

Studies on *Aedes aegypti* Infected in the Larval Stage with the Virus of Yellow Fever.*

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During the course of general transmission studies with *Aedes aegypti*, it became of interest to see whether these mosquitoes could be infected in the larval stage, and retain the infection into adult life. To this end, half-grown larvæ were placed in a bowl containing a mixture of blood serum from a Rhesus monkey at the height of infection with the Asibi strain of yellow-fever virus, and an equal quantity of physiological salt solution. This was shown, by titration in mice, to contain 30,000,000 minimum lethal doses for mice per cc. Pupation commenced after 48 hours. The pupæ were washed and suspended individually in tubes of clean water, and the adults began to emerge 4 days after the first contact with the virus. The first 28 males to emerge were ground up and injected into a Rhesus monkey subcutaneously on the day of emergence. The monkey responded 4 days later with fever, and had virus in its blood stream. It was killed when moribund on the sixth day. Necropsy findings, both macroscopic and microscopic, were typical of yellow fever. Four days later, 15 females, varying in age from one to 4 days, were fed on a normal Rhesus, but failed to infect it. However, 10 days later 29 females from the same lot were able to produce yellow fever by bite in another Rhesus monkey.

To confirm this observation, half-grown larvæ were again placed in 50% serum from an infected Rhesus. This mixture was shown to contain 7,600,000 M.L.D. for mice per cc. In order to prevent the mosquitoes from pupating in the viral suspension, the larvæ were washed 3 times in water at the end of 24 hours, and placed in a bowl of clean water. Pupation commenced the following day, and the first adults appeared 2 days later. Thirty of the first males to emerge were ground up on the day of emergence and the suspension was titrated in mice, by the technic described by one of us.¹

*The studies and observations on which this paper is based were conducted with the support and under the auspices of the Cooperative Yellow Fever Service, maintained by the Brazilian Ministry of Education and Health and the International Health Division of The Rockefeller Foundation.

¹ Whitman, L., *J. Exp. Med.*, 1937, **66**, 133.

Virus was present though the titer was low (284). Thirty more males were tested at 7 and another 30 at 14 days after emergence. The titers had increased considerably (72,500 and 127,500, respectively). In the meantime, 25 four-day-old females fed on a Rhesus monkey, which died of yellow fever.

Using the same technic, we have infected mosquitoes with the mouse-brain-adapted neurotropic strain of the French virus in its 530th to 540th passage in mice.

At this time, it was of interest to determine whether larvæ could be infected when new born and retain the virus throughout the entire aquatic stage. To study this, eggs were placed in serum virus mixtures, and after a few hours those which had not hatched were removed. All resultant larvæ thus had approximately the same exposure to the virus. On the following day, the larvæ were washed 3 times before being placed in a bowl of clean water. Unfortunately, in 3 such experiments the titer of virus in which the eggs were hatched was low, and the adults were subsequently shown to be non-infectious. However, one lot, infected in a suspension of neurotropic virus containing 33,000,000 M.L.D. for mice per cc., was shown, by titration in mice, to have retained the virus during the 7 to 8 days between washing and emergence. From this it would seem that unusually large initial infections are necessary for the larvæ to retain the virus throughout the entire aquatic stage.

The possibility of the transmission of virus through the egg was also considered in this series of experiments. It was thought that, since by the above method males as well as females could be infected, virus might be introduced into the embryos either through the spermatozoa or through the ova, or both. To test this theory, newly-emerged infected males and females were mated, and the females allowed to feed on normal guinea pigs to promote oviposition. Ten days later the first lot of eggs was removed. However, on the assumption that time was necessary for testes and ovaries to become infected, a second lot of eggs was collected over the next 15 days. These had been produced between the tenth and twenty-fifth days of infection. Both batches of eggs were placed in water, and eventually the resultant adults were ground up and injected into both mice and monkeys. No virus was recovered from them.

From this it can be seen that *Aedes aegypti* can be infected in the larval stage with the virus of yellow fever, providing they are exposed to large quantities of virus. The resultant adults, both male and female, are infected. Whether the infection produced in this way is identical with an infection following the ingestion of virus by

feeding during adult life remains to be seen. Nevertheless, this method of infecting mosquitoes offers possibilities for the study of transmission, particularly with insects which can be brought to the laboratory in the egg or larval stage.

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Vascular, Metabolic, and Motility Responses of Uterine Tissue Following Administration of Oestrin.

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Within 30 minutes after the administration of oestrin to ovariectomized rabbits, a maximal hyperemia of the uterine vascular bed takes place.¹⁻⁴ Rhythmic motility of the myometrium does not commence until about the tenth hour after injection of oestrin, and increases progressively until about the twenty-fourth hour.^{5, 6, 2} In addition to these two effects, oestrin also raises the metabolic rate of uterine tissue.⁷⁻¹⁰ The present experiments were performed to ascertain to what extent the initial hyperemia, rise of metabolic activity of the tissue and motility are related to each other.

Two main groups of experiments were performed. In one, the O₂ consumption rate was measured in a group of untreated rabbits (3½ to 4 months of age), 5 of which were intact and the remainder of which (21 animals) were ovariectomized for periods ranging from 2 to 15 days. In the second group, the O₂ consumption rates were measured at intervals of 5, 10, 18, and 24 hours after a single

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