

RESISTANCE AND SUSCEPTIBILITY OF SOME STRAINS OF THE *ANOPHELES GAMBIAE* COMPLEX TO INFECTIONS WITH *PLASMODIUM* SPP.¹ OF RODENTS

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ABSTRACT. *Anopheles gambiae* sp. "A" (strains LSW, 3P6RR, 16CSS and PALA) developed ookinetes of *P. berghei nigeriensis* (N67 strain) in their midguts and all strains except LSW developed oocysts. In strain LSW most of the ookinetes degenerated in the 2-hour period 14-16 hours following the blood meal. In con-

trast, *A. stephensi* and all 4 strains of *A. gambiae* sp. "A" were insusceptible to experimental infections with *Plasmodium vinckei chabaudi* (AS strain). Resistance to infection was probably biochemical in nature and was expressed as differences in individual, strain and species insusceptibility.

INTRODUCTION. It is well known that insusceptibility to infection with malaria parasites can occur in species of mosquitoes which are closely related to other mosquitoes that are good vectors of the disease (e.g. Warren *et al.*, 1963). Garnham (1966) has cited numerous studies to show that in some mosquitoes which were refractory to infection with *Plasmodium* spp. exflagellation of microgametocytes, fertilization and formation of ookinetes and oocysts occurred but the oocysts degenerated. These degenerating oocysts ceased growth at an early stage of development, sclerotization occurred and the oocysts were converted into what are known as Ross's black spores. In other instances (e.g. *Anopheles maculipennis* Meigen infected with *P. gonderi* Sinton and Mulligan, 1933) the oocysts reach maturity but the sporozoites die almost immediately in the haemocoelomic fluid, or very shortly afterwards upon reaching the salivary glands. It has been postulated that a substance toxic to the sporozoites causes their rapid death rather than the absence of a necessary metabolite (Garnham, 1966). In addition, Huff (1934) demonstrated that in strains of *Culex pipiens* Linnaeus refractory to infection with *P. cathemerium* Hartman, 1927 or *P. relictum* (Grassi and

Feletti, 1891) insusceptibility involved degeneration of the zygote. Bennett *et al.* (1966) were able to show that all 3 types of resistance (i.e. against ookinetes, oocysts and sporozoites) occurred depending upon the species of *Anopheles* and strain of *P. cynomolgi* Mayer, 1907.

The purpose of my study was to investigate further the mechanism of susceptibility and insusceptibility to malaria infections in closely related strains of mosquitoes. For this purpose I chose, as an experimental model, strains of species "A" of the *A. gambiae* Giles complex (Davidson *et al.*, 1967) and the rodent malaria parasites *P. berghei nigeriensis* Killick-Kendrick, 1973 (N67 strain) and *P. vinckei chabaudi* Landau, 1965 (AS strain).

MATERIALS AND METHODS. Mosquito strains PALA and 3P6RR are laboratory substrains of an *A. gambiae* sp. "A" strain that was collected at Pala, Upper Volta. These 2 strains have been maintained in the laboratory since 1963. The LSW strain is white-eyed and has been maintained since 1962. It arose from a cross between LSW (originally collected at Lagos, Nigeria and laboratory reared since 1957) and a white-eye strain of *A. gambiae* sp. "A" that was collected at Sokoto, Nigeria.

Mosquito colonies for each experiment were reared from eggs obtained from the Ross Institute. Colonies were kept in an insectary maintained at a temperature of 25 degrees C (± 2 degrees C) in a relative

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humidity of 70-75 percent and subjected to a light-dark regime of 14 hours light and 10 hours dark. Sugar cubes were supplied as the energy source for adults. Larvae were reared in the same insectary as the adults. Larvae were kept in glass rearing bowls containing a chunk of grass sod and were fed ground dog biscuits.

When macro- and microgametocytes were present in large numbers in the blood of an infected mouse, the mouse was strapped to a cork board and laid prone on top of the mosquito cage to allow the mosquitoes to obtain a blood meal. Blood meal weights were determined by weighing individual mosquitoes, before and after a blood meal, on an electro-balance. Mosquitoes were examined for the presence of ookinetes by dissecting out the midgut at varying intervals after a blood meal, making a smear of the midgut contents and staining with Giemsa's stain diluted in saline (Shute and Maryon, 1966). Dissections for oocysts and sporozoites were made in the usual manner.

The N67 strain of *P. berghei nigeriensis* was isolated in Nigeria from *Thannomys rutilans* (Peters, 1876) by Killick-Kendrick *et al.* (1968). This strain is maintained at the Imperial College Field Station by periodic subinoculations of infected blood into white mice. At appropriate intervals the infectivity of this strain is renewed by sporozoite passage through *A. stephensi*

Liston. The AS strain of *P. v. chabaudi* used was obtained (in the form of infected mice and infected *T. rutilans*) from the Institute of Animal Genetics, Edinburgh.

RESULTS AND DISCUSSION. The criterion of susceptibility of the different strains of mosquitoes was the presence of oocysts on the midgut. Unfortunately, too few mosquitoes remained after these dissections to evaluate the significance of the presence or absence of sporozoites in the salivary glands or to determine the infectivity to mice of the sporozoites. Using this criterion for susceptibility, the results (Table 1) for *P. b. nigeriensis* (N67) indicate that strains 3P6RR, 16CSS and PALA of *A. gambiae* sp. "A" were susceptible but that fewer oocysts were present in strains 16CSS and PALA; and no sporozoites were seen in the salivary glands from mosquitoes of the latter strain. Strain LSW, on the other hand, was completely refractory, and mosquitoes of this strain were never found to have oocysts or sporozoites.

In all susceptible strains the number of infected mosquitoes varied from one feeding experiment to another. This variation may have been due to the number of gametocytes in the mouse's blood at the time of the blood meal as well as to varying susceptibility of individual mosquitoes. The size of the blood meal taken by mosquitoes of each strain is unlikely to have influenced the number of mosquitoes

TABLE 1.—Infectivity of *Plasmodium berghei nigeriensis* and *P. vinckei chabaudi* to *Anopheles stephensi* and strains of *A. gambiae*, species "A".

<i>Anopheles</i> species and strain	<i>P. b. nigeriensis</i> stage in mosquito				<i>P. v. chabaudi</i> stage in mosquito			
	No. of feeds	Ookinetes	Oocysts	Sporo- zoites	No. of feeds	Ookinetes	Oocysts	Sporo- zoites
<i>A. stephensi</i>	4	0/7*	1/65	..
<i>A. gambiae</i> , sp. "A", 16CSS	2	3/4	2/14
<i>A. gambiae</i> , sp. "A", PALA	6	23/23	12/36	0/12
<i>A. gambiae</i> , sp. "A", 3P6RR	3	1/4	14/22	5/6	5	0/14	0/11	..
<i>A. gambiae</i> , sp. "A", LSW	6	34/43	0/36	0/10	5	0/36	0/27	..

* Number of mosquitoes positive for this stage of infection/number of mosquitoes examined.

which became infected because there was no significant difference ($p=0.01$) in blood meal size between strains (mean blood meal weights of strains LSW, PALA and 16CSS were 1.0, 1.1 and 1.2 mg, respectively). Hovanitz (1947) has previously shown that with *A. aegypti* (Linnaeus) the number of oocysts of *P. gallinaccum* Brumpt, 1935, on the midgut was directly proportional to the size of the blood meal rather than the latter solely determining the presence or absence of oocysts.

In contrast to *P. b. nigeriensis* (N67), *P. v. chabaudi* (AS) was unable to produce infections in any of the strains of *A. gambiae* sp. "A" and only 1/65 *A. stephensi* developed oocysts of this parasite (Table 1). Wéry (1968) attained infections in 30-70 percent of the *A. stephensi* in his experiments on *P. v. chabaudi*. My failure to infect *A. stephensi* with *P. v. chabaudi* may be due to vector and parasite strain differences, for Bafort (1971) reports that in Antwerp, Belgium he was unable to infect a strain of *A. gambiae* sp. "A" with *P. v. vinckei* whereas other investigators in Liverpool were able to do so by using different strains of the same species of vector and parasite. In addition, the *A. stephensi* colony used in my experiments was heavily infected with the microsporidan *Nosema algerae* Vavra and Undeen, 1970 and it has been shown that infections with *N. algerae* significantly reduce the number of oocysts of both *P. berghei* Hulls (1971) and *P. b. cynomolgi* Ward and Savage (1972) that develop on the midgut of *A. stephensi*.

Ookinetes of *P. v. chabaudi* were not detected in any of the mosquitoes dissected (although they must have been present in the one *A. stephensi* that was positive for oocysts). In one experiment, mosquito strains LSW and 3P6RR were fed on a *T. rutilans* infected with *P. v. chabaudi*. No LSW or 3P6RR were positive for ookinetes (2 LSW and 5 3P6RR dissected) or oocysts (5 LSW and 16 3P6RR dissected). On the same day, Walliker (personal communication) fed a

colony of *A. stephensi* on another *T. rutilans* that was infected with a subinoculum of the *P. v. chabaudi* used above and likewise did not obtain any ookinetes or oocysts in *A. stephensi*. Since *T. rutilans* is the natural host for *P. v. chabaudi*, it was assumed there would be a greater chance for the *A. gambiae* strains to become infected if they fed on a *T. rutilans* infected with *P. v. chabaudi* rather than a white mouse infected with the same parasite. However, *A. stephensi* is known to be susceptible (Wéry, 1968), and without more controls using this species it is still not possible to say whether the strains of *A. gambiae* I used are really refractory to infection with *P. v. chabaudi*. This compares with the relatively large number of mosquitoes in the 4 strains of *A. gambiae* sp. "A" which were positive for ookinetes of *P. b. nigeriensis* even if, as in the case of strain LSW, oocysts did not develop. Normal (Fig. 1, S1-S3) and degenerating (Fig. 1, R1-R3) ookinetes were present in all strains, whether or not oocysts later developed, but in strain LSW the vast majority of ookinetes degenerated during the 2 hour period 14-16 hours after ingestion of the blood meal. Ookinete degeneration appeared to follow the breaking down of the cell wall and the release of the ookinete's contents in the form of a dispersed, amorphous body (Fig. 1, R1-R3). This degeneration phenomenon is similar to that described by Huff (1934) for the degeneration of the (ookinete-like) zygotes of *P. cathemerium* and *P. relictum* in insusceptible *C. pipiens*. Huff regarded the stage between the ookinete and the oocyst (i.e. the stage of the parasite just before and during penetration of the mosquito's midgut wall) to be the zygote. Although Huff did not elaborate, he presumably considered that even though the microgamete had penetrated the macrogamete the 2 nuclei had not yet fused to form a synkaryon and, consequently, a true zygote. This delayed formation of the zygote is in contrast to the results obtained by Bano (1959) in her cytological studies of 7 species of *Plasmodium*. She

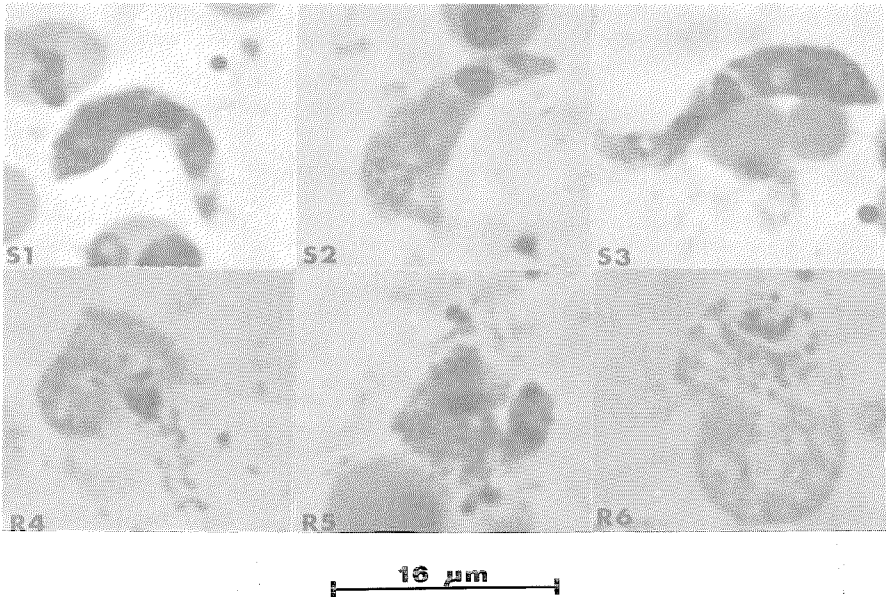


FIG. 1.—Ookinetes of *P. b. nigeriensis* in smears of the midgut contents of *A. gambiae* sp. "A", strains PÅLA (S1-S3) and LSW (R4-R6). Smears made 15 hours after the blood meal. Same scale for all photographs.

concluded that the zygote was formed soon after fusion of the male and female gametes and before the ookinete was fully formed.

It would appear that resistance of some *Anopheles* spp. mosquitoes to infections with species of rodent *Plasmodium* is primarily of a biochemical nature rather than physical (e.g. inability of the ookinete to penetrate the mosquito's midgut wall) and that this resistance includes individual, strain and species insusceptibility. Resistance of this type has previously been reported (e.g. Bafort, 1968; Bennett *et al.*, 1966; Huff, 1934; Ward, 1965; Warren *et al.*, 1963; Wéry, 1968).

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EDITORIAL NOTE

Complaints have been received about questionable advertisements in the December (1973) number of *Mosquito News*. The advertising manager and the editor have assumed that advertisers do not submit inaccurate or misleading material. The matter is being studied, and an explanation will appear in the June number.