Climatic Adaptation in Thermogenesis and Thermal Insulation in Wood Mice (*Apodemus argenteus*)

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Abstract Wood mice (*Apodemus argenteus*) were trapped live at three different altitudes (below 1,000, 1,900, and 2,400 m) during a 1-year period (Feb. 1984–Jan. 1985). After remaining at the trapped locations for 10–14 days, they were transferred into a climatic chamber at an altitude of 610 m. Oxygen consumption ($\dot{V}_{O_2}$) and colonic temperature (T_{co}) were measured at chamber temperatures (T_a) of five steps (30, 20, 10, 0, and -10°C) in freely moving conditions. In response to T_a of 0°C for the mice trapped in winter when their mean local habitat temperature (T_e) were lower than 0°C , there was a significant inverse correlation between $\dot{V}_{O_2}$ and T_e ($r = -0.70$, $p < 0.001$) whereas no significant correlation ($r = 0.23$) was observed in the mice trapped in other seasons when T_e was higher than 0°C . The correlation between T_{co} and T_e was significant ($r = -0.66$, $p < 0.001$) over the entire range of T_e . The pelt weight of the mice trapped at T_e higher than 0°C had a significant inverse correlation with T_e ($r = -0.65$, $p < 0.001$), but not in the mice trapped at T_e lower than 0°C . After measurement of $\dot{V}_{O_2}$ and T_{co} at T_a of 0°C , the mice who had lived in colder habitats (below 0°C) showed 0% mortality, whereas the mortality of the populations which had lived in warmer habitats was 13%. These results suggest that, in wood mice, adaptation to severe cold is established by an enhanced thermogenesis and by an increased insulation of the pelt in moderate cold.

Key words: thermogenesis, thermal insulation, habitat temperature, thermal conductance, pelt weight.

The ecological range of wood mice (*Apodemus argenteus*) extends throughout Japan. In addition, this species is distributed from sea level to an alpine forest zone (over 2,500 m) where very low temperatures prevail during winter. It may thus be concluded that this mouse is highly adaptable to a large spectrum of climatic variables.

SCHOLANDER (1955) proposed that homeotherms might be expected to adapt physiologically to a cold environment by changing body temperature, metabolic rate, and heat loss. Several studies have reported an increasing thermogenesis in cold-acclimatized wild rats (KROG *et al.*, 1955; HART and HÉROUX, 1963; HÉROUX, 1963) and in laboratory animals exposed to cold (MOORE and SIMMONDS, 1966; JANSKÝ *et al.*, 1969). With the exception of wild rats, however, oxygen consumption ($\dot{V}_{O_2}$) at the same environmental temperature did not differ in both warm- and cold-acclimatized wild animals (HART, 1971). It was shown that small mammals had denser fur and their pelage insulation was greater during winter than during summer (HART, 1956; POHL and HART, 1965; BROWN and LEE, 1969). However, in white rats acclimated to continuous low-temperature, no evidence for greater insulation was found (HÉROUX *et al.*, 1959). Some of these differences in the results may be due to the different laboratory conditions in which the animal studies were carried out. We investigated the adaptation of wood mice to their local habitat temperature by studying the correlation between total heat production, brown adipose tissue (BAT) weight, body temperature, pelt weight, and habitat temperature in wood mice captured in the forests and fields in central Japan.

MATERIALS AND METHODS

Animals. A total of 260 wood mice (142 males and 118 females) were trapped alive in the forests and fields at three different altitudes of 610–1,000, 1,900, and 2,400 m in the Yatsugatake Mountains once every month during a 1-year period (Feb. 1984–Jan. 1985). The wild animals trapped were maintained outdoors in metal cages with sawdust for bedding and both food and water given *ad libitum*. The cages were covered on top to protect the animals from direct radiation and rain. After being housed for 10–14 days at the location where they were trapped, the mice were transported to the laboratory located at an altitude of 610 m. The total time elapsed for the transit was approximately 4 h. The mean local habitat temperature (T_e) for 10 successive days after trapping was estimated according to the reports of temperature from the Matsumoto Weather Bureau Station. According to their body weight (14–30 g), 235 adult male and female mice were selected for this experiment.

Measurements. The mice trapped at the same altitude in each month were randomly divided into two experimental groups. The number of animals in each group was from 5 to 12. In the first series of experiments, oxygen consumption ($\dot{V}_{O_2}$) and colonic temperature (T_{co}) were measured in a climatic chamber by changing the test ambient temperature (T_a) from 30 to -10°C by steps of 10°C . First, animals were acclimated at T_a of 20°C for 2 h, then the measurements were started from T_a of 30°C . Five open circuit apparatuses were used to measure $\dot{V}_{O_2}$ of the mice. Each mouse was able to move freely when placed for 30 min in a metabolic chamber (each having a volume of 250 ml), into which air was absorbed at a flow rate of 200 ml/min

from each respirator. The gas content (O_2 and CO_2) of the air from the chamber was measured successively by a gas analyzer (H21, San-ei Sokki) and recorded. All data were corrected to STPD. After measurement of $\dot{V}_{O_2}$, T_{co} was measured by a thermistor probe inserted to a depth of 3 cm beyond the anus of each mouse. Since some animals trapped at T_e higher than $0^\circ C$ showed a progressive decrease of $\dot{V}_{O_2}$ and T_{co} during the measurements at T_a of $-10^\circ C$, we stopped these measurements at $-10^\circ C$ after several experiments were carried out.

In the second series of experiments, new groups of animals were killed with ether and brown adipose tissue (BAT) from the interscapular region was excised and pelts were removed. BAT weight was measured by torsion balance. After each pelt was stuck on corrugated cardboard for 1 month, the dried pelt was weighed. Then the pelt was soaked in chloroform methanol (2:1, v/v) for 2 days to extract lipids, and the dried pelt weight was again measured to estimate the weight of sebum. Next, corium from the pelt was scraped off by knife. Further, to collect hairs, the pelt was soaked in a saturated calcium hydroxide solution for about 7 days. Then hairs obtained and bared skin were immersed in 10% acetic acid to weed out the calcium hydroxide. Finally, these chemicals were washed away by running water. Thermal conductance (C) was established by the relation $C = \dot{V}_{O_2} / (T_{co} - T_a)$ using the data from simultaneous measurements of $\dot{V}_{O_2}$, T_{co} , and T_a ($10^\circ C$). Data were expressed as mean $\pm$ S.E. Linear regression and Student's t -test were used for statistical analysis. The criterion for significance was $p < 0.05$.

RESULTS

Data on body weights of wood mice, recorded at three different locations, and the environmental temperatures (T_e) in which they were trapped, appear in Table 1.

Figure 1 shows $\dot{V}_{O_2}$ of the wood mice measured at $0^\circ C$ in each calendar month. In winter (Jan.), $\dot{V}_{O_2}$ was significantly higher in the mice trapped at 1,900 m than in those trapped at other altitudes. Figure 2 shows the relationship between $\dot{V}_{O_2}$ measured at T_a 's of $-10, 0, 10, 20$, and $30^\circ C$ and T_e in all three groups. As shown in Fig. 3, the relationship between $\dot{V}_{O_2}$ and T_e showed an inflection at T_e of $0^\circ C$, creating the two different sections of the relationship. The regression line obtained in the mice whose T_e was below $0^\circ C$ had a significant correlation coefficient ($r = -0.70, p < 0.001$), while there was no significant relationship between $\dot{V}_{O_2}$ and T_e in the mice whose T_e was higher than $0^\circ C$. The value measured at the four other different T_a also disclosed the same changes as $\dot{V}_{O_2}$ measured at $0^\circ C$ (Table 2). Figure 4 shows the relationship between T_{co} measured at T_a of $0^\circ C$, in the animals captured at different T_e . All data obtained in the mice captured at the three different altitudes fitted on one regression line ($r = -0.66, p < 0.001$).

There was a significant inverse correlation between BAT weight and T_e ($r = -0.69, p < 0.001$; Fig. 5). Figure 6 shows the relationship between the ratio of the total dried pelt weight (which contained the lipid) to body weight and T_e . The regression line obtained at T_e above $0^\circ C$ had a significant inverse relationship

Table 1. Body weights of wood mice trapped monthly at three different altitudes, and estimated average habitat temperatures (T_e).

Month	Tobira* 610–1,000 m**		Shibu* 1,900 m**		Kuroyuri* 2,400 m**	
	Body weight (g)	T_e (°C)	Body weight (g)	T_e (°C)	Body weight (g)	T_e (°C)
Jan	17.4±0.5 (11)	−2.0	16.9±0.8 (8)	−8.4		
Feb	19.4±0.8 (6)	−2.5				
Mar	16.8±1.0 (7)	−0.2				
Apr	16.8±1.1 (4)	7.0				
May	19.3±0.9 (8)	12.5				
Jun	18.0±0.7 (15)	17.0				
	18.3±1.1 (6)	21.0				
Jul			17.2±0.9 (17)	14.3	19.5±0.7 (6)	10.0
			19.5±0.8 (11)	15.0		
Aug	18.0±0.7 (9)	23.0	21.8±1.2 (2)	16.5	18.5±0.7 (19)	11.0
	18.3±1.1 (6)	24.0			19.6±0.8 (9)	12.0
Sep			16.5±1.4 (2)	9.5	23.0±0.8 (8)	5.0
Oct			16.0±0.5 (12)	2.8		
Nov					19.1±0.7 (9)	−5.0
Dec	19.3±1.5 (8)	3.0	17.8±2.9 (15)	−6.0		
	20.5±0.9 (6)	0.5				

Numbers in parentheses indicate sample sizes. Values are mean±S.E.
*: Locations, **: altitudes.

between the two variables, whereas that obtained at T_e below 0°C was not significant. Except for epidermis weight, the other components of pelt weight showed the same changes as the total dried pelt weight (Table 3). The relationship between thermal conductance (C) measured specifically at 10°C and T_e (Fig. 7) showed nearly the same pattern of the changes as $\dot{V}_{O_2}$ measured at 0°C (see Fig. 3). After the measurements of $\dot{V}_{O_2}$ at T_a 's of 0 and −10°C, the animals who had lived in cold habitats (at T_e below 0°C) showed 0% mortality, whereas in other populations 13% died at T_a of 0°C and 25% died at T_a of −10°C, respectively.

DISCUSSION

The data in this report on the thermal adaptation of wood mice trapped at three different altitudes throughout 1 year permit a direct evaluation of the relationships between body weight, metabolism, insulation and habitat environmental temperature (T_e). These are major indicators of climatic adaptation in the wood mice. Bergmann's rule has shown that the relationship between the body weight and the animals' habitat temperature often demonstrates a negative

Fig. 1. Seasonal and altitudinal change of oxygen consumption ($\dot{V}_{O_2}$) measured at 0°C in three populations of wood mice.

Table 2. The relationship between oxygen consumption ($\dot{V}_{O_2}$) of three populations of wood mice measured at various ambient temperatures and habitat temperatures measured at the sites where they were captured and raised.

Ambient temperature ($^\circ\text{C}$)	Habitat temperature ($^\circ\text{C}$)			
	$-8.4-0$		$0-24$	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
-10	-0.47	0.05	-0.12	N.S.
0	-0.70	0.001	0.23	N.S.
10	-0.54	0.01	0.12	N.S.
20	-0.48	0.01	-0.06	N.S.
30	-0.45	0.05	-0.11	N.S.

The correlation coefficients (*r*) were calculated for two regression lines divided at T_e of 0°C .

correlation (BROWN and LEE, 1969). SCHOLANDER (1956) emphasized that large body size was a morphological adaptation to cold climates. However, the body weight of the mice studied here did not correlate with T_e ($r=0.11$, N. S.), indicating that this species does not follow Bergmann's rule. As shown in Figs. 1-3, the altitudes (610-2,400 m) in which the mice had lived had no effect on $\dot{V}_{O_2}$ measured

Fig. 2. The relationship between oxygen consumption ($\dot{V}_{O_2}$) in three populations of wood mice measured at various test temperature (T_a) and habitat temperature (T_e) measured at the sites where they were captured and raised. Symbols for the populations are the same as those in Fig. 1.

at various test ambient temperatures (T_a). A similar observation was made on wild rats living at various altitudes between 0 and 2,700 m (BROWN and LEE, 1969). It is inferred that the chronic moderate hypoxia has no influence on metabolic response in the wood mice. As shown in Fig. 3, the mice captured at T_e lower than 0°C showed a significant inverse correlation between $\dot{V}_{O_2}$ measured at 0°C and T_e , whereas there was no statistically significant relationship between these quantities in the mice captured at T_e higher than 0°C . Therefore, it is possible that the wild mice living at T_e below 0°C could develop a metabolic acclimatization to cold. Mice (*Acomys russatus*) captured in a cold environment could maintain higher $\dot{V}_{O_2}$ and T_{co} at 6°C , but those captured in a hot environment showed a progressive decrease in $\dot{V}_{O_2}$ and T_{co} (HAIM and BORUT, 1981). Our results are similar to those of ROBERTS *et al.* (1966) who reported that $\dot{V}_{O_2}$ increased in wild rodents that lived at severely cold sites.

BAT was reported to be a major site of nonshivering thermogenesis (FOSTER and FRYDMAN, 1979). Also, an increase of BAT in a cold environment was shown to

Fig. 3. The relationship between oxygen consumption ($\dot{V}_{O_2}$) in three populations of wood mice measured at 0°C , and habitat temperature (T_e) measured at the sites where they were captured and raised. The least squares regression lines are plotted. Symbols for the populations are the same as those in Fig. 1.

Fig. 4. The relationship between colonic temperature (T_{co}) in three populations of wood mice measured at 0°C and habitat temperature (T_e) measured at the sites where they were captured and raised. The least squares regression line and the correlation coefficient (r) are shown. Symbols are the same as those in Fig. 1.

Fig. 5. The relationship between brown adipose tissue (BAT) weight in three populations of wood mice and habitat temperature (T_e). The least squares regression line and correlation coefficient (r) are shown. Symbols are the same as those in Fig. 1.

Fig. 6. The relationship between the total pelt weight to body weight in three populations of wood mice and habitat temperature (T_e) measured at the sites where they were captured and raised. The least squares regression lines and the correlation coefficients (r) are shown. Symbols are the same as those in Fig. 1.

be related to the requirement for cold thermogenesis (DIDOW and HAYWARD, 1969). In our experiments, the ratios of BAT to body weight were shown to be increased at lower habitat temperatures (Fig. 5). However, it is uncertain whether the BAT weight alone is an index of the thermogenesis or not, since the correlation patterns between BAT weight and T_e (Fig. 5), and between $\dot{V}_{O_2}$ and T_e (Fig. 3) are different.

Fig. 7. The relationship between the thermal conductance (C) in three populations of wood mice measured at 10°C and habitat temperature (T_e) measured at the sites where they were captured and raised. The least squares regression lines and correlation coefficients (r) are shown. Symbols are the same as those in Fig. 1.

Table 3. The relationship between various pelt components to body weight and habitat temperature at sites where the wood mice were captured.

	Habitat temperature ($^{\circ}\text{C}$)			
	$-8.4-0$		$0-24$	
	r	p	r	p
Total pelt weight	-0.65	0.001	0.05	N.S.
Hair weight	-0.57	0.001	0.34	N.S.
Epidermis weight	-0.21	N.S.	0.22	N.S.
Hair+epidermis weight	-0.34	0.05	0.12	N.S.
Sebum+corium weight	-0.51	0.001	-0.19	N.S.

The correlation coefficients (r) were calculated for two regression lines divided at T_e of 0°C .

BAT begins to function even at only slightly below a thermoneutral zone in small mammals (DONHOFFER and SZELÉNYI, 1965). The lower critical temperature in wood mice was, in our successive measurement of $\dot{V}_{\text{O}_2}$ at 30 to 20°C , just above 25°C . Therefore, thermogenesis in BAT is required even at the warmest season of the year.

In spite of $\dot{V}_{O_2}$ increase caused by the BAT thermogenesis, the reason why the total $\dot{V}_{O_2}$ values in mice whose T_e was higher than 0°C remained constant might be due to the decreased metabolism induced by intensified pelt insulation (Fig. 6). Since the insulative factors may inhibit a remarkable increase in metabolism in rats during exposure to cold (JOY and MAYER, 1968), we assume that the increase of insulation masked the $\dot{V}_{O_2}$ change in wood mice.

We have measured the various components of the pelt. The degree of insulation has been studied by measuring heat conductance (DEAVERS and HUDSON, 1981), hair length (MCCLURE and PORTER, 1983), and hair weight (JOY and MAYER, 1968). There was a highly significant correlation ($r=0.92$) between hair weight and the total pelt weight in all animals. Further, it might be emphasized that the total pelt weight showed high correlation in particular to T_e higher than 0°C (Table 3). The number of hairs in *Apodemus kaup* generally corresponds with that of hair follicles, because new hairs use the same hair follicles as the old hair, except for pile or fur hair (under hair); winter hair increases the under hair and the hair density is higher in winter than in summer (HAITLINGER, 1968), while the ratio of guard hair (upper hair) to under hair in *Apodemus speciosus* increases in summer and vice versa in winter (YANAGIDAIRA and UCHIKAWA, 1987). The increase of the hair weight in a cold environment is considered to depend on the development of the length or thickness of hair. Hair depth is the only important property of hair determining heat loss in nestling (MCCLURE and PORTER, 1983). Considering a role of hair in heat loss, the heat conductance becomes lower as the hair length and thickness increase. Among the same species the amount of heat loss from pelts is lower in winter than in summer (HART, 1956; INNES and LAVIGNE, 1979). Except for the cold factor, hair growth is reported to be influenced by other environmental factors, such as photoperiod (MORRIS, 1961), diet (PINTER, 1978), and hormones (EBLING and JOHNSON, 1964). The decrease of photoperiod in winter may result in an increase in insulation. However, it appears that the effect of the photoperiod on insulation in mice is weak in the present study because the wood mice are nocturnal and therefore stay in dens during natural daylight, and they were raised in a dark cage after capture. Their life habit may have an effect on the hair growth during the seasons. The ratio of the total pelt to body weight at T_e above 0°C was significantly higher in the colder environments than those in the warmer environments, but this tendency disappeared at T_e below 0°C (Fig. 6). We thus speculate that this discontinuity around 0°C represents an important critical temperature between thermogenesis and insulation; this species living at T_e above 0°C would develop an insulated acclimatization to cold. In the present study, T_{co} measured at 0°C did not respond in the same pattern as $\dot{V}_{O_2}$, but the difference in responses between $\dot{V}_{O_2}$ and T_{co} may be due to an enhanced insulation in mice which live at around 0°C . Therefore, we suggest that adaptation to cold in wild wood mice is established by an enhanced thermogenesis in severe cold and by an increased insulation of the pelt in moderate cold.

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