ing females ovipositing in these standing water areas.

ACKNOWLEDGMENTS. The writer expresses his appreciation to the Merced County Mosquito Abatement District for assistance and cooperation in various phases of the study and to Mr. Grafton Campbell, Bureau of Vector Control, for his excellent work in the field aspects of the study.

Literature Cited

LEACH, HOWARD R. 1960. The wildlife and fishery resources in relation to drainage problems in the San Joaquin Valley. Pp. 53–4 in Calif. Dept. of Fish and Game report. 127 pp. Mimeographed.

MCHUGH, ROBERT A. 1958. Interrelationships of plants, mosquitoes and waterfowl in the Great Salt Lake area. Pp. 156–61 in Summary of Investigations No. 15, Communicable Disease Center, Technology Branch, U. S. Public Health Service. 243 pp. (Unpublished records cited with permission.)

ROSAY, BETTINA. 1960. Advances in behavioral studies. Proc. Calif. Mosquito Control Assoc. 28: 88–9.

U. S. DEPT. OF THE INTERIOR, FISH & WILDLIFE SERVICE, REGIONAL DIRECTOR'S OFFICE, PORTLAND, OREGON. 1954. Pp. 7–10 in Wetland Inventory of California. 54 pp. Mimeographed.

TRANSMISSION OF SEMLIKI FOREST VIRUS BY *ANOPHELES ALBIMANUS* USING MEMBRANE FEEDING TECHNIQUES

WILLIAM E. COLLINS [1]

With the Technical Assistance of
Mr. Leo E. Chester and Mrs. Jean R. Westbrook

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 infection of mosquitoes by allowing them to feed through membranes onto virus containing mixtures has been reported by a number of workers. *Aedes aegypti* mosquitoes have been infected with dengue virus using a guinea pig skin (St. John, *et al.*, 1930) and with Chikungunya and Makonde viruses using a bat-wing membrane (Ross, 1956). This mosquito has also been infected with Zika virus (Boorman and Porterfield, 1956), Uganda S virus (Boorman, 1958), and Semliki Forest virus (Nye and Bertram, 1960) using mouse skin as a membrane.

Reported here are the results of studies to infect *Anopheles albimanus* mosquitoes with Semliki Forest virus using an animal derived membrane, and to determine the quantity of virus inoculated during mosquito feeding using this membrane.

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.

[1] Department of Health, Education, and Welfare, Public Health Service, National Institute of Allergy and Infectious Diseases, Laboratory of Parasite Chemotherapy, Section on Epidemiology, P.O. Box 195, Chamblee, Georgia.

The *Anopheles albimanus* mosquitoes were the A-9 strain originally from El Salvador and were obtained through the courtesy of Dr. H. G. Simkover, Shell Development Company, Modesto, California. The colony has been maintained since 1960.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrane[2] on a SFV-defibrinated chicken blood pool. Comparable virus pools for the several experiments were prepared by aseptically harvesting the brains of two mice dying from SFV infection. The brains were ground in three milliliters of Bacto-heart infusion broth (Difco) and centrifuged for 15 minutes at 1500 r.p.m. The supernatant was then diluted 1:9 in the defibrinated chicken blood, warmed to 37° C. and put in a small 1⅛-inch diameter plastic tube, the bottom of which was covered with the membrane. This was then placed on top of a cage containing the mosquitoes which fed directly through the screening and the membrane to obtain the infectious blood meal. Feeding was allowed to continue for 30 to 60 minutes after which 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 honey solution daily on a cotton pledget.

In membrane transmission studies, mosquitoes were caged individually in small plastic tubes, both ends of which were covered with nylon bobbinet. A one milliliter aliquot of defibrinated chicken blood was placed in a small glass tube containing a rubber tipped plunger. The blood was forced to the top of the tube which was then covered with a piece of the Baudruche membrane. This piece of equipment enabled the mosquito to feed down into the blood pool. The blood tube was then held under the mosquito cage until the mosquito had fed. The blood was removed with a hypodermic syringe by piercing the membrane.

In control animal transmission studies, infected 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 four mice. Presence of virus in the chick blood constituted evidence of virus transmission by the mosquito. Cause of mouse deaths was confirmed by serum neutralization tests using the brains from dying mice and hyperimmune chicken serum.

To determine virus titers, mosquitoes were ground individually in a mortar with a one milliliter aliquot of Bacto-heart infusion broth containing 1000 units of penicillin and two milligrams of streptomycin per milliliter. The suspension was centrifuged for 15 minutes at 1500 r.p.m. and serial ten-fold dilutions made. To determine virus titers in the membrane transmission blood pools, the blood was first diluted 1:1 with the broth containing penicillin and streptomycin and then the serial ten-fold dilutions were made. Five 3-week-old mice were inoculated intracerebrally per dilution and the LD_{50}'s were calculated by the method of Reed and Meunch (1938).

RESULTS. A total of 104 mosquitoes were used in the membrane transmission studies. The results of the membrane transmissions are shown in Table 1. Of a total of 81 infected mosquitoes, 30 transmitted SFV in detectable quantities. The feedings were on days 6, 8, 9, 10, 11, 12, 13, and 14 days post infection. The results on days 6 and 8 were similar and have been grouped. The remainder of the feedings have been grouped into 2-day intervals for convenience in tabulating. It is apparent that high levels of transmission (41 to 64 percent) were obtained by day 9 and continued through day 14. The virus titers in those mosquitoes which transmitted the virus in detectable quantities ranged from 5.7 to 7.5 mouse log IC LD_{50}.

The results of the viral titrations of the mosquitoes and blood-membrane trans-

[2] Obtained from Long & Long, Manufacturers of Baudruche Capping, Pad Skins, Kindred Products, 20 Roosevelt Avenue, Belleville 9, N. J.

TABLE 1.—Mean Semliki Forest virus titers and transmission rates in studies with *Anopheles albimanus* mosquitoes using Baudruche membrane and chicken blood pools.

Days post infection	SFV titers (Mouse log IC LD_{50})							
	Mean virus titer in all mosquitoes	Transmitting mosq.		Blood pool		Mean* difference	Positive trans./ attempts	Percen trans.
		Mean	Range	Mean	Range			
6, 8	6.6	6.8	6.3–7.1	2.0	1.5–2.3	4.8	3/31	9.7
9, 10	6.5	7.0	5.8–7.5	2.5	1.2–3.7	4.5	13/22	59.1
11, 12	6.3	6.5	5.7–7.0	2.9	1.5–3.6	3.6	7/17	41.2
13, 14	6.1	6.5	6.3–6.7	2.8	2.0–4.0	3.7	7/11	63.6
Mean	6.4	6.7	5.7–7.5	2.6	1.2–4.0	4.1	30/81**	37.0

* Difference between SFV titer in transmitting mosquitoes and that found in blood pool.
** Totals.

mission pools are also shown in Table 1. The mean viral titers for the transmitting mosquitoes were on all days higher than that of all the infected mosquitoes. There was, however, a considerable amount of overlapping in that some mosquitoes with high titers failed to transmit and some with lower titers transmitted SFV in detectable quantities.

The quantity of virus inoculated through the membrane ranged from 1.2 to 4.0 mouse log IC LD_{50}. The mean difference between the quantity of virus present in the mosquito and that inoculated was higher earlier in the extrinsic incubation period than after longer incubation. For all of the transmitting mosquitoes, the mean difference between mosquito virus content and virus inoculated was on the order of 4.1 mouse log IC LD_{50} or approximately 0.01 percent.

The results of transmissions of SFV to wet baby chicks by *Anopheles albimanus* mosquitoes are shown in Table 2. The mean SFV titer in all the mosquitoes used in these transmissions was approximately the same as in the membrane transmissions with a mean of 6.3 mouse log IC LD_{50} as compared to 6.4. Here again the mean titer of the transmitting mosquitoes was higher than the group as a whole. The rate of transmission was 34 percent as compared to 37 percent for the membrane feedings. The results of transmissions using membranes thus appear to be comparable to those obtained using baby chicks. The virus titers in those mosquitoes which transmitted the virus to baby chicks ranged from 5.5 to 7.3 mouse log IC LD_{50}.

DISCUSSION. Attempts to determine the quantity of virus inoculated during the feeding of mosquitoes have been reported by several workers. Davis (1934) allowed yellow fever-infected *Aedes aegypti* mosquitoes to feed on new-born white mice. The mice were immediately killed, extracted and inoculated into rhesus mon-

TABLE 2.—Transmission of Semliki Forest Virus by *Anopheles albimanus* mosquitoes to wet baby chicks.

Days post infection	Trans.	Percent trans.	Mean SFV titers	
			All mosq.	Trans. mosq.
7	2/7	29	6.0	6.8
10	12/34	35	6.3	6.5
Mean	14/41**	34	6.3	6.6

** Totals.

keys. Titrations showed that the mosquito injected at least 100 infective doses of the virus. A comparison of the amounts of virus in the whole mosquito and in the baby mouse after being fed upon showed that about 1 percent of the total virus content of the mosquito was injected at the time of feeding. Chamberlain, *et al.* (1954) allowed *Aedes aegypti* mosquitoes infected with Eastern encephalitis virus to feed on chicken serum through fresh or fixed chick skins or to feed on a fragment of solid blood agar. Their studies indicated that the majority of infected mosquitoes inoculated less than 100 mouse intracerebral LD_{50} during feeding, and that occasionally 1,000 to 10,000 mouse IC LD_{50} may be injected and rarely 10,000 to 100,000.

In the present work, the use of an animal-derived membrane has enabled the determination of the quantity of virus inoculated by *Anopheles albimanus* mosquitoes infected with Semliki Forest virus. The detectable virus quantities were found to be as high as 4.0 mouse log IC LD_{50}. The transmission rate of 37 percent was comparable with that obtained using baby chicks. The mean of the detectable SFV titers inoculated was 2.6 with a mean difference between the virus present in the mosquito and that inoculated being 4.1 mouse log IC LD_{50}. It is therefore estimated that those mosquitoes which transmit Semliki Forest virus inoculated approximately 0.01 percent of the virus present in their bodies.

SUMMARY. A technique is described using a Baudruche membrane to determine the quantity of Semliki Forest virus inoculated during the feeding of *Anopheles albimanus* mosquitoes. The mosquitoes inoculated detectable virus quantities as high as 4.0 mouse log IC LD_{50} with a mean difference between virus present in the mosquito and that inoculated being 4.1. It is estimated that those mosquitoes which transmit SFV inoculated approximately 0.01 percent of the virus present in their bodies.

References

BOORMAN, J. P. T. 1958. Transmission of Uganda S virus by *Aedes aegypti* Linn. Trans. Roy. Soc. Trop. Med. and Hyg. 52:383–388.

BOORMAN, J. P. T., and PORTERFIELD, J. S. 1956. A simple technique for infection of mosquitoes with viruses. Trans. Roy. Soc. Trop. Med. and Hyg. 50:238–242.

CHAMBERLAIN, R. W., KISSLING, R. E., and SIKES, R. K. 1954. Studies on the North American arthropod-borne encephalitides. VII. Estimation of amount of Eastern equine encephalitis virus inoculated by infected *Aedes aegypti*. Amer. Jour. Hyg. 60:286–291.

DAVIES, A. M., and YOSHPE PURER, Y. 1954. The transmission of Semliki Forest virus by *Aedes aegypti*. Jour. Trop. Med. and Hyg. 57:273–275.

DAVIS, N. C. 1934. Attempts to determine the amount of yellow fever virus injected by the bite of a single infected *Stegomyia* mosquito. Amer. Jour. Trop. Med. 14:343–354.

MCINTOSH, B. M., WORTH, C. B., and KOKERNOT, R. H. 1961. Isolation of Semliki Forest virus from *Aedes (Aedimorphus) argenteopunctatus* (Theobald) collected in Portuguese East Africa. Trans. Roy. Soc. Trop. Med. and Hyg. 55:192–198.

NYE, E. R., and BERTRAM, D. S. 1960. Comparison of natural and artificial infection of *Aedes aegypti* L. with Semliki Forest virus. Virology. 12:570–577.

NYE, E. R., and LIEN, J. C. 1960. Laboratory transmission of Semliki Forest virus by *Aedes togoi* Theo. Trans. Roy. Soc. Trop. Med. and Hyg. 54:263–264.

REED, L. J., and MEUNCH, H. S. 1938. Simple method of estimating fifty per cent endpoints. Amer. Jour. Hyg. 27:493–497.

ROSS, R. W. 1956. A laboratory technique for studying the insect transmission of animal viruses employing a bat-wing membrane demonstrated with two African viruses. Jour. Hyg. 54:192–200.

ST. JOHN, J. H., SIMMONS, J. S., and REYNOLDS, F. H. K. 1930. Transmission of dengue virus from infected to normal *Aedes aegypti*. Amer. Jour. Trop. Med. 10:23–24.

SMITHBURN, K. C., and HADDOW, A. J. 1944. Semliki Forest virus. I. Isolation and pathogenic properties. Jour. Immunol. 49:141–157.

WOODALL, J. P., and BERTRAM, D. S. 1959. The transmission of Semliki Forest virus by *Aedes aegypti* L. Trans. Roy. Soc. Trop. Med. and Hyg. 53:440–444.