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SEASONAL AND GEOGRAPHIC DIFFERENCES
IN COLD RESISTANCE AND HEAT
PRODUCTION CAPABILITIES OF
TWO SPECIES OF PEROMYSCUS

by

LARRY ALVIN DIDOW, B.Sc., M.Sc.

A thesis submitted to the Faculty of
Graduate Studies in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

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September 5, 1972

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ABSTRACT

Cold resistance and heat production capabilities were investigated in white-footed mice (Peromyscus leucopus noveboracensis) and woodland deermice (Peromyscus maniculatus gracilis) collected from the northern and southern portions of their ranges in winter and summer. Oxygen consumption rates and the time these rates could be maintained were measured over a range of low ambient temperatures for each group of acclimatized mice. The range of low exposure temperatures established for each group of mice induced a maximal rate of heat production and resulted in metabolic failure in 1 to 6 hr. The interrelations of ambient temperature, metabolic failure time, and maximal heat production rate were directly related to the previous thermal conditioning of the mice and their geographic distribution.

Winter mice showed the greatest resistance to severe cold stress. They were able to thermoregulate at temperatures 5° to 10 C° lower than summer mice and they were able to maintain their oxygen consumption rate 3.5 to 5.0 times longer at comparable temperatures of severe cold stress. This ability was due primarily to a significantly higher maximal heat production rate in the winter mice. Mice from the more northern latitudes in winter also appeared to have a lower rate of heat loss than the summer mice.

Geographic differences in cold resistance and heat production capabilities corresponded directly to the differences

in climatic conditions in the various study areas. Mean daily minimum temperatures were used as indices of climatic conditions. Mice from the more northerly latitudes, in both summer and winter, showed greater resistance to severe cold exposure than the mice from the most southerly latitude. In winter, a slightly higher maximal heat production rate coupled with a much lower heat loss rate enabled the more northerly groups to maintain their oxygen consumption rates significantly longer at the same low exposure temperatures. In summer, the geographic differences in cold resistance were due to significantly higher maximal heat production rates in the northerly groups. Mice from the northern latitudes in summer could also maintain their maximal oxygen consumption rate at temperatures 5 C° lower than the southern group.

P. maniculatus gracilis and P. leucopus noveboracensis collected from the same geographic areas in summer showed similar cold resistance and heat production capabilities. In winter, the maniculatus had a slightly higher maximal heat production rate and appeared to have a lower heat loss rate during severe cold exposure.

Both species of Peromyscus were maintained under constant laboratory conditions to determine genetic differences in thermoregulatory abilities between the two forms and to evaluate the effects of acclimatization on their innate ability to withstand severe cold. Acclimation to 22° C reduced cold resistance in both the northern and southern

leucopus. Maximal heat production rates were significantly lower than those of the acclimatized mice. Acclimated leucopus also showed very poor positioning behaviour during severe cold exposure. This behaviour increased their rate of heat loss. The cold resistance of acclimated maniculatus was similar to that of the summer maniculatus despite a lower maximal heat production rate. Lower heat loss rates were also indicated in the acclimated maniculatus during cold exposure.

Maximal heat production rates were approximately the same in the acclimated leucopus and acclimated maniculatus; however, the maniculatus could thermoregulate significantly longer at comparable exposure temperatures and maintain their oxygen consumption rate at temperatures 10 C° lower than the acclimated leucopus.

Oxygen consumption responses to noradrenaline injection suggest that nonshivering thermogenesis may account for 45 to 60% of the heat produced during severe cold exposure in all groups of mice.

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INTRODUCTION

Homeostasis in general refers to the stable conditions maintained within living organisms. Such conditions are maintained by regulatory responses of coordinated physiological processes known as homeostatic mechanisms. One of the many homeostatic mechanisms found in birds and mammals is the ability to regulate their body temperature within a very narrow range. These animals are, therefore, known as homeotherms. The physiological processes involved in thermoregulation enable homeotherms to survive in a wide range of climatic conditions. However, thermoregulation, like all physiological processes, is only functional within limits. Therefore, the climatic conditions under which homeotherms exist can be determined by the limits of thermoregulatory responses which compensate for alterations in their internal temperature.

The fundamental criterion for homeothermy is the ability of animals to balance the rates of heat exchange with their environment. The basic thermodynamic process which describes the heat exchange between a homeotherm and its environment can be stated as follows:

$$Q = H_m \pm H_d \pm H_c \pm H_r - H_e$$

where Q = heat content of the animal; H_m = rate of metabolic heat production; H_d = rate of conductive heat transfer; H_c = rate of convective heat transfer; H_r = rate of radiative heat transfer; and H_e = rate of evaporative heat transfer.

Therefore, maintenance of heat balance in homeotherms is done in relation to their metabolism by varying the rates of heat exchange through the physical processes of radiation, conduction, convection, and evaporation. The regulation of internal body temperature in turn is believed to be dependent upon feedback systems which control the production, transfer, and dissipation of heat (Hammel 1968).

Since the maintenance of a constant body temperature depends upon a balance between the rates of heat production and heat dissipation, Scholander et al. (1950a,b,c) recognized three possible avenues for climatic adaptations in homeotherms. These include 1) adjustments of the body-to-air temperature gradient by behavioural orientation to environmental temperature differences in the natural habitat (e.g. inhabiting less stressful microhabitats, nest building, temperature selection, huddling); 2) adjustments in heat dissipation mechanisms such as changes in tissue insulation (vasoconstriction, vasodilatation, and piloerection) and evaporation; and 3) adjustments in heat production rates through metabolic activity. Therefore, under natural environmental conditions, the success of homeotherms in reducing imposed thermal stresses by behavioural and physiological mechanisms not only determines their limits of temperature regulation but also influences their ecological relationships and geographic distributions. Consequently, an understanding of the relationships between an animal and its thermal environment can be derived from a description of

the energetics of heat production and heat dissipation within the functional range and limits of the animal (Hart 1957).

Many studies have been conducted on the effects of climate and temperature on the thermoregulatory abilities of homeotherms. Most of these studies have been summarized in a number of review articles. For example, Hart (1957, 1963) reviewed the energetics of homeotherms in relation to changes in climate and temperature. More recently, Whittow (1971) edited a comprehensive review on the comparative physiology of thermoregulation of mammals. In addition, Chaffee and Roberts (1971) summarized the recent studies devoted to the physiological and biochemical mechanisms associated with temperature acclimation and acclimatization in homeotherms. Studies on the geographic distribution of birds and mammals in relation to their thermoregulatory adaptations are discussed by Scholander et al., (1950a,b,c), Hart (1964), Gates (1969), McNab (1970), and Yarbrough (1970).

It is clear from many of these studies that major changes in the physiology and behaviour of small mammals occur in response to changes in environmental conditions. The temperature-adaptive significance of these physiological changes, however, varies considerably amongst species. Furthermore, these changes may be directly related to the previous thermal history of the animals and their geographic distribution.

Earlier studies have shown that animals previously conditioned to a cold environment are better adapted to withstand cold stress than those conditioned to a warm environment. In addition, many physiological differences have been demonstrated between warm- and cold-adapted animals. Major differences have been documented in such physiological processes as metabolic rates (oxygen consumption rates), tissue insulation (fat content, vascularization, density of fur), hormonal levels, quantity of brown fat, and responses to thermogenic mediators such as nor-adrenaline (see reviews by Hart 1971; Chaffee and Roberts 1971; Himms-Hagen 1967). Thus the thermal conditioning of an animal to a new environment may bring about changes in its physiological processes. These changes are then involved in adjusting the rates of heat production and/or heat transfer to enable the animal to survive within a certain range of environmental conditions. Should these compensatory changes fail to operate sufficiently, the body temperature will not be maintained and the eventual outcome will be death.

One of the approaches to studying the potential influence of climate and temperature upon the thermoregulatory abilities of small mammals during cold exposure is the determination of their thermoregulatory limits (Hart 1957). Such limits may be based upon maximal rates of heat production and heat transfer, survival times, maintenance of body temperatures, magnitude of shivering

and nonshivering thermogenesis, and minimum exposure temperatures. These limits per se do not describe the mechanisms responsible for enhanced survival in the cold. They are, however, indicative of changes in insulation and heat production capacities of small mammals. In addition, they establish the magnitude of change in their thermoregulatory abilities.

Previous studies have shown that cold-adapted rodents extend their thermoregulatory limits beyond that of warm-adapted rodents during cold exposure. For example, Sealander (1951) found the survival times of winter-adapted Peromyscus to be significantly longer than those of summer-adapted mice when exposed to moderate and low temperatures. Greater seasonal changes in cold resistance of Peromyscus were obtained by Hart and Héroux (1953). They found that winter-adapted mice could withstand ambient temperatures 20 C° lower than summer-adapted mice. Similar trends were found between warm- and cold-acclimated Peromyscus (Sealander 1951; Hart 1953), white mice (Hart 1953), and rats (Sellers et al. 1951; Hart 1953) when exposed to low ambient temperatures.

Oxygen consumption rates have also been determined in a number of small mammals exposed to a wide range of environmental temperatures (see reviews by Hart 1957, 1971). Many of these studies have demonstrated that cold-adapted small mammals exhibit an increased capacity to produce heat. Few studies, however, have determined the signifi-

cance of an increased capacity on the extension of lower temperature limits in cold-adapted small mammals. Hart (1953, 1957) found that cold-adapted Peromyscus were able to elevate their oxygen consumption rate significantly higher than that of warm-adapted mice and, as a result, they were able to thermoregulate at much lower ambient temperatures. Further studies have demonstrated that this increased capacity to produce heat in small mammals is primarily through nonshivering thermogenic mechanisms. Héroux et al. (1956), Cottle and Carlson (1956), and Davis et al. (1960) have shown that small mammals, during chronic exposure to cold, decrease the amount of their shivering and consequently, the extra heat production is a result of nonshivering or "chemical" thermogenesis. Similarly, it has been shown that metabolic acclimatization is associated with the enhancement of nonshivering thermogenesis in wild rats during the winter (Héroux et al. 1959; Héroux 1962; Hart and Héroux 1963). More recent studies have demonstrated that noradrenaline is the mediator for nonshivering thermogenesis in several small mammals (see reviews by Himms-Hagen 1967; Chaffee and Roberts 1971). In addition, brown adipose tissue is believed to contribute significantly to this extra heat production in cold-acclimated and acclimatized small mammals (see review by Smith and Horwitz 1969).

Besides increasing their heat production capacities, other small mammals adapted to cold have been shown to

reduce their rates of heat transfer through insulative mechanisms. Winter lemmings, for example, have a lower oxygen consumption rate than summer lemmings at comparable exposure temperatures (Hart and Heroux 1955). This lower heat production rate was attributed to the greater insulative abilities of winter lemmings to reduce heat loss during cold exposure. However, the total effectiveness of this insulative change in winter lemmings was not determined at temperatures lower than -10°C .

Maximal rates of heat production and heat loss have not been well established in small mammals conditioned to various environmental temperatures. Furthermore, the length of time maximal rates of heat production can be maintained is not well documented for small mammals during maximal cold stress. Such data relating maximal heat production, lower thermal limits, changes in body temperature and time would, therefore, provide a more complete understanding of the total energetics and thermoregulatory abilities of small mammals during cold exposure. Thus, the intent of the present study was to examine the interrelationships of these variables and attempt to describe more thoroughly the energetic changes associated with thermoregulatory abilities of small mammals during severe cold exposure.

Furthermore, in view of the evidence accumulated from previous studies, the thermal history and geographic distribution of small mammals are related to the differences

in the energetics and limits of their physiological processes during cold exposure. Therefore, two subspecies of Peromyscus belonging to two different species and distributed over a wide range of environmental conditions, were selected to determine the effect of environmental conditioning and geographic distribution on their thermoregulatory abilities. This study was designed to sample large numbers of mice from various parts of their ranges which have similar and different environmental conditions. Thus, the potential influence of climatic conditions on their distribution was examined by the magnitude of change in their maximal heat production capacities and resistance to cold stress. Controlled experiments were designed to compare the effect of acclimation on the thermoregulatory abilities of the two subspecies and to determine differences, if any exist, in their cold resistance and heat production capabilities. Any demonstrable differences obtained between the acclimated mice would presumably be a reflection of the genetic differences for thermoregulatory abilities in each population of mice from the various geographic areas. In addition, it enables one to evaluate the effects of acclimatization on the thermoregulatory abilities of these mice.

Oxygen consumption responses to noradrenaline were also examined in the two subspecies in relation to their previous environmental conditioning and distribution. This part of the study was designed to estimate the magnitude of nonshivering thermogenesis and to establish its temperature-

adaptive significance in these small mammals conditioned to various thermal environments.

Thus this study provides an overall perspective of the thermoregulatory abilities of small mammals and the changes that may occur in these abilities in relation to the previous environmental conditioning and distribution of the animals. Simple models are constructed to illustrate the potential influence of climate and temperature on the thermoregulatory abilities in representative samples of small mammal populations. These simple models are described by equations derived from changes in the energetics associated with thermoregulation during severe cold exposure. Therefore, estimates of heat production and cold resistance abilities may be compared in species experiencing similar and different environmental conditions.

ANIMALS AND STUDY AREAS

The woodland deer mouse, Peromyscus maniculatus gracilis (LeConte) and the northern white-footed mouse, Peromyscus leucopus noveboracensis (Fischer) were chosen for this study. The ranges of these two subspecies are shown in Fig. 1. Their geographic distribution overlaps from Minnesota eastward through southeastern Ontario and adjacent Quebec to New Hampshire and southern Maine (Hall and Kelson (1959) as amended by Waters (1962) and D.A. Smith (personal communication)). In this zone of sympatry, the two forms are rather similar in appearance and size (Peterson 1966; Baker 1968) and have similar food habits (Cogshall 1928; Hamilton 1941). However, in spite of these similarities, there are certain ecological (Klein 1960), physiological (Riedel 1967), and behavioural (Smith and Speller 1970) differences between the two forms.

In order to obtain a more complete understanding of the physiological adaptations in these two subspecies to gross climatic conditions, the selection of study areas was of prime importance. To obtain mice experiencing different climatic conditions, areas were chosen near the northern and southern portions of the range of each form where climatic conditions were extreme. Furthermore, mice were obtained from areas in the range of overlap to compare thermoregulatory abilities of each form. The four major trapping areas used in this study are shown in Fig. 1 and described below.

Fig. 1. Ranges of Peromyscus maniculatus gracilis (broken line) and Peromyscus leucopus noveboracensis (solid line) in eastern North America. Hatched area shows the region of overlap of the two ranges. Letters represent the trapping locations for Peromyscus used in this study.

- A. Westmoreland County, Virginia.
- B. Ottawa, Ontario.
- C. Algonquin Provincial Park, Ontario.
- D. Lac St. Jean-Saguenay River Area, Quebec.

A. Westmoreland County, Virginia

Westmoreland County is situated between the Potomac and Rappahannock Rivers about 88 km south of Washington, D.C. This general area is primarily under cultivation with large stands of loblolly pine (Pinus taeda), white oak (Quercus alba), black gum (Nyssa sylvatica), and tulip poplar (Liriodendron tulipifera) spread throughout. The main crops grown are corn, soybeans, and wheat which provide an abundant food supply for small mammals inhabiting nearby woodlots or narrow rows of trees and briar bushes that serve as windbreaks between adjacent fields. Only P. leucopus noveboracensis was obtained from this area.

The altitude in this area is about 30.5 m above sea level except for the flats and marshes along the coast which are nearly at mean sea level. There are a large number of tidal creeks and rivers in the area which modify the temperature extremes considerably. The mean monthly temperature ranges from 1° C in January to 25° C in July. The mean daily minimum temperatures for this area are shown in Fig. 2. Westmoreland County has the mildest climate of the four study areas selected. The mean minimum temperature during the winter months is slightly below 0°C while the minimum temperatures during the summer months drop to an average of only 18-20° C. The average snowfall during the winter is about 0.3 m; however, snow seldom stays on the ground for more than a few days at a time. Therefore,

Fig. 2. Mean daily minimum temperatures over the
past 30 yr in the four study areas.

snow does not serve as an insulative cover during the winter.

B. Ottawa, Ontario

The several areas trapped were within 48 km of Ottawa (45°25'N, 75°44'W) in Ottawa-Carleton and Lanark County. This area lies in the Upper St. Lawrence Section of the Great Lakes-St. Lawrence Forest Region (Rowe 1959) and is either under cultivation or wooded with a mixture of coniferous and deciduous trees. Stands of hardwood trees consisting primarily of sugar maple (Acer saccharum) and beech (Fagus grandifolia), or a mixed forest consisting primarily of balsam fir (Abies balsamea) and largetooth aspen (Populus grandidentata) were favourable trapping sites. Some of these forests were inhabited by both species of Peromyscus; however, P. leucopus noveboracensis was the more abundant form trapped.

The altitude in this area ranges from 92 to 122 m above sea level and the climate is moderately temperate. The mean monthly temperature ranges from -11° C to 21° C and the mean minimum daily temperatures are considerably colder than those reached in Westmoreland County, Virginia (see Fig. 2). Snow is present on the ground for at least 4 months during the year and the average yearly snowfall in the Ottawa area is 2.2 m.

C. Algonquin Provincial Park, Ontario

Algonquin Provincial Park is located near the southern edge of the Canadian Shield, between Georgian Bay

and the Ottawa River about 190 km due west of Ottawa. The park consists of 7537 sq km of forested land, lakes and rivers and is used primarily for recreational and lumbering purposes. The park lies in the Algonquin-Pontiac Section of the Great Lakes-St. Lawrence Forest Region (Rowe 1959). Several trapping sites were selected in Canisbay and Sproule Townships, Nipissing District along Highway 60. Small areas of mature hardwoods composed primarily of sugar maple (Acer saccharum), red maple (Acer rubrum), and yellow birch (Betula lutea) with varying admixtures of eastern hemlock (Tsuga canadensis) and white pine (Pinus strobus) were trapped most frequently. These forests were inhabited primarily by P. maniculatus gracilis and a variety of other small mammals.

The elevation of the park ranges from 425 to 520 m above sea level and it has a great influence on the climate. The growing season is short and occasionally frost may occur during the summer. The differences between daily maximum and minimum temperatures are great in this area, especially during the summer with its warm days and cool nights. The mean minimum daily temperatures are about 2 C° lower than those reached in the Ottawa area (Fig. 2); and the mean monthly temperatures range from -12° C to 19° C. Snow cover is present about 5 months of the year and the average annual snowfall is about 2.4 m.

D. Lac St. Jean-Saguenay River Area, Quebec

This study area is found in the Saguenay River

Basin which extends from Lac St. Jean eastward to the St. Lawrence River. The Laurentian Mountains lie to the north and south of the area. Within the basin, the land is fairly flat; some is under cultivation, but most is developed for industrial purposes. Bogs and marshes are very common. This area lies in the Saguenay Section of the Great Lakes-St. Lawrence Forest Region (Rowe 1959). Much of the forested area was burned out at one time or heavily logged; consequently, suitable trapping areas in which large numbers of mice could be obtained were difficult to locate.

Most of the trapping was confined to selected areas within a 40-km radius of Chicoutimi (48°25'N, 76°5'W) in Lac St. John East and Chicoutimi Counties. Small forests consisting primarily of various mixtures of immature trembling aspen (Populus tremuloides), balsam fir (Abies balsamea), white birch (Betula papyrifera), jack pine (Pinus banksiana), white pine (Pinus strobus), white spruce (Picea glauca), black spruce (Picea mariana), and bushy willows (Salix spp.) were trapped. P. maniculatus gracilis was the only form of Peromyscus collected, although a large variety of other small mammals inhabited the forests. A few maniculatus were also collected from similar habitats near St. Simeon (47°50'N, 69°50'W) in Charlevoix East County.

Altitudes in these areas ranged from 122 to 183 m above sea level, considerably lower than the surrounding mountainous areas. The lower altitude and numerous lakes

and rivers in the basin modify temperatures slightly. The mean monthly temperatures range from -16°C in December to 18°C in July. The mean minimum temperatures reached during the summer in this area are very similar to those in Algonquin Park during the summer. In winter, however, the mean minimum temperatures are much more severe in the Saguenay River Basin (Fig. 2). The average annual snowfall ranges from 2.3 to 3.3 m and snow is present for almost 6 months of the year.

Climatic data for the four study areas were obtained from the Meteorological Branch, Canada Department of Transport and the Environmental Data Service, U.S. Department of Commerce.

MATERIALS AND METHODS

A. Sampling Procedures and Maintenance of Animals

To maximize the possibility of discerning temperature-adaptive characteristics, if any exist, in the two species of mice from the four selected areas, mice were divided into three major groups. These were mice acclimatized to winter conditions, those acclimatized to summer conditions, and those acclimated to laboratory conditions.

(a) Acclimatized mice

Both leucopus and maniculatus were live-trapped during the winter and summer months from the respective areas outlined in the previous section. Some maniculatus were collected from the Ottawa area in both summer and winter. These mice, pooled with those collected from Algonquin Park, constituted a representative sample of maniculatus inhabiting the more southerly portion of the Canadian range of P. maniculatus gracilis. Summer mice were collected from July to mid-September in 1969 and 1970 while winter mice were collected from January to mid-March in 1970 and 1971. These months represent the warmest and coldest times of the year to which the mice are exposed in each of the four areas selected.

All mice were live-trapped from their natural environments and transported back to Ottawa for experimental purposes. Sherman-type live traps (7.7 x 7.7 x 30.5 cm), baited with peanut butter, were generally used for all trapping. In order to minimize any physiological changes

in the captured mice, the following precautions were taken. All traps were checked as early as possible in the morning. As Peromyscus are nocturnal, this minimized the time spent in the trap. During the winter, traps were supplied with additional food and terylene nesting material, and they were occasionally checked during the night. Captured mice were then transferred to 23 x 46 x 38 cm wire-mesh cages with food ('Purina' laboratory chow) and water provided ad libitum, and kept outdoors. When sufficient mice were collected from an area away from Ottawa, they were returned immediately and maintained outdoors until tested. The success in trapping enabled cold resistance and metabolic rates to be determined within 2 to 7 days from capture in 95% of the freshly caught mice.

Cold resistance and metabolic rates were determined in each group of mice except for a winter sample from the Lac St. Jean-Saguenay River area. No Peromyscus was collected from this area in spite of considerable trapping effort during the two winters. Similarly, winter samples of maniculatus from the southern portion of their range were difficult to obtain. Consequently, several maniculatus collected near Ottawa in fall were maintained in cages outdoors until mid-winter. This treatment subjected them to 4 months of acclimatization to prevailing outdoor conditions. Sufficient nesting material and food were supplied. These mice constitute about one-half of the winter maniculatus used in this study. There was no apparent difference

between the experimental results for these mice and the naturally acclimatized southern maniculatus; therefore, this pooling seemed justified.

(b) Acclimated mice

Wild-caught mice of both species were maintained in the laboratory under the same controlled conditions for at least 6 months before cold resistance and metabolic rates were determined. This period was considered to be of sufficient duration for any seasonal acclimatization differences to be lost (Hart 1953). Young mice born in the laboratory were also maintained under similar conditions and allowed to reach adult size and pelage before being used for this study. A majority of the acclimated mice tested was born and raised in the laboratory. Thus, any demonstrable differences obtained in their thermoregulatory abilities would presumably be a reflection of genetic variation among the groups of acclimated mice tested.

Mice were housed in wire-mesh cages measuring 23 x 23 x 38 cm or 23 x 46 x 38 cm and kept in groups of 2 to 3 or 6 to 8 individuals, respectively. Wood shavings were placed on the cage floors and a small can was provided for a nest shelter. Food ('Purina' laboratory chow) and water were provided ad libitum. Initially, the mice were held in a large colony-holding room for periods of 6 months or more. Room temperatures ranged from 21° to 26° C. Prior to experimentation, mice were transferred to a more precise temperature-controlled room for a minimum of 3 weeks.

Temperatures were maintained at $22^{\circ} \pm 1^{\circ}$ C. The relative humidity of the room was not controlled. Fluorescent illumination was regulated to a 12-hr (0900 to 2100 hr) photoperiod throughout. All acclimated mice were cared for daily and were believed to be in a healthy state prior to experimentation.

B. Measurement of Cold Resistance

The criterion used to determine cold resistance in Peromyscus was the time for their oxygen consumption rate to decrease by more than 50% of the maximal rate achieved and maintained at any one temperature of severe cold stress. Once this time was reached, the mouse was removed from the cold. Rectal temperatures were taken before and after cold exposure with a thermistor connected to a digital thermometer (Digitemp Instrulab Inc., Dayton, Ohio, U.S.A.). The thermistor was inserted rectally to a depth of 25 to 30 mm. This procedure was carried out within 30 sec after initial disturbance of the mouse and verified whether or not the mouse was in deep hypothermia. If the oxygen consumption of a mouse was maintained at a high level for 6 hr, the experiment was terminated and the mouse was removed from the cold. Food and water were not provided during cold exposure.

A temperature-controlled cabinet was used in subjecting mice to different air temperatures. The cabinet was well insulated and the inside dimensions were approximately 76 x 115 x 56 cm. Temperatures within the cabinet were thermostatically controlled and could be lowered to

-45° C and held constant within $\pm 1^\circ$ C. Light could be provided by two 25W incandescent tubes to facilitate observation of animals through a small window on one side of the cabinet.

Cold resistance was determined in all groups of mice over a range of temperatures from -45° to -15° C. Four temperatures at 5 C° intervals were usually selected for exposure. The range of temperatures to which any one group of mice was subjected depended upon their previous thermal conditioning. Table 1 summarizes the temperatures at which cold resistance and metabolic rates were determined for each group of mice.

C. Measurement of Oxygen Consumption

An open, continuous-flow system was used to determine oxygen consumption rates. The system consisted of two air-flow circuits providing alternate recordings from two respiratory chambers during each experimental session (Fig. 3). In each circuit air was drawn by a pump from outdoors through a respiratory chamber and dried over magnesium perchlorate. It was then passed through a 2:1 mixture of sodium calcium hydrate and ascarite to remove CO₂. Manual manipulation of air-tight stopcocks allowed the partial pressure of oxygen of the air from each respiratory chamber to be successively measured using a Beckman Model C oxygen analyzer (Beckman Instruments, Inc., Fullerton, Calif. U.S.A.). Flow rates were monitored by a Precision wet test meter (Precision Scientific Co.,

Table I. Summary of the mice investigated for cold resistance and metabolic rate determinations.

Species	Area	Group	Exposure Temperature (°C)**	n
<u>Peromyscus</u>	Westmoreland County Virginia	Winter	-43, -38, -33, -23*, -10*	43
<u>leucopus</u>		Summer	-33, -28, -23, -18, -10*, 0*	83
<u>noveboracensis</u>		Acclimated 22°C	-28, -23, -18, -13, 0*	52
	Ottawa, Ontario	Winter	-43, -38, -33, -23*, -10*	65
		Summer	-38, -33, -28, -23, -10*, 0*	64
		Acclimated 22°C	-28, -23, -18, -13, 0*	43
<u>Peromyscus</u>	Algonquin Provincial Park, Ontario	Winter	-43, -38, -33*, -23*, -10*	39
<u>maniculatus</u>		Summer	-38, -33, -28, -23, -10*, 0*	66
<u>gracilis</u>		Acclimated 22°C	-38, -33, -28, -23, -10*, 0*	59
	Lac St Jean- Saguenay River, Quebec	Summer	-38, -33, -28, -23, -10*, 0*	47
		Acclimated 22°C	-38, -33, -28, -23, -10*, 0*	21

* Only metabolic rate determined.

** Temperatures within the respiratory chamber.

Fig. 3. Block diagram showing the two circuit set-up
for measuring oxygen consumption.

Chicago, U.S.A.) and adjusted by a needle valve on a calibrated predictability flowmeter.

The accuracy of the Beckman C oxygen analyzer was ± 0.5 mm Hg. Therefore, to reduce the error in recording oxygen concentrations, air flow rates were set between 200 to 300 cc/min to allow a deflection of the paramagnetic needle on the oxygen analyzer by at least 10 mm Hg. At these flowrates, the time for the air to equilibrate within the respiratory chambers containing a mouse averaged 10 to 15 min. After equilibration, the lag time in the oxygen measuring system in response to changes in oxygen concentration was 5 to 6 min. This system, therefore, enabled one to record high rates of oxygen consumption of a mouse 20 to 25 min after it was placed in the cold. The lower time limit set for the oxygen consumption rate to decrease by 50% of the maximal rate achieved and maintained was 1 hr at the very low temperatures. This lower time limit enabled one to record a high rate of oxygen consumption for 12 to 18 min prior to a decrease in oxygen consumption rate at very low temperatures. Resistance of air flow in each circuit was detected by pressure changes on a manometer and corrected.

Oxygen consumption rates were determined in conjunction with cold resistance as follows: Mice were placed in a cylindrical wire-mesh cage which was coated with latex rubber. This coating was used to minimize the danger of mice freezing to the wire mesh at low test temperatures.

This cage was placed in a glass respiratory chamber which was then sealed with a large rubber stopper. Several pieces of cork were placed on the floor of the respiratory chamber to support the wire-mesh cage. The gap formed allowed urine and feces to fall free of the wire-mesh cage. Two glass tubes were fitted through holes in the metal lid of the wire-mesh cage and the rubber stopper of each chamber. These served as ducts for inflowing and outflowing air. Inflowing air entered near the bottom of the chamber while outflowing air was drawn from the top. This arrangement was designed to minimize thermal layering and accumulation of CO₂ near the bottom of the chamber.

Recording of oxygen consumption began 10 min after a mouse was placed in the cold. The partial pressure of oxygen from each chamber was alternately recorded every 3 min until the oxygen consumption rate decreased more than 50% of the maximal level maintained by each mouse. After exposure, mice were allowed to recover at room temperature before being returned to their cage. No mouse was exposed to more than one temperature during the cold resistance studies. Body weights were taken before and after cold exposure.

To calculate oxygen consumption, the following formula from Depocas and Hart (1957) was used:

$$V_{O_2} = V_E \frac{P_{IO_2} - P_{EO_2}}{P_B - P_{IO_2}}$$

where:

V_{O_2} = oxygen consumption of the mouse per minute.

V_E = volume of CO_2 -free air leaving the respiratory chamber per minute.

$P_{I_{O_2}}$ = partial pressure of oxygen in the air entering the respiratory chamber.

$P_{E_{O_2}}$ = partial pressure of oxygen in the CO_2 -free air leaving the respiratory chamber.

PB = barometric pressure during the experiment.

Gas volumes were corrected to standard conditions of temperature and pressure. The metabolic rate determined for each mouse was obtained by averaging the highest rates of oxygen consumption maintained continuously over a period of time by that mouse during severe cold exposure. Frequent observations were made on the activity and behaviour of the mice during exposure to severe cold stress. In general, activity of mice was minimal. No apparent relation was found between activity and oxygen consumption rate during the cold resistance studies because of the low temperatures selected and the high demand for heat production at these temperatures.

Metabolic rates of mice were also determined at moderate temperatures (-10° and 0° C) where metabolic failure time would definitely exceed the 6-hr limit. In these cases, mice were allowed at least a 1-hr period to adjust to the surroundings before recordings commenced. Oxygen consumption was measured for 1 to 2 hr and the behaviour of the mouse

was observed frequently. Any measurements taken when mice were observed to be active or excited were not used. Therefore, the oxygen consumption rates determined at moderate exposure temperatures represent the rates of heat production of mice in a "resting" or fairly still position. When large numbers of mice were not available, the metabolic rate of one mouse was measured at two different temperatures.

D. Measurement of Temperatures

Temperatures of the cold cabinet, respiratory chambers, and room were monitored concurrently with the measurement of oxygen consumption. These temperatures were initially monitored by five 24-gauge copper-constantan thermocouple wires connected to a manually operated potentiometer. The millivolt readings were converted to degrees Centigrade from a reference table. Later in this study, thermocouples were attached to a 16-channel Speedomax strip chart recorder (Leeds and Northrup Co., Philadelphia, Pa., U.S.A.). Temperatures were automatically recorded to an accuracy of $\pm 0.5^{\circ}\text{C}$ every 3 min.

E. Measurement of Noradrenaline Response

Three groups of mice were tested for their response to noradrenaline. These included leucopus from the Virginia and Ottawa areas and maniculatus collected primarily from the Ottawa area. Mice were tested shortly after capture in summer and winter as well as after acclimation to $22^{\circ} \pm 1^{\circ}\text{C}$ in all three groups. Acclimation procedure was

the same as that used for the cold resistance and metabolic studies. Approximately 30 mice from each group were tested with about 10 mice from each group conditioned to each of the three thermal environments.

Responses to noradrenaline were determined by measuring the maximal metabolic rates achieved after injection. The metabolic apparatus was essentially the same as that previously described with the following modifications: The metabolic chamber consisted of a plexiglass tube 7 cm long and 4 cm in diameter. One end was fitted with a rubber stopper through which glass ducts projected for the inflowing and outflowing air. The other end of the plexiglass chamber was covered with a piece of flexible rubber. A small hole was cut in the rubber to allow the head of a mouse to be easily placed within the chamber. Air flow rates were set between 55 and 80 ml/min and all experiments were carried out at room temperature (20-24° C). All values of oxygen consumption were corrected to STP.

Preparatory to noradrenaline response tests, each mouse was weighed to the nearest 0.1 g and anaesthetized with Nembutal (60-100 mg/kg, intraperitoneally) prior to respiratory measurements. The head of each mouse was placed into the respiratory chamber through the opening in the rubber hood. A pretreatment level of oxygen consumption was recorded until measurements remained constant for at least 15 min.

Subsequently, noradrenaline was injected intra-

peritoneally. Preliminary experiments had shown that for intraperitoneal injection, maximal increases of oxygen consumption were stimulated by dosages greater than 3 mg noradrenaline bitartrate per kg of body weight. Dosages ranging from 3 to 6 mg/kg were used in all groups of mice tested. Control injections of saline stimulated no response in several mice tested.

In conjunction with noradrenaline responses, body temperatures were recorded from a thermocouple inserted about 20 mm in the rectum.

F. Statistical Analyses

Statistical tests were taken from Steel and Torrie (1960) and Smillie (1966). Student's t-test was used to compare means of oxygen consumption rate and metabolic failure time. Measures of central tendency and variability are generally reported as the mean $\pm$ two standard errors ($\pm 2SE$). All linear regressions were done using the method of least squares. Comparisons of regression lines, testing differences in slope and intercept were done as suggested by Smillie (1966).

All statistical analyses were done on the Carleton University XDS Sigma 7 digital computer. Computations of oxygen consumption rates were done on the National Research Council IBM-360 computer according to program File # FP65. This program is available in the Library of the Computing Section, Division of Biology, National Research Council, Ottawa. Both Basic and Fortran IV programming languages were used.

RESULTS

A. Responses of Peromyscus to Cold(a) Behavioural responses

The behavioural responses of the Peromyscus to severe cold in the present experiments, in general, were very similar to those described by Sealander (1953) for P. leucopus noveboracensis. The most dominant pattern of behaviour observed in the present study involved the mice assuming a tightly huddled posture immediately after they were placed in the cold. The limbs, tail, and head were generally compactly tucked beneath the body projecting an almost spherical shape. The pelage in most cases was always erected. Grooming was infrequent; however, licking of the forelimbs and tail was common during the early stages of cold exposure. In addition, frequent turning movements were made; these appeared to be an attempt to achieve a more compact body surface. Such behaviour can be interpreted as an adaptation to reduce the surface area to mass ratio. By reducing this ratio, the animal would also reduce the total amount of heat loss to the environment (Brody 1945). This behaviour was observed in all winter and summer groups of mice as well as the acclimated maniculatus. Acclimated leucopus, on the other hand, appeared to show very poor positioning behaviour during severe cold exposure. They frequently gnawed at the roof of the wire-mesh cage housing them within the respiratory chamber. This required stretching in an upright position,

which in turn exposed a considerable amount of surface area. This behaviour alternated with short periods in the tightly huddled position. The overall behaviour of the acclimated leucopus during severe cold exposure, therefore, appeared to be less efficient in terms of conserving heat.

(b) Oxygen consumption responses

The metabolic responses of Peromyscus to severe cold exposure showed similar patterns in all groups of mice tested. Each mouse exposed to severe cold achieved a high level of oxygen consumption shortly after it was placed in the cold. In most cases, the high rate of oxygen consumption achieved by each mouse was maintained for a certain period of time and then this rate rapidly decreased. This decrease in oxygen consumption rate will be referred to as "metabolic failure" and the time it took for this rate to decrease to one-half of the level previously maintained by each mouse will be referred to as the "metabolic failure time". To illustrate this response in oxygen consumption rate by Peromyscus, 6 representative examples of P. leucopus noveboracensis collected from the southern part of its range (Va.) were selected. Their oxygen consumption rates during long and short term exposures to severe cold stress are shown in Fig. 4a and 4b, respectively. Figure 4a shows that the oxygen consumption during the longer exposures to severe cold remained relatively stable prior to metabolic failure in the winter, summer and acclimated mice. This consistency was the most common pattern

Fig. 4a. Patterns of oxygen consumption rates during long exposures to severe cold stress in a winter, summer, and acclimated leucopus collected from Virginia. Exposure temperatures are shown in parentheses.

Fig. 4b. Patterns of oxygen consumption rates during short exposures to severe cold stress in a winter, summer, and acclimated leucopus collected from Virginia. Exposure temperatures are shown in parentheses.

recorded in the mice tested. In the few exceptions, the oxygen consumption rate of a mouse either fluctuated in a fairly regular manner or showed sporadic deviations for short periods rather than being maintained at a continuous stable level. Figure 4b shows that the pattern of oxygen consumption during the shorter exposures to severe cold was similar to that obtained for the longer exposures. In these cases, a high rate of oxygen consumption was achieved very quickly and maintained at this level for only a very short time before it rapidly decreased. This pattern was also recorded in most Peromyscus during exposures to temperatures at which their metabolic rate failed fairly rapidly. In addition, Fig. 4b shows that the high levels of oxygen consumption rate achieved and maintained by the winter, summer, and acclimated mice corresponded approximately to the levels achieved and maintained by the winter, summer, and acclimated mice, respectively, during the longer exposures to severe cold (Fig. 4a). Thus, the same high rate was achieved within each group of mice whether the metabolic failure time was 1 hr or more. The upper time limit for metabolic failure was set at 6 hr. If metabolic failure did not occur after 6 hr of severe cold exposure, the mouse was removed from the cold. Fewer than 6% of the Peromyscus exposed to severe cold did not show metabolic failure. In these cases, a high level of oxygen consumption was generally maintained at a fairly stable rate for the 6 hr. This response was recorded in most groups of Peromyscus from each

of the four geographic areas; therefore, it was not common to any one group of mice.

(c) Changes in body temperature during cold exposure

Rectal temperatures were recorded before and after severe cold exposure. Table II shows that the rectal temperatures of all Peromyscus prior to cold exposure ranged from 34.9° to 41.0° C while the mean rectal temperature ranged from 37.6° to 40.1° C among the various groups of mice. During severe cold exposure, rectal temperatures decreased an average 20-23 C° in all groups of mice if the oxygen consumption rate failed. The greatest decrease was found in the winter groups of mice (Table II). In contrast, rectal temperatures of mice did not decrease significantly if the oxygen consumption rate was maintained for the 6-hr exposure to severe cold. In the 28 cases of this, the rectal temperatures of mice averaged $39.2^{\circ} \pm 0.4^{\circ}$ C (2SE) prior to cold exposure and only $37.3^{\circ} \pm 0.8^{\circ}$ C after the 6-hr exposure. From these data it was, therefore, assumed that body temperatures of Peromyscus were maintained close to normal if their oxygen consumption rate was maintained at a high continuous rate.

Most Peromyscus recovered after metabolic failure. Their body temperatures returned to normal in about 1 hr at room temperature. The lowest rectal temperature from which a mouse recovered was 10.8° C. Mice recovering from deep hypothermia showed a gradual increase in their breathing rate from an average low of 60 breaths/min to

Table II. Rectal temperatures (T_b in °C) of Peromyscus before severe cold exposure and after metabolic failure.

Group	T_b prior to Cold Exposure		T_b after Metabolic Failure		Mean Decrease in T_b
	Mean	Range	Mean	Range	
<u>Southern leucopus</u>					
a) winter	38.6	(36.0-40.4)	15.6	(10.8-20.9)	23.0
b) summer	39.6	(38.0-41.0)	19.6	(13.5-25.0)	20.6
c) acclimated 22°C	38.9	(36.5-40.5)	18.5	(14.0-23.0)	20.5
<u>Northern leucopus</u>					
a) winter	38.9	(36.4-40.4)	16.9	(11.0-21.4)	21.9
b) summer	39.2	(36.8-41.0)	18.6	(13.0-22.8)	20.8
c) acclimated 22°C	38.6	(36.0-40.0)	17.9	(13.5-22.0)	20.7
<u>Southern maniculatus</u>					
a) winter	39.1	(35.6-40.1)	16.9	(11.7-22.2)	22.1
b) summer	40.1	(37.5-41.0)	18.3	(11.4-24.4)	21.7
c) acclimated 22°C	38.2	(34.9-40.0)	17.8	(11.0-22.0)	20.4
<u>Northern maniculatus</u>					
a) summer	39.4	(37.5-40.9)	18.0	(12.5-23.0)	21.4
b) acclimated 22°C	37.6	(36.0-39.0)	17.2	(12.4-21.2)	20.1

values as high as 200 breaths/min. Vigorous periods of shivering were also common. Some after-effects of severe cold exposure were apparent in all groups of Peromyscus. The most obvious damage of cold appeared to affect the tail and hindlimbs. In several mice, these extremities were considerably reduced in size or lost completely within a few days after exposure.

B. Cold Resistance and Heat Production Capabilities in Relation to Previous Thermal Conditioning

In this section, cold resistance and heat production capabilities in the winter, summer, and acclimated groups of mice are compared within each of the species of Peromyscus. The P. leucopus noveboracensis collected from Virginia and the Ottawa area will be referred to as "southern" and "northern" leucopus, respectively, while the P. maniculatus gracilis collected from the Algonquin Park and Ottawa areas will be referred to as "southern" maniculatus and those collected from the Lac St. Jean-Saguenay River area as "northern" maniculatus.

(a) Metabolic failure times

Cold resistance was based on metabolic failure times and the corresponding temperatures at which the oxygen consumption rate failed between 1 to 6 hr. The lowest temperature to which each group of mice was exposed was that at which the metabolic rate failed in about 1 hr; i.e. that at which thermoregulation was only possible for a short period of time. Such a temperature will be referred to as the

"lower temperature limit of cold exposure". Therefore, differences in either the lower temperature limit of cold exposure or metabolic failure times at comparable exposure temperatures were used to compare the ability of groups of mice to withstand or resist severe cold exposure.

The relationships between ambient temperature and the logarithm of metabolic failure time for all groups of mice are shown in Figs. 5, 6, 7, and 8. All groups of mice showed a rapid decrease in metabolic failure time as the ambient (exposure) temperature decreased. The regression equations describing the relationship between metabolic failure and ambient temperature for the various groups of Peromyscus are in Table III.

In P. leucopus noveboracensis, Figs. 5 and 6 show that the ability of these mice to withstand cold exposure was directly related to their previous conditioning to cold. Winter mice were able to withstand much lower temperatures than either the summer or acclimated mice. A marked difference in cold resistance was demonstrated between the winter and summer mice. In addition to withstanding lower temperatures, winter mice were also able to maintain their metabolic rate significantly longer than summer mice at comparable temperatures of cold stress. For example, in southern leucopus the average time for a winter mouse to reach its metabolic failure point when exposed to -33°C was 201 min. The metabolic rate of a summer mouse exposed to the same temperature failed around 62 min (Fig. 5).

Fig. 5. Relationship between ambient temperature and logarithm of metabolic failure time in southern leucopus during winter (⊙), summer (⊗), and acclimated to 22° C (●). The vertical bars denote $\pm 2SE$ about the mean and the numbers above the bars represent the number of mice examined. Lines were fitted by the method of least squares.

Fig. 6. Relationship between ambient temperature and logarithm of metabolic failure time in northern leucopus collected during winter (⊙), summer (⊗), and those acclimated to 22° C (●). Graphic presentation is the same as in Fig. 5.

Fig. 7. Relationship between ambient temperature and logarithm of metabolic failure time in southern maniculatus collected during winter (⊙), summer (⊕), and those acclimated to 22° C (●). Graphic presentation is the same as in Fig. 5.

Fig. 8. Relationship between ambient temperature and logarithm of metabolic failure time in northern maniculatus collected during the summer (⊗), and acclimated to 22° C (●). Graphic presentation is the same as in Fig. 5.

Table III. Regression equations for the relationship of metabolic failure time and ambient temperature in the various groups of Peromyscus collected from the four geographic areas.

Group	n	Regression equation	Sy.x	r
<u>Southern leucopus</u>				
a) winter	37	$\log_{10} Y = 4.008 + 0.051X$	0.190	0.72
b) summer	66	$\log_{10} Y = 2.997 + 0.037X$	0.162	0.79
c) acclimated 22°C	42	$\log_{10} Y = 2.746 + 0.034X$	0.156	0.77
<u>Northern leucopus</u>				
a) winter	35	$\log_{10} Y = 4.921 + 0.069X$	0.232	0.69
b) summer	52	$\log_{10} Y = 3.285 + 0.042X$	0.196	0.75
c) acclimated 22°C	37	$\log_{10} Y = 3.168 + 0.049X$	0.135	0.90
<u>Southern maniculatus</u>				
a) winter	22	$\log_{10} Y = 5.297 + 0.074X$	0.207	0.70
b) summer	52	$\log_{10} Y = 3.370 + 0.042X$	0.131	0.87
c) acclimated 22°C	46	$\log_{10} Y = 3.434 + 0.044X$	0.170	0.82
<u>Northern maniculatus</u>				
a) summer	38	$\log_{10} Y = 3.523 + 0.048X$	0.259	0.71
b) acclimated 22°C	15	$\log_{10} Y = 3.411 + 0.041X$	0.147	0.85

Symbols used: n = number of mice sampled, Y = metabolic failure time (min), X = ambient temperature (°C), Sy.x = standard error of the estimate, r = regression coefficient.

This difference represented a 3.2-fold increase in time for winter mice. Even at temperatures of -43°C , the average metabolic failure time of winter mice was still longer (67 min) than that of summer mice exposed to -33°C . Similar differences in metabolic failure times were obtained between the winter and summer groups of northern leucopus (Fig. 6). At temperatures of -38°C , the mean metabolic failure time for these winter mice was 169 min while that for the summer mice was 55 min. This represents a 3.1-fold increase in metabolic failure time in winter mice. To further illustrate the ability of winter mice to withstand cold, 4 northern leucopus collected in winter were exposed 9 hr at -33°C without food or water. The metabolic rate failed in only one of these mice before the end of 9 hr. Thus, the ability of winter mice to maintain their metabolic rate, and consequently their body temperature, at temperatures of -33°C was greatly enhanced as reflected by the greater than 5-fold increase in their metabolic failure time over that of mice collected during the summer.

In addition to maintaining their metabolic rate for longer periods of time, northern leucopus in winter were also able to thermoregulate at much lower temperatures than summer mice (Fig. 6). A lower temperature limit of cold exposure was not established in these winter mice because colder temperatures in the respiratory chambers could not be obtained. However, these data do indicate a lower temperature limit of cold exposure for northern

leucopus in winter much lower than -43°C since the average metabolic failure time of 84 min at -43°C was still longer than that of the summer mice exposed to -38°C (Fig. 6).

Figure 7 shows that southern maniculatus in winter also had the greatest resistance to cold stress. Even at -43°C the average length of time for their oxygen consumption rate to fail was still approximately 2 hr. Therefore, the lower temperature limit for minimal thermoregulation could also not be established in these winter mice. However, in spite of this, seasonal comparisons can be made. The changes in cold resistance from summer to winter are demonstrated in southern maniculatus exposed to -38°C . At this temperature, the average metabolic failure time of summer mice was 64 min while that of winter mice was 325 min. Thus, in southern maniculatus, winter mice could last 5.0 times longer than summer mice at temperatures of -38°C before their oxygen consumption rate failed.

The metabolic failure times of Peromyscus collected from each of the three geographic areas in winter were significantly different from those mice collected from the same geographic area in summer ($P < 0.001$). Similarly, a highly significant difference in metabolic failure times was found between the summer and acclimated leucopus collected from each of the two geographic areas ($P < 0.001$). The metabolic failure times of acclimated mice, in both cases, were significantly shorter than those of summer mice when they were exposed to the same ambient temperatures.

In addition, the lower temperature limit of cold exposure for summer leucopus was much lower than that for acclimated mice. For instance, Fig. 5 shows that the lower temperature limit of cold exposure for southern leucopus in summer was -33°C while that for the acclimated mice was -28°C (Fig. 5). Furthermore, when both groups of mice were exposed to temperatures ranging from -18 to -28°C , the mean metabolic failure time of summer mice was 1.6 times longer than that of acclimated mice. Similarly, Fig. 6 shows that in northern leucopus, the lower temperature limit of cold exposure in summer mice was 10°C lower than that of the acclimated mice. The metabolic rate of these summer mice failed in about 50 min at -38°C while that of acclimated mice failed in about 50 min at -28°C . In addition, the metabolic failure times of these summer leucopus were 1.9 and 2.4 times longer than the acclimated mice at comparable exposure temperatures of -28° and -23°C , respectively. Thus, both northern and southern leucopus maintained under constant laboratory conditions showed a significant reduction in their ability to resist severe cold stress as compared to these same groups of mice existing under natural summer conditions.

On the other hand, Figs. 7 and 8 show that the metabolic failure times of summer and acclimated maniculatus were essentially the same during severe cold exposure in both the northern and southern groups respectively. Furthermore, the lower temperature limit of cold exposure was -38°C for both summer and acclimated maniculatus from either geographic area.

These data, therefore, show that summer mice did not significantly change their ability to resist severe cold stress after extended periods of acclimation to constant laboratory conditions.

(b) Maximal oxygen consumption rates

The expression of oxygen consumption rate chosen was ml of O_2 per hr per $W^{0.7}$ where: W = body weight of the animal. Data expressed in this manner have the advantage of correcting for differences in weight when different species are to be compared since metabolism (M) is proportional to W^b where: b = regression coefficient from the equation $M = kW^b$ (Hart 1971). Actual regression coefficients were not determined in the various groups of mice because of the insufficient range in body weights of mice exposed to any one temperature. Thus, a compromise was made between a mean regression coefficient of 0.69 determined for 6 forms of Peromyscus (Hayward 1965b) and that of 0.73 which is the mean interspecies exponent (Brody 1945).

The significant changes previously shown in cold resistance were paralleled by marked changes in oxygen consumption rates. Figures 9, 10, and 11 show the relationships between oxygen consumption rate and ambient temperature in southern leucopus, northern leucopus, and southern maniculatus, respectively, for the winter, summer, and acclimated groups of mice. In northern maniculatus, oxygen consumption rates in relation to ambient temperature were only obtained for summer and acclimated mice. This relationship is shown in Fig. 12 for each group of mice.

Fig. 9. Relationship between ambient temperature and oxygen consumption rate of southern leucopus collected during winter (⊙), summer (○), and acclimated to 22° C (●). The vertical bars represent $\pm 2SE$ about the mean and the numbers above the bars represent the number of mice examined. Curves were fitted by eye.

Fig. 10. Relationship between ambient temperature and metabolic rate in northern leucopus collected during winter (⊙), summer (⊙), and those acclimated to 22° C (●). Graphic presentation is the same as in Fig. 9.

Fig. 11. Relationship between ambient temperature and metabolic rate in southern maniculatus collected during winter (☉), summer (☉), and acclimated to 22° C (●). Graphic presentation is the same as in Fig. 9.

Fig. 12. Relationship between ambient temperature and metabolic rate in northern maniculatus collected during summer (⊙), and acclimated to 22° C (●). Graphic presentation is the same as in Fig. 9.

These data show that the rate of oxygen consumption increased to a maximal level in all groups of mice as the ambient temperature decreased. In addition, the maximal rate of oxygen consumption in each group of mice was achieved over a range of low ambient temperatures. These low temperatures corresponded to those at which the metabolic rate failed in 1 to 6 hr in each of the groups of mice tested. Therefore, the maximal rate of oxygen consumption for any one group of mice could only be maintained for a certain length of time at these low temperatures.

This time is an important factor which must be considered when one interprets the metabolic curves shown in Figs. 9 to 12, especially at temperatures that induce a maximal rate of oxygen consumption. To illustrate, Fig. 9 shows that the maximal rate of oxygen consumption of southern leucopus in winter was achieved at -33°C . Figure 5, on the other hand, shows that the mean time until metabolic failure at -33°C for the same group of winter mice was 218 min. Therefore, at -33°C the maximal rate of oxygen consumption could only be maintained for an average 218 min. Exposure to lower ambient temperatures also induced a maximal rate of oxygen consumption in these same winter leucopus (Fig. 9); however, the length of time this rate was maintained decreased considerably. At -43°C , the mean metabolic failure time for southern leucopus in winter was only 67 min (Fig. 5). Similar interpretations must be made for all metabolic data obtained for each group

of mice during exposure to severe cold stress. Further data on the maximal metabolic rate maintained by each group of mice prior to metabolic failure are in Appendix Table 1.

Figures 9 to 11 show that the maximal rate of oxygen consumption achieved in each group of Peromyscus was directly related to their previous thermal conditioning. Winter mice in all cases achieved the highest oxygen consumption rate. In addition, they were able to achieve this rate at much lower temperatures than either the summer or acclimated mice. A marked difference in oxygen consumption rate was found between the winter and summer groups of mice. Two distinct metabolic response patterns were found for the winter and summer groups of mice.

The first pattern to consider is that shown in Fig. 9 for southern leucopus. In this case, the only difference in oxygen consumption between the winter and summer groups of mice was an enhancement of the metabolic rate in winter mice at lower ambient temperatures. At temperatures of -10°C , the oxygen consumption rate of winter and summer mice was essentially the same. However, as the ambient temperature decreased winter mice continued to increase their oxygen consumption rate while that of summer mice stabilized at a maximal rate (Fig. 9). The mean maximal rate of metabolism achieved by these winter mice was $36.0 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ while that achieved by the summer mice was $30.3 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$. The difference in the maximal metabolic rate between winter and summer mice during

severe cold stress was highly significant ($P < 0.001$; $F=32.5$). In addition to a higher maximal metabolic rate, southern leucopus in winter could achieve a maximal rate of oxygen consumption at temperatures $10\text{ }^{\circ}\text{C}$ lower than the summer mice. (Fig. 9). This reflected on the differences obtained in metabolic failure times between the winter and summer mice. For example, at -33°C the maximal metabolic rate of winter mice was 1.2 times higher than that of summer mice and their metabolic failure time was 3.2 times longer. Thus, the higher maximal rate of oxygen consumption of winter mice could be maintained significantly longer than the lower maximal rate of summer mice at the same exposure temperatures.

The second metabolic response pattern obtained between winter and summer mice was found in southern maniculatus. This pattern is shown in Fig. 11. In this case, winter mice also showed an enhancement of their oxygen consumption rate over that of summer mice at lower exposure temperatures; however, the winter mice maintained a significantly lower oxygen consumption rate at the higher exposure temperatures.

At temperatures of -10°C , the mean metabolic rate of summer mice was $28.0\text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ while that of winter mice was only $20.9\text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ ($P < 0.01$; $t=5.0$). Subsequent exposure to colder temperatures showed a marked increase in the oxygen consumption rate of winter mice while that of summer mice increased only slightly to a maximal

rate (Fig. 11). Between temperatures of -23° and -33° C their metabolic rates were not significantly different; however, at -38° C winter mice achieved a maximal oxygen consumption rate which was significantly higher than that of summer mice exposed to -38° C ($P < 0.01$; $t=3.62$). In addition to this higher oxygen consumption rate at -38° C, winter mice were able to maintain this rate 5.0 times longer than the lower maximal rate could be maintained by summer mice. The mean maximal rates of oxygen consumption achieved and maintained by southern maniculatus in winter and summer were 39.7 and 33.2 ml O_2 /hr.g $^{-0.7}$ respectively. This difference in maximal rates was highly significant ($P < 0.001$; $F=11.9$). Moreover, these winter mice could maintain a maximal rate of oxygen consumption at temperatures 5 C $^{\circ}$ lower than the summer mice (Fig. 11).

In northern leucopus, the metabolic response pattern of the winter and summer groups of mice appeared to be similar to that of southern maniculatus. This is based primarily on the maximal rate of oxygen consumption achieved and the length of time this rate was maintained. To illustrate, Fig. 10 shows that the mean oxygen consumption rates of the winter mice were slightly lower than those of the summer mice at -10° and -23° C. These differences were not significant ($P > 0.05$). However, at temperatures of -23° C summer mice had achieved the maximal oxygen consumption rate while the metabolic rate of winter mice continued to increase as the ambient temperature was lowered. Figure 10

shows that at temperatures of -33° and -38°C , the mean oxygen consumption rates of winter mice were 1.3 and 1.1 times higher, respectively, than those of summer mice ($P < 0.05$). In addition, the length of time these rates were maintained was greater than 5 times longer at -38°C . During severe cold exposure, the mean maximal metabolic rate of northern leucopus was $38.1 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ for the winter mice and $32.6 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ for the summer mice. Winter mice also maintained a maximal rate of oxygen consumption at temperatures 5°C lower than the summer mice (Fig. 10).

Several major differences were also obtained between the oxygen consumption rates of summer and acclimated Peromyscus. Similarly, two basic metabolic response patterns were found between these groups of mice. One pattern was found in both northern and southern leucopus and the other in both northern and southern maniculatus.

P. leucopus noveboracensis acclimated to constant laboratory conditions maintained a much lower oxygen consumption rate than summer mice during severe cold exposure; however, at moderate exposure temperatures their metabolic rates were the same. For example, Fig. 9 shows that the mean oxygen consumption rates of southern leucopus were essentially the same for summer and acclimated mice exposed to 0°C . Under severe cold stress, however, summer mice achieved a slightly higher maximal metabolic rate. In addition, summer mice were able to maintain a maximal rate of oxygen consumption at temperatures 5°C lower than

acclimated mice. The mean metabolic rate achieved by summer mice was $30.3 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ while that for acclimated mice was $26.6 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$. Student's t-test applied at comparable temperatures showed a significant difference only at -28° C ($P < 0.05$; $t=2.21$); however, when straight lines were fitted for each of summer and acclimated mice over the range of temperatures from -18° to -28° C , the lines representing the maximal oxygen consumption rate during severe cold stress were significantly different ($P < 0.001$; $F=7.1$). This difference in maximal metabolic rate also paralleled the difference in metabolic failure times. Over the range of comparable temperatures (i.e. -18° to -28° C), the mean maximal rate of oxygen consumption of summer mice was 1.1 times higher than that of acclimated mice and this rate was maintained 1.6 times longer than the maximal rate of acclimated mice (Fig. 9).

In northern leucopus, Fig. 10 shows that the pattern of oxygen consumption rates of summer and acclimated mice was similar to that just described for southern leucopus. However, the differences between these two groups of mice were much greater than those in southern leucopus. For example, in northern leucopus, the maximal rate of metabolism of summer mice could be maintained at temperatures 10° C lower than that of the acclimated mice. Figure 10 shows that the lowest temperatures at which the maximal rate of oxygen consumption could be maintained by summer and acclimated mice were -38° C and -28° C , respectively.

In addition, the mean maximal rate of metabolism achieved by summer mice was 1.1 times higher than that of acclimated mice; however, this rate could be maintained 1.9 and 2.4 times longer than the maximal rate of acclimated mice at comparable temperatures of -23° and -28° C, respectively (Fig. 10). The maximal rate of metabolism during severe cold exposure was $32.6 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ for summer mice and $29.1 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ for acclimated mice. Regression lines calculated over this range of temperatures for summer and acclimated mice were significantly different ($P < 0.001$; $F=14.5$). At comparable temperatures, t -values were only significant at -23° C ($P < 0.05$; $t=2.64$).

P. maniculatus gracilis acclimated to constant laboratory conditions maintained a slightly lower mean metabolic rate than summer mice in both the northern and southern groups during exposures to temperatures ranging from 0° to -38° C. Figures 11 and 12 show that the oxygen consumption rate of the summer and acclimated groups of mice in both the southern and northern maniculatus, respectively, gradually increased to a maximal level between 0° and -23° C. The rate of increase over this temperature range was approximately $0.50 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}\cdot\text{C}^{-1}$ in all summer and acclimated maniculatus. Comparison of straight lines fitted to these data showed that the acclimated mice maintained significantly lower oxygen consumption rates than summer mice at temperatures ranging from 0° to -23° C ($P < 0.001$; $F=15.0$ for southern maniculatus; $P < 0.01$; $F=5.73$

for northern maniculatus).

Figure 11 shows that in southern maniculatus the mean maximal rate of metabolism achieved by summer mice during severe cold exposure was $33.2 \text{ ml O}_2/\text{hr.g}^{-0.7}$ while that of acclimated mice was $30.7 \text{ ml O}_2/\text{hr.g}^{-0.7}$. Therefore, between temperatures of -23° and -38° C , the mean maximal rate of oxygen consumption achieved by summer mice was 1.1 times higher than that achieved by acclimated mice. Straight lines were fitted to these data and the F-value calculated on the metabolic differences between summer and acclimated mice was significant ($P < 0.01$; $F=7.99$). However, t-tests applied at each of the exposure temperatures showed significant differences only at -23° and -33° C ($P < 0.01$). These slight differences in metabolic rates did not result in any significant difference in the metabolic failure times of these summer and acclimated mice during severe cold exposure (Fig. 7).

Similarly, Fig. 12 shows that in northern maniculatus the maximal metabolic rate maintained by summer mice during severe cold exposure was 1.1 times higher than that of the acclimated mice. This difference, however, was not significant ($F=1.82$).

(c) Total heat production capabilities

An analysis of heat production capabilities was done for each group of Peromyscus during severe cold exposure. This was based on the metabolic failure time and the maximal rate of oxygen consumption maintained prior

to metabolic failure in each group of mice. The maximal rate of oxygen consumption represents either the maximal rate at which oxygen was consumed prior to metabolic failure or the maximal rate at which oxygen was required to maintain the body temperature close to normal. Metabolic failure time, on the other hand, represents the length of time the maximal rate of oxygen consumption was maintained during severe cold exposure. From these data total heat production (total energy expenditure) during severe cold exposure was determined. Oxygen consumption values were converted into energy units ($\text{cal/g} \cdot \text{min}^{-1}$) by multiplying oxygen consumption rate ($\text{ml/hr} \cdot \text{g}^{-0.7}$) by a factor of 0.0788. For this conversion, a value of 4.73 Kcal per litre of oxygen intake was assumed. Corrections for the lower oxygen consumption rate during metabolic failure were not included in the calculations. Therefore, the values for total heat production are maximal values and they are a slight but fairly consistent over-estimation of the total energy expended by each group of mice prior to a failure in their metabolic rate.

Tables IV, V, VI, and VII summarize the heat production capabilities of southern leucopus, northern leucopus, southern maniculatus and northern maniculatus, respectively, during severe cold exposure. Values of metabolic rate expressed on a per gram basis and the mean body weights of each group of mice are also included in each Table to provide easy conversions of metabolic rate and total energy expenditure to any additional desired units. These data show

Table IV. A summary of total heat production in southern leucopus collected in winter, summer, and those acclimated to 22°C.

Group	Metabolic Failure		Total Heat Production until		Maximal Heat Production Rate until		Body Weight	
	Temp. (°C)	Time (min)	Metabolic Failure (ml O ₂ /g)(cal/g ^{0.7})	Metabolic Failure (ml O ₂ /g)(cal/g ^{0.7})	Metabolic Failure (ml O ₂ /g.min ⁻¹)(cal/g ^{0.7} .min ⁻¹)	Metabolic Failure (ml O ₂ /g.min ⁻¹)(cal/g ^{0.7} .min ⁻¹)	n	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Acclimated 22°C	-13	199	33.8	414.2	0.17	2.08	23.8	7
	-18	134	23.7	280.8	0.18	2.10	21.3	14
	-23	90	15.9	189.9	0.18	2.11	21.8	9
	-28	61	10.8	128.2	0.18	2.11	21.4	12
Summer	-18	216	45.9	524.7	0.21	2.43	18.9	16
	-23	142	29.8	338.7	0.21	2.39	18.5	16
	-28	93	18.9	219.7	0.20	2.37	20.1	16
	-33	61	12.4	143.0	0.21	2.36	19.3	18
Winter	-33	208	50.3	608.5	0.24	2.92	22.9	12
	-38	115	26.9	322.9	0.23	2.80	22.4	12
	-43	64	14.7	174.6	0.23	2.73	21.4	13

Table V. A summary of total heat production in northern leucopus collected in winter, summer, and those acclimated to 22°C.

Group	Temp. (°C)	Metabolic Failure		Total Heat Production until		Maximal Heat Production Rate until		Body Weight (g)	n
		Time (min)	Metabolic Failure (ml O ₂ /g)(cal/g ^{0.7})	Metabolic Failure (ml O ₂ /g)(cal/g ^{0.7})	Metabolic Failure (ml O ₂ /g.min ⁻¹)(cal/g ^{0.7} .min ⁻¹)	Metabolic Failure (ml O ₂ /g.min ⁻¹)(cal/g ^{0.7} .min ⁻¹)			
							(1)		
Acclimated 22°C	-13	342	66.3	761.5	0.19	2.23	19.2	8	
	-18	195	38.1	445.7	0.20	2.29	20.4	9	
	-23	111	22.9	257.9	0.21	2.32	17.9	8	
	-28	63	13.5	148.2	0.21	2.34	16.4	11	
Summer	-23	213	50.0	561.2	0.23	2.64	17.8	12	
	-28	132	29.1	338.7	0.22	2.57	20.2	14	
	-33	82	17.2	206.4	0.21	2.53	22.4	15	
	-38	51	11.0	126.5	0.22	2.50	19.1	12	
Winter	-33	427	118.6	1398.8	0.28	3.28	21.0	7	
	-38	192	48.7	572.9	0.25	2.98	20.8	17	
	-43	86	21.7	246.3	0.25	2.85	18.4	14	

Table VI. A summary of total heat production in southern maniculatus collected in winter, summer, and those acclimated to 22°C.

Group	Metabolic Failure		Total Heat Production until		Maximal Heat Production Rate until		Body Weight	
	Temp. (°C)	Time (min)	Metabolic Failure (ml O ₂ /g)(cal/g ^{0.7})	(3)	Metabolic Failure (ml O ₂ /g.min ⁻¹)(cal/g ^{0.7} .min ⁻¹)	(5)	Weight (g)	n
	(1)	(2)		(4)		(6)	(7)	(8)
Acclimated 22°C	-23	260	55.2	622.2	0.21	2.39	18.1	8
	-28	156	33.1	378.2	0.21	2.42	19.0	12
	-33	94	19.8	228.8	0.21	2.44	19.6	13
	-38	56	12.1	138.0	0.21	2.45	18.8	8
Summer	-23	253	58.7	669.6	0.23	2.64	18.8	12
	-28	156	36.3	408.9	0.23	2.62	18.1	14
	-33	96	22.0	250.6	0.23	2.60	18.6	13
	-38	59	13.7	154.0	0.23	2.59	17.9	13
Winter	-38	300	86.6	980.0	0.29	3.27	18.3	8
	-43	128	33.4	379.3	0.26	2.97	18.6	14
	*(-48)	(54)	(13.6)	(154.6)	(0.25)	(2.84)	(18.8)	0

* No experiments were conducted at this temperature. The values in parentheses were predicted from the equations derived for these mice during severe cold exposure.

Table VII. A summary of total heat production in northern maniculatus collected in summer and those acclimated to 22°C.

Group	Metabolic Failure		Total Heat Production until		Maximal Heat Production Rate until		Body Weight	
	Time (min)		Metabolic Failure (ml O ₂ /g)(cal/g ^{0.7})		Metabolic Failure (ml O ₂ /g.min ⁻¹)(cal/g ^{0.7} .min ⁻¹)		(g)	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Acclimated 22°C	-23	288	61.4	698.3	0.21	2.43	18.6	3
	-28	179	39.7	438.5	0.22	2.45	16.9	4
	-33	111	24.4	274.1	0.22	2.47	17.8	4
	-38	69	15.3	170.9	0.22	2.48	17.6	4
Summer	-23	259	59.3	685.1	0.23	2.64	19.6	8
	-28	150	34.9	390.3	0.23	2.62	17.6	12
	-33	86	20.0	223.1	0.23	2.61	17.4	10
	-38	49	11.4	127.7	0.23	2.60	17.9	8

several differences in the heat production capabilities of winter, summer, and acclimated Peromyscus within each species from the northern and southern parts of its range.

i) P. leucopus noveboracensis

The pattern of changes in metabolic failure times, exposure temperatures and maximal oxygen consumption rate among the winter, summer, and acclimated groups of mice was similar in both southern and northern leucopus. Therefore, a description of the effects of previous thermal conditioning on the heat production capabilities of southern leucopus during severe cold exposure also applies to the northern leucopus. Similarly, patterns of heat production capabilities within each group (i.e. either winter, summer or acclimated) were identical in both the northern and southern leucopus. The data for southern leucopus, therefore, will be used to illustrate several major points about the heat production capabilities of P. leucopus noveboracensis during severe cold exposure.

1) Within each group of mice, the metabolic failure time decreased with a decrease in ambient temperature; consequently, the total calories expended prior to metabolic failure also decreased. For example, columns 1,2 and 4 on Table IV show that the metabolic rate of an acclimated mouse would fail around 200 min at temperatures of -13°C and an average $414\text{ cal/g}^{0.7}$ would be utilized during this exposure. At temperatures of -28°C , however, a total of $128\text{ cal/g}^{0.7}$ would be utilized in the 1 hr prior to metabolic

failure. This difference is due to the length of time the maximal heat production rate could be maintained at these temperatures. In addition, these data (column 4, Table IV) show that the total chemical energy available for heat production did not become depleted as the ambient temperature decreased.

2) Within each group of mice, the heat production rate was approximately the same over the range of temperatures representing severe cold exposure (columns 5 and 6, Table IV). Therefore, the decrease in metabolic failure time was proportional only to the increase in heat loss rates as the ambient temperatures decreased.

3) Among the various groups of mice, the total energy expended at comparable exposure temperatures increased with an increase in the thermal conditioning of mice to cold. For example, column 4 of Table IV shows that the total heat produced prior to metabolic failure in summer mice was only $143 \text{ cal/g}^{0.7}$ while that of winter mice was $608 \text{ cal/g}^{0.7}$ when both groups were exposed to -33°C . This major difference in total heat production was a consequence of a longer metabolic failure time and a higher heat production rate of winter mice (columns 2 and 6, Table IV).

4) Among the various groups of mice, the total energy expended at comparable metabolic failure times increased with an increase in the conditioning of mice to cold. At a metabolic failure time of 200 min, winter mice would utilize an average total of $585 \text{ cal/g}^{0.7}$, while summer

and acclimated mice would utilize average totals of 485 and 415 cal/g^{0.7}, respectively. These differences are due to the differences in maximal heat production rates among the winter, summer and acclimated mice.

5) Among the various groups of mice, the maximal heat production rate achieved and maintained during severe cold exposure increased with an increase in the conditioning of mice to cold. Column 6, Table IV shows that winter mice were able to produce more heat per minute than either the summer or acclimated mice during severe cold exposure. Similarly, the heat production rate of summer mice was higher than that of acclimated mice.

ii) P. maniculatus gracilis

The differences in total energy expended prior to metabolic failure in P. maniculatus gracilis were also dependent upon the metabolic failure time and heat production rate within and among the various groups of mice. The effect of previous thermal conditioning on the heat production capabilities of these mice, however, was less pronounced than that shown for P. leucopus noveboracensis, especially between the summer and acclimated groups of mice. For example, column 4 of Table VI shows that the total energy expended by southern maniculatus at comparable temperatures was markedly different between the winter and summer mice but only a slight difference existed between the summer and acclimated mice. The seasonal difference in total energy expended was 6.4 times greater in winter mice

exposed to -38°C than it was for summer mice exposed to the same temperature. This large difference was due to the difference in their heat production rates and metabolic failure times. The heat production rate of winter mice was 1.3 times higher than that of summer mice and this higher rate was maintained 5 times longer than the rate maintained by summer mice when they were both exposed to -38°C (columns 6 and 2, Table VI).

Column 4 of Table VI shows that the total energy expended by summer mice, however, was only slightly more than that expended by the acclimated mice at comparable temperatures. Furthermore, columns 6 and 2 of Table VI show that this small difference was due in part to the higher maximal heat production rate of summer mice since the metabolic failure times of these two groups of mice were essentially the same. Similarly, in northern maniculatus summer mice maintained a higher maximal heat production rate than acclimated mice at the same exposure temperatures (column 6, Table VII). However, the total energy expended by the summer mice prior to metabolic failure was slightly less than that of the acclimated mice. This small difference was due to the shorter times the maximal heat production rate of summer mice could be maintained (column 2, Table VII).

These data show that acclimated maniculatus in each of the northern and southern groups of mice can utilize approximately the same amount of energy as the summer mice prior to metabolic failure even though their heat production

rates were lower than those of the summer mice. This ability, therefore, suggests that the heat loss rate of acclimated maniculatus was much lower than that of summer mice at comparable exposure temperatures since a lower heat loss rate would increase the metabolic failure time and total energy expenditure of an animal during cold exposure.

The total energy expended at comparable metabolic failure times only reflects the differences in the maximal heat production rates among the various groups of maniculatus. Columns 5 and 6, Tables VI and VII show that the maximal heat production rate increased with an increase in the thermal conditioning of mice to cold. Consequently, the total energy expended at comparable metabolic failure times increased as the previous conditioning of mice to cold increased. For example, southern maniculatus acclimated to 22° C would utilize an average total of $598 \text{ cal/g}^{0.7}$ during a 250 min exposure to severe cold stress (i.e. at temperatures inducing the maximal rate of heat production) while summer mice would utilize an average total of $660 \text{ cal/g}^{0.7}$. Winter mice, on the other hand, would utilize an average total of $795 \text{ cal/g}^{0.7}$ in 250 min of severe cold stress. Similarly, in northern maniculatus, the total energy expended by summer mice would also be slightly more than that of the acclimated mice at comparable metabolic failure times because of the higher maximal heat production rate of summer mice. These data for summer and acclimated maniculatus do not indicate any differences in

their heat loss rates. Therefore, when one compares summer and acclimated maniculatus, maximal rates of heat production and metabolic failure times must be compared at the same exposure temperatures in order to obtain a more thorough understanding of their thermoregulatory abilities.

Tables VI and VII also show that the total energy expended within each group of mice decreased as the ambient temperature decreased (columns 4 and 1). In addition, the maximal rates of heat production within each group remained fairly stable as the ambient temperature decreased (columns 5 and 6, Tables VI and VII).

In summary, the total heat production capabilities of Peromyscus were determined from the interrelationships of metabolic failure times, maximal heat production rates, and the lower temperature ranges at which these mice could thermoregulate. Furthermore, these variables changed with respect to the thermal conditioning of the mice. Figures 13, 14, and 15 illustrate the heat production capabilities of southern leucopus, northern leucopus, and southern maniculatus, respectively, and show the effect of previous thermal conditioning on their heat production capabilities during severe cold exposure.

C. Cold Resistance and Heat Production Capabilities in Relation to Geographic Distribution

In this section, cold resistance and heat production capabilities of P. leucopus noveboracensis and P. maniculatus gracilis are compared in relation to their geographic

Fig. 13. Three-dimensional interrelationship of ambient temperature, metabolic failure time, and total heat production in southern leucopus collected in winter (solid line), summer (broken line), and acclimated to 22° C (dotted line). Total heat production values at 60, 100, 200, and 300 min are shown on the hatched area for each group of mice.

Fig. 14. Three-dimensional interrelationship of ambient temperature, metabolic failure time, and total heat production in northern leucopus collected in winter (solid line), summer (broken line), and acclimated to 22° C (dotted line). Total heat production values at 60, 100, 200, and 300 min are shown on the hatched area for each group of mice.

Fig. 15. Three-dimensional interrelationship of ambient temperature, metabolic failure time, and total heat production in southern maniculatus in winter (solid line), summer (broken line), and acclimated to 22° C (dashed line). Total heat production values at 60, 100, 200, and 300 min are shown by the hatched area for each group of mice.

distribution in winter, summer and after acclimation to constant laboratory conditions.

(a) Metabolic failure times

i) Winter mice

Metabolic failure times in relation to ambient temperature for the three groups of winter mice are shown in Fig. 16. Southern maniculatus showed the greatest resistance to cold stress. During severe cold exposure, their metabolic failure times were significantly longer than those of the sympatric northern leucopus ($P < 0.05$; $F=4.02$). Figure 16 shows that, on the average, the time for the oxygen consumption rate to fail in southern maniculatus during severe cold exposure was 1.3 times longer than that of northern leucopus. Similarly, the metabolic failure times of southern maniculatus in winter were significantly longer than those of southern leucopus during severe cold exposure ($P < 0.001$; $F=12.0$). For example, at -38° and -43° C, the metabolic failure times of southern maniculatus were 2.6 and 2.0 times longer than those of southern leucopus, respectively (Fig. 16). Thus the cold resistance of the two species of Peromyscus collected from similar and different geographic areas and acclimatized to winter conditions was significantly different during severe cold exposure.

Similar differences were found between the northern and southern leucopus collected in winter. Figure 16 shows that the metabolic failure times of northern leucopus were 2.0, 1.7, and 1.3 times longer than those of the southern

Fig. 16. Relationship between logarithm of metabolic failure time and ambient temperature in maniculatus and leucopus collected from the northern and southern parts of their ranges in summer and winter.

leucopus at temperatures of -33° , -38° , and -43° C, respectively. The metabolic failure times of northern leucopus were significantly lower than those of southern leucopus over the range of temperatures representing severe cold exposure ($P < 0.01$; $F=6.78$). Actual mean metabolic failure times at comparable exposure temperatures were only significantly different at -33° C ($P < 0.001$; $t=4.56$). Therefore, intraspecific differences in metabolic failure times of winter leucopus were not significant at the extremely cold temperatures. However, when these mice were exposed to temperatures at which thermoregulation was possible for more than 3 hr ($T_a = -33^{\circ}$ C), the length of time before the maximal oxygen consumption rate failed was significantly greater in the northern leucopus.

Winter mice in all cases showed a greater resistance to cold stress, as measured by their metabolic failure times, than summer mice collected from the various study areas (Fig. 16).

ii) Summer mice

Metabolic failure times in relation to ambient temperature for all summer mice collected from the four study areas are also shown by regression lines in Fig. 16. Summer maniculatus from the northern and southern parts of their range showed no significant difference in metabolic failure times between exposure temperatures of -23° and -38° C. Their oxygen consumption rates on the average failed around 1 hr at -38° C and 4 hr at -23° C. The lower temperature

limit of cold exposure was -38°C for all summer maniculatus.

Cold resistance in southern maniculatus and northern leucopus collected from the same geographic area in summer also showed no significant difference in their metabolic failure times nor their lower temperature limit of cold exposure. Figure 16 shows that the average metabolic failure times of southern maniculatus, however, were consistently 1.2 times longer than those of northern leucopus at all exposure temperatures. The average metabolic failure times of northern maniculatus slightly exceeded those of northern leucopus only at -28° and -23°C . Their oxygen consumption rates failed at approximately the same time at temperatures of -33° and -38°C (Fig. 16). The small differences in their metabolic failure times were not significant; thus, cold resistance in northern leucopus was very similar to that of both southern and northern maniculatus when acclimatized to summer conditions.

Figure 16 shows that the metabolic failure times of southern leucopus are markedly different from those of the other groups of summer mice. Their metabolic failure times during severe cold exposure were significantly lower than those of northern leucopus ($P < 0.001$; $F=10.4$) as well as those of both northern and southern maniculatus ($P < 0.001$). In addition, their lower temperature limit of cold exposure was 5°C higher than the other three groups of mice.

iii) Acclimated mice

Peromyscus collected from the various geographic

areas and acclimated to 22° C showed several major differences in their cold resistance during severe cold stress (Fig. 17). Within the maniculatus, no significant difference in metabolic failure times was found between the northern and southern groups of acclimated mice. Both groups could thermoregulate for 1 to 5 hr at temperatures ranging from -38° to -23° C, respectively, before their oxygen consumption rate failed.

Within the acclimated leucopus, however, intra-specific differences in cold resistance were found between the samples from the two geographic areas. Figure 17 shows that the regressions of their metabolic failure times on exposure temperatures converged at their lower temperature limit of cold exposure. In fact, at -28° and -23° C, the metabolic failure times of northern and southern leucopus were the same. However, at -18° and -13° C the average times for the oxygen consumption rate to fail in northern leucopus were 1.6 times longer than those of southern leucopus. These differences at -18° and -13° C were significant ($P < 0.02$). Similarly, the difference between the regression lines representing their metabolic failure times during severe cold exposure was significant ($P < 0.01$; $F=7.87$). Further analysis showed significant differences in the intercepts ($P < 0.001$; $t=33.8$) as well as the slopes of the two lines ($P < 0.05$; $t=2.40$).

Figure 17 shows that the major difference in cold resistance of acclimated mice was between the two species.

Fig. 17. Relationship between logarithm of metabolic failure time and ambient temperature in northern and southern maniculatus and leucopus acclimated to 22° C.

The metabolic failure times of maniculatus were significantly longer than those of leucopus regardless of the geographic area from which they were collected ($P < 0.001$). In addition, the lower temperature limit of cold exposure was -38°C for acclimated maniculatus and only -28°C for acclimated leucopus. To illustrate the magnitude of difference this extension had on cold resistance, the temperatures at which the metabolic rate would fail in about 200 min were compared; southern leucopus would have to be exposed to -13°C as compared to -18°C for northern leucopus. Maniculatus from either geographic area, on the other hand, would have to be exposed to temperatures as low as -28°C for metabolic failure to occur in about 200 min.

Similarly, at all comparable temperatures, the metabolic failure times of acclimated maniculatus were significantly longer than those of acclimated leucopus. For example, maniculatus collected from the same geographic area as leucopus and acclimated to 22°C showed a 2.3 and 2.5 times longer metabolic failure time during exposure to -23° and -28°C ($P < 0.001$).

In summary, the following general conclusions were drawn with respect to cold resistance in summer and acclimated Peromyscus during severe cold exposure:

- 1) Metabolic failure times of all acclimated maniculatus were essentially the same as those of all summer maniculatus and northern leucopus collected in summer (see Figs. 16 and 17). No significant differences were found in their metabolic

failure times at temperatures of severe cold stress.

2) Southern leucopus during the summer showed a significantly lower resistance to cold stress than both groups of acclimated maniculatus. This difference was the same as that described earlier between the northern and southern maniculatus and southern leucopus collected during the summer.

3) Southern leucopus collected in summer were more resistant to cold stress than northern leucopus acclimated to 22° C. At temperatures higher than -23° C, there was no significant difference in their metabolic failure times; however, differences were significant at -28° C ($P < 0.01$). In addition, the lower temperature limit of cold exposure was -33° C in the summer leucopus and only -28° C in the acclimated leucopus.

(b) Oxygen consumption rates

i) Winter mice

Major geographic differences in oxygen consumption rates in relation to ambient temperature among the three groups of winter mice were not as pronounced as those previously described in their metabolic failure times. The oxygen consumption rates of all winter mice exposed to temperatures ranging from -10° to -43° C are shown in Table VIII. The metabolic rate of southern leucopus at -43° C was significantly lower than that of northern leucopus ($P < 0.05$; $t=2.46$), and that of southern maniculatus ($P < 0.02$; $t=2.75$). In contrast, at -10° C, southern leucopus had a significantly

Table VIII. Metabolic rates in relation to ambient temperature in winter Peromyscus from three geographic areas.

Temperature (°C)	Oxygen Consumption Rates (ml O ₂ /hr.g ^{-0.7})*			
	Southern <u>leucopus</u> (n)	Northern <u>leucopus</u> (n)	Southern <u>maniculatus</u> (n)	
-10	25.3 ± 1.9 (8)	22.0 ± 1.4 (8)	20.9 ± 2.0 (4)	
-23	33.9 ± 2.0 (8)	31.2 ± 2.8 (8)	31.2 ± 2.6 (6)	
-33	37.5 ± 2.7 (12)	41.0 ± 2.9 (7)	36.2 ± 3.6 (7)	
-38	37.7 ± 1.4 (12)	37.7 ± 2.3 (17)	42.0 ± 4.1 (8)	
-43	33.9 ± 2.1 (13)	37.9 ± 2.3 (14)	38.4 ± 2.5 (14)	

* Mean ± 2SE

higher metabolic rate than both northern leucopus ($P < 0.02$; $t=2.83$) and southern maniculatus ($P < 0.01$; $t=3.26$). Between temperatures of -23° and -38° C, the oxygen consumption rates of the southern and northern leucopus and southern maniculatus were not significantly different. These data therefore show that although northern leucopus and southern maniculatus in winter maintained a significantly lower oxygen consumption rate than southern leucopus at higher exposure temperatures, they were able to elevate their oxygen consumption rate slightly higher than that of southern leucopus at lower exposure temperatures (Table VIII). The metabolic rates of northern leucopus and southern maniculatus in winter were essentially the same at all temperatures ranging from -10° to -43° C ($P < 0.05$).

At temperatures causing metabolic failure within 1 to 6 hr, the oxygen consumption rates of all winter mice were maximal and these rates were maintained until metabolic failure. The maximal rates of oxygen consumption maintained by winter mice prior to metabolic failure are represented by the upper group of regression lines in Fig. 18a. Between metabolic failure times of 50 and 400 min, southern maniculatus maintained a slightly higher maximal rate of metabolism than northern and southern leucopus. Similarly, northern leucopus had a slightly higher maximal rate of oxygen consumption than southern leucopus. The average maximal oxygen consumption rate maintained by southern maniculatus at metabolic failure times ranging from 50 to

Fig. 18a. Relationship between maximal metabolic rate and metabolic failure time in northern and southern maniculatus and leucopus collected during winter and summer.

Fig. 18b. Relationship between maximal metabolic rate and metabolic failure time in maniculatus and leucopus acclimated to 22° C.

400 min was $39.7 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$ while those for northern and southern leucopus were 38.1 and $36.0 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-0.7}$, respectively. No significant differences were found in the maximal rates of oxygen consumption maintained by these groups of winter mice during severe cold exposure.

Tables IV, V, and VI show that the mean maximal heat production rates of winter mice prior to metabolic failure were 3.09, 2.99, and $2.81 \text{ cal/g}^{0.7}\cdot\text{min}^{-1}$ for southern maniculatus, northern leucopus, and southern leucopus respectively. These rates, in conjunction with total time until metabolic failure, resulted in large differences in the total energy utilized at comparable temperatures. For example, at -38°C , southern maniculatus utilized a total of $980 \text{ cal/g}^{0.7}$ prior to metabolic failure while the total amounts of energy expended by northern and southern leucopus were only 573 and $323 \text{ cal/g}^{0.7}$, respectively. Therefore, at comparable temperatures, southern maniculatus had a much longer metabolic failure time and a slightly higher heat production rate than both northern and southern leucopus; consequently, their total energy expenditure prior to metabolic failure was higher.

At comparable metabolic failure times, however, differences in total energy expenditure were less marked. The average metabolic cost to survive 200 min at temperatures inducing maximal heat production would be 620, 598, and $580 \text{ cal/g}^{0.7}$ for southern maniculatus, northern leucopus, and southern leucopus, respectively. The small differences

would be due entirely to the differences in their heat production rates.

In summary, these data for winter mice suggest that the rate of heat loss in southern maniculatus during severe cold exposure was lower than those of northern and southern leucopus. Similarly, the rate of heat loss of northern leucopus appeared to be lower than that of southern leucopus. Such differences in heat loss rates coupled with the slight differences in maximal heat production rates would account for the large differences found in metabolic failure times and total energy expenditure among the winter mice from the three geographic areas. The differences in maximal heat production rates per se would not account for these differences.

ii) Summer mice

Oxygen consumption rates in relation to ambient temperature for summer mice collected from the four geographic areas are shown in Table IX. There was no significant difference in the oxygen consumption rates of northern and southern leucopus at comparable temperatures. However, with the exception of -10°C , the mean metabolic rate of northern leucopus was higher than that of southern leucopus at all comparable temperatures. In addition to a slightly higher oxygen consumption rate, Table IX also shows that northern leucopus were able to maintain their metabolic rate at a temperature 5°C lower than that at which it was maintained by southern leucopus. The oxygen consumption

Table IX. Metabolic rates in relation to ambient temperature in summer Peromyscus from the four geographic areas.

Temperature (°C)	Oxygen Consumption Rates (ml O ₂ /hr.g ^{-0.7})*					
	<u>Southern</u> <u>leucopus</u>	(n)	<u>Northern</u> <u>leucopus</u>	(n)	<u>Southern</u> <u>maniculatus</u>	<u>Northern</u> <u>maniculatus</u> (n)
0	17.8 ± 1.6	(8)	19.6 ± 2.4	(6)	22.6 ± 1.4	20.0 ± 1.7 (4)
-10	26.5 ± 3.0	(8)	23.9 ± 1.6	(8)	28.0 ± 1.7	25.6 ± 1.8 (5)
-18	29.0 ± 1.6	(16)				
-23	30.6 ± 1.7	(17)	33.4 ± 1.5	(12)	33.7 ± 1.3	32.0 ± 2.3 (8)
-28	31.1 ± 1.6	(16)	32.6 ± 2.7	(14)	31.8 ± 1.5	34.0 ± 2.5 (12)
-33	30.5 ± 1.4	(18)	32.4 ± 2.0	(15)	34.3 ± 1.3	33.3 ± 1.9 (10)
-38			33.2 ± 2.5	(12)	33.1 ± 1.8	33.5 ± 2.3 (8)

* Mean ± 2SE

rates of northern and southern maniculatus were essentially the same at all exposure temperatures (Table IX).

The major difference in oxygen consumption rates between the northern leucopus and southern maniculatus was found only at the higher exposure temperatures. Table IX shows that the oxygen consumption rates of southern maniculatus were higher than those of northern leucopus at -10° and 0° C. The difference at -10° C was significant. Since the oxygen consumption rates of northern leucopus and southern maniculatus were essentially the same at -23° C and lower, there does not appear to be an insulative difference between these two groups of summer mice. The lower oxygen consumption rates of northern leucopus at -10° and 0° C may be due to their positioning behaviour at these temperatures. Northern leucopus maintained a fairly consistent huddled position throughout these exposures while southern maniculatus appeared to spend more time in non-locomotor activity (e.g. grooming). Table IX shows that no such differences in oxygen consumption rates existed between the northern leucopus and northern maniculatus.

The maximal rates of oxygen consumption maintained by summer mice prior to metabolic failure during severe cold exposure are also shown in Fig. 18a. No significant differences were found in the maximal rates of oxygen consumption maintained by northern maniculatus, southern maniculatus, and northern leucopus. The mean maximal metabolic rates maintained by these groups of mice at

metabolic failure times ranging from 50 to 400 min were 33.3, 33.2, and 32.6 ml $O_2/hr \cdot g^{-0.7}$, respectively. The mean maximal oxygen consumption rate of 30.3 ml $O_2/hr \cdot g^{-0.7}$ maintained by southern leucopus between metabolic failure times of 50 and 400 min was significantly lower than that of northern leucopus and the northern and southern maniculatus ($P < 0.001$). Therefore, southern leucopus collected in summer were unable to elevate their oxygen consumption rate and maintain it at the higher level attained by northern leucopus and the maniculatus during severe cold exposure.

The mean maximal rates of heat production maintained by northern leucopus, southern maniculatus, and northern maniculatus during severe cold exposure were 2.56, 2.61, and 2.62 cal/ $g^{0.7} \cdot min^{-1}$, respectively. Furthermore, there were no significant differences in their metabolic failure times during severe cold stress. Therefore, the total energy expended prior to metabolic failure was approximately the same in these three groups of summer mice (see Tables V, VI, and VII). The metabolic failure times of southern leucopus, however, were significantly lower than those of the other groups of summer mice. In addition, their mean maximal heat production rate was only 2.39 cal/ $g^{0.7} \cdot min^{-1}$ (see Table IV). Consequently, the total energy expended prior to metabolic failure by southern leucopus in summer was much lower than that expended by either northern leucopus, southern maniculatus, or northern maniculatus in summer.

iii) Acclimated mice

Several major differences were found in the oxygen consumption rates in relation to ambient temperature among the four groups of mice acclimated to 22° C. Table X shows that the mean oxygen consumption rates of northern leucopus were slightly higher than those of southern leucopus at all temperatures of severe cold stress (between -13° and -28° C). The difference at -13° C was highly significant ($P < 0.001$; $t=5.23$); however, no significant differences were found at the other exposure temperatures.

Similarly, Table X shows that the oxygen consumption rates of northern and southern maniculatus were almost the same at all exposure temperatures except -10° C. This difference at -10° C was significant ($P < 0.05$). The rates of increase in metabolism per C° decrease in temperature from 0° to -23° C was $0.51 \text{ ml O}_2/\text{hr} \cdot \text{g}^{-0.7} \cdot \text{C}^{-1}$ for northern maniculatus and $0.44 \text{ ml O}_2/\text{hr} \cdot \text{g}^{-0.7} \cdot \text{C}^{-1}$ for southern maniculatus. These rates were not significantly different between these two groups of acclimated mice. The oxygen consumption curves for acclimated northern and southern maniculatus were therefore the same between temperatures of 0° and -38° C.

There was no significant difference between the oxygen consumption rates of northern leucopus and southern maniculatus during cold exposure. However, Table X shows that the major difference between these two groups of acclimated mice was the temperatures at which their maximal

Table X. Metabolic rates in relation to ambient temperature in the four groups of Peromyscus acclimated to 22°C.

Temperature (°C)	Oxygen Consumption Rates (ml O ₂ /hr.g ^{-0.7})*					
	<u>Southern</u> <u>leucopus</u>	(n)	<u>Northern</u> <u>leucopus</u>	(n)	<u>Southern</u> <u>maniculatus</u>	<u>Northern</u> <u>maniculatus</u>
0	19.9 ± 1.4	(10)	17.5 ± 1.1	(8)	19.0 ± 2.0	17.5 ± 1.1
-10					24.9 ± 1.7	21.5 ± 1.9
-13	23.8 ± 1.0	(7)	28.1 ± 1.3	(8)		
-18	26.4 ± 2.2	(14)	29.4 ± 1.8	(9)		
-23	27.4 ± 2.8	(9)	29.7 ± 2.3	(8)	29.2 ± 1.8	28.9 ± 3.3
-28	28.0 ± 2.4	(12)	29.2 ± 1.8	(11)	31.4 ± 1.5	31.1 ± 1.7
-33					31.1 ± 1.8	31.4 ± 3.9
-38					31.5 ± 1.5	32.7 ± 3.4

* Mean ± 2SE

oxygen consumption rates were maintained. Southern maniculatus maintained their maximal rate of metabolism at temperatures 10 C° lower than that at which northern leucopus could maintain their maximal rate of metabolism.

The relationships of maximal metabolic rates and metabolic failure times for the acclimated groups of mice are shown in Fig. 18b. The maximal rates of oxygen consumption maintained by northern and southern maniculatus during severe cold exposure were not significantly different. Their mean maximal rates were 31.2 and 30.7 ml O₂/hr.g^{-0.7}, respectively. However, southern leucopus maintained a significantly lower maximal rate of oxygen consumption than northern leucopus between metabolic failure times of 50 and 400 min (P < 0.01; F = 5.75). Their mean rates were 26.6 and 29.1 ml O₂/hr.g^{-0.7}, respectively. The maximal rate of metabolism of southern leucopus was also significantly lower than those of northern and southern maniculatus over the same range of metabolic failure times. Figure 18b shows that the maximal rate of metabolism of northern leucopus was slightly lower than that of southern maniculatus at metabolic failure times ranging from 50 to 400 min. This difference was not significant.

The heat production capabilities of acclimated northern and southern maniculatus during severe cold exposure were very similar. Their mean heat production rates prior to metabolic failure were 2.46 and 2.43 cal/g^{0.7}.min⁻¹, respectively. The mean metabolic failure times of northern

maniculatus were slightly higher than those of southern maniculatus at comparable temperatures; consequently, the total amounts of energy they expended prior to metabolic failure were slightly higher (see Tables VI and VII). These differences were not significant. Similarly, the total amount of energy expended at comparable metabolic failure times were approximately the same in both groups of maniculatus.

Within the acclimated leucopus, the major differences between the northern and southern forms were found during exposure to -13° and -18° C. At these temperatures, the metabolic failure times of northern leucopus were significantly longer than those of southern leucopus and their total energy expenditures prior to metabolic failure were much greater (see Tables V and IV). In addition, the mean maximal rate of heat production of northern leucopus was 1.1 times higher than that of southern leucopus. At -23° and -28° C, the maximal heat production rate of northern leucopus was still 1.1 times higher than that of southern leucopus; however, the differences in metabolic failure times and total energy expenditures between these two groups of acclimated mice were reduced considerably (Tables IV and V). The differences in metabolic failure times and total energy expenditures between the northern and southern leucopus during severe cold exposure appear to be primarily due to the differences in maximal heat production rates. However, at -13° and -18° C, northern leucopus, in addition to a higher maximal heat production rate, may also have a

lower heat loss rate. This could account for the much greater difference in metabolic failure times and total energy expenditures at these temperatures. Such differences in heat loss rates may be due to slight differences in behaviour of the northern and southern leucopus at -13° and -18° C. No insulative differences between these two groups of acclimated mice were indicated at lower exposure temperatures nor during exposure to 0° C.

Tables V and VI show that the mean maximal rate of heat production of southern maniculatus was approximately 1.1 times higher than that of northern leucopus during severe cold exposure. At comparable metabolic failure times, this difference in maximal heat production rate results in a very small difference in total energy expenditure. For example, at metabolic failure times of 100, 200, and 300 min, southern maniculatus would expend approximately 1.1 times more energy than northern leucopus. However, when these two groups of acclimated mice are compared at the same exposure temperatures, southern maniculatus utilized 2.4 and 2.6 times more energy than northern leucopus at -23° and -28° C, respectively (Tables V and VI). This difference in total energy expenditure was due primarily to the significantly higher metabolic failure times of southern maniculatus at these temperatures. For example, at -23° and -28° C, their metabolic failure times were 2.5 and 2.3 times higher than those of northern leucopus. In addition, Tables V and VI show that southern maniculatus

could thermoregulate at temperatures 10 C° lower than northern leucopus. These large differences in total energy expenditure, metabolic failure times, and exposure temperatures do not appear to be a result of the small differences in maximal heat production rates between these two groups of acclimated mice. These data, therefore, suggest the heat loss rates between northern leucopus and southern maniculatus were considerably different during severe cold exposure.

D. Magnitude of Noradrenaline-Induced Thermogenesis

(a) Pattern of response to noradrenaline

Figures 19a and 19b illustrate the typical pattern of increase in oxygen consumption in response to noradrenaline and the corresponding increase in body temperature that was observed in all Peromyscus tested. In the example of the northern leucopus acclimated to 22° C, Fig. 19a shows that oxygen consumption rate increased to a maximum of 5.83 ml O₂/hr.g⁻¹ within 40 min after injection of noradrenaline. This was a 5.4-fold increase over the pre-treatment level of 1.08 ml O₂/hr.g⁻¹. The body temperature increased from a constant anesthetic level of 32.9° C to a maximum of 41.1° C in approximately the same period of time.

Figure 19b shows the oxygen consumption response of a northern leucopus acclimatized to winter conditions. Its oxygen consumption rate increased to a maximum of 9.1 ml O₂/hr.g⁻¹ within 35 min after injection of noradrenaline. This was an 8.6-fold increase over the pre-

Fig. 19a. Oxygen consumption and body temperature before and after intraperitoneal injection of noradrenaline (NA) in a northern leucopus acclimated to 22° C.

Fig. 19b. Oxygen consumption and body temperature before and after intraperitoneal injection of noradrenaline (NA) in a northern leucopus acclimatized to winter conditions.

treatment level of $1.10 \text{ ml O}_2/\text{hr}\cdot\text{g}^{-1}$. During this response, the body temperature rose from an anesthetic level of 31.1° C to a maximum of 41.7° C .

From the individual response curves, such as those shown in Fig. 19, the pretreatment and maximal levels of oxygen consumption were used to determine the magnitude of noradrenaline-induced thermogenesis for each group of Peromyscus.

(b) Magnitude of thermogenic response to noradrenaline

Table XI summarizes the extent to which noradrenaline influenced the oxygen consumption rate of winter, summer, and acclimated Peromyscus collected from two geographic areas. All groups of mice showed a significant increase in their oxygen consumption rates after intraperitoneal injection of noradrenaline. These rates increased to 4.0 to 6.5 times that of the pretreatment level of oxygen consumption maintained by each group of mice.

In southern leucopus, no direct relationship was found between the thermogenic response to noradrenaline and the previous thermal conditioning of these mice. Table XI shows that the response after noradrenaline injection (mean maximal rate of oxygen consumption induced by noradrenaline) of southern leucopus was similar in the winter, summer, and acclimated groups of mice. The pretreatment level of oxygen consumption of the summer mice was significantly higher than that of the winter and acclimated mice ($P < 0.05$). Consequently, their magnitude

Table XI. Oxygen consumption rates before and after noradrenaline injection (3 to 5 mg/kg, intraperitoneally) into winter, summer, and acclimated (22°C) Peromyscus collected from two geographic areas.

Group	n	Mean Body Weight (g)*	Mean Rate of Oxygen Consumption (ml O ₂ /hr.g ⁻¹)**		Magnitude of Response (ratio B/A)
			Pretreatment (A)	Post-treatment (B)	
<u>Southern leucopus</u>					
a) winter	10	24.3 ± 2.1	1.20 ± 0.19	6.21 ± 0.77	5.42 ± 0.63
b) summer	6	22.2 ± 2.1	1.47 ± 0.12	5.70 ± 0.49	3.98 ± 0.53
c) acclimated 22°C	10	21.5 ± 2.3	1.28 ± 0.10	6.18 ± 0.37	4.88 ± 0.33
<u>Northern leucopus</u>					
a) winter	10	22.0 ± 3.0	1.20 ± 0.14	7.68 ± 0.83	6.53 ± 0.84
b) summer	10	22.0 ± 2.4	1.27 ± 0.16	6.31 ± 1.11	4.96 ± 0.64
c) acclimated 22°C	10	21.7 ± 3.3	1.15 ± 0.10	5.32 ± 0.64	4.62 ± 0.41
<u>Southern maniculatus</u>					
a) winter	11	21.3 ± 2.8	1.33 ± 0.13	7.29 ± 0.88	5.55 ± 0.62
b) summer	10	20.1 ± 3.5	1.23 ± 0.11	7.24 ± 0.63	5.95 ± 0.64
c) acclimated 22°C	10	20.7 ± 2.2	1.30 ± 0.19	6.32 ± 0.36	5.11 ± 0.79

* Mean ± 1SD

** Mean ± 2SE

of response (ratio B/A) was much lower than in either the winter or acclimated groups of southern leucopus. During these responses in southern leucopus, rectal temperatures rose from mean levels during anesthetization of 33.6°, 35.0°, and 33.6° C up to a maxima of 42.2°, 41.1°, and 41.4° C in winter, summer, and acclimated mice, respectively.

Table XI shows that the thermogenic responses to noradrenaline of northern leucopus were more related to their previous thermal history than those of southern leucopus. The mean maximal rate of oxygen consumption achieved by the winter mice after noradrenaline injection was significantly higher than that of the acclimated mice ($P < 0.001$; $t=4.09$). Moreover, their magnitude of noradrenaline response was much greater than those of the summer and acclimated groups of northern leucopus. There were no significant differences in the pretreatment levels of oxygen consumption among the three groups of mice. Mean rectal temperatures ranged from 32.6° to 33.2° C prior to noradrenaline injection and rose to maxima of 41.8°, 40.5°, and 40.6° C in these winter, summer and acclimated northern leucopus, respectively after injection.

Table XI shows that within the southern maniculatus, the pretreatment and maximal level of oxygen consumption, and consequently, the magnitude of noradrenaline response were very similar in winter, summer, and acclimated mice. Rectal temperatures of winter mice before noradrenaline injection averaged 33.4° C and rose to an average maximum

of 41.8° C after noradrenaline injection while those of summer and acclimated mice increased from 32.6° and 32.8° C to maxima of 40.7° and 40.6° C, respectively.

The only significant difference among the winter groups of mice was found between the northern and southern leucopus. Table XI shows that the mean maximal rate of oxygen consumption after noradrenaline injection and the magnitude of noradrenaline response of northern leucopus were considerably greater than those of the southern leucopus. The thermogenic responses to noradrenaline were essentially the same in the leucopus and maniculatus collected from similar and different geographic areas in winter.

In summer mice, Table XI shows that the high pre-treatment level of oxygen consumption of southern leucopus resulted in a much lesser response to noradrenaline than those obtained for northern leucopus and southern maniculatus. The mean maximal rate of oxygen consumption achieved by southern leucopus after noradrenaline injection, however, was significantly lower than that of southern maniculatus ($P < 0.01$; $t = 3.87$). Table XI also shows that the mean maximal rate of oxygen consumption of northern leucopus was lower than that of southern maniculatus during noradrenaline response; however, this difference was not significant. Similarly, the magnitude of noradrenaline response of northern leucopus in summer was much lower than that of southern maniculatus.

Among the acclimated groups of mice, Table XI shows

that the pretreatment levels of oxygen consumption and the magnitude of noradrenaline response were approximately the same. The mean maximal rate of oxygen consumption achieved during noradrenaline response by northern leucopus, however, was significantly lower than those achieved by the southern leucopus and southern maniculatus ($P < 0.05$).

Finally, the significance of noradrenaline response as an indicator of nonshivering thermogenesis was evaluated in the various groups of mice. This was done by comparing the maximal rates of oxygen consumption induced by noradrenaline to the maximal oxygen consumption rates maintained during severe cold exposure for all groups of mice (Appendix Table 2). These data show that nonshivering thermogenesis may account for about 45 to 60% of the heat produced in Peromyscus during severe cold exposure. In addition, there was no consistent relationship among the groups of mice from the various geographic areas and their previous thermal conditioning.

DISCUSSION

The understanding of physiological adaptations to natural environmental conditions requires consideration of a complex network of integrating factors, both physiological and environmental in nature. The present study has attempted to describe some of these factors that are related to thermoregulation, and provide a more complete outlook on the thermoregulatory limits and abilities of small mammals from natural populations. In part, this study is an extension of the evidence accumulated from previous laboratory studies; however, the approach taken attempts to synthesize, in one major study, several basic findings obtained from previous less comprehensive studies conducted on small mammals during cold exposure. A study of this nature provides further insight into the thermoregulatory abilities of small mammals in the following ways:

First, it emphasizes the interrelationships of three important thermoregulatory parameters, namely heat production, environmental temperature, and the time a cold stress can be tolerated. Second, it establishes the limits of these parameters for small mammals conditioned to various thermal environments. Differences in these limits are indicative of physiological adaptations responsible for the maintenance of a constant body temperature during cold exposure. Third, it demonstrates the potential influence of climate on the thermoregulatory abilities of small mammals from similar and different geographic areas.

Fourth, it examines the effect of acclimation on the thermoregulatory abilities of natural populations of small mammals. Differences in thermoregulatory abilities are believed to be a reflection of genetic variation within and between species from the various geographic areas.

In the present study, marked differences were found in cold resistance and heat production capabilities of P. leucopus noveboracensis and P. maniculatus gracilis. These differences have been described on the basis of changes measured in their lower temperature limits of cold exposure, maximal rates of oxygen consumption (heat production), and metabolic failure times. These parameters are interrelated and are believed to be valuable indicators of the thermoregulatory abilities of these mice.

A. Metabolic Failure

Progressive lowering of the ambient temperature from 0° C resulted in an increase in oxygen consumption rate to a maximal level in all groups of mice (Figs. 9 to 12). The high rate of oxygen consumption measured for each group of mice was repeatedly achieved over a 15 C° change in temperature; therefore, this rate is believed to represent the maximal rate of heat production possible for each group of mice. Hart (1971) stated that maximal rates of metabolism can only be assumed if the same oxygen consumption is attained at several cold temperatures. At low temperatures inducing maximal heat production, oxygen consumption rates of individual mice were maintained at a fairly stable level

until metabolic failure (Fig. 4a). These maximal rates of heat production would, therefore, be indicative of heat loss rates if the body temperatures of mice were maintained prior to metabolic failure. Continuous body temperatures of mice were not measured during severe cold exposure; therefore, the energetics associated with body temperature maintenance cannot be quantitatively assessed. However, the following relationships among heat production rates, heat loss rates and body temperatures may occur during severe cold exposure:

- 1) Heat production equals heat loss and body temperatures are maintained throughout the entire exposure time. This was indicated in 6% of the Peromyscus exposed to severe cold. Body temperatures averaged only 1 to 2 C° below normal (38° C). Therefore, if metabolic failure did not occur by 6 hr, the maximal rates of heat production are assumed to equal heat loss rates.
- 2) Metabolic failure may result from a depletion of some important metabolic factor. In this case, heat production rates balance heat loss rates until such time that some important factor becomes depleted. Prior to depletion, body temperatures are maintained. Then, heat loss rate exceeds heat production rate and the animal enters a state of hypothermia.
- 3) Metabolic failure results primarily from the effect of temperature. In this case, heat loss rate exceeds heat production rate immediately after the mouse is placed in

the cold. Body temperatures decline at a rate proportional to the differential between heat production rate and heat loss rate at the particular exposure temperature. Further lowering of the temperature increases the differential between the rates of heat production and heat loss, and the body temperature declines more rapidly. Such forced changes in body temperature from normal levels exert feedback effects on physiological thermal defenses, both through Q_{10} relationships and by directly affecting the integrity of neural, hormonal, and cardiovascular thermal control mechanisms (Adams 1971).

Thus, metabolic failure results primarily from an imbalance between the rate of heat production and rate of heat loss. Furthermore, these rates are influenced by the previous thermal conditioning of small mammals from various geographic areas. Consequently, several major differences in heat production capabilities and cold resistance were found in the various groups of mice examined in the present study.

B. Geographic Differences

Geographic differences in thermoregulatory abilities of populations of the same species or of two different species are complicated by the presence of both genetic and nongenetic variation in the populations examined (Hart 1971). The design of the present study enables one to evaluate the potential influence of geographic climates on the thermoregulatory abilities of Peromyscus and enables speculation

on their genetic ability to thermoregulate under conditions of severe cold stress. Mean daily minimum temperatures were used as indices of the climatic conditions in the various geographic areas. These temperatures were believed to be an important environmental factor that affects the thermoregulatory mechanisms of Peromyscus; these mechanisms, in turn, enable them to adapt to cold in their natural environments. Other environmental factors, however, may also influence the ability of these mice to thermoregulate under conditions of cold stress. For example, light, both intensity and photoperiod, may affect the insulative abilities of small mammals and consequently, affect their heat loss abilities. Direct effects of photoperiod on pelage have been demonstrated in several small mammals such as varying hares (Lyman 1943) and ferrets (Hammond 1952). Similarly, quantity and quality of food in various geographic areas may influence the metabolic efficiency and insulative ability of mice inhabiting these areas. The influence of diet has been shown to have numerous effects on heat production and heat loss abilities within and between species. Many of these studies are reviewed by Hart (1971). Thus, geographic acclimatization may result from the effects of many environmental factors which may affect specific mechanisms in small mammals in several different ways so that they show gross differences in their metabolic and insulative abilities during exposure to cold stress.

Previous studies on small mammals from various geo-

graphic areas have demonstrated a variety of intra- and interspecific physiological differences in their thermoregulatory abilities. For example, Ladygina (1952) found that wild house mice, Mus musculus, from a more northerly latitude had higher heat production rates than a more southerly form during exposure to temperatures ranging from 0° to 25° C. Similarly, McNab and Morrison (1963) found that desert subspecies of Peromyscus had better temperature regulation at high temperatures, lower minimal thermal conductance, and lower basal metabolic rates than subspecies from mesic environments.

In the present study, several differences were found in the cold resistance and heat production capabilities within the winter and summer groups of mice from the various geographic areas. These differences in thermoregulatory abilities corresponded directly to the gross climatic conditions in each of the four study areas (Fig. 2). For example, both northern leucopus and southern maniculatus in winter and in summer showed greater resistance to cold stress than the southern leucopus. These mice from the more northerly latitude utilized more energy prior to metabolic failure and could thermoregulate significantly longer than the more southerly form during severe cold exposure. In winter, these differences appear to result primarily from a reduction in heat loss rates in the northern leucopus and southern maniculatus. The oxygen consumption rates of these two groups were much lower than that of southern leucopus

during exposure to moderate temperatures (Table VIII), thus indicating greater insulative abilities in these mice. In addition, the maximal heat production rates maintained by southern maniculatus and northern leucopus were slightly higher than that of southern leucopus (Fig. 18a). Southern maniculatus also had a slightly higher maximal heat production rate and appeared to have a greater ability to conserve heat (as indicated by their significantly longer metabolic failure times during severe cold exposure) than the sympatric northern leucopus. These two abilities would explain the greater resistance to severe cold in the winter southern maniculatus. More direct studies should be conducted on the heat loss abilities of these winter mice. Differences in maximal heat production rates are small and they do not appear to be the main factor contributing to the geographic differences demonstrated in these winter mice.

In summer, the greater resistance to cold stress in the mice inhabiting the more northerly latitudes appears to be directly related to their higher maximal heat production rates. Their higher rates enabled northern and southern maniculatus and the northern leucopus either to thermoregulate at a temperature 5 C° lower than southern leucopus or to thermoregulate approximately 1.5 times longer at the same low temperatures (Fig. 16). Similar oxygen consumption rates were obtained in these groups of summer mice during exposure to moderate temperatures (Table IX).

These similar rates indicate that their overall insulation was also the same. The cold resistance and heat production capabilities of northern and southern maniculatus and the northern leucopus were essentially the same in the summer groups of mice (Tables VII, VI, and V). In addition, mean daily minimum temperatures in these areas in summer are approximately the same (Fig. 2). These findings suggest that the influence of climate on the thermoregulatory abilities of these mice from the northern latitudes is similar during the summer months.

C. Effects of Acclimation

Peromyscus which were conditioned to constant temperatures of 22 °C showed two general patterns of resistance to cold stress and of heat production capabilities. These patterns were characteristic of the two forms of Peromyscus studied. The resistance to cold stress and heat production capabilities of P. leucopus noveboracensis were significantly reduced after acclimation as compared to summer mice. In contrast, P. maniculatus gracilis showed no significant change in cold resistance and only minor changes in heat production capabilities after acclimation.

The large difference in thermoregulatory abilities between summer and acclimated leucopus in both the northern and southern groups of mice resulted from differences in their maximal heat production rates (Tables IV and V) as well as differences in their ability to conserve heat by behavioural mechanisms. The lower maximal heat production

rate of acclimated leucopus coupled with their greater heat loss rate (i.e. poorer positioning behaviour) significantly reduced the length of time these mice could thermoregulate when they were exposed to the same low temperatures as their respective summer groups of mice (Figs. 5 and 6). In addition, the exposure temperatures tolerated by the summer leucopus were 5 to 8 C° lower than those tolerated by the acclimated mice. The acclimation of P. leucopus noveboracensis therefore appears to have had a marked effect on those behavioural mechanisms which are associated with thermoregulation and consequently, it reduces their resistance to cold stress. The behaviour of summer mice during cold exposure was similar to that of all wild-caught mice exposed to cold. Such differences in behaviour between the acclimated and wild-caught P. leucopus noveboracensis may be interpreted as an example of nongenetic variation in this species. The ability for thermoregulatory behaviour may only be induced by one or several fluctuating environmental factors that these mice experience in the wild. Consequently, this behaviour improves their thermoregulatory abilities and may enhance their survival in natural environments. When the acclimatization effects are removed (i.e. acclimation to constant laboratory conditions) or when mice are born and raised in the laboratory, they would not show this thermoregulatory behaviour.

The most striking difference between summer and acclimated maniculatus was the ability of acclimated mice

to thermoregulate approximately the same length of time at the same exposure temperatures as could summer mice even though their maximal heat production rate was considerably lower (Tables VI and VII). This finding suggests that the rates of heat loss in acclimated mice were considerably less during cold exposure. This would increase their metabolic failure time and enable them to withstand the same low temperatures as summer mice. This reduction in heat loss abilities of acclimated maniculatus may be due to an overall increase in insulation. Hayward (1965a) showed that the body composition of 5 races of P. maniculatus increased significantly in fat content when they were brought from their natural environment and acclimated to laboratory conditions. Such changes in body fat may have occurred in the maniculatus used in the present study since the acclimation procedure was similar to that used by Hayward. This would explain the lower rates of heat loss in the acclimated mice. A lower rate of heat loss during severe cold exposure would enable these mice to thermoregulate either for longer periods of time or at lower temperatures.

Thus the above findings suggest that artificial acclimation of Peromyscus may reduce (as in leucopus) or improve (as in maniculatus) cold resistance by behavioural and physiological changes that are not detected in wild populations as natural adaptations. Many more studies are required on the physiological and behavioural changes that

occur in small mammals removed from their natural environment and acclimated to laboratory conditions. Such studies would permit evaluation of the potential influence of environments on the genetic capabilities associated with thermoregulation in small mammals. Furthermore, the removal of environmental effects by prolonged acclimation should indicate the innate abilities of small mammals to thermoregulate during cold exposure.

In the present study, the removal of environmental effects by acclimation showed that innate differences in thermoregulatory abilities existed between the northern and southern leucopus. Northern leucopus showed greater resistance to cold stress than the southern leucopus. This in part was due to their 1.1 times higher maximal heat production rate (Fig. 18b). At very low temperatures (-30° to -20° C), the maximal rates of heat production of these acclimated mice could be maintained approximately the same length of time (Fig. 17). However, at higher temperatures of severe cold stress (-20° to -13° C), northern leucopus maintained their maximal rate of heat production considerably longer than the southern group (Fig. 17). This significant difference in metabolic failure times may be due to a difference in heat loss rate in these two acclimated groups or a rapid depletion of essential metabolic factors in the southern leucopus. Further investigation is required to determine the thermoregulatory mechanisms resulting in this intraspecific difference between the northern and southern

leucopus.

The sympatric groups of Peromyscus also showed marked differences in their thermoregulatory abilities after acclimation to constant laboratory conditions. Southern maniculatus were significantly more resistant to cold stress than northern leucopus. These acclimated mice could withstand temperatures 10 C° lower than the northern leucopus and could maintain their maximal rate of oxygen consumption an average 2.4 times longer at comparable temperatures (Tables V and VI). At each exposure temperature, the oxygen consumption rates of these acclimated mice were approximately the same (Table X). Therefore, these differences in cold resistance must result from a difference in heat loss rates. The poor thermoregulatory behaviour of northern leucopus no doubt plays a major role in significantly reducing their resistance to cold stress. Riedel (1967) also compared these same species after identical acclimation and found the maniculatus to have a greater resistance to severe cold exposure than the leucopus. This was evidenced by the longer times the maniculatus could maintain their body temperatures during cold exposure.

D. Effects of Seasonal Climatic Changes

The physiological adaptations of Peromyscus to cold have been shown in previous studies to be associated with an ability to maintain an increased heat production rate rather than with adaptations to decrease heat loss rates. For example, Hart (1957) found winter-adapted Peromyscus to

have a higher heat production rate than summer-adapted mice at their lower temperature limit (i.e. temperatures at which they could survive for 200 min). Similarly, Peromyscus acclimated to low temperatures have been shown to maintain a higher metabolic rate for a given time than those acclimated to warmer temperatures (Hart 1953b). In addition, both of the above studies showed that the metabolic rates of warm- and cold-adapted Peromyscus were essentially the same at temperatures ranging from their lower incipient lethal level to thermoneutrality. From these data, Hart concluded that no significant change in overall insulation occurs in Peromyscus during their conditioning to cold. Similar conclusions were drawn by Sealander (1951) and Hart (1953a) after examining the pelage density and insulation, respectively, of warm- and cold-adapted mice. On the other hand, Randolph (1971) found the rates of heat loss in the short-tailed shrew to be much lower in winter than those of the same species in summer. This difference in heat loss rates resulted from differences in the amount of locomotor activity during exposures to moderate temperatures (i.e. 26° to 0° C) as well as differences in body insulation. Seasonal changes in insulation have also been demonstrated in several other small mammals (see review by Hart 1971).

In the present study, a marked difference in cold resistance and heat production capabilities was found between the winter and summer groups of mice during severe cold exposure. Both metabolic and insulative adjustments were

demonstrated in response to seasonal changes in climate, especially in mice from the more northerly latitudes. No seasonal change in insulation was evident in southern leucopus. Their metabolic rates were essentially the same during exposure to moderate temperatures (Fig. 5). This finding indicates similar rates of heat loss in these two groups of mice. Winter mice in all cases, however, maintained significantly higher maximal heat production rates than summer mice. Consequently, the winter mice could either thermoregulate longer than the summer mice at the same exposure temperatures or withstand temperatures much lower than the summer mice (Tables IV, V, and VI). For example, the 1.2 times higher maximal heat production rate of southern maniculatus in winter coupled with a lower heat loss rate enabled these mice to either thermoregulate for the same length of time (e.g. 200 min) as summer mice at temperatures 15 C° lower, or thermoregulate 5 times longer at the same low temperatures (Table VI, Fig. 15). The magnitude of change in cold resistance and heat production capabilities from summer to winter was slightly smaller in the northern and southern leucopus than that just described for southern maniculatus. Similar findings were obtained by Hart (1957) in which he found winter Peromyscus to survive temperatures 20 C° lower than summer mice. Hart attributed the extension of the lower temperature limit to a 40% greater heat production rate in winter mice rather than a reduction in their heat loss abilities.

The ability to increase heat production during acclimatization to cold may result from an increase in shivering or nonshivering thermogenesis (Chaffee and Roberts 1971). Vigorous shivering was observed in most summer and acclimated Peromyscus during severe cold exposure. Shivering appeared to be less pronounced in winter mice although the view of mice was often obscured at very low temperatures. No attempt was made to evaluate the degree of shivering per se in the present study. The noradrenaline response data indicate that nonshivering thermogenesis may account for 45 to 55% of the heat produced in both winter and summer Peromyscus during severe cold exposure (Table XII). Similar responses to noradrenaline were obtained by Heldmaier (1971) on a number of cold-adapted small mammals in an age-dependent study. His study was not designed to establish the significance of noradrenaline response in relation to the thermal history of the animals; however, the patterns and values he did obtain after noradrenaline injection corresponded very closely to the findings of the present study. Hissa (1970) compared noradrenaline-induced thermogenesis in warm- and cold-acclimated lemmings and found similar responses in both groups. He concluded that the positive correlation between cold-induced nonshivering thermogenesis and the heat production after injection of noradrenaline previously shown in the rat (Jansky et al. 1969) was not a general phenomenon in cold-adapted mammals. The findings in the

present study tend to support Hissa's conclusion. The temperature-adaptive significance of noradrenaline-induced thermogenesis in small mammals requires further investigation.

The seasonal changes in thermoregulatory abilities of small mammals should enhance their survival despite changes in climatic conditions. The heat production capabilities and cold resistance of Peromyscus in summer and winter suggest that these mice over-compensate for thermogenic demands normally experienced in their natural environments. For example, summer temperatures would rarely drop below freezing in any of the four geographic areas, and yet, the thermoregulatory abilities of summer mice indicate that they are capable of withstanding considerable cold stress during the summer. In winter, mean daily minimum temperatures averaged -15° to -20° C in the most northerly areas selected for this study and only -3° C in the most southerly area (Fig. 2). Moreover, Peromyscus, in general, inhabit microenvironments where they avoid extremes in temperature (Hayward 1965), and they significantly reduce their daily activity during the winter months (R.A. Gaertner personal communication). Furthermore, the ability to undergo daily torpor has been well documented recently in several Peromyscus spp. (Morhardt 1969) as well as P. leucopus noveboracensis (R.A. Gaertner personal communication). Such adaptations are most important relative to the total energy budgets of these mice. There-

fore, the significance of the change in cold resistance and heat production capabilities in mice from summer to winter warrants some explanation. The contribution of an increased heat production rate as an adaptive mechanism to enhance survival during winter can only be postulated. To illustrate, Peromyscus in winter probably leave their nesting sites for intermittent periods of activity. In areas where snow is present throughout the winter, this activity is not confined to the subnivean environment. This was evidenced in the present study by winter trapping experience and observation of tracks on the surface of the snow. Thus, the mice from the four geographic areas may experience considerable stress during their activity periods away from the nest. The cumulative effects of ambient temperature, wind, and humidity may provide conditions in which rates of heat loss of these animals are great during their activity periods. Such conditions would require a high rate of heat production in these animals in order for them to maintain a relatively constant body temperature.

Furthermore, in the northern latitudes, the subnivean microclimate does not exist throughout the entire year. In spring and fall when the snow is absent, Peromyscus may be exposed to relatively low temperatures for long periods of time. Koskina (1957) and Fuller (1967) have cautioned against regarding the presence of snow as being entirely beneficial. They point out that a delayed, wet spring, occasioned by fluctuating temperatures, may cause considerable

stress and mortality to subnivean occupants. Similarly, in the most southerly areas trapped during this study, rain is common during the winter. These wet conditions coupled with wind and near freezing temperatures may also provide situations in which the heat loss rates of mice are great for short periods of time. Consequently, thermogenic demands comparable to those demonstrated in the present study could be encountered in natural environments.

Quantitative data on the environments occupied by Peromyscus are not well documented. Further information on the ecological energetics and behaviour of these animals in natural environments is presently needed. Until such information is available the significance of observed physiological changes cannot be fully appreciated. It is hoped that the knowledge obtained in the present study provides an additional perspective to the seasonal and geographical changes in the thermoregulatory abilities of small mammals and that it serves as a basis for further consideration of the physiological mechanisms and ecological relationships associated with these changes.

LITERATURE CITED

- Adams, T. 1971. Carnivores. In: Comparative Physiology of Thermoregulation. Vol. II Mammals. Edited by G.C. Whittow. Academic Press, New York. pp. 151-189.
- Baker, R.H. 1968. Habitats and Distribution. In: Biology of Peromyscus (Rodentia). Chap. 4. Edited by J.A. King. Amer. Soc. Mammalogists, Spec. Publ., No. 2. pp. 98-126.
- Brody, S. 1945. Bioenergetics and Growth. Reinhold Publ., New York. 1023 pp.
- Chaffee, R.R.J. and J.C. Roberts. 1971. Temperature acclimation in birds and mammals. Ann. Rev. Physiol. 33: 155-202.
- Cogshall, A.S. 1928. Food habits of deer mice of the genus Peromyscus in captivity. J. Mammalogy 9: 217-221.
- Cottle, W.H. and L.D. Carlson. 1956. Regulation of heat production in cold-adapted rats. Proc. Soc. Exp. Biol. Med. 92: 845-848.
- Davis, T.R.A., D.R. Johnston, F.C. Bell and B.J. Cremer. 1960. Regulation of shivering and nonshivering heat production during acclimation of rats. Amer. J. Physiol. 198: 471-475.
- Depocas, F. and J.S. Hart. 1957. Use of the Pauling oxygen analyzer for measurement of oxygen consumption of animals in open-circuit systems and in a short lag, closed-circuit apparatus. J. Appl. Physiol. 10: 388-392.
- Fuller, W.A. 1967. Ecologie hivernale des lemmings et fluctuations de leurs population. La Terre et la Vie 2: 97-115.
- Gates, D.M. 1970. Animal climates (where animals must live) Environ. Res. 3(2): 132-144.
- Hall, E.R. and K.R. Kelson. 1959. The Mammals of North America. Vol. II. Ronald Press, New York. pp. 552-710.
- Hamilton, W.J. Jr. 1941. The food of small forest mammals in eastern United States. J. Mammalogy 22: 250-263.
- Hammel, H.T. 1968. Regulation of internal body temperature. Ann. Rev. Physiol. 30: 641-710.

- Hammond, J. Jr. 1952. Control of reproductive and pelt changes: some experiments with animals kept entirely in artificial light. *J. Agric. Sci.* 42: 293 - 301.
- Hart, J.S. 1953a. The relation between thermal history and cold resistance in certain species of rodents. *Can. J. Zool.* 31: 80-98.
- Hart, J.S. 1953b. Energy metabolism of the white-footed mouse Peromyscus leucopus noveboracensis after acclimation at various environmental temperatures. *Can. J. Zool.* 31: 99-105.
- Hart, J.S. 1957. Climatic and temperature-induced changes in the energetics of homeotherms. *Rev. Can. Biol.* 16: 133-174.
- Hart, J.S. 1963. Physiological responses to cold in non-hibernating homeotherms. *In: Temperature - Its Measurement and Control in Science and Industry* 3: 373-405.
- Hart, J.S. 1964. Geography and season: mammals and birds. *In: Adaptation to the Environment*. Sect. 4. *Handbook of Physiology*. Amer. Physiol. Soc. Washington. Reinhold Publ., New York.
- Hart, J.S. 1971. Rodents. *In: Comparative Physiology of Thermoregulation*. Vol. II. Edited by G.C. Whittow. Academic Press, New York. pp. 1-149.
- Hart, J.S. and O. Héroux. 1953. A comparison of some seasonal and temperature-induced changes in Peromyscus: cold resistance, metabolism, and pelage insulation. *Can. J. Zool.* 31: 528-534.
- Hart, J.S. and O. Héroux. 1955. Exercise and temperature regulation in lemmings and rabbits. *Can. J. Biochem. Physiol.* 33: 428-435.
- Hart, J.S. and O. Héroux. 1963. Seasonal acclimatization in wild rats (Rattus norvegicus). *Can. J. Zool.* 41: 711-716.
- Hayward, J.S. 1965. Microclimate temperature and its adaptive significance in six geographic races of Peromyscus. *Can. J. Zool.* 43: 341-350.
- Hayward, J.S. 1965a. The gross body composition of six geographic races of Peromyscus. *Can. J. Zool.* 43: 297-307.

- Hayward, J.S. 1965b. Metabolic rate and its temperature-adaptive significance in six geographic races of Peromyscus. Can. J. Biol. 43: 309-323.
- Heldmaier, G. 1971. Nonshivering thermogenesis and body size in mammals. Z. vergl. Physiologie 73: 222-248.
- Héroux, O. 1962. Seasonal adjustments in captured wild Norway rats. II. Survival times, pelt insulation, shivering, metabolic and pressor responses to noradrenaline. Can. J. Biochem. Physiol. 40: 537-545.
- Héroux, O., J.S. Hart, and F. Depocas. 1956. Metabolism and muscle activity of anesthetized warm and cold acclimated rats on exposure to cold. J. Appl. Physiol. 9: 399-403.
- Héroux, O., J.S. Hart, and F. Depocas. 1959. Comparison between seasonal and thermal acclimation in white rats. I. Metabolic and insulative changes. Can. J. Biochem. Physiol. 37: 473-483.
- Himms-Hagen, J. 1967. Sympathetic regulation of metabolism. Pharmac. Rev. 19: 367-461.
- Hissa, R. 1971. Calorigenic effect of noradrenaline in the Norwegian lemming, Lemmus lemmus (L.). Experientia 26: 266-267.
- Jansky, L., R. Bartunkova, J. Kockova, J. Mejsnar, and E. Zeisberger. 1969. Interspecies differences in cold adaptation and nonshivering thermogenesis. Fed. Proc. 28(3): 1053-1058.
- Klein, H.G. 1960. Ecological relationships of Peromyscus leucopus noveboracensis and Peromyscus maniculatus gracilis in central New York. Ecol. Monogr. 30: 387-407.
- Koskina, T.V. 1957. Comparative ecology of the red-backed voles in the northern taiga. Materials about Rodent, No. 5 Fauna and Ecology of Rodents. Moscow Society of Naturalists. pp. 3-65. (in Russian, English translation in Boreal Inst., Univ. of Alberta, Edmonton).
- Ladygina, K.M. 1952. Seasonal changes in the response of the house mouse to the effects of environmental temperature. Zool. Zh. 31: 736-743.
- Lyman, C.P. 1943. The control of coat colour in the varying hare. Bull. Mus. Comp. Zool. Harvard. 93: 393 - 461

- McNab, B.K. 1970. Body weight and energetics of temperature regulation. *J. Exp. Biol.* 53: 329-348.
- McNab, B.K. and P.R. Morrison. 1963. Body temperature and metabolism in subspecies of Peromyscus from arid and mesic environments. *Ecol. Monographs* 33: 63-82.
- Peterson, R.L. 1966. *The Mammals of Eastern Canada*. Oxford Univ. Press, Toronto. 463 pp.
- Randolph, J.C. 1971. The ecological energetics of a homeothermic predator. Ph.D. Thesis, Carleton Univ., Ottawa.
- Riedel, D. 1967. Effects of environmental temperature on the body temperature, oxygen consumption and survival of two species of Peromyscus (Rodentia: Cricetidae). M.Sc. Thesis, Carleton Univ., Ottawa.
- Rowe, J.S. 1959. Forest regions of Canada. Can. Dept. Northern Affairs Nat. Resources, Forest Br. Bull. 123.
- Sealand, J.A. 1951. Survival of Peromyscus in relation to environmental temperature and acclimation at high and low temperatures. *Amer. Midl. Naturalist*. 46: 257-311.
- Sealand, J.A. 1953. Body temperatures of white-footed mice in relation to environmental temperature and heat and cold stress. *Biol. Bull.* 104: 87-99.
- Scholander, P.F., R. Hock, V. Walters, F. Johnson and L. Irving. 1950a. Heat regulation in some arctic and tropical mammals and birds. *Biol. Bull.* 99: 237-258.
- Scholander, P.F., R. Hock, V. Walters, F. Johnson and L. Irving. 1950b. Body insulation of some arctic and tropical mammals and birds. *Biol. Bull.* 99: 225-236.
- Scholander, P.F., R. Hock, V. Walters, F. Johnson and L. Irving. 1950c. Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate. *Biol. Bull.* 99: 259-271.
- Sellers, E.A., S. Reichman, and N. Thomas. 1951. Acclimatization to cold: natural and artificial. *Amer. J. Physiol.* 167: 644-652.
- Smillie, K. 1966. *An Introduction to Regression and Correlation*. Academic Press, New York. 168 pp.

- Smith, D.A. and S.W.Speller. 1970. The distribution and behavior of Peromyscus maniculatus gracilis and Peromyscus leucopus noveboracensis (Rodentia: Cricetidae) in a southeastern Ontario woodlot. Can. J. Zool. 48(6): 1187-1199.
- Smith, R.E. and B.A. Horwitz. 1969. Brown fat and thermogenesis. Physiol. Rev. 49(2): 330-425.
- Steel, R.D.G. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York. 481 pp.
- Waters, J.H. 1962. Range of Peromyscus maniculatus in southern New England. J. Mammalogy 43: 102.
- Whittow, G.C. 1971. Comparative Physiology of Thermoregulation. Vol. II. Mammals. Academic Press, New York.
- Yarbrough, C.G. 1971. The influence of distribution and ecology on the thermoregulation of small birds. Comp. Biochem. Physiol. 39A: 235-266.

APPENDIX

Table 1. Regression equations for the relationship of maximal metabolic rate and metabolic failure time in the various groups of Peromyscus collected from the four geographic areas.

Group	n	Regression equation	Sy.x	r
<u>Southern leucopus</u>				
a) winter	37	$Y = 33.48 + 0.0173X$	3.65	0.40
b) summer	66	$Y = 29.55 + 0.0057X$	3.30	0.16
c) acclimated 22°C	42	$Y = 26.98 - 0.0030X$	3.97	-0.06
<u>Northern leucopus</u>				
a) winter	35	$Y = 34.81 + 0.0158X$	4.19	0.51
b) summer	52	$Y = 31.18 + 0.0106X$	3.82	0.29
c) acclimated 22°C	46	$Y = 30.01 - 0.0051X$	2.70	-0.22
<u>Southern maniculatus</u>				
a) winter	22	$Y = 34.90 + 0.0218X$	4.66	0.49
b) summer	52	$Y = 32.69 + 0.0033X$	2.82	0.12
c) acclimated 22°C	46	$Y = 31.31 - 0.0036X$	2.95	-0.13
<u>Northern maniculatus</u>				
a) summer	38	$Y = 32.89 + 0.0024X$	3.52	0.09
b) acclimated 22°C	15	$Y = 31.69 - 0.0032X$	5.34	-0.07

Symbols used: n = number of mice sampled, Y = maximal metabolic rate (ml O₂/hr.g^{-0.7}), X = metabolic failure time (min), Sy.x = standard error of the estimate, r = regression coefficient.

Table 2. Estimations of the degree of nonshivering thermogenesis in various groups of Peromyscus.

Group	A.	B.	Estimated Percentage of N.S.T.** (ratio B/A)
	Maximal Metabolism	Maximal Metabolism	
	during	after	
	Cold Stress	NA Injection	
	(ml O ₂ /hr.g ^{-0.7})*	(ml O ₂ /hr.g ^{-0.7})*	
<hr/>			
<u>Southern leucopus</u>			
a) winter	35.96	16.17	45.0
b) summer	30.32	14.45	47.7
c) acclimated 22°C	26.60	15.51	58.3
 <u>Northern leucopus</u>			
a) winter	38.08	19.41	51.0
b) summer	32.64	15.95	48.9
c) acclimated 22°C	29.12	13.39	46.0
 <u>Southern maniculatus</u>			
a) winter	39.70	18.25	46.0
b) summer	33.18	17.81	53.7
c) acclimated 22°C	30.74	15.69	51.0

* All oxygen consumption data are mean values.

** N.S.T. = nonshivering thermogenesis