THE ADJUSTMENT OF THE SCHIZOGONIC CYCLE OF *PLASMODIUM CHABAUDI CHABAUDI* IN THE BLOOD TO THE CIRCADIAN RHYTHM OF THE HOST

GAUTRET P.*, DEHARO E.*, TAHAR R.*, CHABAUD A.G.* & LANDAU I.*

Summary :

Experiments were performed with *P. chabaudi chabaudi* to investigate the relationship between the development of the parasite and the circadian rhythm of the host. Blood was taken from a donor mouse at 10.00 hours, when it contained mainly young stages and inoculated to receptor mice, either immediately or after 8 hours at +4°C. The inoculum was diluted in order to obtain a more or less extended prepatent period. Thus, by using successively the different mice, parasitemias could be followed during 12 days before the crisis. When parasitemias reached 1%, from day 1 (D1) to D7, depending on the dilution, the parasitic patterns were studied every 6 hours during 2 or 3 days. In mice inoculated at 10.00 hours the rhythm remained unmodified. In mice inoculated at 18.00 hours the infection was at first synchronous (from D1 to D4) but the schizogony occurred between 06.00 and 12.00 hours instead of midnight. From day 4 to day 7 the infection became asynchronous. At day 10 the normal rhythm was resumed and the schizogony occurred around midnight.

KEY WORDS : *Plasmodium chabaudi*. schizogonic rhythm. merozoite latency.

Résumé : RÉAJUSTEMENT DU CYCLE SCHIZOGONIQUE DE *P. C. CHABAUDI* AU RYTHME CIRCADIEN DE L'HÔTE

Une série d'expériences a été réalisée avec P. chabaudi chabaudi *pour étudier les relations entre le développement du parasite et le rythme circadien de l'hôte. Un prélèvement de sang est effectué à 10 heures chez une souris donneuse, lorsque les parasites sont essentiellement représentés par des trophozoïtes jeunes. Il est inoculé aux souris réceptrices, soit immédiatement, soit après conservation pendant 8 heures à +4°C. L'inoculum est plus ou moins dilué ce qui entraîne une période prépatente plus ou moins longue. La parasitémie, avant les phénomènes de crise, peut donc être suivie pendant 12 jours en utilisant successivement les différentes souris. Lorsque la parasitémie atteint 1%, dans un délai de un (J1) à sept jours (J7), selon la dilution, la formule parasitaire est établie toutes les 6 heures pendant 2 à 3 jours. Pour les souris infectées à 10 heures le rythme n'est pas modifié. Pour les souris infectées à 18 heures, l'infection est d'abord synchrone (de J1 à J4) mais la schizogonie se produit entre 6 à 12 heures au lieu de minuit. De J4 à J7 l'infection devient asynchrone. A J10 le rythme habituel est rétabli et la schizogonie se produit vers minuit.*

MOTS CLES : Plasmodium chabaudi. *rythme schizogonique. latence des mérozoïtes.*

INTRODUCTION

The development of *Plasmodium chabaudi chabaudi* in the blood of rodents is relatively synchronous and the timing of the schizogony is set by the circadian rhythm of the host. In normal day/night conditions schizogony occurs between midnight and 03.00 hours (Hawking, Gammage and Worms,1972; Cambie, Landau and Chabaud, 1990). When mice are submitted to an inverted rhythm, the schizogonic rhythm is also inverted (David *et al.*, 1978) and schizogony occurs around midday (Cambie *et al.*, 1990).

The compliance of this species to the host's rhythm is difficult to overcome in the laboratory. Cambie *et al.* (1990) injected infected frozen blood to mice, at various times of the day, and were unable to desynchronize the infection or to modify its rhythm. Whatever the time of inoculation schizogony occurred at midnight in normal day/night conditions (light from 08.00 to 20.00 hours) and at noon, when mice were kept in inverted day/night (light from 20.00 to 08.00 hours). They hypothetized that in normal day-night conditions, most merozoites of *P. c. chabaudi* penetrated into red blood cells (RBC) at midnight, thus setting the time of schizogony. When frozen-thawed blood is inoculated, the infection becomes patent after several days when it has already acquired a normal rhythm, and the intermediate steps of the parasites' adjustment are unknown.

The aim of the present work was to try to clarify experimentally the mechanisms regulating the circadian rhythm of *P. chabaudi.*

We disorganized the parasites' rhythm and followed the parasitemia and the relative proportion of each stage from the time of inoculation to the time the normal rhythm was regained. This was achieved by taking blood from a donor mouse in the morning and keeping it at 4°C for 8 hours before inoculating it to receptor mice. In order to understand and follow the events, it was found necessary to study the parasitemia during at least 10 days and maintain its level

* Laboratoire de Biologie Parasitaire, associé au CNRS (URA 114), et Laboratoire de Protozoologie et Parasitologie Comparée (EPHE), Muséum National d'Histoire Naturelle, 61 rue Buffon, 75231 Paris cédex 05, France.

below 50%. The parasitemias increase from 1 to 50% in 2 to 4 days. Thus the development was followed in several successive batches of mice which had been inoculated with increasingly diluted infected blood.

MATERIALS AND METHODS

Strains

P. c. chabaudi Landau, 1965 (864 VD) from the Central African Republic was isolated from *Thamnomys rutilans*. The strain has since been maintained in the laboratory mice and the deep freeze.

Mice

Male outbred Swiss mice (Iffa Credo-France) were stored in a room artificially lighted from 06.00 hours to 18.00 hours.

Follow-up of the infection

Thin blood smears from the infected mice were taken at six hour intervals, when parasitemias were between 1 and 25 %, fixed with methanol and stained with Giemsa stain. The total parasitemia (percentage of infected cells) and the parasitic pattern (percentage of each parasite stage) were evaluated. Four stages were defined, according to Cambie *et al.* (1990) : ring (R) the smallest intra-cellular stage following the invasion of the erythrocyte by the merozoite; young trophozoite (YT) displaying a larger vacuole, a more developed cytoplasm and an irregular contour, and very faint or no pigment; mid term trophozoite (MT) occupying 1/3 to 1/2 of the host erythrocyte volume, larger nucleus, relatively more abundant cytoplasm, smaller vacuole and a few granules of pigment; old trophozoite (OT) occupying from 1/2 to almost the entire volume of the RBC, densely staining cytoplasm, small vacuole and profuse pigment.

Results plotted in Tables 1-4 are mean values for two mice of each batch, the variations between them being indicated in the adjacent SD column.

Synchronicity index

This index (SD/50) is the ratio between the standard deviation (SD) of the parasitic pattern (percentage of each stage) and the SD of a 100% synchronous infection. It varies between 0 (asynchronicity) and 1 (total synchronicity). This index (SI) is shown in the last column of each table.

Protocol for the infection of mice

Five donor mice were inoculated at 12.00 hours with frozen-thawed infected blood. This procedure was shown by Montalvo-Alvarez *et al.* (1988) to destroy intracellular parasites. Thus the infection is initiated by the surviving stage, the free merozoite. This procedure has since been commonly used to increase the synchronicity of the infections with rodent plasmodia. When parasitemias reached 10%, mice were anaesthetized and blood was taken by heart puncture at 10.00 hours and inoculated to receptor mice. The predominant stage at this time was the YT. In order to obtain a progressively delayed prepatency in the receptor mice, increasing dilutions of blood were used. Two mice were inoculated intravenously (IV) at 10.00 hours (Day 0) with 0.5 ml of the 1/2 diluted blood (10^7 parasites), immediately after it was taken from the donor mice. The rest of the receptor mice received an inoculum taken at 10.00 hours but kept for 8 hours in a refrigerator at 4°C; the following dilutions were injected IV: 1/8 and 1/10000 to two mice each, and 1/100000 IP (to increase further the length of the prepatent period) to six mice.

The development of the parasitemia in mice inoculated at 10.00 was only followed during two days to ensure that it was similar to that observed in routinely blood transmitted infections. With the inoculations performed at 18.00, the parasitemias were followed for 2 or 3 consecutive days, starting on the day they reached approximately 1 % i.e. from day 0 (D0) to D2 for the 1/2 dilution, D0 to D4 for the 1/8 dilution, D4 to D7 for the 1/10000 dilution and D10 to D12 for the 1/100000 dilution.

RESULTS

Immediate inoculation (at 10.00 hours)

Table 1 and Figure 1.1 show the results obtained in mice inoculated at 10.00 hours, immediately after the blood was taken from the donor mice, and diluted to 1/2 before injection. Parasitemias were analysed from D0 to D2.

Parasitic pattern : At 10.00 hours on D0, YTs prevailed. On D1, Rs predominated at 00.01 hours (the peak of Rs occuring normally around 03.00, at midnight they did not exceed 31%). The peak of YTs occurred between 06.00 and 12.00 hours. The MTs were seen at 18.00 hours and OTs at 00.01 hours on D2. The cycle being 24 hours, the schizogony occurred around midnight.

The parasites' development followed in both the donor and receptor mice, showed the normal pattern described by Cambie *et al.* (1990) for *P. chabaudi*. The synchronicity index (SI) was relatively high, between 0.56 and 0.74.

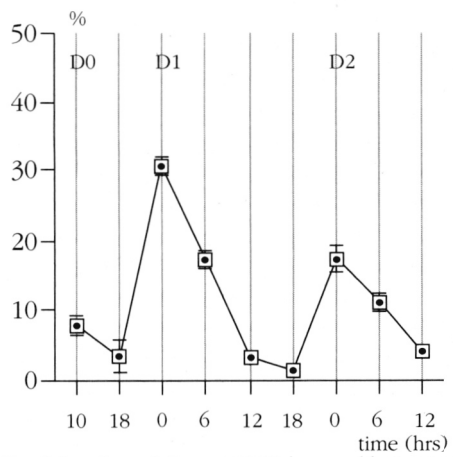

Fig. 1-1. – Inoculation at 10.00 hours, dilution to 1/2.

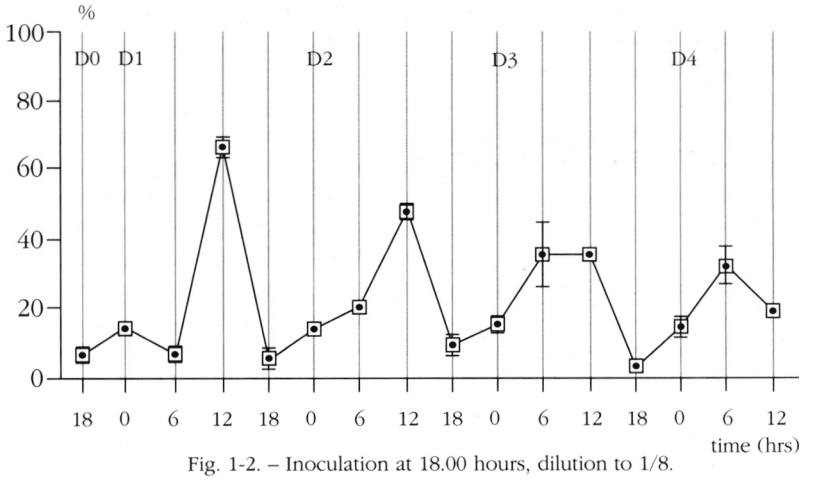

Fig. 1-2. – Inoculation at 18.00 hours, dilution to 1/8.

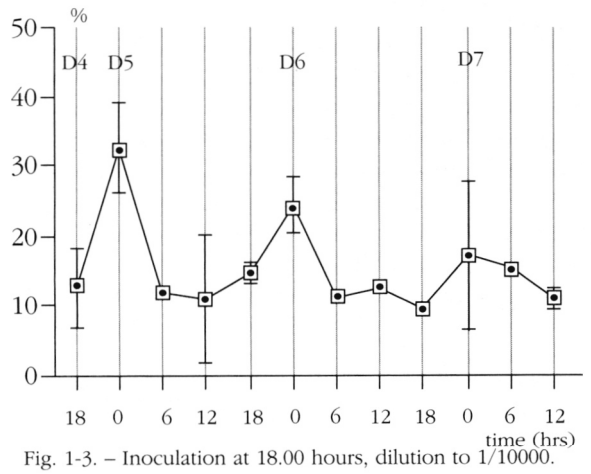

Fig. 1-3. – Inoculation at 18.00 hours, dilution to 1/10000.

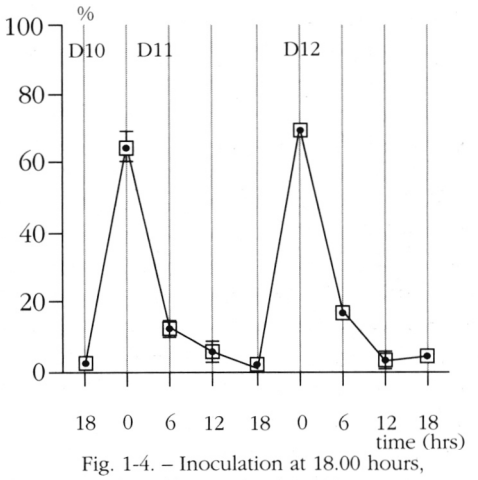

Fig. 1-4. – Inoculation at 18.00 hours, dilution to 1/100000.

Fig. 1 – Follow-up of the percentage of the ring stage of *P. c. chabaudi* as evaluated in two mice at 6 h intervals from D0 to D12
Symbol D : day.

				Mean percentage of each stage.								
	Time	Parasitemia		R		YT		MT		OT		
	hours	%	±SD	%	±SD	%	±SD	%	±SD	%	±SD	Sl
D0	10.00	1.5	0.2	8.0	1.4	80.0	2.8	12.0	4.2	0.0	0.0	0.74
	18.00	2.0	0.3	3.5	2.1	7.5	2.1	65.0	4.2	24.0	0.0	0.56
D1	00.01	2.9	0.1	31.0	1.4	4.5	0.7	3.5	2.1	61.0	4.2	0.54
	06.00	4.9	0.5	17.0	1.4	76.5	3.5	10.5	10.6	1.0	1.4	0.68
	12.00	5.7	0.5	3.5	0.7	67.5	3.5	27.0	1.4	2.0	1.4	0.61
	18.00	7.7	0.9	1.5	0.7	12.5	6.4	73.5	3.5	24.0	5.7	0.64
D2	00.01	10.0	0.4	17.5	2.1	9.0	0.0	10.0	1.4	63.5	3.5	0.52
	06.00	25.6	1.8	11.0	1.4	80.5	2.1	6.5	0.7	2.0	1.4	0.74
	12.00	26.8	1.7	4.0	0.0	68.0	4.2	23.5	2.1	4.5	2.1	0.60

Table 1. - Parasitemia and relative proportion of each stage of *P.c. chabaudi,* ring (R), young trophozoite (YT), mid term trophozoite (MT) and old trophozoite (OT) as evaluated at 6 hours intervals from day 0 (DO) to D2, after inoculation at 10.00 hours of parasited blood diluted to 1/2. SD: standard deviation, Sl: synchronicity index.

				Mean percentage of each stage								
	Time	Parasitemia		R		YT		MT		OT		
	hours	%	±SD	%	±SD	%	±SD	%	±SD	%	±SD	SI
D0	18.00	0.6	0.1	7.5	2.1	79.0	4.2	13.5	2.1	0.0	0.0	0.73
D1	00.01	0.7	0.1	15.5	0.7	10.0	5.7	65.5	6.4	9.0	0.0	0.54
	06.00	0.9	0.1	7.5	2.1	12.5	0.7	13.0	4.2	67.0	2.8	0.56
	12.00	2.0	0.1	68.0	2.8	23.0	7.1	8.0	2.8	1.0	1.4	0.60
	18.00	2.6	0.4	5.5	3.5	77.0	1.4	15.5	4.9	1.0	1.4	0.71
D2	00.01	3.3	0.3	15.0	1.4	14.0	1.4	62.5	6.4	8.0	7.1	0.51
	06.00	3.3	0.1	21.0	1.4	19.5	6.4	6.0	7.1	53.5	0.7	0.40
	12.00	6.3	0.1	48.5	2.1	45.5	5.0	3.0	1.4	3.0	1.4	0.51
	18.00	7.9	0.2	10.0	2.8	58.0	12.7	31.0	15.6	1.0	0.0	0.51
D3	00.01	10.4	0.6	15.5	2.1	6.0	2.8	64.5	3.5	16.5	0.7	0.53
	06.00	16.7	1.6	35.5	9.2	8.5	3.5	4.0	1.4	52.0	4.2	0.49
	12.00	26.8	2.5	35.5	0.7	56.0	1.4	5.0	1.4	3.5	0.7	0.51
	18.00	33.1	0.8	3.0	1.4	35.0	2.8	58.0	0.0	4.0	1.4	0.53
D4	00.01	38.7	4.9	14.0	2.8	6.5	6.4	52.5	9.2	27.0	0.0	0.40
	06.00	46.1	2.8	32.0	5.7	21.5	3.5	5.5	2.1	41.0	4.2	0.31
	12.00	63.8	2.5	19.0	1.4	58.0	4.2	14.5	0.7	8.5	4.9	0.45

Table 2. - Parasitemia and relative proportion of each stage of *P.c. chabaudi*, ring (R), young trophozoite (YT), mid term trophozoite (MT) and old trophozoite (OT) as evaluated at 6 hours intervals from day 0 (DO) to D4, after inoculation at 18.00 hours of parasited blood diluted to 1/8. SD: standard deviation, SI: synchronicity index.

				Mean percentage of each stage								
	Time	Parasitemia		R		YT		MT		OT		
	hours	%	±SD	%	±SD	%	±SD	%	±SD	%	±SD	SI
D4	18.00	1.5	0.1	13.0	5.7	47.5	3.5	20.5	3.5	19.0	5.7	0.31
D5	00.01	2.9	0.1	33.5	6.4	38.0	0.0	11.0	1.4	20.5	6.4	0.25
	06.00	3.7	0.4	12.0	0.0	35.0	14.1	35.5	6.4	17.5	7.8	0.24
	12.00	4.6	0.7	11.5	9.2	42.5	7.8	37.5	2.1	13.5	3.5	0.31
	18.00	7.2	0.4	15.0	1.4	32.0	4.2	28.0	0.0	25.0	2.8	0.15
D6	00.01	11.9	4.2	25.0	4.2	44.0	1.4	17.5	2.1	14.0	0.0	0.27
	06.00	16.9	3.0	11.0	0.0	37.0	14.1	32.5	0.7	19.0	15.6	0.27
	12.00	19.7	1.8	12.5	0.7	36.0	8.5	30.0	0.0	15.0	0.0	0.23
	18.00	24.0	1.4	9.5	0.7	41.0	7.1	28.0	7.1	21.5	0.7	0.26
D7	00.01	28.0	4.2	17.5	10.6	27.5	7.8	23.0	11.3	31.5	6.4	0.12
	06.00	32.1	5.0	15.5	0.7	43.5	2.1	30.5	2.1	10.5	4.9	0.30
	12.00	39.5	2.2	11.0	1.4	44.0	1.4	35.0	14.1	20.5	0.7	0.29

Table 3. - Parasitemia and relative proportion of each stage of *P.c. chabaudi*, ring (R), young trophozoite (YT), mid term trophozoite (MT) and old trophozoite (OT) as evaluated at 6 hours intervals from day 4 (D4) to D7, after inoculation at 18.00 hours of parasited blood diluted to 1/10000. SD: standard deviation, SI: synchronicity index.

	Time	Parasitemia		Mean percentage of each stage. R		YT		MT		OT		
	hours	%	±SD	%	±SD	%	±SD	%	±SD	%	±SD	SI
D10	18.00	1.4	1.8	2.0	0.0	8.5	7.8	62.5	6.4	27.0	1.4	0.54
D11	00.01	2.6	3.3	66.0	4.2	19.0	8.5	2.0	1.4	13.0	5.7	0.56
	06.00	3.3	4.2	13.5	2.1	66.0	1.4	19.0	1.4	1.5	0.7	0.58
	12.00	4.0	4.9	6.0	2.8	61.0	1.4	29.5	4.9	3.5	0.7	0.55
	18.00	3.8	4.5	1.5	0.7	7.5	0.7	70.0	2.8	21.0	2.8	0.62
D12	00.01	9.4	10.7	70.5	0.7	12.5	0.7	2.0	0.0	15.0	0.0	0.62
	06.00	10.8	12.3	17.5	0.7	74.5	0.7	7.5	0.7	0.5	0.7	0.67
	12.00	12.5	14.1	3.5	2.1	59.5	0.7	34.5	0.7	2.0	0.0	0.55
	18.00	14.2	15.9	4.0	0.0	10.0	2.8	64.0	1.4	22.0	1.4	0.54

Table 4. - Parasitemia and relative proportion of each stage of *P.c. chabaudi*, ring (R), young trophozoite (YT), mid term trophozoite (MT) and old trophozoite (OT) as evaluated at 6 hours intervals from day 10 (D10) to D12, after inoculation at 18.00 hours of parasited blood diluted to 1/100000. SD: standard deviation, SI: synchronicity index.

DELAYED INOCULATION (AT 18.00 HOURS)

a) From D0 to D4 : Table 2 and Figure 1.2 show the results obtained in mice inoculated at 18.00 hours with blood diluted to 1/8.

The peak of Rs (Figure 1.2) was progressively displaced from a single peak at 12.00 hours on D1 and D2 to two equal peaks on D3 at 06.00 hours and 12.00 hours (the actual peak occurring probably at 09.00 hours) and a single peak at 06.00 hours on D4. The height of the peaks tended to decrease from D1 to D4 and relatively high percentages of Rs could be seen at 00.01 hours and 06.00 hours. Peaks of other stages (Table 2) were as follows : YTs at 18.00 hours on D1 and D2 and at 12.00 hours on D3 and D4; MTs at 00.01 hours from D1 to D4 tending to decrease and be displaced to 18.00 hours on D3. OTs peaked at 06.00 hours from D1 to D4 and tended to decrease too. The SI decreased, varying from 0.73 on D0 to 0.3-0.45 on D4.

b) From D4 to D7: Table 3 and Figure 1.3 show the data obtained with mice inoculated at 18.00 hours with blood diluted to 1/10000.

The infection in the blood was asynchronous to a large extent (SI comprised between 0.12 to 0.3) but although the differences between the highest and lowest percentages did not exceed 20%, peaks of Rs could already be clearly seen at 00.01 hours.

c) From D10 to D12. The infection became patent only in two mice out of six inoculated. Data are shown in table 4 and Figure 1.4 (dilution 1/100000). The infection was again synchronous (SI comprised between 0.54 and 0.67). Rs predominated at 00.01 hours and peaked around 70%. YTs peaked at 06.00 hours, MTs at 18.00 hours and OTs peak occurred probably between 18.00 and 00.01 hours.

DISCUSSION

The dependency of *P. chabaudi* on the host circadian rhythm is a constant phenomenon observed in common laboratory conditions, when strains are passaged either by subinoculations from mice to mice or by the injection of frozen-thawed blood.

The periodicity of the erythrocytic cycle of *P.c. adami* is very similar to that known for *P.c. chabaudi* (unpublished data).

Our results show that, when the schizogonic rhythm of *P.c. chabaudi* is modified by inoculating a predominant parasitic stage such as YT, which normally develops in the morning, at a different time of the day i.e. 18.00 hours, the schizogonic cycle reverts to its previous rhythm. The adjustment is progressive taking between 7 to 10 days and can be followed by analysing the displacement of the stages' peaks. Indeed, when YTs produced at 06.00 hours are inoculated at 18.00 hours they continue to develop normally, schizogony occurs at 00.01 hours and new YTs are produced at 06.00 hours; the length of the 24 hour cycle remains unchanged and during the two first generations (from D0 to D2), the cycle remains relatively synchronous. Then, a phase of desynchronisation takes place, followed by again a synchronous infection with a different timing, corresponding to the normal cycle as seen in the donor mice.

Cambie *et al.* (1990) assumed that merozoites of *P. chabaudi*, when inoculated at various times of the day, waited until midnight to penetrate into erythrocytes. From our present data it appears that merozoites can be separated into two populations : one penetrating immediately after the bursting of the schi-

zonts and a second waiting until midnight (latent merozoites). The temporary asynchronicity observed on days 4 to 7 is due to the superposition of two schizogonic cycles: one beginning at 18.00 hours and ending at 18.00 hours, the other begining at 00.01 (from the latent merozoites) ending at 00.01. With *P. c. chabaudi*, which has a 24 hours cycle in the blood, the population penetrating at midnight determines a schizogony at midnight the following day and becomes progressively predominant; when the majority of merozoites has penetrated at midnight, the natural rhythm is resumed.

It could be argued that the adjustment may be caused by a latency of the development of one or several intraerythrocytic stages in order to readjust the timing to the circadian rhythm of the host. However when examining the parasitic pattern we see no evidence of such phenomenon: the transformation of each stage into the next one, 3 or 6 hours later, depending on the stage, appears to occur normally, and the multiplication rate is approximately 5 in all experiments.

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