I. INTRODUCTION

Previous studies have demonstrated the existence of an adrenocortical response to acute microwave exposure in rats and rhesus monkeys (Lotz and Michaelson, 1978; Lu, et al., 1980a; Lotz and Podgorski, 1982). The adrenal response was observed only during exposures to a power density that was greater than a threshold power density required to cause a rectal temperature increase greater than 1.5 °C. In the rat, exposures to intensities greater than this threshold caused additional increases in both rectal temperature and circulating corticosterone levels. The dose-response curve for the rhesus monkey was not evaluated with multiple exposure intensities above the threshold for adrenocortical response, but corticosterone levels in the rat were strongly correlated with body temperature above this threshold such that the more the rise in body temperature exceeded 1.5 °C, the greater was the increase in corticosterone levels. The adrenal response was also similar in character and mechanism to the nonspecific adrenocortical "stress" response that has been associated with many environmental stimuli (Lu, et al., 1980b). The earlier experiments with monkeys were conducted with a 24-h experimental protocol that was designed to look for persistent or delayed effects of exposure, including those that might involve the known circadian rhythms in these parameters. However, no delayed or persistent effects of this type were noted. Because the overall response of
the pituitary-adrenal axis to stress has been reported to have a diurnal variation (Gibbs, 1970; Engeland, et al., 1977), and because diurnal variations have also been shown in the sensitivity of different levels of the axis to specific controlling hormones (Kaneko, et al., 1980), this study was designed to compare the effects of identical microwave exposures carried out at two different phases of the circadian cycle on body temperature and circulating cortisol levels of the rhesus monkey. Rectal temperature increases during 8-h microwave exposures were virtually identical for both day and night exposures even though sham and exposed absolute temperatures were substantially lower at night due to the normal circadian rhythm of body temperatures. However, the marked increase in circulating cortisol levels that was observed during the day exposures to 38 mW/cm\(^2\) was completely absent during the night exposures. The absence of a cortisol response during the night exposures may be simply related to the absolute body temperature reached, although more complex circadian influences cannot be ruled out by these data.

II. MATERIALS AND METHODS

A. Animals

Six male rhesus monkeys (Macaca mulatta, 6 to 10 kg) were used in these experiments. These animals were normally housed individually in standard metal primate cages and were maintained on a diet of Wayne Monkey Chow (Allied Mills, Inc., Chicago IL) supplemented with fresh fruit. The illumination in both the normal housing room and the experimental chamber was on a 16:8 light/dark cycle with lights on from 0600-2200. Animals were provided water ad libitum throughout the experiment. Prior to use in this study, the monkeys had been conditioned to prolonged restraint (up to 42 h) in a foamed polystyrene (Styrofoam) restraint chair and had had an indwelling venous catheter surgically implanted in the jugular vein using techniques adapted from those of Herd, et al. (1969) as previously described (Lotz, 1979).

B. Microwave Equipment and Exposure Protocol

The microwave exposures were conducted in a microwave anechoic chamber according to a 24-h protocol. Both the
chamber and the protocol have been described in detail previously (Lotz and Podgorski, 1982). Briefly, the microwave source provided 1.29 GHz pulsed radiation (3 μsec pulse width, pulse repetition rate 337 sec⁻¹) and the animals were irradiated in the far-field zone of a horn antenna while seated in a Styrofoam restraint chair. Power density measurements were averaged over the region occupied by the monkey (crown to rump) and the specific absorption rate was estimated on the basis of previous data (Olsen, et al., 1980) to be 0.107 (W/kg)/(mW/cm²) or 4.1 W/kg for 38 mW/cm² exposures. The resting metabolic rate (RMR) of a rhesus monkey is reported to be about 2.4 W/kg (Bourne, 1975). Exposures were eight hours in length and were conducted from either 1200-2000 (day) or 2200-0600 (night). Hourly blood samples were collected before, during, and after the exposure for a total period of 24-h from 0800 to 0600 the following morning. The samples were drawn remotely (from outside the chamber) via the catheter and were promptly centrifuged. The plasma was separated and stored frozen at -25 °C for later analysis. Body temperature was continuously monitored and recorded hourly using a YSI 402 thermistor probe (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) inserted 10 cm into the rectum of the monkey. Although this probe is not designed for noninterference in microwave fields, no temperature artifacts were observed in measurements accurate to 0.1 °C. These checks were made by cycling the microwave power on and off at each of the power densities used in this study, with the probe in the normal position in the monkey. The chamber was ventilated with room air, with air flow from the back to the front of the chamber with respect to the monkey. Over 30 m³ of air were exchanged each minute, a sufficient rate for an exchange of the chamber volume once per minute, and the velocity of air movement around the animal in the chair was 15 to 30 m/min. The restraint chair covered the back surface of the monkey’s body from the waist down to just above the knee, effectively blocking air flow from the pelvic and upper leg surface areas. The neck was also covered by the upper part of the restraint chair. Ambient temperature was maintained at 24 ± 2 °C (range) and relative humidity averaged 55 ± 17% (mean ± SD) during all experiments.

C. Hormone Analysis

Plasma samples were later thawed and analyzed for levels of cortisol by a competitive protein binding method (Murphy, 1967) and for thyroxine by a radioimmunoassay method
D. Data

Four monkeys were each exposed three times to each condition (sham-day, exposed-day, sham-night, exposed-night). Corresponding values from these three sessions were then averaged to obtain representative values for each subject. The data presented are the means of the values for the four subjects.

III. RESULTS

The effects of microwave exposure on the rectal temperature of these animals for day or night exposures are shown in Figure 1. For the shams, the normal circadian rhythm in deep body temperature is clearly apparent, with the lowest mean temperature occurring at 0100 hours. The pattern of response and magnitude of the temperature increase is virtually identical for both exposure situations, with an average increase of 1.7 and 1.6 °C, respectively, for day and night exposures (Table I). In both cases, the temperature increased steadily for the first two hours of exposure, then stabilized at an elevated level for the remaining six hours of exposure, before returning to sham exposure levels within one to two hours after the exposure ended.

Comparisons of mean rectal temperatures for the sham and exposure conditions for three time intervals are shown in Table I. Values for daytime exposures at 28 mW/cm² from another paper (Lotz and Podgorski, 1982) are included here for comparison purposes. No change in plasma cortisol levels occurred during these day exposures to 28 mW/cm². The three time periods represent the pre-exposure period for day exposures (0800-1200), the period during the day exposure when body temperature was elevated and stable (1500-2000, representing exposure hours three through eight), and the corresponding period during the night exposure when body temperature was elevated and stable (0100-0600). Note that the mean temperature during day exposure to 28 mW/cm² (1500-2000) is exactly the same as the mean temperature during night exposure to 38 mW/cm² (0100-0600). The increase in temperature (ΔT) is, however, quite different for those two periods. In contrast to that
Figure 1. Rectal temperature of rhesus monkeys sham-exposed or exposed to microwaves during the day (1200-2000) or at night (2200-0600). Arrows indicate the beginning and end of the exposure period. The dark bar on the abscissa indicates the dark period of the light cycle.
situation, the temperature increase for both the day and night exposures to 38 mW/cm\(^2\) is practically identical even though the absolute temperature means differ by 0.9 °C.

Table I. Rectal Temperature Comparisons

<table>
<thead>
<tr>
<th>Type of Exposure</th>
<th>Rectal temperature (mean ± SE)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0800-1200</td>
</tr>
<tr>
<td>SHAM</td>
<td>38.0 ± 0.1</td>
</tr>
<tr>
<td>28 mW/cm(^2) (DAY)(^b)</td>
<td>38.0 ± 0.1</td>
</tr>
<tr>
<td>38 mW/cm(^2) (DAY)</td>
<td>38.1 ± 0.1</td>
</tr>
<tr>
<td>38 mW/cm(^2) (NIGHT)</td>
<td>38.0 ± 0.1</td>
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</tbody>
</table>

\(^a\)Values are the mean of hourly measurements for 4 monkeys for times included in the intervals shown.

\(^b\)These data are taken from another study (Lotz and Podgorski, 1982).

*Circulating cortisol levels were significantly altered from sham levels (p < .05).

Circulating cortisol levels in the monkeys are shown in Figures 2A and 2B for day and night exposure sessions, respectively. For day exposures, a clear increase in cortisol levels exists, beginning two to three hours after the onset of exposure. It is readily apparent from Figure 2B that no difference between cortisol levels of sham or exposed monkeys existed during the night sessions. No differences in thyroxine levels existed between sham and exposed conditions for either day or night sessions.
IV. DISCUSSION

These results indicate that the effects of microwave exposure on body temperature and plasma cortisol levels of the rhesus monkey were influenced by the circadian rhythm in these parameters. The increase in body temperature (ΔT) caused by identical exposures conducted either in the day or at night was the same, but the peak level of that temperature was almost 1 °C lower at night. This comparable ΔT suggests that the thermoregulatory response to this microwave exposure was comparable for both day and night exposures, and that this response is consistent with the concepts that have been established from studies of thermoregulatory control mechanisms. The concept of a sustained error signal has been developed to explain body temperature and thermoregulatory responses during a sustained heat load associated with exercise (Stitt, 1979). The work of Nielsen and Nielsen (1965) suggests that the thermoregulatory response to a microwave heat load is essentially equivalent to an exercise heat load. The nocturnal drop in body temperature is thought to be due to a drop in the regulatory set-point at night in animals that are active during the day (Hensel, 1973). Wenger, et al. (1976) demonstrated that the nocturnal lowering of the thermoregulatory set point is accompanied by a lowering of thresholds for sweating and vasodilation in man. The increments in body temperature we observed during microwave exposure, virtually the same whatever the time of day, reflect the constant error signal produced by identical exposures, while the different levels of body temperature reached during irradiation at different times of day reflect the fact that the error signal is added onto a different level of thermoregulatory set point. There is no indication that the microwave exposure has any effect on the control of the basic circadian rhythm of body temperature.

One aspect of this temperature response that is not consistent with the analogy to exercise-induced hyperthermia is the fact that a ΔT in rectal temperature of about 1.6 °C is caused by a rate of microwave heating that is less than twice the resting metabolic rate of the animal. In exercising humans, the degree of hyperthermia is proportional to the relative work load, i.e. to the percentage of the maximum aerobic capacity. Rectal temperatures do not normally exceed 1.5 °C in humans engaged in sustained exercise unless the metabolic rate is more than 5 times the RMR (Saltin and Hermansen, 1966). Although there are few data in the literature on metabolic rates of exercising monkeys, it appears
Figure 2. Plasma cortisol levels in rhesus monkeys sham-exposed or exposed to microwaves. Arrows indicate the beginning and end of the exposure period. The dark bar on the abscissa indicates the dark period of the light cycle.

A. Cortisol levels for exposure during the day (1200-2000).

B. Cortisol levels for exposure during the night (2200-0600).
that rhesus monkeys can exercise vigorously at metabolic rates several times the RMR without causing more than a 1.5 °C rise in rectal temperature (Myers, et al., 1977). An important question, then, is how can a monkey dissipate heat more effectively when exercising than when heat is being generated in his tissues passively by exposure to microwave radiation? It may be that the rhesus monkey is not a good model for humans with which to study thermoregulation during exercise, even though the rhesus monkey is a good model for man with respect to thermoregulation at rest (Johnson and Elizondo, 1979). On the other hand, the rhesus monkey may be a suitable model for exercise-induced hyperthermia, but have a maximum aerobic capacity that is relatively low when compared to the RMR. In the case of a relatively low aerobic capacity, a microwave-induced heat load equivalent to twice the RMR might be predicted to cause a significant increase in body temperature, according to the principle that the degree of hyperthermia is proportional to the relative work load. However, based on the available information, this explanation seems unlikely. If the rhesus monkey is a good model of man for thermoregulation in the exercising or microwave-exposed subject, then other possible explanations for the relative inability to dissipate microwave heat must be considered.

Muscle blood flow may be an important part of the answer to this question, because the initial stimuli to which the blood flow responds is different for the two situations under consideration. Muscle blood flow in the active muscles increases rapidly within seconds of the onset of exercise. This increased blood flow is in response to the increased metabolic demands of the muscle, but the enhanced blood flow also transfers the metabolic heat to the body core where it provides information for the prompt and proportional control of thermoregulatory responses by the hypothalamus. However, at 1.29 GHz, the limbs of the monkey are heated at a rate as much as 5 to 7 times the rate of heating of the body core (Olsen, et al., 1980; Olsen and Griner, 1982). Much of this energy would be deposited in the muscles of the limbs, where blood flow is quite low in the resting animal. This heat could go virtually undetected by either the superficial cutaneous warmth sensors or by the hypothalamus, until muscle temperature had increased sufficiently to cause increased blood flow in the muscles. Based on studies with diathermy, blood flow in a passive muscle does not increase until local temperature reaches about 41 °C (Guy, et al., 1974). Thus, until muscle blood flow increases, the peripheral muscles may be storing a considerable amount of heat. Prior to that transfer, the
thermoregulatory response would be based on incomplete information while the amount of stored heat in the body increased. Once the muscle blood flow increased, the thermoregulatory response would increase in proportion to the additional heat load transferred to the body core, but the legs would probably remain relatively hot throughout the exposure in order to keep their blood flow high enough to carry off enough heat to avoid getting even hotter. It is possible that this extra heat transferred from the legs by the pelvic veins influenced rectal temperature, in a manner similar to that observed by Mead and Bonmarito (1949) in man, to make rectal temperature a biased index of deep body temperature in these microwave-exposed animals. The influence of microwave heating of the peripheral muscles might also explain the difference between the level of heating observed in this study and the level observed by Nielsen and Nielsen (1965), in which the diathermy exposure was limited to the body torso. The whole-body exposed monkeys had a rectal temperature increase of 1.7 °C at a microwave-heat production rate of about twice the RMR, while the human subjects of Nielsen and Nielsen had a rectal temperature increase of only ~ 1 °C at an electromagnetic-heat production rate of ~ 5 times the RMR. Obviously, some information on the temperature or blood flow in the leg muscles and on other deep body temperatures during microwave exposure is needed to test this hypothesis. If this idea holds up to such tests, a given rate of heat production and radiant energy absorption should not warm the leg muscles of exercising monkeys nearly as much as those of irradiated monkeys, because of the much higher blood flow in the exercising muscle.

The surprising result of these experiments was the complete absence of an adrenocortical response during the night exposures, in spite of the equivalent increase in rectal temperature. Earlier reports of both adrenocortical and behavioral effects of microwaves suggested the hypothesis that the interaction of these effects with body temperature was closely correlated with a threshold ΔT during microwave exposure (Lotz and Podgorski, 1982; de Lorge, 1979). The results of these experiments do not support the "critical ΔT" concept, however. These results are more compatible with the idea that the adrenocortical response was only stimulated in the rhesus monkey if the body temperature exceeded a particular critical level between 38.8 °C and 39.7 °C. Cortisol levels were not affected during exposures that raised rectal temperature to 38.8 °C, whether those exposures were to 28 mW/cm² during the day (Lotz and Podgorski, 1982) or to 38 mW/cm² at night, but cortisol
levels were increased during 38 mW/cm$^2$ day exposures that raised rectal temperature to 39.7 °C. The results do not, however, exclude the possibility that the absence of an adrenocortical response during the night exposure was due to complex mechanisms in the CNS control of the rhythmic neuroendocrine system that were unrelated to the body temperature. Even if a higher exposure intensity had been used to increase the rectal temperature to 39.7 °C at night, it is possible that the cortisol response would still not have occurred as it did during day exposures that raised rectal temperature to that absolute level.

The circadian rhythm of glucocorticoid secretion is well defined in most mammals, including man (Gallagher, et al., 1973) and the rhesus monkey (Holaday, et al., 1977). Kaneko, et al. (1980) have described a number of variations in the frequency and amplitude modulation of different levels of the adrenocortical system with respect to the circadian cycle of the rat. The observations of Kaneko, et al. may help to clarify a complex picture in which the adrenocortical response to stress has been reported to be either dependent or independent of the circadian cycle (Gibbs, 1970; Allen, et al., 1975). In those reports that indicate an adrenocortical response that is dependent upon the circadian rhythm, an enhanced response during the peak phase of the cycle has been observed in the rat. For the monkey, the corresponding time of greatest adrenocortical response would be in the morning, when cortisol levels are high. In the study reported here, the "day" microwave stimulus was actually delivered after the morning peak of cortisol levels had begun to decline, but while the levels were still near that peak. The "night" exposure was begun at or near the nadir of circulating cortisol levels and was continued through the period of increasing cortisol secretion to the time of early morning peak levels of cortisol. In actuality, these two periods are contrasted by changes in cortisol secretory rates (Holaday, et al., 1977) as well as cortisol levels in that the secretory rate is low in the early afternoon and high in the early morning. Furthermore, the plasma cortisol in monkeys irradiated during the day rose to, but not above, the early morning peak seen in sham experiments. Thus, these data could be interpreted to mean that small stimuli can increase the cortisol secretory rate up to its highest level in the normal daily range, but much larger stimuli are needed to increase it beyond this range. As shown in Figure 2A, irradiation during the day returned cortisol release to its highest normal daily level, reversing the drop in circulating cortisol, but the mild stimulus of irradiation (Figure 2B) did not alter the cortisol secretory rate at
night when it was already at its normal daily high, and hence produced no change in circulating cortisol levels. This interpretation might be tested in protocols in which the circadian rhythm of body temperature is separated from the pattern of plasma cortisol by infusing ACTH.

Only one other report has been found in which the effects of microwave exposure at different times of the circadian cycle have been studied. Lu, et al. (1980a) observed a suppression of the normal afternoon circadian rise in plasma corticosterone levels of rats exposed to 20 mW/cm² for eight hours. The colonic temperature of the rats was 1.35 °C higher after the exposure than after a sham exposure. The timing of their experiment was comparable, in its phase relationship to the circadian cycle, to the night exposures of rhesus monkeys in this study. However, the expected rise in plasma cortisol was unaffected in our study, but was suppressed during irradiation in the study of Lu, et al.

The hypothesis that the adrenocortical response observed in the day exposures depends on a body temperature in excess of a particular level might be tested by altering the ambient temperature in conjunction with microwave exposure. Other effects of microwave exposure have been shown to be altered by varying environmental temperature during exposure (Deichmann, et al., 1959). Thus, the cortisol response might be stimulated by lower intensity microwave exposure with higher ambient temperature or, conversely, might be negated by lowering the ambient temperature during the exposure that caused the observed effect. More complex experiments of this type using thermodes to alter hypothalamic temperature during exposure could also help clarify this question.

V. CONCLUSIONS

In summary, the results of this study demonstrate an influence of the circadian rhythm on the effects of microwave exposure on plasma cortisol and rectal temperature. The lower rectal temperature during night exposures was presumably due to the lower sham-condition temperature at night, since the temperature increase over sham levels was similar for either day or night exposures. The absence of a cortisol response during night exposures may be simply related to the absolute body temperature reached, although more complex circadian influences cannot be eliminated by these data. Although the results were insufficient to provide a clear understanding of the mechanisms involved, it
was shown conclusively that the responses studied depended not only on the independent variables of microwave exposure selected, but also on the baseline levels of the normal physiological state that existed at the time of exposure.

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Circadian Rhythm and Microwaves


