

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-

quito *Aedes aegypti* (L.) by Hazard *et al.* (1964) has focused attention on the desirability of having more than one group of sterilants available should resistance to the alkylating compounds become a problem. We have therefore evaluated hempa as a chemosterilant for *A. aegypti* by adding the compound to the rearing water of larvae, by administering it in the adult food, and by exposing adult mosquitoes to a residue of the chemical on a variety of surfaces.

MATERIALS AND METHODS. In single tests about 300 larvae of *A. aegypti* were exposed to concentrations of 0.1, 1, 10, 50, or 100 p.p.m. of hempa in the rearing water from the time of the third instar until pupation. Controls were reared in untreated water. Food was added after the first day of treatment. Effects of treatment were determined by confining 25 adult males and 25 adult females from each concentration in organdy-covered cages for 3 to 5 days. During this period, a water-soaked cotton pad and a pad saturated with a 20-percent honey solution were made available to the mosquitoes. At the end of the mating period, the females were allowed to feed on guinea pigs to obtain the blood meal necessary for oviposition. Two days later, pint jars lined with blotting paper and containing 250 ml. of tap water were placed in the cages for oviposition. The eggs were allowed to embryonate and mature on the moist paper for 4 days; then samples of eggs were flooded to determine the hatching rate.

In the adult feeding tests the mosquitoes were segregated by sex within 16 hours after eclosion, while they were still virgin. Twenty-five males or females were placed in each cage and allowed to feed ad libitum on 20-percent honey solution to which a concentration of 1, 0.5, or 0.1 percent of hempa had been added. Controls were given the honey solution without hempa. Each test was replicated twice. Four days later, two cages of each sex of mosquitoes treated at each concentration were mated with each other, and two cages of treated

mosquitoes of each sex were mated with untreated mosquitoes of the opposite sex. Untreated males and females were used as controls. The subsequent procedure was the same as that used for the larval exposure tests.

The residue tests were conducted by exposing virgin one-day-old mosquitoes for 4 hours to deposits of hempa on various surfaces. The deposits were applied as solutions in absolute methanol at 100, 200, and 500 mg. per square foot on glass petri dishes, plastic petri dishes, hardened masonite, and yellow sulfite mimeograph paper as follows: (1) the solution was poured into the glass or plastic petri dishes, and the dishes were rotated until the methanol evaporated; (2) the solution was sprayed onto the masonite panels (6x6 inch) with an atomizer; (3) the solution was pipetted along an edge of the paper (5x6 inch) and allowed to migrate over the entire surface. The controls were handled in the same way except that no hempa was added to the methanol. Distribution of the hempa appeared to be even on the masonite and paper but uneven on the glass and plastic petri dishes. The mosquitoes were confined in the petri dishes by putting on the covers and on the masonite by placing them under overturned petri dishes. For the paper deposit test, the treated papers were inserted in plastic tubes provided with the World Health Organization insecticide resistance test kit. The mosquitoes were then introduced into the paper-lined tubes. After exposure, the mosquitoes were transferred to small cages and mating crosses made. The subsequent procedures were the same as those in the larval exposure tests.

RESULTS. The results of the larval exposure tests are presented in Table 1. Concentrations of 50 and 100 p.p.m. both produced 99-percent sterility, but concentrations of 10 p.p.m. or less had only a slight effect. No larvae died during treatment at any concentration.

The results obtained with hempa administered in the adult diet are given in

TABLE 1.—Effect of hempa in the larval medium on the fertility of *Aedes aegypti*. (Single tests)

Concentration (p.p.m.)	Approximate no. of eggs laid	No. of eggs set	Percent hatch
0	2800	150	97
0.1	2300	102	99
1	2600	76	85
10	2200	94	87
50	2100	66	1
100	2200	72	1

Table 2. Treated females were 86-percent sterile at a concentration of 0.1 percent, completely sterile with a low oviposition rate at a concentration of 0.5 percent, and had complete inhibition of oviposition at a concentration of 1 percent. With males, sterility was no higher (97 percent) at a concentration of 1 percent than at a concentration of 0.1 percent.

TABLE 2.—Effect of hempa in the adult diet on the fertility of *Aedes aegypti*. (Average of 2 tests of 25 males and females each.)

Concentration (percent)	Sex treated	Approximate		
		No. of eggs laid	No. of eggs set	Percent hatch
0	Neither	3800	220	97
0.1	Both	3100	238	97
	Female	2900	241	4
.5	Male	4100	230	14
	Both	27	27	3
	Female	25	25	0
	Male	3500	255	0
1	Both	0	...	3
	Female	0
	Male	4000	230	..

No residual deposit on any surface (Table 3) produced complete sterility in either sex. The 200- and 500-mg. deposits on masonite and the 100- and 200-mg. deposits on paper produced considerable male sterility (10-percent to 49-percent egg hatch), but the 100-mg. deposit on masonite had little if any effect. With treated females, the 500-mg. deposits on plastic and on masonite reduced the percentage of viable eggs to 55 and 66-percent hatches, respectively, but the other treatments were ineffective. The percent

of eggs hatched in the controls ranged from 75 to 95 percent.

DISCUSSION. Although hempa offers a greater measure of safety than the alkylating agents that have been reported as sterilants for *A. aegypti* (tepa, metepa, apholate, and thiotepa), it is also less effective than at least one of these compounds by each method of application. Tepa and apholate induce high sterility in *A. aegypti* at a concentration of 10 p.p.m. in the larval medium; apholate induces complete sterility when it is administered in the adult diet at 0.1 percent; and tepa causes almost complete sterility when the mosquitoes are exposed to residues of 10 mg./sq. ft. on glass (Weidhaas *et al.*, 1961; Weidhaas, 1962 and 1963; Dame and Ford, 1964). However, apholate as a residual treatment and tepa in the diet are no more effective than hempa.

Dame and Schmidt (1964) obtained only 36-percent reduction in fertility with metepa at 10 p.p.m. in the larval medium of this species, 96-percent reduction with 1 percent of metepa in the adult diet (honey solution) and more than 99-percent sterility in adults exposed for 4 hours on glass surfaces treated with 10 mg. of metepa per square foot. White (1964) produced sterility with thiotepa at 2.5 to 5 p.p.m., but also had high mortality at 5 and 10 p.p.m. Bertram (1963) produced high sterility in males and females

TABLE 3.—Effects of exposure to residual deposits of hempa on the fertility of *Aedes aegypti*. (Average of 2 tests of 25 pairs of mosquitoes each.)

Surface	Sex treated	Deposit (mg./sq. ft.)	Approximate no. of eggs laid	No. of eggs set	Percent hatch
Glass	Neither	0	1700	210	75
		100	3900	172	92
	Female	200	2900	162	93
		500	2100	204	84
Plastic	Neither	0	3000	212	94
		100	4700	283	78
	Female	200	4800	270	73
		500	4700	221	66
Masonite	Neither	0	6300	215	97
		100	4700	159	72
		200	5300	250	32
		500	3800	200	23
	Female	100	5400	174	99
		200	5600	252	92
		500	1300	288	55
Paper ^a	Neither	0	3700	235	87
		100	6700	204	49
		200	6200	233	10
	Female	100	5000	284	89
		200	2700	219	78

^a 500 mg./sq. ft. on paper killed both males and females within one hour after they were placed in the tube.

exposed to residual deposits of 20 mg. of thiotepa per square foot on paper. The males sterilized by contact with thiotepa (Bertram, 1963) regained some of their fertility, but males sterilized by contact with tepa (Dame and Ford, 1964) did not.

Thus hempa is unsuitable as a residual treatment and the concentrations required all but preclude its practical use as a larval treatment. However, the chemical might be used in the diet at concentrations ranging from 2 to 5 times those required for apholate.

SUMMARY. Hempa (hexamethylphosphoramide) induced 99-percent sterility in *Aedes aegypti* (L.) when larvae were exposed to 50 p.p.m. in the rearing water from the third instar until pupation. Complete sterility was induced in females allowed to feed on honey solution containing 0.5 percent of hempa, and 97-percent sterility was induced in males at a concentration of 0.1 percent. Exposure for 4 hours to residual deposits of 200 to 500 mg. of hempa per square foot on

paper induced 90-percent sterility in males; less effect was produced in males exposed to 500-mg. deposits on masonite or in females exposed to like deposits or to 500-mg. deposits on glass and plastic.

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AEDES KOMPI VARGAS AND DOWNS 1950, NEW TO THE UNITED STATES

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Aedes (*Finlaya*) *kompi* Vargas and Downs 1950 was described from specimens collected in Tepoztlán, Morelos, Mexico during June, 1949 from tree holes and rock holes. Downs (Pers. comm.) states that his collection from a rock hole near a stream bed was made after a period of heavy rain which filled rock holes not usually containing water.

The author collected *Ae. kompi* pupae on 18 August, 1964, 3 miles southwest of Patagonia, Arizona, in Santa Cruz County. The general area southwest of Patagonia is watered by Sonoita Creek which normally flows all year long. There is a large stand of cottonwoods, willows and sycamores along the creek, providing heavy shade and many tree holes.

The author collected and reared all the larvae and pupae from a single, large willow tree hole containing about 10 liters of dark water. Adults of *Ae. kompi*, *Anopheles barberi* Coq., and *Orthopodomyia kummi* Edwards were taken from the sides of the hole, above the water line and on leaves near the hole. Subsequent rear-

ing of the larvae and pupae yielded *Ae. kompi* 74 ♀♀, 7 ♂♂; *An. barberi* 18 ♀♀, 24 ♂♂; and *O. kummi* 260 ♀♀, 227 ♂♂. Periodic collections from the hole from October, 1964 to August, 1965 yielded specimens of *O. kummi* throughout the year and *An. barberi* from April until termination of sampling at the end of July. No larvae, pupae or adults of *Ae. kompi* were found in other tree holes in the area.

The tree hole from which *Ae. kompi* was collected was unusual for Southern Arizona because of its large capacity and because the accumulation of leafy debris remained damp throughout the year. All of the other tree holes the author has examined in Southern Arizona are dry most of the year, containing water only after the heavy summer rains in July and August.

DESCRIPTION OF *Ae. kompi*. Vargas and Downs (1950) described the immature and adult stages of *Ae. kompi*. The following description deals only with the more conspicuous characters of the larva, pupa and adult separating *Ae. kompi* from other tree hole *Aedes* species, and, except for the adult female, is translated from the original description. The pupa, adult

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