

THE UNIVERSITY OF MANITOBA

DAILY ENERGY EXPENDITURE OF THE RED SQUIRREL  
(TAMIASCIURUS HUDSONICUS): EFFECTS OF SEASONAL  
TEMPERATURE CHANGE

BY

RONALD WALTER PAULS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY  
DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

FEBRUARY, 1979

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## ABSTRACT

Amount and intensity of locomotor activity and time out of the nest were measured for a 1-year period on captive red squirrels (Tamiasciurus hudsonicus) under natural conditions. Daily and annual activity patterns and correlations between activity and meteorological variables were examined. A pronounced annual cycle, in phase with the annual temperature cycle, was found in all three aspects of activity. Daily amount of locomotor activity and time out of the nest were closely correlated with mean daily air temperature. Daily patterns of activity varied seasonally from a distinctly bimodal to a unimodal pattern, with a shift in winter to more midday activity. The behaviour observed was the behaviour expected of animals seeking to reduce or eliminate the effect of air temperature ( $T_a$ ) on daily energy expenditure.

Abdominal temperature ( $T_b$ ) of captive squirrels was monitored by radiotelemetry at naturally occurring  $T_a$  from  $-33$  to  $28^{\circ}\text{C}$ . Abdominal temperature ranged from  $35.9$  to  $41.4^{\circ}\text{C}$ ; it was usually less than  $39^{\circ}\text{C}$  when an animal was inside the nest and greater than  $39^{\circ}\text{C}$  when outside. An increase of  $T_b$  to about  $39^{\circ}\text{C}$  usually occurred before an animal left the nest. Inside the nest  $T_b$  was positively correlated with  $T_a$  outside the nest. It is suggested that inside the nest a reduced  $T_b$  is adaptive as it results in energy conservation while a high  $T_b$  is required outside the

nest for rapid and co-ordinated motor activity.

A laboratory study of the rate of energy expenditure of the red squirrel under controlled conditions indicated total thermal conductance ( $C$ ) of resting summer-acclimatized animals was 120% of  $C$  of winter-acclimatized animals. Thermal conductance of animal plus nest was 40% of  $C$  for winter-acclimatized animals, permitting  $T_b$  regulation at  $-20^{\circ}\text{C}$  with only a slight increase in metabolic rate. Metabolic rate of animals resting at thermal neutral  $T_a$  did not change seasonally. During locomotor activity metabolic rate was a linear function of running speed. Heat generated by exercise did not reduce thermoregulatory heat production at low  $T_a$ , apparently due to increased  $C$  during exercise.

The reliability of heart rate ( $f_h$ ) as an index of oxygen consumption ( $\dot{V}_{O_2}$ ) was assessed. The two variables showed an average correlation of 0.94 within individuals when increased  $\dot{V}_{O_2}$  was elicited either by cold or exercise. There was variability in the regression of  $\dot{V}_{O_2}$  on  $f_h$  within individuals from day to day, within individuals between  $\dot{V}_{O_2}$  elicited by cold or by exercise, and among individuals. However,  $\dot{V}_{O_2}$  of an animal could be estimated from  $f_h$  with reasonable accuracy, using a regression established for that individual. Separate regressions for rest and exercise, and regressions based on more than one day's data will contribute to increased accuracy in estimating  $\dot{V}_{O_2}$ . Over an 8-h period,  $\dot{V}_{O_2}$  of 6 individuals, estimated from

regressions based on one day's data, differed from measured  $\dot{V}_{O_2}$  by 6.9-27.6% (mean = 14%).

Energy expenditure of captive squirrels in large outdoor enclosures was estimated using  $f_h$  telemetry. Regressions of  $\dot{V}_{O_2}$  on  $f_h$ , established separately during both rest and exercise for each individual, were used to estimate  $\dot{V}_{O_2}$  from  $f_h$ , sampled at 5-min intervals over 24-h periods, at various  $T_a$  over the course of 1 year. Mean daily  $\dot{V}_{O_2}$  was  $2.00 \pm 0.11 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ , independent of  $T_a$ . Oxygen consumption of animals both inside and outside the nest increased with decreasing  $T_a$ . The constancy of daily energy expenditure occurred because the proportion of the day spent outside the nest, where  $\dot{V}_{O_2}$  was high, decreased with decreasing  $T_a$  as did locomotor activity.

This study indicates that captive red squirrels, exposed to natural climatic conditions, regulate daily energy expenditure at a constant level, in spite of large seasonal temperature change.

## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Drs. M. Aleksiuk and J.C. Rauch, my supervisors during approximately the first and second half of this study, respectively, for encouragement and support in the planning and executing of this study; to Dr. J.C. Rauch for assistance in the collection of field data and the preparation of this thesis; to Dr. R.M. Evans, my acting supervisor in the final stages of thesis preparation, for constructive criticisms of the thesis manuscript and for expediting the completion of this thesis; to Dr. A.N. Arnason for assistance with statistical aspects of this study; and to Dr. J.G. Eales for constructive criticisms of the thesis manuscript.

Thanks are extended to the Department of Agriculture of the University of Manitoba for permission to construct and use research facilities in their field laboratory adjacent to the University of Manitoba campus.

Research was funded by grants to Drs. M. Aleksiuk and J.C. Rauch from the National Research Council of Canada, a grant to Dr. M. Aleksiuk from the Manitoba Department of Mines, Resources, and Environmental Management, and a grant to Dr. J.C. Rauch from the Research Board of the University of Manitoba. I received financial support in the form of a National Research Council of Canada Scholarship and a University of Manitoba Fellowship. These sources are all gratefully acknowledged.

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# SYMBOLS AND ABBREVIATIONS USED IN THE TEXT

$(A-V)O_2$	diff. difference in oxygen concentration between arterial and venous blood
B	barometric pressure
C	total weight-specific thermal conductance
$F_{E_{O_2}}$	fractional oxygen concentration of dry outflow air from the metabolism chamber
$f_h$	heart rate
$F_{I_{O_2}}$	fractional oxygen concentration of dry inflow air to the metabolism chamber
L	time interval between sunrise and sunset
$P_W$	partial pressure of water vapour in the metabolism chamber
$P_{WI}$	partial pressure of water vapour in the inflow air to the metabolism chamber
RQ	respiratory quotient
S	percentage of time between sunrise and sunset with bright sunshine
SV	stroke volume
t	time
$T_a$	air temperature
$T_{a-1}$	mean air temperature of the day prior to the day for which activity was measured
$T_b$	abdominal temperature
$T_{bb}$	black bulb temperature
$T_c$	air temperature in the metabolism chamber
$T_n$	normal daily mean air temperature
$T_w$	mean air temperature of the week prior to the day for which activity was measured
V	volume of the metabolism chamber

# SYMBOLS AND ABBREVIATIONS cont'd

$V_A$	volume of dry air in the metabolism chamber
$\dot{V}_{AF}$	inflow rate of water vapour to the metabolism chamber
$\dot{V}_{CO_2}$	rate of carbon dioxide formation by the animal
$\dot{V}_E$	outflow rate of dry air from the metabolism chamber
$\dot{V}_I$	inflow rate of dry air to the metabolism chamber
$\dot{V}_{in}$	inflow rate of undried air to the metabolism chamber
$\dot{V}_O$	volume of oxygen in the metabolism chamber
$\dot{V}_{O_2}$	rate of oxygen consumption by the animal
$V_W$	volume of water vapour in the metabolism chamber
$W$	wind speed

A bar over a symbol indicates it represents the mean value for that variable over a specified time period. The character,  $\Delta$ , preceding a symbol denotes "change in ...".

## GENERAL INTRODUCTION

Expenditure of energy made available through the metabolic breakdown of ingested food materials is a continuous and essential process in all living organisms. The rate of energy metabolism of an organism is of interest to biologists both because it integrates many aspects of animal performance and because it is a measure of the total impact of an organism on the energy resources of its environment (Bartholomew 1977a). The rate of energy metabolism of rodents in metabolism chambers may vary from the resting metabolic rate of a post-absorptive individual (basal metabolic rate) to a maximum rate 3.5-8.2 times the basal metabolic rate (Hart 1971). However, very little is known about the daily energy expenditure of undisturbed free-ranging animals in their natural environments.

Major factors contributing to an increase in energy expenditure above the basal level in homeotherms are muscular contraction associated with activity and, in cold environments, heat production for the purpose of thermoregulation. An increase in the rate of energy expenditure necessitates, on average, an increase in the rate of energy intake through behaviour leading to the ingestion of increased amounts of food, as maintenance of energy balance is vital to the long term survival of any organism.

Over the long term, an adult non-reproducing animal regulates its average rate of energy intake to exactly balance its average rate of energy expenditure through physiological mechanisms which drive behaviour that results in food intake. In addition it may be necessary for an animal to regulate behaviourally daily energy expenditure because its capacity for assimilating energy is limited and because the energy resources of its environment are usually limited, at least during certain times of the year.

Behavioural regulation of daily energy expenditure may be accomplished through postponement of essential activities to a time when conditions may be more favourable; less essential activities may be entirely eliminated under environmental conditions where the energy cost of the activity is such that no net benefit is derived from the activity. That this may occur is suggested by evidence indicating animals are capable of adapting behaviour to current environmental circumstances so as to maximize fitness (McFarland 1977). Further regulation of daily energy expenditure may occur through behaviour such as nest construction, microhabitat selection, huddling, or postural adjustment, which reduces the energy cost of thermoregulation in a cold environment (Hart 1971).

Therefore, with respect to behaviour which regulates energy expenditure it appears appropriate to take the approach suggested by McFarland (1977) and view the behaviour

of the animal as controlling the state of the system by virtue of its consequences rather than the traditional approach that the motivational state of the animal drives its behaviour. This approach is taken in the present study in which energy expenditure of adult non-reproducing red squirrels (Tamiasciurus hudsonicus) held in large outdoor enclosures fully exposed to natural climatic conditions in southern Manitoba is examined.

The range of this small rodent extends approximately to the northern edge of the subarctic taiga (see Hall and Kelson 1959). It is semi-arboreal, remaining active in the supranivean environment in winter, except during severe cold (see Pruitt and Lucier 1958). Year-round activity in north temperate and subarctic parts of its range implies that it is well adapted to severe winter cold and large seasonal temperature fluctuation. With the exception of the northern flying squirrel, it is the smallest mammal that remains active above the surface of the snow in winter, and thus faces, along with the northern flying squirrel, the greatest potential cold stress of all boreal and north temperate mammals.

The red squirrel and other small mammals can reduce or avoid cold stress by selecting and/or constructing microhabitats with favourable thermal environments relative to the macroenvironment. However, the degree of protection offered by these microhabitats and, more importantly, the extent of behavioural adjustment for their effective

use is not generally well known, either in the red squirrel, or in other small mammals. These factors, along with seasonal physiological change, will largely determine the effect of seasonal temperature change on daily energy expenditure.

The working hypothesis of this study is that, in spite of large seasonal temperature change in the macroenvironment, seasonal change in daily energy requirements is reduced or entirely avoided by the red squirrel through seasonal and/or temperature modulated behavioural adjustments augmented by seasonal physiological change. This hypothesis was examined by monitoring over a 1-year cycle daily use of the nest, locomotor activity (Part I), and body temperature (Part II). Additionally, seasonal change in total thermal conductance and basal metabolic rate, insulative effectiveness of the nest, and the energy cost of locomotion were examined (Part III). Following a validation study (Part IV), heart-rate telemetry was used to estimate daily energy expenditure at various air temperatures during a 1-year cycle and evaluate the combined effect of behavioral and physiological adaptations on daily energy expenditure.



PART I

Behavioural Strategies Relevant to the Energy  
Economy of the Red Squirrel (Tamiasciurus  
hudsonicus)

## INTRODUCTION

The range of the red squirrel (Tamiasciurus hudsonicus) extends approximately to the northern edge of the subarctic taiga (see Hall and Kelson 1959). It remains active in the supranivean environment in winter, except during severe cold (see Pruitt and Lucier 1958). Year-round activity in north temperate and subarctic parts of its range implies that it is well adapted to severe winter cold and large seasonal temperature fluctuation.

Typical adaptations of small mammals in cold environments include seasonal change in body temperature (hibernation), capacity for non-shivering thermogenesis, and pelt insulation, as well as behaviour which reduces the impact of the macroenvironment on the animal. However, the red squirrel is not known to hibernate; it appears to have a body temperature which is unusually high for a mammal (Irving and Krog 1954; Morrison 1962; Grodzinski 1971); its small size (approx. 200-300 g) limits insulative adaptation and Irving et al. (1955) report no seasonal change in insulation. It is probably capable of an adequate metabolic response to most of the temperatures in its macroenvironment (see Irving et al. 1955), but where energy resources are limited, this is less adaptive than behavioural responses, which are usually energetically less costly.

It is not clear whether the virtual cessation of

supranivean activity at  $-32^{\circ}\text{C}$  by the red squirrel in Alaska (Pruitt and Lucier 1958) and the reduction in supranivean activity with decreasing temperature in northern Alberta (Zirul and Fuller 1971) represent a shift to subnivean activity as implied by Zirul and Fuller, or simply a reduction and cessation of all activity. In either case, it does appear from these studies that adaptive behaviour is an important aspect of the red squirrel's adaptation to the temperature extremes of its environment. For this reason, and because of the paucity of definitive and quantitative data on the behavioural responses of this species to temperature, a study was done of several aspects of behaviour relevant to the energy economy of the red squirrel.

The study was done with animals held outdoors in large wire mesh enclosures in red squirrel habitat in order to permit detailed and accurate measurement of activity while avoiding some of the artificialities of the laboratory environment.

## MATERIALS AND METHODS

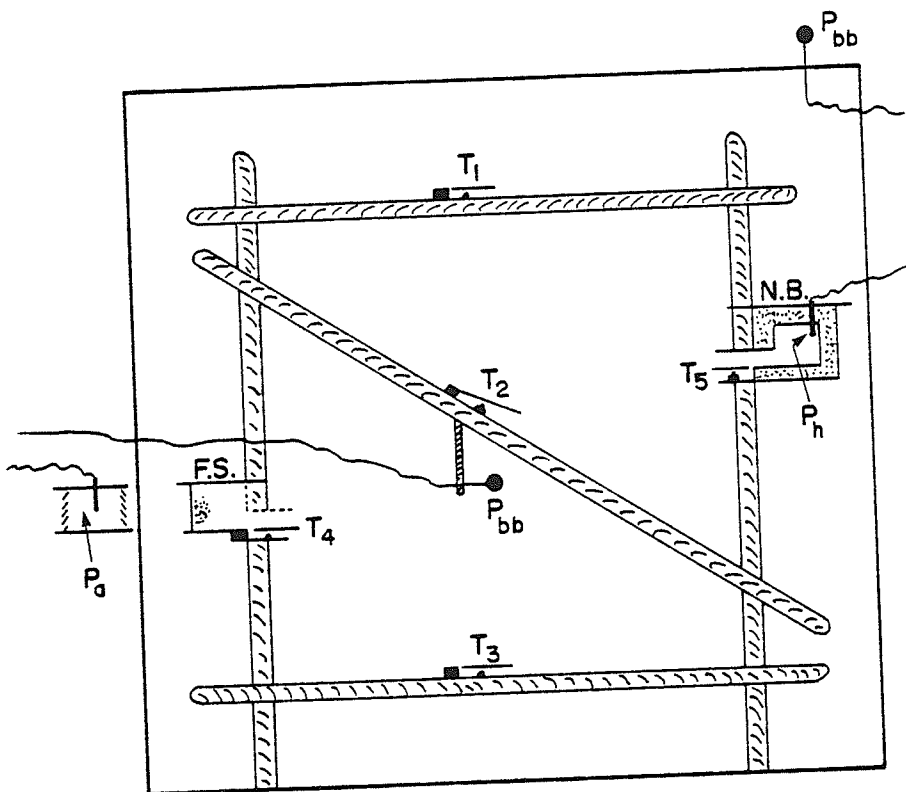
### Animal Care

The animals were live-trapped in the vicinity of Winnipeg ( $50^{\circ}06'N$ ;  $98^{\circ}20'W$ ), and placed individually in 1 of 10 adjacent wire mesh enclosures (80 x 300 x 300 cm). These pens were located in natural red squirrel habitat; they were surrounded and partially shaded by trees. Each contained a plywood nest box (inside dimensions 15 x 15 x 20 cm), insulated at the top and sides with 5 cm of Styrofoam. The bottom of the nest box consisted of wire mesh and 5 cm of fibrewool insulation to permit adequate ventilation. The nesting material provided was used by the animals for building nests in the boxes. Tree limbs were arranged as shown in Fig. 1 in each pen to allow for normal activity by the red squirrel, a semi-arboreal animal. Teklad rat and mouse diet (Teklad Mills, Winfield, Iowa) was supplied ad libitum, and supplemented with 50 g week<sup>-1</sup> of sunflower seeds. Water, crushed ice, or potatoes were provided during times when the animals had no access to snow.

### Activity Measurement

Activity and time out of the nest were recorded for 6 to 10 days per month over a 1-year period. A maximum of 10 individuals were studied at any one time. After 10 months, one animal died and one escaped. They were replaced

Figure 1: Side view of a pen showing the arrangement of tree limbs, treadle locations ( $T_1$ - $T_5$ ), feeding station (F.S.), nest box (N.B.), air temperature probe ( $P_a$ ), black bulb temperature probe ( $P_{bb}$ ), nest temperature probe ( $P_n$ ).



by new individuals. The remaining eight animals were used throughout the study period.

The equipment for recording locomotor activity consisted of five treadles with microswitches per enclosure, connected to an Esterline Angus event recorder located in a hut adjacent to the pens. The arrangement of treadles is shown in Fig. 1. Depression of treadles by active animals resulted in a mark on the recording paper run at a speed of  $15.2 \text{ cm h}^{-1}$ . At this speed, a maximum of 7 marks  $\text{min}^{-1}$  could be resolved. Whenever two or more activity marks were not clearly distinguishable, an estimate of the minimum number of marks was made. Activity, in this study, has been expressed in units, where a unit of activity refers to a single treadle depression or mark on the chart paper. Nest temperature, recorded at 30-min intervals, was used to determine whether a movement through the nest entrance, recorded by the event recorder, was a movement out of or into the nest. From these data, time out of the nest was calculated for each animal.

#### Meteorological Measurements

Air temperature, black bulb temperature, and nest temperature were recorded with a model G820 Grant Recorder (Grant Instruments, Cambridge, England). The locations of all temperature probes are shown in Fig. 1. Air temperature was measured by a probe suspended in a well-ventilated box 130 cm above the ground adjacent to

the pens.

Black bulb temperature was measured because it is a function of air temperature, wind speed, and incident thermal radiation (Pereira et al. 1967), factors, which in conjunction with humidity, define the thermal environment of an animal (Porter and Gates 1969). Wind speed, time of sunrise and sunset, and number of hours of bright sunshine, obtained from the Winnipeg Branch of the Atmospheric Environment Service, were recorded 13 km from the study site.

#### Treatment of Data

To determine possible seasonal changes in activity, monthly means of total locomotor activity, time out of the nest, and intensity of locomotor activity were calculated. The number of locomotor activity units was obtained for each individual for a 24-h period, and the calculated average designated as daily mean. The average of daily means was taken as the monthly mean. Data for time out of the nest were treated similarly. Intensity of locomotor activity was derived by dividing locomotor activity units in a 24-h period by time out of the nest during the same time interval. Daily and monthly means were obtained by the aforementioned method.

To determine the 24-h activity pattern of each animal on each day, the number of activity units in each hour was calculated as a percentage of the total over the 24 h



for that animal. The mean pattern of all animals for each day was calculated from these values. Monthly means were calculated from the daily means.

Correlations between mean daily locomotor activity, mean daily time out of the nest, mean daily intensity of activity and the meteorological variables, defined below, were examined:

1. Variables which are a measure of the current thermal environment:

$\bar{T}_a$  = Mean daily air temperature (mean of hourly temperatures).

$\bar{T}_{bb}$  = Mean daytime black bulb temperature (mean of hourly recordings between sunrise and sunset).

S = Percentage of time between sunrise and sunset with bright sunshine.

$\bar{W}$  = Mean daily wind speed (mean of hourly wind speeds).

2. Variables which are a measure of the immediate thermal history of the animal:

$\bar{T}_{a-1}$  = Mean air temperature of the day prior to the day for which activity was determined.

$\bar{T}_w$  = Mean air temperature of the week prior to the day for which activity was measured.

3. Variables which are a measure of cyclical seasonal changes:

$\bar{T}_n$  = Normal daily mean air temperature (mean

of the mean daily air temperatures of the years 1872-1973).

L = Time interval between sunrise and sunset.

Correlation analysis indicated that variation in air temperature could account for much of the observed variation of activity. Multiple regression analysis was used to see whether the remaining meteorological factors could account for some of the residual variation in activity. The set of meteorological variables which best accounts for the variability in activity was selected by the "step-up method" (Snedecor and Cochran 1967). A variable was included in this set only if its partial regression coefficient was significant at the 95% level. Because of the high intercorrelation between temperature variables, the difference between  $\bar{T}_a$  and each of the other temperature variables was used in the analysis. These differences are meaningful quantities as the variable  $(\bar{T}_{bb} - \bar{T}_a)$  is a measure of the combined thermal effects of wind and thermal radiation (Pereira et al. 1967),  $(\bar{T}_{a-1} - \bar{T}_a)$  and  $(\bar{T}_w - \bar{T}_a)$  are a measure of the deviations of the immediate thermal history of the animal from the current thermal environment, and  $(\bar{T}_n - \bar{T}_a)$  is a measure of the deviation of the current from the expected normal temperature.

## RESULTS

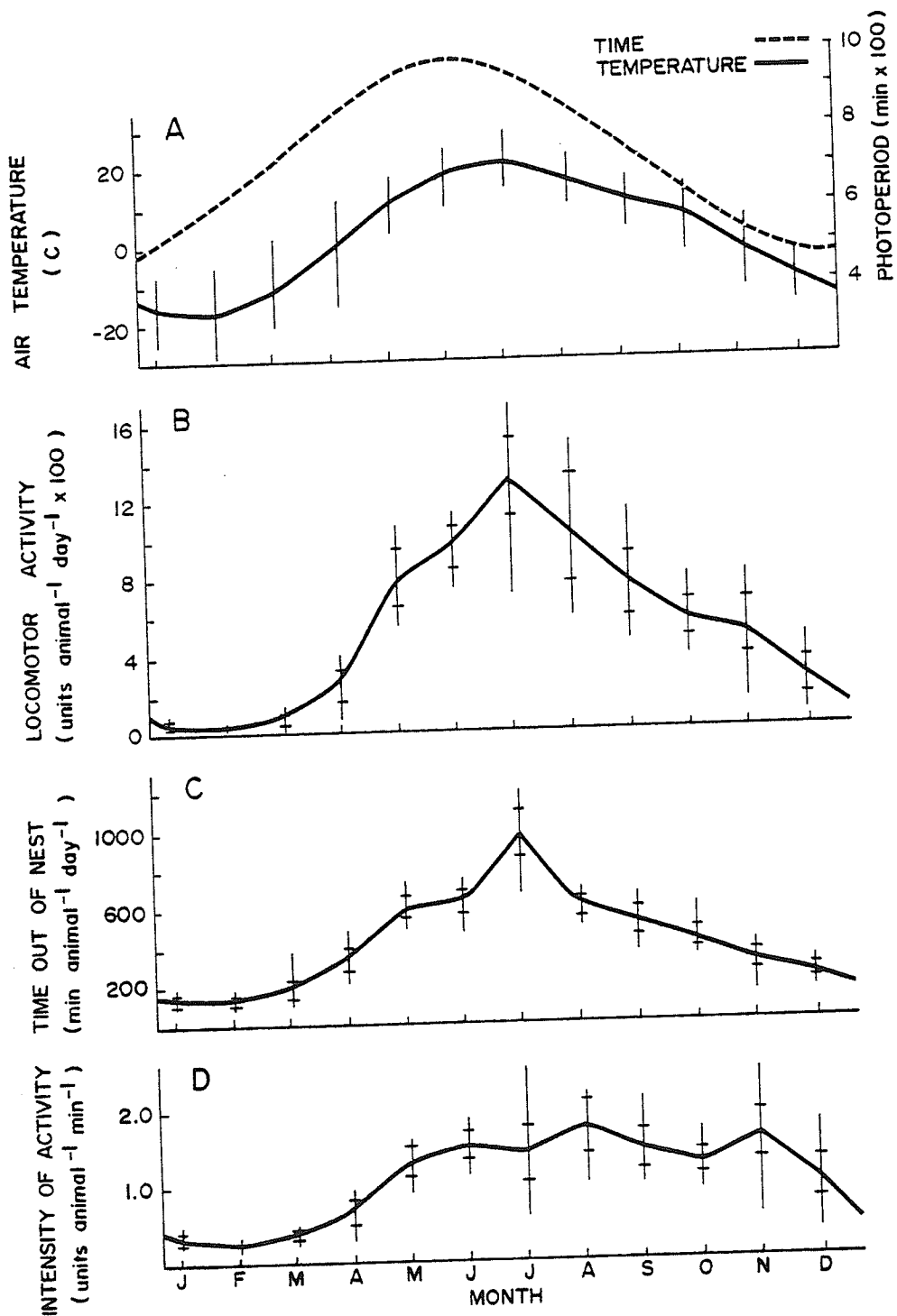
Locomotor activity, and time out of the nest increased markedly between February and July and then declined to become minimal in January (Fig. 2). The annual cycles in locomotor activity and time out of the nest closely paralleled the temperature cycle, but they were slightly out of phase with the photoperiod cycle.

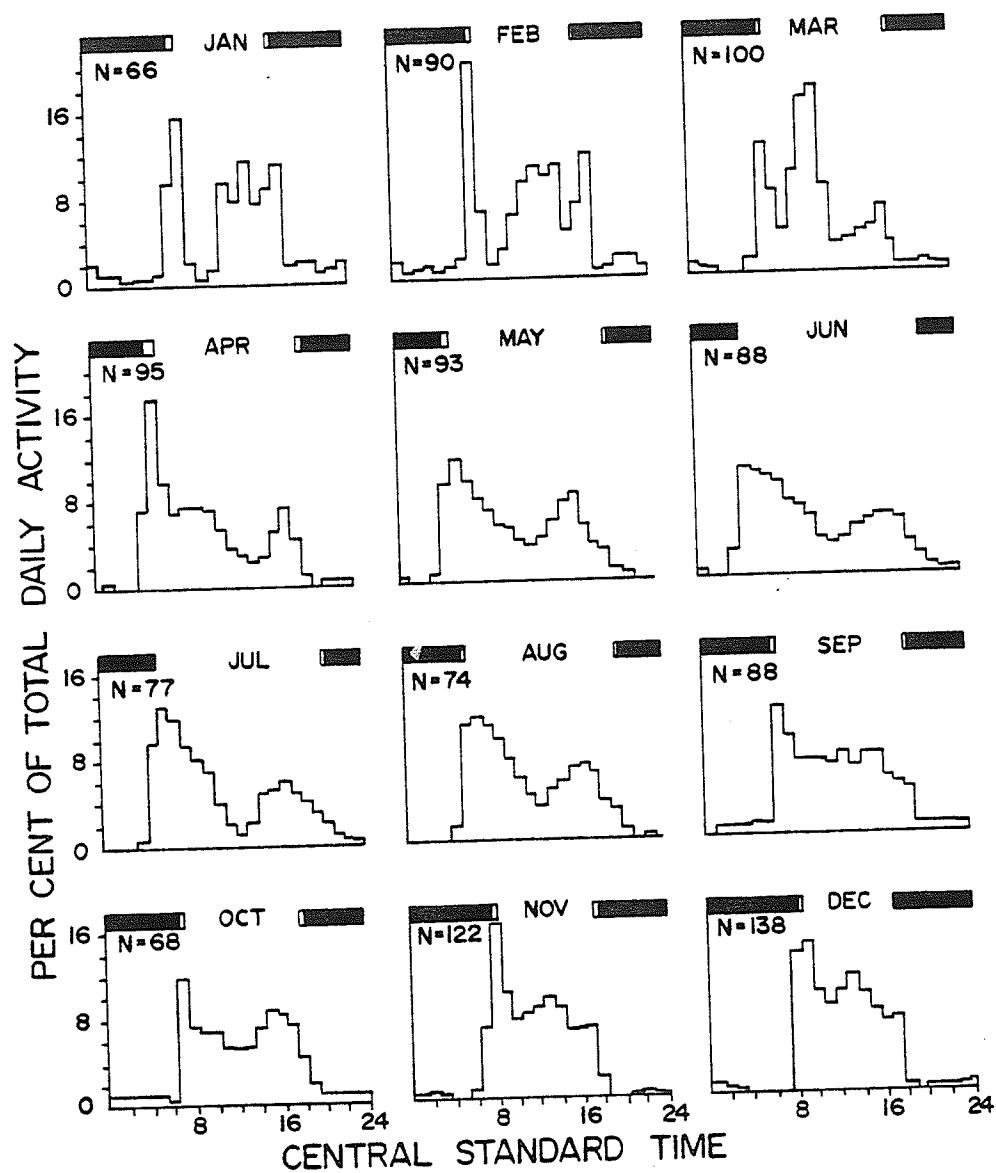
The intensity of activity increased during April and May, did not change significantly between May and November, and declined in December. Between May and November the amount of locomotor activity was directly proportional to time out of the nest.

Mean daily activity patterns for each month (Fig. 3) demonstrate the red squirrel in this area is primarily a diurnal mammal. However some nocturnal activity occurred at all times of the year. Individual records indicated that an animal would be active once or several times during some nights, and remain inactive during other nights. Nocturnal excursions out of the nest were usually 10 min or less in duration, with very little locomotor activity.

Seasonal changes were evident in the pattern of daily activity (Fig. 3). In spring and summer (April to August), the pattern was bimodal with morning and evening peaks. In fall and early winter (September to December), activity was equally distributed throughout the daylight period. In late winter (January to March), a short period of activity

Figure 2. Annual cycles in photoperiod, temperature, and activity. Vertical lines represent range of daily means and horizontal marks 95% confidence intervals. Number of days of activity recorded per month varies from 7 to 12; mean number of animals recorded per day varies from 6.8 to 10.





at sunrise was followed by a quiescent period, with a greater proportion of the day's activity occurring around midday than at other times of the year.

Individual records showed that animals never failed to become active during the day. From January to August, activity usually began between sunrise and 1 h before sunrise. From September to December, activity generally began earlier, in some instances as early as 2 h before sunrise. The time of cessation of activity was more variable, usually occurring between 1 h before and 2 h after sunset in summer, and 3 h before and 0.5 h after sunset in winter. Activity usually became sporadic in the last hours before sunset, with animals leaving the nest for a few minutes at 0.5 to 1-h intervals.

The logarithms of mean daily locomotor activity, time out of the nest, and intensity of activity, show a positive linear correlation with air temperature (Fig. 4). Much variability and some apparent departure from linearity in the data, particularly for intensity of activity, suggest that other variables may affect activity. Correlations of activity with meteorological variables are shown in Table 1.

Multiple regression analyses suggest that, following mean daily air temperature, wind was the next most significant factor affecting time out of the nest, while photoperiod was the next most important factor affecting amount and intensity of activity (Table 2). Mean daily

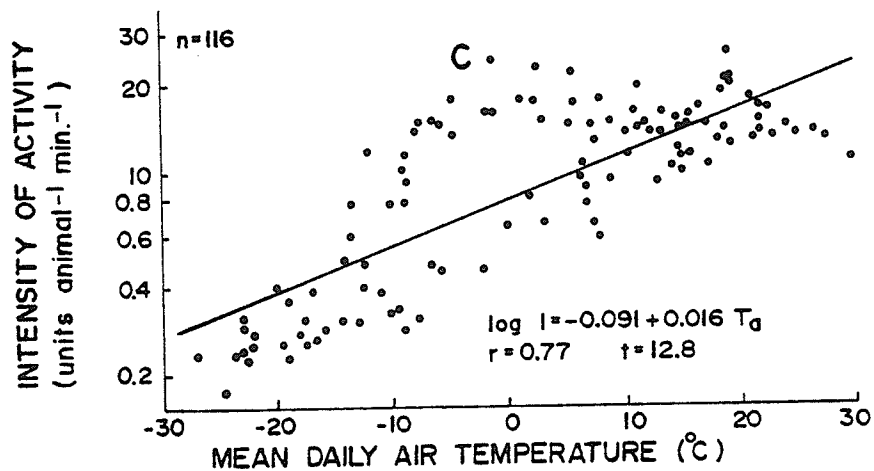
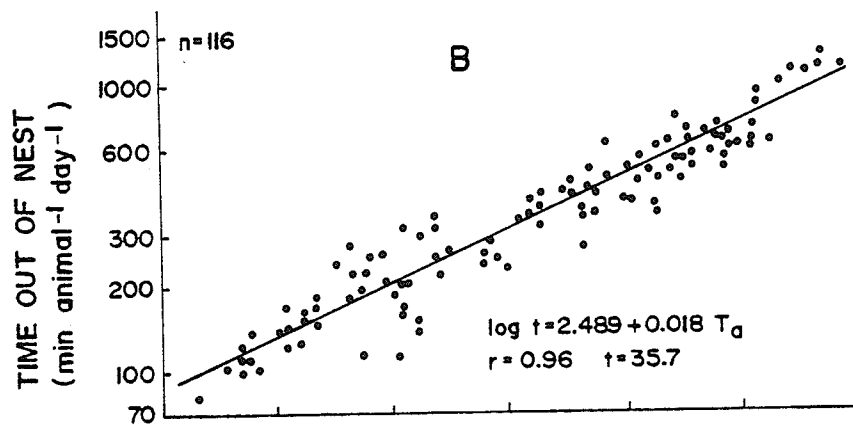
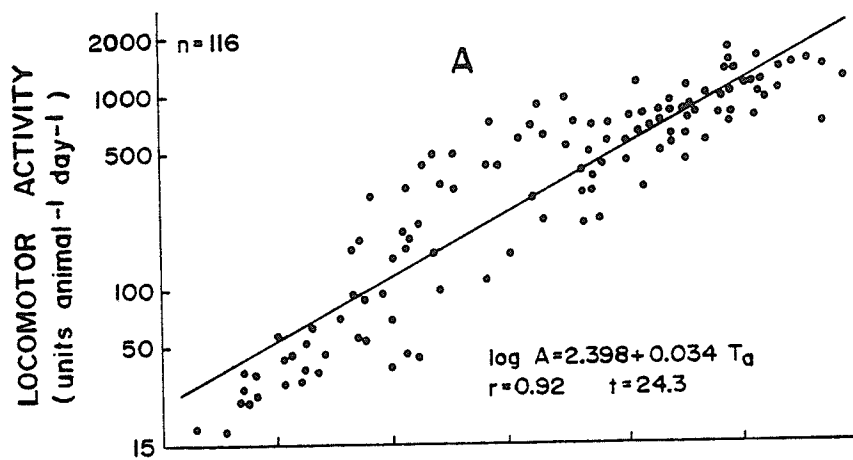




Table 1. Confidence intervals (95%) for the square of the correlation co-efficients between activity and meteorological variables

Independent Variable	Dependent Variable		
	log A (n = 116)	log t (n = 116)	log I (n = 116)
$\bar{T}_a$	0.778-0.885	0.884-0.943	0.527-0.647
$\bar{T}_{bb}$	0.716-0.884	0.884-0.943	0.386-0.638
$\bar{T}_{a-1}$	0.760-0.878	0.821-0.910	0.487-0.712
$\bar{T}_w$	0.799-0.899	0.834-0.918	0.531-0.741
$\bar{T}_n$	0.669-0.826	0.801-0.901	0.373-0.629
L	0.151-0.426	0.190-0.468	0.047-0.281
S	---	---	---
$\bar{W}$	---	---	0.000-0.126

Note: ---, not significant at the 95% level;  
A, locomotor activity; t, time out of nest;  
I, intensity of locomotor activity; other symbols  
as defined in text.

Table 2. Multiple regressions of activity variables on meteorological variables

		log A	log t	log I
	$A_k$	3.227	2.547	0.779
$X_1$	$\bar{T}_a$		$\bar{T}_a$	$\bar{T}_a$
	$b_1$	0.047	0.019	0.028
	$t_1$	25.6	37.0	18.1
	$v_1$	83.8	91.8	59.1
$X_2$	L		$\bar{W}$	L
	$b_2$	-0.0012	-0.0052	-0.0012
	$t_2$	7.69	3.58	9.18
	$v_2$	4.1	0.8	13.4
$X_3$	$\bar{T}_w - \bar{T}_a$		$\bar{T}_w - \bar{T}_a$	$\bar{T}_w - \bar{T}_a$
	$b_3$	0.022	0.0038	0.016
	$t_3$	5.70	2.28	5.06
	$v_3$	2.7	0.3	5.9
	n	116	116	116
	F	363	492	107
	$r^2$	0.907	0.930	0.794

Note: A, locomotor activity; t, time out of nest; I, intensity of locomotor activity;  $A_k$ , the constant in the multiple regression;  $X_i$ , the independent meteorological variables for each regression;  $b_i$ , slope;  $t_i$ , t-value for  $b_i$ ;  $v_i$ , percent variability due to  $X_i$ ;  $r^2$ , multiple correlation co-efficient; other symbols as in text.

air temperature of the preceeding week was the least significant factor affecting each activity variable.

From extensive observation it appeared that the posture the animals typically assumed while resting outside the nest depended on environmental conditions. During cold weather, a nearly spherical shape was generally assumed as the animals sat on their hindlimbs with forelimbs folded against the chest and tail folded against the back. During hot weather the body was frequently extended along the limb of a tree, with the tail extended behind the body or hanging down. At intermediate temperatures, intermediate postures were usually assumed. The spherical shape was sometimes maintained, but with the tail extended, or the body was extended but the tail folded against the back. On cold, windy days, the back, with the tail folded against it, was frequently directed against the wind. On cold sunny days, animals tended to direct the side of the body toward the sun.

## DISCUSSION

The energy required for thermoregulation by the red squirrel is highly temperature dependent at temperatures below about 20°C (Irving et al. 1955). The large annual fluctuations in daily mean temperature of subarctic and north temperate regions could potentially force large variations in daily energy expenditure on this species. This study presents evidence that temperature-modulated behaviour can partially, if not entirely, uncouple daily energy expenditure from temperature. Reduced locomotor activity, less time out of the nest, reduced intensity of activity, postural changes, and a shift in activity to warmer hours of the day, all contribute to reducing energy expenditure at low temperatures.

From February to July the index of total daily locomotor activity increased by a factor of about 40 (Fig. 2). Based on the finding that the net energy cost of running is a function of distance run and is independent of speed (Schmidt-Nielson 1972), it appears likely the energy used for locomotion was reduced by a factor of about 40 during the coldest month. The habit of the red squirrel of caching food in the vicinity of the nest eliminates the need for foraging during cold weather, thus permitting a large reduction in locomotor activity.

Furthermore, a rebudgeting of energy expenditure appears to occur, because while thermoregulatory energy

requirements increase with decreasing temperature, the energy used for locomotion decreases. Alternatively, by increased use of the nest at low temperatures (Fig. 4B) the animal might circumvent a substantial increase in daily energy expenditure for thermoregulation, because the energy required for thermoregulation in the nest when the outside temperature is  $-20^{\circ}\text{C}$  approaches the basal level of energy expenditure, while at the same temperature the energy required for thermoregulation at rest without a nest is 2.8-3.7 times the basal level (Section III). The strong correlation between time out of the nest and temperature suggests precise behavioural regulation of the energy used for thermoregulation.

In addition to a decrease in time out of the nest with decreasing temperature, there was a reduction in the intensity of activity (Fig. 4C). This implies proportionately more of the time out of the nest is spent at rest, making possible more effective use of postural adjustments, by which the animal can reduce exposed surface area, improve insulation, and make most efficient use of its tail as a windbreak.

It can be assumed that the level of activity on the coldest days of the year most closely approaches the minimum level for maintenance. It is then apparent that the squirrels in this study did not minimize activity and thereby energy expenditure throughout the winter months (see Figs. 2 and 4) as might be expected. The following

hypothesis is developed to account for the observation that activity level appears to be a function of temperature (Fig. 4).

Smith (1968) lists the major activities of the red squirrel as feeding, food gathering, territorial defense, predator defense, sexual play, grooming, and gathering nest materials. How an animal partitions its time among these activities will depend, among other factors, on its physiological state, season, time of day, population size, and predator density. Regardless of how time is partitioned among these activities, it appears from this study that the total activity level is at least partially determined by temperature. The following cost-benefit model indicates why a temperature-dependent activity level is an adaptive behavioural response for the red squirrel.

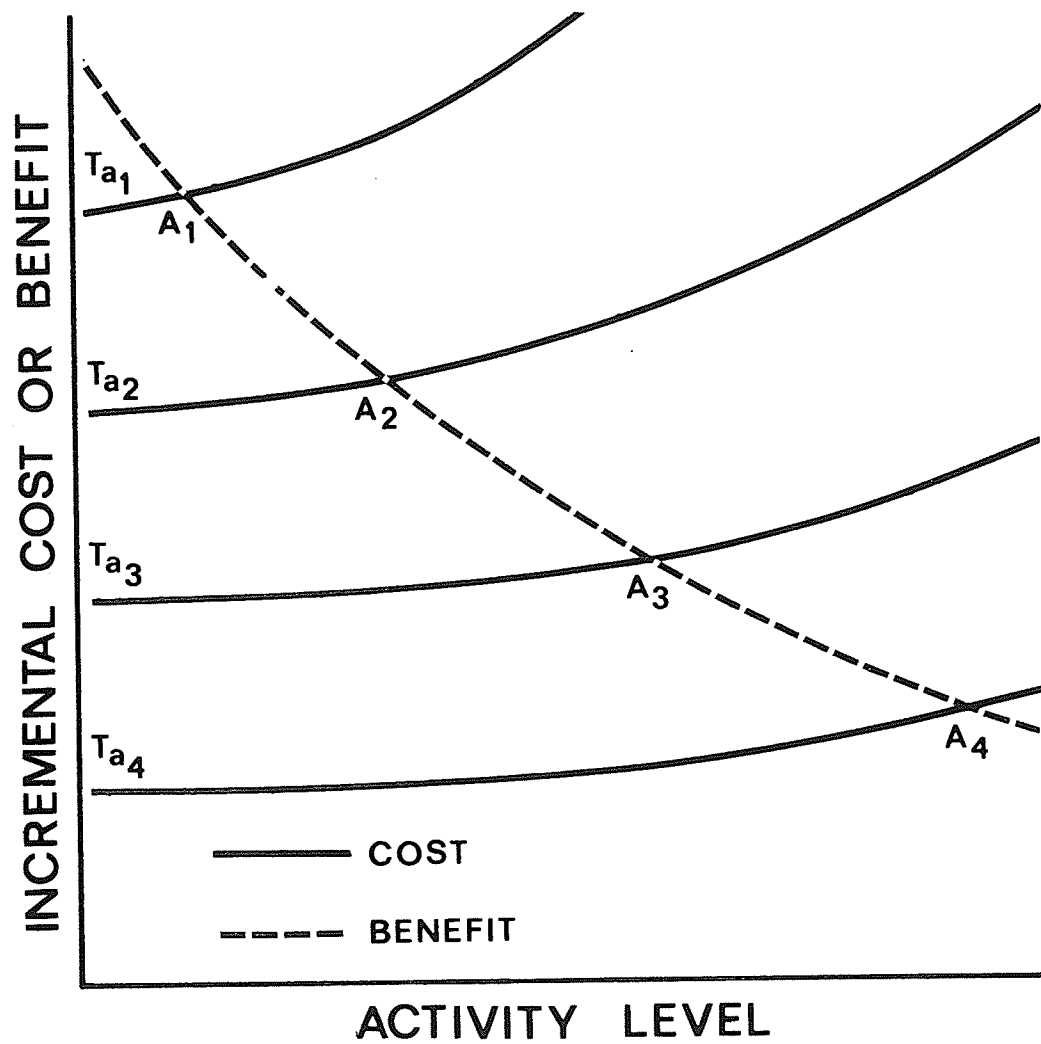
Associated with any type of activity are both costs and benefits. Several factors contribute to the cost of a given activity. The specific cost factors involved may depend on the type of activity, but a cost factor common to most, if not all activity, is the energy cost. This cost includes not only the energy needed for the activity per se, but also the energy needed for thermoregulation. It is assumed the cost of each additional unit of activity, that is, the incremental cost, will be the same as, or greater than the preceeding unit of activity, and during most of the year will depend on temperature. The energy cost of activity is ultimately a cost in

fitness, that is, the probability of survival and reproduction. Although the shape of the cost curve is not known, it will have either a zero or a positive slope (Fig. 5).

The specific benefits associated with any activity will also depend on many factors; however, it is assumed that at levels of activity above some minimum the incremental benefit derived from each unit of activity will be less than from the preceeding unit, i.e. there will be "diminishing returns". It is likely that the benefit curve is less dependent on temperature than the cost curve for most activities, and may be entirely independent of temperature for some. The benefit gained from an activity is also ultimately a benefit in terms of fitness. Again the exact shape of this curve is not known but it will have a negative slope (Fig. 5).

The optimal level of activity is the level at which cost and benefit curves intersect ( $A_i$  in Fig. 5), because for each activity increment up to this level a net benefit is derived, while for each activity increment above this level, a net cost is incurred. An animal maximizes its fitness by engaging in the optimal level of activity, which will decrease with decreasing temperature (Fig. 5).

If a temperature-dependent activity level evolved as a result of the factors discussed, it would have a genetic basis and could persist in captivity even though the





immediate stimuli which orient and direct activity may be absent or altered.

According to this hypothesis temperature is the ultimate cause of the annual activity cycle. It does not follow that temperature must also be the proximate cause. There may be other environmental factors which determine activity level, either directly, or indirectly by entraining an endogenous oscillator. Photoperiod has been implicated as the environmental entraining agent in most studies of endogenous cycles. It seems highly unlikely, however, that the observed annual activity cycle is an endogenous cycle entrained by photoperiod because the two cycles are out of phase with one another and correlations between them are poor (Table 1). However, a delayed response of activity to photoperiod would bring the activity and temperature cycles into phase and would account for the phase relationship and poor correlation between photoperiod and activity. If this argument is correct a close correlation should exist between activity and daily normal temperature, the most probable temperature to be expected on that day. Instead, the strongest correlations occur between activity and those variables that measure day to day temperature change, implying that temperature is the immediate, as well as ultimate factor determining the level of activity.

It has been proposed in this section that temperature influences activity level by affecting the energetic cost of activity. However, wind can also affect the energetic

cost of activity, as it increases the rate of heat loss from animal to environment (Lenz and Hart 1960; Neil 1976). Thus, the slight though significant effect of wind on activity level is consistent with this hypothesis.

The fact that temperature affects time out of the nest more strongly than either the amount or intensity of locomotor activity, accounting for 91.8 vs. 83.8 and 59.1% of the variability, and the fact that wind affects time out of the nest but not locomotor activity, lend support to the above hypothesis, as the energy cost of being out of the nest will be more strongly affected by low temperature and wind than the energy cost of locomotion.

The biological significance of the slight, though statistically significant, effect of photoperiod on the amount and intensity of daily locomotor activity is not clear. It may indicate an endogenous component in the annual activity cycle or a direct effect of photoperiod on activity.

The finding that mean temperature of the preceding week affects activity on a given day suggests changes in activity level do not occur immediately with changes in temperature. An apparent consequence of this delayed response is that activity does not increase during a brief warm period in winter or decrease during a brief cool period in summer as much as would be expected on the basis of temperature. Thereby, the animal avoids squandering its limited energy reserves in winter. A continued high

level of activity during cool periods in summer is probably adaptive, because territorial defense, foraging, food caching, and reproductive activity contribute more to survival than energy conservation.

Many of the behavioural responses to temperature of the captive animals used in this study have been reported and appear qualitatively similar in free-ranging red squirrels. The observation that red squirrels rest in locations exposed to the sun during favourable weather in November and April (Layne 1954; Smith 1968) suggests that they respond behaviourally to take advantage of solar radiation. A shift in activity to mid-day in winter has been reported both in Alaska (Irving et al. 1955) and New York (Layne 1954). A reduction or complete cessation of activity during severe cold has been reported by Dice (1921), Hatt (1929), Hamilton (1939), Pruitt and Lucier (1958), Zirul and Fuller (1971), and has been interpreted by some of these authors as a shift from supranivean to subnivean activity. A graded activity level in response to temperature in the red squirrel as was observed in this study has not been reported, although the data of Zirul and Fuller (1971) and Pruitt and Lucier (1958) lend themselves to this interpretation.

Similar behavioural responses to temperature have been reported in other tree squirrels. A shift of activity to mid-day was observed during the winter in the fox squirrel (Hicks 1949) and the gray squirrel (Thompson 1977).

Brown and Yeager (1945) observed reduced activity in both gray and fox squirrels during cold weather. According to Allan (1942) the reduction in activity occurred in fox squirrels even when in or approaching a period of oestrus. A graded activity level in response to temperature was observed in the southern flying squirrel (Muul 1968). Winter track count data presented by Pullianen (1973) indicate declining activity from November to mid-winter followed by increasing activity from mid-winter to April in the red squirrel (Sciurus vulgaris) of northeastern Lapland.

The results of this study are in apparent contradiction with results obtained by Clarkson and Ferguson (1972) with red squirrels in the laboratory, where activity increased with decreasing temperature. However, as pointed out by the authors, the artificiality of the laboratory environment precludes a direct comparison of their results with the behaviour of the red squirrel in its natural environment.

PART II

Body Temperature Dynamics of the Red Squirrel  
(Tamiasciurus hudsonicus): Adaptations for  
Energy Conservation

## INTRODUCTION

Small endotherms in cold environments face potentially high rates of heat loss and excessive energy expenditure for temperature regulation due to their large surface area relative to body volume and limited pelage insulation. Previous results indicate that temperature-modulated behaviour is an important energy conserving adaptation of the red squirrel (Tamiasciurus hudsonicus) in Manitoba (Pauls 1978; Part I). However, it is likely the red squirrel is also adapted physiologically to seasonal cold.

This section examines the assumption that the red squirrel, although not known to hibernate or enter torpor, has evolved body temperature adaptations for energy conservation in the cold. The limited data on body temperature available for this species (Irving and Krog 1954; Morrison 1962; Grodzinski 1971) have been obtained from freshly-killed animals or from animals in the laboratory and may not indicate normal body temperature of undisturbed animals. Furthermore, the extent of seasonal, daily, and short term variability in body temperature of this species is not known. To obtain this information long-term abdominal temperature measurements of undisturbed squirrels in a semi-natural environment were made by radio-telemetry.

## MATERIALS AND METHODS

Two female red squirrels were held individually in large wire-mesh enclosures (80 x 300 x 300 cm) in a natural red squirrel habitat. The enclosures were at ground level and contained tree limbs to allow for arboreal activity. Insulated nest boxes and nesting materials were provided. Teklad rat and mouse diet (Teklad Mills, Winfield, Iowa) was supplied ad libitum, and supplemented with 50 g week<sup>-1</sup> of sunflower seeds. Water, crushed ice, or potatoes were provided during times when the animals had no access to snow. Further detail on animal care and holding is provided in Part I.

Temperature transmitters (model V, Minimitter Co., Inc., Indianapolis, Ind.), 19 mm in length, 12 mm in diameter, and 2.3 g in weight were implanted in the abdominal cavity of the squirrels under Nembutal anaesthesia (40-45 mg kg<sup>-1</sup>; intraperitoneal injection). At least one week was allowed for recovery before data were collected. Transmitters were removed after 6 months, the approximate life span of the batteries, and replaced with new transmitters. In November, when the transmitters were implanted, the two animals weighed 248 and 263 g, and in April, when they were replaced, they weighed 242 and 271 g.

The signal received from the transmitters consisted of clicks. The click rate varied almost linearly with the temperature of the transmitter from approximately 2.1-2.8

clicks  $\text{sec}^{-1}$  over the temperature range 35 to  $42^{\circ}\text{C}$ , with some variability among transmitters. Before implantation each transmitter was calibrated in a water bath. They were sensitive to a temperature change of less than  $0.2^{\circ}\text{C}$ , but the accuracy of absolute temperature values was limited to the accuracy of the YSI telethermometer (model 43TD) used for calibration. However, the same telethermometer and probe were used for all calibrations, eliminating systematic error from this source from temperature comparisons.

An average of 126 measurements per month (range = 25-292) were taken of body temperature ( $T_b$ ) during all months in a 1-year period except May, June, and July. A  $T_b$  measurement consisted of a recording of the transmitter signal on magnetic tape. These recordings were obtained over 1-10 periods per month, each usually extending over 24 h or longer, by a Sony FM/AM Cassette-Corder (model CF-400A) activated by a timing device for 1-2 min at hourly intervals. The tape was later played back and the click rate was determined by timing 100 clicks. Further  $T_b$  measurements were taken in the same way directly from the audio output of the receiver during visual observation of the animals, made from an adjacent building through a small Plexiglass porthole. There was no indication that the animals were aware of the observer. All  $T_b$  measurements were made on the same two animals, eliminating inter-animal variability as a source of error in making comparisons.



To check for calibration drift 3 of the 4 transmitters that had been implanted were removed from the animals and recalibrated before the batteries were exhausted. No drift was detected.

Air temperature ( $T_a$ ) was determined at the study site with a model G820 Grant temperature recorder (Grant Instruments, Cambridge, England) fitted with a probe suspended in a well-ventilated box 130 cm above the ground. Body temperature measurements were made at  $T_a$  from -33 to 28°C.

Body temperature data for each animal were grouped according to time of day and air temperature (e.g. Table 1). Because inspection of these data did not suggest any substantial differences in  $T_b$  between the two animals and overall means in  $T_b$  differed by less than 0.1°C, the data for the two animals were pooled for this and subsequent analyses.

## RESULTS

Values for  $T_b$  ranged from 35.9 to 41.4°C with an overall mean of 38.5°C (Table 1). Friedman's Test (Gibbons 1976), applied to the data in Table 1, shows significant differences in mean  $T_b$  measured at different times of the day and different  $T_a$  ( $P < 0.01$  for both). Mean  $T_b$  was higher during the day than at night and increased with increasing  $T_a$  up to approximately 20°C.

Body temperature was usually higher than 39°C when an animal was outside the nest during the day and lower than 39°C when it was inside during the night (Fig. 1). Both inside and outside the nest mean  $T_b$  varied with  $T_a$ , but the relationship between  $T_b$  and  $T_a$  differed in several respects in the two situations. Mean  $T_b$  of animals outside the nest during the day (Fig. 1A) showed a slight positive correlation with  $T_a$  at environmental temperatures below 15°C and a stronger negative correlation above this temperature. Mean  $T_b$  of animals inside the nest at night (Fig. 1B) showed a strong positive correlation with  $T_a$  at all environmental temperatures. The regression of  $T_b$  on  $T_a$  below 15°C in Fig. 1A differs significantly ( $P < 0.01$ ) in both slope and elevation from the regression in Fig. 1B (analysis of variance; Snedecor and Cochran 1967). Although the break in the regression (Fig. 1A) was set arbitrarily at 15°C, a break is justified because of the pronounced decline in mean  $T_b$  at a  $T_a$  of about 15°C and because mean

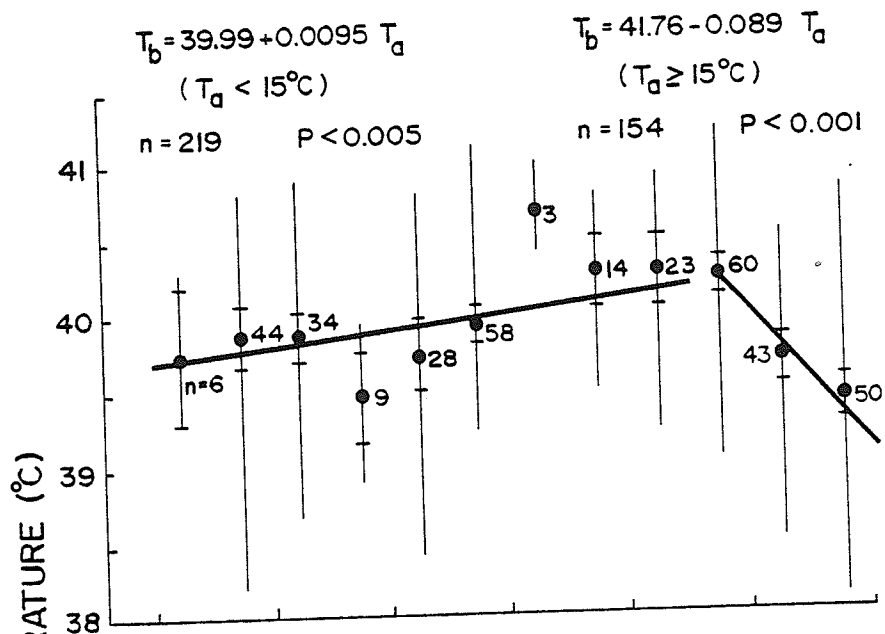
Table 1. Abdominal temperature means based on measurements with animals both inside and outside the nest, grouped according to time of day and air temperature. Data in each block are  $\bar{X} \pm s_x$  (n); range.

Time <sup>a</sup>	Air Temperature <sup>b</sup> (C)						Mean
	$T_a < -20$	$-20 \leq T_a < -10$	$-10 \leq T_a < 0$	$0 \leq T_a < 10$	$10 \leq T_a \leq 20$	$20 \leq T_a$	
1	38.2±0.4 (9) 36.9-39.9	39.1±0.4 (16) 36.6-40.9	38.9±0.3 (15) 37.0-41.0	39.5±0.5 (5) 38.4-41.0	40.3±0.2 (26) 38.3-41.4	40.1 (1)	39.3
2	36.6±0.1 (9) 36.6-37.0	36.6±0.5 (8) 36.6-40.4	38.8±0.2 (30) 36.6-40.2	39.1±0.4 (4) 38.2-39.9	39.5±0.2 (28) 36.8-41.4	39.5±0.2 (4) 38.8-40.4	38.7
3	38.3±0.7 (6) 36.6-40.3	38.5±0.3 (17) 36.7-40.3	38.4±0.2 (24) 36.7-40.6	39.5±0.4 (8) 37.3-40.4	39.3±0.2 (18) 38.5-40.8	40.0±0.2 (15) 37.9-40.1	39.0
4	37.4±0.2 (5) 36.9-38.0	38.4±0.3 (19) 36.5-40.7	39.1±0.3 (21) 36.8-40.6	39.0±0.5 (8) 37.5-41.1	39.6±0.1 (21) 38.5-41.1	39.8±0.2 (20) 37.8-40.8	38.9
5	38.2±0.6 (8) 36.8-40.5	37.5±0.2 (19) 36.3-39.1	38.8±0.2 (33) 36.7-40.8	38.8±0.3 (11) 37.2-39.9	39.8±0.2 (23) 38.0-40.9	39.7±0.1 (23) 38.4-40.6	38.8
6	37.6±0.1 (17) 36.9-38.5	37.6±0.1 (19) 36.7-38.8	38.5±0.1 (42) 37.0-41.0	38.5±0.3 (12) 37.6-40.1	39.0±0.1 (23) 37.9-40.8	39.0±0.2 (6) 38.4-39.6	38.4
7	37.2±0.1 (13) 36.6-38.1	37.7±0.1 (26) 36.7-38.8	37.9±0.1 (31) 37.2-39.2	37.9±0.1 (8) 37.5-38.2	38.7±0.1 (28) 37.6-39.7	38.4 (1)	38.0
8	37.1±0.1 (13) 36.4-37.9	37.7±0.1 (29) 36.9-39.1	37.8±0.1 (28) 36.9-38.6	38.0±0.4 (10) 37.3-41.2	38.8±0.1 (59) 37.6-41.1	39.3 (3) 38.7-40.3	38.1
9	36.9±0.1 (7) 36.2-37.3	37.5±0.1 (32) 35.9-38.7	37.8±0.1 (29) 36.2-39.5	37.8±0.3 (5) 37.5-38.9	38.5±0.1 (24) 37.3-39.2	37.5 (2) 36.8-38.2	37.7
10	37.0±0.2 (8) 36.1-37.9	38.2±0.3 (28) 36.9-40.8	38.6±0.3 (23) 36.3-41.1	39.1±0.3 (10) 37.6-40.8	39.0±0.2 (17) 38.2-40.8		38.4
Mean	37.4	38.1	38.4	38.7	39.3	39.2	38.5

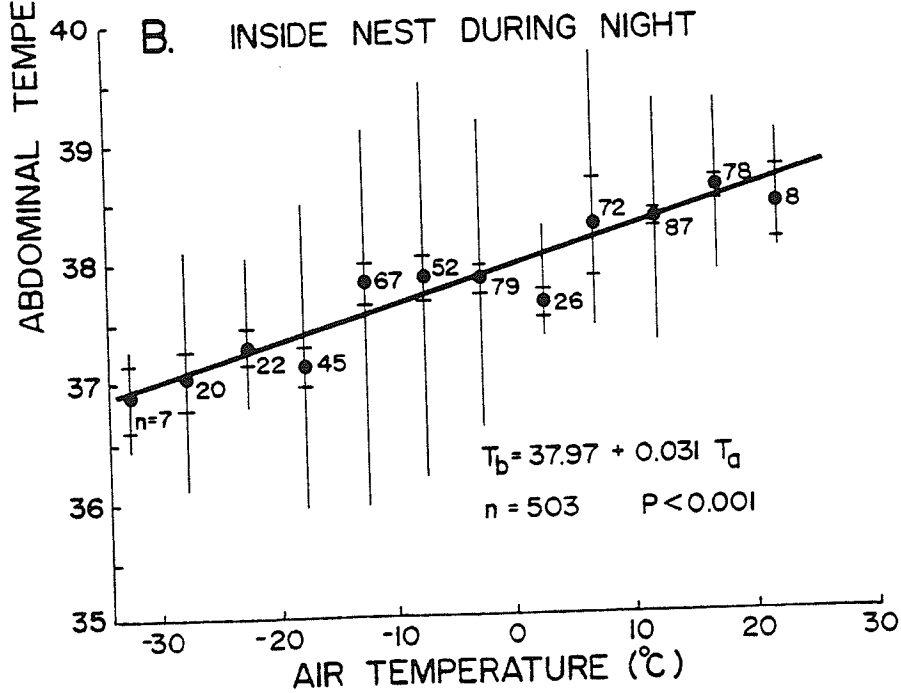
<sup>a</sup> Times 1-5 and 6-10 represent the daylight and nighttime periods, respectively, each divided into 5 equal periods, with time 1 beginning at sunrise and time 6 at sunset.

<sup>b</sup> Air temperature at the time  $T_b$  was recorded.

### A. OUTSIDE NEST DURING DAY



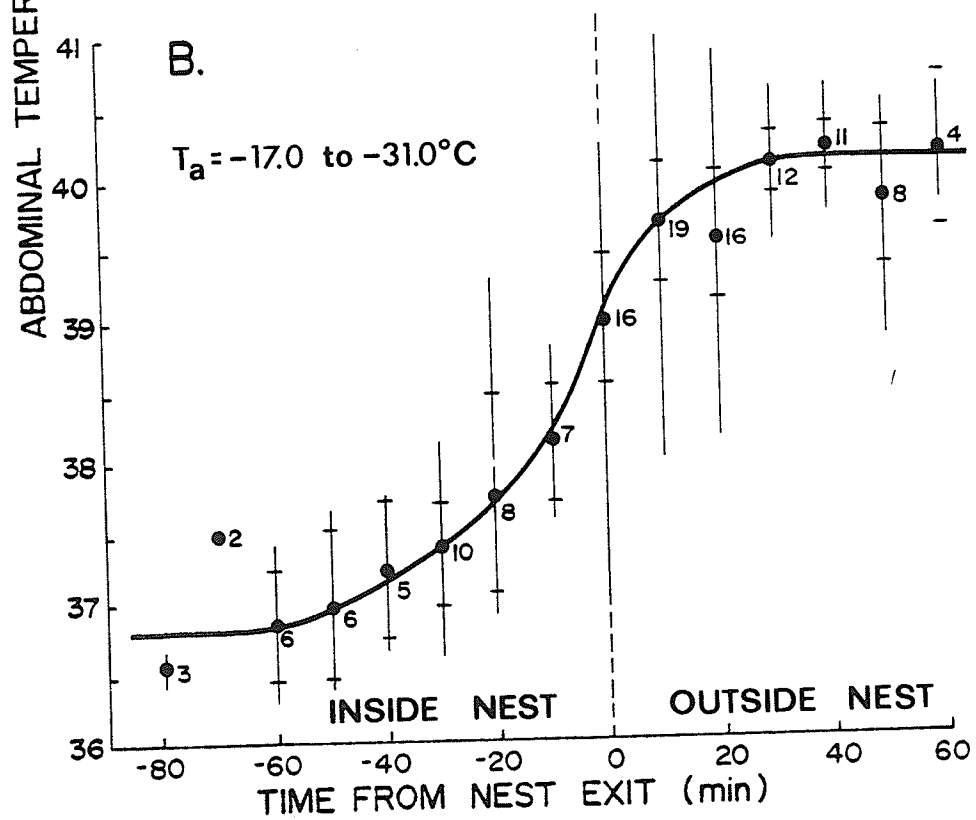
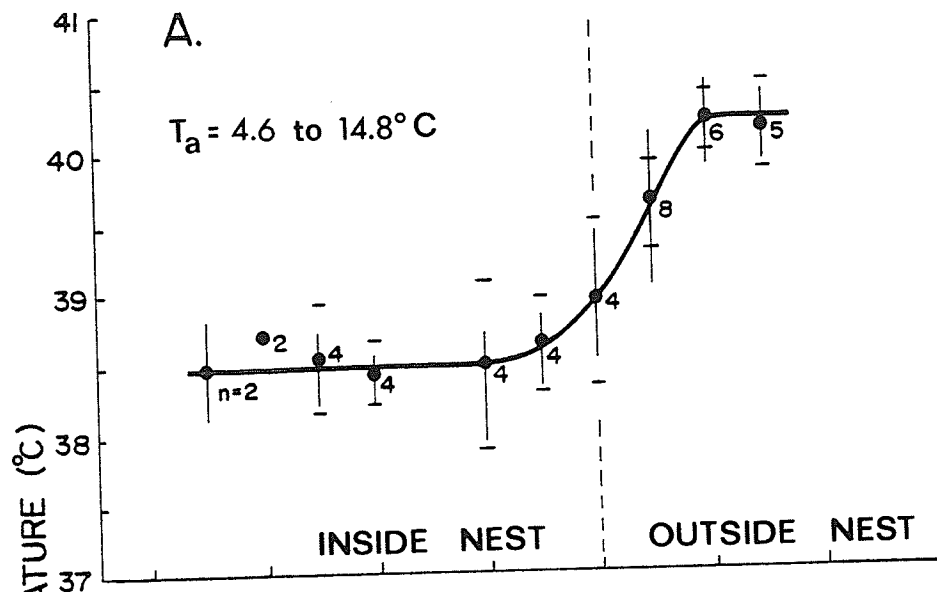
### B. INSIDE NEST DURING NIGHT

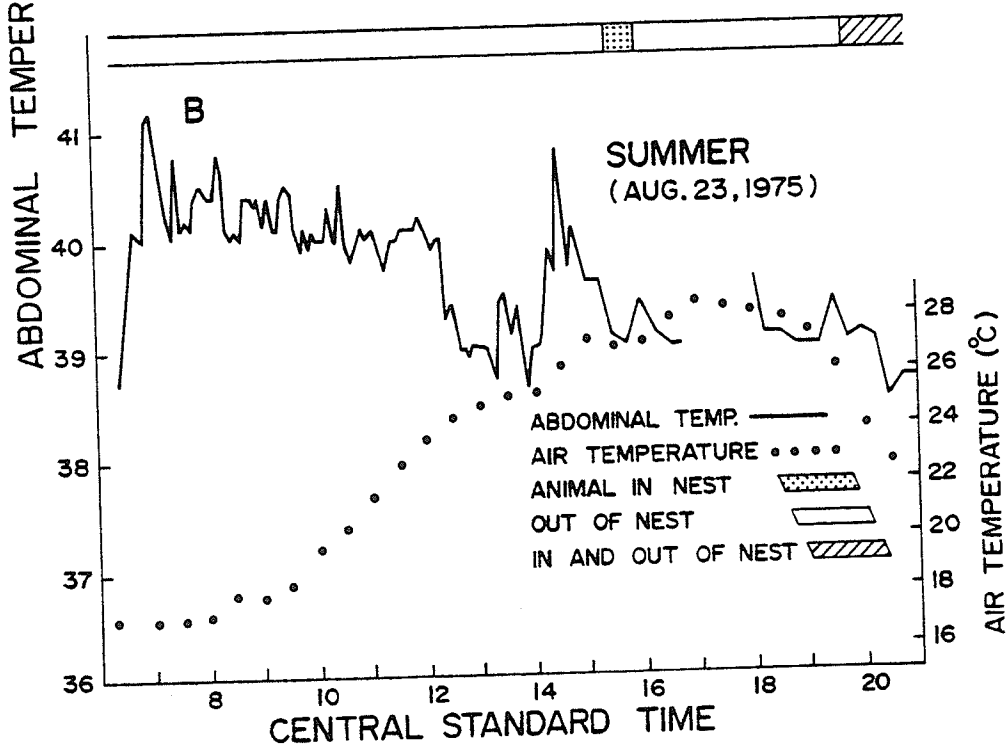
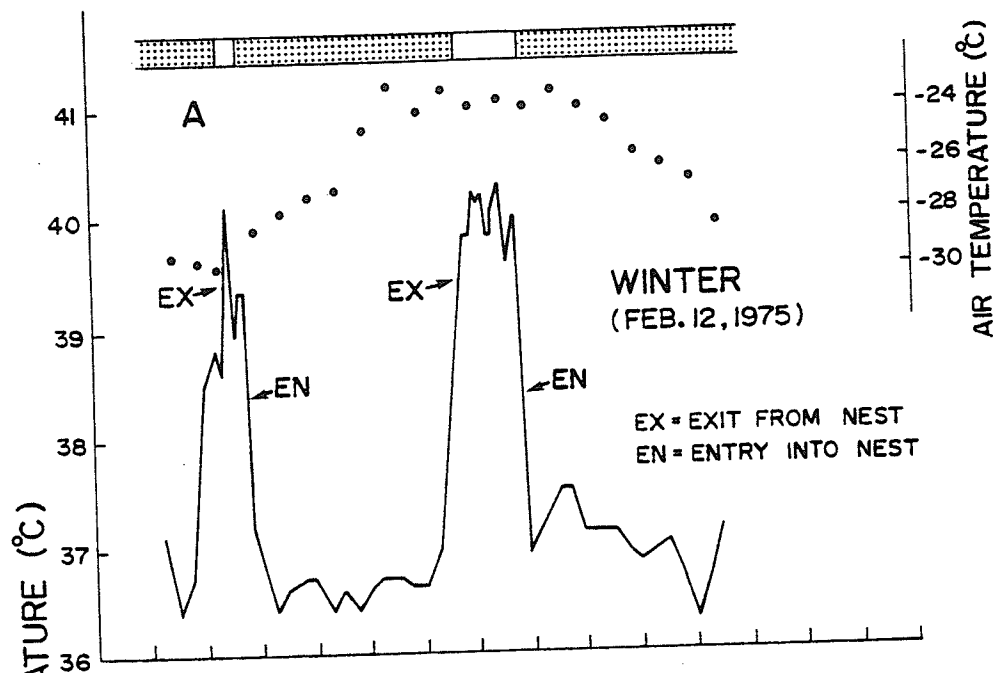


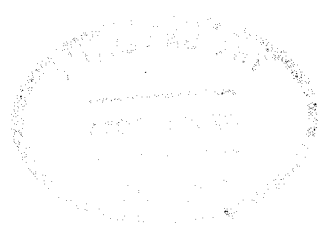
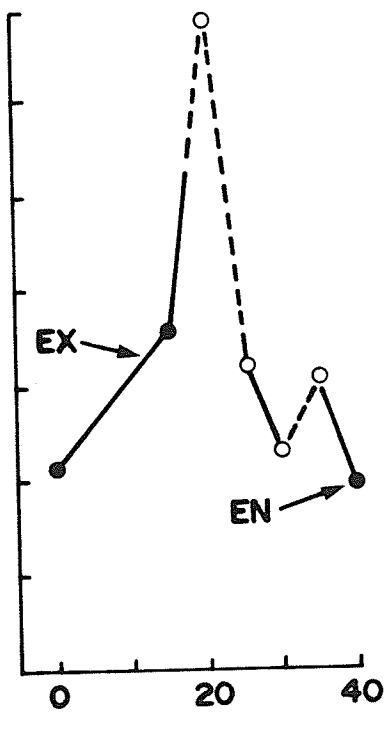
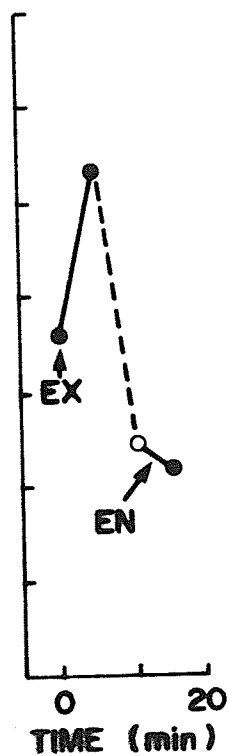
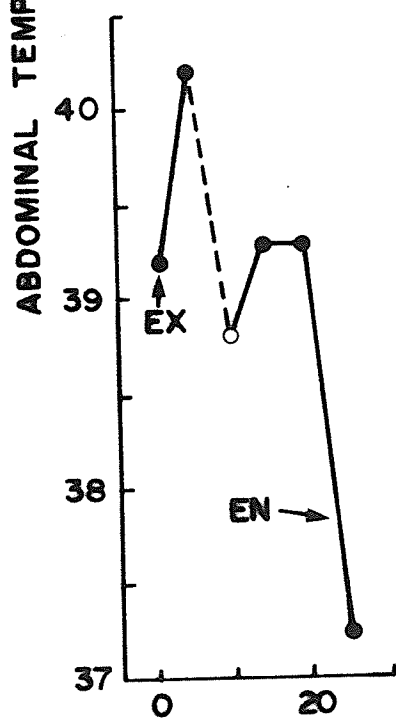
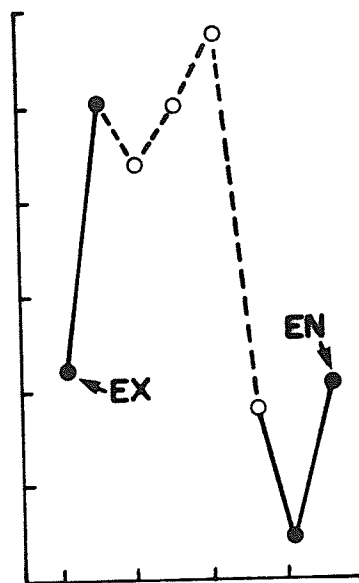
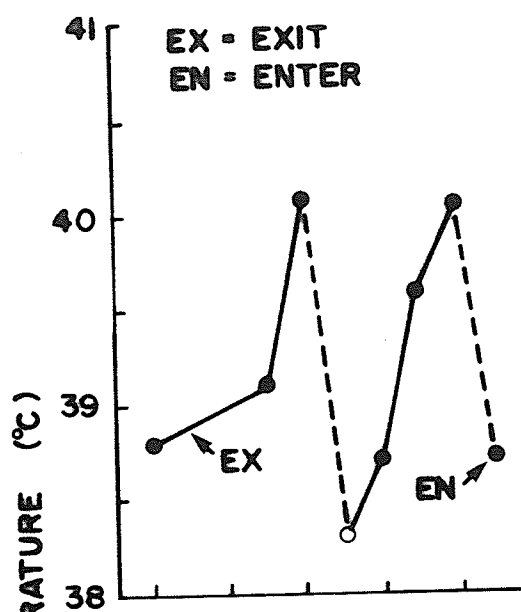
values are based on large numbers of measurements.

Abdominal temperature increased to approximately  $39^{\circ}\text{C}$  before the animals left the nest (Fig. 2). This increase was independent of the initial  $T_b$  in the nest which varied with  $T_a$ . Mean  $T_b$  measured within  $\pm 1$  min of leaving the nest was  $39.2 \pm 0.14^{\circ}\text{C}$  ( $\pm 1$  SE; range =  $38.6$  to  $39.9^{\circ}\text{C}$ ;  $n = 4$ ) at  $T_a$  from  $4.6$  to  $14.8^{\circ}\text{C}$ , and  $38.9 \pm 0.10^{\circ}\text{C}$  ( $\pm 1$  SE; range =  $38.5$ - $39.4^{\circ}\text{C}$ ;  $n = 4$ ) at  $T_a$  from  $-17$  to  $-31^{\circ}\text{C}$  with one exception. In this incident the squirrel left the nest when its  $T_b$  was  $37.2^{\circ}\text{C}$ ; however, it remained motionless at the nest entrance for 17 min while  $T_b$  rose to  $38.8^{\circ}\text{C}$ . When resting  $T_b$  was low as at low  $T_a$ , the increase in  $T_b$  was sometimes initiated as long as 60 min before the animal left the nest. With a higher resting  $T_b$  the time used in raising  $T_b$  was shorter. After leaving the nest, a squirrel usually remained motionless at the nest entrance while  $T_b$  continued to rise. Body temperature rose to a mean value of about  $40.2^{\circ}\text{C}$  at both high and low  $T_a$ .

Several aspects of temperature regulation not evident in pooled data are seen in continuous records of  $T_b$  of individual animals based on recordings at 5 min intervals (e.g. Figs. 3 and 4). At low  $T_a$ , a low  $T_b$  was maintained while the animal was resting in the nest during the day as well as at night. The transition from the high  $T_b$  during activity to the low  $T_b$  during rest in the nest was frequently marked by a rapid decline in  $T_b$  beginning before









the animal entered the nest (Figs. 3A and 4). During activity  $T_b$  fluctuated rapidly both in summer and winter (Fig. 3). From visual observation it appeared fluctuations in  $T_b$  paralleled changes in intensity of activity. When animals were at rest for extended periods of time outside the nest  $T_b$  tended to fluctuate around approximately  $39^{\circ}\text{C}$ , as for example in Fig. 3B, where the animal was at rest for most of the afternoon. This was possibly due to the high  $T_a$ . During intense activity  $T_b$  as high as  $41.4^{\circ}\text{C}$  were recorded. In the winter the animals constructed tunnels in the snow. Entry into a tunnel was followed almost invariably by a rapid decline in  $T_b$  (Fig. 4). Upon emergence from the tunnel,  $T_b$  rose again except when the animal was about to enter the nest.

## DISCUSSION

Some, and possibly most of the variability in  $T_b$  of the red squirrel is related to ambient temperature and activity. It is not believed to result from an inability of the red squirrel to maintain a precise  $T_b$  but rather to be an adaptation for energy conservation.

Different selection pressures have probably been acting on the animal inside than outside the nest to determine optimum  $T_b$ , and these have resulted in the evolution of different "set points" about which body temperature is regulated in the two situations. Inside the nest a reduced  $T_b$  may be advantageous because of the reduction in heat loss to the environment due to a lower temperature gradient from animal to environment. At low  $T_a$  the red squirrel spends a major portion of the day in the nest (Part I) and this small reduction in  $T_b$  may result in a significant reduction in daily energy expenditure. The observation that the depression of  $T_b$  is greatest at low  $T_a$  supports the suggestion that the need for energy conservation is a factor in determining optimum  $T_b$  in the nest. Outside the nest it would appear the selective value of energy conservation resulting from a lower  $T_b$  is outweighed by other factors. The red squirrel, because of its noisy vocalizations, diurnal habit, and arboreal activity, is highly noticeable to predators. It appears to depend less on concealment than on escape to

avoid predation. Escape in an arboreal environment, as well as arboreal activity per se, will be aided by rapid and highly co-ordinated motor activity and these are probably enhanced by an increase in  $T_b$  above the resting level. Because little time is spent outside the nest at low  $T_a$  (Part I), the additional energy required on a daily basis for thermoregulation as a result of the increased  $T_b$  will be slight.

The rate at which the increase from resting to active  $T_b$  occurs is a function of the amount by which the rate of metabolic heat production exceeds the rate of heat loss. At low  $T_a$  the rate of heat loss in the relatively moderate thermal environment of the nest is much lower than it would be outside the nest (Part III) permitting either a more rapid increase in  $T_b$  or an increase with a lower metabolic rate. By raising  $T_b$  before leaving the nest and remaining at the nest entrance for a short time while  $T_b$  continues to rise the red squirrel avoids exposure to predation while its ability to escape is probably less than optimal. This would be particularly important at low  $T_a$  when the metabolic rate required for thermoregulation may approach the metabolic capacity of the animal and the rate of increase in  $T_b$  outside the nest would be very slow. It is of interest that the muskrat also raises  $T_b$  prior to leaving its lodge (MacArther 1977). Thus, this feature of temperature regulation is not peculiar to the red squirrel and may be a feature of temperature

regulation in other small mammals.

The variation of  $T_b$  with intensity of activity outside the nest probably accounts for much of the observed variability of  $T_b$  about the regression of  $T_b$  on  $T_a$  (Fig. 1A). Furthermore, this variation of  $T_b$  with intensity of activity along with the correlation between intensity of activity and  $T_a$  (Part I) probably accounts for the correlation between  $T_b$  and  $T_a$  at  $T_a$  less than  $15^{\circ}\text{C}$ . The reduction of  $T_b$  at  $T_a$  greater than  $15^{\circ}\text{C}$  may also result from a decrease in activity, or alternatively, may reflect a shift in the "set point" (Hammel et al. 1963) for temperature regulation. Such a shift would probably be adaptive for the red squirrel as at high ambient temperature  $T_b$  could rapidly rise to lethal levels during the intense activity associated with territorial defense or escape from a predator. A reduced initial  $T_b$  will increase the possible duration of intense activity. A similar adaptive function has been proposed for the decline in rectal temperature of Peromyscus maniculatus with heat acclimation (Roberts and Chaffee 1976).

The strong correlation between mean  $T_b$  of an animal in the nest and  $T_a$  cannot result from differences in activity, as the animal builds a nest with a chamber just large enough to accommodate itself, leaving little room for activity. It may be a consequence of change in hypothalamic "set point" temperature which may change either seasonally or in direct response to  $T_a$ . However,

an increased thoracic-abdominal temperature difference with decreasing  $T_a$  cannot be excluded as an alternative explanation to a change in "set point" and overall body temperature. During rest in a postabsorptive state more thermogenesis would probably occur in the thoracic than in the abdominal area as a result of cardiac and pulmonary activity. Consequently, thoracic temperature would tend to be higher than abdominal temperature. Red squirrels possess brown adipose tissue, apparently throughout adult life (Aleksiuk 1971). This tissue is located mainly in the thoracic and cervical region in small mammals and has a high thermogenic capacity (Smith and Horowitz 1969). It could contribute to a thoracic-abdominal temperature difference at low  $T_a$ . A local increase in temperature adjacent to this tissue has been observed in the muskrat swimming in cold water (MacArthur 1977).

The higher mean day-time  $T_b$  relative to mean night-time  $T_b$  of the red squirrel is typical of diurnal rodents (Hart 1971). Although a daily cycle in  $T_b$  independent of activity cannot be excluded, the observation that  $T_b$  decreases to approximately night-time levels when the animal is in the nest during the day (Fig. 3A) suggests that most of this diel variation in mean  $T_b$  results from its diurnal activity pattern (Part I).

The rapid decline in  $T_b$  during subnivean activity is unexpected as the subnivean environment usually has more moderate temperatures than the supranivean environment

(Pruitt 1957) and offers protection from wind. This response may be similar to the observed drop in  $T_b$  when cold exposed ground squirrels are suddenly subjected to warm air (Morhardt and Molt 1975). As suggested by these authors, peripheral vasoconstriction, which permits peripheral cooling during cold exposure, may be suddenly relaxed upon entry into a warmer environment. The subsequent influx of peripherally cooled blood to the abdominal area could account for the rapid decline in  $T_b$  both during subnivean activity and upon entry into the nest. Supporting the hypothesis of peripheral cooling is the observation of low tail temperatures in cold exposed red squirrels (Miller 1967).

That  $T_b$  does not decline during activity at low  $T_a$  is surprising as a decline in  $T_b$  during forced exercise has been reported in several mammals (Hart and Heroux 1955; Hart and Jansky 1963). Possibly, the decline in intensity of activity with decreasing  $T_a$  (Part I) is a behavioural adaptation which permits successful thermoregulation during activity at low  $T_a$ .

To permit thorough and detailed measurements over an extended period only two animals were used in this study and both were females. Consequently, it may be argued that the results obtained are not representative. However, since  $T_b$  dynamics of the two animals were similar and differences due to sex are generally small in rodents (Hart 1971), it is held a larger sample would yield

similar results. This view is supported by a subsequent, combined temperature-heart rate telemetry study (Part V) in different individuals of the same species.

### PART III

Energetics of the Red Squirrel: A Laboratory Study of  
the Effects of Temperature, Seasonal Acclimatization, Use  
of the Nest, and Exercise



## INTRODUCTION

The red squirrel (Tamiasciurus hudsonicus), over most of its geographical range, encounters severe seasonal cold which to a small mammal is potentially stressful due to the close coupling between environmental temperature and energy requirements. Small mammals subjected to seasonal cold have, in general, evolved physiological and behavioural adaptations which enhance their capability to maintain a high body temperature at low environmental temperatures and reduce the energy cost of doing so.

Behavioural adaptations of the red squirrel to seasonal cold include increased use of a nest and a reduction in both amount and intensity of locomotor activity at low air temperatures (Part I). Physiological adaptations include a seasonal change in resting body temperature (Part II). Surprisingly, Irving et al. (1955) found no evidence of a seasonal change in overall body insulation. However, their sample sizes were small, their summer and winter acclimatized animals differed considerably in weight, and they had difficulty obtaining oxygen consumption measurements of animals at rest. In apparent contradiction to their results, pelt insulation of red squirrels from the Northwest Territories, Canada, does show seasonal change (Hart 1956).

For these reasons and because results with Alaskan

squirrels may not apply to squirrels in southern Manitoba, an examination of seasonal acclimatization of the red squirrel in this area was a major objective of this part of the study.

A second objective was to examine the potential effect on energy balance of behavioural adaptations previously described (Part I). This required measurement of oxygen consumption of red squirrels at rest in the nest and during exercise at low temperatures, as the combined effect of exercise and cold on metabolic rate of rodents has not yet been firmly established (see Hart 1971).

## MATERIALS AND METHODS

Animals were collected in southern Manitoba, transferred immediately to large outdoor enclosures in the vicinity of the University of Manitoba, Winnipeg, and held there until used for experiments. More detail regarding holding conditions is provided in Part I.

In all experiments, rate of oxygen consumption ( $\dot{V}_{O_2}$ ) was determined using open flow respirometric techniques and a Beckman F-3 oxygen analyzer. The analyzer was calibrated with dry air and a nitrogen-oxygen mixture of known composition. The fractional oxygen content, measured by the oxygen analyzer, of dried outflow air from a metabolism chamber containing an animal was continuously recorded by a strip-chart recorder (Fisher Recordall, series 5000).

Metabolism chambers were housed in a controlled-environment room in which temperature could be regulated to within  $\pm 1.0^\circ\text{C}$  down to  $-20^\circ\text{C}$ . Rates of air inflow, measured with a Matheson model 603 flowmeter, were about  $720\text{ cm}^3\text{ min}^{-1}$  and  $1820\text{ cm}^3\text{ min}^{-1}$  (STP) for resting and exercising animals, respectively. Inflowing air was not dried for resting animals as exposure to dry air could have been stressful. Carbon dioxide was not absorbed from the outflowing air before analysis of its oxygen content. The resulting error in oxygen consumption, as calculated from the fractional oxygen concentration of air flowing out of

the metabolism chamber using equation 5 of Depocas and Hart (1957), is sufficiently low (see Appendix I), considering the objectives of this study, to justify these departures from standard procedure. Unless otherwise stated,  $\dot{V}_{O_2}$  measurements were made during the day, the usual period of activity for the red squirrel. Air temperature ( $T_a$ ) was measured in the metabolism chamber except during measurement of  $\dot{V}_{O_2}$  of animals in the nest when measurement of  $T_a$  outside the nest box permitted calculation of the combined thermal conductance of animal and nest.

Measurement of Minimum  $\dot{V}_{O_2}$  of Summer- and Winter-acclimatized Animals

Five summer-acclimatized adults of both sexes, weighing 190-276 g (mean = 224 g) and 12 winter-acclimatized adults of both sexes, weighing 176-344 g (mean = 231 g) were used for  $\dot{V}_{O_2}$  measurements. These were carried out between June 26 and July 15 with summer-acclimatized animals and between December 15 and February 20 with winter-acclimatized animals.

Summer-acclimatized animals were held in the laboratory at 20°C during the 3-week experimental period. Winter-acclimatized animals were held in the laboratory at 15°C for a maximum of 2 consecutive days and then returned to outdoor holding enclosures for at least 3 days before being used again. Length of photoperiod in the laboratory corresponded to the outdoor photoperiod.

Measurements with summer-acclimatized animals were first made at 30°C, and then at progressively colder  $T_a$  by intervals of approximately 5°C down to -20°C. The procedure was reversed for measurements with winter-acclimatized animals. These different experimental and holding procedures for summer- and winter-acclimatized animals were used to minimize the effects of warm and cold acclimation on winter- and summer-acclimatized animals, respectively, which could potentially occur in the course of the experiments. Measurements at 35°C were attempted last with both summer- and winter-acclimatized animals and were discontinued after several animals appeared heat-stressed.

The metabolism chamber consisted of a 1-gallon (4.5 l) metal paint can with 2-3 cm of wood shavings in the bottom, fitted with ports for air inflow and outflow and temperature measurement. Starting 1 h after the animal was placed in the metabolism chamber fractional concentration of oxygen in the outflow air was recorded continuously for at least 2 h. This procedure was sometimes repeated after the temperature in the controlled environment room was changed by 5°C.

Rate of oxygen consumption during the 12-min period of lowest stable  $\dot{V}_{O_2}$  was selected as the minimum  $\dot{V}_{O_2}$  at a given  $T_a$ . Due to inertial characteristics of the measuring system, a stable recording of fractional oxygen concentration in the outflow air was obtained only after the animal had maintained a resting  $\dot{V}_{O_2}$  for approximately 12 min.

Consequently, a measurement of resting  $\dot{V}_{O_2}$  was obtained only when an animal was relatively inactive for at least 24 min. When this did not occur within the usual 2-h measuring period it was extended up to a maximum of 3 additional hours.

Food was removed from holding cages 1 h before the animal was placed in the metabolism chamber and was not available in the metabolism chamber. As  $\dot{V}_{O_2}$  was not measured during the first hour after an animal was placed in the metabolism chamber and measurements at two ambient temperatures were sometimes made consecutively, fasting time was usually 2-7 h. Fasting the animals until they were in a post-absorptive state was not considered desirable since restlessness induced in small mammals by fasting may cause greater error in measurement of minimum resting  $\dot{V}_{O_2}$  than the calorific action of food which fasting is intended to minimize (Heusner 1971).

Routine rectal temperature measurements were not made before and after  $\dot{V}_{O_2}$  trials as these would have been extremely difficult to do with the red squirrel. Furthermore, a preliminary body temperature investigation using radiotelemetry capsules implanted in the abdominal cavity as described in Part II indicated a stable body temperature of 37-39°C in animals at rest under laboratory conditions at temperatures as low as -30°C.

#### Measurement of Minimum $\dot{V}_{O_2}$ of Animals in a Nest

Five winter-acclimatized animals of both sexes,

weighing 236-295 g (mean = 268 g), were used for  $\dot{V}_{O_2}$  measurements in a nest constructed with similar materials and the same dimensions as the nest boxes used in outdoor behavioural studies (Part I). It was constructed so as to be airtight and fitted with air inlet and outlet ports. With a Plexiglass cover bolted over the entrance it comprised the metabolism chamber.

The air flowing through the nest box during  $\dot{V}_{O_2}$  measurement could potentially remove heat from the nest box interior so that the animals in this nest box would not be in an environment comparable to animals in the outdoor nest boxes (Part I). This problem was avoided by having air flowing into and out of the nest box pass in opposite directions through two parallel adjacent copper tubes with an inner diameter of 0.25 inches (0.64 cm) and a length of 60 cm. The tubes were soldered together along their entire length and insulated with fibrewool. With this counter-current heat exchange system air flowing into the nest box was heated to within less than 1°C of air flowing out of the nest box.

The nest box was attached to a runway measuring 100 x 31 x 36 cm. Each animal was placed in the runway-nest box complex at least 24 h before  $\dot{V}_{O_2}$  measurements commenced. It was provided with burlap which it shredded and used for nesting material, filling the entire nest box, leaving only a central chamber large enough to accommodate the animal. Air temperature at this time was set at

-20°C to promote nest construction by the animal.

Each day, after the initial 24-h period, the nest box was closed after the animal had entered it for the night and fractional oxygen concentration of the outflow air was measured continuously from 21:00 to 9:00 the following day. Minimum  $\dot{V}_{O_2}$  was determined as described on page 54. Measurements of  $\dot{V}_{O_2}$  were made at  $T_a$  intervals of 10°C from -20 to 20°C, the maximum temperature considered consistent with the avoidance of heat stress to the animal. Measurement of  $\dot{V}_{O_2}$  began at -20°C;  $T_a$  was increased at 9:00 following each night's measurements.

#### Measurement of Minimum $\dot{V}_{O_2}$ in Exercising Animals

Five adults of both sexes, weighing 217-270 g (mean = 241 g) were used for measurement of  $\dot{V}_{O_2}$  during rest and exercise in the months of March, April, and May. The animals were held indoors at 20°C and 12L:12D photoperiod.

The exercise chamber consisted of a Plexiglass box measuring 10 x 10 x 38 cm fitted at one end with an electrical stimulator and mounted over a treadmill consisting of a rubber belt mounted on rollers and powered by a 0.2 horsepower (150 watt) Dayton AC-DC series-wound electric motor with a Dayton model 4X599 speed control (Dayton Electric Mfg. Co.). The Plexiglass chamber was fastened with an airtight seal to the housing of the belt and roller assembly; the entire unit comprised the metabolism chamber. Power was transmitted from the motor,



mounted outside the metabolism chamber, to the treadmill via a steel shaft passing through an airtight rubber seal. The belt and roller assembly was carefully designed to eliminate dead air space; total volume of the metabolism chamber was estimated to be 5 l.

After being placed in the treadmill, an animal was allowed to settle down and then forced to run continuously for 20-25 min. A stable recording of  $\dot{V}_{O_2}$  for at least 5 min was taken as representing minimum  $\dot{V}_{O_2}$  at a particular running speed and  $T_a$ . If this was not obtained the experiment was repeated on another day.

Resting  $\dot{V}_{O_2}$  of the same animals in the same apparatus was measured and minimum  $\dot{V}_{O_2}$  determined as described on page 54. These experiments were conducted at  $T_a$  from  $-10^{\circ}\text{C}$ , the lowest temperature at which the treadmill functioned reliably, to  $20^{\circ}\text{C}$ . Experiments at higher temperatures would probably have caused heat stress to the animals. Running speeds of  $0.6\text{--}1.9\text{ km h}^{-1}$  were used. Treadmill speed was checked before and after each experiment by measuring the time required for a given number of revolutions of the treadmill belt.

### Analysis of Data

Rate of oxygen consumption was calculated both as  $\dot{V}_{O_2} W^{-1}$  and  $\dot{V}_{O_2} W^{-0.75}$ , where  $W$  is body weight, for comparisons between different groups of animals or between the same animals in different seasons (e.g. Table 1). This

is in accordance with the recommendation (Hart 1971) that  $\dot{V}_{O_2}$  should be expressed as  $\dot{V}_{O_2} W^{-0.75}$  rather than as  $\dot{V}_{O_2} W^{-1}$  as the latter can result in erroneous conclusions in comparisons between groups of animals with non-uniform weight distributions. Tests of significance with either set of data yielded the same conclusions.

Least-square regressions of  $\dot{V}_{O_2}$  on  $T_a$  were calculated over the range of  $T_a$  where  $\dot{V}_{O_2}$  was clearly sensitive to  $T_a$ . These  $T_a$ , determined from inspection of the data (Figs. 1 and 2), are indicated both on Table 1 and by the range of  $T_a$  covered by the regression lines in Figs. 1 and 2. For animals in the nest (Fig. 1C) a different procedure was followed due to the limited number of measurements where  $\dot{V}_{O_2}$  was sensitive to  $T_a$ . Following a method described in Snedecor and Cochran (1967), a least-square regression was fitted to  $\dot{V}_{O_2}$  at  $T_a$  less than  $0^\circ\text{C}$  and made to intersect the horizontal axis ( $\dot{V}_{O_2} = 0$ ) at  $T_a = T_b$  (approx.  $38^\circ\text{C}$ , Part II). Use of this procedure is based on the assumption that weight-specific  $\dot{V}_{O_2}$  of animals in a nest follows the model:  $\dot{V}_{O_2} = C(T_b - T_a)$ , where  $C$  is a constant representing total weight-specific conductance, and  $T_b$  is body temperature. Minimum metabolic rates of rodents in metabolism chambers generally conform to this model (Hart 1971) as do  $\dot{V}_{O_2}$  of Reithrodontomys megalotis (Pearson 1960) and Microtus longicaudus (Beck and Anthony 1971) in a nest.

The lower critical temperature is defined as the lower limit of the thermal neutral zone, the range of  $T_a$  over

which  $\dot{V}_{O_2}$  is independent of  $T_a$  (Bartholomew 1977b). The lower critical temperature was designated, in this study, as the  $T_a$  at which a regression of  $\dot{V}_{O_2}$  on  $T_a$ , determined as described in the preceeding paragraph, intersected mean  $\dot{V}_{O_2}$  at thermal neutral  $T_a$ . Air temperatures at which  $\dot{V}_{O_2}$  is clearly independent of  $T_a$  were determined from inspection of the data (Figs. 1 and 2) and are indicated in Table 1.

Total thermal conductance was calculated from the relationship:  $C = \dot{V}_{O_2} / (T_b - T_a)$  (Bartholomew 1977b). This procedure is valid if respiratory heat loss is constant and low relative to total heat loss (Hart 1971). This condition may be assumed for homeotherms at low  $T_a$  (McNab 1970). If the regression of  $\dot{V}_{O_2}$  on  $T_a$  at  $T_a$  less than the lower critical temperature extrapolates to  $T_a = T_b$  when  $\dot{V}_{O_2} = 0$ , then  $C$  is numerically equal to the slope of the regression. This procedure was used in determining  $C$  of summer and winter-acclimatized animals as normal resting body temperature of the red squirrel (approx. 38°C, Part II) is included within the interval on the horizontal axis of Fig. 1 bounded by the intersection with the horizontal axis of 1 SE above and below the extrapolated regression line. Because the regression for exercising animals (Fig. 2) did not extrapolate to  $T_a = T_b$  at  $\dot{V}_{O_2} = 0$ ,  $C$  was calculated directly from the relationship:  $C = \dot{V}_{O_2} / (T_b - T_a)$  for both resting and exercising animals in the exercise chamber (Figs. 2 and 3). A  $T_b$  of 38°C during rest and

40°C during exercise was assumed. These estimates are based on radiotelemetry measurements of outdoor animals (Part II).

Mean values have been expressed as  $\bar{X} \pm SE_{\bar{X}}$ . The t-test has been used to test for significant differences between sample means, and analysis of covariance (Snedecor and Cochran 1967) for differences in slope and elevation (i.e. height above the horizontal axis) between regression lines.

## RESULTS

Minimum resting  $\dot{V}_{O_2}$  of summer- and winter-acclimatized animals (Table 1) did not differ significantly ( $P > 0.1$ ) at thermal neutral  $T_a$ . Minimum  $\dot{V}_{O_2}$  at thermal neutral  $T_a$  of animals in nests,  $0.92 \pm 0.06 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ , was significantly less ( $P < 0.01$ ) than the minimum  $\dot{V}_{O_2}$ ,  $1.12 \pm 0.03 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ , calculated from pooled data of summer- and winter-acclimatized animals without a nest.

Total thermal conductance at  $T_a$  less than the lower critical temperature was greater in summer- than in winter-acclimatized animals (Table 1; Figs. 1A and B;  $P < 0.05$ ). Combined C of winter-acclimatized animals with a nest averaged 40% of C of winter-acclimatized animals without a nest (Table 1; Figs. 1B and C).

This seasonal change in C resulted in a seasonal change of  $6^\circ\text{C}$  in the lower critical temperature (Table 1). A further reduction of  $20^\circ\text{C}$  in the lower critical temperature was effected by use of the nest.

The activity increment in  $\dot{V}_{O_2}$ , defined as the difference between  $\dot{V}_{O_2}$  during activity and  $\dot{V}_{O_2}$  during rest at a given  $T_a$  and illustrated by the vertical distance between the regressions of Fig. 2A, remained constant or possibly increased slightly with decreasing  $T_a$ . This is indicated by the marginally significant difference ( $0.01 < P < 0.05$ ) between the slopes of the regressions of  $\dot{V}_{O_2}$  during rest and during exercise on  $T_a$ . This suggests that heat

Table 1. Minimum  $\dot{V}_{O_2}$  at thermal neutral  $T_a$  and total thermal conductance (C) at  $T_a$  less than the lower critical temperature ( $T_{LC}$ ) of resting animals.

	Summer-Acclimatized Animals	Winter-Acclimatized Animals	Animals in a Nest
$\dot{V}_{O_2}$ ( $\text{cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ )	1.09±0.05 (10)	1.13±0.04 (16)	0.92±0.06 (12)
$\dot{V}_{O_2}$ ( $\text{cm}^3 \text{ g}^{-0.75} \text{ h}^{-1}$ )	4.22±0.21 (10)	4.32±0.15 (16)	3.75±0.26 (12)
$T_a$ ( $^{\circ}\text{C}$ )	25 to 30	20 to 30	0 to 20
$T_{LC}$ ( $^{\circ}\text{C}$ )	21	15 <sup>b</sup>	-5
C ( $\text{cm}^3 \text{ O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$ )	0.0621±0.0028 (35)	0.0524±0.0037 (36)	0.0212±0.0060 (9)
C ( $\text{cm}^3 \text{ O}_2 \text{ g}^{-0.75} \text{ h}^{-1} \text{ }^{\circ}\text{C}^{-1}$ )	0.247±0.010 (35)	0.187±0.011 (36)	0.0864±0.0243 (9)
$T_a^c$ ( $^{\circ}\text{C}$ )	-20 to 15	-20 to 10	-20 to -10

<sup>a</sup> Thermal neutral  $T_a$  at which  $\dot{V}_{O_2}$  measurements were made

<sup>b</sup> 14 $^{\circ}\text{C}$  if  $\dot{V}_{O_2}$  expressed as  $\dot{V}_{O_2} \text{ W}^{-1}$ ; 16 $^{\circ}\text{C}$  if  $\dot{V}_{O_2}$  expressed as  $\dot{V}_{O_2} \text{ W}^{-0.75}$

<sup>c</sup> Air temperatures at which thermal conductance was determined.

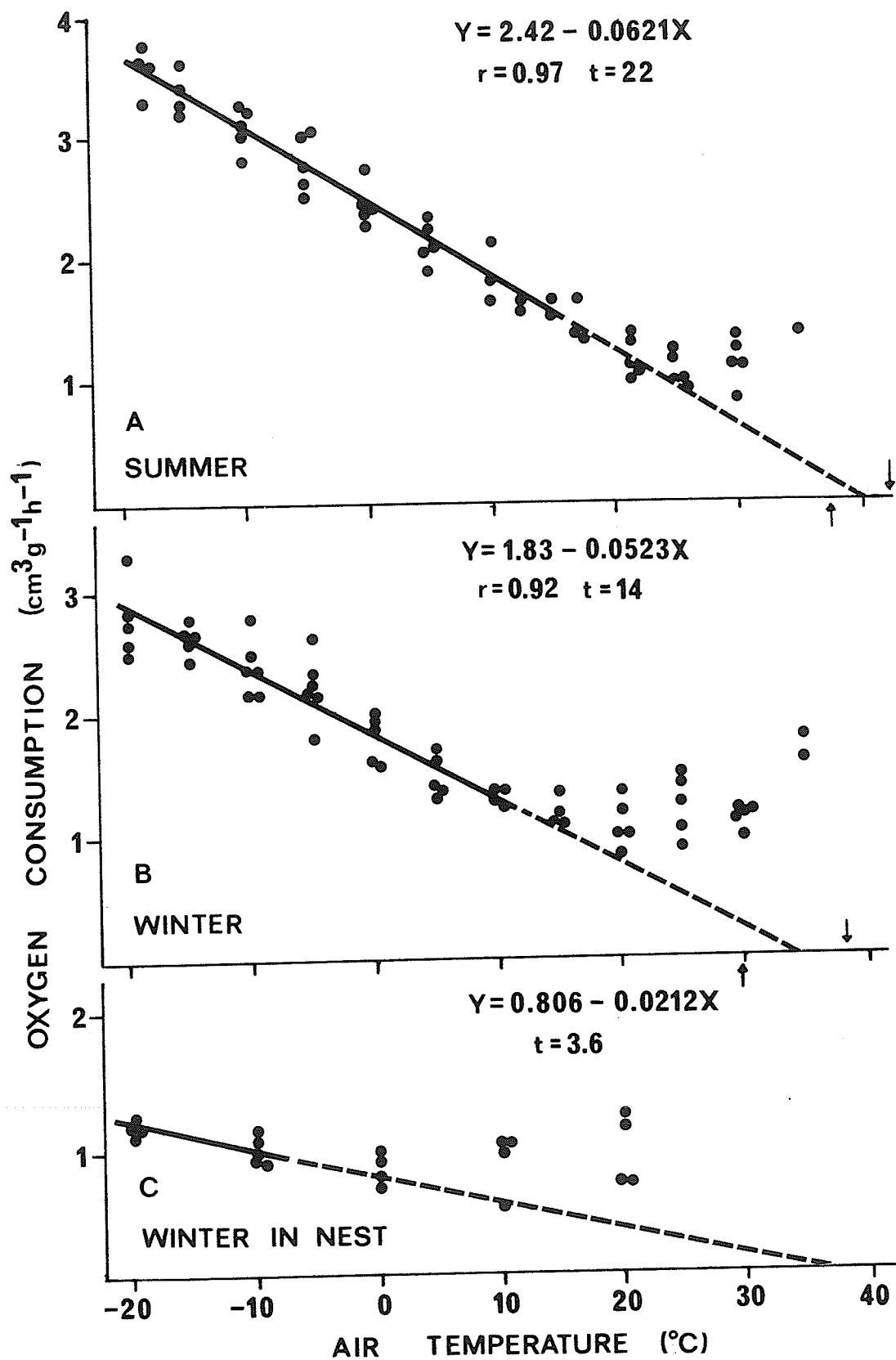
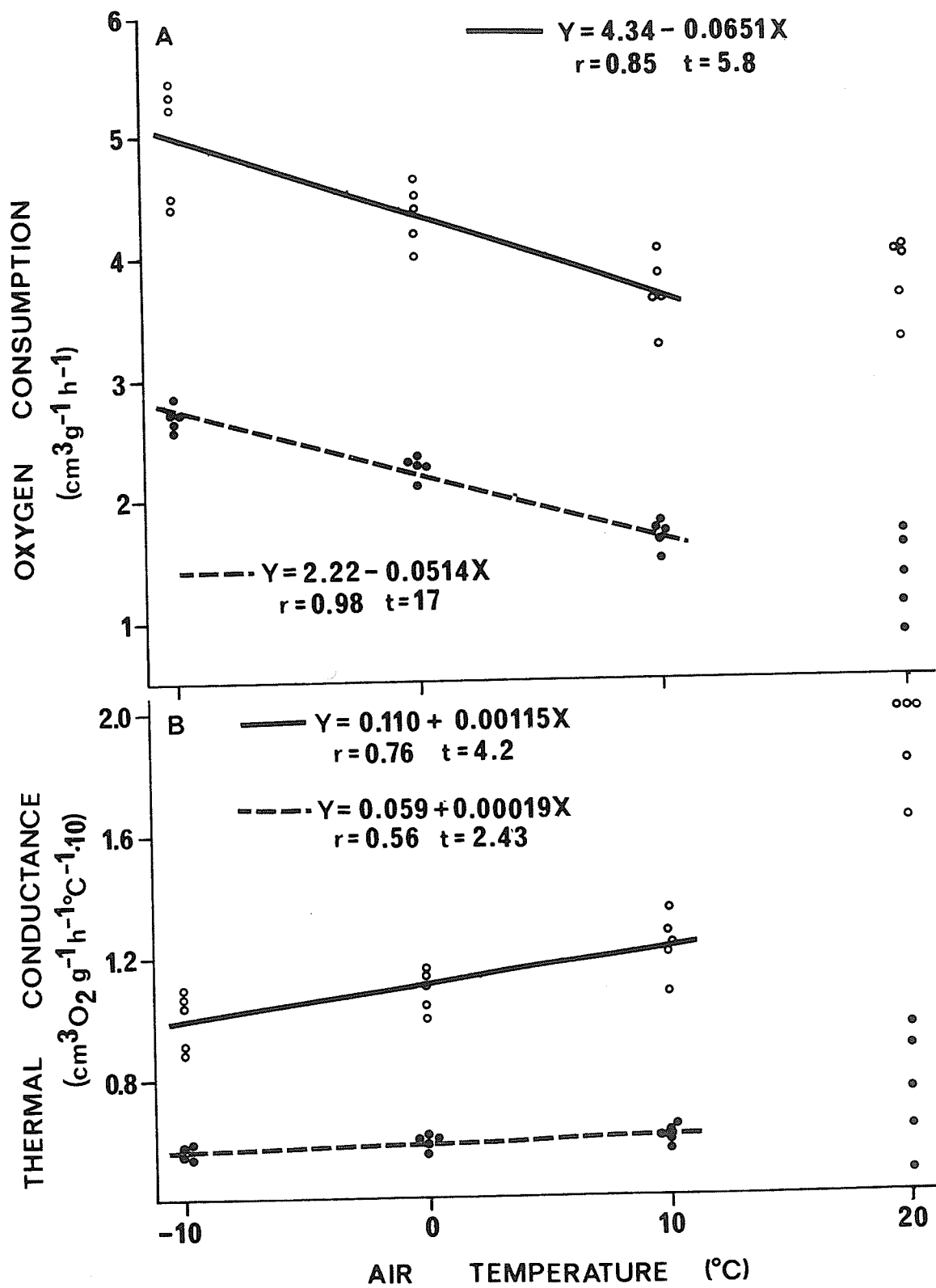


Figure 2. Oxygen consumption and total weight-specific thermal conductance of resting (closed circles) and exercising (open circles) animals at various air temperatures. Solid and broken lines represent least-square regressions over the range of air temperatures indicated by the regression lines. All regressions shown are statistically significant ( $P < 0.05$  for thermal conductance of resting animals,  $P < 0.01$  for the remainder).



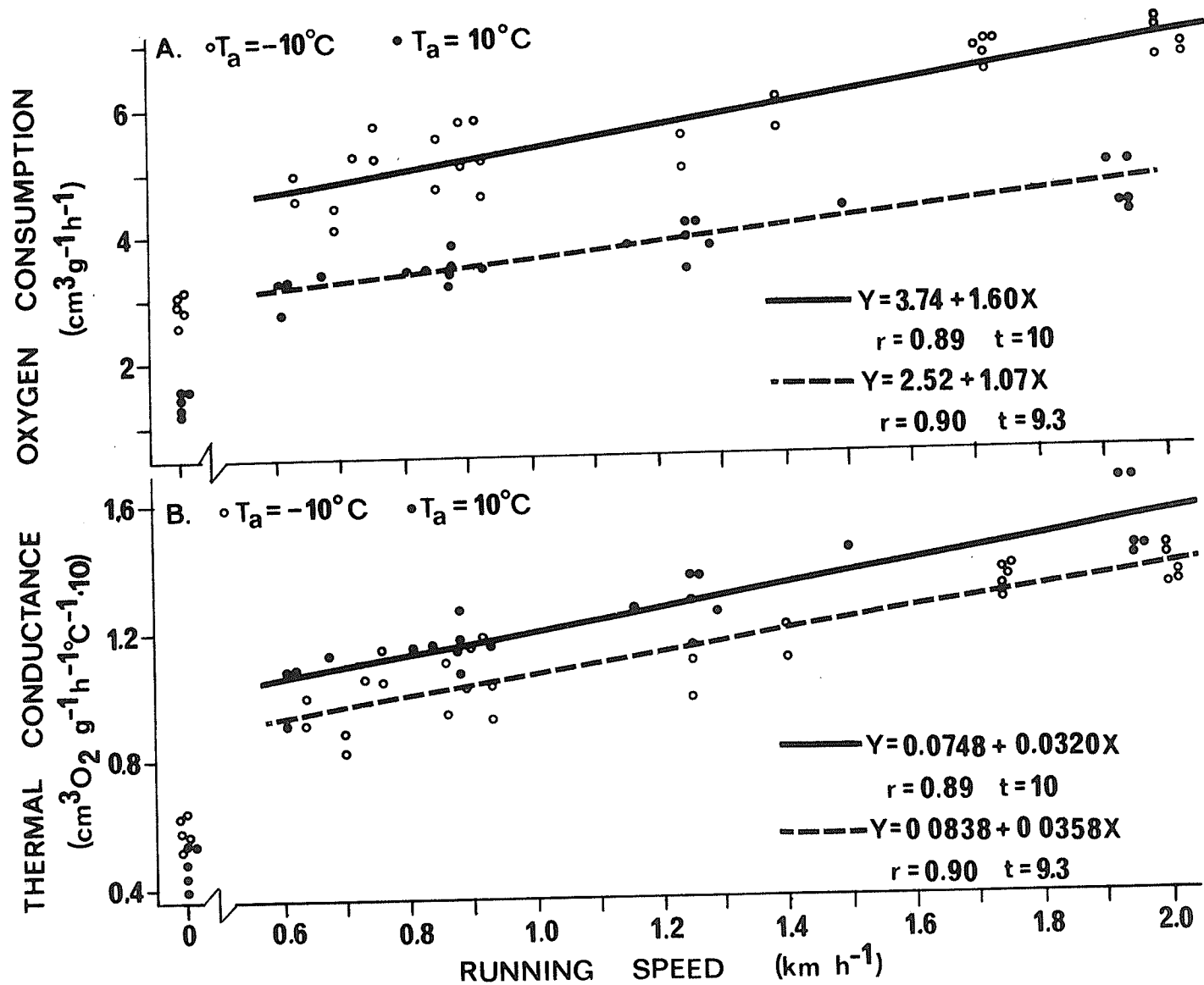


generated by exercise did not replace thermoregulatory heat production. Although the activity increment in  $\dot{V}_{O_2}$  appears greater at 20°C than at lower  $T_a$ , the data are too limited to assume substitution of heat generated by activity for thermoregulatory heat production in the  $T_a$  range 10 to 20°C.

Total thermal conductance of exercising animals was approximately twice that of resting animals in the  $T_a$  range -10 to 10°C (Fig. 2B). Furthermore, with decreasing  $T_a$ ,  $C$  of exercising animals decreases. In contrast and as expected,  $C$  changed only slightly over the  $T_a$  range -10 to 10°C in resting animals (Fig. 2B).

Mean  $\dot{V}_{O_2}$  increased linearly with increased running speed both at -10 and 10°C (Fig. 3A). The thermoregulatory increment in  $\dot{V}_{O_2}$ , defined as the difference between  $\dot{V}_{O_2}$  at 10°C and  $\dot{V}_{O_2}$  at -10°C at a given running speed, is illustrated by the difference in elevation at a given running speed between the regression lines of Fig. 3A. Over the entire range of running speeds tested, the thermoregulatory increment was greater than 1.47  $\text{cm}^3\text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$ , the thermoregulatory increment in animals resting in the exercise chamber (calculated from data illustrated in Fig. 3A). This complements the earlier observation that heat generated by activity did not substitute for thermoregulatory heat production. Furthermore, the thermoregulatory increment in  $\dot{V}_{O_2}$  may have increased with increased running speed as indicated by the

Figure 3. The effect of running speed on oxygen consumption and total weight-specific thermal conductance at 10 and -10°C. All regressions shown are statistically significant ( $P < 0.01$ ).



marginally significant difference ( $0.01 < P < 0.05$ ) between the slopes of the regressions of  $\dot{V}_{O_2}$  on  $T_a$  of animals running at  $-10$  and at  $10^\circ\text{C}$ .

Thermal conductance of exercising animals (Fig. 3B) increased with increased running speed at both  $-10$  and  $10^\circ\text{C}$ . This is consistent with the earlier observation of an increase in the thermoregulatory increment of  $\dot{V}_{O_2}$  with increased running speed. The regressions of  $C$  on running speed at  $-10$  and  $10^\circ\text{C}$  differ significantly in elevation ( $P < 0.01$ ), complementing the earlier observation (Fig. 2A) of a reduction in  $C$  of running animals with decreasing  $T_a$ .

## DISCUSSION

Physiological adaptation to seasonal cold by the red squirrel in Manitoba includes, in addition to seasonal change in resting body temperature (Part II), seasonal change in  $C$  and lower critical temperature.

A seasonal change of approximately 18.5% (see Table 1) in  $C$  of animals inside the metabolism chamber implies a reduction of similar magnitude in heat loss and minimum metabolic rate of animals inside the metabolism chamber at any given  $T_a$  less than the lower critical temperature. Thermal conductance depends on posture, substrate, wind, thermal radiation, and other factors. Consequently  $C$ , as measured in the metabolism chamber, cannot be directly applied to estimating thermoregulatory energy requirements of animals in an outdoor environment (Tracy 1972; Bakken 1976). However, it is reasonable to assume that seasonal change in  $C$  will result in a reduction in energy expenditure at low temperatures for winter-acclimatized animals as compared to summer-acclimatized animals under most environmental conditions.

Of potentially greater significance than seasonal change in  $C$  to the energy economy of the red squirrel is the use of a well-insulated nest. In the laboratory,  $C$ , and therefore heat loss at low  $T_a$  (less than  $-5^{\circ}\text{C}$ ), of a winter-acclimatized animal resting inside a nest was 40% (see Table 1) of  $C$  of a winter-acclimatized animal resting

outside a nest. Consequently, an animal inside a nest can maintain normal body temperature ( $37-39^{\circ}\text{C}$ , Part II) with only a slight increase in metabolic rate above the basal level at macroenvironmental temperatures as low as  $-20^{\circ}\text{C}$ . It is conceivable that a free-ranging squirrel with a nest in the more moderate subterranean or subnivean (see Pruitt 1957) microenvironment could be in a thermal neutral environment inside the nest at any naturally occurring winter macroenvironmental temperature. The extent to which animals in nature use subterranean or subnivean nests during the winter is not known but they have been observed to inhabit middens consisting of uneaten portions of cones from coniferous trees. In this regard it is interesting to note that an animal held in an outdoor enclosure during this study abandoned its nest box during the winter and constructed a subnivean nest.

The seasonal change in  $C$  and lack of seasonal change in minimum resting metabolic rate at thermal neutral  $T_a$  in the red squirrel are typical of small mammals exposed to outdoor environmental conditions (Hart 1957). Hart suggests the lack of seasonal change in resting metabolic rate at thermal neutral temperatures found in most rodents following cold-acclimatization, in contrast to increased metabolic rate following acclimation to continuous cold, indicates mammals in their natural environments do not usually require elevated metabolic rates for extended periods of time. The large proportion of time spent in the

nest at low  $T_a$  by the red squirrel (Part I) and the relatively minor increase in  $\dot{V}_{O_2}$  by animals in the nest at  $-20^{\circ}\text{C}$  (Part III) support Hart's suggestion with respect to the red squirrel. Under these conditions an elevated basal metabolic rate would be maladaptive as it would result in an unnecessary expenditure of energy during the time the animal is in the nest.

Increased C during exercise as compared to rest causes increased heat loss at low  $T_a$ . The increase in heat loss is apparently equal to or greater than the heat generated by the activity per se so that, even during exercise, a decrease in  $T_a$  causes an increase in metabolic rate. As a result heat generated by exercise does not appear to be utilized for thermoregulation. Therefore, activity at low  $T_a$  is energetically costly for the red squirrel as its metabolic rate must be equal to or greater than the sum of the energy cost of activity per se and the energy cost of thermoregulation. As the maximum metabolic rate elicited by exercise is not greater than that resulting from cold exposure in rodents (Hart 1971), it appears likely that over a certain range of  $T_a$  body temperature could be maintained during rest, but not during exercise. An observed decrease in body temperature in lemmings and rabbits during forced exercise but not during rest at low  $T_a$  (Hart and Heroux 1955) supports this suggestion.

The increase in thermal conductance of the red squirrel (nearly 50%, see Fig. 3B) with increased running speed



( $0.6-2.0 \text{ km h}^{-1}$ ) suggests the reduction in intensity of activity of the red squirrel at low  $T_a$  (Part I) may be a behavioural response necessary for thermoregulation during activity at low  $T_a$ . Possibly, at  $T_a$  sufficiently low to tax the thermoregulatory capabilities of the red squirrel, body temperature can be maintained during moderate but not during intense activity, since thermal conductance, and therefore heat loss is probably less during moderate as compared to intense activity. Over a certain range of low  $T_a$  the maximum intensity of activity consistent with the maintenance of body temperature will be related to  $T_a$ , as the maximum metabolic rate elicited by cold is not greater than that elicited by exercise and heat generated by exercise does not replace thermoregulatory heat production (Hart 1971). This may account, at least partially, for the correlation between intensity of activity and  $T_a$  in animals held in outdoor enclosures (Part I).

The results of this study support the concept that heat generated by activity does not replace thermoregulatory heat production. However, the model proposed by Wunder (1975), which assumes that the energy expenditure associated with thermoregulation and that due to activity are additive components of the total metabolic rate, may not adequately describe the combined effects of cold and exercise. Data from this study suggest both that the thermoregulatory component of metabolic rate during activity may be greater than during rest and that the thermoregulatory component increases with increasing intensity of activity.

Bligh and Johnson (1973) suggest metabolic rate during

periods of minimum activity should be considered the best possible estimate of standard metabolic rate of small wild animals as the conditions for measuring basal metabolic rate of these animals can rarely be attained. Metabolic rate based on  $\dot{V}_{O_2}$  in the nest at night is probably the best estimate of standard metabolic rate of the red squirrel. This value ( $0.92 \text{ cm}^3 \text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) does not differ greatly from basal metabolic rate ( $0.83 \text{ cm}^3 \text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) predicted on the basis of body weight from Kleiber's (1975) allometric equation. Irving et al. (1955), in contrast, estimated a basal metabolic rate for the red squirrel which exceeds the predicted value by 80%.

The seasonal change in thermal conductance of the red squirrel observed in this study is at variance with the observation by Irving et al. (1955) of no seasonal change in C of Alaskan red squirrels. Both this difference, and the difference between basal metabolic rate determined in this and Irving's study, may reflect a difference in measuring techniques or an intraspecific latitudinal difference. However, the observation of seasonal change in pelt insulation of another northern population (Northwest Territories, Canada) of red squirrels (Hart 1956) argues against the latter suggestion.

Values obtained in this study for metabolic rates of squirrels running at  $10^\circ\text{C}$  are higher than those obtained under similar conditions by Wunder and Morrison (1974). Unfortunately, information is not available to determine

whether previous and present results differ significantly and whether the difference is due to differences in acclimatization of the animals. Alternatively, the higher metabolic rates obtained in this study may be a consequence of the use of a smaller running chamber which may have altered the natural running gait of the animals.

## PART IV

Heart Rate as an Index of Energy Expenditure in the Red  
Squirrel (Tamiasciurus hudsonicus)

## INTRODUCTION

Energy requirements of the red squirrel (Tamiasciurus hudsonicus) are highly dependent on environmental temperature under laboratory conditions (Part III) which preclude normal behavioural responses to cold. However, considering the temperature-modulated behaviour reported in Part I, and the energetic consequences of such behaviour (Part III), such a dependence of energy requirements on temperature does not appear likely under natural conditions. Crude calculations of daily energy expenditure, based on the time-budget data summarized in Part I, and on the rates of oxygen consumption in a metabolism chamber during rest and activity at various temperatures (Part III), suggest daily energy expenditure may be completely independent of temperature in animals in large outdoor enclosures in a natural environment. Testing this hypothesis requires measurement of energy expenditure with a minimum of disturbance and restraint of the animals so that behavioural and physiological responses to temperature are not altered.

Radiotelemetry provides a technique for obtaining measurements without restraining or disturbing animals. A parameter readily measured by radiotelemetry, and showing some promise as an index of energy expenditure in mammals, is heart rate (Webster 1967; Morhardt and Morhardt 1971; Holter et al. 1976; Lund and Folk 1976; Kautz 1978). The physiological basis for the relationship between

energy expenditure (as measured by oxygen consumption) and heart rate can be expressed as:

$$\dot{V}_{O_2} = f_h \times SV \times (A-V)O_2 \text{ diff.} \quad (\text{Bartholomew 1977a})$$

where  $\dot{V}_{O_2}$  is rate of oxygen consumption,  $f_h$  is heart rate, SV is stroke volume, and  $(A-V)O_2$  diff. is the difference in oxygen concentration between arterial and venous blood. Because rate of oxygen delivery to the body tissues by the blood is a function of 3 variables, a causal relationship between  $\dot{V}_{O_2}$  and  $f_h$  need not exist. Moreover, a relationship, if found to occur, need not be stable, but could vary with the physiological state of the animal or the type of stimulus causing increased  $\dot{V}_{O_2}$ . Consequently, the relationship between  $\dot{V}_{O_2}$  and  $f_h$  can possibly vary both among individuals of a given species, as well as in individuals from day to day.

The objective of this study was to determine whether  $f_h$  can be used to estimate energy requirements of the red squirrel, and if so, under what conditions and with what limitations.

## MATERIALS AND METHODS

Animal Care

Animals were trapped in the vicinity of Winnipeg, Canada ( $50^{\circ}06'N$ ;  $98^{\circ}20'W$ ). They were held in outdoor enclosures in a natural environment as described in Part I for 1-6 months before being brought into the laboratory for experiments. In the laboratory, before and during experiments, the animals were held in a controlled environment room with the temperature adjusted to approximately mean normal outdoor temperature down to the minimum temperature ( $0$  to  $5^{\circ}C$ ) of the animal-holding room. Exceptions were made for the first two days following surgery when the animals were held for a few hours at  $30^{\circ}C$  followed by 1-2 days at  $20^{\circ}C$ . Photoperiod was adjusted weekly according to outdoor photoperiod.

Telemetry of  $f_h$ 

The telemetry system for measuring  $f_h$  consisted of a model TS2-ET FM transmitter and model D2-1C demodulator (Biotelemetry Systems Inc., Rush, New York) and a model CF-400A Sony receiver. The transmitter, along with ECG leads and batteries, weighed about 14 g. The leads supplied by the manufacturer for connecting ECG electrodes to the transmitter were too inflexible to permit unimpaired movement by the animal when implanted subcutaneously. Leads that were flexible, resistant to corrosion and

mechanical breakage, and non-toxic to the animal were constructed using steel piano wire (diameter = 0.13 mm, XLO brand music wire, Johnson Steel and Wire Co., Worcester, Mass.). The wire was inserted into a polyethylene catheter (PE10; inside diameter = 0.28 mm, outside diameter = 0.61 mm; Clay-Adams, New York) filled with a paraffin and beeswax mixture (1:1 ratio) and wound into a tight helix (diameter = 1.8 mm). The ends of the lead were soldered to both electrode and transmitter. The soldered connections and the entire transmitter were coated first with a layer of paraffin and beeswax (1:1 ratio) and then with silicone rubber (382 Medical Grade Elastomer, Dow Corning). The wax prevented penetration by body fluids; the silicone rubber eliminated adherence of body tissue to the transmitter and provided a strong flexible seal at the point of entry of the ECG leads into the transmitter.

The transmitter was implanted in the abdominal cavity through a mid-ventral incision with the animal under Nembutal anaesthesia (40-45 mg kg<sup>-1</sup>, interperitoneal). Along a mid-ventral line from the incision to the anterior end of the sternum the skin was separated from underlying tissue with a blunt probe and scissors for insertion of ECG leads. Two silver/silver chloride electrodes, placed subcutaneously, mid-ventrally, and at anterior and posterior ends of the sternum, were sutured to underlying tissue with surgical silk through small skin incisions. With this electrode placement a strong ECG signal, free of muscle



artifacts, was obtained even during exercise.

#### Simultaneous Measurement of $\dot{V}_{O_2}$ and $f_h$

The relationship between  $\dot{V}_{O_2}$  and  $f_h$  was determined in experiments conducted over approximately a 1-year period using summer-acclimatized animals (June 13 to September 1), winter-acclimatized animals (November 24 to March 10), and animals whose state of acclimatization was considered as uncertain (May 17-20).

The relationship between  $\dot{V}_{O_2}$  and  $f_h$  was examined in animals during rest or minimal activity (hereafter referred to as rest experiments) and during exercise (hereafter referred to as exercise experiments). Temperature was altered to cause variation in  $\dot{V}_{O_2}$  and  $f_h$  in resting animals. The range over which  $\dot{V}_{O_2}$  and  $f_h$  were measured varied between animals as indicated in Fig. 1.

For the duration of rest and exercise experiments mean  $f_h$  and  $\dot{V}_{O_2}$  were determined during consecutive 6-min periods. Mean  $f_h$  in each 6-min period was calculated from 5 counts, each of 0.1-min duration, taken at 1.2-min intervals with an automatic rate counter connected with the demodulator. The QRS wave of the cardiac cycle triggered both the rate counter and a flashing light. With experience and careful attention to the light it was evident whether heart beats were being missed or radio noise was being counted. When this occurred the measurement was rejected, the system adjusted, and the measurement immediately repeated.

Mean  $\dot{V}_{O_2}$  in each 6-min period was calculated from 5 point values at 1.2-min intervals taken from a continuous recording of fractional oxygen concentration in the outflow air from the metabolism chamber. Measurements of fractional oxygen concentration were obtained with the system described in Part III, except that inflow as well as outflow air was dried.

Twenty one animals, 17 male and 4 female, with a mean weight of 224 g (range = 181-281 g) were used in rest experiments. The animals were placed singly in a metabolism chamber consisting of a 1 gallon (4.5 l) metal paint can. As these were not measurements requiring minimum  $\dot{V}_{O_2}$  and since it was desirable to simulate as nearly as possible the conditions under which  $f_h$  would ultimately be used to estimate  $\dot{V}_{O_2}$ , food and water were available to the animals during the experiments. In each experiment, lasting 4-6 h, temperature was varied from -20 to 30°C or vice-versa by increasing or decreasing the temperature setting of the controlled environment room containing the metabolism chamber by 5°C at 20-35 min intervals.

Eleven of the animals used in the preceding experiments, 8 male and 3 female, with a mean weight of 222 g (range = 181-251 g), were used in exercise experiments. These experiments were performed with the animal in a treadmill metabolism chamber identical to that used in Part III, except that a Dayton model 3M50 0.1-horsepower (75 watt) electric motor was used to power the treadmill.

Food was available to the animals up to the time of the experiments but neither food nor water was available to the animals during the experiments as they were of shorter duration than the preceeding experiments and food in the treadmill would have interfered with its operation. Within the constraints imposed by the minimum operating temperature of the treadmill ( $-10^{\circ}\text{C}$ ) and the temperature at which the animals could potentially be heat stressed during exercise ( $20^{\circ}\text{C}$ ), air temperature was varied through the range of ambient temperatures occurring outdoors at the time of the experiments. In each 2 to 3-h experiment, the animal was forced to run continuously for 12-min periods at a speed of  $16.8 \text{ cm sec}^{-1}$  alternated with 12-min rest periods. Data from both resting and exercising periods were included in the analysis.

Both rest and exercise experiments were repeated on different days within a 7-day period with each of 4 of the animals in order to ascertain the extent of day to day variation in the relationship between  $\dot{V}_{\text{O}_2}$  and  $f_h$  within individual animals.

#### Calculation of Oxygen Consumption

Mean  $\dot{V}_{\text{O}_2}$  in each 6-min period was calculated from fractional oxygen concentration in the outflow air from the metabolism chamber. Since  $\dot{V}_{\text{O}_2}$  could and did vary during measurement periods, and since equations suitable for calculation of  $\dot{V}_{\text{O}_2}$  measured in a metabolism chamber

under this condition using open flow respirometry are not available in the literature, an equation appropriate for calculation of  $\dot{V}_{O_2}$  under the experimental conditions of this study was derived (Appendix II). This equation takes the form:

$$\bar{\dot{V}}_{O_2} = \frac{(\bar{F}_{I_{O_2}} - \bar{F}_{E_{O_2}}) \cdot \dot{V}_I - \Delta V_O / t}{1 - \bar{F}_{E_{O_2}} \cdot (1 - RQ)}$$

where

$\bar{\dot{V}}_{O_2}$  = mean rate of oxygen consumption

$\bar{F}_{I_{O_2}}$  = fractional oxygen concentration of dry inflow air

$\bar{F}_{E_{O_2}}$  = mean fractional oxygen concentration of dry outflow air

$\dot{V}_I$  = inflow rate of dry air

$t$  = time of measurement (6 min)

$RQ$  = respiratory quotient (assumed to be 0.85)

The change in oxygen content ( $\Delta V_O$ ) of the metabolism chamber during the measurement period ( $t$ ) was calculated from:

$$\Delta V_O = \Delta F_{E_{O_2}} \cdot V \cdot (1 - P_W/B)$$

where

$\Delta F_{E_{O_2}}$  = change in fractional oxygen content of the outflow air during the measurement period ( $t$ )

$V$  = volume of the metabolism chamber

$B$  = barometric pressure

$P_W$  = water vapour pressure in the metabolism chamber

Water vapour pressure in the metabolism chamber was assumed to be 50% of saturated  $P_W$  approximated within the temperature range used by the formula:

$$P_W = 4.7304 \times 1.0667^{T_c}$$

where  $T_c$  is temperature in the metabolism chamber.

All gas flow and volume measurements were corrected to standard temperature and pressure. An assessment of potential errors due to assumptions made regarding  $RQ$  and  $P_W$  is provided in Appendix II.

#### Determination of Accuracy of Prediction

An indication of the magnitude of random error associated with  $\dot{V}_{O_2}$  estimated from  $f_h$  can be obtained from the standard error of prediction calculated for each regression. However, this standard error is specific to the  $f_h$  sampling regime used in determining the regression. This sampling regime, though well-suited to determining the regression of  $\dot{V}_{O_2}$  on  $f_h$ , was difficult to implement in long-term determinations (e.g. 24 h) of energy expenditure due to the large number and frequency of  $f_h$  measurements involved. This problem was compounded when two individuals were monitored simultaneously as in this study. The magnitude of error when using a less intense rate of sampling was therefore examined using 6 animals weighing on average 231 g (range = 208-255 g).

To assess the accuracy with which mean  $\dot{V}_{O_2}$  can be estimated from  $f_h$  over periods of 1 h and 8-12 h,  $f_h$  was

determined for 0.1-min periods at 5 min intervals and used to estimate  $\dot{V}_{O_2}$ . At the same time  $\dot{V}_{O_2}$  of each animal was measured in the 1-gallon (4.5 l) metabolism chamber. Mean hourly  $\dot{V}_{O_2}$  as well as mean  $\dot{V}_{O_2}$  over 8 or 12 h were calculated from point values, taken at 2.4-min intervals, of fractional oxygen concentration in the outflow air from the metabolism chamber. Temperature in the metabolism chamber varied between 30 and  $-20^{\circ}\text{C}$  as previously described. Food and water were available during the experiment.

#### Treatment of Data and Statistical Analysis

Linear regressions were fitted to  $\dot{V}_{O_2}$  and  $f_h$  data for each animal (e.g. Fig. 1). Where examination of the data indicated that a linear model provided a poor fit, curvilinear regressions of the form:

$$\begin{aligned}\dot{V}_{O_2} &= A + b_1 f_h + b_2 f_h^2 \\ \log \dot{V}_{O_2} &= A + b \cdot (\log f_h) \\ \log \dot{V}_{O_2} &= A + b \cdot f_h \\ \dot{V}_{O_2} &= A + b \cdot (\log f_h)\end{aligned}$$

were calculated. The best fitting regression, according to the correlation coefficient, was selected. In all cases where a quadratic regression provided the best fit, addition of the quadratic component gave a significant reduction in variance ( $P < 0.05$ , Snedecor and Cochran 1967).

The correlation coefficient and standard error of estimate were calculated for the regression of  $\dot{V}_{O_2}$  on  $f_h$  of each animal. For animals where the measurement of  $\dot{V}_{O_2}$

and  $f_h$  was repeated on different days the correlation coefficient and standard error of estimate were based on pooled data for each individual. Standard errors are reported as minimum standard error (occurring at the mean  $f_h$ ) and maximum standard error within the range of measured  $f_h$  for each animal. Standard errors for the means of 2 or more estimated values were calculated according to Acton (1959).

Both variation among individuals and day to day variation within individuals with respect to the regression of  $\dot{V}_{O_2}$  on  $f_h$  were observed. One-way analysis of variance was applied to test whether differences between individuals were real or merely a reflection of daily variation within individuals. This analysis was carried out on 4 animals for which regressions from 2 different days were available for both rest and exercise. The analysis was done first on the slopes of the regressions, and where these did not differ significantly between individuals, on the Y-intercepts of the regressions.

To test the equivalence of regressions of  $\dot{V}_{O_2}$  on  $f_h$  obtained during rest and exercise, t-tests were performed on the slopes of the linear regressions. A paired t-test was used for animals for which linear regressions from both exercise and rest experiments were available, while a simple t-test was used for comparing means including data from animals for which regressions were obtained only during rest or only during exercise.

Even though the regressions of  $\dot{V}_{O_2}$  on  $f_h$  may differ among individuals, a general regression for the species, obtained by pooling data from a number of individuals may be useful when estimating mean  $\dot{V}_{O_2}$  of a number of individuals from  $f_h$ . As neither  $\dot{V}_{O_2}$  nor  $f_h$  are weight-independent variables (Stahl 1967), appropriate corrections of these variables for body-weight ( $W$ ) are necessary when data from individuals differing in  $W$  are to be pooled. Rate of oxygen consumption of mammals is conventionally corrected for  $W$  by dividing  $\dot{V}_{O_2}$  by  $W$  or by  $W^{0.75}$ ; division by  $W^{0.75}$  is recommended by Hart (1971) as it may reduce variability. Because  $f_h$  of mammals is proportional to  $W^{-0.25}$  (Stahl 1967) division of  $f_h$  by this term would appear to be an appropriate correction for  $W$ . Analysis of pooled data was carried out using the variables  $\dot{V}_{O_2}$ ,  $\dot{V}_{O_2} W^{-1}$ ,  $\dot{V}_{O_2} W^{-0.75}$  and  $f_h$ ,  $f_h W^{0.25}$ . The weight corrections providing the best correlation between oxygen consumption and heart rate were used in pooling data.



## RESULTS AND DISCUSSION

A linear relationship between  $\dot{V}_{O_2}$  and  $f_h$  was observed in 15 of the 21 animals tested at rest (Table 1, Fig. 1A). Of the six animals showing a curvilinear relationship (Table 1, Fig. 1B), 5 took the form:

$$\dot{V}_{O_2} = A + b_1 f_h + b_2 f_h^2$$

and one took the form:

$$\log \dot{V}_{O_2} = A + b \cdot f_h$$

In all 11 animals tested during exercise  $\dot{V}_{O_2}$  was linearly related to  $f_h$  (Table 1; Fig. 1C).

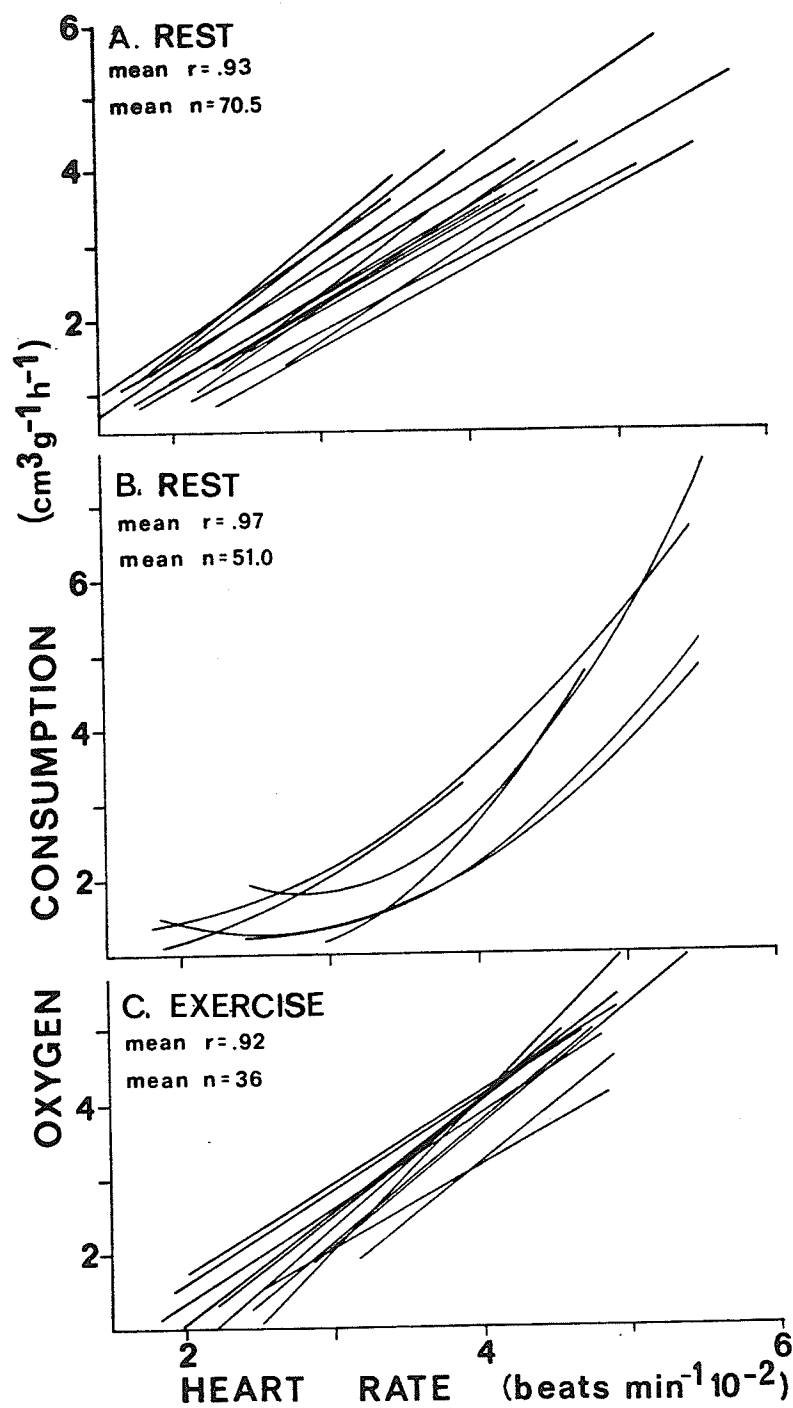
During rest, non-linear regressions of  $\dot{V}_{O_2}$  on  $f_h$  occurred only in summer-acclimatized animals (Table 1); the proportion of animals with non-linear regressions was significantly greater ( $P = 0.012$ , Fisher-Yeates Test, Finney 1948) in summer- than in winter-acclimatized animals. In summer acclimatized animals, non-linear regressions occurred only in animals at rest (Table 1); the proportion of animals with non-linear regressions was significantly greater ( $P = 0.037$ , Fisher-Yeates Test, Finney 1948) in resting than in exercising animals. Apparently, lack of linearity is related both to the state of acclimatization and the stimulus causing increased  $\dot{V}_{O_2}$ . A linear relationship during exercise and a curvilinear relationship during rest (e.g. Fig. 4A and C) in three animals reinforces the latter conclusion.

Although  $\dot{V}_{O_2}$  and  $f_h$  were strongly correlated within

Table 1. Number of animals with linear and non-linear regressions of  $\dot{V}_{O_2}$  on  $f_h$  classified according to seasonal acclimatization and stimulus causing increased  $\dot{V}_{O_2}$ .

	Linear Regressions	Non-linear Regressions
Summer		
Exercise	6	0
Rest	5	6
Winter		
Exercise	5	0
Rest	9	0
Uncertain		
Rest	1	0

Figure 1. Regressions of oxygen consumption ( $\dot{V}_{O_2}$ ) on heart rate ( $f_h$ ) in individual animals during rest (A and B) and exercise (C). Each regression is based on simultaneous measurement of mean  $\dot{V}_{O_2}$  and  $f_h$  during successive 6-min intervals. Mean n indicates the mean of the number of measurements contributing to each regression.



individuals (Table 2), standard errors of estimate were relatively large, particularly when  $\dot{V}_{O_2}$  was not linearly related to  $f_h$ , indicating that a large random error will be associated with estimates of  $\dot{V}_{O_2}$  from single measurements of  $f_h$ . However, this does not preclude accurate estimation of  $\dot{V}_{O_2}$  based on the mean of many measurements.

A potentially more serious error in estimated  $\dot{V}_{O_2}$  is introduced by daily variation in the slope and/or elevation of the regression of  $\dot{V}_{O_2}$  on  $f_h$  (Figs. 2 and 3), as  $\dot{V}_{O_2}$  estimated from  $f_h$  on any day other than the day on which the regression was established, may vary from true  $\dot{V}_{O_2}$  by a non-random or systematic component in addition to the random component. The systematic error component cannot be reduced by increasing the number of measurements contributing to a mean estimated  $\dot{V}_{O_2}$ . Regressions of  $\dot{V}_{O_2}$  on  $f_h$  established on different days for the same individuals (Fig. 3) provide some indication of the potential systematic error which can occur in estimated  $\dot{V}_{O_2}$ . At the minimum and maximum values of  $f_h$  common to both regressions mean differences between the two regressions are 0.34 (range = 0.21-0.47) and 0.59 (range = 0.02-1.62)  $\text{cm}^3\text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$  in regressions for resting animals and 0.40 (range = 0.12-0.75) and 0.54 (range = 0.22-0.76)  $\text{cm}^3\text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$  in regressions for exercising animals.

It can be assumed that regressions of  $\dot{V}_{O_2}$  on  $f_h$  for an individual obtained on different days vary randomly about some average line obtained from measurement of  $\dot{V}_{O_2}$  on  $f_h$

Table 2. Correlation coefficients (r) and standard errors of estimate ( $s_{\hat{y}}$ ) for  $\dot{V}_{O_2}$  versus  $f_h$  within individual animals. Data are mean and range.

	Rest	Exercise
r	0.94 (0.83-0.99) <sup>a</sup>	0.94 (0.72-0.97) <sup>b</sup>
Minimum $s_{\hat{y}}$ (cm <sup>3</sup> O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )		
linear regressions	0.29 (0.17-0.61) <sup>c</sup>	0.38 (0.20-0.57) <sup>b</sup>
curvilinear regressions	0.36 (0.23-0.79) <sup>d</sup>	
Maximum $s_{\hat{y}}$ (cm <sup>3</sup> O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )		
linear regressions	0.30 (0.18-0.62) <sup>c</sup>	0.41 (0.21-0.61) <sup>b</sup>
curvilinear regressions	0.91 (0.58-2.02) <sup>d</sup>	

<sup>a</sup> 21 animals, mean of 64.6 measurements per animal

<sup>b</sup> 11 animals, mean of 36.0 measurements per animal

<sup>c</sup> 15 animals, mean of 70.5 measurements per animal

<sup>d</sup> 6 animals, mean of 51.2 measurements per animal

Figure 2. Regressions of oxygen consumption ( $\dot{V}_{O_2}$ ) on heart rate ( $f_h$ ) in four randomly selected individuals during rest or exercise. Different symbols for a particular animal represent measurements made on different days. Each point represents mean  $\dot{V}_{O_2}$  and  $f_h$  during a 6-min interval.

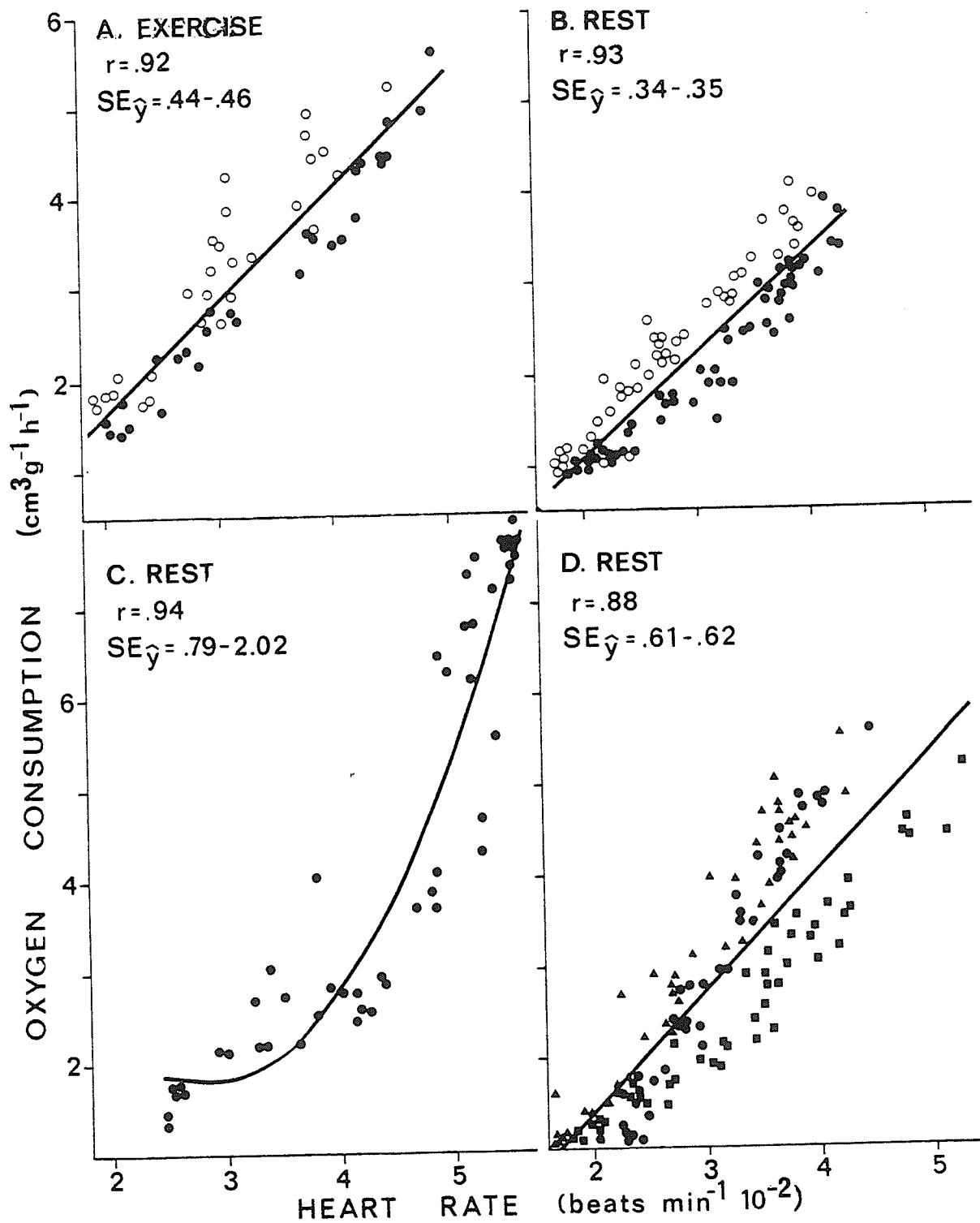
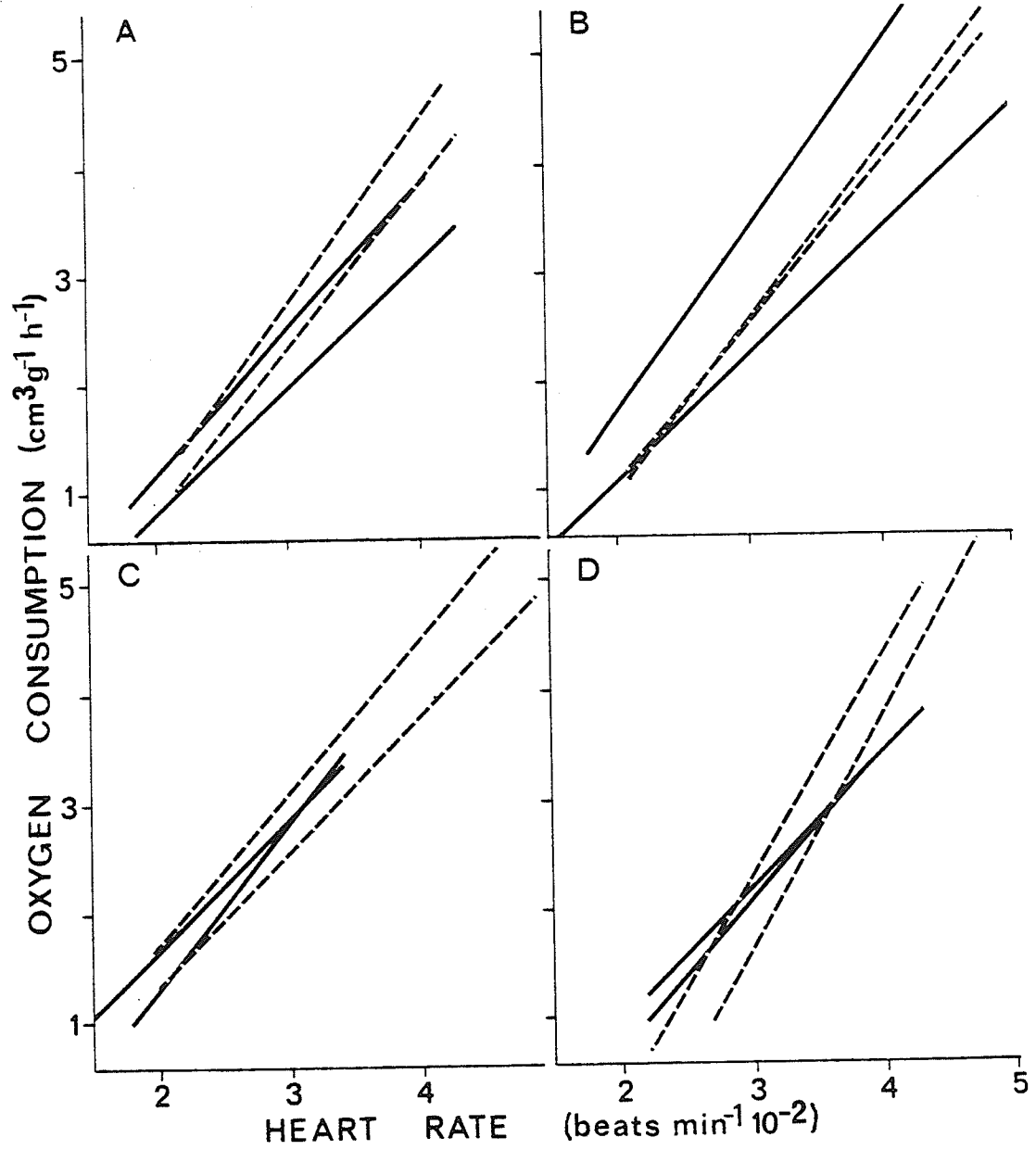




Figure 3. Regressions of oxygen consumption ( $\dot{V}_{O_2}$ ) on heart rate ( $f_h$ ) in four individuals (A-D) during rest (solid line) and exercise (broken line). Each regression line for a given animal is based on data from a different day.



on many days. Therefore, systematic error in estimating  $\dot{V}_{O_2}$  resulting from day to day variation in the regression will usually be less when a regression based on more than one days' data is used to estimate  $\dot{V}_{O_2}$  on a given day or when a regression based on a single day's data is used to estimate mean  $\dot{V}_{O_2}$  over more than one day. It should be almost, if not entirely, eliminated when mean  $\dot{V}_{O_2}$  over several days is estimated using a regression of  $\dot{V}_{O_2}$  on  $f_h$  based on several days' data.

Variability about the regression of  $\dot{V}_{O_2}$  on  $f_h$  is minimized in the case of linear regressions when pooling data from animals differing in body-weight if the weight corrected variables  $\dot{V}_{O_2} W^{-1}$  and  $f_h W^{0.25}$  are used (Table 3). Therefore these variables were used whenever data from different animals were compared or pooled.

The application of heart rate to the estimation of energy expenditure would be greatly simplified if the relationship between  $\dot{V}_{O_2}$  and  $f_h$  were independent of whether increased  $\dot{V}_{O_2}$  is elicited by cold or exercise. The difference in the form of the regressions derived from rest and exercise experiments suggests that such independence cannot be assumed. Further analysis partially supports this conclusion. The mean slope of the regressions obtained during rest ( $n = 15$ ) differed significantly ( $P < 0.05$ , t-test) from the mean slope of regressions obtained during exercise ( $n = 11$ ). Both rest and exercise data showing a linear relationship between  $\dot{V}_{O_2}$  and  $f_h$  were

Table 3. Correlation coefficients of  $\dot{V}_{O_2}$  versus  $f_h$  with pooled data from different animals with and without correction for body-weight (W)<sup>a</sup>

Independent Variables	Dependent Variables					
	Rest			Exercise		
	$\dot{V}_{O_2}$	$\dot{V}_{O_2} W^{-1}$	$\dot{V}_{O_2} W^{-0.75}$	$\dot{V}_{O_2}$	$\dot{V}_{O_2} W^{-1}$	$\dot{V}_{O_2} W^{-0.75}$
Summer-linear						
$f_h$	0.75	0.87	0.84	0.78	0.88	0.86
$f_h W^{0.25}$	0.80	0.89	0.87	0.84	0.89	0.89
Summer-curvilinear						
$f_h$	0.91	0.90	0.90			
$f_h W^{0.25}$	0.90	0.86	0.87			
Winter-linear						
$f_h$	0.67	0.76	0.74	0.88	0.90	0.90
$f_h W^{0.25}$	0.70	0.78	0.76	0.89	0.91	0.90
Overall <sup>b</sup>						
$f_h$	0.75	0.82	0.81	0.84	0.89	0.88
$f_h W^{0.25}$	0.77	0.82	0.81	0.87	0.90	0.90

<sup>a</sup> Sample sizes (number of animals, number of measurements): rest, summer, linear = 5, 345; rest, summer curvilinear = 6, 307; exercise, summer, linear = 4, 162; rest, winter, linear = 9, 599; exercise, winter, linear = 5, 176; rest, overall = 21, 1361; exercise, overall = 11, 398.

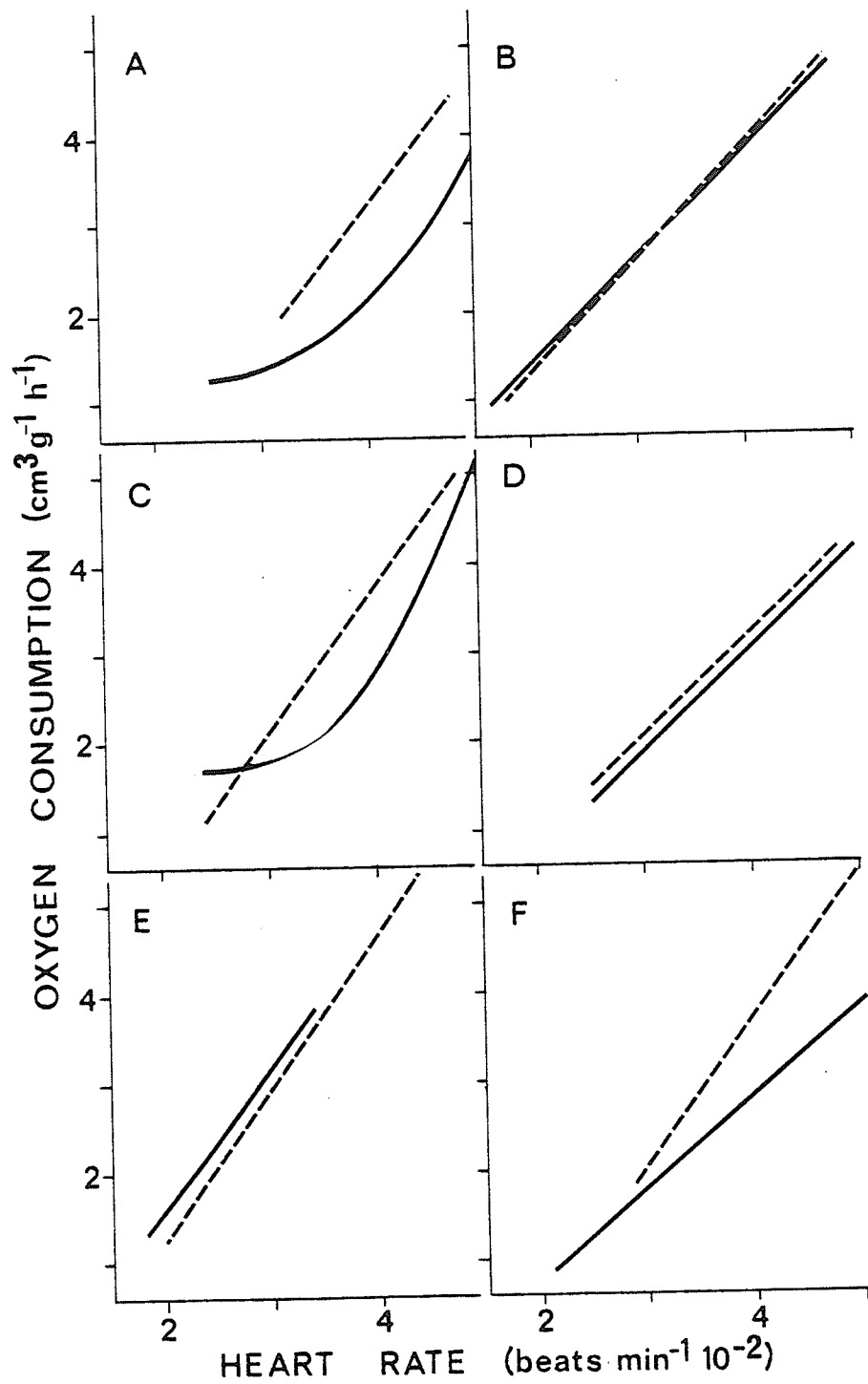
<sup>b</sup> Includes data from animals with a curvilinear relationship between  $\dot{V}_{O_2}$  and  $f_h$  and animals tested in May.

available for 8 animals (Figs. 3 and 4B, D, E, and F). In these animals the slopes of the regressions obtained from rest experiments did not differ significantly ( $P > 0.05$ , paired t-test) from those obtained from exercise experiments.

A different relationship between  $f_h$  and  $\dot{V}_{O_2}$  during exercise than during rest has been observed in white-footed mice (Segrem and Hart 1967). A higher  $f_h$  occurred at the same  $\dot{V}_{O_2}$  when increased  $\dot{V}_{O_2}$  was elicited by cold as compared to exercise. In contrast, Popovic et al. (1969) did not find a marked difference between the effects of exercise and cold on  $f_h$ , A-V diff., and stroke volume of rats.

The amount of time and effort required in using  $f_h$  as an index of energy expenditure would be reduced if a regression of  $\dot{V}_{O_2}$  on  $f_h$  applicable to all animals of the species could be established, thus eliminating the need to determine the regression in each animal used. However, the data suggest that such a regression would be of limited usefulness in estimating  $\dot{V}_{O_2}$  of individual animals due to differences between animals in the regression of  $\dot{V}_{O_2}$  on  $f_h$ . Slopes and Y-intercepts of the regressions of the 4 animals where replicated regressions from 2 different days were available (Fig. 3) did not differ significantly ( $P > 0.05$ , one-way analysis of variance) between animals during rest. However, during exercise the slopes of the regression differed significantly ( $P < 0.05$ ) between animals.

Figure 4. Regressions of oxygen consumption ( $\dot{V}_{O_2}$ ) on heart rate ( $f_h$ ) in 6 individuals (A-F) during rest (solid line) and exercise (broken line).



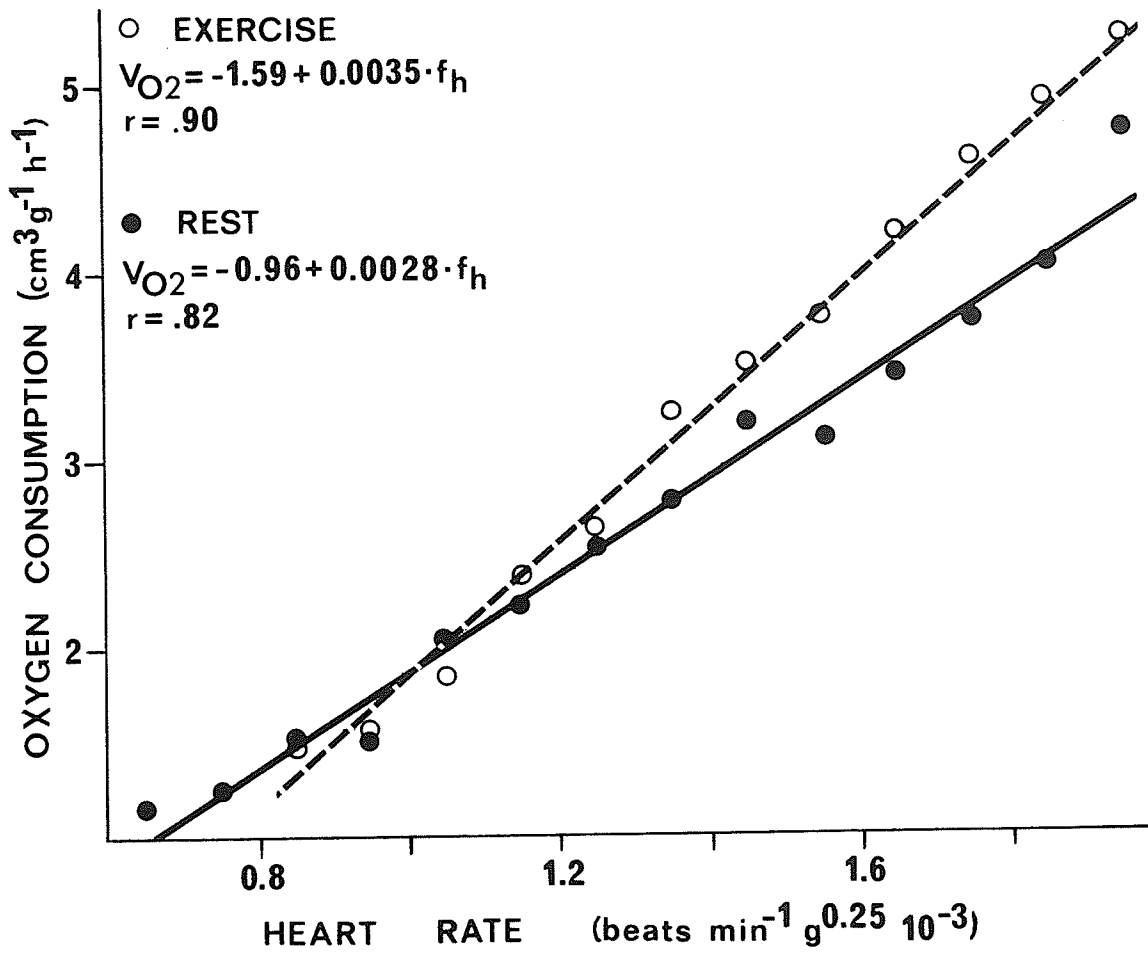
A further observation indicating the need for caution in the use of a general regression for the species is the difference between animals in the form of the regression of  $\dot{V}_{O_2}$  on  $f_h$  during rest (Figs. 2 and 4, Table 1).

Variation in the relationship between  $\dot{V}_{O_2}$  and  $f_h$  between individuals of a species has also been observed to occur in man (Berggren and Christensen 1950; Booyens and Hervey 1960; Malhotra *et al.* 1963), black-tailed prairie dogs (Lund and Folk 1976), mule deer (Kautz 1978), white-tailed deer (Holter *et al.* 1976), sheep (Webster 1967), and several rodent species (Morhardt and Morhardt 1971) indicating that this may be a widespread phenomenon.

In spite of variability between individuals of a species in the relationship between  $\dot{V}_{O_2}$  and  $f_h$ , a general regression for the species may be useful if the error incurred in estimating  $\dot{V}_{O_2}$  of an individual using this regression is within acceptable bounds or if mean  $\dot{V}_{O_2}$  of a number of individuals is being estimated. The regressions of  $\dot{V}_{O_2}$  on  $f_h$  for the red squirrel during both rest and exercise, based on pooled data from all animals tested, are illustrated in Fig. 5. Standard error of estimate during rest over the range in  $f_h$  of 140-560 beats  $\text{min}^{-1}$  for an animal weighing 223 g, the mean weight of animals used in establishing this regression, is  $0.61 \text{ cm}^3 \text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$  for a single estimated value and  $0.16 \text{ cm}^3 \text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$  for the mean of 10 estimated values. During exercise, standard error over the range in  $f_h$  of 220-550 beats  $\text{min}^{-1}$  is  $0.51 \text{ cm}^3 \text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$  for a single estimated value and



Figure 5. Regressions of oxygen consumption ( $\dot{V}_{O_2}$ ) on heart rate ( $f_h$ ) during rest and exercise. The regressions are based on pooled data from 21 animals for rest and 11 animals for exercise. The regression lines are based on the original measured values and the points shown are mean  $\dot{V}_{O_2}$  over  $f_h$  intervals of  $0.1 \text{ beats min}^{-1} \text{ g}^{0.25} 10^{-3}$ .



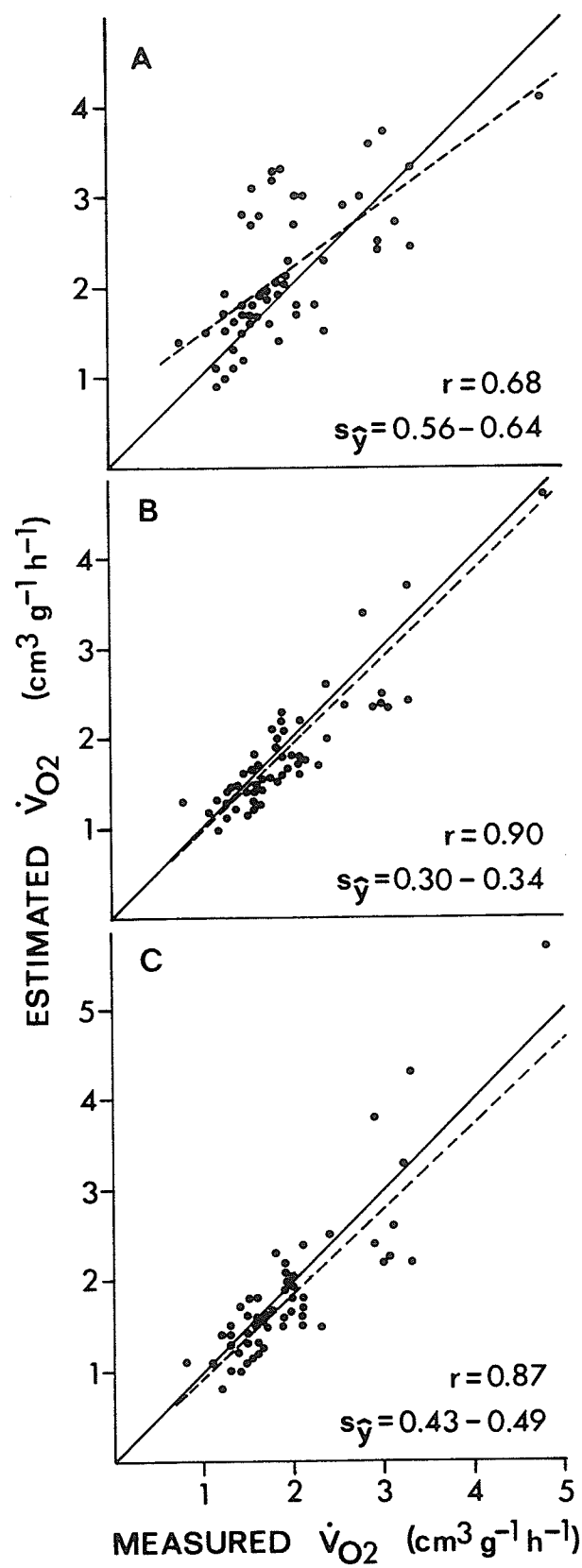
$0.19 \text{ cm}^3 \text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$  for the mean of 10 estimated values.

Mean hourly  $\dot{V}_{\text{O}_2}$  of resting animals, estimated in 6 animals from  $f_h$  readings at 5-min intervals, was significantly correlated ( $P < 0.05$ ) with measured  $\dot{V}_{\text{O}_2}$  (Fig. 6). Random error in estimated  $\dot{V}_{\text{O}_2}$ , as indicated by the scatter of points about the regressions of estimated on measured  $\dot{V}_{\text{O}_2}$  and by the standard error of estimate, was greatest when a regression of  $\dot{V}_{\text{O}_2}$  on  $f_h$  based on pooled data of 21 animals (Fig. 5) was used to estimate  $\dot{V}_{\text{O}_2}$  (Fig. 6A) and least when regressions specific to each animal and based on 2 days' measurement, where available (4 of the 6 animals), were used to estimate  $\dot{V}_{\text{O}_2}$  (Fig. 6B).

Furthermore, when the regression of  $\dot{V}_{\text{O}_2}$  on  $f_h$  based on pooled data from 21 animals (Fig. 5) was used to estimate mean hourly  $\dot{V}_{\text{O}_2}$  of individuals (Fig. 6A), the regression of predicted on measured  $\dot{V}_{\text{O}_2}$  did not pass through the origin ( $P < 0.05$ , Snedecor and Cochran 1967) and had a slope ( $0.73 \pm 0.11$ ;  $\pm 1 \text{ SE}$ ) which differed significantly ( $P < 0.05$ ) from unity, indicating systematic error in estimated  $\dot{V}_{\text{O}_2}$ . At measured  $\dot{V}_{\text{O}_2}$  of 1 and  $4 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ , estimated hourly  $\dot{V}_{\text{O}_2}$  for the six animals was 1.46 and  $3.64 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (Fig. 6A), errors of 46% and 9%, respectively.

When regressions specific to each animal and based on either one or two days' measurements (in 4 of the 6 animals) were used for estimating  $\dot{V}_{\text{O}_2}$  (Figs. 6B and C), the null hypothesis that these regressions of estimated on measured

Figure 6. Mean estimated versus measured oxygen consumption ( $\dot{V}_{O_2}$ ) in 6 animals. Each point represents estimated versus measured  $\dot{V}_{O_2}$  over a 1-h interval in one individual during rest with either 12 points per individual (2 animals) or 8 points per individual (4 animals) in each subfigure. In part A,  $\dot{V}_{O_2}$  was estimated from  $f_h$  using the regression illustrated in Fig. 5. A regression specific to each individual was used in parts B and C. The specific regression was based on 2 days' data in 4 of the 6 animals (B), or on only 1 days' data in all 6 animals (C). Also shown are the line of equality of estimated and measured  $\dot{V}_{O_2}$  (solid line) and a least-square regression (broken line) which in B and C was fitted to pass through the origin.



$\dot{V}_{O_2}$  pass through the origin could not be rejected ( $P > 0.05$ , Snedecor and Cochran 1967) and regressions forced through the origins had slopes ( $0.96 \pm 0.08$  and  $0.94 \pm 0.06$ ;  $\pm 1$  SE) which did not differ significantly ( $P > 0.05$ ) from unity. Standard error of estimate of  $\dot{V}_{O_2}$  was less when regressions of  $\dot{V}_{O_2}$  on  $f_h$  based on two days' data (in 4 of the 6 animals) were used (Fig. 6B) than when regressions based on one days' data were used (Fig. 6C) for estimating  $\dot{V}_{O_2}$ .

Error in mean estimated  $\dot{V}_{O_2}$  of individual animals over 8 or 12 h (Table 4) was least (0.6-16.4%, mean = 7.4%) when regressions specific to each individual and based on two days' measurements were used in estimating  $\dot{V}_{O_2}$ . Somewhat greater error occurred (6.9-27.6%, mean = 14.0%) when a regression specific to each individual but based on one day's measurements was used. Greatest error (3.7-53.2%, mean = 20.2%) occurred when a general regression for the species was used; the error was particularly large (53.2%) in an animal with a curvilinear relationship between  $\dot{V}_{O_2}$  and  $f_h$ .

Mean measured  $\dot{V}_{O_2}$  of the entire sample of 6 animals over 8 or 12 h was  $1.97 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (calculated from Table 4, column 3). Mean  $\dot{V}_{O_2}$  of the same animals in the same period, estimated from  $f_h$  using a regression specific to each animal and based on one days' measurement, was  $1.88 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (calculated from Table 4, column 5), an error of 4.6%. Mean  $\dot{V}_{O_2}$  of the same animals in the

Table 4. Error in mean estimated  $\dot{V}_{O_2}$  over an 8 or 12-h period using regression equations specific to each animal based on either 1 or 2 day's measurements and a general regression for the species to estimate  $\dot{V}_{O_2}^a$

Animal	Time	$\dot{V}_{O_2}$	Estimated $\dot{V}_{O_2}$							
			From Specific Regressions				From a General Regression			
			1 Day		2 Days					
			$f_h$	$\dot{V}_{O_2}$	Error	$\dot{V}_{O_2}$	Error	$f_h$	$\dot{V}_{O_2}$	Error
15	8	1.48	243	1.32	-10.8	1.42	-4.1	957	1.72	16.2
23	8	1.72	205	1.90	10.4	1.73	0.6	808	1.32	-23.2
24	8	2.14	262	1.55	-27.6	1.79	-16.4	996	1.84	-14.0
27	8	2.72	317	3.11	14.3	2.95	8.5	1268	2.62	-3.7
8 <sup>b</sup>	12	2.03	370	1.89	-6.9			1447	3.11	53.2
10	12	1.75	269	1.50	-14.2			1033	1.95	11.4

<sup>a</sup> Units are time (h),  $\dot{V}_{O_2}$  ( $\text{cm}^3 \text{g}^{-1} \text{h}^{-1}$ ),  $f_h$  ( $\text{beats min}^{-1}$  or  $\text{beats min}^{-1} \text{g}^{0.25}$ ), Error (%).

<sup>b</sup> This animal had a curvilinear relationship between  $\dot{V}_{O_2}$  and  $f_h$ .

period estimated from  $f_h$  using a regression based on pooled data from 21 animals (Fig. 5) was  $2.09 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (calculated from Table 4, column 10), an error of 6.1%. Mean measured  $\dot{V}_{O_2}$  over 8 or 12 h of the 4 animals (No. 15, 23, 24 and 27; Table 4) for which 2 days' data were available to establish the regression of  $\dot{V}_{O_2}$  on  $f_h$  was  $2.02 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (calculated from Table 4, column 3). Mean  $\dot{V}_{O_2}$  of the same 4 animals in the same period, estimated from regressions of  $\dot{V}_{O_2}$  on  $f_h$  based on 2 days' measurements, was  $1.97 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ , an error of 2.5%.

Measurements similar to those made in establishing the accuracy of  $\dot{V}_{O_2}$  estimated from  $f_h$  when  $f_h$  is sampled at 5-min intervals were not made in exercising animals. However, the error in estimated  $\dot{V}_{O_2}$  during exercise should be comparable to that observed during rest as variability about the regression of  $\dot{V}_{O_2}$  on  $f_h$ , and its day to day variability, were similar in exercising and resting animals (Fig. 2, Tables 1 and 3).

### Conclusions

The correlation between  $\dot{V}_{O_2}$  and  $f_h$  is sufficiently strong and stable to justify  $f_h$  as an index of energy expenditure in the red squirrel within certain limitations.

Because the relationship between  $f_h$  and  $\dot{V}_{O_2}$  may depend on whether the stimulus for increased  $\dot{V}_{O_2}$  is cold or exercise, it should be determined under both conditions. Estimation of  $\dot{V}_{O_2}$  from the regression obtained during



exercise when the animal is outside the nest and from the regression obtained during rest when the animal is inside the nest should provide more accurate values than estimation from a single regression applied to both conditions.

Estimated  $\dot{V}_{O_2}$  of individual animals is clearly most accurate when a regression of  $\dot{V}_{O_2}$  on  $f_h$  based on at least 2 days' measurements is used. However, the total time and effort required in using  $f_h$  as an index of energy expenditure can be reduced by establishing the regression on the basis of one day's measurements and the accuracy of the estimates obtained using such a regression may be adequate, depending on the information being sought. If mean energy expenditure of several animals is being estimated regressions based on one day's measurements should be adequate.

A general regression for the species is clearly inadequate for estimating energy expenditure of individual animals, due to variability between animals in the relationship between  $f_h$  and  $\dot{V}_{O_2}$ . Error is most likely when  $\dot{V}_{O_2}$  is estimated for an individual with a curvilinear relationship between  $f_h$  and  $\dot{V}_{O_2}$ . Unfortunately, such individuals cannot be identified without making the measurements required in establishing the regression of  $\dot{V}_{O_2}$  on  $f_h$ .

A general regression for the species could be used to estimate mean energy expenditure of a population of animals. If a sufficiently large sample size were used

in establishing the relationship between  $\dot{V}_{O_2}$  and  $f_h$ , and if subsequent estimates of mean energy expenditure were based on a sufficiently large number of individuals, accurate estimates could be expected. Sufficient data are not available from this study to determine minimum sample sizes. However, mean energy expenditure of 6 individuals during 8 or 12 h was estimated from a regression based on 21 animals with an error of only 6.1%.

## PART V

Energy Expenditure of the Red Squirrel in a Semi-natural  
Environment: Estimates from Heart-rate Telemetry

## INTRODUCTION

The daily rate of energy expenditure of small mammals in climates with large seasonal temperature change can potentially undergo large seasonal change. However, small mammals usually use protected microenvironments and make behavioural as well as physiological adjustments which may reduce the impact of seasonal temperature change on daily energy expenditure (Hart 1971).

The amount as well as intensity of locomotor activity and time spent outside of the nest by captive red squirrels exposed to natural climatic conditions are related to air temperature in a way which suggests behavioural adjustments occur to reduce or eliminate seasonal changes in daily energy expenditure (Part I).

Measurements of metabolic rate in the laboratory of exercising animals and animals in nests (Part III) demonstrate that animals exercising at low temperatures must maintain a high metabolic rate while animals resting in nests experience little increase in minimum metabolic rate above the basal level. These results indicate that behavioural adjustments to temperature change (Part I) may reduce or eliminate seasonal changes in energy expenditure.

A decrease in total thermal conductance and resting body temperature during the winter (Parts II and III) will also contribute to the reduction or elimination of seasonal

change in energy expenditure.

This part of the study was designed to test the hypothesis, suggested by the preceding observations, that daily energy expenditure of non-reproductive red squirrels in large enclosures exposed to natural climatic conditions is independent of macroenvironmental air temperature.

Methods for measuring energy expenditure of an animal without confinement in a metabolism chamber or attachment of apparatus for collecting respiratory gases include measurement of food intake, radioisotope techniques employing carbon-14 (carbon dioxide entry rate method) or labelled deuterium oxide ( $D_2^{18}O$  method), calculation of energy expenditure using time-budget data and measurements of oxygen consumption in the laboratory during various behavioural states, mathematical modelling, and radio-telemetry of heart rate.

Measurement of food intake was impractical as the red squirrel caches food supplied to it in many locations, making measurement of uneaten food difficult in large enclosures in an outdoor environment.

The carbon dioxide entry rate method, while successfully used with ungulates (Young et al. 1969; Young 1970; Young and McEwan 1975) is impractical for small mammals as the subject carries a pump for isotope infusion. Furthermore it requires regular sampling of blood or urine, and therefore periodic disturbance of the animal. The  $D_2^{18}O$  method, although successfully used

in small mammals (Mullen 1971, 1973a), requires prohibitively expensive equipment (Mullen 1973b).

Both the time-budget method and mathematical modelling involve extrapolation of energy expenditure under laboratory conditions to the more complex and temporally-variable outdoor thermal environment, making results obtained by these methods potentially subject to substantial error (Tracy 1972; Bakken 1976).

Heart-rate telemetry was selected as the method of estimating energy expenditure in this study. Physical interaction with the subject being monitored can be avoided and a validation study (Part IV) indicates that heart rate is a reliable index of energy expenditure.

## MATERIALS AND METHODS

### Animal Care

Animals were trapped in the vicinity of Winnipeg ( $50^{\circ}06'N$ ;  $98^{\circ}20'W$ ). Prior to having transmitters implanted they were held for 1-6 months in the same outdoor wire mesh enclosures in which heart rate was later measured for the purpose of estimating energy expenditure. This allowed the animals to adapt to the enclosures and construct nests in their nest boxes. The animals were brought into the laboratory either on the day the transmitters were implanted or one day earlier and returned to the outdoor enclosures within 12 days after being brought into the laboratory. The outdoor enclosures, implantation of transmitters, and animal care in the enclosures and in the laboratory have been described elsewhere (Parts I and IV).

Eight males and 2 females, with a mean weight of 228 g (range = 181-255 g) were used.

### Heart-rate and Body-temperature Telemetry

Transmitters capable of transmitting an FM radio frequency signal encoding both heart rate and body temperature information over a distance of 5-10 m were implanted in the abdominal cavities of the animals. Demodulation of the signal to extract the heart rate and body temperature information, counting and digital readout of heart rate, and display of body temperature on

a meter were performed by a demodulator (model D2-1C, Biotelemetry Systems Inc., Rush, New York). The transmitters, telemetry system, and implantation of transmitters have been described in Part IV.

#### Simultaneous Measurement of Rate of Oxygen Consumption

##### ( $\dot{V}_{O_2}$ ) and Heart Rate ( $f_h$ )

To establish the relationship between  $\dot{V}_{O_2}$  and  $f_h$  for each individual, simultaneous measurements of those variables were made in the laboratory as described in Part IV. Since this relationship may depend on whether increased  $\dot{V}_{O_2}$  is elicited by cold or exercise (Part IV), the measurements were carried out, whenever possible, with each animal during rest at temperatures ranging from 30 to  $-20^{\circ}\text{C}$  and during exercise at temperatures approximating outdoor environmental temperature as closely as possible within the range 20 to  $-10^{\circ}\text{C}$ . Measurements with resting animals were carried out at least 5 days after surgery and before measurement of  $f_h$  in the outdoor enclosures. Measurements with exercising animals were carried out at least 7 days after surgery, either before or after measurement of  $f_h$  in the outdoor enclosures.

The regressions of  $\dot{V}_{O_2}$  on  $f_h$  were based on data from 2 days' measurements for two of the 10 animals and on 1 day's measurements for the remainder. The former would have been preferable as it would improve the accuracy of estimated  $\dot{V}_{O_2}$  (see Part IV) but it did not prove to be



practical due to the extra time required and the limited life span of the ECG leads.

#### Estimation of Energy Expenditure from $f_h$

Heart-rate measurements of animals in outdoor enclosures were made from a building with Plexiglass portholes adjacent to the enclosures. Location and activity of the animals were monitored both visually and with the Esterline Angus event recorder and treadle system described in Part I.

Measurement of  $f_h$  began on the day following return of the animals to the outdoor enclosures and at least 11 days after implantation of the transmitters. All measurements of  $f_h$  used in estimating  $\dot{V}_{O_2}$  were made within 17 days of determination of the regressions of  $\dot{V}_{O_2}$  on  $f_h$ ; most were made within 9 days.

Heart rate was measured for 0.1 min at approximately 5-min intervals for eighteen 24-h periods during the course of 1 year. One or two animals (mean = 1.6) were monitored at any given time and each animal was monitored for 1-4 (mean 2.8) 24-h periods.

Proper adjustment of both receiver and demodulator and a sufficiently strong and noise-free transmitter signal were essential for reliable counts of  $f_h$ . The counting process was visually monitored as described in Part IV and repeated when it was apparent that either spurious heart beats were counted or heart beats were

missed by the counter. In the rare event that a reliable count during a given 5-min interval was not obtained, a value was inserted based on mean heart rate in preceding and subsequent samples taking into consideration activity at the time of the missing sample, thus eliminating missing values and facilitating analysis.

The  $\dot{V}_{O_2}$  corresponding to each  $f_h$  count was calculated using the regression of  $\dot{V}_{O_2}$  on  $f_h$  established during exercise for an animal outside the nest and the regression established during rest for an animal inside the nest for 6 of the 10 animals. In the remaining 4 animals, broken ECG leads prevented transmission of heart rate before a regression of  $\dot{V}_{O_2}$  on  $f_h$  during exercise could be obtained. The regression determined during rest was used to estimate  $\dot{V}_{O_2}$  during both rest and activity in these animals. Oxygen consumption of a 228 g animal, the mean weight of animals used in this study, will on average be underestimated during exercise, using the regression established during rest, by about 16% (see Fig. 5, Part IV) at a heart rate of  $500 \text{ beats min}^{-1}$  ( $f_h$  during moderately strenuous exercise). Underestimation of daily energy expenditure will range from a negligible amount when an animal is inside the nest most of the day as at low air temperatures (Part I) to 6.1% at the maximum daily time (9.1 h) spent outside the nest by an animal in this study.

### Measurement of Abdominal Temperature ( $T_b$ )

Abdominal temperature was manually recorded from the meter display of the demodulator at the time of each  $f_h$  count. These measurements provided useful information regarding short term (<24 h)  $T_b$  change but reliable absolute  $T_b$  values were not obtained due to "drift" with time of the transmitter and/or demodulator. Therefore  $T_b$  has been expressed in degrees Centigrade above a baseline resting  $T_b$ , determined from visual inspection of minimum  $T_b$  over each 24-h period for each animal.

### Measurement of Air Temperature ( $T_a$ )

Air temperature was measured hourly using a YSI telethermometer (model 42SC) with a temperature probe suspended in a well-ventilated box 130 cm above the ground adjacent to the enclosures.

## RESULTS

Mean daily estimated  $\dot{V}_{O_2}$ , including both time spent inside and outside the nest, of animals in the outdoor enclosures was  $1.1\text{--}3.3 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (Fig. 1) and was independent of mean daily  $T_a$  ( $r = 0.08$ ,  $n = 28$ ,  $P > 0.1$ ). Overall mean daily  $\dot{V}_{O_2}$  was  $2.00 \pm 0.11 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (mean  $\pm 1$  SE;  $n = 28$  measurements with 10 animals). Inspection of the data indicated that day-to-day variability in mean daily  $\dot{V}_{O_2}$  within individuals was small relative to total variability. Furthermore, a high  $\dot{V}_{O_2}$ , and hence daily energy expenditure, was usually associated with a greater than average time outside the nest and amount of locomotor activity.

When data from animals inside and outside the nest were analyzed separately (Fig. 2) mean  $\dot{V}_{O_2}$  was related to  $T_a$  in both situations. At  $T_a$  less than  $-5^\circ\text{C}$ , mean  $\dot{V}_{O_2}$  of animals inside the nest was significantly correlated with  $T_a$  ( $r = 0.93$ ,  $n = 5$ ,  $P < 0.01$ ); at  $T_a$  greater than  $-5^\circ\text{C}$ , mean  $\dot{V}_{O_2}$  was not significantly correlated with  $T_a$  ( $r = 0.37$ ,  $n = 6$ ,  $P > 0.1$ ) and mean  $\dot{V}_{O_2}$  was  $1.44 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ . Mean  $\dot{V}_{O_2}$  of animals outside the nest was related to  $T_a$  ( $r = 0.93$ ,  $n = 10$ ,  $P < 0.01$ ) through the entire range of  $T_a$  encountered in this study. That mean  $\dot{V}_{O_2}$  of animals inside and outside the nest was dependent on  $T_a$  (Fig. 2) while mean daily  $\dot{V}_{O_2}$  was independent of  $T_a$  (Fig. 1) occurred because of two factors. These were the high metabolic rate of animals

Figure 1. Mean daily oxygen consumption ( $\dot{V}_{O_2}$ ) of animals in outdoor enclosures versus mean daily air temperature. Each point represents mean  $\dot{V}_{O_2}$  over a 24-h period for one animal.

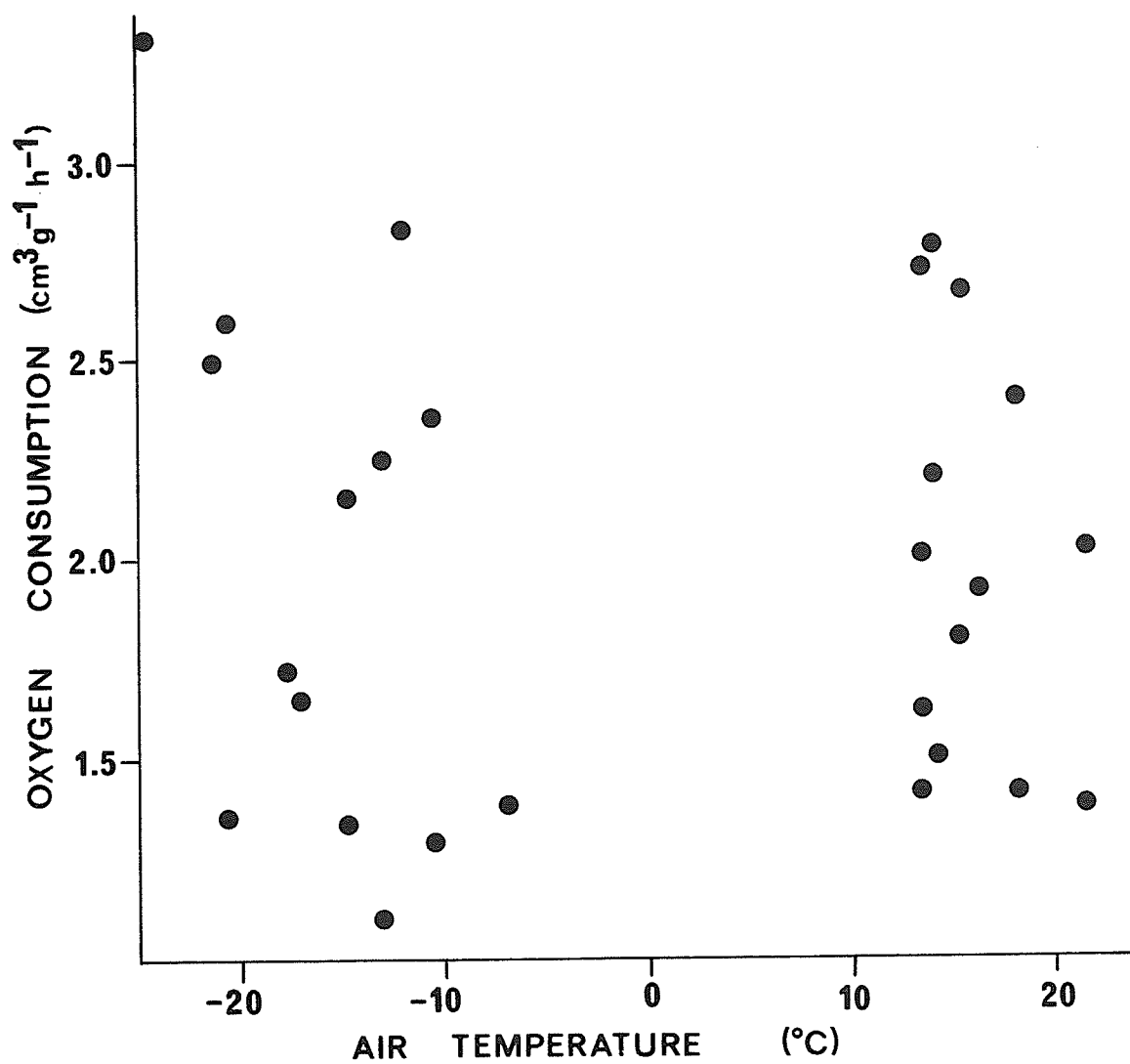
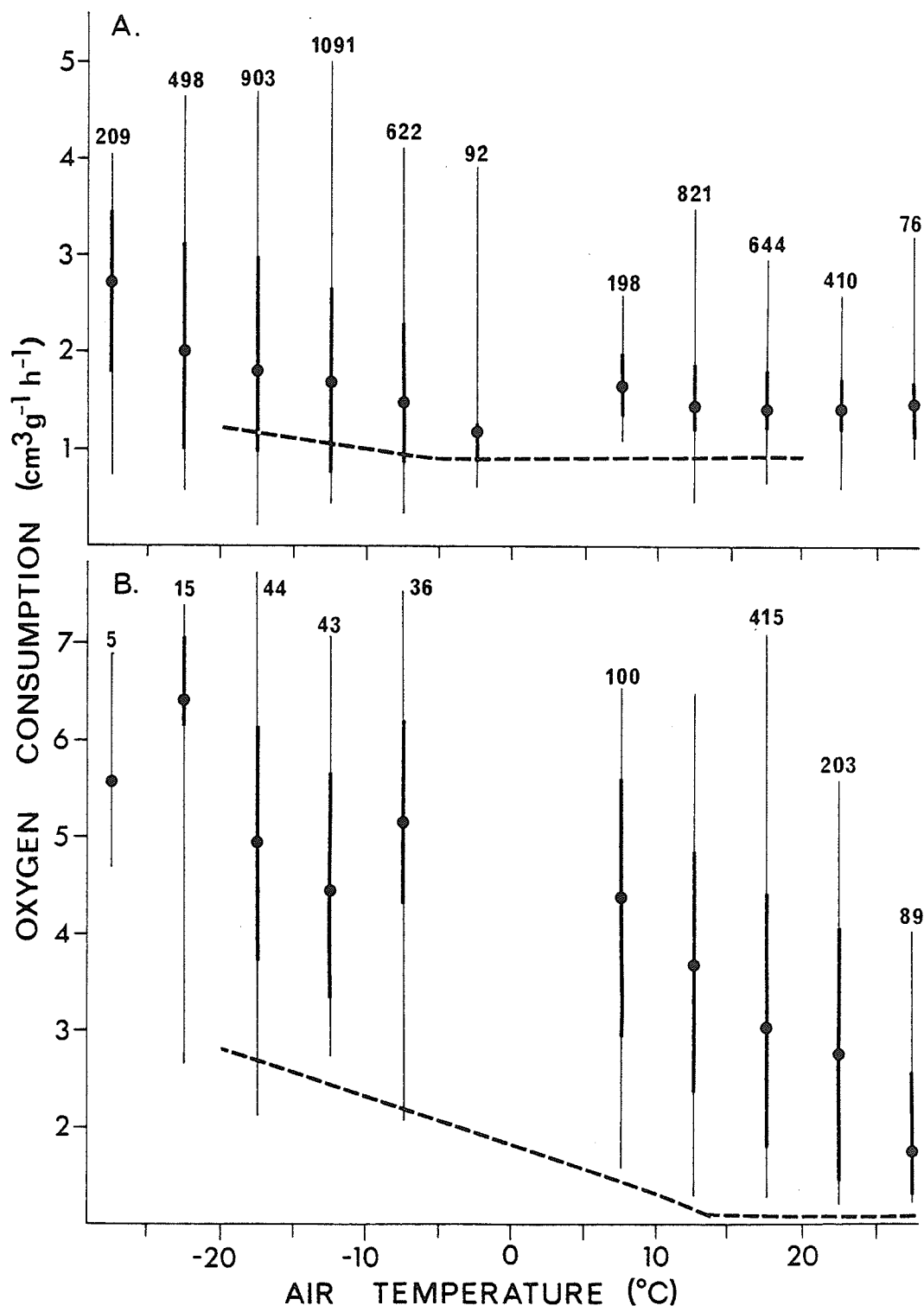


Figure 2. Mean oxygen consumption ( $\dot{V}_{O_2}$ ) of animals in outdoor enclosures in the nest (A) and outside the nest (B) at different air temperatures. Shown are mean  $\dot{V}_{O_2}$  based on  $\dot{V}_{O_2}$  estimates made over  $T_a$  intervals of  $5^\circ\text{C}$  (closed circles), range (light vertical line), range after eliminating the upper and lower 12.5% of values (bold vertical line), and number of measurements contributing to each mean. Broken lines indicate minimum  $\dot{V}_{O_2}$  of squirrels at rest in the laboratory with a nest (A) and without a nest (B) from Fig. 1, Part III.





outside the nest relative to inside the nest (Fig. 2) and the decreasing proportion of the day spent outside the nest with decreasing temperature (Part I).

Minimum  $\dot{V}_{O_2}$  of animals in the outdoor enclosures in the nest at  $T_a$  less than  $0^\circ\text{C}$  appeared to be independent of  $T_a$  and lower than minimum  $\dot{V}_{O_2}$  at  $T_a$  greater than  $0^\circ\text{C}$  (Fig. 2). Furthermore, it appeared from inspection of  $\dot{V}_{O_2}$  of individual animals over 24-h periods (e.g. Figs 3) that  $\dot{V}_{O_2}$  of animals resting in the nest for extended periods at low  $T_a$  was more irregular than that of animals at high  $T_a$ . Thus, while minimum  $\dot{V}_{O_2}$  of animals in the nest was less at low than at high  $T_a$ , frequent transient increases in  $\dot{V}_{O_2}$  at low  $T_a$  resulted in a mean  $\dot{V}_{O_2}$  which increased with decreasing temperature below about  $0^\circ\text{C}$ . It is also of interest that a large number of estimated  $\dot{V}_{O_2}$  of animals in the nest fell below minimum resting  $\dot{V}_{O_2}$  as measured in the laboratory in Part III (Fig. 2).

Outside the nest, both minimum and maximum  $\dot{V}_{O_2}$  tended to increase with decreasing temperature (Fig. 2). Minimum values of  $\dot{V}_{O_2}$  estimated from  $f_h$  corresponded closely with minimum  $\dot{V}_{O_2}$  of laboratory animals without a nest as measured in Part III.

The rate of oxygen consumption of animals in the outdoor enclosures varied widely from minute to minute (Fig. 4) with most peaks occurring during feeding and locomotor activity. Peak  $\dot{V}_{O_2}$  during feeding, when the

Figure 3. Rate of oxygen consumption ( $\dot{V}_{O_2}$ ) of 5 animals in outdoor enclosures. Each point represents  $\dot{V}_{O_2}$  estimated from  $f_h$  counts during 0.1 min taken at 5-min intervals. Also shown are mean air temperature ( $T_a$ ) for the 24-h period in which the data were collected and the time the animal was out of the nest (solid bar).

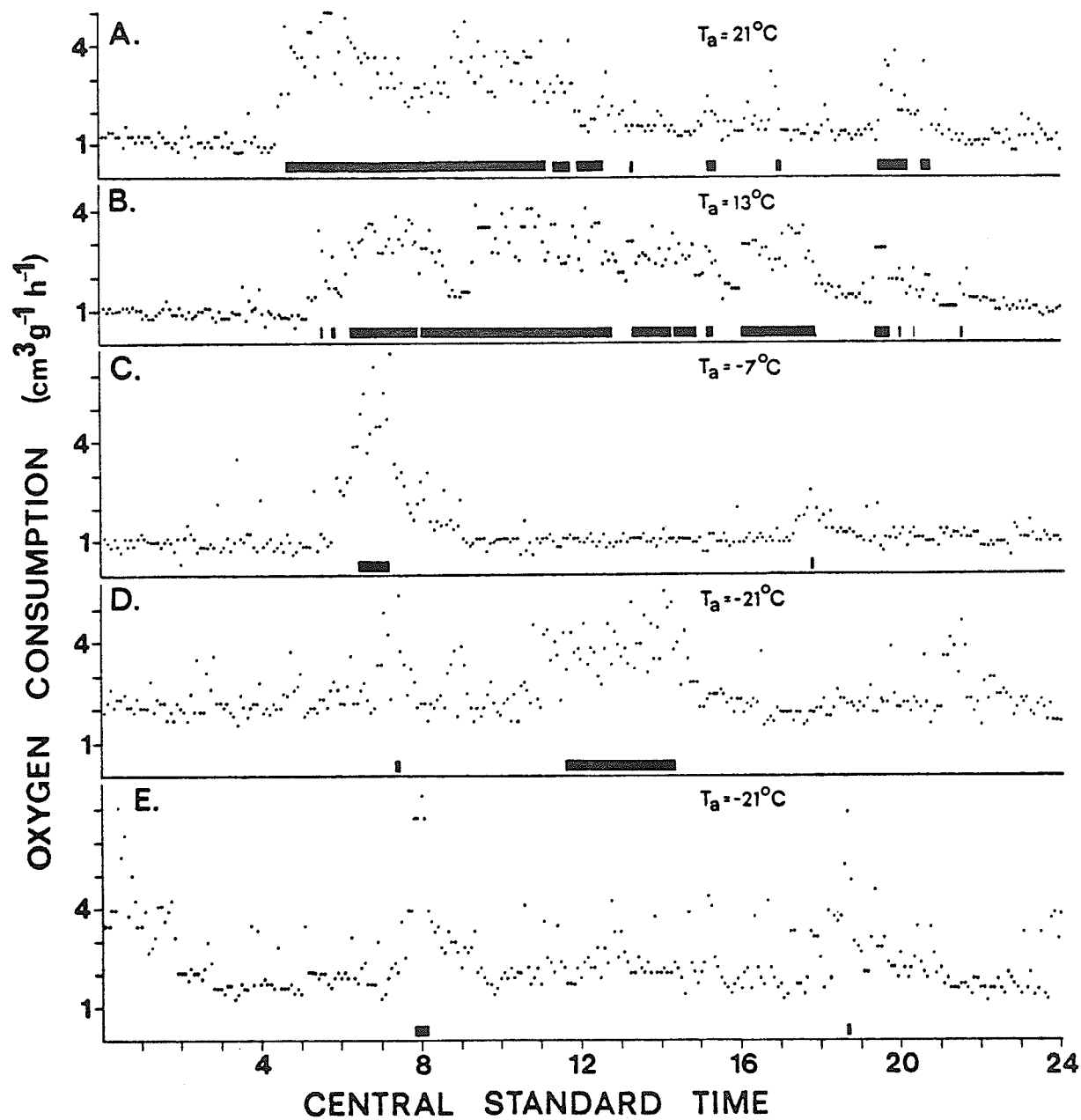
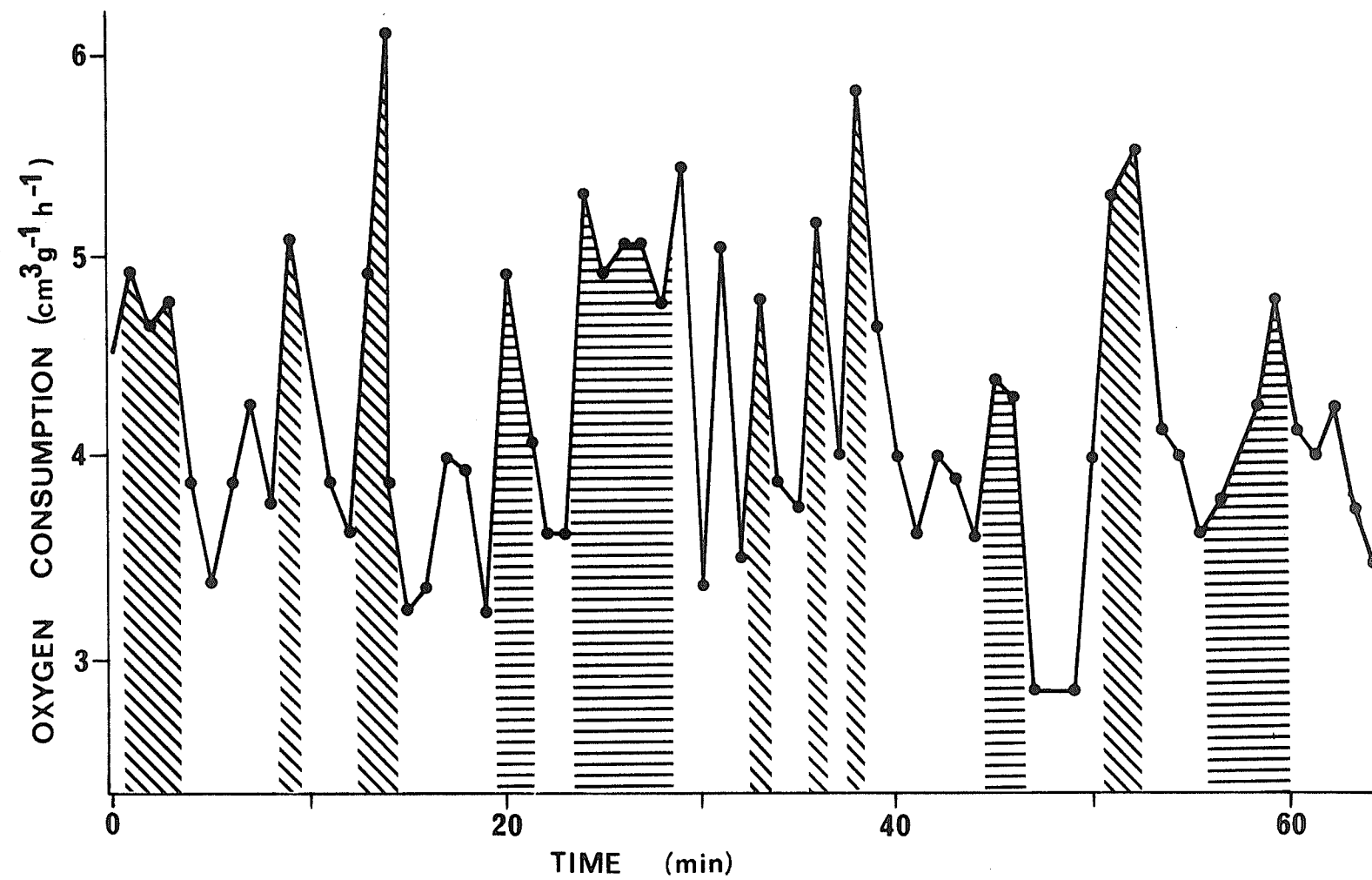


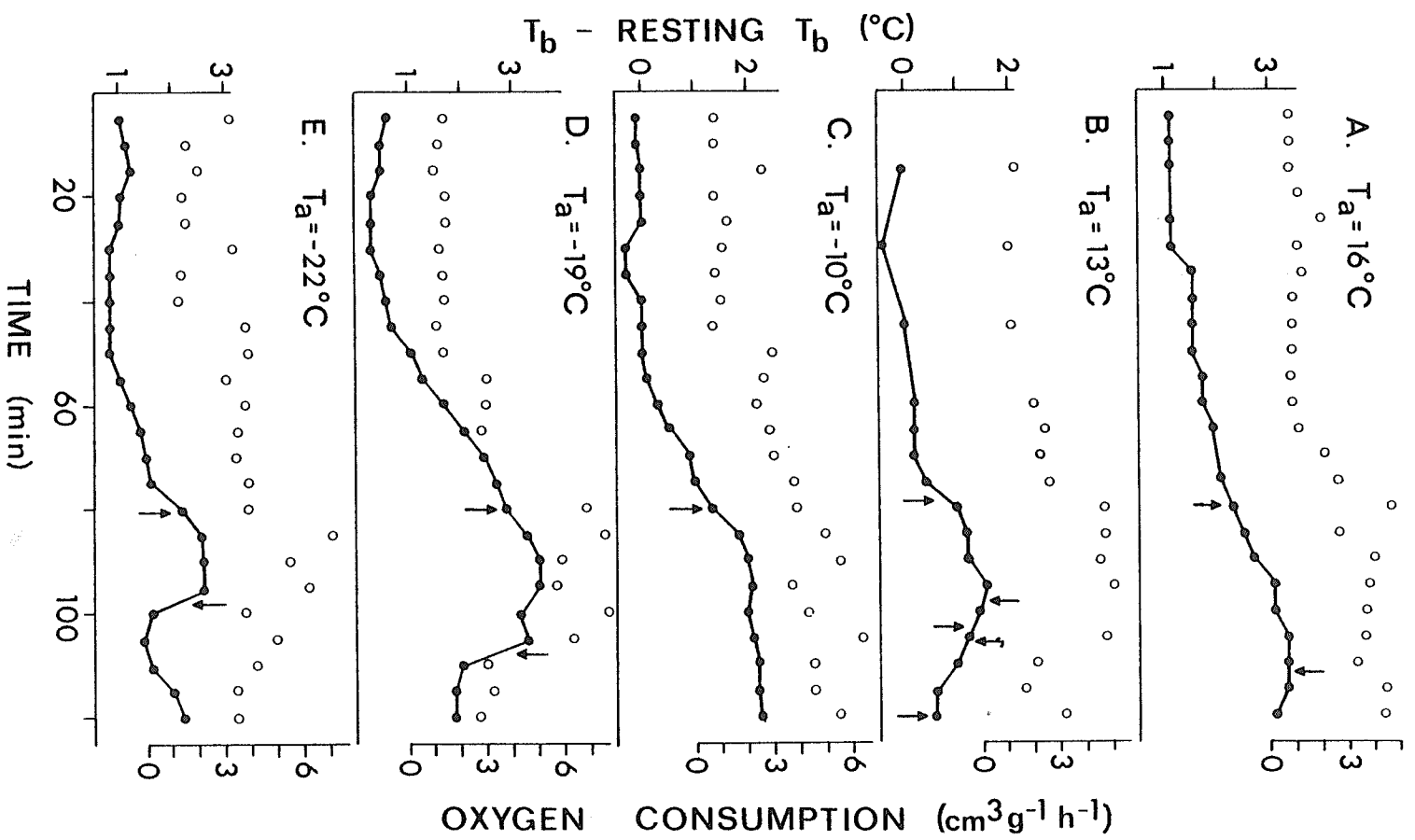
Figure 4. Rate of oxygen consumption ( $\dot{V}_{O_2}$ ) based on heart rate ( $f_h$ ) determined for 0.1 min at 1-min intervals for an animal outside the nest in an outdoor enclosure. The predominant behaviour in each 1-min interval preceding and during the  $\dot{V}_{O_2}$  estimate is classified as locomotor activity (diagonal lines), feeding (horizontal lines), or rest (blank space).



animals typically crouch on their hind legs with the forelegs manipulating the food, was only slightly less than  $\dot{V}_{O_2}$  during locomotor activity.

Simultaneous measurement of  $T_b$  and  $f_h$  (Fig. 5) confirmed the increase of abdominal temperature of animals prior to leaving the nest and the precipitous drop in  $T_b$  after entering the nest (Part II). Furthermore, it appears from Fig. 5, that the increase in  $T_b$  resulted from an increase in metabolic rate which typically occurred well in advance of an animal leaving the nest at low  $T_a$ .

Figure 5. Relationship between change in abdominal temperature ( $T_b$ ; closed circles) and oxygen consumption ( $\dot{V}_{O_2}$ ; open circles) measured before, during, and after out-of-nest activity for five animals (A-E). Change in  $T_b$  is expressed as increase above minimum resting  $T_b$ , determined from inspection of  $T_b$  of each animal over the 24-h period in which the data were collected. Also shown are air temperature ( $T_a$ ), time of exit from the nest ( $\uparrow$ ), and time of entry into the nest ( $\downarrow$ ).





## DISCUSSION

The daily rate of energy expenditure of a homeotherm can potentially be influenced by many physiological and environmental variables, and on the basis of laboratory measurements (Part III), air temperature would appear to be a major variable determining energy expenditure of the red squirrel. However, this part of the study indicates that adult captive red squirrels under non-reproductive conditions eliminate the effect of air temperature on daily energy expenditure and maintain a constant daily rate of energy expenditure throughout the year in spite of large seasonal temperature change. This stability of daily energy expenditure is apparently due to behavioural and physiological responses to temperature and/or season (Parts I, II, and III).

While a constant daily energy expenditure may be a coincidental result of temperature-dependent activity (Part I), it is more likely that daily energy expenditure is regulated by adjustment in the amount of locomotor activity and time out of the nest. Adjustment of these variables so as to maintain a constant energy expenditure would result in an activity level correlated with air temperature as observed in Part I. While this could be viewed as an alternative to the cost-benefit model proposed in Part I to account for temperature-dependent activity, it is not necessarily so as indicated by the following

argument.

The red squirrel has evolved behavioural responses to temperature, probably as a result of the cost-benefit factors discussed in Part I. These result in a reduction of seasonal change in daily energy expenditure. Then, as a result of little seasonal change in energy expenditure, digestive systems and/or metabolic mechanisms may have evolved which function most efficiently at a constant level of energy expenditure. Natural selection would ensure that this would be the level of energy expenditure that, on average, maximizes the individual's fitness (i.e. the probability of survival and reproduction). Selective advantage would then accrue to individuals adjusting behaviourally so as to expend energy at this optimal rate resulting in the evolution of behavioural mechanisms that regulate daily energy expenditure. This could account for the observation that when a given daily energy expenditure is not forced upon a captive animal by low temperature, it is maintained by voluntary activity (Part I).

The captive red squirrels in this study were not subjected to all aspects of temporal variability occurring in the natural environment. The extent to which these affect seasonal change in daily energy expenditure of free-ranging squirrels cannot be estimated from the present study. Possibly, factors such as reproductive activity, gestation, lactation, foraging, food caching,

and territorial defense could modify the constancy of daily energy expenditure noted in the captive animals, resulting in an increased rate of energy expenditure at certain times of the year. Alternatively, these factors could result in a re-budgeting of energy expenditure, via behavioural adjustments, rather than an increase.

In either case, caution must be employed in extrapolating the estimated mean daily  $\dot{V}_{O_2}$  of the captive squirrels in this study to free-ranging squirrels, especially during the reproductive season. During the winter the situation of the non-reproductive captive animals with their ample food supply near at hand approximates more closely that of free-ranging animals with food caches located on their territories, particularly since these territories are small (0.08-1.58 hectares; Smith 1968; Zirul and Fuller 1971; Rusch and Reeder 1978), and the proportion of the total territory used is as small as 2% in mid-winter (Zirul and Fuller 1971).

Estimated daily energy expenditure is remarkably similar in summer and in winter in Microtus agrestis (Hanson and Grodzinski 1970), Microtus arvalis, Clethrionomys glareolus (Grodzinski 1966), Sorex cinereus, Clethrionomys rutilus, Microtus oeconomus (Grodzinski 1971), Apodemus flavicollis (Gebczynski 1966), Apodemus agrarius (Gorecki 1969), Micromys minutus (Gorecki 1971), and Arvicola terrestris (Ashby and Vincent 1976), suggesting that a high rate of energy expenditure for particular functions, such as thermoregulation or reproduction, tends

to cause a re-budgeting rather than an increase in energy expenditure. These data, the results of this study, and the observation by Irving et al. (1955) that most Arctic mammals are sufficiently well insulated that they probably do not require increased energy expenditure for thermoregulation, even at the coldest temperatures occurring in their environments, suggest that it is within the capabilities of many homeotherms, even in climates subject to large seasonal temperature change, to avoid seasonal changes in energy requirements.

The average daily energy expenditure estimated in the present study agrees closely with that by Grodzinski (1971) who based his estimates for the same species on continuous measurement over 24-h periods of metabolic rates of animals in metabolism chambers. However, the values obtained are considerably lower than those by Smith (1968) for an adult male and a lactating female. Smith's (1968) values were based on estimates of food intake in free-ranging animals which could partially account for the increased energy expenditure. However, his estimate of mean daily energy expenditure of the lactating female appears impossibly high, as it is approximately 8 times the basal metabolic rate of non-lactating animals observed in this study. Moreover, it exceeds both the maximum short-term energy expenditure observed in outdoor animals in this study as well as energy expenditure of animals running continuously at  $-10^{\circ}\text{C}$  and  $2 \text{ km h}^{-1}$  in the laboratory

(Part III). Furthermore, maximum metabolic rates of rodents rarely exceed 4-6 times the basal metabolic rate (Hart 1971). Also maintenance costs during lactation are increased by only 82.5% in Microtus arvalis (Migula 1969) and 92% in Clethrionomys glareolus (Kaczmarek 1966).

The observation that minimum  $\dot{V}_{O_2}$  of animals in the nest in the outdoor enclosures does not increase with decreasing  $T_a$ , although mean  $\dot{V}_{O_2}$  does, could possibly result from an inhibition or elimination of thermoregulatory responses during paradoxical or REM sleep as has been noted in the kangaroo rat (Glotzbach and Heller 1976), cat (Parmeggiani et al. 1971, 1973), rabbit (Baker and Hayward 1967), and man (Shapiro et al. 1974). Consequently, during paradoxical sleep, even in a cool environment, metabolic rate tends to fall to a basal level and body temperature may fall below the set point temperature occurring in other sleep states. Subsequently, upon emergence from paradoxical sleep and re-establishment of thermoregulatory responses, a sudden increase in metabolic rate in response to the low body temperature could occur. This may account for the increased short-term variability in  $\dot{V}_{O_2}$  of animals in the nest at low  $T_a$  as compared to high  $T_a$ .

The observation that minimum metabolic rate at low  $T_a$  is less than at high  $T_a$  in animals in nests in the outdoor enclosures suggests that a reduction in basal metabolic rate occurred, possibly due to a reduction in body temperature at low  $T_a$  (Part II). In a thermal neutral

environment, such as may occur in nests even at low macroenvironmental air temperatures, a reduction in the basal metabolic rate would be advantageous in that energy would be conserved.

The observation that  $\dot{V}_{O_2}$  of animals in the nest in the outdoor enclosures falls to lower levels than minimum  $\dot{V}_{O_2}$  of animals in a similar nest in the laboratory may indicate that  $T_b$  in the laboratory does not decrease at low  $T_a$  as it does in outdoor animals (Part II). This suggestion is supported by data (Pauls, unpublished) which indicates that  $T_b$  in the laboratory, in at least some animals, remains at levels typical of active animals even during a 2-h exposure without disturbance to  $T_a$  as low as  $-30^{\circ}\text{C}$ , possibly due to a continued state of alertness in the unfamiliar laboratory environment.

## SUMMARY AND CONCLUSIONS

This study demonstrates that non-reproductive, adult, captive red squirrels in an outdoor environment with easy access to ample food supplies avoid seasonal change in daily energy expenditure in spite of large seasonal temperature change. Various interacting behavioural and physiological features accomplish this independence of daily energy expenditure from macroenvironmental air temperature.

The availability of a well-insulated nest and a reduction in time spent outside the nest with decreasing air temperature are largely responsible for the maintenance of a constant daily energy expenditure. Inside the nest body temperature can be regulated by the red squirrel with little increase in metabolic rate above the basal level at temperatures as low as  $-20^{\circ}\text{C}$ . This is in contrast to the greatly increased metabolic rate of animals without a nest exposed to low air temperatures.

Another contributing factor is a 40-fold reduction in locomotor activity with decreasing air temperature. Apparently with decreasing air temperature there is an increasing shift of energy expenditure from locomotor activity to thermoregulation.

The intensity, as well as amount, of locomotor activity is reduced at low  $T_a$ , possibly due to the high energy cost of activity at low  $T_a$ . Heat generated by activity does

not eliminate the need for thermoregulatory heat production because of increased thermal conductance during activity. Metabolic rate during activity must therefore be equal to, or greater, than the sum of the energy cost of the activity per se and the energy cost of thermoregulation.

The red squirrel appears to make these behavioural responses to temperature on a daily rather than a seasonal basis, as indicated by the close correlation between these behavioural responses and mean measured daily air temperature and their poor correlation with mean normal air temperature.

A reduction in total thermal conductance and resting body temperature during the winter reduces the energy costs of thermoregulation, permitting more activity at a given level of energy expenditure. The reduction in thermal conductance, though not irrelevant to an animal in the nest, is principally important in reducing the metabolic cost of thermoregulation by an animal outside the nest and extending downwards the range of air temperatures at which the red squirrel can thermoregulate while active outside the nest.

The reduction in resting body temperature of the red squirrel, though small relative to that of species entering a torpid state, may be significant to the energy economy of this species due to the large proportion of the day spent resting inside the nest at low air temperatures. A reduction in energy expenditure could occur by one of two means, depending on the thermal regime inside the nest.



In a thermal neutral environment the reduced body temperature may result in a reduction in the basal metabolic rate. In a cooler environment, the energy cost of thermoregulation may be reduced, due to a reduction in the temperature gradient from animal to environment.

This reduced body temperature, although adaptive for an animal resting in a protected microenvironment, apparently is not desirable during out-of-nest activity. The red squirrel avoids exposure outside the nest while body temperature is low by increasing body temperature prior to leaving the nest. This increase is accomplished by an increase in metabolic rate.

Evidently the red squirrel has evolved behavioural and physiological mechanisms by which it maintains a constant daily energy expenditure. The selective advantage of doing so is not clear. It would appear equally adaptive to minimize energy expenditure whenever this does not conflict with the fulfilment of other biological needs. Possibly, in a non-hibernating species, behavioural and physiological mechanisms which maintain a constant rate of daily energy expenditure, provided adequate energy is available in the environment, are more readily evolved than digestive systems and/or metabolic mechanisms which adjust well to seasonal changes in energy expenditure. Alternatively, maintaining a constant daily energy expenditure, even on those days when a lower energy expenditure would serve the needs of the organism, may be

energetically more economical than adjusting to a change in energy expenditure.

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## APPENDICES

## APPENDIX I

Error in Oxygen Consumption Measurements Resulting from  
Water Vapour in the Air Inflow and Carbon Dioxide in the  
Air Outflow of the Metabolism Chamber

Rate of oxygen consumption was calculated according to Depocas and Hart (1957; equation 5). Water vapour in the air inflow and carbon dioxide in the air outflow of the metabolism chamber causes some error in calculated oxygen consumption. The following analysis indicates the magnitude of this error.

The following symbols are used:

$B$  = barometric pressure

$F_{E_{O_2}}$  = fractional oxygen concentration of dry outflow air

$F_{I_{O_2}}$  = fractional oxygen concentration of dry inflow air

$P_{WI}$  = partial pressure of water vapour in inflow air

$\dot{V}_{AF}$  = inflow rate of water vapour

$\dot{V}_{CO_2}$  = rate of carbon dioxide production by the animal

$\dot{V}_E$  = outflow rate of dry air

$\dot{V}_I$  = inflow rate of dry air

$\dot{V}_{in}$  = inflow rate of undried air

$\dot{V}_{O_2}$  = rate of oxygen consumption by the animal

Standard temperature and pressure conditions are assumed throughout the analysis.

The general equation for  $\dot{V}_{O_2}$  in a flow-through system is

$$\dot{V}_{O_2} = \dot{V}_I \cdot F_{I_{O_2}} - \dot{V}_E \cdot F_{E_{O_2}} \quad (1)$$

where

$$\dot{V}_I = \dot{V}_{in} - \dot{V}_{AF} \quad (2)$$

and

$$\dot{V}_E = \dot{V}_I + \dot{V}_{CO_2} - \dot{V}_{O_2} \quad (3)$$

By definition,  $\dot{V}_{CO_2} = RQ \cdot \dot{V}_{O_2}$ . Substituting this expression and equation 2 in equation 3 and rearranging yields

$$\dot{V}_E = \dot{V}_{in} - \dot{V}_{AF} + \dot{V}_{O_2} \cdot (RQ - 1) \quad (4)$$

Substituting equation 2 and equation 4 in equation 1 and rearranging yields

$$\dot{V}_{O_2} = \frac{(\dot{V}_{in} - \dot{V}_{AF}) \cdot (F_{I_{O_2}} - F_{E_{O_2}})}{1 + F_{E_{O_2}} \cdot (1 - RQ)} \quad (5)$$

When  $\dot{V}_{AF} = 0$  (i.e. inflow air is dried) and  $RQ = 1$ , this equation simplifies to

$$\dot{V}_{O_2} = \dot{V}_{in} \cdot (F_{I_{O_2}} - F_{E_{O_2}}) \quad (6)$$

Equation 6, which is identical to equation 5 of Depocas and Hart (1957), was used in calculating  $\dot{V}_{O_2}$  in this study. This value of  $\dot{V}_{O_2}$  can be designated as "estimated  $\dot{V}_{O_2}$ " and  $\dot{V}_{O_2}$  calculated according to equation 5 as "actual  $\dot{V}_{O_2}$ ". If error in estimating  $\dot{V}_{O_2}$  is expressed as the ratio (actual  $\dot{V}_{O_2}$ :estimated  $\dot{V}_{O_2}$ ), then from equation 5 and 6

$$\text{Error} = \frac{\dot{V}_{in} - \dot{V}_{AF}}{\dot{V}_{in}} \cdot \frac{1}{1 + F_{E_{O_2}} \cdot (RQ - 1)} \quad (7)$$

where

$$\dot{V}_{AF} = \dot{V}_{in} \cdot (P_{WI}/B) \quad (8)$$

Potential error in calculated  $\dot{V}_{O_2}$  under the experimental

conditions of this study is indicated in Table 1. Maximum error is 6.5%. However, under usual operating conditions error will be less than maximum.

Table 1. Error, expressed as (actual  $\dot{V}_{O_2}$ :estimated  $\dot{V}_{O_2}$ ), in the calculation of  $\dot{V}_{O_2}$  resulting from water vapour in the air inflow and carbon dioxide in the air outflow from the metabolism chamber.

RQ	Relative Humidity (%)				
	0	25	50	75	100
0.7	1.065	1.059	1.053	1.046	1.040
0.8	1.043	1.036	1.030	1.024	1.018
0.9	1.021	1.015	1.009	1.003	0.997
1.0	1.000	0.994	0.998	0.982	0.976

NOTE: Calculations are based on equations 5, 7, and 8 using the values  $\dot{V}_{in} = 720 \text{ cm}^3 \text{ min}^{-1}$  (STP);  $\dot{V}_{O_2} = 0.9 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$  (STP); weight of animal = 225 g; temperature of air inflow = 20°C; B = 740 mm Hg.

Maximum error occurs at minimum  $\dot{V}_{O_2}$ . If  $\dot{V}_{O_2} = 4 \text{ cm}^3 \text{ g}^{-1} \text{ h}^{-1}$ , error is less than values shown by 0.000-0.006.

## APPENDIX II

Measurement of Oxygen Consumption of an Animal in an Open  
Flow System under Dynamic Conditions



Oxygen consumption of a mammal in a metabolism chamber is conventionally determined in one of two ways. In a closed system oxygen consumption is calculated from the rate of oxygen depletion in the metabolism chamber; in an open-flow system oxygen consumption is calculated from the difference in oxygen concentration of air flowing into and out of the metabolism chamber.

Oxygen consumption of an animal in a closed system can be calculated under dynamic conditions (i.e. during changes in the rate of oxygen consumption by the animal). However, depletion of oxygen in the chamber limits the time over which continuous measurement can be carried out. Increasing the volume of the closed chamber extends the maximum measurement period; unfortunately, it also reduces the sensitivity of the system to short-term changes in oxygen consumption.

An open-flow system can be used only under steady-state conditions if published equations (Depocas and Hart 1957) are used for calculation of oxygen consumption. This requirement makes the open-flow system unsuitable for examination of correlations between heart rate and oxygen consumption in the red squirrel for two reasons. Firstly, the correlation between these two variables during and after a change in both variables is of interest. Secondly, red squirrels rarely maintain a stable level of oxygen consumption for a sufficiently long time to enable the system to reach a steady state. These problems could

be minimized by using a mask rather than a metabolism chamber, thus reducing the time required for the system to reach a steady state. However, training a red squirrel to accept a mask would have been difficult. Another approach sometimes used to reduce the time required for the system to reach a steady state following a change in oxygen consumption is the use of a high flow rate. Unfortunately, the accuracy of measurement will be inversely related to flow rate.

Depocas and Hart (1957) present an equation for calculation of oxygen consumption under dynamic conditions in an open-flow system. However, it provides accurate results only with a metabolism chamber of sufficiently large volume relative to flow rate that it approximates a closed system. Their equation (equation 13) is identical to the one presented for a closed system (equation 15). A large volume open-flow system with a low flow rate, in addition to the previously described disadvantage of a closed system, would have a slow response time due to the low rate of air flow.

To circumvent these problems, a method has been devised of making accurate calculations of oxygen consumption in an open-flow system under dynamic conditions without requiring either a large metabolism chamber with a low air-flow rate, a mask, or a high flow rate.

In an open-flow system the change in fractional oxygen concentration between air inflow and outflow from

a metabolism chamber containing an animal reflects the rate of oxygen consumption by the animal under steady state but not under dynamic conditions. This is because the metabolism chamber acts as an oxygen storage reservoir, a factor which can be ignored under steady-state conditions since the oxygen content of the metabolism chamber remains constant. However, following an increase in oxygen consumption of an animal, the oxygen content of the chamber and therefore the fractional oxygen concentration of the outflow air decreases gradually to a new steady state and vice-versa. By taking into consideration the rate of oxygen storage or depletion from the metabolism chamber as well as the fractional oxygen concentration of the outflow air the rate of oxygen consumption during and following periods of change can be accurately computed. Data required in addition to those needed in steady-state measurements are volume of the metabolism chamber and temperature and water vapour pressure in the metabolism chamber.

#### Terminology and Assumptions

The following derivation is based on a system in which air inflow to the metabolism chamber is metered and fractional oxygen concentration of the air outflow is measured. It is assumed that all gas flow and volume measurements have been converted to standard temperature and pressure unless otherwise stated, and air has been

dried upstream and downstream from the metabolism chamber prior to the taking of inflow and oxygen concentration measurements. It is further assumed that carbon dioxide has been removed from the outflow air or that respiratory quotient is known.

The following symbols are used:

$B$	= barometric pressure
$F_{E_{O_2}}$	= fractional oxygen concentration of dry outflow air
$F_{I_{O_2}}$	= fractional oxygen concentration of dry inflow air
$P_W$	= water vapour pressure in the metabolism chamber (at ambient temperature and pressure)
$RQ$	= respiratory quotient
$T_c$	= air temperature in the metabolism chamber
$V$	= volume of the metabolism chamber
$V_A$	= volume of dry air in the metabolism chamber
$\dot{V}_{CO_2}$	= rate of carbon dioxide formation by the animal
$\dot{V}_E$	= outflow rate of dry air
$\dot{V}_I$	= inflow rate of dry air
$V_O$	= volume of oxygen in the metabolism chamber
$\dot{V}_{O_2}$	= rate of oxygen consumption by the animal
$V_W$	= volume of water vapour in the metabolism chamber

### Theoretical Considerations

During a given time interval,  $t$ , the change in volume of oxygen stored in the the metabolism chamber ( $\Delta V_O$ ) will be

the difference between oxygen added by air inflow ( $= F_{I_{O_2}} \cdot \dot{V}_I \cdot t$ ) and removed by the animal ( $= \bar{V}_{O_2} \cdot t$ ) and the air outflow (approximately  $\bar{F}_{E_{O_2}} \cdot \bar{V}_E \cdot t$ ), where  $\bar{V}_{O_2}$ ,  $\bar{F}_{E_{O_2}}$ , and  $\bar{V}_E$  are mean values during the time,  $t$ . It is assumed  $F_{I_{O_2}}$  and  $\dot{V}_I$  are constant during the time,  $t$ . A more precise measure of oxygen removed by the air outflow would be

$$\frac{\sum (F_{E_{O_2}} \cdot \dot{V}_E) \cdot t}{n} \quad \text{where } F_{E_{O_2}} \text{ and } \dot{V}_E \text{ are measured } n \text{ times during}$$

the time interval,  $t$ . The precision of the estimate, if  $F_{E_{O_2}}$  and  $\dot{V}_E$  vary in a non-linear fashion during  $t$  will be a function of the number of measurements ( $n$ ). That oxygen outflow is only approximated by the expression ( $\bar{F}_{E_{O_2}} \cdot \bar{V}_E \cdot t$ ) is seen from the following argument.

By definition,  $\bar{F}_{E_{O_2}} = (\sum F_{E_{O_2}})/n$  and  $\bar{V}_E = (\sum \dot{V}_E)/n$ .

However,  $\sum (F_{E_{O_2}} \cdot \dot{V}_E)/n \neq (\sum F_{E_{O_2}}/n) \cdot (\sum \dot{V}_E/n)$  when both  $F_{E_{O_2}}$  and  $\dot{V}_E$  vary in a non-linear fashion during  $t$ . The magnitude of the departure from equality depends on the range over which  $F_{E_{O_2}}$  and  $\dot{V}_E$  vary. In animal  $\dot{V}_{O_2}$  experiments, flow rates are usually adjusted so that  $F_{E_{O_2}} > 0.1850$ . Therefore, the potential range over which  $F_{E_{O_2}}$  can vary is approximately 0.1850-0.2095. If  $RQ = 1$ , then  $\dot{V}_E = \dot{V}_I$ , and both  $\dot{V}_E$  and  $\dot{V}_I$  are constant. In this case mean oxygen outflow during the time,  $t$ , is accurately described by the expression ( $\bar{F}_{E_{O_2}} \cdot \dot{V}_E \cdot t$ ) provided an adequate number of measurements of  $F_{E_{O_2}}$  have been made during  $t$ . If  $RQ \neq 1$ , then  $\dot{V}_E \neq \dot{V}_I$ , with the maximum difference occurring at  $RQ = 0.7$ . Then from the relationships  $\dot{V}_E = \dot{V}_I - \dot{V}_{O_2} + \dot{V}_{CO_2}$  and

$RQ = \dot{V}_{CO2}/\dot{V}_{O2}$  it can be shown that  $\dot{V}_E = \dot{V}_I - 0.3\dot{V}_{O2}$ . It is apparent that  $\dot{V}_E$  will be minimized when  $\dot{V}_{O2}$  is maximized. If the minimum permitted value of  $F_{E_{O2}}$  is 0.1850 (through adjustment of flow rate), then the maximum value for  $\dot{V}_{O2}$  is closely approximated by  $(0.2095 - 0.1850) \cdot \dot{V}_I = 0.0245\dot{V}_I$ , and the minimum value for  $\dot{V}_E$  is  $\dot{V}_I - 0.0245\dot{V}_I$  or  $0.9755\dot{V}_I$ . The possible range over which  $\dot{V}_E$  can vary is then from  $\dot{V}_I$  to  $0.9755\dot{V}_I$  or about 2.5%. From sample values it can be demonstrated that the error in the approximation  $(\bar{F}_{E_{O2}} \cdot \bar{V}_E \cdot t)$  for oxygen outflow from the metabolism chamber is negligible. Therefore the approximation  $(\bar{F}_{E_{O2}} \cdot \bar{V}_E \cdot t)$  is assumed to represent oxygen outflow from the metabolism chamber.

Based on the preceding considerations

$$\Delta V_0 = F_{I_{O2}} \cdot \dot{V}_I \cdot t - \bar{F}_{E_{O2}} \cdot \bar{V}_E \cdot t - \dot{V}_{O2} \cdot t$$

By rearrangement

$$\dot{V}_{O2} = F_{I_{O2}} \cdot \dot{V}_I - \bar{F}_{E_{O2}} \cdot \bar{V}_E - \Delta V_0/t \quad (1)$$

The terms  $F_{I_{O2}}$ ,  $\dot{V}_I$ ,  $\bar{F}_{E_{O2}}$  are either known or measured in the experiment. Mean air outflow is given by

$$\bar{V}_E = \dot{V}_I - \bar{V}_{O2} + \bar{V}_{CO2} \quad (2)$$

By definition,  $\bar{V}_{CO2} = RQ \cdot \bar{V}_{O2}$ . Substituting this term in equation 2 and simplifying yields

$$\bar{V}_E = \dot{V}_I - \bar{V}_{O2} \cdot (1 - RQ) \quad (3)$$

Substituting equation 3 in equation 1 and rearranging yields

$$\bar{V}_{O2} = \frac{(F_{I_{O2}} - \bar{F}_{E_{O2}}) \cdot \dot{V}_I - \Delta V_0/t}{1 - \bar{F}_{E_{O2}} \cdot (1 - RQ)} \quad (4)$$

These terms, except for  $\Delta V_0$ , are all known or measured quantities. Calculation of  $\Delta V_0$  is based on the relationship

$$\Delta V_0 = \Delta F_{E_{O_2}} \cdot V_A \quad (5)$$

where  $\Delta F_{E_{O_2}}$  is the change in  $F_{E_{O_2}}$  during  $t$  and  $V_A$  is given by

$$V_A = V - V_W \quad (6)$$

The water vapour content of the metabolism chamber ( $V_W$ ) can be calculated from  $P_W$  in the metabolism chamber which can be directly measured with commercially available equipment (e.g. YSI Dew Point Hygrometer, Fisher Scientific Co.)

Then

$$V_W = V \cdot (P_W/B) \quad (7)$$

Substituting equation 7 in equation 6 and rearranging yields

$$V_A = V \cdot (1 - P_W/B) \quad (8)$$

Substituting equation 8 in equation 5 yields

$$\Delta V_0 = \Delta F_{E_{O_2}} \cdot V \cdot (1 - P_W/B) \quad (9)$$

All these terms are either measured or known. Using  $\Delta V_0$  obtained from equation 9,  $\bar{V}_{O_2}$  for the time interval  $t$  can be calculated from equation 4.

By similar manipulations it can be shown that if  $CO_2$  is absorbed prior to measurement of  $V_{E_{O_2}}$ ,  $\bar{V}_{O_2}$  is given by

$$\bar{V}_{O_2} = \frac{(F_{I_{O_2}} - \bar{F}_{E_{O_2}}) \cdot \dot{V}_I - \Delta V_0/t}{1 - \bar{F}_{E_{O_2}}} \quad (10)$$

where  $\Delta V_0$  is calculated as previously described.

In the limiting case of steady state-conditions,  $\Delta V_O/t = 0$ . Under this condition, equations 4 and 10 are identical to the corresponding equations from Depocas and Hart (1957) for steady-state conditions. Equations 4 and 10 are more generally applicable because they can be applied both to a situation where  $\dot{V}_{O_2}$  is changing and to a system which has not yet reached a steady state following placement of an animal into the metabolism chamber or following a change in  $\dot{V}_{O_2}$  by the animal.

The magnitude of improvement in accuracy of calculated  $\dot{V}_{O_2}$  using the presently derived dynamic-state equation rather than a steady-state equation (the dynamic state equation with the term  $\Delta V_O/t$  omitted) depends on the frequency and rapidity of changes in oxygen consumption by the animal and the relative values of chamber volume and flow rate. Under the experimental conditions of this study (Part IV) the improvement in accuracy can be substantial (Fig. 1).

#### Simplifying Approximations

To simplify measurements it was assumed that RQ was 0.85 and  $P_W$  in the metabolism chamber was 50% of maximum  $P_W$ . Maximum  $P_W$  in the temperature range -20 to 30°C is accurately approximated by the equation

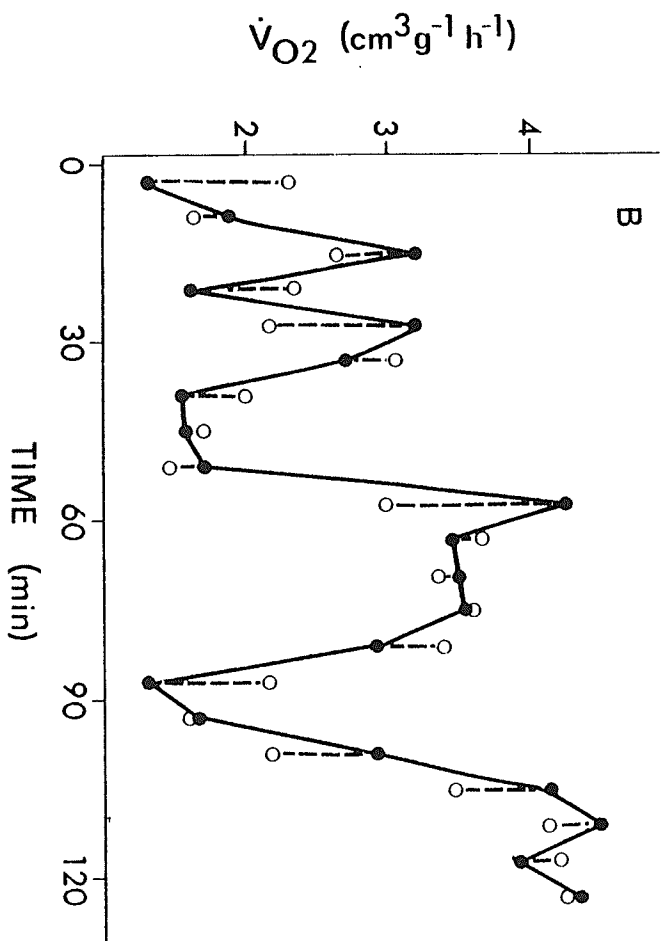
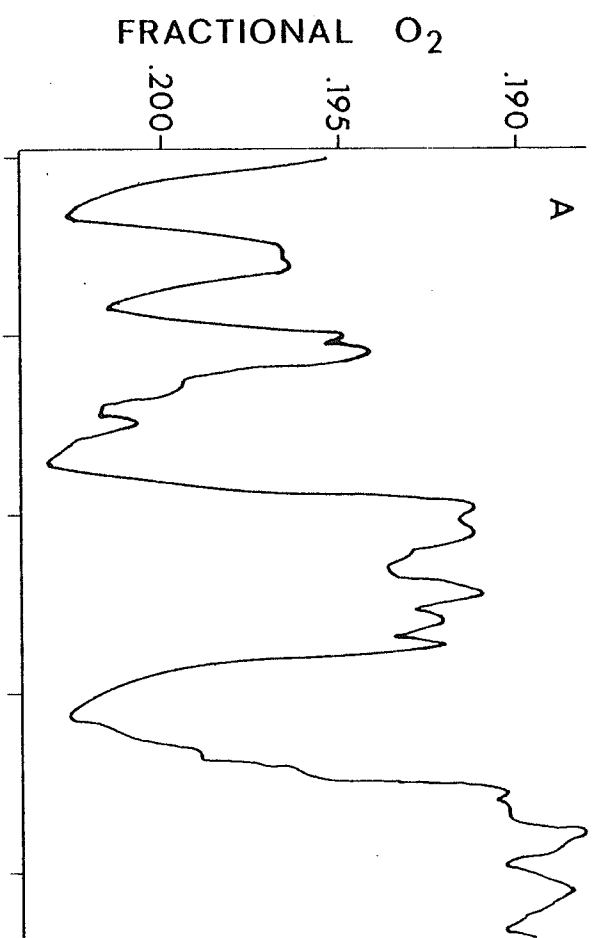
$$P_W = 4.7304 \times 1.0667^{T^{\circ}C}$$

The potential errors introduced by these assumptions are small as indicated by the following analyses:



Figure 1A. Fractional oxygen concentration of dried outflow air from a metabolism chamber containing a red squirrel. Chamber volume ( $V$ ) =  $4000 \text{ cm}^3$ , flow rate ( $\dot{V}_I$ ) =  $1000 \text{ cm}^3 \text{ min}^{-1}$  (ambient temperature and pressure). Measurement methods are fully described in Parts III and IV.

Figure 1B. Mean  $\dot{V}_{O_2}$  (STP) during 6-min intervals based on fractional concentration of oxygen in outflow air from the metabolism chamber as shown in A if oxygen storage in the metabolism chamber is ignored (open circles) and taken into consideration (closed circles). Error resulting from the use of a steady state equation (i.e. storage of oxygen in the metabolism chamber not taken into consideration) under the experimental conditions of this study (Part IV) is shown by broken lines connecting open and closed circles.



1. Error if  $\text{CO}_2$  is not absorbed from the outflow air and  $RQ$  is assumed to be 0.85.

Equation 4 can be rewritten as

$$\bar{V}_{02} = \frac{1}{1 - \bar{F}_{E02} \cdot (1 - RQ)} \cdot X$$

where  $X = (F_{I02} - \bar{F}_{E02}) \cdot \dot{V}_I - \Delta V_0/t$

If  $RQ = 1$ , then  $\bar{V}_{02} = X$ . Therefore the term

$\frac{1}{1 - \bar{F}_{E02} \cdot (1 - RQ)}$  may be considered a correction

factor ( $C_f$ ) for  $\bar{V}_{02}$  when  $RQ \neq 1$ . If  $RQ = 0.85$ , as was assumed in this study, then  $C_f = 1.029-1.032$  over the possible range of values of  $\bar{F}_{E02}$ . Over the potential range of  $RQ$  values (0.7-1.0) the value of  $C_f$  may range from 1.067 to 1.000. Therefore, maximum error resulting from deviation of  $RQ$  from the assumed value, 0.85, is less than 4%.

2. Error if  $P_W$  is assumed to be 50% of maximum  $P_W$ .

Equation 4 can be rewritten as

$$\bar{V}_{02} = \frac{(F_{I02} - \bar{F}_{E02}) \cdot \dot{V}_I}{1 - \bar{F}_{E02} \cdot (1 - RQ)} - \frac{\Delta V_0/t}{1 - \bar{F}_{E02} \cdot (1 - RQ)} \quad (11)$$

$P_W$  enters the equation in the calculation of  $\Delta V_0$ .

Percent error in the second term of equation 11 is equal to percent error in  $\Delta V_0$ . From equation 9 it is apparent that maximum error in  $\Delta V_0$  will occur at maximum  $\Delta F_{E02}$  which is 0.0245. At  $T_c = -20, 5$ ,

and 30°C, maximum  $P_w$  is 1.3, 6.53, and 32.82 mm Hg, respectively. Assuming the volume of the metabolism chamber is 4000 cm<sup>3</sup> (ambient temperature and pressure) and barometric pressure is 750 mm Hg, percent error in  $\Delta V_0$  is  $\pm 0.09$ ,  $\pm 0.44$ ,  $\pm 2.23$ , respectively. Because the second term in equation 11 represents only 1 of 2 terms in  $\bar{V}_{O_2}$ , and it is usually the smaller of the two (Fig. 1), percent error in  $\bar{V}_{O_2}$  will usually be less than half of percent error in  $\Delta V_0$ . It is apparent that error due to deviation of  $P_w$  from the assumed value will be vanishingly small at low  $T_c$  and acceptably low even at 30°C.