

and para-aminobenzoic acid, together with brewer's yeast. Both were men. The fact that the only 2 substantiated results occurred in the group receiving both calcium panto-

thenate and para-aminobenzoic acid may be of importance. Some questionable changes were noted when one or the other drug was given alone.

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Transmission of West Nile Virus by Infected *Aedes albopictus*.

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The West Nile virus,¹ a neurotropic agent related immunologically to the viruses of St. Louis and Japanese encephalitis,² has apparently caused human infection in widely scattered communities in equatorial Africa.³ Comparatively little is known about the natural mode of transmission of this agent;¹⁻³ however, the importance of arthropod vectors in the spread of other neurotropic viruses (see references cited in ⁴) warranted an investigation of their role in West Nile encephalitis. Young hamsters have been shown recently to be suitable for use in experiments dealing with the insect transmission of West Nile virus.⁴ The results presented at this time indicate that *Aedes albopictus* can become infected by feeding on hamsters with West Nile encephalitis and that following an incubation period such mosquitoes can transmit the virus to hamsters by biting. Further tests of transmission with this and other species of mosquitoes and with certain argasid ticks and triatomid bugs are in progress.

Three hamsters, weighing 30 to 40 g, were injected intraperitoneally with a suspension of mouse brain containing 10^8 mouse cerebral lethal doses (CLD) of virus. Twenty-four hours later the animals were anesthetized with sodium barbital given intraperitoneally in doses of 50 mg per 100 g body weight. During

the succeeding 2 hours 76 *Aedes albopictus* were fed on the shaven abdomens of the hamsters. Pooled plasma obtained from the hamsters immediately after the meal contained $10^{4.6}$ CLD of virus; the donors died on the 6th, 7th, and 8th days respectively after intraperitoneal inoculation. Ten insects were killed with tobacco smoke at the end of the donor meal and triturated in 2 cc of saline solution containing 2% normal guinea pig serum; this suspension was centrifuged at 3,000 r.p.m. for 10 minutes after which the clear, claret-colored fluid of the midzone was removed with a capillary pipette. All 6 of the mice injected with this suspension succumbed in 5 to 8 days; suspensions of brain tissue from 2 of these were bacteriologically sterile and were infectious for mice when injected intracerebrally.

The average weight of an adult *A. albopictus*, determined by weighing groups of 40 insects of the same stock and of about the same age as those used in the present experiment, was 2.31 mg when engorged and 1.31 mg when without blood. Therefore, suspensions of the type just described containing mosquito tissue and donor blood were considered as being 10^{-2} dilutions of either insect material or blood depending on which substance was being tested. Although the titration of virus in this lot of mosquitoes was not carried higher than 10^{-2} it was assumed that its titer was the same as that of the donor plasma, namely $10^{-4.6}$.

Forty-three mosquitoes of this lot, after storage for 10 days at 90°F, bit 2 baby hamsters which weighed 25 and 30 g, respec-

¹ Smithburn, K. C., Hughes, T. P., Burke, A. W., and Paul, J. H., *Am. J. Trop. Med.*, 1940, **20**, 471.

² Smithburn, K. C., *J. Immunol.*, 1942, **44**, 25.

³ Smithburn, K. C., and Jacobs, H. R., *J. Immunol.*, 1942, **44**, 9.

⁴ Watson, D. W., and Smael, J. E., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 101.

TABLE I.
Transmission of West Nile Virus by Infected *Aedes albopictus*, Lot III.

Time after donor meal, days	Virus in mosquitoes*	Hamsters bitten	No. of insects fed	Disease in hamsters	Virus isolated from hamsters†
Immediately	>10-2 (approx. 10-4.5)	None			
10	10-5.6	a§ b	43	+	+
18	10-4.6	a b c	5 5 16	+	+
21		a	3	—	‡
24		a	7	+	+
26		a	3	+	+
40		a	2	±	—

* The amount of virus in mosquitoes was determined by intracerebral titration in mice of suspensions of ground insects.

† Hamster brain tissue was injected intracerebrally in mice for isolation of virus.

‡ Not attempted.

§ Each letter designates different hamster.

tively. Ten of the engorged mosquitoes were ground in 2 cc of diluent and the suspension was titrated by injecting groups of 3 mice intracerebrally with serial 10-fold dilutions; the titer was $10^{-5.6}$. One of the bitten hamsters developed weakness and tremors on the 6th day and was moribund on the 7th while the other sickened on the 9th day and was sacrificed on the 10th. An infectious agent pathogenic for mice was isolated from the bacteriologically sterile brain tissue of each hamster. Furthermore, histo-pathological studies of sections of brain from each animal showed lesions of the type seen in hamsters with West Nile encephalitis.⁴ Finally, identification of the first mosquito-transmitted strain of virus was made by preparing an antigen from mouse brains of the 4th passage and demonstrating its capacity to fix complement with specific antibodies in the serum of a hamster hyperimmune to West Nile virus.⁵

A similar successful feeding experiment was made with this same lot of infected mosquitoes after 18 days at 90°F, at which time the titer of virus in the insects was $10^{-4.6}$. In this and later experiments transmission of infection by biting was considered adequately proven if the bitten hamster developed signs of central

nervous system disease within 6 to 10 days and if its bacteriologically sterile brain tissue when injected intracerebrally into mice was capable of inducing a clinical disease like West Nile encephalitis. Insects were stored at 75°F after the 18th day because of the relatively high mortality among the mosquitoes maintained at a constant temperature of 90°F. A summary of the results obtained in feeding experiments carried out with this lot of mosquitoes 10 to 40 days after the initial blood meal is presented in Table I. It is seen that 6 of the 9 hamsters bitten by this group of insects were successfully infected. Furthermore, disease was induced by the feeding of as few as 3 or 5 of these mosquitoes, although positive results were not regularly obtained following the biting of such few insects.

Transmission of West Nile virus was not accomplished in 2 additional experiments similar to the one just described. In one instance, the engorged mosquitoes contained insufficient virus to be detected on intracerebral inoculation in mice. In the other, although a small amount of virus was initially present (one of 6 mice became infected), no virus was demonstrable in mosquitoes stored at 75°F for 10 and 18 days.

Summary. The virus of West Nile encephalitis can be transmitted in the laboratory by the bites of infected *Aedes albopictus*.

⁵ Havens, W. P., Watson, D. W., Green, R. H., Lavin, G. I., and Smadel, J. E., *J. Exp. Med.*, 1943, **77**, 139.