of eastern equine encephalomyelitis from mosquitoes (*Mansonia perturbans*) collected in Georgia. Science 110(2849):141–142.

6. KARSTAD, L. H., FLETCHER, O. K., SPALATIN, J., ROBERTS, R., and HANSON, R. P. 1957. Eastern equine encephalomyelitis virus isolated from three species of Diptera. Science 125(3244): 395–396.

7. CHAMBERLAIN, R. W., SUDIA, W. D., BURBUTIS, P. P., and BOGUE, M. D. 1958. Recent isolations of arthropod-borne viruses from mosquitoes in eastern United States. Mosq. News 18(4):305–308.

8. HAYES, R. O., LAMOTTE, L. C., WHITE, L. A., and BEADLE, L. D. 1960. Isolation of eastern encephalitis virus from the mosquito *Culex restuans* collected in New Jersey during 1959. Mosquito News 20(2):190.

9. WALLIS, R. C., TAYLOR, R. M., and HENDERSON, J. R. 1960. Isolation of eastern equine encephalomyelitis virus from *Aedes vexans* in Connecticut. Proc. Soc. Exp. Biol. & Med. 103: 442–444.

10. CHAMBERLAIN, R. W. 1960. Personal communication. Virus and Rickettsia Section, Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia.

11. SYVERTON, J. T., and BERRY, G. P. 1940. Host range of equine encephalomyelitis susceptibility of the North American cottontail rabbit, jack rabbit, field vole, woodchuck and opossum to experimental infection. Amer. J. Hyg., 32 (2, sect. B):19–23.

12. LUGINBUHL, R. E., SATRIANO, S. F., HELMBOLDT, C. F., LAMSON, A. L., and JUNGHERR, E. L. 1958. Investigation of eastern equine encephalomyelitis. II. Outbreaks in Connecticut pheasants. Amer. J. Hyg. 67(1):4–9.

13. DOW, R. P., REEVES, W. C., and BELLAMY, R. E. 1957. Field tests of avian host preference of *Culex tarsalis* (Coq.). Amer. J. Trop. Med. & Hyg. 6(2):294–303.

14. BELLAMY, R. E., and REEVES, W. C. 1952. A portable mosquito bait-trap. Mosquito News 12(4):256–258.

15. SNEDECOR, G. W. 1946. Statistical methods, 4th ed. Iowa State College Press, Ames, Iowa. xvi 485 pp.

16. THOMAS, L. A., EKLUND, C. M., and RUSH, W. A. 1958. Susceptibility of garter snakes (*Thamnophis* spp.) to western equine encephalomyelitis virus. Proc. Soc. Exp. Biol. 99(3): 698–700.

17. THOMAS, L. A., and EKLUND, C. M. 1960. Overwintering of western equine encephalomyelitis virus in experimentally infected garter snakes and transmission to mosquitoes. Proc. Soc. Exp. Biol. 105:52–55.

18. CHAMBERLAIN, R. W., SIKES, R. K., NELSON, D. B., and SUDIA, W. D. 1954. Studies on the North American arthropod-borne encephalitides. Amer. J. Hyg. 60(3):278–285.

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

organic chemicals) and 1-phenyl-2-thiourea (M. W. 152.22, Eastman organic chemicals) was established so that concentrations of each chemical were obtained as follows: 0, .1, .01, .001, .0001, 00001 M. Two hundred ml. of each concentration of the two solutions were placed in flasks with .1 gm. of Purina rabbit feed pellet and 50 second-stage larvae of *Aedes aegypti,* and maintained for observation at room temperature (70° ± 4° F.). For the first four days larval mortality was recorded each 24 hours, and pupae and adults that developed in the low concentrations of the compound were harvested for further study. Adult mosquitoes were transferred to lantern chimney cages, given sucrose solution for food, allowed a three-day mating period; and then the females were given a blood feeding. These mosquitoes were maintained until egg deposition, the eggs then conditioned and tested for hatchability. The procedure was then repeated twice for replication, and the results summarized and compared to those obtained from controls.

RESULTS. The higher concentrations of both chemicals in the larval medium (.1, .01 M) were insecticidal, killing all of the larvae within four days. Larval mortality in both chemical solutions at .001M concentrations was quite variable, ranging from 3 to 88 percent among the six replicates. Larvae surviving in this concentration and in the .0001 M and .00001 M concentrations of both phenylthiourea and the 4-chlororesorcinol exhibited signs of lack of melanin formation. They also remained semi-transparent and failed to develop the brown cuticular coloration of the control larvae.

A more pronounced effect was observed in the growth of the larvae. The developmental period was extended to 15–21 days as compared to 7–10 days to pupation of the control larvae. There was considerably more pupal mortality in the treated groups than in the controls. This amounted to as much as 40 percent in those reared in .001 M. 4-chlororesorcinol. Adult mosquitoes from larvae reared in this solution failed to live more than 10 days. Only 4 females of a total of 83 adult mosquitoes that developed after rearing in the 4-chlororesorcinol solution lived beyond 5 days and developed eggs. These eggs, however, after oviposition, conditioning and testing for hatchability, proved to be viable and produced vigorous larvae. Mortality in the pupal and adult stages of mosquitoes from larvae grown in concentrations below .0001M phenylthiourea was not exceptionally more than among controls. Phenylthiourea treatment of larvae likewise did not affect the fertility or longevity of the adult mosquitoes.

Pupae and adult mosquitoes, developing from larvae that exhibited inhibition of melanin formation during the larval stage retained normal coloring in the pupal and adult stages so that it was impossible to distinguish a difference between those and treated or control groups.

DISCUSSION. In the experiments with 4-chlororesorcinol of Kull, *et al.* (1) there was the complete inhibition of melanin in regenerating fin of one species of fish. However, in another species of the same genus, the melanin inhibition was not complete—suggesting that even in closely related fish the pigment producing cells converted tyrosine to melanin under different conditions. In their experiments when the treated fish were transferred from medicated to untreated water, the melanin inhibition was reversible and the regenerating tissue became black. However, there was no extended investigation of the post-treatment effects. These effects on survival, growth and reproduction are of considerable interest inasmuch as little is known of the activity of tyrosine in the reproduction and longevity of insects. Mosquitoes, particularly males that feed only on carbohydrates in the adult stage theoretically accumulate the protein requirements for reproduction only during their larval life in the aquatic medium. This, together with their short generation time, makes them exceptionally good

experimental tools for the investigation of enzyme-inhibiting compounds *in vivo*.

While tyrosine is an amino acid that is not generally considered essential for growth of insects (Gilmore (3)), the addition of two compounds of known anti-tyrosinase activity at sub-insecticidal concentrations more than doubles the time necessary for development to the pupal stage. Likewise, the increased pupal mortality and greatly reduced longevity of adults following larval treatment with 4-chlororesorcinol indicate either a carry-over of toxicity or the induction of a severe nutritional deficiency.

As pointed out by Brown (4) phenylthiourea is of considerable interest in that it is selectively insecticidal against DDT-resistant flies. Chemicals with a similar mode of action that are more insecticidal then phenylthiourea may be of considerable importance if they are also selectively effective against DDT resistant insects. While 4-chlororesorcinol did not affect the fertility of *A. aegypti,* it is worthwhile to note that solutions of this compound are larvicidal at concentrations that are non-toxic to fish; therefore, studies utilizing DDT-resistant larvae are suggested.

Regardless of their larvicidal potential, chemicals that effectively lengthen the larval growth stage in the aquatic medium may be useful in enhancing biological control. The longer mosquito larvae are in contact with parasitic fungi in the water and are exposed to predatory fish, the better chance these agents have to produce an effect on the larval population; 4-chlororesorcinol may be useful for this purpose since it significantly lengthens larval developmental time at dilutions that are sub-toxic to fish. It has the additional advantage of rapid dissociation in aqueous solution; so the possibility of providing a cumulative residual toxicant to endanger wildlife is minimal.

References

1. KULL, F. C., BONORDEN, R., and MAYER, R. L. 1954. Inhibition of melanin formation in-vivo by 4-chlororesorcinol. Proc. Soc. Exp. Biol. & Med. 87:538–540.

2. KULL, F. C., GRIMM, M. R., and MAYER, R. L. 1954. Studies on inhibition of tyrosinase. Proc. Soc. Exp. Biol. and Med. 86:330–332.

3. GILMORE, P. 1961. The Biochemistry of Insects. Academic Press, New York, 343 pp.

4. BROWN, A. W. A. 1961. The challenge of insecticide resistance. Bull. Ent. Soc. Amer. 7(1): 6–19.