NSL 64-29-13

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

(Contract NASw-812)

A SUMMARY OF PROGRESS
1 January 1966 through 31 December 1966

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PRINCIPAL INVESTIGATOR

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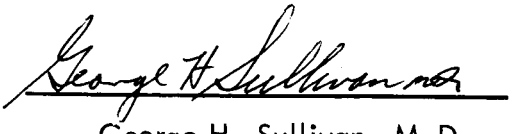

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PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR SPACE RESEARCH

SUMMARY

This contract was undertaken to establish the feasibility and practicality of using pocket mice as subjects for biological experiments in space; and to identify specific experiments in which their particular physiological attributes could be used to study biological phenomenon. Sufficient baseline data on hematology, cytogenetics, circadian periodicity, radiation response, thermoregulation and breeding have been obtained to warrant the use of pocket mice in many experiments in which conventional mice, rats or hamsters are prepared to study the effect of space residence on mammals. The pocket mouse has the added advantage of small body size and low life support requirements promulgated early in these investigations.

Under this contract, sufficient physiological data were obtained to effect the design and prototyping of experiment hardware for studying the effect of orbital flight on circadian periodicity. The prototype hardware was developed under contract NASr-1191, and the prototype equipment was used as a laboratory tool to study circadian periodicity under a portion of NASw-812. Those studies clearly showed that the experiment hardware configuration did not perturb the biological parameters being measured.

The major portion of the contracted effort during 1966 was spent on examining Perognathus as a classical hibernator and studies on hypothermia and thermoregulation were initiated. While some of the studies have been completed, Perognathus appears to display a seasonal response which is in the process of study. Also in progress are a series of comparative studies of circadian periodicity within the family Heteromyidae which will be reported at a later time.

The following pages provide a more detailed account of accomplishments under contract NASw-812: Section I is the manuscript of a paper on thermoregulation recently accepted for publication. Section II, is a summary of laboratory work directly pertinent to the consideration of Pocket Mice as classical hibernators. Section III is a tabulation of publications in the open literature resulting from research supported in part or in whole by NASw-812.

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SECTION I

"Temperature Regulation in the Little Pocket Mouse,

Perognathus longimembris"

MANUSCRIPT OF A PAPER ACCEPTED FOR PUBLICATION

IN THE JOURNAL OF COMPARATIVE BIOCHEMISTRY

AND PHYSIOLOGY

Temperature Regulation in the Little Pocket Mouse, *Perognathus longimembris*

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This work has been supported by NASA Contracts NASr-91 and NASw-812 to Northrop Space Laboratories, Hawthorne, California. Part of the work was done in the research laboratory of the senior author at the University of Southern California, Dept. of Biological Sciences, Los Angeles.

Abstract:

(1) 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.

(2) P. longimembris showed good thermoregulation when not torpid. A variation of 1-4.5° in colonic temperature did not vary significantly over the range of T_A 2-34° C. Mice acclimatized to 22-24° C showed resistance to change in T_B when exposed to cold, heat and continuously changing T_A.

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

(4) P. longimembris simulated a following of Newton's law of cooling when metabolizing at a minimum maintenance level, i.e. conductance was constant at ambient temperatures below 33° C. Active mice had a variable conductance which decreased with T_A.

(5) Mice entering and arousing from torpor go through several phases of temperature change. During cooling conductance was greater than, and during warming, less than in normothermic mice in a minimum maintenance state.

(6) Resting metabolism was 0.53 and conductance was 0.78 of certain mammalian standards, which is consistent with a T_B reduced below 37° C.

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 and 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 and Cade (1957) and Chew et al. (1965), P. 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 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, 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 and 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 and Cade (1957) on P. longimembris and of Tucker (1965a, 1965b) 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 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.

Body temperatures were measured with bead thermistors ($\sim$ 0.6 mm^3) implanted subcutaneously in the middorsal region and inserted 2 cm into the colon, and by small telemeters (17 x 9 x 5 mm; $\sim$ 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 disc 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 megacycles with a temperature dependent repetition rate of 150/sec to 500/sec. Temperature resolution is to 0.1°C, with a shift of about 0.5° in six months.

During temperature measurements animals were confined in 1000-ml tall-form beakers submerged in a water bath, or in 20 x 7.5 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 two hours 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°. After exposure to 37°, mice were exposed 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 $\sim$ 20 ml O_2. In measurements with the respirometer, the mice were alternately measured one day at an experimental T_A and rested one day at room temperature,

until the range of 5°, 15°, 25° and 35° C was completed. During measurement periods mice were confined in 100-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

Fig. 1 Fig. 2 Table 1

Fig. 1 shows the colonic temperatures of P. longimembris during 24-48 hr exposures at different T_A. Fig. 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.

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° than at 2° and 32°C.

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 30°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° salivated markedly; mice often everted their cheeck 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° within 2 hours. 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.

Fig. 3

Fig. 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° 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.

Core-to-subcutaneous temperature gradient

Fig. 4

Fig. 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°, $p < 0.001$; for T_A 25-39°, $p < 0.05$. 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: T_A 0-14° C, $T_B = 36.59 - 0.0055T_A$; T_A 14-28°, $T_B = 36.44 - 0.0041T_A$; T_A 28-34°, $T_B = 33.64 + 0.081T_A$; T_A greater than 34°, $T_B = 1.519 + 1.034T_A$.

Metabolic rates of normothermic animals.

Fig. 5

Fig. 5, a 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. 5, a) 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° exposure. Minimum maintenance metabolism (Fig. 5, b) 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 hr = $12.68 - 0.268T_A$ (standard error of estimate, 0.12; n = 27). Minimum maintenance rate was: ml O_2/g hr = $10.91 - 0.277T_A$ (standard error of estimate, 0.08; n = 27).

At T_A 35° the average maintenance rate was 2.94 ml O_2/g hr, and minimum maintenance rate was 2.06 ml O_2/g hr.

Metabolic rate was also measured with the Beckman analyzer for ten mice (ave. 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_A30.0°, 1.12 ± s.e. 0.075 ml O_2/g hr; T_A 32.5°, 1.07 ± 0.059 ml O_2/g hr; T_A 36.0°, 1.45 ± 0.13 ml O_2/g hr. The value of 1.07 ml O_2/g hr at T_A 32.9° is taken as the best approximation to basal metabolic rate. These measurements also indicate the zone of thermal neutrality is no more than 33 ± 1.5° 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.

Tables 2, 3 The analysis is summarized in Tables 2 and 3.

Metabolism and T_B during torpor.

Body temperature and metabolism were measured for three P. longimembris during five cycles of entry and arousal from torpor. One cycle is shown

Fig. 6 in Fig. 6.

In these five cycles the maximum cooling constants averaged K = 0.0321, and maximum warming constants averaged K = 0.0399. The difference between constants for consecutive coolings and warmings was significant: d = 0.0078 ± s.e. 0.0019, $p < 0.01$.

Cooling of dead mice.

The cooling characteristics of freshly dead individuals of three

Table 4. species of Perognathus are shown in Table 4.

DISCUSSION

Regulation of body temperature

P. longimembris is clearly a good temperature regulator in the range T_A 2-32°C. Although T_B varied 1-4.5° during recording periods of 2-36 hr for normothermic animals, a precision of regulation is not lacking if one considers the problems which small size imposes.

P. longimembris may have somewhat more stable normal body temperatures than several other species that have been studied, although exact comparison is confounded by differences in procedures of measurement and exposure. In the present study the mean square was 0.55° for 142 measurements at 10 minute intervals during 2 hours on six P. longimembris at T_A 22° and 27°C. Calculations from the statistics of Morrison and Ryser (1959) give a mean square of 4.1° for 207 measurements on 27 Peromyscus leucopus; their measurements were taken over a period of days on mice free in their cages at T_A 23-27°C. Similarly, a mean square of 1.05° was calculated for 163 measurements on nine Zapus hudsonius kept at T_A 20°C (Morrison and Ryser, 1962). Bartholomew and Cade (1957) found less variation of colonic temperature in P. longimembris acclimatized 10 days at T_A 19-23°C, than found in the present work for telemetered animals at T_A 22°, i.e. s.e. of 0.14° for 14 mice, and 0.36° for 9 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 intraindividual 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 and 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 and Ryser, 1959), Zapus hudsonius (Morrison and 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 and Cade (1957) found that this species had lower T_B at T_A 2.5-5.3°C than at T_A 19-23°. Their measurements were made after 10 days 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 20 min transition to 37°, it was 50 minutes before abdominal temperatures averaged 37° for the group. The T_B of one mouse did not rise to 37° in the 2-hr exposure. Then, after a 30 min transition to T_A 39°,

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 = 38.2° in 60 min, another warmed to 39.0°, while a third reached a lethal T_B of 41.9° 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°/ 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 3.5-9 degrees/g hr between the expected and actual body temperatures would require the evaporation of 5.5-14 mg H_2O/g hr, 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 ($\sim$ 600 mm^3 vs $\sim$ 0.6 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 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 and Ryser (1959) and others have shown that the measured colonic temperature varies with the depth of insertion of the thermocouple or thermistor. Bartholomew and 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° 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° 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 suggestes 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° 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 to 4.0° 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 Fig. 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°, since $T_c - T_s$ remains at about 1°, 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°. 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°, $T_c - T_s$ is relatively stable at 3°. 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 temperature 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 it 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.

Musser and 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 2 days at T_A 10°C (Table 2, 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 and 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 #B3 (Table 2, 3) up to the time of its entry into prolonged torpor. In mouse #B4 there was a lack of coincidence prior to and between two short periods of torpor. In mouse #B3 there was a complete positive association of mean hourly levels of T_B and of oxygen consumption (Table 3). However, in #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 #B3 and #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. 5, b) extrapolates to 39.4°C, which is high in the normal range of T_B. But, the extrapolation is reasonable considering that the 5% confidence limits of the slope of the regression rate 0.248 to 0.306.

The mice of Fig. 5, at their minimum metabolic rate, had a thermal conductance that was constant below LCT at about 0.28 ml O_2/g hr °C. 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 hr °C 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 and Ryser (1951): cal/ g hr °C = 4.8 $wt(g)^{-0.50}$.

The linear regression of average maintenance metabolism on T_A (Fig. 5, line a), however, extrapolates to 44.2° 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: $C = \frac{H.P.}{T_B - T_A}$. 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 = 2.5; T_A 32°, c = 1.3; T_A 25°, C = 0.58; T_A 15°, C = 0.43; T_A 5°, C = 0.42. 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 LCT, 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/g hr = $0.379T_B$ - 2.77. The rate of warming averaged 0.3°

C/min, once T_B reached 25°. This is only half the rate found by Bartholomew and Cade (1957) for the same species, and even less than the warming rate for Sicista betulina (Johansen and 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° or less of T_A. The theoretical minima, as determined during periods of maintained torpor at different ambient temperatures were: at T_A 10-22°C, minimum ml O_2/ g hr = $0.027T_A - 0.177$; T_A 22-32°, minimum ml O_2/ g hr = $0.048T_A - 0.68$.

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 in 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 = 0.82 cal/ g ^{o}C; 1 ml O_2 consumed ≡ 4.7 cal; $\Delta 1^{o}C\ T_B \equiv 0.174$ ml O_2/ g. The thermal conductance necessary to achieve the observed cooling or warming was estimated as: $C\ (\text{ml } O_2/\text{ g hr}^{o}\text{C}) = \frac{\text{observed H.P. + } O_2 \text{ equivalent of } \Delta T_B}{T_B - T_A}$. 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 hr^{o}C 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 facilities heat accumulation.

Physical insulation.

Dead P. longimembris cooled at rates equivalent to an average conductance of 0.32 ml O_2/ g hr^{o}C; 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 = 0.14 ml O_2/ g hr^{o}C (which is equivalent to 0.66 cal/ g hr^{o}C or 0.30 cal/cm^2 hr^{o}C) may approximate the reciprocal of maximum physical insulation of the little pocket mouse. However, variation of 0.23 to 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 and 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 (^oC) = 4.7(M/C) + 32.2, 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 hr; this is 0.53 of the value predicted by the general relationship developed by Kleiber (1961): ml O_2/ g hr = 3.5 $wt^{-0.25}$. The conductance of P. longimembris in a minimum maintenance state, 0.28 ml O_2/ g hr^oC, is 0.78 of that predicted by the equation of Morrison and Ryser (1951): C = 1.02 $wt^{-0.50}$. Following the procedure of McNab (1966), T_B predicted by his formula is then 35.4^oC, i.e. a reduction below the mammalian "standard" of 37^o 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^oC; 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 and Ryser (1959), it is not possible to be more conclusive about the temperature relationships of the little pocket mouse without much more information.

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Legends for figures.

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).

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 minute transition between temperatures, except there was an overnite break in the sequence between measurements at T_A 12° and T_A 17°C. The data plotted for T_A 37° are for the 2nd hr of exposure only; data for 39° are for a 1-hr exposure only.

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.

Legends, 2.

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.

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 1. Body temperature at different ambient temperatures.

T_B measured by telemeter in abdominal cavity during 2-hr exposure.

P. longimembris (n=6), ave wt 11.55 g (range 9.0-14.0 g)

T_A (°C)	2	7	12	17	22	27	32	37[b]	39[c]
Mean ave 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 ave T_B (°C)	0.90	1.67	2.05	2.33	2.59	1.82	1.23	1.05	1.88
Total range of ave 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[a]	0.10	0.40	0.48	0.53	0.61	0.52	0.11	0.26	———

P. alticola (n=3) wts 15.5, 15.7, 22.2 g

T_A (°C)	2	7	12.5	17	22.5	27	32	37
Mean ave 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 ave T_B (°C)	1.46	1.66	2.00	2.63	2.80	2.53	2.30	2.03
Total range of ave 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

a - 12-14 measurements at 10 minute intervals on each mouse at T_A 2-37°, 6 measurements at T_A 39°; mean square = sum deviations^2/degrees freedom

b - ave and s.e. for 2nd hour only, when T_B was stabilized

c - 1-hr exposure only

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 #B3	Mouse #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[a]	0.93	0.79
minima of T_B and metabolism[a]	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

a - calculated for those hours when there was enough variation to determine clearly minima and maxima

Table 3. Mean hourly metabolic rate and T_B for *P. longimembris* #B3 at T_A 10°C. Data for normothermic hours only.

n	ml O_2/ mouse/ hr	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

Table 4. Cooling characteristics of freshly dead Perognathus spp.

Species	Body wt (g)	Surface area[a] (cm^2)	Cooling constant (K)	Conductance (cal/ cm^2 min ^oC)
P. longimembris	8.18	17.82	0.0362	0.0138
	8.14	17.76	0.0350	0.0133
P. formosus	21.70	34.26	0.0210	0.0110
	19.68	32.09	0.0239	0.0134
	19.51	31.90	0.0204	0.0104
P. californicus	31.68	44.14	0.0189	0.0133
	28.18	40.81	0.0210	0.0120
	25.68	38.35	0.0219	0.0122

a - calculated as, Surface area (cm^2) = 9 $wt(g)^{0.67}$

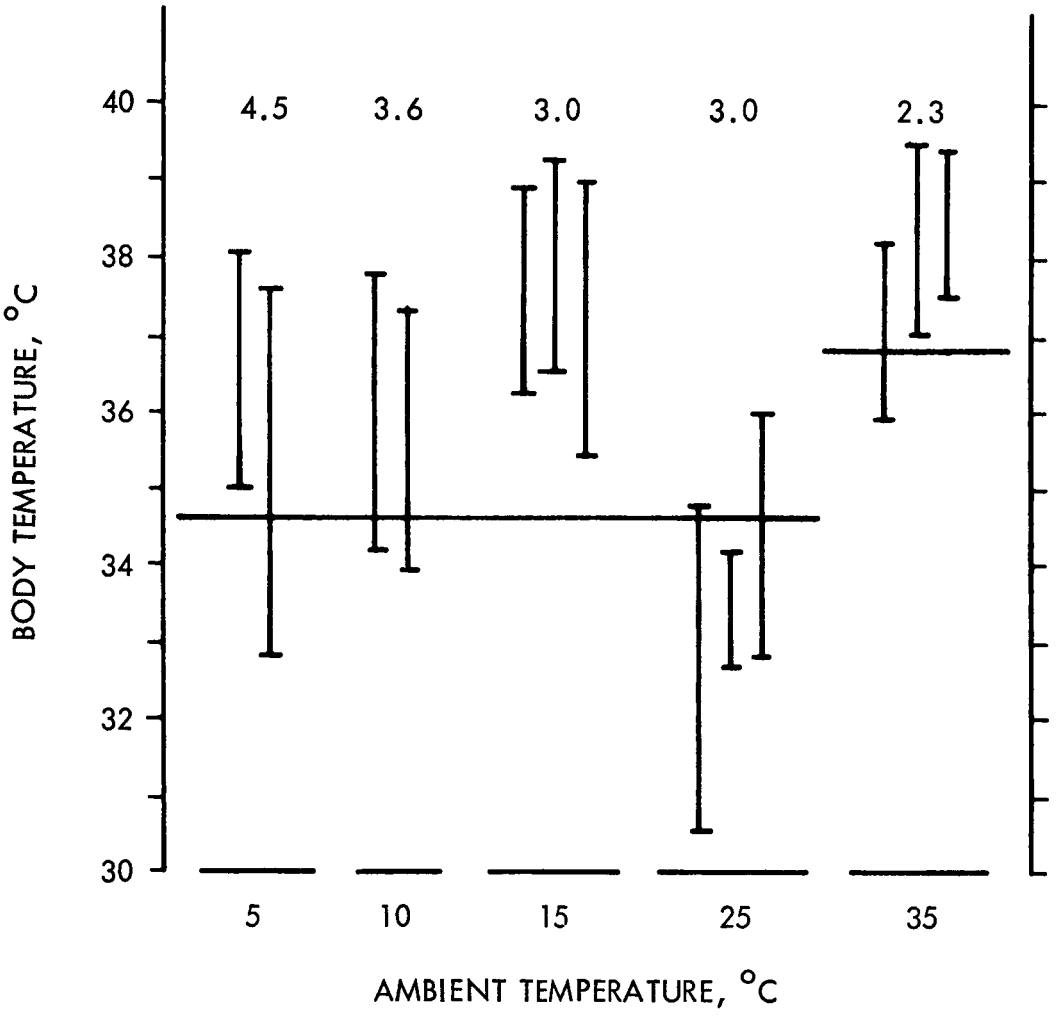

FIGURE 1 BODY TEMPERATURE OF P. LONGIMEMBRIS KEPT 24 - 48 HR AT DIFFERENT AMBIENT TEMPERATURES; T_B RECORDED CONTINUOUSLY FROM BEAD THERMISTOR 2 CM INTO COLON. OPEN CIRCLE IS THE AVERAGE T_B AND VERTICAL LINE IS THE RANGE FOR EACH ANIMAL DURING ITS NORMOTHERMIC PERIODS. HORIZONTAL LINE IS MEAN AVERAGE T_B OF ANOTHER GROUP OF SIX POCKET MICE AS MEASURED BY TELEMETER IN BODY CAVITY (MICE OF FIG. 2 AND TABLE 1).

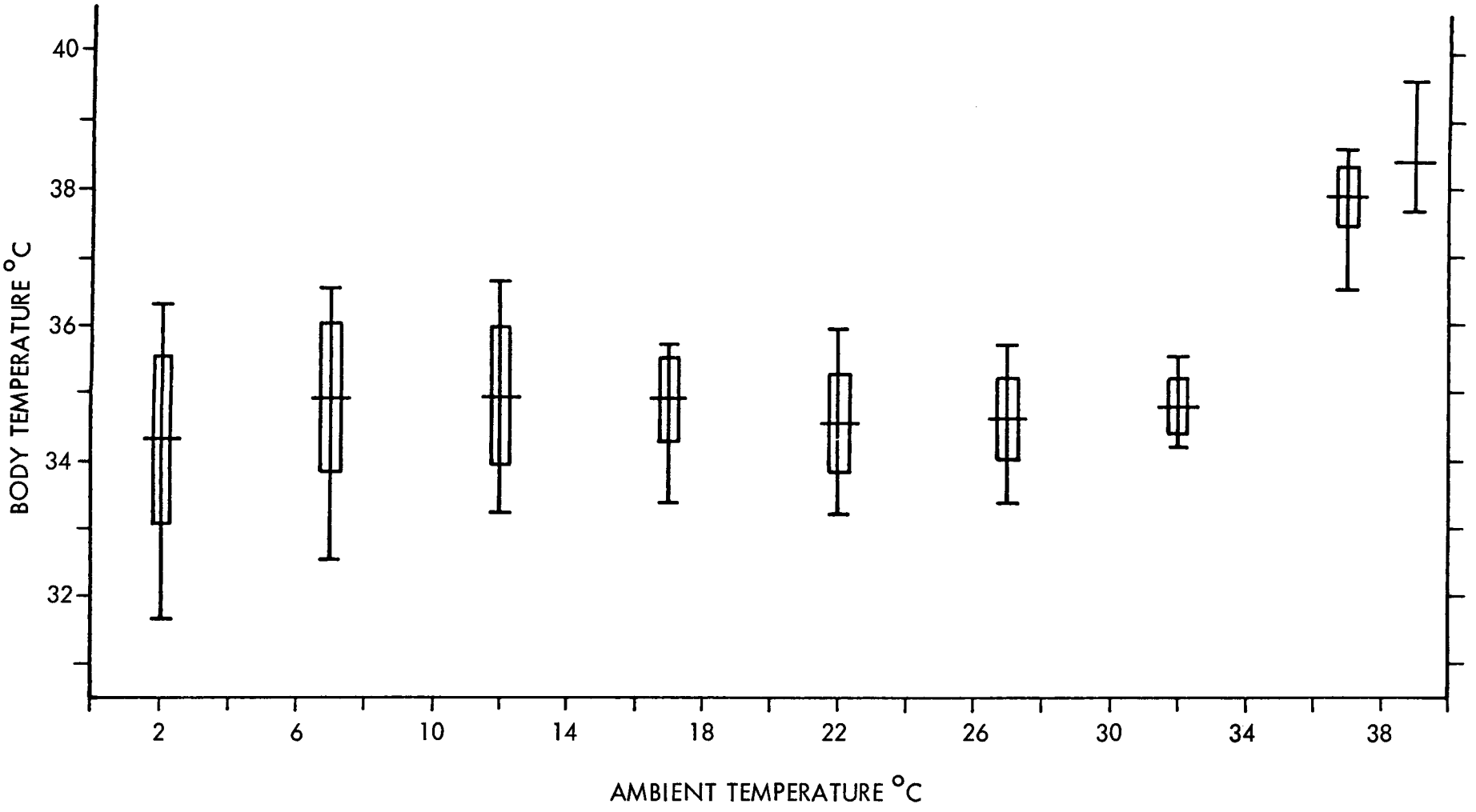

FIGURE 2 RELATIONSHIP OF MEAN AVERAGE ABDOMINAL TEMPERATURE TO AMBIENT TEMPERATURE FOR SIX P. LONGIMEMBRIS. HORIZONTAL LINE IS MEAN AVERAGE T_B FOR THE SIX MICE, VERTICAL BAR IS $\pm$ 2 S.E., AND VERTICAL LINE IS RANGE OF AVERAGE ABDOMINAL TEMPERATURES. MICE WERE EXPOSED 2 HR AT EACH TEMPERATURE, PROCEEDING FROM 2° TO 37°C, WITH A 20 - 30 MINUTE TRANSITION BETWEEN TEMPERATURES, EXCEPT THERE WAS AN OVERNITE BREAK IN THE SEQUENCE BETWEEN MEASUREMENTS AT T_A 12° AND T_A 17°C. THE DATA PLOTTED FOR T_A 37° ARE FOR THE 2ND HR OF EXPOSURE ONLY; DATA FOR 39° ARE FOR A 1-HR EXPOSURE ONLY.

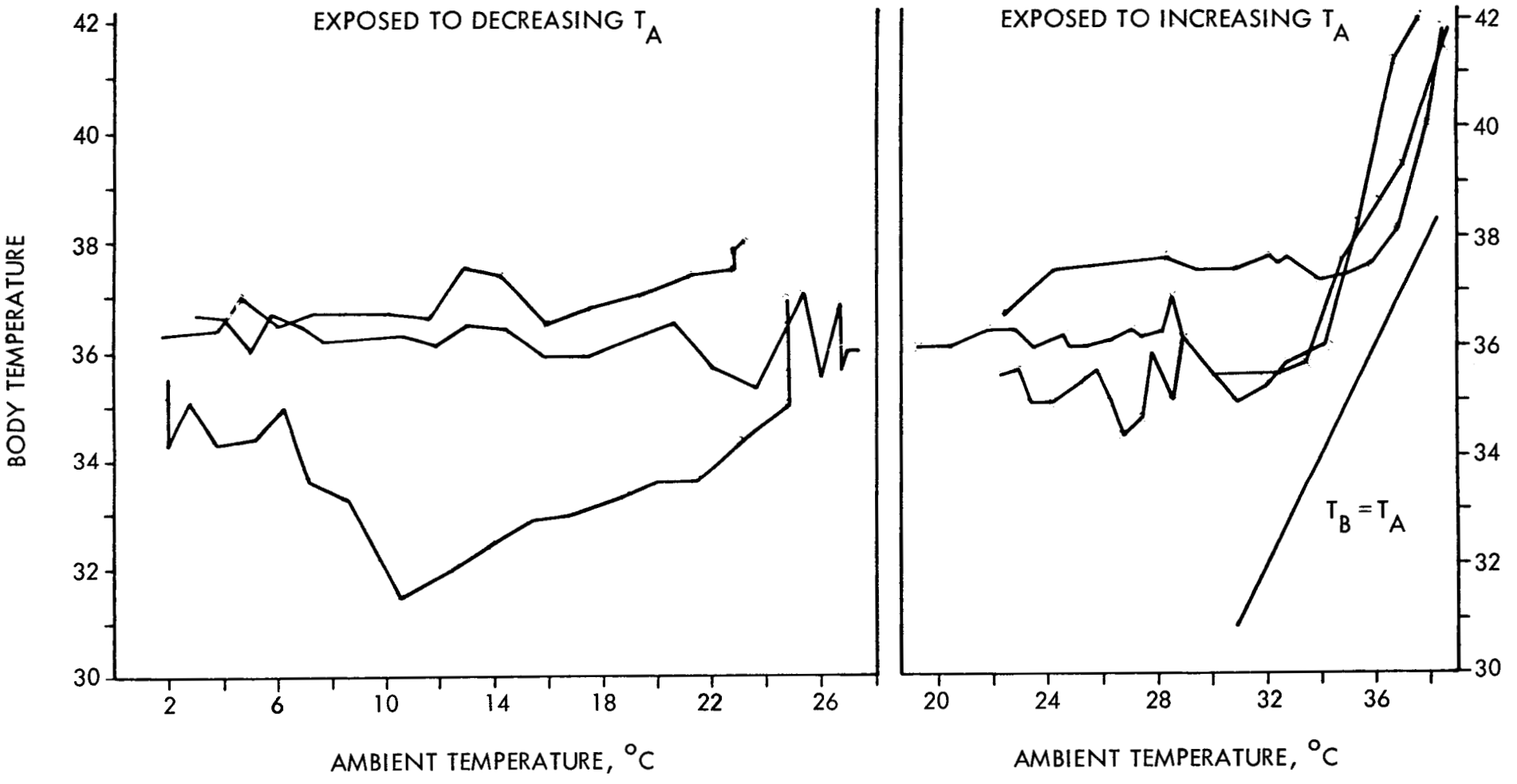

FIGURE 3 COLONIC TEMPERATURES OF SIX P. LONGIMEMBRIS EXPOSED TO CONTINUOUSLY CHANGING AMBIENT TEMPERATURE OVER A PERIOD OF 3.5-6 HR; THREE MICE EXPOSED TO DECREASING T_A AND THREE EXPOSED TO INCREASING T_A. PLOTTED POINTS ARE AVERAGE BODY TEMPERATURES FOR CONSECUTIVE 15 MINUTE PERIODS.

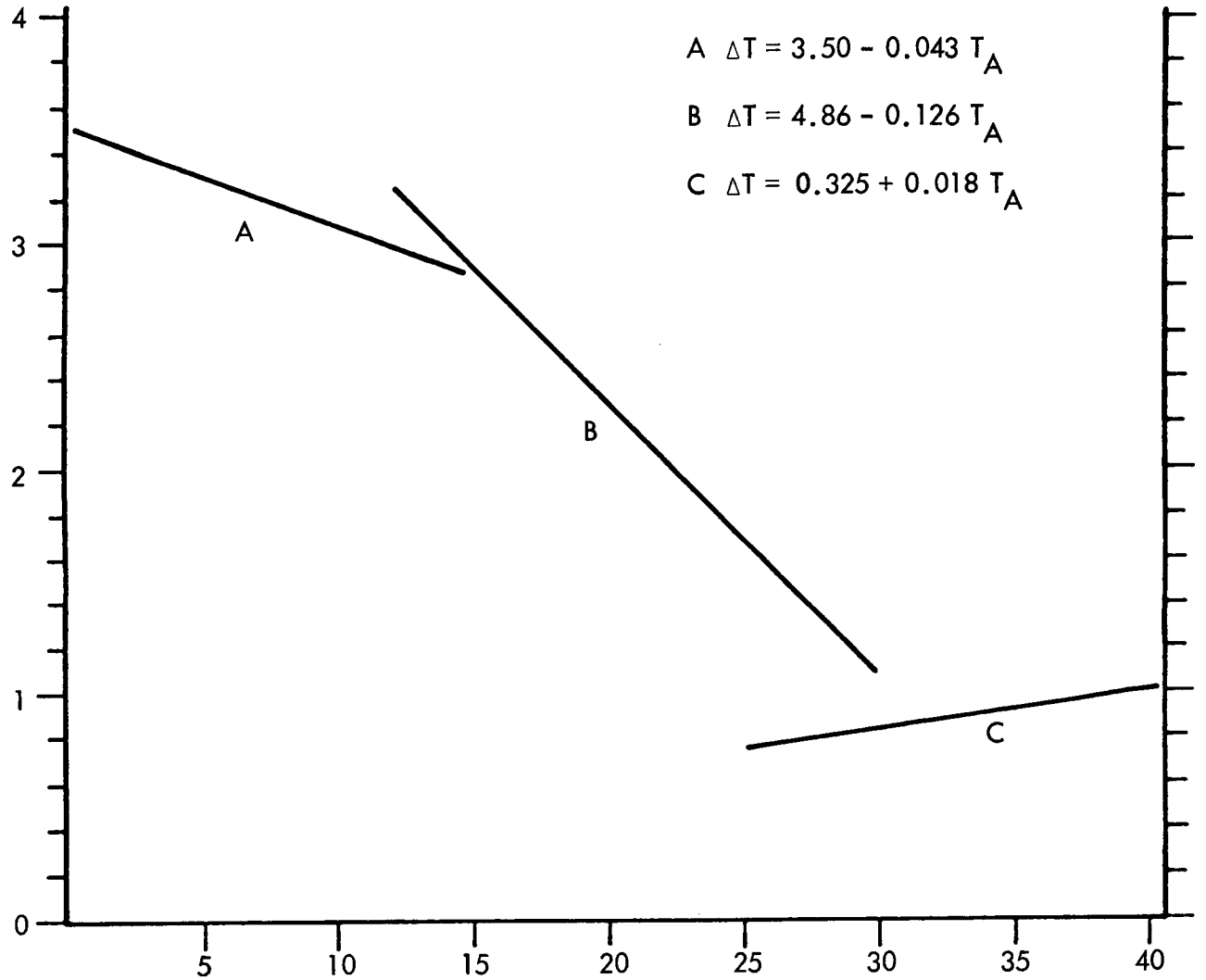

FIGURE 4 RELATIONSHIP OF THE GRADIENT: COLONIC TEMPERATURE (T_C) MINUS SUBCUTANEOUS TEMPERATURE (T_S), TO AMBIENT TEMPERATURE (T_A) FOR THE SIX MICE OF FIG. 3. VALUES FELL INTO THREE GROUPS REPRESENTED BY THE THREE LINEAR REGRESSION LINES.

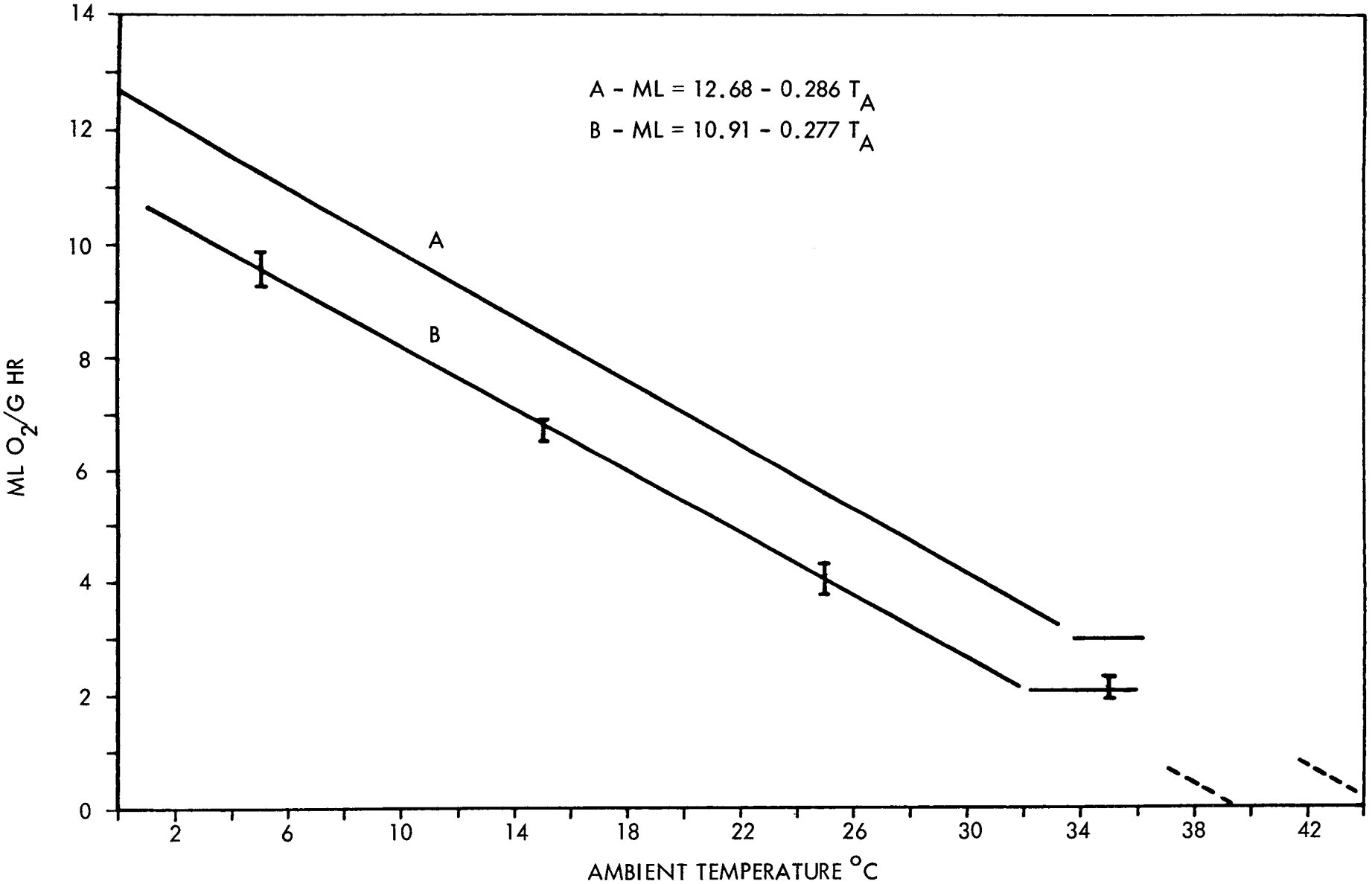

FIGURE 5 OXYGEN CONSUMPTION OF P. LONGIMEMBRIS. (A) AVERAGE MAINTENANCE METABOLISM AND (B) MINIMUM MAINTENANCE METABOLISM OF NINE MICE AS MEASURED IN MANOMETRIC RESPIROMETER; (C) MEAN MINIMUM METABOLISM FOR 5-MIN PERIOD FOR TEN POSTABSORPTIVE MICE, AS MEASURED WITH BECKMAN OXYGEN ANALYZER. CIRCLES ARE MEAN VALUES; VERTICAL LINES FOR (B) ARE ± 2 S.E.

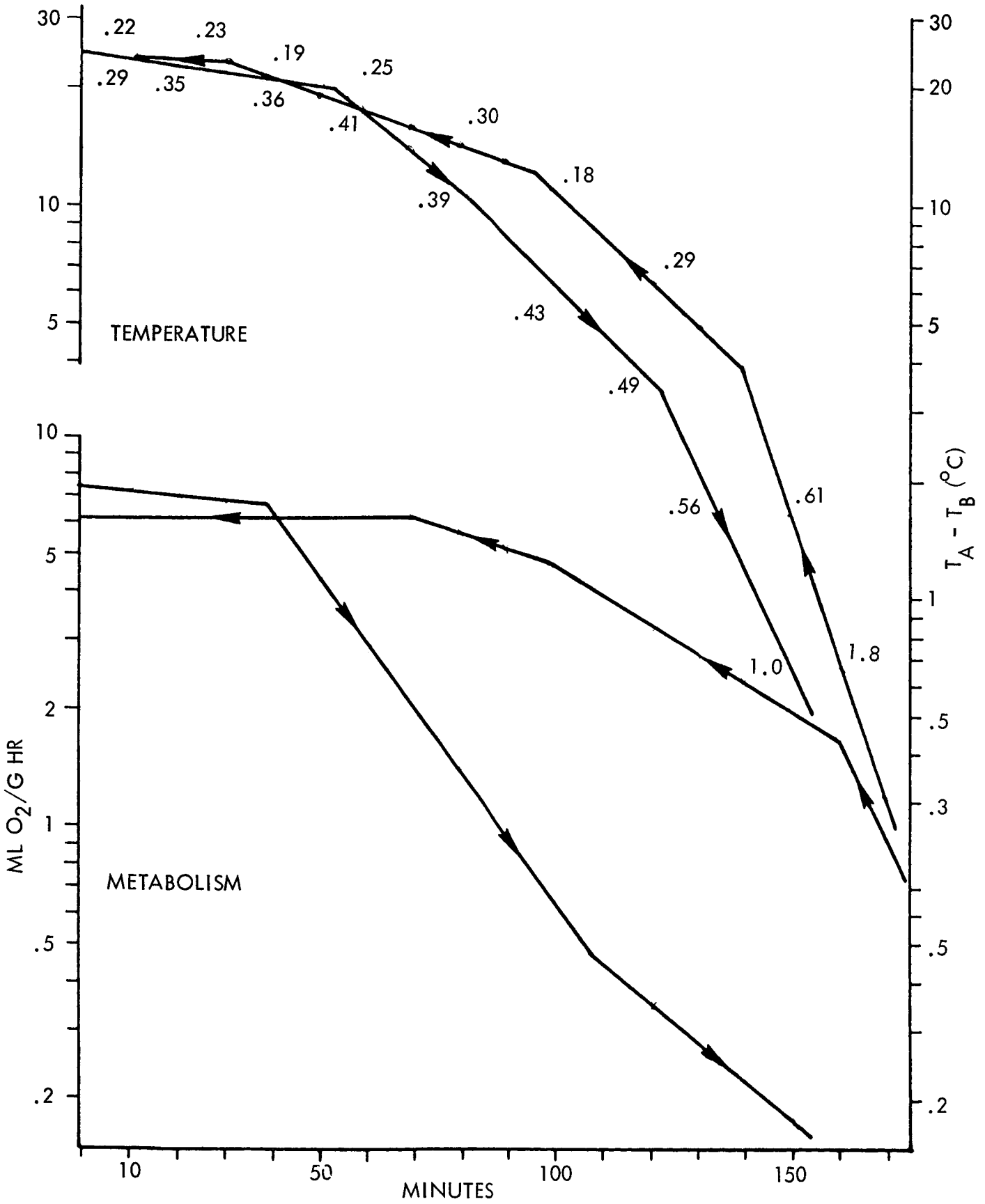

FIGURE 6 BODY TEMPERATURE AND OXYGEN CONSUMPTION OF ONE P. LONGIMEMBRIS DURING ENTRY AND SUBSEQUENT AROUSAL FROM TORPOR WHILE AT T_A 10°C. VALUES ADJACENT TO TEMPERATURE CURVES ARE THERMAL CONDUCTANCES ESTIMATED AT VARIOUS POINTS IN CYCLE (SEE TEXT).

SECTION II

SUMMARY OF POCKET MOUSE

HIBERNATION STUDIES

HIBERNATION CHARACTERISTICS IN PEROGNATHUS LONGIMEMBRIS

Kyllikki Grubel

I. INTRODUCTION

It has been established that Perognathus longimembris has a circadian rhythm of torpidity throughout the year (Bartholomew et al., 1957, Chew et al., 1965). The pocket mice are able to go into torpidity and arouse spontaneously every 24 hours. They also may stay in torpidity for a few days without arousing when kept in a cold environment (+10°C).

The present work was designed to determine whether there is a seasonal factor influencing the torpidity pattern of the pocket mice and whether their 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. Their brown fat performs a thermogenic role during arousal. And in most hibernators, the thyroid glands are involuted during hibernation season. This report summarizes the results of experiments in progress designed to establish whether these systems are present in P. longimembris and whether there are seasonal variations in these parameters.

II. MATERIALS AND METHODS

The animals used for the present work were randomly chosen individuals of the species P. longimembris. The age of the animals varied from 1½ to 3½ years, and both males and females were used. During their captivity the mice had been kept in individual one-gallon jars, which contained about one inch of sand. Ambient temperature was held constant at ca. +22°C, and 12 hours light-dark photoperiod was provided. The mice were fed a mixture of rolled oats, bird seed and sunflower seed ad libitum and an occasional piece of lettuce or apple, but no water. All the mice had been captured near Whitewater, California.

A. Blood flow rate and colonic temperature during arousal

1) Apparatus. A scintillation probe (Nuclear-Chicago DS 5), which was connected to a rate-meter (Nuclear-Chicago 1620 B) and further to a recorder (Nuclear-Chicago R 1000), was placed in a vertical position underneath a working table. On the table above the scintillation probe was a standard size (5 x 10 x 20 cm) lead brick, with a 5 mm diameter hole drilled through its center. The lead brick was topped by a fitted pine-wood board.

2) Method. When an experiment was started, a mouse was removed from its jar, and tied onto the board ventrally in such a position that the left thigh was directly above the 5 mm hole in the lead brick. A thermistor probe was placed in the colon (ca. 2 cm deep) to obtain a continuous record of the body temperature. 2 μc of I^{131} in 0.2 - 0.5 ml of 0.9% NaCl solution was then injected into the musculature of the left thigh above the scintillation probe. The recorder thus provided a continuous record of I^{131} clearance from these peripheral muscles, thereby giving an indication of the blood flow rate at site.

In handling the data obtained from these experiments, time zero was chosen to be the time of iodine injection. To achieve the temperature records (Fig. 1) a reading was taken from the original records at 5 min. intervals. The iodine clearance record in the arousing mice formed a two-part curve. The first part indicated negligible blood flow. The second part showed abrupt increase in blood flow rate. The point of change was taken to indicate the length of time that peripheral vasoconstriction was maintained.

3) Animals. One group of nine mice was kept in normal room temperature and used for controls. Two groups of mice were placed in a constant temperature room with a temperature of +10°C and a 12-hour light-dark cycle. The fall group (October, 6 mice) had food at will. The winter group (February, 9 mice) was first given food, but since after four days of cold exposure only one animal had become torpid, the food and sand were removed from the jars. The animals then became torpid, with the exception of one mouse that died.

B. Amount of brown fat

For this study, each mouse was first weighed and then anesthetized with ether. All brown fat was removed from the mouse and weighed immediately. From these figures, the brown fat percentage of total body weight was calculated.

Two groups of mice were studied: 1) a group of ten pocket mice kept in normal room temperature, with food ad libitum; 2) a group of ten mice kept at +10°C, with food ad libitum for three to six days before sacrifice.

C. Histological appearance of the thyroid glands

For these observations, the same individual mice were used as for Part B, brown fat measurements. Due to the small size of P. longimembris, the removal of the thyroid glands was performed under the microscope. Also, in the subsequent handling of these tissues the small size called for special methods. When the tissues were embedded in paraffin, the following steps were taken: the thyroid glands were embedded in the ordinary manner into colorless paraffin in a mold 3 x 3 x 2 mm. The aluminum foil was then peeled off the paraffin, and the small paraffin cube with the thyroid glands in it was subsequently embedded into colored paraffin in a regular size mold.

The tissues were sectioned 6μ thick and stained with hematoxylin and eosin. The diameter of the follicles and the thickness of the epithelium were measured.

III. RESULTS

A. Colonic temperature and peripheral vasoconstriction during arousal

The results of these experiments are illustrated in Figures 1 and 2. The colonic temperature of the control mice stayed between +32°C and +36°C. No peripheral vasoconstriction was observed in these mice.

The arousal of the torpid mice in October forms a colonic temperature curve characteristic of hibernating animals. There is definite vasoconstriction in the peripheries, the average time being 38.3 min. The fastest arousing mouse released the peripheral blood flow 15 min. after beginning of the experiment. The mouse with the longest arousing time maintained this vasoconstriction for 65 min.

However, in February when the mice had to be starved before they would become torpid, only one out of eight mice aroused. The average time

of vasoconstriction was only 11.2 min. The only mouse that did arouse, rewarmed at about the same rate as the slowest arousing mouse in October. This mouse maintained the peripheral vasoconstriction for 10 min.

B. The amount of brown fat

The body weights, brown fat weights and the percent of brown fats relative to body weights are shown in Figure 3. As is seen in this figure, the differences in the body weights and the absolute brown fat weights are 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 $\pm$ 0.9% and in the group of cold exposed mice 3.3 $\pm$ 0.4% of body weight.

C. The thyroid glands

The histological appearance of the thyroid glands is different in the two groups of mice. In the control group, the follicles are large (diameter 59.5 $\pm$ 11.7μ) and filled with colloid. The epithelial cells are squamous, the average thickness of the epithelium being 4.5 $\pm$ 0.8μ. In general, the thyroids of the control group in the latter part of January may be described as involuted.

On the other hand, the thyroid glands of the cold exposed mice at the same time of the year appeared very active. The center parts of the glands were not organized into follicles. The cells were crowded, and little or no colloid was present. The remainder of the gland consisted of small follicles with the average diameter of 38.9 $\pm$ 6.1 μ. The epithelial cells were cubical or cylindrical in shape, and the thickness of epithelium was greater than in the control group, 9.9 $\pm$ 1.7 μ. The data are illustrated in Figure 4. It shows that the two groups obviously have differences in their thyroid gland histology.

IV. DISCUSSION

The purpose of this study was to determine whether the physiology of torpor in P. longimembris is similar to the physiology of "classical" hibernators. Obviously, the data available at the present stage do not permit any conclusive deductions. But they do suggest (1) that P. longimembris does behave as a "classical" hibernator, and (2) that there may be a seasonal rhythm in the torpidity 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 previous authors (Bullard et al., 1962, Lyman et al., 1963) in the true hibernators. The fact that they seem to be less apt to go into torpidity, are unable or very slow to arouse and maintain their vasoconstriction for a relatively short time when trying to arouse in early February is possibly due to the late season. Bartholomew et al. (1957) state that the potentiality for hibernation (or aestivation) in P. longimembris presumably exists throughout the year, and that there is no difference in arousals during the year. But he did his experiments between June and September and aroused the animals in 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. The possibility of seasonal effects on the torpidity pattern of P. longimembris has been previously suggested by Chew et al. (1963). They found that P. longimembris kept at +10°C for nine months on a constant 12-hr photoperiod showed the greatest incidence of torpidity in the winter. The works of other authors have pointed towards the possibility of peripheral vasoconstriction during arousal in pocket mice. Eisenberg (1963) says that during arousal, coordination is attained first in the forelimbs while control over the hindquarters lags some minutes behind. Bartholomew et al. (1957) found that rectal temperature lagged slightly behind the oral temperature during arousal. Our results seem to confirm these statements.

Several authors have stated that the thyroid glands are involuted during hibernation (e.g., Kayser, 1961; Hoffman, 1964). 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. This might be explained by the late season when the mice would tend to maintain their normal body temperature rather than go into hibernation under exposure to the stress of a cold environment. But if this would be the case, it would mean that there is some difference in the system of hibernation and aestivation. This would be the opposite of what Bartholomew et al. (1957) states. There is disagreement among various authors as to the state of the thyroid glands during hibernation, and Kayser (1961) 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 shown by Smith and Hock (1963) and Kauppinen et al. (1964, abstr.). 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. SUMMARY

1) The arousal from torpidity was studied in P. longimembris in October and early February. During arousal, the colonic temperature and the blood flow rate in a hind limb were observed. In October, the temperature increment formed a curve, which is typical of "classical" hibernating mammals. The blood flow rate in the hind limb indicated peripheral vasoconstriction. In February, seven out of eight mice failed to arouse. Their peripheral vasoconstriction was maintained for a relatively short time, if at all.

2) The amount of brown fat and the histological appearance of the thyroid glands were studied in late January. The amount of brown fat in control animals was 2.0% of body weight. In cold exposed mice, it was as much as 3.3%. The thyroid glands in control mice appeared passive and in cold exposed mice, active.

3) The sparse data do not allow definite conclusions, but the results of these experiments suggest that the physiology of torpor in P. longimembris is similar to that of "classical" hibernators, and that there is a possibility of a seasonal factor affecting their torpidity pattern.

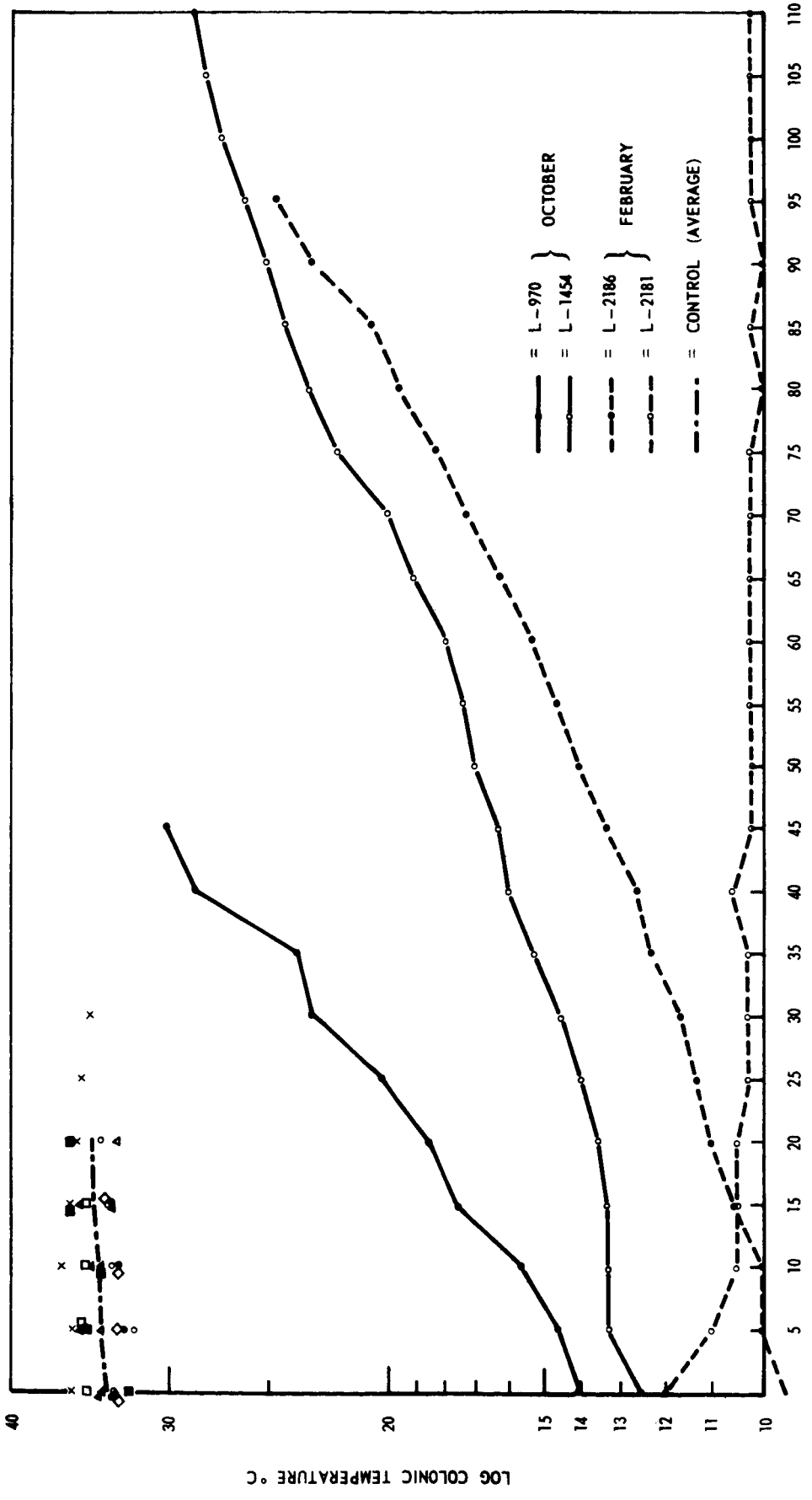

Figure 1. Rate of body warming during arousal in Perognathus longimembris.

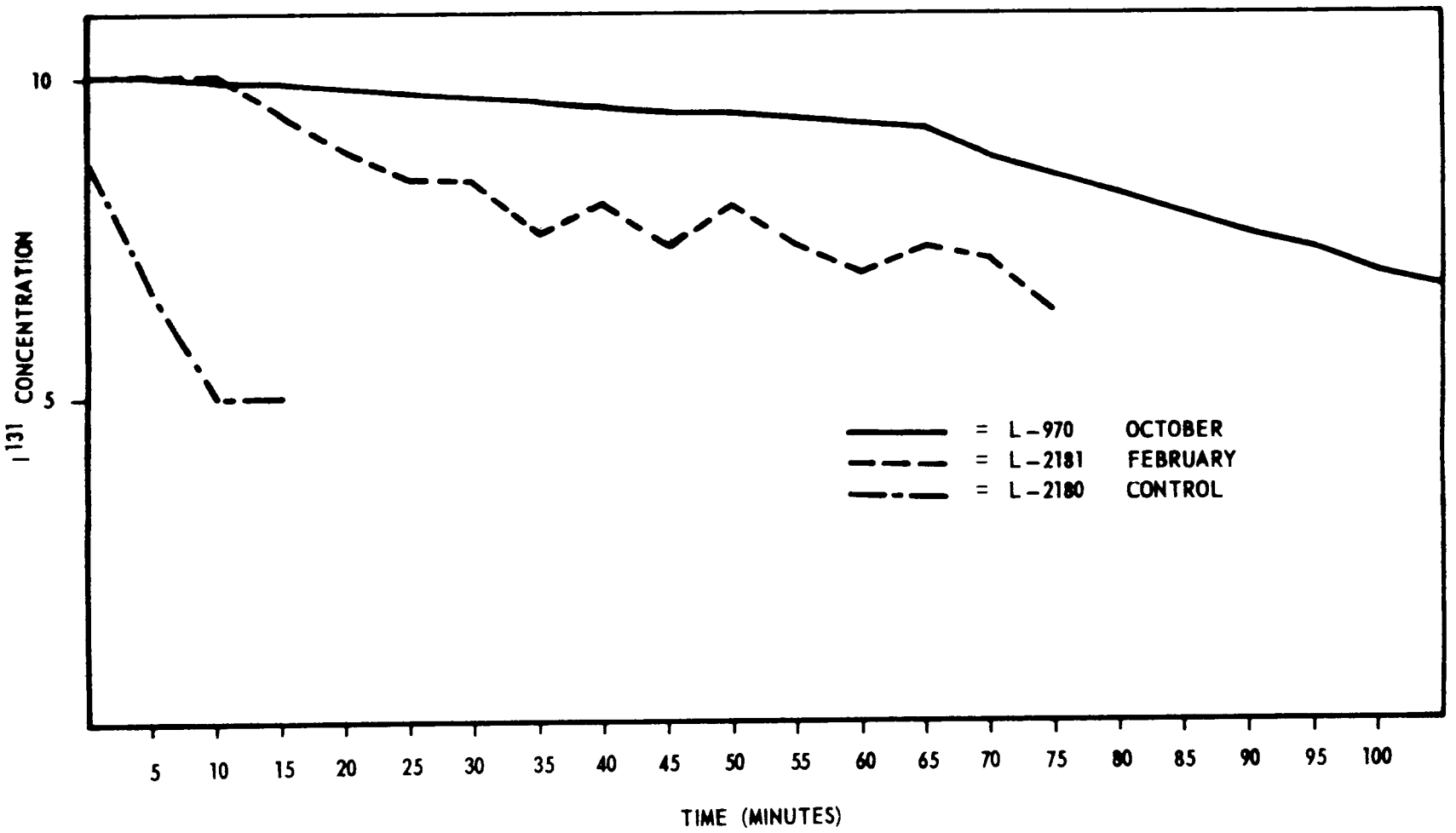

Figure 2. Vasoconstriction in Perognathus longimembris as demonstrated by clearance rate of I^{131} from hind limb muscles.

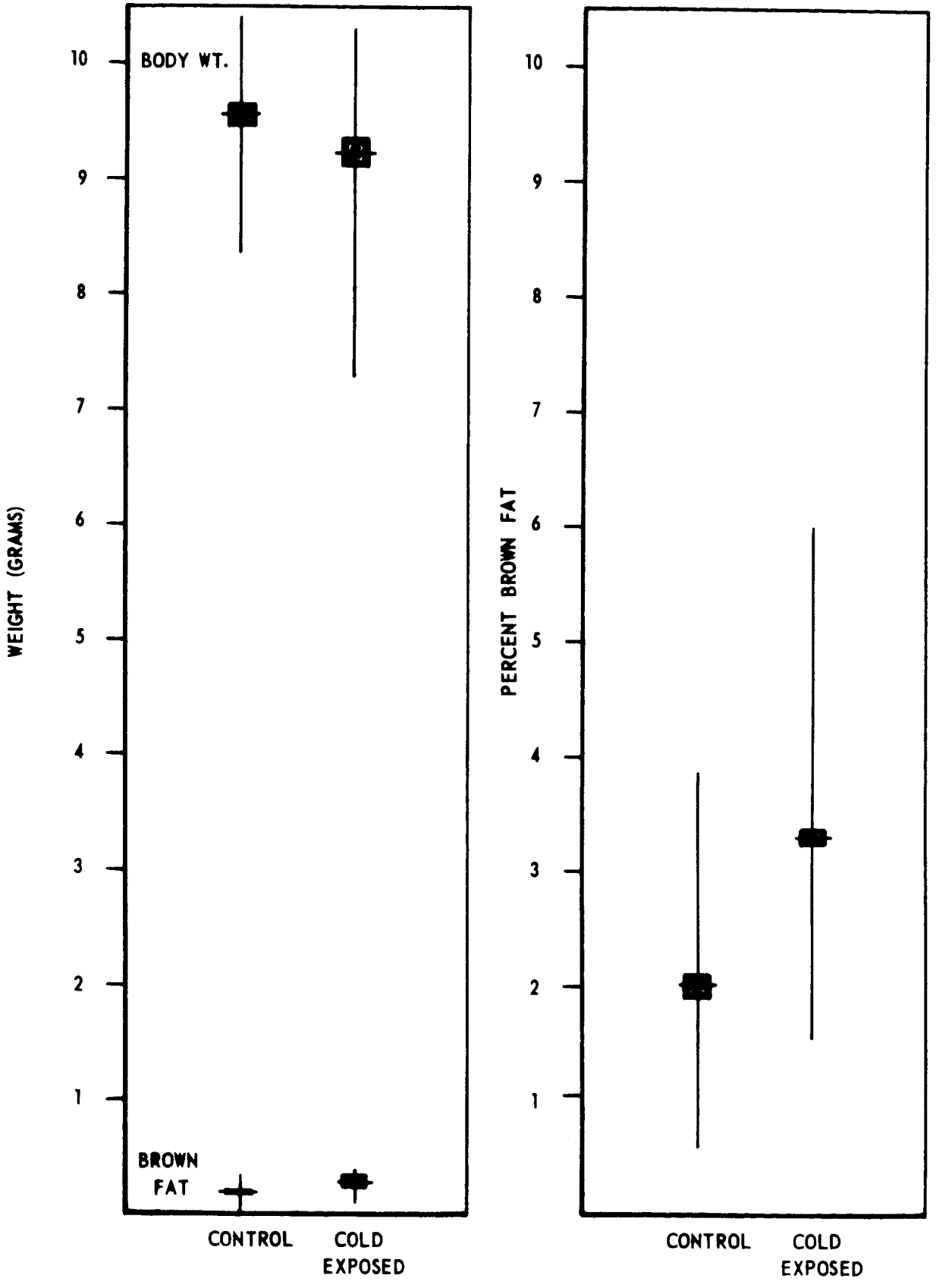

Figure 3. Proportion of brown fat to body weight in Perognathus longimembris (10 mice/group).

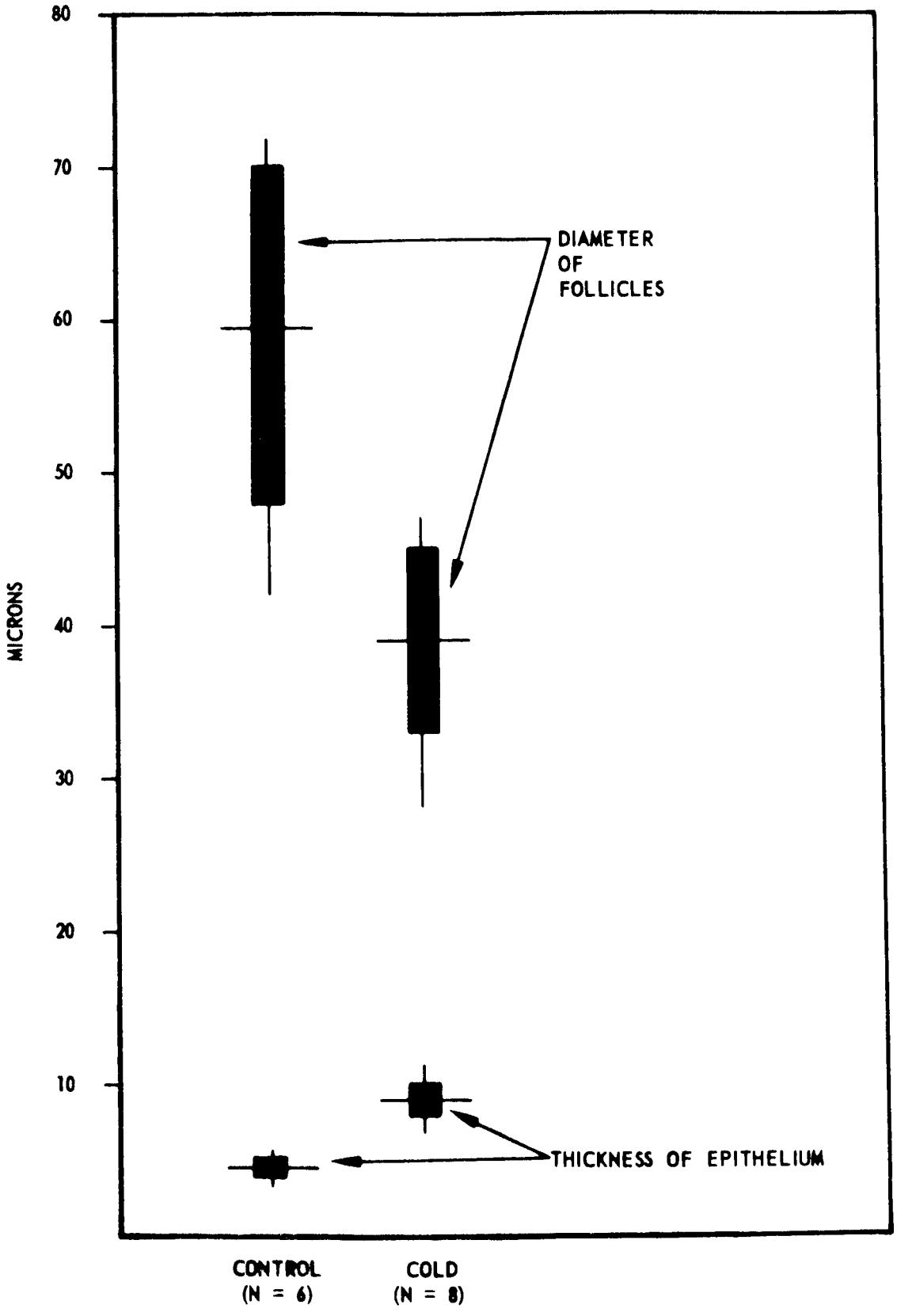

Figure 4. Thyroid gland structure in cold exposed and control groups of Perognathus longimembris.

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PRELIMINARY REPORT OF FREE RUNNING RHYTHMS OF BODY TEMPERATURE IN PEROGNATHUS LONGIMEMBRIS AT 22°C AND 10°C

Page Hayden

I. INTRODUCTION

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 yearly cycle of hibernation vs. normal activity.

The Little Pocket Mouse (Perognathus longimembris) undergoes cyclic periods of depressed metabolism of a circadian nature (Chew, Lindberg and Hayden, 1965) and also an annual cycle of increased expression of this rhythm in any given number of animals (unpublished observation in the laboratory) which corresponds with field observations. 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 more closely resembling those found in nature than previously used. The most pertinent factors are: time of year, surplus of food, constant dark, isolation from noise and low temperature.

II. METHODS AND MATERIALS

The experimental plan was to constantly monitor the body temperature of a group of pocket mice that were to be maintained in dark at three temperature levels (22°C, 10°C and 5°C) for three weeks at each level. Temperature monitoring telemeters were surgically implanted within the peritoneal cavity and were not tied to any organ. 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 12 hour light (0600-1800) and 12 hour dark (1800-0600) photoperiod. Animals used in this experiment were selected because frequent 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 minutes.

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.

III. 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.

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.

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, 22°C and 10°C respectively. The onset and arousal from torpor during a 24-hr period are plotted in Figure 1. Periods of torpor were observed initially during the light portion of the regimen and were generally evident by the fourth or fifth day. After the constant 24D was initiated on the 7th day, all animals exhibited daily periods of

torpor. These torpor periods lasted from four hours to the entire 24-hr period. In some cases, multiple periods of torpor were observed (#5 on day 30, Fig. 1).

The duration of sequential periods of torpor is plotted in Figure 2. 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 (#3, Fig. 2). The maximum time spent in continuous torpor was 4200 minutes (72 hours) at 10°C. One animal exhibited five sequential periods of torpor of over 3700 minutes (61 hours) each. At 22°C, 69% of the torpor periods were from 200 to 800 minutes (3-13 hours), with 30% being from 400 to 600 minutes (6.6-10 hours), and 9% were from 1800 to 2200 minutes (30-36 hours). At 10°C, 24% of torpor periods were from 200 to 800 minutes, 32% were greater than 2200 minutes (= max time in torpor at 22°C) and 11% were from 3600-4200 minutes (60-70 hours).

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 reacted to the decrease in ambient temperature (from 22°C to 10°C) with a <u>decrease</u> in duration of torpor (compared to the duration of torpor at 22°C). This decrease in duration of torpor is evident for 3-5 torpor periods after the temperature change. Two animals (Fig. 2, #3 and #7) were unusual in that they underwent two lengths of torpor periods, approximately 600 minutes and 1600-2000 minutes, during the 22°C ambient temperature. The long periods were generally separated by one or two short periods. One of these animals (#3) was unique in the relatively long torpor period that was maintained during both the 22°C and 10°C temperature regimens. This long period, however, was evident less frequently at 22°C than at 10°C. At the latter temperature, it was the daily mode. Another animal (#6, Fig. 2) maintained a relatively constant short duration of torpor in both 22°C and 10°C. In general, as was expected, the duration of torpor was prolonged in 10°C as compared to 22°C.

The times of entry and arousal from torpor are distinctive points in the life processes of this animal and can be used as phase markers of the metabolic circadian rhythm. In the 22°C temperature regimen, entry and arousal from torpor had a strong, well-defined rhythm (e.g., Fig. 3).

Several days after the termination of the 12L-12D light regimen and commencement of constant dark, all entered into free-running metabolic rhythms. These free-running periods varied from 22 hrs to 23 hrs and 41 minutes. The arousal from torpor seemed to be a more predictable and consistent marker than entry at ambient temperature of 22°C. The temperature drop to 10°C on day 29 apparently caused a severe disturbance in the pattern of a stable periodicity. One animal (Fig. 4) regained a well-defined stable pattern after showing a disturbance for about seven to eight days. Several animals changed from a less than 24-hr rhythm to a greater than 24-hr rhythm. With one animal, it was not manifested until five days after the temperature change and was a transient phenomenon lasting about seven days.

The arousal of an animal at a time in 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 (Fig. 1, #4, 10°C, body temperature = ∽11°C).

Table I is a summary of free-running periods calculated from both entry and arousal from torpor and subjective estimates of the accuracy of the rhythm by the two phase markers. Transients of rhythms induced by the temperature decrease are evidenced by polyphasic nature of free-running period and by gross changes in length of period. In all but one individual there was a degradation of accuracy of the rhythm.

IV. DISCUSSION

This study again emphasizes the metabolic lability of P. longimembris (Chew, Lindberg and Hayden, 1965). 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 (Chew and Butterworth, 1964).

	22°C		10°C	
	Entry	Arousal	Entry	Arousal
#3	-good- τ = 22 hr 27 min	-excellent- τ = 22 hr 27 min	-poor- very erratic	-fair- (2 phases) τ = 22 hr 27 min τ = 24 hr 8 min
#4	-good- 1st 12 days τ = 24 hr --- after 12 days τ = 22 hr 52 min	-excellent- 1st 9 days τ = 24 hr --- after 9 days τ = 23 hr 30 min	-excellent- τ = 22 hr 34 min	-excellent- τ = 22 hr 10 min
#5	-good- τ = 22 hr 40 min	-good- τ = 23 hr	-fair- τ = 24 hr 19 min	-fair- τ = 24 hr 19 min
#6	-fair- τ = 23 hr 16 min	-good- τ = 23 hr 41 min	-good- (2 phases) τ = 22 hr 41 min τ = 23 hr 24 min	-poor- 1st half --- -good- 2nd half τ = 23 hr 35 min
#7	-good- 1st 9 days τ = 24 hr --- after 9 days erratic τ = ~ 22 hr	-good- 1st 9 days τ = 24 hr --- after 9 days erratic τ = ~ 22 hr	-good- (3 phases) τ = 23 hr 30 min τ = 26 hr 24 min τ = 23 hr 42 min	-good- more erratic but same form

Table 1. Summary of P. longimembris free-running period at two different ambient temperatures. Period length and subjective estimate of accuracy based on entry and arousal from torpor.

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 hrs 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 strong 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 is impossible 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 known 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 (Strumwasser, 1960).

The pocket mice used in this experiment probably had undergone test drops necessary to go torpid in an ambient temperature of 22°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 22°C to 10°C generally did not immediately increase the duration of torpor, but <u>decreased</u> the time in torpor for several days. The first torpid period of one animal (Fig. 2, #5) 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 (Twente and Twente, 1965) 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 (Pengelley and Fisher, 1961) 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 22°C to 10°C, and yet in other animals the duration increased only 2 times. One animal (Fig. 2, #3) had a duration of torpor in 22°C that was more typical of that shown in 10°C. 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 #4, Fig. 1, day 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 (Chew, Lindberg and Hayden, 1965), and the present data indicate that the rhythm functions during extended periods of torpor with body temperatures of 11°C.

V. SUMMARY

1. Perognathus longimembris undergo daily periods of torpor even when not stressed, i.e., food plentiful and temperatures relatively high.

2. A continuous torpor of about 3 days was observed at 10°C and may represent a maximum for this species at this temperature.

3. A period of increasing duration of torpor was observed at the beginning of the experiment and may represent temporal "test-drops".

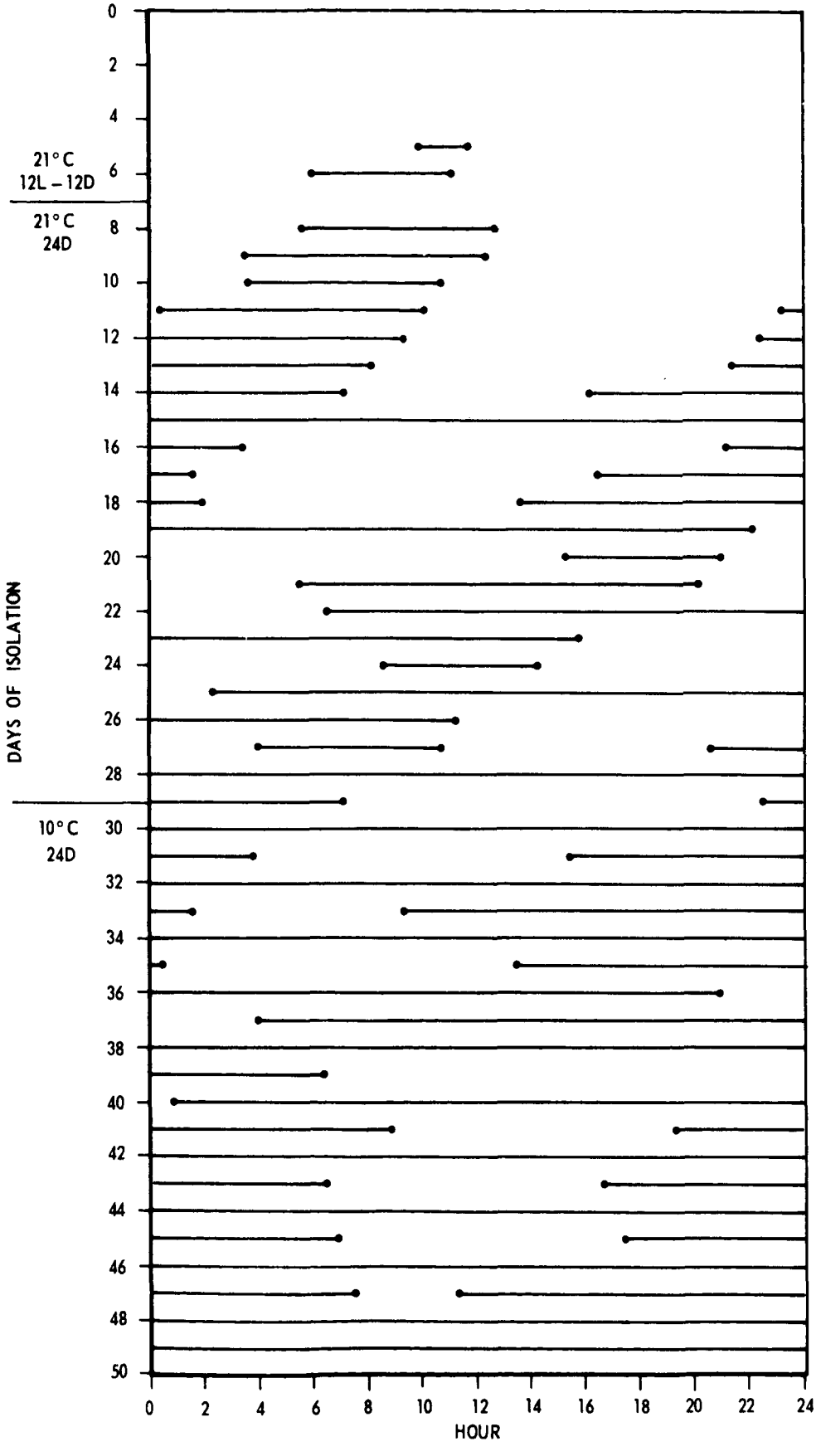
4. An ambient temperature decrease of 12°C initially decreased the duration of torpor.

5. At an ambient temperature of 10°C, the duration of torpor was from 2 to 6 times longer than at 22°C.

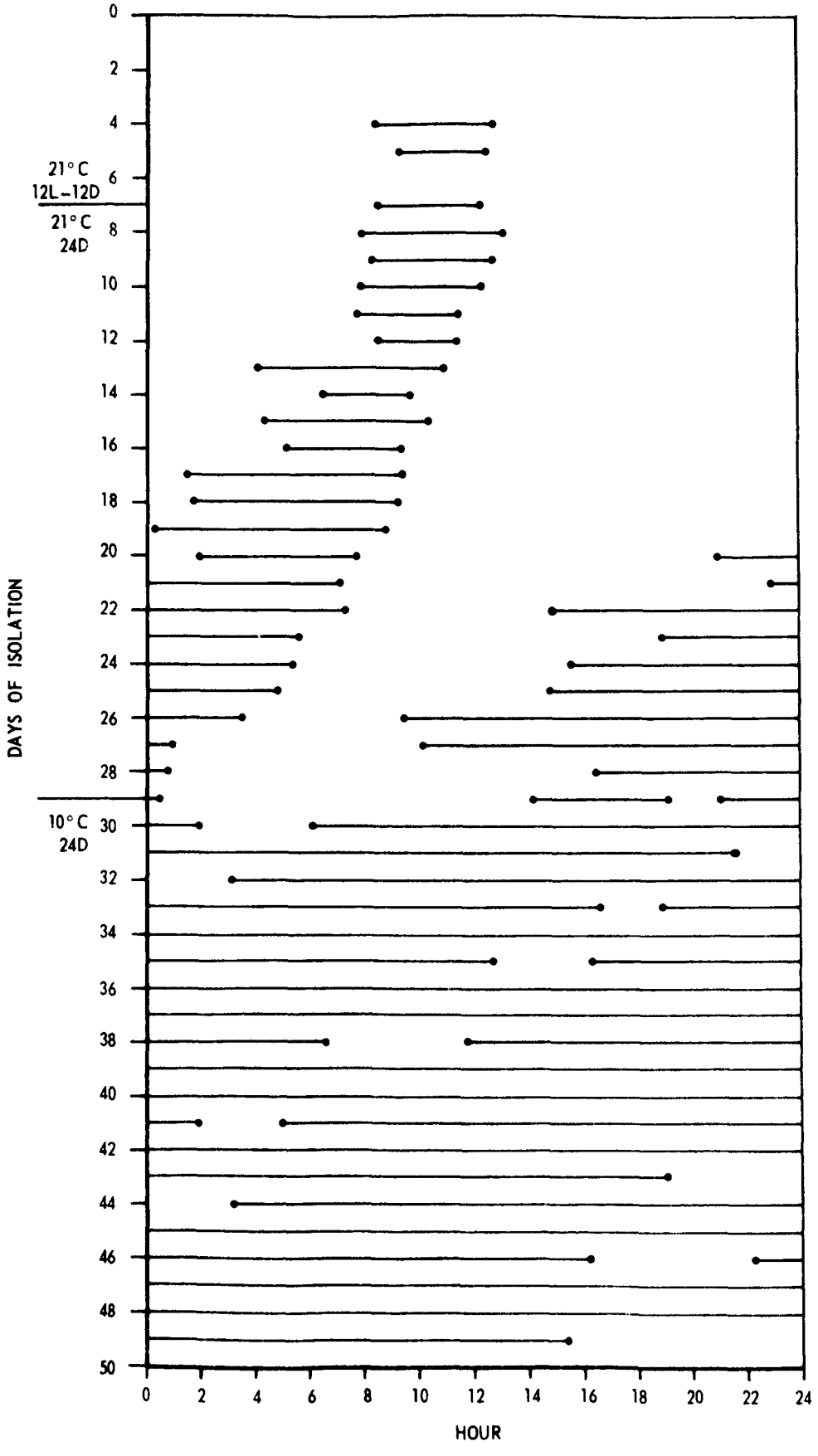
6. A strong circadian rhythm is involved in the time of arousal from a deep torpor (hibernation).

7. The temperature change resulted in a disturbance of the circadian rhythm and was, in general, less precise at the lower temperature.

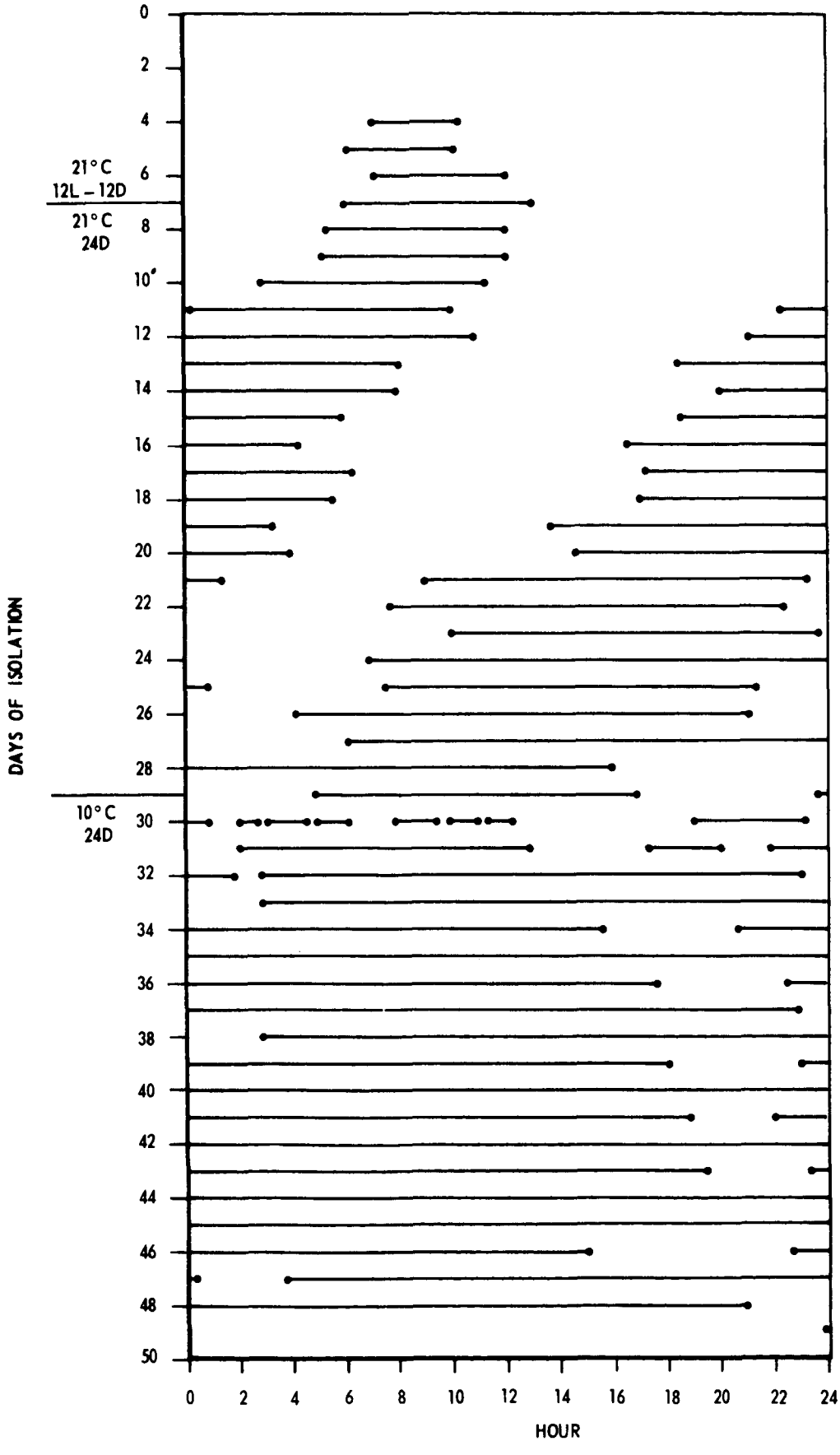
Figure 1 - Torpor periods of Perognathus longimembris maintained with excess food, constant dark, in isolation, during two temperature regimens. The dark bar represents the period of the day in which body temperature was near ambient temperature, and the absence of the bar represents normal body temperature.



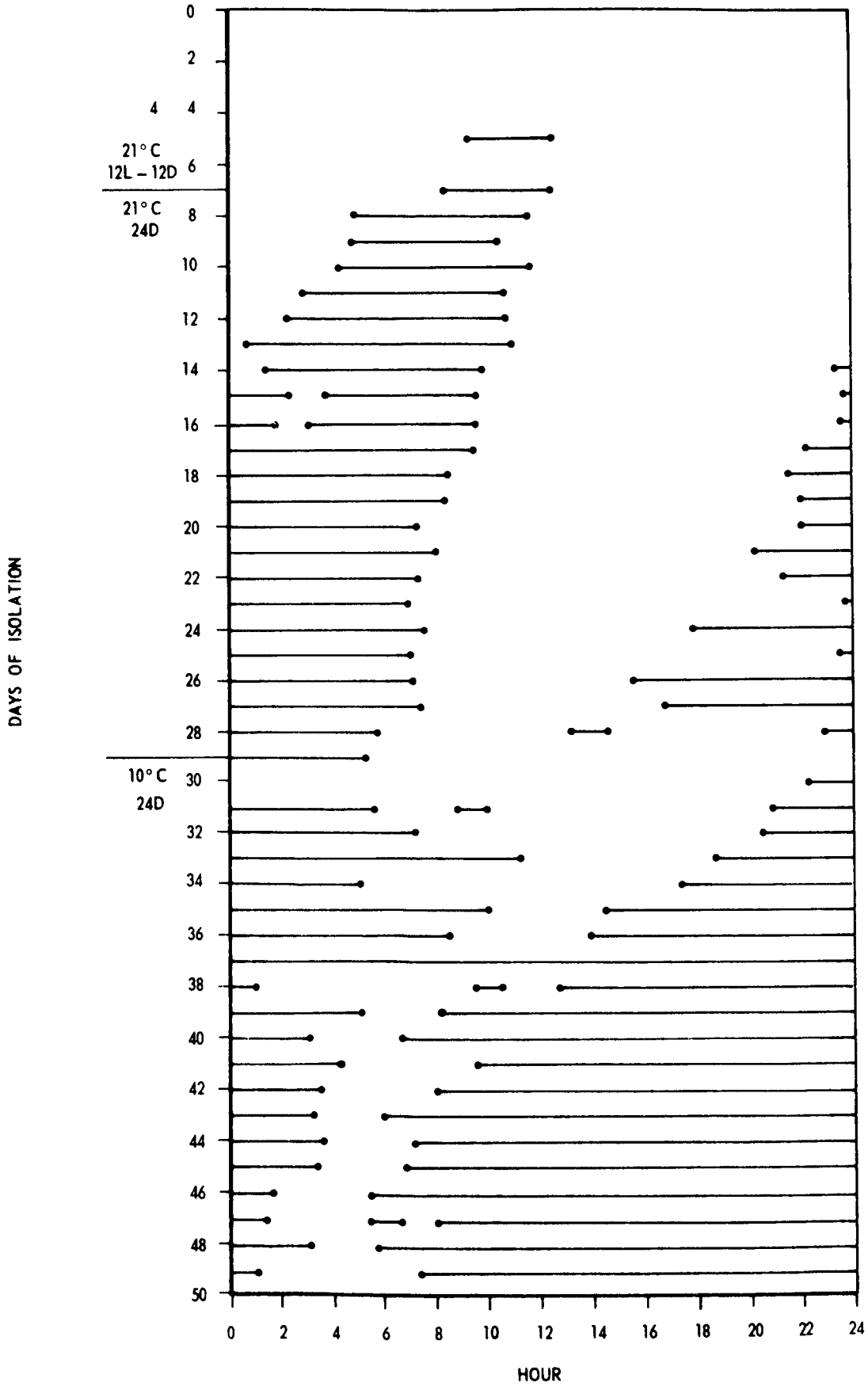
P. longimembris #3 (L-1520 ♀)



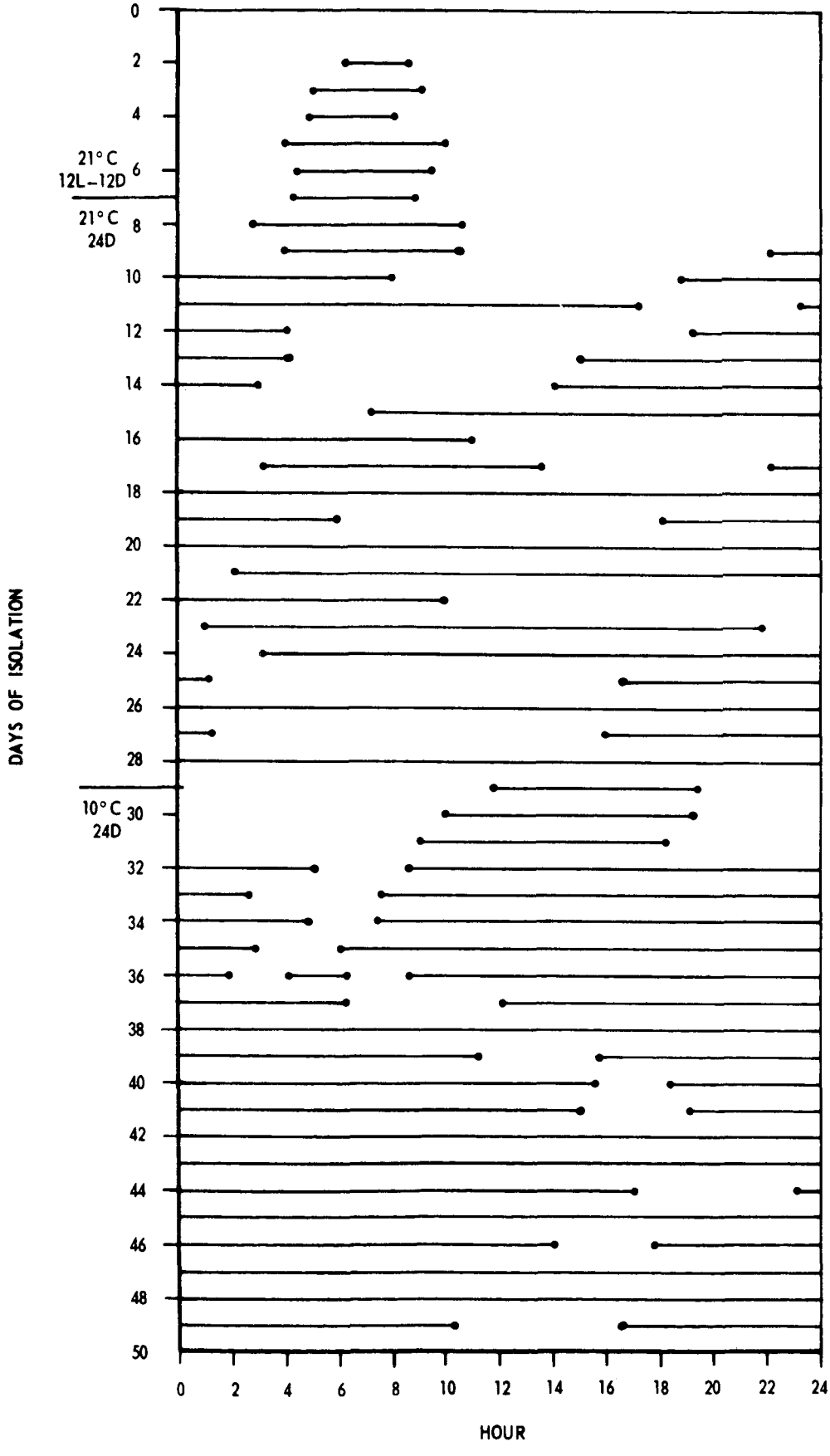
P. longimembris #4 (L-1532 ♂)



P. longimembris #5 (L-1579 ♀)

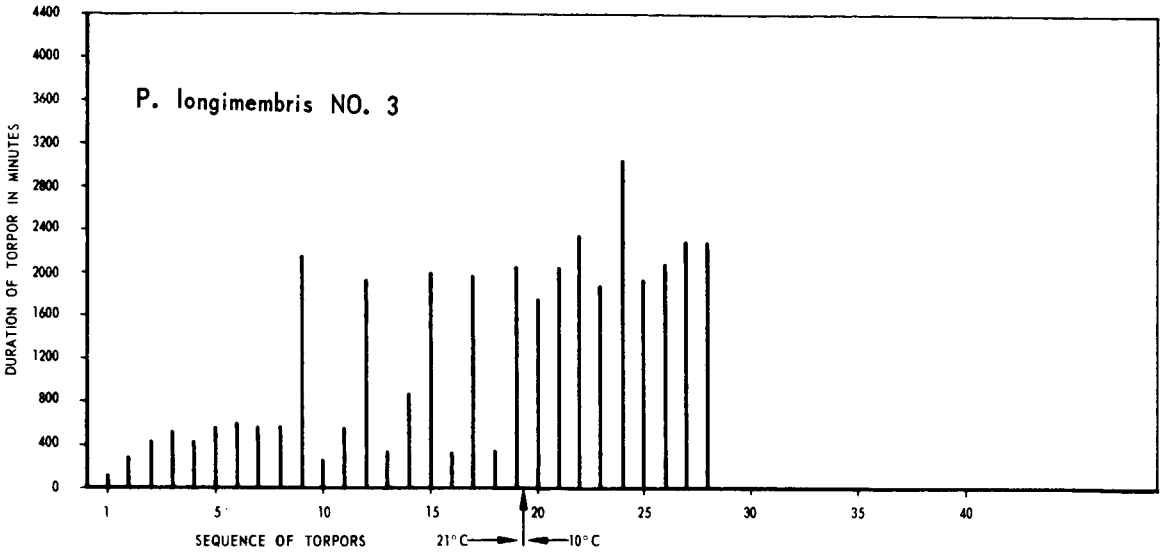


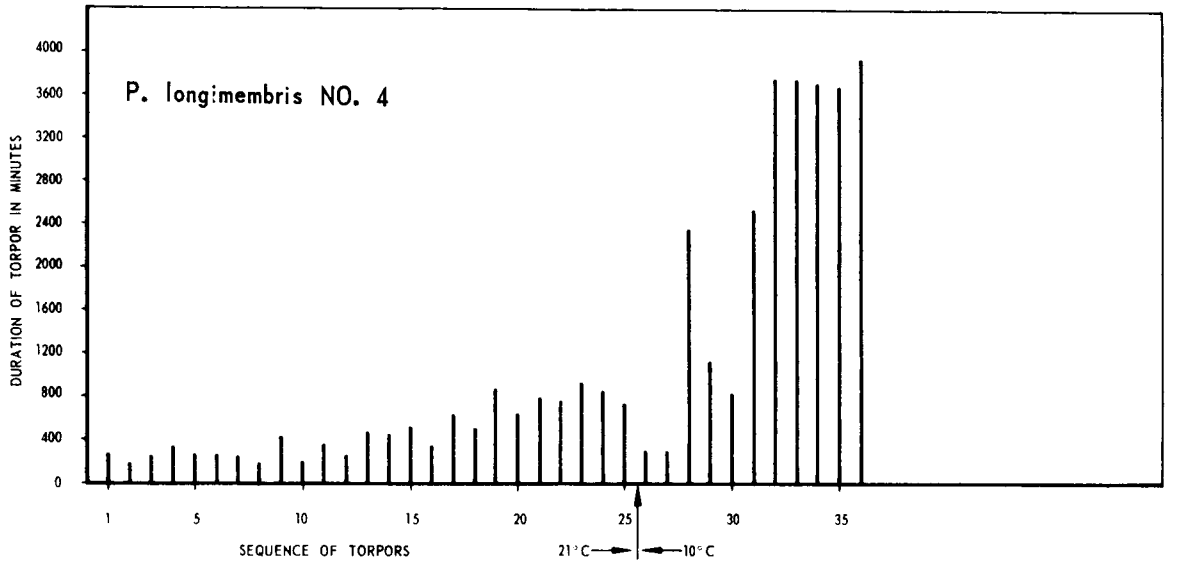
P. longimembris #6 (L-1822 ♀)

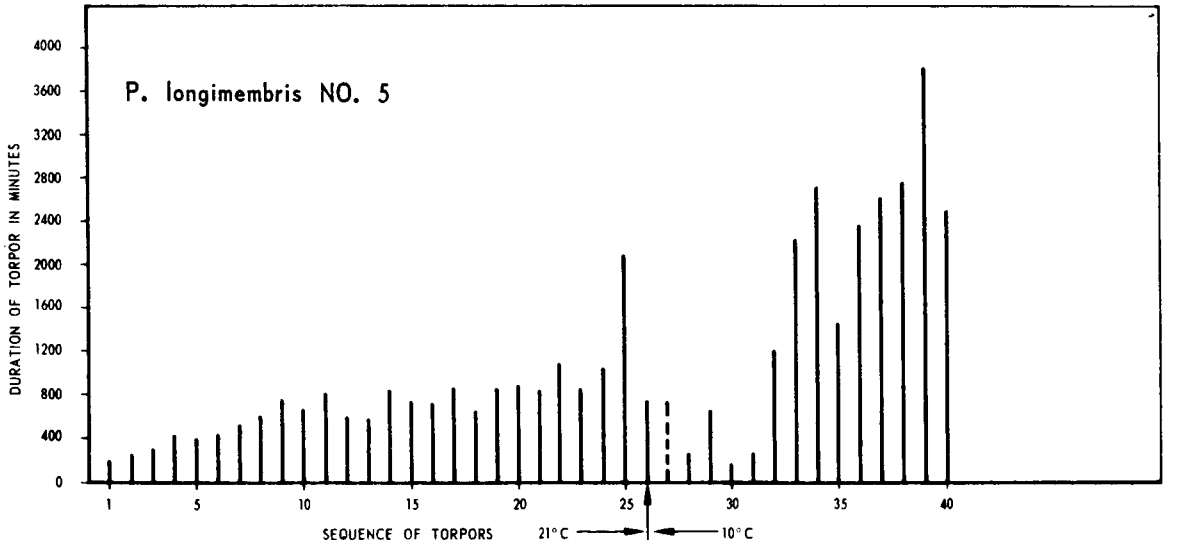


P. longimembris #7 (L-1425 ♀)

Figure 2 - Duration of individual torpor periods of P. longimembris


P. longimembris NO. 3
DURATION OF TORPOR IN MINUTES
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
40
SEQUENCE OF TORPORS
21°C
10°C


P. longimembris NO. 4
DURATION OF TORPOR IN MINUTES
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
SEQUENCE OF TORPORS
21°C
10°C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DURATION OF TORPOR IN MINUTES
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3600
3200
2800
2400
2000
1600
1200
800
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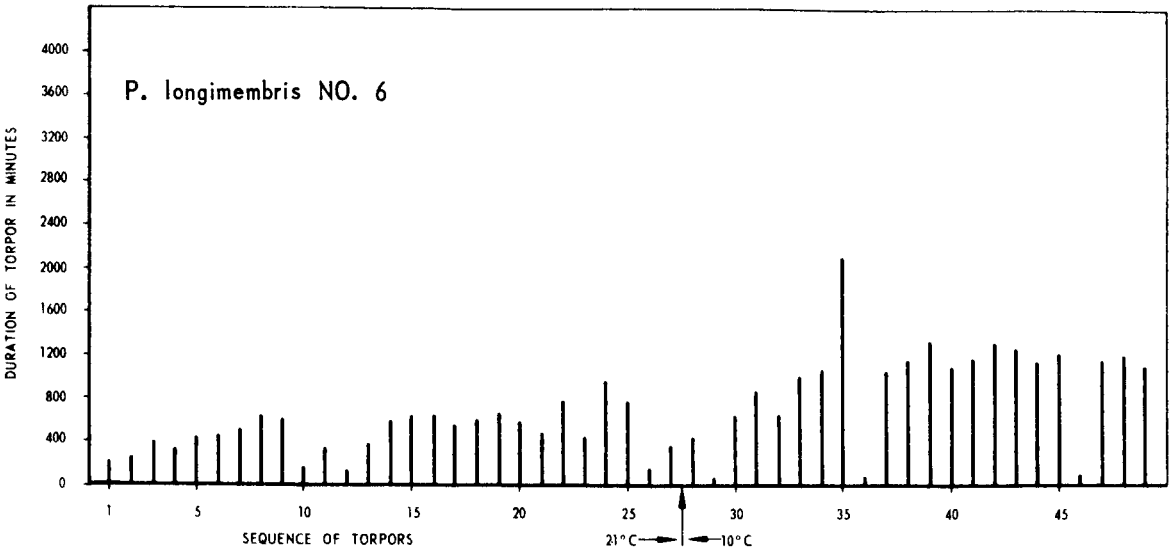
40
SEQUENCE OF TORPORS
21°C
10°C


P. longimembris NO. 6
DURATION OF TORPOR IN MINUTES
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
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10
15
20
25
30
35
40
45
SEQUENCE OF TORPORS
21°C
10°C

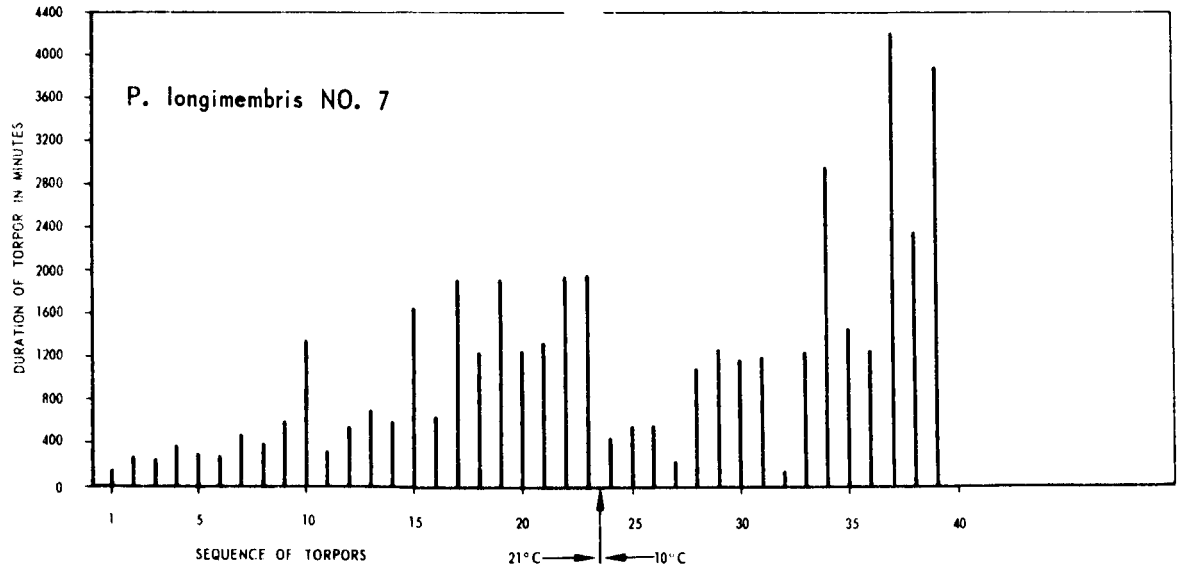

P. longimembris NO. 7
DURATION OF TORPOR IN MINUTES
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
40
SEQUENCE OF TORPORS
21°C
10°C

Figure 3 - The times of entry and arousal from torpor of P. longimembris #4 during two temperature regimens. Note decrease of period length in 10°C ambient and maintenance of accurate rhythm.

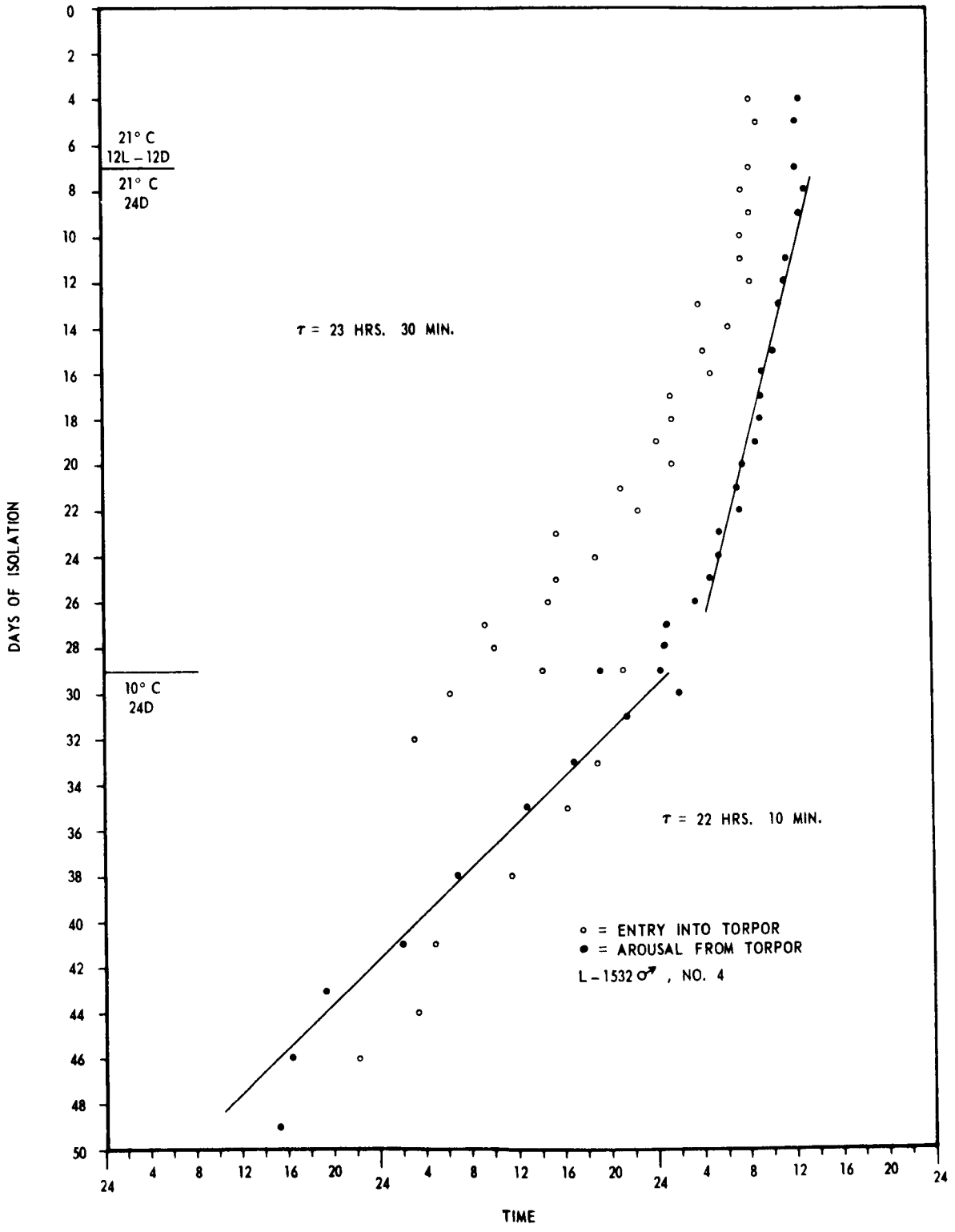
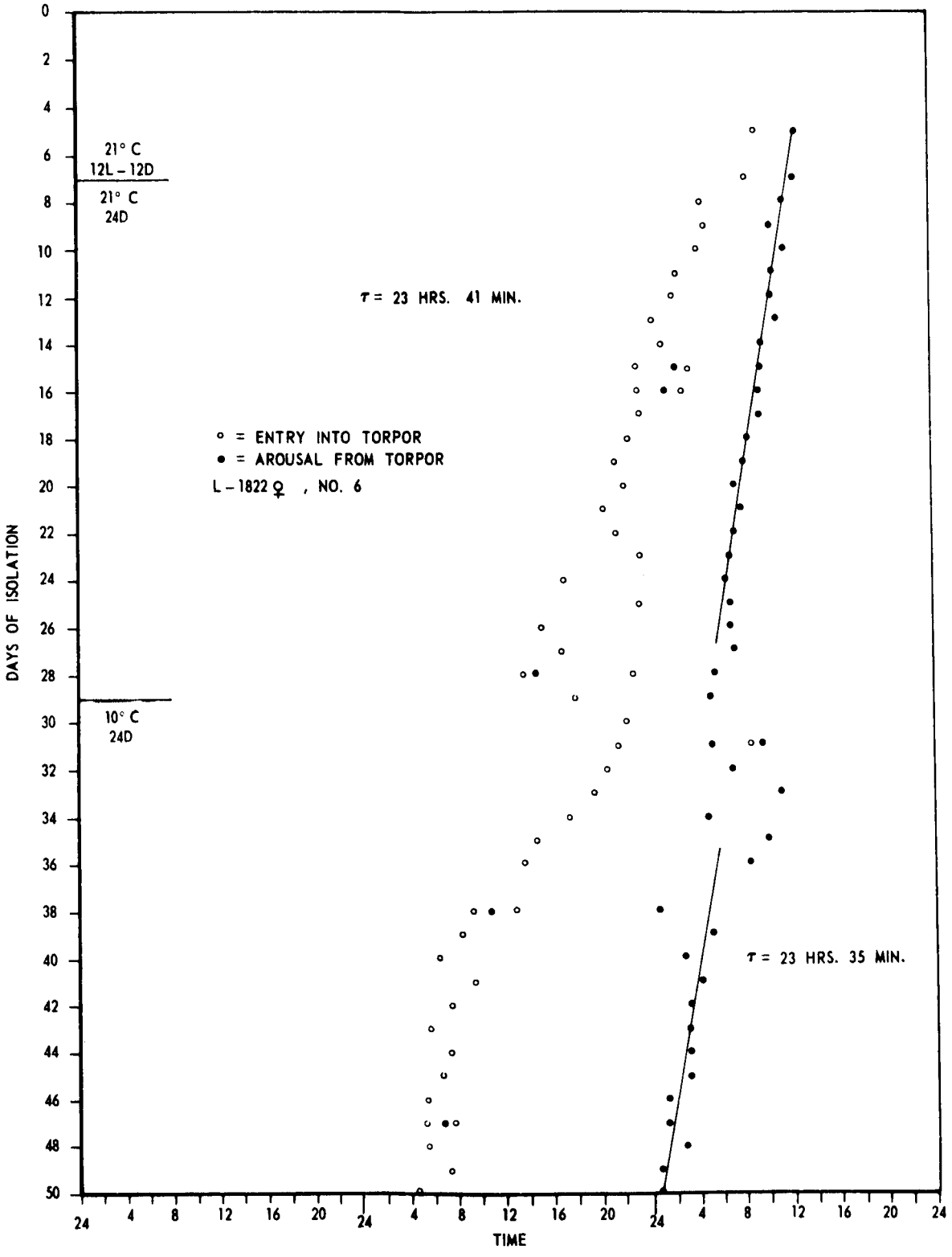

21° C
12L – 12D
21° C
24D
τ = 23 HRS. 30 MIN.
10° C
24D
τ = 22 HRS. 10 MIN.
o = ENTRY INTO TORPOR
● = AROUSAL FROM TORPOR
L – 1532 ♂ , NO. 4
DAYS OF ISOLATION
TIME
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
24
4
8
12
16
20
24
4
8
12
16
20
24
4
8
12
16
20
24

Figure 4 - The times of entry and arousal from torpor of P. longimembris #6 during two temperature regimens. Note disturbance of rhythm at temperature change and re-establishment after about 10 days.


21° C
12L – 12D
21° C
24D
10° C
24D
τ = 23 HRS. 41 MIN.
τ = 23 HRS. 35 MIN.
° = ENTRY INTO TORPOR
• = AROUSAL FROM TORPOR
L – 1822 ♀ , NO. 6
DAYS OF ISOLATION
TIME

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ANATOMY OF THE BRAIN OF PEROGNATHUS LONGIMEMBRIS

Kyllikki Grubel

I. INTRODUCTION

When an animal enters hibernation, certain changes take place in its body functions. The body temperature decreases from normothermic levels, ~ 37°C, down to 0-2°C above the ambient temperature. Oxygen consumption and basal metabolic rate decline. Heat production disappears. 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 (Hoffman, 1964). 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 (Hoffman, 1964). 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 (Bullard and Funkhouser, 1962). The thermogenic brown fat tissue rewarms the blood circulating through it (Smith and Roberts, 1964), and thus aids in the rapid rewarming of heart, brain and respiratory muscles. The important role of brown fat in arousal of hibernators was shown by Smith and Hock in marmots (1963) and Smalley and Dryer in bats (1963). It appears, though, that the activation of brown fat tissue is under nervous control (Kauppinen, Bullard and Smith, 1964), 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.

II. MATERIALS AND METHODS

Four adult mice of species Perognathus longimembris were lightly anesthetized with ether and then killed by decapitation. The brains of the animals were extirpated, cut crosswise in two parts and placed in toluidine blue fixative and stain (Davenport, 1960). After about one week's fixation period, the brains were frozen and sectioned in 50 μ thick slices. From the four sectioned brains, the best slides were chosen to compose one representative brain of P. longimembris. From this representative brain, photographs were taken from sections at 1 mm intervals. The location of each section in question is marked as a vertical line in the drawing of a medial sagittal section of a brain (fig. 1). The scale of Figure 1 is 15:1, and the scale of the cross section photographs and line drawings (figs. 2-8) is 28:1.

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 used is that found in König and Klippel (1963).

III. RESULTS

The results are presented in the following seven photographs and corresponding line drawings of the cross sections of the brain of P. longimembris (figs. 2-8).

LEGENDS TO THE FIGURES

Figure 1. Drawing of a medial sagittal section of the brain of P. longimembris. Scale 15:1. Vertical lines 1 - 7 mark the corresponding cross section 1 - 7, (figs. 2 - 8).

Figures 2a - 8a. Photographs of cross sections of the brain of P. longimembris. Scale 28:1.

Figures 2b - 8b. Line drawings of cross sections of the brain of P. longimembris. Scale 28:1.

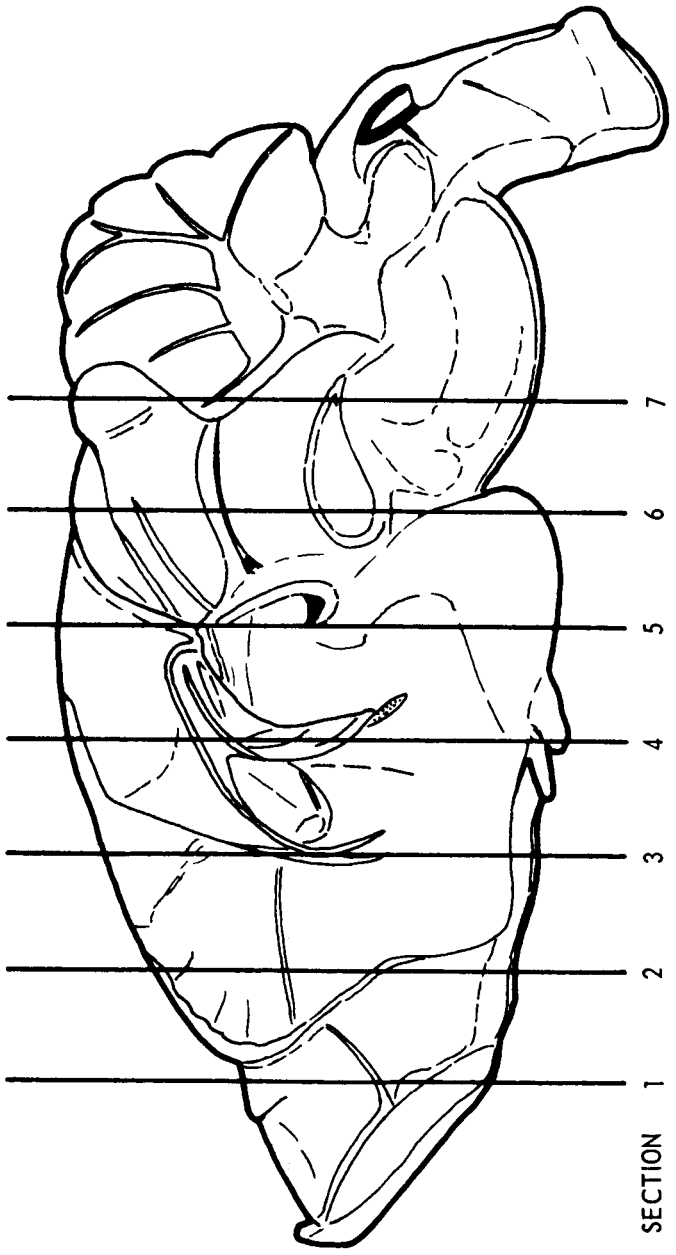


FIGURE 1

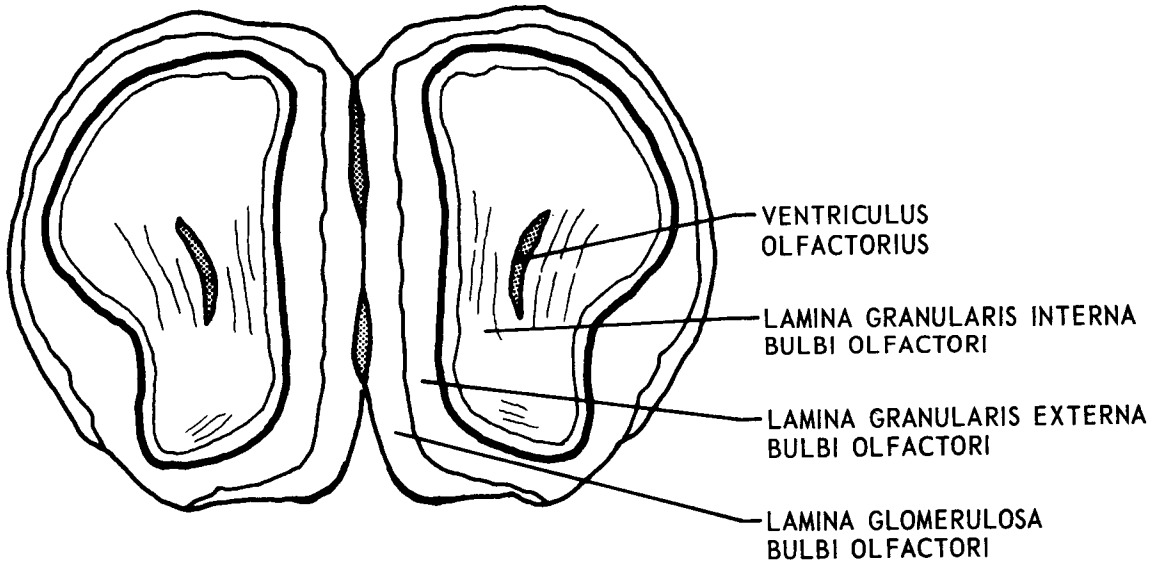
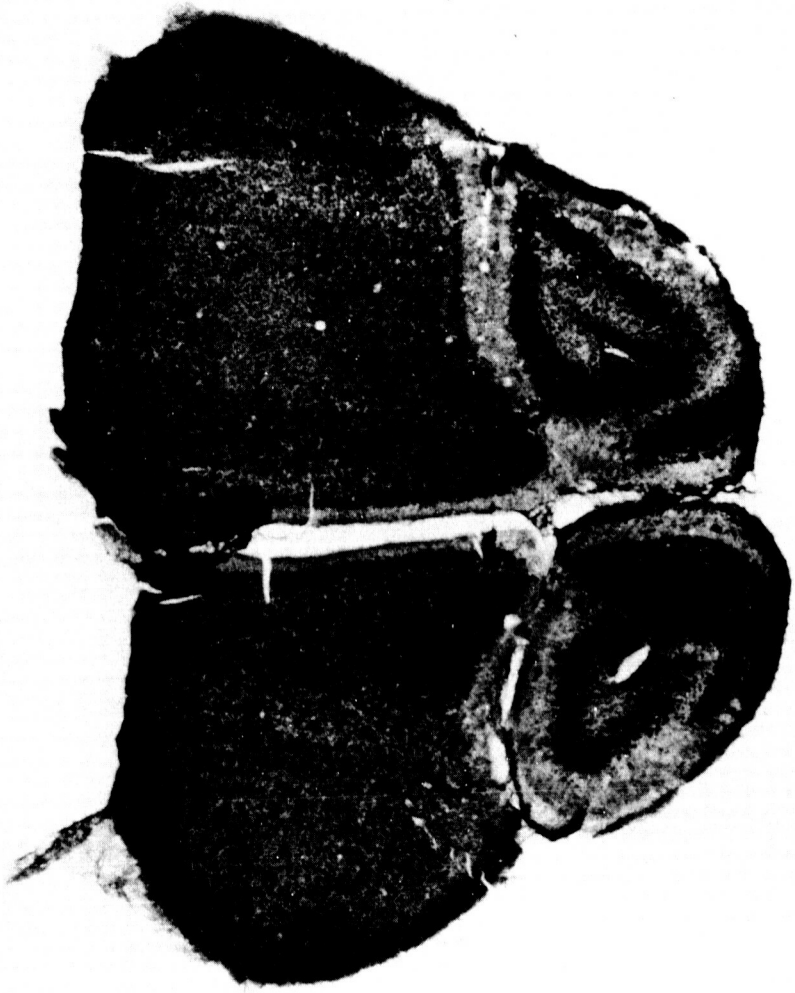

VENTRICULUS
OLFACTORIUS
LAMINA GRANULARIS INTERNA
BULBI OLFACTORI
LAMINA GRANULARIS EXTERNA
BULBI OLFACTORI
LAMINA GLOMERULOSA
BULBI OLFACTORI

FIGURE 2



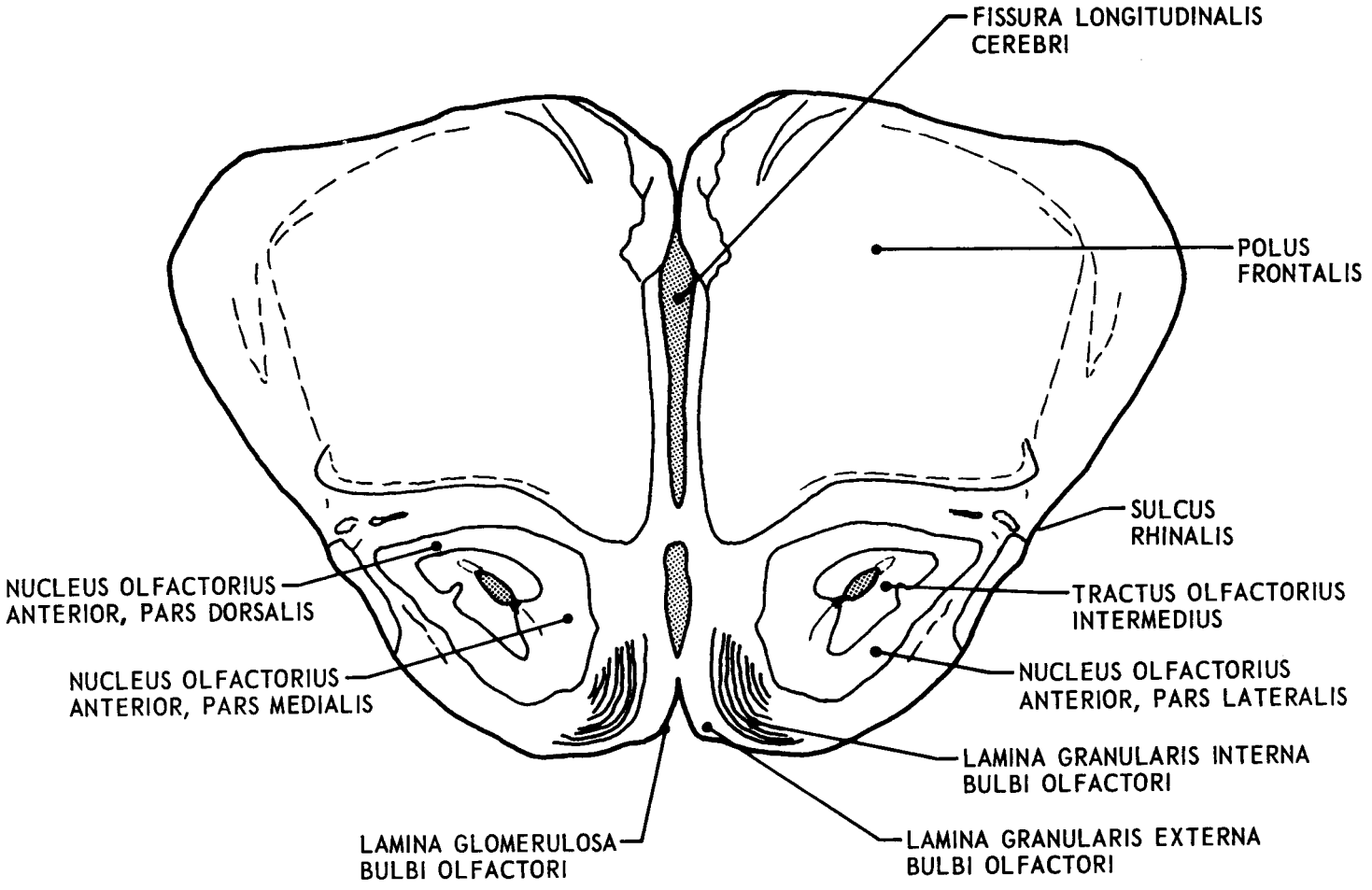
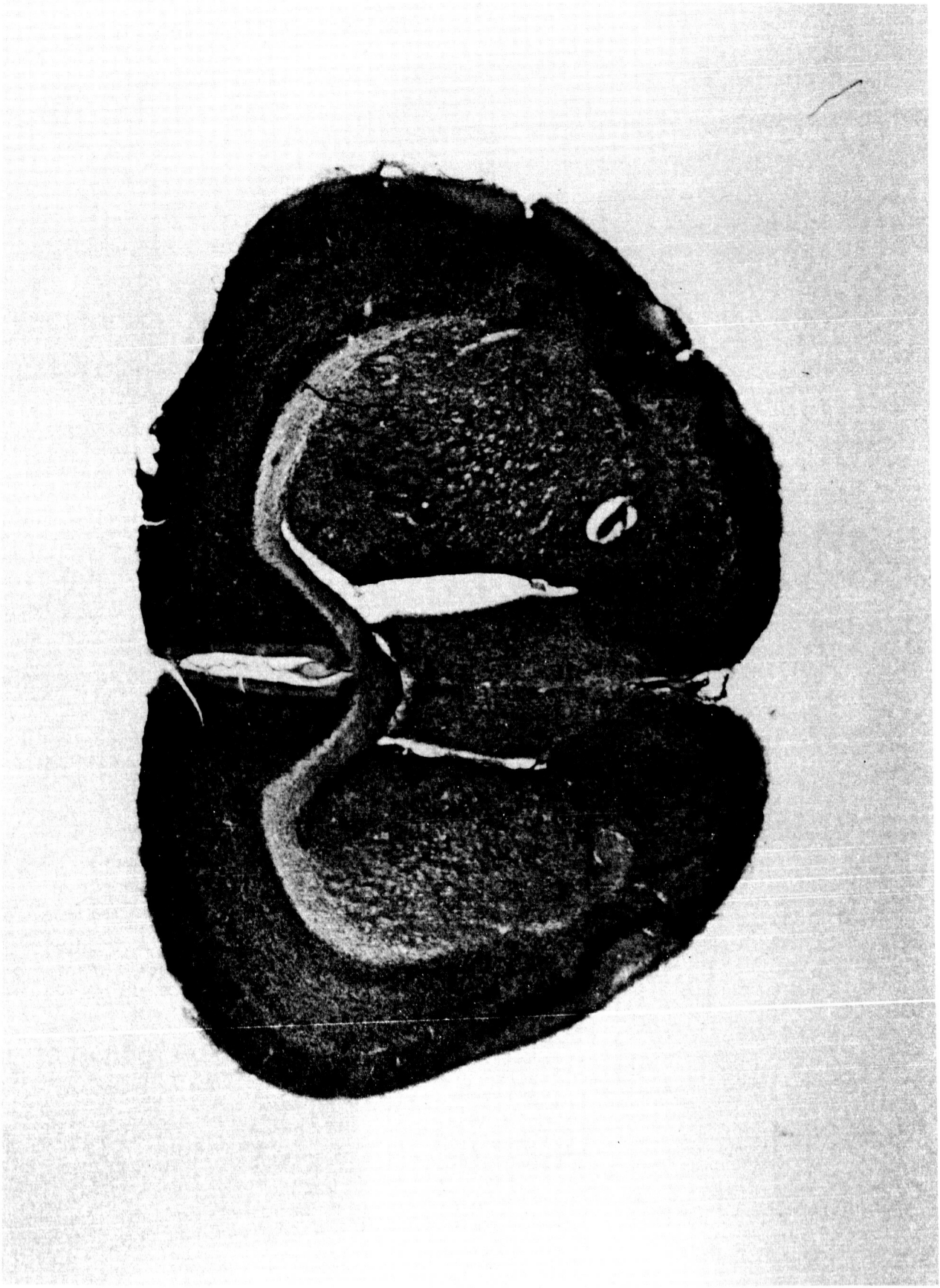

FISSURA LONGITUDINALIS CEREBRI
POLUS FRONTALIS
SULCUS RHINALIS
TRACTUS OLFACTORIUS INTERMEDIUS
NUCLEUS OLFACTORIUS ANTERIOR, PARS LATERALIS
LAMINA GRANULARIS INTERNA BULBI OLFACTORI
LAMINA GRANULARIS EXTERNA BULBI OLFACTORI
NUCLEUS OLFACTORIUS ANTERIOR, PARS DORSALIS
NUCLEUS OLFACTORIUS ANTERIOR, PARS MEDIALIS
LAMINA GLOMERULOSA BULBI OLFACTORI

FIGURE 3



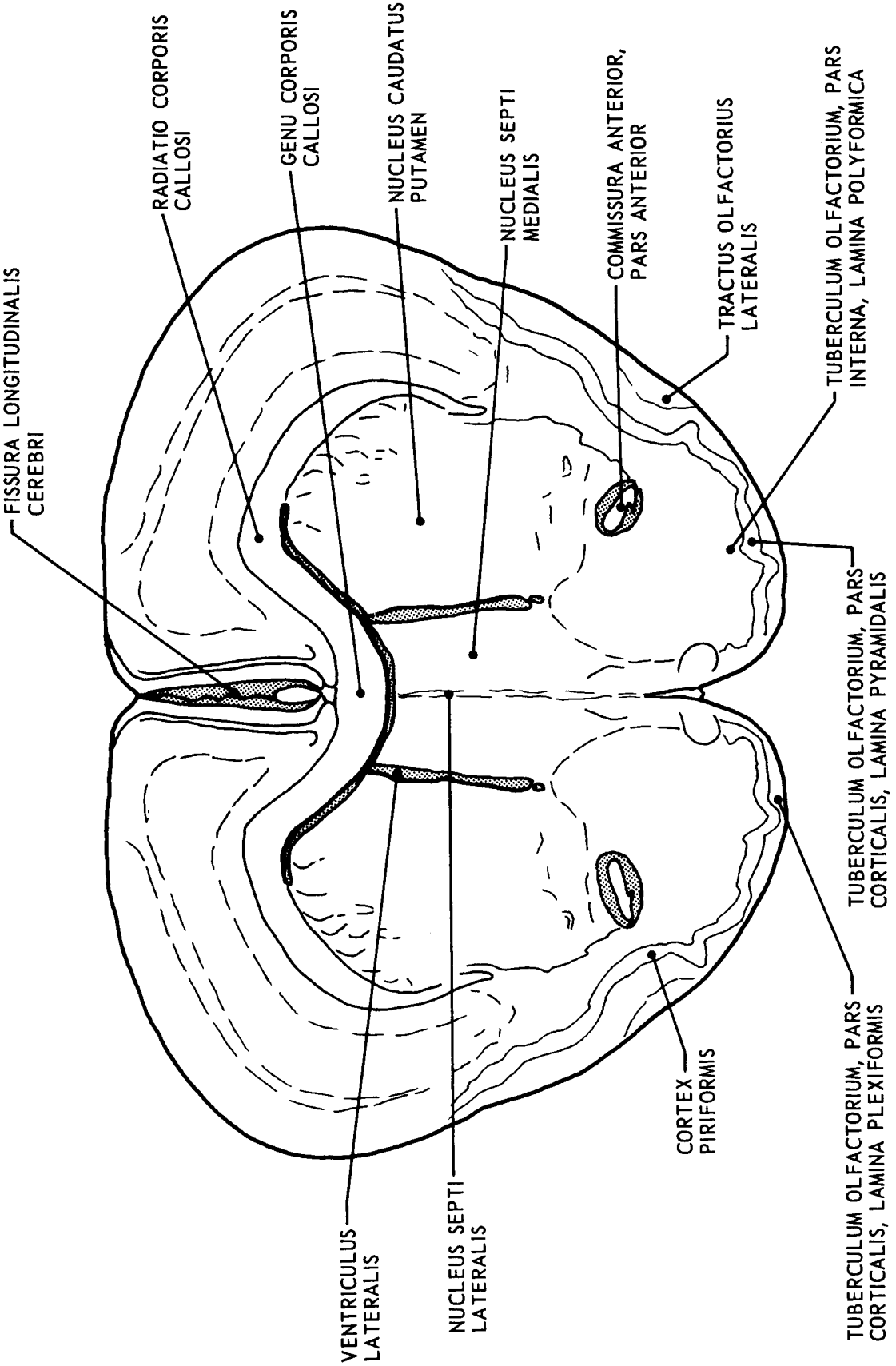
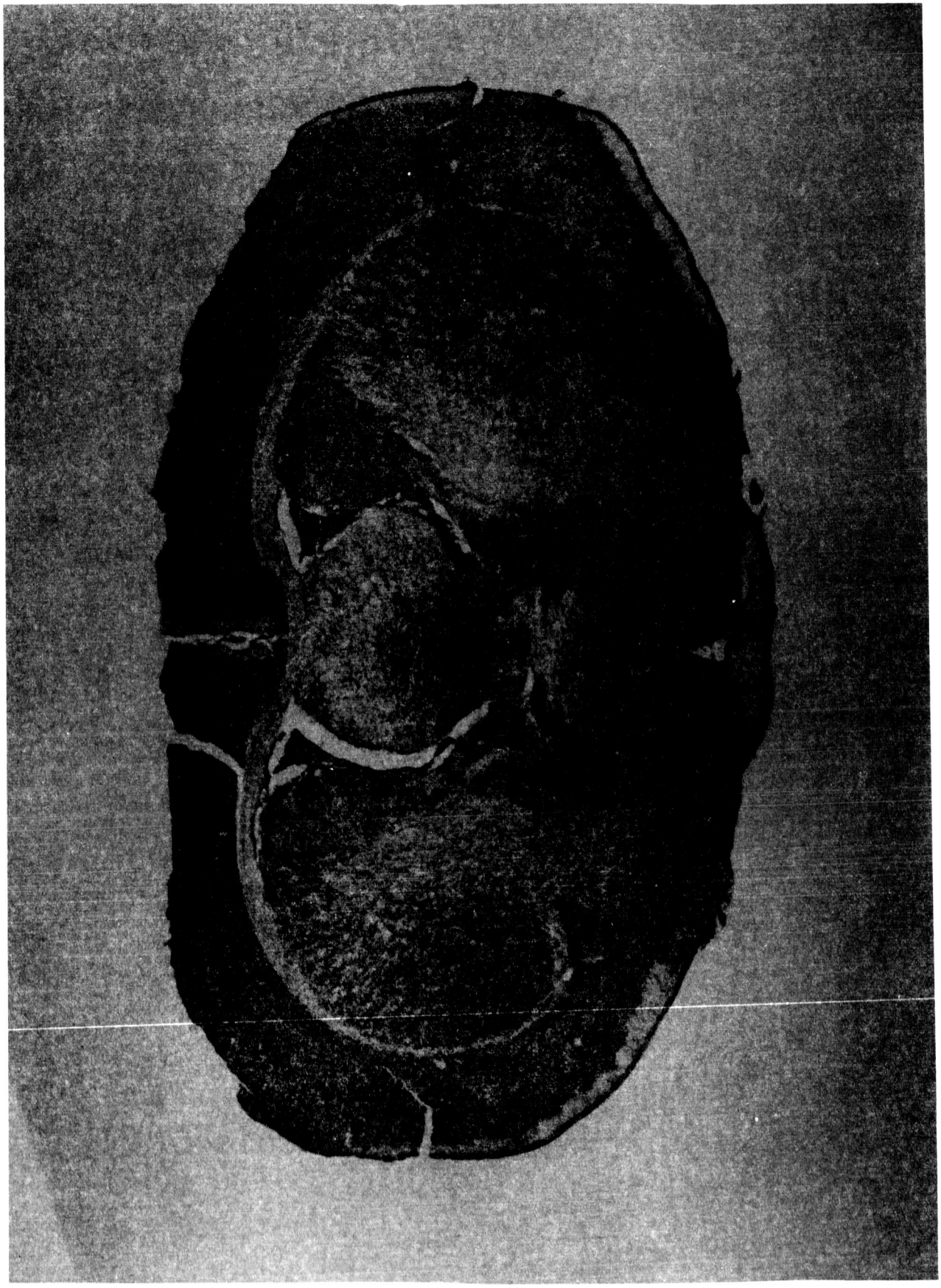

FISSURA LONGITUDINALIS CEREBRI
RADIATIO CORPORIS CALLOSI
GENU CORPORIS CALLOSI
NUCLEUS CAUDATUS PUTAMEN
NUCLEUS SEPTI MEDIALIS
COMMISSURA ANTERIOR, PARS ANTERIOR
TRACTUS OLFACTORIUS LATERALIS
TUBERCULUM OLFACTORIUM, PARS INTERNA, LAMINA POLYFORMICA
VENTRICULUS LATERALIS
NUCLEUS SEPTI LATERALIS
CORTEX PIRIFORMIS
TUBERCULUM OLFACTORIUM, PARS CORTICALIS, LAMINA PYRAMIDALIS
TUBERCULUM OLFACTORIUM, PARS CORTICALIS, LAMINA PLEXIFORMIS

FIGURE 4



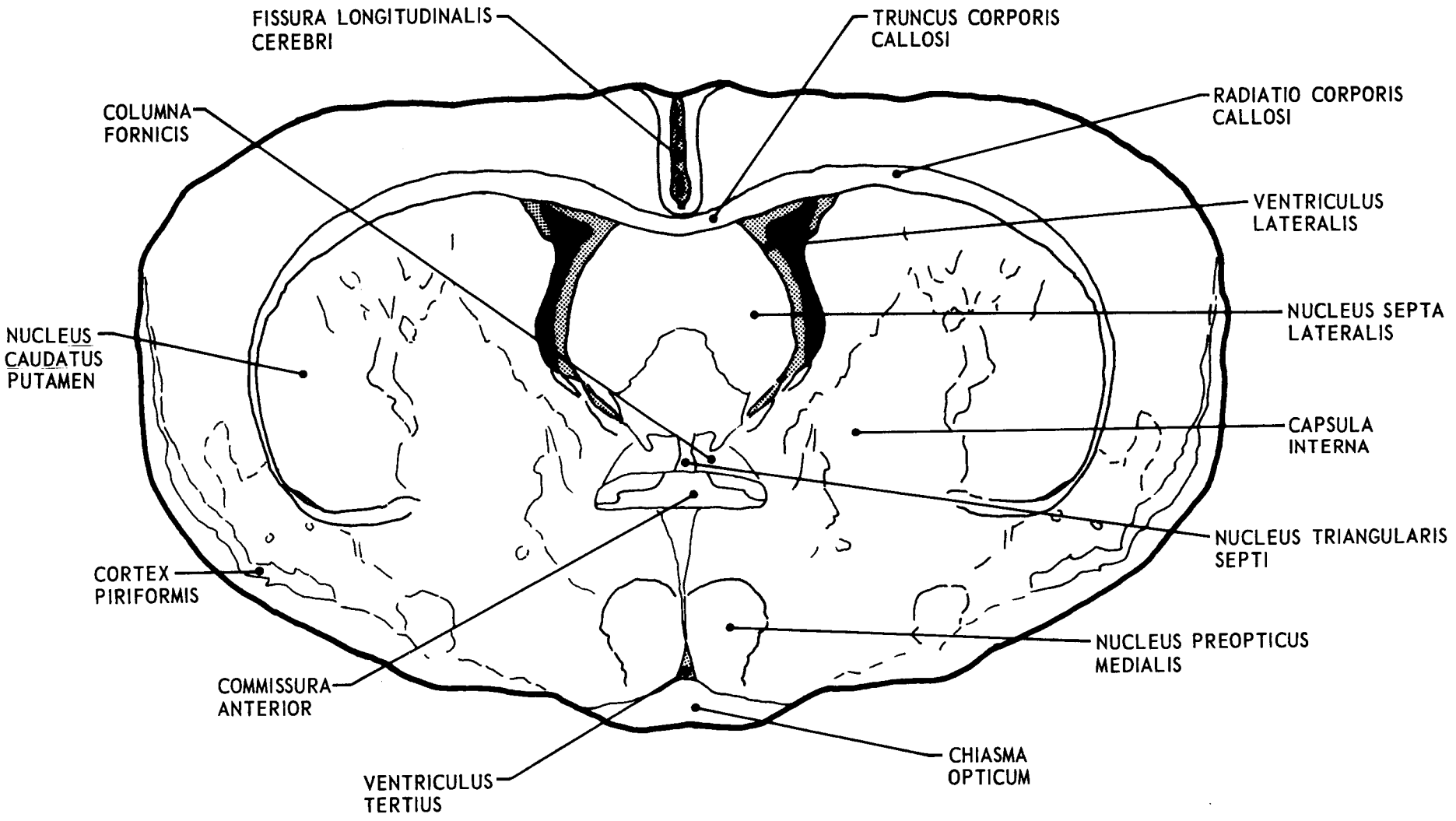
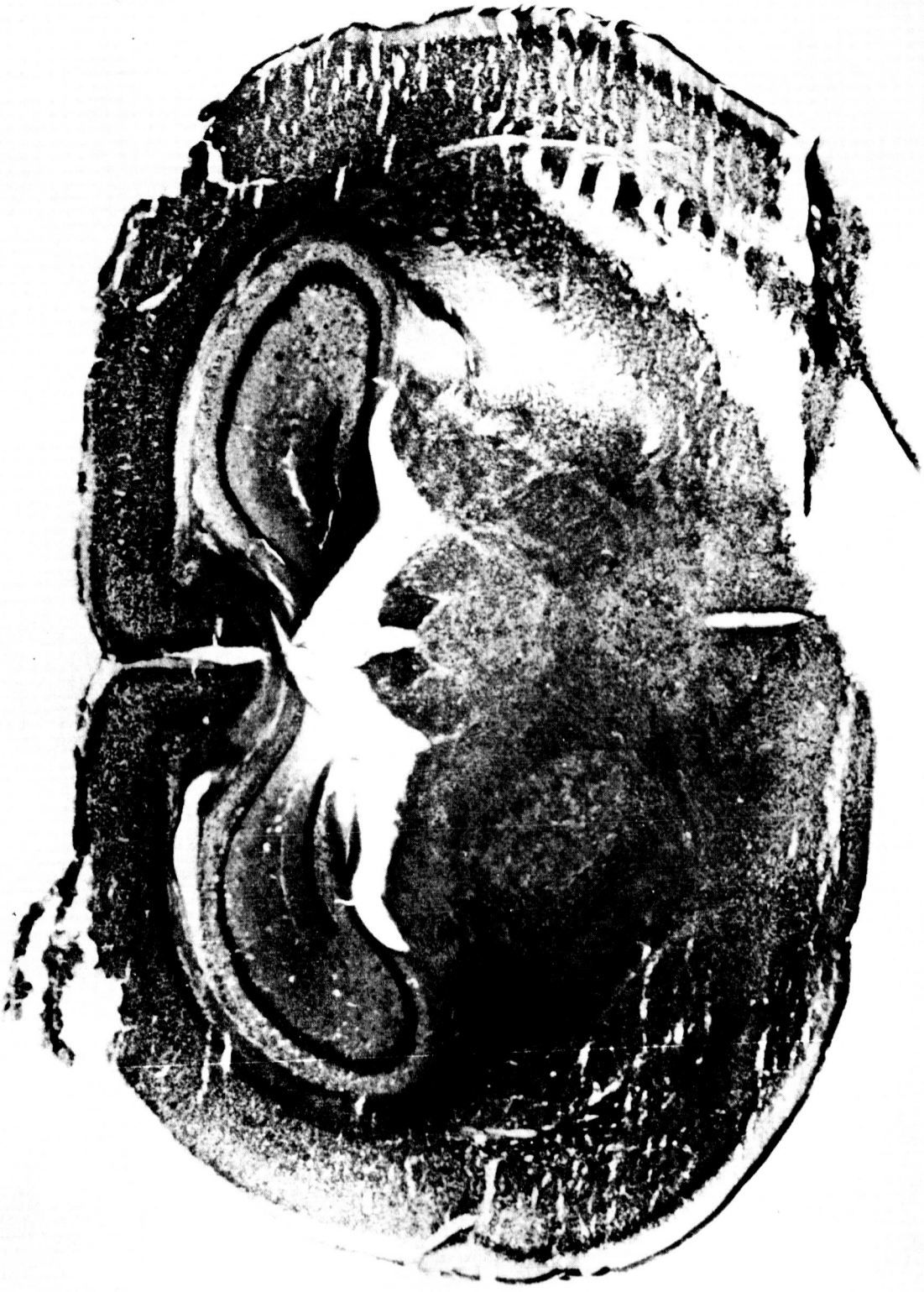

FISSURA LONGITUDINALIS CEREBRI
TRUNCUS CORPORIS CALLOSI
COLUMNA FORNICIS
RADIATIO CORPORIS CALLOSI
VENTRICULUS LATERALIS
NUCLEUS CAUDATUS PUTAMEN
NUCLEUS SEPTA LATERALIS
CAPSULA INTERNA
NUCLEUS TRIANGULARIS SEPTI
CORTEX PIRIFORMIS
NUCLEUS PREOPTICUS MEDIALIS
COMMISSURA ANTERIOR
CHIASMA OPTICUM
VENTRICULUS TERTIUS

FIGURE 5



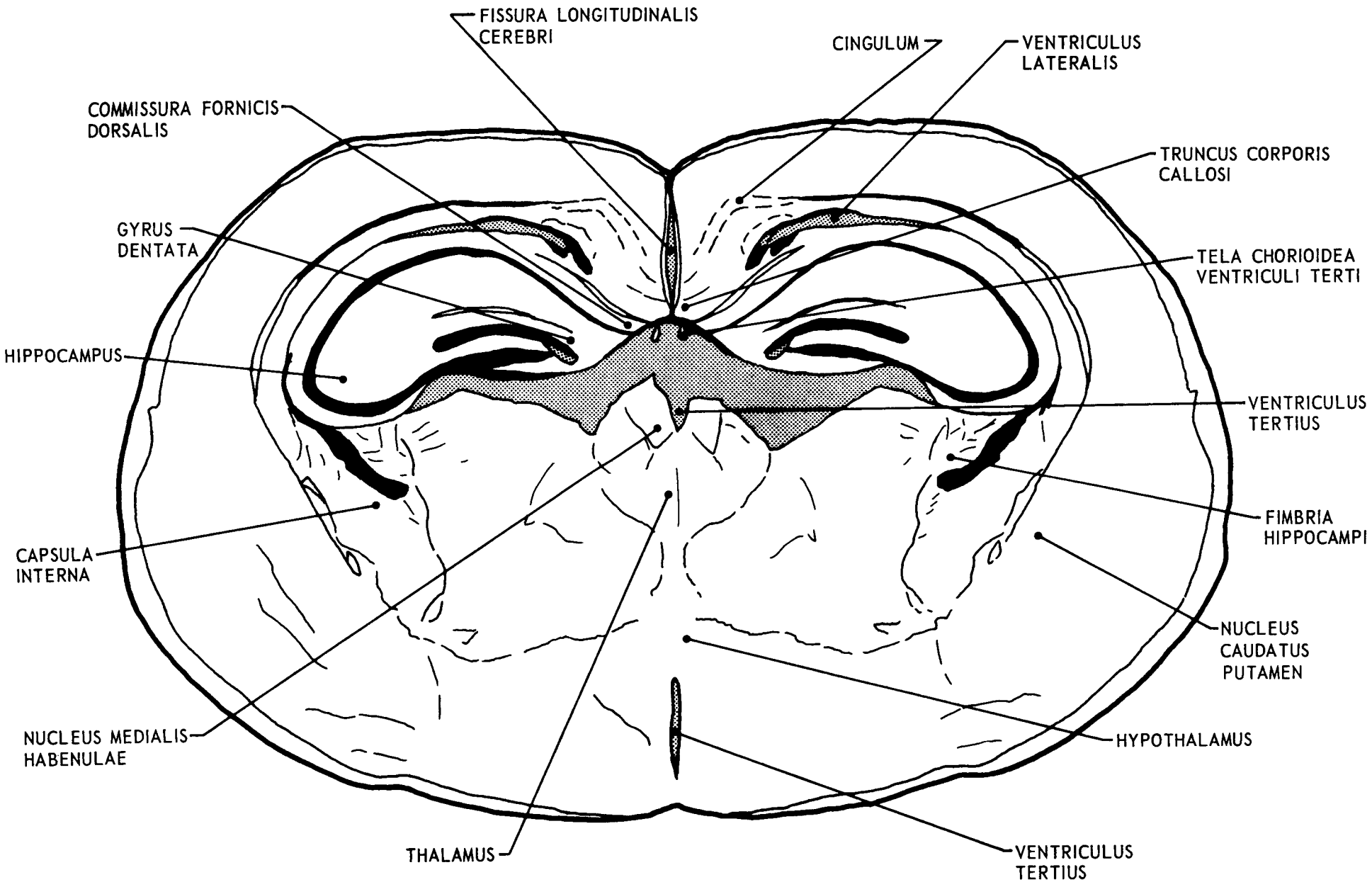
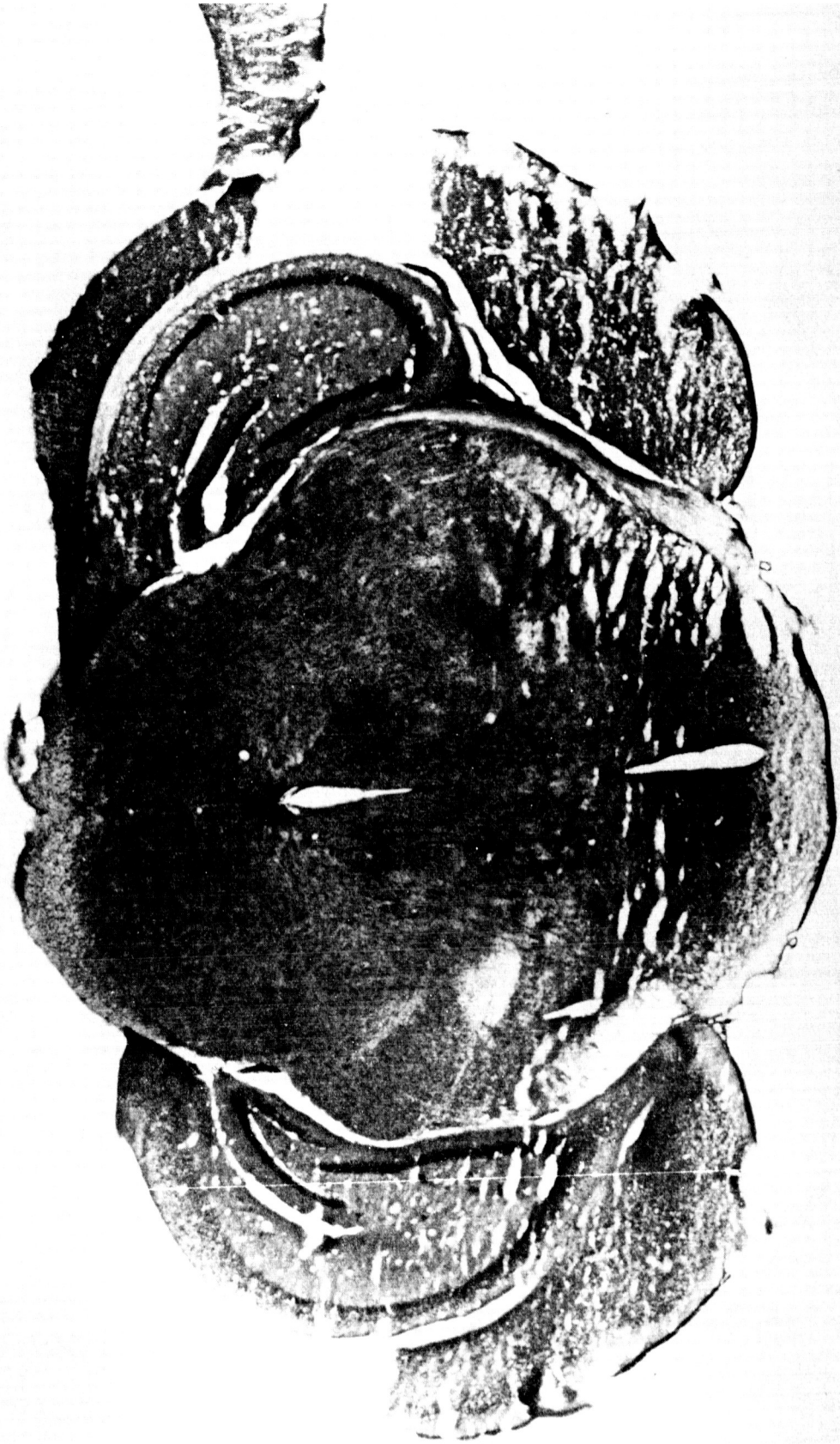

FISSURA LONGITUDINALIS CEREBRI
CINGULUM
VENTRICULUS LATERALIS
COMMISSURA FORNICIS DORSALIS
TRUNCUS CORPORIS CALLOSI
GYRUS DENTATA
TELA CHORIOIDEA VENTRICULI TERTI
HIPPOCAMPUS
VENTRICULUS TERTIUS
FIMBRIA HIPPOCAMPI
CAPSULA INTERNA
NUCLEUS CAUDATUS PUTAMEN
NUCLEUS MEDIALIS HABENULAE
HYPOTHALAMUS
THALAMUS
VENTRICULUS TERTIUS

FIGURE 6



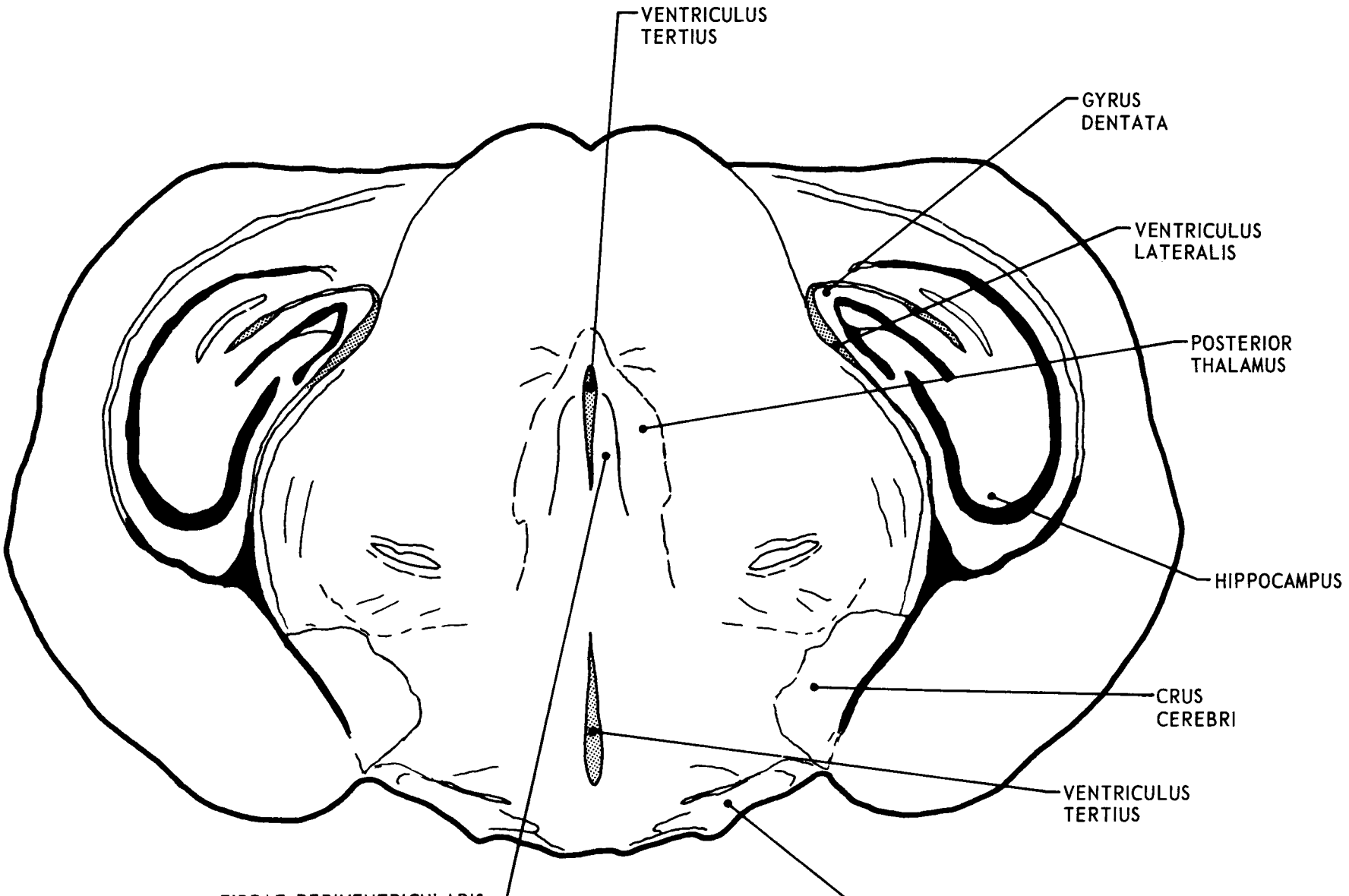
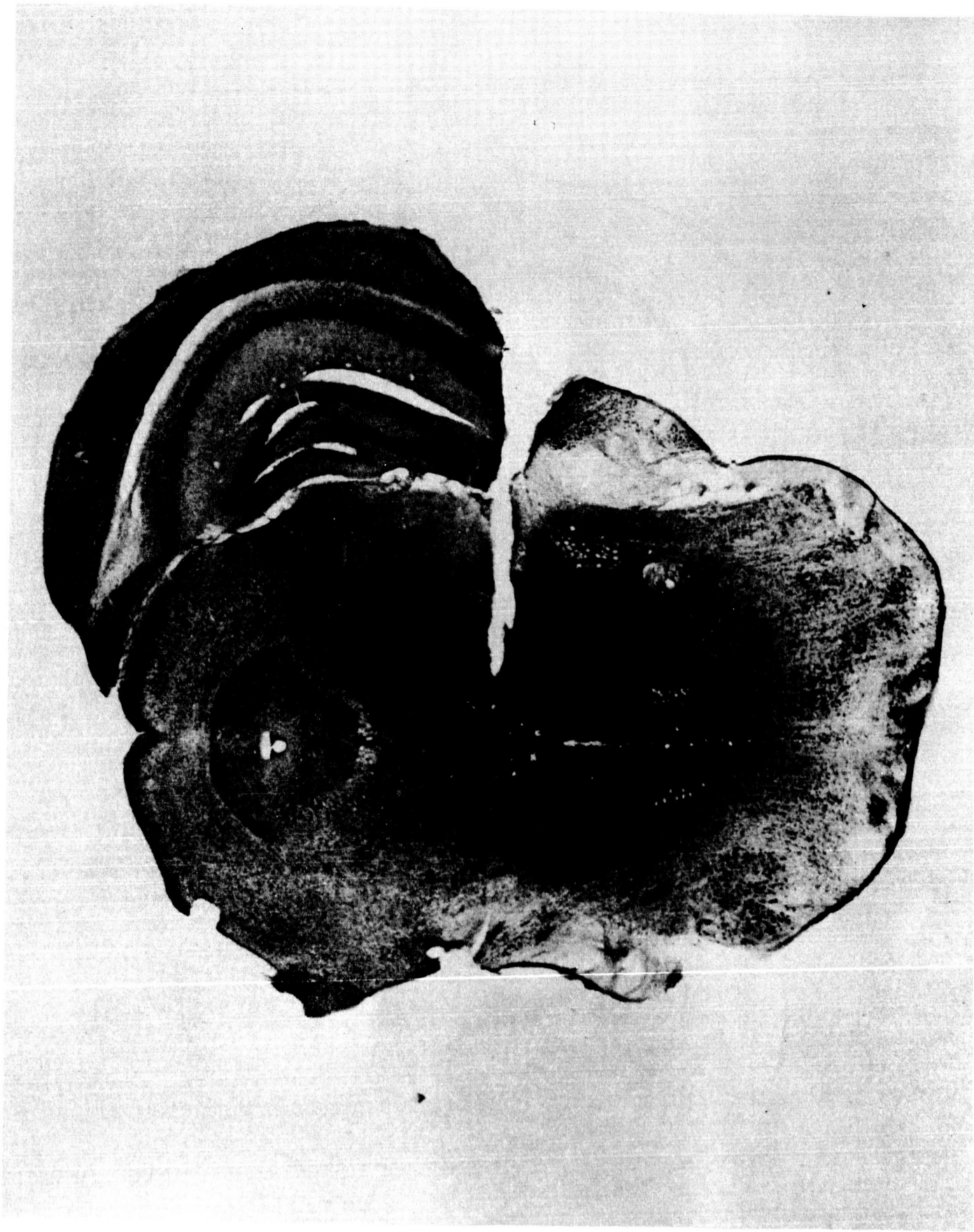

VENTRICULUS TERTIUS
GYRUS DENTATA
VENTRICULUS LATERALIS
POSTERIOR THALAMUS
HIPPOCAMPUS
CRUS CEREBRI
VENTRICULUS TERTIUS
TRACTUS OPTICUS
FIBRAE PERIVENTRICULARIS HYPOTHALAMI

FIGURE 7



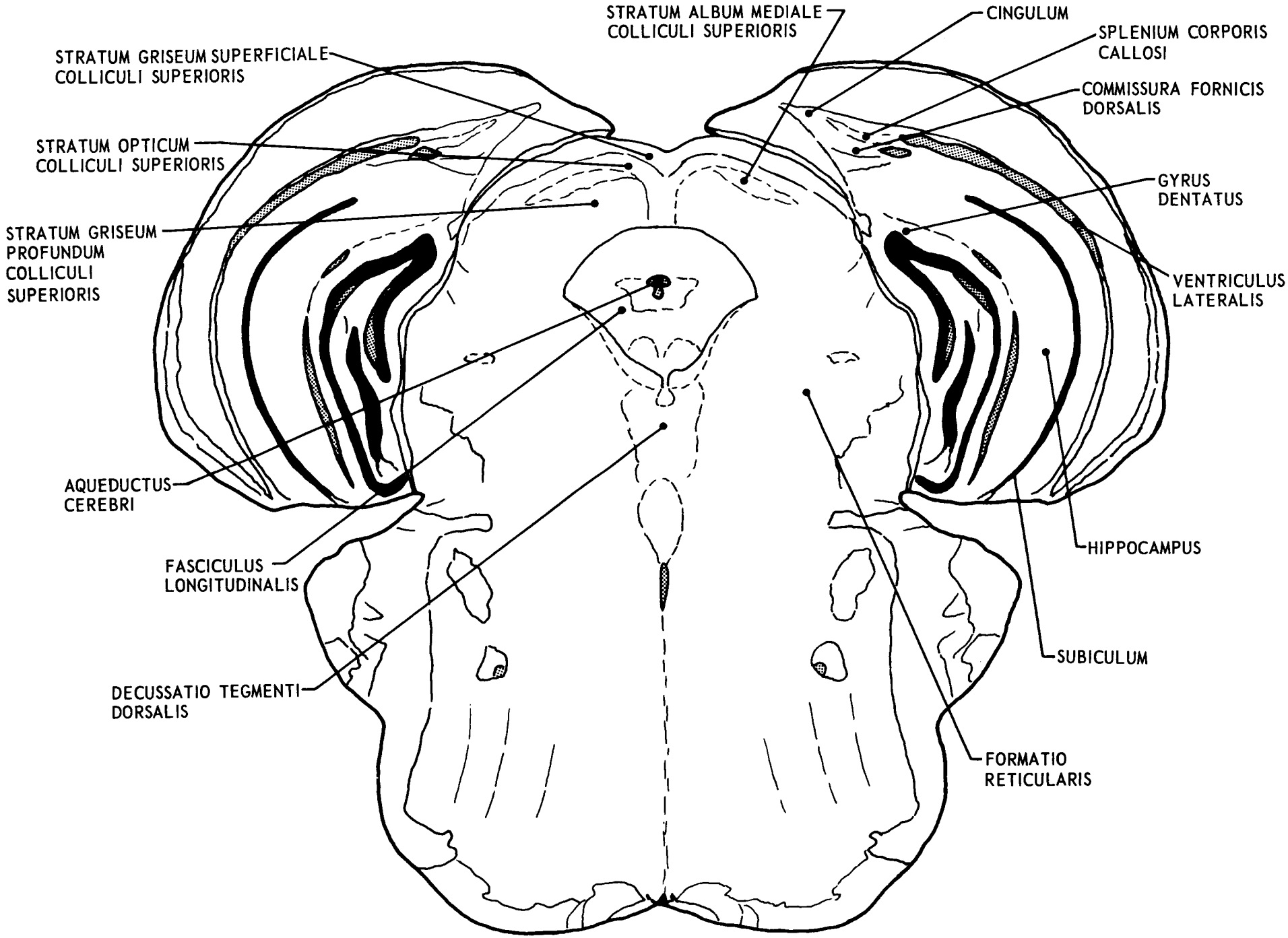

STRATUM ALBUM MEDIALE COLLICULI SUPERIORIS
CINGULUM
SPLENIUM CORPORIS CALLOSI
COMMISSURA FORNICIS DORSALIS
STRATUM GRISEUM SUPERFICIALE COLLICULI SUPERIORIS
STRATUM OPTICUM COLLICULI SUPERIORIS
GYRUS DENTATUS
STRATUM GRISEUM PROFUNDUM COLLICULI SUPERIORIS
VENTRICULUS LATERALIS
AQUEDUCTUS CEREBRI
HIPPOCAMPUS
FASCICULUS LONGITUDINALIS
SUBICULUM
DECUSSATIO TEGMENTI DORSALIS
FORMATIO RETICULARIS

FIGURE 8

IV. SUMMARY AND CONCLUSION

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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SECTION III

PUBLICATIONS IN THE OPEN LITERATURE RESULTING FROM RESEARCH SUPPORTED IN WHOLE OR IN PART UNDER CONTRACT NASw-812

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1964 Gambino, J. J. and R. G. Lindberg, Response of the pocket mouse to ionizing radiation, Rad. Res. 22(4):586-597 (Aug)

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