PLASMODIUM VINCKEI VINCKEI, *P. V. LENTUM* AND *P. YOELII YOELII* : CHRONOBIOLOGY OF THE ASEXUAL CYCLE IN THE BLOOD

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Summary :

The biological rhythms of *Plasmodium vinckei vinckei*, *P. v. lentum* and *P. yoelii yoelii* i.e. synchronicity, duration of the erythrocytic cycle, timing of the schizogony and of the penetration of merozoites into red blood cells, were studied in the Swiss white mouse. Two different methods of synchronisation were used: the freezing-thawing of parasitized blood and the inoculation of a single parasitic stage, separated from the other stages by centrifugation through a Percoll®-Glucose gradient. The duration of the schizogonic cycle of *P. v. vinckei* and *P. v. lentum*, two highly synchronous subspecies, was 24 hours. With *P. v. vinckei* the timing of the schizogony was independent of the circadian rhythm of the host and was set by the time of inoculation. With *P. v. lentum* the timing of the schizogony and merozoites penetration into red blood cells depended both, on the hosts rhythm and the time of inoculation of frozen-thawed blood : schizogany occurred at 18:00 if the inoculum was injected at 06:00 or 12:00, and at 06:00 if injected at 18:00 or 00:01. *P. y. yoelii* a naturally asynchronous parasite was synchronized by means of a Percoll®-Glucose gradient. The duration of its intraerythrocytic cycle was found to be 18 hours, similar to that of the other subspecies of *P. yoelii*.

KEY WORDS : *Plasmodium vinckei vinckei. Plasmodium vinckei lentum. Plasmodium yoelii yoelii.* duration of the erythrocytic cycle. timing of the erythrocytic cycle. synchronicity. biologicol rhythms.

Résumé : CHRONOBIOLOGIE DU CYCLE SCHIZOGONIQUE ÉRYTHROCYTAIRE DE *PLASMODIUM VINCKEI VINCKEI*, *P. V. LENTUM* ET *P. YOELII YOELII*.

Etude des rythmes biologiques de la schizogonie érythrocytaire de Plasmodium vinckei vinckei, P. v. lentum *et* P. yoelii yoelii.

Les caractères étudiés sont le synchronisme plus ou moins marqué des différents stades, la durée des cycles, les horaires des schizogonies et la chronologie de la pénétration des mérozoïtes dans les hématies.

Deux techniques de synchronisation ont été utilisées: l'inoculation de sang infectant congelé puis brutalement décongelé ou bien l'inoculation d'un seul stade parasitaire, séparé des autres stades par centrifugation sur gradient de Percoll- glucose.

La durée du cycle des deux sous-espèces très synchrones Plasmodium v. vinckei *et* P. v. lentum *est de 24 heures.*

Avec P.v. vinckei, *l'horaire de la schizogonie est indépendant du rythme circadien de l'hôte et est déterminé par l'heure de l'inoculation. Avec* P. v. lentum, *l'horaire de la schizogonie et de la pénétration des mérozoïtes dans les hémoties dépend à la fois du rythme circadien de l'hôte et de l'heure d'inoculation du sang décongelé: la schizogonie survient à 18 heures si l'inoculation est faite à 6 heures, ou à midi et à 6 heures si l'inoculation est faite à 18 heures ou à minuit. La sous-espèce très asynchrone* P. y. yoelii *a été synchronisée par la technique du gradient Percoll- glucose. La durée du cycle est de 18 heures, identique à celle des autres sous-espèces de* P. yoelii.

MOTS CLES : Plasmodium vinckei vinckei. P. v. lentum. P yoelii yoelii. *Durée du cycle érythrocytaire. Horaire du cycle érythrocytaire. synchronisme. rythmes biologiques.*

INTRODUCTION

The rodent malarias have been used as models for studying various aspects of the biology of malaria parasites for more than 40 years. Recent work (Montalvo *et al.*, 1988, Cambie *et al.*, 1990, Landau *et al.*, 1990) has shown that chronobiological data are of great importance because the biological rhythm of the rodent parasites may vary from one species to the other, or even from one subspecies or strain to the other. The consequence of these notions is that, in many cases, an accurate interpretation of experimental results cannot be achieved without taking into account these chronobiological data. In this work, we have investigated the duration of the asexual erythrocytic cycle and the "timing niche" (Cambie *et al.*, 1990) of 2 subspecies of *P. vinckei* and one of *P. yoelii*. Two methods of synchronization have been employed according to the strain : 1) *P. vinckei* : freezing-thawing of infected blood which increases the natural synchronicity of the strain (Montalvo *et al.*, 1988); 2) *P. yoeli* : centrifugation through a Percoll®-Glucose gradient (Deharo *et al.*, 1994) which results in a synchronous infection for at least 2 schizogonic cycles.

MATERIAL AND METHODS

STRAINS

– *Plasmodium vinckei vinckei* Rodhain, 1952, (67) a synchronous parasite from Zaïre.

– *Plasmodium vinckei lentum* Landau, Michel, Adam and Boulard, 1970, (194 ZZ L) a synchronous parasite from the Congo Republic.

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– *Plasmodium yoelii yoelii* Landau and Killick-Kendrick, 1966, (265 BY), an asynchronous parasite from the Centralafrican Republic.

Methods of synchronization

– *Freezing-thawing of infected blood* : Rapid freezing and thawing before injecting infected blood from a donor to a recipient mouse was shown (Montalvo Alvarez *et al.*, 1988) to destroy most intra-cellular parasites and to allow in the recipient mouse an infection initiated by the only surviving stage, the free merozoite. The method has since been used for studies on chronotherapy with *P. v. petteri* (Cambie *et al.*, 1991, Caillard *et al.*, 1992 and 1993). In the present study the method was employed to determine the relationships between the time of inoculation and the time of schizogony of *P. v. vinckei* and *P. v. lentum*. In all experiments, mice were inoculated intraperitoneally with the same amount of blood from a frozen aliquoted stock. It is impossible to determine the number of merozoites inoculated by this technique but all mice, in a given experiment, received the same inoculum.

– Percoll®-Glucose gradient: the technique was devised (Deharo *et al.*, 1994) for synchronizing *P. yoelii*. Parasitized blood (*P. y. yoelii*) was centrifuged through a discontinous gradient of Percoll®-Glucose and separated into two main layers: a layer containing old parasites : Mid-term trophozoite (MT); Old trophozoite (OT); Schizont (S) and a layer containing only Rings (R) and Young trophozoites (YT). The latter was inoculated intravenously into mice in which a patent, synchronous infection, evolved over the first two schizogonic cycles.

Course of parasitemias

Male Swiss outbred mice (Iffa Credo, France) were used for the follow-up of the infection. Thin smears were made from tail blood, fixed in methanol and stained by Giemsa stain. Parasitemias were evaluated by examining 2000 red blood cells. The parasitic pattern can be expressed as the relative proportion of each stage: Rs, YTs, MTs, OTs and Ss according to the classification by Cambie *et al.*, 1991. The evolution of the parasitemia and the parasitic pattern was followed in *P. v. vinckei* from day 4 and in *P. v. lentum* from day 8 post inoculation, when parasitemias reached 1 % or more. In *P. y. yoelii*, infections were immediately patent. Blood smears were performed at 3 or 6 hours interval during 48 or 54 hours and all experiments were stopped before the parasitic crisis, as described by Bastien *et al.* (1986).

RESULTS

P. v. VINCKEI

Two batches of 3 mice each were inoculated with frozen-thawed infected blood, one at 12:00, the other at 00:01. The evolution of the parasitic pattern can be followed in the curves of Fig. 1. The peak of the stages contained from 60 to 70 % of that stage. When the inoculation was performed at 12:00, Rs peaked at 12:00, YTs at 18:00, MTs at 00:01, OTs at 06:00. When the inoculation was performed at 00:01, Rs peaked at 00:01, YTs at 06:00, MTs at 12:00, OTs at 18:00. The interval between peaks was consistently 24 hours.

In conclusion, the duration of the schizogonic cycle is 24 hours; the timing of the schizogony is independent of the circadian rhythm of the host and depends only on the time of inoculation. Schizogony occurred 24 hours post-inoculation and this indicates that the penetration of merozoites inoculated with the frozen blood is immediate.

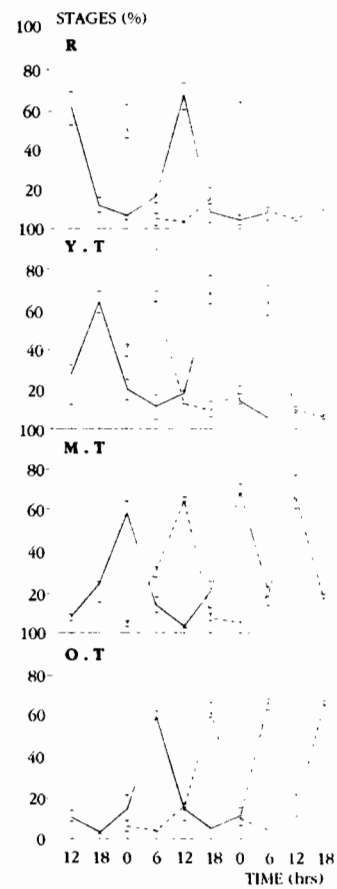

Fig. 1 – Follow-up of the parasitic pattern (mean percentages of each stage) of *P. v. vinckei*. Time of inoculation: 12:00 (full line) and 00:01 (intermittent line).

P. V. LENTUM

Four batches of three mice each were inoculated with frozen-thawed infected blood, at different times: 06:00, 12:00, 00:01 and 18:00. The evolution of the parasitic pattern is represented in the curves of Fig. 2. Peaks of stages, except rings, reached from 60 to 70 % of that stage. Peaks of Rs did not exceed 34% of Rs due to the fact that the duration of the stage is 3 hours only and that blood smears were performed every 6 hours. In mice inoculated at 18:00 and 00:01, Rs peaked at 06:00, YTs at 12:00, MTs at 18:00, OTs at 00:01. In mice inoculated at 06:00 and 12:00, Rs peaked at 18:00, YTs at 00:01, MTs at 06:00 and OTs at 12:00. The curves showing the evolution of stages in the mice inoculated at 06:00 are mean values for two mice; the third one in the batch reached crisis time before the end of the experiment and was discarded. The interval between peaks was consistently 24 hours.

In conclusion, *P. v. lentum* has a synchronous development, the duration of a schizogonic cycle is 24 hours, the timing of the schizogony depends on the time of inoculation and also on the circadian rhythm of the host. When mice were inoculated at 06:00 or 12:00, schizogony occurred around 18:00, while it occurred around 06:00 in mice inoculated at 00:01 or 18:00.

P. Y. YOELII

Four mice were inoculated intravenously at 13:00 with Rs and YTs concentrated in a layer of the Percoll®-Glucose gradient. The parasitemia and parasitic pattern were evaluated at 3 hours intervals during the 54 hours post-inoculation and results are plotted in Table I. The stage composition of the inoculum injected at 13:00 was 63 % Rs and 37 % YTs. In Fig. 3, the curve represents the evolution of the percentages of Rs during the 54 hours follow-up. The peaks of Rs occurred at 13:00 (time of inoculation), at 07:00 on Day 1, and at 01:00 on Day 2. They were separated by an interval of 18 hours. Other stages' peaks, as can be seen in Table I are also 18 hours apart. However, the height of the peaks tended to decrease as the infection became less synchronous (after the second cycle), as shown in table I where it can be seen that, 54 hours post-inoculation, all stages could be found.

In conclusion, the duration of a schizogonic cycle of *P. y. yoelii* is 18 hours: the parasite remains synchronous during the first two cycles; afterwards particular stages do not predominate so evidently.

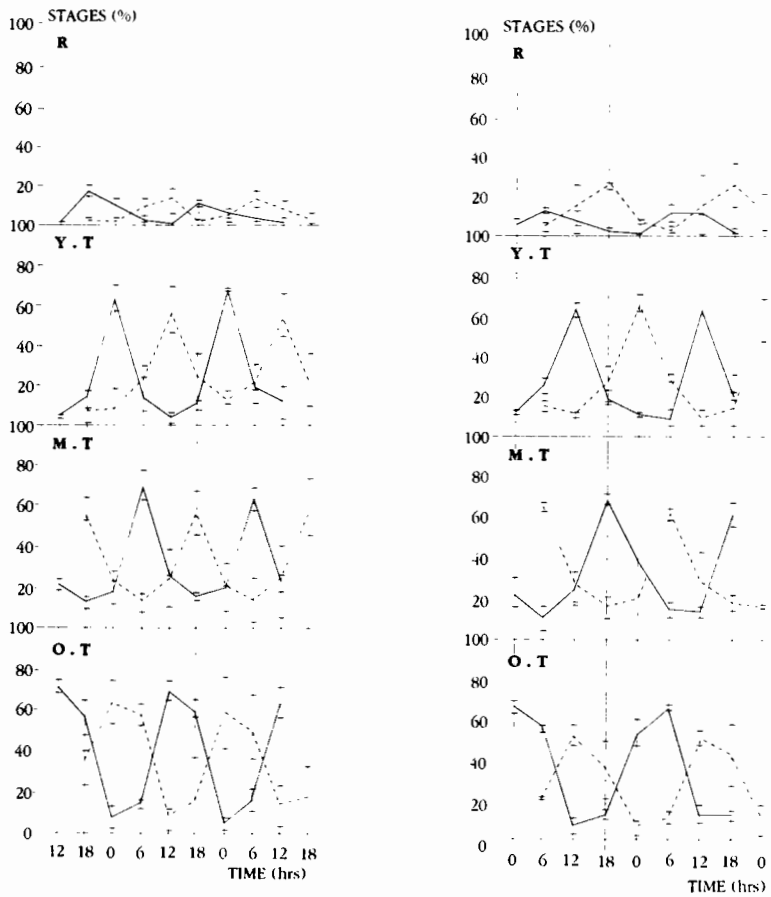

Fig. 2 – Follow-up of the parasitic pattern (mean percentages of each stage) of *P. v. lentum*; left: Time of inoculation 12:00 (full line) and 18:00 (dashed line). right: Time of inoculation: 00:01 (full line) and 06:00 (dashed line).

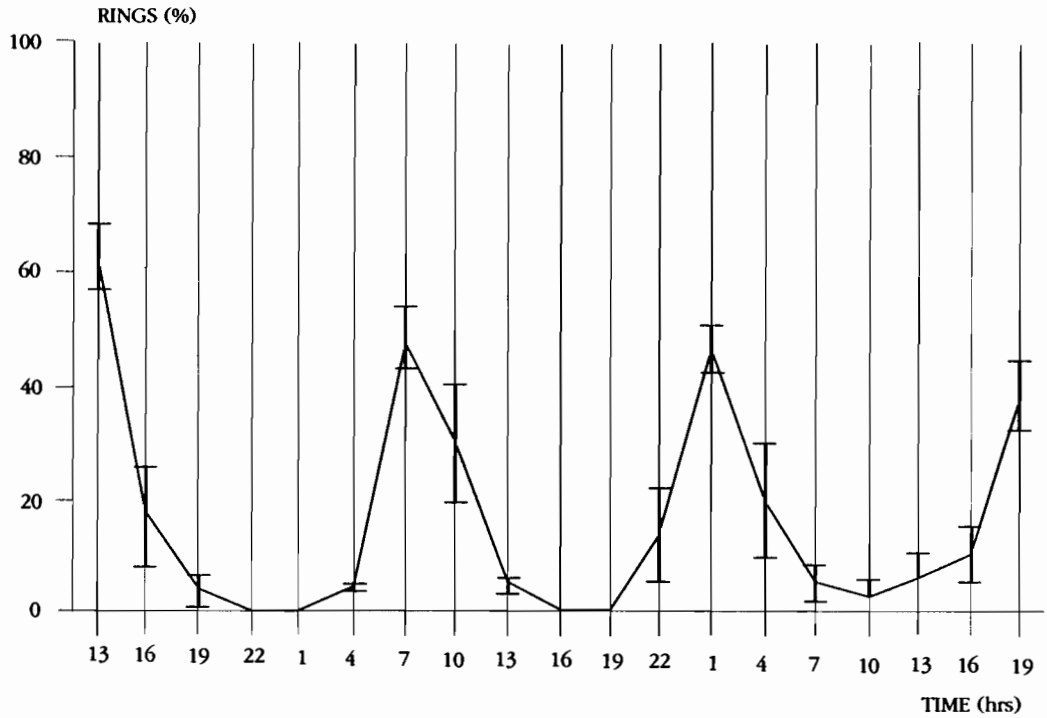

Fig. 3 – Follow-up of the rings percentage of *P. y. yoelii*.

TIME (hrs)	PARASITEMIA %	± SD	R %	± SD	Y.T %	± SD	M.T %	± SD	O.T %	± SD	S %	± SD
13:00	0.1	0	62.7	5.8	37.3	5.8	0	0	0	0	0	0
16:00	0.1	0	17.3	8.7	70.7	12.5	12	9.6	0	0	0	0
19:00	0.1	0	3.7	2.5	26.7	5.8	67	3	2.7	3.1	0.7	1.2
22:00	0.1	0	0	0	8.7	2.3	63.3	12.7	27.3	15.4	0.7	1.2
01:00	0.2	0.1	0	0	3.3	3.1	23.7	5.5	71.7	10.4	1.3	2.3
04:00	0.2	0.1	4.3	0.6	1.7	2.9	12	6.1	61.3	10.3	20.7	4.5
07:00	0.3	0.1	48	5.6	21.7	1.5	3	0	16	8.5	11.3	4.7
10:00	0.4	0.2	29.7	10.1	57.7	4	7.3	5.5	2.7	3.8	2.7	2.3
13:00	0.5	0.2	4.7	1.2	31	4.6	57.3	2.3	5	1.7	2	1.7
16:00	0.5	0	0	0	13.3	4.2	64	6	22	8.7	0.7	1.2
19:00	0.7	0.2	0	0	3.7	0.6	23.7	7.8	72.7	9	3.3	4.2
22:00	0.7	0.2	13.3	8.1	10	7.2	10.7	5.9	55	6.2	10.3	3.8
01:00	1.5	0.3	46	3.5	22	10.6	7	3.6	15	4.6	10	7.21
04:00	2.8	1.9	19	9.9	52.7	7.8	11.3	1.2	7.7	3.8	9.3	2.3
07:00	2.9	1.8	4.7	3.1	34	6.9	47.7	0.6	12	2	1.7	2.1
10:00	1.9	0.5	2	3.5	13.7	10	54.7	4.2	27.7	7.4	2	3.5
13:00	1.9	0.7	4.7	5	9	2.7	27.3	7.1	52.3	5.1	6.7	5.8
16:00	2	0.6	9.3	5.1	20	4.6	19	7	44.7	10	7	3.6
19:00	2.6	1	37	6.2	21.3	6	14.3	4	22	10.6	5	5

Table 1 – Follow-up of the parasitemia and parasitic pattern (percentage of each stage) of *P. y. yoelii*.

DISCUSSION

The present study demonstrates that very closely related parasites like the subspecies of *P. vinckei* may show important chronobiological differences. In all the subspecies studied, the length of the schizogonic cycle and the duration of each stage appeared to be identical. The main differences concern the relationships of the parasites with the circadian rhythms of the host. It has been shown (Montalvo *et al.*, 1988) that, in ordinary laboratory conditions, the timing of the schizogony of *P. v. petteri* was independent of the hosts' rhythm. The present study indicates that *P. v. vinckei* shows the same behaviour. In both cases merozoites from the frozen thawed inoculum penetrate immediately into red blood cells and establish the time of schizogony which occurs 24 hours post-inoculation. On the contrary the cycle of the closely related *P. v. lentum* is under the control of both, the time of inoculation, like *P. v. petteri* and *P. v. vinckei,* and the hosts rhythm, like *P. chabaudi* (Cambie *et al.*, 1990) which is unusual, parasite cycles normally being governed either by one or the other. Whatever the time of inoculation, merozoites of *P. v. lentum* appear to wait for the favourable time to penetrate and the subsequent cycle is set with schizogony occuring either at 06:00 or at 18:00. We have no definite explanation for this duality of timing. It bears perhaps a relationship with the biology of the vectors biting twice during the night, at dawn and dusk. Finally, *P. y. yoelii* differs little from the other *yoelii* subspecies *killicki* and *nigeriensis* (Deharo *et al.*, 1994), the cycle of which also last 18 hours. The existence of a "timing niche" is very common in polyparasitic infections by sporozoans. The 12 species of Isospora described by Grulet *et al.*, 1982 in the domestic sparrow occupy a different localisation in the gut but also their oocysts are formed and excreted at a definite time of the day, different from one species to the other. The analysis of available data on the chronobiology of the rodent malarias lead to the conclusion that differences exist not only between parasites coexisting in the same host but also between the subspecies of a same species occuring in different localities. It would be very surprising if the biology of the human malarias differed very much from that of the other animal malarias. In fact workers are increasingly aware of the biodiversity of the human parasites in the field; the duration of the schizogonic cycle, the timing of the different stages, the degree of synchronicity are variable parameters of which it is important to be aware, for chemotherapeutic or epidemiological studies.

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