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The Physiology of Daily Torpor in White-footed Mice (*Peromyscus* spp.)

by

Josef Emil Morhardt, IV

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

Thesis Director's signature: [Signature]

Houston, Texas

May, 1968
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INTRODUCTION

The body temperatures of birds and mammals may rise well above low environmental temperatures due to metabolic heat production. Because the source of body heat is internally derived these animals may be considered endothermic. In contrast, most lower vertebrates maintain a large body temperature gradient with the environment only when energy is absorbed from the environment, and therefore may be considered primarily ectothermic.

Although the body temperatures of endothermic animals may increase independently of external heat, the precision with which the body temperatures are regulated varies from species to species. In many higher orders of mammals (carnivores, ungulates and most primates) and in most orders of birds, the variability of core temperature is small, less than 3°C about the mean (Eisentraut, 1934, 1960; Bligh and Harthoorn, 1965; King and Farner, 1961) and never falls sufficiently low to render the animals behaviorally lethargic. These animals have been traditionally considered to be "higher warm bloods" (Eisentraut, 1960) or homeotherms (Prosser, 1961).

The body temperature variability is more than 3°C in many of the lower mammals (Marsupialia, Monotremata, Insectivora, Edentata, Tubulidentata, Chiroptera), in some species of rodents and fissipeds, and in a few families of birds (Hirundinidae, Caprimulgidae, Micropodidae, Cuculidae, Cathartidae, and Trochilidae), (Lasiewski and Thompson, 1966; Hudson and Bartholomew, 1964; Johansen, 1962; Heath, 1962; Kayser, 1961; Warren, 1960; Nouvel, 1958, cited by Kayser, 1961; McAtee, 1947). These animals have been considered "lower warm
babbles" (Eisentraut, 1960) or, in the terminology of Prosser (1961), heterotherms.

Body temperature fluctuations in heterotherms may remain relatively small throughout much or even all of the animal's life. However from time to time, usually in response to some environmental stimulus or physiological condition, the body temperature may fall enough to make the animal behaviorally lethargic. It is this lethargy accompanying low body temperature that is considered to be a state of torpidity or torpor (Hudson and Bartholomew, 1964).

It is generally considered that torpid animals either retain or quickly recover physiological control of the cardiovascular and respiratory systems and other activities necessary for metabolic homeostasis and temperature regulation (Lyman and Chatfield, 1955). A torpid animal therefore rewarms without the aid of external heat, and physical disturbance is sufficient to initiate arousal, or to prevent torpor from even occurring. Torpidity is consequently to be distinguished from hypothermia experienced by any endothermic animal exposed to cold in excess of its thermoregulatory ability, treated with drugs which interfere with temperature regulation, or starved to the point that there are insufficient metabolic energy reserves to allow temperature maintenance.

The most pronounced and most thoroughly studied torpor is that of mammalian hibernators whose body temperatures fall nearly to 0°C for as long as two weeks (Kayser, 1961). Some other species engage in a torpor in which the body temperature does not fall so low, or remain low so long, or both. A great many terms have been applied to these states of torpor which for one reason or another are not considered to
be hibernation. They include estivation (Hudson and Bartholomew, 1964), Halbwach (Herter, 1934, cited by Kristoffersson and Soivio, 1964a), Winterruhe (Eisentraut, 1934), carnivorean lethargy (Hock, cited by Hoffman, 1964), seasonal lethargy (Hock, 1960), partial hibernation (Morrison, 1960), and semi-torpidity (Folk, 1949, 1951; Panuska, 1959).

From the variety of terms that have been applied, it is apparent that heterothermic animals exhibit many patterns of temperature regulation. Hudson and Bartholomew (1964) have suggested that "The various patterns of dormancy shown by birds and mammals form a continuum..." including the shallow but prolonged torpor of wintering bears, daily torpor which in some animals involves a lowering of the body temperature of only a few degrees and in others profound lowering almost to freezing, and finally to the extremes of classical hibernation of ground squirrels and marmots in which the body temperature may approach 0°C for two weeks or more at a time (Kayser, 1961).

The temperature regulation of a number of species of mice of the genus Peromyscus has been systematically examined (Kendeigh, 1945; Sealeander, 1953; Morrison and Ryser, 1959; Murie, 1961; McNab and Morrison, 1963). These studies have shown that the body temperatures of some white-footed mice (Peromyscus spp.) are exceedingly labile under conditions of close confinement or low ambient temperature, however there is no indication that any of the animals studies were capable of rewarming without the application of exogenous heat. For example the body temperatures of some P. leucopus noveboracensis fell as low as 25°C when the animals were exposed to ambient temperatures between 0°C and 15°C, and the body temperature was maintained at
that level for several hours with no indication that it was likely to fall any lower (Morrison and Ryser, 1959). These authors referred to *P. l. noveborascensis* as a heterotherm, or an incipient hibernator, even though arousal did not occur unless the ambient temperature was raised.

Sealander (1953) exposed two *P. leucopus* to ambient temperatures of -12°C and -23°C, eliciting body temperatures of 10°C and 23°C respectively from which both animals recovered when placed at room temperature (which provided exogenous heat).

Murie (1961) observed individuals of *P. maniculatus* with body temperatures of 33.3°C at ambient temperatures ranging from 4.7°C to 21.6°C, and Kendeigh (1945) noted that a *P. maniculatus gracilis* was able to recover from a body temperature of 23.5°C when warmed.

McNab and Morrison (1963) found that subspecies of *P. crinitus*, *P. maniculatus*, *P. eremicus*, *P. truei*, and *P. californicus* all had body temperatures within a few degrees of 36°C at ambient temperatures between 10°C and 30°C, but that at ambient temperatures of 3°C, individual *P. crinitus* and *P. eremicus* had body temperatures below 15°C.

In field studies Howard (1951) found *P. leucopus* and *P. maniculatus* lethargic (body temperature unmeasured) in artificial nest boxes during winter, and Michael Smith (personal communication) found *P. polionotus* in their burrows with body temperatures as low as 10°C. In these field studies as in the laboratory studies, no attempt was made to determine if the animals could rewarm themselves without application of external heat. The ability to do so is one of the primary distinctions between natural torpor and induced hypothermia (Hudson and Bartholomew, 1964).
Since in none of these instances did the animals reawarm without an increase in the environmental temperature, it is likely that rather than being torpid they were being chilled beyond their metabolic capabilities of heat production. This possibility is supported by the observation that *P. leucopus* progressively improved their ability to maintain body temperatures at normothermic levels during successive exposures to low ambient temperatures (Morrison and Ryser, 1959), and by the fact that *P. maniculatus* and *P. leucopus* could withstand ambient temperatures as low as -30°C after prolonged cold acclimation (Hart, 1953; Hart and Heroux, 1953).

*P. eremicus* however, has been shown to undergo a typical cycle of daily torpidity in the laboratory when fed a limited food ration (MacMillen, 1965). Well fed animals maintained their body temperatures between 33°C and 38°C even when exposed to ambient temperatures as low as 5°C. Similarly, *P. truei*, *P. boylei*, and, to a lesser extent, *P. californicus*, underwent daily cycles of torpidity only when deprived of food (Hudson, 1967).

Since *Peromyscus* apparently become torpid only when they have been starved (MacMillen, 1965; Hudson, 1967) the fact that torpidity has been observed in only three species may be the result of the normal laboratory practice of supplying animals with food ad libitum. The number of species shown to tolerate low body temperatures, even though arousal requires exogenous heat, suggests that many species may become torpid under appropriate conditions, and invites a comparative examination of a number of species.

The present study is a comparative examination of the body temperature, and of temperature related physiological parameters, of five
species of *Peromyscus* under conditions of partial and complete food deprivation, at a variety of environmental temperatures. It is intended to determine whether torpidity occurs in all five species, and if there are physiological differences in the control of torpor of the various species. As a means of determining the relative control exercised during episodes of torpor in *Peromyscus*, the relationships between body temperature and ambient temperature, heart rate, breathing rate and metabolic rate are examined. In addition, the control of heart rate is studied by infusion of drugs.

In order to study temperature regulation and other physiological parameters during torpor it is crucial to use experimental conditions which do not disturb the animals. Since torpid animals often arouse when disturbed by the attachment of electrodes or thermocouples, or by the injection of drugs (Lyman and O'Brian, 1963) it has been necessary to develop techniques of chronic implantation of recording leads and catheters.

There are a number of studies of body temperature, heart rate, breathing rate, or some combination of the three recorded from implanted electrodes in undisturbed animals during torpor. These represent a fairly broad cross section of animals showing patterns of torpor ranging from classical hibernation to very shallow forms of torpor. The animals studied include the following: ground squirrels (Strumwasser, 1959a, 1959b, 1959c, 1960; Lyman and O'Brian, 1960, 1963; Popovic, 1964), hamsters (Pohl, 1964), hedgehogs (Kristoffersson and Solvio, 1964b), woodchucks (Lyman, 1958), several species of bats (Leitner, 1966; Stones and Wiebers, 1966; Reite and Davis, 1966; Herreid and Schmidt-Nielson, 1966), some small mice (Johansen and
Krog, 1959; Hudson, 1965; MacMillen, 1965; Chew et al., 1967), a
marsupial phalanger (Bartholomew and Hudson, 1962a), and several
Studies of the effects of drugs on the cardiovascular systems of un-
disturbed torpid animals have been made only in ground squirrels

Techniques of chronic implantation of thermocouples, electrodes
and catheters have been used throughout the present study to avoid
disturbance to the animals during torpor.
MATERIALS AND METHODS

The species examined include *Peromyscus crinitus stephensi*, *P. eremicus fraterculus*, *P. maniculatus sonoriensis*, and *P. boylei rowleyi* from southern California, and *P. leucopus texanus* from south Texas. The animals were housed in a windowless room with a light regime of 13 hours of light followed by 11 hours of darkness. The ambient temperatures were 23°C to 26°C. The animals were fed Purina Laboratory Chow *ad libitum* supplemented occasionally with white millet, sunflower seed, and lettuce. Water was always available.

Under these conditions mature *P. crinitus* weighed 20.1 ± 0.9 grams (+ 95% confidence limits), *P. eremicus* weighed 23.9 ± 2.6 grams; *P. maniculatus* weighed 22.5 ± 1.4 grams; *P. boylei* weighed 33.1 ± 4.6 grams; and *P. leucopus* weighed 23.9 ± 2.5 grams.

*P. maniculatus* and *P. leucopus* reproduced prolifically whether paired, or caged in groups of 15 or more. *P. eremicus* reproduced seldom and only when pairs were caged separately. Neither *P. crinitus* nor *P. boylei* reproduced in the laboratory.

Torpor was induced in all of the species by reducing the daily food ration. Water was always provided *ad libitum*. The food ration was adjusted daily to increase the occurrence of torpor and minimize weight loss. If torpor was occurring regularly but was accompanied by regular weight loss the food ration was increased. If torpor was not occurring, the daily ration was decreased.

To test for any relationship between duration of torpor and the daily food ration, five *P. eremicus* were maintained at a constant ambient temperature of 25°C for 17 days with a constant daily food
ration of 1.5 grams millet, and for another 10 days with the increased daily ration of 2.0 grams millet. The animals were placed in 1 pint vacuum flasks and a thermocouple was placed in the bottom of each flask. Because of the shape of the flasks, a resting or torpid animal sat directly over the thermocouple. The occurrence and duration of torpor was determined from the temperature recorded under the animals.

Carcass analysis of total lipid fraction (fraction extractable in petroleum ether) was accomplished by cutting the freshly killed animal into small pieces with shears and homogenizing the pieces in a Waring blender along with 400 ml acetone for 5 minutes. The homogenate was dried in an oven at 103°C to 105°C to a constant weight (24 hours). The dried material, transferred to a dried weighed, fat-free soxhlet extractor thimble, was extracted with petroleum ether (ligroin) for 7 hours. Further time in the soxhlet apparatus did not increase the amount of extracted substances. The thimble was dried for 24 hours at 103°C to 105°C and weighed. The difference between the final dry weight and the dry weight before extraction was considered to be the total body lipid fraction.

Body and ambient temperatures were recorded from 30-gauge loop type copper-constant in thermocouples insulated with enamel except for the 3 millimeter soldered overlap at the thermocouple junction which was left uninsulated. Temperatures were continuously recorded from 2 to 16 thermocouples (2 seconds/thermocouple) on a Leeds and Northrup Speedomax G variable potentiometer with a range of -5.0°C to 50°C, and an accuracy of ± 0.1°C according to the manufacturer.
Thermocouples were surgically implanted in animals anesthetized with 70 to 100 mg/kg sodium pentobarbital (Nembutal). The thermocouple was threaded into the peritoneal cavity, dorsal to the gut at the level of the kidneys. The leads were threaded subcutaneously to a point dorsal to the scapulae where they left the body. A second thermocouple was often implanted in the neck musculature dorsal to the vertebral column at the base of the skull with the leads leaving the body adjacent to those of the abdominal thermocouple.

For drug infusions a polyethylene catheter (I.D. = 0.28 mm, O.D. = 0.61 mm) was implanted beside the abdominal thermocouple with an open end in the peritoneal cavity. The thermocouple wires and the catheter were threaded through holes in a small plastic plate which was subsequently sutured to the skin to prevent twisting of the wires within the body. A 6 cm length of latex tubing (I.D. = 0.12 inch, O.D. = 0.18 inch) was slipped over the leads and catheter and was fastened to the plastic plate. This arrangement protected the leads from twisting and chewing.

The implanted animal was placed in a 1 liter clear plastic jar containing 2 cm to 3 cm crushed corn cob filler, and the latex tubing was passed through a small hole in the top of the jar. A pin through the tubing prevented the end from slippind down into the jar and allowed relatively free rotation, and thus freedom of movement to the animal.

A third thermocouple was placed in a glass tube on the inside of the jar to monitor the ambient temperature.

The entire apparatus was transferred to a refrigerator equipped
with a thermostat device (Yellow Springs Instrument Co. Thermistemp) which maintained the ambient temperature inside the animal container within ± 0.5°C of the level at which it was set.

Freshly implanted animals were kept at room temperature with limited rations of sunflower seed until they became torpid, usually two to five days after implantation. Thereafter, in the experiments designed to determine the lowest ambient temperature suitable for torpor, the ambient temperature was lowered every two or three days in decrements not exceeding 3°C.

Continuous measurements of body temperature were recorded throughout 155 periods of torpor from 19 animals (Table 1).

For oxygen consumption measurements the animals were transferred to smaller (420 ml) airtight containers equipped with ports for the thermocouple leads and compressed air. Compressed air, dried over silica gel was introduced at a known rate of flow near the top of the chamber and removed at the level of the animal. After removal it was again dried by silica gel and the carbon dioxide removed with Ascarite. The effluent was continuously analyzed for oxygen content in a Beckman G-2 oxygen analyzer. The oxygen consumption was calculated using the method of Depocas and Hart (1957) for open circuit systems.

Oxygen consumption during torpor was recorded from 2 P. leucopus (3 periods of torpor), 1 P. eremicus (1 period of torpor) and 1 P. crinitus (4 periods of torpor). Body temperature was recorded along with oxygen consumption in P. crinitus.

The two implanted thermocouples could be used as electrodes
for recording the electrocardiogram (EKG) and the breathing rate without interfering with the recording of body temperature. The electrodes were connected to an E & M impedance pneumograph for recording breathing rate on an E & M Physiograph. The impedance pneumograph measures changes of impedance between the two recording electrodes during ventilation.

From the impedance pneumograph the original biopotential was transferred by an interconnecting cable to an E & M cardiac preamplifier which amplified the signal sufficiently to be recorded as an electrocardiogram on the Physiograph. The heart rate was determined from the electrocardiogram either directly by counting the heart beats during one minute intervals, or indirectly through the use of an E & M electronic cardiotachometer. The cardiotachometer functions by driving a recording pen downward at a constant rate until a heart beat (the ORS complexes of the EKG) intervenes, then automatically resetting the pen. The distance that the pen travels downward before being reset is proportional to the amount of time between beats. The cardiotachometer trace can be read directly as heart rate provided the recording is calibrated against a standard rate provided by special circuitry in the cardiotachometer.

Heart rates were recorded from a total of 59 periods of torpor. The animals used include 4 P. eremicus (2 and 6 periods of torpor), 4 P. leucopus (2, 2, 3, and 4 periods of torpor) and 9 P. maniculatus (1, 1, 2, 2, 2, 4, 4, and 11 periods of torpor).

For determination of the relationship between heart rate, breathing rate, body temperature, and ambient temperature during
torpor, 35 periods of torpor from 11 fully torpid animals were chosen
(24 recordings were unusable for technical reasons). The animals used
include 2 P. eremicus (2 and 2 periods of torpor), 2 P. crinitus
(3 and 3 periods of torpor), 2 P. leucopus (1 and 2 periods of torpor)
and 3 P. maniculatus (1, 1, and 9 periods of torpor).

Isotonic saline, atropine sulfate (Mallinckrodt, 10 mg/kg), and
acetylcholine chloride (Merck, 25 mg/kg) were infused through the
indwelling peritoneal catheters into fully torpid, undisturbed animals.
At the time of infusion a 30-gauge hypodermic needle was inserted
into the open end of the catheter and the desired amount of solution
was introduced with a syringe.

In the case of the saline solution, 0.06 ml was introduced. The
volume of atropine and acetylcholine solution varied between 0.01
and 0.02 ml depending on the weight of the animal, but following
infusion of either drug, a 3 mm column of air was introduced into the
catheter followed by sufficient isotonic saline solution to bring the
total volume injected to 0.06 ml. The total volume of the catheter
was less than 0.03 ml so that the additional saline completely displaced
the drug. An air bubble is considered more satisfactory than oil or
other substances in maintaining a separation between adjacent
solutions in polyethylene tubing of this diameter (Lyman and O'Brian,
1963).

All drugs were initially infused into normothermic animals and
the dosages of the drugs used during torpor were approximately the
minimum amounts which resulted in the expected response in the
normothermic animals.
All statistical procedures used are from Steele and Torrie (1960). Where confidence limits are established, the number of degrees of freedom is based on the number of measurements (normally the number of periods of torpor from which measurements were taken) rather than the number of animals measured.
RESULTS

I. Body Weight, Stored Lipids and Occurrence of Torpor.

With two exceptions (one *P. maniculatus* and one *P. crinitus*), torpor was not observed in animals provided with excess food. Torpor could be induced in both implanted and unimplanted animals of each species by limiting or withholding food for a day or more.

A rough idea of the magnitude of weight loss necessary to induce torpor is provided from 6 *P. maniculatus* which were implanted and kept at room temperature without food until they entered torpor for the first time. They weighed 91.1%, 88.9%, 88.3%, 83.5%, 79.5%, and 77.4% of their initial body weights at the end of the first period of torpor. Also, five *P. eremicus*, unimplanted and kept in vacuum bottles equipped with thermocouples weighed 94.1%, 83.4%, 80.1%, 75.4%, and 73.3% of their initial body weights at the end of their first period of torpor. The daily body weights of seven animals, implanted and periodically entering torpor, are listed in Table 1.

Once the body weight dropped to a level compatible with torpor, it could remain relatively constant, decrease, or increase gradually while torpor occurred daily (Table 1). There was no critical weight below which torpor invariably occurred. Animals given food *ad libitum* rapidly increased their weights. For example, *P. leucopus* 9 (Table 1) given excess food for four days gained back nearly all the weight lost in 16 days of partial fasting.

As an independent index of the amount of fasting necessary to induce torpor, the total body lipids were determined by carcass analysis from (a) control animals, (b) animals torpid for the first time, and
TABLE 1

Body weights of seven animals implanted with thermocouples and partially starved to induce torpor.

The initial weight is the body weight at the time the animals were implanted, and the weights taken at the end of each day thereafter are listed below the initial weight. Weights taken at the end of a day on which the animal became torpid are marked with an asterisk (*). Dashes (----) indicate days on which the body temperatures were not recorded, or the weight was not taken, usually because of difficulty with the recording leads.
### TABLE 1

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(c) animals that had been using torpor daily for long periods of time and had finally failed to arouse, apparently dying from starvation. The results are shown in Table 2 along with the weights of the liver and the right interscapular brown fat pad. The weight-relative amount of total extractable lipids and of the liver was significantly greater ($p < .05$) in the control animals than in the starved animals but that from the animals torpid for the first time was intermediate between the control and starved values and not significantly different from either. The interscapular brown fat pad was too small to dissect in the starved animals so no comparison was possible.

II. Body Temperature.

Figures 1 and 2 are recordings of the body temperature of a single *P. crinitus* on three consecutive days when torpor occurred at an ambient temperature of 19.9°C (Fig. 1) and on two consecutive days at an ambient temperature of 15.5°C (Fig. 2). These five periods of torpor were chosen as samples representative of the torpor in all the species examined.

Before entrance into torpor the body temperature often remained above 36°C with no indication that torpor was about to occur until entrance had actually begun (Figs. 1B, 2A,B). Small fluctuations of the body temperature of one or two degrees were common to all of the animals whether about to enter torpor or not.

Equally often, however, entrance did not begin abruptly, and the body temperature underwent a gradual and erratic decline (Fig. 1A) or a series of dips of several degrees C (Fig. 1C) before the final drop into torpor.
TABLE 2

Weight of the body lipids extractable with petroleum ether, weights of the liver, and of the right interscapular brown fat pad, expressed as percent fat free body weight \( \pm t_{0.05} \left( s_x / n \right) \), the 95% confidence limits.

The control animals include 11 P. maniculatus, 1 P. eremicus, and 1 P. leucopus. The animals torpid for the first time were unimplanted, the body temperatures being checked daily with a rectal thermister. They include 4 P. maniculatus. The starved animals, implanted and using torpor daily prior to death, include 2 P. maniculatus, 1 P. eremicus and 1 P. leucopus.
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (N = 13)</th>
<th>FIRST TIME TORPID (N = 4)</th>
<th>STARVED (N = 4)</th>
</tr>
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<tr>
<td>TOTAL BODY LIPIDS</td>
<td>21.8 ± 7.9 %</td>
<td>12.0 ± 14.0 %</td>
<td>3.3 ± 0.8 %</td>
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<tr>
<td>LIVER WEIGHT</td>
<td>6.3 ± 0.5 %</td>
<td>5.6 ± 1.6 %</td>
<td>5.2 ± 0.3 %</td>
</tr>
<tr>
<td>INTERSCAPULAR BROWN FAT PAD (RIGHT PAD) WEIGHT</td>
<td>0.34 ± 0.12%</td>
<td>0.20 ± 0.23 %</td>
<td>---------------</td>
</tr>
</tbody>
</table>
FIGURE 1

The body temperature of *P. crinitus* No. 10 recorded from an implanted abdominal thermocouple on three consecutive days on which torpor occurred. The ambient temperature ($T_A$) on all three days was 19°C.
FIGURE 2

The body temperature of *P. crinitus* No. 10 recorded from an implanted abdominal thermocouple on two consecutive days on which torpor occurred. The ambient temperature (\(T_A\)) on both days was 15.5°C.
During the initial stages of entrance, the body temperature usually fell smoothly at a steadily decreasing rate giving a straight line when the logarithm of the gradient between the body temperature and the ambient temperature (log delta T) was plotted as a function of time. This exponential decrease of body temperature occasionally persisted for a number of hours until the animal was fully torpid* (Fig. 1B), but more commonly the rate of decline was abruptly decreased toward the end of entrance which resulted in a higher sustained body temperature during torpor (and a larger delta T) than would have been predicted had the initial exponential decline not been interrupted (Figs. 1C 2A, B, 3). Two animals (one P. leucopus and one P. crinitus) consistently retained the exponential rate of cooling long enough that the body temperature undershot what was apparently the optimum sustained level, because immediately after the completion of entrance, the body temperature increased two or more degrees before stabilizing.

Figure 2 shows a common situation in which the body temperature of a fully torpid animal was maintained at different levels on successive days of torpor at the same ambient temperature.

During torpor the body temperature sometimes remained relatively constant (+ 0.1°C) for several hours (Fig. 1B, C) but more often it continued to gradually decrease, increase, or fluctuate (Figs. 2B, 3) until arousal. Fluctuations of the body temperature occurred in the absence of any known disturbance, but they could also be elicited by

*A fully torpid animal was arbitrarily defined as one in which the body temperature during entrance initially declined rapidly then leveled to a rate of change of less than 0.1°C/hour. Hence, neither entrance in Fig. 1A is considered to have been complete.
FIGURE 3

The body temperature ($T_B$), ambient temperature ($T_A$) and heart rate (HR) from *P. maniculatus* No. 17 during a single period of torpor. The body temperature was recorded from an implanted abdominal thermocouple, and the heart rate was calculated from the electrocardiogram recorded from two implanted electrodes.
P. maniculatus 17

Temperature (°C)

Time of Day (Hours)

Beats/Minute

TB

TA

HR
mild disturbances such as loud noise, shaking of the animal container, or abrupt changes of the ambient temperature. If the disturbance was severe enough, the animal aroused.

Arousal in response to disturbance was usually abrupt. An undisturbed arousal began slowly and the body temperature described a sigmoid curve when plotted as a function of time (Figs. 1B, 2A, 2B), increasing to a peak arousal rate then gradually slowing as the normal body temperature was reached.

The rates of increase of body temperature during arousal are given in Table 3. While there were striking differences in the mean arousal rates between certain individuals, the mean rates of each species were not significantly different from one another. The maximum rate observed for *P. eremicus* was 0.43°C/minute; for *P. leucopus*, 0.42°C/minute; for *P. crinitus*, 0.51°C/minute; for *P. boylei*, 0.54°C/minute; and for *P. maniculatus*, 0.64°C/minute.

Toward the end of arousal the body temperature increase often slowed to the point that it took several hours to increase from 33°C to 36°C. However, if the animals were disturbed there was an immediate and rapid increase to 37°C or 38°C. When food was placed in the container during torpor the animals began to eat very early in arousal (at body temperatures as low as 22°C) and the body temperature always increased rapidly to the normothermic level of 36°C to 38°C.

The body temperature remained somewhat independent of the ambient temperature during torpor (Fig. 2). If the ambient temperature was lowered, the body temperature usually immediately increased slightly then fell slightly (Fig. 3) or remained at the increased level. If the ambient temperature was lowered enough, arousal occurred.
TABLE 3

Arousal rates calculated from the fastest 15 minute portion of each arousal and presented with the 95% confidence limits.

The mean arousal rate for each species is calculated from the mean rates of the individuals of that species.
<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>AROUSAL RATE (°C/min ± (t .05) (s₀))</th>
<th>N (Measurements)</th>
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<td><em>P. crinitus</em></td>
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<tr>
<td>8</td>
<td>0.18 ± 0.06</td>
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<tr>
<td>10</td>
<td>0.20 ± 0.05</td>
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<td>3</td>
<td>0.28 ± 0.08</td>
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<tr>
<td>4</td>
<td>0.34 ± 0.10</td>
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<tr>
<td>Mean</td>
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<td><em>P. maniculatus</em></td>
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<tr>
<td>22</td>
<td>0.15 ± 0.11</td>
<td>3</td>
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<tr>
<td>17</td>
<td>0.19 ± 0.06</td>
<td>3</td>
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<tr>
<td>4</td>
<td>0.22 ± 0.03</td>
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<tr>
<td>6</td>
<td>0.37 ± 0.05</td>
<td>25</td>
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<tr>
<td>10</td>
<td>0.44 ± 0.05</td>
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<td>9</td>
<td>0.29 ± 0.06</td>
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<td>7</td>
<td>0.30 ± 0.05</td>
<td>6</td>
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<td>5</td>
<td>0.31 ± 0.02</td>
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<td>0.38 ± 0.51</td>
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<td>Mean</td>
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<td>6</td>
<td>0.22 ± 0.00</td>
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<td>3</td>
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<td>0.48 ± 0.02</td>
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<tr>
<td>Mean</td>
<td>0.34 ± 0.32</td>
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The relationship between the lowest body temperature for each period of torpor and the ambient temperature is shown for each species in Figure 4. Within the range of ambient temperatures examined there was an overall positive correlation between the body temperature during torpor and the ambient temperature \( r = 0.69, p < .01 \) established by *P. maniculatus, P. boylei*, and *P. eremicus*. The body temperatures of torpid *P. leucopus* and *P. crinitus* were not significantly correlated with the ambient temperature.

The body temperature of only a single animal fell to within 1°C of the ambient temperature and all individuals and species maintained a large range of delta T's during torpor.

As the ambient temperature was lowered the animals became less tractable and often chewed the thermocouple leads, prematurely ending the experiment. Individuals of all species became torpid at ambient temperatures as low as 12.5°C but below that most animals refused to enter torpor. If food rationing was continued, and the animals were unable to free themselves of the thermocouple leads, the body temperatures eventually fell nearly to the ambient temperature. In this state, no amount of disturbance would cause an animal to arouse, and even if removed to room temperature (or to 37°C) and fed, most of the animals died.

Of the animals that did become torpid at low ambient temperatures the lowest body temperatures observed in torpid animals capable of arousal were 13.4°C for a single *P. maniculatus*, 17°C for all other *P. maniculatus, P. leucopus* and *P. crinitus*, 18°C for *P. boylei*, and 19°C for *P. eremicus*.

Figure 5, calculated from the data in Figure 4 shows the relation-
The lowest body temperatures recorded from each period of torpor in which the animals became fully torpid (see text) plotted as functions of the ambient temperature. The diagonal line indicates the limit at which the body temperature ($T_B$) would equal the ambient temperature ($T_A$).
Regressions of the delta T (the difference between the body temperature and the ambient temperature in °C) as functions of the ambient temperature. The regression lines are fitted by the method of least squares. The total regression is the summed data from all species. \( r \) = the correlation coefficient. ** means highly significant (\( p < .01 \)), * means significant (\( p < .05 \)), and ns means not significant.
**Graph showing the relationship between Ambient - Body Temperature (ΔT) and Ambient Temperature (°C).**

- **PMANICULATUS**: $r = -0.92^{**}$, $y = -0.55x + 13.8$
- **PLEUCOPUS**: $r = -0.56^{**}$, $y = -0.74x + 18.4$
- **P. BOYLEI**: $r = -0.48$ns, $y = -0.30x + 11.9$
- **P. CRINITUS**: $r = -0.45^{**}$, $y = -0.55x + 14.8$
- **P. EREMICUS**: $r = -0.44^{*}$, $y = -0.40x + 13.0$
- **TOTAL**: $r = -0.67^{**}$, $y = -0.53x + 14.4$
ship between the delta T and the ambient temperature during torpor for
each species and for all species combined. There is a significant
negative correlation between the delta T and the ambient temperature
for all species except _P. boylei_. The slope of the regression line
of only _P. leucopus_ is significantly different from that of the total
regression (t test, p < .01).

III. Duration of Torpor.

The maximum duration of an individual period of torpor observed
in this study was 670 minutes, and individuals of each species were
torpid for single periods of at least 525 minutes. The mean duration
for each species was much less (Table 4).

The duration of single periods of torpor was significantly corre-
lated with low body temperature during torpor (Table 4). The duration
decreased as the body temperature increased in all species except _P.
eremicus_, and the summed partial correlation between low body temperature
and duration, holding delta T constant was significant (p < .01).

The duration of torpor of _P. crinitus_ and _P. leucopus_ decreased
with increased delta T, as did the summed partial correlation between
duration and delta T, holding body temperature constant (p < .01).

The multiple correlation coefficient, which indicates the corre-
lation between the duration and the combined effects of the body tem-
perature and the delta T, was highly significant (p < .01) but weak
(Table 4). The body temperature was 1.8 times as important as the
delta T in contributing to the regression.

Of the five animals given a constant subnormal food ration (1.5 grams
of millet daily) for 17 days, all 5 lost weight rapidly at first, then
TABLE 4

The correlation between the duration of torpor (as measured from the beginning of the fall of body temperature to the beginning of arousal) and the body temperature, and the gradient between the ambient temperature and body temperature (delta T).

N = number of periods of torpor; n = number of animals. ** means highly significant (p < .01). * means significant (p < .05). NS means not significant. A negative correlation coefficient indicates that as the body temperature, or delta T decreased the duration increased. A positive correlation coefficient indicates that as the body temperature or delta T decreased the duration also decreased.
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>N(n)</th>
<th>Duration versus body temperature</th>
<th>Duration versus delta T</th>
<th>Mean Duration</th>
<th>Maximum Duration</th>
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<tr>
<td>P. crinitus</td>
<td>33(4)</td>
<td>-0.512**</td>
<td>-0.499**</td>
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<td>570</td>
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<td>P. boylei</td>
<td>12(4)</td>
<td>-0.716**</td>
<td>-0.523NS</td>
<td>260</td>
<td>525</td>
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<tr>
<td>P. maniculatus</td>
<td>29(5)</td>
<td>-0.543**</td>
<td>+0.059NS</td>
<td>440</td>
<td>650</td>
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<tr>
<td>P. leucopus</td>
<td>27(6)</td>
<td>-0.600*</td>
<td>-0.691*</td>
<td>350</td>
<td>675</td>
</tr>
<tr>
<td>P. eremicus</td>
<td>12(4)</td>
<td>+0.643*</td>
<td>-0.224NS</td>
<td>375</td>
<td>670</td>
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<tr>
<td>POOLED DATA</td>
<td>113(23)</td>
<td>-0.526**</td>
<td>-0.326**</td>
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<tr>
<td>(Partial</td>
<td></td>
<td></td>
<td></td>
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<td>correlation)</td>
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<td>correlation)</td>
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</tbody>
</table>
more slowly, with periods of a day or more in which weight was gained. Torpidity occurred on the fifth day of restricted food in 4 of the animals, and on the sixth day in the last, thereafter occurring on an average of two thirds of the days until the larger food ration (2.0 grams millet/day) was instituted. During the 10 days of the larger food ration, torpor occurred only sporadically, although there were no significant gains in weight by any of the animals.

The durations of the periods of torpor were quite variable, ranging from 60 to 470 minutes, even though the food ration remained constant. Initially the durations were not correlated with the daily fluctuations of body weight; but as the experiment progressed, and body weights became progressively lower, there was a general tendency for durations to increase.

IV. Metabolic Rate.

Figure 6 is a representative recording of oxygen consumption during torpor.

Prior to entrance, the metabolic rate fluctuated rapidly but not differently from that of a normally active animal. At the beginning of entrance body temperature and metabolic rate fell nearly simultaneously. It was not possible to determine accurately the relative chronology of the decline of oxygen consumption and body temperature because the two functions were recorded on different recorders at very low chart speeds. Once begun, the decline of metabolic rate was proportionately more rapid than that of body temperature so that a level of oxygen consumption characteristic of a fully torpid animal was reached while the body temperature was still falling. As illustrated in Fig. 7,
The body temperature ($T_B$) and oxygen consumption (MR) of *P. crinitus* No. 6 during a single period of torpor. The body temperature is recorded from an implanted abdominal thermocouple. The oxygen consumption is calculated from the percentage of oxygen removed from air forced past the animal. The ambient temperature ($T_A$) was 12.5°C.
FIGURE 7

The oxygen consumption of *P. crinitus* No. 6 during entrance and arousal from the period of torpor in Figure 5 plotted as a function of body temperature.
Metabolic Rate (cc O₂/gm/hr)

Body Temperature (°C)

P. crinitus 6
- o entrance
- • arousal
the metabolic rate fell as an exponential function of body temperature down to a body temperature of 26°C, but as the body temperature fell from 26°C to 18°C the metabolic rate changed very little. During arousal the reverse situation occurred. The metabolic rate increased proportionately more rapidly than the body temperature in early arousal.

V. Heart Rate and Breathing Rate.

Figures 8 and 9 illustrate the heart rates and breathing rates respectively of two normothermic P. maniculatus at selected ambient temperatures between 2°C and 38°C. Both the heart rates and breathing rates were lowest when the ambient temperature was between 28°C and 33°C, and increased rapidly as the ambient temperature was shifted below 28°C or above 33°C. The low heart rates of 400 to 500 beats/minute increased to 750 to 800 beats/minute at ambient temperatures below 10°C and to 700 to 750 beats/minute at ambient temperatures above 37°C. The low breathing rates were 80 to 150 breaths/minute, increasing to 300 to 400 breaths/minute below 10°C and to 250 to 300 breaths/minute above 37°C. Although it was not systematically examined, this general relationship between the heart rate, breathing rate and the ambient temperature was similar for the other species in the present study.

There was no special pattern of heart or breathing rates which occurred before entrance into torpor. During entrance the mean heart rate and breathing rate fell rapidly and reached the level characteristic of fully torpid animals while the body temperature was still declining (Fig. 10). Thus, during the final 3°C or 4°C of body temperature decline the mean heart and breathing rates remained
FIGURE 8

The heart rates from two normothermic *P. maniculatus* plotted as functions of the ambient temperature. Closed circles = *P. maniculatus* No. 35, open circles = *P. maniculatus* No. 36.
FIGURE 9

The breathing rates from two normothermic *P. maniculatus* plotted as functions of the ambient temperature. Closed circles = *P. maniculatus* 35, open circles = *P. maniculatus* 36. The breathing rates are calculated from the impedance pneumogram recorded from implanted electrodes. Ample time was allowed at each ambient temperature for the breathing rate to stabilize.
FIGURE 10

The body temperature ($T_B$), heart rate (HR) and breathing rate (BR) recorded from *P. maniculatus* No. 6 during a single period of torpor at an ambient temperature ($T_A$) of 15°C.
relatively constant.

In fully torpid undisturbed animals, heart rates and breathing rates were correlated with both the body temperature and the delta T (Table 5). The lowest mean heart rate recorded from a torpid animal was 70 beats/minute from *P. maniculatus* with a body temperature of 20.6°C at an ambient temperature of 19.0°C. The lowest mean breathing rate was 10 breaths/minute from *P. maniculatus* with a body temperature of 20.1°C and an ambient temperature of 15.0°C.

During arousal the heart rates and breathing rates both increased abruptly so that at each body temperature during arousal the rates were considerably faster than at the same body temperature during entrance. Plots of heart rate or breathing rate as functions of body temperature result in mirror-image curves (Figs. 11 and 12) similar to those of oxygen consumption plotted as a function of body temperature (Fig. 7).

Included in Figures 11 and 12 are the mean heart rates and breathing rates of two *P. maniculatus* implanted with EKG electrodes, heavily anesthetized with pentobarbital sodium (Nembutal, 120 mg/kg) and cooled at ambient temperatures of 15°C and 5°C respectively. The heart rates and breathing rates of the anesthetized animals fell between those of the animals entering and arousing from torpor and both heart beats and breaths occurred at very regular intervals.

During the majority of the time spent in torpor no action potentials from skeletal muscle obscured the EKG and impedance pneumograph traces. Breaths remained regularly spaced with no pause between exhalation and inhalation unless the breathing rate was less than 100 breaths per minute. Below this rate there were usually pauses between each breath, and breaths often appeared in groups of two or more followed by periods
TABLE 5

Partial and multiple correlation coefficients of the relationships between log heart rate and body temperature, log heart rate and delta T, log breathing rate and delta T.

** means highly significant (p < .01). * means significant (p < .05). Data from all species were pooled because of the relatively small sample sizes (N = 35 for heart rate; N = 20 for breathing rate).
<table>
<thead>
<tr>
<th></th>
<th>Partial Correlation with Body Temperature Holding Delta T Constant</th>
<th>Partial Correlation with Delta T Holding Body Temperature Constant</th>
<th>Multiple Correlation with Delta T and Body Temperature</th>
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<td>Log. Heart Rate</td>
<td>$r = 0.69^{**}$</td>
<td>$r = 0.72^{**}$</td>
<td>$R = 0.87^{**}$</td>
</tr>
<tr>
<td>Log. Breathing Rate</td>
<td>$r = 0.46^{*}$</td>
<td>$r = 0.57^{**}$</td>
<td>$R = 0.71^{**}$</td>
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FIGURE 11

The heart rates of _P. maniculatus_ No. 6, during the period of torpor shown in Figure 10, plotted versus the body temperature during entrance (open circles) and arousal (closed circles). The closed triangles represent the pooled heart rates of two _P. maniculatus_ anesthetized with sodium pentobarbital (Nembutal) and cooled.
FIGURE 12

The breathing rates of *P. maniculatus* No. 6, during the period of torpor shown in Figure 10, plotted versus the body temperature during entrance (open circles) and arousal (closed circles). The closed triangles represent the pooled breathing rates of two *P. maniculatus* anesthetized with sodium pentobarbital (Nembutal) and cooled.
of apnea usually less than 10 seconds in duration. Pauses occurred only after exhalation. No pattern of breathing was characteristic of a particular species, and all of the patterns were observed in single individuals during single periods of torpor.

Each animal had a characteristic pattern of heart beats during torpor. Figure 13 is a representative recording from an individual of each species. Pronounced irregularities of heart rate with skipped beats were common to P. maniculatus and P. leucopus. Skipped beats occurred only occasionally in P. eremicus, and were not seen at all in P. crinitus. P. eremicus No. 13 consistently had the most pronounced periods of tachycardia of any of the animals. The other 3 P. eremicus had heart beat patterns similar to those of P. eremicus No. 13 during the periods of slow heart rate. Both of the P. crinitus also had arrhythmic heart beat patterns, but with very little grouping of beats.

Neither intraspecific nor interspecific trends in arrhythmia were apparent and a single individual could have a highly arrhythmic heart beat pattern during one period of torpor and much less arrhythmia during another period.

Periodically throughout torpor, particularly toward the end of entrance, the breathing rate increased transiently. Such an increase was followed by an increased heart rate and shivering (or movement) action potentials on the electrocardiogram. These periods during which the mean heart rate increased are marked by arrows on Figure 10. Figures 14 and 15 are actual recordings of the EKG, impedance pneumograph and cardiotach traces from each of four species during typical periods of increased activity.

Normally, after the breathing rate had increased for a few seconds
FIGURE 13

Electrocardiograms during torpor from representative individuals of four species. The ambient temperatures ($T_A$) and body temperatures ($T_B$) are indicated for each eanimal. Attention is called to the large amount of cardiac arrhythmia present during torpor. Electrocardiograms were recorded from implanted electrodes.
FIGURE 14

The electrocardiograms (EKG), breathing rates, and heart rates from representative *P. maniculatus* and *P. leucopus* during torpor when there was increased activity of the skeletal musculature and the heart. The small deflections of the impedance pneumograph trace are not breaths but 60 Hz. interference from external electrical sources. The large deflections of the impedance pneumograph trace when skeletal muscle action potentials have begun to obscure the EKG indicate large changes of impedance accompanying movement. The body temperature ($T_B$) and ambient temperature ($T_A$) are shown.
FIGURE 15

The electrocardiograms (EKG), breathing rates, and heart rates from representative *P. eremicus* and *P. crinitus* during torpor when there was increased activity of the skeletal musculature and the heart. The small deflections of the impedance pneumograph trace are not breaths but 60 Hz. interference from external electrical sources. The large deflections of the impedance pneumograph trace when skeletal muscle action potentials have begun to obscure the EKG indicate large changes of impedance accompanying movement. The body temperature \((T_B)\) and ambient temperature \((T_A)\) are shown.
Peremicus 13  \( T_A = 15 \text{C} \)  \( T_B = 21 \text{C} \)

EKG

Breaths

Beats/Minute

P. crinitus 3  \( T_A = 24 \text{C} \)  \( T_B = 26 \text{C} \)

EKG

Breaths

Beats/Minute

10 SECONDS

10 SECONDS
the animal made postural adjustments which obscured the impedance pneumograph trace, and the muscle action potentials were often great enough to interfere with the cardiotach operation. After the muscle action potentials subsided, the EKG resumed its irregular pattern and there was usually a period of apnea 5 to 30 seconds long. The apnea was either terminated by another period of rapid breathing as in Figure 14, or by resumption of the pattern present before the disturbance.

A period of abruptly increased heart rate accompanied by movement action potentials was always preceded by rapid breathing, but a period of rapid breathing was not necessarily followed by any change in the heart rate.

In order to examine the possibility that the cardiac arrhythmia during torpor resulted from autonomic nervous activity reaching the heart, atropine sulfate and acetylcholine chloride were infused through indwelling intraperitoneal catheters into fully torpid, undisturbed animals. Isotonic saline solution was used as a control infusion.

Figure 16 illustrates the change in heart rate after infusions of saline. The responses of the different animals were varied though most of the animals were unaffected by the physiological saline. In only one instance (P. eremius 18) did the infusion result in partial arousal. The body temperatures of all of the other animals remained within 1°C of the body temperature at the time of infusion.

Following atropine infusion (Figure 17) the heart rates of all of the animals abruptly increased and in some cases, remained at the increased level up to the termination of the experiment. Accompanying the increased heart rate was a decrease of cardiac arrhythmia. The heart beats normally became evenly spaced between 10 and 20 minutes
FIGURE 16

The heart rates (plotted as percent of the heart rate at the time of infusion) before and after the infusion of isotonic saline solution into the peritoneal cavities of torpid *Peromyscus* through indwelling catheters. All animals except *P. eremicus* No. 18 were fully torpid (see text). Electrocardiograms were recorded from implanted electrodes and heart rate was calculated.
The heart rates (plotted as percent of the heart rate at the
time of infusion) before and after the infusion of atropine
sulfate solution into the peritoneal cavities of fully torpid
Peromyscus through indwelling catheters. Electrocardiograms were
recorded from implanted electrodes.
after the infusion, then slowly returned to the pre-infusion level of arrhythmia. Arousal occurred in only one instance (*P. maniculatus* 4), and the body temperatures of the other animals remained within 1°C of the body temperature at the time of infusion.

Infusion of acetylcholine (Fig. 18) was followed by an immediate decrease in the mean heart rate which was accomplished by general bradycardia and many skipped beats. Shortly after the initial slowing, the heart rate began to increase with both a decrease in the number of skipped beats, and a general tachycardia. Complete or partial arousal was accompanied by an increase in body temperature greater than 6°C in every instance.
FIGURE 18

The heart rates (plotted as percent of the heart rate at the time of infusion) before and after the infusion of acetylcholine chloride (ACh) into the peritoneal cavities of fully torpid Peromyscus through indwelling catheters. Electrocardiograms were recorded from implanted electrodes.
DISCUSSION

I. Body Weight, Stored Lipids and Occurrence of Torpor.

If conditions of disturbance and ambient temperature are within the ranges suitable for torpor, then the factor which probably has the greatest effect on whether or not torpor will occur is food availability. Most animals known to enter torpor will do so when fasted (Hudson and Bartholomew, 1964) and Peromyscus are no exception. Furthermore, many animals, like Peromyscus apparently do not enter torpor at all unless partially starved (Davis, 1966; Pengelley and Kelley, 1966; Hudson, 1965, 1964; Peiponen, 1965; Lasiewski and Dawson, 1964; Carpenter, 1963; Bartholomew and Cade, 1957; Marshall, 1955; Koskinies, 1949). Evidently then, torpidity which occurs only in response to actual starvation is not uncommon and will probably be shown to occur in many more small animals as more species are examined.

It is not known whether the physiological effects of fasting which cause torpor result from depletion of total energy reserves, or from relatively short term changes such as accumulation of certain metabolites or temporary depletion of oxidizable substrate. There is evidence in different species for both possibilities. Certain birds do not become torpid until as much as 34% of their initial body weight has been lost through starvation (Lasiewski and Dawson, 1964; Peiponen, 1965) while the woodchuck enters torpor when starved and relative thinness or fatness has little effect (Davis, 1966).

Since Peromyscus normally lose more than 10% of their initial body weights prior to entrance into torpor and animals above a certain weight do not become torpid, it appears that the absolute fatness is,
in some way, linked to the onset of torpor in *Peromyscus*. However, the obesity of *Peromyscus* in the laboratory (Morhardt and Hudson, 1965) is often considerably more than that of the same species in the field (Hayward, 1965) so that percent loss of weight in the laboratory may not be the same weight loss necessary to induce torpor in the field. For example Hayward (op. cit.) found that the mean field weight of *P. maniculatus sonoriensis* (14.1 grams) was only 68.3% of the mean laboratory weight (20.6 grams). In other words, the animals would have to lose 31.7% of their initial laboratory weights to resume normal field weight. No animal in the present study lost this large a percentage of the laboratory weight before entering torpor for the first time. It is suggested that animals in the field may be of sufficiently low weight to enter torpor with little or no fasting.

The weight of stored body lipids provides a reasonable index of reserve energy available to an animal and has been measured to determine the rate of depletion of stored energy reserves during prolonged hibernation in the European hedgehog (Weinland, 1925). The mean total body lipid content prior to torpor in *Peromyscus* was greater than that of freshly caught field animals (Hayward, 1965). Starvation sufficient to cause significant weight and lipid loss may be necessary only in unusually fat animals, and torpor may be resorted to by animals in the field at any time the food intake is insufficient to prevent weight loss.

II. Body Temperature.

Variable body temperature is common in normothermic nonstressed *Peromyscus* (McNab and Morrison, 1963) so that many of the fluctuations of body temperature prior to entrance into torpor represent normal
activity. Fluctuations of a larger magnitude which result in a gradual drop of several degrees which returns to normal levels before entrance have been recorded in the poorwill (Bartholomew et al., 1962) the California ground squirrel (Strumwasser, 1959a), the pygmy mouse (Hudson, 1965) and to a lesser degree in the woodchuck (Lyman, 1958), as well as in *Peromyscus*.

An exponential decrease of body temperature during entrance has been pointed out only in hummingbirds (Lasiewski and Lasiewski, 1967) where it was considered to represent a Newtonian cooling curve and interpreted to mean that "...the rate of entry is determined by physical phenomena...". This interpretation may reasonably be extended to that portion of cooling which is exponential in *Peromyscus*, particularly since there are no indications of shivering or periodically increased metabolic rate during this phase. These activities might be expected if the animal were periodically changing the amount of control exerted over the body temperature.

At the time when shivering, movement, and transiently increased heart and breathing rates are observed in *Peromyscus* entering torpor, there is usually a noticeable deviation of the body temperature from the exponential cooling curve. Varying degrees of muscle tone and periods of shivering do occur throughout entrance in the California ground squirrel (Strumwasser, 1959b) and are accompanied by "...plateaus and gentle steps of declining (body) temperature..." rather than the initial smooth cooling curve observed in *Peromyscus*. Shivering then acts "...as a brake slowing down the rate of fall of temperature..." (Strumwasser, op. cit.) throughout entrance in the squirrel but primarily toward the end of entrance in *Peromyscus*. 
Since the exponential fall in body temperature of all individuals of all five species was interrupted toward the end of entrance and widely variable delta T's occurred throughout torpor it appears that the body temperatures of Peromyscus are regulated throughout torpor. The fact that the body temperature does not passively follow changes in the ambient temperature supports this view.

The regulation of the body temperature well above the ambient temperature while torpid at a wide range of ambient temperatures distinguishes Peromyscus from most heterotherms which either do not regulate the body temperatures at all during torpor, or begin to regulate only if the ambient temperature is so low that it threatens to force the body temperature below the critical arousal temperature.*

In the former category are a number of birds including (along with their critical arousal temperatures) four species of hummingbirds at 13°C (Lasiewski, 1963), the poorwill at 15°C (Howell and Bartholomew, 1959), and the white-throated swift at 20°C (Bartholomew et. al., 1957).

The animals which apparently regulate their body temperatures only if threatened with a specific low temperature include the European hedgehog at 4.5°C (Kristoffersson and Siovio, 1964b), several bats at 5°C (Henshaw and Folk, 1962; Reite and Davis, 1966), several ground squirrels at 6°C to 12°C (Pengelley and Kelly, 1966; Pengelley, 1964; Bartholomew and Hudson, 1960; Strumwasser, 1959a) and two species of pocket mice at 13°C to 15°C (Wang, unpublished masters thesis, Tucker, 1962).

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*The body temperature below which an animal can no longer spontaneously arouse and will die unless, or in some species, even though artificially rewarmed (MacMillen, 1965).
There are a few heterotherms like *Peromyscus* however which do maintain a variable and sometimes large gradient between the ambient temperature and the body temperature during torpor. Included are a pygmy possum (Bartholomew and Hudson, 1962), the eastern chipmunk (Panuska, 1959; Cade, 1963; Jameson, 1964), the Mexican free-tailed bat (Herreid, 1963) the pygmy mouse (Hudson, 1965), the Madagascar hedgehog (Kayser, 1960), the European nightjar (Peiponen, 1965, 1966) and the inca dove (MacMillen and Trost, 1967).

The lowest body temperatures observed during torpor in the present study, with the exception of a single *P. maniculatus*, were above the critical arousal temperatures of all known heterotherms except the pygmy mouse (20°C) and the white-throated swift (20°C).

In the California ground squirrel (Strumwasser, 1959a) the critical arousal temperature is not fixed for each individual animal, but apparently reflects the state of physiological preparedness for torpor. Thus the animal makes several approaches toward what appears to be a limiting body temperature during each period of torpor and during subsequent periods of torpor the limiting level becomes lower. Strumwasser (op.cit.) considers these successively deeper periods of torpor to be "test drops" as the animal becomes prepared for seasonal hibernation.

*Peromyscus* are clearly not making test drops in the same sense as the California ground squirrel because the level to which the body temperature falls in *Peromyscus* is not necessarily related to previous experience. It is possible, however, that the levels of body temperature that are maintained during torpor reflect daily changes in the critical arousal temperatures, and in turn, daily changes in the physiological ability to withstand low body temperatures.
There are some interesting discrepancies between MacMillen's (1965) data on the body temperature of *P. eremicus* during torpor and that reported in the present study. MacMillen found that *P. eremicus* would enter torpor at ambient temperatures below 10°C but that the delta T was never greater than 3°C and decreased as the ambient temperature was lowered. In all animals in the present study the delta T increased as the ambient temperature was lowered and on only two occasions out of 25 periods of torpor from 5 *P. eremicus* did the body temperature come within 3°C of the ambient temperature. The discrepancy is difficult to explain. The most likely possibility seems to be that MacMillen's animals were starved to the point that they were incapable of maintaining more than a very small delta T and incapable of preventing the body temperature from dropping below the 15°C level that MacMillen considers to be the critical arousal temperature. In further support of this explanation the body temperatures of the *P. eremicus* in the present study did not fall below 19°C even though the animals became torpid at ambient temperatures as low as 9°C, while MacMillen's animals consistently had body temperatures as low as 16°C.

The arousal rates of *Peromyscus* are generally slower than those from other species of approximately the same weight. When the arousal rates are calculated from published data in the same way that the rates were calculated in the present study, a kangaroo mouse which weighed 15.2 grams aroused at a rate of 1.1°C/minute (Bartholomew and MacMillen, 1961), a birch mouse weighing 11 grams aroused at 1.4°C/minute (Johansen and Krog, 1959), a California pocket mouse weighing 21 grams aroused at 0.88°C/minute (Tucker, 1962, 1965a, 1965b) and a murine opossum weighing 14 grams aroused at a rate of 0.66°C/minute (Morrison and
McNab, 1962).

The significance of arousal rates is not obvious and may simply reflect the maximum metabolic rate available to an animal, which in turn reflects the level at which the normothermic animal must produce heat to maintain a normal body temperature at ambient temperatures below the thermal neutral zone (Hudson and Bartholomew, 1964).

It is extremely interesting that there are no great differences in the way in which the body temperature is related to the ambient temperature during torpor among the five species of Peromyscus. Small differences, such as the critical arousal temperatures, deviation of P. leucopus from the regression in Figure 5, or the maximum arousal rates, are probably all attributable to individual variation rather than species variation. The five species appear to be essentially homogeneous in their capacity for torpor.

III. Duration of Torpor.

The duration of a single period of torpor ranges from a few hours in animals which use daily torpor (Hudson and Bartholomew, 1964) to several weeks or more in animals which use seasonal torpor (Lyman and Chatfield, 1955). Hudson and Bartholomew (op.cit.) point out that "it is unreasonable to force all birds and mammals which are capable of becoming periodically dormant into two categories, daily or seasonal..." because there are species which show intermediate periodicities of torpor.

In the majority of animals which remain torpid for periods of time no longer than a day, torpor occurs during the normal period of daily inactivity and is over before the time in which the animal would normally begin to seek food (Lasiewski, 1963; Ruschi, 1949; Morrison, 1962; Hudson, 1965; Tucker, 1962; Leitner, 1966; MacMillen and Trost, 1967).
The characteristic arousal from torpor at feeding time is particularly evident in the European nightjar which can become torpid twice daily, arousing at dusk and at dawn to feed on insects, but becoming torpid in between (Peiponen, 1966). Behaviorally, there is a very real distinction between the daily torpor of these animals, including Peromyscus, in which torpidity does not interfere with the animal's normal daily activities, and other patterns of torpor including seasonal and intermediate forms, such as that of the pygmy possum (Bartholomew and Hudson, 1962), in which the daily cycle of activity is abandoned.

The duration of torpor in the species of Peromyscus examined here is habitually less than 8 hours, and was in no case as long as 12 hours, suggesting that these animals are limited to daily torpor. MacMillen (1965) has suggested that P. eremicus might "...enter a prolonged state of torpor lasting several days to a week or more..." but that suggestion is based on data from obviously moribund animals incapable of spontaneous arousal and seems an unlikely proposition considering the data presented here.

Since daily torpor occurs in Peromyscus and other species in response to food deprivation and serves to reduce the amount of energy needed to maintain weight (Tucker, 1962), the duration of torpor, if it were the primary factor determining the amount of energy conserved during torpor, might be expected to be influenced by the amount of food available. The pygmy mouse remained torpid a larger percentage of the time when the food ration was small than when it was large (Hudson, 1965) and the California pocket mouse remained torpid for increasingly long periods of time as the food ration was diminished (Tucker, 1962), thus confirming the expectation. In both of these species, at the level of
ambient temperature at which the experiments were performed, the body
temperature fell predictably close to the ambient temperature and the
delta T was relatively constant throughout the series of experiments.
In *Peromyscus* neither the body temperature, nor the delta T is predict-
able and since the level of both of these influences the rate of
oxygen consumption during torpor (Hudson and Bartholomew, 1964); it is
not surprising that the duration of torpor is independent of the daily
food ration.

In a number of animals which become torpid on a seasonal basis the
duration of individual periods of torpor is understandably not influenced
by food availability since food may be completely unavailable for
months. This is the case when the source of metabolic energy is stored
depot fat (Kayser, 1961). In a few instances, food may be available
in excess as is usual for the hamster (Pengelley, 1967). In animals
where duration is not related to food availability, duration is related
to the body temperature or to the ambient temperature. The relationship
is such that the greater the metabolic rate during torpor, the shorter
the duration. Arousal is postulated to occur sooner at high ambient
temperatures because of an increased rate of production of unwanted
metabolites (Fisher, 1964), a depletion of some necessary substance
which must be replaced upon arousal (Twente and Twente, 1965) or an
increased sensitivity to endogenous (Strumwasser, 1964) or exogenous
(Twente and Twente, 1966) stimuli.

It is interesting that the duration of torpor of *Peromyscus* is
similarly related to the absolute body temperature, because it suggests
that the duration of torpor in animals which use only daily torpor may
be limited by physiological mechanisms of the same type that apparently
limit the duration of periods of seasonal torpor.

The relationship between duration of torpor and the delta T has not been systematically examined elsewhere, but there is evidence that in a few species an increased delta T shortens the duration (Wang, unpublished master's thesis; Pengelley and Kelly, 1966). If, as most of the work exploring the relationship between duration and body temperature suggests, the duration is shortened by an increased metabolic rate, then it is reasonable that the duration of torpor should be inversely related to the delta T. The present data are consistent with this idea.

IV. Metabolic Rate.

The mean level of oxygen consumption before entrance, shown in Figure 7, is considerably higher than the mean value of about 5 cc O_2/gram hour determined for _P. crinitus stephansi_ in the resting state at an ambient temperature of 12.5°C (McNab and Morrison, 1963). A similarly high metabolic rate prior to entrance is shown for _P. eremicus_ (MacMillen, 1965), suggesting that the animals are relatively active until immediately prior to entrance. If this is true, then much of the initial decline of metabolic rate is simply due to a cessation of activity and a passive decrease of metabolic rate to the basal level. In addition to the initial passive decline of metabolic rate however, the oxygen consumption continues to fall proportionately much more rapidly than the body temperature. If there were not an abrupt decrease in the rate of decline of oxygen consumption near a body temperature of 25°C, the metabolic rate during torpor would extrapolate to half of the level that is actually observed.
It is possible that the metabolic rate during the entire initial phase of entrance falls passively in a temperature-dependent way and the change of rate near 25°C indicates the resumption of metabolic regulation that prevents the body temperature from falling to near the ambient temperature, or below the critical arousal temperature. The temperature-rate function during the initial phase of cooling ($Q_{10} = 4.1$) is somewhat greater than would be expected from the passive behavior of isolated tissues (South, 1958; Meyer and Morrison, 1960) and considerably greater than that of the California pocket mouse during cooling ($Q_{10} = 1.6 - 3.2$) (Tucker, 1965a). Morrison (1960) has suggested a model in which an equally rapid cooling function might be explained as passive behavior in the Arctic ground squirrel. In other species as well, the decline of metabolic rate during entrance may be explained in terms of passive behavior (Strumwasser, 1959b; Tucker, 1965b).

Alternatively it is possible that the metabolic rate is being actively depressed below the temperature dependent level during the initial phase of entrance, and that the body temperature falls primarily as a result of a decreased metabolic rate. Once the metabolic rate reaches a level characteristic of torpor it ceases to fall any further and is maintained at roughly constant levels while the body temperature continues to decline. Similar data from the golden hamster was interpreted to indicate active depression of oxygen consumption during entrance (Lyman, 1948). In support of the possibility of active depression of the metabolic rate there is independent evidence that the oxygen consumption of several small animals may be forced below the basal level, even in the zone of thermal neutrality (Hudson, unpublished; Popovic and Popovic, 1956; Lasiewski, 1963). It seems reasonable that
the ability to abruptly lower the metabolic rate would be useful to facilitate entrance into torpor, and it seems likely that such facilitation is being used by *Peromyscus*.

V. Heart Rate and Breathing Rate.

In all species so far observed the heart rate falls prior to any decline of the body temperature during entrance into torpor (Lyman, 1965). In the thirteen-lined ground squirrel the breathing rate also falls before any decline in heart rate (Landau, 1956). Lyman and O'Brien (1963) conclude that "...these vital functions are being repressed by something more than changes in temperature alone...".

In animals as small as *Peromyscus* it is difficult to observe the chronology of the initial depression of the body temperature, heart rate, breathing, and metabolic rate because the large surface area relative to the body weight is conducive to rapid cooling, and therefore measurable changes of body temperature occur much more rapidly than in the larger animals after any metabolic change. Instead, the relative rates of decline during entrance and of increase during arousal were determined. These resulted in mirror-image relationship of rate-temperature curves, and similar results have been observed in the European hedgehog and in ground squirrels (Dawe and Morrison, 1955; Strumwasser, 1959b).

Strumwasser (1959b, 1960) suggested that this relationship indicates that the heart rate is not passively dependent of the body temperature but is driven below the temperature-dependent level during entrance by parasympathetic activity, and above the temperature-dependent level during arousal by sympathetic activity. To evaluate this possibility
it is necessary to measure the temperature-dependent heart rate, therefore the heart must be isolated from central nervous system influence. Rushmer (1962) notes that (in dogs) anesthetic agents cause most of the spontaneous fluctuations of heart rate to disappear as though the cardiovascular system were completely severed from the central nervous system, and Nash et al. (1956) have shown that sodium pentobarbital (Nembutal) has a vagal blocking action. Barbiturates are also known to depress respiration (Lumb, 1963).

Since Peromyscus cooled under heavy pentobarbital anesthesia have heart rates and breathing rates intermediate between the rates of animals entering and arousing from torpor, there must be an active depression of heart rate and breathing rate during normal entrance into torpor and active increase during normal arousal from torpor.

The heart rate in woodchucks cooled under Nembutal anesthesia is also always more rapid at any given body temperature than the heart rate of animals entering torpor (Lyman, 1958). The very even spacing of heart beats and breaths in Peromyscus under Nembutal further indicates that irregularities and arrhythmias during entrance result from central nervous system activity rather than from direct effects of cooling on the heart.

Cardiac arrhythmia during torpor occurs in most animals (Johansson, 1967) but is usually expressed simply as the occurrence of skipped beats, or in relative terms. The extreme variation in the amount of arrhythmia from animal to animal, and during different periods of torpor in individual Peromyscus has not been demonstrated in other species. The fact that the arrhythmia is variable, particularly within the same animal suggests that central control of the heart is maintained
throughout torpor.

The obvious synchronization of breathing, heart rate, and muscle action potentials periodically throughout torpor in *Peromyscus* has also been observed in the California ground squirrel (Strumwasser, 1959c) and supports the idea that central control is retained during torpor.

The nature of central control of the heart during torpor has been examined in the hamster (Biewald and Raths, 1959) and the ground squirrel (Lyman and O'Brien, 1960, 1963). By stimulating the vagus nerves of hibernating hamsters while recording the EKG, Biewald and Raths (1959) demonstrated that below 10°C to 12°C the heart was unresponsive to vagal stimulation. Lyman and O'Brien (1963) showed that in the thirteen-lined ground squirrel stimulation of the vagus nerve during deep torpor had no effect on the heart rate.

By blocking the parasympathetic nervous system of the thirteen-lined ground squirrel with atropine, Lyman and O'Brien (1960, 1963) demonstrated that the variability of the heart rate and the skipped beats during entrance and the early part of torpor were mediated by parasympathetic activity. The variability of heart rate decreased as the animal became more fully torpid so that some time after entrance atropine, which exerts its influence on the heart principally by blocking pre-existing vagal activity (Ahlquist, 1965), failed to have any noticeable effect. In *Peromyscus* the heart beats are irregularly spaced throughout torpor, and atropine infusion always caused an increase in heart rate and a decrease in beat to beat variability. It therefore appears that in *Peromyscus* the parasympathetic nervous system is active throughout torpor in contrast to the ground squirrel and the hamster.
Attempts to slow the heart of the thirteen-lined ground squirrel during torpor with the parasympathomimetic drug acetylcholine met with failure (Lyman and O'Brian, 1963) because there was invariably an increase in heart rate before the drug had time to reach the heart. The increase in heart rate was apparently due to hyper-sensitization of the peripheral skeletal musculature to acetylcholine during torpor or to reflex correction of acetylcholine induced vasodilation, rather than to any effect on the heart itself. In Peromyscus during torpor the heart responded to infused acetylcholine in exactly the same way that it did in the active animal. The initial pronounced slowing probably results from action of the drug directly on the heart. The subsequent tachycardia appears to be a reflexive response to lowered blood pressure which probably accompanies the acetylcholine-induced bradycardia. The heart then is definitely sensitive to the vagal neurohormon, acetylcholine, during torpor. This fact reinforces the probability that the heart rate remains under autonomic control during torpor.

It must be noted that thirteen-lined ground squirrels torpid with body temperatures similar to those recorded for Peromyscus are actually still entering torpor. During entrance, their response to atropine infusion is similar to the response of fully torpid Peromyscus. Furthermore, it apparently takes at least 10 hours for the ground squirrels to become fully torpid (Lyman and O'Brian, 1960, 1963) and show no parasympathetic activity, and Peromyscus normally do not remain torpid for as long as 10 hours. Physiologically Peromyscus appear to remain, throughout torpor, in a state similar to that of entrance in the thirteen-lined ground squirrel.
During torpor the heart rate is positively correlated with the body temperature in hummingbirds which have a delta T consistently smaller than 1.2°C (Lasiewski, 1964), in the pygmy mouse with a delta T smaller than 3.2°C (Hudson, 1965), and in the European hedgehog when the delta T is less than 2.0°C (Kristoffersson and Soivio, 1964a). The similar correlation in *Peromyscus*, although significant, is not very strong and indicates that the heart rate during torpor is not strictly temperature dependent. The relative independence is emphasized by the fact that after atropine injections the heart rate can increase to over 200% of the pre-injection level with practically no change in the body temperature. Lyman and O'Brien (1963) observed similar atropine-induced increases in heart rate in torpid thirteen-lined ground squirrels while the body temperature continued to decline.

The breathing rate of *Peromyscus* during torpor is even more independent of the body temperature than is the heart rate. The breathing rate declines during entrance into torpor and is therefore positively related to the body temperature, but the breathing rates from fully torpid animals are weakly negatively correlated with the body temperature. There is no obvious reason why a negative relationship between body temperature and breathing rate should exist during torpor.

The heart rates and breathing rates of small, non-torpid birds and mammals are strongly influenced by the ambient temperature, increasing as the ambient temperature is shifted above or below certain critical levels (Odum, 1945; Bartholomew and Hudson, 1962, Bartholomew et al., 1962, Lasiewski and Dawson, 1964; Hudson, 1964, 1965). Bartholomew and Hudson (1962) have noted that these critical levels correspond to the upper and lower critical temperatures of the thermal neutral zone in several
species. Indeed, the ambient temperature range at which the lowest non-torpid heart, and breathing rates were recorded in the present study correspond to the thermal neutral zone as determined by oxygen consumption measurements (McNab and Morrison, 1963). As the ambient temperature is lowered, heart and breathing rates increase as the delta T increases in normothermic animals.

In the torpid bats *Myotus lucifugus* and *Lasiurus borealis* (Reite and Davis, 1966), and in the European hedgehog (Kristoffersson and Soivio, 1964b) the delta T remains small and constant unless the ambient temperature falls below 5°C. While the delta T is small and constant, the heart rate and delta T increase simultaneously and the heart rate becomes a function of the delta T as it is in normothermic animals. This suggests that the same factors which maintain physiological control of the body temperature in normothermic animals are active in bats and the hedgehog during torpor if the ambient temperature falls below 5°C. In torpid *Peromyscus* the delta T is variable throughout the entire range of ambient temperatures examined, and over this range the heart rate is a function of both delta T and body temperature. It therefore appears likely that the same physiological mechanisms which control the heart rate in normothermic animals remain active throughout torpor in *Peromyscus* at all ambient temperatures.

The breathing rates of torpid *Peromyscus* are correlated with the delta T in the same way as are the heart rates. When the delta T increases so does the breathing rate. A similar correlation has been observed in the European hedgehog (Kristoffersson and Soivio, 1964b); the only
other species in which the relationship has been systematically examined. The same situation exists in normothermic *Peromyscus* and it appears that the control mechanisms that regulate the breathing rate during torpor are identical to those functional in the normothermic animal.
SUMMARY

Five species of white-footed mice, *Peromyscus maniculatus sonoriensis*, *P. leucopus texanus*, *P. crinitus stephensi*, *P. eremicus fraterculus* and *P. boylei rowleyi*, when exposed to ambient temperatures between 0°C and 23°C without food periodically entered a state of torpidity in which their body temperatures fell below the normal activity levels of 35°C to 40°C. Before entering torpor the animals lost between 6% and 32% of their initial body weight. Some animals torpid for the first time retained a considerably larger percentage of their total body weight in the form of lipids than did animals which had been starved to death, indicating that total depletion of depot fat was not a prerequisite for torpor.

During entrance into torpor the body temperature rapidly fell toward the ambient temperature until it was within 1°C to 23°C of the ambient temperature. The difference between body temperature and ambient temperature (delta T) was variable at any ambient temperature, but the mean delta T increased as the ambient temperature was lowered.

The lowest body temperatures from which animals spontaneously aroused were 13.4°C for *P. maniculatus*, 17°C for *P. leucopus* and *P. crinitus*, 18°C for *P. boylei*, and 19°C for *P. eremicus*. Although arousal from torpor occurred spontaneously, it could also be elicited by mild disturbance. Maximum arousal rate for any individual was 0.64°C/minute and the mean rate for all arousals was 0.29°C/minute.

The maximum duration of an individual period of torpor was 670 minutes and the mean duration of all periods of torpor was 340 minutes.

During entrance heart rates, breathing rates, and oxygen consumption
fell rapidly and reached levels characteristic of fully torpid animals while body temperatures were still declining. During arousal these rates increased abruptly so that at each body temperature during arousal the rates were higher than at the same body temperature during entrance.

In the fully torpid, undisturbed animal, heart rates and breathing rates were correlated with both the body temperature and the delta T. The lowest mean heart rate was 70 beats/minute and the lowest mean breathing rate was 10 breaths/minute.

The heart beat pattern during torpor was arrhythmic with occasional skipped beats. Breaths were normally evenly spaced. Periodically throughout torpor there were short periods of activity beginning with an increased breathing rate, then an increased, and more rhythmic heart rate, finally partially obscured by muscle action potentials accompanying shifting about by the animal. When the muscle action potentials ceased there was generally a period of apnea lasting as long as 30 seconds, and a gradual decrease in heart rate and increase in cardiac arrhythmia.

Cardiac arrhythmia during torpor was blocked, and heart rate increased, by intra-peritoneal infusion of the parasympatholytic drug atropine sulfate, suggesting that vagal activity was partially responsible for the low heart rates present during torpor. The heart rate was abruptly slowed by infusion of the parasympathomimetic drug, acetylcholine chloride, demonstrating that the heart retained its sensitivity to parasympathetic activity during torpor.

The data presented in this thesis strongly suggest that the ability of successfully use torpidity is widespread in the genus Peromyscus. The short duration of torpor suggests that if torpor occurs in nature
it must serve as a very short-term means of conserving energy, occurring in place of, or as an extension of normal inactivity during sleep.

Physiologically, torpidity in Peromyscus resembles a deep sleep. There is a profound lowering of the body temperature and slowing of physiological processes, but there is no evidence at all to suggest a temporary abandonment of regulation. Body temperature, heart rate, and breathing rate, as well as skeletal musculature all appear to be closely regulated during torpor, and torpidity in Peromyscus should be thought of as a well controlled, by no means accidental or inadequate, form of temperature regulation.


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