J. therm. Biol., Vol. 4, pp. 251 to 255
Pergamon Press Ltd 1979. Printed in Great Britain

THE CIRCADIAN RHYTHM OF BODY TEMPERATURE OF UNRESTRAINED OPOSSUMS, *DIDELPHIS VIRGINIANA**

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(*Received* 18 *September* 1978; *revised* 4 *January* 1978; *accepted* 11 *January* 1979)

Abstract—1. The circadian rhythms of body temperatures of 4 female opossums maintained at a constant temperature ($T_a = 21 \pm 0.5°C$) were studied by means of radiotelemetry. Two of these opossums were also studied during cold exposure ($T_a = 7 \pm 0.5°C$).

2. The circadian rhythm showed a mean variation between day and night readings of 1.70° at 21°C ambient temperature and 3.19° at 7°C ambient temperature.

3. At 21°C ambient temperature, the mean minimum daily body temperature ranged from 32.91° to 35.60°C (mean 34.42°C); the mean maximum daily body temperature ranged from 34.79° to 37.37°C (mean 36.12°C).

4. During 7°C ambient temperature, the mean minimum daily body temperature ranged from 31.30° to 36.41°C (mean 33.86°C); the mean maximum daily body temperature ranged from 35.10° to 39.00°C (mean 37.05°C).

5. The highest body temperatures all occurred at night and were usually associated with activity.

6. A method of clarifying the reporting of body temperature data is suggested.

INTRODUCTION

WE ARE unaware of any studies of circadian rhythms of the opossum, *Didelphis virginiana*, in which body temperatures have been measured continuously or have been collected from unrestrained animals in both natural and caged environments. Body temperatures have been obtained from rectal measurements of restrained opossums by Higginbotham & Koon (1955) using thermocouples, and by McManus (1969) using rectal thermometers. McManus (1969) reported that no daily fluctuation in body temperature occurred. However, variable day and night temperatures determined for the Central American opossum, *Metachirus nudicaudatus* (Morrison, 1946), and radiotelemetry studies showing circadian rhythm of body temperature with other marsupials (Guiler & Heddle, 1974; Brown & Dawson, 1977) lead us to anticipate the presence of a circadian rhythm of body temperature in the opossum. The present study was undertaken to investigate this issue.

MATERIALS AND METHODS

Four female opossums, designated *O*, *P*, *Q* and *R*, were used in this investigation. All animals had been obtained from Florida, had been pregnant, and had been housed in the animal colony for 2 months after lactating had ceased, before measurements were taken. Their mean weight was 2.37 kg. Each animal was housed in the laboratory at separate times from March through August, 1977 and was fed on Purina "Puppy-O" chow; the high calcium in this chow is needed by opossums.

Radiocapsules (Mini-Mitter Company, Inc., P.O. Box 88210–G, Indianapolis, IN 46208, Model L) were implanted in the peritoneal cavity on the ventral abdominal wall of each opossum 4–7 days before the first temperature readings were taken. The radiocapsule transmitted pulses with a frequency which is a function of temperature. The pulse frequency was calibrated with a mercury thermometer in a sereological water bath. The curve of best fit equating body temperature with pulses/min was obtained using a Fortran program (STEPREGN) for a step-wise regression analysis. The resulting relationship between body temperature (T_b) and pulses/min (y) was:

$$T_b = 3.77537 + 0.45509\,y - 0.00124\,y^2.$$

All body temperature measurements were obtained by this equation.

The signal from the radiocapsule was transmitted by a Citizen Band Radio (Midland International Corporation, Type No. 13–701B) and was tape-recorded (model Panasonic RQ–309AS) through a time switch (Honeywell time activator Type R883B) as a series of 60-s events every 30 min. The pulses were timed with a stopwatch; automatic pen recording on paper was not appropriate due to periodic static caused by sunspot activity and adjacent laboratory electrical interference.

Each of the 4 opossums was housed for 9–12 days in a constant temperature room ($T_a = 21 \pm 0.5°C$) in a steel cage approximately 54 × 54 × 76 cm. In addition, opossums *Q* and *R* were housed in a cold chamber ($T_a = 7 \pm 0.5°C$) for 5–7 days in a smaller cage approximately 33 × 35 × 56 cm. Upon removal from the cold chamber, they were maintained in the same cage at 21°C. The body temperature of each opossum was determined every 30 min while the animal was maintained at air temperature of 21° and 7°C. Throughout the investigation the conditions were a light cycle of L:D 9:15 (0830–1730 h) and a relatively constant humidity.

Periodic regression analysis of body temperature data

In order to improve the description and validity of our results, we examined the data using a periodic Fourier regression analysis developed by Bliss (1958, 1970). A computer program developed by Sharma (1968) performed all

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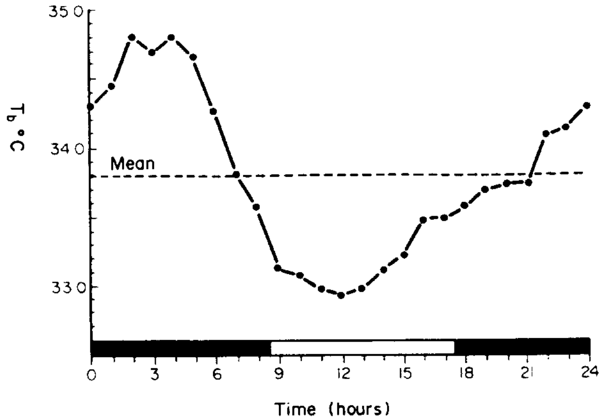

Fig. 1. Mean hourly body temperatures of opossum R at control temperature (n = 9 days).

the mathematical operations. The Fourier series was fitted to the body temperature measurements across days for each opossum at 21°C and for the 2 opossums maintained at 7°C. A separate regression analysis was conducted for each animal using 24 measurements taken every 60 min on the hour and 24 measurements taken every 60 min on the half hour.

RESULTS

Circadian rhythm at 21°C ambient temperature

All 4 opossums exhibited prominent daily rhythms. The mean body temperature for each hour of the day for opossum R is shown in Fig. 1. These graphed data are typical of the other 3 animals; nocturnal mean body temperatures were higher than the day-time mean body temperatures. Maximum body temperatures existed almost exclusively (96%) during hours of darkness (1730–0830 h), and minimum body temperatures had their greater incidence (80%) during daylight hours (0830–1730 h). Furthermore, minimum body temperatures not recorded during daylight hours occurred shortly after the onset of darkness (1800–1930 h).

Sample observations indicated that all 4 opossums slept predominantly during daylight hours and were rarely active during this time. On the other hand, the opossums were usually found feeding in the latter hours of the evening (2100–2400 h). The mean maximum body temperatures all occurred during hours of darkness while two of the minimum body temperatures occurred during daylight hours and two occurred (opossums O and P) shortly after the onset of darkness (Table 1).

Circadian rhythms at 7°C ambient temperature

The circadian rhythms of body temperature were not upset when opossums Q and R were maintained at 7°C. However, the range from mean minimum to mean maximum body temperature increased from 2.05° to 2.59°C and from 1.88° to 3.80°C for opossums Q and R respectively. The mean hourly body temperatures for opossum R before, during and after cold exposure at 7°C are shown in Fig. 2.

The decline in body temperature for opossum Q was different at 7° compared to 21°C and different from the other 3 opossums in that the low body temperature anticipated at 0400–1000 h showed instead several periodic elevations of temperature (Fig. 3). Opossums Q and R exhibited different thermoregulation responses at 7°C. Both minimum mean and maximum mean daily body temperatures were clearly elevated for opossum Q whereas the minimum mean daily body temperature was depressed for opossum R and the maximum mean daily body temperature was not significantly elevated (Table 1).

Behavioural responses

The 4 opossums varied consistently in their behavioural responses at the same T_a in the same cage. Opossums O and P tended to present aggressive dis-

Table 1. Mean maximum and minimum body temperatures and their ranges for 4 opossums at control and cold temperatures

Animal	T_a (°C)	Mean max. T_b (°C)	Hour	Mean min. T_b (°C)	Hour	Range (°C)
O	21	35.78 ± 0.08*	0100	34.69 ± 0.10*	1800	1.09
P	21	37.37 ± 0.40	0400	35.60 ± 0.18	1730	1.77
Q	21	36.55 ± 0.19	0130	34.50 ± 0.18	1600	2.05
R	21	34.79 ± 0.12	0400	32.91 ± 0.22	1230	1.88
$\bar{X}$		36.12		34.42		1.70
Q	7	39.00 ± 0.28	2000	36.41 ± 0.37	0400	2.59
R	7	35.10 ± 0.12	2030	31.30 ± 0.29	0930	3.80
$\bar{X}$		37.05		33.86		3.19

* S.E.M.

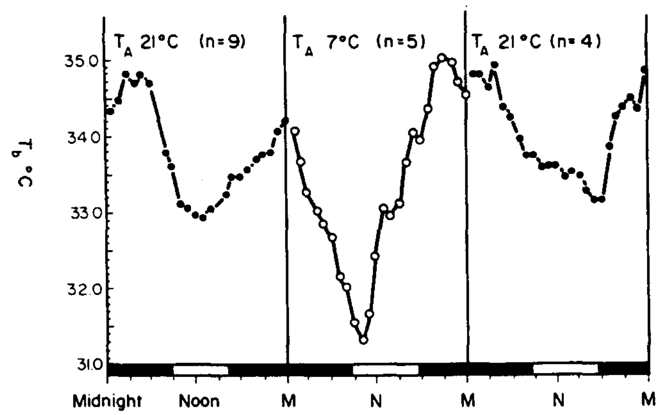

Fig. 2. Mean hourly body temperatures of opossum *R* before, during, and after cold exposure. The increased range in body temperature during cold exposure and the maintained circadian rhythm are clearly evident.

plays if awakened or if approached during feeding. On similar occasions opossum *Q* tended to be hyperactive, either running around the cage or climbing into a "safe" corner. On the other hand, opossum *R* tended to be very docile; this may explain the lower body temperature of this animal (Table 1).

When the opossums were housed at 7°C, they initially tended to explore the new cage and frequently exhibited aggressive displays. In contrast to her usual sleeping behaviour at 21°C ambient temperature, opossum *R* was noted to be awake during the mid-periods of daylight in addition to late evening. Examination of the data for mean body temperature at 7°C ambient temperature shows that the circadian trough was at 0900 h compared to 1200 h during 21°C ambient temperature. Similarly the maximum mean body temperature at 7°C occurs during 2000 h compared to 0400 h at 21°C (Figs 2 and 4).

At 21°C the resting body positions of all opossums were quite variable with curling up on the side being the most predominant position. No characteristic position of the tail was noted. During exposure to 7°C ambient temperature, both opossums tended to curl more tightly than at the higher temperature and to pull the tail and feet closer and sometimes under the body. Piloerection of the fur was evident especially around the shoulder regions; visible shivering in the first 2–3 days in the cold chamber was observed.

Necessary changes in operating techniques

We had intended to house all 4 opossums in the cold chamber but opossums *O* and *P* "grew" the radiocapsule through their abdominal wall in approx. 3 weeks and hence could not be used at the lower T_a. Opossum *P* was sacrificed for closer examination of this phenomenon. The radiocapsule was visibly protruding from the skin and the underlying muscle was intact; there was no indication of an opening in the abdominal musculature. The opening in the skin contained several silk sutures which could be lifted out easily. One silk loop which had fastened the radiocapsule to the ventral abdominal wall was still intact; this loop must have passed through all the abdominal musculature. Upon examining the interior, there was one slight adhesion, completely non-

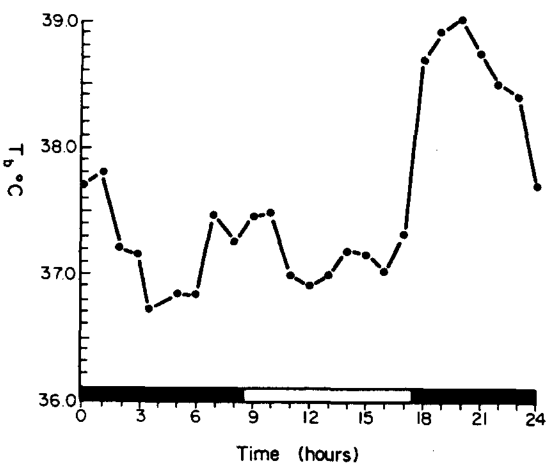

Fig. 3. Mean hourly body temperatures of opossum *Q* during cold exposure ($n = 5$ days). The low temperature settings predicted from 0400 to 1000 h show instead periodic elevations.

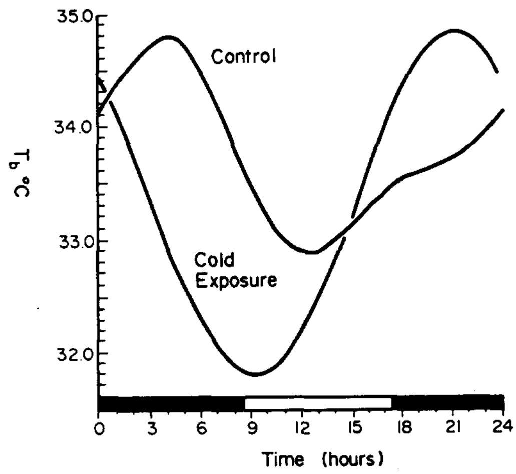

Fig. 4. Least squares fitted curve for hourly body temperatures of opossum *R* at control temperature (using two successive harmonics) and cold temperature (using one harmonic).

complicatory, attached at the original incision. There was no evidence of incision or passageway for the radiocapsule through the abdominal wall. A layer of yellow fat had been deposited at the location of the incision. In essence, this was a remarkable healing process to take place within 3 weeks.

The operating procedure was modified for securing the radiocapsule in the peritoneal cavity on the ventral abdominal wall of opossums *Q* and *R*. The radiocapsule was secured below the muscle incision so that movement out through the abdominal wall and linea alba was prevented.

DISCUSSION

Higgenbotham & Koon (1955) and McManus (1969) used thermocouples and mercury thermometers respectively to measure the rectal temperature of restrained opossums. In this investigation radiocapsules were secured in each abdomen following calibration with a mercury thermometer. Data were collected from 4 opossums over a period of 9–12 days at 21°C ambient temperature and from 2 of these opossums for 5–7 days during cold exposure at 7°C ambient temperature.

There are two prime reasons for using radiotelemetry to collect T_b data. First, there are inherent problems in comparing measurements in the literature which have been obtained using rectal probes and which are not resting body temperatures. For example, if one attempts to relate body temperature to size of species, it is basal T_b not mean daily T_b which should be utilized (Folk *et al.*, 1977). Second, the use of rectal probes which restain the animal will elevate the T_b. Poole & Stephenson (1977) further elaborate on this shortcoming of rectal temperature measurement.

The mean body temperature amplitude of 1.70°C (T_a = 21°C) for the circadian rhythm of *Didelphis virginiana* as determined in this investigation is comparable to the daily fluctuation of body temperature (variable T_a) noted by Morrison (1946) for the Central American opossum, *Metachirus nudicaudatus*. This investigation would clearly contradict McManus' (1969) findings when he noted that there were no daily fluctuations in the body temperature of the opossum.

In a summarizing paper dealing with "primative mammals", Dawson (1973) reports a body temperature range of 34.0°–36.5°C with a mean of 35.0°C for the opossum. (This value was obtained from the South American opossum *Didelphis fensis* which is superficially indistinguishable from *Didelphis virginiana* and has similar habits.) McManus (1969) presents similar results for the mean body temperature of female opossums at 25°C of 35.65 ± 0.65°C. Our calculated overall mean for daily body temperatures (35.22 ± 0.38°C) and range (33.78°–36.34°C) at 21°C ambient temperature are comparable to values described by the above authors.

The findings of this investigation are comparable with more recent radiotelemetry studies of the circadian rhythms of body temperatures of Australian marsupials by Guiler & Heddle (1974) and by Brown & Dawson (1977). The former researchers showed that the daily mean variation was 2.2°C (variable T_a) for Tasmanian devils, *Sarcophilus harrisii*; the latter researchers obtained a daily mean variation of 1.0°–2.5°C (T_a = 16–31°C) for red kangaroos, *Megaleia rufa*. Guiler & Heddle's (1974) results also showed that the body temperature of Tasmanian devils reached a maximum during the hours of darkness, following the characteristics of a nocturnal species (Morrison, 1965). The present investigation yielded compatible body temperature findings from the opossum which is also a nocturnal marsupial. Hulbert & Dawson (1974) have suggested that earlier findings by Morrison (1962, 1965) showing elevated nocturnal temperatures could have been caused by handling the animals when measuring body temperature while they were active. The variation in body temperature observed in the opossum from the present study is clearly not caused by subjecting the animals to any stress.

The finding that the mean hourly body temperatures were significantly different on different days is most unusual. Prior to the second half of this century, it was generally accepted that marsupials were primitive or inferior homeotherms. Recent studies (discussed in Dawson, 1973), however, have clarified the position that marsupials are excellent homeotherms and use similar mechanisms to maintain their body temperature as those of advanced eutherians. Although we have no record of reported variation in mean body temperature across days for other animals, marsupial or otherwise, the non-systematic nature of these variations (see Fig. 5) convinces us that they are not an artifact of the experimental set-up.

A final point which arises from this investigation is the need for some clarification in reporting body

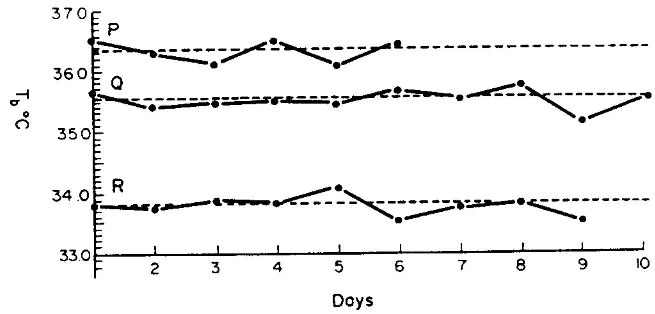

Fig. 5. Mean body temperatures of opossums *P*, *Q* and *R* on successive days at control temperature. The dashed line represents the mean T_b for all days for each animal.

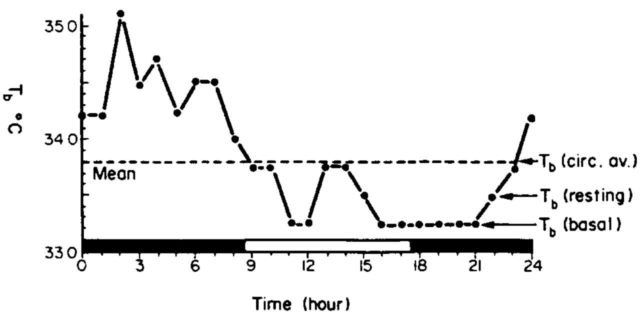

Fig. 6. Hourly body temperatures for opossum *R* at control temperature on day 1 illustrating three different measurements: $T_{b(\mathrm{basal})}$ and $T_{b(\mathrm{resting})}$ are measurements taken at a specific time; in contrast $T_{b(\mathrm{circ.av})}$ is the mean of all body temperatures over 24 hr (the circadian average).

temperature measurements. We suggest that body temperature be reported in three specific ways:

$T_{b(\mathrm{basal})}$: This is the resting or sleeping body temperature which is the lowest during 24 h, and is due to the physiological setting of the animal. It is found only at the time of the circadian trough.

$T_{b(\mathrm{resting})}$: This is the body temperature when the animal is asleep or resting and can be recorded as $T_{b(r_1)}$, $T_{b(r_2)}$, etc. $T_{b(r)}$ may be the same as $T_{b(\mathrm{basal})}$ or it may occur at any time on the circadian curve.

$T_{b(\mathrm{circ.av})}$: This is the body temperature of the animal averaged over 24 h. It combines resting, sleeping, exercise and activity values.

These three specific body temperature measurements are illustrated in Fig. 6 which is a graph of the hourly body temperatures for opossum *R* at 21°C ambient temperature on day 1. The $T_{b(\mathrm{resting})}$ measurement was recorded when the animal was sleeping at 2200 h. We believe that this manner of reporting body temperature data can greatly clarify comparative research in this area of thermal biology.

In summary, the opossum exhibits a circadian rhythm of body temperature with a daily range comparable to those found in other marsupials.

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