Lysozyme activity in tissues of guinea pigs

Tissue	Activity[a] mean ± SEM (n = 8)
Stomach fundus	2205 ± 217
Stomach pylorus	2489 ± 236
Duodenum	8.4 ± 0.8
Jejunum	2.9 ± 0.3
Ileum	2.3 ± 0.2
Cecum	33 ± 6.2
Ascending colon	15.8 ± 3.2
Transverse colon	3.4 ± 0.7
Descending colon	3.6 ± 0.4
Rectum	4.1 ± 0.4
Bone marrow	614 ± 55
Lung	330 ± 51
Spleen	347 ± 76
Kidney	1008 ± 95
Serum	35 ± 5.2[b]

[a]Chicken egg white lysozyme equivalent μg/mg protein; [b]chicken egg white lysozyme equivalent μg/ml.

activity in the fundus and pylorus. The mean activity in the stomach was 71 times that of the next highest gastrointestinal tissue, the cecum, and over 300 times greater than the mean activity of the three segments of the colon.

The activities of lysozyme in the nongastrointestinal tissues of guinea pigs were rather moderate, being greater than the levels in cattle, a species that is relatively lysozyme deficient[6], but substantially less than the levels in rabbits, a species with relatively high lysozyme activity[13,14]. The relatively high activities of lysozyme in the bone marrow, lung and spleen probably represent lysozyme in the leukocytes in these tissues, whereas the high activity in the kidney is a result of lysozyme that has been reabsorbed by tubule cells after being filtered by glomeruli. Evidence has been presented that gastrointestinal lysozyme of herbivores is an isozyme of lysozyme and is the product of a gene that is distinct from the gene that codes for the lysozyme of other tissues[4,15,16]. Associated with this novel isozymic nature of gastrointestinal lysozyme, a unique functional activity has been hypothesized: lysozyme in the gastrointestinal tract digests cellulolytic bacteria that pass into the lysozyme-containing segment from an anterior fermentation organ[4,5,7].

However, because this study has demonstrated that the gastrointestinal lysozyme of guinea pigs is localized in the stomach and because it is known that the fermentation organ in guinea pigs is the cecum, it can be concluded that gastrointestinal lysozyme, at least in guinea pigs, and therefore possibly also in other herbivores, does not function in the digestion of bacteria from the fermentation organ. This conclusion is contradictory to recent hypotheses[4,5,7] regarding the function of gastrointestinal lysozyme in herbivores.

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0014-4754/86/050542-02$1.50 + 0.20/0
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Seasonal changes in the critical arousal temperature of the marsupial *Sminthopsis crassicaudata* correlate with the thermal transition in mitochondrial respiration[1]

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9 May 1985

Summary. During periods of torpor *Sminthopsis crassicaudata*, a dasyurid marsupial, regulated its body temperature above about 16.3 °C in summer and 13.0 °C in winter. Animals with lower body temperatures were unable to arouse. Liver, heart and brain mitochondrial succinate:cytochrome c reductase showed a thermal transition at 16 °C in summer and at 12.5 °C in winter. Thus the lowest regulated body temperature was just above the temperature where changes were detected in mitochondrial respiration.
Key words. Marsupial; torpor; season; critical arousal temperature; mitochondrial respiration; thermal transition.

A marked increase in the Arrhenius activation energy (E_a; a measure of the slope of the log enzyme activity against the reciprocal of the absolute temperature) below a critical temperature (T*) has been described for many mammalian membrane-associated enzymes. For example, in liver and heart mitochondria from homeotherms and/or normothermic (summer active) hibernators, T* is observed at 20–23 °C for succinate oxidase, succinate:cytochrome c reductase, succinate oxidase linked H^+ ejection and mitochondrial Ca^{2+} uptake[2–10]. During the torpid state of heterothermic and hibernating mammals, the lowered body temperature (T_b) is associated with a significant lowering, or even a disappearance of T* for these and several other membrane-associated enzymes, when compared to homeothermic species and normothermic hibernators in summer[2–5,8–10]. In preparation for hibernation various membrane-associated respiratory enzymes of liver mitochondria from ground squirrels exhibit a lowering of T* from 20 to about 12 °C. Furthermore, a constant E_a during the hibernating state suggests that T* is lowered below the minimum T_b of 2 to 5 °C which ground squirrels experience[9,10]. Since homeothermic mammals which exhibit a T* at about 20 °C are unable to survive hypothermia at body temperatures which do not threaten heterothermic species, the lowered T* evident during hibernation/torpor may be an important factor in the ability of many small mammals to enter into, and arouse from, the torpid state. Thus the T* may be a determinant of the 'critical arousal temperature' below which animals

Arrhenius parameters and Q_{10} of mitochondrial succinate; cytochrome c reductase of *Sminthopsis crassicaudata* in summer and winter

Season	Organ	(n)	T* (°C)	Ea1 (kcal/mole)	E_a2	Q_{10}
Summer	Liver	(4)	16.0 ± 0.6	6.1 ± 0.1	22.2 ± 2.2	1.40 ± 0.02
	Heart	(4)	16.3 ± 0.5	7.4 ± 1.0	20.0 ± 2.3	1.50 ± 0.08
	Brain	(3)	15.7 ± 0.7	7.9 ± 0.8	17.2 ± 2.9	1.52 ± 0.06
	Mean		16.0 ± 0.3	7.0 ± 0.5	20.0 ± 1.4	1.47 ± 0.03
Winter	Liver	(4)	12.0 ± 1.4	10.7 ± 1.5	25.4 ± 3.9	1.84 ± 0.13
	Heart	(4)	13.5 ± 1.5	11.3 ± 1.2	24.7 ± 2.8	1.85 ± 0.10
	Brain	(4)	12.0 ± 1.6	13.8 ± 1.4	24.1 ± 0.7	2.09 ± 0.13
	Mean		12.5 ± 0.8	11.9 ± 0.8	24.8 ± 1.5	1.92 ± 0.07
Summer vs winter			$p < 0.005$	$p < 0.001$	$p < 0.05$	$p < 0.001$

Data are expressed as the mean ± SE from the number of individuals shown in brackets. The Q_{10} was measured in the temperature region above T*.

are unable to rewarm by means of endogenous heat production. During torpor, T_b is regulated at a specific lower 'set point'. At ambient temperatures below this 'set point' an increase in both metabolic rate and T_b have been observed in birds and placental mammals[11,12]. These phenomena have been suggested as a means of preventing the animals from reaching a fatal temperature or a temperature from which the arousal time would carry some ecological penalty[11]. However, no suggestion for a possible biochemical mechanism has so far been advanced.

In this study we have tested whether a marsupial species regulates its body temperature during periods of torpor in a manner similar to placental mammals, and whether the Arrhenius critical temperature for mitochondrial membrane-associated enzyme activity is in some way correlated with the 'set point' temperature. The species used was the fat-tailed dunnart, *Sminthopsis crassicaudata*, a small insectivorous marsupial of the family Dasyuridae. It is distributed over much of southern Australia in a range of habitats and has been observed to undergo torpor in both field and laboratory situations[13,14].

Materials and methods. Adult *S. crassicaudata* were obtained from a laboratory colony. They were housed individually in outside enclosures under natural photoperiod and temperature fluctuations. The animals were maintained on water and processed pet meat ad libitum with occasional dietary supplements of *Tenebrio* larvae and an egg gelatine mixture.

Rates of oxygen consumption ($\dot{V}_{O2}$) were measured over a range of ambient temperatures (T_a) during the austral summer and winter. Animals were placed in 3 l metabolic chambers which had ports for thermocouples and incurrent and excurrent air, through which air was drawn at metered flow rates of 0.2–0.4 l/min. $\dot{V}_{O2}$ was determined from the difference between the oxygen content in two parallel circuits, one being a room air reference, the other coming from the chamber containing the animal. A Servomex Model OA 184 paramagnetic oxygen analyzer was used. Calibration was initially done by reducing the pressure in the analysis cells to check for linearity of response, thereafter by using nitrogen, ambient air or a calibrated gas mixture. The difference in the fractional concentration of oxygen between the gas streams was used to calculate the rate of oxygen consumption using equation 3a of Withers[15]. All gas volumes were corrected to STPD. For the determination of mass specific rates, the animals were weighed immediately before and after the experiment and the interpolated values were used in the calculations. The measurements were conducted in a quiet controlled temperature room that was acoustically isolated from the recording equipment. 62 tests of 21.2 ± 1.9 h (mean ± SD) were conducted in summer and 40 tests of 20.1 ± 2.6 h duration in winter. Additional measurements over several hours were taken from normothermic resting animals at temperatures around and above their respective zones of thermoneutrality. To induce torpor, food and water were not provided during the periods of measurement. Only measurements in which a constant $\dot{V}_{O2}$ of at least 30 min duration during periods of torpor occurred, were included.

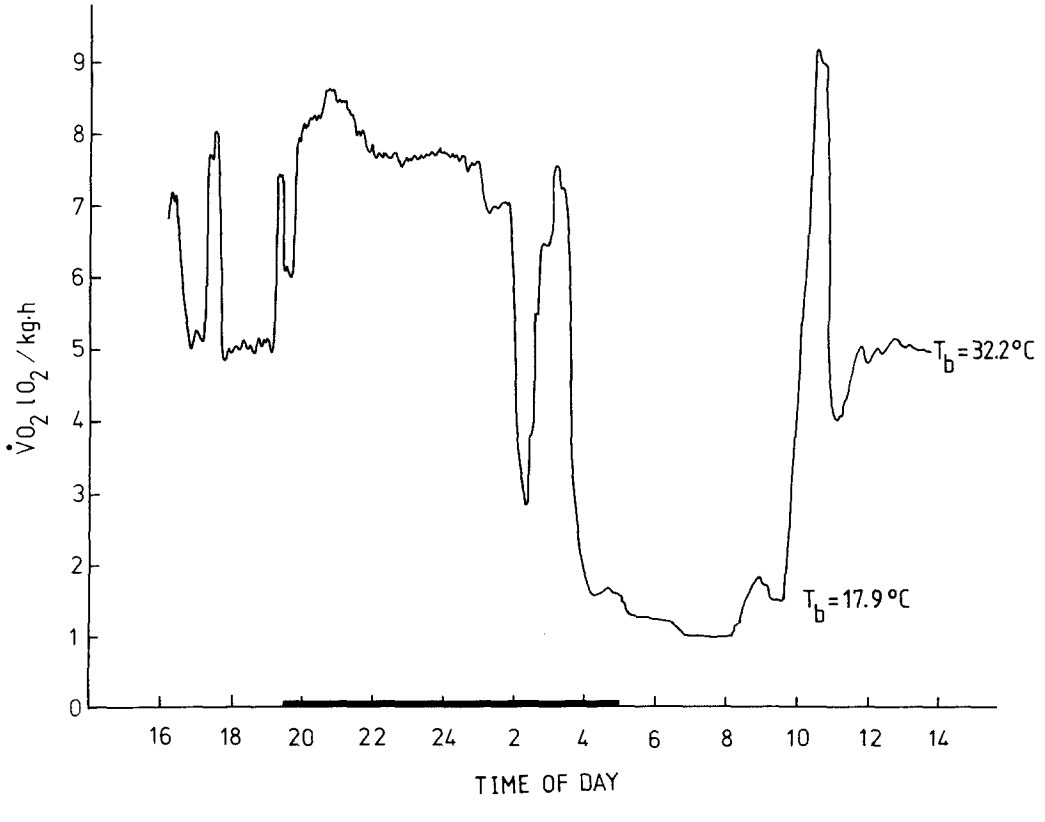

Figure 1. The rate of oxygen consumption ($\dot{V}_{O2}$) of an individual *S. crassicaudata* measured in summer over an 21-h period at a constant ambient temperature of 12 °C. The abscissa represents the local time and the dark bar indicates the period of darkness. Body temperatures (T_b) are indicated in °C.

Rates of oxygen consumption of normothermic resting animals were measured during at least 30 min of inactivity with a variation in $\dot{V}_{O2}$ of less than 5% over 15 min. Body temperature measurements were taken with a calibrated 0.5 mm diameter copper-constantan thermocouple inserted 25 mm into the rectum.

The temperature-activity profile of succinate:cytochrome c reductase was determined for liver, heart and brain mitochondria of *S. crassicaudata*. Eight animals (four in summer, four in winter) were sacrificed while they were torpid or shortly after they had aroused from torpor and the tissues were immediately removed. Mitochondria were isolated by differential centrifugation and the measurement of activity for mitochondria from individual animals were carried out at temperatures between 2 and 38 °C, essentially as previously described[4,16]. Arrhenius plots were fitted by least squares regressions on log transformed data[17]. All other lines were fitted by linear regression analyses and differences in elevation, slope and y-intercept were determined using the derived t- and F-values. Means of samples in the text are expressed ± SD.

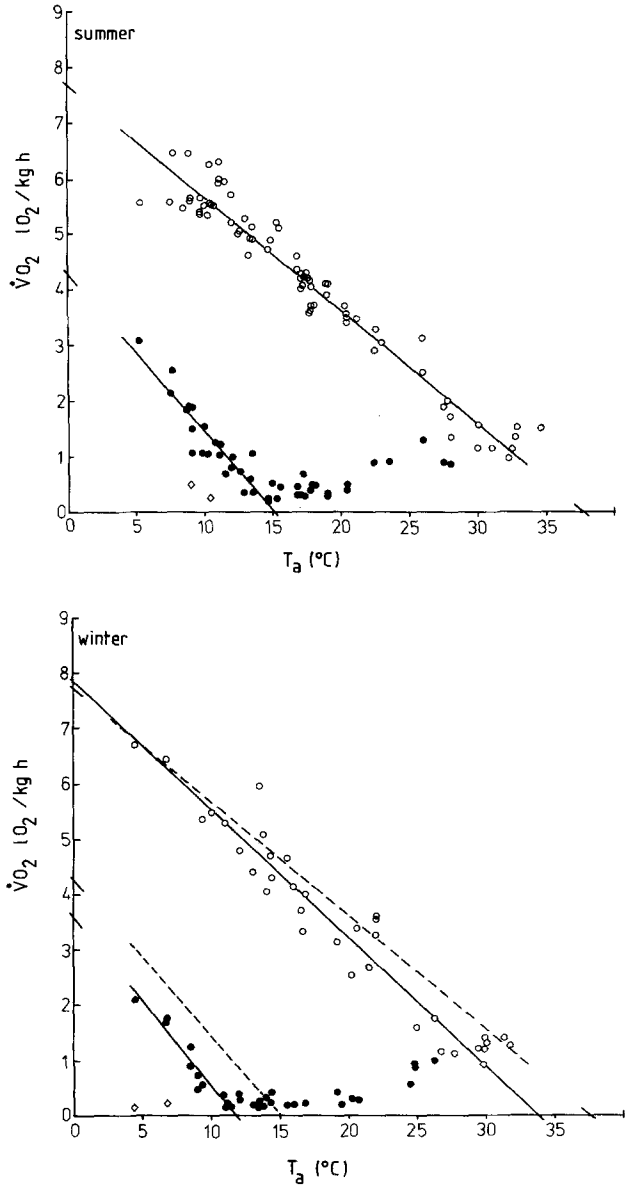

Figure 2. The rate of oxygen consumption ($\dot{V}_{O2}$) of *S. crassicaudata* (16 males, 12 females) at different ambient temperatures (T_a). Measurements were taken in summer and winter. The mean body mass was 17.7 ± 2.1 g (summer) and 17.3 ± 2.5 g (winter). The symbols indicate normothermic inactive animals (○), animals in torpor (●) and in hypothermia (◇). The equations for the linear regressions for normothermic inactive animals below thermo-neutrality were: $y = 7.82–0.231\ x$; $r = 0.96$; summer, $y = 7.69–0.204\ x$; $r = 0.96$; winter, and for torpid animals at T_a below the minimum T_b: $y = 4.28–0.285\ x$; $r = 0.92$; summer, $y = 3.58–0.305\ x$; $r = 0.95$; winter. The dashed lines in winter represent the linear regression for summer animals.

Results and discussion. Temperature regulation during torpor. Sminthopsis crassicaudata showed strong diurnal fluctuations in $\dot{V}_{O2}$ As shown for one individual measured at T_a 12 °C during summer (fig. 1), $\dot{V}_{O2}$ decreased after an initial activity period to normothermic resting values (5.0 l O_2/kg h) at 18.00–19.00 h. Shortly after the lights were switched off, $\dot{V}_{O2}$ increased to an activity peak value of 8.63 l O_2/kg h. The period of activity lasted for several hours and the animal entered torpor after a single 'test drop'. A minimum $\dot{V}_{O2}$ value of 1.0 l O_2/kg h was observed between 06.45 and 08.10 h. At the time when T_b was measured, $\dot{V}_{O2}$ was slightly increased and T_b was 17.9 °C. After the disturbance by the T_b measurement, $\dot{V}_{O2}$ increased rapidly to an arousal peak value of 9.2 l O_2/kg h which was followed by a second normothermic inactive period with a $\dot{V}_{O2}$ of about 5.1 l O_2/kg h and T_b was 32.2 °C.

The effect of T_a on $\dot{V}_{O2}$ from individual normothermic inactive animals and the minimum levels during torpor (fig. 1) measured in summer and winter are shown in figure 2. In normothermic inactive animals, $\dot{V}_{O2}$ was temperature-dependent and increased in a linear manner with decreasing T_a. During summer the resting metabolism was significantly higher in elevation ($p < 0.001$; F-test) than in winter. For the $\dot{V}_{O2}$ minima, a steady decrease was observed with decreasing T_a. The lowest $\dot{V}_{O2}$ values were observed between T_a 15 and 20.5 °C in summer with a mean of 0.39 ± 0.14 l O_2/kg h ($n = 17$). This was significantly higher ($p < 0.01$, t-test) than the minima at T_a 11–21 °C in winter (mean 0.27 ± 0.10 l O_2/kg h; $n = 19$). However, no significant differences between summer and winter could be detected in ΔT ($T_b - T_a$) during torpor in the respective ranges of T_a (data not shown). In summer a linear increase in $\dot{V}_{O2}$ below T_a 14 °C was observed which intersected the abscissa at 15.0 °C. In winter this linear increase was observed below T_a 11 °C and the intersection with the abscissa was 11.7 °C. The linear regressions for both lines were significantly different in elevation ($p < 0.001$; F-test). The lowest individual T_b measured during torpor was 16.3 °C in summer and 13.0 °C in winter.

Three animals in summer (T_a 5.3, 9.0 and 10.4 °C) and two animals in winter (T_a 6.8 and 4.4 °C) were observed in torpor for a period of 2–3 h. Thereafter $\dot{V}_{O2}$ steadily declined and was not maintained at a constant level like in other individuals. These animals had body temperatures of 11.6 and 14.1 °C (summer), 10.5 and 8.1 °C (winter), and could not arouse after a disturbance. The third animal in summer (T_a 5.3 °C) died during hypothermia; all the other animals could arouse after partial rewarming to 18–20 °C.

$\dot{V}_{O2}$ of torpid animals increased when they were cooled below a critical temperature. The mean T_a where an increase in $\dot{V}_{O2}$ followed cooling was 15.0 ± 0,9 °C ($n = 7$) in summer which was significantly higher ($p < 0.001$; t-test) than the 11.5 ± 0.8 °C ($n = 7$) in winter. Cooling resulted in arousal in half of the observations, while the other individuals remained in torpor but with an increased metabolic rate.

A correlation between the critical arousal temperature and the thermal transition in mitochondrial respiration. To examine the possible correlation between T* and the critical arousal temperature during torpor, the temperature activity profile of succinate:cytochrome c reductase of liver, heart and brain mitochondria was determined. As shown in figure 3, T* occurred at about 16 °C (summer) and 12 °C (winter) for all three tissues. The T* and slopes of the lines relating enzyme activity to the reciprocal of the absolute temperature (a measure of the apparent

Arrhenius activation energy of the enzyme system), were statistically similar in all tissues both above and below T* and therefore were combined (table). The mean for T* from the three tissues measured in summer animals (16.0 °C) was significantly higher than the 12.5 °C measured in winter animals and both values were lower than the T* of 22.6 °C reported for liver and heart mitochondrial succinate oxidase and succinate:cytochrome c reductase from 11 homeothermic species[3]. Thus for animals examined in both summer and winter, T* occurred at about 1 °C below the temperature at which a thermoregulatory increase was observed in the metabolism during torpor. Since the difference between T_b and T_a during torpor of *S. crassicaudata* is greater than 1 °C it appears that T_b is regulated at a temperature just above the temperature at which a change in the respiratory enzyme is observed (i.e. T*). The seasonal alteration of T* in *S. crassicaudata* was accompanied by a lower value for E_a1 (7.0 kcal/mole; above T*) and E_a2 (20.0 kcal/mole; below T*) in summer than in winter (E_a1, 11.9 kcal/mole; E_a2, 24.8 kcal/mole) (table). The Q_{10} above T*, which is within the body temperature of the animals, was 1.47 in summer and 1.92 in winter.

The dasyurid marsupial *S. crassicaudata* regulates its body temperature above a critical level during periods of torpor in a manner similar to placental hibernators[12] and torpid humming birds[11]. The metabolic rate during torpor is significantly lower in winter than in summer as has been observed in garden dormice[18]. Furthermore during winter the thermoregulatory increase in the metabolic rate during torpor in *S. crassicaudata* occurs at a lower temperature than during summer.

The temperature-activity profile of the mitochondrial membrane respiratory enzyme shows a lower thermal transition (T*) in winter and a concomitant increase in the apparent activation energy or the Q_{10} (above T*) when compared with the summer animals. It has been suggested that T* has as its basis some temperature-induced change in the molecular ordering of membrane lipid components[19,20] and this may play a role in limiting the survival of endotherms during hypothermia. Subsequently, the seasonal changes in the Arrhenius parameters of *S. crassicaudata* may be attributed to modified membrane lipids. Since the nature of the dietary lipid intake markedly affects the lipid composition as well as the thermal response of the mitochondrial membrane respiratory enzymes in rats[6,7], this view appears to have strong support. However, a seasonal alteration in the protein component of the mitochondrial electron transport chain also must be considered.

The critical arousal temperature of *S. crassicaudata* in both seasons correlates with the transition temperature in mitochondrial respiration and supports the view that during torpor T* is in some manner maintained at a temperature below the minimum regulated T_b experienced by these animals[3,21]. The Q_{10} of the mitochondrial respiratory enzymes in the temperature range above the minimum T_b is greater in summer than in winter and correlates with the greater reduction in the metabolic rate during torpor in the winter animals. Furthermore it has been suggested that the increased Q_{10} may facilitate arousal[22]. Thus an alteration in the thermal response of mitochondrial respiration provides a possible biochemical explanation as to the mechanism by which heterothermic animals survive prolonged periods of torpor, conserve energy whilst torpid, and are able to arouse from torpor by means of endogenous heat production.

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1 Acknowledgments. This work was supported by a Flinders University Research Scholarship Award to F. Geiser. We thank Prof. J.H. Bennett for the supply of animals and M. O'Driscoll for the animal maintenance.

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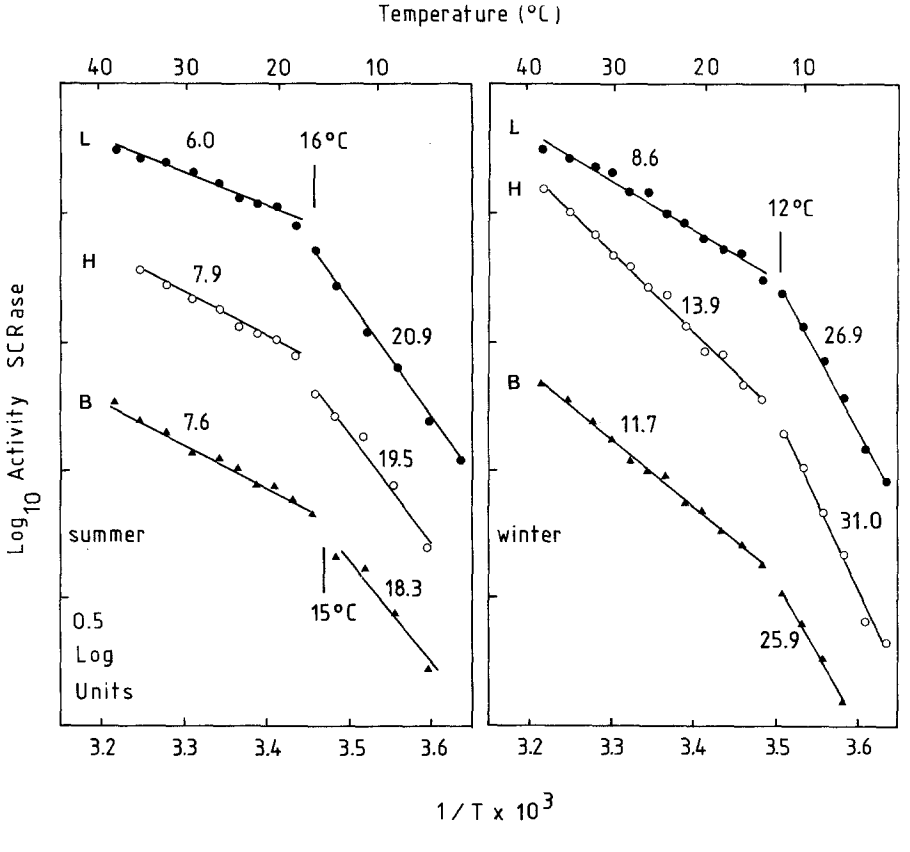

Figure 3. The effect of temperature on succinate:cytochrome c reductase activity of liver (L ●), heart (H ○), and brain (B ▲) mitochondria from *S. crassicaudata* in summer and winter. The lines were fitted to the data by the method of least squares[17]. The correlation coefficients for each linear region above and below T* were between −0.97 and −0.00. The numbers adjacent to the lines are the Arrhenius activation energies (E_a; E_a1 above T*; E_a2 below T*) in units of kcal/mole.

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0014-4754/86/050543-05$1.50 + 0.20/0
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Activity-induced thermogenesis in lean and genetically obese *(ob/ob)* mice

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Summary. Motor activity was approximately 60% lower in genetically obese than in lean mice, during three consecutive hours at thermal neutrality. It is suggested that this must have contributed to the lower heat production measured in the obese mice and that activity-induced thermogenesis contributes significantly to differences in energy expenditure between the genotypes, at least in the short-term.
Key words. Activity; genetics; obesity; thermogenesis.

In previous investigations on energy expenditure and obesity, considerable attention has been focussed on possible differences in the various components of resting metabolism both in man[1-3] and in rodents[4,5]. This emphasis is in accord with the fact that a large proportion of energy utilized by the body is spent on resting metabolism. However, variations in resting metabolism tend to be small compared with the influence of activity on metabolic rate[6-9], and yet the role of spontaneous activity has been relatively neglected and examined in only a few studies of obesity[3]. Differences in activity could be of importance not only in obese subjects but also in lean subjects during over-feeding, since they might help to explain any apparent discrepancy between actual and predicted weight-gain during over-feeding[10]. In the present investigation, the contribution made by motor activity to energy expenditure has been studied in lean and genetically obese *(ob/ob)* mice living undisturbed at thermal neutrality.

Materials and methods. Mature mice of the strain C57BL/6-*ob*/01a were investigated. Mean b.wt ± SEM were 35 ± 1.8 g for the lean (n = 12) and 54 ± 2.5 g for the obese mice (n = 9). The animals were housed separately at 28 ± 1 °C on a 12 h light : 12 h dark cycle (light = 07.30–19.30 h) and food (CRM, Labsure, Christopher Hill Group Ltd, RHM) and water were available ad libitum.

Simultaneous measurements of energy expenditure and motor activity were made on each animal over a 3-h period. Energy expenditure was measured using an open-circuit indirect calorimeter housed in a temperature-controlled room at 28 ± 0.1 °C[11]. The conditions were as similar as possible to the animal's usual living conditions: the clear perspex chamber was similar in size to the box in which it usually lived and the animal's own bedding of wood shavings was used. The calorimeter was continuously ventilated at 750 ml/min and a change-over box directed ingoing or outgoing air to a paramagnetic oxygen analyser (Type OA184, Taylor Servomex Ltd, Crowborough, Sussex). The principle of the system was similar to that used in this laboratory for human subjects[12]. Animals were accustomed to the procedure by being placed in the chamber on three earlier occasions, each of several hours. They remained undisturbed during the measurements and food and water were available ad libitum.

Mean values of oxygen consumption were obtained for every 1-h period of measurement and converted to STP. Errors associated with measurement of oxygen consumption alone can be minimized by converting it to heat production[13,14]. Several suggestions for the conversion factor have been made[15-18] and the average value of 20.5 J/ml O_2 was used in the present investigation.

Motor activity was monitored while the subject occupied the respiration chamber by two independent methods, one visual and the other using a modified Doppler-type burglar alarm[19]. The visual method used a closed-circuit television system and a score was given for each 5-min period, the technique being based on the activity-diary used for man[20]. Activity was allocated to one of five categories and given a score (shown in parentheses): no activity (0) e. g. lying quietly with no movement; minor activity (1) e.g. sitting, with a little movement; feeding or drinking (1);

Motor activity (visual score/h) and heat production (J/$g^{0.67}$ per h) of lean (n = 12) and obese (n = 9) mice during three consecutive hours at 28 °C (mean values ± SEM)

Measurement and hour of observation	Lean	Obese
Motor activity		
Hour 1	219 ± 15.1	108 ± 11.5
Hour 2	185 ± 13.2	56 ± 13.9
Hour 3	140 ± 14.4	44 ± 11.1
Heat production		
Hour 1	238 ± 9.8	146 ± 7.0
Hour 2	206 ± 8.8	120 ± 8.0
Hour 3	172 ± 7.8	101 ± 6.8