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THE RELATION BETWEEN SPONTANEOUS ACTIVITY, METABOLIC RATE AND THE 24 HOUR CYCLE IN MICE AT DIFFERENT ENVIRONMENTAL TEMPERATURES

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SUMMARY

1. Oxygen consumption rates and levels of spontaneous activity were recorded simultaneously for mice, both singly and in groups, over 24 hr periods at temperatures ranging from 8 to 37° C.

2. There was marked 24 hr variation in both metabolic rate and activity, with maxima during the night; the amplitude of the variation diminished at the lower environmental temperatures.

3. At 28–33° C environmental temperatures, increased activity was associated with an increased oxygen consumption rate.

4. At 8 and 15° C, increased activity was accompanied by only a small increase in oxygen consumption.

5. These results show that thermogenesis from spontaneous activity can take the place of thermoregulatory heat production in the cold.

INTRODUCTION

Exposure to cold causes an increase in metabolic rate in homoeothermic animals; the magnitude of the response depends on the species and on the levels of metabolic and insulative adaptation (Barnett & Mount, 1967). Exercise also increases the metabolic rate, and this gives rise to the question of whether the extra heat production associated with exercise in the cold can replace, in whole or in part, the additional heat production required for thermoregulation under cold conditions.

The relation of metabolic rate to forced activity has been investigated in mice by Hart (1950, 1952) and Jánský (1959), and in rabbits and lemmings by Hart & Héroux (1955); in these experiments a constant metabolic increment for work was found at all temperatures. However, later work with rats (Hart, 1958; Hart & Jánský, 1963) has shown that, in some circumstances, thermogenesis by activity can be substituted for

thermoregulatory heat production. Similar results, showing substitution, have been found in man by Lefèvre & Auget (1931), who postulated the partial substitution hypothesis.

In the present paper, the problem is re-examined in mice, under conditions in which the animals' spontaneous activity has been measured instead of a forced level of exercise at a given rate. The experiments have been carried out over 24 hr periods in order to take into account the marked daily metabolic cycle exhibited by mice.

METHODS

Animals. Female laboratory stock albino mice were used, either singly or in groups. The mice were housed at 23° C in plastic mouse boxes; water and pelleted food (diet 41B) were available *ad libitum*. Mature animals were used, with an age range of 112–203 days and a weight range of 27·0–34·7 g. The experiments took place between May and September, so that there was probably little seasonal effect (Jánský, 1959).

Oxygen consumption determination. Oxygen consumption was used as the measure of metabolic rate or heat production. Determinations were made in a simple closed system comprising a rectangular brass chamber, volume 13 l., immersed to its top rim in a constant temperature (±0·2° C) water-bath, closed by an insulated lid seated in a paraffin seal, and connected to a recording spirometer containing oxygen. A wire cage (20 × 16 × 16 cm) inside the chamber contained the mice, which were free to move without restraint. Two tiered trays, each 16 × 8 cm, containing soda asbestos for the absorption of carbon-dioxide, were placed beside the cage. A small fan kept the chamber air stirred, whilst the cage lid shielded the mice from forced convection draughts.

Oxygen was supplied from a recording spirometer holding about 200 ml. Contacts on the counter-weight of the recording spirometer operated relays and thence two valves at preset points on the bell's travel. The opening of one valve allowed the recording spirometer to refill from a large reservoir containing oxygen at the same temperature and under an excess pressure of 3–4 cm water. The other valve automatically isolated the chamber from the recording spirometer to prevent transmission of the refill pressure to the chamber with a consequent delay in recording. Both valves were made of Perspex tubes in paraffin seals, and operated simultaneously, one opening whilst the other closed.

The consumption of oxygen was indicated on a kymograph by a pen attached to the spirometer counter-weight. The rate of consumption was determined from the slope of the trace between refills, in this way providing a series of values for the 24 hr period. Variations in ambient temperature and pressure were indicated on the same chart by a trace from a control spirometer which was connected to a thermobarometer chamber immersed in the same water-bath as the mouse chamber. The two systems, mouse chamber and recording spirometer, and thermobarometer chamber and control spirometer, were adjusted so that both spirometer traces moved in a parallel fashion when subjected to changes in temperature and pressure without an animal in the chamber. The adjustment was made by varying the volume of the thermobarometer chamber. The control spirometer tracing was then used as the base line for the estimation of oxygen consumption from the slope of the recording spirometer traces. The volumes of oxygen used were converted to s.t.p.

Spontaneous activity. The mouse cage inside the chamber rested on an aluminium tray, to catch urine and faeces. The tray, in turn, rested on two coiled horizontal springs and was balanced on a partially inflated football bladder, which was connected to a tambour recording on the kymograph chart. The system could be adjusted so that mouse movements were detected as irregularities in the tambour trace. Using dividers to measure the trace, activity

was calculated as the percentage of time for which the mice were active, during an interval between two spirometer refills or over a period where the rate of oxygen consumption was constant. In this way, simultaneous oxygen consumption and activity could be measured; no reliance was placed on the amplitude of the trace, which varied with the adjustment of the system.

Course of experiments. The mice were weighed, and rectal temperature measured with a 36 s.w.g. copper–constantan thermojunction inserted for 2–3 cm. The animals were put in the cage with food and water provided *ad libitum*; the activity recording system was adjusted, and the soda asbestos trays placed in position. The chamber was then closed by the lid, leaving one vent open, and the air-stirring fan started. When the chamber and water-bath temperatures had equilibrated, the chamber was connected to the spirometer.

Each experimental run lasted 22–23 hr, beginning at about 10 a.m. At this time of day mice are inactive, with a relatively low metabolic rate. It was thus possible to get a good estimate of the 24 hr oxygen consumption for a run lasting 22–23 hr by estimating the oxygen consumption for the intervening 1–2 hr from the mean of the first and last figures of the run. These figures were usually close together (see Fig. 1).

The chamber temperatures used were close to 8, 15, 29, 31, 33, 36 and 37° C; the temperatures were measured by thermocouples. At the end of each run, the mice were weighed and their rectal temperatures were measured.

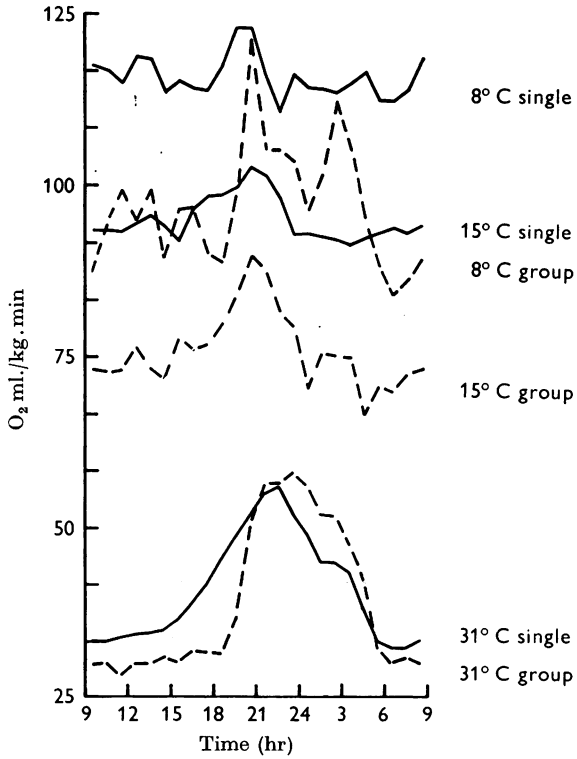

Fig. 1. Mean oxygen consumption rates, ml./kg.min, over the 24 hr period for both single mice and groups at environmental temperatures of 8, 15 and 31° C. Single mice: continuous lines; groups of mice: interrupted lines. Note that ordinate scale does not extend to zero.

RESULTS

The 24 hr pattern of oxygen consumption, calculated on an hourly basis, is shown in Fig. 1 for both single mice and groups of mice at environmental temperatures of 8, 15 and 31° C. The results obtained at 29 and 33° C were similar in pattern to those at 31° C, but have been omitted for clarity. Table 1 gives the mean 24 hr rates for each mouse or group at given temperatures.

TABLE 1. Mean 24 hr values for oxygen consumption rates at various environmental temperatures for albino mice, both singly and in groups

	Mean environmental temperature (° C)	Mean body weight (g)	Mean O_2 consumption rate (ml./kg.min)
Single	8	29·9	115·8
Group	8	29·4	96·1
Single	15	29·7	94·1
Group	14	29·7	74·8
Single	29	31·7	41·7
Group	29	31·9	40·5
Single	31	31·7	40·8
Group	31	29·7	38·7
Single	33	30·4	33·3
Group	33	29·5	29·4
Single	36	28·6	34·5
Group	—	—	—
Single	37	28·6	41·0
Group	—	—	—

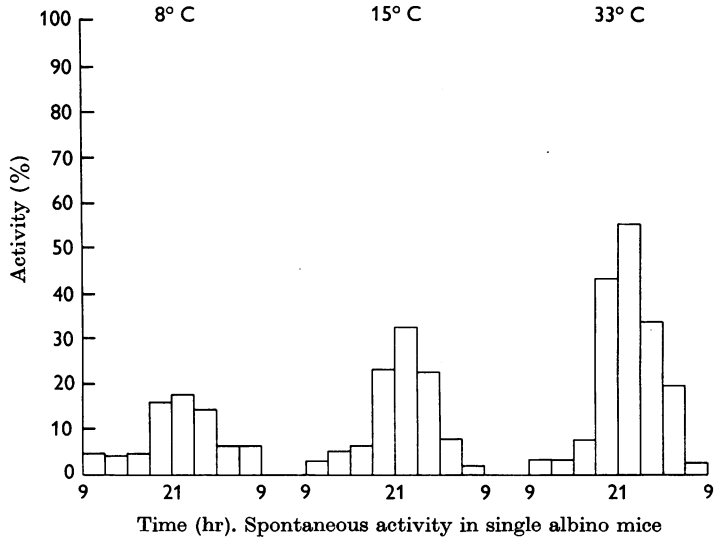

Fig. 2. Percentage of time during which single mice were active, in 3 hr periods during the 24 hr, at 8, 15 and 33° C.

Figure 2 shows for single mice how the percentage of time during which they were active is related to time of day in 3 hr intervals, over a range of temperatures. Figure 3 gives corresponding values for groups of mice.

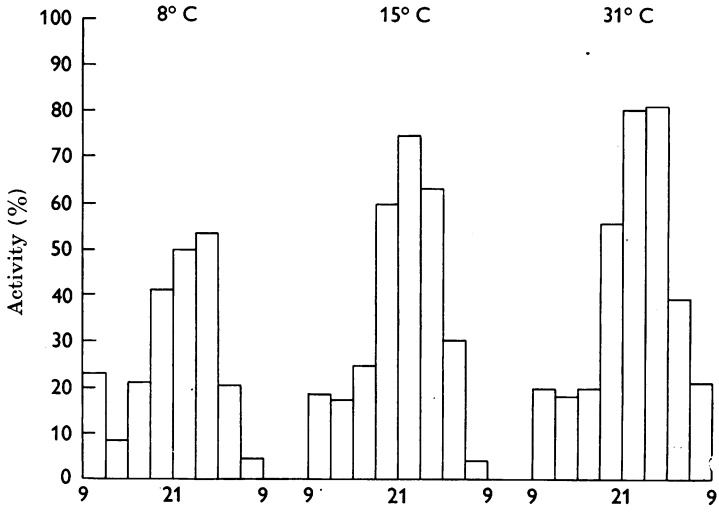

Fig. 3. Percentage of time during which groups of mice were active, in 3 hr periods during the 24 hr, at 8, 15 and 31° C.

Figures 4 and 5 give the regressions of log oxygen consumption rate on percentage activity for single mice and groups of mice respectively; the regression coefficients are given in Table 2. The intercept gives the predicted oxygen consumption rate at zero activity, and corresponds to the resting metabolic rate. The regressions for the single mice are highly significant statistically, and show a highly significant difference between 8 and 15° C on the one hand, and 29, 31 and 33° C on the other ($P < 0{\cdot}001$). Measurements on groups of mice, however, showed considerable within-group variation on successive occasions, and when this was taken into account the corresponding difference for the groups was not significant ($P = 0{\cdot}1$), although the trends shown in Table 2 and Fig. 5 for the group results were similar to those from the single mice. In addition, the oxygen consumption rates at zero activity predicted from the regressions showed apparently as close a correspondence with the 5–7 a.m. measured rates in the groups as in the singles, suggesting that the group regressions were in fact reliable estimates.

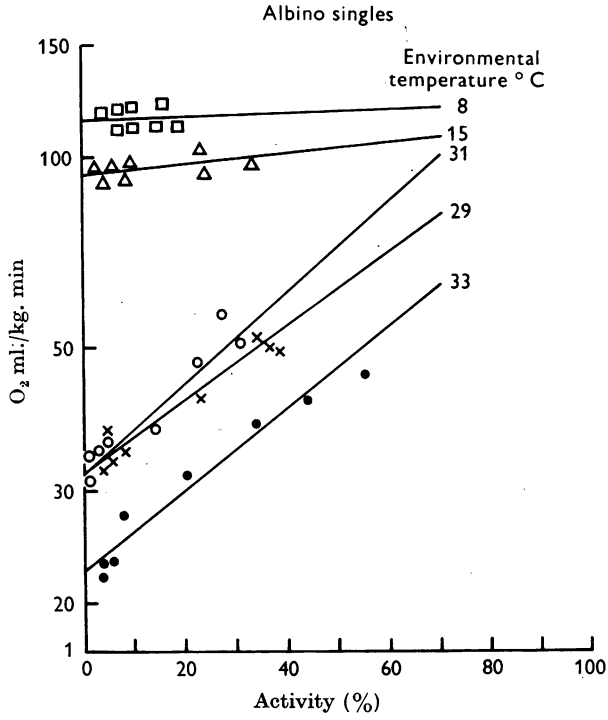

Fig. 4. Regressions of log oxygen consumption rates (ml./kg.min) on percentage of time active for single mice, at environmental temperatures of 8, 15, 29, 31 and 33° C.

DISCUSSION

The much smaller regression coefficient for oxygen consumption rates on percentage activity at the lower temperatures indicate that increased activity in the cold does not add nearly as much to the metabolic rate as it does at higher temperatures. The results of these experiments support the partial substitution hypothesis to the extent that thermogenesis from spontaneous activity is not necessarily added to thermoregulatory heat production in the cold. In the cold there is still an increase in oxygen consumption as activity increases, but this would be expected even if the substitution were total. This is because activity increases heat loss as a result of decreased over-all thermal insulation (due particularly to increased convective losses and to peripheral vasodilatation), and this leads to a rise in heat production.

These results do not at first sight agree with Hart's (1950, 1952) findings that in mice there was no substitution by the heat produced during wheel running at a forced rate. However, an alternative conclusion can be drawn from Hart's (1952) results if they are set out as in Fig. 6. Here both

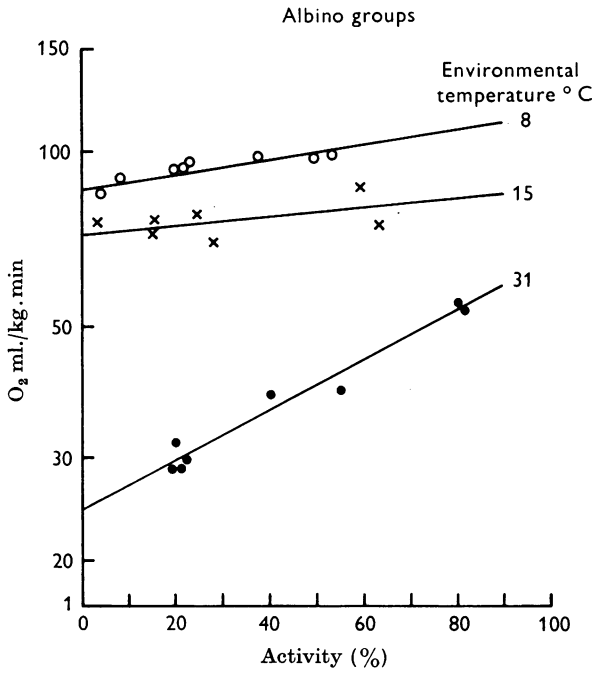

Fig. 5. Regressions of log oxygen consumption rates (ml./kg.min) on percentage of time active for groups of mice, at environmental temperatures of 8, 15 and 31° C.

TABLE 2. Regression coefficients for log oxygen consumption rates on percentage of time active (see Figs. 4 and 5)

Environmental temperature (° C)	Regression coefficient × 10^3	Zero activity intercept O_2 ml./kg.min	O_2 ml./kg.min 5–7 a.m.
	Single mice		
8	0·35*	114·4	112·4
15	0·89*	93·8	93·0
29	5·88*	32·0	32·0
31	7·13*	31·8	32·5
33	6·49*	22·3	26·8
	Groups		
8	1·27	86·0	84·2
14	0·76	71·7	70·1
31	4·25	24·5	30·2

* Significant at 0·1 % level.

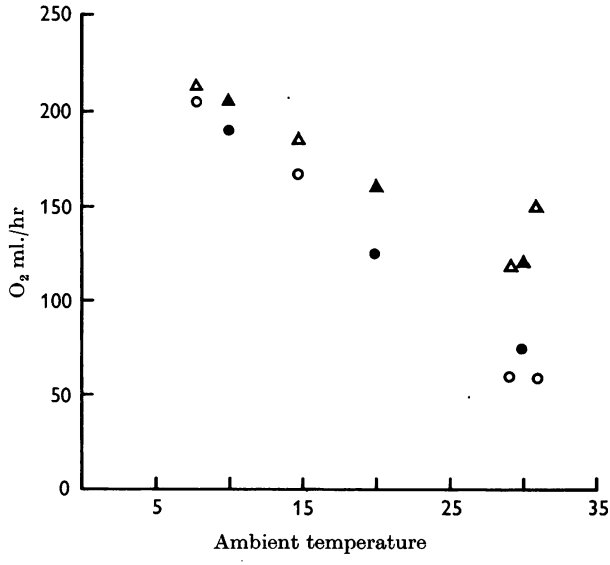

Fig. 6. Oxygen consumption rates (ml./hr) of mice at different activity levels over a range of environmental temperature. Results from Hart (1952): ● zero activity, ▲ activity at 4 cm/sec; present results: ○ zero activity, △ active during 50 % of time.

Hart's results and those of the present paper are given as oxygen consumption against ambient temperature for both active and inactive mice. Figures corresponding to 50 % activity from the present results agree closely with those from Hart's activity level of 4 cm/sec. As the temperature falls both sets of results show that the active and inactive lines tend to converge, indicating a smaller increment for a given level of exercise in the cold. In fact, Hart (1952) did find a significant increase in the metabolic increment due to activity (10 cm/sec) at 30° C, but not at lower temperatures.

Results from Hart (1958) and Hart & Jánský (1963) suggest possible reasons for the discrepancy between Hart's earlier conclusions and those put forward in his later discussions. Hart (1958) referred to observations on warm- and cold-acclimated rats running in a treadmill. In the warm-acclimated rats, the exercise increment in metabolism was small at −4° C and large at 30° C; in the cold-acclimated rats the increments were large at both temperatures. Further, the warm-acclimated rats showed a fall in colonic temperature during exercise in the cold, whereas the cold-acclimated rats showed a rise. The interpretation suggested by Hart is that in the warm-acclimated rats, which produce heat by shivering, exercise thermogenesis is substituted for shivering thermogenesis, although not enough heat is produced to offset the fall in body insulation and the

associated rise in heat loss. In cold-acclimated rats, however, the heat production of exercise instead of being substituted for the existing non-shivering thermogenesis is added on.

Hart & Jánský (1963) later extended these observations. They found that whereas warm-acclimated rats became hypothermic when exercised below 10° C, rats acclimated to the cold did not succumb to hypothermia during exercise until the environmental temperature dropped to −20° C. They pointed out that the extension of the temperature range for activity in cold-acclimated rats could be due to the development of non-shivering thermogenesis, which, unlike shivering, is not eliminated by exercise.

It is possible that a similar explanation may apply to the present results which show activity thermogenesis being substituted for thermoregulatory heat production, since the mice used were accustomed to 23° C ambient temperature and were more likely therefore to be far less cold-acclimated than if they had been at a temperature close to 0° C. Over the course of the 24 hr periods, mice at 8 and 15° C, the temperatures at which substitution apparently occurred (see Figs. 4 and 5), showed no systematic fall in rectal temperature (see Table 3). Their temperatures were not measured continuously, however, so that a fall in body temperature during the nocturnal period of high activity cannot be ruled out.

TABLE 3. The means and standard errors of rectal temperatures of single mice taken at the beginning and end of 24 hr oxygen consumption measurements at several environmental temperatures

Environmental temperature (° C)	Rectal temperature (° C)		Number of observations
	Initial	Final	
8	37·1 ± 0·5	36·4 ± 0·3	4
15	37·0 ± 0·3	36·9 ± 0·1	4
29	36·4 ± 0·6	36·3 ± 0·3	4
31	37·0 ± 0·1	36·9 ± 0·2	6
33	36·7 ± 0·3	36·8 ± 0·1	5

In this connexion, the mice used by Hart (1950) were kept at 6° C for 3 weeks before the experiment, and possibly would not show the substitution effect because they were cold-acclimated, whereas those used in the later experiments (Hart, 1952) were kept at 20° C.

Figures 2 and 3 show the relative independence of activity and oxygen consumption at the lower temperatures as compared with the higher. Although the marked 24 hr variations in metabolism in the warm shown in Fig. 1 may be due largely to variation in activity, the same is not true at lower temperatures. At 8° C the 24 hr variation in single mice is very considerably reduced; the resting metabolism is as high as the metabolism when the animals are active, which is in accordance with the substitution hypothesis.

Müller-Beissenhirtz & Ohnesorge (1966) recently measured oxygen consumption rates and spontaneous activity (measured on a wheel) simultaneously in mice during $2\frac{1}{2}$ hr periods. They used environmental temperatures of 15, 20, 25, 30 and 35° C. The regression of oxygen consumption rate on level of motor activity was 2–3 times higher at 35° C than it was at the other temperatures. This finding appears to be in the same sense as the present result, although wheel turning and our values for percentage of time active may give different measures of activity.

Grouping mice together does not affect the results when it is recognized that the presence of the group effectively means a rise in environmental temperature for the individual mouse. As can be seen from Fig. 1, the level of oxygen consumption for a group at 8° C is roughly the same as for a single mouse at 15° C, showing that grouping reduces the cold stress by 7° C. The peaks are present in the groups at 8 and 15° C because during activity the group breaks up and the mice behave as single individuals in their thermoregulatory responses. The effect of the group in modifying the influence of the environment is seen also in chickens (Kleiber, 1961) and pigs (Mount, 1960), and even putting two mice together has been demonstrated by Prychodko (1958) to decrease the usual cold-induced rise in food consumption.

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