Pathology of *Anopheles stephensi* After Infection with *Plasmodium berghei berghei*

I. Mortality Rate

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**Summary.** The mortality of *P. berghei*-infected *Anopheles stephensi* females can be about 30% higher during the first three days than in normal blood-fed mosquitoes. As expected the mortality is higher after feeding on highly infected mice but also depends on the date of feeding and the temperature. Infected mosquitoes kept at 25°C die more often than those kept at 21°C. On the other hand sporozoite production needs the low temperature of 21°C. So the sporozoite production rate falls with increasing temperature, and the mortality rate increases.

**Introduction**

The development of malaria parasites in vertebrates has been investigated in avian as well as in rodent malaria, and most intensively in human malaria. However, thorough systematic investigations on the pathology of mosquitoes as malaria vectors is lacking (isolated observations can be found in international papers): Ross (1910) found the lives of mosquitoes infected with *Plasmodium*

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to be shortened. Buxton (1935) made similar observations on *Culex* infected with *Proteosoma praecox*, De Buch (1936) and Thompson and Huff (1944) on *Culex* and *Aedes* infected with *Plasmodium rhadinurum* (summarized by Maier, 1976). Parasitisation obviously leads to a disease of the vector, which may result in a high mortality. The disease also affects the biological and physiological processes.

For instance in the model *Culex* infected with *P. cathemerium*, mortality appears to be mainly due to the disruption of the midgut epithelium through the penetration of the ookinetes (Maier, 1973). Furthermore, various parasites are known to impair the fertility of their insect hosts.

There exist as yet no extensive studies on the deleterious effects of *Plasmodium* on the species *Anopheles*. In this paper, the reaction of *Anopheles stephensi*, a major malaria vector of man, to infection with *Plasmodium berghei berghei* has been investigated. The influence of various temperatures on breeding and keeping has also been determined, as it plays a major role in *P. berghei* (Vanderberg and Yoeli, 1966).

**Material and Methods**

The investigations were carried out on the model system *A. stephensi/P. berghei*. The *Anopheles* strain was kindly supplied by Prof. F. Kuhlow (Hamburg, Tropeninstitut). We also thank the late Prof. W. Kretschmar and Dr. H.M. Seitz (Tropenmedizinisches Institut, Tübingen) for having placed the gametocyte-forming strain of *P. berghei berghei* (Anka) at our disposal.

*Anopheles Breeding*

*A. stephensi* was bred at a constant temperature of 25°C ± 0.5°C and a relative humidity of 80% ± 5%, and a 16:8 light-dark photophase. The larvae were reared in 40 x 25 cm breeding dishes in boiled water. The larvae were fed on ground crispbread and shrimp grist. The pupae were collected daily from the breeding dishes. The adults emerged in flight cages 50 x 50 cm and were immediately provided with 7.5% glucose solution ad libitum. For oviposition the female mosquitoes were allowed to feed on white mice, previously anaesthetized with Nembutal (Abbott, Belgium). To check the mortality rate about 300 pupae with cuticles not yet stained were collected right after pupation from the breeding dishes and isolated in tubes of 1.5 cm diameter and 10 cm length, containing 3 ml water and stopped with gauze. After emergence the imagines were placed together in small cages (20 x 20 x 20 cm).

About 80–100 adult females and 40–50 adult males of exactly the same age could thus be used for the experiment. The dead female mosquitoes were collected daily at the same hour from the cages and their number was noted. For the infection of *A. stephensi* with *P. berghei berghei* white mice were infected with 1 x 10⁷ parasitized erythrocytes. To maintain the capacity for gametocyte formation, 4- to 6-week-old rats were regularly infected with sporozoites. The blood of mice, inoculated with parasitized rat erythrocytes, was kept at −70°C and used for the infection of mice in the following experiments.

**Results**

Observations on the formation of the *Anopheles* colony had shown that the quality of the food provided influences the survival rate of the mosquitoes of the species *A. stephensi*. To determine the effect of various regimens on