Studies on Malarial Sporozoites. II. Effect of Age and Dosage of Sporozoites on Their Infectiousness

Richard J. Porter, Raymond L. Laird and Elizabeth M. Dusseau

Department of Tropical Diseases, School of Public Health, University of Michigan, Ann Arbor, Michigan

EPITOME

The significance of dosage of sporozoites in malarial infections has not been accessible to accurate quantitative study. The inoculation methods of Porter, Laird and Dusseau, however, furnished a tool for investigation of the problem. Exploitation of this opportunity has shown a rather consistent relationship between the numbers of sporozoites inoculated and the intensity of resulting infections. Further, and more important, it has revealed a progressive decrease in numbers and infectiousness of sporozoites during their sojourn in mosquitoes.

Sporozoites of *Plasmodium gallinaceum* were obtained from *Aedes aegypti* or *Aedes albopictus* mosquitoes kept at about 28°C (27–30°) for various periods after infection. Chickens were inoculated intravenously with known numbers of these sporozoites and the intensities of the ensuing infections were compared on the basis of the proportion of parasitized erythrocytes six days after inoculation. Experiments with serial dilution showed that the parasitemias on the sixth day were proportional to the dosage of sporozoites. Therefore, all data were converted to a single value, the number of parasitized erythrocytes (per 10,000) on the sixth day, per million sporozoites inoculated. The relative constancy of this ratio for a given preparation, regardless of the actual dosage of sporozoites, makes it suitable for comparison between different preparations.

Figure 1 shows the relationships between duration of infection in mosquitoes and (S/M) number of sporozoites recovered per mosquito dissected, (P/S) parasitemia per million sporozoites inoculated and (P/M) parasitemia per mosquito inoculated. All curves are based on weekly logarithmic averages. A total of 109 lots of mosquitoes is included. Three
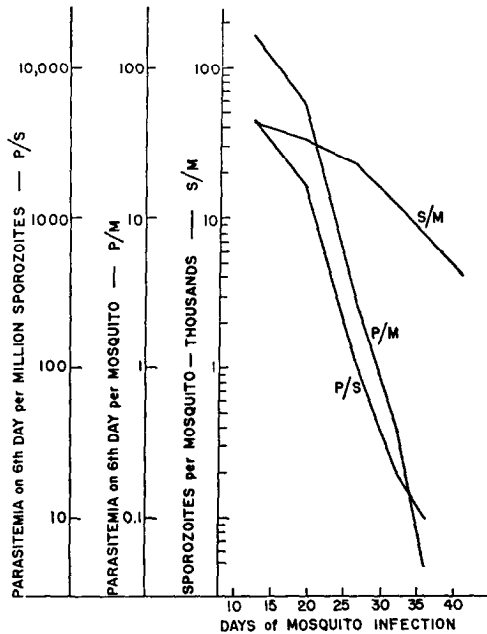
FIG. 1. Numbers and infectivity of sporozoites in relation to duration of infection in mosquitoes. Logarithmic means.

facts, also supported by the additional data recorded in the *Amplification* (see below), are apparent from the graph. The number of sporozoites recovered per mosquito fell about 90 % in 4 weeks. The intensity of infection in chicks per million sporozoites inoculated declined at a rate of about 90 % per week until, after 4 weeks, very large doses of sporozoites often failed to produce infection. Finally, the intensity of infection per mosquito inoculated decreased during 4 weeks to about one ten-thousandth its original value.

Samples of mosquitoes tested during the first week (9 to 15 days after infection) showed a mean value of 4200 parasitized erythrocytes (per 10,000) per million sporozoites inoculated. The extreme range was 700-20,000, and 30 of the 36 samples gave values between 2000 and 10,000. This must represent the optimum infectivity of sporozoites of *Plasmodium gallinaceum* with the technique used. Since a calculated dose of 50 sporozoites did not always cause infection, many sporozoites must be non-infective under these conditions. The relative consistency of the data, however, suggests that the proportion of non-viable sporozoites is fairly constant.

Previous work of Boyd and others has produced roughly quantitative evidence of a decline in infectivity of sporozoites during their stay in mosquitoes. The present data show for *Plasmodium gallinaceum* that such a decline clearly exists and that it is a result of loss in both numbers and infectivity of sporozoites.

AMPLIFICATION

MATERIALS AND METHODS

The procedures for infection of chicks with sporozoites have been discussed extensively elsewhere (Porter, *et al.*, 1952). *Aedes aegypti* and, to a small extent, *A. albopictus* were reared by the general methods of Shannon and Putnam (1934). They were infected within a week of emergence by feeding on chicks harboring a blood-passage line of the 8-A strain of *Plasmodium gallinaceum.* Infected mosquitoes were kept at 27–30°C (mean 28°) with cooked raisins or slices of apple as a maintenance diet.

For dissection, mosquitoes were immobilized with minimal exposure to anesthetic ether. The heads were cut off, and a bit of thoracic contents was squeezed out into 0.85% sodium chloride solution. The tissue thus removed usually contains the salivary glands. Glands from each lot of mosquitoes were suspended in 0.50 ml of heparinized normal chick blood (0.85% saline solution, 0.05 cc; powdered heparin, about 20 mg; blood, 0.45 ml) in a glass roller mill rotated for 30 minutes.

Dried, Giemsa-stained films of the preparation were examined to determine the ratio of sporozoites to erythrocytes. At least 10 sporozoites were counted unless a total of 10,000 erythrocytes was reached first. This gives a figure (*s* sporozoites per 10,000 erythrocytes) with a probable error of 20% or less for values of 10 or more. For smaller values, the probable error is progressively larger. The total number of sporozoites in the suspension can be calculated from the sufficiently accurate assumption that 0.45 ml of normal chick blood contains about 1,000,000,000 erythrocytes. Each suspension contained the salivary glands from 9 to 177, usually 10–20 mosquitoes. Aliquots of the preparation were inoculated intravenously to White Rock or White Leghorn chicks, 2–7 days old, in groups of four or more.

Parasitemias were determined from daily blood films of inoculated chicks, the values obtained on the sixth day after inoculation being used for comparison between different groups. When these values were too low they were calculated from the parasitemias on the seventh day, which, in these experiments, were about 10 times those for the sixth day.

SUPPLEMENTARY RESULTS

Figure 2 shows the numbers of sporozoites recovered per mosquito in 109 samples of *A. aegypti* and 8 of *A. albopictus*, plotted against the duration of infection in mosquitoes. The considerable variation is probably a result of both variation in initial level of infection and irregular recovery of sporozoites by the suspending method. It does not obscure the trend, which clearly reveals a progressive decline in numbers of sporo-

zoites with increasing duration of infection. This trend is particularly obvious in the curve of logarithmic means, calculated weekly beginning with the ninth day (the last value represents all samples from the 37th through the 48th day).

Eighteen lots of mosquitoes were tested both before the 18th and after the 28th day. Fifteen showed a reduction in number of sporozoites per mosquito between the earlier and later tests. The mean reduction for all mosquito lots was 50 %, with a standard error of 12.1 %.

Table I shows two experiments in serial dilution of suspensions of sporozoites. In both cases the mosquitoes were dissected 13 days after infection. In experiment A the original suspension contained 35 million sporozoites from 177 mosquitoes. Dilutions were made in saline solution containing about 1 % blood. Each group contained six chicks. In experiment B the preparation contained four million sporozoites and dilutions
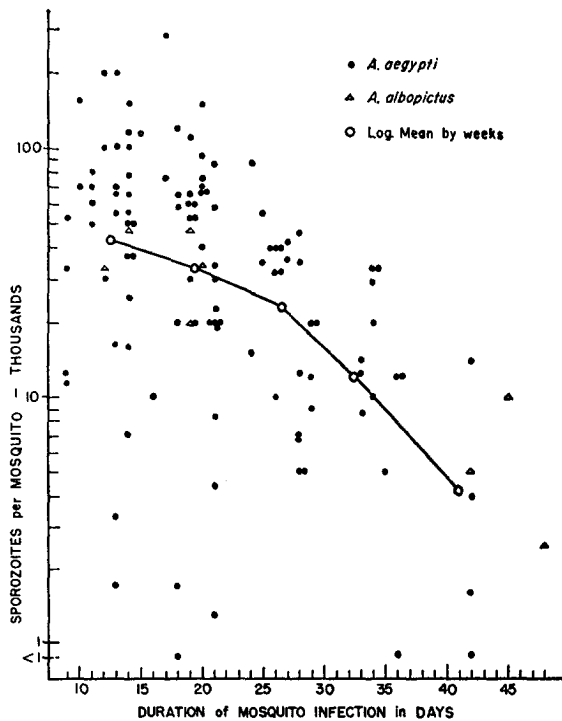

FIG. 2.   Numbers of sporozoites in relation to duration of infection in mosquitoes.

TABLE I

Sporozoites inoculated	Experiment A		Experiment B	
	Mean parasitemia on 6th day	Parasitemia per million sporozoites	Mean parasitemia on 6th day	Parasitemia per million sporozoites
500,000	1275	2550	820	1640
50,000	177	3540	136	2720
11,250			35	3110
5,000	14	2800		
500	2.2*	4400	1.7*	3400

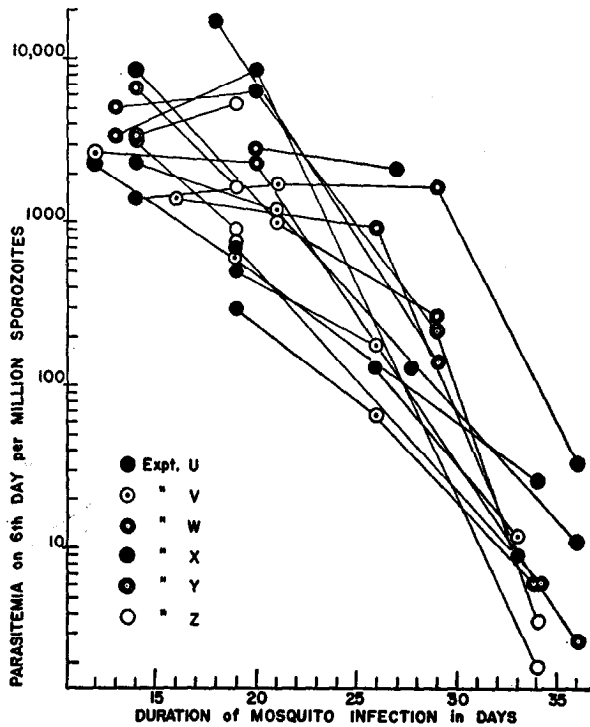* Calculated from parasitemia on the 7th day, see Materials and Methods section.

FIG. 3. Infectivity of sporozoites in relation to duration of infection in mosquitoes. Values for the same lot of mosquitoes tested in different experiments are connected by solid lines.

were made with saline solution. The first group contained three chicks, the others, eight to ten each.

In experiment A six chicks were inoculated with 50 sporozoites each; three became infected. With doses of 5 and 0.5 sporozoites each, none became infected. The data from these two experiments show that the figure obtained by dividing the 6th day parasitemia by the number of sporozoites inoculated is relatively constant for a rather wide range of actual sporozoite dosages.

Figure 3 presents data from six experiments on infectivity of sporozoites in relation to the time elapsed after the infecting feed. Each point on the graph represents a value for a group of four or more chicks inoculated with sporozoites from nine or more mosquitoes. The values (number of parasitized cells per 10,000 erythrocytes on the sixth day, per million
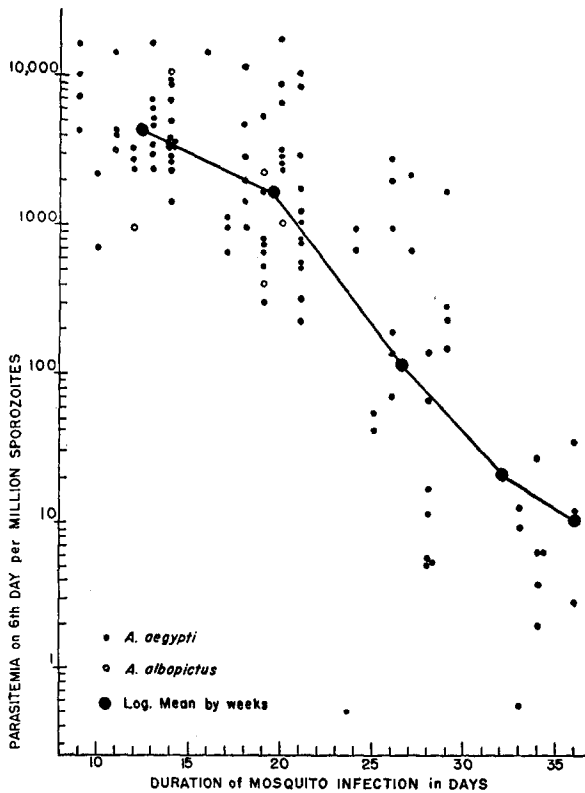

Fig. 4. Infectivity of sporozoites in relation to duration of infection in mosquitoes.

sporozoites inoculated) for each experiment are represented by a distinctive symbol, so that infectivities of different lots of mosquitoes in one experiment may be compared. Lines connect the values for samples of each lot tested in different experiments, so that the change in infectivity of one lot from experiment to experiment may be observed. It is clear that within each experiment the relative infectivity of sporozoites decreases with age of the infection in mosquitoes. Similarly, the infectivity of samples from the same lot, as determined in serial experiments, falls progressively with increase in duration of the infection.

Figure 4 gives the same data for all 109 mosquito samples tested, including those shown in Fig. 3. The logarithmic means show a progressive decrease in infectivity at a rate of about 90 % a week. By the fifth week from the infecting feed the infectivity of sporozoites was so slight as to be nearly undetectable. In fact, in addition to the data shown in Fig. 4, there were two lots tested at 35 days and six tested at 44–48 days which produced no infection in inoculated chicks.

The data presented apply principally to *Aedes aegypti*. Those on *A. albopictus* are insufficient in numbers to justify conclusions other than that they show no discrepancies from those for *A. aegypti*.

DISCUSSION

The data presented confirm the over-all loss in infectivity reported by others for human malarias. While mosquitoes have transmitted *P. vivax* up to ten weeks after infection (James, 1926) and *P. falciparum* to 55 days (Mayne, 1919, according to Boyd, 1949), there are reports of initially infective mosquito lots which became negative in as short a time as 23 days with *P. vivax* (Mitzmain, 1916) and 40 days with *P. falciparum* (Boyd et al., 1936). In some of these studies it appeared that the loss of infectivity resulted from depletion of sporozoites by repeated feeding of the mosquitoes, but in other instances (Barber, 1936; Boyd et al., 1936) microscopic studies indicated degeneration of sporozoites in the salivary glands.

The types of quantitative methods used in the present investigation have not been applied to primate malarias and are probably not appropriate. Rough measures of the viability of the sporozoites have, however, been utilized. Mitzmain (1916) found an increase in length of prepatent periods with successive applications of *A. punctipennis* harboring *P. vivax*. Boyd and Stratman-Thomas (1934) with P. *vivax* and Boyd et al. (1936) with *P. falciparum* reported a progressive decrease in percentage of infections in exposed paretics with increase in duration of infection in

A. quadrimaculatus kept at 4–7°C. No takes were obtained with *P. vivax* after 50 days nor with *P. falciparum* after 40 days from the completion of sporogony (total time from infecting feed not given).

The principal difference between the present work and that reported by others is that, by inoculation of known numbers of sporozoites, we have been able to distinguish between loss in numbers and loss of infectivity of sporozoites and to show that, in *P. gallinaceum*, at least, both occur. Since our mosquitoes received no blood meals after the infecting feed, it is clear that repeated feeding on animals was not responsible for the decline in infectivity. It should be pointed out that our data do not distinguish between effects of age of infection and those of age of the host mosquitoes. However, with *P. vivax* James (1926 and 1927) reinfected mosquitoes which had lost their infectivity. Since there are no major discrepancies in any of the work discussed it may be supposed that the general effects of duration of mosquito infection are similar for primate and avian malarias. We may conclude tentatively for all malarias that sporozoites decrease in numbers and infectivity during their stay in mosquitoes and that these decreases are not primarily a result of age of the mosquitoes nor of depletion of sporozoites by repeated blood meals.

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