COMPARATIVE INFECTIVITY OF A STRAIN OF *PLASMODIUM FALCIPARUM* FROM PANAMA TO THREE SPECIES OF *ANOPHELES* AS STUDIED BY MEMBRANE FEEDING

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Studies on the comparative infectivity of *Plasmodium falciparum* to *Anopheles quadrimaculatus, A. albimanus* and *A. freeborni* have been reviewed and reported previously (Collins, 1962 and Collins *et al.*, 1963). The present report concerns the result of similar infectivity studies of a strain of *P. falciparum* from Panama and the same three species of *Anopheles,* with a comparison of direct mosquito feeding on patients and feeding on a blood pool through a membrane.

METHODS AND PROCEDURES. The Panama D strain of *P. falciparum* was originally recovered from a mestiza of El Limon, Transisthmian Highway, Panama, in 1948 (Jeffery *et al.*, 1950). Following subcurative drug therapy in the laboratory, this strain was found to be resistant to pyrimethamine (Burgess and Young, 1959). In the linear passage as described by Burgess and Young (1959), the inoculation was from patient 1243 to 1300 and 1332 to the present patient, 1338. This patient was infected by intravenous inoculation of parasitized blood which had been preserved frozen in a dry ice chest (—78° C) for 940 days.

The *A. quadrimaculatus* (Q-1 strain) was originally from the Southeastern United States and has been maintained in our laboratory since 1941. The *A. albimanus* (A-9 strain) was originally from El Salvador and was obtained through the courtesy of Dr. H. G. Simkover, Shell Development Company, Modesto, California. The colony has been maintained by us since 1960. The *A. freeborni* (F-1 strain) was from Marysville, California, and has been maintained in our laboratory since 1944.

The patient was an adult Negro male being treated for neurosyphilis. The asexual parasites were first found 22 days after inoculation and the gametocytes appeared 11 days later. The comparative feedings were during the first gametocyte wave, on the eighth through the twelfth days of gametocytemia.

Three- to five-day-old adult female mosquitoes were caged in lots of 50 to 125 in pint ice cream carton cages and allowed to feed through the screened top either on the patient's arm or on a blood pool through a membrane. For the membrane feedings, 20 ml of blood were drawn by venipuncture into a heparinized syringe at the time of direct mosquito feeding. Ten ml of heparinized blood were placed in each of two feeding containers, constructed by replacing the bottoms of half-pint ice cream cartons with Baudruche (untreated) membrane.[1]

The blood meal was offered to the mosquitoes by placing the membrane in contact with the screen top of the mosquito cages. The membrane was warmed for a period of 20 seconds every 5 minutes during the 15-minute feeding period by resting the container on water heated to 37°C, after which the membrane was blotted dry on a towel and the container replaced on the mosquito cage. In order to feed three species of mosquitoes on only two blood pools, two easily differentiated species, *A. quadrimaculatus* and *A.*

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albimanus were caged together during the membrane feeding.

Engorged mosquitoes were transferred to pint-carton holding cages and incubated at 78° F. to 80° F. The mosquitoes were offered daily a 5 percent honey solution in a cellulose sponge. Seven or eight days after the blood meal, the mosquitoes were dissected and the guts microscopically examined for presence and number of oocysts. Dissections of glands at 12 to 15 days, as well as prior experience with the strain of parasite and the mosquito used, demonstrated the maturation of gut infections to the sporozoite stage.

RESULTS. The results of the comparative feedings using the membrane feeding technique are shown in Table 1. Both the *A. quadrimaculatus* and *A. albimanus* were more heavily infected by membrane feeding than by direct feeding on the patient. The *A. freeborni* were infected to a lesser degree when compared to the patient feeding, but it was still the most heavily infected species. Presumably, the lesser infection in this species is due to the characteristic slowness with which they feed. The majority of the *A. freeborni* mosquitoes were observed to feed in the 10–15-minute period whereas the *A. quadrimaculatus* and *A. albimanus* fed more in the 0–5-minute period. Our studies have previously shown that infection is drastically reduced if the blood is held for more than 30 minutes and is rarely infective if held for more than one hour prior to feeding.

The results of the comparative infectivity studies using the patient feeding method are shown in Table 2. The *A. freeborni* had a higher percentage of infection than the *A. quadrimaculatus* mosquitoes, which in turn had a higher percentage of infection than the *A. albimanus* mosquitoes. The gut infection index (average number of oocysts per 100 guts) followed the same pattern.

The relationships between the membrane feedings and the patient feedings are shown on Table 3. The results have been converted so that the feedings by *A. quadrimaculatus* on the patient equals 100. The *A. freeborni* would appear to have the greatest vector potential of the three mosquitoes tested. Although the gut infection index was higher in the *A. quadrimaculatus* than in the *A. albimanus*, the ratio of oocysts per positive gut (181:100 and 288:136) would possibly indicate a greater vector potential of the latter species.

DISCUSSION. The infection of mosquitoes by membrane feeding techniques with *Plasmodium* spp. has been previously reported by Yoeli (1938) using *Anopheles elutus* and *P. falciparum* and by Bishop and Gilchrist (1946) using *Aedes aegypti* and *P. gallinaceum*. The comparison of the infectivity of a strain of *P. falciparum* to three species of *Anopheles* as measured by the membrane-feeding technique and by patient-feeding (Table 3) indicated some similarities but also some variation in the results. In general, the two species which fed rapidly through the membrane had higher gut infection indices than those feeding directly on patients, which suggests the possible use of this technique to obtain more heavily infected mosquitoes. This would be very useful when the feedings are on patients with low gametocyte densities. Certainly, in those individuals upon whom it is difficult to feed mosquitoes, the membrane-feeding technique offers an alternative method for mosquito infection.

The studies of Jeffery *et al.* (1950) indicate that two strains of *A. albimanus*, one from Panama and one from the Florida Keys, were both more susceptible to infection by the Panama strain of *P. falciparum* than was *A. quadrimaculatus* (Q-1 strain). The results reported here on direct patient feeding indicate that the strain of *A. albimanus* from San Salvador (A-9) has a lesser gut infection index than does the *A. quadrimaculatus* but a higher ratio of oocysts per positive gut.

These results extend those reported previously (Collins, *et al.*, 1963) that with the four strains of *P. falciparum* thus far examined, the *A. freeborni* are the most susceptible to infection. In contrast with

TABLE 1.—Infectivity of *P. falciparum* (Panama D strain) to three species of *Anopheles* using the membrane feeding technique.

Comparative feeding	*Anopheles quadrimaculatus* P/D †	Percent inf.	GII ‡	Ave. no. oocysts per pos gut	*Anopheles albimanus* P/D †	Percent inf.	GII ‡	Ave. no. oocysts per pos gut	*Anopheles freeborni* P/D†	Percent inf.	GII ‡	Ave. no. oocysts per pos gut
A	15/25	60	152	2.5	3/8	38	75	2.0	..	..	..	..
B	6/25	24	68	2.8	..	..	..	..	..	..	..	..
C	8/25	32	64	2.0	9/25	36	208	5.9	8/10	80	970	12.1
D	12/30	48	93	2.3	4/30	13	50	3.8	20/25	83	596	6.5
Totals *	35/80	44	103	2.3	16/63	26	136	5.4	..	..	..	..
Totals **	20/55	36	80	2.2	13/55	24	122	5.2	28/35	80	703	8.8

* Comparative feedings with *A. quadrimaculatus* and *A. albimanus*.
** Comparative feedings with *A. quadrimaculatus, A. albimanus* and *A. freeborni*.
† P/D—Positive mosquitoes/total number dissected.
‡ GII Gut infection index=average number of oocysts per 100 guts.

TABLE 2.—Comparative infectivity of *P. falciparum* (Panama D strain) to three species of *Anopheles* by direct patient feedings.

Comparative feeding	*Anopheles quadrimaculatus* P/D †	Percent inf.	GII ‡	Ave. no. oocysts per pos gut	*Anopheles albimanus* P/D †	Percent inf.	GII ‡	Ave. no. oocysts per pos gut	*Anopheles freeborni* P/D †	Percent inf.	GII ‡	Ave. no. oocysts per pos gut
A	6/25	24	40	1.7	2/12	17	50	3.0	..	..	..	..
B	5/25	20	36	1.8	0/10	0	0	..	..	..	..	..
C	9/25	36	64	1.8	6/25	24	92	3.8	19/20	94	1085	11.4
D	10/30	33	60	1.8	6/30	20	37	1.8	22/25	88	1132	12.9
E	11/30	37	43	1.2	2/30	7	27	3.0	4/6	67	350	5.2
Totals *	41/135	30	49	1.6	16/107	15	43	2.9	..	..	..	..
Totals **	30/85	35	55	1.6	15/85	16	49	3.0	45/51	88	1022	11.6

* Comparative feedings with *A. quadrimaculatus* and *A. albimanus*.
** Comparative feedings with *A. quadrimaculatus, A. albimanus* and *A. freeborni*.
† P/D—Positive mosquitoes/total number dissected.
‡ GII Gut infection index=average number of oocysts per 100 guts.

TABLE 3.—Relationships between infectivity of *P. falciparum* (Panama D strain) to three species of *Anopheles* by patient and membrane feeding using *A. quadrimaculatus* as a standard (=100).

Mosquito strain	Gut infection index ratio		Oocysts per positive gut ratio	
	Patient feeding	Membrane feeding	Patient feeding	Membrane feeding
Q-1	100	187	100	136
A-9	88	171	181	288
F-1	1848	1183	738	517

the results of the three previous strains studied, the *A. albimanus* was infected to a greater degree with the Panama D strain *of P. falciparum* as compared with the standard *A. quadrimaculatus*. These results agree with the findings of Jeffery *et al.*, (1950) that strains of *P. falciparum* are more infective to coindigenous strains of *A. albimanus*.

SUMMARY. Comparative studies on the infectivity of a strain of *Plasmodium falciparum* from Panama to *Anopheles freeborni*, *A. quadrimaculatus* and *A. albimanus* using membrane-feeding and patient-feeding techniques indicated that the first was the most heavily infected. All species were infected with parasites using the membrane-feeding technique with the latter two species having heavier infections from membrane-feeding than from feeding directly on the patient. The usefulness of membrane-feeding to infect mosquitoes with *P. falciparum* is indicated.

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