THE USE OF A MEMBRANE FEEDING TECHNIQUE TO DETERMINE INFECTION AND TRANSMISSION THRESHOLDS OF SEMLIKI FOREST VIRUS IN *ANOPHELES QUADRIMACULATUS* AND *ANOPHELES ALBIMANUS*

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The Semliki Forest virus (SFV) was first isolated from a group of *Aedes abnormalis* mosquitoes from Uganda (Smithburn and Haddow, 1944) and later isolated from *Aedes argenteopunctatus* collected in Portuguese East Africa (McIntosh, *et al.*, 1961). Semliki Forest virus belongs to Group A of the arthropod-borne viruses.

The virus has been shown to be transmitted by *Aedes aegypti* (Davies and Yoshpe Purer, 1954, and Woodall and Bertram, 1959) and by *Aedes togoi* (Nye and Lien, 1960) using suckling mice as the transmission animal. The virus has also been shown to be transmitted by *Anopheles albimanus* and *A. quadrimaculatus* (Collins, 1963) using wet baby chicks as the transmission animal.

Reported here are the results of studies to determine the infection and transmission thresholds of SFV for *A. albimanus* and *A. quadrimaculatus* using the membrane feeding technique.

METHODS AND PROCEDURES. The virus was Semliki Forest virus (SFV), strain R-1-1, mouse brain passage 12, obtained through the courtesy of Dr. Telford Work, Communicable Disease Center, Atlanta, Georgia.

The *Anopheles quadrimaculatus* mosquitoes were the Q-1 strain which was obtained from Technical Development Laboratories, CDC, Savannah, Georgia and maintained in our laboratory since 1959.

The *Anopheles albimanus* were the A-9 strain originally obtained from San Salvador and maintained in our laboratory since 1960.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrane on serial 10-fold dilutions of SFV in fresh heparinized human blood. The brains of six moribund mice were ground in three milliliters of Bacto-heart infusion broth (DIFCO) and centrifuged for 15 minutes at 1500 r.p.m. Serial 10-fold dilutions of the supernatant were made in broth. For mosquito feeding, one part of each dilution was added to four parts of blood; this was then warmed to 37° C and placed on the membrane which formed the bottom of a ½ pint feeding cup. The cup was then placed on top of the cage containing the mosquitoes. The feeding period was 15 minutes after which time the engorged mosquitoes were transferred to holding cages and stored in an incubator at 25° C. to 26° C. The mosquitoes were fed 5 percent Karo solution daily on a cotton pledget.

After 10 days of extrinsic incubation, mosquitoes were allowed to feed individually on wet baby chicks. Approximately 48 hours later, blood samples were taken by cardiac puncture and the blood inoculated intracerebrally into 4 mice. Presence of virus in the chick blood constituted evidence of virus transmission by the mosquito.

Samples of mosquitoes were collected and killed by freezing immediately after feeding and after 10 days of extrinsic incubation. These were stored in a me-

chanical freezer at —65° C. to —70° C. until titrated. To determine virus titers, mosquitoes were ground individually in a mortar with a 1 ml. aliquot of Bacto-heart infusion broth containing 1000 units of penicillin and 2 milligrams of streptomycin per ml. The suspension was centrifuged for 15 minutes at 1500 r.p.m. and serial 10-fold dilutions were made in the broth. Five 3-week-old mice were inoculated intracerebrally per dilution and the LD_{50}'s calculated by the method of Reed and Meunch (1938).

RESULTS. Three separate experiments were made, two of which involved transmission studies. The results of these experiments have been grouped according to the SFV titers originally ingested by the mosquitoes. The relationship between the virus ingested by *A. quadrimaculatus* mosquitoes and that present after 10 days of extrinsic incubation is shown in Table 1. The infection threshold was at approximately 3.2 mouse log IC LD_{50} and the 50 percent infection level at approximately 4.7 mouse log IC LD_{50}. The positive SFV titers ranged from 2.3 to 7.3 with 13 of the 67 positive mosquitoes (19 percent) having titers of 5.0 or greater.

The relationship between the virus ingested by *A. albimanus* and that present after 10 days of extrinsic incubation is shown in Table 2. The infection threshold was at approximately 3.3 mouse log IC LD_{50} and the 50 percent infection level at approximately 5.4 mouse log IC LD_{50}. The positive SFV titers ranged from 3.2 to 7.1 with 27 of the 31 positive mosquitoes (87 percent having titers of 5.0 or greater.

The results of the transmission studies are shown in Table 3. The mean positive SFV titers are for all the mosquitoes which were allowed to feed. In these tests, a total of 3 *A. quadrimaculatus* and 6 *A. albimanus* transmitted SFV. The trans-

TABLE 1.—Relationship between Semliki Forest virus ingested by *A. quadrimaculatus* mosquitoes and that present after 10 days of extrinsic incubation.

Initial			10 Day			
SFV * Ingested	Pos./ Tested	Range of SFV Titers	Pos./ Tested	Percent infected	Positive SFV Titers Mean	Positive SFV Titers Range
6.9	4/4	6.3–7.3	11/13	85	5.1	3.3–7.3
5.9	16/16	4.6–7.0	37/40	92	4.0	2.3–7.0
4.7	12/12	3.8–5.1	15/30	50	3.2	2.3–5.0
4.0	4/4	3.5–4.3	3/10	30	2.7	2.7
3.2	4/4	3.1–3.3	1/10	10	2.7	2.7
2.0	4/4	1.2–2.7	0/10	0	...	...

* Mean of initial SFV titers (mouse log IC LD_{50}).

TABLE 2.—Relationship between Semliki Forest virus ingested by *A. albimanus* mosquitoes and that present after 10 days of extrinsic incubation.

Initial			10 Day			
SFV * Ingested	Pos./ Tested	Range of SFV Titers	Pos./ Tested	Percent infected	Positive SFV Titers Mean	Positive SFV Titers Range
6.7	4/4	6.1–7.0	3/3	100	6.5	5.7–7.0
6.0	8/8	5.3–6.5	13/16	81	5.8	3.2–6.9
5.2	12/12	4.8–6.0	5/12	42	6.7	6.3–7.1
4.2	12/12	3.3–5.0	9/30	30	5.5	4.0–6.2
3.3	4/4	3.0–3.8	1/10	10	5.0	5.0
2.7	1/4	2.7	0/10	0	...	...

* Mean of initial SFV titers (mouse log IC LD_{50}).

Table 3.—Effect of initial SFV titer on virus transmission by *Anopheles quadrimaculatus* and *A. albimanus*.

Anopheles quadrimaculatus				*Anopheles albimanus*			
		SFV Titers				SFV Titers	
Initial SVF Titer	Trans./ Attempts	Mean Pos.*	Trans. Mosq.	Initial SFV Titer	Trans./ Attempts	Mean Pos.*	Trans. Mosq.
6.9	3/13	5.1	7.3, 6.7, 6.3	6.7	1/3	6.5	5.7
5.9	0/39	4.0		6.0	3/16	5.8	6.0, 6.0, 5.7
4.7	0/20	3.2		5.2	2/12	6.7	7.1, 6.8
4.0	0/10	2.7		4.2	0/30	5.5	

* Mean positive SFV titer for all mosquitoes allowed to feed.

mission threshold for the *A. quadrimaculatus* was between 5.9 and 6.9 mouse log IC LD_{50} and for the *A. albimanus* was between 4.2 and 5.2 mouse log IC LD_{50}. The titers of the transmitting mosquitoes ranged from 5.7 to 7.3.

Discussion. The use of the membrane feeding technique to determine infection and transmission thresholds has herein been shown to be feasible using Semliki Forest virus and *Anopheles quadrimaculatus* and *A. albimanus*. Its extension to other mosquito-arbovirus systems would appear to be practicable thus acting as an adjunct to the use of experimental animals with circulating viremias.

As applied to the present system, it appears that the SFV infection thresholds for *A. quadrimaculatus* and *A. albimanus* are essentially equal whereas *A. albimanus* has a lower transmission threshold than does *A. quadrimaculatus*. In addition, the higher percentage of the positive *A. albimanus* having titers of 5.0 mouse log IC LD_{50} or greater would confirm the previous suggestion (Collins, 1963) that *A. albimanus* has a greater vector potential for SFV than does *A. quadrimaculatus*.

Summary. A technique is described whereby serial 10-fold dilutions of Semliki Forest virus were fed upon by *Anopheles quadrimaculatus* and *A. albimanus* mosquitoes. The infection thresholds for these mosquitoes were approximately equal, being 3.2 and 3.3 mouse log IC LD_{50}. The transmission threshold for the *A. quadrimaculatus* was between 5.9 and 6.9 and for the *A. albimanus* was between 4.2 and 5.2 mouse log IC LD_{50}. It is postulated that the latter mosquito has a greater SFV vector potential.

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