

## *Plasmodium chabaudi*: Effect of Antimalarial Drugs on Gametocytogenesis

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Buckling, A., Crooks, L., and Read A. 1999. *Plasmodium chabaudi*: Effect of antimalarial drugs on gametocytogenesis. *Experimental Parasitology* 93, 45–54. The proportion of asexual blood-stage malaria parasites that develop into transmission stages (gametocytes) can increase in response to stress. We investigated whether stress imposed by a variety of antimalarial drugs administered before or during infection increased gametocyte production (gametocytogenesis) *in vivo* in the rodent malaria parasite, *Plasmodium chabaudi*. All methods of drug treatment greatly reduced the numbers of asexual parasites produced during an infection but resulted in either no reduction in numbers of gametocytes or a smaller reduction than that experienced by asexuals. We used a simple model to estimate temporal variation in gametocyte production. Temporal patterns of gametocytogenesis did not greatly differ between untreated and prophylaxis infections, with rates of gametocytogenesis always increasing as the infection progressed. In contrast, administration of drugs 5 days after infection stimulated increased rates of gametocytogenesis early in the infection, resulting in earlier peak gametocyte densities relative to untreated infections. Given the correlation between gametocyte densities and infectivity to mosquito vectors, and the high frequency of subcurative drug therapy and prophylaxis in human populations, these data suggest that antimalarial drugs may frequently have only a small effect on reducing malaria transmission and may help to explain the rapid spread of drug-resistant genotypes. © 1999 Academic Press

*Index Descriptors and Abbreviations*: Apicomplexan parasite; *Plasmodium chabaudi*; malaria; gametocytogenesis; gametocytes; postinfection (pi); antimalarial chemotherapy; antimalarial prophylaxis; quinine, pyrimethamine, chloroquine; red blood cells (rbc); dimethyl sulfoxide (DMSO); standard error of the mean (SEM); analysis of variance (ANOVA).

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## INTRODUCTION

Mammalian malaria parasites (*Plasmodium* spp.) undergo cycles of asexual replication in red blood cells, with a small proportion of the asexual parasites producing nonreplicating transmission stages, gametocytes. *In vitro* studies have demonstrated that gametocyte production (gametocytogenesis) of the human malaria parasite *Plasmodium falciparum* is upregulated in response to a variety of stimuli, most of which create conditions unfavorable for asexual growth (Mons 1985; Carter and Graves 1988; Sinden *et al.* 1996). If increased gametocytogenesis is a general response to unfavorable conditions, it may be expected following novel stresses, such as antimalarial drugs (Buckling *et al.* 1997).

Antimalarial drugs by definition kill malaria parasites. However, if drugs are used in doses which fail to kill all parasites, at least some survivors may suffer ill effects and be stimulated to produce gametocytes. Indeed, subcurative therapy is common (Wernsdorfer 1994), as is partially protective prophylaxis, as evidenced by the high proportion of individuals with low drug concentrations detected in blood and urine (Koella *et al.* 1990; Hogg *et al.* 1995). Because of the generally positive correlation between gametocyte density and infectivity to the mosquito vector (Carter and Graves 1988; Buckling *et al.* 1997; Taylor and Read 1997; Taylor *et al.* 1997), stress-induced gametocyte production may limit the impact of antimalarial drugs on malaria transmission. Further, if drug-resistant parasites are more likely to suffer drug-imposed stress without dying, stress-induced gametocytogenesis may help explain the rapid spread of drug-resistant genotypes.

A number of clinical studies have investigated the effect of antimalarial drugs on gametocytogenesis in humans (reviewed in Buckling *et al.* 1997). These are, however, necessarily ambiguous: for ethical reasons, drug doses must be curative and there can be no untreated controls. Any changes in gametocyte densities may therefore represent the natural progression of the infection. However, a series of controlled experiments on the effect of subcurative therapy with chloroquine in the rodent malaria, *Plasmodium chabaudi*, have been performed. These showed that drug therapy more than doubled the proportion of circulating parasites that were gametocytes, induced early peak gametocyte densities and, importantly, had no impact on infectivity to mosquitoes (Buckling *et al.* 1997).

Here we report further investigations into drugs induced gametocytogenesis of *Plasmodium in vivo*, which address the following issues. First, is increased gametocytogenesis and the earlier appearance of peak gametocyte densities in response to subcurative drug therapy independent of the mode of action of antimalarial drugs? We used pyrimethamine, quinine, and mefloquine, which have different parasite-killing mechanisms. Pyrimethamine ultimately inhibits DNA synthesis. The mechanisms of action of the other drugs are not fully understood but, like chloroquine, interference with hemoglobin metabolism is believed to be involved (reviewed in Butcher 1997). Second, does increased gametocytogenesis occur following different types of sublethal prophylaxis? We used pyrimethamine and chloroquine to test this. Third, how does gametocytogenesis temporally vary during the course of an untreated *in vivo* infection, and how is this pattern altered by drug treatment? For consistency and to avoid ambiguity, we use the term 'therapy' when drugs were administered to already infected mice, 'prophylaxis' when drugs were administered prior to infection, and 'treatment' to include both prophylaxis and therapy.

## MATERIALS AND METHODS

All work was carried out in accordance with the Animals (Scientific Procedures) Act 1986 (UK). Male C57/BL/6J mice (Harlan-Olac, England) aged 8–14 weeks were infected with  $1 \times 10^6$  rbc infected with a drug-naive *P. chabaudi* clone (either CR or ER [Beale *et al.* 1978] from the WHO Registry of Standard Malaria Parasites maintained at the University of Edinburgh, UK) in a 0.1-ml intraperitoneal inoculum of 50% Ringer's solution (27 mM KCl, 27 mM CaCl<sub>2</sub>, 0.15 M NaCl), 45% heat-inactivated calf serum, and 5% 200 units/ml heparin solution. Mice, age-matched within experiments, were housed in cages of two to four animals at a temperature of  $25 \pm 1^\circ\text{C}$  with a 0700 to 1900 h light cycle and provided with 41B rat and mouse maintenance diet

(Harlan-Teklad, England) and water containing 0.05% pABA, *ad libitum*.

Chloroquine solution was prepared from a stock of 40 mg/ml chloroquine sulphate (Nivaquine) diluted in distilled water. Quinine and mefloquine solutions were prepared by dissolving their hydrochlorides in distilled water, except in the cases in which pyrimethamine (base) was used in experiments, in which case all drugs were dissolved in DMSO. Drug solutions were administered orally using a lubricated catheter in approximately 0.1-ml volumes; doses are shown in Table I. Drug treatment occurred on day 5 pi between 1600 and 1700 h and prophylaxis approximately 1 h before parasite inoculation (day 0 pi). Control mice were given 0.1 ml distilled water or DMSO, depending on

TABLE I  
Details of Experiments and Analyses

Experiment <sup>a</sup>	Experimental block <sup>b</sup>	Clone	Treatment	Dose (mg kg <sup>-1</sup> ) <sup>c</sup>	<i>n</i>
<i>Quinine chemotherapy</i>					
1	1	CR	Untreated	—	5
			qu-treated	200	4
2	2	ER	Untreated	—	4
			qu-treated	200	4
3	3	ER	Untreated	—	4
			qu-treated	200	6
<i>Pyrimethamine chemotherapy</i>					
1	1	CR	Untreated	—	5
			py-treated	2	4
3	2	ER	Untreated	—	4
			py-treated	5	5
<i>Mefloquine chemotherapy</i>					
5	1	CR	Untreated	—	4
			mf-treated	9	4
6	2	ER	Untreated	—	4
			mf-treated	9	3
<i>Pyrimethamine prophylaxis</i>					
1	1	CR	Untreated	—	5
			py-treated	1	4
4	2	ER	Untreated	—	6
			py-treated	2	5
<i>Chloroquine prophylaxis</i>					
4	1	ER	Untreated	—	6
			cq-treated	6	5
5	2	CR	Untreated	—	4
			cq-treated	4	5
6	3	ER	Untreated	—	4
			cq-treated	4	3

*Note.* qu, quinine; py, pyrimethamine; mf, mefloquine; cq, chloroquine; proph, prophylaxis; *n*, number of mice.

<sup>a</sup> Six experiments were carried out, each including a variety of treatments; experiment number thus identifies experimental blocks run concurrently.

<sup>b</sup> Each treatment was compared with untreated infections in two to three of these experiments, resulting in two to three 'Experimental blocks' replicating the relevant comparisons.

<sup>c</sup> mg/kg mouse weight.

the solvent used for the drugs in each experiment. Details of individual experiments are shown in Table I.

Thin blood smears from the tail vein were taken approximately every second day from day 4 or 5 pi and Giemsa-stained, and asexual parasites were counted per  $1.5 \times 10^3$  rbc. At low asexual densities parasites were counted per  $10^4$  rbc. Mature gametocytes were counted per  $1.25 \times 10^4$  rbc. Parasite densities were calculated from rbc densities, measured by flow cytometry (Coulter Electronics) every second day, multiplied by parasites per rbc.

We used a simple exponential growth model to estimate the proportion of asexual parasites that produced gametocytes on individual days (see Fig. 1). The synchrony of parasite replication in *P. chabaudi* infections (Cox 1988) simplifies matters. The maturation period of *P. chabaudi* gametocytes is approximately 2 days (Gautret *et al.* 1997), such that a proportion,  $g$ , of the asexual parasites at day  $t$  will produce gametocytes that will mature on day  $t + 2$ . *P. falciparum* gametocytes become less vulnerable to immune and drug clearance as they mature, probably because they express less immunogenic antigens and have reduced metabolic activity (Carter and Graves 1988). Age-dependent mortality of *P. chabaudi* gametocytes is likely to be similar. It is therefore assumed that survival probability ( $s$ ) in the first 24 h after asexual densities were measured is the same for gametocytes and asexuals. This period encompasses the time taken for asexual parasites to mature and rupture (schizogony), releasing the gametocyte or asexual progeny. Schizogony occurs around midnight in clones ER and CR under the employed light–dark cycle (McLean 1986), approximately 7 h after parasite densities at time  $t$  were measured. Zero gametocyte mortality is assumed in the second 24-h period.

The half-life of mature *P. chabaudi* gametocytes is estimated to be less than 12 h (A. Buckling, unpubl. data), so that few mature gametocytes are likely to contribute to the gametocyte population of the following day. If we assume that asexual parasites produce  $m$  progeny parasites which develop into all gametocytes or asexuals (as is the

case for *P. falciparum* [Bruce *et al.* 1990]), the number of gametocytes on day  $t + 2$  ( $G_{t+2}$ ) can be written as

$$G_{t+2} = sgmA_t, \tag{1}$$

where  $A_t$  refers to the number of asexuals on day  $t$ .

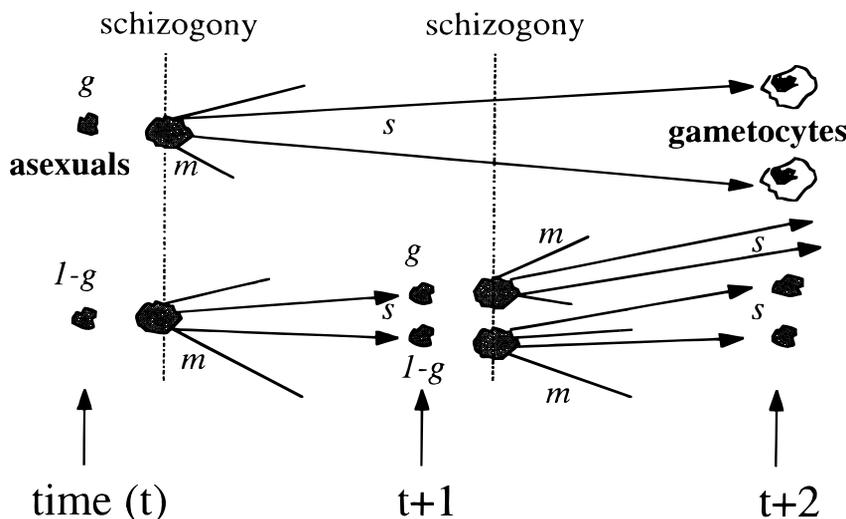
The asexual cycle of *P. chabaudi* is 24 h (Cox 1988), so that two asexual cycles occur during the maturation time of a gametocyte. Assuming that both asexual mortality,  $s$ , and the probability of an asexual parasite producing gametocytes,  $g$ , are constant during the 2-day period, the number of asexuals on day  $t + 2$  ( $A_{t+2}$ ) can be written as

$$A_{t+2} = s^2(1 - g)^2m^2A_t, \tag{2}$$

The proportion of parasites that produced gametocytes can be estimated from observed densities of asexuals and gametocytes by combining Eqs. (1) and (2):

$$g = \frac{\frac{G_{t+2}}{A_t}}{\sqrt{\frac{A_{t+2}}{A_t} + \frac{G_{t+2}}{A_t}}} \tag{3}$$

Some of the simplifying assumptions used to derive this approximation may not always hold. In particular, it is likely that rates of gametocyto-genesis ( $g$ ) and survival of parasites ( $s$ ) will differ between the two rounds of asexual replication that occur for each estimate of  $g$ . Values of  $g$  were generally very low (see below); differences in  $g$  between the two periods will thus have only a small effect on the estimates. Differences in parasite survival may however be very large, potentially under-estimating or over-estimating  $g$  when mortality is greatest during



**FIG. 1.** Schematic showing parameters of the model (see text). A proportion ( $g$ ) of asexual parasites measured at time  $t$  will produce gametocytes when they reach maturity. Mature parasites undergo schizogony, producing  $m$  progeny. A proportion of parasites ( $s$ ) will survive until  $t + 1$ . Surviving asexual parasites then repeat the cycle. Surviving gametocytes undergo no further mortality, reaching maturity at  $t + 2$ .

the first or second period, respectively. However, by considering the extremes of biologically feasible parameter estimates, Crooks *et al.* (in preparation) demonstrate that the current estimates of gametocytogenesis are a reasonable approximation.

Drug treatment may more strongly affect asexual parasites than gametocytes. Pharmacokinetic data, which we summarize under Discussion, make it unlikely that this will cause consistent bias in our estimates of  $g$  during the bulk of the infection. However, with some caution, we also use Eq. (3) to estimate gametocyte conversion rate immediately following chemotherapy on day 5 pi, when biologically relevant drug concentrations still occur. The calculation of gametocytogenesis makes the assumption that gametocytes between 24 and 48 h (when they reach maturity) have zero mortality but that mortality before this is the same as for asexual parasites. These assumptions are probably not greatly violated by drug-imposed mortality. Specific data for *P. chabaudi* are not available, although all the drugs used are inactive against mature *P. falciparum* gametocytes but kill immature forms (Desjardins *et al.* 1988). Moreover, mortality during the first 24 h includes the approximately 7 h it takes for asexual parasites to mature. Given the rapid absorption and subsequent parasite-killing activity of these drugs (at least in people [Desjardins *et al.* 1988]), drug-induced mortality is likely to be greatest for these pre-schizont asexual parasites, probably overshadowing any possible differences in mortality of the gametocyte or asexual progeny produced by the survivors of the drug treatment. The possibility that gametocytes that reached maturity on day 6 pi (which might not have been affected by the drug therapy on day 5 pi) would result in a significant overestimation of drug-induced gametocytogenesis is also unlikely. The half-life of *P. chabaudi* gametocytes is less than 12 h (A. Buckling, unpubl. data); thus, very few of the small number of mature gametocytes present on day 6 pi would contribute to the population on day 7 pi.

The following summary measures were determined for each infection.

(1) *Total asexuals.* This was estimated as the total number of parasites circulating in the peripheral blood between days 7 and 18 pi for drug therapy comparisons and between days 0 and 18 pi for prophylaxis comparisons. Parasite densities prior to day 7 pi were not used for the former because the effects of drug treatment on gametocytogenesis would not be apparent, and by day 18 pi, the vast majority of parasites had been cleared by their hosts. Estimates of total parasites between these days were obtained by integrating under the parasite density through time curves for each infection. This is a reasonably accurate measure of total numbers of asexuals (between the appropriate time periods) given the 24-h asexual cycle of *P. chabaudi* (Cox 1988).

(2) *Total gametocytes.* An index of the total number of gametocytes produced during an infection was estimated between the same time periods as for the asexual parasites by integrating under the gametocyte density through time curves. This measure will be reasonably accurate, as the half-life of gametocytes is less than 12 h and measures were taken every second day. The sum of the gametocyte densities on each day is likely to be well correlated with total transmission probability: gametocyte densities at any point in time correlate with both the proportion of mosquitoes infected and parasite densities within mosquitoes.

(3) *Proportion of gametocytes.* This index was calculated as the proportion of total parasites [(1) + (2)] that were gametocytes (2) for each infection.

(4) *Day of peak gametocyte density.*

(5) *Daily rates of gametocytogenesis.* Rates of gametocytogenesis were calculated using equation (3) for all drug-treated and the appropriate untreated infections on days 5, 8, 10, and 12 pi, except where

asexual densities on those days were determined to be zero. Rates were additionally calculated for days 14 and 16 pi in prophylaxis experiments, when, unlike the drug-therapy infections, parasite densities were consistently at a detectable level.

Prior to analysis, estimates of total asexuals and total gametocytes were  $\log_{10}$ -transformed, and proportions of gametocytes were square-root arcsin-transformed, to bring the distributions close to normal. The effect of each method of treatment relative to the appropriate control infections (i.e., separate analyses were carried out for quinine-therapy, pyrimethamine-therapy, mefloquine-therapy, pyrimethamine prophylaxis, and chloroquine prophylaxis infections) on each of these measures was determined by ANOVA using GLIM 4, with TREATMENT (drug+, drug-) and EXPERIMENTAL BLOCK (each method of treatment was compared with untreated infections two to three times; see Table I) fitted as factors. The interaction term was removed from the statistical model if nonsignificant ( $P > 0.05$ ) (Crawley 1993). Analyses of days of peak gametocyte density for each infection were carried out as above, except that the data for all drug therapy experiments were pooled together, and likewise for all the prophylaxis data.

Daily rates of gametocytogenesis ( $g$ , in Eq. 3) were highly overdispersed and in no way approximated a normal distribution, regardless of transformation. The data consisted of many zeros and very low values, as well as much higher values (Fig. 2). Even the use of nonparametric ranking procedures is unlikely to provide an appropriate solution to this problem, given that variation in the very low values will often represent the presence or absence of a single gametocyte in the surveyed microscopic fields. A nominal classification system was therefore used, with each rate of gametocytogenesis classified as high or low, based on whether the value was less or greater than the median of the data set. The drug therapy and prophylaxis data sets were analyzed separately. To control for multiple sampling from the same infection, the median of a data set was calculated as the median of the medians for each infection within the data set. Data for each day was analyzed using  $\chi^2$  tests of independence (with Yates's correction for continuity) to determine whether the proportion of infections that had high rates of gametocytogenesis (median  $> 0.008$  and  $0.003$ , for drug therapy and prophylaxis comparisons, respectively) differed between all drug therapy and the appropriate untreated infections, and all prophylaxis and the appropriate untreated infections.

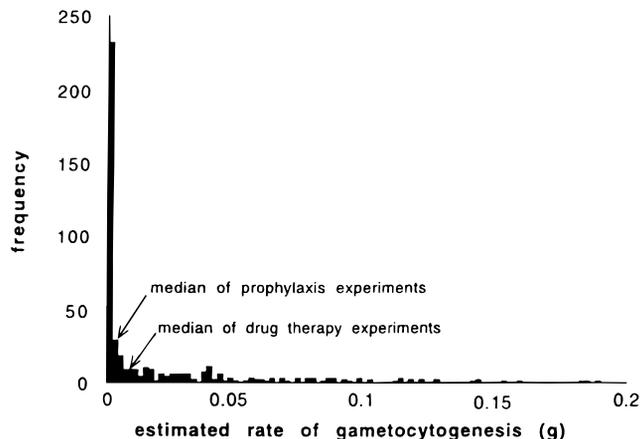


FIG. 2. Histogram of rates of gametocytogenesis at all time points, in all infections.

## RESULTS

Statistical tests of the main effects of drug therapy and prophylaxis on total asexual parasites and gametocytes produced during an infection and the proportion of total parasites that were gametocytes are summarized in Table II. Subcurative chemotherapy was achieved. Quinine, pyrimethamine, and mefloquine administered on day 5 pi reduced total asexuals by approximately 75% in all cases. However, gametocyte densities were not correspondingly reduced: total number of gametocytes was reduced by about 25% by quinine and was not significantly affected by the other drugs. The proportions of parasites that were gametocytes were approximately four times higher following quinine and pyrimethamine therapy than in untreated infections. There was no significant change in the proportion of gametocytes following mefloquine therapy (Figs. 3 and 4).

Treatment prior to infection also had a greater impact on asexuals than on gametocyte densities. Following both pyrimethamine and chloroquine prophylaxis, the total number of asexual parasites appearing in an infection was half that in untreated infection but total gametocyte numbers were not significantly lower. The resultant proportion of gametocytes was not significantly different from untreated infections following chloroquine prophylaxis but was almost twice that of untreated infections following pyrimethamine prophylaxis (Figs. 5 and 6).

Both clones CR and ER were used in at least one experimental block each for all comparisons between treated and untreated infections. Significant differences between experimental blocks ( $P < 0.05$ ) occurred for total gametocytes

TABLE II

Main Effects of Treatment for the Analyses of Total Asexuals, Gametocytes, and the Proportion of Gametocytes; Effects of Experimental Block Were Controlled

Method of chemotherapy	Total asexuals	Total gametocytes	Proportion gametocytes
<b>Treatment</b>			
Quinine	$F_{1,23} = 51.2^{**}$	$F_{1,23} = 6.17^*$	$F_{1,23} = 5.18^*$
Pyrimethamine	$F_{1,15} = 23.8^{**}$	$F_{1,15} = 2.07$	$F_{1,15} = 16.2^{**}$
Mefloquine	$F_{1,12} = 26.3^{**}$	$F_{1,12} = 2.2$	$F_{1,12} = 2.67$
<b>Prophylaxis</b>			
Pyrimethamine	$F_{1,16} = 22.7^{**}$	$F_{1,16} = 0.76$	$F_{1,17} = 5.67^*$
Chloroquine	$F_{1,21} = 61.74^{**}$	$F_{1,23} = 3.93$	$F_{1,23} = 0.15$

Note. All methods of treatment were analyzed against the appropriate untreated infections.

\*  $P < 0.05$ .

\*\*  $P < 0.001$ .

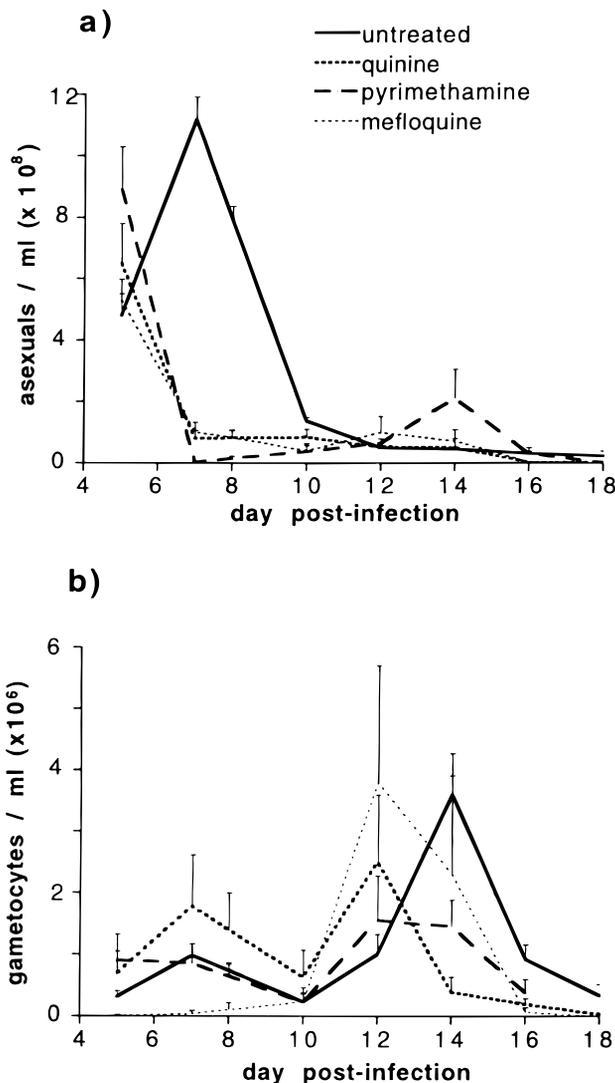


FIG. 3. Mean + 1 SEM asexual densities (a) and gametocyte densities (b) through time for infections drug treated on day 5 pi and untreated controls. Data are pooled across experiments.

and the proportion of gametocytes in the mefloquine therapy experiments and for total asexuals, total gametocytes, and the proportion of gametocytes for both prophylaxis experiments. In all cases, these effects could be attributed to significantly higher values in clone CR than clone ER. There were no other significant differences between blocks for either of these measures. The magnitude of the difference between untreated and prophylaxis infections differed between experimental blocks (experimental block by treatment interactions;  $P < 0.05$ ) for total asexuals and total gametocytes in the pyrimethamine prophylaxis experiments and for total

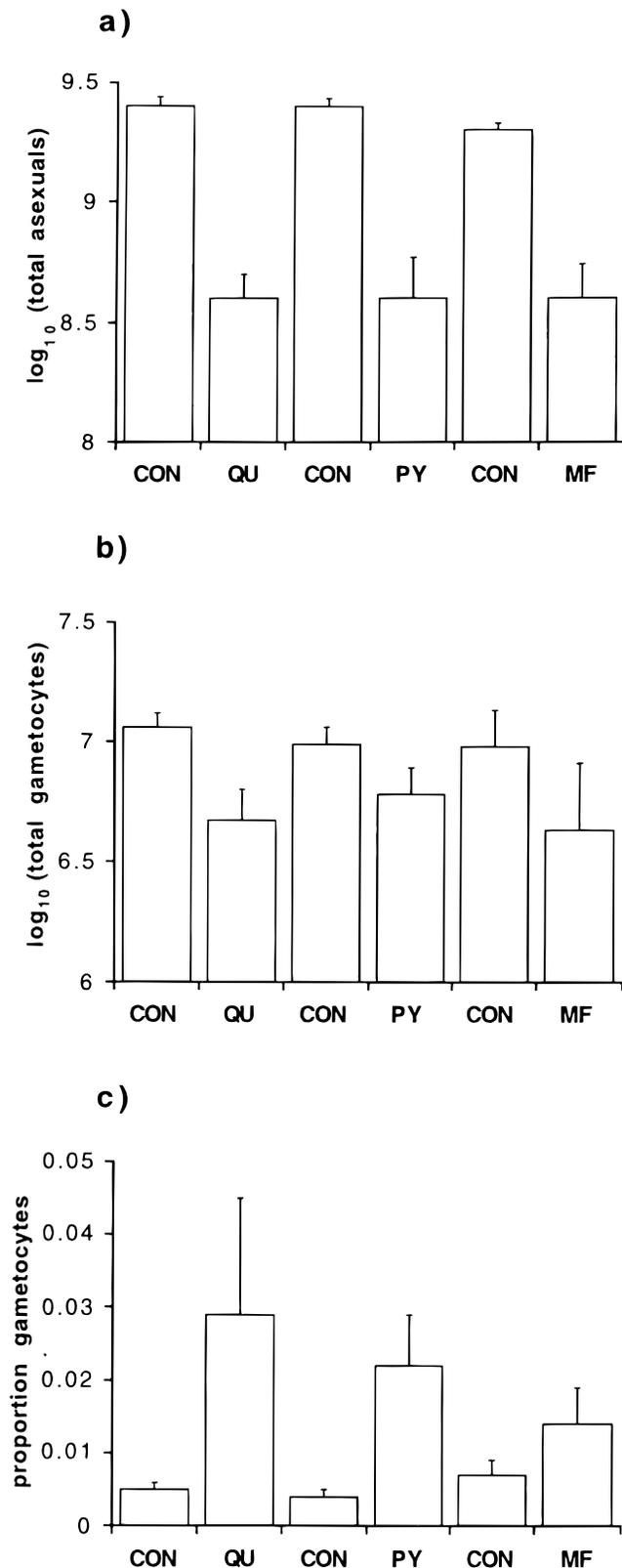


TABLE III

Results of Analyses of the Proportions of Infections with 'High' (Greater than the Median) Rates of Gametocytogenesis on Each Day

Day pi	Drug-therapy vs untreated	Prophylaxis vs untreated
5	$\chi^2_1 = 10.2^{**}$	$\chi^2_1 = 0.91$
8	$\chi^2_1 = 0.98$	$\chi^2_1 = 0.27$
10	$\chi^2_1 = 4.75^*$	$\chi^2_1 = 3.96^*$
12	$\chi^2_1 = 2.01$	$\chi^2_1 = 1.31$
14		$\chi^2_1 = 0.033$
16		$\chi^2_1 = 0$

\*  $P < 0.05$ .

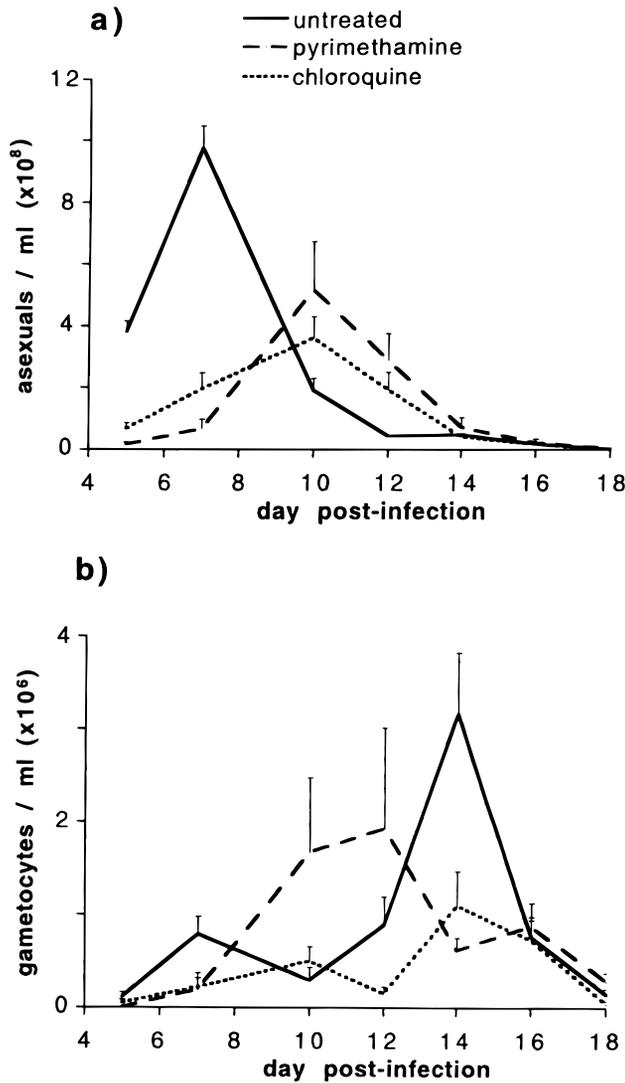
\*\*  $P < 0.01$ .

asexuals in the chloroquine prophylaxis experiments, although the treatment effects were always in the same direction within experimental blocks. These effects can be attributed to different drug doses being used in the different experimental blocks. There were no other significant experimental block by treatment interactions ( $P > 0.1$ ).

Drug treatment on day 5 pi resulted in peak gametocyte density occurring on average 3 days earlier than in untreated infections (days 11 and 14 pi, respectively; Fig. 3,  $F_{1,45} = 9.0$ ,  $P < 0.01$ ). Treatment prior to infection did not induce a change in the timing of peak gametocyte densities (peaks in untreated and prophylaxis infections occurring on average between days 13 and 14 pi; Fig. 5,  $F_{1,36} = 0.06$ ,  $P > 0.1$ ). There were no significant experimental block or interaction effects ( $P > 0.1$  in all cases).

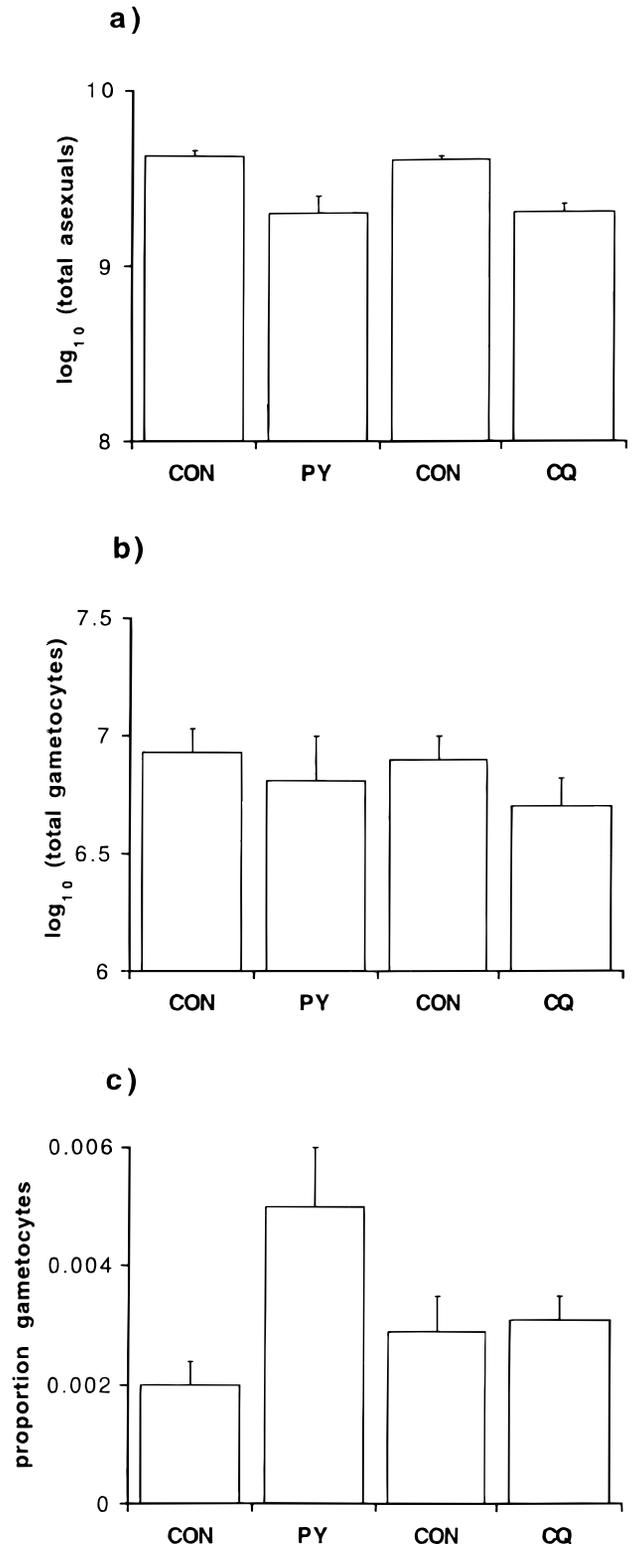
Statistical tests of the effects of drug treatment on the estimates of daily rates of gametocytogenesis are summarized in Table III. Rates of gametocytogenesis increased as the infection progressed, with most conversion rates below the overall median early in the infection and most above the median toward the end (Figs. 7 and 8). This pattern differed quite markedly following chemotherapy: almost 50% of infections had rates of gametocytogenesis above the median immediately following drug treatment (on day 5 pi) and 80% had rates above the median (compared with 40% in untreated infections) on day 10 pi (Fig. 7). In general, the different drugs generated similar patterns of gametocytogenesis through time, except on day 5 pi when, unlike quinine-

**FIG. 4.** Mean + 1 SEM total asexual parasites (a), total gametocytes (b), and proportion of parasites that were gametocytes (c) for infections drug treated on day 5 pi and untreated controls. CON, control; QU, quinine therapy; PY, pyrimethamine therapy; MF, mefloquine therapy.



**FIG. 5.** Mean + 1 SEM asexual densities (a) and gametocyte densities (b) through time for drug prophylaxis and untreated controls. Data are pooled across experiments.

and pyrimethamine-treated infections, no mefloquine-treated infections had high rates of gametocytogenesis (Fig. 7). Prophylaxis had little effect on patterns of gametocytogenesis through time. The only exception was on day 10 pi, when conversion rates above median were substantially more frequent than in untreated infections (60 and 20%, respectively; Fig. 8). Patterns of gametocytogenesis based on the proportion of infections that had high rates of gametocytogenesis were very similar to patterns of mean gametocytogenesis (compare Figs. 7 and 8 with Fig. 9).



**FIG. 6.** Mean + 1 SEM total asexual parasites (a), total gametocytes (b), and proportion of parasites that were gametocytes for all drug prophylaxis and untreated controls. CON, control (untreated); PY, pyrimethamine prophylaxis; CQ, chloroquine prophylaxis.

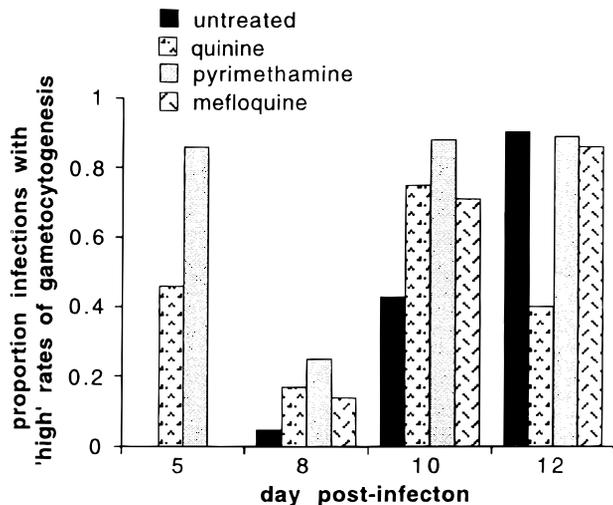


FIG. 7. Proportion of infections with 'high' (greater than median) rates of gametocyto-genesis for infections drug treated or not on day 5 pi.

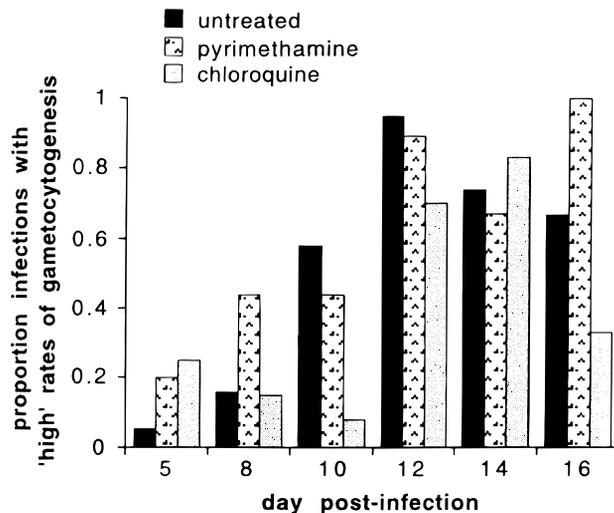


FIG. 8. Proportion of infections with 'high' (greater than median) rates of gametocyto-genesis following prophylaxis or sham drug treatment.

## DISCUSSION

All drugs, whether used as therapy or prophylaxis, reduced the number of asexual parasites produced during infections. However, gametocyte densities were not markedly reduced following pyrimethamine and mefloquine therapy or prophylaxis with either chloroquine or pyrimethamine. Quinine therapy did result in a small decrease in total gametocytes (approximately 25%, relative to untreated infections) but that was less than the associated reductions in asexual parasites. These data support the view that, during the course of an infection, a given number of asexual parasites produce more gametocytes following drug treatment.

An alternative possibility, that our observations are due to increased mortality of asexuals relative to gametocytes, can be ruled out. The antimalarial drugs that we used are known to be more damaging to asexual parasites than to mature gametocytes (Desjardins *et al.* 1988). However, the effective half-life of mefloquine in mice is estimated to be 18 h (Desjardins *et al.* 1988) and while detailed pharmacokinetic data from mice are not available for the other drugs, extrapolation from human data suggests that this will be the longest half-life of the drugs used (Peters 1987). For all drugs, preliminary studies showed that concentrations 25% of those actually used had no effect on asexual growth. Changes in the relative death rates of gametocytes and asexuals through drug use is therefore only of any potential

importance during the first 2 days following drug administration, a period excluded from our calculation of total numbers of parasites (assuming a maximum half-life of 18 h, after 36 h maximum drug concentration, = original concentration  $\times 0.5^2$ ). It also seems highly unlikely that other sources of differential mortality may affect our interpretations. Differences in stage-specific immune mortality due to drug treatment would require the existence of an as yet unknown

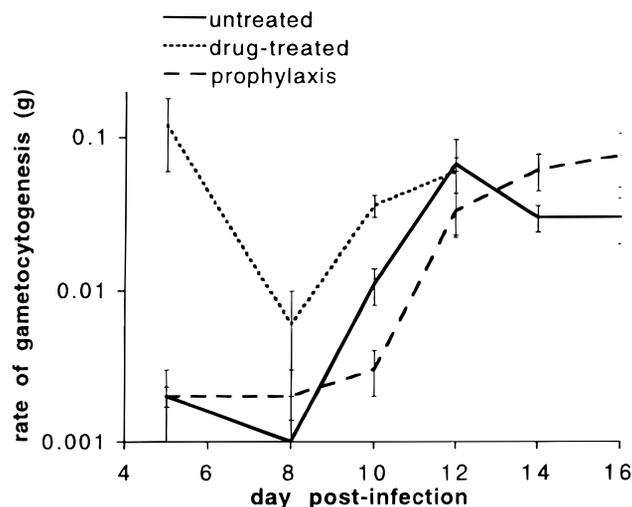


FIG. 9. Mean  $\pm 1$  SEM (approximation) rates of gametocyto-genesis through time for pooled drug-therapy, prophylaxis, and untreated infections.

gametocyte-specific clearance mechanism (Taylor and Read 1997), which is suppressed by all the antimalarial drugs used.

In all infections, rates of gametocytogenesis remained low and then rapidly increased up to much higher values toward the end of the infection. Alterations in daily rates of gametocytogenesis were more pronounced when drug treatment occurred during rather than before infection, presumably because therapy is more damaging to asexual parasites (as indicated by the greater reduction in total numbers of asexuals). Notably, there was an immediate elevation in gametocytogenesis following administration of pyrimethamine and quinine to infected mice. Such a rapid response to stressful conditions is consistent with studies on *P. falciparum* *in vitro* (Carter and Miller 1979; Brockelman 1982; Bruce *et al.* 1990).

This immediate increase was not, however, observed following mefloquine therapy. It is possible that malaria parasites do not respond to mefloquine-imposed stress. Alternatively, the effect occurred but was not detected because gametocyte densities in mefloquine-treated infections were generally below the detection threshold on day 7 pi. Mefloquine is much faster acting than quinine and especially pyrimethamine (Desjardins *et al.* 1988), so that fewer parasites will have survived on day 5 pi to produce the gametocytes observed on day 7 pi. Chemotherapy with chloroquine, a similarly fast-acting drug, generates almost identical gametocyte dynamics (Buckling *et al.* 1997). More generally, daily rates of gametocytogenesis calculated over 2-day periods will have been underestimated if mortality of asexual parasites is greater during the first 24 h than the second. This was likely the case immediately following treatment with all the antimalarial drugs used.

*P. chabaudi* appears to increase gametocytogenesis when conditions are unfavorable for asexual replication. In addition to drug treatment, gametocytogenesis increased as the infection progressed and the parasite numbers were suppressed by the host immune system (Fig. 9). Similarly, parasites from longer established *P. falciparum* infections of Gambian children (as determined by the presence of mature gametocytes) had rates of gametocytogenesis six times higher than parasites from younger infections (Smalley *et al.* 1981). Increased investment into transmission with 'age' of the infection is consistent with theoretical predictions of the dynamics of *Plasmodium* infections within vertebrate hosts (Koella and Antia 1995) and of organisms' life histories in general, if transmission is considered analogous to reproduction (Stearns 1992).

*P. chabaudi* infections in mice have qualitatively similar dynamics to *P. falciparum* infections in humans, with peak gametocyte densities occurring after peak asexual densities

(Carter and Graves 1988) and, at least *in vitro*, *P. falciparum* gametocytogenesis is also more frequent when asexual growth is inhibited in general (Carter and Miller 1979; Brockelman 1982; Carter and Graves 1988; Bruce *et al.* 1990; Sinden *et al.* 1996), and specifically by antimalarial drugs (Buckling *et al.* 1999). Our results may therefore have implications for antimalarial drug use against *P. falciparum* in the field. First, reductions in transmission bought about by subcurative antimalarial treatment may be much less than expected from reductions in asexual densities if average rates of gametocytogenesis are simultaneously increased. Second, drug therapy may stimulate high rates of gametocytogenesis to occur earlier than in untreated infections, resulting in earlier high infectivity. Such a phenotype will potentially increase transmission under conditions in which the speed of transmission is a major component in parasite fitness (e.g., during epidemics). Third, if drugs are used in doses that are lethal to drug-sensitive but not resistant parasites, changes in gametocyte production in response to drug-imposed stress may help to explain the rapid spread of drug resistance (Peters 1987). Drug treatment may further enhance transmission as a result of reduced host immunity through decreased exposure to the parasite (Graves *et al.* 1988), increases in per gametocyte infectivity (Butcher 1997), and genetic changes in patterns of gametocytogenesis (Bishop 1954; Lines *et al.* 1991; Handunnetti *et al.* 1996).

Partially effective vaccines may also stress parasites, at least in the short term. If so, they too may generate compensatory gametocyte production similar to that observed in this study. *In vitro*, *P. falciparum* gametocyte production increased following the addition of serum and lymphocytes from *P. falciparum*-infected children (Smalley and Brown 1981) and anti-*P. falciparum* antibody (Ono *et al.* 1986). More generally, facultative changes in parasite life histories may render medical and veterinary intervention less effective than anticipated.

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