

## ARTICLES

HOST PREFERENCES OF *CULISETA MELANURA* AND ALLIED MOSQUITOES<sup>1</sup>RICHARD O. HAYES<sup>2</sup>

The role of mosquitoes as vectors of eastern encephalitis (EE) is at present poorly known. In Massachusetts, EE virus has been isolated from only one species of naturally infected mosquito, *Culiseta melanura* (1). This arthropod is generally considered to be the principal enzootic vector of EE (2), but not in all areas (3). Other mosquitoes or even other arthropods are thought to transmit the virus to man and horse (2, 4). Among the mosquitoes occurring in Massachusetts that may be involved in EE transmission are five species which have been found naturally infected elsewhere: *Mansonia perturbans* (5), *Anopheles crucians* (6), *Culex salinarius* (7), *Culex restuans* (8), and *Aedes vexans* (9, 10).

The general hypothesis regarding transmission of EE is that wild birds are the principal reservoirs, though it appears from laboratory data (11) and field data (12) that small mammals could also be involved as reservoirs. Presumably any mosquitoes or other haematophagous arthropods that serve as primary enzootic vectors of EE would show a high preference for feeding on birds, and any

endemic vectors that transmit EE to man and domestic animals would be species which feed readily on mammals as well as birds. The primary objective of the present study was to obtain information on the relative attractiveness of various species of amphibians, reptiles, birds and mammals for *C. melanura* and associated mosquitoes. Previous investigations (13) had shown that if potential hosts were exposed in traps and the bait animal were accessible to the trapped mosquitoes for feeding, the hosts could be compared with respect to both the number of mosquitoes attracted and the percent of mosquitoes which obtained blood.

**MATERIALS AND METHODS.** The observations were made between July 6 and September 12, 1959, in Raynham, Massachusetts, at a rural residence with a long history of EE virus activity. The exact site was less than 200 yards from Hockomock Swamp, the largest freshwater swamp in the state. The animals used as bait in the mosquito traps were obtained in Raynham or other nearby towns. They included seven species of birds, eight of mammals, six of reptiles, and four of amphibians (Table 1).

The cylindrical traps, operated in a horizontal position, were made from lard cans of 120-pound capacity (Fig. 1). They were similar to previous bait traps (13, 14) with two exceptions: the dimensions of the conical entrance baffles placed in the two ends, and the device for holding the test animal. The baffles, made of galvanized wire screening of 14 x 18 mesh, were 10½ inches in diameter at the base and 8½ inches high. The entrance holes, each ¾ inch in diameter and reinforced by a metal ring to insure permanence of

<sup>1</sup> This investigation is part of a joint study on eastern encephalitis by the Division of Communicable Disease, Massachusetts Department of Public Health, and the Communicable Disease Center, U. S. Public Health Service. The assistance of Mr. Frank M. Mack and Mr. Robert Frazer in the field and laboratory phases of this study, the statistical counsel provided by Dr. Richard G. Cornell and Mr. Elmer C. Hall, and the analytical review of the data by Dr. Richard P. Dow and Dr. A. D. Hess are gratefully acknowledged.

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TABLE 1.—Attraction and engorgement of four common mosquito species in tests with twenty-five vertebrate species conducted at Raynham, Massachusetts during 1959<sup>1</sup>

Vertebrate species	No. of trap nights	<i>Culiseta melanura</i>		<i>Culex pipiens</i>		<i>Culex salinarius</i>		<i>Aedes canadensis</i>	
		A. <sup>2</sup>	E. <sup>3</sup>	A.	E.	A.	E.	A.	E.
<i>Birds</i>									
Catbird	1	63	0	..	..	109	29	19	10
Chicken, 4-5 week	11	66	85	58	94	27	70	12	97
Grackle, common	5	116	64	99	78	3	90	11	98
Pheasant, immature	7	195	80	106	86	81	93	12	86
Pigeon, domestic	5	255	38	92	88	150	45	52	46
Sparrow, English	2	87	32	..	..	188	48	3	100
Starling, immature	3	299	21	..	..	87	48	25	13
All birds	34	153	55	86	85	76	57	11	58
<i>Mammals</i>									
Hat, little brown	4	25	0	18	18	10	0	2	50
Chipmunk, eastern	4	75	9	..	..	92	48	40	42
Mouse, white-footed	4	24	0	10	0	49	42	8	55
Rabbit, eastern cottontail	6	63	28	27	46	120	55	6	93
Rabbit, domestic	4	9	25	..	..	8	58	8	90
Rat, Norway	4	22	11	..	..	8	63	27	46
Squirrel, eastern gray	4	89	14	37	23	112	33	7	100
Volc, redback	5	64	1	24	19	47	7	1	80
All mammals	35	48	12	24	26	66	45	18	62
<i>Reptiles</i>									
Snake, eastern garter	4	2	0	0	0	7	0	2	11
Snake, eastern milk	4	1	100	3	61	0	0	0	0
Snake, northern water	8	10	35	15	56	19	54	1	50
Turtle, eastern box	4	9	0	20	28	24	46	1	0
Turtle, eastern painted	4	1	0	0	0	9	17	5	74
Turtle, eastern spotted	4	1	0	..	..	6	29	11	91
All reptiles	28	5	21	10	49	14	41	2	69
<i>Amphibians</i>									
Bullfrog	4	8	0	..	..	46	11	3	22
Frog, green	4	20	0	..	..	22	16	19	12
Salamander, redback	4	10	0	..	..	6	0	11	0
Toad, American	4	1	0	0	0	5	0	4	0
All amphibians	16	10	0	0	0	20	11	10	8

<sup>1</sup> Total number of trap nights when each mosquito was present was: *C. melanura*, 113; *C. pipiens*, 47; *C. salinarius*, 95; *A. canadensis*, 109.

<sup>2</sup> Average number attracted per trap night when at least one specimen of the species was taken in any trap. Dashes indicate mosquito species absent during all trials with the vertebrate.

<sup>3</sup> Percent of mosquitoes engorged (all trap nights combined). Dashes indicate mosquito species absent during all trials with the vertebrate.

the aperture size, were about  $3\frac{1}{2}$  inches apart when the baffles were in position. The pocket for holding the test animal was  $10\frac{1}{2}$  inches wide by  $6\frac{1}{2}$  inches high. It was made of nylon mesh (28 threads to the inch) and provided with a 2-inch muslin collar with four button holes. It could be inserted and removed through a hole, 6 inches in diameter, which was cut

in the top of the trap. The pocket, placed in a transverse position in the trap, was supported by flaring the collar over the outer surface, holding it in place with a sheetmetal gasket  $1\frac{1}{2}$  inches wide, and clinching both collar and gasket to the trap by two bolts fastened on the inside. A sheetmetal cover (not shown in Fig. 1) which closed the hole and thus prevented



FIG. 1.—Mosquito trap opened at end to show a pigeon in the nylon pocket. The metal cover has been removed from the hole in the top through which the bait is introduced.

mosquitoes from reaching the host animal without entering through the baffles, was secured by two additional bolts. These, unlike the first two, were fastened externally. The underlying purpose of this hatchway was to permit removal of the bait animal to a safe place before anesthetizing the trapped mosquitoes. Four of these traps were used.

Except for the turtles, which were placed unrestrained in the nylon pocket, the host animals were immobilized to reduce their interfering with mosquito feeding. Each bird was placed in the toe of a nylon stocking, which had been cut off just above the heel. The bird's back rested on the sole of the stocking, and its feet were bound by the top of the stocking and secured together by an elastic band. Thus immobilized, the bird was placed on its back in the nylon pocket. In addition to immobilizing the bird, this procedure increased the surface area available to the mosquitoes; an unrestrained bird would stand in the cloth pocket, and

its feet would provide very little body area susceptible to mosquito attack. The amphibians tested also were immobilized by using stockings. The mammals and the snakes were restrained by wrapping them in galvanized hardware cloth of  $\frac{1}{4}$ -inch mesh. The larger hosts so wrapped (rat, squirrel, rabbit, and snakes) would not fit in the nylon pocket and were placed on the bottom of the mosquito trap. The white-footed mice tended to damage the nylon pocket by gnawing, so they, too, were placed on the bottom of the trap.

The traps, restraining materials, and nylon pockets were washed after each use to remove animal odor. Mammals generally fouled the traps with urine, so a pad of cotton was placed on the floor of the trap to absorb it.

To overcome bias in the mosquito collections which would result from trapping at four different sites, the traps were suspended from the four horizontal arms of a rotary (13) that was turned by an elec-

tric motor at the rate of 0.2 revolution per minute. Each arm of the rotary extended 4 feet from the vertical axis, and each trap was about 5 feet above the ground.

The experimental design used for each test was derived from a randomly formed Latin square (15). The basic design provided for a 4-night test with each of four species of host animal exposed in a different trap on each successive night. Nine 4-night tests were made, each with three or four different species representing from one to three classes of vertebrates. The different species of host and any controls were changed nightly with respect to both position and sequence. Each trap, however, was always hung from the same arm of the rotary. With one exception each test was run from about 4 p.m., E.D.T., to about 8 a.m., E.D.T., on four consecutive nights. Four of the tests included, as a control in place of one host, either an unbaited trap (7 trap-nights) or a trap containing 50 adult *Culex pipiens* (4 trap-nights).

A difference from previous rotary tests of host preference was the attempt to obtain more representative information on a given species of vertebrate by exposing a different individual of the selected species each night. It was not always possible to obtain four species of a given species, however, and some individual hosts were tested from 2 to 6 nights.

**RESULTS.** Each of the 25 selected vertebrate species was exposed successfully from 1 to 11 times. A few of the birds and mammals, but none of the reptiles or amphibians, either died or became free of the restraining material during the testing. All data from these unsuccessful trials were discarded.

Thirteen different species of mosquitoes were caught in the 113 successful trials of traps baited with vertebrates. There were large totals of four species (7,168 *Culiseta melanura*, 4,450 *Culex salinarius*, 2,030 *C. pipiens*, and 1,198 *Aedes canadensis*), and smaller totals of four others (100 *Culex restuans*, 100 *Mansonia per-*

*turbans*, 77 *Culiseta minnesotae*, and 48 *Culiseta morsitans*). The five remaining species were taken in very small numbers (Five engorged *Aedes aberratus*, one engorged and one unengorged *Aedes excrucians*, and one unengorged *Aedes vexans* were taken in mammal-baited traps; one *A. aberratus*, one *Aedes caniator*, and one *Aedes triseriatus*, all engorged, were taken in bird-baited traps.)

Three species of bird, three of mammal, one of reptile, and three of amphibian were tested on nights when no *C. pipiens* were trapped, and no more tests of these vertebrates were made after *C. pipiens* became abundant. Similarly, various vertebrates were tested on nights when some species of mosquitoes were apparently absent. Therefore, in calculating the average number of a species of mosquito attracted to a given vertebrate, the data have been limited to observations made on those nights when at least one specimen of the mosquito was trapped (even in an unsuccessful trial). This restriction tends to distort somewhat the average attraction in the scarcer mosquitoes by making it proportionately higher than in those species that were common throughout the season.

In addition to average attraction of a given mosquito to a particular vertebrate there is a second measure of host preference in the percentage of engorgement total engorged times 100 divided by total trapped. All or most of the mosquitoes that fed were conspicuously engorged perhaps because of the general use of restraining materials which favored interrupted feeding.

In general, the warm-blooded vertebrates were much more attractive to the mosquitoes than the cold-blooded vertebrates, the birds were more attractive than the mammals, and the amphibians were more attractive than the reptiles (Table 1, Figure 2). The figures for engorgement are certainly affected by the restraint of the test animals but indicate that as a class, the birds were more acceptable as a source of blood than the mammals. I

the cold-blooded vertebrates, which usually attracted very small numbers of mosquitoes, the reptiles were more acceptable than the amphibians. In fact, there was no feeding at all on two of the amphibians, the red-backed salamander and the American toad.

The average number of *C. melanura* attracted to a bird, 153, was the highest for any mosquito on any class of host. The engorgement of *C. melanura* was highest on birds, though only 55 percent. Even so, there were 2,878 specimens which fed on birds, the highest number of any mosquito feeding on any class of host. *C. melanura* was attracted less frequently to mammals and engorged on them at a low rate (only 12 percent). *C. melanura* followed the general pattern of higher attraction to amphibians than reptiles but lower engorgement on amphibians than on reptiles; in fact, this mosquito did not feed at all on amphibians.

The average attraction of *C. pipiens* showed high preference for birds over mammals similar to that of *C. melanura*, and the percentage of engorgement on birds, 85, was the highest rate in any of the four common mosquitoes for any class of vertebrate. Like *C. melanura*, it fed to a limited extent on reptiles, but did not feed at all on the one amphibian (American toad) against which it was tested.

*C. salinarius* was quite different in its behavior. The average attraction to (76 mosquitoes) and engorgement on (57 percent) birds as a class were not significantly different from mammals as a class (66 and 45, respectively). This mosquito was not, therefore, primarily a bird feeder as are *C. melanura* and *C. pipiens*. *C. salinarius* also fed to a limited extent on both reptiles and amphibians.

*A. canadensis*, like *C. salinarius*, was not primarily attracted to birds; the number attracted to mammals was higher than for any other class of vertebrates. The engorgement rates for reptiles, birds, and mammals were high, but the rate for amphibians was low.

To explore the reliability of the figures for average attraction of the four common mosquitoes to the four classes of vertebrates, each separate single-night comparison of two vertebrates of different classes was ranked for each species of mosquito as won, lost, or tied (excluding tied with zero score) according to the total numbers of the mosquitoes in the two traps. The mammal was more attractive than the bird, or equally attractive, 4 out of 21 times in *C. melanura*, 9 out of 21 times in *C. salinarius*, and 14 out of 21 times in *A. canadensis*. (There were only four bird-mammal comparisons in *C. pipiens*.) The amphibian was more attractive than the reptile, or equally attractive, 3 out of 5 times in *C. melanura*, 2 out of 8 times in *C. salinarius*, and 6 out of 8 times in *A. canadensis*. The only other departures from the ranking determined by class averages were 10 out of 52 comparisons in *A. canadensis*. All of these exceptions are seen to correspond very neatly with the decrease in ornithophily from *C. melanura* and *C. pipiens* through *C. salinarius* to *A. canadensis*.

Four mosquitoes were represented by only 48 to 100 specimens (*Culex restuans*, *Mansonia perturbans*, *Culiseta minnesotae*, and *C. morsitans*). They were similar in having higher attraction to, and engorgement rates on, warm-blooded than cold-blooded vertebrates (table 2); however, the percentage engorgement of *C. restuans* on mammals (16) was outstandingly low in comparison with that for the other three species (64 to 84).

When the four traps used in all the tests were "baited" with 50 adult *C. pipiens* and operated on successive nights as controls, they contained only 44, 44, 41, and 43 *C. pipiens* at the end of the respective testing periods. In the course of the testing, two *C. melanura* entered one trap and three *C. melanura* another. The fact that the loss of *C. pipiens* from the traps was uniform is considered more important than the escape of a few specimens which were not host-attracted to begin with. The average number of *C. melanura* entering each of these traps (1,

2) may be compared with the average numbers of the mosquitoes entering the unbaited control traps in 7 nights of testing (based on the nights when each species was known to be present: 5 *C. salinarius*, 4 *A. canadensis*, 1 *C. pipiens*,

0.3 *C. melanura*, and 0.2 *C. minnesotae*. The relatively high averages of *C. salinarius* (higher than the average attraction to reptiles) and *A. canadensis* (higher than the average attraction to amphibians) agree with the observation that these

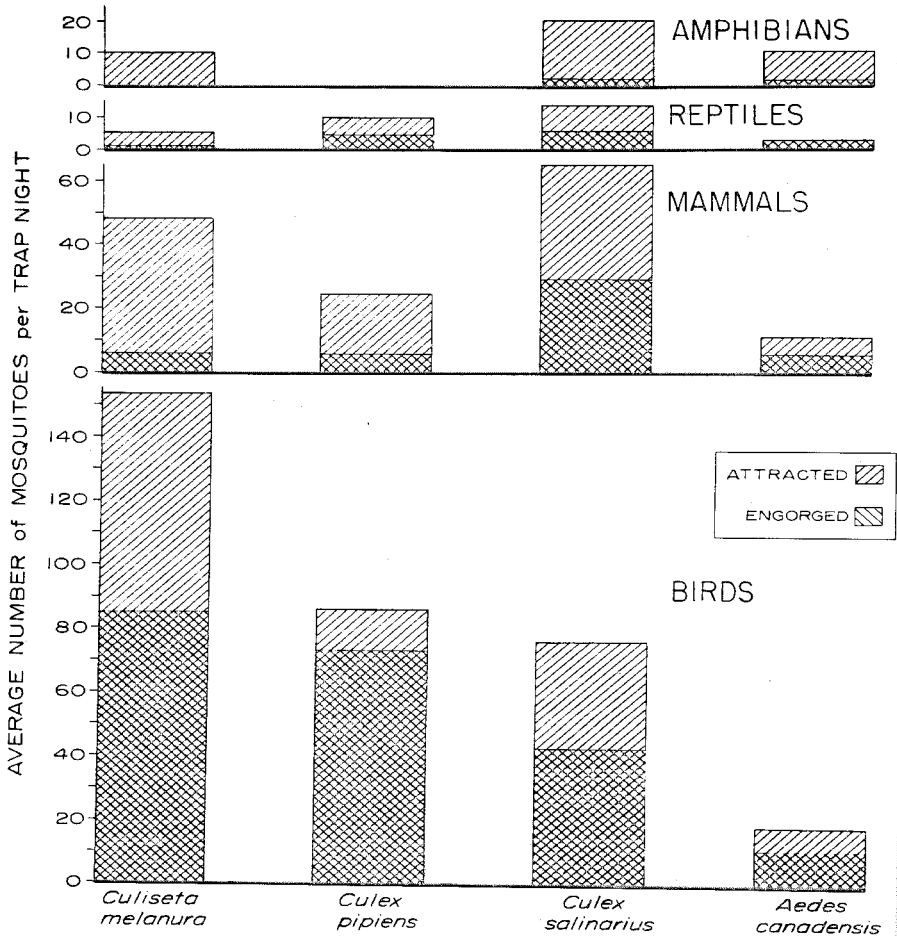


Fig. 2.—Average number of four mosquito species attracted to and engorged on vertebrates per trap night at Raynham, Massachusetts during 1959.

species were more generally attracted to all four classes of vertebrates than were *C. melanura* and *C. pipiens*.

DISCUSSION. The average attraction and engorgement of the four common mosquitoes for the four classes of vertebrates can be discussed with considerable confidence, and the evidence is quite conclusive that *C. melanura* and *C. pipiens* were

attracted to and fed upon birds much more than any of the other three classes of vertebrates. There were suggestions of significant differences between some vertebrate species within a class (e.g., engorgement rates for chicken vs. starling, and for cottontail vs. vole); however, because of the many variables involved, it is not believed that valid conclusions

TABLE 2.—Attraction and engorgement of four mosquito species in tests with twenty-five vertebrate species conducted at Raynham, Massachusetts during 1959<sup>1</sup>

Vertebrate species	<i>Culex restuans</i>		<i>Mansonia perturbans</i>		<i>Culiseta minnesotae</i>		<i>Culiseta morsitans</i>	
	A. <sup>2</sup>	E. <sup>3</sup>	A.	E.	A.	E.	A.	E.
<i>Birds</i>								
Catbird	2	0	..	..	..	..	..	..
Chicken, 4-5 week	1	80	2	100	1	100	1	100
Grackle, common	0	0	3	93	1	100	1	100
Pheasant, immature	4	100	2	100	2	100	1	88
Pigeon, domestic	3	42	2	80	2	92	3	91
Sparrow, English	2	0	1	100	4	22	0	0
Starling, immature	0	0	0	0	4	92	2	80
All birds	1.8	70	2.0	96	1.9	81	1.3	89
<i>Mammals</i>								
Bat, little brown	0	0	1	0	0	0	1	0
Chipmunk, eastern	1	0	..	..	2	75	0	0
Mouse, white-footed	2	0	1	100	1	0	..	..
Rabbit, eastern cottontail	2	33	2	77	3	75	1	50
Rabbit, domestic	1	100	2	88	0	0	..	..
Rat, Norway	2	0	3	100	1	0	..	..
Squirrel, eastern gray	0	0	0	0	3	56	1	75
Vole, redback	8	13	1	33	1	100	1	100
All mammals	1.5	16	1.4	84	1.7	64	1.0	64
<i>Reptiles</i>								
Snake, eastern garter	1	0	0	0	1	0	..	..
Snake, eastern milk	0	0	0	0	0	0	0	0
Snake, northern water	1	20	1	100	0	0	1	75
Turtle, eastern box	1	33	0	0	0	0	1	0
Turtle, eastern painted	1	0	0	0	0	0	..	..
Turtle, eastern spotted	0	0	..	..	1	100	0	0
All reptiles	0.4	22	0.1	100	0.1	50	0.3	60
<i>Amphibians</i>								
Bullfrog	0	0	1	0	0	0	1	0
Frog, green	0	0	2	0	1	0	0	0
Salamander, red-backed	0	0	0	0	1	0	0	0
Toad, American	0	0	0	0	0	0	0	0
All amphibians	0	0	0.4	0	0.2	0	0.1	0

<sup>1</sup> Total number of trap nights when each mosquito was present was: *C. restuans*, 89; *M. perturbans*, 76; *C. minnesotae*, 70; *C. morsitans*, 61.

<sup>2</sup> Average number attracted per trap night when at least one specimen of the species was taken in any trap. Dashes indicate mosquito species absent during all trials with the vertebrate.

<sup>3</sup> Percent of mosquitoes engorged (all trap nights combined). Dashes indicate mosquito species absent during all trials with the vertebrate.

can be made concerning differences between species within any one of the four classes of vertebrates.

No other tests of host preference are known to have involved so many species of mosquitoes, but there is no apparent indication of interference between the different kinds, and in *C. melanura* at least, the percentage found engorged in each trap shows no relation to the total number of other species of mosquitoes.

With regard to the epidemiology of eastern encephalitis, the results not only support the hypothesis that *C. melanura* feeds primarily on birds, but show that, because it feeds also on mammals, it must be considered a potential endemic as well as enzootic vector of EE virus. Moreover, though it did not feed upon amphibians, it did engorge upon reptiles and thus could be involved in the over-winter transmission cycle if, as recently suggested for western encephalitis virus (16, 17), snakes were indeed the winter reservoir.

*C. pipiens* and *A. canadensis* have not yet been shown to be involved in the transmission of EE. Laboratory tests indicate that *C. salinarius* has a poor vector potential for EE virus (18), but engorged specimens collected from swamps have been found to contain EE virus, which could have been in the recently ingested blood rather than in the mosquito tissues (7). Though *C. salinarius* might have little importance as an interepidemic vector, its tendency to feed on mammals as well as birds would give it great potential importance during an epidemic season even if only as a mechanical transmitter.

*C. restuans* and *M. perturbans* have been found naturally infected with EE. The limited amount of data on these two species at least indicates that they feed on both birds and mammals and to some extent on reptiles.

**SUMMARY.** Seven species of birds, eight of mammals, six of reptiles, and four of amphibians were exposed as bait in mosquito traps in southeastern Massachusetts to ascertain the species and numbers of mosquitoes which would be attracted to

and feed upon each type of animal. Four mosquito species (*Culiseta melanura*, *Culex pipiens*, *Culex salinarius*, and *Aedes canadensis*) were trapped in sufficient numbers to permit broad classification of the mosquito-host relationships. All were attracted to and fed to a limited extent on reptiles. All but *C. pipiens* also were attracted to amphibians, but only *C. salinarius* and *A. canadensis* fed upon them. Both *C. melanura* and *C. pipiens* were attracted to and fed upon birds much more than mammals, but *C. salinarius* and *A. canadensis* showed no significant difference between birds and mammals. Two other mosquitoes that have been reported naturally infected with EE, *Mansonia perturbans* and *Culex restuans*, were trapped in small numbers only. Both were attracted to warm-blooded more than to cold-blooded vertebrates, and both fed, at least to some extent, on all classes of vertebrates except amphibians.

The data support the hypothesis that *C. melanura* is the primary enzootic vector of eastern encephalitis, maintaining the summer reservoir in wild birds by transmitting the virus from one bird to another. It also could be a vector in summer or winter transmission cycles involving wild mammals or reptiles.

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## THE EFFECT OF PHENYLTHIOUREA AND 4-CHLORORESORCINOL ON *Aedes Aegypti* LARVAE

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**INTRODUCTION.** During the course of experiments with sub-lethal concentrations of compounds that inhibit various enzyme systems in insects, two chemicals known to block tyrosinase were tested in larval medium of *Aedes aegypti*. Phenylthiourea and 4-chlororesorcinol were reported by Kull, *et al.* (1), to be effective in inhibiting melanin formation in the regenerating fin of platy fish during *in-vivo* experiments. However, while little toxicity to the fish was indicated, no observations of post-treatment effects on growth and reproduction were made. For

this purpose it was postulated by the author that other aquatic animals, such as rapidly growing mosquito larvae, would be excellent tools for tests with enzyme-blocking compounds. Consequently this study using low concentrations of these compounds was conducted, utilizing *Aedes aegypti* larvae as test animals. The object of this paper is to report the *in-vivo* inhibition of melanin formation and the growth-retarding effect of these chemicals.

**METHODS.** A series of solutions of 4-chlororesorcinol (M. W. 144.56, Eastman