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Effects of Chronic Infusion of Lipopolysaccharide on Food Intake and Body Temperature of the Rat[1]

BARBARA O'REILLY, ARTHUR J. VANDER AND MATTHEW J. KLUGER[2]

Department of Physiology, The University of Michigan Medical School
Ann Arbor, MI 48109

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O'REILLY, B., A. J. VANDER AND M. J. KLUGER. *Effects of chronic infusion of lipopolysaccharide on food intake and body temperature of the rat.* PHYSIOL BEHAV 42(3) 287–291, 1988.—Unrestrained male Sprague-Dawley rats were infused for seven days with a low (2.45 μg/hr) or high (9.81 μg/hr) concentration of *E. coli* lipopolysaccharide (LPS). Compared to control (saline-infused) rats, food intake in the LPS-infused rats remained depressed for the entire infusion period. Despite this long-term suppression of food intake, fever was observed only during the daytime hours for the first two days of infusion. No significant increase in nighttime body temperature was observed. These data indicate that although tolerance to LPS occurred in rats with regard to its fever-inducing effect, tolerance with respect to its anorexigenic action did not occur.

Biotelemetry Fever Endotoxemia Circadian rhythms Body weight Activity

ENDOTOXEMIA results in the initiation of a stereotyped host reaction termed the "acute phase response" [8, 10, 19]. Among the many metabolic, physiologic and behavioral alterations associated with the acute phase response are the development of fever [6,13] and a reduction in food appetite [5, 11, 15, 25]. For many years it was assumed that infection- or endotoxin-induced anorexia was the result of the development of fever (see review by Brobeck [4]); however, McCarthy *et al.* [15,16] have shown that the reduction in food intake following the injection of lipopolysaccharide (LPS) or interleukin-1 into rats was not related to the elevation in body temperature.

There is an extensive literature on the effects of repeated injections of LPS on the development of "tolerance"; that is, after the second or third injection of LPS, the experimental animal (generally the laboratory rabbit), no longer develops a fever (see, for example [2,26]). Roberts *et al.* [20] infused endotoxin into rabbits and found that some rabbits developed fevers "which lasted as long as the pump continued to operate" (ten days). In their abstract, the authors stated that the rabbits did not "appear to become tolerant to the endotoxin, as they do following repeated daily bolus injections." Other acute phase changes were apparently not measured. Fish and Spitzer [7] have performed endotoxin-infusion experiments with rats, but their dose (0.3 mg/100 g body weight) was large enough to produce morbidity, as indicated by the development of both hypothermia and hyperlactacidemia, a fall in hematocrit, and a progressive leukocytosis. This dose of endotoxin was sufficiently high to result in the death of several rats.

The present study was conducted in order to characterize the pattern of food intake, body weight, body temperature, and activity during chronic endotoxemia using doses that do not produce indications of sickness or endotoxin shock. These variables were monitored in conscious unrestrained rats for 31 days. On days 11 through 18 the rats received a continuous infusion of sterile saline or LPS.

METHOD

Animals

Eighteen specific pathogen-free male Sprague-Dawley rats weighing 150 grams at the start of the experiment were obtained from Charles River Breeding Laboratories, Inc., Portage, MI. Rats were housed individually in a temperature-controlled room at 27±1°C, the thermoneutral zone for rats. A photoperiod of 12 hr light (0600 to 1800 hr) and 12 hr dark (1800 to 0600 hr) was maintained throughout the experiment. Tap water and rodent chow (Purina, 1001) were provided ad lib.

Measurement of Body Temperature and Activity

Body temperature and activity were measured using battery-operated biotelemetry devices (Model VMFH, Mini-Mitter, Inc., Sunriver, OR) implanted intraperitoneally into 9 rats three days prior to the start of the experiment.

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[2]Requests for reprints should be addressed to Dr. Kluger.

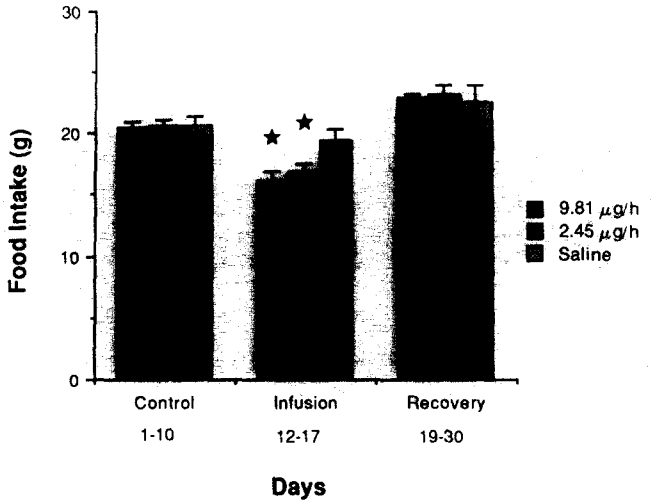

FIG. 1. Average daily food intake in rats infused with saline or LPS (9.81 μg/hr or 2.45 μg/hr) for Control, Infusion, and Recovery periods. Data collected on day 11 (the day of implantation of the Alzet pumps) and day 18 (theoretically, the pumps would have stopped infusing 10 hr into this day) were omitted. Asterisks indicate significant differences from saline-infused rats.

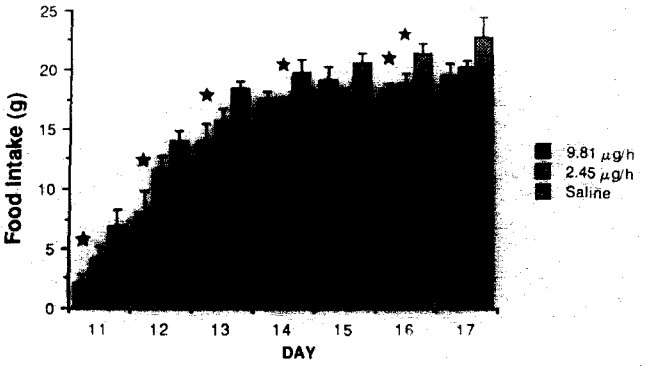

FIG. 2. Average daily food intake in rats during the 7 days of infusion with saline or LPS (9.81 μg/hr or 2.45 μg/hr). Asterisks indicate significant differences from saline-infused rats.

Each transmitter was calibrated prior to implantation. Both deep body temperature and activity were monitored by signals received by a mounted antenna placed under each animal's cage and fed into a peripheral processor (Dataquest III, Data Sciences, Inc.) connected to an IBM-PC. Temperature and activity counts were recorded at 30 minute intervals 24 hours a day for 31 days. See Scales and Kluger [23] for further details regarding the telemetry system.

Measurement of Food Intake and Body Weight

Between 11:30 and 13:30 of each day all rats and their food bins were weighed using a Sartorius 1206 MP electric digital scale. Food intake was measured by subtracting the weight of the food bin on each day from the previous day. The food bins were a type that retained most of the powder that results when rats gnaw food pellets, thereby minimizing errors due to noningested food. Since there was little difference among the groups in activity, it is unlikely that there would have been significant differences among the groups in food spillage.

Intraperitoneal Infusion of Bacterial Lipopolysaccharide (LPS)

After the rats were weighed on day 10 of the experiment, they were divided into three groups so that the mean weights of the groups were within 1 gram. On day 11, the rats were anesthetized with methoxyflurane, and Alzet osmotic pumps (2ML-1; 5-cm length by 1.4-cm diameter; reservoir volume 2.0 ml) were implanted intraperitoneally. These pumps deliver approximately 10 microliters of solution per hour for 7 days starting 4 hours post-implantation. The pumps were filled with either a sterile pyrogen-free 0.9% solution of sodium chloride (saline) or one of two concentrations of LPS (from *Escherichia coli* #0111:B4, phenol extract, Sigma Chemical) in saline. The rats were moving around their cages normally and had regained normal body temperature within 4 hours following implantation.

Six rats were implanted with saline-filled pumps (the "SAL" group). Six rats were implanted with pumps containing a LPS concentration of 250 micrograms/ml (the "LOW" group); this concentration was equivalent to an infusion rate of 2.45 μg/hr. Six rats were implanted with pumps containing an endotoxin concentration of 1000 micrograms/ml (the "HIGH" group); this concentration was equivalent to an infusion rate of 9.81 μg/hr. Three rats in each group had biotelemetry devices implanted prior to the start of the experiment.

Data Analysis

Day 1 to day 10 of the experiment was considered the "Control" period. Day 11 to day 18, during which time the rats were infused with saline or LPS, was considered the "Infusion" period, and days 19 through 30 were "Recovery" days. For analysis of temperature and activity data, "daytime" refers to all data collected between 06:00 and 18:00 hr; "nighttime" refers to all data collected between 18:00 and 06:00 hr. The mean change in daytime (or nighttime) body temperature during infusion was calculated by subtracting the average of the mean temperature for 12 hours on the last three control days (days 8 to 10), from the daytime (or nighttime) temperature for any desired infusion day. The mean relative activity during infusion was calculated by dividing the average of the raw counts on any given daytime or nighttime infusion day by the average of the mean on the last three control days (days 8 to 10). We chose the last three days prior to experimentation since this represented an extremely stable baseline to compare subsequent days.

All data were analysed using the computer service of the Michigan Terminal System. To determine whether infusion of LPS resulted in statistically significant changes in food intake, body weight, temperature or activity, the Student's *t*-test was used. For multiple comparisons, analysis of variance was used; intergroup comparisons were then made using Scheffe allowances. We have chosen only to compare food intake between LPS-infused groups and the saline-infused group. Because of the relatively small sample sizes, and the nature of statistics using ANOVA with Scheffe allowances, the decreases in food intake that were observed each day were not always statistically significant. To determine whether the rates of increase in body weight were significantly different, one-way analysis of covariance was used. All ± values represent standard errors of the mean.

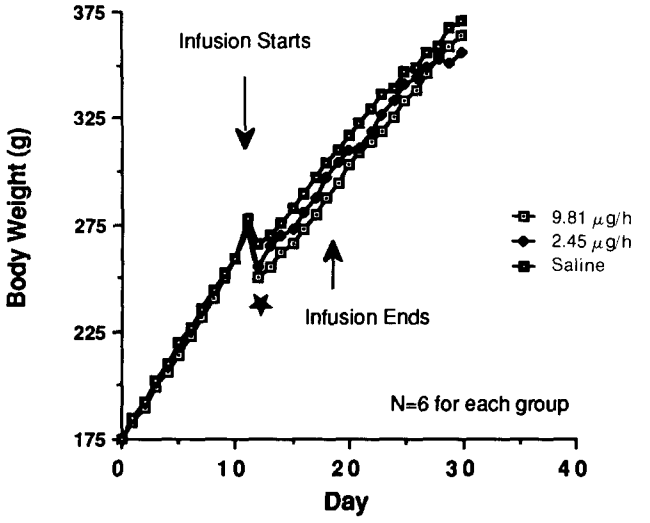

FIG. 3. Average daily body weight for rats infused with saline or with LPS (9.81 μg/hr or 2.45 μg/hr). Alzet pumps were implanted on the morning of day 11 and the infusion lasted for ca. 7 days. Asterisks indicate significant differences from saline-infused rats.

RESULTS

Food Intake

During the first 10 days of the experiment (the "Control" period), food intake was not different among the 3 groups (Fig. 1). The mean daily food intake for the entire infusion period was significantly lower in both the HIGH-infused ($p<0.01$) and LOW-infused ($p<0.02$) compared to the SAL-infused group (Student's t-test). During the Recovery period (day 19 through 30), food intake among the 3 groups was virtually identical. As shown in Fig. 2, food intake during the 24 hours following implantation of the Alzet pumps (day 11) fell in all three groups; however, the decline in food intake in the HIGH LPS-infused group was greater than in the SALINE-infused group. This magnitude of decrease in food intake in the HIGH LPS-infused group was generally seen on each day during the infusion period ($p<0.05$ on days 11, 12, 13, and 16, ANOVA, Scheffe allowances) (Fig. 2). For the LOW LPS-infused group food intake tended to be lower than that of the SAL-infused group on each day, but the differences were significant only on days 14 and 16 ($p<0.05$, ANOVA, Scheffe allowances).

Body Weight

During the "Control" period (days 0 to 10), the mean daily weights and the daily average weight gains of the rats in each group were similar (Fig. 3). From day 11 (when the Alzet pumps were implanted) to day 12, the weight of the SAL rats decreased by 10.1±2.0 g. This decrease in weight was significantly less ($p<0.01$) (ANOVA, Scheffe allowances) than that in the HIGH group, which lost 21.5 g (±2.37), or than that in the LOW group ($p<0.05$), which lost 19.2 g (±2.1). During the 7 days of infusion, mean body weight of the HIGH LPS-infused group remained depressed compared to the SAL-infused group (body weight increased 12.5±5.8 g in the HIGH LPS-infused group compared to 30.0±3.4 g in the SAL-infused group; $p<0.03$, Student's t-test). Although the body weight of the LOW LPS-infused group tended to remain depressed compared to the SAL-infused group, this difference was not statistically significant ($p>0.15$). After the initial decline in body weight on day 11 to 12, the rates of weight gain among the three groups were similar ($p>0.66$, analysis of covariance).

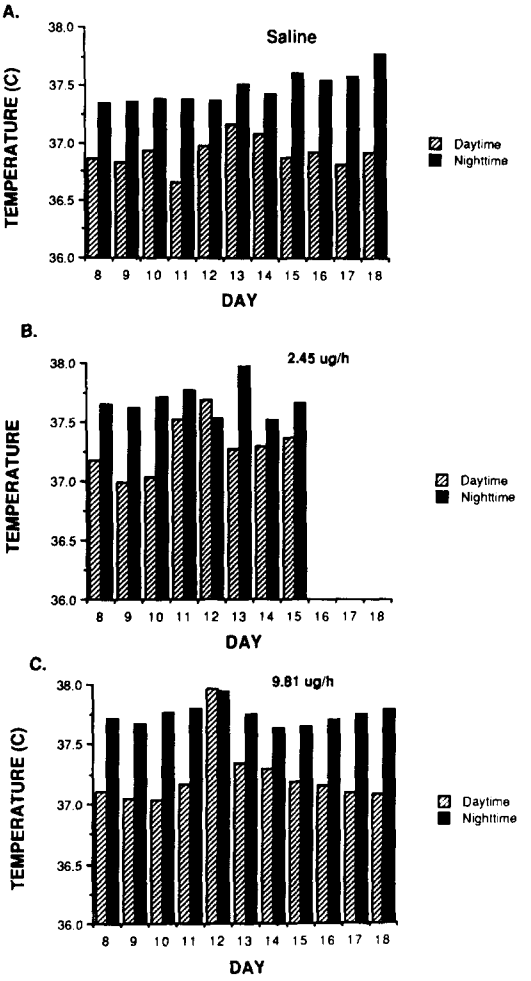

FIG. 4. Average daytime and nighttime body temperatures of rats infused with saline (A) or with LPS (B and C) (9.81 μg/hr or 2.45 μg/hr). By day 15, 2 of the 3 transmitters in the group receiving 2.45 μg/hr LPS stopped working; as a result, data from this group are omitted for days 16 to 18. When the batteries start to run-down, generally the temperature signals (frequency) becomes erratic; the information regarding activity seems to be transmitted for several days to weeks afterwards.

Body Temperature

Rats normally have pronounced circadian rhythms in body temperature (see, for example [22–24]). The average daytime and nighttime body temperatures for rats infused with saline or LPS is shown in Fig. 4. Even though infusion of LPS resulted in a reduction in food intake and body weight for several days, the only significant differences in body temperature between the SAL-infused and LPS-infused groups occurred during the daytime hours between the SAL and LOW on days 11 (the day of surgery) and 12, and between the SAL and HIGH on day 12 ($p<0.05$, ANOVA, Scheffe allowances) (Fig. 5A). Despite the continuous infusion of LPS, no significant increase in nighttime body temperature was observed (Fig. 5B).

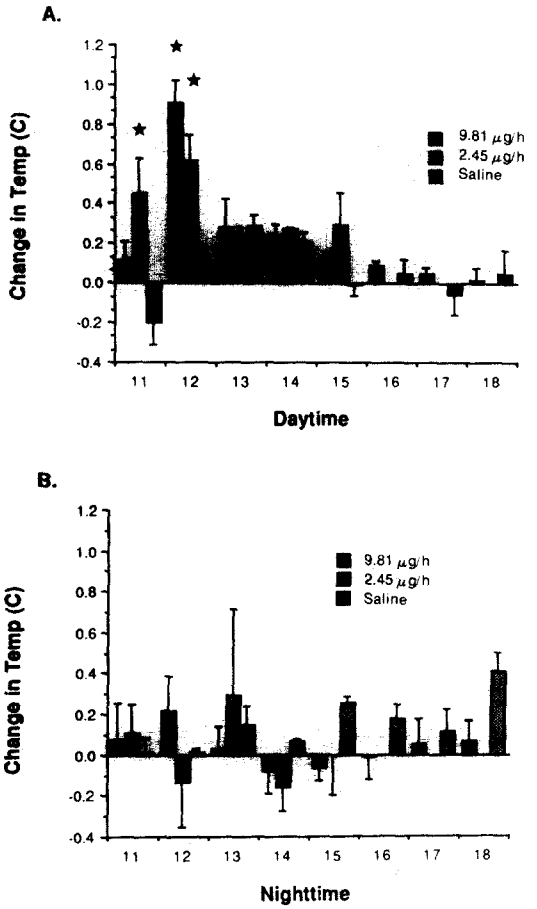

FIG. 5. Average changes in temperature for rats infused with saline or LPS (9.81 μg/hr or 2.45 μg/hr). (A) Mean daytime temperature. The mean temperature from 6:00 to 18:00 hr each day is subtracted from the average of 06:00 to 18:00 hr during the last three control days (days 8 to 10). Asterisks indicate significant differences from saline-infused rats. (B) Mean nighttime temperature. The mean temperature from 18:00 to 6:00 hr each night is subtracted from the average of 18:00 to 6:00 hr during the last three control days (days 8 to 10). By day 15, 2 of the 3 transmitters in the group receiving 2.45 μg/hr LPS had stopped working; as a result, data from this group are omitted for days 16 to 18.

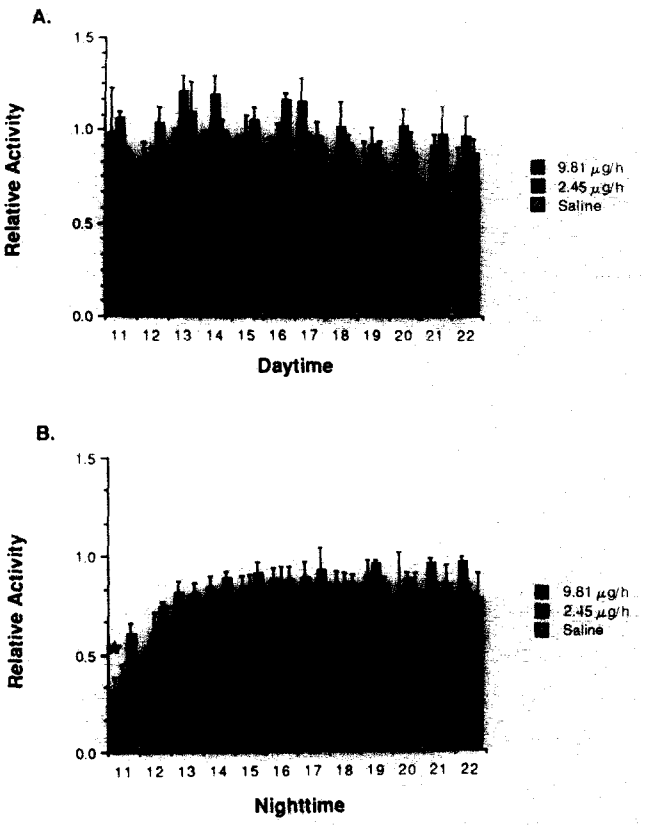

FIG. 6. Relative activity for rats infused with saline or LPS (9.81 μg/hr or 2.45 μg/hr). Values above 1.0 indicate greater activity than on control days; values below 1.0 indicate less activity than on control days. (A) Relative daytime activity. The mean activity from 6:00 to 18:00 hr each day is divided by the average of 06:00 to 18:00 hr during the last three control days (days 8 to 10). (B) Relative nighttime activity. The mean activity from 18:00 to 6:00 hr each night is divided by the average of 18:00 to 6:00 hr during the last three control days (days 8 to 10). Asterisks indicate significant differences from saline-infused rats.

Activity

Prior to implantation of the pumps, there were no differences in absolute activity counts among the three groups. Implantation of the Alzet pumps resulted in a reduction in nighttime activity in all three groups (Fig. 6). On day 11 (the day of surgery), there was a significantly greater decrease in relative nighttime activity in the HIGH LPS-infused group compared to the SAL-infused group ($p<0.05$, ANOVA, Scheffe allowances). After day 11, there were no significant differences among the three groups.

DISCUSSION

Infusion of LPS resulted in a rapid decrease in food intake and body weight greater than that observed in the SAL-infused group. During the first 24 to 36 hr after beginning the infusion the rats also developed a fever, but only during the daytime hours. This initial increase in body temperature (and, presumably, increase in metabolic rate) may account for part of the reduction in body weight compared to the saline-infused controls. Interestingly, even though body temperature returned to normal by the second day of infusion with LPS, food intake (for both LPS-infused groups) remained lower for the seven experimental days. These results indicate that although tolerance to LPS occurred in rats with regard to its fever-inducing effect, tolerance with respect to its anorexigenic action did not occur.

Although food intake remained depressed, the rate of increase in body weight in the LPS-infused groups was virtually identical to that in the SALINE-infused group. Except for the HIGH LPS-infused group during the nighttime following implantation of the infusion pumps, activity was not significantly different between the SAL and LPS-infused groups. Therefore, these data suggest to us that the LPS-infused groups may have been more efficiently utilizing the ingested food. It is, however, important to note that the LPS-infused groups remained about 10 g lighter than the SALINE-infused group. It is possible that this lower body weight simply resulted in a reduction in resting metabolic rate so that weight gain was normal despite decreased food intake. It would be interesting to compare 24 hour energy expenditures between LPS- and SALINE-infused rats.

Several studies have indicated that body weight is regulated around a "set point" [17,18], perhaps through the control of energy utilization. One study, by Levitsky *et al.* [14], showed that within a few days after food deprivation had

ended, the body weights of rats were similar to that of controls without there being complete compensation for the loss of food that had occurred during deprivation. Several reviews document studies of the high capacity that obese mice and rats possess for retention of dietary energy [3,9], and it is believed that a lower maintenance energy requirement of obese mice is a major factor contributing to their high efficiency of energy retention [21].

The infusion of LPS in the present study may have resulted in a reduction in set-point for body weight. By day 31 of the experiment, 11 days after the osmotic pumps had stopped infusing solution, the mean body weights of the two LPS groups were approaching values similar to those of the saline controls (see Fig. 3). Studies that monitor the body weight beyond 31 days would be needed to determine whether the LPS-infused groups undergo another change in set-point that brings their weight up to that of the SALINE-infused group. The suppression in food intake may have resulted from some direct actions of the LPS on the gastrointestinal tract, or indirectly through alteration in some metabolite or hormone concentration; but, despite the continued suppression in food intake, once body weight had fallen to the reduced set-point, the rate of weight gain proceeded normally.

It is interesting that despite the continued infusion of LPS, significant fevers were only observed during the daytime hours. As demonstrated here and elsewhere [22–24], body temperature is highest at night in the rat and lowest during the daylight hours. In a recent study, Scales and Kluger [23] have shown that much of rise in body temperature that occurs at night can be blocked by administration of antipyretic drugs such as sodium salicylate or indomethacin, both inhibitors of prostaglandin synthesis. These drugs had no effect on daytime body temperature of rats. Based on these data, it was suggested that the circadian rhythm in body temperature might be equivalent to a cyclical fever, dependent on the synthesis of prostaglandins within the central nervous system. If both the nighttime rise in body temperature and infection- or LPS-induced fever is dependent on synthesis of prostaglandins, it is not surprising that the magnitude of the change in body temperature (i.e., fever) that occurred during the nighttime in the LPS-infused rats was smaller than this change in body temperature during the daytime. A decrease in nocturnal fever in rats injected with brewer's yeast was recently reported by Satinoff's laboratory (presented as an abstract by M. Price, S. Kent and E. Satinoff at the Neuroscience meetings, 1987). A similar circadian reduction in fever was reported by Kluger for pigeons infected with live *Pasteurella multocida* ([12]; see page 117). Pigeons, generally active during the daytime hours, have a pronounced rhythm in body temperature with the peak occurring during these daylight hours. During infection, the change in body temperature from control days was considerably higher during the daytime than during the nighttime. If the circadian changes in body temperature are attributable to a circadian rhythm in prostaglandins, there may also be a similar circadian rhythm in either the circulating or tissue concentration of the putative mediators of fever: interleukin-1, tumor necrosis factor, or other endogenous pyrogen.

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