equivalent to those obtained by assay of each hormone separately.

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Transmission of WEE Virus to Snakes by Infected *Culex tarsalis* Mosquitoes.* (31452)

LOUIS P. GEBHARDT, G. JOHN STANTON, AND STEPHEN DE ST. JEOR
Department of Microbiology, College of Medicine, University of Utah, Salt Lake City

Previous studies have shown that *Culex tarsalis* mosquitoes will readily feed on snakes (Thamnophis spp) under laboratory conditions (Gebhardt *et al*(1); Thomas(2), and that a WEE virus titer in snakes as low as 2.7×10^2 PFU (plaque forming units) per ml of blood will infect 31.2% of the mosquitoes feeding (Gebhardt *et al*), which is equivalent to at least 1.0 PFU per infectious blood meal.† The present report deals with the transmission of WEE virus to snakes by infected *C. tarsalis* mosquitoes.

Materials and methods. Baby chicks were infected by subcutaneous injection of 2×10^4 PFU of chick cell cultured WEE virus. Mosquitoes *(C. tarsalis)*‡ were fed on these baby chicks, the virus allowed to maturate for 14 days, then 12 snakes (Thamnophis spp) were exposed to these infected vectors. After taking a blood meal from the snakes, the mosquito's head and thorax was removed and triturated with measured amounts of ground glass in complete Melnick's culture medium. A total of 2 ml of culture fluid was added during the grinding. During the trituration process the samples were frozen and thawed several times in acetone-dry ice. These samples were stored at −27 to −30°C until tested for virus. Snakes were bled from the heart at various times after the mosquito had taken a blood meal and the bloods diluted 1:10 with complete Melnick's culture medium and stored at the above temperatures until tested for virus. All snakes were about the same size, thus approximately the same age. Snakes were maintained at room temperature, 72-76°F. Virus assays were made on monolayer cultured chick embryo cells, using 60 × 10 mm Falcon plastic petri dishes. Melnick's culture fluid with phenol red to which was added 5 g/liter of lactalbumin hydrolysate and 5% calf serum, with 100 units of penicillin and 100 μg streptomycin per ml was used. Plaque forming units (PFU) were determined after incubating the inoculated monolayers at 37°C under 5% CO_2 for 30-36 hours with an agar overlay. Plaques were counted after adding neutral red and incubating for 12 to 18 hours at room temperature.

Results. These results (Table I) clearly show that not all snakes are equally susceptible to WEE virus injected by an infected mosquito during a blood meal. The results

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† The female mosquitoes from our colony average 3 mg of blood per meal.

‡ Larvae to start our colony were obtained through the courtesy of Dr. A. Hess, Field Station PHS Laboratory, Greeley, Colo. in 1961.

TABLE I. Number of *C. tarsalis* Mosquitoes Biting Snakes, Virus Titer (PFU) of the Mosquitoes at the Time of Biting the Snake, and Snake Blood Titers (PFU) at Various Times After Being Bitten by Infected Mosquitoes.

Snake No.	No. of mosquitoes feeding on snakes	Date snake bitten by WEE-infected mosquito and virus titer of mosquitoes, PFU	Date snakes bled after mosquito feeding (days in parentheses)	WEE virus titer of snake blood, PFU
34	1	8/24/65 2.5×10^5	8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (16) 10/13/65 (50)	0* 0 0 0 0
42	1	8/24/65 1.5×10^5	8/25/65 (1) 8/26/65 (2) 8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28)	1.66×10^2 0 1.2×10^2 5×10^1 1.66×10^2 2.55×10^5 0
43	1	8/25/65 7.5×10^4	4 bleedings 8/25 to 8/30 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28) 10/13/65 (50)	0 5×10^1 1.45×10^2 0 0
44	1	8/24/65 1.75×10^4	8/25/65 (1) 8/26/65 (2) 8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28) 10/13/65 (50)	3.83×10^3 1.0×10^6 2.1×10^7 1.05×10^5 1.9×10^8 1.55×10^2 0 0
46	1	8/25/65 1.05×10^4	3 bleedings Aug.; 3 bleedings Sept.; 1 bleeding Oct.	0
47	1	8/24/65 4.25×10^4	8/25/65 (1) 8/26/65 (2) 8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28) 10/13/65 (50)	0 0 2.3×10^4 3.0×10^4 2.0×10^3 1.83×10^3 0 0
128	1	8/25/65 1.6×10^4	4 bleedings Aug.; 3 bleedings Sept.; 1 bleeding Oct.	0
257	1	8/24/65 8.5×10^3	4 bleedings Aug.; 3 bleedings Sept.; 1 bleeding Oct.	0
50	2	8/24, 25/65 1. 1.8×10^4 2. 7.35×10^4	8/25/65 (1) 8/26/65 (2) 8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28)	0 0 0 0 0 1.3×10^6 0
262	2	8/24/65 8/25/65 1. 6.59×10^4 2. 5×10^2	4 bleedings Aug.; 3 bleedings Sept.	0

(Continued next page)

TABLE I (continued).

Snake No.	No. of mosquitoes feeding on snakes	Date snake bitten by WEE-infected mosquito and virus titer of mosquitoes, PFU	Date snakes bled after mosquito feeding (days in parentheses)	WEE virus titer of snake blood, PFU
51	3	8/24/65 1. 5.25×10^4 2. 7.0×10^4 3. 4.0×10^4	8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28)	2.9×10^7 2.1×10^7 1.95×10^6 1.5×10^4 0
252	3	8/24/65 2 mosq. 8/25/65 1 mosq. 1. 1×10^2 2. 1.3×10^5 3. 9.67×10^4	8/25/65 (1) 8/26/65 (2) 8/27/65 (3) 8/30/65 (6) 9/ 3/65 (10) 9/10/65 (17) 9/21/65 (28)	1.66×10^2 3.33×10^3 1.22×10^7 2.0×10^5 2.4×10^4 1.4×10^6 3.33×10^2

* 0 = no virus isolated from snake blood.

also show that the viremia in snakes varies, not only with the time of virus appearance, but also the quantity of virus produced. The final virus titer in the snake blood apparently bears little relationship to the amount of virus present in the mosquito at the time of the blood meal. Two snakes bitten by 3 infected mosquitoes each appeared to produce a more sustained viremia than when a single mosquito transmitted the virus. In general, each *C. tarsalis* mosquito appears to maturate about the same total amount of virus after an infectious blood meal, the low being 1×10^2, a high of 2.5×10^5 and a mean of 2.2×10^4 PFU per mosquito for 18 mosquitoes.

Discussion. It is not clear why some snakes do while others do not develop a viremia when bitten by a WEE-infected mosquito harboring sufficient virus to produce an infection. Whether it is the resistance of the particular snake or some fault in the mosquito regurgitating mechanism is not known. Some of the snakes (44, 51, 252) produced an immediate viremia, while others showed a lag of two or more days. Snake 50 behaved rather peculiarly in that virus was not detected in the blood until 17 days after being bitten by two infected mosquitoes. This snake then developed a high virus titer (1.3×10^6 PFU/ml blood) with a loss of viremia within 10 days.

Summary. Of 12 snakes offered as blood meal sources for 18 infected mosquitoes, seven of these poikilothermic animals responded with a viremia. Six developed titers of 10^5 or more, while one developed a viremia of only 1.45×10^2 PFU per ml of blood. Two developed titers of 10^7 and one 10^8 PFU per ml of blood. Five developed a viremia within one to three days, while one was delayed to the 9th day and one was delayed to the 17th day after being bitten. The virus content of the mosquito biting the snake appeared not to be a determining factor whether the snake became infected or not. When snakes were exposed to a single infected mosquito each, 4 of 8 became viremic. When snakes were exposed to 2 or 3 infected mosquitoes each, 3 of 4 became viremic.

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