PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 196–204 (1984)

Circadian Temperature Rhythm and Circadian–Circaseptan (about 7-Day) Aspects of Murine Death from Malaria[1] (41788)

SALVADOR SÂNCHEZ DE LA PEÑA, FRANZ HALBERG, HANS-GEORG SCHWEIGER, JOHN EATON, AND JOHN SHEPPARD

University of Minnesota, Minneapolis, Minnesota 55455 Max-Planck-Institut für Zellbiologie, Ladenburg bei Heidelberg, Federal Republic of Germany

Abstract. About-7-day (circaseptan) and circadian rhythms were sought and found in host–parasite relations of mice infected with *Plasmodium berghei.* Five inbred male DBA mice, about 18 weeks of age, were implanted with transsensors for temperature telemetry. Core temperature, monitored every 10 min for 3 days before the intravenous or intraperitoneal inoculation of 10^5 infected erythrocytes and thereafter until death, was analyzed by cosinor. A statistically highly significant circadian rhythm exhibited similarly synchronized acrophases. Core temperatures on the days immediately after malarial infection were mostly within the range of temperatures observed before injection. A mesor-hypothermic stage preceded death by several days. In a second study, 24 male BALB/c and 42 male DBA mice, 12 weeks of age, housed in three rooms on different regimens of light and darkness (alternating at 12-hr intervals), staggered by 8 hr, were inoculated ip with 10^4 infected erythrocytes, one-half at noon, the other half at midnight, within 0.5 hr of blood withdrawal. Thus, one endeavored to cover six circadian host stages (02, 06, 10, 14, 18, and 22 hr after light-on). At 54 and 51% overall mortality (irrespective of inoculation time), a circadian rhythm in susceptibility to malaria was demonstrated in these mice by the single cosinor fit of a 24-hr period ($P < 0.003$ and < 0.020, respectively). The single cosinor fit of a 7-day period further demonstrated a circaseptan rhythm ($P = 0.014$) in the mortality of both strains following the inoculation of *P. berghei.* The acrophase (360° = 168 hr) was at −325° from the inoculation time with 95% confidence limits extending from −276 to −378°. Such predictable time relations of *P. berghei* to its murine host await the exploration of mechanisms underlying the circadian and infradian (7-day) rhythmicities here demonstrated and quantified with their uncertainties. Irrespective of mechanisms, information on such periodicities may also guide attempts to optimize treatment by timing according to the interactions of plasmodial virulence and host resistance that remain to be quantified separately.

Malaria is undergoing a resurgence and, in many areas, is becoming resistant to antimalarial drugs. For this reason and others, pathogen–host interrelationships are attracting increased attention (1). Relatively few efforts, however, have been made to investigate temporal aspects of the host in relation to the parasite (2–9), even if the organism's (9) and the microorganism's (11–14) circadian time structure are each separately well-recognized in textbooks for students and researchers (14).

As yet, rhythm analysis (10) has not been extended to a study of cyclic events like fever attacks in the case of (tertian or other) malaria, which have been well-known for at least several thousand years (9). By 1886, Golgi (15) correlated the multiplication cycles of *Plasmodium malariae* within erythrocytes with paroxysms in patients with quartan malaria. Such cyclic paroxysms may require a high degree of synchronization which remains a high-priority goal in continuous cultures (1) and may be a contribution of synchronizing effects exerted by the host upon the parasite, as well as *vice versa.* Since the mammalian host is characterized by a spectrum of circadian, circaseptan (7-day) (10), and other rhythms with differing periods, one might test whether a synchronization of malaria can be achieved by entrainment of the parasite by the host's rhythm spectrum, quite apart from interactions (such as those by Rowson–Parr virus) with *Plasmodium chabaudi,* which exhibit at least a single circaseptan cycle in the percentage of infected red cells (16). Synchronization of the life cycle of such parasites re-

[1] Supported by the National Institutes of Health (GM-13981) and National Cancer Institute (CA-14445).

0037-9727/84 $1.50
Copyright © 1984 by the Society for Experimental Biology and Medicine.
All rights reserved.

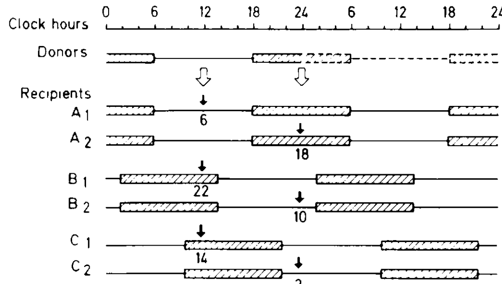
quires an extreme accuracy and uniformity of cellular–temporal organization. The mechanisms underlying this behavior of strains of *Plasmodium* which induce cyclic fever attacks (9) remain to be elucidated.

Some strains of *Plasmodium* (including *P. berghei*) are not known to show signs of synchronization. As background to this study, by 1948 it was noted that white mice succumb to what was just then recognized as *P. berghei* in 11–15 days (17, 18). The period of 11–15 days is a double circaseptan period, or in frequency terms, we are dealing with a so-called circaseptan frequency demultiplication or frequency division, a phenomenon of interest in chronobiologic study design (10). The question may be raised whether an apparent lack of synchronization may be due to the failure to standardize procedures for rhythm analysis. If rhythms could be demonstrated, their characteristics could serve as reference standards in studies of the peculiarities of the host, the parasite, and their interactions in determining the fate of a malarial infection. Answers to questions about rhythms may lead more generally to a better understanding of host–parasite relationships. This study was undertaken in order to find out whether the mortality of mice from *P. berghei* reveals any signs of temporal organization in the range of circadian (20- to 28-hr) and infradian (longer than 28-hr) periods.

Materials and Methods. *Plasmodium berghei* (NYU-2 strain) was maintained and passed in Swiss albino mice by inoculation of infected red cells as described earlier (19). In the first study, the donors of plasmodia were these albino mice; the donors in the second study were DBA/Jax mice, to which *P. berghei* had been passed in the interim. The mice were bled under ether anesthesia from the axillary vein. Blood was aspirated into a sterilized and heparinized glass pipet and transferred into a heparinized plastic tube. A stained blood smear was also prepared for enumeration of infected cells. At the first transfer from albino to DBA mice, which corresponded to the time of inoculation of the first study, 75% of the red blood cells were found to be infected.

For the first study, 5 DBA/Jax mice were singly housed in a room maintained in light alternating with darkness at 12-hr intervals (LD12:12), with Purina Laboratory Chow and deionized water freely available. The animals were implanted with transsensors for the monitoring by telemetry of core temperature every 10 min, as described earlier (20). The room was maintained at 24 ± 1°C and ~50% relative humidity. After 1 week of standardization when the animals were 18 weeks of age, doses of 10^5 infected red blood cells were inoculated at 12 hr after light-on (HALO). Three males received the inoculum intraperitoneally; two other males intravenously. Temperature was monitored in these animals until death. Results were analyzed by a so-called chronobiologic serial section (21), involving the fit by least squares of a 24-hr cosine curve and providing point-and-interval estimates for the following rhythm parameters: the mesor (the rhythm-adjusted mean), the amplitude (a measure of the extent of change, namely one-half of the peak-to-trough difference in the cosine curve best approximating all data), and the acrophase (a measure of the timing of rhythm, namely a lag from zero-time (light-on) of the peak in the function best approximating all data).

A second study used 66 mice from two different strains: 42 DBA and 24 BALB/c, all males, about 5 weeks ± 3 days of age. The mice were housed 2 per cage and standardized in light (L) and darkness (D) alternating at 12-hr intervals (LD12:12) in three rooms. All three rooms were kept at a temperature of 24 ± 1°C and humidity of 50%. Deionized water and food (Purina Chow) were freely available to the mice. The lighting regimens in the three rooms were staggered by 8 hr so that light was on in room A from 0600 to 1800 central standard time (CST), in room B from 1400 to 0200, and in room C from 2200 to 1000 (Scheme I). These regimens were maintained for 7 weeks prior to the start of the experiment and throughout its duration. On June 14–15, 1982, the animals were injected intraperitoneally with a fixed dose of 10^4 parasitized cells in 0.1 ml of saline-diluted blood at two different times (Scheme I) separated by 12 hr, 1200 and 0000 clock-hr. At each of these two times, a donor from room A was anesthetized ~15 min prior to inoculation. The blood obtained from the axillary vein was diluted in 0.9% NaCl solution, counted, and stored in an ice bath until inoculation within 30 min into the subgroups of animals from the three



SCHEME I. Time schedule for harvesting and inoculating parasitized erythrocytes. Blood was drawn from a *P. berghei*-parasitized donor mouse at noon (12) and from another at midnight (24). (⇩). Recipient mice, kept on three different light–dark regimes (A, B, and C), were inoculated at six different circadian hours (↓). Bars: dark span; lines: light span. The numbers give circadian hours for the respective groups.

rooms. The animals from the three subgroups were injected in alternation (Scheme I) in order to keep the time elapsed from blood collection to the injection of the recipient as similar as possible. The infection was thus administered at each of six different circadian stages, approximated by 02, 06, 10, 14, 18, and 22 HALO.

Scheme I must not be misunderstood to indicate that the same animals were injected at two different times; any one animal was injected only once. Separate groups of animals from each of the rooms were injected at different times. Thus, Scheme I shows that parasitized blood harvested from donors killed at noon (6 HALO), kept in light from 0600 to 1800, could be given to recipients in room A at 6 HALO, to animals in room B at 22 HALO, and to animals in room C at 14 HALO. By the same token, donors killed at midnight, that is at 18 HALO, allowed malaria inoculations not only at 18 HALO in room A, but also at 10 and 2 HALO in rooms B and C, respectively. As Scheme I shows, by killing mice at only 2 clock-hr, one could promptly infect mice at six circadian stages (if, as documented by temperature rhythmometry, the circadian systems had adjusted to the lighting regimens).

Mortality was assessed every 4–8 hr. Data were analyzed by the least-squares fit of trial periods with results presented by cosinor methods (21).

Results. A more or less apparent circadian bioperiodicity characterizes the time course of (telemetered) core temperature after the malarial injection. This bioperiodicity is revealed by Fig. 1, which shows each measurement as a dot for a so-called macroscopic inspection. The time of inoculation of *P. berghei* is shown as a vertical line in this figure. Any change following the inoculation (Fig. 1, vertical line) can readily be scrutinized, until death. The two mice receiving the *Plasmodium* intravenously (Figs. 1a and b) died before the animals receiving the *Plasmodium* intraperitoneally (Figs. 1c, d, and e). After inoculation, no elevations are seen in the core temperatures above the prior upper limit in four of the five mice, with the fifth animal showing two elevations in an incomplete record. This finding is in agreement with the results of much earlier work on agents which are pyrogens in human

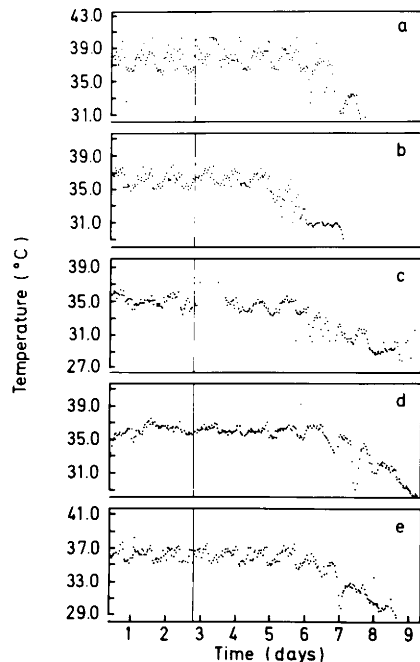


FIG. 1. Time course of intraperitoneal temperature in mice after infection with *Plasmodium berghei.* Elderly inbred DBA mice were telemetered before and after inoculation, intravascularly (a and b) or intraperitoneally (c–e) of *P. berghei* (NYU-2 strain). Each measurement is shown as a dot for so-called macroscopic inspection. Time of inoculation indicated by vertical line. Note mesor-normothermic and mesor-hypothermic stages in all five records.

beings and certain other species; in rodents (22), such agents usually fail to induce fever, defined as elevation above the usual reference range.

The naked eye can discern, at least in parts of the record, an about-24-hr or circadian variation (Fig. 1). The fit of a 24-hr cosine curve as a so-called chronobiologic serial section (21) to these data (not shown) validates the occurrence of a rhythm during most, though not all, of the record (by the rejection of the assumption of a zero-amplitude, i.e., of no rhythm). The acrophases of the temperature rhythm have thus been analyzed (Table I). A reasonable degree of circadian synchronization is found among the records of the different animals before inoculation. Of 10 acrophases (for 2 consecutive days on each of five mice), 9 are between −234 and −281°, i.e., within −47°; the 10th is at −1° and could also be written as −361°. Thus, all 10 acrophases are within 127°. Such relative circadian metabolic synchronization gauged by core temperature is also seen during most of the stage following inoculation, which does not yet involve a drop in core temperature (Fig. 1).

The naked eye can indeed discern two stages following malaria inoculation (Fig. 1): first, a span during which there is no change in range or in overall rhythm-adjusted mean, i.e., a mesor-normothermic stage; second, a span during which temperature drops progressively, i.e., a mesor-hypothermic stage. This decrease in mesor is clearly not a phase-shift of the rhythm known to occur following endotoxin injection in mice (22).

In view of the limited scatter of the temperature acrophases which were used as a metabolic marker rhythm characteristic (Table I), one might expect some degree of host synchronization to have prevailed at the time of inoculation; hence, one can anticipate that the time of inoculation may play an important role in survival as well (23). This anticipation is confirmed by the demonstration of a statistically significant 24-hr synchronized circadian rhythm in the resistance to *P. berghei* of mice of both strains tested. Original results are summarized at a time when about 50% of the mice, irrespective of inoculation time, had died (54 and 51% in the case of the BALB/c and DBA mice, respectively) as shown in Fig. 2a. At this time, the numbers of survivors are expressed as a percentage value of the total number of animals per group at each circadian stage of inoculation.

From a technical viewpoint, it is important to note that these results on a circadian rhythm are not due to the progression of time after the drawing of blood from the donor, so that progressively older blood is being used for inoculation. Fresh (parasitized) blood was used on each occasion within 30 min of its collection. Thus, the aging of blood cannot account for the circadian rhythm here documented.

The circadian survival data are analyzed by a so-called single cosinor (Fig. 2b). In this plot, the length of a vector represents the amplitude as a measure of the extent of predictable

TABLE I. CORE TEMPERATURE ACROPHASE OF *MALE* MICE BEFORE AND AFTER INOCULATION OF ERYTHROCYTES INFECTED WITH *Plasmodium berghei*

See Fig.	Days in relation to inoculation time (=0)									
	−2	−1	0	+1	+2	+3	+4	+5	+6	+7
					Intravenously inoculated					
1a	(−260°)	(−234°)		(−287°)	(−254°)	(−276°)	(−268°)	(−316°)		
1b	(−251°)	(−249°)		(−17°)	−259°	(−257°)	(−294°)	−307°	(−296°)	
					Intraperitoneally inoculated					
1c	(−264°)	(−239°)		(−248°)	(−232°)	(−246°)	(−245°)	−315°	(−339°)	−245°
1d	(−1.4°)	(−281°)		(−352°)	−281°	−250°	−229°	(−260°)	(−198°)	(−351°)
1e	(−260°)	(−253°)		—	(−246°)	(−226°)	(−288°)	(−257°)	(−88°)	(−110°)

Note. Acrophase given in degrees, 360° ≡ 24 hr (ref: light-on); underlined values associated with mesor-hypothermia; values in parentheses from cosine fit associated with rejection of zero-amplitude (no-rhythm) assumption.

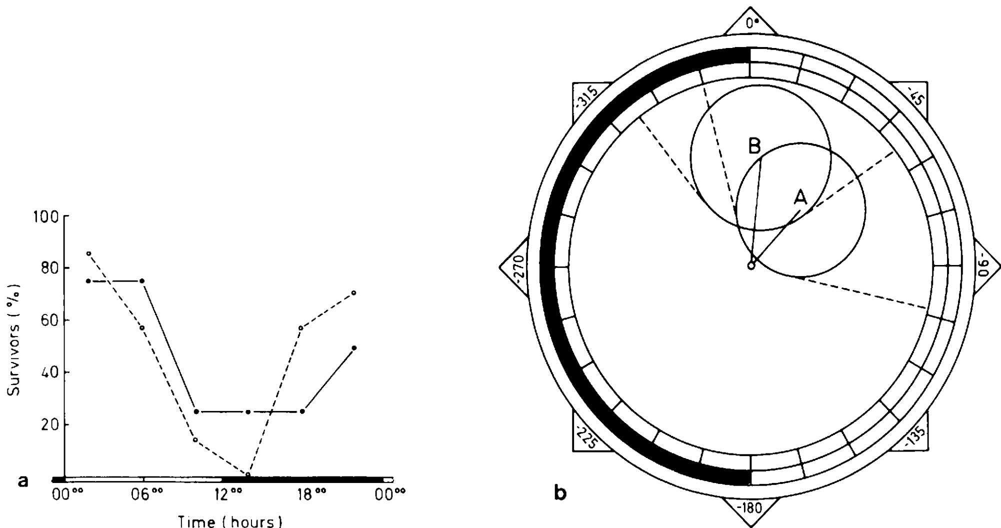


FIG. 2. Circadian rhythm in susceptibility of mice to *P. berghei*. BALB/c (○; four/timepoint) and DBA (●; seven/timepoint) were inoculated ip with 10,000 parasitized erythrocytes at one of six different circadian host stages, defined by standardization in light (empty bar) and darkness (full bar) alternating at 12-hr intervals (a). Evaluation at 54% and 51% overall mortality. For the two groups, the percentage rhythm is 90 and 93; the P is 0.033 and 0.020; the mesor (in percentage survivors) ± SE is 46.0 ± 5.2 and 47.0 ± 4.8; the amplitude (in percentage survivors) + 95% confidence limit (CL) is 30.0 (4.3–56.0) and 41 (11.9–71.0), and the acrophase + CL (with 360° ≡ 24 hr and 0° = L-on) is at −44° (−345 to −103°) and at −6° (−321 to −52°), respectively (b).

change; the angle of the vector is a measure of timing, the acrophase. The area around the vector tip represents a 95% confidence region for the amplitude–acrophase pair. This region does not cover the center of the display, i.e., the pole, a circumstance which documents the statistical significance of the rhythm. In terms of timing, the acrophases for the two strains being compared are 38° apart. This is equivalent to a difference in acrophase of 2 hr and 32 min, since 360° is equated for the analysis to 24 hr, and hence 15° ≡ 1 hr (1° = 4 min).

Further, we determined whether the pattern of times of death exhibits, in addition to any circadian aspects, some rhythms with a frequency lower than 1 cycle in 28 hr. Indeed, the display of the data from the two strains shows a peak clearly at ~7 days postinoculation for the C mice (Fig. 3a) and multiple peaks at 7, 14, and 21 days for the DBA mice (Fig. 3b). Statistical analysis of the data reveals a statistically significant circaseptan rhythm (Fig. 3c).

Discussion. A circaseptan (about 7-day) rhythm in mortality from malaria can be viewed in the context of other findings. A rhythm with similar period has also been found in the urinary 17-ketosteroid excretion of a healthy man over ~15 years: a period that differed slightly but with statistical significance from exactly 7 days was found to characterize this variable after the self-administration of testosterone suppositories (24). A consistent deviation of this period from precisely 7 days (24) suggests that one is dealing with the expression of an endogenous, maybe a genetically anchored frequency, rather than with a phenomenon impressed upon the organism by the routines of the social week.

Following the removal of one kidney and a 30-min ischemia of the remaining kidney, a dramatic circaseptan change in the remaining renal tissue was demonstrated (25). Over the years, Hildebrandt (26, 27) described a host of circaseptan variations, emphasizing their exogenous nature. With respect to mechanisms, Vollrath *et al.* (28) documented changes in a pineal enzyme that had a circaseptan frequency. More recently, he wrote that "the curves exhibited distinct troughs in the Friday–Saturday region, independently of whether the experiments started on a Monday

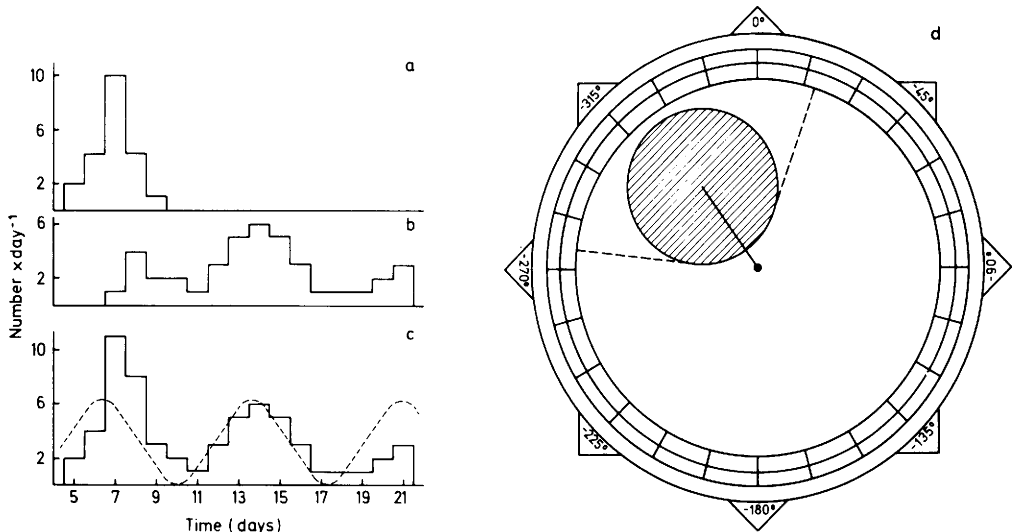


FIG. 3. Circaseptan rhythm in mortality following inoculation of *P. berghei* into mice. BALB/c (a) and DBA (b) mice were inoculated, data were pooled (c) and summarized by single cosinor with the fit of a 7-day period (d). $P = 0.014$; percentage rhythm = 51. In deaths per day, the mesor ± SE is 3.1 ± 0.6 and the amplitude is 3.2, with 95% confidence limits (CL) from 0.7 to 5.7. The acrophase (with 360° = 168 hr) is at −325° from inoculation time, with 95% CL from −276 to −378°.

or a Wednesday" (29). Circaseptan rhythmicity also characterizes the rejection of human kidney (30) and rodent skin, kidney, heart, or pancreas allografts (31–33). In the absence of any known socioecologic 7-day bioperiodicity, frequency response curves to shifts in the lighting regimen implemented at intervals varying from 2 to 11 or more days show the presence of circaseptan frequencies in face flies (34) and a unicell (35) that has presumably been around for several hundred million years. Moreover, in the enucleated unicell, studied on two occasions in the spring, the circaseptan frequency doubles into about two cycles per week (35). This finding suggests that the nucleus plays a role in the phenomenon of physiologic frequency division (or demultiplication), an observation of interest in chronobiologic study design (10, 33). A relative temperature compensation has also been demonstrated for the period of a circaseptan rhythm in *Folsomia candida,* the springtail (36), over a range from 15 to 25°C.

With respect to circadian bioperiodicity in host–parasite relations, the larvae of the nematode worm, *Wuchereria bancrofti,* emerge into the circulating blood by night in nocturnally resting diurnally active human subjects, as described before the turn of the century by Manson ((37); cf. also (38, 39)). In the human host on a similar schedule, the microfilariae of *Loa loa* have an oppositely timed circadian pattern (40). It is also known that the circadian bioperiodicity of *W. bancrofti* microfilariae can be shifted by changes in the routine of living (41) and that this microfilarial bioperiodicity can be synchronized by manipulating the adrenal cortex, e.g., by suppressing it transiently with Δ^1-9α-fluorocortisol (5, 6). Upon removal of suppression, the microfilarial rhythms appear to be more closely synchronized than before suppression.

With respect to the circadian stage dependence of endotoxin effects as well, a periodicity in the organism's ability to withstand its lethal effect has been repeatedly reported and recently reviewed (42).

Turning to *P. berghei,* Vandenberg *et al.* (43) presented data on the percentage of parasitized red blood cells at different times after inoculation. On inspection, these data show a peak at 6 and again at about 14 days. Statistical analyses of these and other data, however, were not done from the viewpoint of establishing any rhythm, even though the bioperiodicity of human malaria is among the

best-known organoleptically described phenomena.

It is against this background that our finding of circadian and circaseptan rhythms in the death of mice injected with *P. berghei* must be assessed. The mouse infected intraperitoneally with *P. berghei* constitutes a laboratory model for looking at circadian and circaseptan periodic mechanisms underlying immune phenomena, a topic recently reviewed (44). The present data do not allow extrapolation beyond the age, sex, inbred strain, dose, and kind of malarial inoculum and rhythmic components investigated. This qualification is made because of the remarkable seasonality recorded for several forms of malaria (3). Moreover, circannual periodicity modulates circadian and circaseptan rhythms and rhythms with other frequencies, not only in human beings (44), but also in several species investigated thus far in the laboratory (45).

The temperature data of this study demonstrate to the naked eye (Fig. 1) the well-known bioperiodicity of this variable before and after inoculation (10, 20). Cosinor analyses, however, are needed to quantify the extent of interindividual synchronization, shown in Table I. A consistent change in the timing of the circadian temperature pattern, reported for the effect of several endotoxins in rodents, does not take place with the inoculum used (22). The major effect of the *P. berghei* infection in the mice studied is a mesor-hypothermia, rather than the fever accompanying symptoms associated with malaria in human beings and several other nonrodent species. More generally, hypothermia from agents that are pyrogens in human beings is extensively documented in rodents (22, 42), as is the fact that the same dose of the same agent, such as an endotoxin, differs dramatically in its lethality as a function of the circadian stage at the time of infection (46).

The data presented here also suggest that the *Plasmodium*-infected mouse is a model in which the relative roles of either or both *P. berghei* and murine host can be explored for intermodulation between circadian, circaseptan, and other frequencies. These intermodulations may point to mechanisms underlying natural changes in the course of an infection of current interest (47), as a complement or alternative to the homeostatic view that fever, rather than being a harmful by-product of infection, constitutes a phenomenon enhancing resistance and increasing the chances of survival. On the basis of extensive comparative physiologic studies (48–53), Kluger *et al.* have come to regard fever as an ancient ally against disease in many species, but possibly exceptions are associated with each rule. Whenever fever is being studied from such viewpoints or others, one should focus, with longitudinal and/or concomitant controls for 24-hr and longer spans, upon the mesor, acrophase, and other rhythm characteristics, rather than solely upon temperature behavior for a few hours following the administration of some agent. In other words, since a temperature rhythm is demonstrable before inoculation, one must assess the time course of any temperature alteration with a view of the limits and characteristics of the circadian rhythm.

Huff (54) attempted to study the course of pyrexia in *P. relictum*-infected canaries and found striking differences (up to 6°C) in the temperatures taken at day and at night, in infected and healthy birds. Reportedly, these changes "bore no relationship at all to the schizogony of the parasite" (10). This is not surprising if only the mean temperature and the extent of change rather than timing as well, along several frequencies, is being considered (10). Indeed, changes in timing may occur in the absence of changes in mean. In Huff's birds, the mean temperature of the birds infected with *P. relictum* was 42.8 and that of the healthy ones 42.7°C.

It is pertinent then that in canaries, no temperature rhythm alteration was found to accompany the times of greatest segmentation of the asexual forms of four kinds of avian malarial parasites. It seems important in such work, however, to quantify rhythms with inferential statistical methods at each frequency examined, by measures of the extent and timing of change (the amplitude and acrophase) as well as the mesor, as was done earlier in a different context in this journal (55). Such methods, previously used extensively to quantify circadian rhythms, are particularly useful in this presentation to document the circaseptan rhythm of a rather great degree of generality (25–36, 43, 44, 56). Other aspects of the periodicity of malaria are still being discussed (57), but will have to be considered in the future in their relations to the malarial

parasite. Huff deserves credit for reporting that *P. matutinum* in birds is reportedly highly synchronous in a 24-hr cycle, with the schizonts maturing at a regular hour (0800) (58). Recent books on malaria (57), however, as yet make no explicit reference to intermodulating rhythms, a circumstance almost certainly due to the fact that models such as the mouse infected with *P. berghei* or others have not heretofore been scrutinized by a rhythmometric approach to the multiple frequencies that characterize the relation of the malarial parasite to the mammalian host. The monitoring of a spectrum of host rhythms and the as-one-goes analysis of changes in parameters is now feasible, not only in the laboratory by telemetry as implemented herein and earlier (20), but also with miniaturized ambulatory solid-state devices for human use, e.g., after ambulatory surgery (59).

1. Smith EA, Erickson JM. In: Kreier JP, ed. The Agency of International Development Program for Malaria Vaccine Research and Development in Malaria. New York, Academic Press. Vol 3:p331, 1980.
2. Taliaferro WH, Taliaferro LG. Alteration in the time of sporulation of *Plasmodium brasilianum* in monkeys. Amer J Hyg **20**:50–59, 1934.
3. Gill CA. The Seasonal Periodicity of Malaria and Mechanism of the Epidemic Wave. London, J. and A. Churchill, p136, 1938.
4. Demina NA. Contribution a l'étude de l'influence de regime nycthemeral de l'hote sur l'evolution de la multiplication de *Plasmodium relictum*. Riv Malariol **38**:27–44, 1959.
5. Engel R, Halberg F, Dassanayake WLP, De Silva J. 24-hour rhythms in blood eosinophils and *Wuchereria bancrofti* microfilariae before and after Δ_1-9αfluorocortisol. Nature (London) **181**:1135–1136, 1958.
6. Engel R, Halberg F, Dassanayake WLP, De Silva J. Adrenal effects on time relations between rhythms of microfilariae and eosinophils in the blood. Amer J Trop Med Hyg **11**:653–663, 1962.
7. Garnham PCC. Malaria Parasites and other Haemosporida. Oxford, Blackwell, 1966.
8. Hawking F, Worms J, Gamage K. 24- and 48-hour cycles of malaria parasites in the blood: Their purpose, production and control. Trans R Soc Trop Med Hyg **62**:731–760, 1968.
9. Garnham PCC. Malaria in its various vertebrate hosts. In: Kreier JP, ed. Malaria. New York, Academic Press. Vol 1: pp95–139, 1980.
10. Halberg E, Halberg F. Chronobiologic study design in everyday life, clinic and laboratory. Chronobiologia **7**:95–120, 1980.
11. Edmunds LN, Halberg F. Circadian time structure of Euglena: A model system amenable to quantification. In: Kaiser H, ed. Neoplasms—Comparative Pathology of Growth in Animals, Plants and Man. Baltimore, Williams & Wilkins, pp105–133, 1981.
12. Ehret CF. Action spectra and nucleic acid metabolism in circadian rhythms at the cellular level. Symp Quant Biol **25**:149–158, 1960.
13. Halberg F, Conner RL. Circadian organization and microbiology: Variance spectra and a periodogram on behavior of *Escherichia coli* growing in fluid culture. Proc Minn Acad Sci **29**:227–239, 1961.
14. Giese AC. Cell Physiology. Philadelphia, Saunders, 5th ed, p609, 1979.
15. Golgi C. Sulla infenzione malarica. Arch Sci Med **10**:109–135, 1886.
16. Cox FEG. A comparative account of the effects of betamethasone on mice infected with *Plasmodium vinckei chabaudi* and *Plasmodium berghei yoelii*. Parasitology **68**:19–26, 1974.
17. van den Berghe L. The newer medical research in the tropics. In: 4th Int Cong Trop Med Malaria Vol 2: pp1436–1438, 1948.
18. Bruce-Chwatt LJ. Introduction to rodent malaria. In: Killick-Kendrick R, Peters W, eds. Rodent Malaria. New York, Academic Press, p xi, 1978.
19. Etkin NL, Eaton JW. Malaria-induced erythrocyte oxidant sensitivity. In: Brewer GJ, ed, Erythrocyte Structure and Function. New York, Liss, p219, 1975.
20. Halberg F, Nelson W, Runge WJ, Schmitt OH, Pitts GC, Tremor J, Reynolds OE. Plans for orbital study of rat biorhythms. Results of interest beyond the Biosatellite program. Space Life Sci **2**:437–471, 1971.
21. Halberg F, Carandente F, Cornelissen G, Katinas GS. Glossary of chronobiology. Chronobiologia **4**(Suppl. 1):1–189, 1977.
22. Halberg F, Spink WW. The influence of brucella somatic antigen (endotoxin) upon the temperature rhythm of intact mice. Lab Invest **5**:283–294, 1956.
23. Radha E, Halberg F, Theologides A. Circadian state dependence of murine survival time after transplantation of a lymphosarcoma. Chronobiologia **9**:343–344, 1982.
24. Halberg F, Engeli M, Hamburger C, Hillman D. Spectral resolution of low-frequency, small-amplitude rhythms in excreted 17-ketosteroid; probable androgen-induced circaseptan desynchronization. Acta Endocrinol (Kbh) (Suppl) **103**:5–54, 1965.
25. Hübner K. Kompensatorische Hypertrophie, Wachstum und Regeneration der Ratteniere. Ergeb Allg Pathol Pathol Anat **18**:1–80, 1967.
26. Hildebrandt G. Hygiogenese: Grundlinien einer therapeutischen Physiologie. Therapiewoche **27**:5384–5397, 1977.
27. Hildebrandt G. Chronobiologische Grundlagen der Prävention und Rehabilitation. Z Angew Bäder Klimaheilkd **25**:326–346, 1978.
28. Vollrath L, Kantarjian A, Howe C. Mammalian pineal gland: 7-day rhythmic activity? Experientia **31/4**:458–460, 1974.

29. Vollrath L. Circadian and infradian activity rhythms in the mammalian pineal body. In: Aschoff J, Daan S, Groos GA, eds. Vertebrate Circadian Systems. Berlin, Springer-Verlag, p141, 1982.
30. DeVecchi A, Halberg F, Sothern RB, Cantaluppi A, Ponticelli C. Circaseptan rhythmic aspects of rejection in treated patients with kidney transplant. In: Walker CA, Winget CM, Soliman KFA, eds. Chronopharmacology and Chronotherapeutics. Tallahassee, Florida, A & M Foundation, p339, 1981.
31. Ratte J, Halberg F, Kühl JFW, Najarian JS. Circadian variation in the rejection of rat kidney allografts. Surgery **73**:102–108, 1973.
32. Halberg J, Halberg E, Runge W, Wicks J, Cadotte L, Yunis E, Katinas G, Stutman O, Halberg F. Transplant chronobiology. In: Scheving LE, Halberg F, Pauly JE, eds. Chronobiology. Thieme, Stuttgart; Igaku Shoin Ltd, Tokyo, p320, 1964.
33. Halberg F, Halberg E, Herold M, Vecsei P, Günther R, Reinberg A. Toward a clinospectrometry of conventional and novel effects of ACTH 1-17—Synchrodyn[R]—in rodents and human beings. In: Takahashi R, Halberg F, Walker C, eds. Toward chronopharmacology. Oxford, Pergamon Press, p119, 1982.
34. Hayes DK, Halberg F, Teslow T. Replicated frequency of lighting regimen shifts and aging in the face fly reveals circadian–circaseptan interaction. Physiologist **23**:146, 1980.
35. Schweiger HG, Halberg F. Can a unicell measure the week and an isolated cytoplasm measure half a week? Not SIBioC **6**:525–526, 1982.
36. Cutkomp LK, Halberg F, Cornelissen G. Temperature effect on infradian oviposition rhythms in the springtail, *Folsomia candida* (Willem). In: Proc XV Int Conf Int Soc Chronobiol, Minneapolis, Minn, Sept 14–19, 1981, in press.
37. Manson P. Additional notes on filaria sanguinis hominis and filaria disease. Med Rep Shanghai, Spec Ser, Issue 18, pp31–51, 1880.
38. Hawking F, Thurston JP. The periodicity of microfilariae. I. The distribution of microfilariae in the body. Trans R Soc Trop Med Hyg **45**:307–328, 1951.
39. Hawking F, Thurston JP. The periodicity of microfilariae. II. The explanation of its production. Trans R Soc Trop Med Hyg **45**:329–340, 1951.
40. Hawking F. Periodicity of microfilariae of *Loa loa*. Trans R Soc Trop Med Hyg **49**:132–142, 1955.
41. Mackenzie S. X. Miscellaneous specimens. 1. A case of filarial haematochyluria. Trans Pathol Soc **33**:394–410, 1882.
42. Kovats TG, Szabo I, Halberg F. Circadian changes in contact hypersensitivity of CBA mice to dinitrochlorobenzene. In: Takahashi R, Halberg F, Walker C, eds. Toward Chronopharmacology. Oxford, Pergamon Press, p281, 1982.
43. Vanderberg JP, Nussenzweig RS, Most H. Further studies on the Plasmodium berghei–Anopheles stephensi–rodent system of mammalian malaria. J Parasitol **54**:1009–1016, 1968.
44. Levi F, Halberg F. Circaseptan (about-7-day) bioperiodicity—spontaneous and reactive—and the search for pacemakers. Ric Clin Lab **12**:323–370, 1982.
45. Levi F, Halberg F, Chihara G, and Byram J. Chronoimmunomodulation: circadian, circaseptan and circannual aspects of immunopotentiation *or* suppression with lentinan. In: Takahashi R, Halberg F, Walker C, eds, Toward Chronopharmacology, Proc 8th IUPHAR Cong and Sat Symp, Nagasaki, July 27–28, 1981. Oxford/New York, Pergamon Press, p289, 1982.
46. Halberg F, Johnson EA, Brown BW, Bittner JJ. Susceptibility rhythm to *E. coli* endotoxin and bioassay. Proc Soc Exp Biol (NY) **103**:142–144, 1960.
47. Marshall E. NYU's malaria vaccine: Orphan at birth? Science **219**:466–467, 1983.
48. Kluger MJ. The evolution and adaptive value of fever. Amer Sci **66**:38–43, 1978.
49. Kluger MJ, Bernheim HA, Vaughn LK, Foster MA, D'Alecy LG. Evolution and adaptive value of fever. In: Cooper KE, Lomax P, Schonbaum E, eds. Behavioral Thermoregulation, Drugs, Biogenic Amines and Body Temperature, 3rd Symp on the Pharmacology of Thermoregulation, Banff, Alta, 1976. Basel, Karger, pp75–83, 1977.
50. Kluger MJ. Fever in the frog *Hyla cinerea*. J Therm Biol **2**:79–81, 1977.
51. Grieger TA, Kluger MJ. Fever and survival: The role of serum iron. J Physiol **279**:187–196, 1978.
52. Kluger MJ. Letter to the editor: Fever vs. hyperthermia. N Engl J Med **299**:555, 1978.
53. Bernheim HA, Kluger MJ. Endogenous pyrogen-like substance produced by reptiles. J Physiol **267**:659–666, 1977.
54. Huff CG. Relation between malarial infections and body temperatures in canaries. Amer J Hyg **29C**:149–154, 1939.
55. Reinberg A, Zagula-Mally ZA, Ghata J, Halberg F. Circadian rhythm in duration of salicylate excretion referred to phase of excretory rhythm and routine. Proc Soc Exp Biol (NY) **124**:826–832, 1967.
56. DeLisi C, Hiernaux J, Halberg F. Circaseptan component of mammalian antibody production in response to immunization. Chronobiologia **10**:119, 1983.
57. Kreier JP (ed). Malaria. New York: Academic Press, Vol 1:p416; Vol 2:p329; Vol 3:p346, 1980.
58. Huff CG. A new variety of Plasmodium recticulum from the robin. J Parasitol **23**:400–404, 1937.
59. Halberg E, Fanning R, Halberg F, Cornelissen G, Wilson D, Griffiths K, Simpson H. Toward a chronopsy: Part III. Automatic monitoring of rectal, axillary and breast surface temperature and of wrist activity; effects of age and of ambulatory surgery followed by nosocomial infection. Chronobiologia **8**:253–271, 1981.

Received July 28, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted October 11, 1983.