

nated as the CDC Entomological Chill Table and useful for processing mosquitoes for virus isolation studies, is described and details of construction are given. The chill table consists of a surface plate which is cooled by a coil beneath it. A household type refrigeration unit is used

to provide cooling. The temperature of the cooling surface is regulated by a thermostat. Three of these chill tables have been in use at the Arbovirus Vector Laboratory for over a year and have proved to be reliable and convenient to use.

TRANSMISSION OF THE RO STRAIN OF *PLASMODIUM CYNOMOLGI* BY *A. STEPHENSI*, *A. QUADRIMACULATUS* AND *A. LABRANCHIAE ATROPARVUS*

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Plasmodium cynomolgi is a tertian malaria parasite which was first described by Mayer (1907) from a *Macaca irus* monkey brought to Germany from Java. It was redescribed by Sinton and Mulligan (1932-1933) from parasites found in a *Macaca irus* said to be from Singapore.

The transmission of strains and subspecies of *P. cynomolgi* has been studied by a number of investigators. Transmission of the parasite by mosquito bite has been demonstrated using *Anopheles freeborni* (Eyles 1960a), *A. quadrimaculatus* (Hawkins *et al.*, 1948), *A. labranchiae atroparvus* (Shortt and Garnham, 1948), *A. aztecus* (Garnham, 1959), and *A. stephensi* (Ramakrishnan and Mohan, 1962). In addition, transmission has been obtained by the intravenous inoculation of infected salivary glands from *A. albimanus* (Eyles, 1960b) from *A. kochi*, *A. lesteri*, *A. letifer*, *A. maculatus*, *A. philippinensis* and *A. sundaicus* (Warren *et al.*, 1963).

The present studies were made to investigate the RO strain of *P. cynomolgi*

in regard to its infectivity to and transmission by the five species of *Anopheles* being used in this laboratory. Previous reports of transmission of this strain have been limited to *A. freeborni* (Schmidt, 1964).

MATERIALS AND METHODS. The RO strain of *P. cynomolgi* was isolated in December 1960 from a *Macaca mulatta* monkey imported from the Burma-Assam area. After isolation it was maintained by serial monkey-mosquito (*A. freeborni*) transfers (Schmidt, 1964) until February 1964, when the strain was established in this laboratory in an *M. mulatta* monkey (No. C-159) by the intravenous inoculation of 64 pairs of infected salivary glands. Subsequently this strain has been maintained in *M. mulatta* monkeys either by the intravenous inoculation of sporozoites or of parasitized blood.

The Q-1 strain of *A. quadrimaculatus*, originally from Southeastern United States, has been maintained in the laboratory since 1941. The F-1 strain of *A. freeborni* from Marysville, California, has been maintained as a laboratory colony since 1944. The *A. stephensi*, originally from India, and the *A. labranchiae atro-*

¹ With the technical assistance of Harvey Akins and Peggy Sue Stanfill.

TABLE 1.—Comparative susceptibility of five species of *Anopheles* to infection by the RO strain of *Plasmodium cynomolgi*.

Day of Patency	<i>A. freeborni</i>			<i>A. stephensi</i>			<i>A. l. atroparvus</i>			<i>A. quadrimaculatus</i>			<i>A. albimanus</i>		
	P/D*	%**	GII***	P/D	%	GII	P/D	%	GII	P/D	%	GII	P/D	%	GII
18	25/25	100	23,668	10/25	40	16,624	MONKEY C-159†			8/25	32	152	1/25	4	8
19	25/25	100	20,092	25/25	100	36,628	16/25	64	4,036	17/25	68	1,868	0/25	0	0
20	25/25	100	19,040	17/25	68	20,748				14/25	56	1,276	0/5	0	0
6	26/30	87	2,220	2/25	8	28	MONKEY C-172			20/27	72	6,737	0/10	0	0
7	17/25	68	8,376	13/21	61	6,643	13/25	52	756	17/25	68	1,532	0/16	0	0
9	6/25	24	1,216	7/9	77	7,800	14/24	58	4,391	7/25	28	308	0/5	0	0
10	12/25	48	656	6/25	24	372	4/11	36	591	7/25	28	140	0/9	0	0
8	25/25	100	19,768	19/25	76	5,976	MONKEY C-183			24/25	96	5,976			
11	13/25	52	388	2/25	8	24	23/25	92	14,396	0/25	0	0	0/25	0	0
Totals	174/230	76	10,372	101/205	49	10,830	73/135	54	4,391	114/227	50	2,019	1/120	<1	1.7

* Positive mosquito guts/number dissected.

** Percent infection.

*** Gut infection index (average number of oocysts per 100 guts).

† Monkeys=*Macaca mulatta*.

parvus, originally from England, have been maintained in our insectary since 1963, when they were obtained from the London School of Hygiene and Tropical Medicine, through the courtesy of Mr. G. Davidson. The A-9 strain of *A. albimanus* originated in San Salvador and has been maintained in our laboratory since 1960.

Caged mosquitoes were allowed to feed directly on infected monkeys. Engorged mosquitoes were maintained in the insectary at 78-80° F., receiving daily feedings of 5 percent Karo syrup in a cellulose pledget. Dissections for oocyst counts were done beginning on the sixth day after the blood meal, and examinations of salivary glands for the presence of sporozoites were initiated three days later. For transmission attempts, the mosquitoes were allowed to feed directly on the monkey and were subsequently dissected and examined for presence of sporozoites in the salivary glands.

RESULTS. Susceptibility Studies. To determine the comparative susceptibility of the five anopheline species to the RO strain of *P. cynomolgi*, a total of nine separate feedings were made on three infected *M. mulatta* monkeys. Six of the nine feedings included all of the species, while in the remainder only four of the five anophelines were included. Two of the three monkeys used as donors were sporozoite-inoculated; in the third the infection had been induced by inoculation of fresh parasitized blood. The results of these comparative feedings are shown in Table 1.

Four of the species tested, *A. freeborni*, *A. stephensi*, *A. labranchiae atroparvus* and *A. quadrimaculatus*, were readily infected with the RO strain of *P. cynomolgi*. While in percent of mosquitoes infected, *A. freeborni* appeared superior to *A. stephensi*, the average numbers of oocysts found in these two species were approximately equal. These had approximately 2.4 times the average number of oocysts found in the *A. labranchiae atroparvus* and 5.1 times that found in the *A. quadrimaculatus*. Only one *A. albimanus* mos-

quito was found to be infected, this specimen having only two oocysts.

Transmission Studies. Since it had been previously shown that *A. freeborni* was capable of transmitting this strain of *P. cynomolgi* (Schmidt, 1964), transmissions were attempted only with *A. quadrimaculatus*, *A. stephensi*, and *A. labranchiae atroparvus*.

Monkey C-159 was fed upon by *A. quadrimaculatus* mosquitoes on the 15th day of patency. The gametocyte count was 4 males and 15 females per 50 W.B.C. These mosquitoes had a gut infection index of 4,448. After 10 days of extrinsic incubation, approximately 250 of these mosquitoes were allowed to bite monkey C-172. This monkey had a prepatent period of nine days.

Monkey C-183 was inoculated with fresh parasitized blood. *Anopheles stephensi* mosquitoes were allowed to feed on the eighth day of patency when the gametocyte count was 6 males and 22 females per 50 W.B.C. After 13 days of extrinsic incubation, 11 of these mosquitoes were allowed to feed on monkey T-1. Microscopic examination of the salivary glands of these mosquitoes indicated that only two contained sporozoites. The prepatent period in this monkey was 11 days.

Anopheles labranchiae atroparvus mosquitoes were also fed on monkey C-183 on the eighth day of patency. After 13 days of extrinsic incubation, seven of these mosquitoes were allowed to feed on monkey T-2. Microscopic examination of the salivary glands of these mosquitoes indicated that five contained sporozoites. The prepatent period in this monkey was 10 days.

DISCUSSION. From these studies, it appears that *Anopheles freeborni*, *A. stephensi*, *A. labranchiae atroparvus*, and *A. quadrimaculatus* are all readily susceptible to infection by the RO strain of *P. cynomolgi*. This strain had been previously transmitted by *A. freeborni* (Schmidt, 1964). In addition, the ease with which transmission was obtained using the other three species of mosquitoes

would further indicate the usefulness of this strain of *P. cynomolgi* in malaria investigations.

SUMMARY. *Anopheles freeborni* and *A. stephensi* were highly susceptible to the RO strain of *Plasmodium cynomolgi*. *A. labranchiae atroparvus* and *A. quadrimaculatus* were also susceptible, but the intensity of infection was somewhat lower. *A. albimanus* was virtually insusceptible.

Transmission of the RO strain to *Macaca mulatta* monkeys was accomplished by the bites of infected *A. stephensi*, *A. quadrimaculatus*, and *A. labranchiae atroparvus*.

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HEMPA AS A CHEMOSTERILANT FOR THE YELLOW-FEVER MOSQUITO *Aedes Aegypti* (L.) (DIPTERA: CULICIDAE)

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In recent years, chemosterilization of insects has been investigated extensively as a new method of insect control. Much research has centered on alkylating agents such as tepa, metepa, or apholate, all derivatives of aziridine. However, several other groups of chemicals, including the dimethylamine derivatives, have also shown promise. Hempa (hexamethylphosphoramide), one of the dimethylamine derivatives, has had considerable attention because: (1) it is not an alkylating agent; (2) it is an effective sterilant for house flies (*Musca domestica* L.) (Chang *et al.* 1964; LaBrecque *et al.* in

press); and (3) it has relatively low toxicity for mammals. The acute oral minimum lethal dose for rats was reported to be 2640 mg./kg. by Chang *et al.* (1964). Kimbrough and Gaines (in press) found it to be 2650 mg./kg. for male rats and 3360 for females. Testicular atrophy was caused by single doses as low as 1000 mg./kg. (but not by a dose of 500 mg./kg.) and by a diet that contained 750 p.p.m. for 45 days (40 to 80 mg./kg./day). Dosages of 25 mg./kg./day for 56 days did not affect male fertility. In addition, the experimental induction of resistance to apholate in larvae of the yellow-fever mos-