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Seasonal Changes in Thermogenesis, Organ Weights, and Body Composition in the White-Footed Mouse, *Peromyscus leucopus*

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Summary. 1. Seasonal adjustments in wild-caught *Peromyscus leucopus* include autumn increases in basal metabolic, nonshivering thermogenesis, and interscapular brown fat and decreases in weights of gonads, liver, adrenal glands, and total lipid. Body weight and nonextractable dry weight do not change.

2. Basal metabolic rate, nonshivering thermogenesis, and interscapular brown fat increase following initial cold exposure in mid-September and are maintained at similar levels through January.

3. There is a positive correlation between nonshivering thermogenesis and interscapular brown fat, and negative correlations for both nonshivering thermogenesis and interscapular brown fat with body weight.

4. These seasonal changes in wild-caught *P. leucopus* are compared with laboratory studies on the effects of chronic exposure to differences in temperature and photoperiod on these characters. It is concluded that disparities between the effects of cold acclimatization and cold acclimation could result from the influence of additional environmental cues, such as photoperiod, in cold acclimatized mice.

Introduction

Seasonal changes in temperature require that small arctic and temperate mammals make physiological and behavioral adjustments in order to maintain a constant body temperature. The nature of these adjustments has been established through numerous laboratory studies (see reviews by Barnett and Mount, 1967; Chaffee and Roberts, 1971); these investigations have shown that physiological adjustments to cold exposure include shifts in body composition, hypertrophy of specific organs, and a general increase in thermogenesis. However, information on seasonal modification of these physiological characters in wild populations of small mammals is less complete. Heroux (1963) examined changes in thermogenesis and organ weights by comparing winter and summer-caught Norway rats. More recently, Didow and Hayward (1969) assessed changes in brown fat content in the meadow vole at monthly intervals, and Alesksiuck and Frohlinger (1971) have monitored seasonal modification of thyroid activity, brown fat, and organ weights in the muskrat. At present, no study has examined the multiple responses associated with physiological acclimatization[1] to cold in small mammals live-trapped during summer, fall, and winter.

Furthermore, comparison of acclimated and acclimatized rats has revealed a number of discrepencies between animals exposed to temperature differences in the laboratory and those exposed to seasonal changes in the natural environment (Heroux, 1963). These disparities could be due to the effects of additional environmental cues, such as photoperiod, in the natural environment. Thus, it was the purpose of the present investigation 1. to monitor seasonal changes in thermogenesis (basal metabolic rate, nonshivering thermogenesis), organ weights (interscapular brown fat, liver, adrenal glands, gonads), and body composition in wild-caught *Peromyscus leucopus,* and 2. to determine the relative effects of differences in temperature and photoperiod on expression of these characters, with the intention of better understanding the nature of environmental cueing in acclimatized *P. leucopus.*

Material and Methods

Acclimatized Mice

Six samples of wild-caught *P. leucopus* were live-trapped from two woodlots near Iowa City, Iowa on the following dates: September 7, 9, 1970 ($n = 8$), September 29 ($n = 9$), October 22 ($n = 9$), November 24, 25 ($n = 15$), January 10, 12, 19, 1971 ($n = 9$), and July 2, 4, 1971 ($n = 17$). Sherman traps were set in early evening and collected within three hours, except in January when traps were checked every 45 minutes. All mice were at least 50 days of age, since only animals with subadult or adult pelage were collected (Layne, 1968). Mice were maintained overnight on a light-dark cycle which approximated the natural photoperiod for that time of year. Summer-caught mice were held in the laboratory at 26° C; fall and winter-caught animals were kept at 5° C. Animals were individually caged and food (Wayne Lab Blox) and water provided *ad libitum;* cotton was supplied as a source of nesting material. Measurements on mice were made within 18 hours of capture.

Prior to measurement, mice were kept at room temperature (22° C) for 2 to $2^1/_2$ hours without food, then anesthetized with 25% urethane (5 ml/kg body weight IP). These animals were placed in a metabolism chamber in a metabolism chamber in a 31° C water bath and permitted to equilibrate for 20 to 35 minutes. Metabolic rate (under anesthesia) was assessed as a change in mm Hg O_2/time in a closed circuit system as measured with a Beckman oxygen analyzer (Model D2). Measurements were taken every 5 min until three consecutive values were consistent. Metabolic rate in ml O_2/g/hr was estimated using the mean of the three measure-

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[1] In this study, acclimatization refers to the response of organisms to seasonal changes in the natural environment, whereas acclimation refers to the response of organisms to treatment differences in the laboratory.

ments. A 25 g mixture of "Drierite" and "ascarite" adsorbed water vapor and carbon dioxide, respectively, and a modified aquarium pump circulated air through the closed system. Anesthesia was necessary to minimize activity and facilitate quick handling during estimation of nonshivering thermogenesis (see following paragraph).

The "norepinephrine test" was used to index the extent of nonshivering thermogenesis (Brück, 1970). Nonshivering thermogenesis was assessed as the metabolic response above the "basal" metabolic rate[2] following SC injection of 6 mg norepinephrine (Levophed, Winthrop Laboratories)/kg body weight. The extent of nonshivering thermogenesis was expressed as the absolute increase in ml/O_2/g/hr at 31° C above "basal" metabolic rate. All metabolic measurements were made between 12:00 p.m. and 5:00 p.m.

After estimation of nonshivering thermogenesis the mice were killed, and wet weights of the female reproductive tract, testes, and adrenal glands determined. The liver was excised, washed, and dried at 80° C in an oven. The interscapular fat pad was excised, dried in an 80° C oven, and the lipid extracted in a 2:1 chloroform: methanol solution. The solution was changed the following day; then, on day 3 the fat pad was dried and weighed. Little change in weight occurred after two changes in the extraction solution which indicated that little extractable lipid remained. Lipid-free dry weight of interscapular brown fat was employed because it most accurately estimates the thermogenic capacity of brown adipose tissue (Hayward, in: Chaffee and Roberts, 1971).

Carcasses were dired in a Thelco vacuum oven (Model 19; Precision Instruments) at 90° C under −15 mm Hg pressure for 36 hours. The dried carcass was crushed, and total lipid content estimated by extraction with a 2:1 chloroform: methanol solution (after Folch *et al.*, 1957). Each day for two days the extraction solution was decanted and replaced with fresh solution. The sediment was then dried and the difference between dry weights taken as an estimate of total extractable lipid. The remaining sediment is referred to as the nonextractable dry weight and is primarily composed of minerals and protein.

In addition, daily minimum air temperature at ground level was recorded with a maximum-minimum thermometer from September, 1970, through January, 1971, and during late June and early July, 1971.

Acclimated Mice

Mice were laboratory reared under a long day photoperiod (16:8LD) at 26° C; the stock was originally derived from a wild-caught population trapped near Iowa City, Iowa. One hundred 4-month old male and female mice were assigned to one of the following treatment groups: i) warm acclimated (26° C) under a long day photoperiod (16:8LD), ii) warm acclimated under a short day photoperiod (9:15LD), iii) cold acclimated (5° C) under a long day photoperiod, or iv) cold acclimated under a short day photoperiod. Following 12 weeks chromic acclimation, thermogenic variables, organ weights, and body composition were assessed according to the techniques previously described.

The relative effects of photoperiod, temperature, and their possible interaction were determined using analysis of variance (least squares method).

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[2] Metabolic rate under anesthesia does not adhere to the criteria established for assessing basal metabolic rate, and the term is used here merely to be consistent with previous literature on estimating the extent of nonshivering thermogenesis.

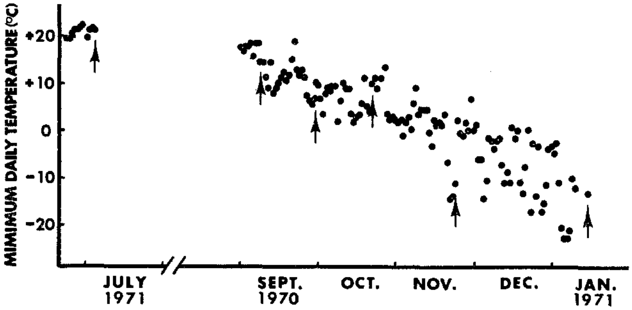

Fig. 1. Daily minimum air temperature (taken at ground level) for Iowa City, Iowa during 1970–1971. Arrows represent approximate dates when wild populations of *Peromyscus leucopus* were sampled

Results

Acclimatized Mice

Records of daily minimum temperature (Fig. 1) indicate that the first cold wave of late summer occurred after the September 7, 9th sampling of wild-caught *P. leucopus*. A second abrupt decrease occurred during the third week of September but prior to the September 29th sample of mice. Minimum temperatures then steadily decreased through mid-January; both November and January samples of mice were trapped on evenings when the minimum temperature was below −10° C. However, minimum daily temperature is only a rough estimate of the degree of cold stress experienced by small mammals under natural conditions since other environmental factors (e.g. wind velocity and sky temperature) also influence factors (e.g. wind velocity and sky temperature) also influence heat exchange between a mammal and its environment (Gates, 1962).

Comparison of means for physiological characters of mice trapped in July and January indicate the following increases in January-trapped mice: 1. 24% ($t=2.20$; d.f. $=23$) for basal metabolic rate (Fig. 2A); 2. 100% ($t=6.41$; d.f. $=20$) in extent of nonshivering thermogenesis (Fig. 2B); and 3. 85% ($t=6.82$; d.f. $=24$) in lipid-free interscapular brown fat/g body weight (Fig. 2C). Conversely, adrenal wet weight/g wet body weight (Fig. 2D) decreased 43% ($t=4.27$; d.f. $=24$) and gonad wet weight (Fig. 2E) decreased 95% ($t=5.41$; d.f. $=20$) when winter-caught mice are compared with summer animals. Only slight differences occurred between summer and winter-caught *P. leucopus* for wet body weight (Fig. 3A), dry body weight (Fig. 3B), nonextractable dry weight (Fig. 3C),

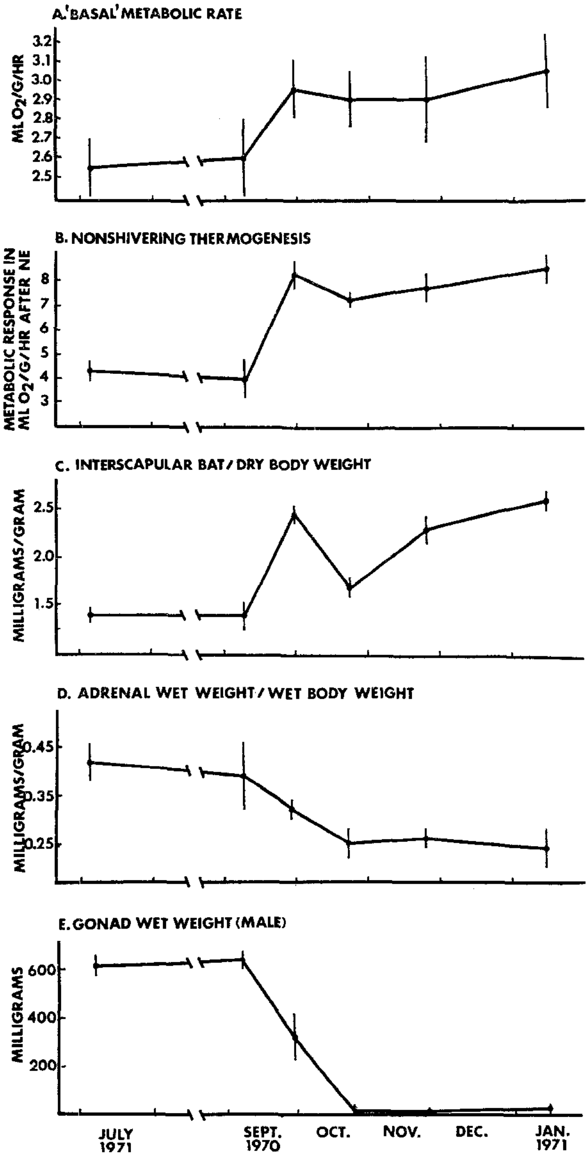

Fig. 2A—E. Mean values for physiological variables associated with seasonal acclimatization in 67 wild-caught *Peromyscus leucopus*. The vertical line through each mean represents one standard error of the mean

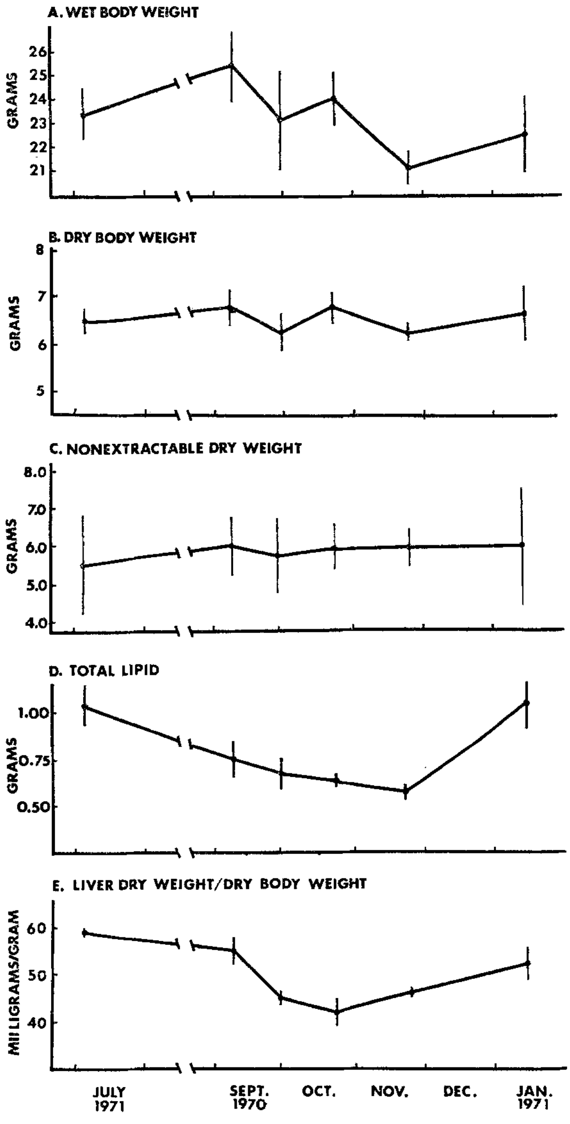

Fig. 3A—E. Mean values for physiological variables associated with seasonal acclimatization in 67 wild-caught *Peromyscus leucopus*. The vertical line through each mean represents one standard error of the mean

Table 1. Pooled within treatment correlation matrix for physiological variables associated with thermoregulation in wild-caught *Peromyscus leucopus*

	1.	2.	3.	4.	5.	6.	7.	8.
1. Wet body weight (g)	1.00[a]							
2. Dry body weight (g)	0.94*	1.00						
3. Nonextractable dry weight (g)	0.91*	0.97*	1.00					
4. Total extractable lipid (g)	0.60*	0.64*	0.44*	1.00				
5. Basal metabolic rate (ml O_2/g/hr)	−0.13	−0.09	−0.19	0.21	1.00			
6. Nonshivering thermogenesis (ml O_2/g/hr)	−0.48*	−0.43*	−0.48*	−0.06	0.12	1.00		
7. Interscapular brown fat (mg/g dry weight)	−0.48*	−0.54*	−0.51*	−0.29	0.13	0.58*	1.00	
8. Liver weight (mg/g dry weight)	0.04	0.11	−0.15	0.10	0.12	0.03	0.25	1.00

* $p < 0.01$.

[a] Degrees of freedom = 49.

total extractable lipid (Fig. 3D), and liver dry weight/g dry body weight (Fig. 3E).

Although summer-winter comparisons are useful in understanding cold acclimatization, additional samples of fall-caught animals provide a more complete representation of seasonal acclimatization. For example, following initial cold exposure in mid-September, *P. leucopus* exhibits pronounced increases in basal metabolic rate (Fig. 2A), the extent of nonshivering thermogenesis (Fig. 2B), and hypertrophy of interscapular brown fat (Fig. 2C). Further increases in these variables did not occur during late fall and early winter. Similarly, the substantial decreases in adrenal gland weight (Fig. 2D) and gonad weight (Fig. 2E) as observed in winter-caught mice (relative to summer) occurred during late September and October. These pronounced decreases in adrenal gland and gonad weights coincided with the fall molt in *P. leucopus*. However, the fall samples of wild-caught mice also suggest that initial cold acclimatization during fall differs from cold acclimatization during winter for specific characters. Relative to both summer and winter-caught mice,

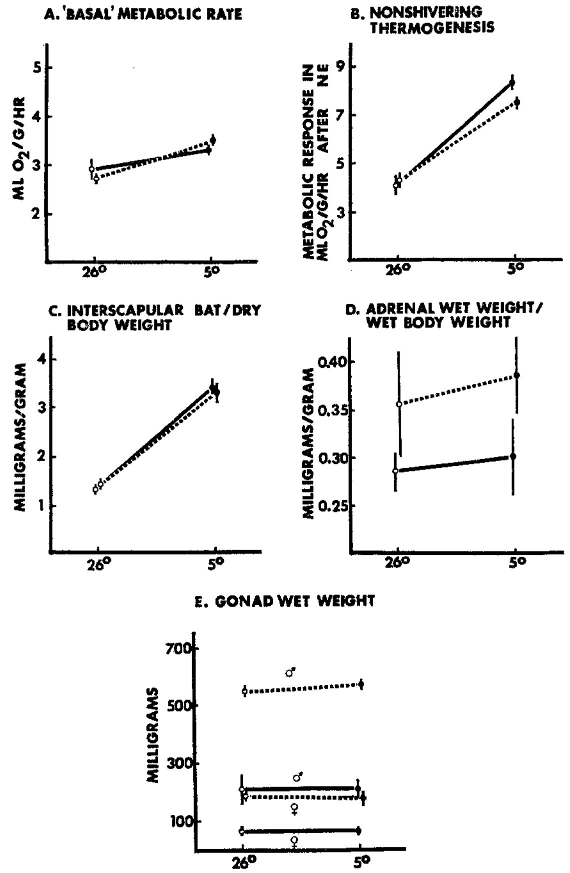

Fig. 4A—E. Mean values for variables associated with physiological thermoregulation for 100 *Peromyscus leucopus*. Mice were simultaneously exposed for twelve weeks to either 26° C (○) or 5° C (●) and either long (---) or short (——) photoperiod. The vertical line through each mean represents one standard error the mean

there is a decrease in total extractable lipid (Fig. 3D) and liver weight/g dry body weight (Fig. 3E) in fall-caught mice.

In addition to seasonal changes in physiological characters, assessment of multiple variables on individual mice permitted estimation of correlations between physiological characters. Examination of correlations, pooled within sample, reveal substantial associations between the extent of nonshivering thermogenesis and weight of lipid-free interscapular brown fat (Table 1). There is also a pronounced inverse

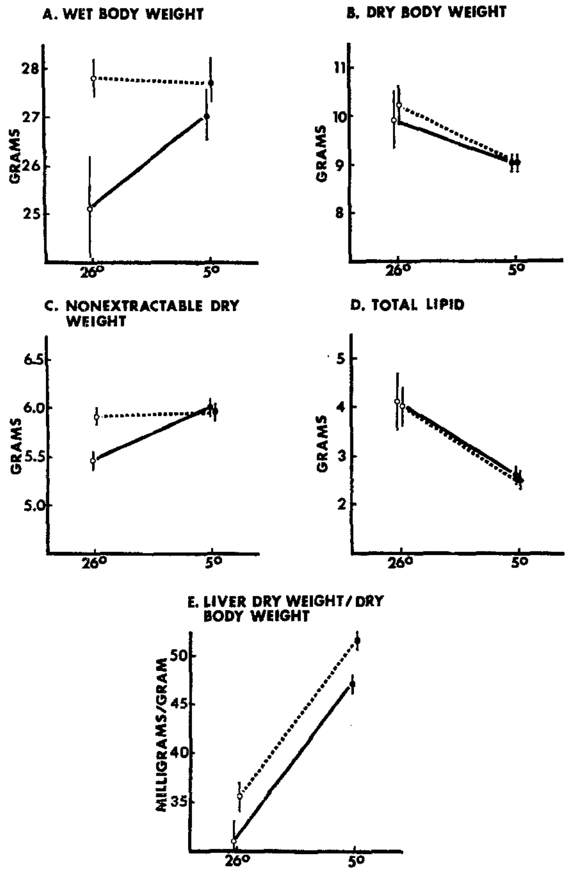

Fig. 5A—E. Mean values for variables associated with physiological thermoregulation for 100 *Peromyscus leucopus*. Mice were simultaneously exposed for twelve weeks to either 26° (○) or 5° C (●) and either long (---) or short (——) photoperiod. The vertical line through each mean represents one standard error of the mean

relationship between both nonshivering thermogenesis and interscapular brown fat and body weight. However, a similar inverse relationship between basal metabolic rate and body weight is not statistically significant. The remaining positive correlations indicate the extent of relationship between two measures of body weight and the major components of body weight (they are included here largely for comparative purposes with other species and will not be discussed *per se*). Liver weight/g dry body weight, adrenal weight/g wet body weight, and basal metabolic rate exhibit no statistically significant correlation (at the 0.05 level of probability) with the other physiological characters assessed.

Acclimated Mice

Comparison of means, pooled across photoperiod, for warm and cold acclimated mice indicates that the following increases occurred with cold exposure: 1. 17% ($t = 3.58$; d.f. $= 92$) for basal metabolic rate (Fig. 4A); 2. 93% ($t = 10.50$; d.f. $= 91$) for extent of nonshivering thermogenesis (Fig. 4B); 3. 144% ($t = 13.46$; d.f. $= 96$) for interscapular brown fat/g dry body weight (Fig. 4C); 4. 6% ($t = 3.71$; d.f. $= 96$) for nonextractable dry weight (Fig. 5C); and 5. 40% ($t = 11.70$; d.f. $= 96$) for liver dry weight/g dry body weight (Fig. 5E). Conversely, cold acclimated mice exhibited an 11% decrease ($t = 2.73$; d.f. $= 96$) in dry body weight (Fig. 5B) and a 38% decrease ($t = 4.26$; d.f. $= 96$) in total lipid (Fig. 5D) relative to warm acclimated animals. Weights of the adrenal glands (Fig. 4D) and gonads (Fig. 4E) were not affected by differences in temperature.

Finally, comparison of means, pooled across temperature, for mice treated under long and short photoperiod indicates the following decreases occurred under short photoperiod: 1. 24% ($t = 1.90$; d.f. $= 96$) for adrenal gland wet weight/g wet body weight (Fig. 4D); 2. 62% ($t = 5.42$; d.f. $= 46$) for testicular weight and 63% ($t = 9.22$; d.f. $= 49$) for weight of the female reproductive tract (Fig. 4E); and 3. 11% ($t = 3.24$; d.f. $= 96$) for liver weight/g dry body weight (Fig. 5E).

Discussion

Substantial increases in basal metabolic rate (Fig. 2A), non-shivering thermogenesis (Fig. 2B), and interscapular brown fat (Fig. 2C) occur following seasonal cold exposure. These results agree with previously described increases in non-shivering thermogenesis for winter-caught Norway rats (Heroux, 1963), and hypertrophy of brown fat in wild populations of muskrat (Alesksiuk and Frohlinger, 1971), and meadow vole (Didow and Hayward, 1969). Furthermore, the extent of the increase in nonshivering thermogenesis in natural populations of *P. leucopus* during winter is similar to values following laboratory acclimation at 5° C (Fig. 4B). Acclimated mice exhibited an increase of 7.9 ml O_2/g/hr above basal rate following injection of norepinephrine while the average increase of fall and winter-caught animals was 8.0 ml O_2/g/hr. The absence of additional increases in thermogenic variables with decreases in seasonal temperatures (Fig. 1) during late fall and winter suggests a limit for these characters in wild populations of *P. leucopus*. A similar physiological limit for nonshivering thermogenesis has been reported in the laboratory rat (Jansky *et al.*, 1967). They found that the extent of the calorigenic reponse to norepinephrine injection was inversely related to acclimation temperature with a maximal response at —4° C. Thus, not only does this field study on *P. leucopus* suggest that a similar limited response of nonshivering thermogenesis (and other thermogenic variables) is present in wild populations, but it also implies that modification of behavioral thermoregulation is required with seasonal decreases in ambient temperature, since a maximal thermogenic response in this species occurs in early fall. It should be mentioned that burrow temperatures of *Peromyscus sp.* seldom approach freezing (Hayward, 1965); thus, similarity of values for the extent of nonshivering thermogenesis in wild-caught mice and animals acclimated at 5° C is understandable.

It is especially notable that the basal metabolic rate of wild-caught *P. leucopus* increased following initial cold exposure (Fig. 2A). Heroux (1963) did not observe an increase in winter-caught Norway rats and recently attributed the observed increase in basal metabolic rate in acclimated rats to a pathological response associated with cold acclimation (Heroux, 1970). However, these data on *P. leucopus* clearly demonstrate a substantial increase in basal rate following initial cold exposure in nature and thus agree with a similar increase in cold acclimated mice (Fig. 4A).

Comparison of adrenal gland and liver weights in acclimated and acclimatized mice do suggest that specific seasonal adjustments differ from responses associated with temperature acclimation. Cold acclimation usually induces increases in certain visceral organs, such as heart, liver, and adrenal glands (Emery *et al.*, 1940; Heroux, 1963). In *P. leucopus,* liver weight increased while adrenal gland weight remained the same following 12 weeks chronic exposure at 5° C (Figs. 5E and 4D, respectively), while in wild-caught mice, both liver weight and adrenal gland weight sharply decreased during early fall (Figs. 3E and 2D, respectively). A similar disparity between acclimated and acclimatized rats with respect to these organ weights has been reported by Heroux (1963). However, since natural populations are adapted to multiple seasonal changes, discrepancies between cold acclimation and cold acclimatization may be attributed to the simultaneous effect of additional environmental changes (e.g., photoperiod) in wild populations. Differences in photoperiod do effect change in both liver weight and adrenal gland weight in *P. leucopus* (Figs. 5E and 4D, respectively). Thus, seasonal differences in photoperiod (and undoubtedly other seasonal changes in the natural environment) could effect change of these organ weights in natural populations of mice and thereby, at least in part, account for discrepancies between cold acclimation and cold acclimatization.

It is also evident from seasonal changes in total lipid (Fig. 3D) and liver weight/g dry wet weight (Fig. 3E) that acclimatization during fall may differ from winter acclimatization. This is especially noticeable in total lipid where, following a gradual decrease in total lipid during fall, a pronounced increase occurs between late November and mid-January. Whether this abrupt increase in lipid during early winter is a natural phenomenon in this species or due to sampling error is presently unclear. Acclimation studies on *P. leucopus* indicate that cold exposure results in lipid utilization (Fig. 5D), and the initial decrease in lipid during late summer and fall may be due in part to greater cold exposure. However, this laboratory study does not suggest the environmental cueing, if any, responsible for the sharp increase in total lipid during winter. It is possible that lipid deposition in *P. leucopus* may be controlled by a "circannian" rhythm. Pengelley and Kelly (1966) have demonstrated that aspects of hibernation in *Citellius* sp. are controlled by a "circannian" rhythm. They found that wild-caught ground squirrels exhibited an annual weight cycle and entered hibernation at approximately the usual time of year, even though they were maintained under constant environmental conditions for more than a year. Other studies have also reported increased lipid deposition in winter-caught mammals (Heroux, 1963; Beumer, 1970), although the exact time of year during which this increase occurred was not established.

Seasonally changing photoperiod is probably responsible for both regression of the reproductive system (Fig. 2E) and the fall molt in wild populations of *P. leucopus*. Differences in photoperiod had a major effect on the reproductive system whereas differences in temperature had little effect (Fig. 4E). Whitaker (1940) also reports an effect of photoperiod on the reproductive system in this species. Similarly, acclimation studies have demonstrated that short photoperiod induces the "fall" molt in this species, although simultaneous cold exposure accelerates the rate of molting relative to warm acclimated, short photoperiod mice (Lynch, 1973).

Finally, simultaneous assessment of multiple characters on individual mice permitted a better understanding of the relationships between physiological characters. For example, this study demonstrated a positive association between lipid-free dry weight of interscapular brown fat and extent of nonshivering thermogenesis (Table 1). Thus, mice with large deposits of brown fat also exhibit greater nonshivering thermogenesis. This within species correlation and a similar positive correlation across species (Hayward, in: Chaffee and Roberts, 1971) provide additional support that brown fat is a thermogenic tissue. Furthermore, inverse relationships on both nonshivering thermogenesis and interscapular brown fat/g dry body weight with body weight indicate that smaller mice tend to exhibit greater nonshivering thermogenesis and have greater quantities of brown fat on a per gram basis than do larger mice. A similar negative correlation between body weight and hoarding behavior in *P. leucopus* has been reported (Lynch, 1972), suggesting that body weight may be of major influence in determining expression of both physiological and behavioral characters related to thermoregulation and winter survival From an evolutionary point of view, associations between characters are meaningful because natural selection, by acting through differential reproduction of individuals, may alter multiple phenotypes simultaneously. Thus, estimation of phenotypic correlations between variables is a first step in understanding to what extent these associations have a common genetic basis. Breeding studies are presently being conducted to assess the extent to which a common genetic basis contributes to phenotypic correlations between variables associated with mammalian thermoregulation.

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