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TEMPORARY LOSS OF *PLASMODIUM* GAMETOCYTES INFECTIVITY DURING SCHIZOGONY

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SUMMARY

Experiments with *P. y. nigeriensis* and *Anopheles stephensi* fed through a membrane showed that gametocytes infectivity was impaired by the addition of hemolysed serum to parasitized blood. The same phenomenon occurred when mouse hemoglobin, bovine hematin or hemolysed mouse blood were added to normal serum.

In vivo, when *A. stephensi* was fed on a mouse infected with a synchronous strain such as *P. vinckei petteri,* gametocytes lost much of their infectivity at the time of schizogony and recovered it a few hours later. This phenomenon should be taken into account when experiments on gametocytes infectivity or transmission are performed. These results confirm the involvement of toxic and non specific, rather than immunological, factors in the blockage of gametocytes infectivity.

RÉSUMÉ : **Diminution temporaire de l'infectivité des gamétocytes de *Plasmodium* au moment de la schizogonie.**

L'expérimentation avec *P. yoelii nigeriensis* transmis par nutrition artificielle à *Anopheles stephensi* montre une baisse d'infectivité des gamétocytes en présence de sérum hémolysé. Ce phénomène se retrouve si on ajoute au sérum normal de l'hémoglobine de Souris, de l'hématine bovine ou du sang de Souris hémolysé.

In vivo, lorsque *A. stephensi* est gorgé directement sur Souris infectée par une souche synchrone comme *P. vinckei petteri* il apparaît que l'infectivité des gamétocytes baisse au moment de la schizogonie puis remonte dans les heures qui suivent. Ce phénomène doit être pris en considération dans les expérimentations concernant l'infectivité des gamétocytes et confirme l'implication de facteurs toxiques non spécifiques plutôt qu'immunitaires dans le blocage de l'infectivité des gamétocytes.

It was previously shown that: *a*) gametocytes infectivity of *Plasmodium gallinaceum* (Eyles, 1951) and *Plasmodium yoelii nigeriensis* (Bastien *et al.,* 1987) raises gradually as the rate of parasitaemia increases and falls suddenly at the time of crisis or pre-crisis (Landau *et al.,* 1979, Bastien *et al.,* 1987), *b*) infectivity is recovered as soon as gametocytes are transfered to a clean mouse (Landau *et al.,* 1979), *c*) serum from a heavily infected mouse, near crisis, inhibits *in vivo* (Petit *et al.,* 1982) and *in vitro* (Bastien *et al.,* 1987) gametocytes infectivity.

While attempting to identity the seric inhibitory factor mentioned above with a series of experiments performed by artificial feeding, we found out that a simple hemolysis of the serum from uninfected mice had a strong inhibitory effect.

Critical analysis of more than 20 experiments performed by artificial nutrition to test several gametocytes infectivity potentially inhibitory products, revealed that normal serum from uninfected mice used as control had a strong inhibitory effect when a slight accidental hemolysis occurred during its preparation.

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Thus, we attempted to reproduce intentionally a similar result by adding hemolysis products *in vitro* to blood with infective gametocytes, or injecting them *in vivo* to mice; slightly hemolysed serum, hemolysed uninfected blood, hemoglobin (Sigma) or bovine hematin (Sigma) were used.

At the time of crisis which has a well established inhibitory effect on gametocytes infectivity, hemolysis is massive. Hemolysis also occurs during schizogony and experiments were devised to investigate gametocytes infectivity before, at the time, and after a synchronous schizogony such as that of *P. vinckei petteri.*

I — EFFECT OF HEMOLYSIS PRODUCTS ON THE *in vitro* INFECTIVITY OF GAMETOCYTES

Materials and methods

Hemolysed serum: blood was left 24 hours at + 4° C, centrifuged at high speed (400 rpm), aliquoted and frozen at − 70° C.

Hemolysed RBC were obtained by freezing blood at − 70° C.

Hemoglobin, hematin, and hemolysed blood were added to normal uninfected mouse serum in artificial feeding experiments, and to saline when injected to mice.

Membrane feeding experiments lasted 15 minutes at the maximum, at the end of which 20 to 30 mosquitoes were usually well engorged.

In order to duplicate experiments, pools of several gametocyte donor mice were used.

Experiments and results

Experiment 1: Mosquitoes were fed on blood from a mouse with a 17 % parasitaemia mixed with an equal volume of either normal non hemolysed serum from a clean mouse or slightly hemolysed serum.

In two different experiments the mean numbers of oocysts in 20 mosquitoes at day 7 was respectively 3 and 4 with the hemolysed serum against 237 and 183 with the normal serum.

Experiment 2: Hemolysed blood from a clean mouse had the same effect. Dosages of hemoglobin were made and the following results were obtained:

— control serum (Hb = 130 mg/dl): 49 oocysts,

— hemolysed serum (Hb = 230 mg/dl): 5 oocysts,

— hemolysed uninfected RBCs (Hb = 280 mg/dl): 1 oocyst.

Experiment 3: Two dilutions of hemolysed serum with normal serum (1/2 and 1/3) were tested. Results are summarized in *Table I.*

Oocyst numbers were significantly lower ($P < 0,01$, Mann-Whitney's test U) than in the control experiments only when the hemolysed serum was undiluted but figures obtained with diluted serum also suggest an inhibitory effect.

TABLE I. — *Infectivity of parasitized blood, mixed with different proportions of hemolysed serum.*

SERUM DILUTION	HEMOGLOBIN mg/ 100ml	EXPERIMENT1 MEAN OOCYST NUMBER Parasitaemia 17%	EXPERIMENT2 MEAN OOCYST NUMBER Parasitaemia 13%
S 100%	130	447	439
S 66%, H 33%	160	395	305
S 33%, H 66%	180	365	340
H 100%	230	250	267

S = non hemolysed serum; H = hemolysed serum.

Experiment 4: The addition of mouse hemoglobin or bovine hematin to normal serum had also an inhibitory effect.

Blood from infected mice was mixed with equal quantities of:

— non hemolysed serum from a clean mouse (control),

— the same control serum added with 1.5 or 3 mg of mouse hemoglobin (corresponding to a lysis of 2 to 4 % of RBCs),

— the same control serum added with 1.5 or 3 mg of hematin.

All experiments were duplicated.

Results are summarized in *Table II*: there was a significant difference between the mean numbers of oocysts in Anopheles fed on blood + pure serum and those fed on blood + serum with added hemoglobine or hematin.

TABLE II. — *Infectivity of a pool of parasitized mouse blood mixed with a solution of serum + hemoglobin or hematin.*

Experiment	1	2	3	4
Parasitaemia	12%	3%	11%	5%
S (control)	50	16		
S + 1,5 mg Hb	NT	7*		
S + 3 mg Hb	32*	5*		
S (control)			55	10
S + 1,5 mg Ht			16*	2*
S + 3 mg Ht			6*	5*

Figures indicate the mean number of oocysts per Anopheles. S = mouse serum; Hb = hemoglobin; Ht = hematin; % parasitaemias = mean values of pooled blood; NT = not tested; * = significantly ($p < 0,01$) different from controls (Mann-Whitney U test).

In conclusion, these experiments drew the attention to the potential role of hemolysis and the need of *in vivo* experiments.

II — SCHIZOGONY AND DECREASE OF INFECTIVITY *in vivo*

Materials and materials

P. vinckei petteri was chosen because of its synchronism and also because the timing of schizogony is set up by the time of

TABLE III. — *Inhibition of gametocytes infectivity by schizogony independently of the mice circadian rythm.*

MOUSE	FEEDING TIME	SCHIZOGONY TIME	MEAN NUMBER OF OOCYSTS	PARASITAEMIA %
1	9H	12H	25	4,8
	15H	12H	8 *	12
	9H	12H	5 *	18-crisis
2	9H	12H	2	4
	15H	12H	0.3	8
	9H	12H	8 *	13
3	9H	12H	3	3
	15H	12H	0.7	8
	9H	12H	3	9
4	21H	0H	20	10
	3H	0H	6 *	25
	21H	0H	0.4	crisis
5	21H	0H	3	5
	3H	0H	2	14
	21H	0H	0.2	crisis
6	21H	0H	32	6
	3H	0H	14	16
	21H	0H	58	17

Mice 1, 2 3 were inoculated at 12 hours; mice 4, 5, 6 were inoculated at 0 hour.

* = significantly different ($p < 0,01$) from previous feed (Mann-Whitney U test).

TABLE IV. — *Variations of gametocytes infectivity according to time: before, during and after schizogony.*

MOUSE	1	2	3	4	5	6	7	8	9	10	11
TIME OF FEED											
10H	1.2	1.2	0.5	7.6	2.0	2.0	0.6	2.5	13.7	2.1	0.2
% parasitaemia	1.6	1.7	1.2	4.9	3.3	5.3	1.3	3.8	3.5	3.5	2.1
16H	0.2	0.3	0.2	1.7	0	0.5	0.1	0.7	0.8	1.6	0.1
% parasitaemia	2.5	3.0	3.0	NT	5.0	8.0	2.0	5.0	6.1	5.1	3.2
10H	3.1	4.3	19.6	9.9	8.8	20.9	1.8	5.0	22.6	26.3	1.2
% parasitaemia	5.0	6.0	6.0	NT	10.9	11.8	4.1	11.2	11.0	11.0	6.0

Time of schizogony = 13 hours-16 hours.
Percentages of parasitaemia were evaluated at the beginning of each feed.

inoculation with frozen blood (Montalvo *et al.,* 1988).

Mice were inoculated either at midnight or at noon (experiment 5) or at 13 hours (experiment 6). Five to seven days later, twenty to thirty Anopheles were fed three times on each mouse, before, during, and after schizogony.

Numbers of oocysts were estimated at day 7 after the blood meal.

Before each feed, parasitaemias of donor mice were estimated in order to confirm the occurrence of schizogony at the expected time or that of the crisis.

RESULTS

Experiment 5: Results are detailed in *Table III.*

The number of oocysts was always lower when Anopheles were fed during schizogony than before schizogony; decrease of infectivity was accentuated if crisis occurred during the third feed (mice 1, 4, 5). When crisis was still remote (mice 2, 3, 6) infectivity rose again after schizogony.

Experiment 6: The 11 mice used in this experiment had relatively low parasitaemias (maximum at the end of experiment = 11,8 %) and crisis was not expected before one or two days.

Results are detailed in *Table IV.*

There was always a decrease of infectivity to mosquitoes at the time of schizogony, followed by a rise 18 hours later.

CONCLUSIONS

The results detailed above:

a) show that gametocytes infectivity is inhibited:

— by several components of the RBC *in vitro,*

— temporarily by schizogony *in vivo,*

b) confirm that inhibition of gametocytes infectivity is not an immunological phenomenon.

Schizogony as well as crisis causes the destruction of numerous parasitized RBCs and the release of various erythrocytic and parasitic products in the plasma; those are very likely to be toxic for the gametocytes.

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