INTRODUCTION

In general, the course of a malaria infection is monitored by examining daily made Giemsa stained smears. However, the shape of the corresponding curve, displaying the total parasitaemia as a function of time, does not reflect the detailed evolution of the schizogonic cycle. This implies that important relevant data remain hidden if only daily observations are made. Against this background we present the analysis of total and differential parasitaemia curves of *P. chabaudi* in their rodent host based upon hourly made thin blood smears. *P. chabaudi chabaudi* is an interesting experimental model (Landau et al., 1993) and shares a number of biological features with *P. falciparum* (Jarra & Brown, 1985; Gilks et al., 1990). Besides, *P. chabaudi chabaudi* infections are synchronous, with a period of 24 hours, and depend on the circadian rhythm of the host (Gambie et al., 1990; Landau et al., 1990, 1991).

MATERIALS AND METHODS

MICE

Six-week-old, female, inbred, pathogen-free, CBA/Ca mice (Kingman, England) were kept in plastic translucent cages at 21 °C and 50 % relative air humidity. Their photoperiodic rhythm was set by a regimen of 12 hours light (13 h 00-01 h 00)/12 hours dark (01 h 00-13 h 00). The mice were handled conform with national and international legislation and guidelines.
PARASITE AND INFECTION

The virulent IP-PC1 strain of *P. chabaudi chabaudi* was inoculated into a CBA/Ca mouse. When a parasitaemia of 30-50 % was reached and late schizonts predominantly occurred, 1 ml of blood was suspended in RPMI 1640 (GibcoBRL) supplemented with 10 % foetal calf serum (FCS; GibcoBRL), and centrifuged. The recovered erythrocytes containing mature schizonts were resuspended in the same medium and incubated at 37 °C for 30 minutes to liberate the merozoites. After centrifugation, the supernatant containing the merozoites was centrifuged again, and the brownish pellet was resuspended in 250 μl of PBS (pH 7.3) and immediately i.v. injected into another mouse at 10 h 00. When its parasitaemia had reached a level of about 10 % (ring forms), 10^6 infected red blood cells in sterile Alsever medium (pH 6.2) were i.v. injected into 4 receptor mice at 11 h 00. The experiment was started when the parasitaemia of these mice reached a level of 5-10 % and about 30 %, respectively.

TOTAL AND DIFFERENTIAL PARASITAEMIA

After Giemsa staining of thin blood smears, the total parasitaemia (number of infected red blood cells expressed as a percentage of the total number of red blood cells) and the differential parasitaemia (numbers of ring forms, trophozoites, and schizonts, expressed as percentages of the total number of infected red blood cells) were determined. Ring forms were defined as the stages developing between the attachment of merozoites to the erythrocytes and the disappearance of the ring specific vacuole, while trophozoites did not display such a vacuole and their cytoplasm was larger than the one of the ring shaped parasites. Schizonts occupied most of the host erythrocyte cytoplasm, with a very dense and/or dividing nucleus. For each smear a minimum of 100 infected red blood cells was counted.

RESULTS

TOTAL PARASITAEMIA CURVES

The detailed time course of the total parasitaemia of mice with an initial parasitaemia of 5-10 % is given in figure 1a. By the end of the dark period a plateau level with a constant parasitaemia of ca. 12.5 % was reached, which lasted the full length of the light period plus 2-3 hours of the dark period. At the end of the plateau the parasitaemia showed a distinct decrease with a minimum level roughly corresponding with the middle of the second dark period. Then it rose sharply and reached a new plateau level with a parasitaemia of ca. 37.5 %.

INVASION RATES

Invasion rates were obtained by dividing the values of two consecutive plateau levels. Figure 1a yields a rate of about 3, while a value of about 2 is deduced from figure 1b. In previous experiments (B. Chimanuka, unpubl. obs.) we obtained higher invasion rates of 10 and 8, when initial parasitaemias were 0.1 and 1 %, respectively. The correlation between initial parasitaemia and invasion rate is clearly negative (r = - 0.87).

DIFFERENTIAL PARASITAEMIA CURVES

The development of ring forms, trophozoites, and schizonts, corresponding with the total parasitaemia curves of figure 1a, is shown in figure 2. Gametocytes...
were not observed throughout this experiment. The duration of the developmental stages was estimated at 12 hours for the rings, 8 hours for the trophozoites, and 4 h for the schizonts. The proportion of ring forms rose sharply at the beginning of the experiment and remained almost constant at a level of about 95% until the end of the first light period. Then it decreased to reach a minimum level of 10% in the middle of the second dark period. The proportion of trophozoites on the other hand was on the decrease when the observations begun and stayed at a very low level (< 5%) during the light period. Then it increased to reach a peak level of about 75% of the total number of parasites by the middle of the second dark period. The schizont curve showed peaks at 25-30% and 40-45%, each of them occurring 7-8 hours after the onset of the dark periods, i.e. 2-3 hours after the appearance of the trophozoite peaks. In between, the proportion of schizonts did not exceed 5% of the total number of infected erythrocytes. The differential distribution curves showed a cyclic pattern, as illustrated by the observations made after the first 24 hours of the experiment.

**DISCUSSION**

Analysis of the total parasitaemia curves (Fig. 1) reveals the duration and the periodicity of the schizogonic cycle, the existence of a plateau, indications of schizont withdrawal from the peripheral blood, the timing of the rise of the parasitaemia, and the invasion rate of the merozoites. The initial increase of the total parasitaemia (Fig. 1a) during the second half of the dark period corresponds with the appearance of ring forms (Fig. 2) after the release of merozoites by mature schizonts. When the total parasitaemia reaches a plateau, ring forms are predominant and account for at least 95% of the infected erythrocytes (Fig. 2). Other authors (Hommel et al., 1982; Boyle et al., 1983; Cox et al., 1987; Gilks et al., 1990) mentioned only part of these possibilities or used the curves for different purposes. The position of the parasitaemia drop at the end of each plateau phase perfectly coincides with the rising slope of the schizont peak. Similar drops were observed with *P. chabaudi adami* by Gautret (1993). This phenomenon has been explained by the disappearance of schizonts from the peripheral blood and their sequestration in the inner organs, mainly the liver and the spleen (Cox et al., 1987; Gilks et al., 1990). This peripheral withdrawal was estimated at 8-10% of the preceding plateau level.

The apparent discrepancy observed between the duration of the presence of ring forms in this paper (12 h) and in other reports (6 h) (Cambie et al., 1990; Caillard et al., 1992; Gautret et al., 1995) is due to the fact that our ring forms coincide with the combined ring and young trophozoite forms described by the mentioned authors.

Schizonts of other species such as *P. falciparum* and *P. berghei* are sequestered in the cerebral capillaries and are not frequently seen in the peripheral blood (Kwiatkowski & Greenwood, 1989; Lambert & Grau, 1989). This explains why severe *P. falciparum* malaria attacks can even occur with little or no evidence of parasites in the examined blood films (Carme et al., 1989). It means that a more pronounced drop in the total parasitaemia curve can be expected in the case of *P. falciparum*. In principle, this phenomenon could be developed as a diagnostic parameter for the onset of cerebral *P. falciparum* malaria, as sequestration is the key initiating event for this pathology (Warrell, 1987).

![Graph](image-url)
The invasion rate with a value of 2, deduced from figure 1b), corresponds with the one given by others (Boyle et al., 1983). However, invasion rates of about 10 and 8 obtained in other experiments (B. Chimpanuka, unpubl. obs.) correspond with the mean number of merozoites produced per mature schizont (Landau, 1965). The negative correlation between invasion rate and initial parasitaemia could be explained by the fact that at high parasitaemia levels less erythrocytes are available for invasion because many of them are lysed at each schizogony, immune reactions are more pronounced (Jayawardena, 1981), multiple invasion of red blood cells occurs, and free merozoites could aggregate both within themselves and with pigment and membranes, as has been shown for Plasmodium falciparum (Vernot & Wasserman, 1990).

The timing of the administration of antimalarial drugs to the target parasite stage has been proposed by many authors (Yayon et al., 1983; Cambie et al., 1991; Caillard et al., 1992; Landau et al., 1992). In such a strategy, the parasitaemia curves presented in this report could become an important tool.

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