/Robert
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Kinetics and efficiency of *Plasmodium falciparum* development in the midguts of *Anopheles gambiae*, *An. funestus* and *An. nili*

The early sporogonic development of *Plasmodium* occurs in the lumen of the anopheline midgut and involves gametes, zygotes and ookinetes. An immunofluorescent method, using a monoclonal antibody (MoAb) specific for the 25-kDa protein (Pfs-25) expressed on the *P. falciparum* gamete–ookinete surface, may be used to detect infected mosquitoes in the field (Robert *et al.*, 1995). In the present study, the same method has been used to evaluate the densities and determine the stages of *P. falciparum* in the midguts of naturally infected anophelines within 30 h of the infective bloodmeal on gametocyte carriers.

The holo-endemic study site was the hamlet of Ndonzengue, near the village of Mbebe (4°N, 11°E), Cameroon, in the rainforest bordered by the Sanaga river. *Anopheles gambiae* s.s. was the only species of the *An. gambiae* complex present. Wild anopheline mosquitoes were collected at night just after they had fed on one of four *P. falciparum* gametocyte carriers, using a transparent tube (8 × 1 cm) for each fly, and then maintained at 27.5 ± 0.5°C. Midguts were dissected at various times post-bloodmeal (pbm), homogenized, mixed with the labelled Pfs-25 MoAb and completely screened, between slide and coverslip, with a fluorescent light microscope (Robert *et al.*, 1995) and always within 45 min of dissection.

As the kinetics of the early sporogonic cycle of *P. falciparum* were similar in all three mosquito species studied (i.e. *An. gambiae* s.s.; *An. funestus*; and *An. nili*), the results presented here (Fig.) are pooled for all 351 anophelines investigated.

Although no round forms were observed before 20 min pbm (probably because the Pfs-25 protein is not expressed on the gametocyte surface), they were observed in the midgut lumen from 30 min pbm, in about 80% of the anophelines. Round forms still represented a large proportion of stained parasites in mosquitoes dissected 14–18 h pbm, perhaps then corresponding to microgametocytes (usually 25% of all gametocytes) or to unfertilised macrogametes, but were not found >27 h pbm. The protruding round forms known as retorts or pre-ookinetes were observed from 6 h pbm but decreased in abundance (as a percentage of stained parasites) from 13 h, supporting the idea that the round-form–retort transformation does not occur after 13 h. Ookinetes were first seen at 12 h pbm, increased in abundance up to 16 h and then decreased in abundance from 20 h. These observations agree with those of Garnham (1966), who stated that ookinete formation took 12–18 h, and those of Robert *et al.* (1995), who deduced that ookinetes would appear at 12–15 h and did not observe any ookinetes in gravid anophelines.

In immunofluorescent method used, the stained parasites remain alive and apparently continue their normal development, even when removed from the mosquito midgut. In the present study, some round-form–retort and retort–ookinete transformations were observed under the microscope, between slide and coverslip.

The development of the wild strains of malarial parasites observed in the present study appeared to be very fast compared with that observed with the NF54 strain which has been maintained *in vitro*; ookinetes of the latter strain only appear 24 (Meis *et al.*, 1992; Chege and Beier, 1994) or even 31 h (Vaughan *et al.*, 1992) pbm.

There appear to be optimal times pbm for detecting particular stages of *P. falciparum* during its early development in the vector; ideally, midguts from wild-caught anophelines should be examined 1–5 h pbm for round forms, 9–11 h pbm for retorts and 16–18 h pbm for ookinetes. Research on the stages of the parasite which are present within 30 min

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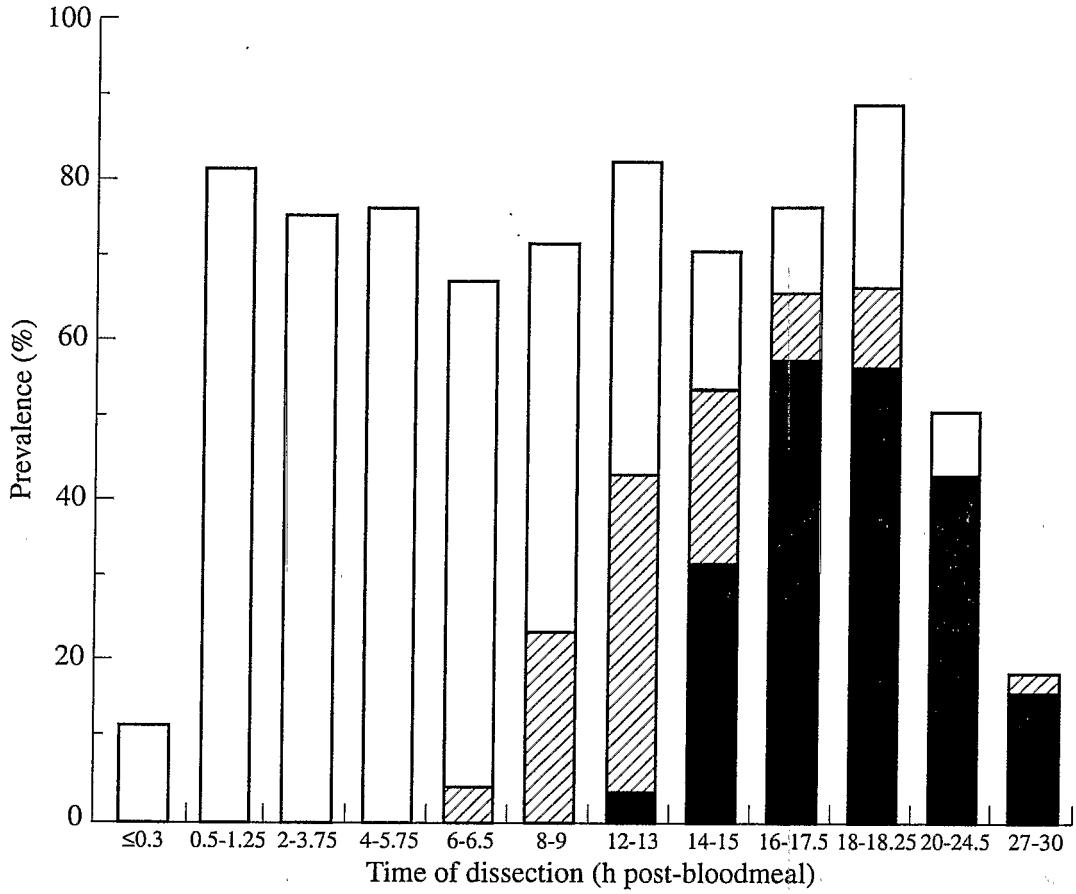

Fig. Prevalence of midgut infections with the round forms (□), retorts (▨) and ookinetes (■) of *Plasmodium falciparum* in anopheline mosquitoes fed on one of four volunteers. The results are shown for 12 periods from 0.3 to 27–30 h post-bloodmeal, at which eight, 21, 61, 37, 18, 28, 28, 31, 42, 17, 44 and 16 mosquitoes were dissected.

of the infective feed should not be based on detection of Pfs-25 protein.

If the immunofluorescent technique is used on the intact walls of midguts removed from anophelines 40–56 h after an infective feed, intermediate stages between ookinetes and young oocysts may often be observed in contact with the walls. The significant decrease in the percentage of anophelines apparently infected after 20 h may simply be due to ookinetes leaving the lumen of the midgut and penetrating the midgut epithelium from 20 h onwards.

The prevalences and intensities of infection with round forms, retorts and ookinetes were also determined in 168 mosquitoes (*An. gambiae* s.s., *An. funestus* or *An. nili*) fed on one of the four gametocyte carriers, an 18-year-old volunteer, over a period of 17 days. No asexual stages could be detected in the peripheral blood of the volunteer at the start of the study but screening of a thick smear of 5 μl blood did reveal 41 *P. falciparum* gametocytes (8.2/μl) of which 10 were microgametocytes. Although the prevalences of infection with round forms or ookinetes were higher for *An. gambiae* and *An. funestus* than for *An. nili* (Table 1), this may be because *An. nili* takes a relatively small bloodmeal, and not because *An. nili* is less susceptible to infection by the same inoculum. The smaller meal taken by *An. nili* may also explain why this species is usually found to have relatively low sporozoite indexes.

In each of the three anopheline species investigated, both the prevalence of infection and the intensity of infection tended to decrease as the malarial parasites developed (Tables 1 and 2). Most round forms (90% of

TABLE 1

Prevalence of midgut infections with the round forms, retorts and ookinetes of Plasmodium falciparum *in anopheline mosquitoes, after one bloodmeal on a gametocyte carrier*

Species	*Round form*	*Retort*	*Ookinete*
Time of dissection (h post-bloodmeal)	0.5–4.5	8–13	15–18
PREVALENCE (% of dissected mosquitoes)			
An. gambiae	83 (19/23)	89 (16/18)	62 (13/21)
An. funestus	80 (12/15)	43 (3/7)	60 (3/5)
An. nili	74 (25/34)	32 (6/19)	54 (14/26)

TABLE 2

The intensities of infection with the round forms, retorts and ookinetes of Plasmodium falciparum *in anopheline mosquitoes, after one bloodmeal on a gametocyte carrier*

Species	*Round form*	*Retort*	*Ookinete*
Time of dissection (h post-bloodmeal)	0.5–4.5	8–13	15–18
MEAN (S.D.) INTENSITY (no. of forms/infected mosquito) AND [no. of parasites observed]			
An. gambiae	3.10 (2.35) [59]	2.25 (1.34) [36]	2.78 (1.88) [36]
An. funestus	3.50 (2.15) [42]	2.67 (2.08) [8]	1.00 (0.00) [4]
An. nili	2.56 (1.39) [64]	1.67 (0.52) [10]	1.79 (1.19) [25]

those in *An. gambiae* and 70% of those in *An. nili*) of the wild strain of *P. falciparum* from the volunteer apparently developed successfully into ookinetes. This success is far better than that observed by Vaughan *et al.* (1992, 1994), when they fed the G3 strain of *An. gambiae*, through a membrane, on the NF54 strain of *P. falciparum*. In this experimental system, there were 40- and 316-fold decreases in parasite numbers in the transition from the macrogametocyte to the ookinete stage.

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REFERENCES

CHEGE, G. M. M. & BEIER, J. C. (1994). *Journal of the American Mosquito Control Association*, **10**, 419–422.

GARNHAM, P. C. C. (1966). *Malaria Parasites and other Haemosporidia.* Oxford: Blackwell Scientific.

MEIS, J. F. G. M., WISMANS, P. G. P., JAP, P. H. K., LENSEN, A. H. W. & PONNUDURAI, T. (1992). *Acta Tropica*, **50**, 227–236.

ROBERT, V., LE GOFF, G., ESSONG, J., TCHUINKAM, T., FAAS, B. & VERHAVE, J. P. (1995). *American Journal of Tropical Medicine and Hygiene*, **52**, 366–369.

VAUGHAN, J. A., NODEN, B. H. & BEIER, J. C. (1992). *Journal of Parasitology*, **78**, 716–724.

VAUGHAN, J. A., NODEN, B. H. & BEIER, J. C. (1994). *American Journal of Tropical Medicine and Hygiene*, **51**, 233–243.

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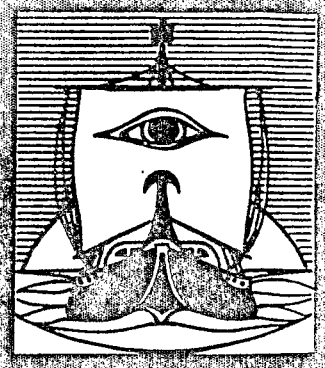

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