

Evaluation of the Sporonticidal Effect of Pyrimethamine-Sulfadoxine,  
Quinine, and Quinine-Pyrimethamine against *P. falciparum*

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**OBJECTIVE :** To determine the effect of three therapeutic regimens for malaria upon the subsequent development of sporogonic forms of *P. falciparum* in mosquitoes fed on infected patients.

**BACKGROUND :** Fansidar, the fixed combination of pyrimethamine and sulfadoxine, has been shown to be effective against the asexual forms of chloroquine resistant and chloroquine sensitive strains of *P. falciparum* as well as *P. vivax* malaria in many parts of the world. It is currently used in Thailand as an alternative regimen by the clinics of the National Malaria Eradication Project and is widely used in hospitals and clinics throughout the country. A number of studies have proven its effectiveness as a schizonticide, but it has not been thoroughly evaluated as a gametocytocide and sporonticide. Chin and Rattanarithikul<sup>1</sup> reported that 47% of *A. balabacensis* fed on gametocytemic patients following single-dose therapy developed sporozoites. Serum levels of the drug in the donor patients at the time of the mosquito feedings were not reported by these authors.

Quinine has always been considered to have no effect on gametocytes or upon the development of *P. falciparum* in the mosquito host. In the past, pyrimethamine was thought to effectively interrupt the sporogonic cycle, however, since resistance of asexual forms of the parasite to pyrimethamine is common in the region, it is reasonable to expect that the drug's effect as a sporonticide has diminished.

In view of the widespread use of Fansidar in the therapy of malaria in Southeast Asia and its emergence as a drug of choice for chloroquine resistant malaria in many parts of the world, the effect of this drug upon the infectivity of gametocytes needs clarification. Epidemiologically, it is essential to be aware of the need for the additional use of a sporonticidal drug such as primaquine in combination therapy.

**DESCRIPTION :** Patients presenting to the outpatient clinics of the National Malaria Eradication Project and the district hospital in Phraphuttabat, who were at least 15 years of age and had blood smears positive for *P. falciparum*, were considered eligible for the study. Eligible patients who volunteered were assigned to one of three treatment groups; Group A: Patients were treated with a single dose of Fansidar, two tablets (total 50 mg. pyrimethamine and 1.0 mg. sulfadoxine); Group B: Patients were treated with quinine, 650 mg. every eight hours for six days; Group C: Patients received quinine as in Group B, above, but in addition, were given pyrimethamine 50 mg. daily for the first three days. Fansidar, either alone or in combination with quinine, is a standard therapy for *P. falciparum* used in the outpatient clinic and on the wards in Phraphuttabat hospital and in the clinic operated by the National Malaria Eradication Project.

Medications were administered by the nursing staff under the supervision of the study physicians. At the conclusion of the 21-day study period patients from Groups B and C were given two tablets of Fansidar.

Parasite counts were performed and blood drawn for pyrimethamine and sulfadoxine levels before treatment was begun on day 0, daily in hospital, and on days 5, 10, 15 and 20 after treatment. Mosquito feeds were performed on days 0, 5, 10, 15 and 20, using previously unfed *A. balabacensis* from the colony of the SMRL Phraphuttabat insectary. Patients were asked to return to the laboratory for follow-up. If necessary, they were followed at home. Ten percent of the mosquitoes fed on the patients were dissected six to ten days after feeding, and all surviving mosquitoes were dissected on day 15, regardless of the results of the previous dissection.

Serum levels of the antimalarial drugs are being determined at the biochemistry laboratory of the SMRL.

**PROGRESS :** One hundred and three patients were initially admitted to the study, but 28 of these were not included in the final tabulation of the data. Patients were eliminated for the following reasons :

1. No gametocytes at any time during their course (11 patients)
2. No gametocytes after day 0 (3 patients)
3. Change in therapeutic regimen (1 patient)
4. No follow-up after day 0 (13 patients)

A total of seventy-five patients was included in analysis of the data. The Fansidar treatment group comprised 35 patients, the quinine group 28, and the quinine-pyrimethamine group 12. Choice of therapy was not random, but was often dictated by the clinical condition of the patient. Patients with severe infections were not considered eligible for single-dose oral therapy, and were treated with quinine instead. This fact is reflected in the higher average asexual parasite count in the quinine and quinine-pyrimethamine groups (See Table 1).

Gametocytemia on admission was similar for the three groups, however, the Fansidar-treated group subsequently developed much higher levels. Whether this phenomenon was due to stimulation by Fansidar or was the result of suppression by quinine is not clear. (Figure 1).

Positivity of mosquito dissections seems to have been related to gametocyte levels, and when the average gametocyte counts were compared with percentages of mosquitoes showing development of parasites, the relationships were similar for all three groups (Figure 1).

Over 12,800 mosquitoes were allowed to feed upon the 75 study patients. Of the 60% of *A. balabacensis* that engorged on these patients, 4,880 survived and were dissected and examined for malaria parasites (Table 2). Fourteen percent of the dissected mosquitoes were found to be positive. Measurements of oocyst diameters were made to determine if normal parasite development took place. Guts of mosquitoes fed on patients before treatment or on days 5, 10, 15 or 20 following treatment were examined. Mosquitoes that fed on Day 5 on Fansidar-treated patients had oocysts that were larger than those seen in mosquitoes that fed on quinine-treated patients on the same day (Table 3). Also of interest is the fact that these oocyst diameters are larger than those reported by Coatney et al. (1971) in *A. freeborni* and *A. quadrimaculatus* mosquitoes. More measurements are required to determine if this apparent difference of growth rate is significant or the result of inadequate sampling. Sporozoites were not found in all lots of mosquitoes positive for oocysts. Thus, development to the sporozoite stage occurred in 21/25 lots fed on Fansidar-treated patients as compared with 9/10 lots fed on quinine-treated patients and 4/5 lots fed on quinine-pyrimethamine treated patients (Figure 2). Sporozoites, when seen, appeared to be normal in morphology; however, it is not known whether they were infective.

Determinations of serum levels of quinine, pyrimethamine, and sulfadoxine are not complete, but correlation of these data will be included in the final analysis of these results.

<sup>1</sup>Chin, William and Rattanaarithikul, Manop. The Evaluation of the Presumptive and Radical Treatments against Falciparum Malaria in Thailand. SE Asian J. Trop. Med. Pub. Hlth. 4: 400-406, 1973.

Table 1. Gametocytemia

Drug	Day	No. Pts.	Parasite Counts Mean (S.D.)	
			Asexual	Sexual
FANSIDAR	0	35	12622 (17562)	234 (365)
	5	35		644 (1067)
	10	31		802 (981)
	15	28		326 (387)
	20	22		120 (156)
QUININE	0	28	78387 (125932)	225 (451)
	5	28		211 (252)
	10	25		72 (117)
	15	18		24 (100)
	20	15		6 (8)
QUININE-PYRIMETHAMINE	0	12	58426 (104365)	440 (663)
	5	12		526 (896)
	10	12		227 (385)
	15	11		52 (100)
	20	6		13 (23)

Table 2. Mosquito Feeding and Dissection Data

Day	Engorgement (%)	Survival (%)	Positive Mosquito Feeds (%)	
			Lots	Individuals
Fansidar	74	57	39	14
	61	50	55	33
	63	55	53	27
	57	60	23	7
	55	64	10	2
Mean (S.D.)	62 (7.4)	57 (5.3)	36 (19.4)	17 (13.1)
Quinine	68	68	15	6
	50	63	32	15
	62	67	5	1
	56	69	22	1
	62	70	0	0
Mean (S.D.)	52 (29.7)	67 (2.7)	15 (12.9)	5 (6.3)
Quinine-Pyrimethamine	62	80	43	29
	47	75	45	29
	56	71	27	14
	83	79	17	7
	94	71	33	1
Mean (S.D.)	68 (19.5)	75 (4.3)	33 (11.6)	16 (12.7)

Table 3. Fansidar Sporonticidal Study  
 Mean Oocyst Diameters Seven Days Post-Feeding

Drug	Day Post - Treatment	Mean Diameter in Microns	
		Minimum (S.D.)	Maximum (S.D.)
None	0	26 (5.1)	37 (4.6)
Fansidar	5	29 (10.0)	39 (11.6)
Fansidar	10	26 (2.7)	37 (6.4)
Fansidar	15	27 (0)	35 (2.3)
Quinine	5	18 (0)	29 (2.7)
Quinine	10	—	38 (0)

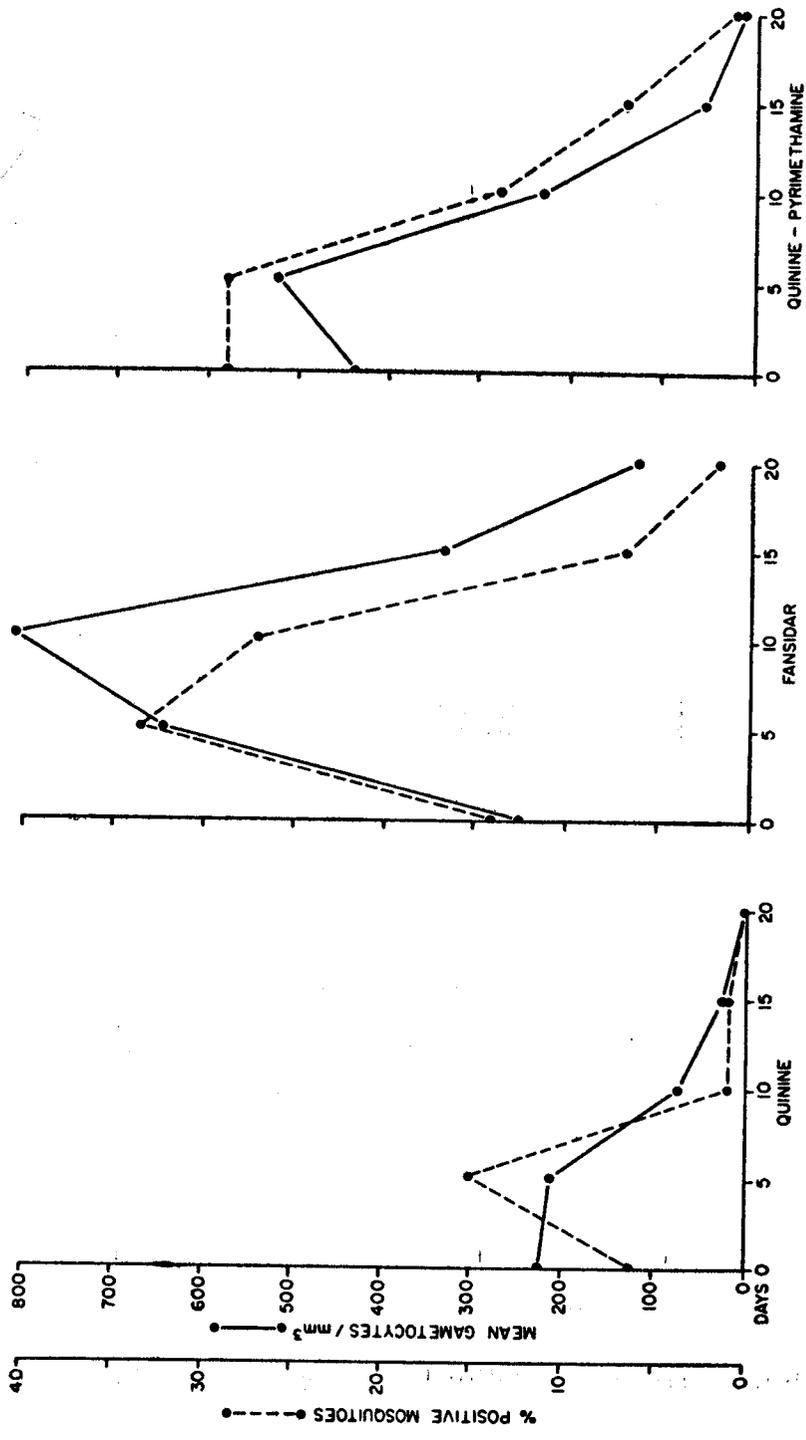


FIGURE 1. GAMETOCYTEMIA AND POSITIVE MOSQUITO FEEDS

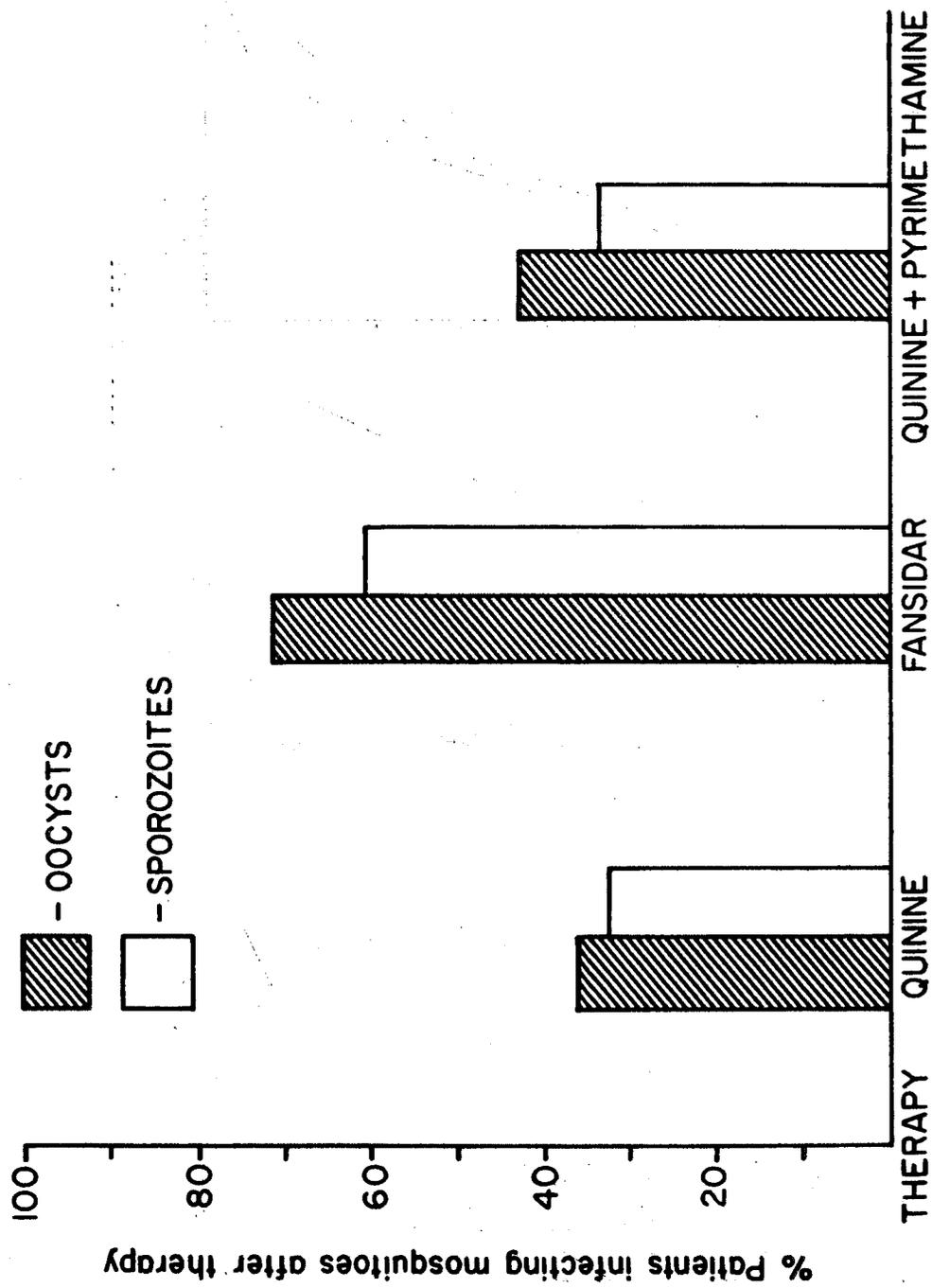


FIGURE 2. MOSQUITOES INFECTED AFTER FEEDING ON TREATED PATIENTS