in rodents. This has been shown for the pygmy mouse, *Baiomys taylori* (6–9 g) (Hudson, 1965), the harvest mouse, *Micromys minutus* (5–9 g) (Smirnov, 1957), and several species of deer mice, *Peromyscus* spp. (16–22 g) (Morrison & Ryser, 1959; MacMillen, 1965).

As in the pygmy mouse, the diurnal fluctuation of body temperature in a small mouse frequently comes to the point of torpor (Hudson, 1965). As shown by Bartholomew & Cade (1957) and Chew *et al.* (1965), *Perognathus longimembris* is easily induced to become torpid by limiting its food supply or subjecting it to ambient temperatures below 20°C. *P. longimembris* has a greater facility for torpor than most of the small rodents that have been studied; it can arouse spontaneously from T_B at 10°C, without mortality, if its energy reserves have not been seriously reduced, and most individuals can arouse from a T_B of 5°C. *B. taylori* has an arousal threshold of $T_B = 17$°C (Hudson, 1965), and the California pocket mouse,

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P. californicus (22 g), has a threshold of $T_B = 15°C$ (Tucker, 1965a). The 7–16 g birch mouse, *Sicista betulina*, of the Arctic can arouse from $T_B = 4°C$ (Johansen & Krog, 1959).

The present study was made coincident with a comparison of the metabolic rates of eight species of *Perognathus* (Chew *et al.*, 1963). Temperature regulation was studied in detail only for *P. longimembris*; a few observations were made on several other species. This work complements that of Bartholomew & Cade (1957) on *P. longimembris* and of Tucker (1965a, b) on the larger *P. californicus* (22 g).

MATERIALS AND METHODS

Animals

Live specimens of *P. longimembris* were collected at Whitewater Canyon and Pearblossom, California; *P. alticola* from Palmdale, California; *P. formosus* from Lathrop Wells, Nevada; and *P. californicus* from Coalinga, California. Mice were kept individually in 1-gal jars, with a substrate of sand or granulated absorbent clay. A mixture of parakeet seed, rolled oats and sunflower seed was provided in surplus; small amounts of vegetable greens were given occasionally. The animal room was kept at 20–24 C, 45–55 per cent relative humidity, and at a photoperiod from 0600 to 1800 hr P.S.T.

Body temperatures were measured with bead thermistors ($\sim 0{\cdot}6$ mm^3) implanted subcutaneously in the middorsal region and inserted 2 cm into the colon, and by small telemeters ($17 \times 9 \times 5$ mm; ~ 600 mm^3) inserted in the abdominal cavity. The leads of the colonic thermistor were taped to the tail and protected by a light metal spring; the leads from the subcutaneous thermistor were passed through a plastic disk sewn to the skin of the neck.

The telemeters are of a blocking oscillator type, developed at Northrop Space Laboratories. They have a useful life of approximately 6 months. The pulse carrier center frequency is about 5 Mc/s with a temperature-dependent repetition rate of 150–500/sec. Temperature resolution is to 0·1°C, with a shift of about 0·5° in 6 months.

During temperature measurements animals were confined in 1000-ml tall-form beakers submerged in a water bath, or in $20 \times 7{\cdot}5$ cm plastic containers in a constant temperature incubator. Mice were provided sand substrate and food as in their storage jars.

One group of ten mice with implanted thermistors was exposed to constant ambient temperatures for periods of 24–48 hr. In some cases oxygen consumption and temperature were recorded simultaneously, and several mice went through one or more periods of torpor during the recording period. Another six mice with implanted thermistors were subjected to continuously changing ambient temperatures. Temperature was reduced from about 27° to 2°C over a period of 4–6 hr, or was increased from about 16° to 38°C over a period of 3·5–6 hr.

Six mice with implanted telemeters were exposed to each of nine ambient temperatures in the range 2–39°C. Beginning with 2°C, mice were exposed for 2 hr at each temperature: 2, 7, 12, 17, 22, 27, 32 and 37°C. There was a 20–30 min transition period between different temperatures, except there was an overnight return to room temperature between exposures to ambient temperatures of 12° and 17°C. After exposure to 37 C, mice were exposed for 1 hr to 39°C.

Metabolic rate

Oxygen consumption was measured by use of a Beckman paramagnetic oxygen analyzer, or a manometric respirometer (Chew *et al.*, 1965). Oxygen consumption was continuously recorded with the Beckman analyzer, while the respirometer recorded the time for consumption of successive units of ~ 20 ml O_2. In measurements with the respirometer, the

mice were alternately measured 1 day at an experimental T_A and rested 1 day at room temperature, until the range of 5°, 15°, 25° and 35°C was completed. During measurement periods mice were confined in 1000-ml beakers with food and substrate as in their storage bottles.

Conductance

Thermal conductance was estimated from metabolic and temperature measurements. Heat loss was also measured in terms of rate of cooling of freshly killed mice. For the latter measurements, the live animal was kept at the desired T_A several hours until its body temperature was stable. Chloroform was then introduced into the air stream; the animal died quickly, usually without a change in posture. Colonic and subcutaneous temperatures were recorded as the dead body, resting in a natural position, cooled to T_A.

Temperature change constants, for dead mice and for torpid live animals, were calculated as

$$K = \frac{\ln T_1 - \ln T_2}{\Delta t},$$

where T_1 and T_2 are body temperatures (°C) at the beginning and end of the time period, Δt (min).

RESULTS

Body temperature in relation to ambient temperature

Figure 1 shows the colonic temperatures of *P. longimembris* during 24–48 hr exposures at different T_A. Figure 2 shows the abdominal cavity temperatures for other *P. longimembris* during 2-hr exposures. Table 1 summarizes measurements for the six mice of Fig. 2, and also gives measurements for three *P. alticola*.

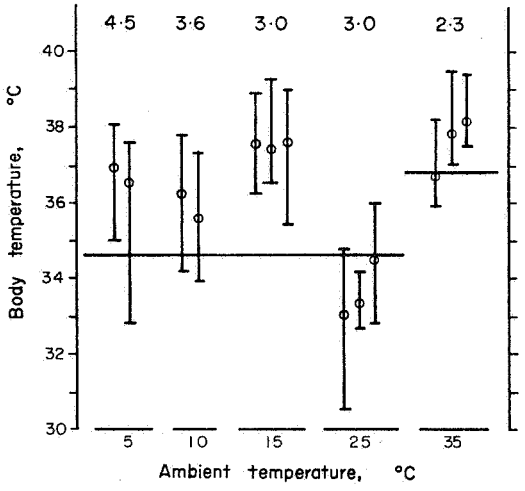

FIG. 1. Body temperature of *P. longimembris* kept 24–48 hr at different ambient temperatures; T_B recorded continuously from bead thermistor 2 cm into colon. Open circle is the average T_B and vertical line is the range for each animal during its normothermic periods. Horizontal line is mean average T_B of another group of six pocket mice as measured by telemeter in body cavity (mice of Fig. 2 and Table 1). Numbers at top are mean ranges, °C.

TABLE 1—BODY TEMPERATURE AT DIFFERENT AMBIENT TEMPERATURES. T_B MEASURED BY TELEMETER IN ABDOMINAL CAVITY DURING 2-HR EXPOSURE

P. longimembris (n = 6), av. wt. 11·55 g (range 9·0–14·0 g)									
T_A (°C)	2	7	12	17	22	27	32	37*	39†
Mean av. T_B (°C)	34·31	34·93	34·97	34·93	34·58	34·65	34·79	37·89	38·41
S.E.	0·63	0·56	0·51	0·32	0·36	0·30	0·20	0·23	—
Mean range of av. T_B (°C)	0·90	1·67	2·05	2·33	2·59	1·82	1·23	1·05	1·88
Total range of av. T_B (°C)	34·0–34·9	34·2–35·9	34·1–36·1	33·7–36·0	33·1–35·6	33·8–35·6	34·2–35·4	37·6–38·6	38·0–39·8
Mean square variation within individuals‡	0·10	0·40	0·48	0·53	0·61	0·52	0·11	0·26	—
P. alticola (n = 3), wt. 15·5, 15·7, 22·2 g									
T_A (°C)	2	7	12·5	17	22·5	27	32	37	
Mean av. T_B (°C)	36·17	35·73	36·17	34·60	34·73	35·27	36·23	40·13	
S.E.	0·17	0·41	0·44	0·21	0·11	0·52	0·09	0·33	
Mean range of av. T_B (°C)	1·46	1·66	2·00	2·63	2·80	2·53	2·30	2·03	
Total range of av. T_B (°C)	34·8–36·9	33·6–36·9	34·3–37·8	32·5–36·3	32·8–37·1	32·8–37·6	34·2–37·9	36·7–40·8	

* Av. and S.E. for second hour only, when T_B was stabilized.

† 1-Hr exposure only.

‡ Twelve to fourteen measurements at 10-min intervals on each mouse at T_A 2–37°C, six measurements at T_A 39°C; mean square = sum deviations²/degrees freedom.

Abdominal temperature of *P. longimembris* did not vary significantly with ambient temperature from T_A 2° to 32°C. In this range mean average abdominal temperature was 34·68°C. Abdominal temperatures of the small sample of three *P. alticola* were significantly lower at T_A 16° and 22·5°C than at 2° and 32°C.

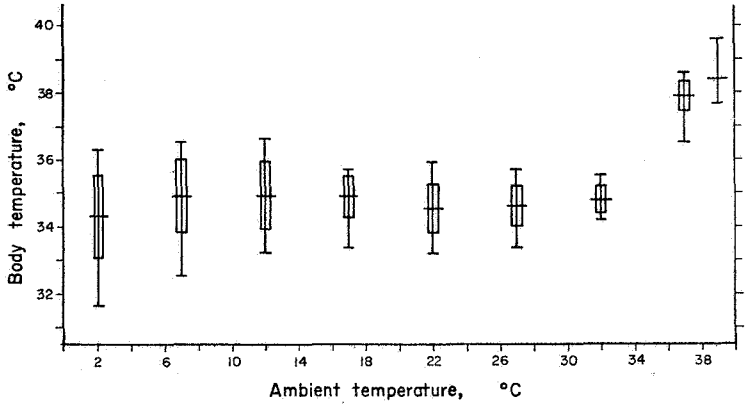

FIG. 2. Relationship of mean average abdominal temperature to ambient temperature for six *P. longimembris*. Horizontal line is mean average T_B for the six mice, vertical bar is ± 2 S.E., and vertical line is range of average abdominal temperatures. Mice were exposed 2 hr at each temperature, proceeding from 2° to 37°C, with a 20–30-min transition between temperatures, except there was an overnight break in the sequence between measurements at T_A 12° and T_A 17°C. The data plotted for T_A 37°C are for the second hour of exposure only; data for 39°C are for a 1-hr exposure only.

P. longimembris showed decreasing intragroup variation of T_B as T_A increased from 2 to 32°C, but conversely showed increasing intra-individual variation (Table 1).

Above T_A 32°C, both species showed a rise of T_B with T_A. Some *P. longimembris* stabilized their temperatures at T_A 39°C during a 1-hr exposure, and briefly (15 min) tolerated colonic temperatures as high as 42·0°C. The one animal that recovered from a T_B of 42·0°C salivated markedly; mice often everted their cheek pouches at T_A 35–39°C. Another mouse survived two rises of colonic temperature to 15-min peaks of 41·8° and 41·3°C within 2 hr. Two *P. longimembris* died at colonic temperatures of 41·9° and 42·5°C, after about 15 min at these T_B.

Three *P. alticola* exposed to T_A 40°C died within an hour. Their abdominal temperatures at death were sharply defined as 43·2°, 43·0° and 44·0°C.

Figure 3 shows the colonic temperatures of *P. longimembris* exposed to continuously changing ambient temperature over periods of 3·5–6 hr. Two of three mice exposed to declining T_A had an initial decrease in T_B, but then stabilized or increased T_B. Similarly, the two animals of Fig. 1 exposed to 10°C showed a drop in T_B for the first 1·5–4 hr, but then returned to their initial T_B. None of three mice subjected to increasing T_A showed a change of T_B, until T_A of about 34·5°C.

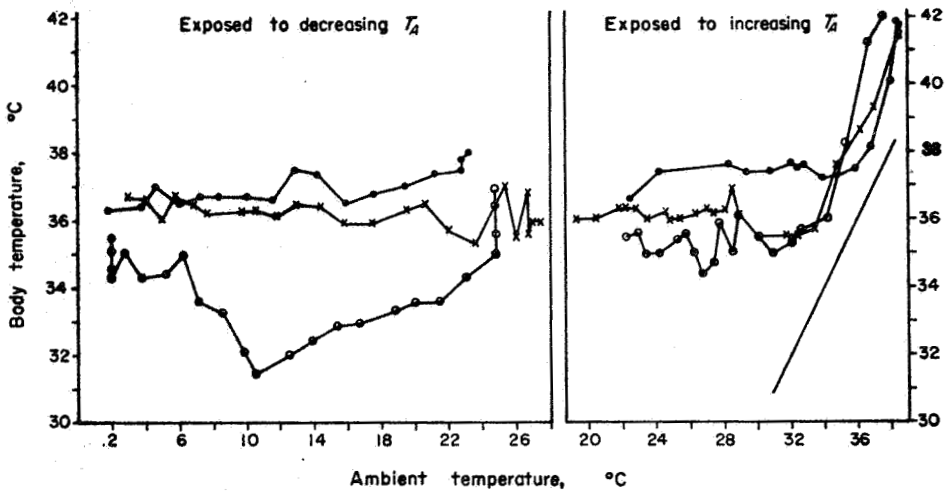

FIG. 3. Colonic temperatures of six *P. longimembris* exposed to continuously changing ambient temperature over a period of 3·5–6 hr; three mice exposed to decreasing T_A and three exposed to increasing T_A. Plotted points are average body temperatures for consecutive 15-min periods.

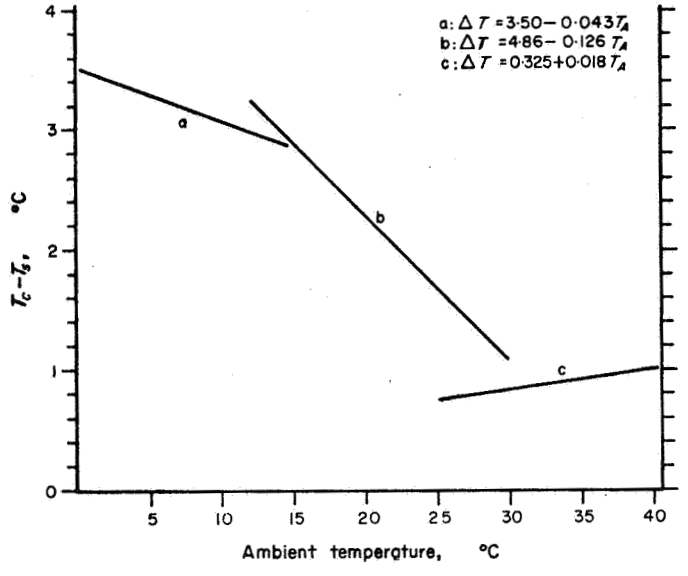

FIG. 4. Relationship of the gradient: colonic temperature (T_c) minus subcutaneous temperature (T_s), to ambient temperature (T_A) for the six mice of Fig. 3. Values fell into three groups represented by the three linear regression lines.

Core-to-subcutaneous temperature gradient

Figure 4 shows the difference between deep colonic temperature and subcutaneous temperature, in relation to ambient temperature, for the six mice subjected to continuously changing T_A. The slopes of the regression lines are all significantly different from zero: for T_A 2–15°C, P is slightly less than 0·05; for T_A 12–30°C, $P<0{\cdot}001$; for T_A 25–39°C, $P<0{\cdot}05$. The point of transition from one slope to another varied with the animal, hence the overlapping temperature ranges for which regressions are calculated in Fig. 4.

The regression of colonic temperature (T_B) on T_A for the mice of Fig. 4 was

$$\begin{aligned} T_A\ 0\text{–}14°C, &\quad T_B = 36{\cdot}59 - 0{\cdot}0055T_A; \\ T_A\ 14\text{–}28°C, &\quad T_B = 36{\cdot}44 - 0{\cdot}0041T_A; \\ T_A\ 28\text{–}34°C, &\quad T_B = 33{\cdot}64 + 0{\cdot}081T_A; \\ T_A \text{ greater than } 34°C, &\quad T_B = 1{\cdot}519 + 1{\cdot}034T_A. \end{aligned}$$

Metabolic rates of normothermic animals

Figure 5a and b shows the metabolic rates for nine *P. longimembris*, as measured in the manometric respirometer. These mice had an average weight of 8·23 g (range 8·0–8·5). As defined here, average maintenance metabolism (Fig. 5a)

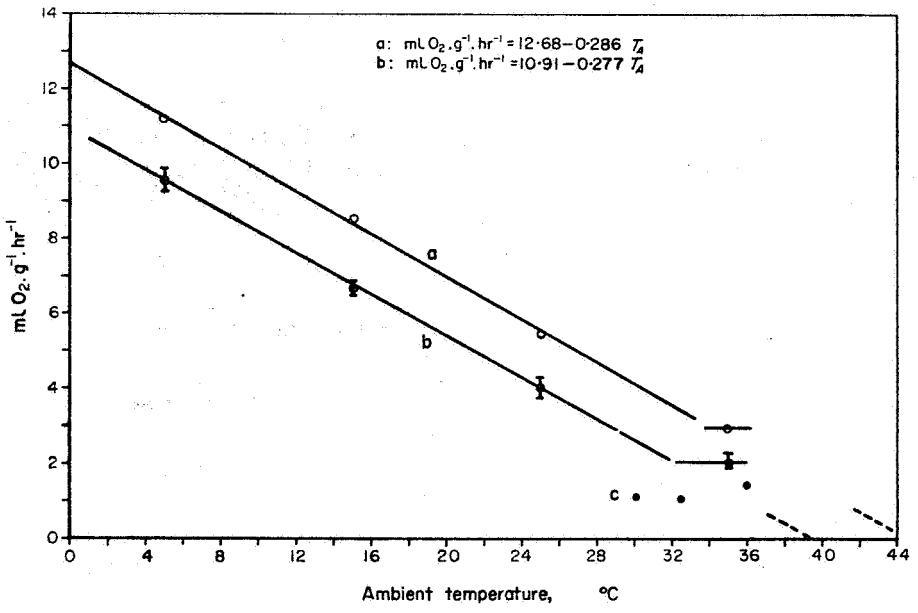

FIG. 5. Oxygen consumption of *P. longimembris*. (a) Average maintenance metabolism and (b) minimum maintenance metabolism of nine mice as measured in manometric respirometer; (c) mean minimum metabolism for 5-min period for ten postabsorptive mice, as measured with Beckman oxygen analyzer. Circles are mean values; vertical lines for (b) are ±2 S.E.

is the average rate for the entire 23-hr recording period at a particular ambient temperature, excluding any obvious periods of torpor. Four of nine mice were torpid for a time during the 15°C exposure; all nine were torpid for a period during the 5°C exposure. Minimum maintenance metabolism (Fig. 5b) is the rate calculated during the slowest consumption of one 20-ml unit of oxygen in the respirometer, again excluding periods of torpor. The maximum time for consumption of one unit ranged from an average of 14·9 min at 5°C to 67·7 min at 35°C.

Below 30°C, average maintenance metabolism had a regression on T_A of: ml $O_2 . g^{-1} hr^{-1} = 12{\cdot}68 - 0{\cdot}286 T_A$ (standard error of estimate, 0·12; $n = 27$). Minimum maintenance rate was: ml $O_2 . g^{-1} hr^{-1} = 10{\cdot}91 - 0{\cdot}277 T_A$ (standard error of estimate, 0·08; $n = 27$).

At T_A 35°C the average maintenance rate was 2·94 ml $O_2 . g^{-1} hr^{-1}$, and minimum maintenance rate was 2·06 ml $O_2 . g^{-1} hr^{-1}$.

Metabolic rate was also measured with the Beckman analyzer for ten mice (av. wt. 8·89 g, range 7·8–10·4 g), while they were postabsorptive, at three points in the range of T_A 29–36°C. The average minimum rates for 5-min periods were: at T_A 30·0°C, $1{\cdot}12 \pm$ S.E. 0·075 ml $O_2 . g^{-1} hr^{-1}$; T_A 32·5°C, $1{\cdot}07 \pm 0{\cdot}059$ ml $O_2 . g^{-1} hr^{-1}$; T_A 36·0°C, $1{\cdot}45 \pm 0{\cdot}13$ ml $O_2 . g^{-1} hr^{-1}$. The value of 1·07 ml $O_2 . g^{-1} hr^{-1}$ at T_A 32·9°C is taken as the best approximation to basal metabolic rate. These measurements also indicate that the zone of thermal neutrality is no more than $33 \pm 1{\cdot}5$°C.

Metabolic rate and body temperature

The correlation of metabolic rate and colonic temperature was analyzed for two *P. longimembris* at T_A 10°C (those of Fig. 1) for which there were 36–48 hr of continuous records of both T_B and oxygen consumption. The analysis is summarized in Tables 2 and 3.

TABLE 2—METABOLIC RATE AND T_B OF TWO *P. longimembris* KEPT 2 DAYS AT T_A 10°C. DATA FOR NORMOTHERMIC PERIODS ONLY

	Mouse No. B3	Mouse No. B4
Hours normothermic	15	36
Mean body temperature (°C)	36·25	35·58
Extreme range T_B, absolute	34·2–37·8	33·9–37·4
degrees	3·6	3·5
Mean hourly range T_B (°C)	1·5	1·0
Coincidence of hourly		
maxima of T_B and metabolism*	0·93	0·79
minima of T_B and metabolism*	0·81	0·83
Time interval (min) between		
extremes of metabolism and T_B		
between maxima	0·7	2·3
between minima	1·9	2·9

* Calculated for those hours when there was enough variation to determine clear minima and maxima.

TABLE 3—MEAN HOURLY METABOLIC RATE AND T_B FOR *P. longimembris* NO. B3 AT T_A 10°C. DATA FOR NORMOTHERMIC HOURS ONLY

n	ml O_2 (mouse)$^{-1}$ hr^{-1}	T_B (°C)
1	63·6	35·6
2	66·4–68·2	35·8
4	68·3–70·0	35·9
5	70·1–71·9	36·4
2	72·0–73·8	36·6
1	74·8	37·1

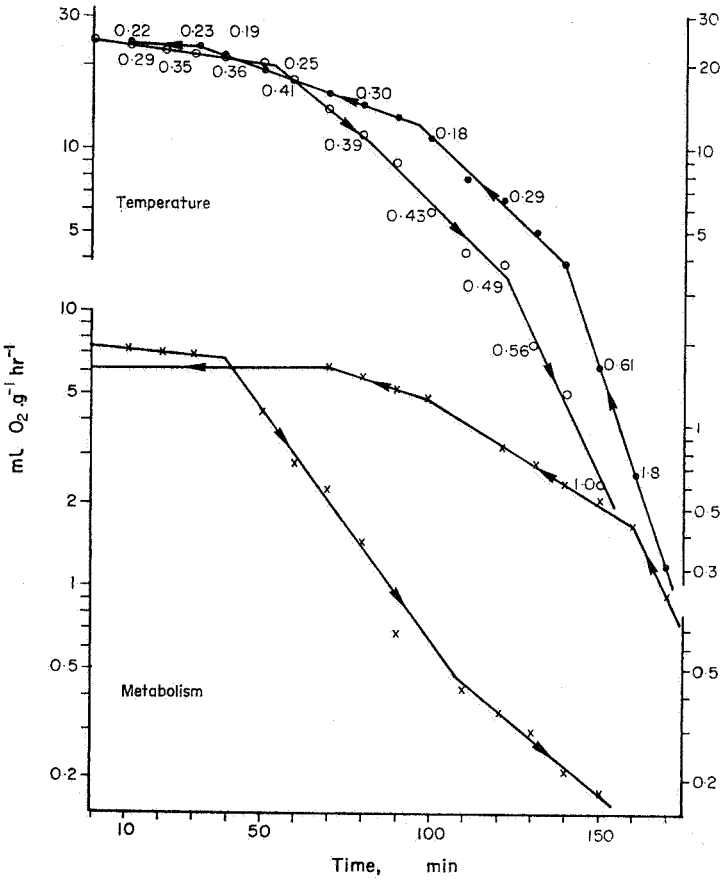

FIG. 6. Body temperature and oxygen consumption of one *P. longimembris* during entry and subsequent arousal from torpor while at T_A 10°C. Values adjacent to temperature curves are thermal conductances estimated at various points in cycle (see text).

TABLE 3—MEAN HOURLY METABOLIC RATE AND T_B FOR *P. longimembris* NO. B3 AT T_A 10°C. DATA FOR NORMOTHERMIC HOURS ONLY

n	ml O_2 (mouse)$^{-1}$ hr^{-1}	T_B (°C)
1	63·6	35·6
2	66·4–68·2	35·8
4	68·3–70·0	35·9
5	70·1–71·9	36·4
2	72·0–73·8	36·6
1	74·8	37·1

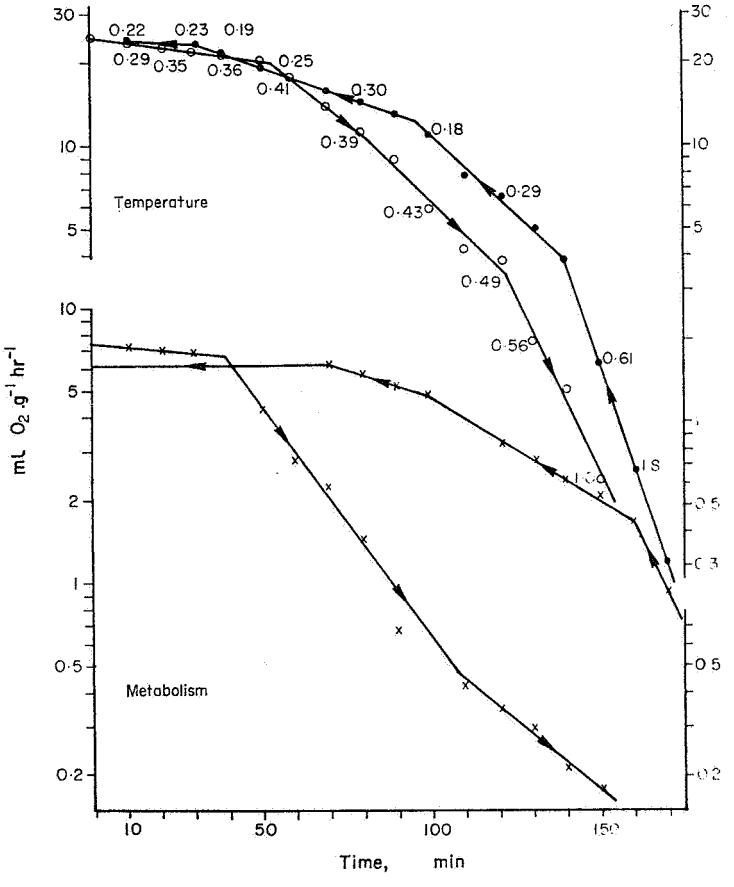

FIG. 6. Body temperature and oxygen consumption of one *P. longimembris* during entry and subsequent arousal from torpor while at T_A 10°C. Values adjacent to temperature curves are thermal conductances estimated at various points in cycle (see text).

found less variation of colonic temperature in *P. longimembris* acclimatized for 10 days at T_A 19–23°C than found in the present work for telemetered animals at T_A 22°C, i.e. S.E. of 0·14°C for nine mice, and 0·36°C for nine mice respectively.

P. longimembris showed good resistance to cold. Mice acclimatized to room temperature did not show any drop in T_B during 2-hr exposures to T_A down to 2°C. Although there was more spread of the mean body temperatures of individuals at the lower ambient temperatures (as shown by S.E. of group means, Table 1), there was less intra-individual variation, i.e. more stability of T_B, at the lower ambient temperatures, as shown by the mean squares and ranges of Table 1.

There were no significant differences in mean average T_B of *P. longimembris* over the range T_A 2–32°C (Fig. 2, Table 1). The same was generally true for ten subspecies of *Peromyscus* studied by McNab & Morrison (1963). But most small rodents have shown some depression of T_B at low T_A: slight in *Peromyscus eremicus* (MacMillen, 1965) and *Perognathus californicus* (Tucker, 1965a); moderate to considerable in *Peromyscus leucopus noveboracensis* (Morrison & Ryser, 1959), *Zapus hudsonius* (Morrison & Ryser, 1962), *Micromys minutus* (Smirnov, 1957) and *Mus musculus* (Hart, 1951).

If they had been exposed to experimental T_A for longer times, the present animals might have shown a lowering of T_B, since Bartholomew & Cade (1957) found that this species had lower T_B at T_A 2·5–5·3°C than at T_A 19–23°C. Their measurements were made after 10 days of exposure.

When *P. longimembris* were exposed to continuously changing T_A two of three mice showed an initial drop in T_B when T_A was decreasing, but soon stabilized or increased their core temperature (Fig. 3). None of three mice showed any significant change in T_B as T_A increased up to 34°C.

Body temperature of *P. longimembris* rose when ambient temperature was increased above 34°C. However, the rate of increase above normal T_B was slower than expected. For the six mice of Fig. 2, after 2 hr at T_A 32°C, and a 20-min transition to 37°C, it was 50 min before abdominal temperatures averaged 37°C for the group. The T_B of one mouse did not rise to 37°C in the 2-hr exposure. Then, after a 30-min transition to T_A 39°C, it was 30 min before the group mean T_B rose to ambient. There was considerable variability of response at T_A 39°C. Four of six mice had stabilized their body temperatures or nearly so at the end of 60 min; one mouse warmed to only $T_B = 38{\cdot}2$°C in 60 min, another warmed to 39·0°C, while a third reached a lethal T_B of 41·9°C in that time.

From average minimum maintenance metabolism and conductances measured for *P. longimembris*, a warming rate of 5·5–11°C/hr is expected at ambient temperatures above normal body temperature. Warming rates of only 1·8°C/hr were observed for the mice of Table 1 and Fig. 2 at T_A 37° and 39°C. There were no apparent radiation or conduction heat sinks available to the mice during the measurements. To dissipate the heat associated with the difference of $\sim 3{\cdot}5$–9°C.g^{-1} hr^{-1} between the expected and actual body temperatures would require the evaporation of 5·5–14 mg H_2O.g^{-1} hr^{-1}, or 2·8–7 mg H_2O/ml O_2 consumed. Rates of evaporation in the range 3–4 mg H_2O/ml O_2 have been

observed for several desert rodents at ambient temperatures 37–40°C (unpublished material). Higher rates can probably be achieved for short periods of time by an increase in ventilation of respiratory and nasal surfaces, and by evaporation from the surfaces of everted cheek pouches in addition to the general body surface. The present mice were not observed to salivate; however, others did during more extreme exposures.

Body temperature measured by a telemeter in the abdominal cavity was usually lower than that measured with a small thermistor in the colon (see Fig. 1). Undoubtedly, the telemeter, because of its much larger size (~ 600 mm^3 versus $\sim 0{\cdot}6$ mm^3 for the thermistor), is giving more of an average temperature for the whole body volume, while the thermistor is measuring the temperature of a relatively small volume of tissue in the body core. Hart (1951) found that the average temperature of the whole body of house mice (measured in a calorimeter) was about 2°C lower than the temperature of the body core. The measurements of Smirnov (1957) show the differences that can exist between different parts of the external surface of a small rodent.

When speaking about body temperature, in the case of small rodents in particular, it is necessary to specify where in the body the temperature was measured and with what kind of sensor. Morrison & Ryser (1959) and others have shown that the measured colonic temperature varies with the depth of insertion of the thermocouple or thermistor. Bartholomew & Cade (1957) found that rectal temperatures taken manually with a thermocouple were 1°C higher than those measured by thermocouples implanted in the body core. The present work shows that temperatures telemetered from the abdominal cavity are not comparable in absolute terms with those measured with thermistors.

McNab (1966) proposed that a body temperature of 35°C is the lowest compatible with good thermoregulation in a 2-hr exposure at 10°C, and is inadequate for longer exposures or lower ambient temperatures. The mice of Fig. 2 had abdominal temperatures averaging 34·6°C during a 4·5-hr exposure at T_A 2° and then 7°C. Two other mice had mean colonic temperatures of 36·3° and 35·6°C over 48-hr exposure at T_A 10°C (Table 3). Thermoregulation was good in both cases, except when the mice were torpid. Obviously, however, the meaning of "body temperature" and "good" thermoregulation is open to interpretation.

Core-to-subcutaneous temperature gradient

When $T_c - T_s$ differences are plotted against T_A, for the six mice exposed to continuously changing T_A, there are three groupings of values, represented by the three regression lines of Fig. 4. This suggests that *P. longimembris* has four zones of temperature regulation. These are, speculatively, as follows

(1) In the range of T_A 34–39°C, heat production is minimum in an inactive animal. Since $T_c - T_s$ remains at about 1°C throughout the range, heat transport to the superficial tissues and heat loss through the surface remain maximum. Equilibrium of T_B occurs passively when T_B increases to the point where the surface-to-air temperature gradient is sufficient to make heat loss equal to minimum

heat production. This equilibrium occurs when core temperature is about 1·5–4·0°C above T_A (Fig. 3).

Metabolic rate reaches an absolute minimum near T_A 33°C. Above this T_A, metabolism increases, and this complicates the achievement of equilibrium. The mice of Figs. 3 and 4 were not allowed to reach an excited state; they usually remained prostrate, so heat production of increased muscular activity was not a problem.

(2) Below T_A 34°C there is a constant rate of increase of heat production (Fig. 5a). In the range of T_A 34° down to 30°C, since $T_c - T_s$ remains at about 1°C, probably there is little change in circulation of heat to the surface. As T_A decreases, heat balance is achieved partly by increased heat production and partly by cooling of the body to a lower equilibrium temperature.

(3) Below T_A 30°C, core temperature remains constant, but $T_c - T_s$ increases from about 1° at T_A 30° to 3° at T_A 17°C. This suggests that there has been a reduction in circulation to the superficial tissues, and that heat balance is now being partly achieved by cooling of the surface and consequent reduction in heat loss from the surface.

(4) Below T_A 17°C and down to at least T_A 2°C, $T_c - T_s$ is relatively stable at 3°C. This suggests that in addition to previous adjustments, heat balance is now being achieved by some additional reduction in total thermal conductance. Conductance is gradually reduced (integrated insulation is increased) as T_A decreases.

The reality of the different $T_c - T_s$ relationships is given particular credence by two mice of Fig. 3 that were warmed to near the lethal point, allowed to cool rapidly, then rewarmed and recooled; these mice had the same $T_c - T_s$ values for specific ambient temperatures when warming and cooling. Also, the mouse of Fig. 3 that had the greatest drop in core temperature during exposure to decreasing T_A had the same $T_c - T_s$ values as the other mice. The consistency of the temperature gradient for different ranges of T_A suggests that the little pocket mouse may integrate its metabolism to this gradient, as some mammals seem to integrate to skin temperature (Hart, 1964). It may also be significant that the region of implantation of the subcutaneous thermistor bead is rich in brown fat tissue, an active heat-producing tissue.

Musser & Shoemaker (1965) similarly have found for two species of *Peromyscus* that deep colonic and superficial colonic temperatures did not vary linearly with T_A, but that the difference between them increased sharply from T_A 30° to T_A 20°C. This is about the same region of greatest gradient change for the present mice.

Metabolic rate and body temperature

There is evidence for *P. longimembris* that variation in T_B depends upon variation of metabolic rate. In mice kept for 2 days at T_A 10°C (Tables 2 and 3) T_B, like metabolism, showed a greater range in 24 hr than in hourly periods. There was a high degree of coincidence of hourly extremes of metabolism and T_B, with the temperature maxima and minima lagging several minutes behind those of metabolism. The true lag was something greater than the value in Table 2, due

to lag in recording oxygen concentration in an analyzer about 1 m downstream from the animal chamber, and due to time for mixing of atmospheres in the chamber. McNab & Morrison (1963) found variations of T_B in *Peromyscus* spp. associated with changes in activity.

The coincidence of metabolism and temperature extremes persisted in Mouse No. B3 (Tables 2 and 3) up to the time of its entry into prolonged torpor. In Mouse No. B4 there was a lack of coincidence prior to and between two short periods of torpor. In Mouse No. B3 there was a complete positive association of mean hourly levels of T_B and of oxygen consumption (Table 3). However, in No. B4, temperature and metabolism levels changed in opposite directions (15 hr) more often than in the same direction (11 hr). Hart (1951) found that physical activity in mice at low T_A can decrease T_B below that of a resting animal, in spite of differences in metabolic level. This might be the reason for the difference between animals No. B3 and No. B4.

Thermal conductance of normothermic mice

Considering the number of physiological and physical factors that influence the heat loss of a small rodent, it is surprising that a linear relationship is so often obtained between metabolic rate and T_A, and that this relationship often extrapolates to a temperature within the range of normal T_B at theoretical zero metabolism. When such a relationship occurs in animals with a constant T_B over the range of T_A involved, it means that all the factors affecting heat loss have integrated in such a fashion as to simulate Newtonian cooling of an inanimate object, i.e. cooling rate proportional to $T_B - T_A$.

P. longimembris simulates Newtonian cooling in a particular instance only, e.g. when metabolizing at the minimum maintenance rate. The regression relating minimum rate to T_A (Fig. 5b) extrapolates to 39·4°C, which is high in the normal range of T_B. But the extrapolation is reasonable considering that the 5 per cent confidence limits of the slope of the regression are 0·248–0·306.

The mice of Fig. 5, at their minimum metabolic rate, had a thermal conductance that was constant below the lower critical temperature (L.C.T.) at about 0·28 ml $O_2 . g^{-1} hr^{-1} (°C)^{-1}$. This is the same as the value of 0·27 found by Pearson (1960) for 9-g harvest mice, *Reithrodontomys megalotis*, but less than the 0·40–0·48 measured by Hudson (1965) for 6·4-g pygmy mice, *Baiomys taylori*. Tucker (1965a) found a value of 0·19 for 22-g *Perognathus californicus*. All these species, except *B. taylori*, fit the general curve presented by Lasiewski (1963) relating conductance to body size in small birds and mammals. A thermal conductance of 0·28 ml $O_2 . g^{-1} hr^{-1} (°C)^{-1}$ is 93 per cent of the value (0·30) predicted by the curve of Lasiewski (1963), but is only 78 per cent of the value (0·36) predicted by the formula of Morrison & Ryser (1951): $cal . g^{-1} hr^{-1} (°C)^{-1} = 4·8$ wt $(g)^{-0·50}$.

The linear regression of average maintenance metabolism on T_A (Fig. 5a), however, extrapolates to 44·2°C at zero metabolism, which is several degrees above

lethal T_B. In this case Newtonian cooling is not simulated. Thermal conductance can be calculated by the formula

$$C = \frac{\text{H.P.}}{T_B - T_A}.$$

If heat production (H.P.) is taken as the average maintenance rate of Fig. 5, and T_B is taken as the abdominal temperatures of the mice of Fig. 2, then the following values of C result: T_A 35°C, $C = 2{\cdot}5$; T_A 32°C, $C = 1{\cdot}3$; T_A 25°C, $C = 0{\cdot}58$; T_A 15°C, $C = 0{\cdot}43$; T_A 5°C, $C = 0{\cdot}42$. Such a variation of conductance is consistent with the interpretation of Fig. 4, relating the gradient of core-to-subcutaneous temperature to ambient temperature.

A comparison of minimum and maintenance metabolism rates (Fig. 5) suggests that while the amount of energy needed to maintain T_B increases linearly with T_A below L.C.T, the additional amount of energy expended in voluntary activity by a mouse in a small chamber, with food *ad libitum*, remains constant. Since the two regressions are essentially parallel, that for average metabolism extrapolates to a temperature well above normal T_B at theoretical zero metabolism, and the active animal must have a variable conductance. Hart (1952) found that house mice in small metabolism chambers with food had a constant energy expenditure for voluntary activity at T_A 10–30°C.

Conductance and metabolism during cycles of torpor

While dead mice followed Newton's Law of Cooling, and had the same cooling constant from $T_B = 35$°C down to where $T_B \simeq T_A$ (Table 4), live mice entering or recovering from torpor went through several phases of temperature change with differing constants (Fig. 6).

An analysis of the simultaneous measurements of T_B and metabolism of three *P. longimembris* during cycles of torpor showed that during warming from a T_B of 10°C, the rate of metabolism was at or near the theoretical maximum dictated by T_B up to the point where $T_B = 18$°C; then metabolism progressively decreased. The theoretical maximum estimated by Tucker (1965b) for *P. californicus* was taken as the standard for *P. longimembris*, i.e. max ml $O_2 \cdot g^{-1}\ hr^{-1} = 0{\cdot}379T_B - 2{\cdot}77$. The rate of warming averaged 0·3°C/min, once T_B reached 25°C. This is only half the rate found by Bartholomew & Cade (1957) for the same species, and even less than the warming rate for *Sicista betulina* (Johansen & Krog, 1959) which went from T_B 6° to 35°C in 30–40 min.

During cooling the rate of metabolism of these three *P. longimembris* dropped rapidly, but did not approach the theoretical minimum until T_B was within 3°C or less of T_A. The theoretical minima, as determined during periods of maintained torpor at different ambient temperatures, were:

T_A 10–22°C, minimum ml $O_2 \cdot g^{-1}\ hr^{-1} = 0{\cdot}027T_A - 0{\cdot}177$;

T_A 22–32°C, minimum ml $O_2 \cdot g^{-1}\ hr^{-1} = 0{\cdot}048T_A - 0{\cdot}68$.

The metabolism of these *P. longimembris* was unlike that of the *P. californicus* studied by Tucker (1965b), in that the latter achieved a minimum rate near the start of entry into torpor by a "shutting off the thermostat" type of response. However, there are too few measurements to allow one to determine if there is a true species difference. Lindberg (1966) measured abdominal temperature of six *P. longimembris* during cycles of torpor repeated daily for 30 days. He found that the rate of temperature decline at the start of torpor can vary within the individual from day to day, from a relatively gradual decline to an abrupt decline suggesting a "turning off the thermostat". The rate of decline is undoubtedly influenced by the previous and simultaneous conditions imposed upon the pocket mouse. The effect of different conditions in the laboratory needs to be carefully assessed before it can be determined if "shutting off the thermostat" is or is not the probable response in nature.

Heat balances were estimated for two *P. longimembris* during entry and recovery from torpor. The following constants were used: heat content of body $= 0{\cdot}82$ cal.g^{-1} $(°C)^{-1}$; 1 ml O_2 consumed $\equiv 4{\cdot}7$ cal; Δ 1°C $T_B \equiv 0{\cdot}174$ ml O_2/g. The thermal conductance necessary to achieve the observed cooling or warming was estimated as

$$C\ [\text{ml } O_2.g^{-1}\,hr^{-1}\,(°C)^{-1}] = \frac{\text{Observed H.P.} + O_2 \text{ equivalent of } \Delta T_B}{T_B - T_A}.$$

Conductance for different points in the cooling–warming cycle are illustrated in Fig. 6. During cooling, conductance ranged from about 0·35 early in cooling to 0·5–1·0 late in cooling. In all cases estimated conductance was higher than the 0·28 ml.g^{-1} hr^{-1} $(°C)^{-1}$ for animals in a minimum maintenance state. The higher conductances are within the range of values estimated for active normothermic mice. In the first phase of warming conductance was also high, 0·6–2·0 but thereafter was usually less than 0·28 and averaged 0·23. As is logical, cooling animals somehow effect an increase in conductance, which facilitates heat loss; warming animals decrease conductance, which facilitates heat accumulation.

Physical insulation

Dead *P. longimembris* cooled at rates equivalent to an average conductance of 0·32 ml $O_2.g^{-1}\,hr^{-1}\,(°C)^{-1}$; estimated conductances ranged from 0·14–0·23 immediately after death to 0·39–0·41 when T_B was nearing T_A. The value, $C = 0{\cdot}14$ ml $O_2.g^{-1}\,hr^{-1}\,(°C)^{-1}$ [which is equivalent to 0·66 cal.g^{-1} hr^{-1} $(°C)^{-1}$ or 0·30 cal.cm^{-2} hr^{-1} $(°C)^{-1}$] may approximate the reciprocal of maximum physical insulation of the little pocket mouse. However, a variation of 0·23–0·40 could have been due simply to changing posture of the body, as has been found for torpid *P. californicus* (Tucker, 1965a). Although live *P. longimembris* had higher conductances when entering torpor (0·35–1·0) than dead mice, they cooled more slowly. This is a consequence of the heat production of the live animal, which must be dissipated along with the initial heat content of the tissues.

Freshly dead *P. longimembris* cooled more rapidly than the larger species, *P. formosus* and *P. californicus* (Table 4), as is expected from geometric relationships alone. The heat flux of these three species was in the same range as that measured for the Arctic rodents, *Clethrionomys rutilus* (14·3 g) and *Microtus economis* (20·4 g) by Morrison & Tietz (1957). This suggests that the pocket mice of temperate and warm deserts might have a physical insulation equal to that of Arctic rodents. However, comparison is confounded by the fact that the measurements on the Arctic species were made on rewarmed carcasses that had been frozen and which did not have normal pelage.

Body temperature, metabolic rate and conductance

In an analysis of body temperatures of mammals, McNab (1966) proposes that T_B is a function of metabolic rate and conductance, and that it can be predicted as

$$T_B\ (°C) = 4{\cdot}7\ (M/C) + 32{\cdot}2,$$

where M and C are the relative metabolic rate and relative conductance respectively.

For the present *P. longimembris*, basal metabolism is approximated by the value 1·07 ml $O_2 . g^{-1}\ hr^{-1}$; this is 0·53 of the value predicted by the general relationship developed by Kleiber (1961): ml $O_2 . g^{-1}\ hr^{-1} = 3{\cdot}5\ wt^{-0{\cdot}25}$. The conductance of *P. longimembris* in a minimum maintenance state, 0·28 ml $O_2 . g^{-1}\ hr^{-1}\ (°C)^{-1}$, is 0·78 of that predicted by the equation of Morrison & Ryser (1951): $C = 1{\cdot}02\ wt^{-0{\cdot}50}$. Following the procedure of McNab (1966), T_B predicted by his formula is then 35·4°C, i.e. a reduction below the mammalian "standard" of 37°C is expected because of the lower metabolic rate, which is only partly compensated by a lower conductance (or higher insulation) than expected. The observed body temperatures do show a reduction below 37°C; the observed abdominal temperatures are somewhat lower than predicted T_B, 35·4°C, while the colonic temperatures are in general somewhat higher than predicted.

In view of the many factors that can influence absolute body temperature of a small mammal, as shown by the detailed study of one species by Morrison & Ryser (1959), it is not possible to be more conclusive about the temperature relationships of the little pocket mouse without much more information.

SUMMARY

Abdominal, colonic and subcutaneous body temperatures (T_B) were measured in mice exposed to various ambient temperatures (T_A). Thermal conductance, physical insulation and metabolic rate were measured in normothermic, torpid and dead animals.

P. longimembris acclimated to 22–24°C are good thermoregulators when not torpid. A variation of 1–4·5°C in colonic temperature is consistent with their small adult size, 8–11 g. Mean abdominal temperature did not vary significantly over the range of T_A 2–34°C. The mice showed good resistance to change in T_B when exposed to cold, heat and continuously changing T_A.

The core-to-subcutaneous temperature gradient varied with T_A in a definite pattern that suggests four zones of temperature regulation. There was a high correlation of body temperature with metabolic rate.

P. longimembris simulated a following of Newton's Law of Cooling when metabolizing at a minimum maintenance rate, i.e. their conductance was constant [0·277 ml $O_2 \cdot g^{-1}$ hr^{-1} $(°C)^{-1}$] at T_A below 33°C (ml $O_2 \cdot g^{-1}$ $hr^{-1} = 10{\cdot}91 - 0{\cdot}277$ T_A). Active mice, however, had a variable conductance which decreased with T_A.

Mice entering and arousing from torpor went through several phases of temperature change. During cooling, conductance was greater than normal and during warming it was less. Warming constants averaged, $K = 0{\cdot}040$ and cooling constants, $K = 0{\cdot}032$.

Resting metabolism was 53 per cent and conductance was 78 per cent of certain mammalian standards; this is consistent with a T_B reduced below 37°C.

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TORPOR IN THE LITTLE POCKET MOUSE, PEROGNATHUS LONGIMEMBRIS

Page Hayden, R. G. Lindberg, and K. Grubel

The daily and seasonal drop in the body temperature of warm-blooded animals has been an area of increased documentation and quantitative measurement in recent years. Several species of birds (1,2,3) and many orders of mammals contain members that undergo hibernation or related conditions, most easily recognized by a decrease in the body temperature. The order Rodentia contains by far the most number of species that undergo this condition (4). Torpor has been intensively documented in several species of the family Heteromyidae (5,6,7,8,9,10,11). The thermoregulative behavior of many species of this group, however, is unknown or known only through very scanty and casual observations or adduced through circumstantial evidence. Our work with the little pocket mouse as a tool in space experimentation has yielded data pertinent to the hibernative nature of this species.

I. Annual Weight Variation

A group of 23 animals was periodically weighed at approximately monthly intervals from April 1966 to April 1967. The group was composed of 11 adult field trapped animals at least a year old and 12 were animals of the same age that had been bred and reared in the laboratory.

The animals were housed in gallon glass bottles with a sand substrate and supplied with a mixed seed diet. The animals were maintained in a windowless room with a LD 12:12 (0600-1800 hours light) photoperiod, using the existing overhead flourescent fixtures as light source. Humidity was relatively constant but extremes during the year were 40-75% RH. Temperature was controlled and remained near 22°C except for several short but inevitable increases due to mechanical and/or electrical failures.

Four of the field trapped animals died during the year. Figure 1 is a presentation of the weight variation throughout the year. It would appear that the average weight does increase slightly during the fall-winter portion of the year and decreases late winter-early spring. The observed

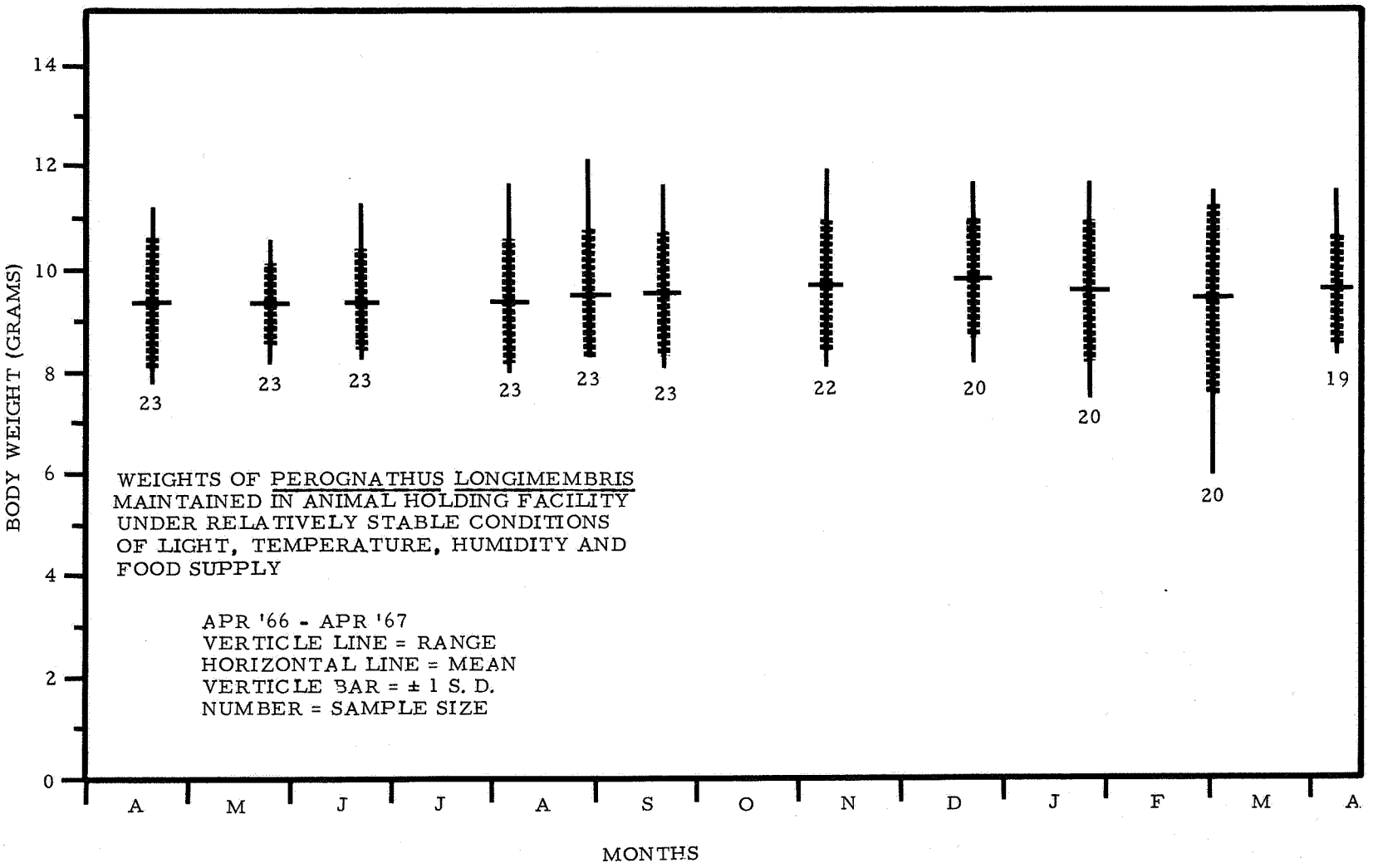


Figure 1 Annual weight variation of P. longimembris maintained under constant conditions of light and temperature.

average fluctuation was 0.5 gm (9.3-9.8 gm). This fluctuation seems to be less than expected if the little pocket mouse does anticipate a period of the year when periods of extended torpor or hibernation occur. Hock (12) documented a 50% increase in body weight of Arctic ground squirrels from May to October. Pengelly and Fisher (13,14) found that in a constant photoperiod and ambient temperature, golden-mantled ground squirrels maintained their annual weight cycle for up to 2 years. However, all hibernators do not manifest the prehibernation response as a weight change by an increase of adipose tissue. Lyman (15) has demonstrated that the golden hamster actually stores food and that the "food horde" is an essential part of the prehibernation behavior.

It has been observed in our laboratory that pocket mice which show a predilection for periodic torpor also manifest a high degree of "orderliness" in their living chambers. The various types of seeds are sorted, as to kind, in individual piles and in some cases the fecal pellets and seed chaff are sorted. This kind of behavior may be equivalent to the hording behavior of hamsters.

II. Annual Spontaneous Daily Torpor at Low Constant Ambient Temperature

A group of 31 mixed-sex little pocket mice were maintained in a relatively constant temperature regimen of 10 $\pm$ 0.5°C for a year (Nov 1962 - Dec 1963) and periodically observed for torpor. The animals were housed and fed the same as stated above. No attempt was made to control humidity which ranged from 60-90% RH. The light regimen was not rigid, a fluorescent light was on from 0800-1700 hours every day (weekends excepted), but laboratory light and diffused natural light were able to enter the chamber through a small window.

The animals were observed usually once daily in the morning. Animals showing arhythmic breathing, periodic apnea and sluggish response to stimuli were noted as being torpid. Several deep body rectal temperatures of these "observed torpid" animals were taken with a thermistor probe and were found to be 1-1.5°C above the ambient temperature of the cold chamber.

On several occasions, animals that were observed to be torpid in the morning were observed at hourly intervals throughout the day. The general pattern was that these animals remained torpid until late afternoon. Only rarely did an animal go torpid late in the day. This observation has been thoroughly documented (10).

Figure 2 summarizes the frequency of observed torpor in a group of 31 P. longimembris. Over seventy percent of the animals were observed torpid from 11-50 times, with only one animal each being observed torpid less than 10 times or greater than 111 times.

Figure 3 is an expression of observed torpid periods per animal-observations with relation to time of year. It will be noted that the fall-winter months have an incidence of torpor that is from 2-6 times higher than the rest of the year. It is important to note, however, that some individuals demonstrate daily torpor throughout the year. These individuals were specifically selected for subsequent studies of circadian periodicity.

Under the conditions of the experiment (constant temperature, excess food, relatively constant photoperiod), the little pocket mouse (P. longimembris) expresses an annual variation of spontaneous periods of torpor with the highest incidence in the winter. This observation is in agreement with field data that document the disappearance of this species during the winter months (16).

Studies of diurnal torpidity in pocket mice (5,7) indicate that these periods of torpor may be elicited with a reduced food supply, but the present data document a seasonal response that is not directly associated with food scarcity. Further, data from other experiments at this laboratory show an increased tendency towards daily incidence of torpor when the animal voluntarily restricts its activity to an insulated nest chamber in the presence of a low ambient temperature (10°C).

The greatest occurrence of torpor was observed in the laboratory during that portion of the year when environmental stresses (i.e., extreme

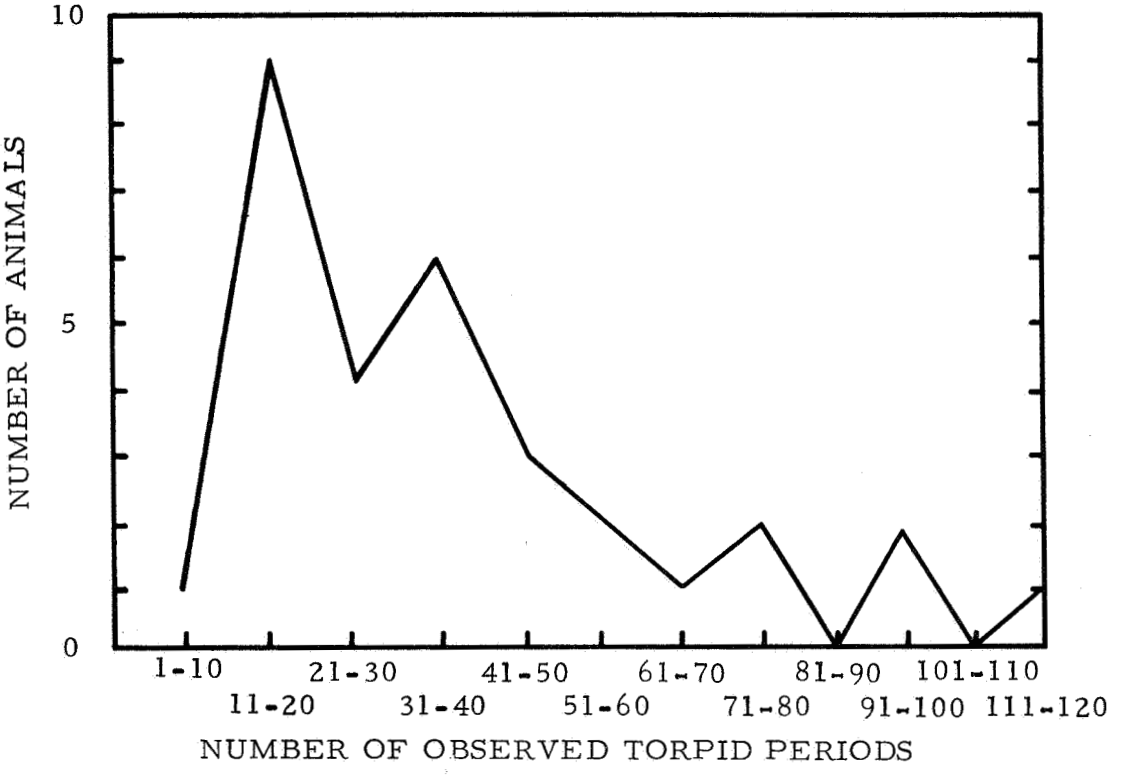


Figure 2 Frequency of observed torpor in a group of 31 Perognathus longimembris.

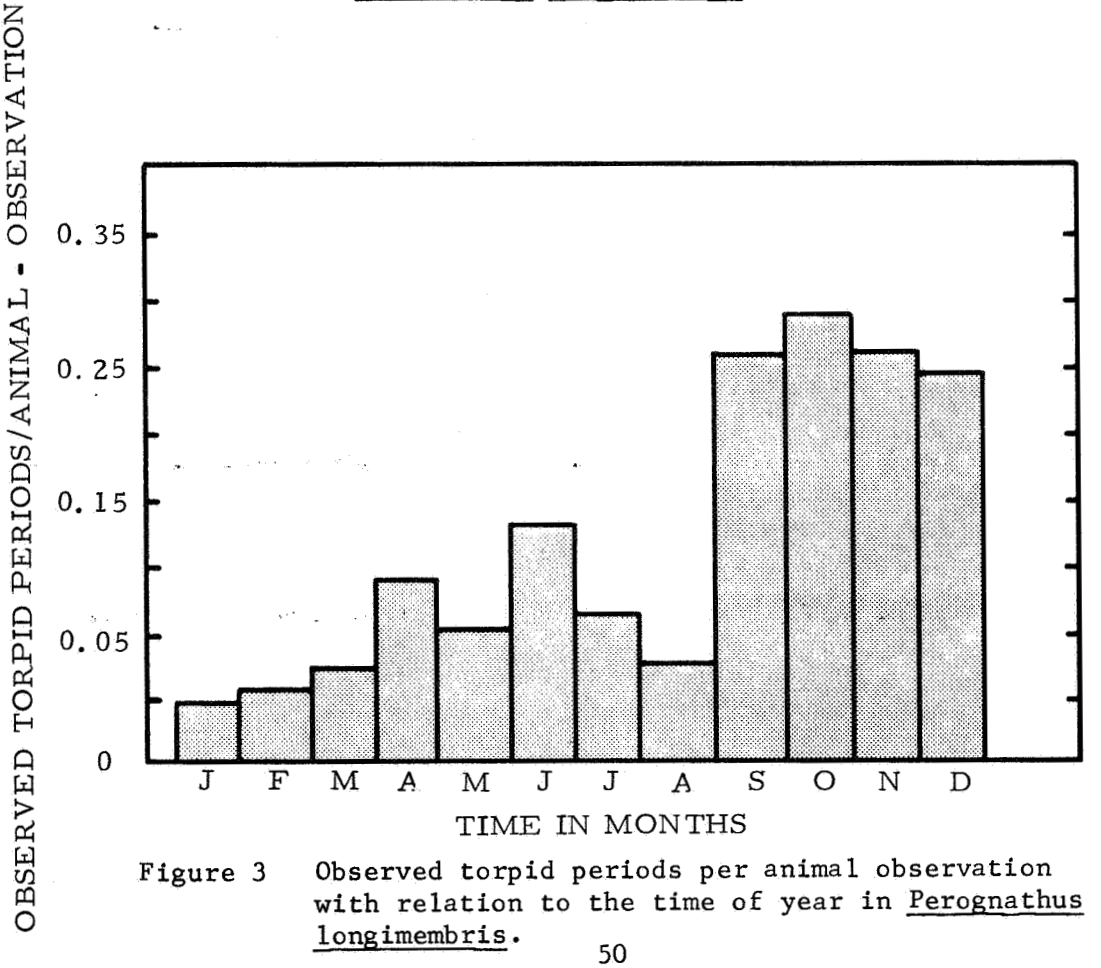


Figure 3 Observed torpid periods per animal observation with relation to the time of year in Perognathus longimembris.

ambient temperature and low food supply) are probably the greatest in a natural condition. It would appear that this response is a manifestation of some "biological clock" that cues the species to the annual cycle of weather and food availability.

In summary, it appears that there is an annual cycle of a physiological state in which torpor is a common feature. This state is marked by an increase in body weight with an associated behavioral trait of food sorting and storing. The disappearance of the little pocket mouse from above ground activity during the winter months is replaced with completely troglodytic existence in which periods of torpor are alternated with burrow rearrangement and manipulation of food stores.

III. Sequence and Duration of Torpor Under Optimum Conditions for Hibernation at Two Temperature Regimes

The study of hibernation has both a physiological and a temporal facet. The yearly life cycle of a "typical" hibernator involves three major segments: (1) preparation for hibernation, (2) hibernation, and (3) arousal and reproduction. During the hibernation period, the typical hypothermic state is broken periodically, and the body temperature is raised to normal. These arousals occur in a rhythmic manner every few days or weeks, depending on the species and environmental conditions. These rhythmic arousals with immediate re-entry into the hibernating state are different phenomena from the overall annual cycle of hibernation vs. normal activity.

In the laboratory the little pocket mouse (Perognathus longimembris) undergoes cyclic periods of depressed metabolism of a circadian nature (11) and also an annual cycle of increased expression of this rhythm in any given number of animals. In nature, these animals disappear from above ground activities from October until late January and have been thought to hibernate. It was the purpose of this phase of the study to document the metabolic rhythm of torpor and activity under conditions closely resembling the environment of a burrow dwelling animal in nature. The

most pertinent factors were assumed to be: time of year, surplus of food, constant dark, isolation from noise and low temperature.

The experimental plan was to monitor the body temperature of a group of 7 pocket mice maintained in constant dark at three temperature levels (21°C, 10°C and 5°C) for three weeks at each level. Temperature monitoring telemeters were surgically implanted within the peritoneal cavity. The animals were allowed to recuperate from the implantation for 8 days, by which time the incision had healed, and the animal appeared to be normal. They were then held for 7 days at 22°C with a photoperiod of LD 12:12 (0600-1800 hours). The animals were maintained under constant darkness (24D) for the remainder of the experiment. Animals used in this experiment were selected because observations indicated a tendency to periodic torpor.

All experimental chambers were provided with a half-inch substrate of desert sand, 35 gm of sunflower seeds and a handful of dry grass for bedding. The individual animal chambers were placed in a light-proof constant temperature room and were semi-isolated from each other by open front boxes constructed of acoustical tile. After 29 days of continuous isolation, the food supply was replenished and a small amount of dry grass added. Entry into the constant temperature room was made with the aid of a ruby red light (photographic safe light) and care was taken to keep direct illumination of animals at a minimum. Previous experience has indicated that Perognathus cannot entrain to this portion of the spectrum or intensity of illumination. Total entry time was about 7 min.

The animals were kept at 5°C longer than the allotted time period. However, because of multiple failures of a portion of the data recording system, unreliable data were obtained during the 5°C portion of the experiment. It is significant, however, that the animals at 5°C did undergo periodic torpor and survived the 36-day exposure.

Results. Two animals were found dead when the chamber was entered on the 29th day. One animal had escaped from its monitoring chamber and presumably had starved. The other animal was dead in its monitoring chamber, with no obvious cause of death.

a. Food Consumption: At the termination of the experiment, the remaining five mice appeared to be in excellent health, even though a general weight loss was noted. All animals lost weight, with an average of 1.3 gm (range 0.8 - 1.8 gm). Food consumption averaged 21.1 gm (range 14.8 - 23.8 gm) with three of the five consuming approximately 23.7 gm of sunflower seeds during the 86 days of the total experiment. These range values of 0.17 - 0.27 gm food/day are 1/3 to 1/5 of the amount required by the animals to maintain normal body temperature.

b. Sequence and duration of torpor: For the purpose of this paper torpor is defined as the decline in body temperature to within one or two degrees above the ambient temperature, 21°C and 10°C respectively. The onset and arousal from torpor during a 24-hr period are plotted in Figure 4. Periods of torpor were observed initially during the light portion of the regimen and were generally evident by the fourth or fifth day. After constant darkness (24D) was initiated on the 7th day, all animals exhibited daily periods of torpor. These torpor periods lasted from 4 hr to the entire 24-hr period. In some cases, multiple periods of torpor were observed (Fig. 6).

The duration of sequential periods of torpor is plotted in Figures 9 and 10. It appears that the periods of torpor progressively lengthen until about the 8th or 9th day; however, one animal reached a plateau after four periods of torpor (L-1520, Fig. 9). The maximum time spent in continuous torpor was 4200 min (70 hr) at an ambient temperature of 10°C. One animal exhibited five sequential periods of torpor of over 3700 min (61 hr) each. At 21°C ambient, 69% of the torpor periods were from 200 to 800 min (3-13 hr), with 30% being from 400 to 600 min (6.6-10 hr), and 9% were from 1800 to 2200 min (30-36 hr). At 10°C ambient, 24% of torpor periods were from 200 to 800 min, 32% were greater than 2200 min (equals maximum time in torpor at 21°C) and 11% were from 3600-4200 min (60-70 hr).

A decrease in ambient temperature and concomittant decrease in the ultimate body temperature during torpor does not initially increase the length of torpor exhibited by the animal. Four of the five animals

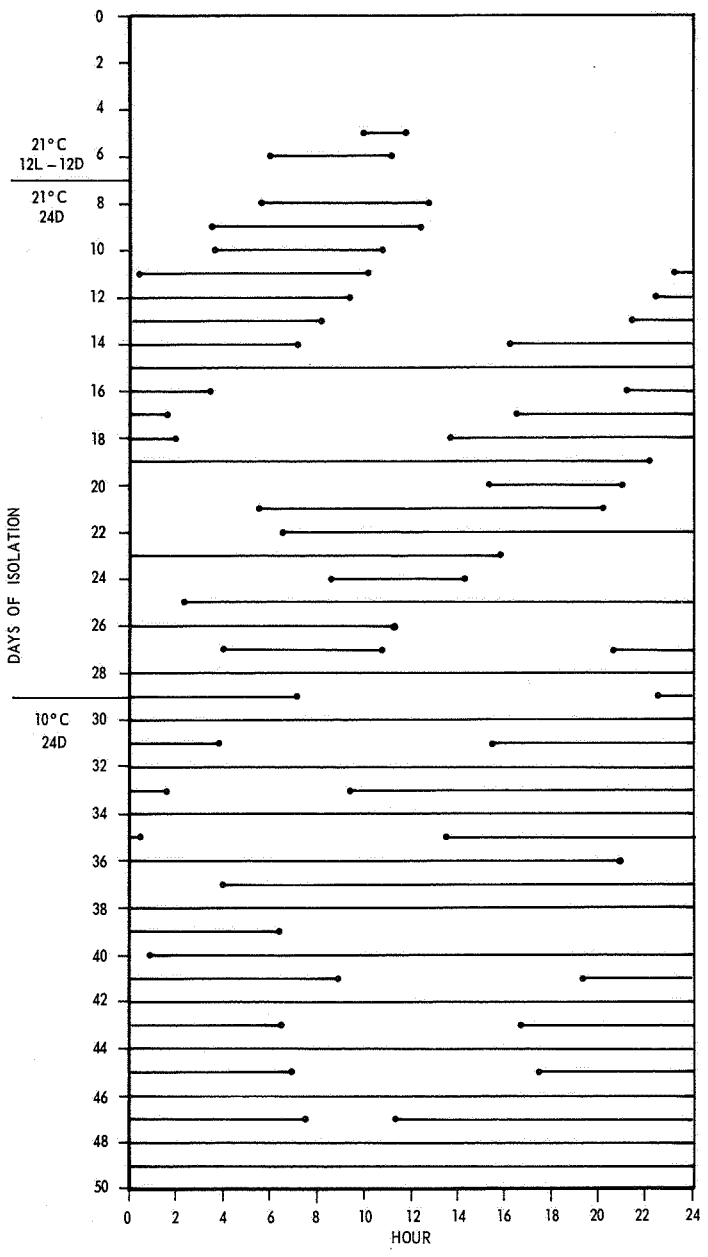


Figure 4 Animal No. L-1520♀

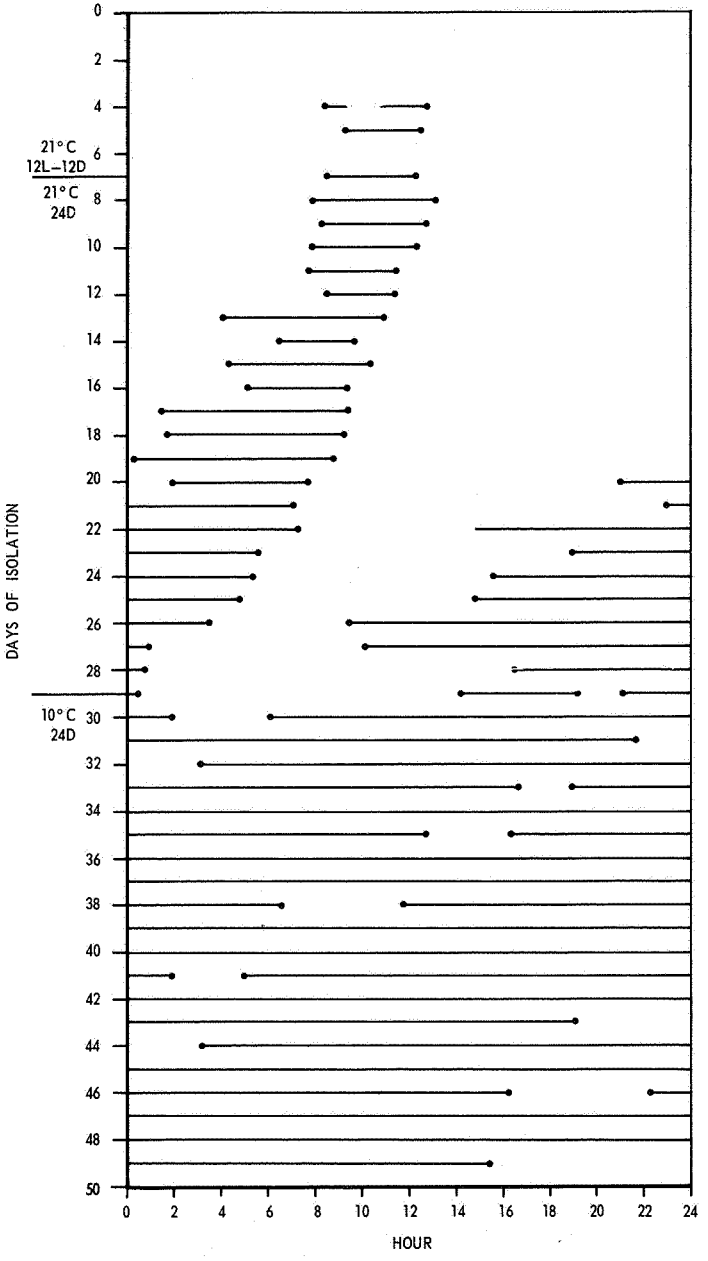


Figure 5 Animal No. L-1532♂

Torpor periods of P. longimembris maintained with excess food, constant dark, in isolation, during two temperature regimens. The dark bar represents the period of the day in which body temperature was near ambient temperature, and the absence of the bar represents normal temperature.

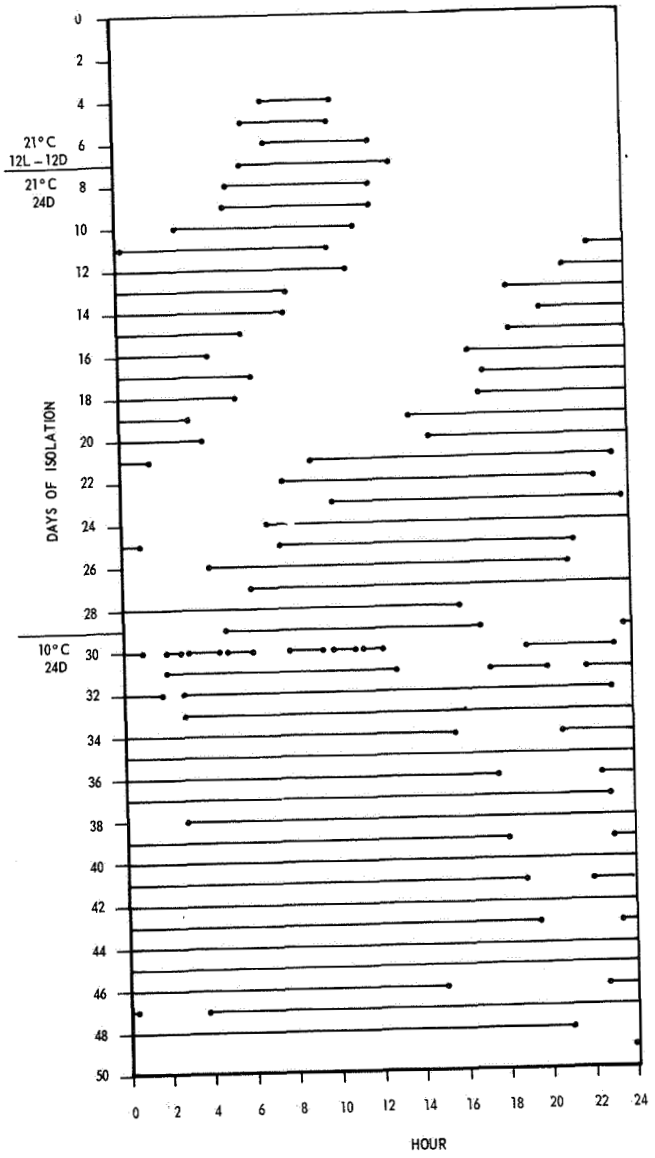


Figure 6 Animal No. L-1579♀

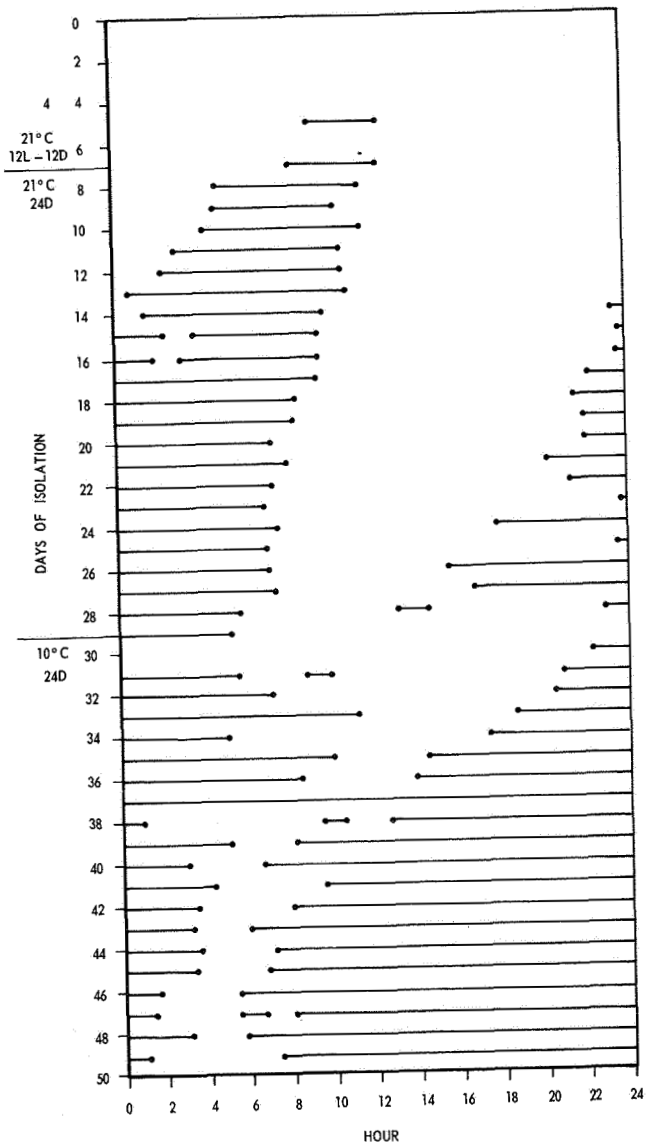


Figure 7 Animal No. L-1822♀

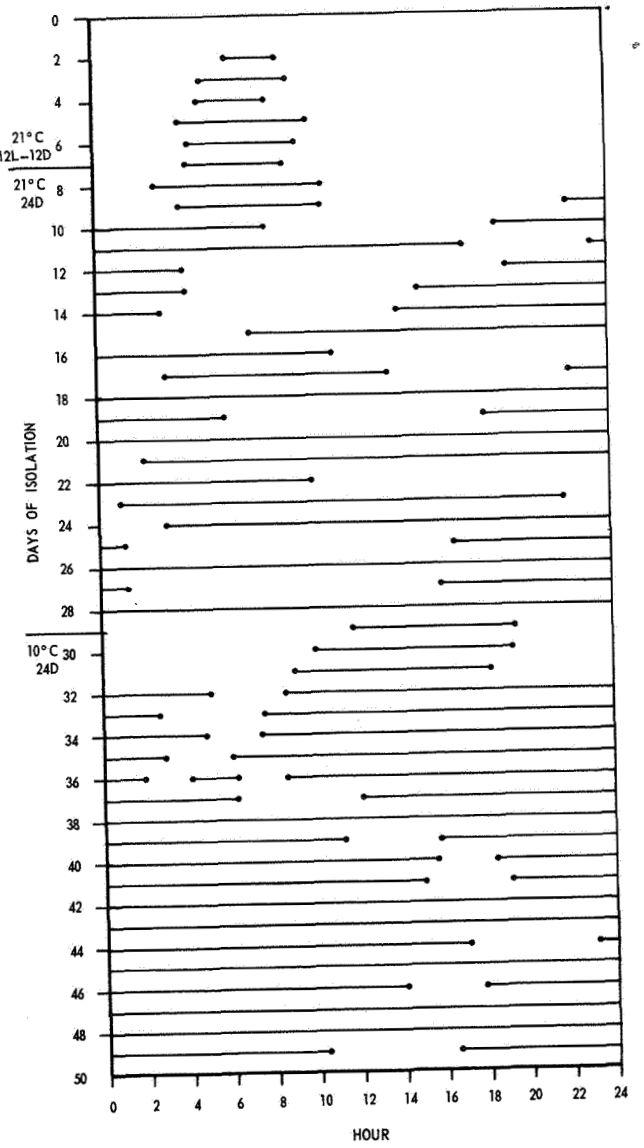


Figure 8 Animal No. L-1425♀

Torpor periods of P. longimembris maintained with excess food, constant dark, in isolation, during two temperature regimens. The dark bar represents the period of the day in which body temperature was near ambient temperature, and the absence of the bar represents normal temperature.

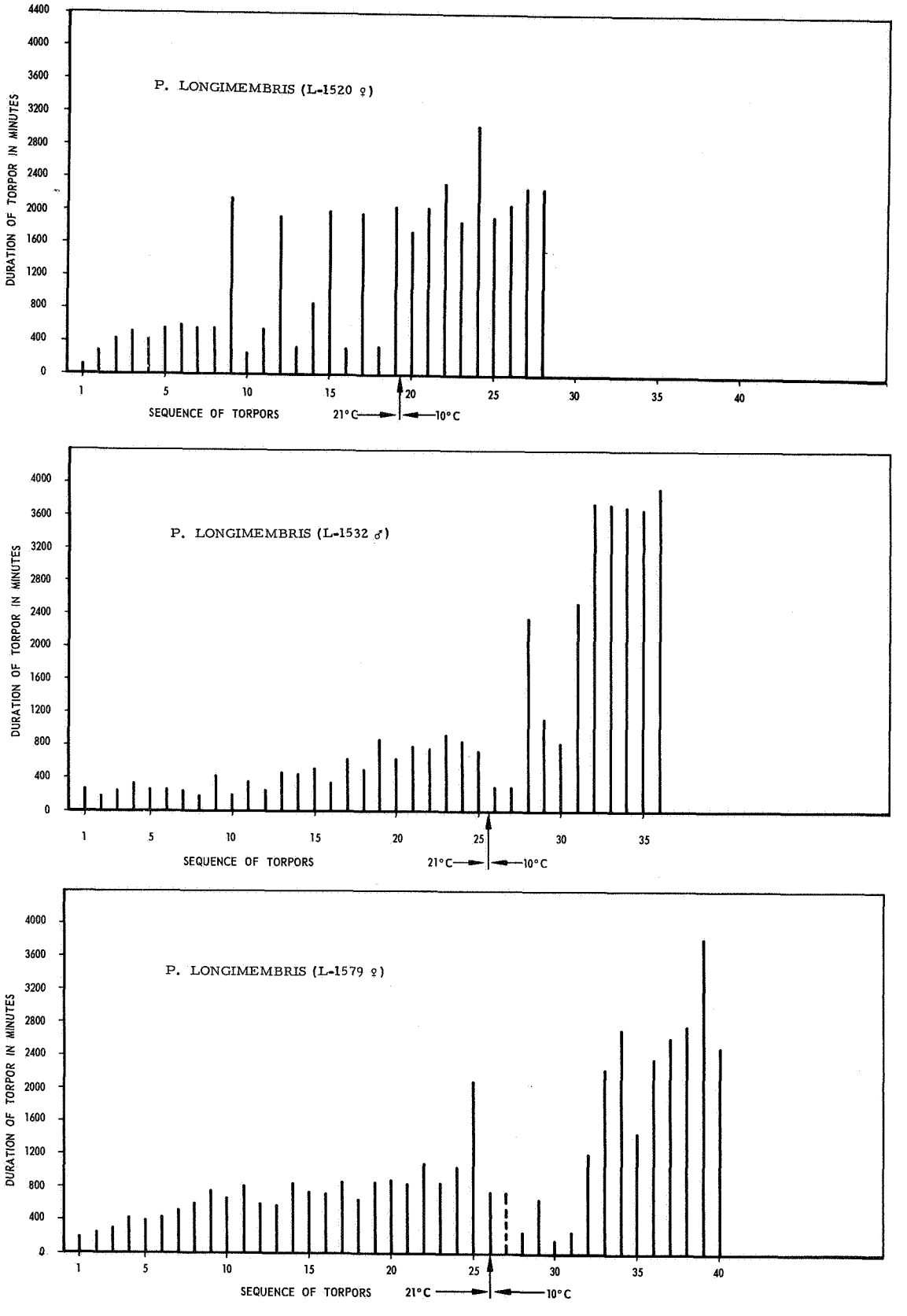


Figure 9 Duration of individual torpor periods of P. longimembris in two temperature regimens.

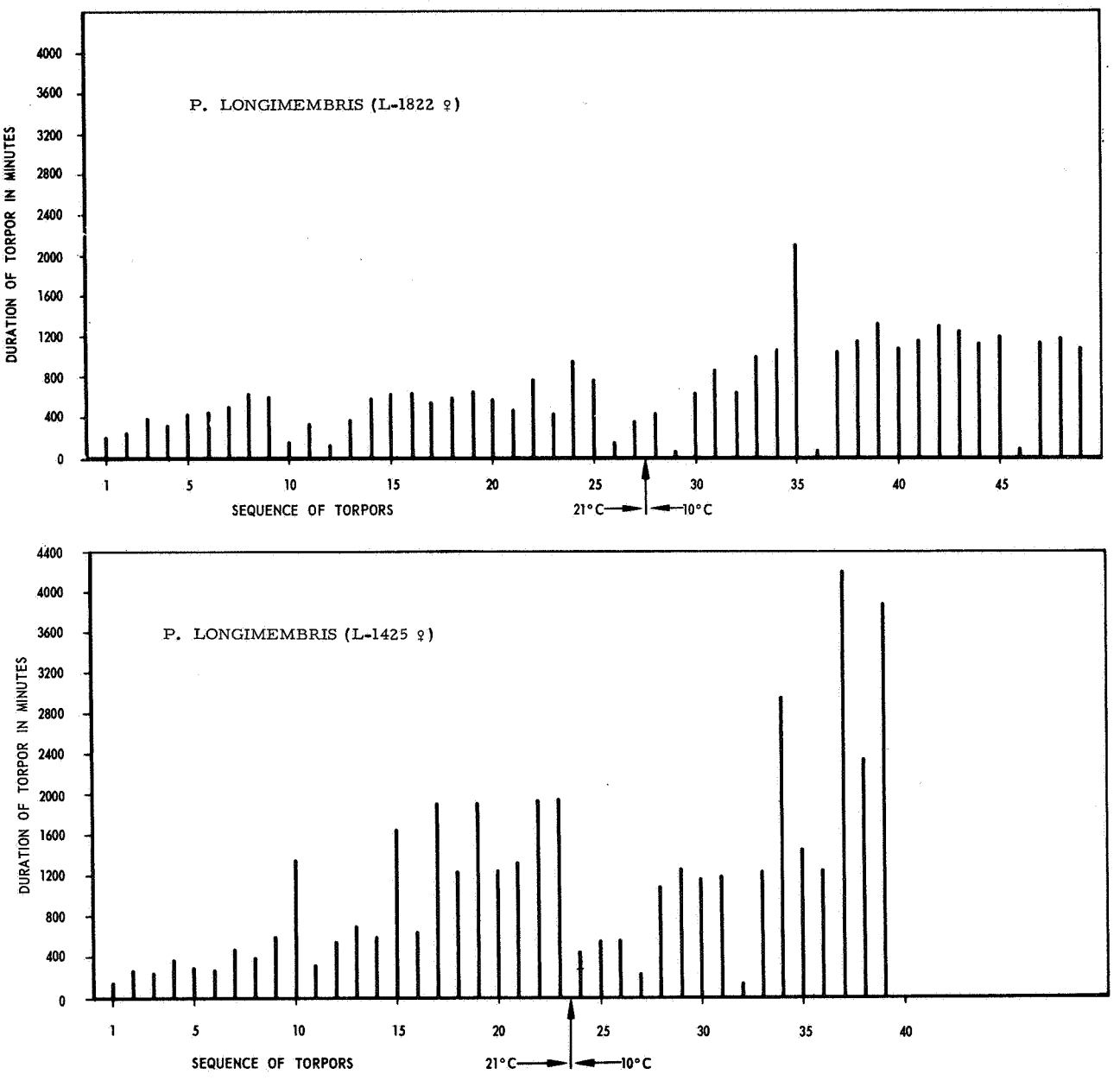


Figure 10 Duration of individual torpor periods of P. longimembris in two temperature regimens.

reacted to the decrease in ambient temperature (from 21°C to 10°C) with a decrease in duration of torpor (compared to the duration of torpor at 21°C). This decrease in duration of torpor is evident for 3-5 torpor periods after the temperature change. Two animals (Fig. 9, L-1520 and Fig. 10, L-1425) were unusual in that they underwent two lengths of torpor periods, approximately 600 min and 1600-2000 min, during the 21°C ambient temperature. The long periods were generally separated by one or two short periods. One of these animals (L-1520) was unique in the relatively long torpor period that was maintained during both the 21°C and 10°C temperature regimens. This long period, however, was evident less frequently at 21°C than at 10°C. At the latter temperature, it was the daily mode. Another animal (L-1822, Fig. 10) maintained a relatively constant short duration of torpor in both 21°C and 10°C. In general, as was expected, the duration of torpor was prolonged in 10°C as compared to 21°C.

Discussion. This study again emphasizes the metabolic lability of P. longimembris (11). Prolonged periods of natural torpor were evidenced when the animal was not stressed, i.e., food was provided in excess at all times, natural substrate and bedding material available, a reasonable degree of isolation, temperature relatively high and normal gaseous atmosphere. The metabolic lability probably reflects the seasonal cycle of hibernative behavior of this species, although it appears in some animals throughout the year. The experiment was carried out during that portion of the year when mice in the field are absent from activity above ground (i.e., cannot be trapped) and presumably are undergoing periods of reduced metabolism (16).

The duration of individual torpor periods (hibernation) was longer in this experiment than has been observed in previous experiments. The experimental conditions of isolation from periodic noise, constant dark, surplus food, sufficient time to acclimate and time of year probably contributed to the maximum time spent in torpor. The observed maximum of 70 hr may represent the limits of natural continuous hypothermia that this small mammal can undergo at an ambient temperature of 10°C. It was

unfortunate that very little data were derived from the 5°C portion of the experiment, but there was a stong indication that duration of torpor was increased, and the circadian component of arousal was still in operation at this temperature.

The weight loss of the animals was greater than expected but did not appear to affect the general well being of the animals. At the termination of the experiment, all had sleek coats, bright eyes, showed normal activity and are still living. It is possible that there was preferential use of body fat as an energy source, even though food was available at all times. It was not possible to tell if the weight loss was gradual or if it was lost incrementally within the three temperature regimens.

When a typical hibernator (ground squirrel) enters hibernation, it undergoes periods of body temperature depression know as "test drops". These drops occur over a period of a few days to weeks and are characterized by each drop being slightly lower in temperature than the previous one. It is thought that these drops are a kind of acclimation of metabolic processes associated with hibernation and arousal (17).

The pocket mice used in this experiment probably had undergone test drops necessary to go torpid in an ambient temperature of 21°C. It is interesting to note, however, that in most cases there was a sequential increase in duration of torpor at the beginning of the experimental period, and this may represent a kind of temporal "checkout" of prolonged hypothermic metabolism and functioning of arousal processes with time.

The change from 21°C to 10°C generally did not immediately increase the duration of torpor, but decreased the time in torpor for several days. The first torpid period of one animal (Fig. 9, L-1579) was characterized by a series of entries and arousals from torpor as if the animal's temperature dropping below a critical level immediately aroused the animal to normal body temperature. It is possible that these were "test-drops" to acclimate the animal to the new low temperature.

It has been suggested in the literature (18) that duration of hibernation is a direct function of body temperature during hibernation. The

arousal from hibernation might be initiated by a build up of specific metabolites (13) as the rate of metabolism is governed by the temperature of the tissues. If this is true in pocket mice, it is difficult to explain how the duration of torpor could be increased from 4 to 6 times in some animals, when the temperature was decreased from 21°C to 10°C, and yet in other animals the duration increased only two time. One animal (Fig. 9, L-1520) had a duration of torpor at 21°C ambient that was more typical of that shown at 10°C ambient. This would indicate that the animal could either limit the production of the specific metabolites involved with arousal or could regulate the threshold of the metabolite sensor(s). The duration of hypometabolism does not seem to be a direct function of when the animal arouses, as evidenced, for example, by animal L-1532, Fig. 5, days 30-49. It apparently made little difference if the animal was torpid for one day or two days, for arousal occurred at the appropriate time with regard to the previous arousal. The presence of a circadian rhythm in this species has been documented (19), and the present data indicate that the rhythm functions during extended periods of torpor with body temperatures of 11°C.

IV. Changes in Blood Flow, Brown Fat Accumulation, and Thyroid Activity During Various Hibernative Conditions

This phase of documentation of torpor in the little pocket mouse was undertaken to assay certain physiological parameters associated with the torpidity pattern of the little pocket mouse and whether its torpidity is comparable to the hibernation phenomenon of the "classical" hibernators. During the first part of arousal, the true hibernators maintain a differential vasoconstriction in order to enhance the rapid rewarming of the brain and the vital organs in the thorax and their brown fat performs a thermogenic role during arousal. In most hibernators, the thyroid glands are involuted during hibernation season.

Clearance rate of radioactive iodine from peripheral tissue, gross dissection of brown fat, and histological observation of thyroid glands were undertaken.

The peripheral vasoconstriction response was evaluated by immobilizing an animal over a small collimation aperture in a shielded scintillation probe. TwoμCi of I^{131} in 0.2-0.5 ml of 0.9% NaCl solution was injected into the musculature of the hind leg directly above the hole in the probe shield. The movement of radioactive material away from the sensing area of the probe (hence lower radioactivity readings) was used as a measure of the tissue vascular response.

Of the 9 control mice kept at normal room temperature, no peripheral vasoconstriction was observed. There was a steady reduction of decline of radioactive material with time. In October (fall of the year sample), 6 mice were maintained at 10°C and torpid animals were tested for vascular response during arousal. In this group there was a definite vasoconstriction in the peripheries, with the average time being 38.3 min (Fig. 11). The fastest arousing mouse released the peripheral blood flow 15 min after injection of isotope as opposed to the longest arousing mouse which maintained vasoconstriction for 65 min.

A group of 9 animals was maintained at 10°C in February (late winter sample) but only one animal entered torpor. This group was then deprived of food in order to induce torpor. Of this group, only one mouse aroused and regained normal body temperature with a vasoconstriction time of 10 min. It rewarmed at about the same rate of the slowest arousing mouse in the October sample. The average constriction time for this group was 11.2 min.

The brown fat and thyroid studies were based on a group of 20 animals. One group of 10 animals was maintained at normal room temperature; the others were maintained at 10°C for 3-6 days before sacrifice. The animals were sacrificed with ether, thyroids removed, and all brown fat excised and weighed immediately. The thyroids were fixed, mounted in paraffin, sectioned at 6μ, and stained with hematoxylin.

The body weights, brown fat weights and the percent of brown fats relative to body weights are shown in Figure 12. As is seen in this figure, the differences in the body weights and the absolute brown fat weights are

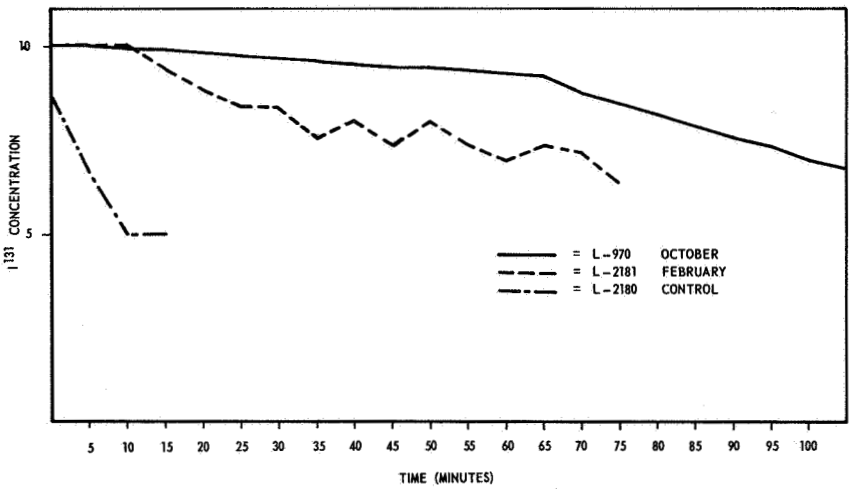


Figure 11 Vasoconstriction in P. longimembris as demonstrated by clearance rate of I^{131} from hind limb muscles.

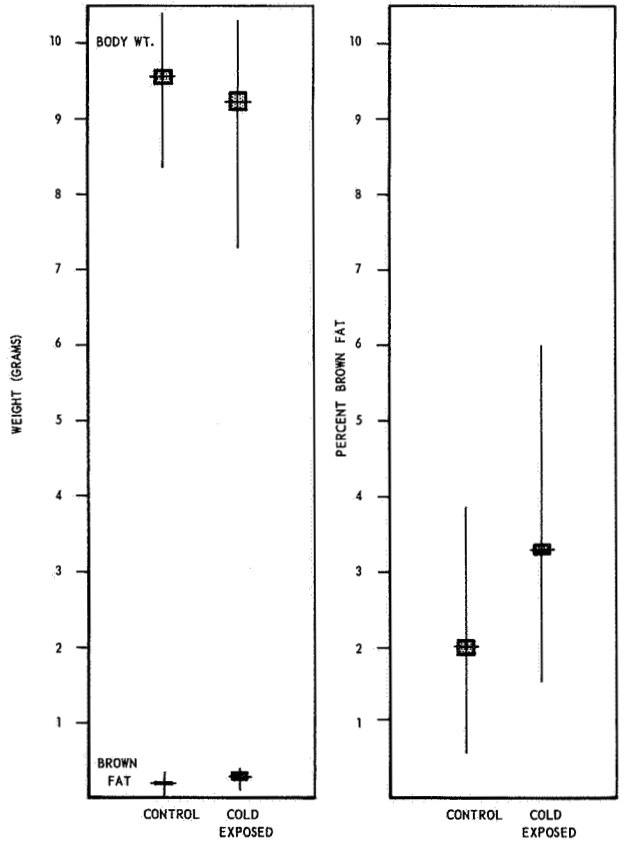


Figure 12 Proportion of brown fat to body weight in P. longimembris (10 mice/group). Verticle line equals range, horizontal line equals mean, and shaded area equals plus and minus one standard error.

not significant. But the relative amount of brown fat is significantly larger in the cold exposed mice. The amount of brown fat in the control mice was 2.0 ± 0.09% and in the group of cold exposed mice 3.3 ± 0.04% of body weight.

The thyroid glands appear to be different in the two groups of mice. In the control group, the follicles are large (59.5 ± 11.7 μ dia) and filled with colloid. The epithelial cells are squamous, with an average thickness of 4.5 ± 0.8 μ. The thyroid gland of this group may be described as involuted. However, the thyroids of the cold exposed mice at the same time of year appeared very active. The central portion of the gland was not organized into follicles, cells were crowded with little or no colloid present. Those follicles present were smaller (38.9 ± 6.1 μ dia), with cuboidal or columnar epithelial cells averaging 9.9 ± 1.7 μ.

The data obtained during these investigations suggest (a) that P. longimembris does behave as a "classical" hibernator, and (b) that there may be a seasonal effect in the torpor pattern of P. longimembris. The arousal pattern and simultaneous peripheral vasoconstriction seen in these mice in October confirm the hypothesis that the organism would have the same types of physiological mechanisms as has been observed by other investigators (20,21) in the true hibernators. The fact that they seem to be (a) less apt to go into torpor, (b) have impaired arousal, if at all, and (c) maintain their vasoconstriction for a relatively short time when trying to arouse in early February, is possibly due to a seasonal effect. Bartholomew et al (5) state that the potentiality for hibernation (or estivation) in P. longimembris presumably exists throughout the year, and that there is no difference in arousals during the year. His experiments were conducted between June and September and the animals aroused at room temperature, whereas our arousal experiments were conducted in an ambient temperature of +10°C. These differences in the experiments would account for the different results.

Several authors have stated that the thyroid glands are involuted during hibernation (22,23). The appearance of the thyroid glands of the

control group in late January seem to agree with this. The thyroid glands of the cold exposed mice appeared more active. However, there is disagreement among various authors as to the state of the thyroid glands during hibernation, and Kayser (22) concludes: "Hibernation is usually accompanied by an underfunctioning of the thyroid, but small sized hibernators may hibernate even though their thyroids show the morphological signs of active glands and though their blood contains active forms of iodine elaborated by the thyroid."

The importance of brown fat to hibernators during their arousal has been well documented (24,25). Our data on the amount of brown fat in P. longimembris show that these mice have a relatively large amount of brown fat and that their brown fat readily responds to cold stimulus.

V. Glucose Metabolism in P. longimembris During Arousal from Torpor

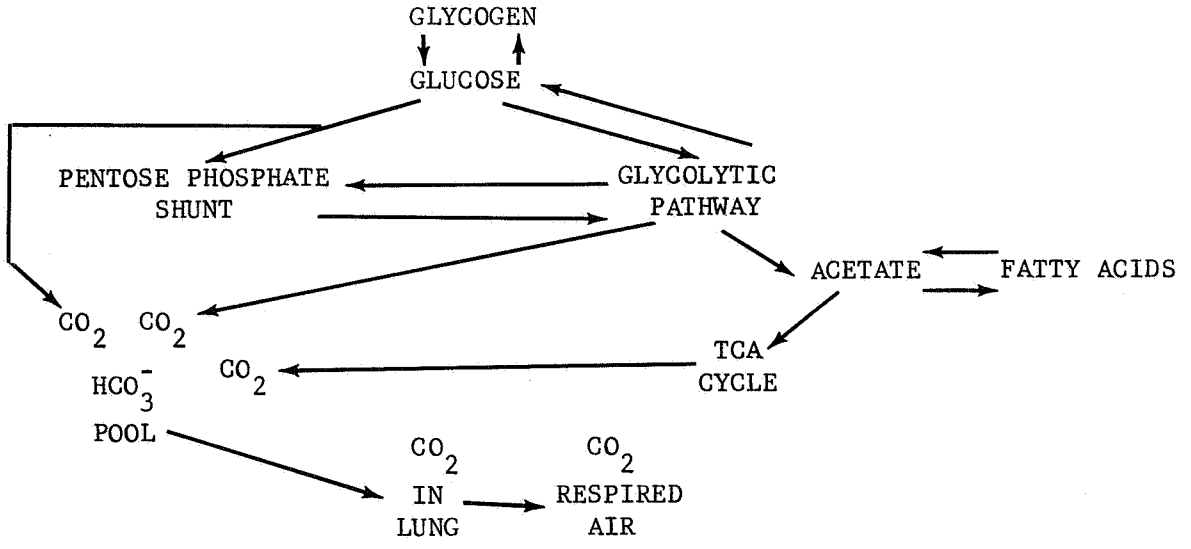
The analysis of the dynamic processes of metabolism of an intact animal have been greatly advanced through the use of intermediary products of metabolism tagged with carbon-14. The injection of radioisotopically labelled compounds into various metabolic schemes and tracing of the radioactive molecules has given much information regarding pathways, pool sizes and reaction rates.

Radiorespirometry is a technique in which labelled compounds of intermediary metabolism are given to an organism and the evolution of labelled carbon in carbon dioxide is monitored. Most carbon molecules eventually are released from living bodies as carbon dioxide.

The intermediary metabolism of glucose in a mammalian system involves the glycolytic (Embden-Meyerhof) pathway and tricarboxylic acid (Krebs) cycle. These pathways present not only the sources of energy for the ultimate phosphorylation of ATP but are a common center for other areas of metabolism. Another pathway, the so called pentose phosphate (hexose monophosphate) shunt has been identified in glucose metabolism. The operation of this latter pathway has been studied through the differences in the rate of liberation of specific carbon atoms in the glucose molecule (26).

In the glycolytic route, carbons 1 and 6 of glucose are both converted to the methyl carbon of pyruvic acid while in the pentose phosphate pathway carbons 1 and 6 are handled differently. Most investigations have concluded that the pentose phosphate pathway appears to occupy a subsidiary place in the total glucose metabolism.

The following scheme is a simplified representation of the various pathways and compounds that are involved with glucose metabolism in mammals.



It was the aim of this investigation to document the presence and shift of these pathways in a small mammal during arousal from deep torpor. During arousal it would seem that, of the myriad possible reactions and equilibria that could take place within an animal, all would be shifted toward greater efficiency in the production or degradation of energy bearing compounds.

Glucose labelled at either the 1 position in the glucose molecule or at the 6 position was injected into a series of pocket mice (Perognathus longimembris). The mice were either in normal mammalian physiological state (body temperature = 37°C) or were in deep torpor (body temperature = 3-4°C).

A total of 40 individual determinations were made on a group of 10 pocket mice (5♂ and 5♀). Time and apparatus limitations necessitated a 5 day period to obtain data on the group. Therefore, individual animals received a single injection of glucose about once every 7 days.

The mice were injected intraperitoneally (i.p.) with 1 μCi of glucose - ^{14}C in 0.2 ml isotonic glucose solution (5%). It was assumed that injections spaced 7 days apart would have little or no effect on the animal or the route of metabolism. By using the same animals, variation was reduced as each acted as its own control.

Mice were induced into deep torpor by removing food and isolating them in a refrigerator over night. Their body temperature was about 4°C when injected and placed into the monitoring chamber. If arousal was not initiated by the injection, body temperature gradually increased until it reached room temperature or until arousal was initiated.

The expired respiratory gases were monitored by an infrared CO_2 analyzer and a vibrating-reed electrometer. The respired $^{14}CO_2$ from the animal passed through the ion chamber connected to the electrometer, and produced an ion current exactly proportional to the instantaneous activity of the air sample. Concentration of CO_2 in air stream was monitored because any variation due to metabolic variation would cause "artificial" peaks to appear in the expired air radioactivity record.

The entire experimental system consisted of (a) animal chamber, (b) drying tube with magnesium perchlorate, (c) electrometer, (d) infrared CO_2 analyzer, (e) potassium hydroxide gas scrubber, (f) lithium hydroxide bed, and (g) vacuum source. Gas flow through the system was regulated at 150 ml/min.

The respiratory pattern of $^{14}CO_2$ in normal body temperature pocket mice from both the 1 and 6 positions of labelled glucose is given in Figure 13. The pattern is typical in that radioactive carbon dioxide appears in the expired air almost immediately, a plateau is maintained for a period of time, followed by a gradual decline and eventual total

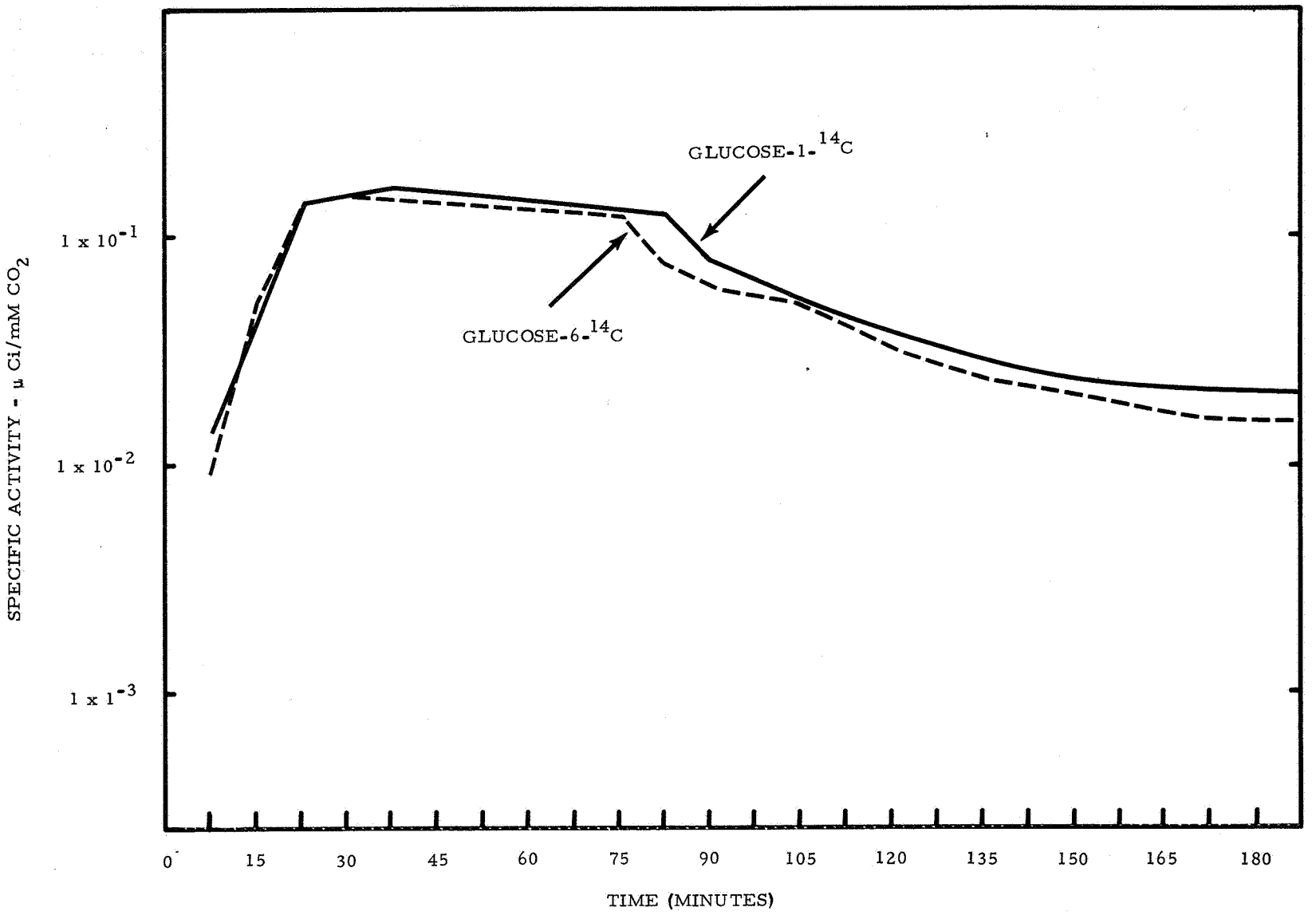


Figure 13 $^{14}CO_2$ respiratory patterns of normal pocket mice (P. longimembris) given 1μCi glucose -^{14}C labeled at the 1 or 6 position. Zero time equals time of injection.

elimination. Because of the high metabolic rate of this small animal (10 gm), the excretion pattern is much compressed compared to a human where the plateau is not reached until about 90 min.

The respiratory patterns indicate the metabolic difference of the labelled positions. The carbon from the one position is excreted in greater quantities per unit time and appears to maintain this higher excretion rate longer after reaching the peak specific activity.

The evaluation of the metabolic response of pocket mice during arousal from torpor has certain complexities and uncertainties associated with the metabolic lability of this organism. All animals were torpid with a body temperature of 4-5°C at the time of isotope injection. In some cases arousal was initiated immediately, while others did not arouse for as long as 113 min. If the onset of the major peak of CO_2 production is used to indicate the arousal process, the animals used in the 1-^{14}C position had a mean time to arousal of 55 minutes (range 0-113 min) while the group used in the 6-^{14}C position had a mean time of 22 minutes (range 0-46 min). It would seem meaningless to compare these groups using time zero as time of injection of labelled glucose, as individuals in the groups wold be in very different metabolic states.

Normalization of individual animals to the onset of the major peak of CO_2 production, or the transition from torpidity to normothermia, seemed a more logical approach to the evaluation of data. Figure 14 presents the respiration pattern of $^{14}CO_2$ in mice when normalized.

It appears from this data that at the time of arousal the excretion of radiocarbon from the 1-^{14}C position is greater than the 6 position. This disparity during the early stages of arousal is greater than the differences observed at later time periods. Peak specific activity is reached earlier and the higher rates of excretion are maintained longer.

If certain assumptions are valid it would seem that there is a shift in the enzymatic equilibrium to allow more glucose to be metabolized in the pentose phosphate shunt during arousal from torpor than is "normally" metabolized via this route. It is not implied that this is an "on-off"

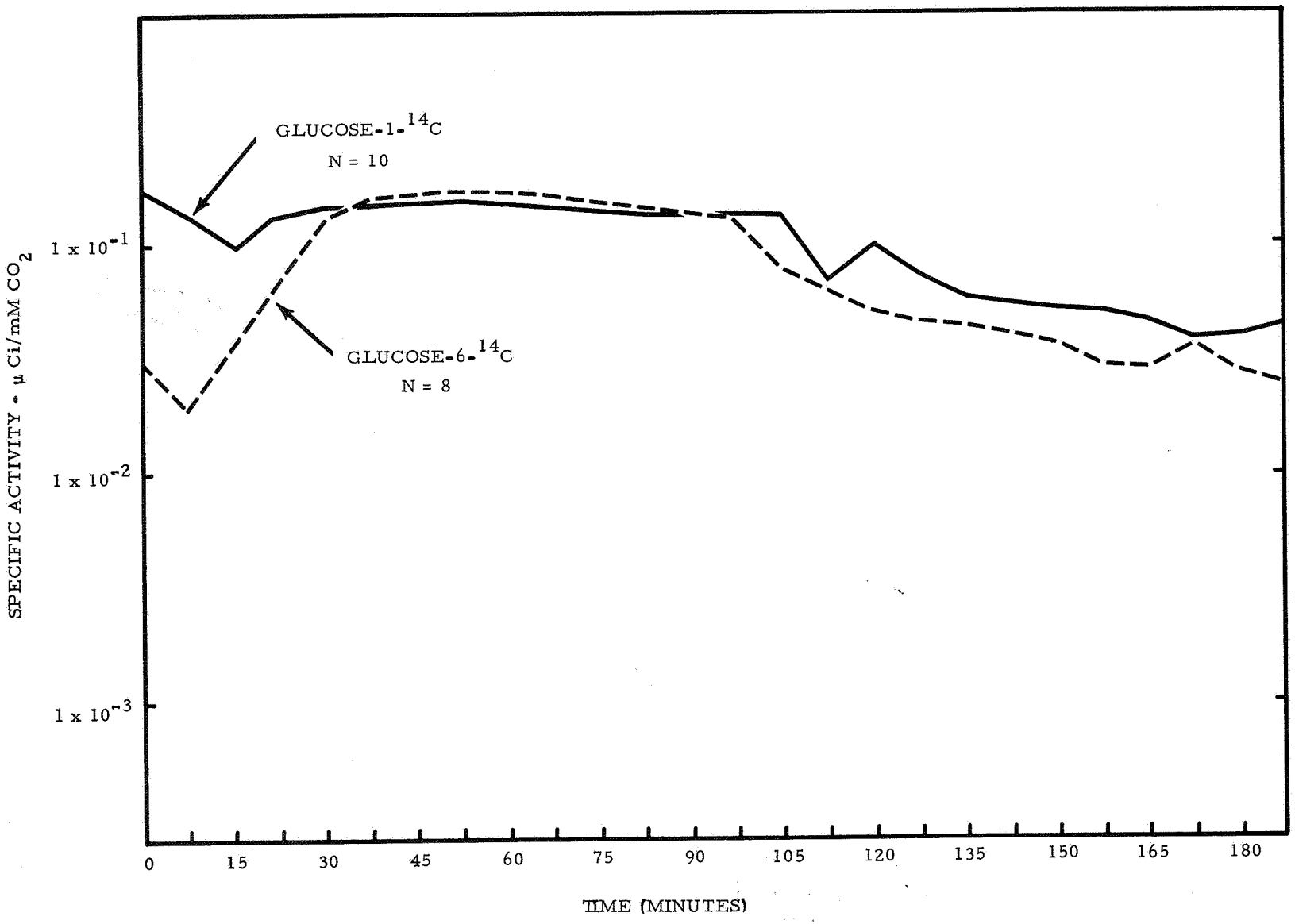


Figure 14 $^{14}CO_2$ respiratory patterns of torpid pocket mice (P. longimembris) given 1 μ Ci glucose - ^{14}C labeled at the 1 or 6 position. Data normalized to zero as onset of arousal from torpor.

shunt activated only in times of high metabolic requirement. The normal occurence is documented in "normal" metabolism (Fig. 13). Although the prime importance of the pentose phosphate cycle has been thought to be associated with the production of 5-carbon units for DNA synthesis, it may supplement 3 and 6 phosphated carbon units to the glycolytic pathway during maximum energy requirements. This "alternate" pathway also supplies reduced NAD to the cytochrome system for phosphorylation of ADP.

VI. Summary

1. The little pocket mouse Perognathus longimembris undergoes spontaneous diurnal torpor throughout the year.
2. The incidence of torpor is highly variable between individuals but there is a significant increase in the frequency and duration of torpor in the fall and winter months.
3. Coincident with the increased incidence of torpor in the fall and winter is a slight increase in body weight ($\sim$ 0.5 gm). The fluctuation in weight seems to be less than expected if, as in the arctic ground squirrel, the little pocket mouse does anticipate a period of the year when periods of extended torpor or hibernation occur.
4. At an ambient temperature of 10°C the duration of torpor was from 2 to 6 times longer than at 21°C.
5. A period of increasing duration of torpor was observed at the beginning of the experiment and may represent temporal "test drops."
6. Continuous torpor of about 3 days was observed at ambient temperatures of 10°C and may represent a maximum for this species at this temperature.
7. Data show that the amount of brown fat in P. longimembris is relatively large and that the fat deposits readily respond to a cold stimulus by increasing in amount.
8. Similarly the thyroid glands appeared more active in cold exposed mice compared to thyroids of mice maintained at room temperature.

9. Metabolism of C^{14} labelled glucose during arousal from torpor show a tendency for glucose labelled at the 1 position to be preferentially metabolized over glucose labelled at the 6 position. It would appear that there is a shift in the enzymatic equilibrium to allow more glucose to be metabolized in the pentose phosphate shunt during arousal from torpor than is normally metabolized via this route.

10. The arousal pattern and simultaneous peripheral vasoconstriction observed in P. longimembris in the fall confirm the hypothesis that this species has the same physiological mechanisms for arousal from daily torpor as has been observed in the classical seasonal hibernators.

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TOLERANCE OF THE LITTLE POCKET MOUSE (PEROGNATHUS LONGIMEMBRIS) TO HYPOXIA

J. J. Gambino and Page Hayden

In an effort to produce hypoxia in Perognathus longimembris as part of a radiation effects problem we found that this species responds to decreased available oxygen as do other mammalian hibernators. That is, as ambient oxygen is reduced, the pocket mouse adjusts to the potentially damaging situation by reducing its body temperature. This response occurs only if the oxygen decrease is slow enough, allowing the animal time to cool. Cooling rate must be sufficient to reduce its metabolic need for oxygen to below that which is available. In this way, pocket mice, like most hibernators, exhibit "hypoxic tolerance."

When body temperature reaches ambient, however, continued reduction of ambient oxygen can be as disastrous to the pocket mouse as it is to non-hibernators. That is, tissue and cellular anoxia quickly ensue and death occurs within a very short period.

The purpose of this series of experiments was to find the best method of producing hypoxia in pocket mice, to determine criteria for judging the severity of hypoxia, and to ascertain the maximum duration of hypoxia in this species that is compatible with survival.

Materials and Methods

Adult Perognathus longimembris of both sexes were used. These animals had been in our holding facility for 6 months to 1 year and were all young adults. Hypoxic conditions were produced by exposing animals to a nitrogen-oxygen mixture at a pressure of one atmosphere. The exposure chamber was a 1000 ml spoutless beaker with a rubber stopper, fitted to take incurrent and excurrent air tubes and temperature sensors. Oxygen flow was varied to obtain the desired oxygen concentration in the mixture. A gas flow of 400-600 ml/min was sufficient to ensure rapid equilibrium when pO_2 was changed. Outflow oxygen concentrations lagged only slightly behind changing flow

concentrations. A Beckman expanded scale pH meter with an oxygen adapter for a polarographic oxygen sensor was used to measure inflow and outflow oxygen concentration.

Deep body temperature and ambient temperature were simultaneously recorded in most of the trials. Body temperature was obtained with a thermistor rectal probe. Experiments were conducted at room temperature (~ 24°C) and at 10°C. A constant temperature water bath was used to maintain the 10°C temperature.

Protocol for each trial differed slightly. Usually the animal was instrumented and allowed to acclimate in the chamber for ½ to 1 hr before hypoxic conditions were started. Oxygen was then incrementally decreased and visual observations were made as the hypoxic conditions became increasingly severe. Since techniques for measuring *in vivo* intra and extracellular oxygen tension in small mammals are extremely difficult, clinical signs and mortality were used to judge the degree and time limits of hypoxia.

Results

Table 1 presents data on individual pocket mice which were subjected to either acute or stepwise reduction of oxygen in a nitrogen-oxygen breathing mixture at either room temperature (~ 24°C) or at 10°C at approximately one atmosphere pressure. Eleven animals exposed to acute reduction to 5.0% oxygen survived for periods ranging between 14 and 140 hr with no apparent stress or hypoxia damage. Low ambient temperature was not required for this prolonged survival.

Acute depression of amibient oxygen to levels below 5.0%, however, was not well tolerated. Death occurred within 23 min in one animal (L-1152) administered 4.0% oxygen directly from air. In another (L-1158) death occurred within 6 min after reducing the chamber atmosphere from air directly to 3.3% oxygen. Complete anoxia was tolerated less than 10 min in one animal (L-1142) which had been made hypothermic by prior 5.0% oxygen treatment (See also Fig. 1, Chart 4).

Of the 11 animals that were placed on 5.0% oxygen, several were kept there for survival studies and others were subjected either gradually or sharply to even lower oxygen partial pressures. Results show that survival is marginal below about a 3.0% oxygen concentration. One animal (L-1146) survived 36 min at 2.8% oxygen after a period of 3½ hr at 5%. It was in severe hypoxia stress prior to restoration of air. Another (L-1155) died after 35 min in 2.6% oxygen. It, too, was in severe hypoxia stress. Survival in oxygen concentrations below 3.0% is possible in Perognathus but only after the animal is acclimated by a slow stepwise oxygen reduction. Survival time appears to be approximately 20 to 30 min.

In anticipation of radiobiological studies a series of experiments was carried out on groups of Perognathus to determine how well groups of 10 or more animals administered hypoxia in a single chamber tolerate stepwise reduction of oxygen to 2.6%. Table 2 summarizes the response in these groups. During these group exposures animals were severly stressed at the lower oxygen levels. Signs of anoxia included cyanosis, hyperventilation, gasping, struggling, and finally apnea. Several animals that had stopped breathing long before termination of the experiment and appeared dead when removed from the chamber gasped after several minutes in air. Some of these were subsequently revived in 100% oxygen.

The low oxygen concentration (2.6-2.8%) was chosen for group exposures to insure that all animals would become hypoxic. This oxygen concentration is marginal for survival in this species and every animal in the chamber appeared severely stressed by this procedure. Of 40 animals treated in this manner, 28 survived. All survivors, however, appeared active and healthy over an observation period of several months.

Deep body temperature was continuously monitored in a number of individual Perognathus exposed to reduced oxygen concentration while in a 10°C ambient chamber. Figures 1 and 2 show the manner in which body temperature falls when available oxygen is reduced. Figure 1 presents records of animals that were subjected to acute reduction from normal to 5.0% oxygen. In all cases, in a 1-2 hr period, body temperature fell to within a degree or two of ambient. (Records shown in figures are thermistor

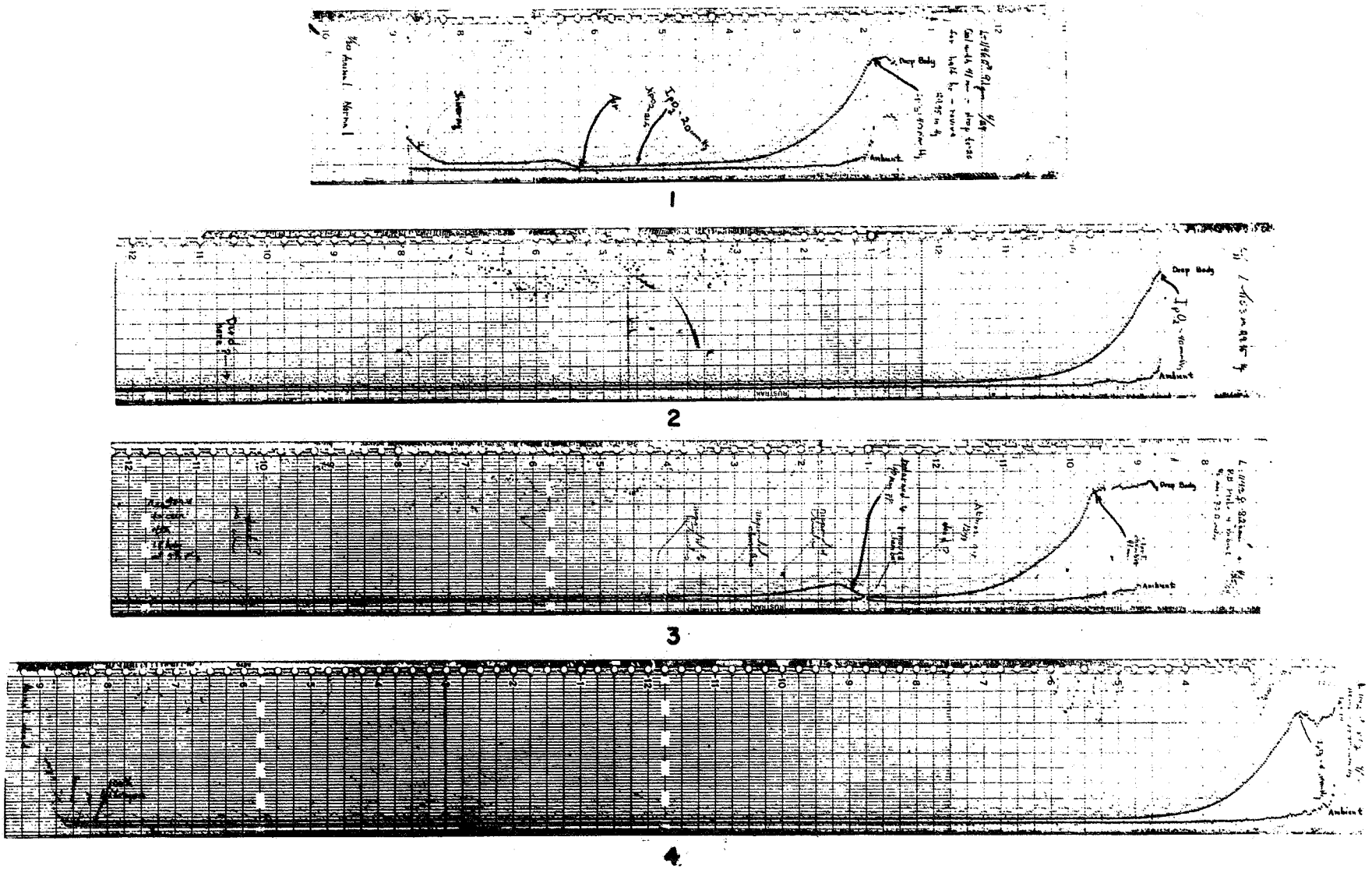

Figure 1 Deep body temperature* of P. longimembris administered 5% oxygen in nitrogen at an ambient of 10°C. (* Relative measurement from uncorrected thermistor readings.)

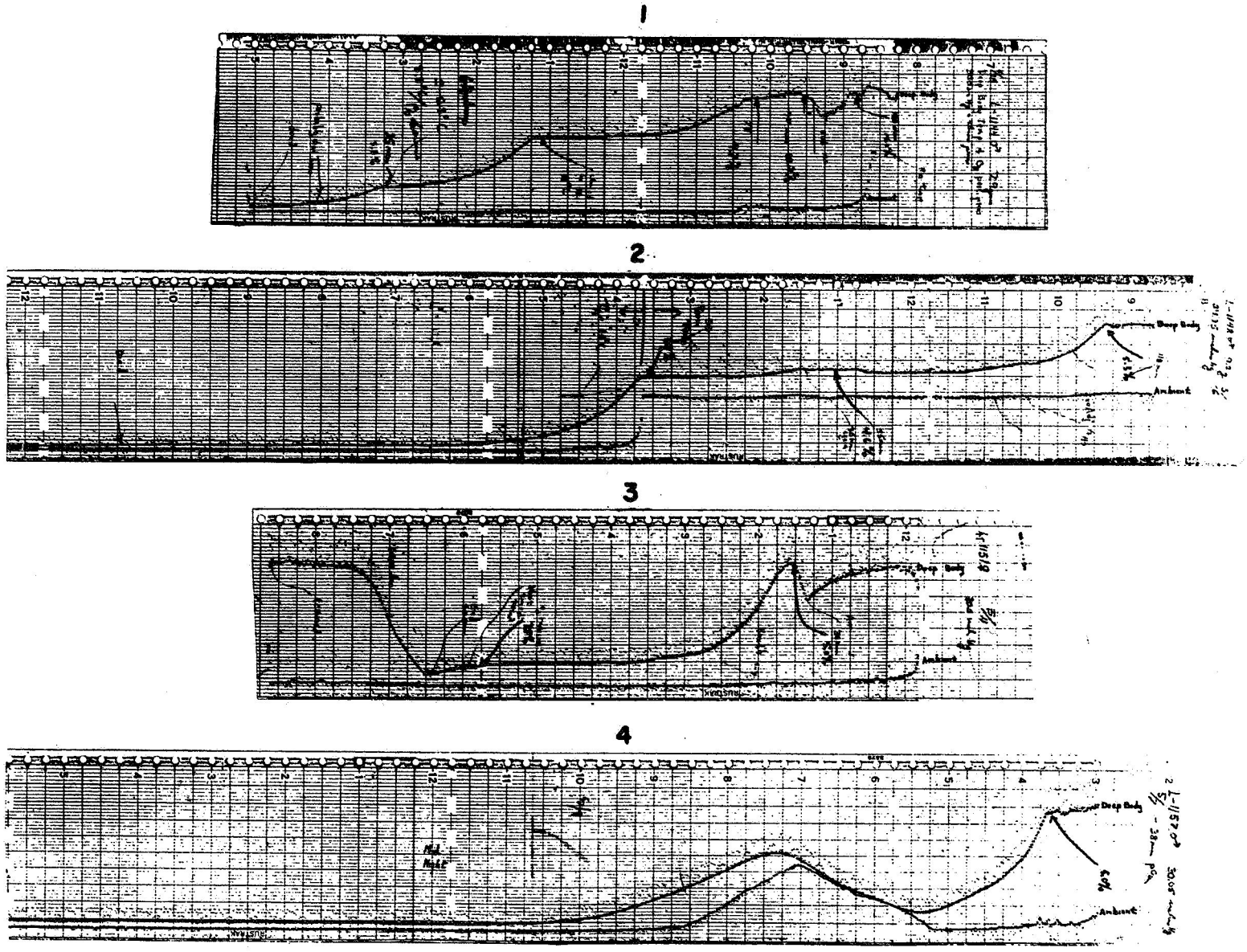

Figure 2 Deep body temperature* of P. longimembris administered various concentrations of oxygen in nitrogen at 22°C or 10°C ambient temperature. (* Relative measurement from uncorrected thermistor recordings.)

TABLE I

Response of individual pocket mice to acute or stepwise reduction of oxygen in a nitrogen-oxygen breathing mixture

Animal No. & Sex	Ambient Temp. °C	Step 1 pO_2	Step 1 Duration	Step 2 pO_2	Step 2 Duration	Step 3 pO_2	Step 3 Duration	Reason for Terminating Experiment
L-1155 ♂	22	5.0%	1 hr 27m	3.9%	3hrs 13m	2.6%	35 m	Died
L-1154 ♂	22	5.0%	21 hrs	4.8%	1 hr 35m			Experimenters Choice
L-1156 ♂	22	5.0%	2 hrs	4.0%	1 hr 40m	3.2%	40 m	Experimenters Choice
L-596 ♀	22	5.0%	140 hrs					Experimenters Choise
L-1152 ♂	22	4.0%	23 m					Died
L-1143 ♀	10	5.0%	2hrs 40m	21.0%*	8 m	5.0%	18 hrs	Experimenters Choice
L-1151 ♀	10	5.0%	4hrs 30m	4.0%	30 m			Experimenters Choice
L-1153 ♀	10	5.0%	14 hrs					Died
L-1157 ♂	10	5.0%	77 hrs					Died (malfunction of heater)
L-1146 ♂	10	5.0%	3hr 25m	2.8%	36 m			Experimenters Choice
L-1158 ♀	10	3.3%	< 6 m					Died
L-1142 ♂	10	5.0%	17 hrs	0%	< 10 m			Died

* Animal appeared dead but was found to be alive - experiment resumed (see Fig. 1, Chart 3).

records and show only relative differences between body temperature and ambient. Values must be converted from calibration curves to show actual temperatures). During the period of temperature drop the animal gradually reduces its activity until it finally becomes torpid. At this time it appears to be in a natural hypometabolic state. When normal oxygen concentration is restored, there is an immediate increase in body temperature and the animal returns to normal in about two hours (Fig. 1, Chart 1).

In Figure 2, Chart 1 the animal was in a 10°C environment and oxygen was reduced gradually from normal air concentration to 14% and subsequently down to 3.3%. Body temperature remained normal in an oxygen concentration as low as 12.3%. It dropped off gradually with each succeeding decrease in oxygen concentration with the animal apparently making good adjustment down to 3.3% pO_2. For some unknown reason the oxygen had drifted in this trial and at the end of the first hour after starting the 3.3% oxygen mixture, the excurrent concentration read 2.0%. The animal appeared severely stressed and oxygen was returned to about 4.0%. The animal did not recover; however, autopsy revealed the heart still active suggesting that resusitation, had it been applied, may have been successful.

In Chart 2 of Figure 2 the animal was started at 22°C ambient and administered 5.3% oxygen. Body temperature fell to 26.0°C but remained substantially above ambient (22.3°C). This represents a drop in body temperature of about 10°C. The oxygen was decreased to 3.9% and the chamber plunged into the cold water bath reducing the ambient to 10°C. The animal appeared to make the normal adjustment to the low oxygen concentration at low temperature; however, it died within 7 hr.

Chart 3 of Figure 2 shows one animal that was placed in 5.0% oxygen at 10°C ambient. The body temperature of this animal did not reach ambient; however, oxygen was further reduced to 3.9%. After a short period in 3.9% oxygen, air was restored and the animal returned rapidly to normal body temperature.

Chart 4 of Figure 2 shows an animal (in 5.0% oxygen at 10°C ambient) that was subjected to two malfunctions of the heating unit in the water

TABLE 2

Response of groups of pocket mice to stepwise reduction of oxygen in a nitrogen-oxygen breathing mixture

No. of animals	pO_2 mmHg	$\%O_2$ (Approx.)	Duration (min.)	Mortality	Comments
10	41	5.3	3	2 dead	T_A = 22.8° - 23.8°C
	160*	21.0	1		
	81	10.5	41		
	61	7.6	33		
	45	5.9	27		
	40	5.3	70		
	33	4.3	67	1 dead	
	20	2.6	20	2 dead	Experiment terminated - air restored, 5 of 10 survived
10	83	10.8	38		T_A = 22.8° - 23.8°C
	63	8.0	37		
	42	5.5	85		
	33	4.3	70	1 dead	
	21.5	2.8	20		Experiment terminated - air restored, 9 of 10 survived
20	100	13.1	30		T_A = 19° - 20°C
	80	10.5	30		
	50	6.5	30		
	40	5.3	30		
	30	4.0	45		
	20	2.6	20	6 dead	Experiment terminated - air restored, 14 of 20 survived

* Several animals appeared severely stressed. Opened chamber for less than one minute, then started oxygen reduction more slowly.

bath causing a severe rise in temperature. The first malfunction occurred only a short while after the experiment started; however, the malfunction corrected itself and the animal survived. The second occurred after 77 hr, killing the animal (not shown in the record). It is noted in this chart that the animal's body temperature coincides with ambient as the heating occurred but lagged as the chamber returned to low ambient temperature.

Discussion and Summary

The ability to survive exposure to lowered environmental oxygen is not the same in all mammals. For most adult mammals, hypoxic environmental conditions quickly result in a chain of events leading to tissue hypoxia, reduced cellular oxygen tension and, if oxygen lack is severe enough, death.

Some mammals, on the other hand, are able to withstand remarkably long periods in extremely low ambient oxygen levels. Many hibernating mammals have this ability, which is based on body cooling and keeping oxygen "demand" below "supply". According to Van't Hoff's law, body cooling decreases metabolic oxygen requirement and thereby prevents or postpones anoxia damage.

This ability, as it is seen in hibernators, appears to be an amplification of a phenomenon common in many other mammals. In Wistar rats, for example, as environmental oxygen is reduced, body cooling occurs and hypoxia survival is enhanced. The ability is not nearly as well developed in the rat as it is in the hibernating ground squirrel (1). At room temperature both rats and ground squirrels survive at least 2 hr in a 5.5% ambient oxygen concentration. During this period body temperature of the rats dropped 9°C. In a 4.1% oxygen atmosphere rats died in 30 min, while ground squirrels survived for at least 2 hr. The ground squirrel showed a 10°C decrease in body temperature in this atmosphere.

Ability to cool may not be the whole story. The hamster is able to survive 4.1% oxygen for 2 hr, with only half the temperature drop exhibited by ground squirrels (1). It has been suggested that in hibernators mechanisms other than passive body cooling are operating. High oxygen affinity

of hibernator hemoglobins, unusual vasomotor activity, increased anaerobic metabolism are listed among the adaptations possibly available to the hibernator.

Perognathus may be classed as a hibernator. Indeed, it has the ability to undergo periods of hypometabolism and appears to possess many of the adaptive mechanisms of hibernators. Our data suggests, also, that Perognathus responds to reduced oxygen in a manner that is reminiscent of hibernators. Its return to normal, however, appears dissimilar. Ground squirrels in a 7°C chamber made hypothermic by hypoxia do not return to normal when normal atmospheric gas concentration is restored but remain in a "hibernation" state (1). They arouse only after chamber temperature is increased to 15°C. It was suggested the these animals may be in a state of "neutral" hibernation but not "endocrine" hibernation (1). In contrast, in hypoxia-induced hypothermia in P. longimembris, arousal occurs immediately after normal atmospheric oxygen is restored.

Interestingly enough, because there is difficulty in making in vivo intra-cellular oxygen measurements in small rodents, there is little comparative data on survival of hibernators and non-hibernators after the onset of tissue hypoxia. In Perognathus, if the reduction in oxygen is sudden and severe, survival time is extremely short. This demonstrates that the rate of oxygen reduction is very important for survival. If the rate at which oxygen levels are reduced exceed the rate at which metabolic needs are reduced, oxygen lack is certainly evident in Perognathus. This suggests that the hibernator may not be tolerant to hypoxia at the cellular level. Their "hypoxia tolerance" may be based solely on their efficiency in reducing metabolic need for oxygen.

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HYPOXIA INDUCED HYPOTHERMIA IN THE GENUS PEROGNATHUS

Page Hayden

The classification of mammals into the broad categories of "hibernator" and "non-hibernator" is based upon the individual species ability to spontaneously re-warm from body temperatures that are lower than "normal". These abnormal body tempetatures may be evoked by depriving the animal of oxygen so that the heat producing processes are curtailed while essential metabolic reactions continue. The lowering of body temperature, in turn, establishes new requirements and equilibrium of all processes involved in a particular thermal realm. This continued cycling of decreased oxygen concentration and resultant cooling of the animal can be pursued to nearly any level of depressed body temperatures desired. Upon restoration of normal oxygen concentration, the "hibernator" (or facultative homeotherm) possesses specialized physiological mechanisms, i.e., brown fat, selective vasoconstriction, basic nerve differences, etc., by which it can re-warm to normal body temperature. While the obligate homeotherm may live for a time at reduced body temperature, it will eventually die. Not only is life limited at reduced temperature, there is also a temporal limit at which the animal may remain at the reduced temperature and be revived successfully (1).

Within the genus Perognathus (pocket mice) are species that are known to express daily periods of moderate hypothermia or even deep hypothermia (dependent upon prevailing ambient temperature) in relatively unstressed laboratory conditons (food, bedding and nest chamber). One of these species (P. longimembris) expresses an annual winter period of inactivity in its natural state. P. californicus has been documented to express a laboratory induced moderate hypothermia in direct response to decreased food supply. This torpor in P. californicus is interesting in that the lower limit of survival is 15°C (2). The species apparently cannot re-warm if its body temperature falls below the critical value.

The report of a lower temperature limit for survival in torpid P. californicus aroused interest in determining thermal limits for other species within the genus. It also raised the related question of possible

correlations between oxygen-binding characteristics of the blood and the various degrees of faculatative homeothermy observed in Perognathus sp. Of equal interest was the possibility of relating one or more of these characteristics to the high resistance to ionizing radiation exhibited by several members of this genus, thereby providing a clue to the mechanism of radiation resistance in Perognathus.

Methods and Materials

The equipment used for producing, regulating and monitoring the response of confined, but not restrained, animals consists of six major components: animal chamber, cooling unit, oxygen monitor, body temperature readout, 2 pen analog recorder, and gas supply.

The animal chamber is made of lucite with a double wall water jacket construction for temperature control and is provided with heat exchange coils and humidifier for incurrent gas mixtures. An antenna system is fitted to a subfloor of the acutal animal chamber to receive signals transmitted from a telemeter implanted abdominally in an animal (3). The signal is amplified, integrated and analog output fed to one pen of the recorder.

Various respiratory atmospheres can be made using two vernier valves and bottled gases under three stages of regulation. Pure breathing oxygen and 95% nitrogen with 5% carbon dioxide are mixed and supplied, at the rates of 100-200 ml/min, to the animal chamber. A small bypass shunt goes to the oxygen analyzer (Beckman F3) and monitors the mixture constantly. The O_2 analyzer output is fed to one channel of the recorder.

The animal chamber is maintained below ambient (6-7°C in most cases) by circulating water from a cooling source which can be regulated through adjustments of back-pressure in the refrigerating gas. Thermostatic adjustment devices could not be used since that caused perturbation in the temperature monitoring system.

In all experiments, animals were supplied with a piece of paper towel for urine absorption and sunflower seeds for food.

Results and Discussion

Hypoxic Hypothermia - Table 1 is a summary of the response of P. formosus to hypoxic induced hypothermia. Of the six animals run, one showed a spontaneous re-warming from a deep body temperature of 7.5°C (F-C, 6 June). However, in four of the six animals, re-warming did not take place when the deep body temperature was between 10-11°C. One animal had successfully re-warmed from 12.5°C several days later. This animal had a deep body temperature near 7°C for 14 hr, yet maintained its capacity to be re-warmed and live. Three of the four animals that showed no re-warming died before 15 hr from the time of restoration of normal atmosphere. This low temperature viability is apparently not directly associated with an immediate successful low temperature recovery as compared in F-597 and F-B, Table 1.

P. fallax response to hypoxia induced hypothermia is summarized in Table 2. One animal was unable to re-warm from 16.6°C and was dead before 15 hr at 7°C. Four other animals could not re-warm between temperatures of 11.9-12.7°C. Two of these animals had previously re-warmed from 14.8°C and 14.3°C. After a series of progressively deeper hypothermic experiences, a P. fallax re-warmed from a temperature of 9.2°C (F-824, Table 2), and there is stong evidence that this same animal would have been able to arouse from a temperature near 7°C, as noted in the remarks (Table 2). This animal underwent an arousal from 9.2°C to normal temperature and, after a period, entered a spontaneous torpor. A spontaneous re-warming from this latter torpor only reached 17.8°C when the animal re-entered torpor and died before 12 hr. The point is that the re-warming processes were successful in raising the body temperature up out of the critical range. The decline and eventual death of the individual were probably associated with the depletion of energy stores during the immediately preceding thermal stresses.

Extensive work with the species P. longimembris has given much data upon the hibernative ability to arouse from low body temperature. However, in Table 3, only the experiments with protocol sufficiently similar to the other species is presented.

Table 1. Hypothermia in P. formosus

Animal	Date of Hypoxic Exposure	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm	Max. Warming Rate °C/min	Total Time to Normal T_B min	Remarks
F-C female	13 May 31 May 6 June	9.8 8.0 5.0	19.0 17.0 9.0	19.0 17.0 7.5	0 0 20	0.270 0.150 0.103	94 265 515	1. 300 mins required to warm to T_B = 13°C. 2. T_B stabilized for 30 mins at 25°C, forming plateau in rewarming curve.
F-597 male	7 Aug 9 Aug	5.6 5.0	12.5 10.9	12.5 T_A=~7°C	0 No Arousal	0.139 -----	270 ---	1. T_B essentially stabilized at 10.8°C for 140 mins when air restored. T_B decreased to near T_A for 14 hrs. No visible respiration, removed to room temp, rewarmed to normal body temp.
F-595 male	3 Aug	7.0	11.2	T_A=~7°C	No Arousal	-----	---	1. Cooling rate uneffected by restoration of air, dead before 15 hrs.
F-1060 female	8 Aug	7.8	10.0	T_A=~7°C	No Arousal	-----	---	1. Dead before 15 hrs.
F-B female	5 June 7 June	6.5 5.0	14.0 10.0	14.0 T_A=~7°C	0 No Arousal	0.208 -----	225 ---	1. Cooling rate uneffected by restoration of air, dead before 15 hrs.
F-748 female	1 June	5.8	15.0	15.0	0	0.116	230	

The lowest temperature from which immediate arousal from induced hypothermia occurred, in this series of experiments, was 9.1°C. Several other lower body temperatures were reached but no arousals were initiated up to a 36 hr residence at the low temperature. This does not imply that an arousal was not possible, just that immediate arousal did not occur. Even at 10.6°C (Table 3, L-1749, 28 June) arousal may be delayed for several hrs. From other experiments with this species, it is known that spontaneous arousal from natural deep torpor is possible to at least 5°C (under proper conditions probably lower). No deaths were encountered during the extended periods of $T_B = \sim T_A$ nor during the rewarming process at room temperature. It will be noted that these experiments took place in the summer months when expression of hibernative ability is at a low.

The data from experiments with P. amplus are summarized in Table 4. Rewarming from a depressed body temperature as low as 7.5°C occurred in this species, although this was manifest in only one of three animals exposed (Table 4, #6, 8 February). In each case the animals were exposed to several hypoxic experiences and resultant drop in body temperature during a 3-4 week period. One animal (Table 4, #4, 30 January) was induced into deep torpor (T_B = 7.5°C) and with the exception of what appeared to be three partial arousals of 20.2, 18.9 and 15.5°C during the first 6 hrs, was maintained in deep torpor for 141 hr. After the animal was removed from the cold chamber it was noted that the animal had blood in the urine and that the site of an i.p. injection bled rather profusely. However, it appeared normal after one day and is now healthy and alive in the laboratory. No deaths occurred in this group during extended residence at low T_B's.

The responses of P. parvus to induced torpor are presented in Table 5. Of the five animals used in these experiments, three attained body temperatures of 7-8°C and 2 aroused from this deep torpor. One remained in deep torpor for 92 hrs and did not arouse even after rather violent handling. In all body temperatures above 10°C rewarming was immediate and rapid. One animal (Table 5, F-688, 28 September) entered a spontaneous torpor upon exposure to the low ambient temperature of the experimental chamber. It aroused from T_B = 7.5°C after 31 hr and again was torpid for 69 hr before

Table 2. Hypothermia in P. fallax

Animal	Date of Hypoxic Exposure	Method:* 1. Step 2. Single 3. Rebreath	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm min	Maximum Warming Rate °C/min	Total Time to Normal T_B min	Remarks
F-824	24 Aug	1.	8.0	25.7	25.2	5	0.172	70	
	29 Aug	1.	7.5	23.7	23.7	0	0.399	41	
	31 Aug	1.	5.0	18.6	18.6	0	0.433	-	1. Total time not available, as terminated before T_B stabilized.
	1 Sept	3.		13.8	13.8	0	not deter.	not deter.	
	12 Sept	2.	5.5	13.8	13.8	0	0.273	160	
	14 Sept	2.	5.0	11.9	10.5	30	0.356	235	
	18 Sept	2.	5.0	9.2	9.2	0	0.386	190	1. After 12 hr of normal body temp., animal entered a spontaneous torpor for 9 hr (T_B = ~7°C). A spontaneous arousal occurred but animal only warmed to 17.8°C and again cooled to ambient. It died before 12 hr in this torpor.
F-617	6 Sept	3.		12.5	T_A=~7°C	no arousal	-	-	1. Remained at T_B =~7°C for 4 hr. 2. Artificially warmed to T_B = ~17.4°C, replaced in chamber, again cooled to ambient, dead before 15 hr.
F-869	25 Aug	1.	8.5	12.7	T_A=~7°C	no arousal	-	-	1. Rate of cooling after air restored less than in hypoxic mixture. 2. Dead before 3 hr after low T_B.
F-4	14 Aug	3.		15.8	14.8	17	0.400	not deter.	1. Terminated before T_B stabilized.
	15 Aug	1.	7.5	11.9	T_A=~7°C	no arousal	-	-	1. Cooling rate retarded after air restored. 2. Dead before 15 hr after low T_B.
F-867	21 Aug	3.		26.0	26.0	0	not deter.	-	1. Rewarm record erratic, data not good.
	23 Aug	1.	6.8	16.6	T_A=~7°C	no arousal	-	-	1. Cooling rate retarded after air restored. 2. Dead before 15 hr after low T_B.
F-829	5 Sept	1.	6.5	16.8	16.8	0	0.222	135	
	8 Sept	2.	5	14.7	14.3	9	0.300	180	
	11 Sept	2.	4.8	12.1	T_A=~7°C	no arousal	-	-	1. After 3 hr at T_B =~9°C, resp. rate 12/min. After 18 hr, no visible resp., warmed to 15°C, respiration erratic. HR = 40. 2. Retained at room temp., dead before 15 hr.

* Step, single and rebreath refer to method of reduction of oxygen content in chamber; step = sequential reductions in increments; single = direct change to reduced level; rebreath = reduction by animal in a sealed chamber.

** T_B = deep body temperatures.

Table 3. Hypothermia in *P. longimembris*

Animal	Date of Hypoxic Exposure	Method:* 1. Step 2. Single 3. Rebreath	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm min	Maximum Warming Rate °C/min	Total Time to Normal T_B min	Remarks
L-1749	27 June	1	13.75	31.6	31.6	0	0.190	27	
	28 June	1	11.70	13.7	10.6	110	0.183	310	1. Arousal 2 hr after return to normal air.
	12 July	1	8.30	19.9	19.9	0	0.487	35	
	12 July	1	8.00	20.4	20.4	0	0.367	40	
	29 Aug	1	8.60	11.6	11.6	20	0.200	230	
L-1946	1 July	1	13.75	9.3	8.2	-	-	-	1. No arousal in 3 hr. Terminated.
	8 July	1	10.00	17.0	17.0	-	0.397	60	
	11 July	1	7.25	9.3	9.2	10	0.458	160	
L-1650	29 June	1	14.00	21.1	8.0	-	-	-	1. Terminated after 16 hr continuous torpor; artificially rewarmed; again entered torpor.
	13 July	2	7.25	10.4	10.4	30	0.400	350	1. Entered spontaneous torpor after 9.5 hr.
	13 July	Spontaneous	21.00	-	10.7 (Auditory Stimulation)	0	0.272	210	1. Animal stimulated after 4.5 hr in torpor.
L-1603	5 July	1	11.25	25.0	25.0	10	0.650	19	
	5 July	1	7.00	7.6	7.6	-	-	-	1. Terminated after 17 hr continuous torpor at 7.6°C, rewarmed at room temperature, normal.
L-1570	7 July	1	10.75	7.5	7.5	-	-	-	1. Terminated after 14 hr continuous torpor at 7.5°C; rewarmed at room temperature, normal.
L-1577	23 Aug	1	12.5	9.6	8.0	-	-	-	1. Terminated after 36 hr continuous torpor; rewarmed at room temperature, normal.
L-1604	25 Aug	1	4.1	11.7	11.7	0	0.178	Only rewarm to 28.3 135	1. Aroused and entered torpor again.

* Step, single and rebreath refer to method of reduction of oxygen content in chamber; step = sequential reductions in increments; single = direct change to reduced level; rebreath = reduction by animal in a sealed chamber.

** T_B = deep body temperature.

Table 4. Hypothermia in P. amplus

Animal	Date of Hypoxic Exposure	Method:* 1. Step 2. Single 3. Rebreath	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm min	Maximum Warming Rate °C/min	Total Time to Normal T_B min	Remarks
#2	5 Jan	1	7.75	25.3	24.7	0	0.405	45	
	5 Jan	1	7.75	21.7	21.1	5	0.337	70	
	11 Jan	1	7.50	19.2	19.2	5	0.321	87	
	16 Jan	1	3.00	15.2	15.0	10	0.332	145	
	6 Feb	1	5.00	8.0	7.5	-	-	-	1. No arousal, torpid 16 hr, removed to room temp to rewarm.
#4	3 Jan	2	8.00	31.0	31.0	0	0.334	18	
	9 Jan	1	6.30	20.2	18.0	-	-	-	1. Partial rewarm to 24.5°C, cooled to 18.0°C, terminated after 2.5 hr. A hysteresis band of 0.5°C upon exposure to T_A = 7°C.
	15 Jan	1	3.25	14.9	7.5	-	-	-	1. Entered torpor, 2 partial arousals to 19.5°C and 18.4°C (within 5 hr), terminated after 17 hr, returned to room temperature to rewarm.
	30 Jan	1	3.00	13.4	7.5	-	-	-	1. Entered torpor, 3 partial arousals to 20.2, 18.9, 15.5°C (within 6.5 hr). Removed to room temp after 141 hr of torpor, appeared normal after 1-2 days.
#6	4 Jan	1	7.50	27.1	26.9	5	0.325	38	
	10 Jan	1	4.30	17.3	16.9	5	0.400	76	
	5 Feb	1	4.75	7.7	7.7	10	0.287	250	1. Remained at normal body temp for 10½ hr, entered spontaneous torpor, returned to room temp after 3 hr to rewarm.
	8 Feb	1	3.50	8.0	8.0	30	0.338	210	1. Entered spontaneous torpor 9.5 hr after arousal, aroused after 16.5 hr, again entered torpor but terminated after 4 hr.
#8	8 Jan	1	7.25	20.0	19.6	10	0.380	96	
	12 Jan	1	6.00	17.5	17.5	0	0.383	90	
	22 Jan	1	3.00	19.5	19.5	0	0.328	78	1. Entered spontaneous torpor after 11.5 hr, for 17 hr seemed to be regulating temp at about 15.5°C, then drifted down to near ambient, terminated after 11 hr, T_B= T_A, rewarmed at room temperature.
	7 Feb	1	3.00	12.6	12.6	-	-	-	1. Gradual rewarm to 14.6°C in 7 hr, cooled again, removed after 16 hr.

* Step, single and rebreath refer to method of reduction of oxygen content in chamber; step = sequential reductions in increments; single = direct change to reduced level; rebreath = reduction by animal in a sealed chamber.

** T_B = deep body temperature.

Table 5. Hypothermia in P. parvus

Animal	Date of Hypoxic Exposure	Method:* 1. Step 2. Single 3. Rebreath	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm min	Maximum Warming Rate °C/min	Total Time to Normal T_B min	Remarks
#5	23 Oct	1	5.25	15.9	15.9	0	0.318	77	
	26 Oct	1	4.70	10.0	10.0	0	0.356	130	
	30 Oct	1	10.00	14.0	7.5	-	-	-	1. Animal remained in constant torpor for 92 hr after normal atmosphere restored. Did not arouse after handling at 40 hr T_B=~7.5°C during torpor. 2. Returned to room temperature for rewarming.
#4	13 Oct	1	4.30	14.6	14.6	0	0.400	82	1. Entered spontaneous torpor 10½ hr after arousal from induced torpor. 2. Returned to room temp for rewarm after 35 hr of T_B =~7°C. 3. Cheyne-Stokes respiration, 15-20 resp/min then no respiration for ~8 min.
	17 Oct	1	4.00	11.0	11.0	0	0.500	89	
	24 Oct	1	3.70	11.2	11.2	0	0.585	81	
F-698	11 Oct	1	7.50	18.8	19.4	10	0.408	50	
	16 Oct	1	5.00	11.1	10.0	12	0.407	165	1. Entered spontaneous torpor 5½ hr after arousal from induced torpor. Returned to room temperature for rewarm after 10 hr T_B=~7°C.
	25 Oct	1	3.00	8.0	8.0	15	0.386	130	
#1	12 Oct	1	6.50	15.4	15.4	0	0.382	77	
F-688	28 Sept	No hypoxia spontaneous torpor with cold.	21.00	-	7.5	-	0.289	190	1. Entered torpor after 3 hr, T_A = 7°C; in torpor 31 hr, T_B = 7-8°C; arousal for 1 hr; re-entered torpor for 69 hr; removal and rewarmed at room temperature.

* Step, single and rebreath refer to method of reduction of oxygen content in chamber; step = sequential reductions in increments; single = direct change to reduced level; rebreath = reduction by animal in a sealed chamber.

** T_B = deep body temperature.

it was removed to room temperature to rewarm. No deaths resulted from experimental treatment.

Table 6 presents the temperature data derived from the species P. penicillatus. Only one animal was able to arouse from a deep body temperature of less than 10°C (9.9°C). Arousal from this temperature occurred after seven previous hypothermic experiences. When normal oxygen concentration was restored to animals with a deep body temperature of from 12-16°C, the rewarming trend did not occur or was delayed up to 1½ hrs. Three deaths occurred in this group during a relatively short residence of $T_B = \sim T_A$ (Table 6, #7, 19 December; #5, 16 November; #4, 8 November).

The data from experiments with P. baileyi are presented in Table 7. The lowest temperature from which this species rewarmed was 13.7°C. There is evidence, however, (Table 7, #5, 23-28 February; #2, 27 February; #7, 27-29 February) that this species can regulate its metabolism (and temperature) above some critical level during torpor in the presence of a much lower ambient temperature. When regulation during torpor is abandoned (i.e. Table 7, #7, 29 February) the animal assumes the ambient temperature and eventually dies. This regulation of depth of torpor was also observed once in P. amplus (Table 4, #8, 22 January). Death occurred, or probably would have occurred, in all animals that had body temperatures below 12-13°C.

Hypothermic Adaptation - It is difficult not to speculate on the effect of the previous hypothermic experiences of the animal that aroused from low temperatures. Would these animals have aroused from these low temperatures if they had been exposed to them without "benefit" of the previous arousals? There is evidence that the animal probably had gained some advantage through these trial exposures, an "adaptation to hypothermia." It has been demonstrated that white rats previously experiencing hypothermia have an improved operant behavior with regard to the acquisition of external heat. These experienced rats acquired an instrumental technique of artificially warming faster and from a lower body temperature (25°C vs 29°C) than the hypothermic novice (4).

Table 6. Hypothermia in *P. pencillatus*

Animal	Date of Hypoxic Exposure	Method:* 1. Step 2. Single 3. Rebreath	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm min	Maximum Warming Rate °C/min	Total Time to Normal T_B min	Remarks
#1	14 Nov	1	8.75	18.3	18.3	0	0.280	82	
	20 Nov	2	10.00	17.3	17.3	0	0.223	96	
	5 Dec	2	7.00	10.6	7.3	-	-	-	1. Run terminated after 1 hr; animal continued to cool at -0.055°C/min.
	7 Dec	2	10.00	14.1	12.0	20	-	-	1. Record erratic during rewarm.
T_A=5°C	13 Dec	1	9.00	9.9	5.7	-	-	-	1. Run terminated after 56 min; animal continued to cool at 0.075°C/min. Animal allowed to rewarm at room temperature (24°C).
T_A=5°C	14 Dec	2	9.00	12.7	9.9	94	0.140	307	1. Temperature stabilized for 83 min at minimum temperature.
	18 Dec	2	9.00	14.5	14.5	0	0.200	120	
	20 Dec	2	9.00	12.3	9.9	45	0.185	230	1. Entered spontaneous torpor after 10½ hr from induced hypothermia (T_B= ~T_A). No respiration, resuscitated, rewarmed at room temperature, survived.
#7	29 Nov	1	10.00	19.9	19.9	0	0.209	85	
	12 Dec	1	9.00	16.9	16.9	32	-	-	1. After initiating warming trend, took 140 min to warm 2.6°C; remainder of data lost.
	15 Dec	1	5.50	18.2	18.2	0	0.273	95	
	19 Dec	2	7.00	12.8	ambient	-	-	-	1. 145 min after normal air returned, animal dead. Cooling trend not halted after normal air returned.
#5	13 Nov	1	11.0	25.3	25.3	0	0.144	76	
	16 Nov	1	7.5	19.1	ambient	0	-	-	1. Animal found dead next morning; probably died about 3½ hr after air restored. Cooling trend only slightly halted by returning normal air.
#4	8 Nov	1	10.0	22.4	22.4	0	0.200	72	1. Entered spontaneous torpor (10½ hr after normal air returned). Found dead after 10 hr of torpor. Noted alive at T_B=9.5°C, 3½ hr in spontaneous torpor.
#3	15 Nov	Oxygen Conc. not Reduced.	21.0	17.1	17.1	-	-	-	1. Animal abnormal; later noted to have respirator difficulty.

* Step, single, and rebreath refer to method of reduction of oxygen content in chamber; step = sequential reductions in increments; single = direct change to reduced level; rebreath = reduction by animal in a sealed chamber.

** T_B = deep body temperature.

Table 7. Hypothermia in *P. baileyi*

Animal	Date of Hypoxic Exposure	Method:* 1. Step 2. Single 3. Rebreath	Final O_2 Conc %	T_B at Return to Normal O_2 °C	Lowest T_B** Attained °C	Time Lag to Rewarm min	Maximum Warming Rate °C/min	Total Time to Normal T_B min	Remarks
#5	17 Feb	1	6.75	17.6	17.6	20	0.148	248	
	22 Feb	spontaneous torpor	----	23.0	23.0	--	0.211	---	1. Spontaneous torpor with arousal before induced torpor.
	22 Feb	1	6.00	18.0	18.0	0	0.273	82	
	23 Feb	spontaneous torpor	----	----	17.4	--	0.230	--	1. Entered spontaneous torpor 7½ hr after return to normal air. 2. Animal maintained minimum T_B=15.3°C in T_A=7°C for 13 hr, arousal to normal T_B when disturbed and food presented.
	28 Feb	spontaneous torpor	----	----	14.4	--	0.251	--	1. Entered spontaneous torpor after 10 hr at 7°C, maintained minimum temperature of 14.4°C for 8 hr, returned to room temp to rewarm.
#1	19 Feb	1	7.25	17.0	16.3	100	0.160	388	
	24 Feb	1	7.00	12.3	7.5	---	-----	---	1. No change in cooling rate when normal air restored, dead before 15 hr (probably within several hr).
#2	21 Feb	1	9.50	14.0	7.5	---	-----	---	1. No change in cooling rate when normal air restored. 2. Removed after 3 hr, not breathing $T_B \rightleftharpoons T_A$, resuscitated under lamp, normal next morning.
	28 Feb	2	9.25	17.7	7.5	---	-----	---	1. Maintained at T_B=16°C for 6 hr when normal air restored, cooled to ambient, dead before 7 hr.
#7	20 Feb	1	6.25	14.0	13.7	10	0.214	160	
	26 Feb	1	7.50	20.5	20.5	0	0.284	67	1. Entered spontaneous torpor 9 hr after air restored.
	27 Feb	spontaneous torpor	----	----	16.5	--	0.189	---	1. Animal maintained minimum T_B= 16.5°C for 4 hr, aroused when disturbed to give food.
	29 Feb	1	6.50	18.6	18.6	0	0.318	76	1. Entered spontaneous torpor 6 hr after normal air returned, apparent regulation of depth of torpor for 15 hr, regulation abandoned, $T_B \rightleftharpoons T_A$, dead before 12 hr.

* Step, single and rebreath refer to method of reduction of oxygen content in chamber; step = sequential reductions in increments; single = direct change to reduce level; rebreath = reduction by animal in a sealed chamber.

** T_B deep body temperature

This "adaptation to hypothermia" may be the laboratory equivalent of the naturally occurring "test drops" observed in animals preparing for hibernation. A related phenomenon has been observed in P. longimembris, in that periods of torpor increase in length to the maximum value of 72 hr (5). There is a gradual increase in the length of a naturally occurring torpor, and appears to be a "temporal" adaptation to the extended hypothermic state.

The significance of the conditioning to hypothermic states may be important to the definition of the zone of response available to a particular species, but it seems to be unimportant in bridging the responses between potential hibernators and obligate homeotherms. It is highly improbable that a highly experienced white rat could assume the responses of even a novice facultative homeotherm in a hypothermic situation.

Maximum rewarming rates and body temperature - arousal time relationships. - The rate at which a torpid animal returns its body temperature to normal mammalian temperatures is a measure of the total integrated effort of all mechanisms of heat production and heat conservation, whether it be chemical, physiological or behavioral. The maximum rates of rewarming are used in this study as an index of the general ability of a species of pocket mice to regain thermostasis from a condition of induced hypothermia.

This maximum rate was determined by fitting a line to the steepest portion of the rewarming records which usually occurred within a 7-10°C range somewhere between a body temperature of 23-34°C. These maximum rates of rewarming for body temperatures below 22°C are given in Table 8 for the species studied.

The deep body temperatures in torpor and the total time to rewarm to ~ 37°C for the various species is given in Figure 1. It appears that parvus, amplus, and longimembris have time-temperature curves that at low temperatures are less asymptotic to the time axis than the other species, and means that arousal is possible within a rather well defined time period. The decision to include longimembris in this group is based on more complete knowledge of this species than could be implies from the data as presented.

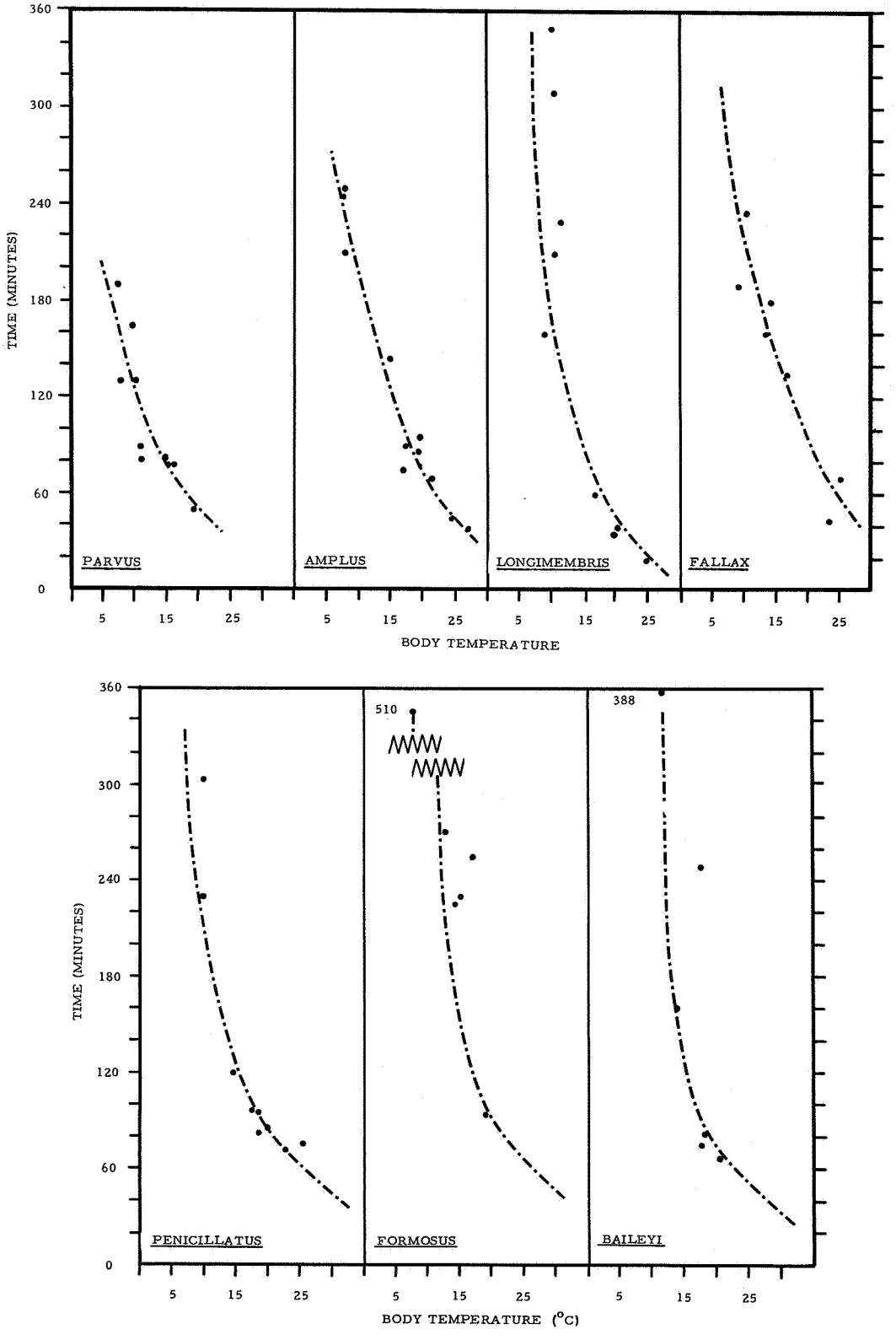

Figure 1 Deep body temperature in torpor and its relationship to total time required to rewarm to normal body temperature in various species of pocket mice.

A notable exception to this grouping is an arousal in formosus from 7.5°C which took 8½ hr to complete. No explanation is presented other than some members of the species have metabolic tenacity.

It is realized that maximum rates of warming analysis neglects the volume-area relationships but is sufficient to point out the variations in intrageneric warming rate between animals of even the same weight range. It would seem that the animals are divided into two groups; longimembris, amplus, parvus and fallax, formosus, penicillatus, baileyi. With the exception of formosus this dichotomy is evident in the taxonomy of this family.

The taxonomy of this group is family Heteromyidae, subfamily Perognathinae, genus Perognathus and two subgenera Perognathus and Chaetodipus. The subgenera are separated by pelage characteristics of either soft or harsh pelage and seveal other characteristics. Although formosus is classified with the soft pelage group (Perognathus) it is also an exception to the characteristics of the group by having naked soles on the hind feet and supraoccipitals with lateral indentations by mastoids. The species of the subgenus Chaetodipus are in general more southern in geographic distribution than those of the subgenus Perognathus.

It would appear that the taxonomic classification based on pelage is closely paralleled by the physiological measurements of ability to rewarm from body temperatures below normal mammalian temperatures. The apparent reduced efficiency of the rewarming process in the Chaetodipus group may be associated with impaired insulation (increased conductance) and not with the heat production per se.

A cursory low powered microscopic examination of the pelage of longimembris and baileyi as examples of both groups revealed definite variation in pattern of hair growth. Longimembris is characterized by small bundles of follicles and associated hair scattered over the surface of the skin in a rather close spaced uniform pattern. In contrast the follicles and associated hair in baileyi is arranged in linear groups of up to 8 follicles, with large open areas of skin in between the alternating linear

Table 8 - Maximum warming rate of various species of pocket mice (Perognathus) arousing from hypoxia induced hypothermia in an ambient temperature of 7°C.

Species	Wt	Range °C/min	Warming Rate $\bar{x} \pm$ S. E. °C/min	°C/hr/kg
P. longimembris	10.5	0.178-0.650	$0.359 \pm .015$	2048
P. amplus	16.9	0.230-0.400	$0.334 \pm .005$	1184
P. parvus	22.7	0.318-0.585	$0.416 \pm .009$	1098
P. fallax	26.2	0.222-0.433	$0.330 \pm .014$	754
P. formosus	20.7	0.103-0.270	$0.164 \pm .011$	475
P. penicillatus	25.8	0.140-0.280	$0.200 \pm .005$	464
P. baileyi	41.9	0.148-0.284	$0.213 \pm .006$	305

groups. This latter arrangement would seem to be a more effective arrangement for controlling dissipation of internal heat than effective retention.

The subgenus Chaetodipus seems to show pelage morphological adaptations which are not particularly suited to aid physiological processes of re-animation from reduced body temperatures. Although the ability to rewarm from various depths of torpor is found in species of this subgenera, their geographic distribution may be strongly regulated by the extreme of temperature encountered in their micro-environment. Moderate depths of torpor ($20 \pm 4°C$, estivation) seem to be compatible with the general physiology of this group. This is in contrast to the physiological adaptations of the Perognathus group (i.e., parvus, longimembris, amplus) which apparently can re-animate from deep torpor (5-8°C or lower) and can occupy geographic localities where the lower temperature limit of the micro-environment is less critical.

Extended residence at low body temperature. - The quest for the extension of a normal life span through the suppression or slowing of metabolic rate, i.e., suspended animation, is an ancient one and has taken on new interest in the space age. Although homeotherms can survive decreased body temperature there are severe temperature and time limits placed on survival. For example, a white rat lives 9-10 hr when cooled to a body temperature of 15°C (clinical survival). It can live only if rewarmed during the first 5 hr (biological survival). If the body temperature is in the 2-8°C range clinical survival is about 1.5 hrs. Although the exact cause of hypothermic death is not known, it is postulated to be associated with hemoconcentration.

The hibernator, whether it be a "classical" hibernation, i.e., ground squirrel, or a species of "facultative" homeotherms, i.e., pocket mice, must possess mechanisms to permit relatively long term low temperature survival. The clarification of these mechanisms of extended hypothermia may be in the intensive study of the reasons for periodic arousals of animals during extended periods of torpor.

The survival of pocket mice with reduced body temperatures varies from a few hours to well over one hundred and seems to be characteristic of the

species. An inspection of Tables 1 through 7 will reveal that time of hypothermic death follows the taxonomic dichotomy of the genus, again with the exception of formosus.

No deaths occurred in the Perognathus group with longimembris held at T_B = 7.5°C for as long as 36 hr in this series of experiments.

Earlier work had indicated the longimembris probably has a maximum induced hypothermic limit compatible with survival of near 36 hr. However, since that time, successful restoration of normal body temperature has been made after 86 hr at a body temperature of near 7.5°C. Three healthy survivors of 70, 72 and 86 hr have been maintained in the laboratory for a number of months. This upper value well surpasses the maximum duration of a natural hypothermic state so far observed in the laboratory under optimum conditions as to time of year, isolation and environmental temperature (5).

Species of parvus were observed in continuous torpor for as long as 69 hr with no fatalities and probably would have gone longer if run had not been terminated.

No deaths were observed in amplus, although one animal was held at a low body temperature for 141 hrs. Several observations on this animal during rewarming suggest that the mechanism of death in the group may be of a different nature than those suggested for death of homeotherms at low temperature (1). If blood was actually in the urine and not from some superficial irritation of the urethral area, it would indicate a deterioration of the membrane in the glomeruli of the kidney. The observation of profuse bleeding at an injection site implies slow clotting time and is in accord with observations of other hibernating species during dormancy.

Death occurred rather quickly among members of the Chaetodipus group. Although in most cases a precise time of clinical death could not be assigned, it appeared to be approximately 2-8 hrs after reaching a deep body temperature of 7-8°C. Time of death unfortunately can only be stated as "before 15 hours," in most cases.

In the case of fallax "biological death" was passed before 18 hours in that an animal was alive after 18 hours but died during the artificial rewarming process.

Summary

The data acquired on pocket mice, members of the genus Perognathus, indicate that the ability to spontaneously rewarm from artificially induced hypothermia is similar to that response usually associated with the "classical" hibernators (arousal from 11°C for ground squirrels vs 23°C for white rats). This response, however, is species associated, with a rather wide variation within the genus.

Survival time in deep hypothermia varies from a few hours to about 6 days within the genus. Both ability to rewarm at low temperature and survival in hypothermia seem to follow the taxonomic dichotomy at the subgenus level (Perognathus vs Chaetodipus). Several species gave strong indication that a well regulated metabolism could be maintained in moderate torpor in order to maintain the species above the critical temperature of rewarming.

The supposition that "torpor is genus wide" is probably true within specific limits.

Perognathus parvus may be an ideal subject for future work in the areas of circadian rhythm studies, hibernation and induced hypothermia. It has all the attributes of P. longimembris plus being large enough to accomodate the necessary instrumentation for sophisticated telemetry research.

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HEMOGLOBIN OXYGEN AFFINITY IN THE GENUS PEROGNATHUS

Page Hayden

The data acquired on pocket mice, members of the genus Perognathus, indicate that the ability to spontaneously rewarm from artificially induced hypothermia is similar to that response usually associated with the "classical" hibernators. However, there is rather wide variation in the rate of rewarming between different species (1).

These observations raise the question of possible correlations between oxygen-binding characteristics of the blood and the various degrees of faculative homeothermy observed in Perognathus. Of equal interest was the possibility of relating the oxygen-binding characteristics of the blood to the high resistance to ionizing radiation exhibited by several members of this genus (2).

The binding of oxygen to the hemoglobin molecule has certain characteristics that are described by the so-called oxygen dissociation curve of blood. The sigmoid shape of a typical dissociation curve is associated with the interaction of the iron contained in the 4 heme portions of the hemoglobin molecule. The most important single characteristic that is used to compare the dissociation curves of various animal species is the point (in concentration of oxygen expressed in mm Hg) at which half of the hemoglobin is saturated with oxygen ($Hb = HbO_2$). This value of T_{50} or $T_{\frac{1}{2}}$ sat is a measure of the relative degree of the tendency of the hemoglobin to join with oxygen in a loose association.

The affinity of the hemoglobin for oxygen is significant in two processes (a) the uptake of oxygen in the lungs and (b) the unloading of oxygen in the tissues. The examples of the shifting of the dissociation curves to the left (more affinity) of certain mammals at high altitude permit the hemoglobin to become fully saturated with oxygen at a partial pressure at which the blood of other mammls is only partially saturated (3). Another

example of the left displacement is the fetal blood as compared to that of the maternal organism (4). Similar relationships have been described for the developing chick and for the larva of the bullfrog as compared to the adult (5,6).

All of these animals have in common an environment of relative oxygen scarcity, which is being met by a blood adapted to take on a full load of oxygen at a lower oxygen pressure.

However, it has been documented that there is a characteristic shift of the curve to the right with the diminishing size (weight) of the animal which seems to be an adaptation to the metabolic need for oxygen and, therefore, directly related to the unloading of oxygen in the tissues (7). It has been calculated that oxygen must be supplied to the tissues of a mouse 15 times faster than to a horse. This increased need for high oxygen is in part met in the mouse by decreasing the diffusion distance from capillary to cell (increased capillaries per square millimeter cross section) and maintaining a higher difference in oxygen tension at the capillary and the cell (an increased capillary level).

Recent work on various species of rodents, as to position of dissociation curve and species ability to extract oxygen from hypoxic atmosphere tends to confirm the hypothesis of size-oxygen affinity relationship (8,9). Several notable exceptions existed and were explained on the basis of certain ecological factors in these species.

The effect of pH changes and the shifting of the dissociation curve indicates that the smaller the animal the greater the sensitivity to pH shift (10). It has also been observed that a decreasing oxygen affinity is accompanied by an increasing Bohr effect (11).

It has been hypothesized that mammals that hibernate have hemoglobin which bind oxygen at low pressures. This suggestion is complicated by the fact that temperature exerts a strong influence on the position of the dissociation curve. Human hemoglobin binds oxygen so tightly at low temperatures that it becomes almost useless as a carrier at 15°C (shifted to the left).

It was the aim of the present study to determine the dissociation curves for a number of species of pocket mice (Perognathus) and evaluate the results in the light of present knowledge concerning response to low body temperatures, natural torpor and response to ionizing radiation.

Materials and Methods

The evaluation of the oxygen binding capacity of the blood of the rodents required the development of micro-techniques to handle the small quantity of blood that was available from the smaller species (i.e., P. longimembris weighing 10 gm). The alternative was to pool blood aamples for the macro-techniques available. This, in turn, would require the exsanguination, and consequent sacrifice, of relatively large numbers of the smaller species (which were in limited numbers).

The micro-technique developed utilized the Natelson Microgasometer (Scientific Industries, Queens Village, N. Y.). The instrument is a modified and minaturized classical Van Slyke manometric apparatus for gas analysis. Gases are released from a liquid sample, and by selective absorption of specific gases and changes in total volume, at a standard pressure, the amount of a specific gas can be determined.

A 0.5 ml syringe that had been rinsed with heparin was used to take blood samples by cardiac puncture. All animals were lightly anesthetized with Metofane (Pitman-Moore) and given 0.4 ml of sterile physiological saline i.p. immediately before blood sampling. The blood sample was maintained in an ice bath prior to tonometric equilibrium with known concentration gases.

The tonometric vessels were 3 ml conical bottom plastic beakers (Auto Analyzer, Techincon Instrument Corp.) fitted with rubber dropper bulbs so that gases could be injected via hypodermic needles. The elongate squeeze portion of the bulb was filled with fine glass wool - saturated with water so that incoming dry gases would be humidified. The excurrent port needle penetrated the dropper near the beaker lip and extended approximately one-half way into the beaker to assure good mixing of the residual

and incurrent gases. All beakers were lined with a coating of silicon grease to present a hydrophobic surface on which to place the blood sample. Although 0.03 ml of blood was required by the analyzer, 0.05 ml of blood was placed in each tonometric vessel. Known concentration oxygen mixtures, varying from 10-90 mm partial pressure oxygen with 41 mm carbon dioxide and makeup nitrogen (obtained from and analyzed by Matheson Corp.) were passed through the tonometers in two 200 ml flushes separated by a 10-min equilibrium period. Total equilibrium time was 20 min in a 37.0°C constant temperature incubator. During equilibrium, the tonometers were rotated in a Bryan-Garrey blood pipette rotor. Before placing the blood samples in the microgasometer, they were covered with a 3 mm layer of de-aerated capyrlic alcohol injected into the sealed chamber.

All time schedules were rigidly observed to preserve a common temporal baseline. All microgasometric analyses were preceeded by a daily reagent and instrument check via determination of oxygen in air, and all blood sample determinations included a complete procedural check with a nitrogen-carbon dioxide blood equilibration.

Experimental values for oxygen content expressed a percent of maximum capacity of the blood, at the various concentrations of oxygen, were plotted on linear graph paper. A curve was fitted to the mean values (N=5, except P. californicus N=3), and the point at which half of the hemoglobin was saturated ($T_{\frac{1}{2}}$ sat) was determined.

It should be emphasized that all determinations were made on whole, undiluted and unbuffered blood at 41 mm CO_2 pressure. Thus the blood was kept as close to conditions existing in the living animal as practicable under experimental conditions, except for the inevitable aging of the sample during the several hours that elapsed during the determination of the dissocation curve.

Results and Discussion

Dissociation curves for 8 species of pocket mice are presented in Figure 1. All dissocation curves exhibit the characteristic sigmoid shape

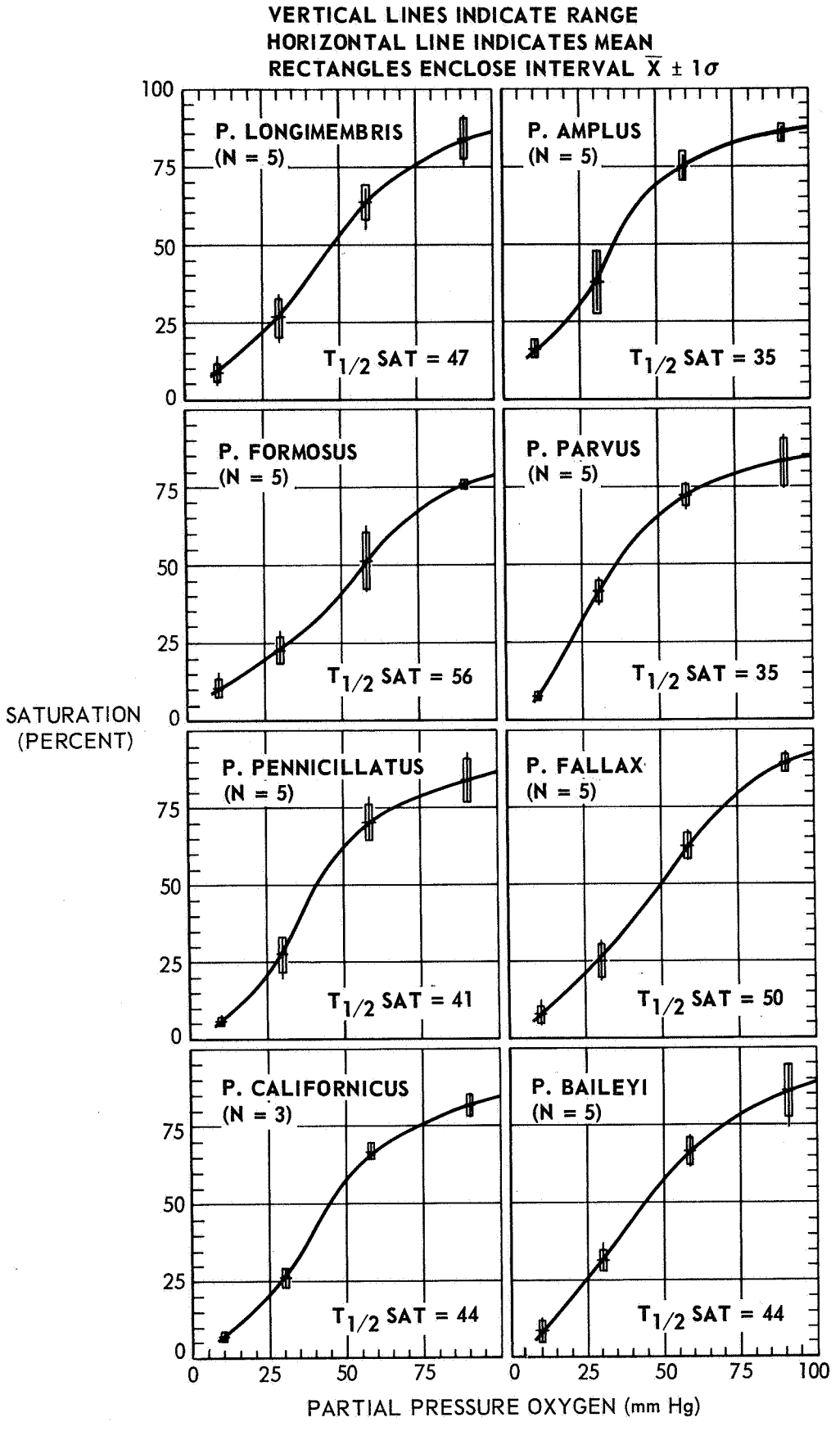

Figure 1. Oxygen dissociation curves of various species of pocket mice.

to varying degrees. Table 1 is a tabulation of the $T_{\frac{1}{2}}$ sat values for the species under consideration.

Table 1 Hemoglobin half saturation values for 8 species of pocket mice (Perognathus)

Species	No.	Weight (gm)	$T_{\frac{1}{2}}$ sat (mm Hg)
P. longimembris	5	10.5 ± 0.9	47
P. amplus	5	16.9 ± 3.4	35
P. formosus	5	20.7 ± 1.5	56
P. parvus	5	22.7 ± 6.7	35
P. penicillatus	5	25.8 ± 1.9	41
P. fallax	5	26.2 ± 3.4	50
P. californicus	3	30.4 ± 2.4	44
P. baileyi	5	41.9 ± 9.8	44

Studies of the ability of seven species of pocket mice to rewarm from hypoxia induced hypothermia have revealed that parvus, amplus and longimembris can arouse from deep hypothermia (deep body temperature = ~ 7°C). These same species apparently are able to sustain long-term reduced hypothermia.

The species formosus, fallax, penicillatus and baileyi all have rewarming limits of from 10-13°C. However, some individuals of formosus are able to rewarm from a deep body temperature of near 7°C, but with a reanimation time of near 8 hr.

The rate of rewarming and the long-term residence at low body temperature seem to follow the subgeneric classification of the Perognathus group and the Chaetodipus group.

It appears that the ½ saturation values (Table 1) do not follow the hypothermic response of the respective species. Parvus and amplus have low saturation values (high affinity) and good re-animation characteristics. Yet longimembris, apparently equal in low temperature characteristics, has one of the higher ½ saturation values (low affinity).

On the basis of preliminary work we postulated earlier that relatively high saturation values (47 to 56) would be advantageous to species at low temperatures. If the dissociation curve is shifted to the left with low temperature, those animals that require a relatively high partial pressure of oxygen (high $T_{\frac{1}{2}}$ sat) may have a dissociation curve that is still in the usable range at low temperature. This is opposed to those that have a high binding capacity (low $T_{\frac{1}{2}}$ sat) at normal body temperature but are unable to utilize the transported oxygen at low temperature because it is bound tightly to the hemoglobin. Apparently this is not a tenable postulate.

Therefore, we conclude that the hibernative characteristics of these species of rodents do not correlate directly with the oxygen binding characteristics of the blood.

It will be noted from Table 1 that there was only a four-fold change in body weight from the lightest to the heaviest species; yet there was a 60% change in the $T_{\frac{1}{2}}$ sat values (35-56). It would seem that this group of animals generally does fall within the expected range of $T_{\frac{1}{2}}$ sat values, from 10-40 gm should be 41 to 46 mm Hg (7). But several values fall outside the range with a $T_{\frac{1}{2}}$ of 35 mm equal to about 600 gm. The higher values of 47, 50, 56 agree fairly well with experimental values of white mouse, deer mouse, house mouse, kangaroo rat (49, 50, 52, 53 respectively, Table 2) (7,9).

The prairie dog has blood that has a great affinity for oxygen ($T_{\frac{1}{2}}$ sat = 22 mm Hg) and it has been hypothesized to be associated with its fossorial nature which may present hypoxic situations. However, the same paper ignores the kangaroo rat which is equally fossorial with a $T_{\frac{1}{2}}$ sat = 53 mm Hg. All of the pocket mice in the present study are fossorial and some spend considerable periods of time underground. The interpretation of the fossorial nature and high blood affinity for oxygen would be more complete if it were noted that prairie dogs are gregarious and live in colonies, as opposed to the other species which are solitary. It would seem that a social underground existence could result in hypoxic conditions.

Table 2 A Comparison of $T_{\frac{1}{2}}$ Sat Values of Various Rodents and Lagomorphs

		Weight gm	$T_{\frac{1}{2}}$ SAT: Schmidt-Nielson and Larimer 1958	$T_{\frac{1}{2}}$ SAT: Hall 1966
1.	White rat (Rattus norvegicus)	245	33	38
2.	Hamster (Mesorcricetus auratus)	88	29	29
3.	Cotton rat (Sigmodon hispidus)	162	40	39
4.	Guinea pig (Cavia porcellus)	375	34	30
5.	White mouse (Mus musculus)	30	49	--
6.	House mouse (Mus musculus)	18	--	52
7.	Deer mouse (Peromyscus sp.)	50	50	--
8.	Kangaroo rat (Dipodomys merriami)	47	--	53
9.	Jack rabbit (Lepus californicus)	2,045	--	23
10.	Cottontail rabbit (Sylvilagus floridanus)	2,242	--	31
11.	Prairie dog (Cynomys ludovicianus)	1,200	--	22

The resistance to ionizing radiation exhibited by several members of this genus, P. longimembris, P. formosus and P. parvus, apparently cannot be correlated directly with the oxygen transport system as delineated by the dissociation curve of whole blood. These resistant species of pocket mice fall into the extremes of the $T_{\frac{1}{2}}$ sat values 35 and 56, with the most resistant species, P. longimembris, in between.

Summary

1. The $T_{\frac{1}{2}}$ sat values of the oxygen dissociation curve of whole blood determined on eight species of pocket mice generally fall within the expected range based on size. The blood of P. parvus and P. amplus have a greater affinity, and P. formosus and P. fallax have a lesser affinity for oxygen than predicted.
2. Variation of hemoglobin-oxygen affinity at the species level is as great as the variation in intraspecific comparisons.

3. There appears to be no direct correlation between high blood-oxygen affinity and ability to re-warm from induced deep hypothermia in pocket mice.

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RESPONSE OF POCKET MICE TO ENVIRONMENTAL EXTREMES

The little pocket mouse, Perognathus longimembris, has been proposed for space biology research to study the stability of circadian systems divorced from terrestrial conditions. The problem of designing experiment hardware in which to conduct the experiment required a quantitative description of the environment which the hardware must maintain. Further, the significance of failure of the hardware to meet the requirements had to be determined in terms of the particular biological end point under study. This paper summarizes a series of pilot experiments undertaken primarily to obtain engineering data, but which nevertheless relate to the response of P. longimembris to environmental extremes.

I Water Balance and Food Consumption in Two Humidity Regimes

Members of the genus Perognathus although adapted to desert life, probably are not subjected to such extreme dryness as we suspect. A major portion of the day is spent in a plugged burrow where the humidity is near 100% and they venture out in the evening when the temperature is relatively low and the humidity high. The sensible and the insensible water loss are directly related to the environmental humidity and to the body temperature of the mouse.

During some laboratory experimentation, animals may be placed under conditions that cause a negative water balance with resultant dehydration and weight loss. Questions have arisen on several occasions as to the transient nature of this weight loss and if a completely dry environment is incompatible with survival of this non-water drinking species.

Materials and Methods. - Two groups of 10 mice each were selected at random from the animal holding facility and brought into the laboratory. One group was placed on the shelf and exposed to conditions of 50-60% relative humidity. The other was fitted with lids so that dry air could be supplied near the bottom of the bottle, and waste air exhausted at the top. The air was dried by passing it through magnesium perchlorate and was metered

to the individual bottles at about 200-250 ml/min from a manifold system. Humidity readings from a Serdex recording Hygrothermograph indicated essentially zero humidity at room temperature.

Each animal was given a weighed amount of sunflower seeds, wheat and rye seed. All animals were weighed every 3-4 days and food consumption was determined twice during the 46 days of the study.

Results and Discussion. - The weights of the two groups are plotted in Figure 1. Both groups lost weight rather rapidly at first but leveled out after a few days in their respective regimes. The animal holding facility has a higher humidity, in general, than in the laboratory and probably explains the weight loss of the group maintained on the shelf. The overall weight loss after 46 days of 7% and 11%, or a 4% greater weight loss of those maintained in dry air does not seem excessive. However, the possible method of maintaining this small difference is interesting. Table 1 is a summary of the kinds and amount of food eaten.

Table 1. Summary of kinds and amount of food eaten by two groups of pocket mice on different humidity regimes (Mean $\pm$ 1 SD).

Regime	No. of Mice	Avg.Wt. (gm)	Food Consumed			
			Total		per gm - mouse	
			Wheat & Rye	Sunflower	Wheat & Rye	Sunflower
Dry - < 1% RH	10	8.59	22.4$\pm$5.81	36.7$\pm$3.96	2.61$\pm$0.67	4.27$\pm$0.46
Normal - 50-60% RH	10	9.38	30.3$\pm$7.26	35.5$\pm$3.30	3.23$\pm$0.88	3.78$\pm$0.35

It would appear that the animals in dry air preferred more sunflower seeds in their diet than did those in normal air. The total food consumed in each case was nearly equal (6.88 gm/gm mouse-dry vs. 7.01 gm/gm mouse-normal). The preference for sunflower seeds in the low humidity group may

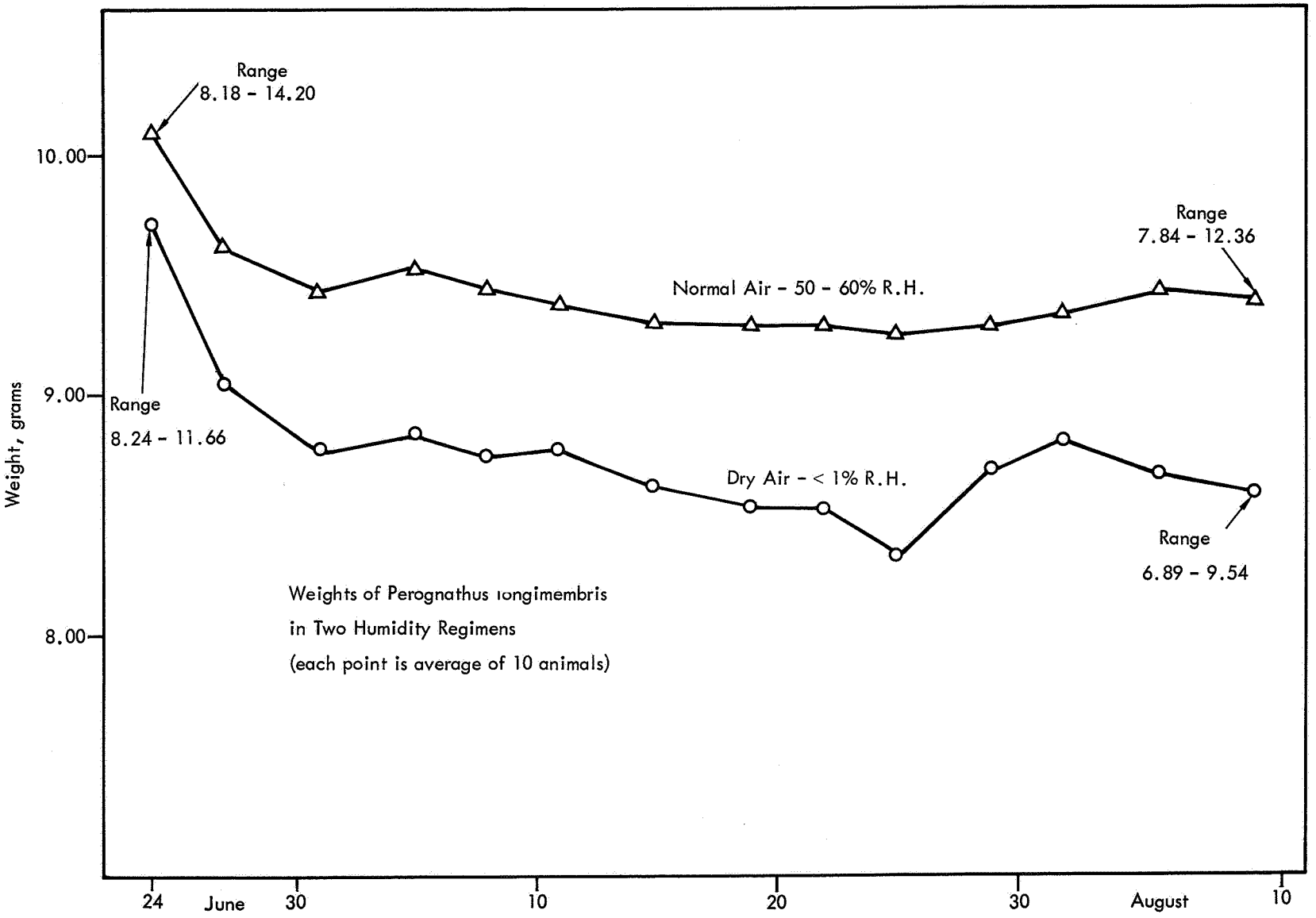

Figure 1 Weights of two groups of pocket mice maintained in different humidity regimes with excess food for a period of 46 days.

be a reflection of the greater demand for water. Sunflower seeds have a greater percentage oil content and would yield more water upon metabolism.

Summary. - Pocket mice can live in an essentially dry environment with relatively little weight loss, if an adequate diet of high oil content seeds is provided.

II Survival and Weight Loss in 100% Oxygen at Reduced Pressure

One solution to the problem of maintaining life in a viable atmosphere in a closed system, has been to use pure oxygen at reduced pressure to approximate the partial pressure of oxygen under normal conditions. The approach has certain engineering advantages. Except for minor difficulties, the absence of nitrogen and trace gases seem to have no short-term adverse effects. The reduced total pressure, however, increases the rate of water loss and could completely offset the balance in water metabolism of a non-drinking species of animal such as Perognathus sp.

The following discussions are summaries of various experiments that bear upon this general question.

100% O_2 at 5 psi, flow through O_2. - Two mice were maintained under reduced pressure for 17 days in lucite chambers 2 inch by 10 inch, with a flowing-through at 100 ml/min. Actually, 4 mice were started, but two were removed on the eighth day to provide more freedom of activity, and to more closely approximate the proposed design of a space experiment. The pressure was returned to normal four times for weight checks during the run. One of the animals removed on the eighth day had lost 36% of its initial weight (as opposed to about 20% for the other animals) and it died two days after removal. This animal initially weighed the least, and was noted to be excessively nervous and "jumpy".

The weights of the animals are plotted in Figure 2 at the termination of the experiment; the animals were 7% and 14% less than starting weight, and it appears that they would eventually regain lost weight. The data

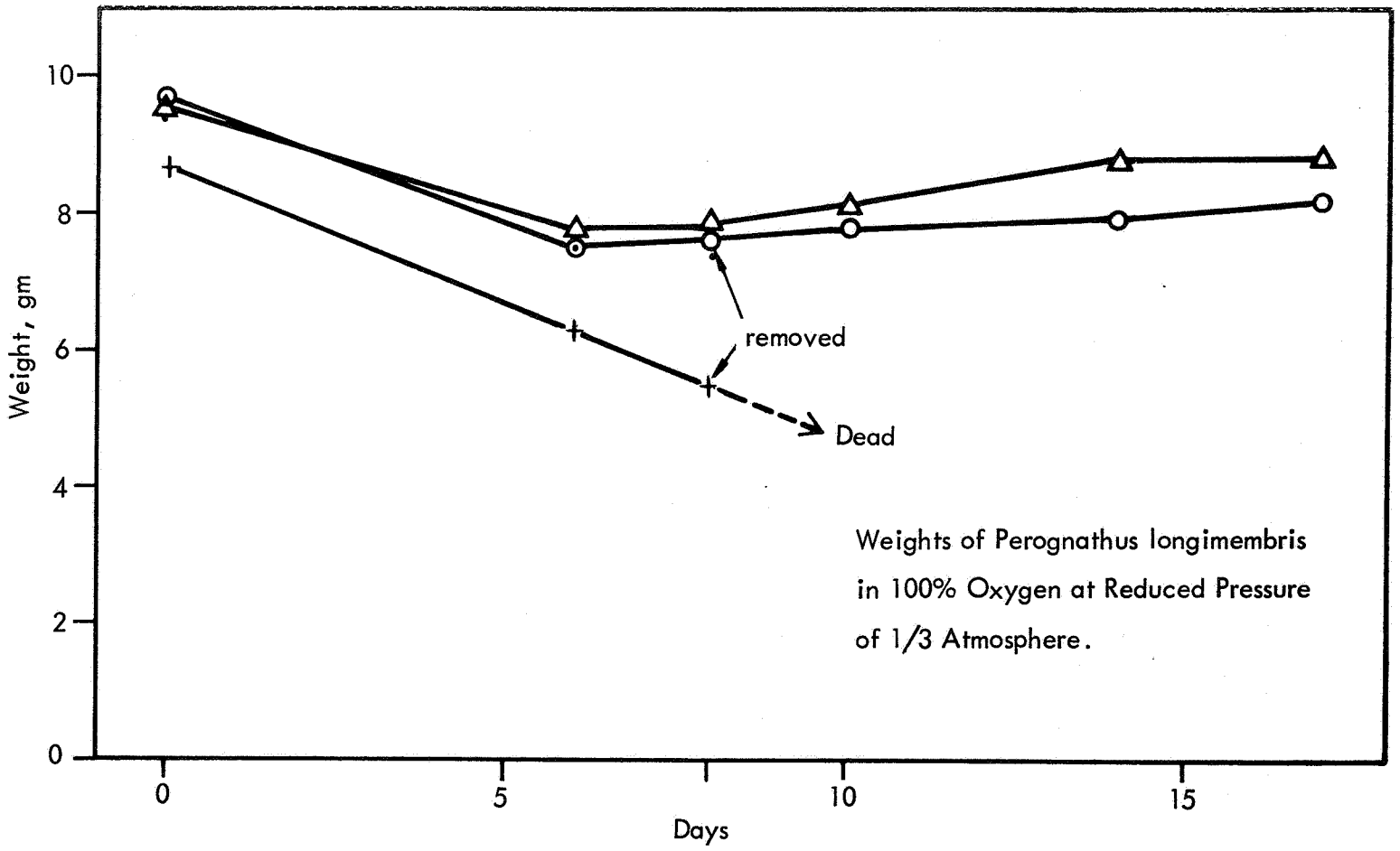

Figure 2 Weights of P. longimembris maintained in 100% oxygen, reduced pressure for 17 days. Two animals removed on 8th day, one of which was obviously distressed and died 2 days later.

suggest that there may be a minimum weight animal that can be used. Lightweight animals have no "fat buffer" that may be preferentially metabolized during an adaptation period. The light animals may become dehydrated and eventually die.

100% O_2 at 5 psi, no flow. - The possibility of using a single chemical compound to provide oxygen and remove carbon dioxide and water vapor from a sealed environment is very attractive from an engineering aspect. Potassium superoxide is this kind of chemical and has been explored for these possibilities. However, it has the disadvantage that a slight overproduction of oxygen occurs during its use.

The feasibility of using KO_2 as the environment-stabilizing agent with 100% O_2 at reduced pressure was tested. A series of nine chambers were constructed with porous plastic floors, so that potassium superoxide would be in close proximity to the animal. Boric acid powder and charcoal were also used to absorb or react with ammonia or other noxious gases. In one group of animals, a water supply and wick were added to try to aleviate the dehydrating effect of the superoxide. All chambers were flushed with 100% O_2 and were maintained at an absolute pressure of 5 psi via a cartesian diver and a vacuum source.

A 14-day experiment indicated that the group which contained the humidifier had the least weight loss (7% vs 11%) and all animals appeared to be in excellent health. Observations of animals during experiment indicated that periodic torpor was manifested by the rodents, and the amount of food remaining at the end of experiments corroborated this.

Initial experimentation using potassium superoxide with no control of atmosphere composition, gave data to document the stability of the circadian periodity of pocket mouse over a wide range of environment conditions, including oxygen concentration. Figure 3 and Table 2 are records of torpor in a sealed system with potassium superoxide.

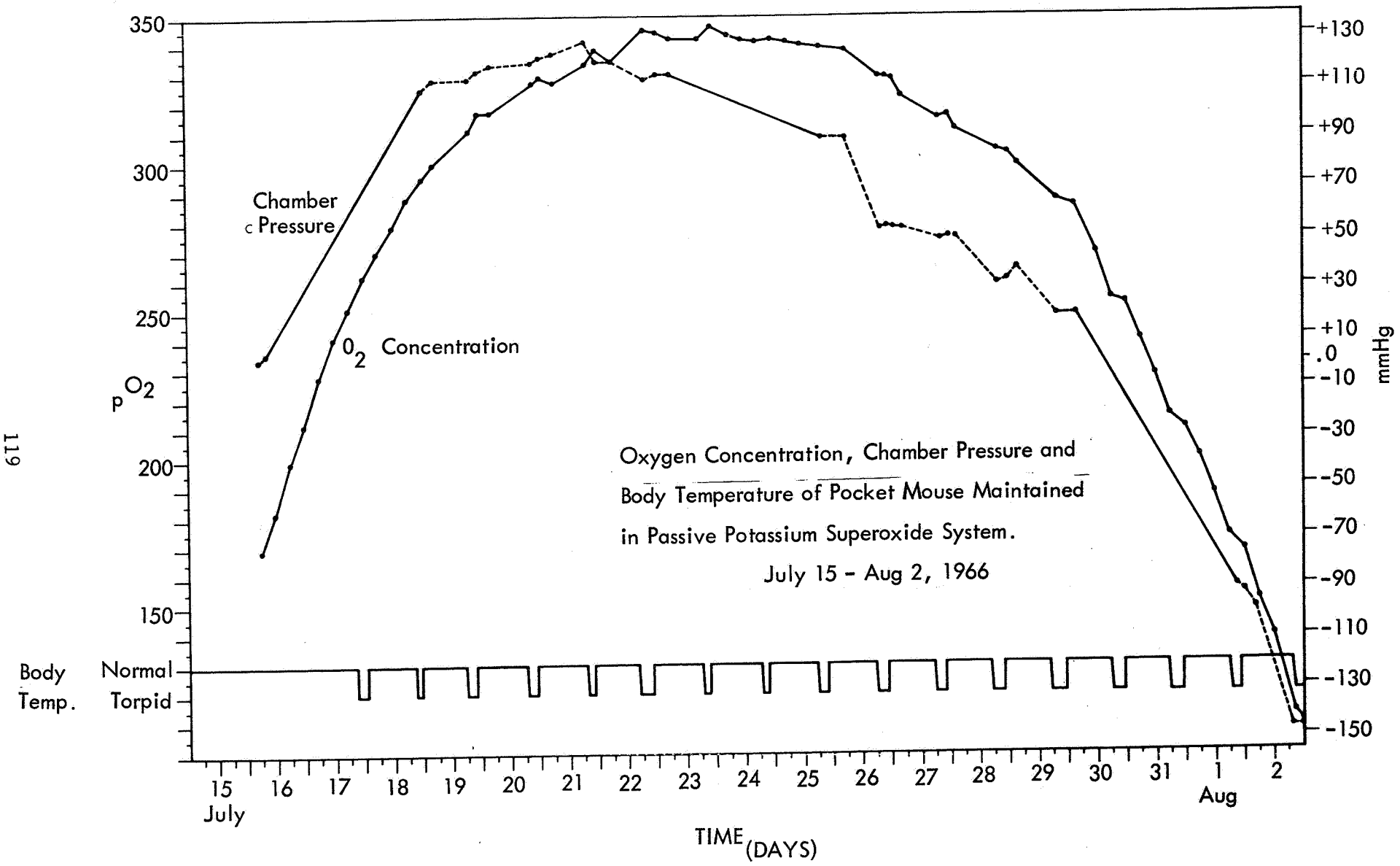

Figure 3 Atmospheric measurements and body temperature of a P. longimembris sealed in a chamber for 18 days with potassium superoxide as oxygen supply, carbon dioxide and water absorber.

Table 2 - Daily entry and arousal from spontaneous torpor of *Perognathus longimembris* maintained in closed system with potassium superoxide as oxygen supply.

Date	Time of Arousal	Duration of Torpor (min)	Oxygen Concentration
July 16	----	0	
17	1300	250	20.9 - 41.8%
18	1030	100	
19	1010	280	
July 20	1040	250	
21	1020	250	
22	1000	360	
23	0940	210	41.8 - 45.8%
24	0950	190	
25	1005	305	
26	1005	285	
27	1015	310	
July 28	1035	360	
29	1115	410	
30	1110	330	41.8 - 14.3%
31	1050	350	
Aug. 1	1000	260	
2	1100	240	

Mechanical control of atmosphere composition using KO_2. - If KO_2 is to be used in a mixed gas-system (nitrogen 80%, oxygen 20%) at normal pressure, the over-production of oxygen must be controlled in some manner. If excess pressure is bled off, the concentration of oxygen still increases. One approach to controlling the atmosphere is by using the pressure increase to mechanically decrease the production of oxygen (i.e., cover KO_2 source) and increase the absorption of CO_2, i.e., uncover a LiOH source. This was accomplished by sensing a pressure change with a rolling diaphragm device referenced to atmospheric pressure. Mechanical linkage to two reactive beds of KO_2 and LiOH controlled the concentrations of O_2 and CO_2.

The results were marginally successful, in that good control was maintained for short periods of time (1-2 days), but the porous nature of the diaphragm caused the gaseous atmosphere to shift concentrations during an extended period.

Summary - The specialized physiology of pocket mice seems to be amenable to experimentation in 100% oxygen at reduced pressure. Potassium superoxide can be used as an atmospheric regenerative source in a sealed system at reduced pressures. Water balance of this animal is probably stressed to near its limit, but biological adaptations apparently are made. However, a simple non-mechanical manipulation of water vapor can aleviate the dehydrating effect of the environment.

ANATOMY OF THE BRAIN OF PEROGNATHUS LONGIMEMBRIS

Kyllikki Grubel

When an animal enters hibernation, certain changes take place in its body functions. The body temperature decreases from normothermic levels, ~ 37°C, down to 0.5-2°C above the ambient temperature. Oxygen consumption and basal metabolic rate decline. Heart rate and respiratory rate decrease. The order in which these things can be observed to take place varies somewhat from species to species, but body temperatures appear to be subservient to the changes in respiration, heart rate or oxygen consumption (1). These phenomena are, however, only a result, not the cause, of entry into hibernation. Entry into hibernation is not a simple passive abandonment of temperature regulation, but rather thermoregulatory mechanisms become readjusted at this time. It has been suggested that these changes can be mediated only via the autonomic nervous system. Certain biochemical adjustments or a process of acclimatization takes place in the central nervous system of the prepared hibernator.

As hibernation ensues, certain specific structures or pathways are stimulated to regulate and coordinate the physiological changes as temperatures drop. Throughout hibernation, the peripheral nervous system appears to have an increased sensitivity to certain stimuli. During this period, certain subcortical areas remain functional, ensuring regulation of temperature and of cardiac and respiratory function, while the higher centers become reduced in activity but may maintain a certain minimal function (1). The temperature regulation is governed primarily by the thermodetectors of the hypothalamus, and it is possible that some biochemical changes take place in this area of the brain prior to the entry into hibernation. These speculative biochemical changes in the hypothalamus may be the triggering factor for entry into hibernation.

Much more factual information is available on the arousal phase of hibernation than on the entry phase. As arousal commences, the thoracic and brown fat temperature, heart rate, respiratory rate and cardiac output increase rapidly. Peripheral vasoconstriction restricts the increased

blood flow mostly to the heart muscle, brown fat, and respiratory muscles and possibly the brain (2). The thermogenic brown fat tissue rewarms the blood circulating through it (3), and aids in the rapid rewarming of the heart, brain and respiratory muscles. The important role of brown fat in arousal of hibernators was shown by Smith and Hock in marmots (4) and Smalley and Dryer in bat (5). It appears, though, that the activation of brown fat tissue is under nervous control (6), and that the thermoregulatory centers of hypothalamus trigger the spontaneous arousals. It is most likely that some biochemical changes take place in the hypothalamus during hibernation and that these changes would cause the initiation of arousal.

This report describes the anatomy of the brain of P. longimembris. The task was undertaken to provide orientation in the internal structures of the brain and to establish the feasibility of studying the neurological basis of entry into and arousal from hibernation using P. longimembris as an experimental animal.

Four adult mice of species Perognathus longimembris were lightly anesthetized with ether and then decapitated. The brains of the animals were extirpated, cut crosswise in two parts and placed in toluidine blue fixative and stain (7). After one week, the brains were frozen and sectioned at 50 μ. From the four sectioned brains, the best slices were chosen as representative of the brain of P. longimembris. From this series, photographs were taken from sections at 1 mm intervals. The location of each section is marked as a vertical line in the drawing of a mid-sagittal section of a brain (Fig. 1). Photographs and line drawings of the series are shown in Figures 2 through 8.

The small size of the pocket mouse precluded the use of standard stereotaxic apparatus to establish coordinates. The anatomical structures of the brain were identified, and the nomenclature is that of König and Klippel (8).

The anatomy of the brain of P. longimembris does not differ markedly from that of the albino rat. However, the small size of the animal and its brain dictates the need for development of special handling techniques and equipment if the species is to be used for neurophysiological research.

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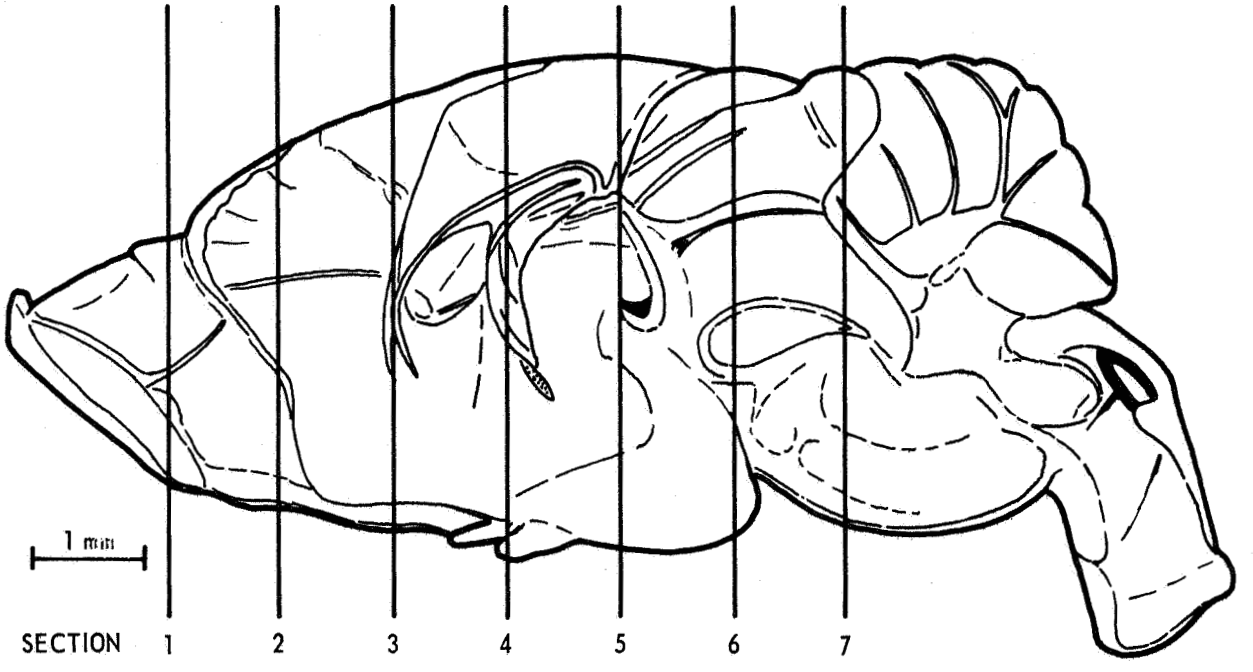
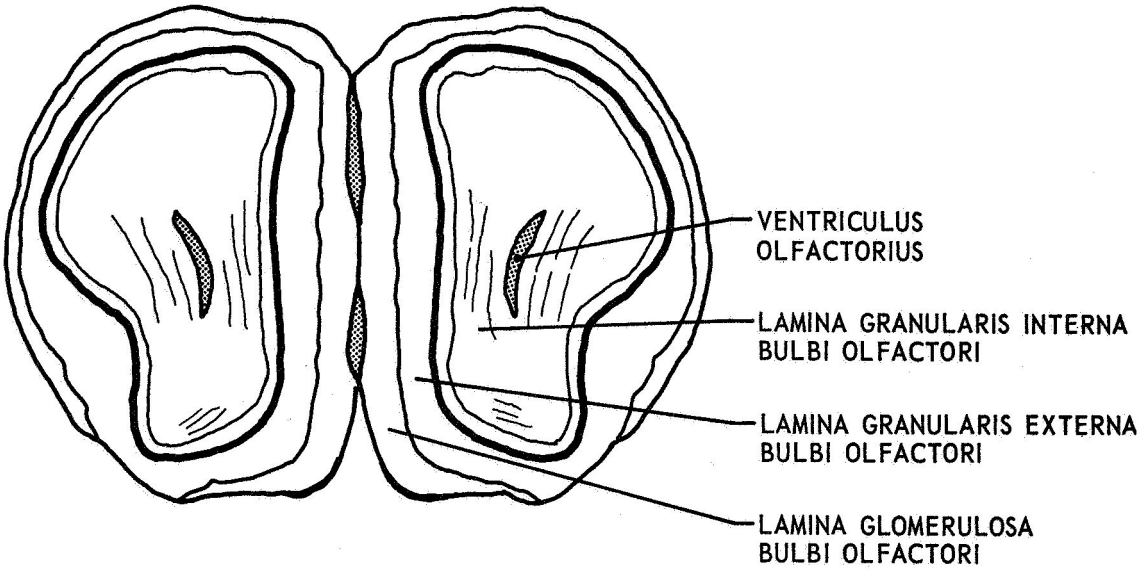


Figure 1 Drawing of a medial sagittal section of the brain of P. longimembris. Vertical lines 1-7 mark the corresponding cross section 1-7 (Figures 2 through 8).



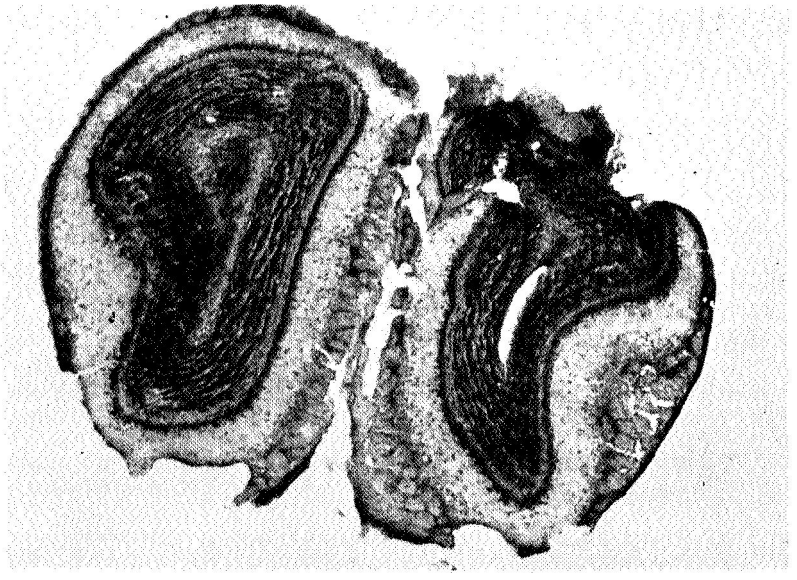


Figure 2 Brain cross section 1.

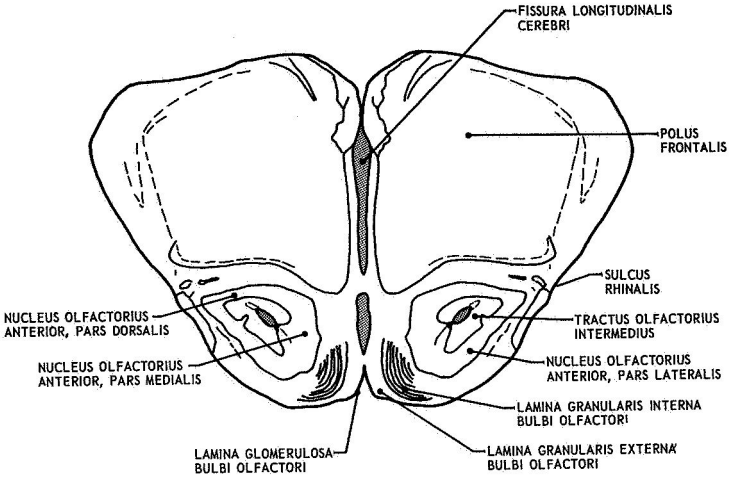


FIGURE 3

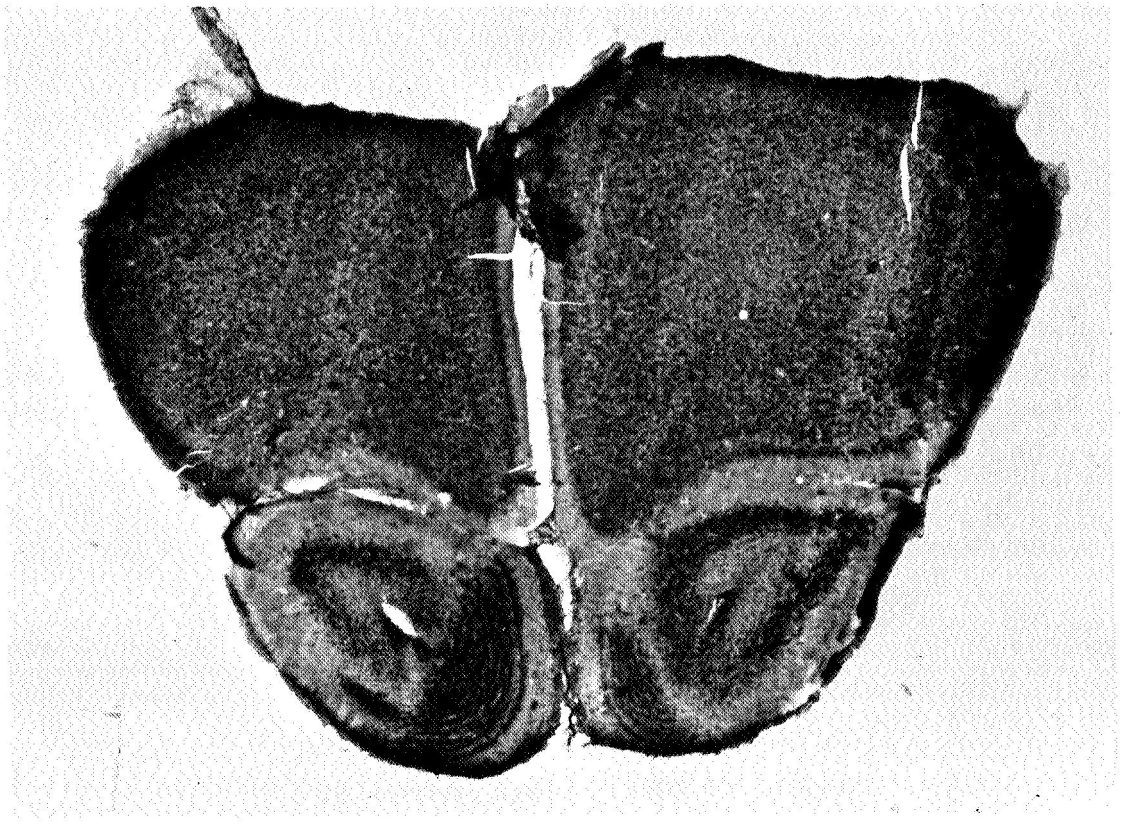


Figure 3 Brain cross section 2.

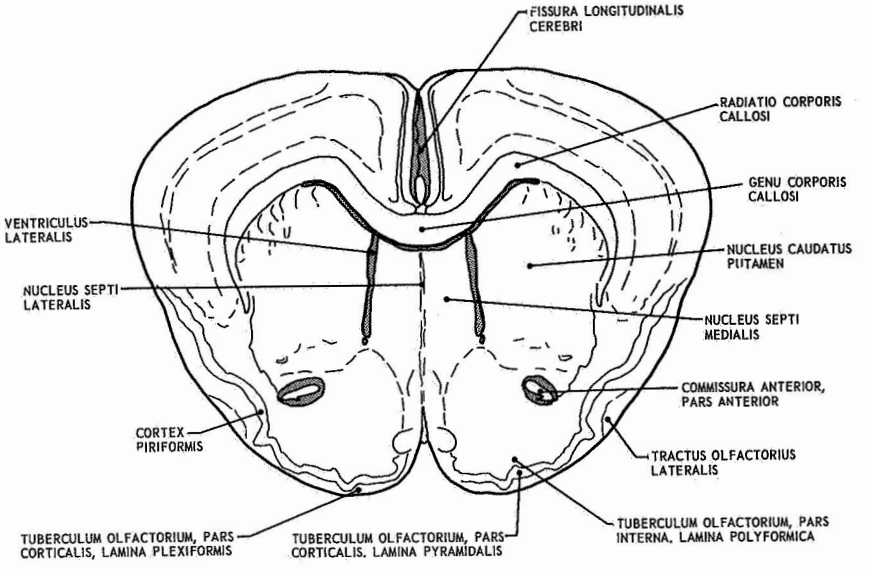


FIGURE 4

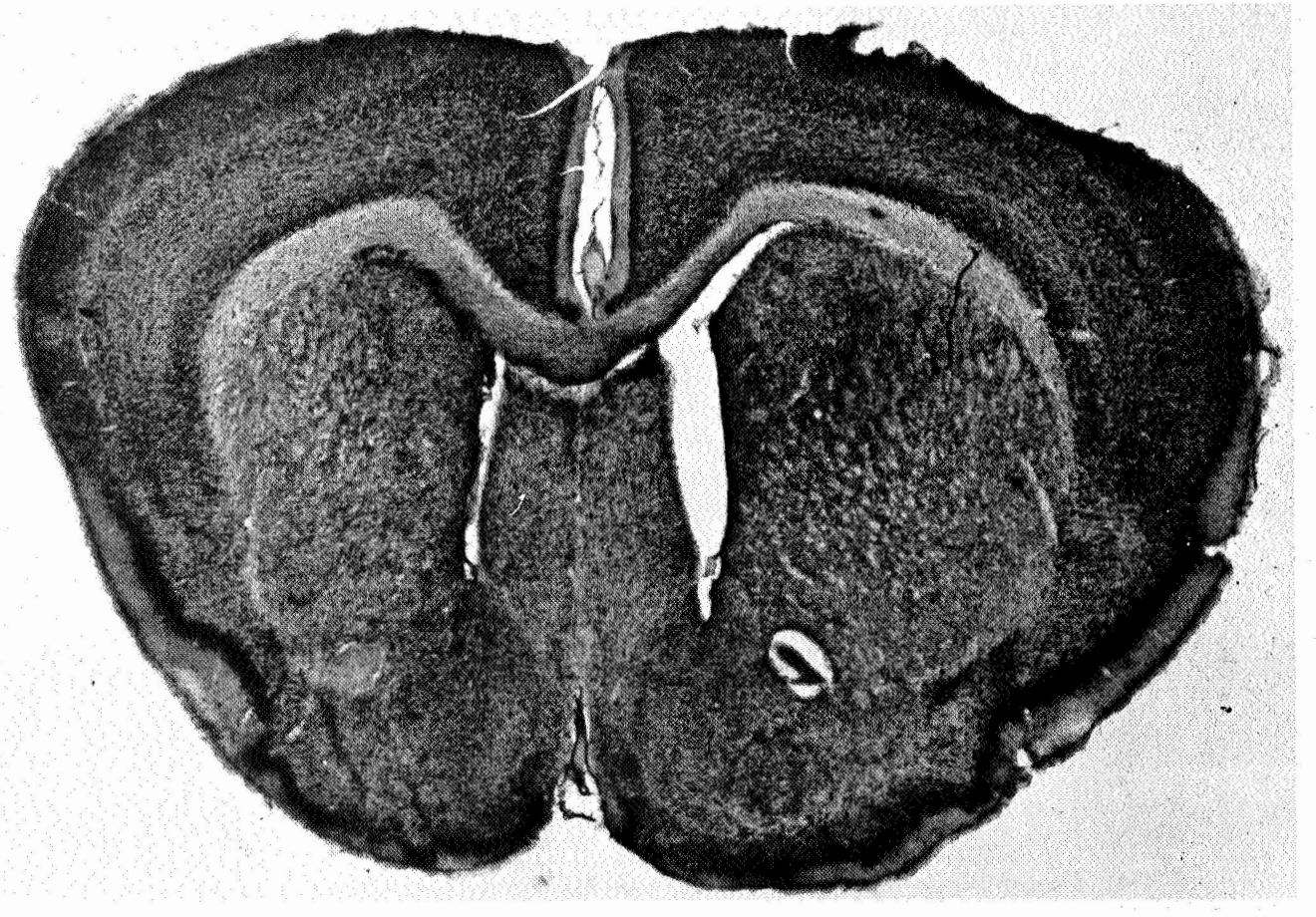
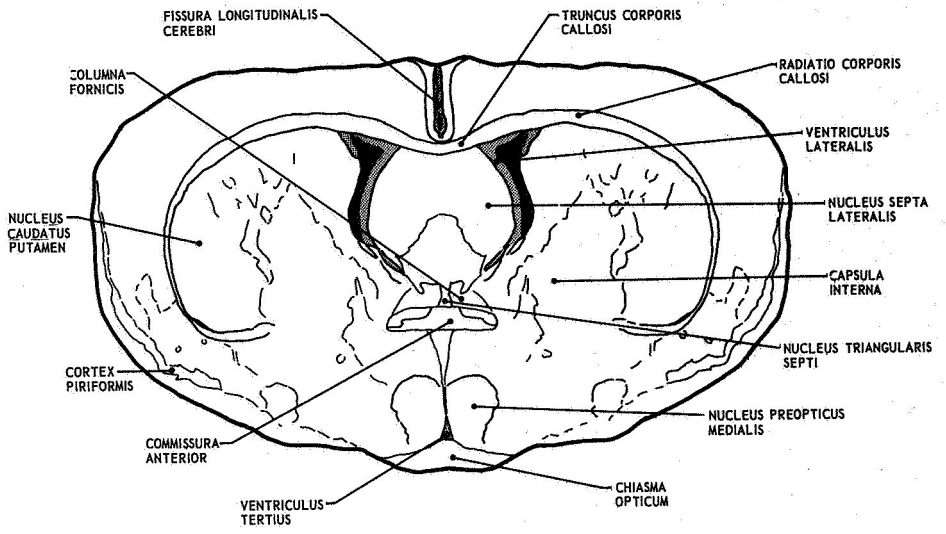


Figure 4 Brain cross section 3.



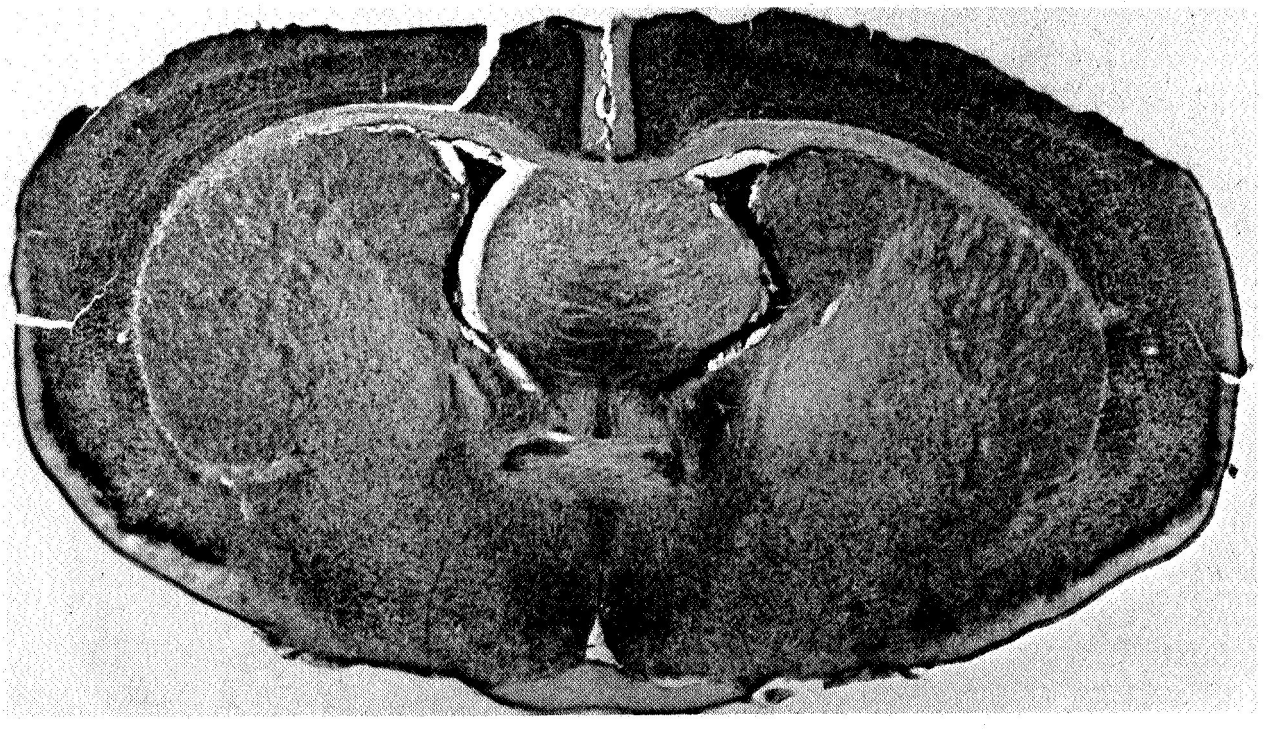
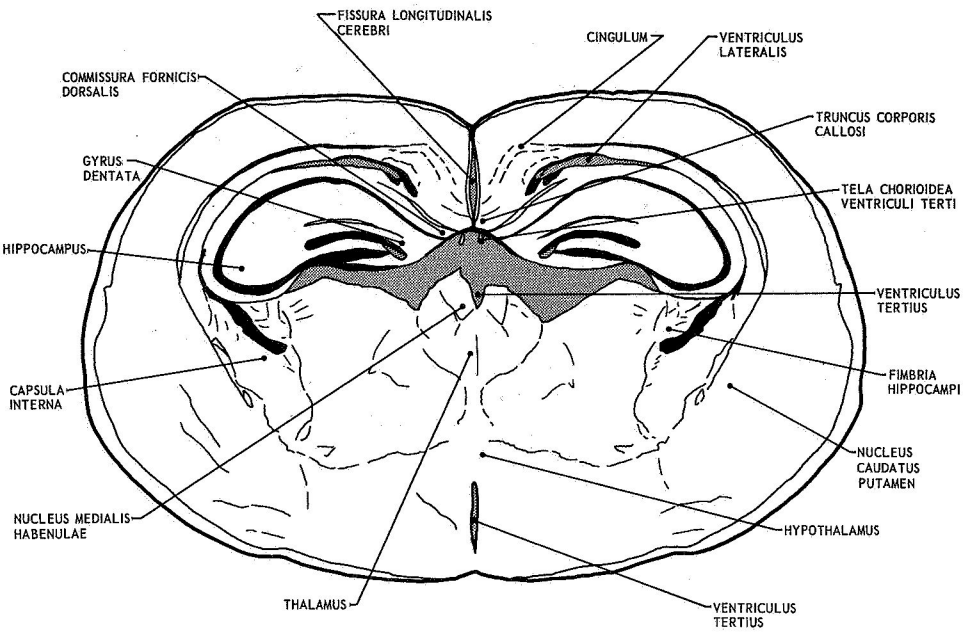


Figure 5 Brain cross section 4.



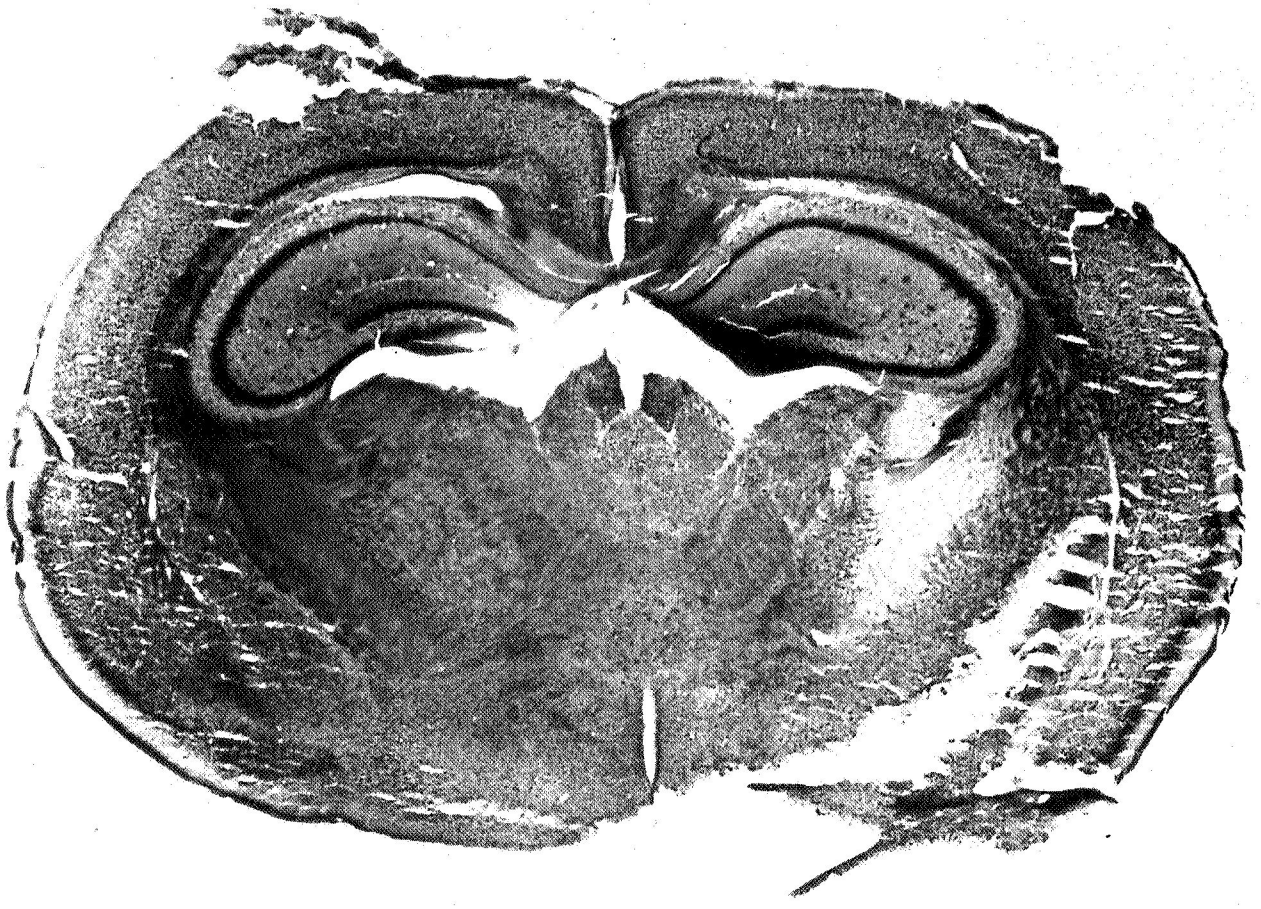
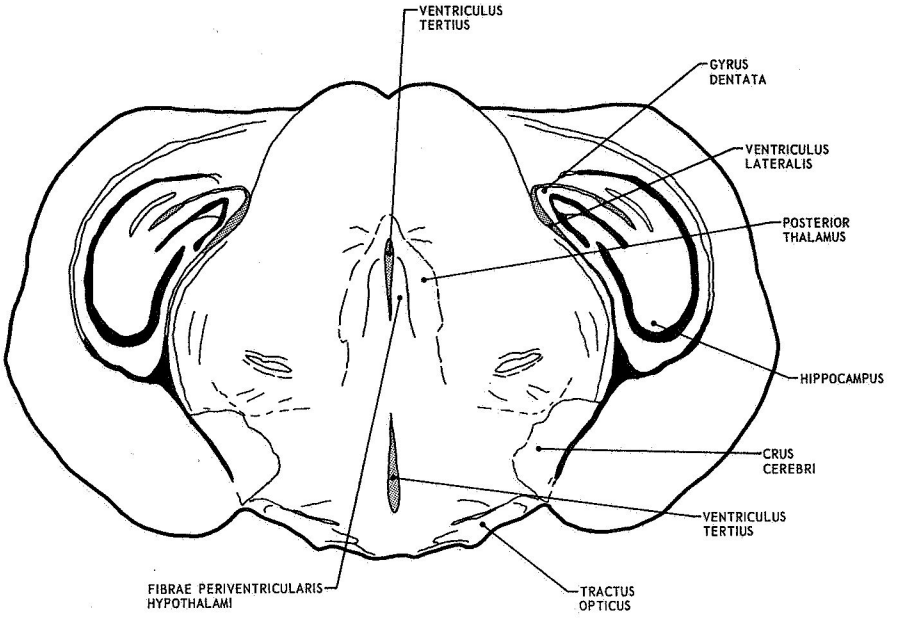


Figure 6 Brain cross section 5.



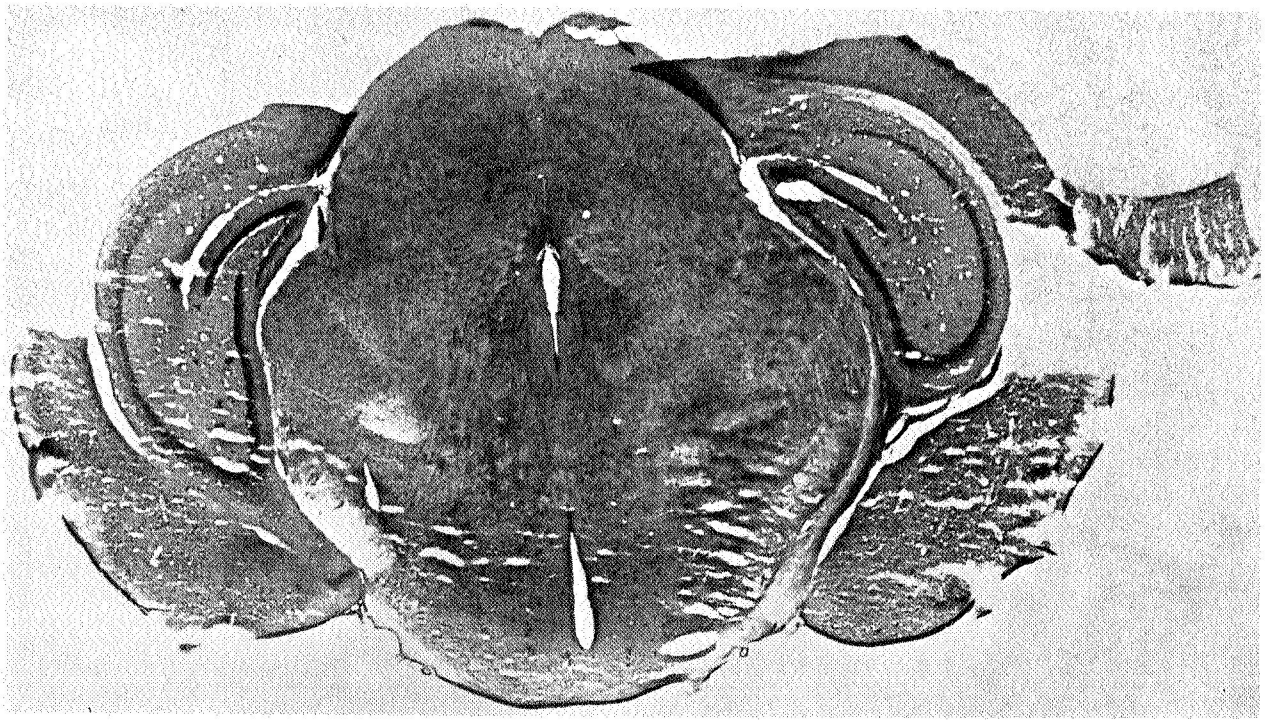
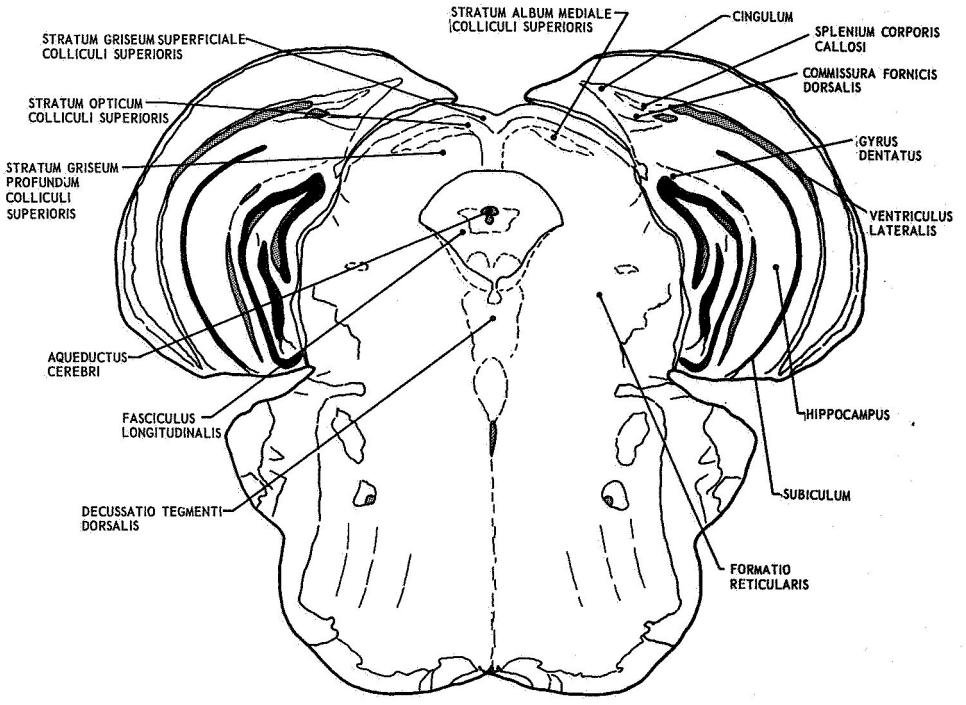


Figure 7 Brain cross section 6.



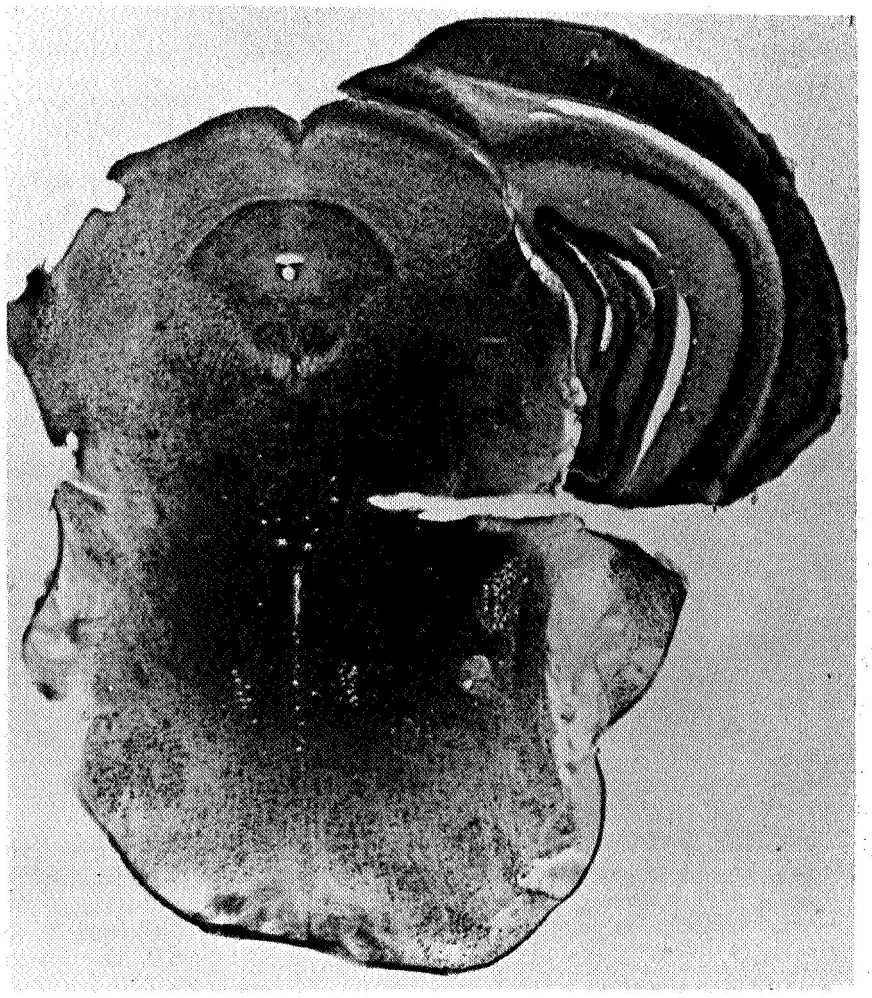


Figure 8 Brain cross section 7.

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CIRCADIAN RHYTHM OF METABOLIC RATE IN POCKET MICE

BY ROBERT M. CHEW, ROBERT G. LINDBERG AND PAGE HAYDEN

ABSTRACT: *Perognathus longimembris*, under a variety of conditions, demonstrated essentially a 24-hr rhythm in oxygen consumption. The amplitude, correlation with light cycle, and duration of periods of high and low metabolism varied. In September, mice provided with food and held at 22–24° C had a rhythm closely synchronized with a 12-hr light cycle (high in dark); mice were hypometabolic 12.5% of the days. In May, mice were more often out of synchrony and were hypometabolic 32.5% of the days. Without food at 22° C, mice became torpid every day; the first period of torpor began in the dark, and the rhythm of oxygen consumption was thereafter reversed. At 10° C without food, mice failed to arouse to a normal metabolic rate every day; sometimes they were deeply hypometabolic for 48–56 hr. A circadian rhythm of reduced amplitude continued, however, and arousals to normal rate occurred approximately on multiples of 24 hr. Arousals continued to occur in mice without food at 10° C despite exposure to or state of animals under the following conditions: constant darkness with or without sound, air with 4.5% CO_2, obesity, 9 months' prior conditioning to 10° C, 1,400 R acute whole-body gamma radiation. Mice acclimatized to 10° C had the shortest periods of normal metabolism and obese mice the longest. In *P. longimembris* (mean wt 10.7 g), *P. inornatus* (14.6 g) and *P. formosus* (20.5 g) at 10° C without food, frequency and extent of hypometabolism varied inversely with body weight.

INTRODUCTION

Many species of mammals have strictly defined nocturnal or diurnal activity patterns in nature. These patterns are one of the obvious factors of functional organization in biotic communities. In a few instances (e.g., Pearson, 1960)

activity rhythms of mammals in nature have been quantitatively studied. Many species have been studied in the laboratory (see Aschoff, 1962).

Usually there is an obvious adaptive phase relationship between rhythms of behavior and cycles of environmental factors, and the behavior patterns can shift in time to re-establish normal phase relationships with experimentally altered environmental factors. Activity rhythms are accompanied by rhythms of body temperature and metabolic rate. These and other rhythmic physiological processes have definite phase relationships (Halberg, 1960).

The rhythm of *Perognathus longimembris* is noteworthy because of the extreme relative amplitude of its cycle and the paucity of information on metabolic cycles *per se* in mammals. Pearson (1947) found night–day ratios of oxygen consumption ranging from 1.17 to 4.0 for seven species of small mammals that are rhythmically active and lesser ratios of 1.0 to 1.09 for species that are not. Circadian rhythms of metabolism have been demonstrated for *Perognathus californicus* (Tucker, 1962), *Myotis myotis* and *Glis glis* (Pohl, 1961) and *Citellus tereticaudus* (Hudson, 1964). Hart (1950, 1952) has studied the diel metabolic cycle of white mice, and Heusner (1957) the cycle of white rats. Bartholomew and Cade (1957) described the lability of body temperature of *P. logimembris*, and its invariable entry into torpidity when deprived of food. The characteristics of torpidity of a variety of rodents in the family Heteromyidae have been reviewed by Cade (1964).

Much work has been directed toward the investigation of timing mechanisms of biological rhythms (for example, Withrow, 1959; Cold Spring Harbor Symposia, 1960; Wolf, 1962; Aschoff, 1963; Bünning, 1964; Harker, 1964). The observation that a rhythm may persist in the absence of obvious environmental cues, such as light and temperature cycles, suggests that either the rhythm is inherent or is dependent upon less obvious environmental periodicities. The special metabolic characteristics of *Perognathus longimembris*, together with its small size, present a promising situation for experimental study of rhythms of endothermic organisms while they are in prolonged outer space residence beyond the "reach" of terrestrial cues.

MATERIALS AND METHODS

Live specimens of *P. longimembris* were collected at Whitewater Canyon, Deep Springs Valley, Barstow and Pearblossom, California, and from Lathrop Wells, Nevada. Specimens of *P. inornatus* were collected near Coalinga, California, and those of *P. formosus* from Lathrop Wells, Nevada. Mice were kept individually in gallon jars, with a substrate of sand or granulated absorbent clay. A mixture of parakeet seed, rolled oats and sunflower seed was provided in surplus; small amounts of vegetable greens were given occasionally. The animal room was kept at 20–24° C, 45–55% relative humidity, and at a photoperiod from 0600 to 1800 HR PST. Oxygen consumption was measured in two ways: (1) in an open-system with a Beckman G-2 Paramagnetic Oxygen Analyzer (POA) and (2) in a closed-system automatic manometric respirometer. The Beckman POA continuously measures and records the oxygen content

of an airstream passing from an animal chamber. A single animal or group of mice can be measured at a time. Oxygen consumption, as ml O_2/g hr, is calculated from the rate of airflow, change in oxygen concentration and animal weight. All oxygen values herein are corrected to 0° C and 760 mm Hg.

The respirometer has nine independent closed-system units. In each unit an oxygen-containing atmosphere is continuously circulated through the animal chamber and carbon dioxide and water vapor absorbers. As oxygen is consumed, the pressure drops within the system; after a unit drop in pressure, a sensing manometer triggers a solenoid system and oxygen is replenished or refilled. The time of each refilling is recorded by an event recorder. Oxygen consumption is calculated from the known volume of the unit refill, the number of refills per unit of time and the weight of the animal. Refill volumes of about 20 ml and 10.5 ml were used with 1,000 cc and 280 cc animal chambers, respectively. In both methods, the animal chambers are kept in a constant-temperature water bath.

Since the study was oriented toward the use of *Perognathus* for biosatellite experiments in which it is technically desirable to have a one-gas system, most of the measurements in the respirometer were made with an atmosphere varying from 80 to 90% oxygen and 10 to 20% nitrogen, and at 760 mm Hg pressure.

Some measurements were made with the mice kept in air. In a series of comparisons, there were no significant differences in the metabolic rates of mice kept for at least 7 days in either air or in 80 to 90% oxygen.

The respirometer method has the advantage of providing results on nine animals simultaneously; also, measurements can be made conveniently over as long a time as desired. However, the respirometer cannot measure short-term changes in oxygen consumption, since it is limited to the time needed for an animal to consume 10.5 or 20 ml of oxygen and thereby record an event. The Beckman POA method measures oxygen consumption continuously, so that all changes are recorded. The humidity of the airstream flowing through the chamber in the POA method can be regulated to any desired value, while only dry air can be used in the respirometer. The two methods of measurement are complementary and give comparable results.

The metabolism of *P. longimembris* was measured in several series of experiments. One series dealt with the effect of varying stress conditions on the metabolic rhythms of mice acclimatized to 20–24° C, a food surplus and a 12-hr photoperiod, when the mice were abruptly put under the measurement conditions. The stress sequence was: Group 1, moderate temperature (24° C), with food, in the fall of the year; Group 2, 22° C with food, in the spring; Group 3, high temperature (35° C), with food and water; Group 4, 22° C, without food; Group 5, low temperature (10° C), without food. In all these experiments a 12-hr photoperiod was continued.

In another series of experiments, several factors were tested for their ability to suppress the rhythm of animals acclimatized to 20–24° C and 12-hr photoperiod, when the mice were suddenly exposed to the double stress of 10° C

and starvation. Mice of Group 6 were chosen for high body weight and obesity, and put into continuous darkness; half of Group 7, also in continuous darkness, was isolated from external sounds, half was not isolated; Group 8 was continued on a 12-hr photoperiod, but was exposed to air with 4.5% CO_2. Mice of Group 9 were acclimatized to 10° C for 9 months, before being measured at 10°C without food.

Because of the probability of radiation exposure in space experiments, the effect of 1,400 R of acute whole body gamma radiation (Co^{60}) on the metabolic rhythm was tested in two groups. Group 10 was measured at 22° C, with food, after radiation; and Group 11 was measured at 35° C, with food and water. An ambient temperature of 35° C was used for Group 11 since the high temperature in itself would prevent hypometabolism due to hypothermia.

In order to obtain some idea of species differences in rhythms, particularly species of different body weights, *P. longimembris*, *P. inornatus* and *P. formosus* were compared under the same conditions of 10° C, no food, dry air atmosphere and continuous darkness. The three groups had mean body weights of 10.7, 14.6 and 20.5 g, respectively. Another group of *P. inornatus* was measured collectively under the same conditions, except in air saturated with water vapor.

When the change from one metabolic rate category to another is gradual, it is often impossible to exactly determine the beginning and end of successive low and high phases of a rhythm. This impreciseness magnifies our error in statistical comparisons; certain comparisons were analyzed for significance at the 5% level.

We have used the midpoints of periods of high and low metabolism in the calculation of periodicities. Midpoints can be estimated with greater reliability than the limits of a phase of metabolism, and are expected to be more constant than either the beginnings or ends of metabolic phases. When a pocket mouse has a normal metabolic rate for most of the time, then the midpoints of the metabolic lows are the most discrete events in the record. When a mouse is principally hypometabolic, then it is the periods of arousal that are the most conspicuous rhythmic events.

The threshold of hypometabolism is taken as the minimum maintenance metabolic rate. For *P. longimembris* this is calculated as: $Y = 10.127 - 0.279X$, where Y is ml O_2/g hr, and X is ambient temperature over the range of 2–32° C (Chew et al., 1963). Metabolic rate categories used herein are defined as follows:

Ambient temp. ° C	Metabolic rate categories, ml O_2/g hr			
	Normal metabolism	Shallow hypometabolism	Moderate hypometabolism	Deep hypometabolism
24	> 3.4	3.4–2.5	2.5–1.0	< 1.0
22	> 4.0	4.0–2.5	2.5–1.0	< 1.0
10	> 7.3	7.3–2.0	2.0–0.5	< 0.5

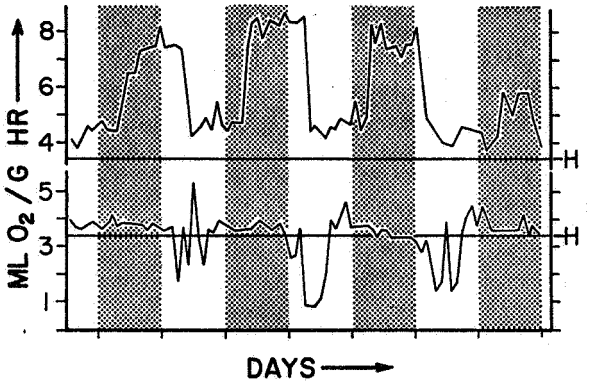

FIG. 1.—Metabolic rhythms of *P. longimembris* at 24° C with food. Two representative mice of Group 1; H is threshold of hypometabolism and dark bars are 12-hr periods of darkness.

RESULTS AND DISCUSSION

Experiments with Perognathus longimembris

Mice kept under "moderate" conditions.

The mice of Groups 1 and 2 (see Table 1) were kept at 24° and 22° C, respectively, with food, on a 12-hr photoperiod, in a dry atmosphere of 80 to 90% oxygen.

In Group 1, measured 6–10 September, six of seven mice showed a definite metabolic rhythm, in phase with the photoperiod. The mean interval between midpoints of metabolic lows was 23.9 hr. In all instances the daily minimum rate occurred during the light period. The mean midpoint of periods of low metabolism was at 1300 HR. In half the instances the entire period of low metabolism was within the light, while in half it persisted into the dark phase of the lighting cycle, but not longer than 3 hr.

As shown in Fig. 1, there were two types of rhythms with respect to amplitude. In one type the pocket mouse never became hypometabolic. At night, when the mouse was active, its metabolism was considerably above the hypometabolic threshold, while during the day metabolism declined abruptly to near this threshold. In the other pattern the animal became hypometabolic during one or more light periods. For Group 1 as a whole, metabolic rates dropped into the hypometabolic range during 12.5% of the periods of low metabolism. One of the seven mice in Group 1 did not show a metabolic rhythm. This animal died soon after the end of the measurements and is not included in Table 1.

In Group 2, measured from 30 April to 14 May, all nine mice showed circadian rhythms. The mean interval between midpoints of hypometabolic lows was 25.8 hr (SE $\pm$ 0.77); this is not significantly different from 24 hr. The

TABLE 1.—*Summary of measurements for different groups of* P. longimembris

Experimental conditions	Period of metabolic rhythm mean ± SE, in hr (range)	Mean duration in hr[f]	INCIDENCE OF PERIODS OF LOW METABOLISM, %				Incidence of hypometabolism % of days	Mean minimum hypometabolic rate ml O_2/g hr
			Within light	Light→ dark	Within dark	Dark→ light		
Group 1. 6–10 Sept. 1962. n = 6/7[a], 8.8 g[b], 24° C, with food, 12-hr photoperiod, dry 80–90% oxygen.	23.9 ± 0.28[e] (22.5–25.3)	Lows 9.1	50	50	0	0	12.5	
Group 2. 30 April–14 May 1963. n = 9/9, 9.0 g, 22° C, with food, 12-hr photoperiod, dry 80–90% oxygen.	25.8 ± 0.77[d] (15.1–33.7)	Lows 5.3	57	7	14	21	32.5	0.52 (0.33–0.82)
Group 3. 6–12 Nov. 1963. n = 7/7, 10.0 g, 35° C, with food and water, 12-hr photoperiod, dry air.	24.1 ± 1.02[e] (13.7–32.1)		90	0	11	0	0	
Group 4. 21–25 May 1963. n = 6/7, 9.1 g, 22° C, without food, 12-hr photoperiod, dry 80–90% oxygen.	24.8 ± 0.67[e] (21.0–28.9)		12	20	12	56	100	0.32 (0.13–0.46)
Group 5. 19–26 Feb. 1963. n = 6/9, 9.2 g, 10° C, without food, 12-hr photoperiod, 80–90% oxygen saturated with water vapor.	23.2 ± 0.57[e] (17.9–25.3)	PDH 50.0 (3) NM 5.7 (18)	18	47	35	0	100	0.12 (0.06–0.16)
Group 6. 31 July–7 Aug. 1963. n = 7/8, 10.7 g, 10° C, without food, continuous darkness, dry air.	23.7 ± 0.79[e] (20.3–27.9)	PDH 47.9 (5) NM 12.0 (8)	0	75	25	0	100	0.10 (0.06–0.10)
Group 7. 26 Nov.–4 Dec. 1962. 9.5 g, 10° C, without food, continuous darkness, dry air.								
a. isolated from sound. n = 4/4.	24.7 ± 0.28[e]						96.8	0.10 (0.08–0.12)
b. not isolated from sound. n = 4/4.	25.5 ± 0.79[e]						92.9	0.12 (0.09–0.16)

TABLE 1.—*Continued*

Experimental conditions	Period of metabolic rhythm mean ± SE, in hr (range)	Mean duration in hr[f]	INCIDENCE OF PERIODS OF LOW METABOLISM, %				Incidence of hypometabolism % of days	Mean minimum hypometabolic rate ml O_2/g hr
			Within light	Light→dark	Within dark	Dark→light		
Group 8. 19–25 March 1963. n = 5/6, 8.5 g, 6 mice in series, 10° C, without food, 12-hr photoperiod, nesting material, dry air with 4.5% CO_2.	22.5[e]							
Group 9. 25–30 July 1963. n = 5/8, 8.6 g, cold conditioned animals, 10° C, without food, 12-hr photoperiod, dry air.	23.9 ± 0.65[e] (20.9–26.8)	PDH 56.1 (5) NM 2.4 (9)	11	33	56	0	100	0.11 (0.05–0.20)
Group 10. 8.3 g, 22° C, with food, 12-hr photoperiod, dry 80–90% oxygen, 1,400 R whole body exposure.								
25 March–1 April 1953. n = 7/7.	24.6 ± 0.56[c]		14	24	8	54	54.8	0.48 (0.33–0.73)
1–8 April 1963. n = 8/8.	24.0 ± 0.49[c]						51.8	
Group 11. 9.2 g, 35° C, with food and water, 12-hr photoperiod, dry air, 1,400 R whole body exposure.								
a. 20–27 Nov. 1963. n = 8/8.	23.5 ± 0.99[c]		63	0	38	0	0	
b. 20 Nov.–18 Dec. 1963. n = 4/8.	24.0 ± 0.28[c]							

a—surviving number/initial number.
b—mean body wt at beginning of experiment.
c—period calculated on basis of midpoints of all phases of low metabolism whether within normal or hypometabolic range.
d—period calculated on basis of midpoints of hypometabolic periods only.
e—period calculated on basis of periods of deep hypometabolism only.
f—PDH, prolonged deep hypometabolism, sustained more than 24 hr; NM, normal metabolism; Lows, periods of low metabolic rate (usually within normal metabolism range).

incidence of hypometabolism was 30.2% the first week and 35.2% the second week. Individual mice varied in their frequency of hypometabolism from one to eight instances during the 2-week period.

For Group 2 as a whole, the midpoints of the hypometabolic periods averaged 1015 HR; 76.6% of the hypometabolic periods had their midpoints in the light and 57.1% were entirely within the light. However, in five mice which had sequences of daily hypometabolism, the intervals between midpoints were almost always greater than 24 hr; consequently the midpoints shifted forward in time. As a result 24.4% of hypometabolic periods had midpoints within the dark and 14.3% were completely within the dark. In about 35% of the days metabolism was clearly out of phase with lighting, occasionally as much as 180 degrees.

Group 2 was much more variable than Group 1. This suggests a seasonal variation in the metabolic lability of *P. longimembris*, independent of temperature and light. Chew et al. (1963) found that *P. longimembris* kept at 10° C for 9 months on a constant 12-hr photoperiod showed the greatest incidence of torpidity (i.e., lability) in the winter. The same has been found for *P. penicillatus* and *P. baileyi* (Hudson, 1964).

In nature, *P. longimembris* apparently spends long periods underground during the winter (Chew and Butterworth, 1964). There is no information on the metabolic state of the mice during such times. In limited laboratory experiments with *P. longimembris* kept at 10° C in aquaria with artificial burrows (thermos bottles), some mice did stay in their burrows for weeks. During this time they were not continuously torpid, but aroused every day or so, or remained normometabolic for days (Chew et al., 1963). Trapping results suggest that during late spring and summer *P. longimembris* are probably active on the surface every night. They then have daily contact with the natural light cycle and their activity can thus become entrained by this factor.

In Group 2, the greater incidence of hypometabolism coincided with greater variation of phasing of metabolism and lighting. This suggests that whenever there is changing lability of metabolism of *P. longimembris* in nature, such as is speculated to occur in spring, the occurrence of torpor may interfere with the entrainment of activity by photoperiod.

Mice kept in an abnormally warm environment.

Mice measured at 35° C (Group 3) had a rhythm with a period of 24.1 hr, in phase with the 12-hr photoperiod as in Group 1. The form of the metabolic curve was modified by the high ambient temperature. Usually, as shown in Fig. 2, the metabolic rate increased continuously during the dark period. The peak metabolism occurred either late in the dark period (35.5% of cycles) or early in the light period (64.5%). Metabolism decreased abruptly when the lights came on, or within the next 2 hr. In 89.9% of cycles the metabolic low occurred in the light, with a mean midpoint at 1405 HR.

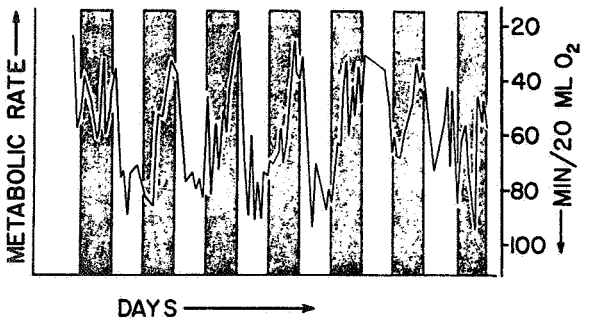

FIG. 2.—Metabolic rhythm of *P. longimembris* at 35° C with food. One representative mouse of Group 3. Dark bars are 12-hr periods of darkness. Metabolic rate is in terms of minutes (plotted inversely) for mouse to consume unit refill of oxygen (20 ml).

Since 35° C is near the upper limit of thermoneutrality of *P. longimembris* (Chew et al., 1963), the continuous rise of metabolism in darkness is undoubtedly the result of progressive hyperthermia in active animals. At 35° C, heat from muscle contraction added to basal heat production would increase body temperature. A rise in body temperature would further increase metabolism, hence leading toward a self-perpetuating trend. In no instance, however, did a mouse reach the point of uncontrolled hyperactivity, as occurs when *P. longimembris* become hyperthermic at ambient temperatures of 37–39° C. Hyperthermia could cause metabolism to remain high for some time after activity was reduced in response to light.

Mice under metabolic stress.

Deprivation of food.—The nine mice of Group 4 were subjected to the same moderate conditions as Group 2, except that they were not given any food. Seven of these mice had been included in Group 2 a week earlier, so a direct comparison is possible. There are several obvious contrasts. The mice became hypometabolic every day when deprived of food, as compared to 32.5% of days when with food. With food the seven mice had a mean minimum metabolic rate of 0.52 ml O_2/g hr (SE ± 0.068); without food the mean was 0.32 ml/g hr (SE ± 0.041). The difference is not statistically significant.

When they had food, the mice were hypometabolic principally in the daytime (64.2% of instances). Without food, however, five of six mice became hypometabolic during the first period of darkness, which began only 3 hr after the start of the experiment (see Fig. 3). One mouse remained normometabolic through this night and became hypometabolic the next day. Possibly starvation caused an advance in the occurrence of the first hypometabolic period. The metabolic rhythm then persisted at approximately this new "setting" for the 4 days of the experiment; 78% of subsequent periods of hypometabolism began in the dark.

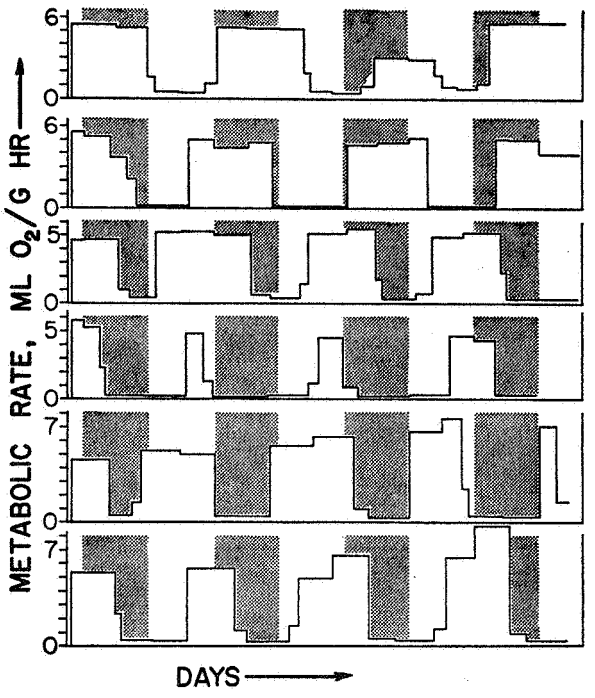

FIG. 3.—Metabolic rhythms of *P. longimembris* starved at 22° C. All surviving animals of Group 4. Dark bars are 12-hr periods of darkness.

In Group 4, the mean interval between midpoints of hypometabolic periods was 24.8 hr (SE ± 0.67).

One mouse died without arousing from a second, prolonged hypometabolic period. This animal had the highest initial metabolic rate of all mice in the group. Another mouse died 4 days after the end of the experiment. The fact that this animal had the lowest initial body weight, a high initial metabolic rate and a delayed entry into its first hypometabolic period may have contributed to its failure to recover from the stress of the experiment. These and similar observations of several other groups suggest that survival and recovery are enhanced by an early entry into a cycle of hypometabolic periods, before excessive use of energy reserves has occurred.

Deprivation of food and cold stress.—Group 5 was exposed to a double metabolic stress, the lack of food and the low ambient temperature of 10° C. The mice were kept in an atmosphere saturated with water vapor in order to reduce evaporative water loss, but this did not obviously increase survival.

In contrast to the mice that were subjected to only food-deprivation stress (Group 4), each mouse of Group 5 lost the pattern of daily arousals to a normal metabolic rate and was hypometabolic for more than a day on one or more

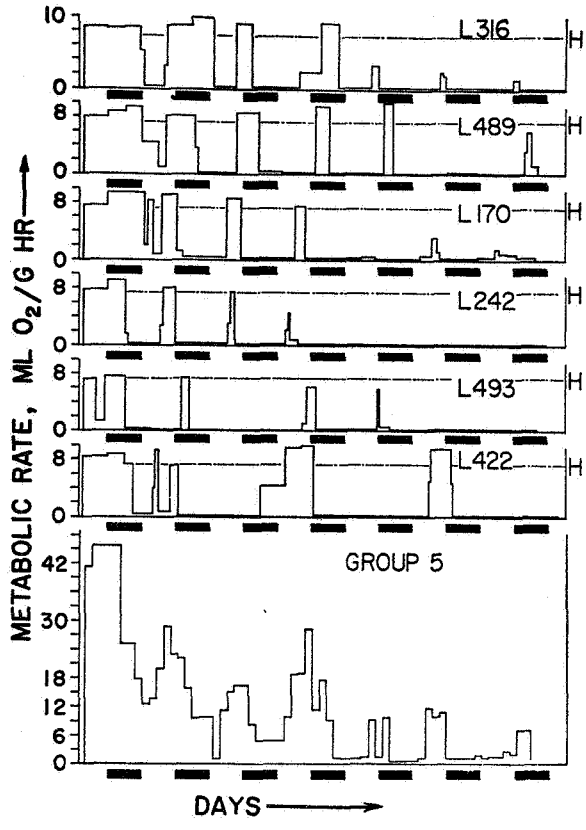

FIG. 4.—Metabolic rhythms of *P. longimembris* starved at 10° C. Surviving animals of Group 5. Summation graph at bottom and graphs of individual mice above. H is hypometabolic threshold. Dark bars are 12-hr periods of darkness.

occasions. The same thing occurred for mice of other groups kept at 10° C without food.

There were some instances when torpor was sustained more than 24 hr at a level of deep hypometabolism; the mean duration of these periods of prolonged deep hypometabolism (PDH) are given in Table 1. Usually, as shown in Fig. 4 and 5, the periods of deep hypometabolism were interrupted by periods of moderate hypometabolism. Thus, a circadian rhythm with suppressed amplitude is still discernible. These lesser peaks of metabolism were used along with peaks of normal metabolism in the calculations of periodicities of rhythms.

When torpor persisted without discernible rhythm, it was assumed that there was a fusion of two or more daily periods of deep torpor. For a rhythm with a 24-hr period, the interval between midpoints should be in the sequence of 36, 48, 60, 72 hr, as 1, 2, 3, 4 arousals are "skipped," respectively. Intervals approximating these durations occurred in the records for Groups 5, 6 and 9.

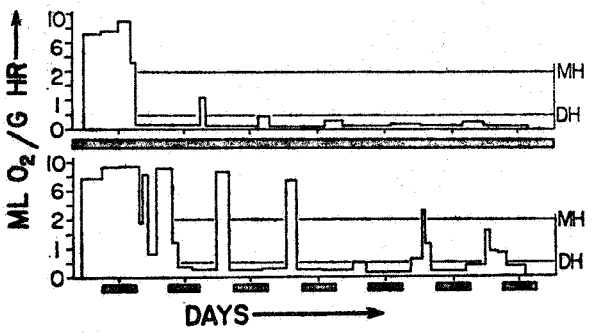

FIG. 5.—Suppressed metabolic rhythms of *P. longimembris* starved at 10° C. Two representative mice; upper graph of mouse in continuous darkness, lower of mouse on 12-hr photoperiod.

Periodicity was estimated in such instances by dividing the observed duration by 1.5, 2.0, 3.0, respectively.

The mice of Group 5 had a mean interval between midpoints of hypometabolism of 23.2 hr (SE ± 0.57). The depth of hypometabolism was significantly greater for the mice starving at 10° C than for those at 22° C, a mean minimum metabolic rate of 0.12 ml O_2/g hr (SE ± 0.016) as compared to 0.52 (SE ± 0.068).

For Group 5, the discrete periods of normal metabolism are the best basis for analysis of the phasing of metabolism and light. All six of the mice that survived the experiment entered their first torpor during the last half of the first night or the early hours of the next day. The 17 periods of normal metabolism that occurred after the first torpor may be classed as follows: six were completely within the 12-hr dark period (as normally expected); one began in the dark and continued into the light; three were completely in the light and seven began in the light and continued into the dark. Eleven of the midpoints of the normal metabolic periods were in the dark, while six were in the light. There is no definite entrainment of metabolic arousals by photoperiod; there is only a tendency for periods of normal metabolism to occur at night. In Group 5, as in Group 4, the first hypometabolic period may have "reset" the metabolic rhythm and shifted it out of phase with the light cycle.

Attempts to suppress metabolic rhythm.

None of the conditions tested prevented arousals from deep hypometabolism on a circadian rhythm. There were effects on amplitude and duration of high and low phases of metabolism.

Obesity.—Mice of Group 6 had a mean initial weight of 10.7 g (13–29% greater than Groups 5, 7 and 9). They had a mean duration of normal metabolic periods of 12.0 hr, significantly greater than the values for Groups 5 and 9.

Continuous darkness.—Continuous darkness did not significantly alter any of the measures studied (Table 1). If the metabolic rhythms of the mice were

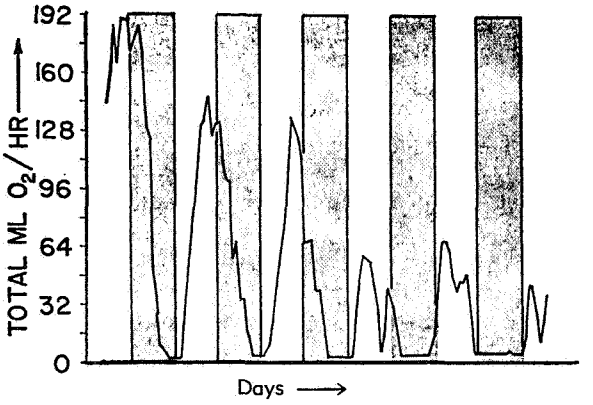

FIG. 6.—Metabolic rhythm of Group 8. Six *P. longimembris* starved at 10° C on 12-hr photoperiod in air with 4.5% CO_2. Metabolism measured collectively and plotted on group basis rather than per gram.

truly free-running in the dark, i.e., free from entrainment by light and other external factors, the rhythms were not significantly different from a 24-hr rhythm.

The most sensory-deprived mice, Group 7B, kept in continuous darkness and isolated from external noises, showed no loss of rhythm. One individual had one of the most precise 24-hr periodicities recorded.

High CO_2 atmosphere.—The metabolism of the six mice of Group 8 was measured collectively; the mice were in individual beakers connected in series. The mice were given nesting material, but no food, and were exposed to 4.5% carbon dioxide in dry air. Carbon dioxide has anesthetic properties, and Petter and Mostachfi (1957) speculated that its accumulation in a small nest chamber may induce torpor in the ground squirrel, *Spermophilopsis leptodacytlus*, in nature.

Five of the six mice survived the 6-day exposure. As shown in Fig. 6, a rhythm of arousal and torpor persisted. There was a progressive decline in the peaks of metabolism, presumably as some animals skipped a particular arousal or only partly aroused. A similar decline has been observed in other group-measurement experiments, with both high oxygen and air atmospheres. Probably six mice participated in the first recorded arousal and the equivalent of only one in the last arousal. The first metabolic peak began in the light and continued into the dark. Since the peak-to-peak interval averaged 22.5 hr, the metabolic peaks occurred progressively earlier in the light phase.

In Fig. 6, the sharpness of the metabolic peaks indicates a high degree of synchrony of the rhythms of individual mice; a secondary peak can be interpreted from the 4th day onward, with a period of 21.5 hr. Less synchrony is evident in the summed results of the individually measured mice of Group 5 (see Fig. 4).

Acclimatization to cold.—The mice of Group 9 were chosen from those kept for 9 months at 10° C, on the basis of their having had the higher incidences of torpidity during the last 4 months at 10° C (April to July). Without food these mice had periods of normal metabolism that lasted an average of only 2.35 hr, significantly shorter than for Groups 5, 6, 7 and 9.

During 9 months at 10° C in individual jars containing a small amount of nesting material and a surplus of food, individuals of *P. longimembris* were frequently tropid but repeatedly aroused. They had a very high survival rate since they were able to feed during arousals and thus restore their energy stores. All mice starved at 10° C during measurement periods had one or more periods of prolonged deep hypometabolism. The means for the duration of these periods ranged from 47.9 to 56.1 hr; maximum times for different groups ranged from 63 to 101 hr. During repeated arousals the starving animals depleted their energy reserves, sometimes to the point of going into prolonged torpor that ended in death (or presumably would have if the experiment had not been terminated).

Effect of irradiation.

The mice of Group 10 were irradiated just before the measurements were begun and then measured for 6.5 days, removed for weighing and then returned for a further 7-day measurement of metabolism. *P. longimembris* has an LD_{50} at 30 days of 1,510 R of ionizing radiation, in comparison to 628 R for CF_1 white mice (Gambino and Lindberg, 1964; Gambino et al., 1965).

The periodicity of the metabolic rhythm of the irradiated mice was not significantly different from 24 hr. The irradiated mice did show a greater incidence of hypometabolism than Group 2, 53.1% versus 32.5%. This may be the result of radiation damage, or it may be chance variation. Individual mice of Group 10 varied from 0% to 100% incidence of hypometabolic periods. Most of the hypometabolic periods began in the dark and ended in the light (54%), while for Group 2, most occurred entirely within the light (57%). Some of the irradiated mice showed a shift of their rhythm after being disturbed for weighing on the 7th day. The hypometabolism midpoints were an average of 2.8 hr later the second week. No shift of this magnitude was observed for Groups 2 and 11, which were similarly weighed in the middle of a 2-week measurement period.

Mice of Group 11, irradiated with 1,400 R and then kept at 35° C, are directly comparable with the control Group 3, measured just previously. All mice survived the first week after irradiation; four mice died after 8 or 9 days. The total group for the first week (Group 11A) and the four mice that survived for 4 weeks (Group 11B) had the same periodicity as the controls. The irradiated mice did have higher average maximum metabolic rates than the controls, 5.19 ml O_2 g/hr (SE ± 0.15) versus 4.45 ml O_2/g hr (SE ± 0.087).

Comparison of Perognathus *species*

Under conditions of 10° C, dry air, continuous darkness and no food, *P. longimembris, P. inornatus* and *P. formosus* all showed circadian metabolic

TABLE 2.—*Comparison of the metabolic rhythms of* P. longimembris, P. inornatus *and* P. formosus. *All groups measured at 10° C, without food, in continuous darkness and in dry air*

Species	Mean wt, g (range)	Rhythm period[b] mean ± SE, hr	Duration of PDH, hr	Deep hypometabolism, % of days
longimembris n = 7/8[a]	10.7 (10.5–11.7)	23.7 ± 0.79	47.9 (n = 5)	100
inornatus n = 4/4[d]	14.6 (13.0–17.4)	24.8 ± 0.75		83
formosus n = 2/8	20.5 (16.9–28.2)	25.0 ± 0.76		61

a—surviving number/initial number.
b—mean interval between midpoints of hypometabolic periods.
c—PDH, prolonged deep hypometabolic periods persisting more than 24 hr.
d—one animal died 2 days after end of experiment without arousing from torpor.

rhythms. The results (Table 2) suggest that metabolic lability decreases with body size. There is an inverse relationship between incidence of hypometabolism and body weight. In five instances, mice of the smallest species, *P. longimembris*, remained hypometabolic for more than 24 hr and then spontaneously aroused. There were no instances of prolonged torpor in the larger species, except those which ended in death.

Tolerance for hypometabolism at 10° C also may vary inversely with body weight. Six of eight *P. formosus* went into a sustained torpor and died after periods of 3.8 to 5.7 days. Of the two that survived, one showed a consistent circadian rhythm of deep torpor and arousal, while the other remained normometabolic. Tucker (1962) observed that *P. californicus* (mean wt 20.9 g) cannot arouse spontaneously when body temperatures are below 15° C. In another heteromyid genus, *Dipodomys, D. merriami* cannot recover from body temperatures below 12–15° C (Lipp and Folk, 1960).

The poor survival of *P. longimembris* at 10° C in some experiments suggests that 10° C is near the arousal threshold for the species. The present experiments are not a fair test, however, of what pocket mice can do if they have food available during arousal periods.

Another group of *P. inornatus* was kept without food at 10° C, in continuous darkness and in air saturated with water vapor. The mice were kept in individual chambers connected in series and were given nesting material. The record (Fig. 7) can be analyzed as a rhythm with 21–25-hr intervals (mean 23.2 hr). After the 3rd day a lessening of the synchronization of animals disrupted the clear-cut rhythm. There was a 100% incidence of deep hypometabolism. As with *P. longimembris* groups, there was a progressive decline in the metabolic peak each day as a result of failure of some animals to arouse daily.

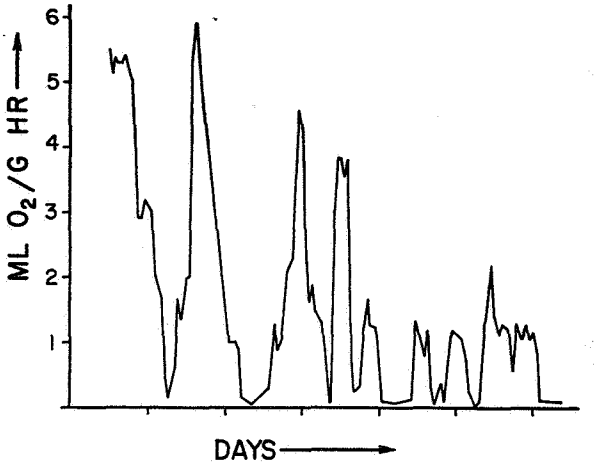

FIG. 7.—Metabolic rhythm of group of six *P. inornatus* starved at 10° C in continuous darkness in air saturated with water vapor, 20–26 Feb. 1963.

THE ADAPTIVE VALUES OF A PERSISTING 24-HR METABOLIC RHYTHM

A persistent rhythm which brings about arousals from torpor on a circadian period or multiple thereof can have physiological and behavioral advantages. Periodic arousals can insure the correction of physiological changes that occur during torpor.

The present records for *P. longimembris* showing suppressed circadian metabolic cycles in mice undergoing prolonged deep torpor (see Fig. 4 and 5) directly support the hypotheses of Folk (1957) and Folk et al. (1958) that: (1) certain species of hibernators have rhythms of deep and shallow hibernation; (2) when an arousal-stimulus coincides with the shallow phase, the animal may arouse; (3) if the metabolic rhythm is entrained by photoperiod before hibernation begins, the animal will tend to arouse to normal activity during that part of the diel environmental cycle to which it is adapted. Folk and his associates found circumstantial evidence for this in ground squirrels, *Citellus tridecemlineatus*. Records in Pohl (1961) show the same thing for the metabolism of hibernating dormice, *Glis glis*.

P. longimembris in the laboratory took 4–7 days to shift their motor activity pattern into phase with a reversed light-dark cycle (Hayden, 1965). Pocket mice in nature that happened to arouse from hibernation during the day could take as long to get back into synchrony. In the laboratory, motor activity persisted in "inappropriate" lighting situations until the phase shift of activity was completed. If the same thing occurred in nature, the disadvantage to the individual could be fatal

Present results strongly suggest that the stresses of starvation and cold exposure can upset the synchronization of metabolism (activity) and photoperiod. For example, "unstressed" Groups 1 and 3 had midpoints of high

metabolism (activity peaks) of 0100 and 0200 HR, respectively (which is normal), while stressed Groups 4, 5, 6 and 9 had midpoints of 1900, 1745, 1815 and 1820 HR, respectively (abnormal in that much of the activity occurred before the beginning of darkness). Usually the shift of midpoints was due to an early occurrence of the first hypometabolic period by about 6 hr, which "set the clock of activity forward" by this amount. Folk (1958) found a similar shifting of activity peaks by 6–10 hr in *C. tridecemlineatus* exposed to cold.

Such an effect of stress could decrease the survival of pocket mice in nature. However, an abrupt stress is probably infrequent in nature, particularly by starvation. When a stress develops gradually, as with the gradual decline of availablity of food, correct phasing of activity and lighting may be retained. A lack of synchronization in winter would not be critical for *P. longimembris* that remain in their burrows. However, a lack of synchronization in the spring, when mice are beginning to emerge from burrows, could be serious.

Theoretically it would be advantageous for a pocket mouse to be able to go into long-sustained deep torpor without arousals when food was not available. Avoidance of the energy expense of arousals would prolong survival. There is no evidence that such an ability exists. In the present study no mouse remained torpid for longer than 4.2 days. The work of Morrison (1960) suggests that the body size and metabolic characteristics of *P. longimembris* probably make it incapable of long-term torpidity from one "good" season to the next.

The pattern of relatively brief arousals from torpor on a 24-hr rhythm does allow effective conservation of a minimal food supply. For example, the mice of Group 6 were deeply hypometabolic for 78.8% of the experimental period, moderately hypothermic for 2.9% and normally metabolic for 19.1% of the time. The hypometabolism resulted in a saving of 80.4% of the estimated metabolic cost of maintaining a normal metabolic rate for the entire time.

ACKNOWLEDGMENTS

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THE STABILITY OF THE FREE-RUNNING CIRCADIAN PERIOD OF BODY TEMPERATURE IN THE LITTLE POCKET MOUSE (P. longimembris)

R. G. Lindberg and P. Hayden

Introduction

The occurrence of diurnal periodicity in the metabolic rate of pocket mice has been well documented (1,2,3). This periodicity is frequently manifest as a deep torpor in which body temperature approaches ambient. The phenomenon is a particularly useful marker for analytical studies of circadian periodicity and requires a minimal statistical test for establishing both the precision and persistence of the rhythm as a function of various environmental variables.

The manifestation of a persistent biological rhythm under constant conditions is analogous to self-sustaining oscillations under constant conditions experienced in physical systems. In biology they are referred to as "free running" periods and those with periods approximating the period of the earth's rotation are designated "circadian" (4). Characteristically, circadian periods are innate and precise but are not a fixed characteristic of the individual organism. These circadian rhythms, however, are open to spontaneous and induced shifts within a range typical of the species.

Period lengths in pocket mice have been established for the species Perognathus longimembris and P. formosus. Analysis of periods of activity in 25 P. longimembris showed that 68% had a circadian period of activity between 23-24 hr, with a maximum range in the population between 21 and 24 hr. In the case of 12 P. formosus 75% had activity periods between 23 hr 40 min and 24 hr 20 min, with a maximum range in the population of 21 hr 45 min and 24 hr 20 min.

The purpose of this study was to better define the free-running period of Perognathus longimembris and to determine the stability of the rhythm when the mice were contained in prototype experiment hardware. This hardware was designed to study the effects of space residence on circadian systems.

Methods

Measurement of Circadian Period (τ). - Changes in metabolic rate were studied first in terms of oxygen consumption. The methods and results are summarized in the literature (1). Representative metabolic rhythm of oxygen consumption derived from this technique for one P. longimembris under constant conditions (i.e. free-running in constant dark) is given in Figure 1. This figure depicts the arousal from torpor as a discrete event moving across the 24-hour "window" of observation. While the data provided ample evidence of circadian periodicity, the respirometric procedures used did not provide adequate resolution of the rate of oxygen consumption during non-torpid periods and this monitoring approach was abandoned.

A small temperature transmitter was developed by Northrop Corporation early in this contract and used extensively to study thermoregulation in pocket mice (5). This transmitter implanted in the abdominal cavity provided a means of continuously monitoring body temperature with an accuracy of $\sim 0.1°C$ for a period of several months (6). This monitoring method was used for subsequent studies of circadian periodicity of body temperature in pocket mice. Figure 2 is an example of the kind of data obtained with this system in which temperature data are collected at ten-minute intervals. The figure also shows the primary marker used (i.e., midpoint of arousal), and the manner in which the time at which the event occurs can be used to define the precision of the circadian period (τ). The midpoint of entry into torpor is also a useful marker.

Some attempts were made to monitor animal activity utilizing running wheels, cage mounted microphones (i.e., piezoelectric sensors), or frequency of changes in the signal strength of the implanted temperature transmitter. The pocket mice demonstrated unreliable running wheel behavior. The cage mounted microphone monitored gross activity and did not distinguish between different kinds of behavior. In addition, the microphones proved extremely difficult to adjust in terms of sensivity to movement. As a consequence, the animal activity data obtained by these two methods, while informative, lacked the definition desired and were discontinued.

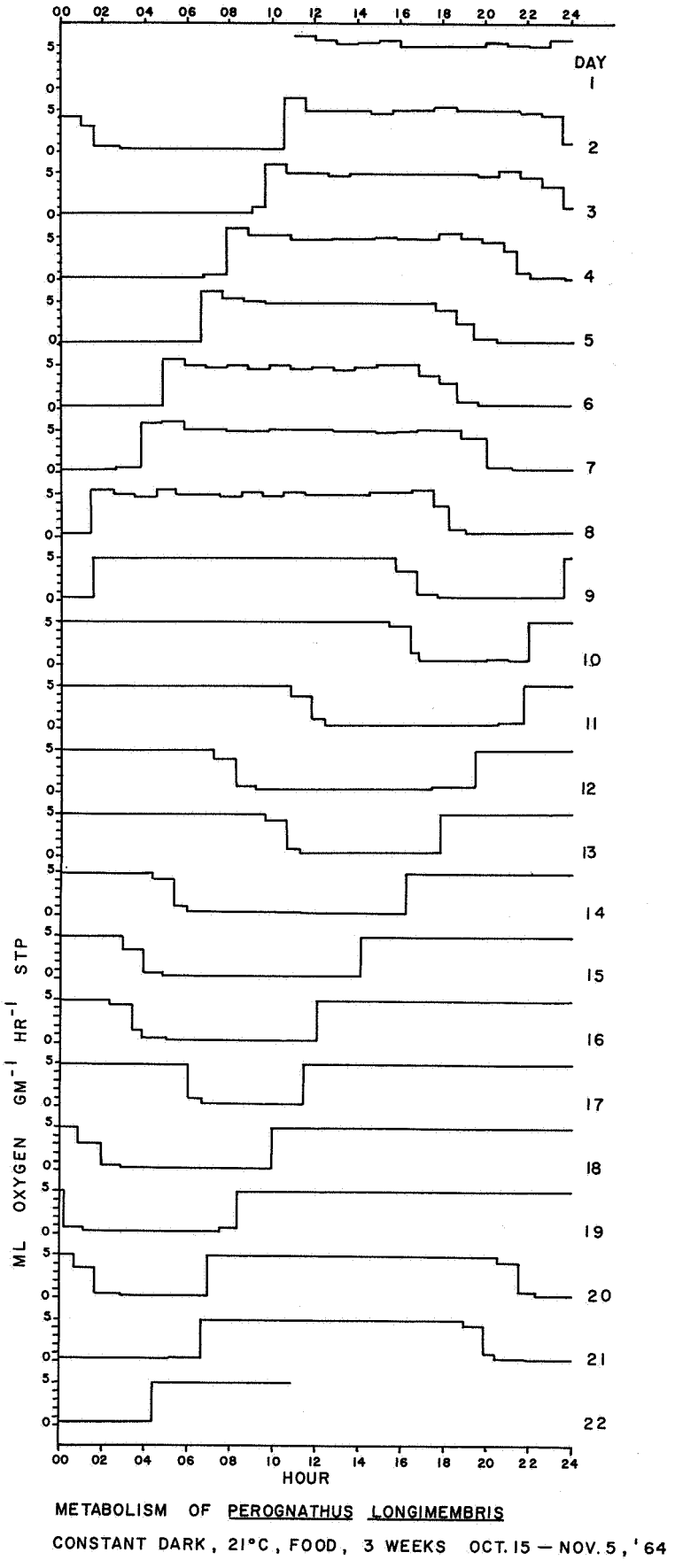

Figure 1 Oxygen utilization of a P. longimembris maintained in a respirometer simulating proposed space experiment hardware for three weeks at 21°C, constant dark. Actual values of oxygen consumption during normometabolic periods are plotted from day 1-9. Days 10-22 normometabolic values are averages for that period.

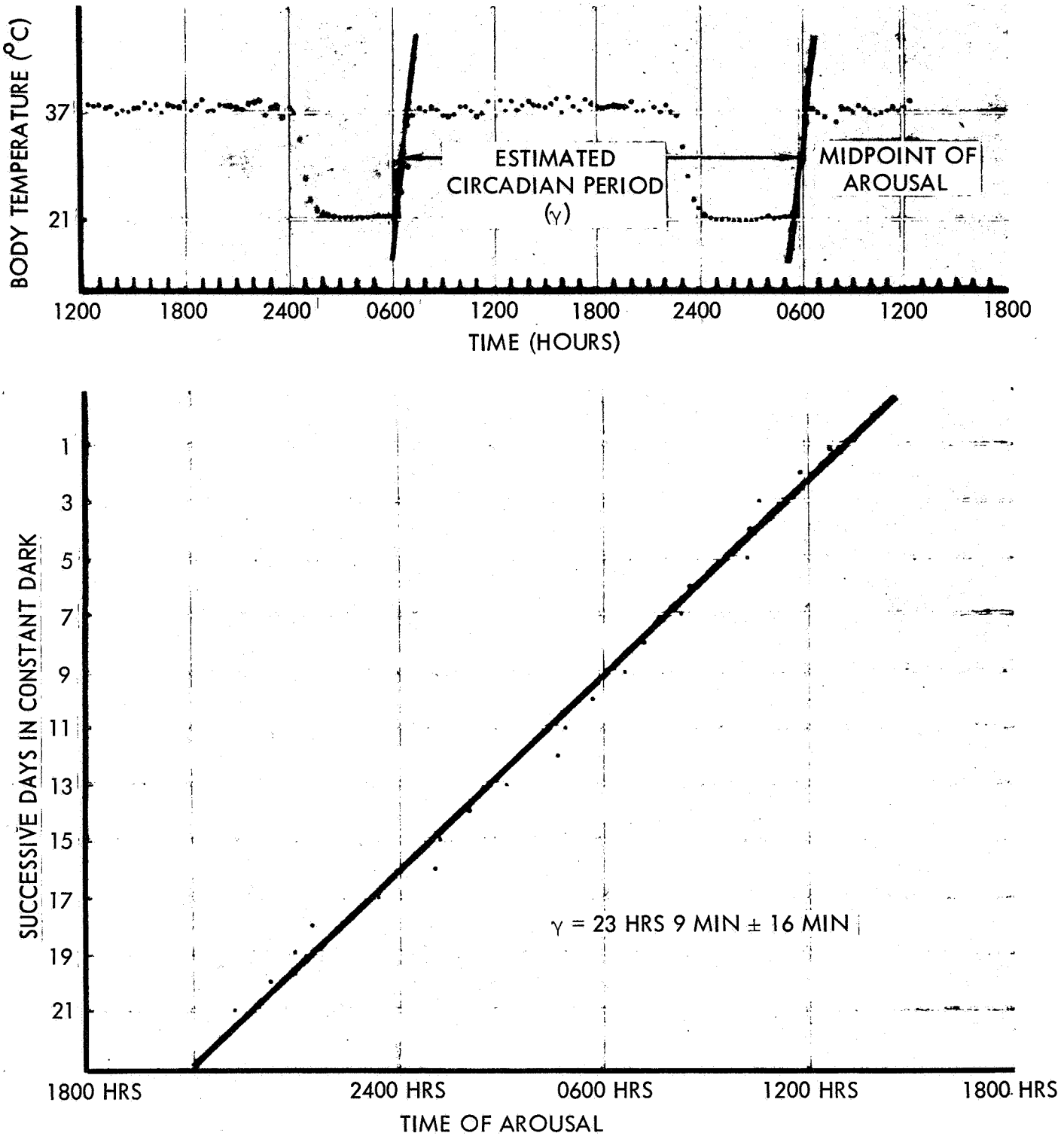

Figure 2 Determination of the free-running circadian period of body temperature in the little pocket mouse (P. longimembris) under conditions of constant dark and constant temperature.

The third method of measuring animal activity utilized the inevitable changes in signal strength received from the implanted temperature transmitter brought about by changes in the relative position of the transmitter coil and the antenna. The method is promising, but a reliable monitoring system was evolved too late in this contract to result in publishable data.

Experiment Conditions. - Studies of circadian periodicity were primarily undertaken in a walk-in, light-tight, constant temperature room fitted with semi-isolated compartments for animal chambers. The compartments were open on the front. The animal chambers were 12 inch square lucite boxes fitted with a running wheel and antenna system. Sawdust was provided for animal grooming and urine absorption. Each compartment contained an incandescent bulb which provided a white light intensity of ~ 25 ft-c at floor level. The lights were controlled from outside the constant temperature room. Although no "white" noise was provided, an air circulating blower within the constant temperature room masked extraneous noises that occurred outside the chambers. Pocket mice do not drink water; they excrete small amounts of waste and store surplus food. These characteristics make it possible to isolate experiments for long periods (one to two months).

Some studies were undertaken utilizing prototype experiment hardware developed for space biology research under contract No. NASw-1191 (7) (Fig.3). The principal difference in experiment setup was that the experimental animals were individually housed in sintered polyethylene tubes (1 3/8 inch i.d. x 15 inches long) treated by the manufacturer with a hydrophylic coating which enhanced urine absorption. All experiments in the space hardware were conducted in constant dark.

All animals used in this study were trapped in the field in the spring of the year and judging from size and pelage were young adults. In the laboratory the animals were maintained individually in gallon bottles with a sand substrate. A mixture of sunflower seeds, wheat, rye, oats, and millet was provided in excess at all times. The animal holding facility was air conditioned with a temperature of 20-24°C, 40-60% relative humidity, and a photoperiod of 12 hr light and 12 hr dark (light: 0600-1800 hr PST).

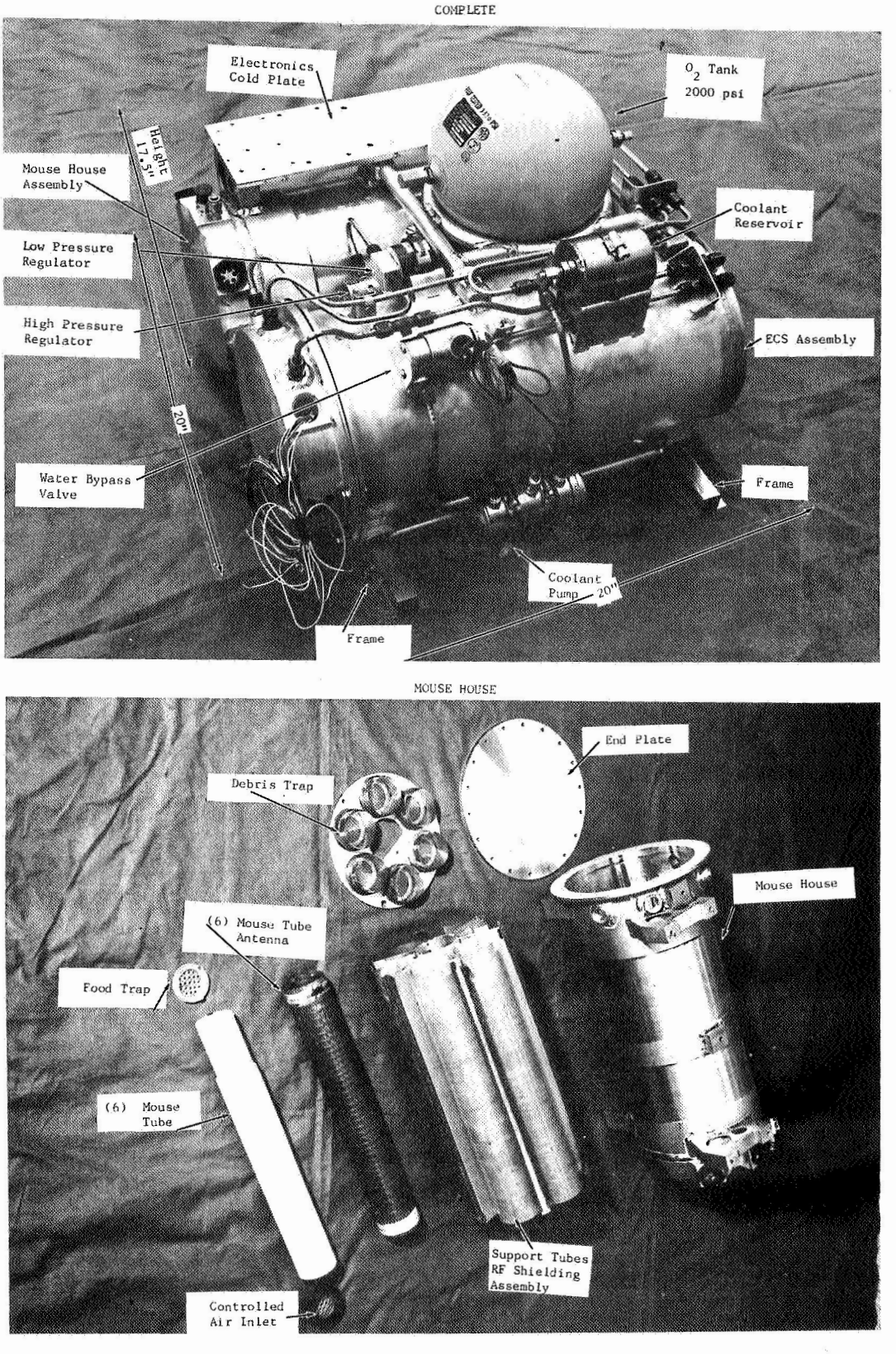

Figure 3 Upper: Assembled prototype hardware designed to study the stability of circadian systems in pocket mice during earth orbital missions (7).

Lower: Animal housing assembly disassembled.

Occurrence of Torpor within the Genus Perognathus. - A survey was made to determine how many of the available species of pocket mice displayed a daily torpor useful to the study of circadian periodicity. Temperature transmitters were implanted in the abdominal cavity of P. longimembris, P. formosus, P. alticola, P. californicus, P. penicillatus, P. parvus and P. amplus. All demonstrated a reasonably precise circadian period measured in terms of the midpoint of arousal from torpor (Figures 4 and 5). While the tendency for daily torpor appears to be a common trait within the genus, the body temperature fluctuations during normothermic periods in some cases appear strikingly different between various species.

Validity of Time of Arousal as Circadian Marker. - The question immediately arose as to whether the fluctuations noted above were rhythmic in nature and whether they were in any way coupled to the frequency of diurnal torpor. A time frequency analysis of P. formosus data was done at Princeton University and implied that despite appearances the fluctuations were non-rhythmic and made up a negligible part of the frequency spectrum (8).

Concurrently, experiments were undertaken at Northrop to determine the effect of ambient temperature on the pattern of normothermic temperature fluctuations in normal animals and animals which were shaved to upset their normal thermoregulation.

Two species of pocket mice, P. formosus ($\sim$ 20 gm) and P. longimembris ($\sim$ 10 gm), were implanted with temperature telemeters and exposed to several ambient temperatures varying from 10 to 35°C. The animals were maintained at each temperature regimen from one to several days. Analog temperature recordings were obtained and actual temperatures determined from the individually calibrated telemeters. Animals were then lightly anesthetized and shaved from head to rump and lateral line to lateral line. These shaved animals (decreased insulation, increased conductance) were then again exposed to the same series of temperature regimens.

Representative body temperature data are given in Figures 6 and 7 for P. longimembris and P. formosus. The temperature recordings presented are from late afternoon to early evening. This is the time period of the day

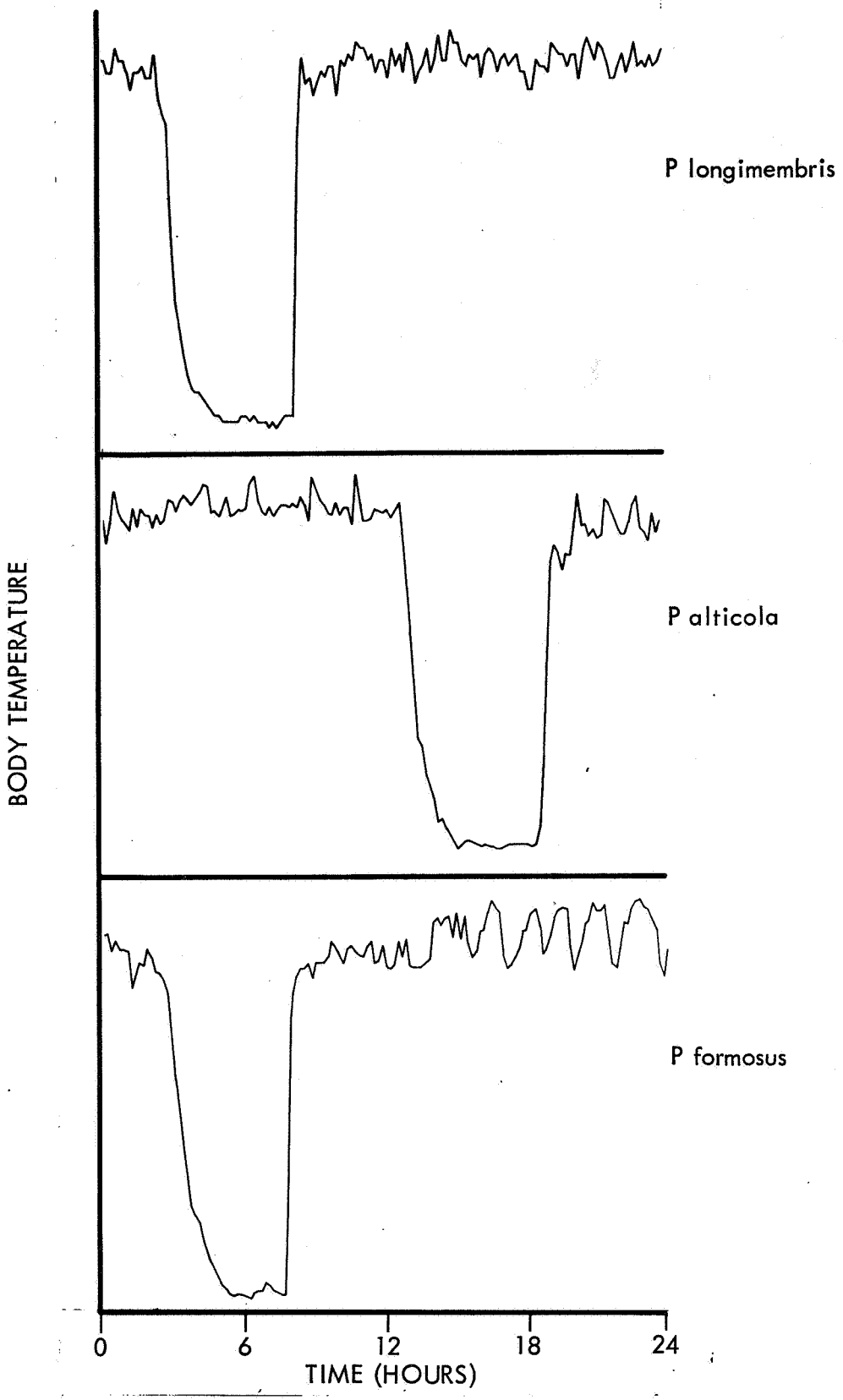

Figure 4 Representative patterns of daily fluctuations of body temperature in three species of Perognathus. Time of temperature drop is not significant in this series since the examples were selected from "free-running" animals. The temperature span is from 21°C to 37°C.

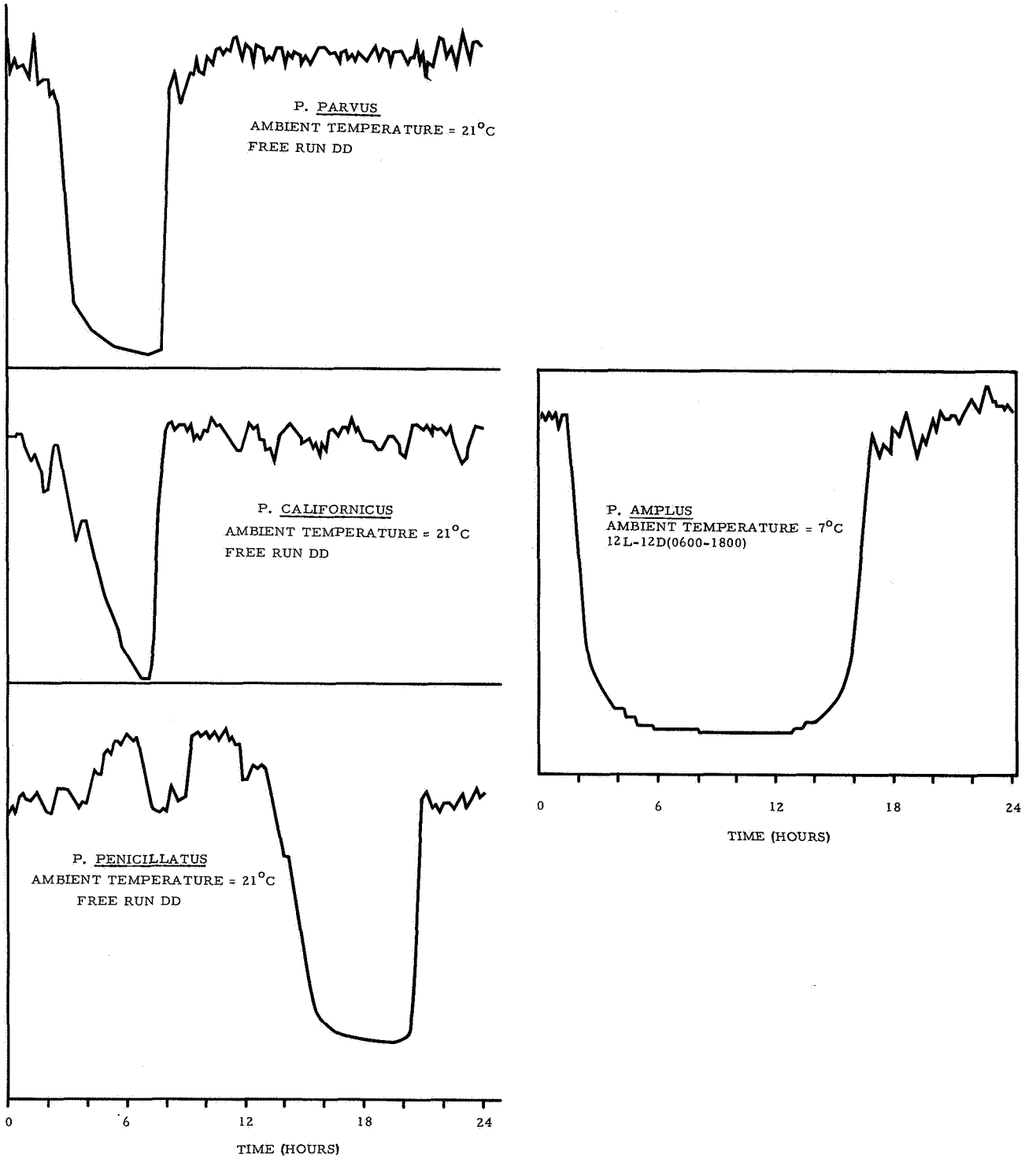

Figure 5 Representative patterns of daily fluctuations of body temperature in four species of Perognathus. Data obtained from "free-running animals, 21°C, constant dark, with the exception of P. amplus which was entrained to 12L-12D (0600-1800) and at an ambient of 7°C.

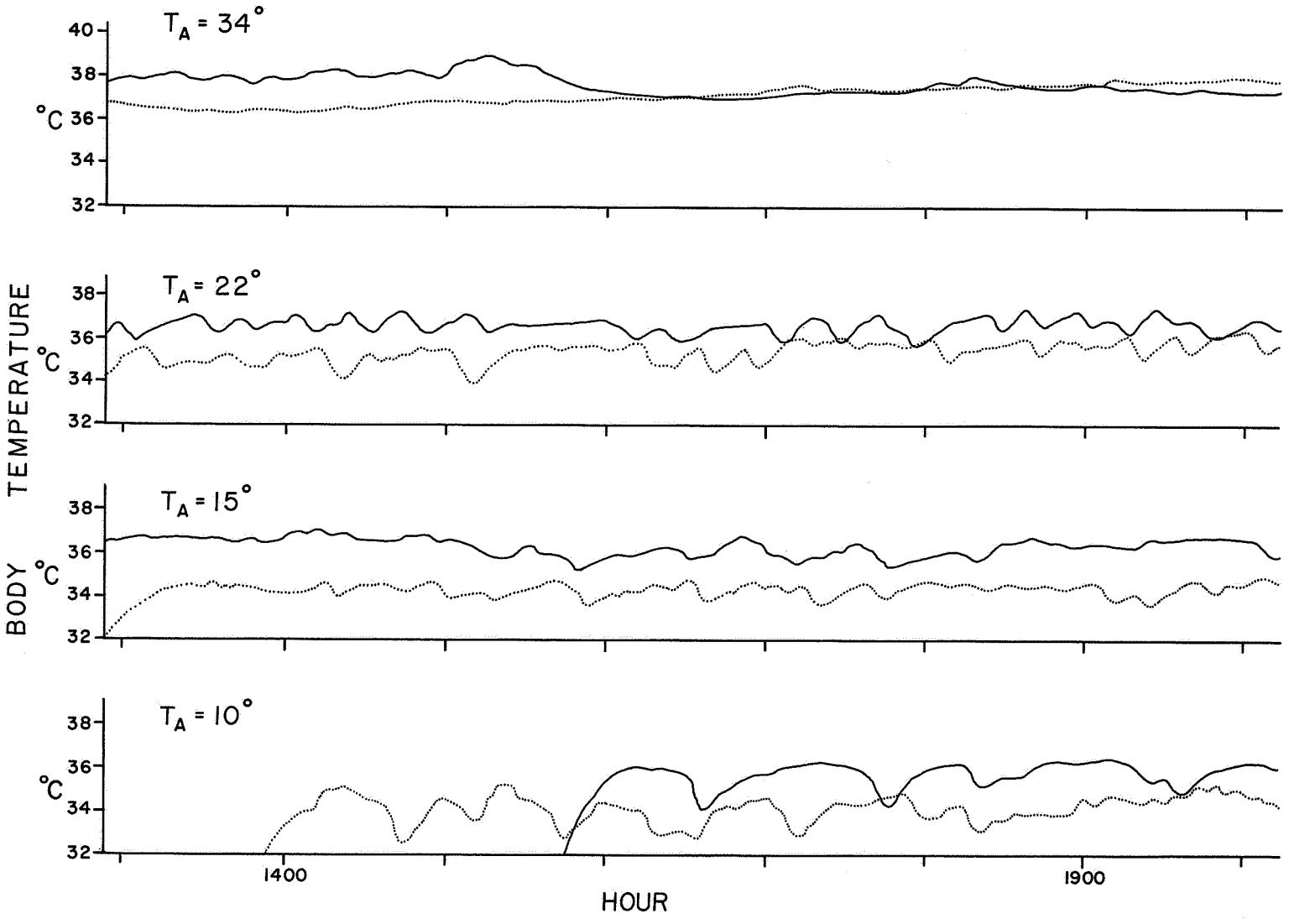

Figure 6 The effect of reduced insulation (shaved animals) on the expression of body temperature variations during a normothermic period in P. longimembris. Solid line indicates body temperature fluctuations in normal condition. Broken line indicates body temperature fluctuations in shaved condition. Arousal from torpor is indicated where temperature data intersects base line.

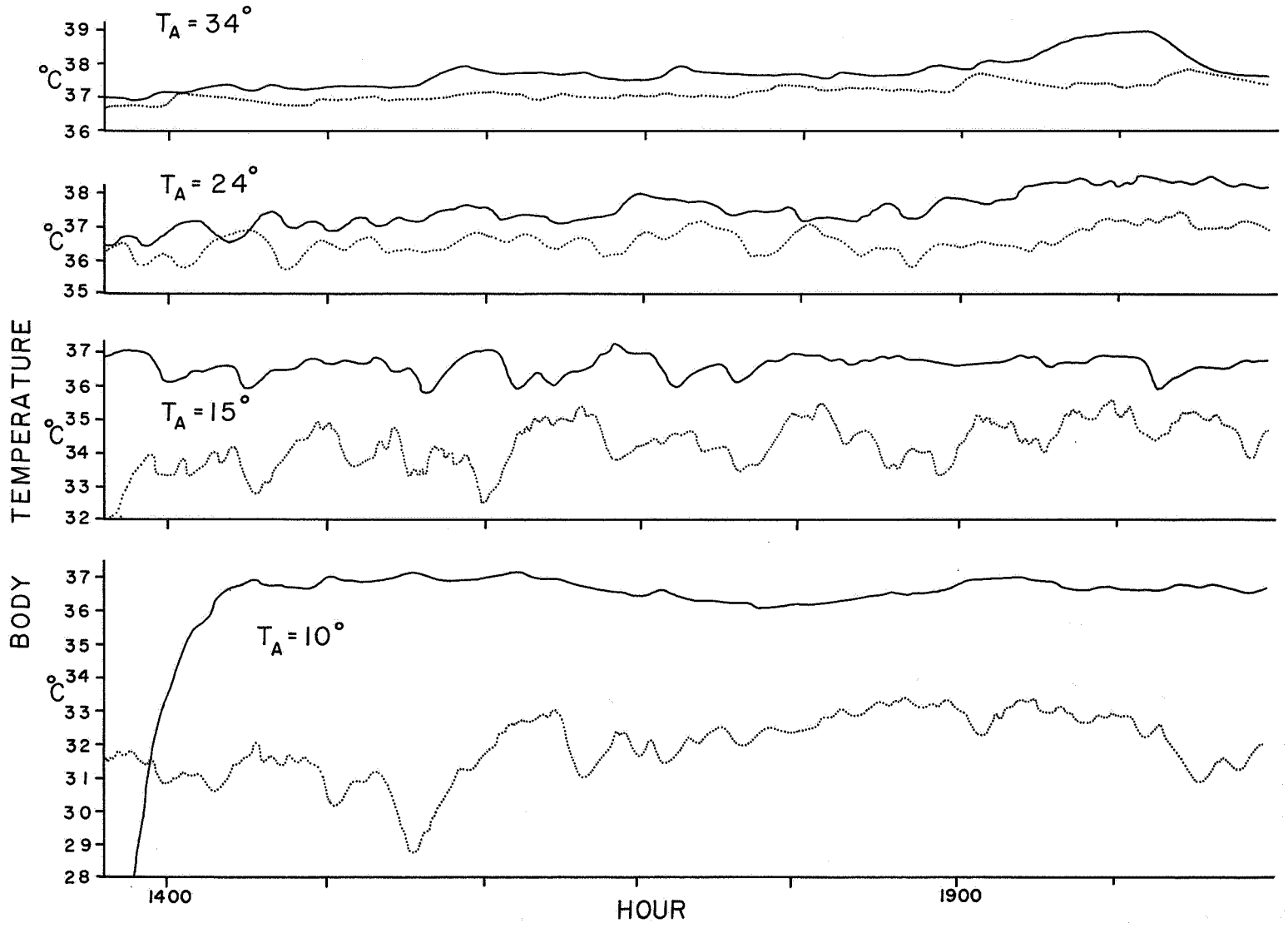

Figure 7 The effect of reduced insulation (shaved animals) on the expression of body temperature variations during a normothermic period in P. formosus. Solid line indicates body temperature fluctuations in normal condition. Broken line indicates body temperature fluctuations in shaved condition. Arousal from torpor is indicated where temperature data intersects base line.

when the animals have aroused from torpor (if expressed) or is the normal termination of the daily rest - activity cycle. Animals are characteristically awake, preening, eating, or if resting, alert. Although some major activity does normally occur during the latter part of this period, the physical size of the monitoring chamber precluded any excessive activity.

There are obvious changes in the gross pattern of temperature regulating between shaved and unshaved animals. The overall trend is for temperature regulation, in the extremes of ambient temperature (35°C and 10°C), to be more "smoothed" in the fully insulated animals. In those with decreased insulation, the regulation band is depressed with a lowered average temperature.

Definitive studies of the effect of shifting the thermoregulation pattern on the circadian period were not done. However, the independence of the phenomenon is strongly indicated by one shaved P. longimembris which underwent torpor and aroused precisely "on time" during two successive days at an ambient temperature of 15°C and aroused only 2 hr after 34 hr of continuous torpor. A change in ambient temperature from 15°C to 10°C occurred during the 34-hr torpid period yet the animal aroused on schedule.

During the development of an activity sensor utilizing the change in signal strength of the implanted temperature transmitter, analog recordings were taken which superimposed "activity" signals on a baseline of temperature data (Figures 8, 9). It is readily apparent that there is a high degree of correlation between the peaks of body temperature during the normothermic period and animal activity.

On the basis of the above observations our decision to ignore (for the present) the relative high frequency of temperature fluctuations during normothermic period in the analysis of circadian markers such as time of entry or arousal from torpor seems justified. It appears that much of the temperature fluctuation is due to random activity of the animal. This latter phenomenon is under more definitive study.

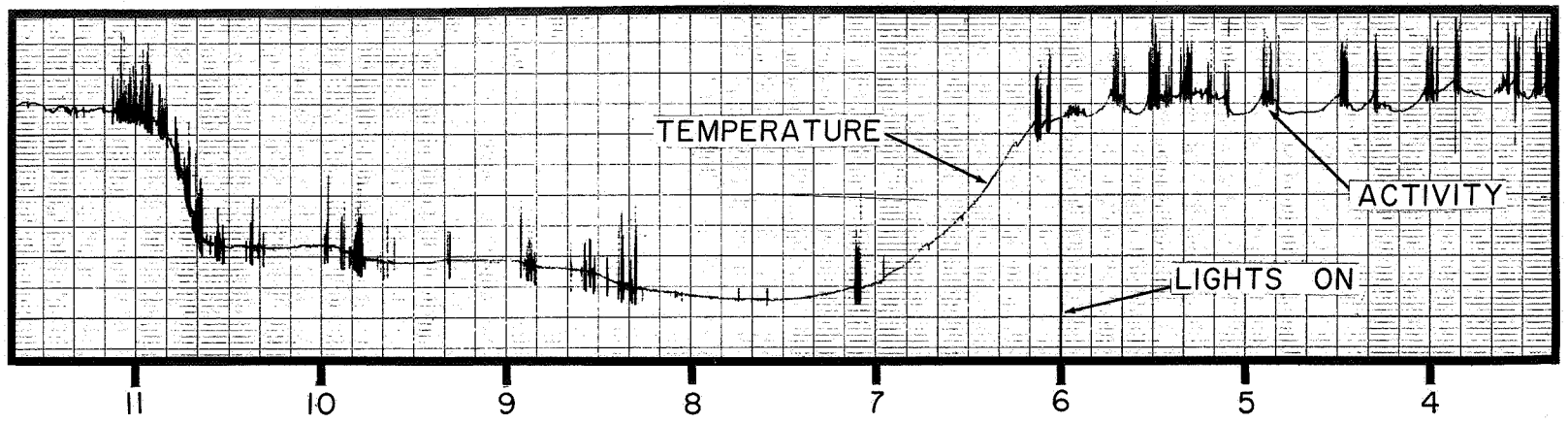

Figure 8 An analog recording of body temperature (base line) including a period of shallow torpor in P. longimembris. Periods of activity have been superimposed on the temperature data and are shown as spikes. Animal movement was sensed by changes in signal strength from the implanted temperature transmitter.

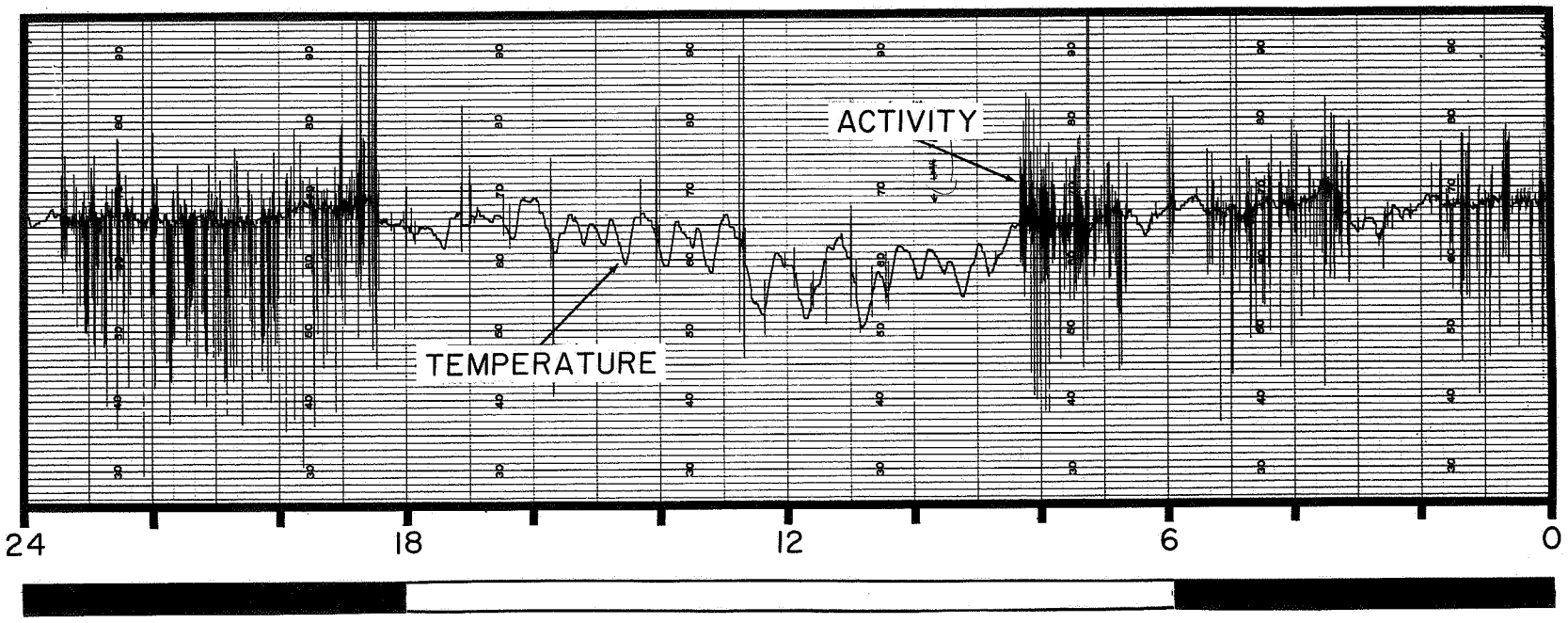

Figure 9 An analog recording of body temperature (base line) in P. longimembris. Periods of activity have been superimposed om the temperature data and are shown as spikes. Animal movement was sensed by changes in signal strength from the implanted temperature transmitter. While the animal did not go torpid as in Figure 8, the period of rest is clearly shown. Dark period is indicated by black bar and light period by open bar under time scale.

Results

Free-Running Circadian Period (TFR). - A group of five P. longimembris were implanted with temperature transmitters and subjected to conditions thought to closely resemble those occurring in the natural burrows of animals during the fall and winter. The pertinent environmental factors were constant dark, low temperature, surplus of food, isolation from noise, and natural bedding (grass) and substrate (fine sand). The experiment was conducted during October, November, and December.

The initial conditions of the experiment were an ambient temperature of 21°C with LD 12:12 (0600-1800). After seven days the photoperiod was changed to constant dark (DD) with no change in ambient temperature. Without disturbing the animals, 28 days later the ambient temperature was dropped to 10°C in DD and maintained for 50 days. However, because of mechanical failure in the recording system, reliable data was obtained for 20 days only.

All animals showed periods of torpor alternating with normothermic body temperatures characteristic of the species. The times of arousal were used as phase reference points (φR) and are plotted for each animal to assess the persistence and precision of the circadian period. (Figures 10 through 14).

Characteristically, free running circadian periods show after effects of the environmental regimen immediately preceding the steady state free-run being studied. These after effects or transients, in the form of variable period lengths, always precede attainment of a new steady state and have been observed in P. longimembris (4).

Several days after the termination of the LD 12:12 light regimen and commencement of constant dark, all entered into free-running metabolic rhythms. These free-running periods varied from 23 hr 2 min to 25 hr 42 min. Transients were induced by the temperature decrease from 21°C to 10°C. During the 10°C regimen free-running periods varied from 22 hr 4 min to 25 hr 26 min. In most animals there seemed to be a degradation of the precision of the rhythm at the lower temperature.

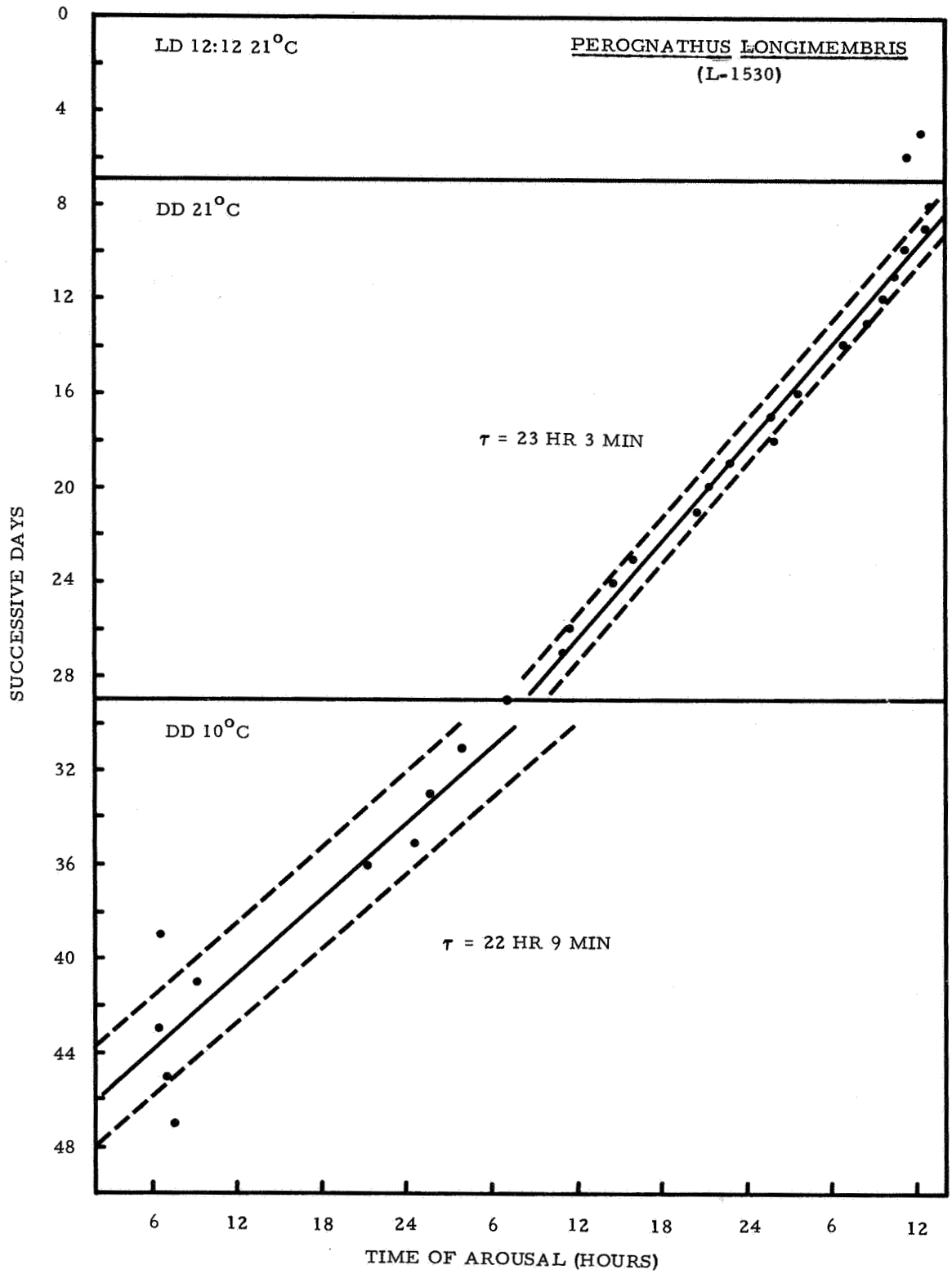

Figure 10 The stability of the free-running circadian period of body temperature in P. longimembris exposed to a step drop in ambient temperature. The animals were undisturbed and except for the change in photoperiod to DD on day seven and the temperature drop on day 29 were maintained in a constant environment. The dotted lines delineate ±1 S.E.

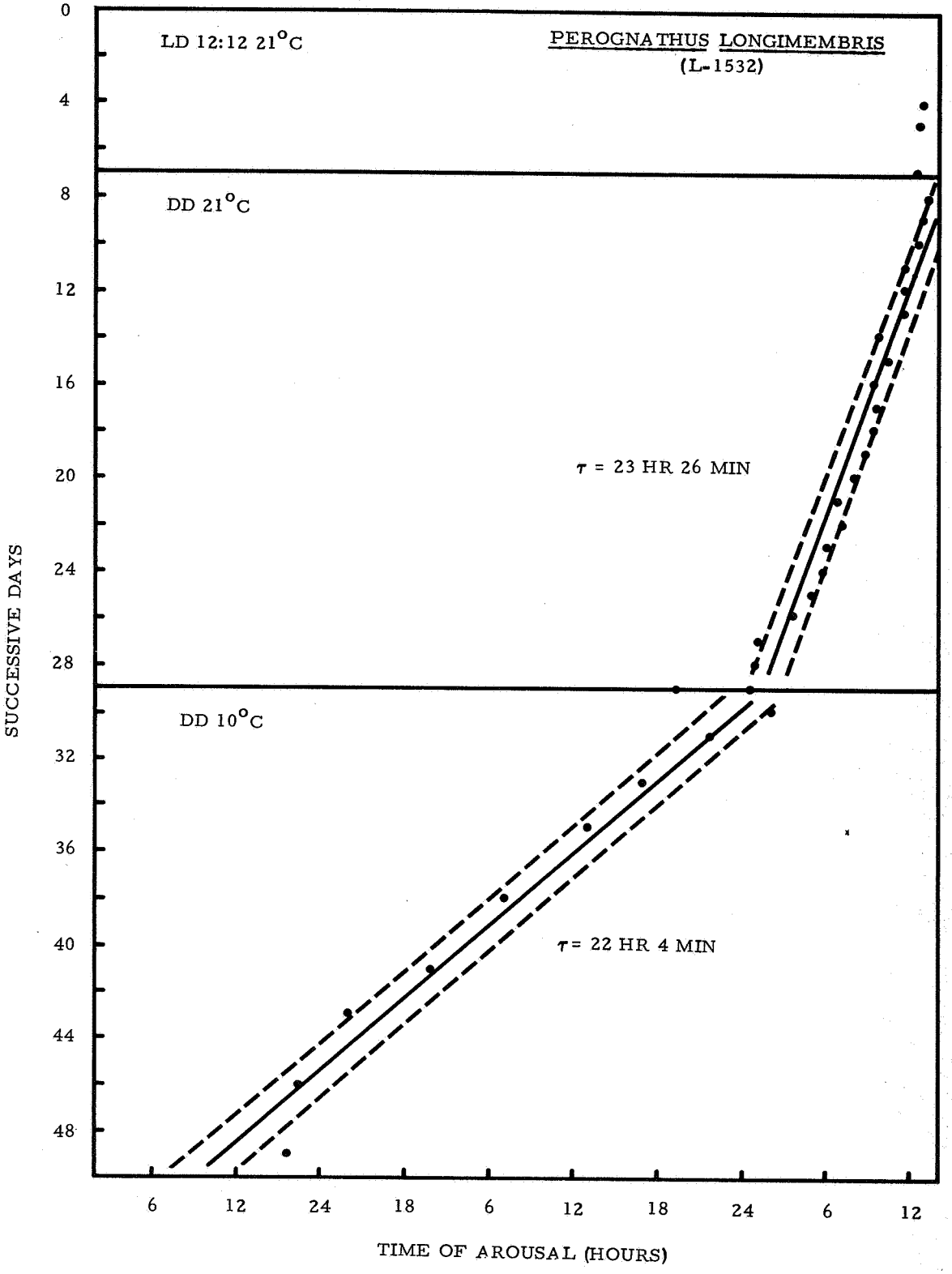

Figure 11 The stability of the free-running circadian period of body temperature in P. longimembris exposed to a step drop in ambient temperature. The animals were undisturbed and except for the change in photoperiod to DD on day seven and the temperature drop on day 29 were maintained in a constant environment. The dotted lines delineate ±1 S.E.

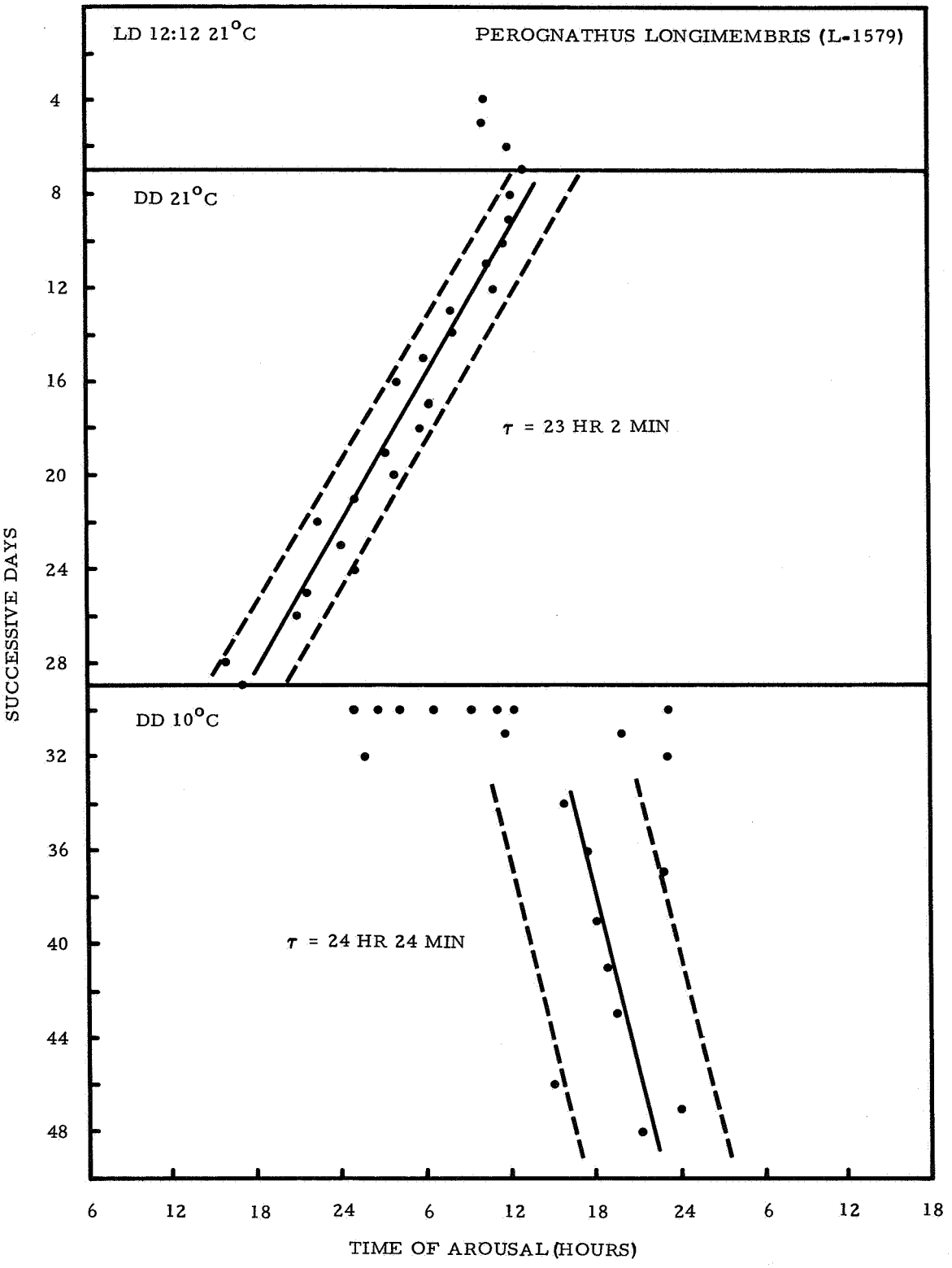

Figure 12 The stability of the free-running circadian period of body temperature in P. longimembris exposed to a step drop in ambient temperature. The animals were undisturbed and except for the change in photoperiod to DD on day seven and the temperature drop on day 29 were maintained in a constant environment. The dotted lines delineate ±1 S.E.

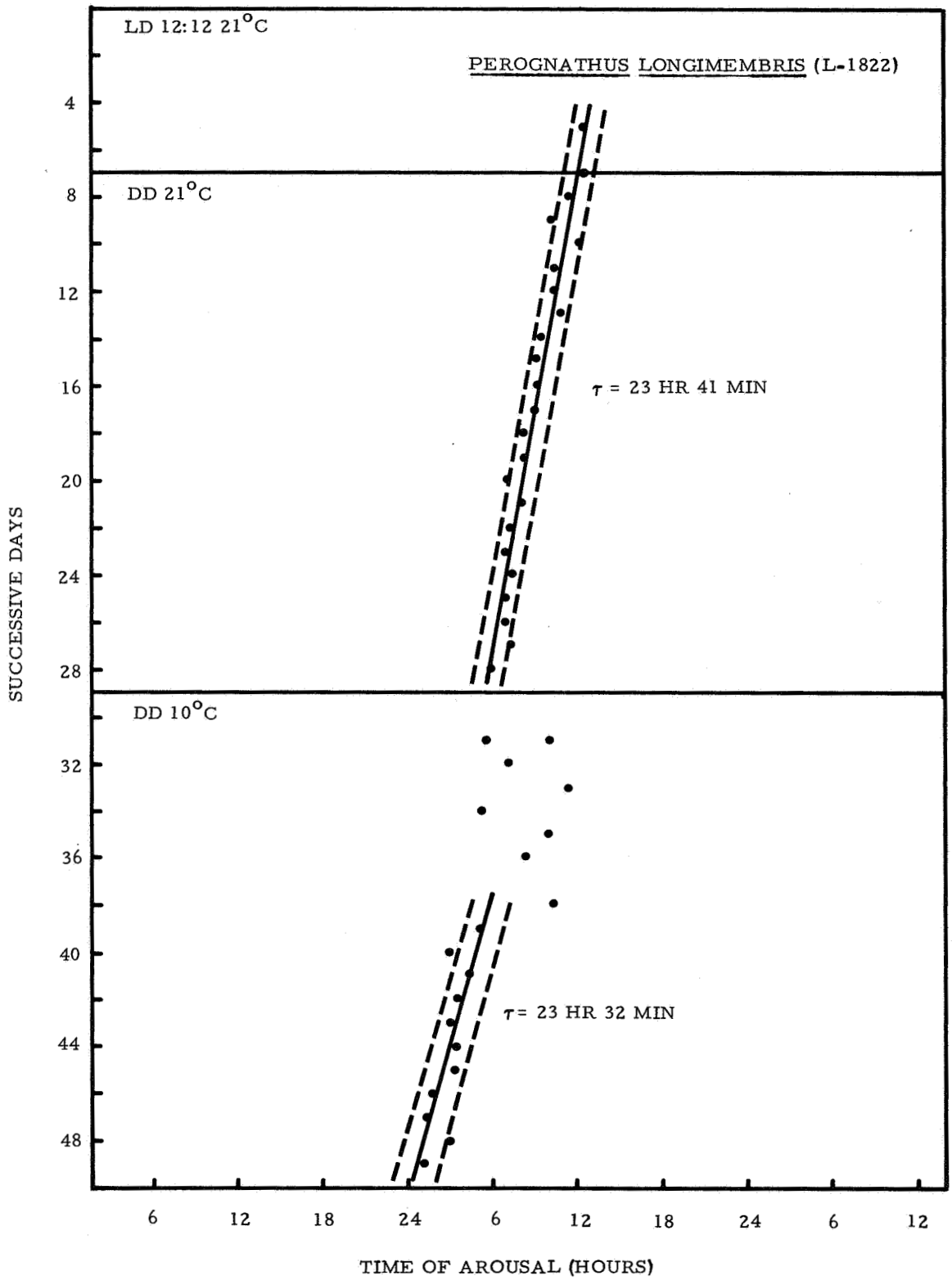

Figure 13 The stability of the free-running circadian period of body temperature in P. longimembris exposed to a step drop in ambient temperature. The animals were undisturbed and except for the change in photoperiod to DD on day seven and the temperature drop on day 29 were maintained in a constant environment. The dotted lines delineate ±1 S.E.

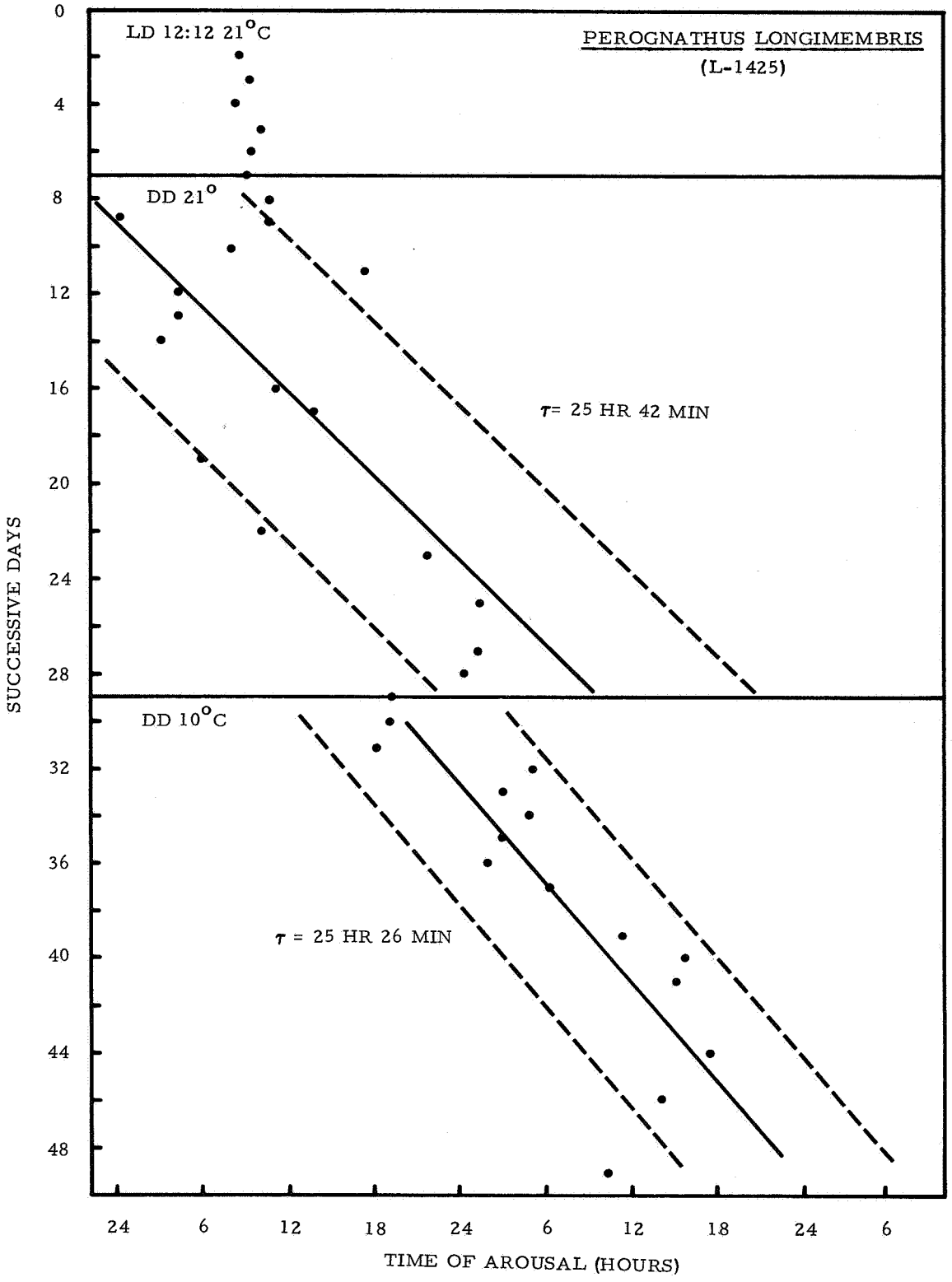

Figure 14 The stability of the free-running circadian period of body temperature in P. longimembris exposed to a step drop in ambient temperature. The animals were undisturbed and except for the change in photoperiod to DD on day seven and the temperature drop on day 29 were maintained in a constant environment. The dotted lines delineate ±1 S.E.

One animal regained a well defined stable pattern after showing a disturbance for about 7 to 8 days (Figure 13). One animal changed from a less than 24-hr rhythm to a greater than 24-hr rhythm (Figure 11).

The arousal of an animal at a time in proper phase with the established circadian rhythm after one or two days in a continuously hypothermic state was a rather common occurrence and in some animals had an amazing accuracy (Figure 11, 10°C ambient: body temperature ~ 11°C). The relative independence of the circadian period to temperature is indicated by the low Q_{10} derived from comparing the period at 21°C ambient with the period at 10°C ambient. It should be noted that while the animals are torpid their body temperatures approach ambient. The precision of the circadian period, however, is relatively unaffected.

Effect of Confinement in Prototype Space Hardware on the Free-Running Period. - Pocket mice have been proposed to study the effect of space residence on the stability of circadian systems. The experiment hardware concept involves housing the mice in individual porous plastic tubes for approximately thirty days (Figure 3). Little information is available as to the possible disturbance or degradation of the daily period of torpor under such conditions of confinement. Figure 15 demonstrates the occurrence and persistence of daily torpor for one mouse maintained in the prototype experiment hardware during qualification testing of the equipment during contract NASw-1191.

It was the objective of this experiment to compare the free-running period of animals maintained in laboratory equipment with their free-running period expressed while confined in the space experiment hardware.

Initially, 12 P. longimembris were studied for 21 days at an ambient temperature of 21°C and constant dark (DD) following seven days at 21°C and a 12 hr light, 12 hr dark photoperiod (LD 12:12 0600-1800). Of these animals, five which demonstrated the most frequent incidence of torpor were selected to be placed in the space hardware. The space hardware was designed to accommodate six animals but one tube was used to monitor ambient temperature with a reference telemeter.

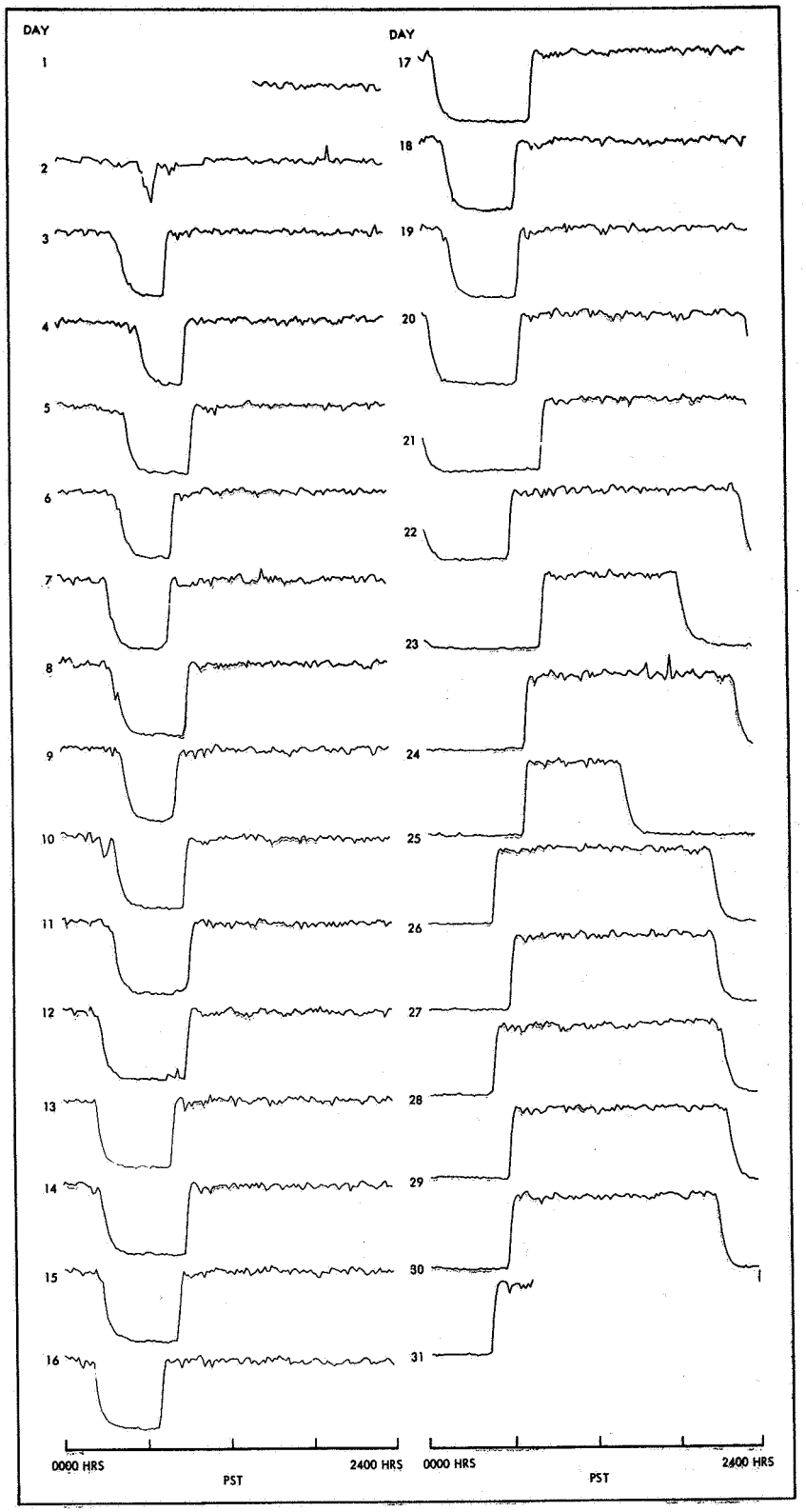

Figure 15 Free running circadian period of body temperature in a pocket mouse, P. longimembris, maintained for 30 days in an experiment package prototype operated under simulated space conditions (temperature scale: max 37°C; min 21°C).

Of the five animals placed in the space hardware, two displayed torpor on the first day, two more by the fourth day, and the last on the sixth day. Data were collected for 21 days and are summarized in Figure 16 through 20.

Stability of the free-running period was demonstrated in three of five animals (Figs 17, 19, 20). There was no apparent degradation of period in the confines of the space hardware. To the contrary, there appears to be a tendency toward a more precise period. Animal No. L-640 (Fig. 16) seemed to undergo a true change in period. Unfortunately, due to equipment failure data obtained in laboratory chambers and those obtained in the space hardware are not directly comparable (for this individual) in that activity data were used to determine period in laboratory chambers and body temperature data were used in the space hardware. It would seem, however, that activity and body temperature must be closely coupled, or one is faced with the impossible situation of a torpid animal running on a wheel. It is interesting to note that this particular animal in the space hardware had a period of 24 hr 28 min and is one of the few P. longimembris that we have observed to have a period greater than 24 hours.

Despite the encouraging data summarized above, our experience indicates that animals must be carefully selected for the experiments and manipulated in the proper manner. For example, at the time that the 12 animals were placed in the laboratory chambers in preparation for the above experiment, five animals randomly chosen were placed directly in the space hardware. Data were lost on one because of equipment failure. Two demonstrated a typical peridicity of torpor (Fig. 21), and two failed to undergo torpor. When removed from the space hardware and placed in laboratory chamber, the two which had demonstrated good torpor became sporadic and in a 21-day period underwent torpor once in one animal and three times in another. The two animals which failed to undergo torpor in the space hardware also failed to demonstrate torpor in the laboratory chambers. The data obtained from this experiment is summarized in Table 1. The poor showing of these first five animals placed in the space hardware is no doubt associated with the fact that the animals were not selected on the basis of a known tendency for daily torpor. This phenomenon has been demonstrated to be highly varia-

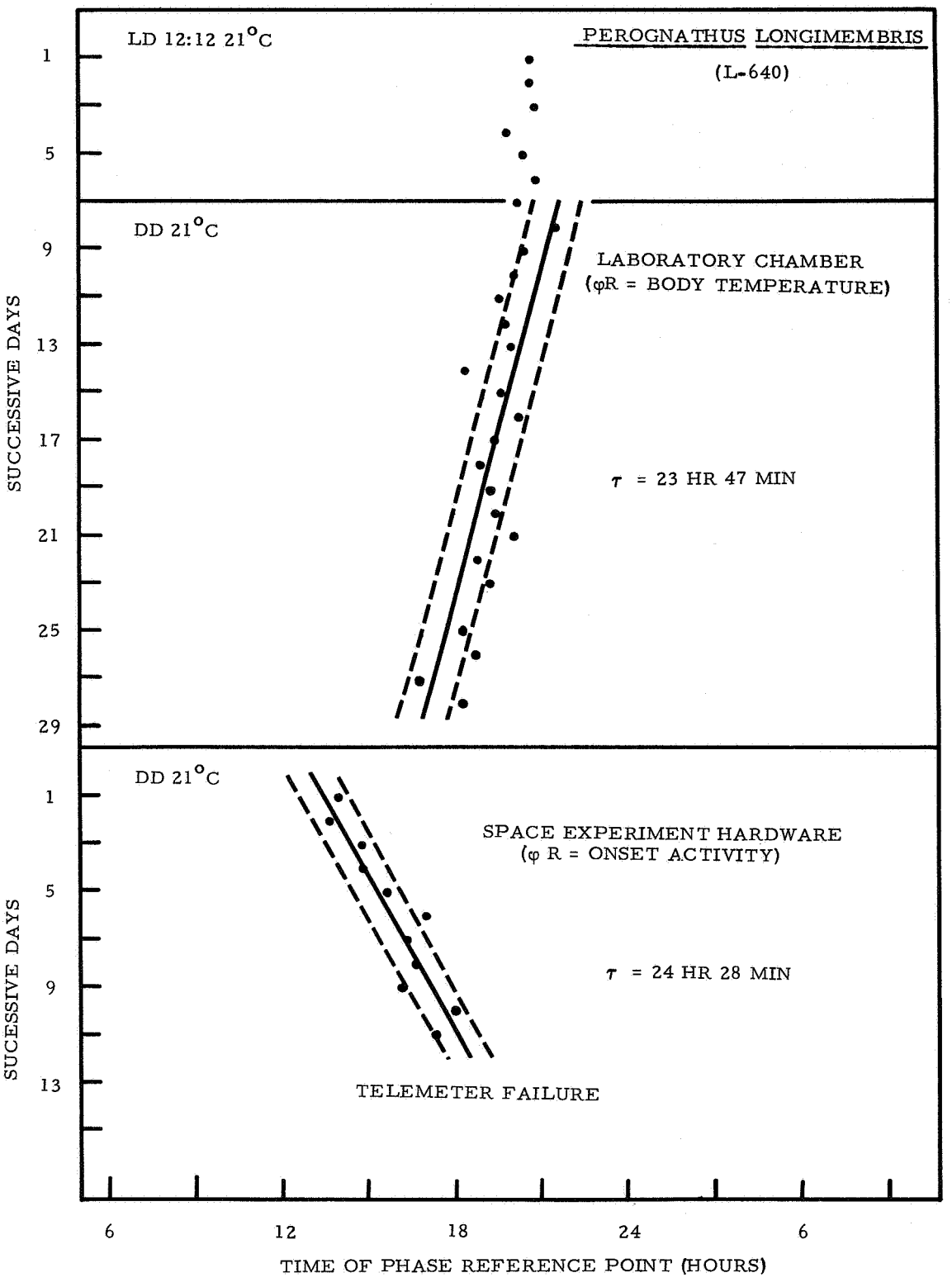

Figure 16 A comparison of the "free-running" circadian periods in P. longimembris maintained in a laboratory chamber and subsequently in space experiment hardware.

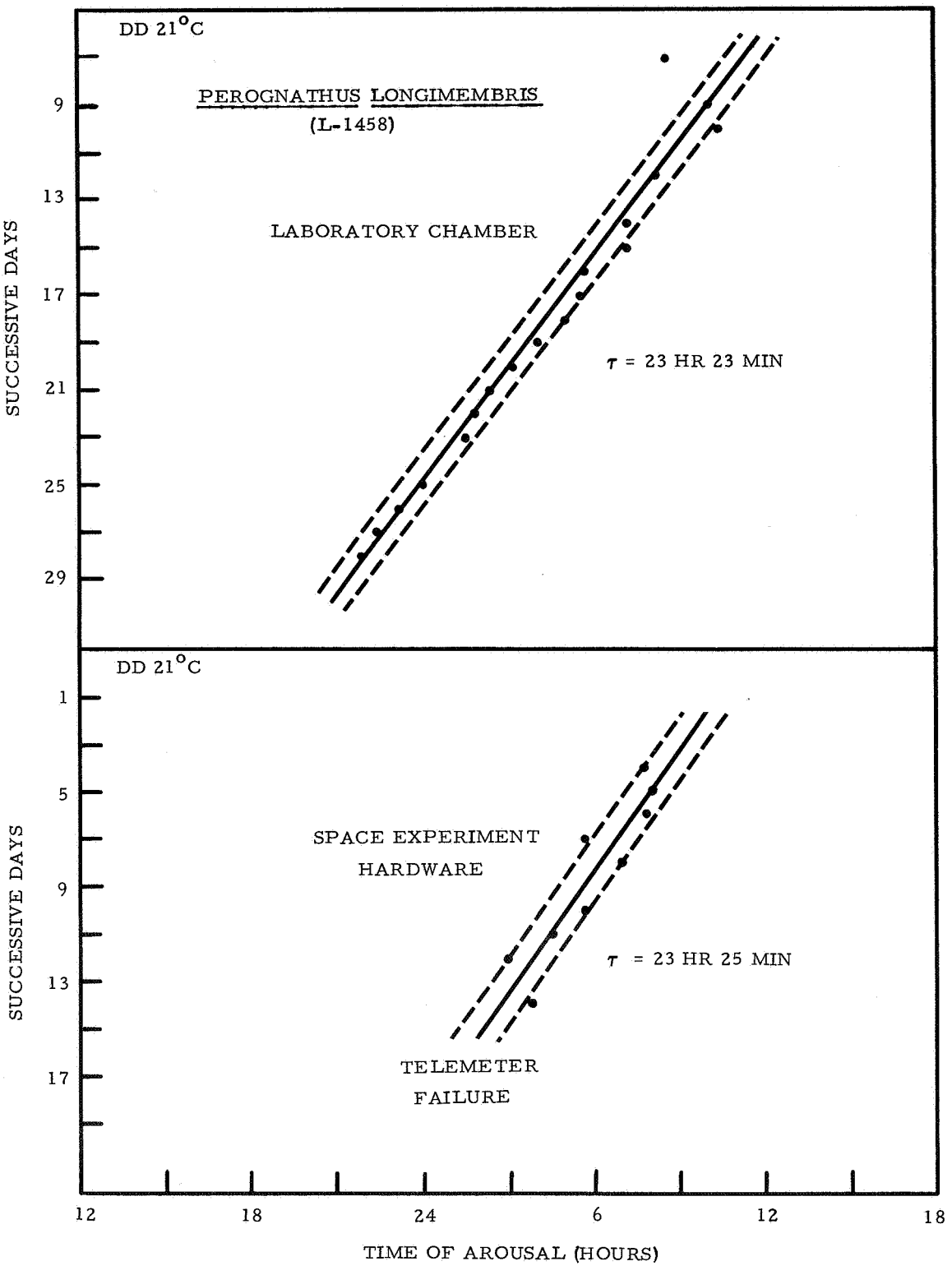

Figure 17 A comparison of the "free-running" circadian period of body temperature in P. longimembris maintained in a laboratory chamber and subsequently in space experiment hardware.

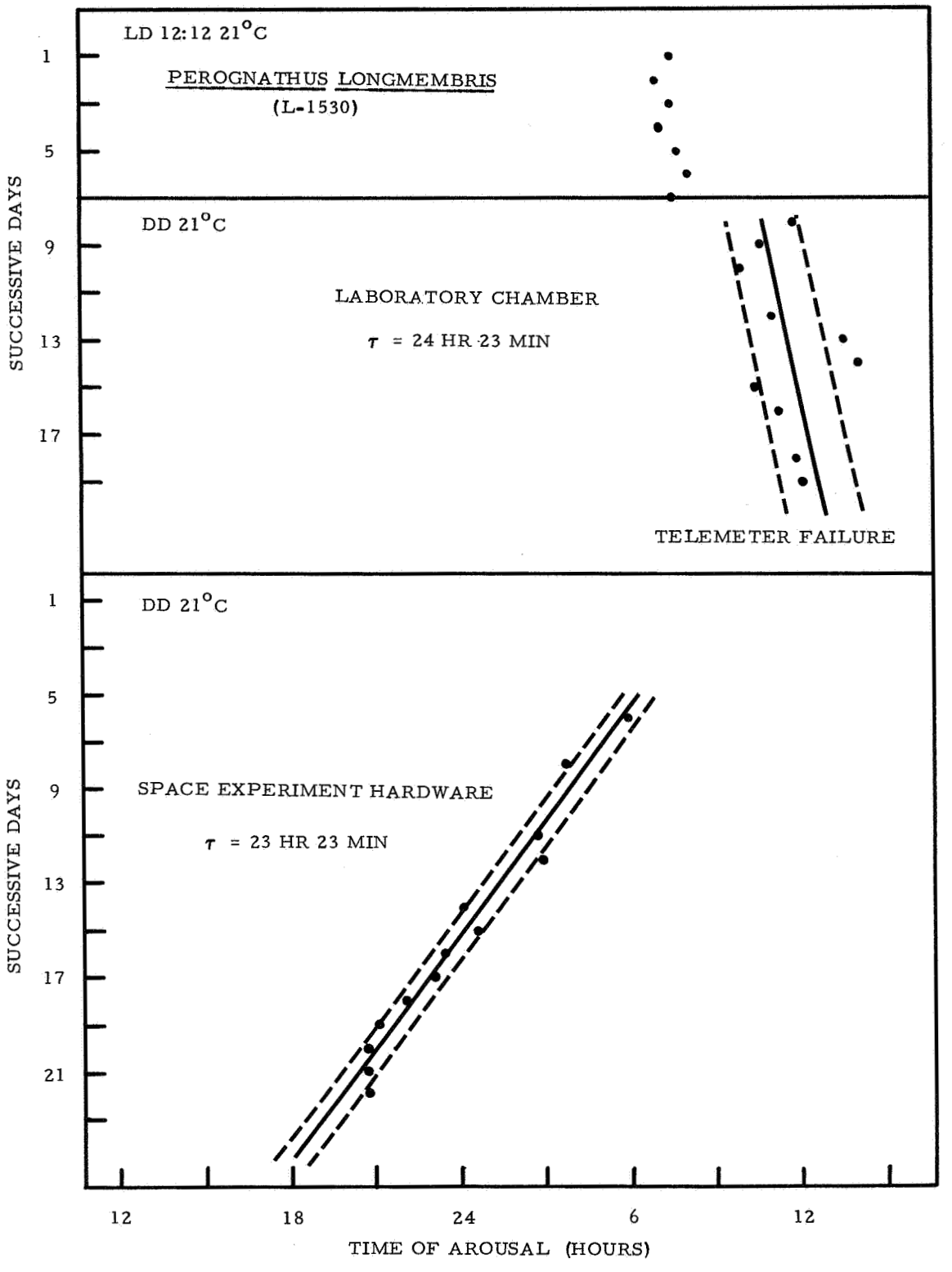

Figure 18 A comparison of the "free-running" circadian period of body temperature in P. longimembris maintained in a laboratory chamber and subsequently in space experiment hardware.

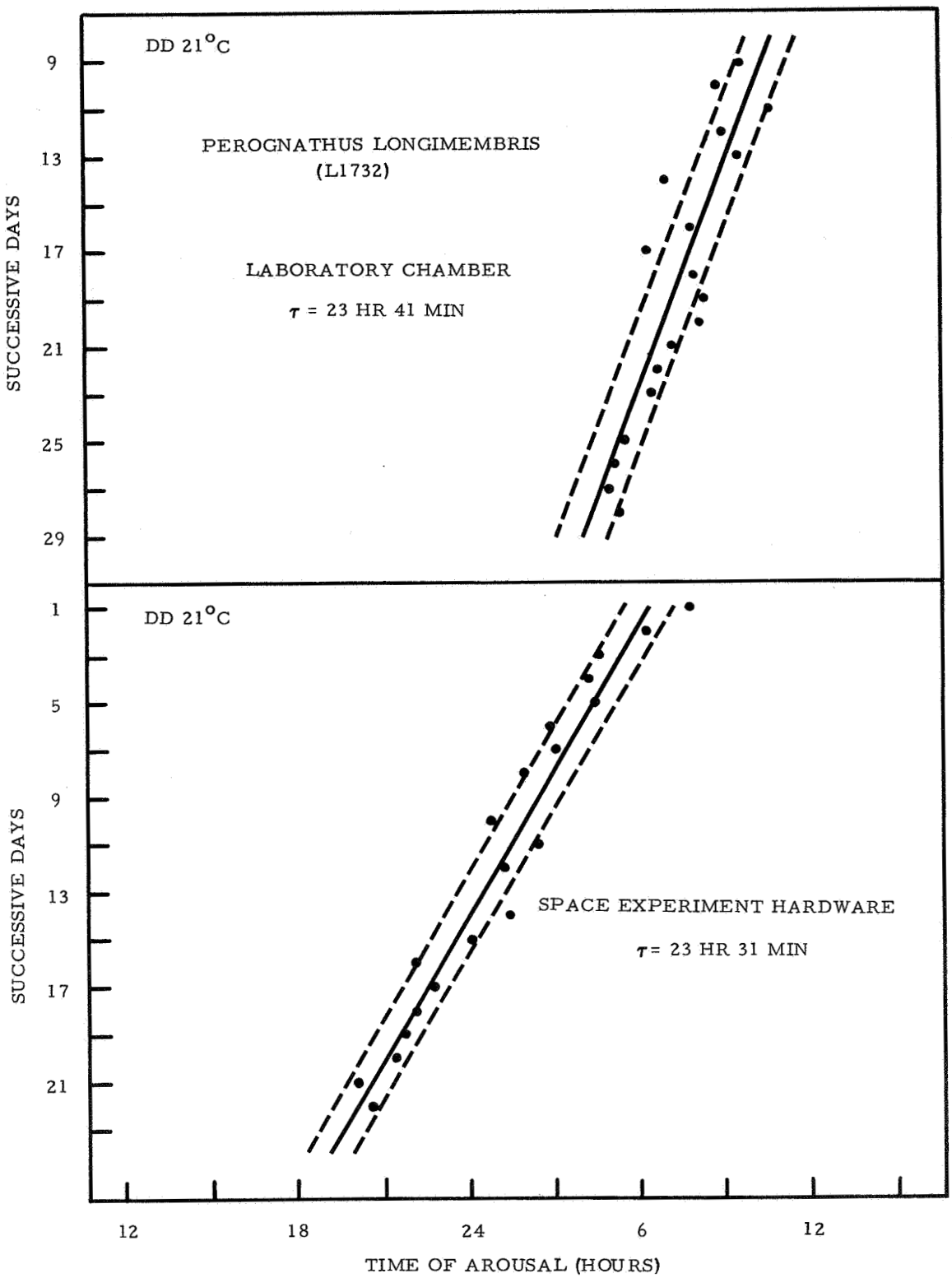

Figure 19 A comparison of the "free-running" circadian period of body temperature in P. longimembris maintained in a laboratory chamber and subsequently in space experiment hardware.

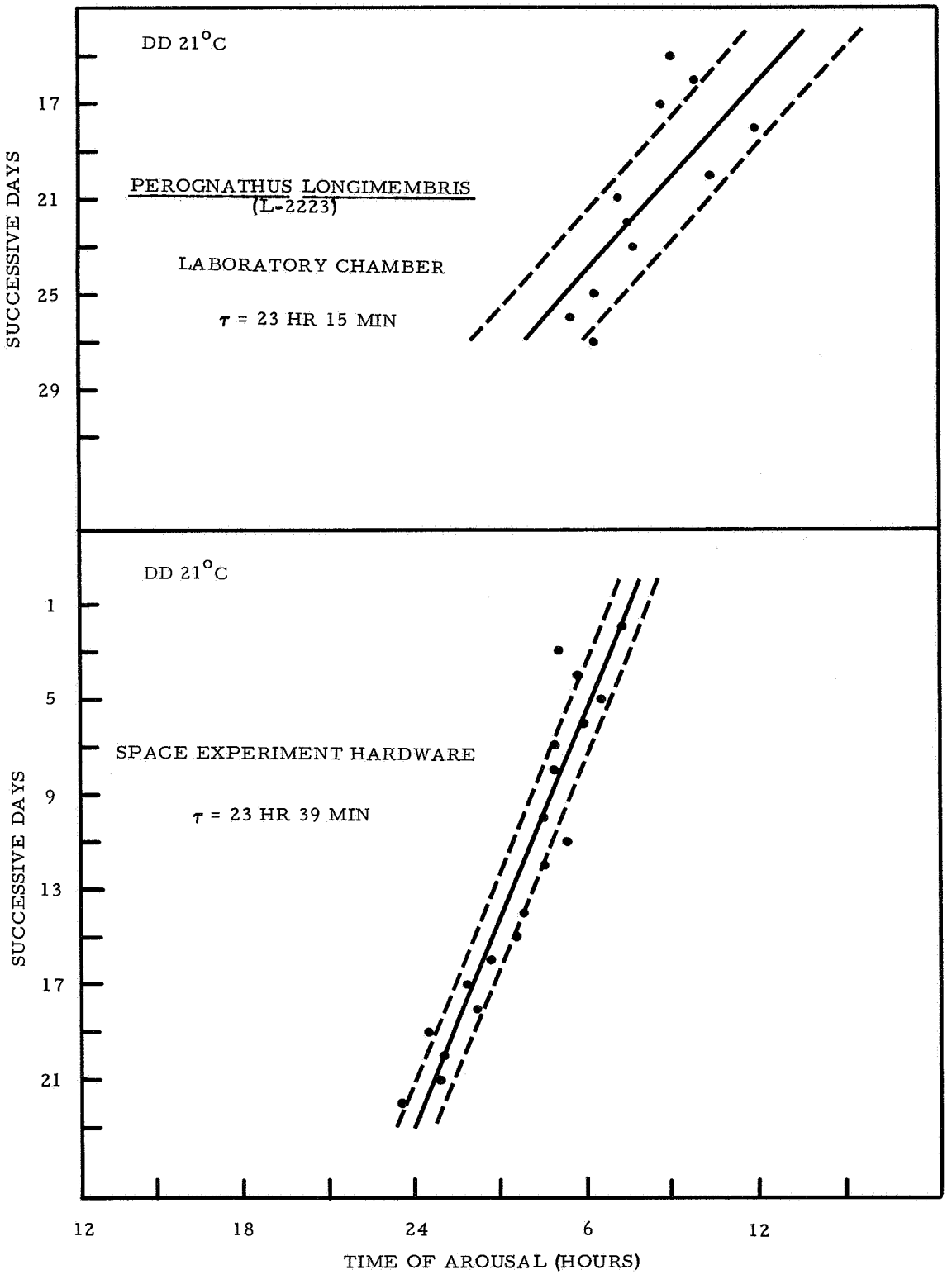

Figure 20 A comparison of the "free-running" circadian period of body temperature in P. longimembris maintained in a laboratory chamber and subsequently in space experiment hardware.

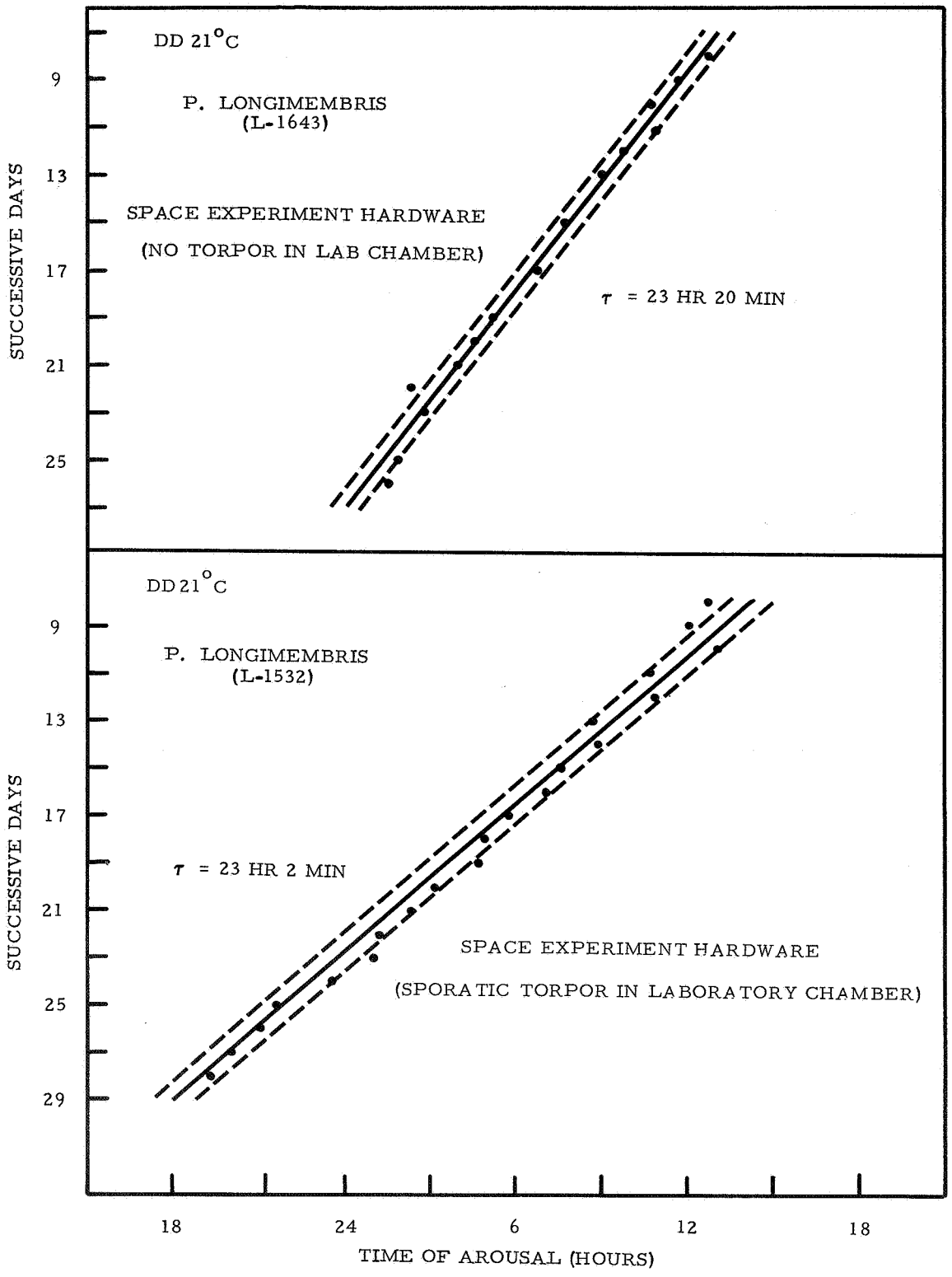

Figure 21 Expression of the "free-running" circadian period of body temperature in two P. longimembris maintained in space experiment hardware.

ble between individuals during the time of year in which the experiment was run (9).

The most important and pertinent observations to come from this experiment are that animals with a documented history of torpor, survive and continue to exhibit an established circadian periodicity for at least 3 weeks in space hardware; the free-running period of daily torpor is relatively fixed as measured in both laboratory chambers and space hardware; and conditions of housing in the space hardware seem to enforce a more rigorous expression of the circadian periodicity of torpor.

Table 1 - Comparison of τ as determined in laboratory chambers and in space hardware configuration

Animal #	Laboratory Chambers Sept. 26 - Oct. 28, 1966	Space Hardware Nov. 10 - Dec. 1, 1966
L-2223	23 hrs., 15 mins.	23 hrs., 39 mins.
L-1732	23 hrs., 41 mins.	23 hrs., 31 mins.
L-1530	24 hrs., 23 mins.	23 hrs., 23 mins.
L-1458	23 hrs., 23 mins.	23 hrs., 25 mins.
L-640	23 hrs., 47 mins.*	24 hrs., 28 mins
Animal #	**Space Hardware Sept. 26 - Oct. 28, 1966**	**Laboratory Chambers Nov. 10 - Dec. 1, 1966**
L-632	Not torpid	Not torpid
L-2262	Not torpid	Not torpid
L-1396	Data doubtful torpors, but electrical noise	Sporatic torpor (5 times)
L-1643	23 hrs. 20 mins.	Torpid once
L-1532	23 hrs. 2 mins.	Sporatic torpor (3 times)

* determined from onset of running wheel activity, torpor sporatic.

Effect of LL and DD on Period Length. - The effects of constant light or constant dark on the free-running period of an animal depends upon whether the species is nocturnal or diurnal. This relationship has been formalized

as "Aschoff's Rule" which states that nocturnal animals demonstrate a period of less than 24 hr in conditions of constant dark and greater than 24 hr in constant light. The reverse is true of diurnal animals.

Analysis of gross motor activity data from 8 Perognathus longimembris indicated that the average circadian period of activity under conditions LD 12:12 was 24 hr 1 min. In constant dark the average period was 23 hr 49 min, and in constant light the average period was 25 hr 57 min. P. longimembris obviously follows Aschoff's rule and behaves as a nocturnal animal.

Phase-reversal. - Early experimentation with P. longimembris using gross motor activity and oxygen consumption indicated that they can complete a 180° phase shift of the circadian period in 7 days or less by reversing the photoperiod from 12 hr light and 12 hr dark to 12 hr dark and 12 hr light. One animal completed the light rephasing in four days be delaying the phase reference point by two major shifts of 5 hr each (φR = onset of activity). In all cases gross activity was inhibited during the reversed light period, with an associated increase during the dark period.

Using the midpoint of arousal from torpor as the phase reference (φR), phase shift was studied in a group of 5 P. longimembris implanted with temperature transmitters and exposed to various photoperiods. Reversal of the 12 hr light 12 hr dark photoperiod was undertaken. In all cases φR was delayed ($-\Delta\varphi$) so that the rhythm of torpor was rephased to the light period within 4-8 days. No difference was detected in the kind of response to the two different light regimen reversals (subjective day to subjective night and its reverse).

Summary

1. The phenomenon of daily torpor has been demonstrated in seven species of pocket mice (Perognathus).
2. The usefulness of the time of arousal as a phase reference point for the study of circadian periodicity in this genus is clearly established.

3. Studies of the free-running circadian period (τFR) of body temperature in P. longimembris and P. formosus were shown to be both persistent and precise.

4. The τFR of body temperature in P. longimembris was studied as functions of ambient temperature and in two cage configurations, one of which was prototype experiment hardware proposed for space biology research. The τFR was shown to be persistent and stable in all cases but there was some evidence of degradation of the precision of the period at low ambient temperatures (10°C).

5. The τFR of body temperature in P. longimembris is less than 24 hr in constant dark and greater than 24 hr in constant light.

6. Phase reversal of τFR in P. longimembris occurs within 4-8 days upon reversal of the photoperiod. In all cases the phase reference point was delayed irrespective of whether the photoperiod reversal occurred by subjective day lengthening or night lengthening.

7. The data presented endorse the use of P. longimembris to study the stability of circadian systems in space both in terms of reliability of the biological data to be obtained and in terms of experiment hardware proposed for experiemtn execution.

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ENTRAINMENT OF THE FREE-RUNNING CIRCADIAN PERIOD OF BODY TEMPERATURE TO CYCLIC PRESSURE CHANGES

P. Hayden and R. G. Lindberg

Introduction

Light and temperature are the most obvious environmental factors that can entrain the circadian rhythm of organisms. Other environmental factors such as noise, feeding regimens and social interaction have not been rigidly tested as synchronizers of biological rhythms (1). Most organisms, when placed under constant light and temperature conditions, show rhythms with periods which differ consistently from 24 hr.

Locomotor activity and tidal rhythm of a sandbeach isopod has been demonstrated to be most likely associated with mechanical stimulation by wave agitation (2). The locomotor activity of house sparrows can be entrained by cyclic presentation of recorded bird song (3). Of ten birds experimentally treated, in this latter study, three met the rigorous criteria of entrainment as stated by the investigator. Sound, in this experiment, became a true "zeitgeber."

Evidence for a true "zeitgeber" requires that (a) the free-running period of experimental individuals be demonstrated, (b) the rhythm examined must be forced to the same frequency as the stimulus, and (c) it must be shown that the phase of the rhythm has been shifted during entrainment (3).

By these criteria we believe that we have demonstrated the entrainment of the circadian rhythm of body temperature in a pocket mouse (P. longimembris) to cyclic environmental pressure changes.

Method

Body temperature was monitored by a small temperature transmitter implanted within the abdominal cavity of eight Perognathus longimembris several months prior to this experiment. Constant environment conditions were provided by a walk-in constant temperature, light-tight incubator.

Experimental conditions were 20°C, 24 hr dark (DD), and a pressure cycle of 12 hr at normal ambient pressure and 12 hr at ambient pressure + 1.3 psi. Four animals were housed in sealed chambers connected to solenoid valves attached to the excurrent air flow system located outside of the experimental chamber. Pressure was varied by a clock mechanism and a water manometer without disturbing the animals. The remaining mice were housed in plastic boxes in the same environment but experienced no pressure change.

The minimum maintenance required by pocket mice permitted the incubator containing the experiment to be sealed for the entire experimental period (42 days). Exposure of both experimental and control groups to unknown or uncontrolled stimuli (barometric pressure changes, cosmic ray fluctuation, laboratory noise, etc.) should have been the same. Cyclic pressure changes began eight days after initiation of the experiment.

Results

Our results show that, of the four animals receiving cyclic pressure variations from Day 8 through 29, two entrained (Fig. 1,2); two did not entrain but did respond to pressure changes during torpor (Fig. 3,4). Three control animals manifested free-running periods of less than 24 hr, with no perturbation of their respective periods during the time interval the experimental groups were exposed to a cyclic pressure change (Fig. 5,6,7). One control animal spontaneously drifted from a period of less than 23 hr to about 25 hr in 42 days (Fig. 8). Table 1 is a summary of the body temperature rhythm of both groups of animals. Period estimates are based on time of arousal from torpor.

The phase relationship to the stimulus of the two entrained animals is interesting. In one (Fig. 3), the increase of pressure stimulates the animal to arouse from torpor most of the time. However, the animal anticipated the pressure increase about one-third of the time. The other animal (Fig. 4) had a much different phase relationship with the pressure change. This animal initiated arousal about 2.5 hr after the pressure transition.

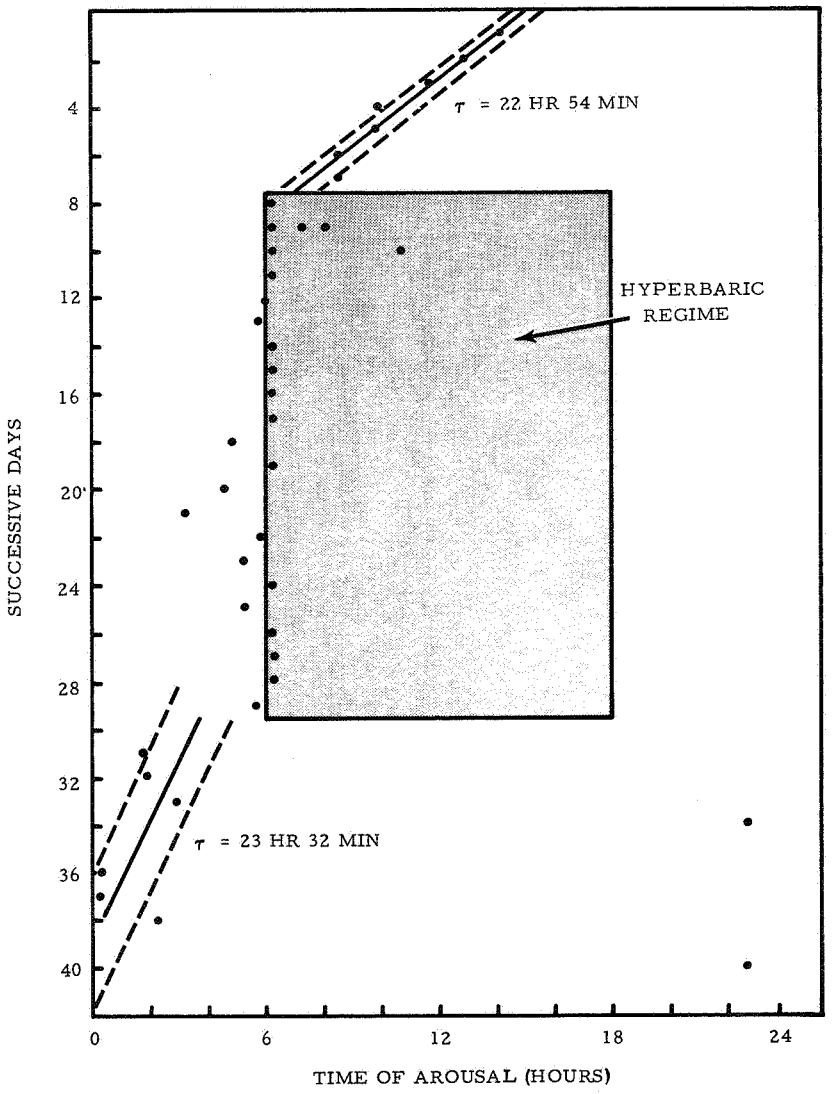

Figure 1

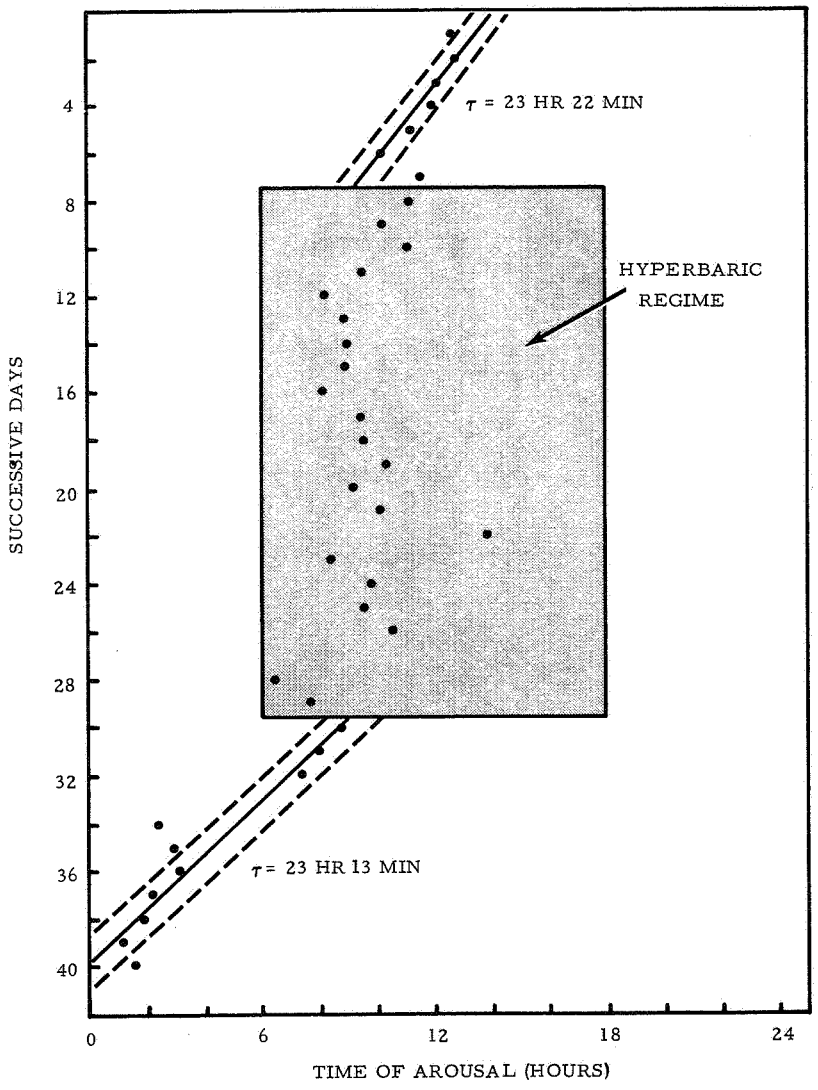

Figure 2

Arousal from torpor of individual Perognathus longimembris maintained in constant dark and constant temperature. A 12 hour cycle hyperbaric regime was imposed from day 8 to day 29.

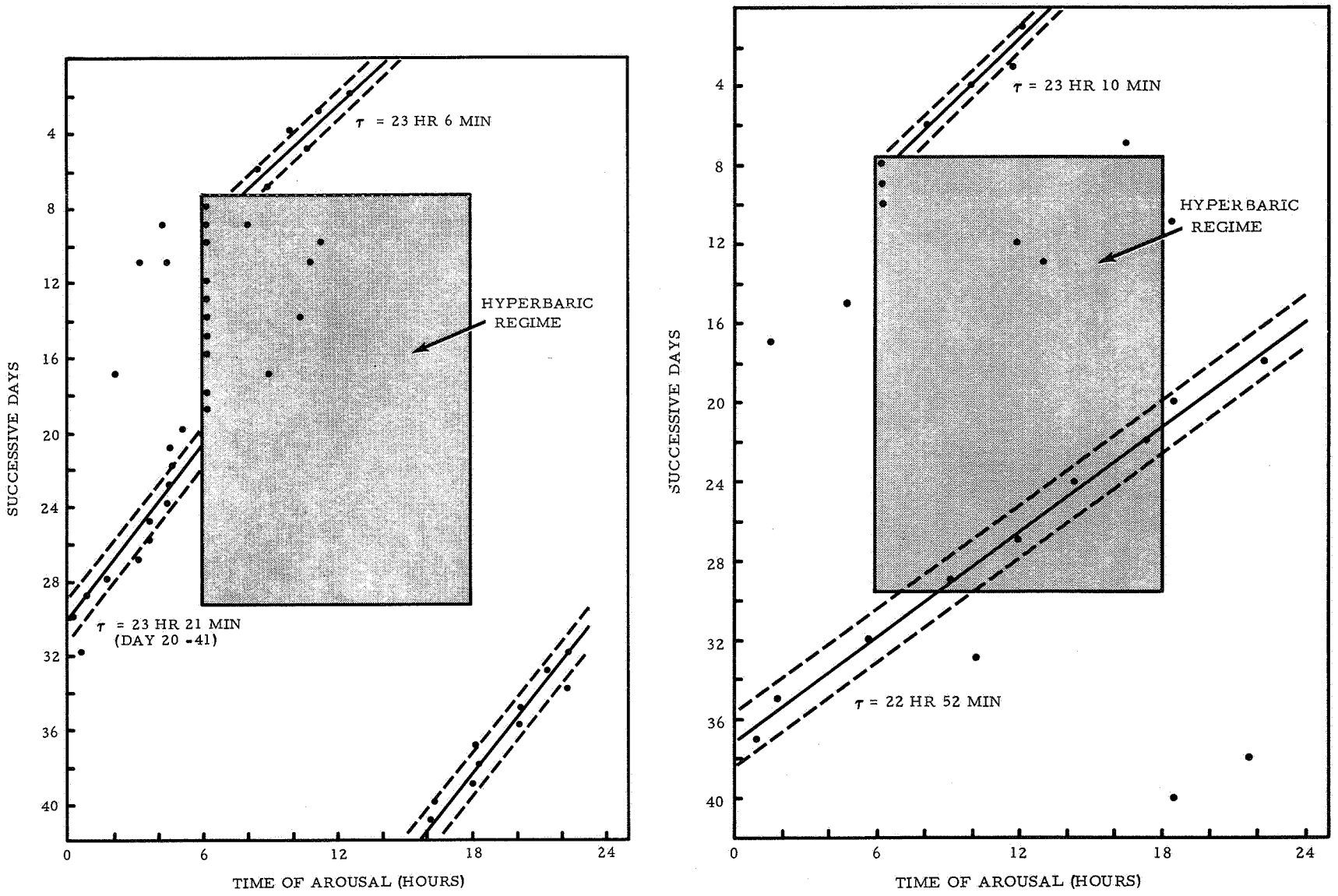

Figure 3

Arousal from torpor of individual Perognathus longimembris maintained in constant dark and constant temperature. A 12 hour cycle hyperbaric regime was imposed from day 8 to day 29.

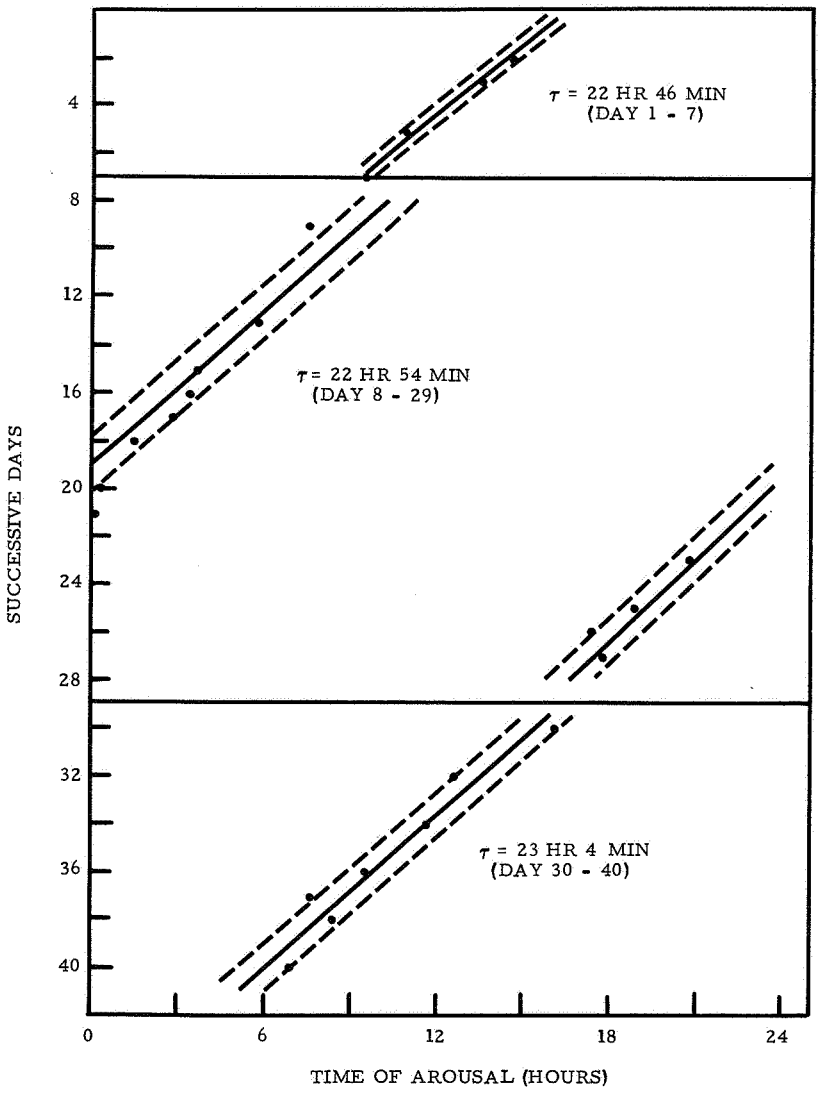

Figure 5

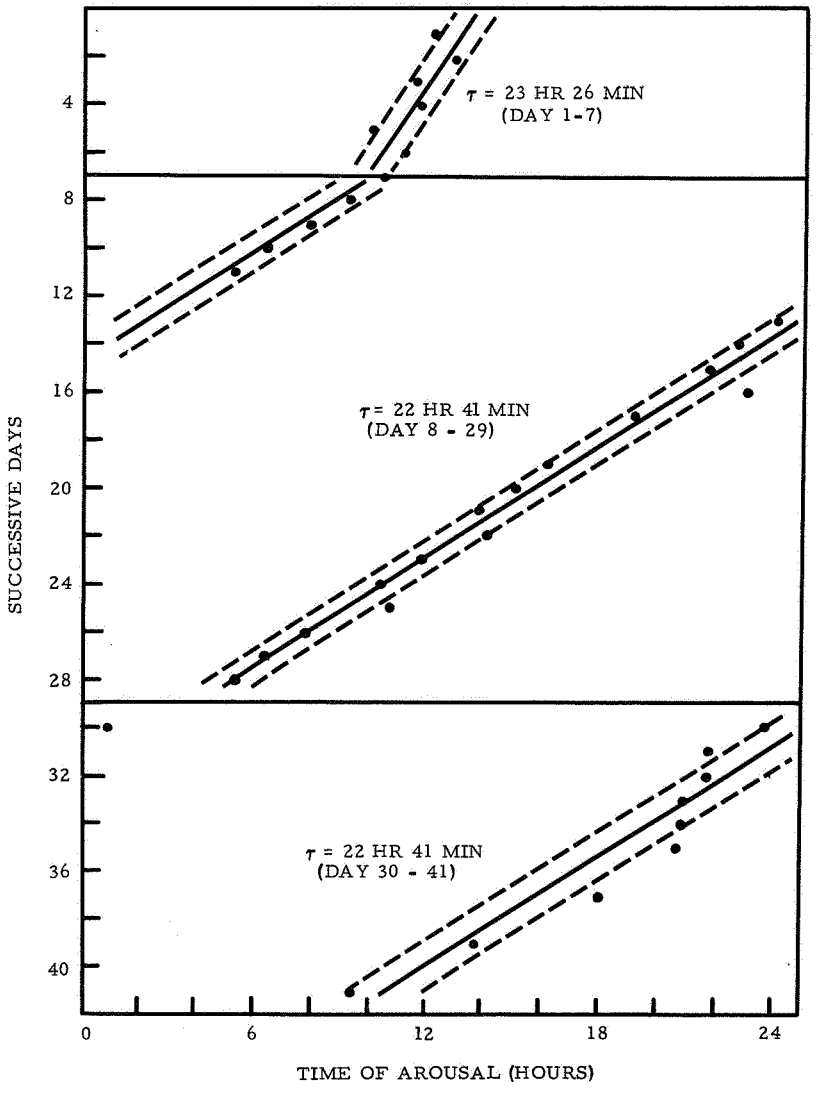

Figure 6

Arousal from torpor of individual Perognathus longimembris maintained in constant dark, constant temperature, and constant pressure as controls to Figures 1-4.

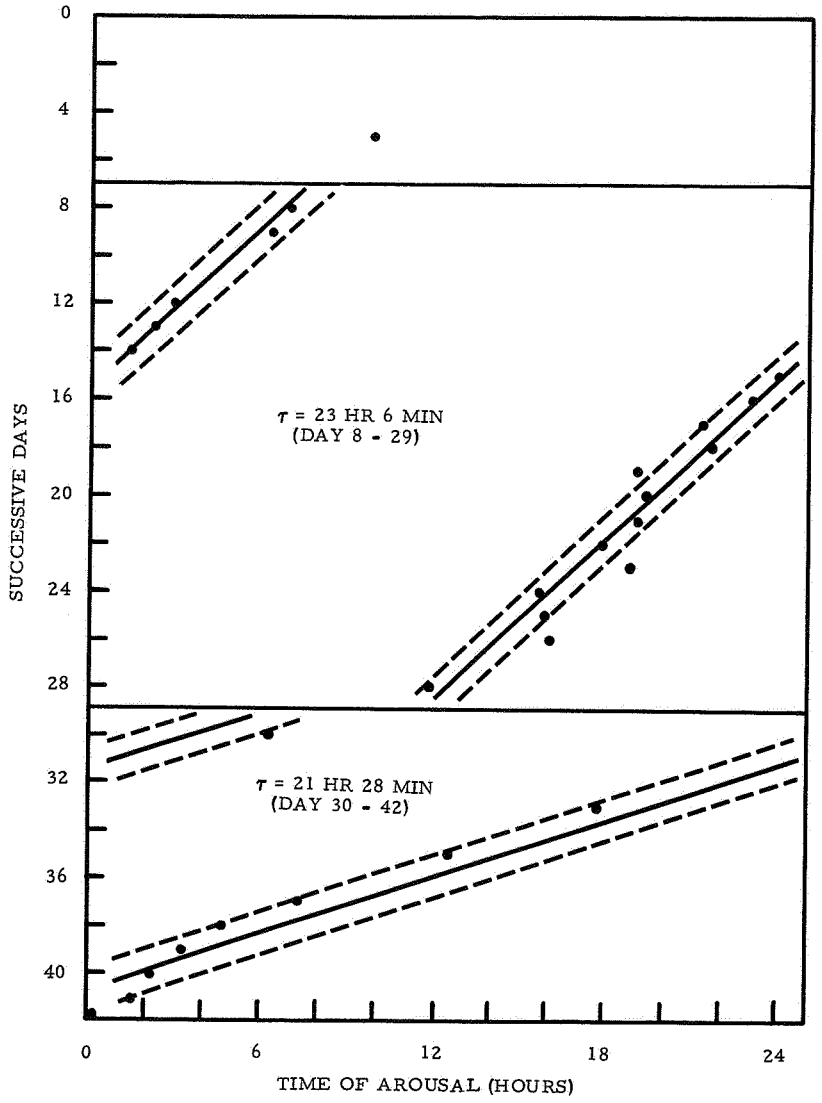

Figure 7

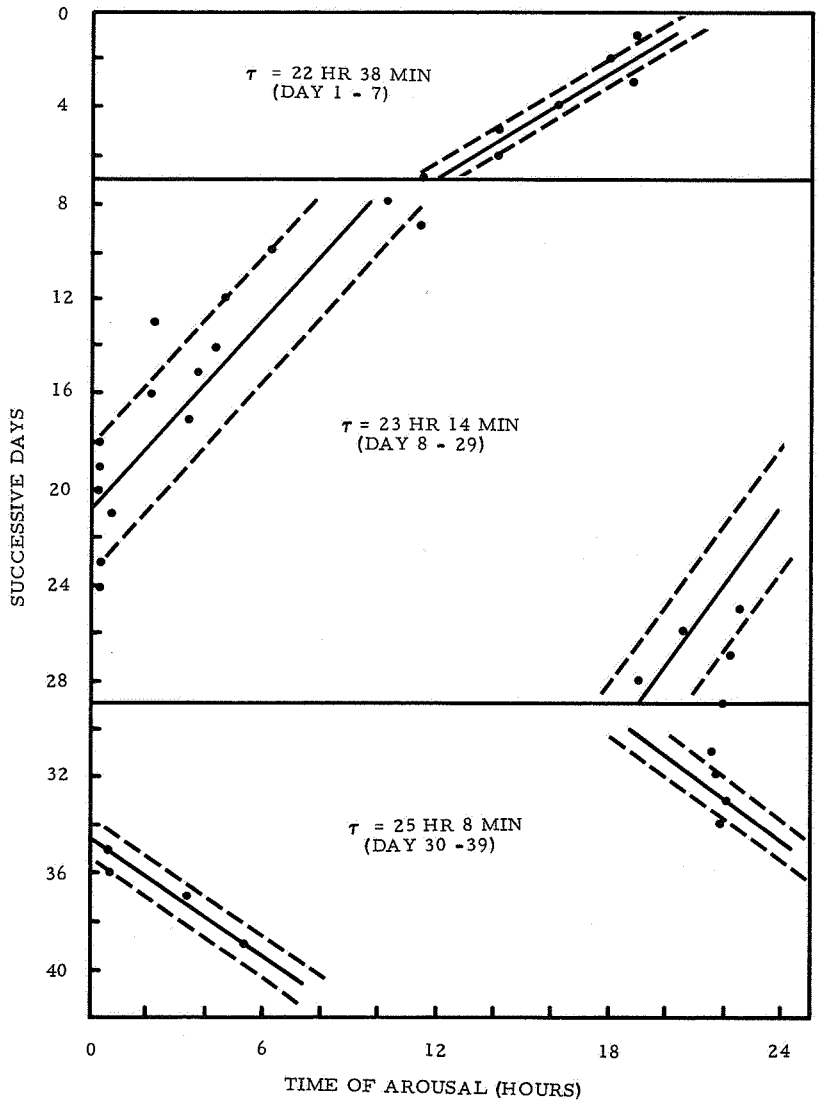

Figure 8

Arousal from torpor of individual Perognathus longimembris maintained in constant dark, constant temperature, and constant pressure as controls to Figures 1-4.

Table 1 - Summary of free-running periods of P. longimembris exposed to similar constant conditons, except for cyclic pressure changes in one group.

	Day 1-7	Day 8-29	Day 30-41	Animal #
Cyclic	22 hr 54 min	entrained	23 hr 32 min	3
Pressure	23 hr 22 min	entrained	23 hr 13 min	4
Changes	23 hr 6 min	disturbed	23 hr 21 min	2
Day 8-29	23 hr 10 min	disturbed	22 hr 52 min	1
Normal	22 hr 46 min	22 hr 54 min	23 hr 4 min	7
	23 hr 26 min	22 hr 41 min	22 hr 41 min	8
		23 hr 6 min	21 hr 28 min	11
	22 hr 38 min	23 hr 14 min	25 hr 8 min	6

Discussion and Summary

The pressure transition from ambient to 1.3 psi overpressure corresponds to an altitudinal downward displacement of about 2000 feet, or a water submersion of approximately 3 feet. The pressurization of the experimental chambers occurred in 25-30 seconds. This rather rapid pressure change was probably sufficient to cause discomfort in the ears of the pocket mice. On several occasions after the termination of the experiment, torpid animals were observed during the positive and negative pressure transition. Positive pressure usually elicited brief pawing at the ears, and negative transition usually no response. Judging from the torpor records, the negative pressure change did not seem to be as disturbing as the positive change. The entrained animals frequently underwent the pressure change without disturbing torpor. The positive pressure transition, or the period of higher pressure, seems to have the same entraining effect as the stimulus of "lights off" for nocturnal animals in a photoperiod regimen.

The role of ambient barometric pressure as a determinant of physical and metabolic activity in several plants and in invertebrates has been

documented by the laboratory of F. Brown (4,5,6,7). Recently, it has been reported that laboratory mice show greater motor activity when the barometric pressure increases (8). This study did not rule out other factors that were strongly associated with barometric pressure (e.g., humidity, pO_2, etc.). A study on humans indicated that changes away from a prevailing average pressure were associated with an increased proportion of subjects showing signs of onset of sleep as judged by EEG examination (9).

Barometric pressure has been postulated as one of the "pervasive geophysical factors" that is present in all experimental setups in which the investigator has attempted to provide "constant conditions" for his experimental subjects. Our study seems to minimize the effect of barometric pressure as an entraining force. Our entraining pressure was many times greater than any barometric shift and only entrained half of the sample used. It is possible that the discomfort experienced during the change to positive pressure is the primary "zeitberger" rather than pressure. If this latter is true, then it is conceivable that an animal could just as well be entrained to an electric shock. This has yet to be demonstrated. It has been stated that in the absence of the strongest entraining stimuli of light and temperature, any stimulus that an organism can perceive is a potential entraining force. We believe that we have demonstrated this with pressure.

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Response of the Pocket Mouse to Ionizing Radiation[1]

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INTRODUCTION

Pocket mice (genus *Perognathus*) have been proposed as particularly suitable subject matter for space biology research. Their unusual physiology allows significant compromises in the life support requirement for mammals in biosatellites. The resultant savings in payload weight, coupled with the small size of the animals, permits experimental designs with statistically significant numbers of animals and good reliability.

Since there is a dearth of physiological data on the pocket mouse, baseline data points had to be established prior to designing satellite experiments. Among the baseline studies undertaken was the response of the pocket mouse to ionizing radiation.

It was anticipated that the radiation response of *Perognathus* would be similar to that of other small mammals. This expectation was based on the fact that the closely related kangaroo rat has an $LD_{50(30)}$ comparable to that of the CF_1 mouse (*1*). In the course of these studies, however, the pocket mouse was found to demonstrate a high degree of radiation resistance. In this respect it differs greatly, not only from its close relatives, but from all other mammals.

MATERIALS AND METHODS

Pocket mice are heteromyid rodents indigenous to the arid regions of Western United States and parts of Mexico. Taxonomically, *Dipodomys* (kangaroo rat), *Microdipodops* (kangaroo mouse), and *Perognathus* (pocket mouse) are grouped in the subfamily Perognathinae (Heteromyidae). This grouping reflects not only morphological similarities but also ecological and physiological ones. For example, the ability to subsist on dry seeds with no requirement for drinking water or succulent foods appears to be a physiological characteristic the three genera have in common (*2*).

The capability of becoming hypothermic under certain adverse environmental conditions also occurs among the heteromyids. Lack of food and low environmental

[1] Portions of this work were supported by NASA Contract NaSr-91.

temperature is the trigger for this phenomenon, which has been documented in species of *Perognathus* and *Microdipodops* (*3*, *4*). There are no reports of naturally occurring hypothermia in the genus *Dipodomys*, although it can be induced.

The genus *Perognathus* includes twenty-six species. *Perognathus longimembris*, weighing approximately 8.5 gm, is among the smallest of the genus, it is, indeed, one of the smallest mammals. *P. formosus*, weighing approximately 20 gm, compares favorably in size to the common laboratory mouse. Pocket mice are available in large numbers, easily live-trapped, and tractable when brought into the laboratory. They are particularly easy to maintain by virtue of their small size and their ability to exist on dry food and to produce concentrated body wastes.

Animals were selected according to sex and weight from a large collection of *Perognathus* which were live-trapped in the field and maintained in our laboratory. From field data and available information on population dynamics of the pocket mouse, it is assumed that approximately 80 % of the animals used in this study were just under 1 year old at the time of irradiation. The others were just under 2 years old.

Survival Studies—X-rays

One hundred and twenty-five (125) healthy adult male *P. longimembris* with a mean weight of 8.7 gm (range 7.4 to 10.5 gm) were segregated from the main colony and divided into five groups of 25 each (Table I). Group assignments were made from a table of random numbers (animals are numbered consecutively as they arrive from the field). In this manner groups are randomized as to age and collection site of the mice.

Four groups received a single exposure to whole-body irradiation at doses of 400, 600, 800, and 1000 r delivered from a 250-kvp 15-ma X-ray machine[2] (0.47 mm Cu inherent, 65.0 cm TOD, and 20.52 r/m in dose rate). A control group was handled similarly to the irradiated groups except for the actual irradiation.

Animals were transported to and from the radiation site, a round trip of approximately 20 miles, and irradiated in compartmentalized plastic boxes. The animals were in these boxes for approximately 4 hours. Although their movement was limited, ventilation was adequate as judged by their apparent comfort. Because of the usual amount of handling required in getting the animals to the radiation source, a group of 25 White Swiss mice were also subjected to 600 r of whole-body irradiation at the same time and in the same manner as were the pocket mice. In addition, a control group of White Swiss mice were retained.

During irradiation, the boxes were placed on a rotating device which ensured a uniform distribution of radiation over 99 % of the field. Animals were oriented in the compartments in such a manner that irradiation was received dorsoventrally.

[2] Use of the radiation facilities at the Department of Nuclear Medicine and Biophysics, Laboratory of Nuclear Medicine and Radiation Biology at the University of California at Los Angeles, is gratefully acknowledged.

TABLE I

EXPERIMENTAL DESIGN

Dose (r)	*Species*	*Sex*	*Number*
	X-Ray Survival		
0	*P. longimembris*	♂	25
400	*P. longimembris*	♂	25
600	*P. longimembris*	♂	25
800	*P. longimembris*	♂	25
1000	*P. longimembris*	♂	25
	Co^{60} Survival		
0	*P. longimembris*	♂	25
0[a]	*P. longimembris*	♂	25
800	*P. longimembris*	♀	25
1200	*P. longimembris*	♂	25
1400	*P. longimembris*	♂	25
1600	*P. longimembris*	♂	25
1800	*P. longimembris*	♂	25
2000	*P. longimembris*	♂	25
0	*P. formosus*	♂ and ♀	9
0[a]	*P. formosus*	♂ and ♀	24
600	*P. formosus*	♂ and ♀	10
1200	*P. formosus*	♂ and ♀	10
1400	*P. formosus*	♂ and ♀	23
1600	*P. formosus*	♂ and ♀	24
1800	*P. formosus*	♂ and ♀	9
	Hematological Studies		
0	*P. longimembris*	♂	25
0	*P. longimembris*	♀	49
0	*P. longimembris*	♀	25
400	*P. longimembris*	♂	25
400	*P. longimembris*	♀	25
1400	*P. longimembris*	♂	10
1400	*P. longimembris*	♀	50

[a] Control groups are duplicated because irradiations were performed on three different dates.

After irradiation the animals were returned to our laboratory and restored to their original caging. All pocket mice in our laboratory are maintained in individual gallon-size wide-mouth jars containing 2 to 3 inches of sand. A mixture of grass seed ("parakeet" seed), rolled oats, and sunflower seeds is made available *ad libitum*. No drinking water is required.

Temperature control in the laboratory is set at 22°C. Normally, at night it is maintained between 20° and 22°C. During the day it varies between 22° and 24°C, with occasional excursions to 25°C. Relative humidity is maintained at 50 ± 5%.

During the first month post-irradiation, animals were checked twice daily. Dead animals were autopsied as soon after death as possible.

Survival Studies—Co^{60} Radiation

P. longimembris. Seven groups of 25 adult male *P. longimembris* were established as described for the X-ray studies. Mean weight of the animals was 8.3 gm (range 6.0 to 11.9 gm). Radiation doses of 1200, 1400, 1600, 1800, and 2000 r were delivered from a 10,000-curie Co^{60} source at a dose rate of 102 r/min. One group of 25 adult female *P. longimembris* was administered 800 r from the same source and in the same manner. Mean weight of females was 8.5 gm (range 6.8 to 10.2 gm). Appropriate control groups were retained. All other techniques and conditions for these studies were as described above for the X-ray studies.

P. formosus. Thirty-eight (38) adult *P. formosus* of both sexes were divided into four groups of 9 or 10 each and subjected to 600, 1200, or 1800 r of Co^{60} radiation. One group was retained as a control group. The mean weight of these animals was 18.7 gm (range 15.5 to 21.7 gm). In a second radiation series, 71 *P. formosus* of both sexes were divided into three groups of 23 or 24 each. One group was subjected to 1400 r of Co^{60} irradiation, another to 1600 r, and a third group was retained as controls. The mean weight of these animals was 20.4 gm (range 14.0 to 28.1 gm). Radiation was delivered and techniques used were as described for *P. longimembris.*

Hematological Studies

Fifty adult male *P. longimembris* with a mean weight of 8.8 gm (range 7.7 to 10.9 gm) were segregated from the main colony and divided into two groups in the same manner as described in the X-ray studies.

One group of 25 animals received 400 r of whole-body X-irradiation. A group of 25 controls were handled identically to the irradiated except for the actual irradiation.

On days 1, 3, 5, 7, and 9 postirradiation, blood samples were taken from 5 irradiated and 5 control animals. Different animals were used on each of the days so that each animal was bled just once. Peripheral blood samples were obtained by the method of tail transection. Total erythrocytes, leukocytes, and differential counts were made, and microhematocrits were obtained.

In a second blood series, four groups of adult female *P. longimembris* were established. One group of 25 animals received 400 r of Co^{60} radiation; a second group containing 50 animals received 1400 r. A group of 50 manipulation controls and 25 laboratory controls were retained. The mean weight of these animals was 8.1 gm (range 6.5 to 11.3 gm). Total erythrocyte, leukocyte, and platelets counts, differentials, hemoglobins, and hematocrits were obtained over the 10-day period immediately after irradiation.

Sufficient numbers of animals were irradiated or used as controls in each group so

that no animal was bled more than once. Daily blood samples of five manipulation controls over the 10-day period were obtained. Ten 1400-r animals were bled on alternate days during the period: days 1, 3, 5, 7, and 9. Five 400-r and 5 laboratory control animals were bled on days 2, 4, 6, 8, and 10.

Total erythrocyte and leukocyte counts, differentials, and microhematocrits were obtained throughout the period. Hemoglobin determinations were made on the first and second days and on the ninth and tenth days postirradiation. Platelet counts were made on several control animals and a small number of irradiated animals on the ninth and tenth days.

The Coulter electronic counter was used for total counts. Phase microscopy was used for direct platelet counts. Hemoglobins were determined by the acid-hematin method. Slides were stained with Wright stain for differential counts.

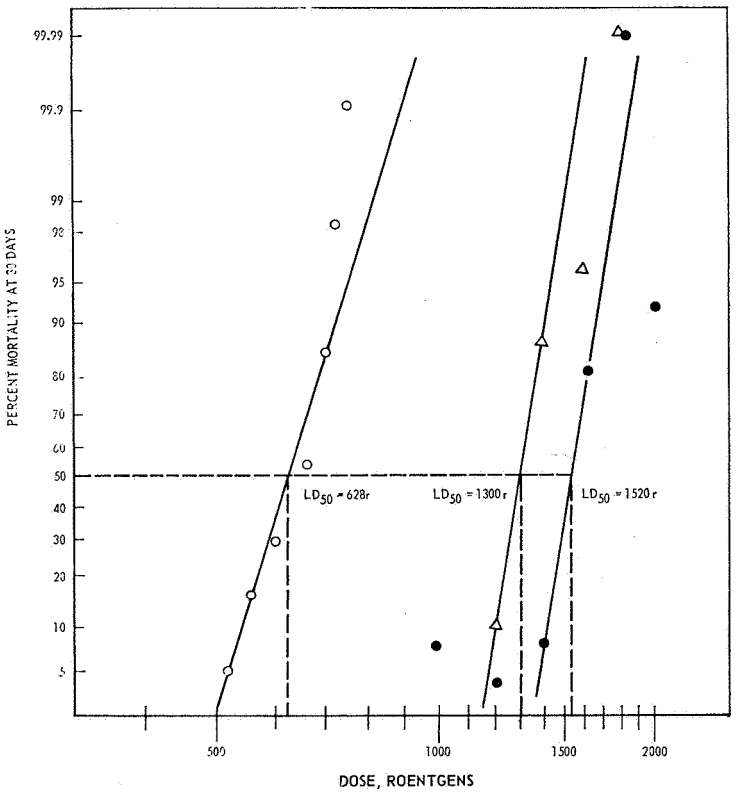

FIG. 1. Acute mortality of mice exposed to varying doses of ionizing radiation. Abscissa, $\log_{10}$ scale; ordinate, probability scale. Open circles—♀ CF_1 mice (data of Patt *et al.*, *5*); triangles—*Perognathus formosus*; filled circles—*Perognathus longimembris*.

RESULTS

Probability plots for acute dose-mortality data are shown in Fig. 1. The $LD_{50(30)}$ value obtained for *P. longimembris* is 1520 r; that for *P. formosus* is 1300 r. In Fig. 1, mortality curves for these two species are compared with that for female CF_1 mice from the data of Patt *et al.* (*5*). The $LD_{50(30)}$ for CF_1 mice is 628 r. The ST_{50} for the White Swiss mice irradiated at 600 r in this experiment was 19 days.

Acute mortality curves for *Perognathus* species had the typical sigmoid shape exhibited by dose-mortality data for other mammals. There were no deaths prior to 6 days, and, as shown in Fig. 1, significant numbers of deaths occurred in high-dose groups only. That is, it was necessary to administer doses in excess of 1200 r to obtain significant early deaths in *P. formosus*. Similarly, doses in excess of 1400 r were necessary to obtain significant numbers of deaths in *P. longimembris*.

Continued observations on survival for periods up to 29 weeks postirradiation indicate only a gradual decline in survival in any of the radiation groups. Animals irradiated at 1000 r, for example, have 80% remaining at 29 weeks; those receiving 1400 r have 75% remaining at 21 weeks.

Gross autopsy findings of those animals that died during the acute period reveals that deaths were probably due to either acute respiratory disease, gastrointestinal damage, or hemorrhages. Internal bleeding, either gastrointestinal or intracranial, was observed in approximately 70% of the dead animals. Complete autopsy results will be the subject of a subsequent report when all the irradiated and control animals are dead.

Tables II to V present various blood values of irradiated and control pocket mice.

TABLE II

BLOOD VALUES OF 25 ADULT MALE *Perognathus longimembris* AND 25 CONTROLS SAMPLED IN GROUPS OF 5 ON DAYS 1, 3, 5, 7, AND 9 AFTER SUBLETHAL TOTAL-BODY X-IRRADIATION

Group	*Day*	*Erythrocytes/ mm³, × 10⁻⁶*		*Leukocytes/ mm³, × 10⁻³*		*Lymphocytes (%)*		*Hematocrit (%)*	
		$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	*Range*
400 r total-body irradiation	1	14.0	± 1.7	6.4	± 2.1	55	± 16	55	(50–59)
	3	14.1	± 1.4	5.5	± 2.8	55	± 21	53	(50–57)
	5	12.5	± 2.7	5.1	± 1.6	89	± 8	52	(50–52)
	7	13.4	± 1.6	4.7	± 1.0	75	± 25	53	(52–54)
	9	12.5	± 2.8	8.3	± 2.9	65	± 28	57	(52–56)
Nonirradiated controls	1	13.6	± 1.7	7.5	± 4.7	76	± 8	52	(49–55)
	3	14.2	± 0.7	8.8	± 2.2	74	± 13	53	(50–56)
	5	13.8	± 0.9	11.4	± 2.3	83	± 5	53	(47–59)
	7	14.3	± 1.7	9.1	± 3.0	70	± 16	56	(54–58)
	9	13.6	± 1.6	10.3	± 3.2	78	± 16	56	(53–58)

TABLE III

TOTAL BLOOD CELL COUNTS OF POCKET MICE (*Perognathus longimembris*) SERIALLY SAMPLED DURING THE 10-DAY PERIOD IMMEDIATELY AFTER TOTAL-BODY Co^{60} IRRADIATION

Day postirradiation	*400 r*		*1400 r*		*Manipulation control*		*Laboratory control*	
	$\overline{X}$	σ	$\overline{X}$	σ	$\overline{X}$	σ	$\overline{X}$	σ
	Erythrocytes (cells/mm³, × 10⁻⁶							
1			14.0	2.5	14.8	1.7		
2	13.8	1.9			14.5	0.4	13.4	1.8
3			12.3	1.6	13.0	0.5		
4	12.8	1.0			13.1	0.8	13.4	1.8
5			12.7	0.8	12.5	1.1		
6	12.1	0.7			11.5	1.0	12.0	1.0
7			13.2	1.5	13.3	0.7		
8	15.9	2.3			13.8	1.7	15.5	2.2
9			11.5	2.7	13.8	0.3		
10	12.6	1.6			13.6	1.1	13.1	0.5
	Leukocytes (cells/mm³, × 10⁻³)							
1			3.9	1.9	6.6	4.5		
2	6.9	2.1			9.6	3.3	11.4	5.9
3			3.0	1.3	8.1	4.1		
4	2.6	1.1			8.4	3.9	8.8	6.5
5			1.6	0.8	9.3	4.9		
6	5.3	3.8			6.8	2.2	7.0	1.7
7			1.5	0.9	7.5	2.0		
8	5.9	2.2			7.3	2.0	6.9	2.6
9			0.9	0.7	6.0	3.6		
10	7.0	5.3			5.4	2.5	6.6	3.7

Inspection of erythrocyte data suggests that no marked depression of erythropoiesis occurred during the early postirradiation period. Slight reductions in mean values of erythrocyte counts, hematocrits, and hemoglobins are seen in animals that were administered 1400 r. These reductions are not statistically significant even at 9 days postirradiation. They suggest, however, that at the 1400-r dose level the pocket mouse may demonstrate a typical radiation anemia at approximately the same time that it occurs in other mammals after just sublethal irradiation. Stippled and nucleated erythrocytes were observed on blood slides of irradiated animals as early as the first day postirradiation.

As is typical of mammals, leukocytes of the pocket mouse respond to irradiation within 24 to 36 hours. This early response is manifested as a general leukopenia (Fig. 2). Since total leukocyte counts are normally extremely variable, it seems reasonable to present the data as in Fig. 2, comparing counts of irradiated animals

TABLE IV

PER CENT LYMPHOCYTES OF POCKET MICE (*Perognathus longimembris*) SERIALLY SAMPLED DURING THE 10-DAY PERIOD IMMEDIATELY AFTER TOTAL-BODY Co^{60} IRRADIATION

Day postirradiation	*400 r*		*1400 r*		*Manipulation control*		*Laboratory control*	
	$\overline{X}$	*Range*	$\overline{X}$	*Range*	$\overline{X}$	*Range*	$\overline{X}$	*Range*
1			46	(20–87)	75	(40–95)		
2	56	(22–95)			92	(88–95)	83	(64–94)
3			39	(12–81)	70	(68–85)		
4	64	(59–73)			83	(77–92)	85	(78–92)
5			96	(90–100)	79	(72–91)		
6	73	(68–81)			85	(78–92)	87	(81–91)
7			90	(60–100)	77	(65–88)		
8	70	(40–92)			73	(63–88)	73	(46–84)
9			68	(42–100)	77	(49–95)		
10	69	(60–76)			68	(58–75)	69	(58–79)

with a range of values (the stippled area) which represents the normal variability in control values. Mean values for 400-r animals fall below the normal control range on the fourth day only. Mean values for 1400-r animals fall below the normal control range on every day tested.

Differential counts reveal that the leukopenia reflects an initial lymphopenia followed by a marked but transient neutropenia at both the 400-r and the 1400-r dose levels.

Differential counts also indicate that the lymphopenia is a result of the prompt disappearance of small lymphocytes after irradiation. Large lymphocytes outnumber small lymphocytes in blood smears on the first through the seventh day after 1400 r of irradiation. Large lymphocytes predominate on the fourth, sixth, and eighth days after 400 r of irradiation, but not on the second and tenth days. On the whole, small lymphocytes were found in greater numbers than large lymphocytes in blood smears of controls.

Platelets were markedly depressed in 4 of 5 1400-r animals. Four of these animals had counts ranging from 0 to 25,000 per mm^3 on the ninth day postirradiation; one had a normal count (978,000 per mm^3). Platelet counts of nine control animals ranged between 300,000 and 993,000 per mm^3. The one 400-r animal whose platelets were counted had a value of 705,000 per mm^3 at 10 days postirradiation.

DISCUSSION

Blood responses of the pocket mouse to total-body irradiation follow the same general pattern seen in other mammals (*6*). It is necessary, however, to administer to *Perognathus* much larger radiation doses to produce changes of comparable

TABLE V

SELECTED BLOOD VALUES OF POCKET MICE (*Perognathus longimembris*) SAMPLED DURING THE 10-DAY PERIOD IMMEDIATELY AFTER TOTAL-BODY Co^{60} IRRADIATION

Day post-irradiation	*400 r*			*1400 r*			*Manipulation control*			*Laboratory control*		
	$\bar{X}$	*Range*	*No.*	$\bar{X}$	*Range*	*No.*	$\bar{X}$	*Range*	*No.*	$\bar{X}$	*Range*	*No.*
					Hematocrit (%)							
1				56	50–61	2	57	54–60	2			
2	54	50–56	4				58	53–61	5	53	49–58	5
3				51	46–58	15	53	52–55	4			
4	49	45–54	5				57	55–60	5	53	49–58	5
5				52	46–54	8	55	47–61	5			
6	53	50–56	5				55	53–56	5	53	50–56	5
7				52	46–58	13	55	53–58	4			
8	56	51–60	4				51	49–54	4	56	51–60	4
9				46	22–56	9	57	54–62	4			
10	55	51–58	4				55	52–57	5	55	51–58	4
					Hemoglobin (gm/100 ml)							
1				17.9	15.9–20.4	4	16.9	16.1–18.4	3			
2	16.2	15.2–17.3	5				17.2	15.3–18.8	5	15.9	14.9–16.8	5
9				13.8	7.2–16.0	6	17.2	16.9–17.6	4			
10	16.2	13.8–17.7	3				17.6	16.0–18.7	5	17.9	16.5–18.9	5
					Platelets/mm³, × 10⁻⁴							
9				20.4	0–97.8	5	59.0	43.5–73.0	3			
10	70.5	—	1				64.1	30.0–98.2	2	83.4	59.8–99.3	4

magnitude. For example, hematological responses of *Perognathus* administered 400 r of total irradiation resemble those of the kangaroo rat which was administered 50 r (*1*). *Perognathus* administered 1400 r demonstrates hematological changes comparable to those in other mammals receiving 400 to 500 r of total-body irradiation (*7*).

Depending on dose, maximum leukocyte depression occurs between 4 and 9 days postirradiation. Differential counts indicate that leukocyte changes reflect an initial lymphopenia which is followed in a few days by a marked reduction in peripheral neutrophiles. This course is normal for small mammals after sublethal irradiation (*8*).

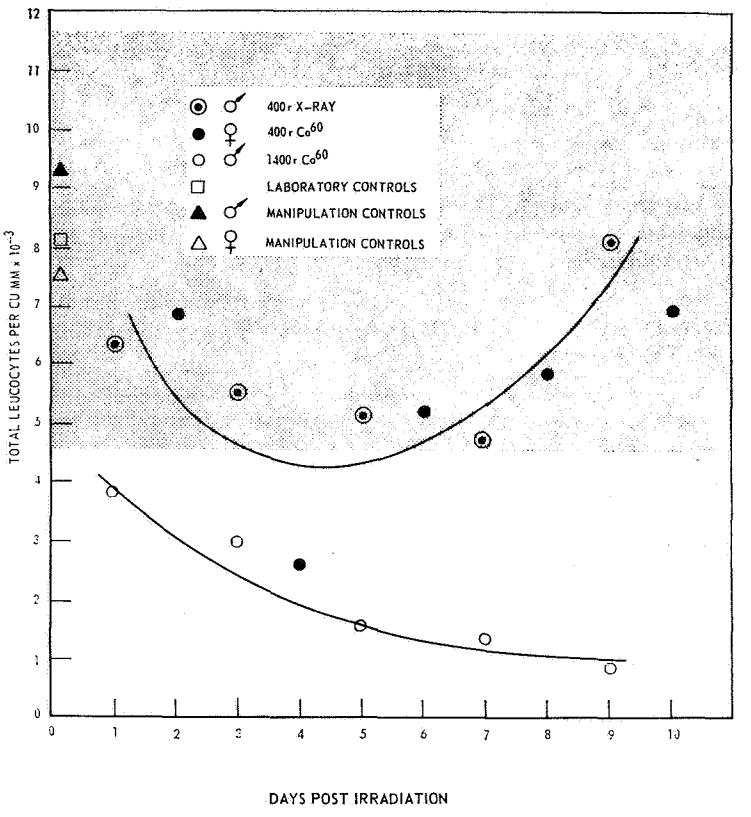

FIG. 2. Early hematological changes in peripheral blood of *Perognathus longimembris* after total-body irradiation. Shaded area represents ±1 standard deviation of laboratory control mean. Curves fitted by inspection.

Perognathus survives doses of acute radiation significantly higher than those survived by other mammals, when the irradiation is delivered under comparable conditions. The ST_{50} reported for *Dipodomys*, for example, is 10.3 days at a delivered dose of 550 r (*1*). In *P. formosus* and *P. longimembris*, 1600 r and 1800 r, respectively, are required to obtain approximately the same ST_{50}.

Since *Perognathus* has the capability of going hypothermic, a comparison might logically be made between it and other small mammalian hibernators. It is well documented that the state of hibernation confers protection against the lethal effects of radiation (*9*). On the other hand, mortality of hibernators when irradiated while in a normothermic condition follows closely that of any other mammal (*9*). In other words, the ability to hibernate does not appear to confer a special ability to resist the effects of radiation, if the irradiation is delivered while the animal is normothermic.

In contrast to other hibernators, the radioresistance noted in *Perognathus* occurs when the animal is irradiated in a normothermic state. There is insufficient evidence to conclude at this time that the resistance is either related or unrelated to the ability of *Perognathus* to hibernate.

Reported $LD_{50(30)}$ values among the mammals range from a few hundred to several hundred roentgens when the irradiation is delivered as a single acute dose and at a comparable rate (*10*). Variation in $LD_{50(30)}$ values among the mammals probably reflects a number of real and experimentally produced differences between the species investigated. Despite the wide differences in sensitivity, certain basic mechanisms of death are quite similar. At doses less than 1000 r, death results from hematopoietic failure and occurs within the first 3 or 4 weeks, but not earlier than the second week after irradiation. At doses greater than 1000 r, death is the result of irreparable damage to the gastrointestinal epithelium and occurs within the first week or two postirradiation. Much higher dose levels (5000 to 10,000 r) produce extremely early CNS death. $LD_{50(30)}$ values are not derivable from such high doses.

The significance of $LD_{50(30)}$ values reported for *Perognathus* is the fact that they survive doses (>1000 r) that are uniformly fatal to other mammals via the gastrointestinal syndrome. Second, they survive doses after which, even if by some life-saving treatment other mammals are protected from the gastrointestinal death, hematopoietic failure is certain to ensue and ultimately cause death. Methods of protecting against hematopoietic death are known but are not remarkably successful if applied after high-dose irradiation.

The two- to threefold increase of $LD_{50(30)}$ values for *Perognathus* over those for other mammals suggests that both hematopoietic and gastrointestinal damage are ameliorated. Hypoxic hypoxia produces the same protection in other mammals. Dehydration is also protective (*11*). Postirradiation hypothermia delays irradiation damage. These agents are mentioned from the long list that is known to alter radiation response, because there is some evidence that one or all of these may be acting in *Perognathus*. Follow-up work in this laboratory is designed to elucidate the mechanism of radioresistance in *Perognathus*.

SUMMARY

1. Several hundred pocket mice representing two species, *Perognathus longimembris* and *P. formosus*, were administered single doses of whole-body X- and γ-radiation. Exposure doses of 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000 r were used.

2. Radiation at the highest dose levels only (>1200 r) resulted in significant numbers of acute (30-day) deaths. The $LD_{50(30)}$ for pocket mice in this experiment (1300 r for *P. formosus*; 1520 r for *P. longimembris*) is two to three times that reported for related mammalian forms.

3. Postirradiation hematological changes as evidenced by total and differential

blood counts, hematocrits, hemoglobins, etc., corroborate the extreme radiation insensitivity reflected in survival curves. Leukocyte depression is maximum at 5 days postirradiation. At 1400 r the magnitude of the leukocyte depression is of the same order as that observed in other rodents receiving 400 to 500 r.

4. Since radiation was administered while the animals were in a normothermic state, the low mortality suggests a high degree of natural radiation resistance. This resistance may be related to certain unique physiological adaptations of the pocket mouse that enable it to survive in the desert environment.

5. In contrast, the kangaroo rat, which is a desert form with similar adaptations, has an LD_{50} corresponding closely to that of other small mammals.

6. These results suggest that *Perognathus* may be the most radioresistant of any mammal tested to date.

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A Search for Mechanisms of Radiation Resistance in Pocket Mice[1]

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INTRODUCTION

Members of the pocket mouse genus, *Perognathus*, withstand whole-body irradiation doses up to 1400 rads without appreciable mortality (*1*). Several other wild rodents—notably *Peromyscus polionotus*, *P. gossypinus*, *Sigmodon hispidus*, *Reithrodontomys humulis*, and the Mongolian gerbil, *Meriones unguiculatus*—are now listed as exhibiting a degree of radiation resistance approaching that of *Perognathus* (*2*, *3*).

The experiments reported here were designed to study the question of radiation resistance in *Perognathus*. The work was done in six parts as reported herein, and an attempt is made to amalgamate the results of the various experiments in a common discussion.

MATERIALS AND METHODS

All animals used in these studies were adult, field-trapped *Perognathus longimembris*, weighing between 8 and 10 gm. They were maintained in our holding facility for at least 6 months prior to use. Subgroups were established from our main holding colony by random methods. Details of maintenance and the method of forming experimental groups were reported earlier (*1*).

Radiation was delivered from a 5000-Ci Co^{60} source at dose rates that varied with the experiment. These are reported under the special methods of each experiment. Both ferrous sulfate and phosphate glass dosimeters were used.

After irradiation all groups were returned to the holding facility and maintained as described earlier. They were checked twice daily, in the morning and in late afternoon, and observed for signs of radiation sickness and for deaths. Dead animals were autopsied to determine gross pathology.

Modes of radiation death in *P. longimembris* are categorized under three broad

TABLE I

SURVIVAL OF POCKET MICE AFTER 1400 RADS OF TOTAL-BODY γ-IRRADIATION WHILE IN 100% OXYGEN AT 3 ATM. OR AFTER RECOVERY FROM SPLENECTOMY

Group	*Initital number*	*Treatment*	*Dose (rads)*	*30-day survival (%)*
1	9	100% O_2, 3 atm	1400	67
2	10	100% O_2, 3 atm	0	100
3	10	Normal atmosphere	0	100
4	12	Normal atmosphere	1400	75
5	9	Splenectomized	1400	45

[1] This work was supported by Contract NASw-812, Office of Space Sciences, NASA.

headings based on gross autopsy findings. These are gastrointestinal, hemopoietic, and respiratory death. These categories are not easily differentiated. However, gastrointestinal death is generally characterized by: (1) diarrhea as evidence by caked feces and sand in perianal region, (2) intestine containing yellowish mucus or bloody fluid, (3) stomach empty or containing mucus only, and (4) rapid deterioration of viscera after death.

Hemopoietic death is characterized by: (1) periorbital bleeding, (2) generalized or local petechiae—often of axillary and neck region, but sometimes in stomach, intestine, or intracranial, (3) massive intracranial hemorrhages, and (4) massive hemorrhages in other regions of body.

Respiratory death is characterized by: (1) bloody crusts around nares and mouth, (2) bloody fluid in pleural cavity, and (3) large portions of lungs hemorrhagic, consolidated or hepatized.

The Oxygen Effect

The presence of oxygen in a living system during exposure normally enhances the damaging effect of ionizing radiation. Conversely, removing oxygen from the system—producing hypoxia in an animal—protects against radiation damage.

If *Perognathus* is radiation-resistant by virtue of some ability to make vital cells hypoxic during exposure, the resistance might be abrogated by forcing oxygen into tissues. In this experiment animals were administered midlethal irradiation while breathing 100% oxygen at 3 atm pressure. It was hypothesized that survival of any of these animals would argue strongly against a physiological hypoxia mechanism.

Special methods (O_2 *at 3 atm*). Four groups of animals were established. One group of 10 animals received 1400 rads of total-body Co^{60} irradiation while maintained at 3 atm pressure in 100% oxygen. These animals were maintained under these conditions for 30 minutes prior to the irradiation to ensure tissue oxygen saturation. A second group of 10 animals received the same treatment except for the irradiation. One irradiated animal died during depressurization.

One group of 12 animals served as irradiation controls, receiving 1400 rads of total-body irradiation but no other treatment. A final group of 10 animals served as handling controls. Dose rate in this experiment was 37 rads/min.

Results. The percentage of survival at 30 days postirradiation is shown in Table I. Deaths in the normal atmosphere-irradiated group followed the normal time course for pocket mice. No deaths occurred prior to 8 days postirradiation, and few after the third week. Deaths in the oxygen-irradiation group occurred in the fourth week. Most of the animals that survived the acute period are still alive approximately a year later.

No deaths occurred in the nonirradiated control groups during this period.

Physiological Hypoxia

Visceral hypoxia resulting from stress response to handling during the preirradiation period could result in protection against both hemopoietic and gastrointestinal death. Splenic involvement as a source of hemopoietic "seed" tissue could be tested by irradiating splenectomized mice. If the spleen does provide necessary hemopoietic precursor cells to "re-seed" bone marrow in intact postirradiation *Perognathus*, its absence should prove fatal to the splenectomized *Perognathus*. Survival of any splenectomized pocket mice after midlethal irradiation would argue against a visceral hypoxic mechanism.

Special methods (*splenectomies*). One group of 10 animals was splenectomized 5 to 10 days prior to the irradiation date. Nine survivors of this group were administered 1400 rads of irradiation in air at normal pressure.

These splenectomized mice were irradiated at the same time as the mice in the oxygen experiment; therefore, the same controls were used.

Results. Of the 9 splenectomized mice, 4 survived the 30-day acute period (Table I). Survivors remain alive at 1 year postirradiation.

Metabolic Rate Effect

Perognathus normally undergoes daily periods of metabolic depression with concomitant hypothermia. The animals may undergo excursions of body temperature ranging from 37.5°C down to room temperature (*4*). This suggests that the high $LD_{50(30)}$ values of *Perognathus* may be merely the result of delayed lethality. To test this possibility a number of animals were prevented from undergoing hypothermia by maintaining high ambient temperature during the 30-day period immediately after total-body midlethal irradiation.

Special methods (*respiration at high ambient temperature*). Immediately after receiving 1400 rads of total-body irradiation at a dose rate of 37 rads/min, 7 *P. longimembris* were placed in a multichannel continuous-recording respirometer. The ambient temperature in the animal chambers was maintained at 35°C. The mice remained in the chambers for the entire 30-day postirradiation period, except for one brief period of cleaning. Oxygen consumption was recorded automatically during the entire period.

TABLE II

MEAN OXYGEN CONSUMPTION OF NORMAL AND IRRADIATED *Perognathus longimembris* MAINTAINED AT 35°C DURING PERIOD IMMEDIATELY AFTER EXPOSURE

Treatment	*Number of animals*	*Oxyygen consumption (Mean ± S.D., ml/gm-hr STP)*		
		Mean, high	*Mean, low*	*24-hr. mean*
Nonirradiated 6-day period (values for days 1–6)	7	4.45 ± 0.23	1.36 ± 0.07	2.12 ± 0.05
Irradiated (1400 rads)				
Surviving >30 days (values for days 1–9)	3	5.19 ± 0.29	1.31 ± 0.16	2.07 ± 0.23
Surviving <12 days (values for days 1–8)	4	5.39 ± 0.67	1.35 ± 0.14	2.28 ± 0.07

A control group was subjected to the same conditions, but for 6 days rather than 30 days.

Results. Of the 7 animals maintained at high temperature, 3 survived the radiation and the 30-day period thereafter. These 3 animals have survived beyond one year postirradiation. The only sign of late radiation effects in these and other high-dose radiation survivors is graying. The 4 animals that died in the respirometer died between the eighth and twelfth days.

The mean high O_2 consumption of irradiated mice was somewhat higher than that of controls (Table II).

Combined Effect of Radiation and Hypometabolism

To test the possibility that fortuitous transient reduction of body temperature during exposure might increase postirradiation survival in *Perognathus*, a group was irradiated while torpid. Immediately after irradiation the animals were returned to room temperature and maintained under normal conditions until their death.

Special methods (*hypometabolism*). Four groups of 12 male animals each were established. Food was withheld from all groups for 24 hours prior to radiation. In the afternoon of the day before irradiation, all four groups were placed in plastic compartmented boxes. Two of the groups were then placed inside a cooling unit set up in the radiation source room where they remained overnight. The other two groups were kept at room temperature (22°C).

Within an hour after the animals were placed in the cooling unit, the temperature within the unit was reduced from room temperature to 9°C. The temperature remained at 9°C for the duration of the experiment.

TABLE III

COMPARISON OF 30-DAY SURVIVAL OF POCKET MICE IRRADIATED AT SEVERAL DOSE LEVELS WHILE NORMOMETABOLIC (22°C AMBIENT) OR HYPOMETABOLIC (9°C AMBIENT)

Whole-body Co^{60} dose (rads)	*Initial number of mice*	*Number dead at postirradiation day:* 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30	*Per cent surviving*
2000 Normometabolic	25	2 3 1 4 3 1 3 1 5	8
1800 Normometabolic	25	2 1 2 4 1 4 3 2 2 1	0
1700 Normometabolic	12	1 2 4 1 2 1 1	0
1700 Hypometabolic	12	5 3 3 1	0
1600 Normometabolic	25	1 1 1 2 3 2 1 3 2 3 1	20
1400 Normometabolic	12	1 1 1 1	67
1400 Normometabolic	48	2 1 1 4 3 1 2 1 1 3	61
1300 Normometabolic	25	1 1	92

One group maintained torpid at 9°C and one group maintained active at room temperature (22°C) were simultaneously administered 1700 rads of Co^{60} radiation at a dose rate of 36.2 rads/min. The other two groups were held as hypothermia and normal controls. The total time of radiation exposure was 48 minutes, after which time all the animals were returned to their individual cages at normal temperature, and food was restored.

The hypothermic animals were observed just prior to and after irradiation. Prior to irradiation they all were deeply torpid. One animal showed signs of awakening when the lid of the cooler was removed just after irradiation. It took approximately 1 hour for all the animals that were made hypothermic to return to a normal active state after being returned to room temperature.

Results. Table III shows the mortality pattern in the two irradiated groups (1700 rads normometabolic and 1700 rads hypometabolic) and compares these with mortalities reported previously (*1*). Mean survival time was the same for both groups—hypometabolic 10.0 days, normometabolic 10.8 days. No controls died during the same time period.

Gastrointestinal hemorrhages were seen at autopsy in all but one of the irradiated animals. In that one, early death was attributed to respiratory failure, possibly owing to an infection antedating the radiation. In several instances both severe gastrointestinal damage and respiratory disease were noted at autopsy. In addition, as noted on other occasions in postirradiation necropsy of pocket mice, massive intracranial hemorrhages were seen in 4 of the 24 irradiated mice.

Irradiation during Hypoxia

Since artifically induced tissue hypoxia is a known means of producing radiation resistance in mammals, a group of *P. longimembris* were irradiated while they were in severe hypoxia. Survival of irradiated hypoxic mice was compared to survival of mice administered the same dose while in a normal oxygen environment.

Special methods (hypoxia). Sixty pocket mice were segregated from our holding colony and randomly divided into three groups of 20 each. One group received 2100 rads of total-body irradiation in air; the second group received 2100 rads of total-body irradiation while hypoxic; and the third group received hypoxia only.

Pocket mice proved to be extremely tolerant to low ambient oxygen. In order to produce tissue hypoxia in these animals, ambient oxygen had to be reduced to below 5%. Reduction of oxygen was accomplished by altering the flow of oxygen in a nitrogen–oxygen breathing mixture piped into a Lucite exposure chamber. The degree of hypoxia was judged by clinical signs and by survival studies in control groups. Preliminary studies revealed that 75% of a normal population of pocket mice would survive for 25 to 30 minutes in 2.6% oxygen at room temperature. This oxygen concentration was judged sufficient to ensure tissue hypoxia in all the mice.

TABLE IV

SURVIVAL OF POCKET MICE RECEIVING 2100 RADS OF TOTAL-BODY IRRADIATION WITH AND WITHOUT HYPOXIA
(2.6% O_2 in N_2, 25 min)

Group	*Initial number*	*Treatment*	*Number surviving exposure period*	*Per cent surviving at 30 days*
A	20	Irradiation + hypoxia	8[a]	40
B	20	Irradiation only (in air)	20	0
C	20	Hypoxia only	14	70

[a] The high mortality during irradiation in this group suggests that the combined stresses were too severe, or perhaps our preliminary data on hypoxia tolerance were insufficient. There is also a possibility that occlusion of the air line may have occurred briefly during the oxygen reduction period. In spite of the severe mortality during irradiation, the fact that all 8 survivors are still alive whereas all the unprotected mice died before the sixteenth day postirradiation indicates that the hypoxia treatment was successful.

Irradiation was delivered at a dose rate of 105 rads/min. The total dose of 2100 rads was delivered in about 20 minutes.

The group irradiated under hypoxia was returned to a normal oxygen atmosphere immediately after irradiation. The total period in 2.6% oxygen was less than 25 minutes. The hypoxia control group had identical treatment except for the irradiation. The group irradiated without hypoxia was handled similarly and was administered the same dose under the same conditions as the irradiation-hypoxia group except, of course, for the hypoxic atmosphere.

Results. Survival of mice in this experiment is shown in Table IV. All mice that received 2100 rads without hypoxia died in the eighth through the fifteenth day, with a mean survival time of 11 days. The most common autopsy finding in this group was large amounts of bloody fluid in the small intestine. In addition, respiratory infection characterized by large foci of consolidation or hepatization was seen in 7 of the 20 dead mice. Intracranial hemorrhages were seen in 4 animals.

Only 8 of the 20 mice administered the irradiation under hypoxia survived the exposure period. However, these 8 (40% of the initial group) are alive and, except for graying, appear healthy at 1 year postirradiation. During the period in which the unprotected irradiated mice were dying, one of the irradiated-hypoxia animals appeared lethargic for about a day. Aside from this single observation, hypoxia alone or hypoxia and irradiation did not affect the survivors as judged by outward signs.

Response to Massive Gamma Irradiation

Pocket mice were administered massive doses of γ-irradiation to determine whether they follow the pattern of central nervous system death familiar in other mammals.

Special methods (massive rapid doses). Four groups of 10 adult *P. longimembris* each were established. Three of these groups were administered radiation doses ranging from 8.5 to 24.1 krads. One group was kept as controls.

To obtain massive irradiation, the animals were lowered into a 5000-Ci Co^{60} source composed of twelve 8-inch-long Co^{60} needles arranged to form a cylindrical basket 12 inches to diameter. The dose rate within the source was 5.2 krads/min. A transit-time dose of 8.5 krads was accumulated while the samples were being raised and lowered into the source. Total dose was determined by adding dose received while within the source to transit-time dose.

Of the three groups exposed, one received only the transit-time dose of 8.5 krads. A second group received the transit-time dose plus 1 minute of exposure within the Co^{60} source, accumulating a total of 13.7 krads. The third group received 24.1 krads during a 3-minute exposure within the Co^{60} source.

Results. The animals administered 8.5 krads exhibited no locomotor or other behavioral effects either immediately postirradiation or at any time thereafter. Mean survival time in this group was 7.8 days, with all deaths occurring between the fifth and tenth days.

Animals administered 13.7 krads and 24.1 krads all showed early locomotor disturbances. Ataxia was noticed immediately after the animals were transferred from exposure cages back to their individual cages. Some showed extreme sensitivity to handling or other mechanical stimuli. For example, they reacted violently to prodding with a pencil. Others showed uncommon aggressive behavior in the first 6 hours after irradiation. Behavioral effects were most pronounced in the highest dose group (24.1 krads). This group had a mean survival time of about 1 day. All but 1 of the animals died before 21 hours postirradiation. One survived an additional 24 hours.

Mean survival time in the group receiving 13.7 krads was 7.9 days; 8 of the 10 died between the seventh and the eleventh days. Two died earlier, 1 on the first day and the other on the fourth day postirradiation.

No controls died during this experiment.

Autopsies were performed on all animals. There were no grossly observable pathological conditions in the 24.1-krad group. The brain and vital organs of thoracic and abdominal cavity of these animals all appeared normal.

All animals that died between the fifth and eleventh days showed signs of severe gastrointestinal bleeding. In most instances the small intestine was full of bloody fluid. There were no signs in any of these animals of intracranial hemorrhages as is often seen in pocket mice administered 1500 to 2000 rads.

A dose-survival curve based on these data and data from preceding reports is shown in Fig. 1 (*1*).

DISCUSSION

Postirradiation recovery depends on survival of a critical number of regenerative or proliferative cells in systems vital to the integrity of the organism (*5*). In turn,

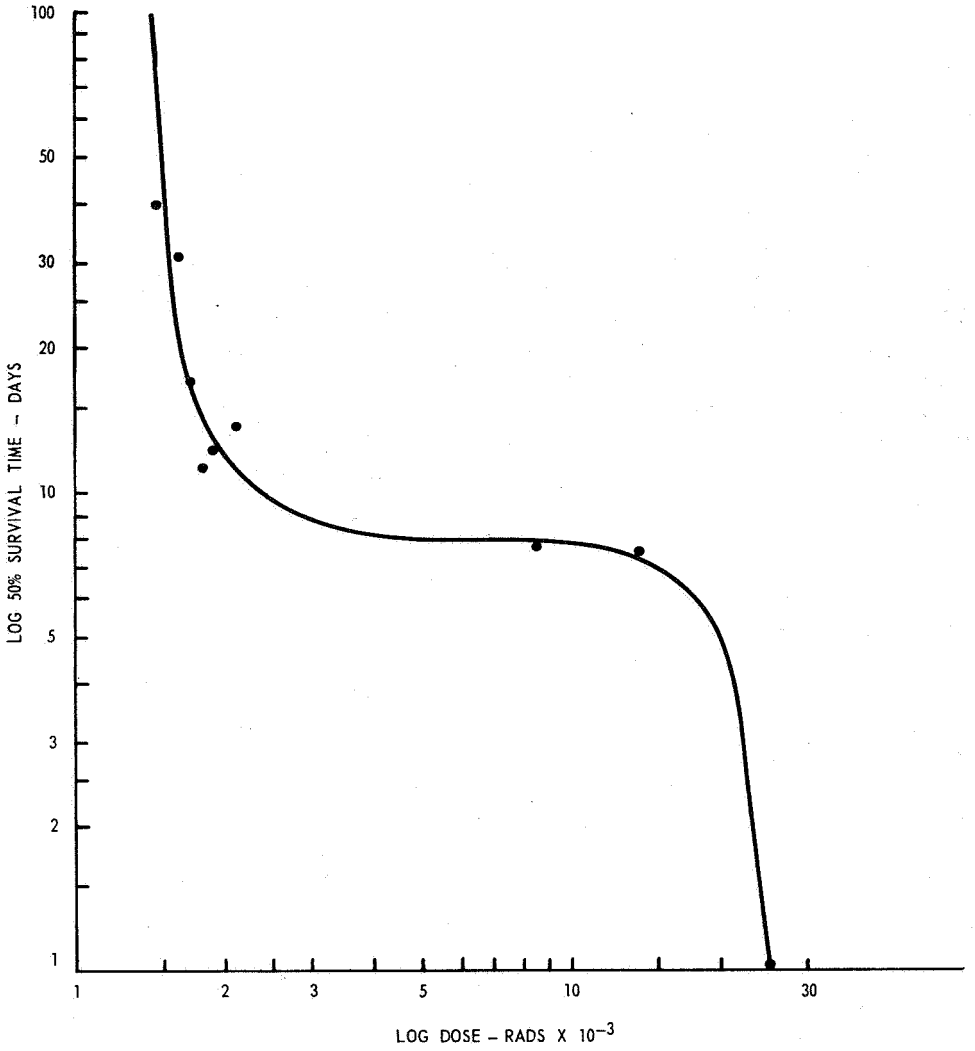

FIG. 1. Survival times of pocket mice after various doses of γ-irradiated (curve visually fitted).

recovery or death of these vital cells reflects certain intrinsic characteristics which confer greater or lesser radiosensitivity. For example, radiation lethality in cells has been correlated with nucleic acid content, nuclear size, and ploidy (*6*).

Variations in radiation sensitivity of cells may also reflect alterations in the internal milieu. The protective effect of low oxygen tension is an example of this kind of control over radiation response. In rodents, hypoxia is known to confer protection by causing significant reductions in oxygen tension within both the spleen and the gut.

In this study, removing the spleen of pocket mice prior to irradiation did not significantly alter their ability to survive a midlethal dose. Furthermore, forcing oxygen into tissues by high-pressure oxygen treatment, designed to override pockets of low oxygen tension, did not enhance the lethality of the delivered dose. These results suggest that explanation of pocket mouse radiation resistance cannot be based on generalized or local tissue hypoxia.

This conclusion is reinforced by results of the converse experiment in which very low oxygen concentrations enhanced survival in *P. longimembris* exposed to an otherwise lethal dose of whole-body irradiation. It was hypothesized that, if a natural hypoxia mechanism was already operating, the mechanism would be "saturated"; therefore, added hypoxia (via hypoxic hypoxia) would not increase protection. In this experiment all animals that were administered 2100 rads without hypoxia protection died within 3 weeks, whereas all those surviving exposure to 2100 rads while hypoxic are alive 1 year later.

Conclusions based on any of the above experiments taken singly would be tenuous. Taken together, however, they provide sufficient reason to reject the hypothesis that pocket mice survive high levels of acute irradiation by having local hypoxia in critical radiosensitive tissue during irradiation.

It is possible that intermittent periods of hypothermia protract the acute period postirradiation, thereby raising the $LD_{50(30)}$ but not the $LD_{50(>30)}$. Ultimate recovery is more meaningful than $LD_{50(30)}$ values in instances where natural hypothermia is possible. However, the fact that several pocket mice did survive 1400 rads, even when forced to maintain active metabolism for the 30-day acute period, argues against postirradiation hypothermia's playing a large role in *Perognathus* radiation resistance.

In hibernation or in artificially produced deep hypothermia (both produce a hypometabolic state), expression of radiation injury is inhibited until the animal is warmed to normal temperature. The "protective" effect of hypometabolism is only temporary, however, since rewarming of irradiated animals allows manifestation of the injury in much the same manner as it appears in normometabolic counterparts (*7*).

There is good evidence that prolongation of survival by hibernation has little to do with eventual recovery from radiation injury. Indeed, it has been demonstrated that animals kept continuously in hibernation after high-dose irradiation eventually succumb to radiation injury. It is apparent that lowered metabolic rate postpones but does not significantly affect the final outcome of lethal radiation exposure.

In most instances where nonhibernators have been made hypometabolic, radiation protection is attributed to the concomitant severe hypoxia rather than to the low metabolic state (*8–10*). In fact, when hypoxia is avoided in these kinds of experiments, protection is not conferred (*4*). Hypothermia, then, may be protective, but only when attended by tissue hypoxia.

There was no reason to expect that a hypometabolic state during exposure would alter the response of *Perognathus* to lethal irradiation. However, since this genus exhibits large excursions of body temperature under standard environmental conditions, it appeared judicious to study the effect of combined hypometabolism and radiation in this species. As was anticipated, there was no significant difference in survival between normometabolic and hypometabolic *Perognathus*.

Data derived from high-dose exposures suggest that pocket mice are just as susceptible to extremely high-dose irradiation as are conventional mice. There is some indication that pocket mice may be slightly more susceptible at the extreme dose levels. Conventional mice and rats survive for 24 to 48 hours after doses as high as 20,000 to 30,000 rads, whereas pocket mice survive for less than 1 day.

Response of pocket mice to irradiation appears to be modified in dose ranges where continued functioning of cell renewal systems is important for survival. At specific exposure dose levels, both hemopoietic and gastrointestinal damage are less severe in *Perognathus* than in other mammals. Damage to the gastrointestinal mucosa is responsible in a large measure for early deaths in mammals after radiation doses ranging between 1000 and 10,000 rads. This portion of the survival curve is termed the "gut death" plateau.

Closer scrutiny of the "gut death" portion of the pocket mouse survival curve may be instructive. In this dose range, pocket mice have a mean survival of 7 to 8 days, whereas conventional mice have a mean survival time between 3 and 4 days. In this respect pocket mice are similar to germfree mice, which have a mean survival time of 7 days (*11*). McLaughlin suggests that survival of germfree mice in the "gut death" plateau results in part from a prolonged life span of intestinal epithelial cells in these animals.

Judging from gross pathology, severe gastrointestinal damage occurs in pocket mice receiving doses greater than 1000 rads. However, histological studies now in progress show persistent villi for at least a week after a single irradiation dose of 1500 rads. Perhaps natural prolonged life span of intestinal epithelial cells in *Perognathus* allows survival in the first critical period. High resistance to bacteremia after massive invasion of tissues by gut flora may also play a role in the early response of *Perognathus*. Roderick shows that general fitness appears to be an indicator of radioresistance in mice (*12*).

The peripheral blood picture of irradiated *Perognathus* is similar in kind, but not in degree, to that of other mammals administered comparable doses (*1*). It takes about twice the dose to produce the same effect in pocket mice as in conventional mice.

At doses greater than 1000 rads, deaths occurring in pocket mice during the second and third week may be attributed to hemopoietic failure and complications caused by damage in the gut epithelium and capillary net, allowing massive hemorrhaging into the intestinal lumen. This hypothesis is supported by demonstrations of extremely low platelet counts in pocket mice at 10 days postirradiation (*1*).

Failure of doses in the 500- to 1000-rad range to cause hemopoietic death in pocket mice may be attributed to factors outlined by Russell *et al.* (*13*). Precursor cells may be especially radioresistant because of a gentic makeup which allows them to withstand the initial radiation insult. Rapid regeneration capability coupled with low destruction rates would tend to maintain hemopoiesis at the level necessary for life.

It is possible to conclude at this time that at least three unrelated mechanisms may be acting to confer high radiation resistance on pocket mice: one, persistent intestinal epithelium; two, natural resistance to common pathogens; and three, radioresistance of hemopoietic stem cells.

SUMMARY

In a series of experiments designed to elucidate the role of hypoxia in radiation resistance of *Perognathus*, several groups of *P. longimembris* were subjected to Co^{60} irradiation under various conditions. One group was administered 100% oxygen at 3 atm during irradiation. Another was splenectomized prior to irradiated. These two groups received a total-body dose of 1400 rads. A third group was administered 2100 rads while in severe hypoxia. Results suggest that hypoxia mechanisms are not responsible for the remarkable radiation resistance of *Perognathus*.

Effects of body temperature on radiation response were tested in two ways. Animals were administered 1400 rads and were forced to maintain normal active body temperature during the entire 30-day postirradiation period. Others were administered 1700 rads while hypometabolic (body temperature 9°C). Postirradiation survival in pocket mice was not significantly altered by changes in metabolic processes either during or after irradiation.

Pocket mice were exposed to massive Co^{60} irradiation: 8.5, 13.7, or 24.1 krads. Animals receiving the 8.5- or 13.7-krad doses died in 5 to 11 days. Those administered 24.1 krads died within 1 day.

Radiation resistance in pocket mice appears to be related to unusual capabilities within specific regenerative systems, rather than to physiological processes related to their ability to undergo hypothermia.

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MITOTIC RHYTHMS IN POCKET MOUSE INTESTINE

J. J. Gambino and T. Y. Tagami

Mitotic rhythms or diurnal variations in mitotic activity are reported in a number in in vivo mammalian cell populations (1-4). Among these are the corneal epithelium, epidermis, oral and esophageal epithelium, bone marrow, adrenal cortex and regenerating liver. Most observations of mitotic rhythms have been made in conventional laboratory rodents. However, epidermal and bone marrow rhythms have been reported in man as well (5).

Several authors have reported the absence of mitotic thythms in the intestinal mucosa of rodents (2,6). It has been suggested that the presence or absence of a rhythm is correlated with turn-over rate of the tissue in question. In general, slowly dividing tissues show diurnal variations, whereas rapidly dividing populations do not (6).

The rodent genus Perognathus contains members which show considerable daily variation in body temperature. P. longimembris, for example, may undergo daily periods of torpor during which time its body temperature drops to nearly ambient and remains there for several hours (7). This species also has a 5 to 7 day transit time for intestinal villi epithelial cells (8). Based on these two observations, a mitotic rhythm in Perognathus intestinal mucosa might be anticipated. The following experiments were performed to answer this question.

Pocket mice (genus Perognathus; family Heteromyidae) were maintained individually in gallon bottles with a sand substrate. A mixture of sunflower seeds, wheat, rye, and millet was provided in excess at all times. The pocket mouse has no requirement for drinking water. The animal quarters were air-conditioned with a 20-24°C, 40-70% relative humidity, and a photoperiod of 12 hr light and 12 hr dark (light: 0600-1800 hr PST).

In the first experiment we used field collected animals which were in our holding facility for approximately 2 years. Forty female mice were selected randomly from the holding colony and brought into the main laboratory where they were again divided randomly into 8 subgroups of 5 mice each.

Starting at noon the following day, subgroups were killed at 4-hr intervals by ether anesthesia. The entire small intestine was removed and fixed in Bouin's solution. Five micron cross sections were prepared from a portion of the duodenum removed 10 mm distal to the pyloric sphincter. The slides were stained with hematoxylin-esoin.

In the second experiment, performed a year later, 26 field collected animals were maintained in our colony for three weeks prior to sacrifice. They were randomly divided into two groups, each containing 13 mice (5♂ and 8♀). One group was sacrificed between 2400 hr and 0100 hr, and the other between 0400 hr and 0500 hr the same day. To avoid disturbing the others in the group, animals slated for sacrifice were taken to an adjoining room and killed by a blow on the head. The entire small intestine was removed quickly and fixed in Helly's solution. A 5 mm segment of the duodenum, 5 mm from the pyloric sphincter was taken for sectioning. Duplicate sets of 5 μ cross sections were made. One set was stained in hemotoxylin-eosin and the other in feulgen-fast green.

We scored mitotic figures on 3 entire cross sections for each animal. The cross sections contained approximately 36 crypts. Cell counts on several representative sections and on material prepared by the squash technique provided a value for total cells scanned per animal. We estimate that between 600 and 800 cells can be viewed in a 5 μ section; therefore 1800 to 2400 crypt cells were scanned for each animal.

This method of cell counting was used because of certain peculiarities in the histo-anatomy on P. longimembris intestine. Most noticeable among these are the extremely shallow, bulbous form of the intestinal crypts, as contrasted to the elongate structure of those in conventional laboratory rodents. This peculiarity makes selection of mid-saggital sections and identification of the proliferative compartment more difficult in the pocket mouse than in conventional rodents. Total cell counts obtained by this method no doublt include an unknown percentage of non-proliferative cell; therefore, expressing the values as a mitotic index would be unjustified.

Scoring was done on randomized and coded slides. Sections for scoring were selected at intervals to insure that the same crypts were not scanned twice.

The data are presented as absolute counts (i.e., number of mitoses per cross section of duodenum). We believe absolute counts coupled with an estimate of the total cell population, give a value which is meaningful for comparing individuals of the same population with regard to time-related changes in mitotic activity.

Results are shown in Figure 1. In both experiments the mean value obtained at the 0400 to 0500 hr sacrifice period was greater when compared with means obtained for the other sacrifice periods. In both experiments P lies beyond 0.02 for the calculated "t" value.

We conclude, therefore, that a peak in mitotic activity occurs in pocket mouse intestinal crypt cell populations between 0400 and 0500 hr. When the photoperiod is based on a 0600 to 1800 hr lights on regimen, this time period corresponds to an AZT 22:00 to 23:00.* Locomotor activity and deep body temperature data available on this species shows this time to be toward the end of the nightly active period. Peaks of metabolic high and metabolic low in pocket mice on the light regime used occur at about 2300 hr and 0900 hr, respectively (7).

Observation of a wave of mitotic activity in the intestinal epithelium of an animal which undergoes daily torpor is not too surprising. This suggests that daily periods of lowered metabolic activity imposes synchrony on a dividing population. There is ample evidence of experimental synchronization of cell populations <u>in vitro</u> by temperature shock (2). The slow turn-over rate of pocket mouse intestinal epithelium also favors establishment and maintenance of a diurnal rhythm in mitotic activity (6).

The difference between our results with pocket mice and the findings of others using conventional rodents may also reflect difference in technique.

* Arbitrary Zeitgeber Time - scale runs from 00.00 to 24.00, with 00:00 defined as onset of light.

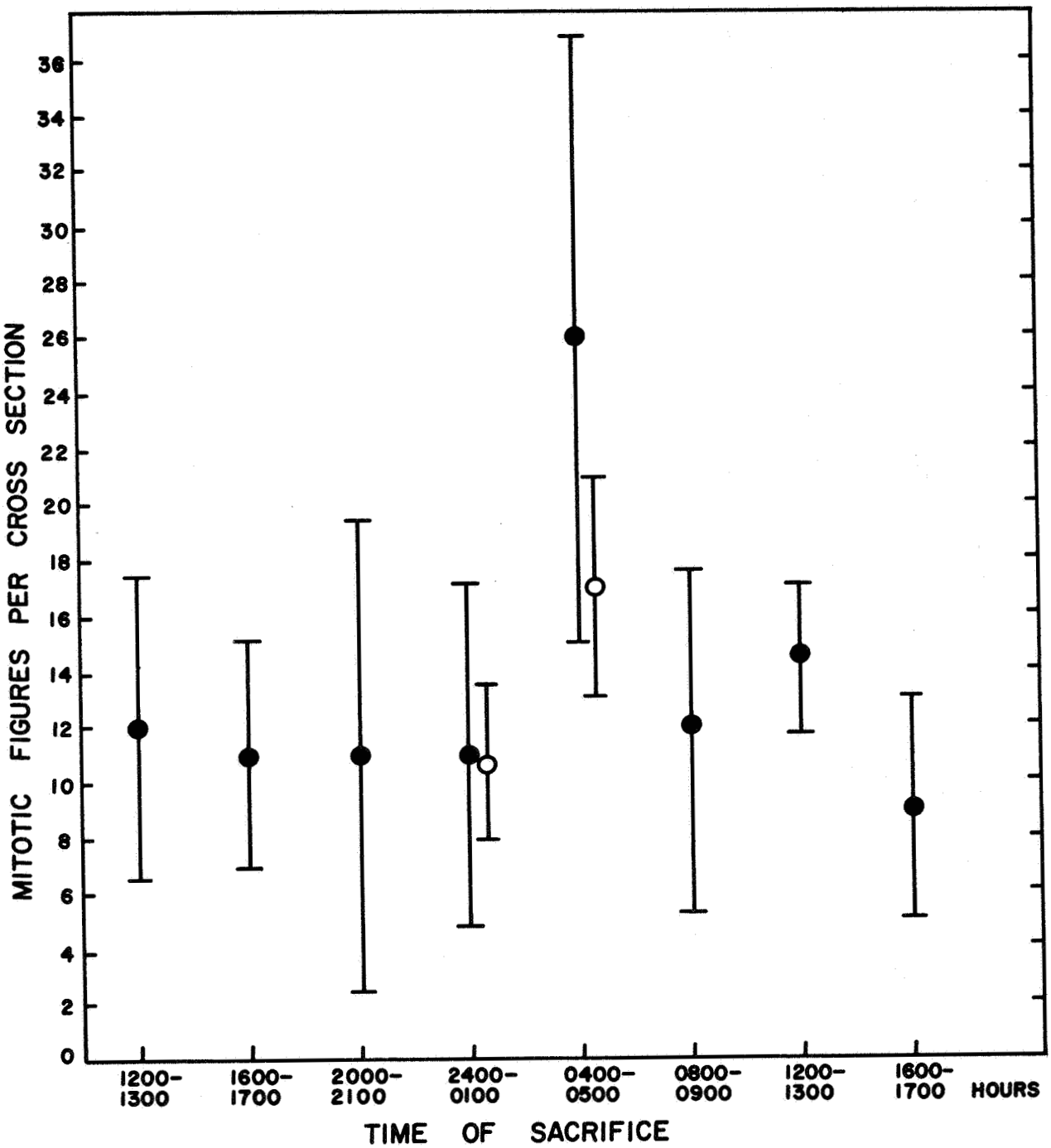

Figure 1 Mitotic activity in duodenal crypts of Perognathus longimembris. Filled circles represent means of counts on 5 animals at each sacrifice period. Open circles show means of counts on 13 animals at each point. Standard deviations of the means are indicated.

We did not use colchicine to halt mitoses at metaphase for scoring as others have done (6). The colchicine method is used primarily to improve counting statistics. It may also mask the true rhythmic nature of mitotic activity in a tissue (9). We feel that the omission of colchicine in the present study enhances its validity.

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POCKET MOUSE SURVIVAL AFTER GAMMA RADIATION DURING METABOLIC HIGH AND LOW PERIODS

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ABSTRACT

Two groups of pocket mice (Perognathus longimembris) were exposed to Co^{60} irradiation at 2 different times of the day. Irradiations were performed at times selected to coincide with the middle of the metabolic high period (23:30 hrs) for one group, and the metabolic low (0900 hr) for the other. Those exposed during the metabolic low period had a significantly higher mortality rate ($P \leqq 0.02$).

Circadian rhythmicity in sensitivity to whole body exposure to ionizing radiation was demonstrated earlier in Swiss-Webster and C_3H strain mice and in "Carworth Farms" rats (1). Others reported no evidence of cyclic variation in response to radiation in either CF_1 or ICR mice, or in Sprague-Dawley rats (2,3). The question of diurnal variation in response to radiation is unresolved; however, if it exists, the importance of such a phenomenon to radiology is uncontested.

Pocket mice can be used to advantage in radiosensitivity versus time-of-day experiments because they exhibit well defined periodicity in body temperature, metabolic rate, and activity. We have described and reported elsewhere the phase and wave-form of these interrelated parameters on a large number of pocket mice (4). We have also established 30 day LD_{50} doses for P. longimembris for x-ray, gamma and neutron irradiation (5).

It should be noted, however, that the LD_{50} values are based on irradiation delivered within the normal working day (i.e., between 0900 to 1500 hr). Therefore, we exposed two groups of pocket mice to a dose of Co^{60} irradiation which we predicted would cause 100% deaths in 30 days. One group was irradiated during the metabolic high period and the other group during a metabolic low. We believe the results are germane to the question of diurnal response to radiation in mammals.

Field collected pocket mice were housed in an air-conditioned facility with the temperature maintained at 20-24°C, and relative humidity at 40-70%. Lights was turned on at 0630 hr and turned off at 1730 hr PST. The mice were maintained individually in gallon bottles with a sand substrate. A mixture of sunflower seeds, wheat, rye, oats and millet was provided in excess at all times. The pocket mouse has no requirement for drinking water.

Gross motor activity data was the basis for selecting the times of irradiation. Figure 1 shows activity of 28 P. longimembris during the first several days in constant dark after entrainment to the 0630 to 1730 hr lights-on regimen. The metabolic low of pocket mice on this regimen occurs at AZT*:02.50; whereas, the metabolic high is at AZT:17.00. Oxygen

* Arbitrary Zeitgeber Time - scale runs from 00.00 to 24.00, with 00.00 defined as onset of light.

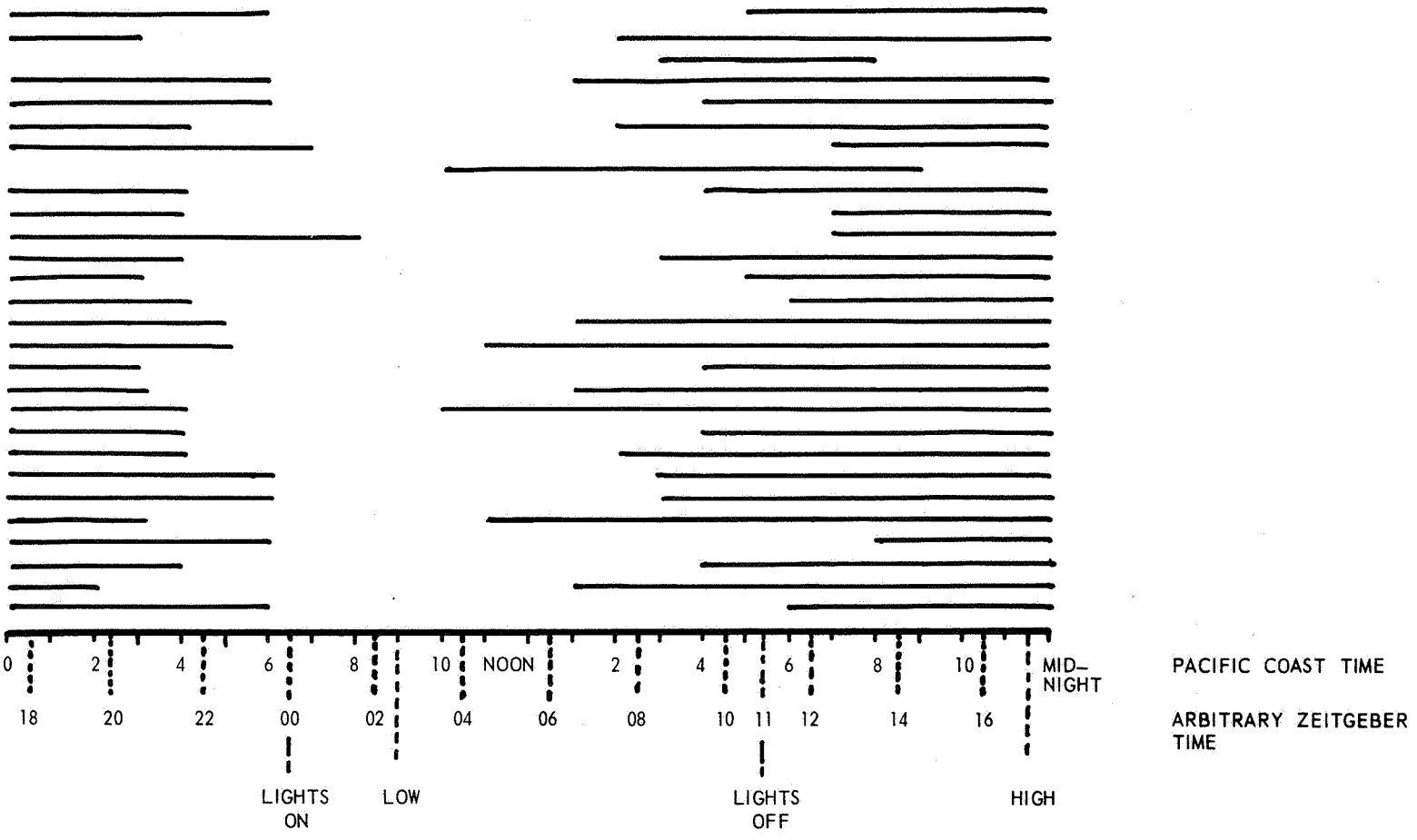

Figure 1 Periodic locomotor activity of pocket mice entrained to a LD 11:13 (0630-1730) light regimen. Each line represents active period of a single animal.

consumption data and body temperature data obtained with implanted temperature telemeters confirmed these times (4).

As in most other organisms, pocket mice exhibit individual variation in circadian activity; therefore, further selection was made to insure that all animals used in this study were in phase. Records were kept on over 300 animals for one week prior to the day of irradiation. From these we selected a group of 100 animals which were regularly in torpor or inactive between 0900 and 1000 hr. These were randomly divided into four subgroups of 25 animals each. Sexes were equally represented in the subgroups.

One group was irradiated during the metabolic low period, 0900 hr (AZT:02.50), and the other group was irradiated at 2330 hr (AZT:17.00) during their metabolic high. A sham-irradiated control group was included at each exposure.

One hour before exposure, mice were placed in compartmented plastic boxes and transported to the nearby Co^{60} irradiation facility. Irradiation was delivered from a 5000 Ci source at a dose rate of 37.8 rads/min calibrated with NBS ion chamber standards and ferrous sulphate dosimeters. Total dose delivered was 1500 rads. The dose was monitored during the exposure with silver-activated phosphate glass dosimeters. The accuracy of the total dose delivered is within $\pm$ 5%. Delivery of identical radiation doses to the two groups was insured by precisely locating the exposure chambers and by accurately timing the exposure period.

After irradiation, animals were observed twice daily for mortality over a period of 30 days.

Since it is well documented that non-specific stress may alter radiation response, special care was taken to handle the day and night groups identically. In spite of these precautions, animals in both groups exhibited moderate agitation during the packaging and transport procedures. As judged by outward appearances, however, stress was minimal.

Pocket mice which received the irradiation at 0900 hr had a ST_{50} of 11.6 days. There were no survivors in this group beyond 18 days (Figure 2).

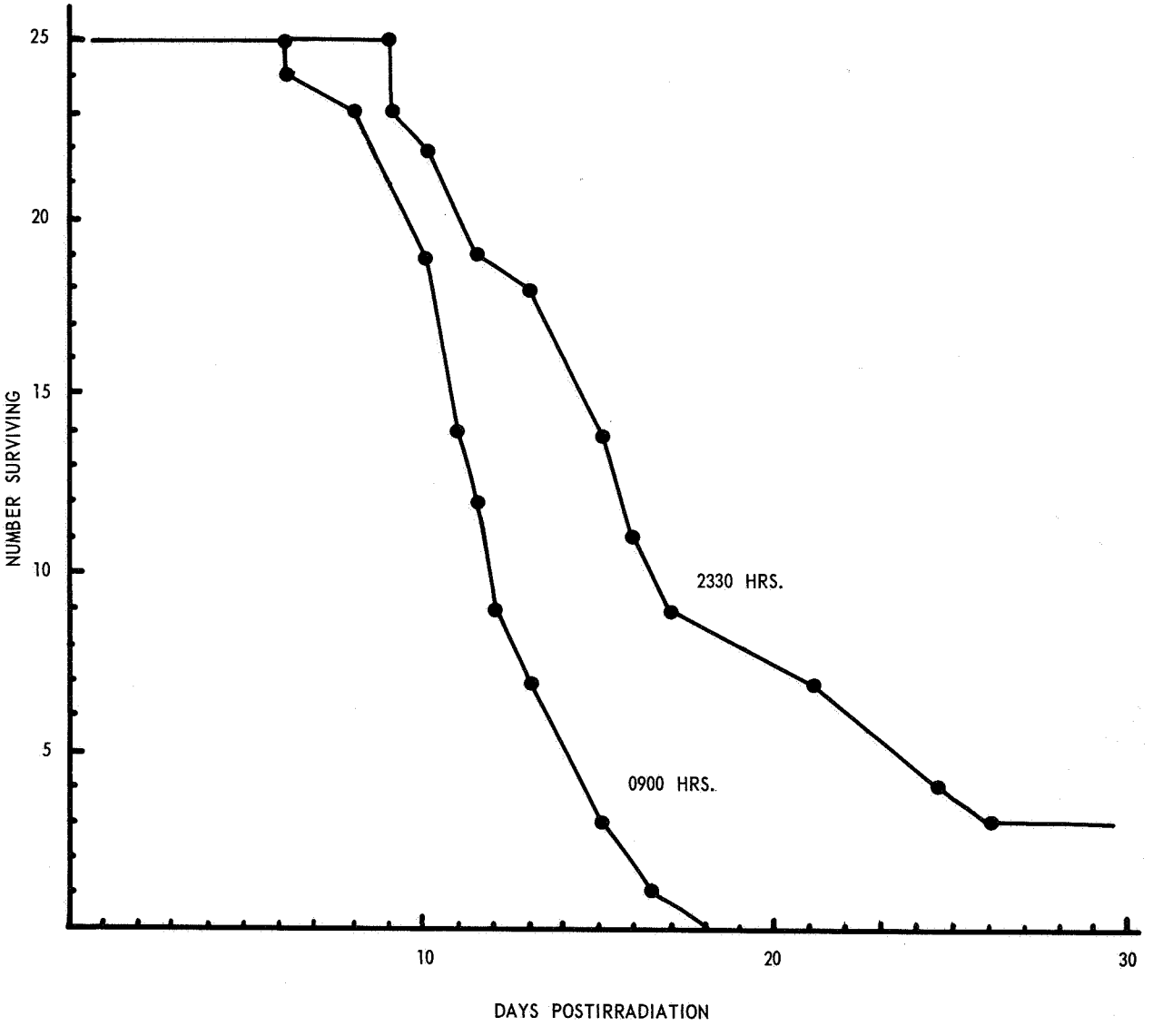

Figure 2 Survival in two groups of pocket mice administered whole body Co^{60} radiation at two different times of day.

Pocket mice administered the same dose under identical conditions except for the time of exposure (2330 hr) had a ST_{50} of 15.1 days. At the end of the 30-day period three of the night-irradiated group were still alive. None of the sham-irradiated controls died during the same period.

Survival data were plotted on probability paper with percent survival as the ordinate and the logarithm of time as the abscissa (Fig. 3). The method of Litchfield was applied to test the difference between mean survival times and the slopes of the survival curves (6). At the $P \leqq 0.02$ level the mean survival times are different and the two curves deviate from parallelism. These results suggest that under the conditions described, pocket mice irradiated during their active period live significantly longer than those irradiated during their inactive period.

Contrary to what was found in certain other rodent species, enhanced survival in pocket mice occurred in the night-irradiated group. Swiss-Webster and C_3H strains of mice were more sensitive to whole body irradiation delivered at 0200 hr (AZT:19.00) than at any other time in the diurnal cycle. Laboratory rats were more sensitive when irradiated at 2100 hr than at 0900 hr (1). This reversal, however, is perhaps less important than the corroboration of a diurnal effect. Our results support the contention that time of exposure does indeed alter the response of some rodents to whole body radiation.

Rugh et al (3) and Straube (2), on the other hand, contend that there is no significant difference in survival of CF_1 mice and Sprague-Dawley rats irradiated at various times of the day. There is some evidence, however, that a difference did occur in these latter studies, but went undetected by the authors (1).

Although Rugh did not confirm the existence of diurnal variation in sensitivity in CF_1 mice, he did concede that the idea of minor diurnal fluctuations in radiosensitivity is plausible. This, he notes, is suggested by a slightly higher mortality in his mice irradiated at 6 a.m. than at noon. He explains this difference as the result of night acitivity which normally occurs in these animals. To substantiate this argument Rugh

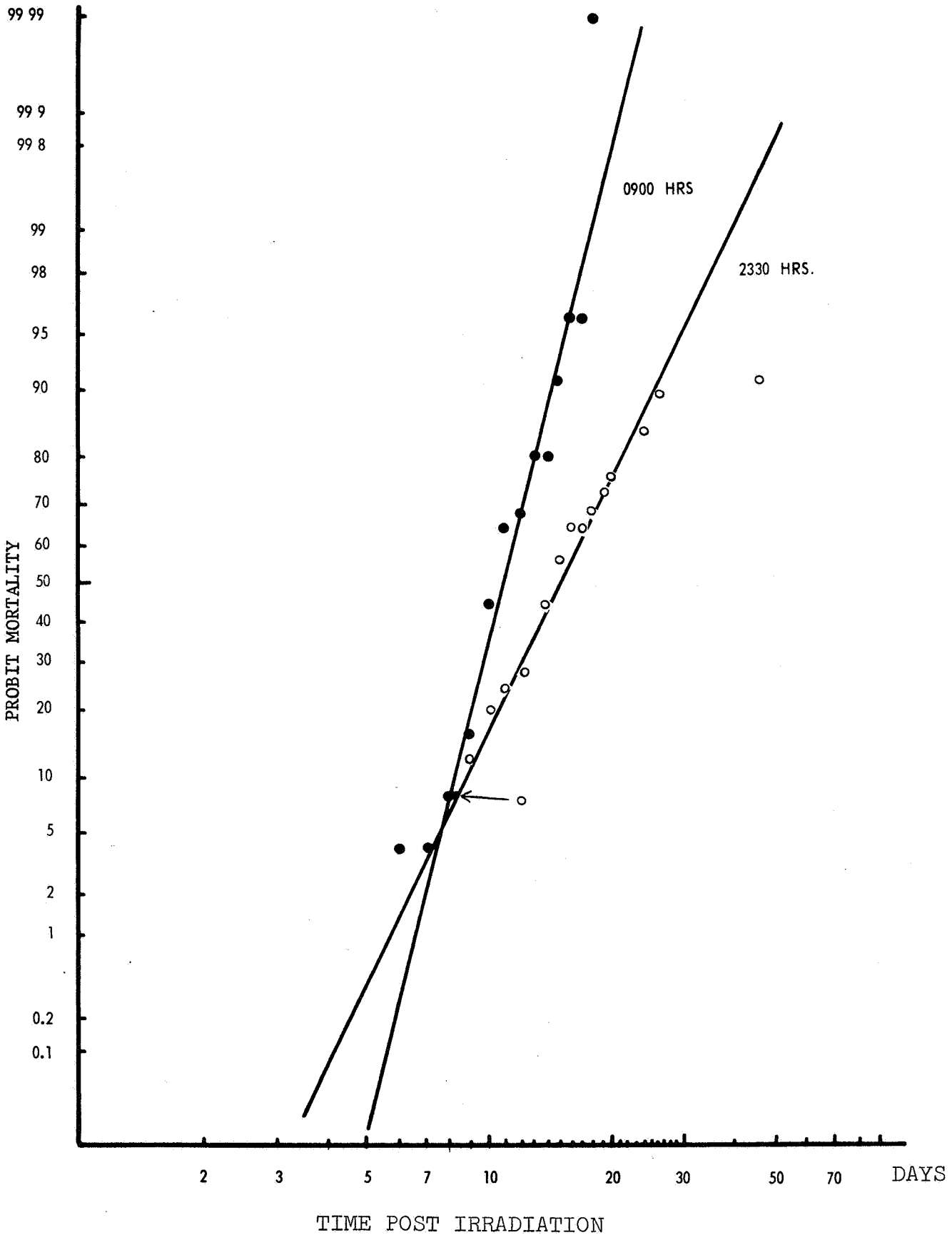

Figure 3 Probit transformation of survival data of pocket mice administered 1500 rads Co^{60} radiation at two times of day.

references the work of Kimeldorf et al in which exercise to exhaustion was shown to enhance the deleterious effect of irradiation (7). Benjamin and Peyser suggest similar response in mice administered lethal irradiation during exploratory activity or forced activity (8). Vacek, on the other hand, proposes an opposite effect of normal physiological acitivity on radiation response (9). He shows that rats having a lower oxygen consumption during irradiation die sooner than those having a higher oxygen consumption.

In this respect we are in accord with Vacek. Pocket mice irradiated during the morning hours are assumed to have a lower average oxygen consumption than their night counterparts. They also had a shorter survival time. Since we did not measure oxygen consumption during irradiation, we can only suggest this relationship between metabolic activity and survival in pocket mice. Other work, however, indicates that minor fluctuations in metabolic rate as judged by body temperature, unless accompanied by tissue hypoxia, may have very little bearing on radiation response (10).

Although minor rhythmic fluctuations in metabolic rate may not have direct bearing on radiation response, metabolic rate may be useful as an index of other cyclic activity within the organism. Pizzarello et al suggest that metabolic or locomotor acitivity may be useful as a "marker" when studying cyclic radiosensitivity (1). But, according to him, the ultimate search should be for physiological rhythms which may be directly related to radiosensitivity at a given phase in a given photoperiod.

We feel that this search should be directed toward elucidating circadian rhythms in cell proliferative activity; particularly in regenerative populations of hemopoietic tissue and the gastrointestinal epithelium. Both in vitro and in vivo studies on synchronously dividing cell populations have revealed differences in radiosensitivity relative to the phase of the cell cycle exposed (11). Conclusions regarding radiosensitivity depend upon the tissue being examined and endpoints used. Judging from chromosomal damage, and from cell survival based on colony-forming ability, the S (synthesis) phase is considered the most resistant phase in the mammalian cell cycle (11).

It may be significant that counts of mitotic figures in pocket mouse intestinal epithelium revealed more post-metaphasal figures in animals sacrificed between 0400 hr and 0500 hr than at any other time in the diurnal cycle (12). Estimates of time durations of the G_1, S, G_2, and M phases of pocket mouse intestinal crypt cells have been made (13). Based on this information it appears fortuitous that the time chosen for the night-irradiation (2330 hr) based on gross activity was also the time when proportionally more crypt cells would be in the S phase, and hence more radioresistant.

Indeed, the evidence for this rationale is tenuous. However, it is presented as a model of the kind of experiement that can be designed to relate gross locomotor activity with internal physiological rhythms; and, thereby, to establish an explanation for observed differences in sensitivity among certain mammals to whole body radiation delivered at various times of the diurnal cycle.

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14. Work supported by NASA contract NASw-812.

RELATIONSHIPS BETWEEN CELL POPULATION KINETICS AND RADIATION RESISTANCE IN POCKET MICE (HETEROMYIDAE: PEROGNATHUS)

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Abstract

Pocket mice (Perognathus longimembris), subjected to 1000 and 1500 rads whole-body Co^{60} irradiation, were sacrificed in groups of 5 on days 1, 2, 5, 6 and 7 post-irradiation. Degenerative changes were noted in their intestinal crypts within hours after irradiation. Except for slight reduction in size, villi remained intact throughout the observation period. Regeneration of mucosal epithelium was prompt; restoring villi to normal by the 7th day. Tritiated thymidine studies indicate a villus transit time of 5.7 days in intestinal epithelial cells of pocket mice. Cell-cycle time of crypt cells was 16 hr. There is evidence of prolongation of G_2 after $T\text{-}H^3$ injection. Partial synchrony of the crypt cell population is also evident.

Introduction

Earlier we suggested that pocket mouse radiation resistance might be due, in part, to a slow rate of loss of epithelial cells from the intestinal villi (1). One consequence of a slow sloughing rate would be a slower rate of villus attrition and, effectively, protection from the gastrointestinal syndrome. This suggestion was predicated on the fact that no deaths occur in pocket mice during the first week after high dose whole body irradiation, a time when conventional mice and rats succumb to the gastrointestinal syndrome. Furthermore, dose survival time curves of pocket mice and germfree mice show remarkable similarities, with the plateau representing gastrointestinal death displaced upward and shortened for both forms (1,2,3). Since this shift of the plateau was attributed to certain peculiarities in cell population dynamics of the intestinal mucosa of germfree mice, similar mechanisms were sought in pocket mice.

Reported here are the results of early post-irradiation damage to the intestine, and normal cell-cycle and villus transit times of intestinal mucosal cells.

Methods and Materials

Field trapped, adult Perognathus longimembris, which had been maintained in our laboratory for approximately one year were used. Ambient temperature and humidity were maintained at 20-24°C and 50-70%, respectively. The animals were individually housed and fed a mixture of assorted grains and seeds. Pocket mice have no requirement for drinking water. Lights were turned on at 0630 hr and off at 1730 hr PST.

Twenty-five male pocket mice were used in the first experiment. They were divided into 3 groups: two groups were given a single, whole body dose of either 1000 rads or 1500 rads, and one was kept as control.

Irradiation was delivered from a 5000 curie Co^{60} source at 24.5 rads/min for the 1000-rad dose, and at 37.8 rads/min for the 1500-rad dose. Ferric sulphate and phosphate glass dosimetry was used.

Animals were sacrificed at selected intervals up to one week post-irradiation. Sections of jejunum were removed and fixed in 10% formalin, sectioned at 7μ and stained with haematoxylin-eosin.

For the second experiment 20 P. longimembris of both sexes were randomly divided into 5 groups of 4 mice each. All 20 animals were administered intraperitoneally 10 μCi of tritiated thymidine (Schwarz Bioresearch, Inc. Lot #1402, Sp. Ac. 0.36 Ci/mM) in 0.1 ml physiological saline.

At 12, 24, 72, 120 and 168 hours post-injection, four animals were sacrificed by ether anaesthesia and a section of jejunum was fixed in Bouin's.

Autoradiographs were prepared in the usual manner with Kodak NTB-2 nuclear track emulsion*. Some were developed after 10 days and some after 30 days exposure. Haematoxylin-eosin stain was used after development.

Two slides were prepared for each animal, providing ample material for scanning. The percentage of villus height traversed by labelled cells was judged by scanning all the sections prepared for each animal and measuring the distance traveled by the most advanced labelled cell front. This "front" was sufficiently representative of all the labelled cells on any particular slide.

Forty pocket mice, P. longimembris, were used in the third experiment. Each mouse was given 10 μCi of tritiated Thymidine in 0.1 ml physiological saline intraperitoneally at the start of the experiment. All injections were made within a 30-min period between 0710 and 0740 hr. The animals were sacrificed at closely spaced time-intervals 0.5 to 24 hours post-injection. The mice were killed by a blow on the head and a section of the small intestine, extending from the pylous to the duodenal flexure, was quickly removed and placed in Helly's fixative.

* Appreciation is expressed to Dr. A. C. Upton and William D. Gude of Oak Ridge National Laboratory for the preparation of these autoradiographs.

Cross sections (4μ) were cut, prestained by the Feulgen reaction and autoradiographs (ARG) prepared by the dipping method (Kodak NTB-2). After two weeks exposure the slides were scanned and all labelled mitotic figures (metaphase-anaphase) were scored. Six entire cross sections from each animal were scored. The sections were selected to insure separation by at least 100 μ of tissue. At a later date sections were recut at 3 μ and repeated ARG's were made. The percentage labelled mitosis on these slides were the same as on the 4 μ sections.

Results

The intestinal mucosa of pocket mice sacrificed at 6 and 15 hr after receiving 1000 rads and 1500 rads total body gamma irradiation showed the usual degenerative changes. Villi remained intact but the villus epithelial cells showed increased cytoplastic vacuolation and some distortion in size and shape. The size and staining qualities of the nuclei appeared unchanged. Cells of the jejunum crypts showed much greater changes than those of the villi. Their cytoplasm was highly vacuolated and considerable nuclear damage was evident. Chromatin debris and cellular fragments filled the crypts. Few mitotic figures could be seen, and those that were visible appeared abnormal.

At 30 to 39 hr post-irradiation the villi were still intact, although they appeared to be slightly shortened. Nuclear and other cellular debris was cleared from most of the crypts, and a few mitotic figures were observed.

By the 4th day post-irradiation, crypts appeared normal except for an increased rate of mitosis as compared with controls.

Between the 5th and 7th days, movement of regenerated epithelial cells up the villus was well marked by the characteristic basophilic staining of new cells. It was at this time that differences in the 1000-rad and 1500-rad groups were seen. By day 6 the replacement of villus epithelium was completed in the 1000-rad animals, while the 1500-rad animals showed the regeneration wave only part way up the villus. It was difficult to differentiate between control slides and slides of either of the irradiated groups on day 7.

During the course of the post-irradiation period, neither irradiated group showed appreciable degeneration of the villus. At these dose levels villus epithelial cells tended to remain intact on the villus core until replaced by regenerated cells.

Figure 1 shows the percentage of villus height traversed by labelled cells at intervals in the 7 days following injection with tritiated thymidine.

Figure 2 compares transit times of pocket mice, germfree mice and conventional mice (5). The points plotted in Figure 2 for the pocket mouse were obtained by averaging the distances represented by bars in Figure 1. These data demonstrate a longer villus transit time for pocket mouse epithelial cells, than for either germfree or conventional mice. The estimated times for reaching the villus tip are 2.1 days for conventional mice, 4.3 days for germfree mice, and 5.7 for pocket mice.

Examination of the labelled mitoses curve of intestinal crypt cells of P. longimembris reveals the presence of labelled cells within 1 hr post-injection of T-H^3 (Fig. 3). Considerable scatter in data points on the ascending limb of the curve and a reduced percentage of labelling is also evident. Peak labelling of 80% occurs at 4 hr post-injection. Between 12 and 16 hr post-injection the percentage of labelled mitoses drops to, and remains near zero. The second peak in labelled figures is reached at 20 hr post-injection. Based on midpoints of the ascending portions of the curve, the estimated total cell cycle time for longimembris intestinal crypt cells is 16 hr.

Discussion

Damage to the gastrointestinal mucosa is responsible, in a large measure, for early deaths in mammals after whole body radiation in the dose range between 1000 and 10,000 rads. Death is generally attributed to denudation of the intestinal mucosa and attendant sequelae.

In germfree mice and in several species of wild rodents (including pocket mice), all of which show some degree of resistance to whole body

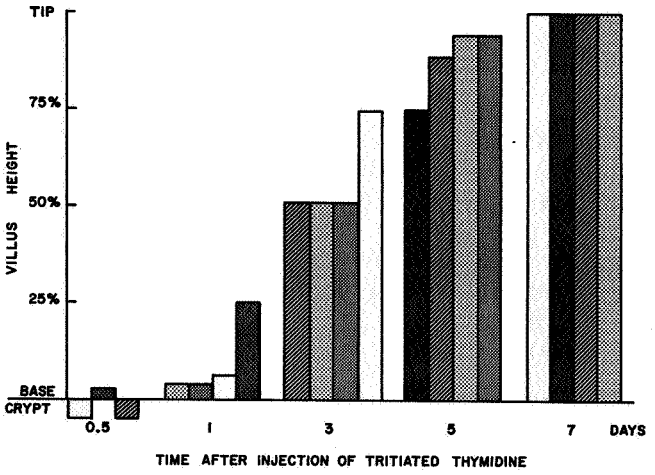
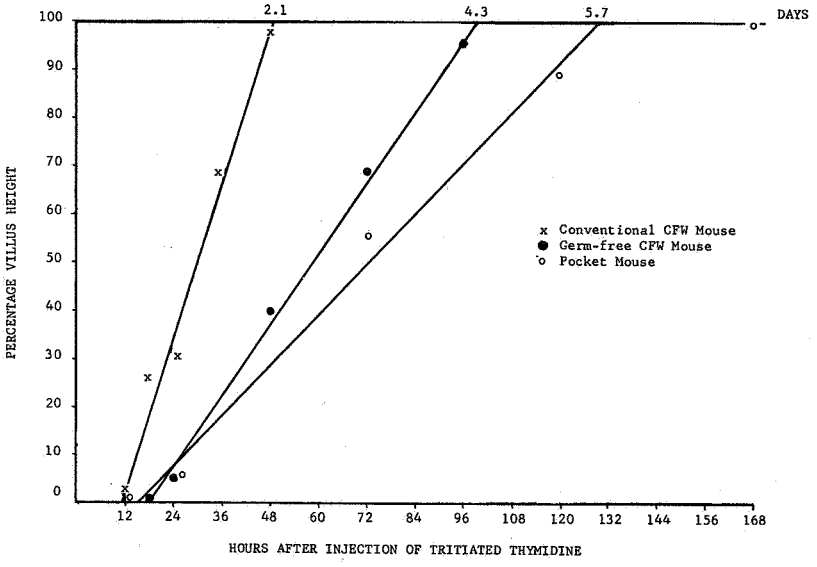


Figure 1 Progress of thymidine-H^3-labeled cells on villi of pocket mice jejunsum expressed as percentage of villus height traversed by the labeled cell "front." Each bar represents one animal.

Figure 2

Progress of thymidine -H^3- labeled cells on villi of pocket mice compared with values for germfree and conventional mice reported by Matsuzawa and Wilson (Rad. Res. 25:15-24, 1965).



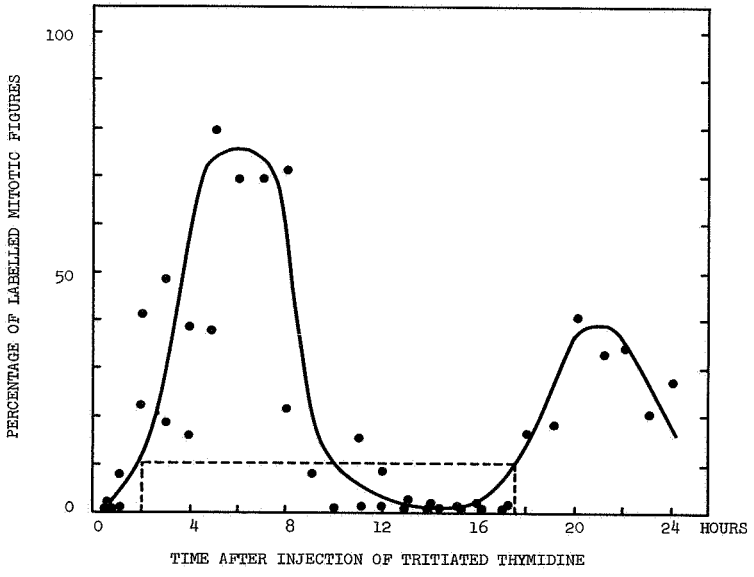


Figure 3 Labeled mitoses curve in jejunal crypts of Perognathus longimembris.

irradiation exposure, the gastrointestinal syndrome is either delayed or bypassed completely (1-6). It has been suggested that enhanced survival in germfree mice reflects a normally slow sloughing rate of mucosal cells (2,3). However, decreased cellular activity, low mitotic rate, and the absence of gut flora may all contribute to the net effect of enhanced survival in germfree mice (3).

P. longimembris has an even longer intestinal mucosal cell transit time than does the germfree mouse. Moreover, radiation deaths seldom occur earlier than 7 or 8 days in this species (1). These facts suggest that much of the radiation resistance of P. longimembris may be attributed to maintenance of the integrity of the gastrointestinal mucosa during the critical week post-irradiation. Histological evidence corroborates this point.

The estimated total cell-cycle time of 16 hr for pocket mouse duodenal crypt cells is within the normal range reported for other rodents (7). Precise measures of the phases of the cell-cycle cannot be obtained, because the labelled mitoses plateau did not reach 100% and data points are scattered on critical parts of the percentage labelled curve.

The possible causes of reduced labelling and scatter requires some comment. If, for example, errors in technique can be eliminated, several physiological explanations are available. It is possible that a prolonged G_2, caused by T-H^3-induced labelling. These lagging, unlabelled G_2 cells are still present when the labelled cohort passes into mitosis (9).

Pocket mice have an extremely labile body temperature, flucuating as much as 5 to 6°C. When warming or cooling (i.e., coming out of, or going into torpor), they may exhibit changes in core temperature as great as ½° C/min (8). Body temperatures of this magnitude usually require efficient blood shunts, which could produce local regions of hypemia. An analogous situation occurs in conventional mice, where reduced T-H^3 labelling has been attributed to a labile blood supply to the organ in question (7). Circulation to conventional mouse duodenum is known to be particularly sensitive to the stress of handling during T-H^3 injection. Reduced labelling results from the unavailability of the T-H^3 to cells in the organs or tissues with the reduced blood supply.

Circadian flucuations in body temperature, which occur in P. longimembris, would also tend to reduce percentage labelling. This would be particularly influential if the labelled compound was injected at a time when the animals were going into their metabolic low period. Animals in this study were injected at the beginning of the daily inactive period. It may well be that the observed variation in labelling index during the first hours after injection reflects this lowered metabolic activity.

Daily flucuations in body temperature would also tend to synchronize mitoses in certain tissues of the body. The importance of mitotic rhythms in radio-critical tissues is obvious. If, for example, radiation is delivered to a synchronous population of cells during their least sensitive phase (S), and if the population happened to be bone marrow or gut stem cells, significant reductions in 5- and 30-day deaths would be noted.

In summary. Reduced labelling in pocket mouse intestinal crypt cells suggests the presence of circulatory shunts which may become active as a result of handling during injection. Scatter in data points on the labelled mitosis curve indicates that pocket mouse cells have wider variation in the duration of cell cycle phases than do other rodents studied. Both of these phenomena can be related to the ability of the pocket mouse to undergo large and rapid excursions in body temperature. The possible effect of diurnal torpor on mitotic rhythms in radio-critical tissues has profound implications on whole body radiosensitivity in the mammals.

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LONG TERM SURVIVAL OF POCKET MICE AFTER X-RADIATION

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Abstract

Pocket mice, Perognathus longimembris, which received single, whole body exposure to 400, 600, 800 and 1000 R x-radiation were maintained for the duration of their life span and studied for late-effects. Mortality in irradiated groups was compared with that of untreated pocket mice similarly maintained. Life-span data on untreated groups indicate that the pocket mouse is a long-lived rodent, with a median survival time in captivity of 42 months. Radiation-produced life-shortening ranging between 0.45 and 0.75 days per roentgen was noted. Life-shortening was dose-dependent, and for a given dose comparable to that seen in other rodents.

There are abundant data on long-term survival of conventional rodents after single, whole body exposure to ioninzing radiation (1). On the other hand, there is a dearth of information of long-term survival of wild rodents after irradiation.

A series of x-ray exposures to a group of heteromyid rodents, Perognathus longimembris, several years ago proved to be less damaging than anticipated. The large number of survivors in radiation groups that received doses ranging from 400 to 1000 R, provided the opportunity to observe long-term survival and late-effects in this species.

This report presents longevity data obtained on four groups which received x-irradiation and two groups which were maintained under identical conditions but having no radiation exposure. In addition, several incidental notes on late-effects in Perognathus are presented.

Pocket mice used in these studies were field collected and maintained in our animal facility for a period of 6 months to a year before use. One hundred animals were randomly divided into 4 subgroups of 25 each and given single, whole body exposure to 400, 600, 800, and 1000 R of x-radiation. Details of exposure and animal maintenance were reported earlier (2).

Two of the mice in the 1000 R group died within the 30-day acute period following irradiation. All others survived the acute period and lived long enough to be considered "long-term" survivors. These animals were maintained in our animal facility and observed routinely for the remainder of their lives. Two other groups of 25 pocket mice each were similarly maintained over the same period. These latter two groups received no irradiation, and were used to obtain longevity data on pocket mice in captivity under the conditions of our laboratory.

Figure 1 compares survival in the two untreated groups with those receiving x-radiation. The median survival time (ST_{50}), and related life-shortening data are given in Table 1. Figure 2 shows graphically the relationship between dose and life-shortening. Based on an average ST_{50} of 42 months for the two untreated groups, life shortening in the irradiated

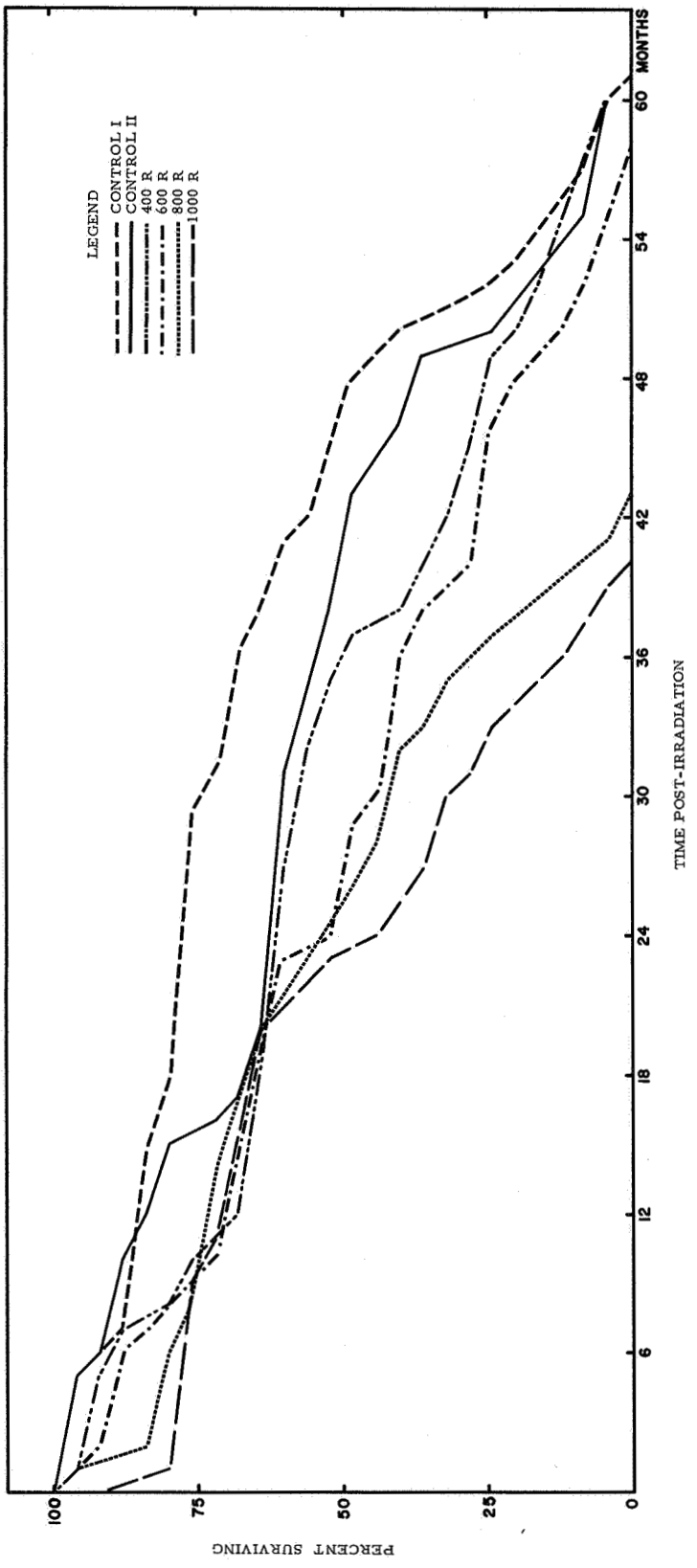

Figure 1 Long term survival in pocket mice, P. longimembris, after exposure to X-irradiation.

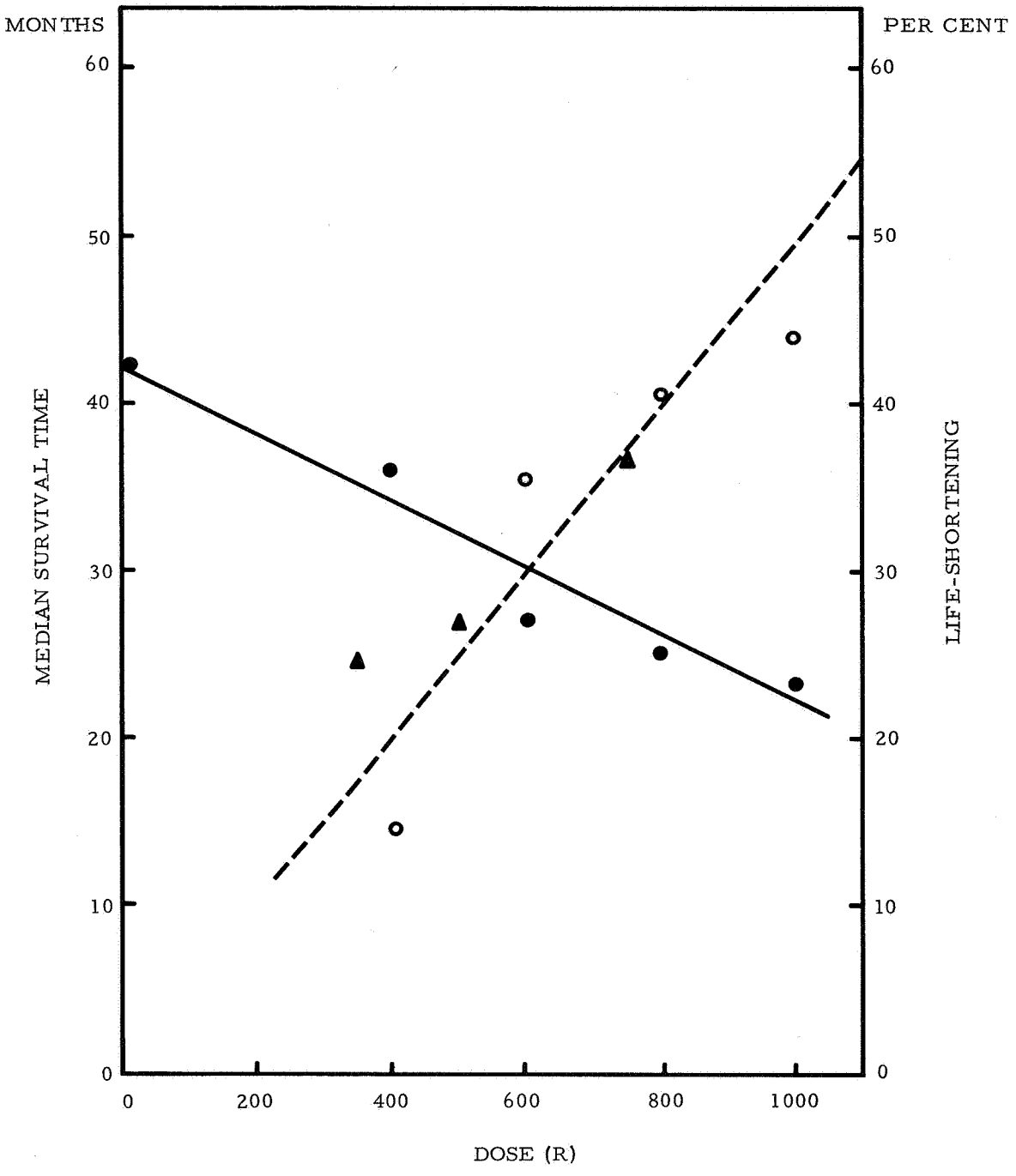

Figure 2 Median survival time vs dose for P. longimembris (filled circles, left ordinate).

Percent life-shortening vs dose for P. longimembris (open circles, right ordinate).

Life-shortening vs dose for $1C3F_1$ mice (triangles) - data of Cosgrove et al ref. 5.

groups was 14.2, 35.5, 40.5 and 43.0% for the 400, 600, 800, and 1000 R doses, respectively. In terms of days per roentgen, life-shortening ranged between 0.45 and 0.75.

During the course of the study incidental notes were made of other late effects. No significant degree of epilation was observed in any of the animals. Greying however, was observed in all irradiated groups. As in other mammalian species, greying is dose-dependent in the pocket mouse. Animals receiving 1000 R became completely grey at the time of the first molt after exposure. This occurred within two to four months afer irradiation. In these groups greying became progressively more pronounced as the animals aged, although those which received the lower doses (400 and 600 R) never became totally white, as did the 800 and 1000 R-animals. The 400 R-animals did not exhibit greying until about 3½ to 4 years after exposure.

Table 1 - Longevity of Perognathus longimembris as influenced by irradiation (25 animals in each group)

X-ray Exposure (R)	ST_{50}	Life-Shortening *		
		%	days	days/R
0	44	-	-	-
0	40	-	-	-
400	36	14.2	180	0.45
600	27	35.5	450	0.75
800	25	40.5	510	0.64
1000	23.5	43.0	525	0.53

* Based on an average of 42 months for the median survival time of the 2 untreated groups.

Casual observations of cataract incidence in the post-irradiation period were also made. Since the primary objective of the study was to obtain longevity data, it appeared unwise to subject the animals to any treatment that might jeopardize their lives. Therefore, cataract inspection was

superficial, allowing recognition of only the most advanced opacities. Nevertheless, sufficient data were collected to indicate that by the time 50 to 75% of each irradiated group was dead, all of the survivors had opacities.

Tissue pathology slides were prepared on a small number of the animals in the study. Examination of these slides revealed none of the classic late sequelae such as renal lesions, neoplasms, or foci of chronic infection.* Additional material is currently under study and will be reported later.

Pocket mice are long-lived in captivity. One Perognathus longimembris is reported as having lived 8 years (3). Records on a group of 210 mice born and reared in our laboratory indicate a median survival time of 32 months (4). Non-irradiated field collected pocket mice used in the present study had a median survival time of 42 months. The longest-lived individuals in non-irradiated groups and in the 400 R irradiated group lived beyond 61 months post-irradiation.

Although pocket mice have an $LD_{50/30}$ nearly twice that of conventional mice, long-term survivors show the same life shortening in terms of days life-shortened per roentgen as do conventional mice. For the total doses used in the present study, life-shortening ranged between 0.45 and 0.75 days/roentgen. This range compares well with values found by Cosgrove et al in $1C3F_1$ mice, which ranged from 0.47 to 0.64 days/roentgen (5). Radiation-produced life shortening appears to be proportional to life span, with the longer-lived species having the same percentage life-shortening, following a given dose, as does the shorter-lived species.

* Slides were prepared by W. D. Gude and pathological examinations performed by Dr. G. E. Cosgrove of the Biology Division, Oak Radge National Laboratory. Their able assistance is greatfully acknowledged.

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PROPOSED EXPERIMENT TO STUDY THE CIRCADIAN PERIODICITY OF POCKET MOUSE BODY TEMPERATURE, HEART RATE, AND ACTIVITY IS SPACE

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Objectives

The purpose of the experiment is to determine whether prolonged space flight will affect the circadian periodicity of a mammalian system. Specifically, the question to be asked is: whether the circadian rhythm of body temperature, heart rate and activity in pocket mice changes when all geophysical variables other than light or temperature are either removed or sensed by the animals with periods other than 24 hours.

Significance

The ubiquitous nature of periodic functions in biological material suggests that rhythmicity is a fundamental quality of life. Evidence from plant research has demonstrated that upsetting the normal pattern of rhythmicity by manipulation of the photoperiod can cause serious consequences. Biomedical research on humans has revealed periodicity of many clinical indicators ranging from fluctuations in body temperature to cation excretion, each of which signals changes in physiological state. Evidence is now strong that the life process involves a system of rhythmic events, likened to a system of oscillators, which couple to produce periodicities of various lengths. Thus cyclic biochemical events that account for the spontaneous discharge of neurons couple to produce rhythmic bursts that may have a period of less than a second, while the events associated with thermoregulation couple to produce a cycle of change in body temperature that may approximate twenty-four hours. The class of biological rhythmicity which has a period of about twenty-four hours is referred to as diurnal, or more

popularly circadian. The fact that this period approximates the length of an earth day raises the question as to whether this kind of periodicity is a manifestation of physiological processes that have evolved in an earth environment or whether there is a circadian "cue" resulting from some geophysical event that entrains the biological system. If indeed geophysical phenomena are responsible for circadian periodicity, then it is reasonable to assume their involvement in longer cyclic events such as tidal, lunar, and seasonal periodicity in biological material. Proponents of the theory that biological periodicity or "time keeping" is controlled primarily by internal or endogenous factors modified by environmental cues have demonstrated the stability of biological periodicity in spite of changing geophysical forces and argue that it is unnecessary to postulate an undefined geophysical phenomenon to interpret their data. Proponents of an external or exogenous time keeper appear willing to accept the concept of endogenous rhythmicity but argue that the correlation of circadian tidal, lunar and seasonal periodicity with geophysical events is prima facie evidence for an external "time keeper."

If the circadian rhythms of man are in any way coupled with terrestrial cues, the probability of his satisfactory performance on prolonged space missions would be low. Within this context, studies of circadian rhythms in mammals in space can have as high a priority as the required measurement of ionizing radiation in space.

The significance of this proposed experiment is that study of the persistence and stability of circadian systems divorced from the physical environment of earth will

a. Provide insight into a most fundamental characteristic of life.

b. Test the dependency of circadian periodicity on the geophysical environment.

c. Provide data pertinent to evaluation of the risks associated with extended space flight by man.

d. Provide data pertinent to the design of future space biology experiments, particularly with regard to the adequacy of ground controls for flight experiments.

Disciplinary Relationship

The first formal record of biological rhythms was probably by the astronomer De Mairan, who in 1729 described diurnally periodic leaf movements in plants held in constant conditions. The literature is now replete with evidence of diurnal periodicity in many forms of life at many levels of organization. Present day research emphasizes on one hand, the mechanism of the so-called "biological clock," and on the other, the coupling of the clock to environmental stimuli. Whether control of the period of the clock is predominantly a physiological or environmental phenomenon is debatable on the basis of existing data. The study of biorhythms in space theoretically would permit resolution of the question as to whether terrestrial stimuli indeed set the period or simply change its phase.

The principal evidences for an external stimuli setting the period of the circadian rhythm are: (1) the remarkable persistence and precision of the rhythm in organisms kept in constant environments apparently free of stimuli that are capable of entraining a self-sustaining oscillation, and (2) the remarkable insensitivity of the free running period to the level of temperature (temperature compensation). If the control of the period were truly endogenous (metabolic), changes in temperature - it has been argued - should have produced pronounced changes in period in poikilothermic organisms. On the basis of these observations, Dr. Frank Brown of Northwestern University has postulated a precise 24-hour component in the circadian rhythm resulting from entrainment to some "pervasive geophysical force."

Evidence for endogenous time keepers totally accounting for circadian periodicity independent of geophysical stimuli remains controversial.

Experiment Approach

Premise. - The proposed experiment will test the premise that some "pervasive geophysical force" does entrain the circadian periodicity of organisms and that as a consequence circadian systems removed from terrestrial stimuli will degrade. The experiment studies the persistence and precision of the circadian period in a mammal. Specifically, the question

to be asked is: whether the circadian rhythm of body temperature, heart rate or activity changes when all geophysical variables are either removed or sensed by the animals with a period other than 24 hours. The only impressive evidence of control of circadian organization by an unknown periodic variable will come from the observation of an animal's free running self-sustaining oscillation (circadian period) equal to 24 hours, after the animal has been entrained to periods not equal to 24 hours. Statistical constraints in determining significant shifts in circadian periods, and the presence of precise 24-hour components in the data point to the desirability of an experiment lasting for as long as possible (2-3 weeks minimum). This, coupled with the need to place the experiment away from any residual coupling to the earth's cycles, points to the desirability of a spacecraft placed in solar and/or lunar orbit as well as earth orbit. If a circadian periodicity perists in earth orbit but decays in distant solar orbits, we would have direct evidence, available from no other combination of experiments, that geophysical periodicities are essential inputs for maintenance of circadian organization.

Experiment. - A minimum of three pocket mice individually housed will be flown in conditions of constant darkness and temperature (21°C) for a minimum of 100 days, after having been entrained to a period of 22.5 hours in the laboratory. Their body temperature, heart rate and activity will be monitored at 10-min intervals continuously for the duration of the experiment.

Digital data will be collected and stored, broadcast to earth on command, recollected on magnetic tape and the tape, after some degree of manipulation, will be processed by an existing computer program for data reduction.

The resulting length and precision of the circadian period of each end point from each animal will be compared with the period of that same animal established prior to space flight. The data will be examined for evidence of entrainment to a precise 24-hour period by a sophisticated frequency spectral analysis. The computer program is presently written

for high statistical confidence and dictates a minimum requirement for 21 days of continuous data.

It is proposed that two ground "control" groups be run concurrently with the flight experiment. One group would be in flight hardware, and the second group in an animal holding facility.

Housekeeping data (ambient temperature, atmospheric pressure and partial pressure of oxygen) will be required to document the consistency of the environment which the experiment requires.

Validity of Technique. - The techniques of monitoring changes in body temperature in small mammals via implanted transmitters, and the reduction of these data to meaningful studies of circadian rhythm phenomenon via computer analysis, have been well proven in the laboratory by both coinvestigators. The pocket mouse has been demonstrated to be a suitable experimental subject by both investigators.

Baseline or Control Data

The precision and length of the circadian period must be established with high statistical confidence for each animal selected for this study. This requires a minimum study of 30 days prelaunch. Since the objective of the experiment is to test for unknown "pervasive geophysical forces," it is essential that ground controls be run simultaneously with the flight experiment. However, it should be understood that because of the variation in periodicity between individuals, any changes that should occur will in all probability be of different magnitudes and possibly different direction. The controls may therefore provide more qualitative than quantitative data.

Two control groups are anticipated. The first is a handling control with three to six pocket mice in flight hardware modules; the second will be six mice undisturbed in the animal holding facility.

Baseline circadian rhythm data for pocket mice as well as their life support requirements have been intensively studied both at Northrop Corporate

Laboratories and Princeton University. The data are summarized in the following contract reports and publications.

Contract Reports

1) "Investigation of Perognathus as an Experimental Organism for Research in Space Biology" NASr-91 Final Report, August 1963
NASw-812 Progress Report, December 1964
" " December 1965
" " December 1966

2) "Development and Flight Qualification of a Biosatellite Experiment Package to Study Circadian Rhythms in Pocket Mice" NASw-1191 (June 1966).

3) Contract NASr-223 between NASA and Princeton Univ. (no title) Annual Report 1 February 1965 - 31 January 1966 and 1 February 1966 - 31 January 1967.

4) "Feasibility Study for Conducting Biological Experiments Aboard a Pioneer Spacecraft" NAS2-4491 (February 1968).

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1) Circadian Rhythm of Metabolic Rate in Pocket Mice, R. M. Chew, R. G. Lindberg and P. Hayden, J. Mammalogy 46:477-494, 1965.

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Animal Temperature Sensing for Orbital Studies on Circadian Rhythms

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THE first formal record of biological rhythms is probably by the astronomer De Mairan who, in 1729, described diurnally periodic leaf movements in plants held in the dark. The literature is now repleat with evidence of diurnal periodicity in many forms of life at many levels of organization.[1] Present-day research emphasizes on one hand the mechanism of the so-called "biological clock", and on the other hand the coupling of the clock to environmental stimuli. However, few precise 24-hr rhythms have been documented; periods may vary from 22 to 26 hr, depending upon the species studied. A variation of 1 or 2 hr is not unusual between individuals of the same species, and a few experiments have been involving crossing strains of different rhythms. As a result, the phenomenon is commonly called "circadian" rhythm, meaning "about a day." Many individual rhythms, usually not in phase, can be measured within a single organism, and in some way they probably integrate to form the dominating circadian rhythm of the total organism.

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Whether the setting of the clock is predominantly a chemical, physical, biochemical, or environmental phenomenon is debatable on the basis of existing data. The study of biorhythms in space theoretically would permit some resolution of the question as to whether terrestrial stimuli indeed set the period or simply change its phase. Since the purists argue that a satellite in orbit 200–300 miles above the earth's surface may still be within the influence of "pervasive geophysical forces" that may be sensed by biological material, a heliocentric orbit would be highly desirable. A circumlunar orbit would be a compromise, since periodic approach to the earth might entrain biological rhythms and mask the effects being sought.

If the circadian rhythms of man are in any way coupled with terrestrial cues, the probability of his satisfactory performance on prolonged space missions will be low. Within this context, studies of circadian rhythms in deep space can have as high a priority as the required measurement of ionizing radiation in space.

Data from metabolic studies on pocket mice show that Perognathus longimembris has a circadian metabolic rhythm that can be detected at both moderate (22°–24°C) and low (10°C) environmental temperatures, at high and low humidities, in the dark or under normal photoperiod, with and without food, in normal atmospheres and 100% oxygen, and in both individually housed and grouped mice.[2] Placing these animals in earth orbit should elucidate the effects of exogenous factors that may influence a persistent endogenous rhythm. Whereas the most obvious exogenous cues to be studied are weightlessness and orbital period, the experimental design is easily adapted to provide for the input of almost any specific environmental stimuli in the isolation of space.

Experiment Definition

Colin S. Pittendrigh of Princeton University and R. G. Lindberg of Northrop Space Laboratories (NSL) have pro-

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posed that six Perognathus be flown in conditions of constant darkness and temperature at about 22°C for a minimum of 21 days. Their body temperatures and locomotory activities will be recorded continuously, the former by intraperitoneally implanted temperature transmitters and the latter by either amplitude modulation of the transmitter signal or magnetometer or capacitor "gates" along the length of the container. The data will be subjected to frequency spectrum analysis, which shows for grounded animals a clear circadian component. The question is whether the circadian rhythm of either temperature or locomotion changes when all geophysical variables other than light and temperature are either removed or sensed by the animals within a period of about 90 min instead of 24 hr. Each animal flown will have been ground tested for its capacity to withstand launch and preorbital flight conditions, and its frequency spectrum will have been measured for one month before flight.

Perognathus is almost uniquely suited for this experiment. It has an unusually large (3–5°C) amplitude to its daily temperature cycle; weighs 8–10 gm, and, most significantly, it has no water demands. Urine production is extremely low (0.05 ml/24 hr), and the feces are dry and odorless (0.01 gm/day). The problems of watering and waste removal for mammals in zero g are therefore either completely eliminated or easily solved.

Each mouse will be individually housed in a thin-walled aluminum cylinder 12 to 18 in. long and 1.5 in. in diameter containing 30 g of loose sun flower seeds (30-day supply). An oxygen-nitrogen atmosphere at 14.7 psi, or an oxygen atmosphere at 5 psi, will flow through the tube at a rate of ~0.03 ft³/min, at a constant temperature (~75°F) and constant relative humidity (~50%). At the exhaust end of the tube, coarse screening will prevent the seeds from being blown from the tube under zero-g conditions, but will permit the escape of feces (0.05–0.1 gm/24 hr) and seed particles into a debris trap. The tube will be lined with a mesh screen impregnated with an absorbant asbestos-cellulose material. The lining serves the following four purposes: 1) absorption of urine (0.05 ml/24 hr), 2) grooming, 3) foot hold for the mice, and 4) shielding for electronic instrumentation. Ambient temperature will be monitored by a thermistor in the exhaust air of each tube. Body temperature will be monitored by an implanted telemeter device and a loop antenna running the length of the cylinder. Locomotory activity can be monitored. The thermistor, antenna, and motor activity leads, if present, will feed into an electronic instrumentation package, which in turn will feed into a data storage device and, on demand, into the satellite telemetry system. It is not essential that the six individual cylinders be close together or that the data instrumentation package be located immediately adjacent to the tubes.

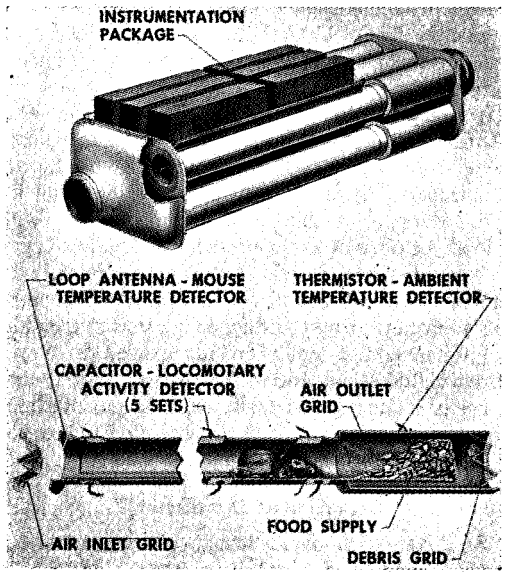

Fig. 1 Concept of an experimental package to study circadian rhythm in pocket mice during prolonged orbital flight.

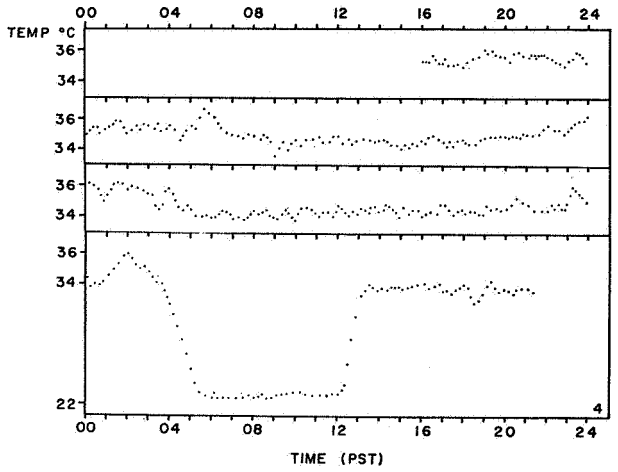

Fig. 2 Daily pattern of body temperature change in Perognathus longimembris entering into a period of hypothermia.

A graphic concept of the minimum experiment package for monitoring both temperature and activity (i.e., without data handling and environmental control system) is shown in Fig. 1. The six tubes are assembled in two layers of three tubes each. The instrumentation packages are mounted on a common panel adjacent to the tubes. A constant quality atmosphere from the spacecraft environmental control system enters the assembly through a manifold and is exhausted through a manifold at the opposite end. Mounting brackets will be provided at each end of the assembly to match the mounting requirements of the spacecraft. A "self-contained" package for a 30-day mission, incorporating data handling and storage and an environmental control system, is estimated to weigh less than 25 lb, occupy less than 1 ft³ of space, and utilize 12 w of power. The experiment design is adaptable to earth, lunar, or heliocentric orbits provided that a telemetry link is available negating the requirement to recover the experiment package.

Data Handling System

The main function of the data handling system is the measurement of temperature from each animal. These data are accurately converted into a convenient format, accumulated in a buffer storage device, and retrieved and transmitted to the ground station on command via the spacecraft telemetry system. To achieve the experiment objectives, the temperatures must be measured with a resolution of 0.1°C. This temperature resolution must be preserved, together with its corresponding time base accuracy, through the chain of events up to and including ground data reduction. Such a system is best implemented by using digital techniques. The advantages of a pulse code modulation (PCM) system are obvious.

A thermal sensitive telemeter implanted in the animal will transmit mouse body temperature to within 0.05°C. These tiny telemeters (Fig. 2), developed at NSL, have been successfully used at NSL and Princeton University with pocket mice to generate data of the type and quality required for the orbital experiment. They do not encumber the animals with wires, harnesses, or restricted movement, and they lend themselves to sterile surgical implant techniques. Since only a single data point was required for each 5-min time interval, and since there is an inherent time lag constant of the sensor (thermistor), it appeared that continuous carrier operation

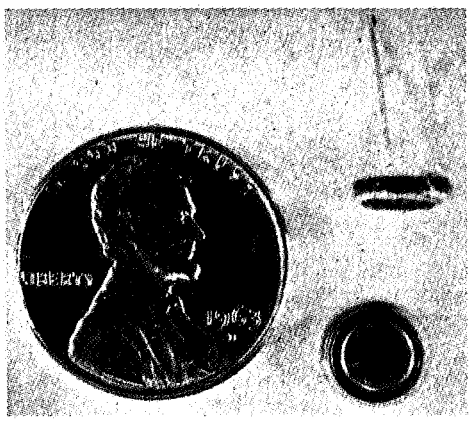

Fig. 3 Prototype of NSL temperature transmitter prior to welding of mercury cell and final encapsulation in paraffin.

would add little to the character of the transmitted data, but would impose a needless load on the primary power source. A simple method of pulsed data transmission was therefore explored in which the "off time" of the rf carrier would result in conserving primary power.

The simplest and most direct approach to the problem was the relaxation type of "squegging" oscillator circuit. Briefly, this simple circuit may be described as a modification of a conventional inductive capacitance oscillator. If the capacitance-resistance (CR) combination controlling the base bias is increased, the time required for the bias to again adjust itself to the small reduction in the total loop gain becomes longer until a point is reached where the oscillations decay spontaneously. If the total loop gain is now increased by increased feedback, the amplitudes of the oscillations themselves become oscillatory. As in the previous case the oscillation again decays, the base bias leaks away at a rate determined by the capacitor-resistor combination, whereupon strong oscillations start once more. Oscillations build up very rapidly, generating a bias that eventually rises to a level too high to maintain them, cutting the base off, and the oscillations again die away. This mode of operation is the so-called "squegging" circuit. In such a circuit, if the capacitance C is held constant, and the R value consists of a varying resistance sensor, then the pulse rate or squegging frequency becomes a function of temperature.

Laboratory tested system performance of a typical temperature sensing transmitted evolved from the squegging circuit is as follows: temperature resolution, 0.05°C to 0.1°C; life expectancy, 250 days (at room temperature); primary powered source, mercury cell; carrier center frequency, 4.5 Mc; radiation patters, figure "8," normal to, and omnidirectional in plane of, transmitter inductance; pulse rate, 100/sec to 500/sec; peak power input, 15 mw; volume, 0.25 cm^3 (ready for implant); weight, 0.75 g; and encapsulation material, paraffin. Figure 3 shows a prototype of one such transmitter subassembly prior to welding of mercury cell and final encapsulation.

Temperature pulses from each of the six channels are fed into the data processor, which basically consists of a 10-bit binary counter (1023-count capacity), a 10-bit shift register, and the required logic elements to organize the temperature data into the required format. Pulses from the temperature subsystem are gated into the data processor by 10-sec timing pulses from the programer where timing accuracies are maintained to 0.01%. At a nominal temperature, the telemeter pulse rate is approximately 350 pps and produces a total of 3500 counts in 10 sec. The binary counter recycles every 1024 counts, and at the end of 10 sec has recycled three times and the registered count will be in the fourth cycle. In effect, this constitutes a 12-bit digital system where the first two significant bits (or first 3072 counts) are not recorded since they are always known. The resolution of the system is about 0.025°C, providing a total ambiguity of 0.05°C

A programer sequentially selects each channel at the proper time and shifts its data into the tape recorder. The main elements of the programer are as follows:

1) Clock: a 10-kc crystal control oscillator is divided to produce pulses at several lower frequencies. These pulse trains are used for the timing and synchronization of the events controlled by the programer.

2) Channel selector: each stage of a six-stage ring counter sequentially enables a set of three gates which controls the operations required to shift data out of a particular data processor and to reset it to accept the next temperature data point.

3) Shift pulse generator: this circuit is a four-stage binary counter preset to the count of 10. Upon the receipt of a reset pulse, it generates a train of 10 shift pulses (at 2/msec) for each group of 10 to be shifted into storage.

4) Word selector: a ring counter, with a stage for each word in the data frame, generates pulses to recycle the shift pulse generator and produces control pulses for resetting the particular data processor being interrogated.

5) Recorder control: the data storage tape recorder is turned on 10 msec prior to the recording of the first word in each frame to allow the tape transport to come up to operating speed. The control circuit is a flip-flop that drives the control circuits in the tape transport; it is turned off at the end of the last word in each frame.

6) Barker code sync generator: a word at the beginning of each frame is used to synchronize data processing systems with the recorded data; the Barker code also establishes bit synchronization.

7) Housekeeping data: An analog-to-digital converter is used to digitize the several analog voltages from the temperature and pressure transducers and from electronics circuits being monitored.

8) Recorder and controls: The biorhythms experiment dictates the use of data storage because data are taken every 5 min but are transmitted to ground stations only once per satellite orbit. The digital data are stored in serial form on one track of a two-track tape recorder. The second track records clock pulses to insure bit synchronization during data reduction. A 0.25- or 0.50-in. tape will be used in the form of a continuous loop about 36 in. in length. A single head for each track will serve for both record and playback. No erase head is required since the record head always saturates the tape during the record mode. Two control systems are required; one to start and stop transport in the record mode, and one to play back on command. On command to playback, the transport advances the tape in the record mode to the end of the loop (at splice). This erases any bits left on the remaining tape at the end of the last orbit. When the end of the loop is reached, the recorder automatically switches to play-back mode and the tape continues to advance feeding "non-return to zero" PCM data to the spacecraft telemetry system. The tape recorder continues to operate in play-back mode through several cycles to insure a good transmission and ground station recording. The time required for one playback of the data taken during one orbit is ~10 sec. On command the recorder switches back to the record mode the next time it reaches the starting point on the tape loop. The tape speed for both record and playback is approximately 3 in./sec, and the packing density is approximately 200 bit/in. Tape speed is not critical, since a time reference is used on the adjacent track.

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