

FEBRILE TEMPERATURES CAN SYNCHRONIZE THE  
GROWTH OF *PLASMODIUM FALCIPARUM* IN VITRO

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The cardinal symptom of human malaria is fever, which is associated with the rupture of intraerythrocytic schizonts and the appearance of their progeny in the circulation. Malaria parasites undergo repeated cycles of intraerythrocytic growth and tend to grow in synchrony with each other in vivo. The result is paroxysmal fever that recurs with the same periodicity as the asexual erythrocytic growth cycle of the parasite: for *Plasmodium falciparum* and *Plasmodium vivax* this is 48 h. The mechanism of parasite synchronization, which is central to this phenomenon, remains poorly understood (1-3).

The biological significance of the host fever response in malaria is open to speculation. Malaria fever appears to be mediated, at least in part, through the endogenous pyrogen cachectin/TNF, which is secreted by monocytes at or shortly after schizont rupture (reference 4 and Kwiatkowski, D., J. G. Cannon, K. R. Manogue, A. Cerami, C. A. Dinarello, and B. M. Greenwood, submitted for publication). There is accumulating evidence that TNF might mediate host defense against the malaria parasite (reviewed in reference 5) but it is not known whether fever is itself protective. One fundamental question is whether the growth of malaria parasites is affected by exposure to febrile temperatures, which often exceed 40°C in the acute illness. This paper reports that exposure to 40°C can inhibit the growth of *P. falciparum* in erythrocytic culture in vitro and that periodic fluctuations of temperature, such as occur in natural infection, are capable of synchronizing the parasite population.

Materials and Methods

*Parasites.* Established isolates of *P. falciparum* (Gam 83-1 and Gam 85-372) were maintained in continuous erythrocytic culture in vitro at 37°C according to the method of Trager and Jensen (6). Growth in these cultures was asynchronous. For those experiments that required synchronized starting cultures, these were prepared by two cycles of sorbitol lysis (7) followed by Percoll density gradient centrifugation (8).

Wild parasite isolates were obtained from heparinized blood freshly collected from Gambian patients with malaria.

*Experiments.* Parasites in erythrocytic culture were incubated in candle jars at 37°C, at 40°C, or at 37°C and 40°C on alternate days. At the start of each experiment, 100- $\mu$ l aliquots of parasitized erythrocytes were dispensed into 35-mm tissue culture dishes with 2.5 ml of RPMI plus Hepes, glucose, hypoxanthine, gentamicin, and 10% nonimmune human serum. Medium was changed and Giemsa-stained thin films were made every 24 h.

*Assessing Parasite Development.* Rings, trophozoites, and schizonts were identified according to conventional criteria but, in order to improve resolution, intermediate stages of develop-

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ment were also classified as follows: R (young rings), very thin rim of cytoplasm; RT (older rings and some young trophozoites), prominent central vacuole but thicker rim of cytoplasm, pigment absent or barely visible; T (mature trophozoites), conspicuous pigment, one nucleus; TS (early schizonts), nucleus dividing, or two nuclei; S (schizonts), more than two nuclei.

### Results

Established isolates of *P. falciparum*, which had previously been maintained in continuous erythrocytic culture at 37°C, failed to grow at 40°C (Fig. 1). Wild parasite isolates were also cultured directly from five patients with malaria. Within the first growth cycle, schizonts appeared pyknotic and hyposegmented in cultures incubated at 40°C compared with those at 37°C. After two growth cycles, no viable parasites were seen in the 40°C cultures, whereas the parasitemia in each of the 37°C cultures (mean 1.4%, SE 0.3%) equaled or exceeded the initial parasitemia (mean 1.0%, SE 0.2%).

To determine the effect of temperature on different stages of parasite development, erythrocytic cultures were prepared containing only young intraerythrocytic parasites (rings), and were then subjected to 40°C for all or part of the ensuing 48-h growth cycle (Table I). When assessed at 48 h, parasites exposed to 40°C during the first half of the growth cycle (rings → trophozoites) had grown almost as well as control cultures maintained at 37°C, whereas those exposed to 40°C during the second half of the cycle (trophozoites → schizonts → rings) had formed pyknotic schizonts and generated a diminished number of new rings. Parasitemia in the latter cultures remained markedly depressed after a further 24 h of incubation at 37°C (data not shown).

Similarly, when asynchronous cultures (i.e., cultures containing a mixed population of parasites at different stages of the erythrocytic cycle) were incubated at 37°C or at 40°C for 24 hours, at the end of this period the 40°C cultures contained fewer healthy-looking schizonts and markedly fewer rings than the 37°C cultures, whereas both sets of cultures contained a similar number of trophozoites (data not shown). In other words, when initially asynchronous cultures were transiently exposed to

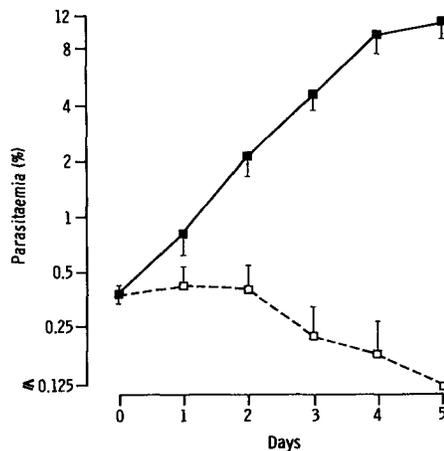


FIGURE 1. Growth of *P. falciparum* in erythrocytic culture at 37°C (■) and at 40°C (□). Results of seven experiments expressed as geometric means with standard errors. In three experiments, the 40°C cultures appeared dead within 5 d; for statistical purposes, they were assigned a parasitemia of 0.05%.

TABLE I  
*Effect of Incubating P. falciparum at 40°C for the First or Second Half of the 48-h Erythrocytic Cycle*

Culture	Incubation temperature (°C)		Multiplication of parasitaemia (between 0 and 48 hrs)
	0-24 h	24-48 h	
a	37°	37°	4.1 (2.9-5.6)
b	40°	40°	0.4 (0.1-0.5)
c	40°	37°	3.6 (3.0-4.3)
d	37°	40°	0.6 (0.5-0.7)

Cultures contained only young rings (0.6-1.2% parasitaemia) at the start of the experiment. Results of five experiments expressed as geometric means with 95% confidence intervals. Difference in growth between cultures c and d is significant ( $p < 0.01$  by two-tailed paired  $t$  test on logarithmically transformed data).

40°C, thus selectively disrupting the latter half of the erythrocytic cycle, the surviving parasites were more synchronous than the starting population.

To investigate the effect of periodic fever on parasite development, initially asynchronous cultures were incubated at 37°C and at 40°C on alternate days, in simulation of the 48-h cycle of tertian malaria fever. Parasite development was compared in paired cultures whose temperature cycles were directly out of phase (Fig. 2). Within 48 h, parasite development in these cultures became synchronized, and acquired a fixed relationship to the temperature cycle; i.e., cultures contained predominantly trophozoites after a day of incubation at 40°C, and predominantly young rings after a day of incubation at 37°C (Figs. 2 and 3). The overall rate of parasite growth in these cultures, once synchronized, was similar to that of control cultures maintained at 37°C, which remained asynchronous.

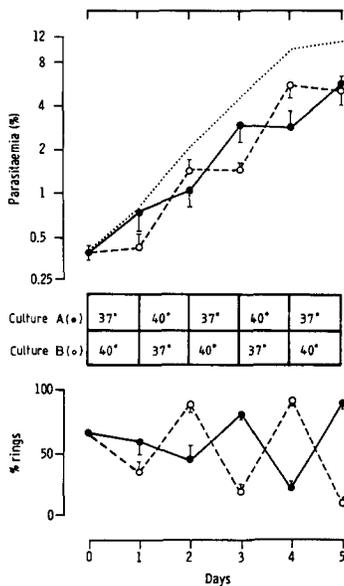


FIGURE 2. Erythrocytic cultures of *P. falciparum* incubated at 37°C and at 40°C on alternate days: seven experiments. Starting cultures were asynchronous. Culture A (●) was at 40°C on days 2 and 4; and culture B (○) was at 40°C on days 1, 3, and 5. (*Top panel*) Growth rates expressed as geometric means with standard errors. Dotted line represents mean growth of control cultures maintained at 37°C. (*Bottom panel*) Percentage of parasites at the ring stage of development (stages R and RT as defined in Materials and Methods) expressed as arithmetic means with standard errors. Cultures A and B differ significantly from 2 d onwards ( $p < 0.01$  by paired  $t$ -test).

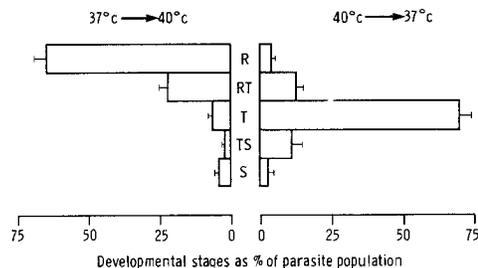


FIGURE 3. Synchronization of cultures incubated at 37°C and 40°C on alternate days: distribution of developmental stages at the time of temperature change from 37°C to 40°C (left) and from 40°C to 37°C (right). R, young rings; RT, older rings/early trophozoites; T, mature trophozoites; TS, early schizonts; S, schizonts. 20 pairs of observations from the experiments shown in Fig. 2 (cultures A and B: days 3, 4, and 5) expressed as arithmetic means with standard errors.

### Discussion

These data indicate that febrile temperatures are capable of markedly inhibiting the growth of *P. falciparum* in erythrocytic culture. It has been demonstrated that this is due to disruption of the latter half of the asexual erythrocytic cycle, and morphological appearances suggest that developing schizonts are particularly vulnerable.

It has also been observed that initially asynchronous cultures become synchronized when exposed to 40°C on alternate days in simulation of the 48-h fever cycle of malaria. The synchronization appears to be caused by periodic disruption of the latter part of the erythrocytic cycle by exposure to 40°C. In these experiments, cultures contained predominantly middle-aged intraerythrocytic parasites (trophozoites) after 24 h of exposure to 40°C, and predominantly young intraerythrocytic parasites (rings) 24 h later when the experimental temperature was due to rise again. This temporal relationship between parasite development and the temperature cycle is remarkably similar to that observed in natural infection. Once synchronized, cultures grew well in spite of periodic exposure to 40°C.

These results suggest that fever and parasite synchronization may have a more complex biological relationship than has hitherto been recognized. Classical studies of human malaria demonstrated (a) that the malaria parasite has a recurrent erythrocytic growth cycle of fixed duration; (b) that malaria parasites tend to develop synchronously in vivo; and (c) that the host fever response is triggered by paroxysmal schizont rupture at the end of each cycle, or some closely associated event (1). These factors, in combination, determine the periodic nature of malaria fever and its characteristic association with the appearance of rings. The present data indicate that febrile temperatures are capable of inhibiting parasite growth, but that rings are relatively temperature-resistant and that a synchronized parasite population can exhibit good growth despite periodic exposure to febrile temperatures. The data also suggest that exposure to febrile temperatures might act to promote parasite synchronization in vivo.

These findings raise the possibility that synchronization may be, in part, an adaptation of the parasite population to the fever response of the host.

### Summary

To investigate the possibility that the host fever response in malaria may affect parasite development, we studied the effect of temperature on *Plasmodium falciparum* in erythrocytic culture in vitro. Growth was markedly suppressed at 40°C compared with 37°C, due to disruption of the second half of the 48-h erythrocytic cycle. How-

ever, young intraerythrocytic parasites, which are highly exposed to fever during natural infection, appeared to develop normally at 40°C. Because of the differential temperature sensitivity within the erythrocytic cycle, asynchronous cultures could be synchronized by transient elevations of temperature. Pronounced synchronization was observed when cultures were exposed to periodic elevations of temperature that simulated the 48-h fever cycle of tertian malaria. These findings indicate that malaria fever might act to promote parasite synchronization in vivo.

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