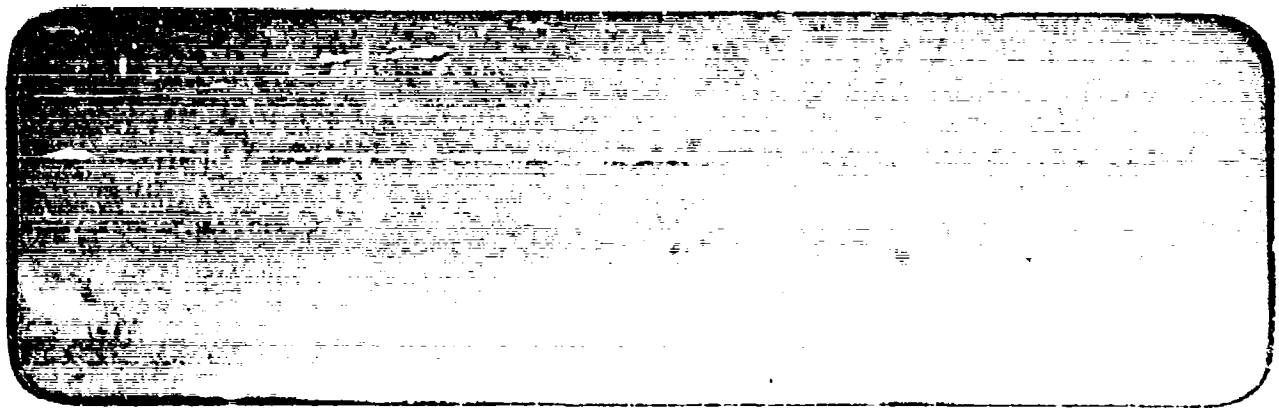

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE Vol. 16, No. 4

Printed in U.S.A.

EFFECT OF APHOLATE AND METEPA ON *AEDES AEGYPTI* INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS*

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The use of chemical agents for inhibiting reproduction in arthropod pests is currently being studied by a number of investigators. Among the many radiomimetic compounds being investigated, those of the aziridine group of alkylating agents have received the most attention. Commonly used compounds in this category are apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis(1 - aziridinyl) - 1,3,5,2,4,6-triaza-triphosphorine) and metepa (tris(1-2 methylaziridinyl) phosphine oxide). Several authors have recently reviewed the subject.[1-3] One aspect of chemical sterilization that has received little attention is its effect on the transmission potential of arthropod vectors. Although several workers[4-6] have studied the effects of irradiation on the susceptibility of arthropod vectors to plasmodia, the only paper dealing with chemical sterilizing agents is that of Altman[7] on the effect of tepa on plasmodial infection and subsequent transmission in *Aedes aegypti*. The present study is an attempt to determine what effect such treatment of an arthropod vector could have upon its susceptibility to an arbovirus and the subsequent transmission of the agent.

MATERIALS AND METHODS

A. aegypti mosquitoes used in this study were obtained originally from The Rockefeller Foundation and had been maintained in laboratory culture for numerous generations. All operations with the mosquitoes, except when otherwise noted, were carried out at 27°C ± 1° and at 78 to 80% relative humidity. The virus was the Trinidad strain of Venezuelan equine encephalomyelites virus (VEE).

The experiment was designed to compare fecundity and mortality as well as rates of virus infection and transmission of treated and untreated adult mosquitoes. The treatments were: 24-hour exposure to 10% sucrose solutions containing 0.25% metepa, 0.025% metepa, 0.25% apholate, or 0.025% apholate. The untreated vectors were fed a solution containing 10% sucrose only. Three-inch-square gauze pads saturated with the appropriate solution were placed on separate gallon ice-cream cartons, each containing 125 newly emerged, unfed females. The concentration of the solutions on the pads was not maintained but increased with evaporation during the 24 hours the pads were on the cartons. Four days later the females were exposed to a mixture of three parts of a VEE virus solution, four parts washed, packed, guinea-pig RBC's, and three parts of a 1.0 molar sucrose solution. Drops of this mixture were distributed evenly over the gauze of all the cartons, and the mosquitoes were allowed to feed freely for 1 hour. The virus-meal was then removed. A day after exposure to the virus-meal, all cartons were equipped with feeders containing 10% sucrose solution, and 50 male *A. aegypti* were added. Mortality counts of the females were made every 2nd day. The cartons were supplied with oviposition containers; these were changed every 4th day, when the females were given a blood-meal on anesthetized adult mice. Twelve days after exposure to the virus-meal, 50 females were removed from each of the cartons and tested for virus infection. For transmission assay, each mosquito was given access to a 2- to 4-day-old suckling mouse on the gauze top of a pint holding carton for 2 hours. Deaths occurring in mice on the 2nd day after exposure were attributed to virus infection. After feeding on suckling mice, the test mosquitoes were frozen and held at −70°C until assayed for infection. For assay, each mosquito was triturated with a micromortar and pestle in 1.0 ml of beef-heart infusion broth containing 2 mg of streptomycin and 200 units of penicillin. After light centrifugation, 0.03 ml of the supernatant fluid was inoculated intracerebrally into each of five 21- to 23-day-old albino Swiss mice. The mice were observed for 7 days after inocula-

* In conducting the research reported herein, the investigators adhered to "Guide for Laboratory Animal Facilities and Care," established by the committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

tion. The death of three or more of the five mice on days 4 through 7 after inoculation was accepted as evidence of vector infection. Observations of mortality and oviposition-data collection were continued with the remaining female mosquitoes for 30 days after exposure to the virus. Four separate studies were conducted.

RESULTS

No eggs were deposited by female mosquitoes after treatment with the 0.25% concentration of either apholate or metepa. The four groups treated with 0.025% apholate deposited a total of 3,214 eggs during the collection period, but the groups treated with 0.025% metepa produced only 165 eggs. In contrast, from each of the four untreated groups more than 30,000 eggs were obtained (Table 1). Larvae were obtained from the eggs deposited by the groups treated both with 0.025% metepa and apholate. However, the percentage of viable eggs produced by these groups was much lower than that of untreated groups. There was no trend toward the recovery of fecundity by treated females through the observation period.

The 0.25% treatment levels of metepa and apholate resulted in higher mortality. This was especially marked in the period shortly after the treatments, as shown in Table 2. During the 16-day period between the chemical meal and the transmission assay, 21% mortality was recorded in the groups treated with 0.25% metepa and 8% in those with 0.25% apholate. These results were in contrast to the mortality rates of 4, 3, and 2% in the groups treated with 0.025% metepa and apholate, and in the untreated groups, respectively. Thus, on a basis of concentration, metepa was a somewhat more effective sterilizing agent than apholate, but apparently was more toxic.

The results of the tests of transmission and infection, summarized in Table 3, show that treatment with 0.25% metepa and 0.25% apholate increased the transmission percentages on an average of 18.0 and 22.6%, respectively. The transmission averages of the groups treated with 0.025% apholate and 0.025% metepa were 1.3 and 3.1% higher, respectively, than the 56.8% average of the control groups. Similarly, the average infection percentages of the groups treated with 0.25% metepa and apholate are 92.5 and 94.0%, in contrast to the 79% average of the control groups and the 80.5% average of both the groups treated with 0.025% apholate and metepa.

The increase in the rates of transmission and

TABLE 1

Number of eggs deposited by A. aegypti *treated with metepa and apholate*

Treatment	Day of collection								Total
	4	8	16	20	24	28	32	36	
0.25% Apholate	None								
0.25% Metepa	None								
0.025% Apholate	247	866	218	71	101	3	65	33	1,604
	137	16	0	19	6	29	0	0	207
	365	90	111	5	6	2	0	0	579
	549	183	68	1	3	6	8	6	824
0.025% Metepa	0	0	0	0	0	0	0	0	0
	41	0	0	0	0	0	0	0	41
	76	3	0	0	0	0	0	0	79
	44	0	1	0	0	0	0	0	45
Untreated controls	>5,000	>5,000	>5,000	>5,000	4,231	4,397	4,652	3,753	>37,857
	>5,000	>5,000	>5,000	1,802	4,709	4,641	2,667	2,729	>31,548
	>5,000	>5,000	>5,000	>5,000	4,591	4,436	4,898	4,194	>38,119
	>5,000	3,414	>5,000	>5,000	>5,000	3,197	2,482	3,379	>32,472

TABLE 2

Mortality of A. aegypti *females after treatment with apholate and metepa* *

Treatment	1-12 days		13-24 days		25-36 days		1-36 days	
	No. dead	%	No. dead	%	No. dead	%	No. dead	%
0.25% Apholate	10		14		9		33	
	10		17		15		42	
	14	9.8	5	15.1	16	30.5	35	50.7
	15		2		25		42	
0.25% Metepa	37		13		7		57	
	34		5		8		47	
	20	24.8	14	22.7	15	30.9	49	68.7
	33		8		12		53	
0.025% Apholate	5		11		19		35	
	5		8		23		36	
	5	3.4	5	9.9	20	30.9	30	40.4
	2		4		17		23	
0.025% Metepa	5		8		10		23	
	4		4		18		26	
	9	4.4	6	7.5	19	22.6	34	33.7
	4		3		11		18	
Untreated controls	4		2		12		18	
	2		5		13		20	
	3	2.6	5	6.1	12	16.5	20	24.7
	4		5		7		16	

* The percentage of mortality is based on total number of females alive at the beginning of each period, but only calculations for day 1 to 12 include the females used in the tests of transmission and infection.

infection in the groups of mosquitoes treated with the high levels of chemical sterilizing agent represents a substantial increase in vector potential.

DISCUSSION

Results of the experiment show that treating *A. aegypti* females with chemical sterilizing agents significantly affected ($P = .01$) both their infection and transmission percentages. Treatments, appropriately timed, can raise substantially both the rates of infection and of transmission. However, in experiments not presented here, *A. aegypti* females when treated with either apholate or metepa at concentrations of 0.5% and 0.1% after exposure to VEE virus showed no significant difference from controls in rates of infection after sterilizing treatment, although their rates of transmission were lower. There is evidence that this effect was due to the toxicity of the chemical for the mosquitoes. It was also observed that such mosquitoes, especially when fed high concentrations or several meals of these chemicals, were sluggish and often unsuccessful in locating and feeding on host mice. On the other hand, in the experiments presented, the effect of the agents was similar to that observed following irradiation. As shown in Table 3, the effect was manifested in higher rates of infection and transmission. The specific mechanisms of the chemical sterilizing agents on infection are not known. In a review of this subject, Ross[8] noted that such compounds are believed to disrupt many metabolic processes and probably alter directly the chromosomal nucleic acids. Such basic metabolic disruption might well be responsible for a change in susceptibility to virus infection, which could result in both the infection of more individuals and in the infection of more cells within the individual.

TABLE 3

Effects of metepa and apholate on VEE virus infection and transmission by A. aegypti

Replicates	0.25% Apholate		0.25% Metepa		0.025% Apholate		0.025% Metepa		Untreated	
	Infected	Transmitted	Infected	Transmitted	Infected	Transmitted	Infected	Transmitted	Infected	Transmitted
A	48/50*	41/49	48/50	38/49	34/50	22/50	37/50	27/50	39/50	25/47
B	48/50	40/50	45/50	38/50	42/50	28/50	43/50	33/50	38/50	26/50
C	47/50	38/50	47/50	41/50	46/50	39/48	39/50	28/47	40/50	31/44
D	45/50	39/50	45/50	32/50	39/50	26/50	42/50	30/50	41/50	26/49
Totals	188/200	158/199	185/200	149/199	161/200	115/198	161/200	118/197	158/200	108/190
	94.0%	79.4%	92.5%	74.9%	80.5%	58.1%	80.5%	59.9%	79.0%	56.8%

* Number of mosquitoes positive over number tested.

Studies with mosquitoes and houseflies have shown that metepa is distributed rapidly and nonselectively[9] and that almost all ingested metepa is metabolized within 24 hours.[10] Thus, the 3-day interval between the removal of the chemical meal and the ingestion of the virus suggests that the observed increases in rates of infection were not due to a direct action of the chemical on the virus, unless the meal were stored in the diverticula of the mosquitoes. The effect of the time of the feeding of the chemical in relation to exposure to the virus appears to be a critical variable and should be studied further.

Smith *et al.*[3] have suggested the field use of chemical sterilizing agents as a potential method for the eradication of arthropod pests. The experiments presented here are not sufficiently extensive to warrant any accurate projection of the results to a field situation, but they do suggest caution in such application. The balance of other virus-vector interrelations may be even more dramatically affected by these agents than the VEE virus–*A. aegypti* model studied here. If a large-scale field application of sterilizing agents were conducted, the balance of an endemic viral zoonosis could be affected.

SUMMARY

Groups of *Aedes aegypti* female mosquitoes were fed 0.25 and 0.025% concentrations of apholate and metepa. Four days later they were fed a solution containing VEE virus and sweetened blood. Male mosquitoes were introduced also at this time. Treatment with the 0.25% concentrations of either sterilizing agent completely inhibited oviposition. Both the total number of eggs and the percentage of viable eggs in the groups treated with 0.025% concentrations were a fraction of those deposited by the untreated groups. Mortality in the treated vectors, especially those given the 0.25% concentrations, was higher than that in the untreated mosquitoes.

The lower concentrations of sterilizing agents had no demonstrable effects on the susceptibility of the vectors to VEE virus or on subsequent transmission of the virus. Susceptibility to virus infection and the ability to transmit the disease were significantly influenced by the higher concentrations of both compounds. The results suggest that other mosquito vector-arbovirus combinations could demonstrate similar or increased potentials.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. Cordia Harris and Mr. Jerry Love for technical assistance.

REFERENCES

1. Lindquist, A. W., 1961. Chemicals to sterilize insects. *J. Wash. Acad. Sci.*, *51:* 109–114.
2. Knipling, E. F., 1962. Potentialities and progress in the development of chemosterilants for insect control. *J. Econ. Entomol.*, *55:* 782–786.
3. Smith, C. N., LaBrecque, E. C., and Borkovec, A. B., 1964. Insect chemosterilants. *Ann. Rev. Entomol.*, *9:* 269–284.
4. Ward, R. A., Bell, L. H., and Schneider, R. L., 1960. Effects of X-irradiation in the development of malarial parasites in mosquitoes. *Exper. Parasitol.*, *10:* 324–332.

5. Taliaferro, W. H., Taliaferro, L. G., and Simmons, E. L., 1945. Increased parasitemia in chicken malaria (*Plasmodium gallinaceum* and *Plasmodium lophurae*) following X-irradiation. *J. Inf. Dis., 77:* 158-176.
6. Torzian, L. A., 1953. The effect of X-irradiation on the immunity of mosquitoes to malarial infection. *J. Immunol., 71:* 202-206.
7. Altman, R. M., 1963. The effects of tepa on *Plasmodium gallinaceum* in *Aedes aegypti*. *Am. J. Hyg., 77:* 221-227.
8. Ross, W. C. J., 1962. Biological alkylating agents. Fundamental chemistry and the design of compounds for selective toxicity. 232 pp. Butterworths, London.
9. Dame, D. A., and Schmidt, C. H., 1964. Uptake of metepa and its effect on two species of mosquitoes (*Anopheles quadrimaculatus*, *Aedes aegypti*) and house flies (*Musca domestica*). *J. Econ. Entomol., 57:* 77-81.
10. Plapp, F. W. Jr., Bigley, W. S., Chapman, G. A., and Eddy, G. W., 1962. Metabolism of methaphoxide in mosquitoes, house flies and mice. *J. Econ. Entomol., 55:* 607-613.