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Observations Concerning *Culex pipiens* as a Possible Carrier of St. Louis Encephalitis.

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It has been shown by Casey and Broun that St. Louis encephalitis cases appear to occur with higher incidence in those areas of the city and county which are adjacent to small streams and open ditches.¹ This suggests the possibility of a water breeding insect as a possible transmitting agent. Transmission of the equine types of encephalomyelitis by mosquitoes from animal to animal has been demonstrated by Kelser², Simmons, Reynolds and Cornell,³ and Merrill, Lacaillade and Ten Broeck.⁴

Webster, Clow and Bauer⁵ demonstrated the St. Louis encephalitis virus could be taken into the body of the *Anopheles quadrimaculatus* mosquito and retained for the duration of their lives. The virus containing mosquitoes, however, did not infect mice or monkeys by biting. Attempts were made subsequent to the 1933 epidemic to transmit encephalitis from human being to human being by the bite of the various species of mosquito without success.⁶

Since *Culex pipiens* is the most common type of mosquito in the St. Louis area, we have studied the ability of this mosquito to become infected with the virus of St. Louis encephalitis.

Mice infected with St. Louis encephalitis by intraperitoneal injection of heavy doses of virus have been shown by Webster and his co-workers to have a considerable concentration of the virus in the circulating blood for a period of five hours after the injection. In our experiments 1 cc of a 1/10 dilution of virus containing brain was injected intraperitoneally. The mouse was then placed in a specially built biting cage where he was exposed to a number of mosquitoes for a period of 5 hours after dark in a quiet room.

¹ Casey, A. E., and Broun, G. O., *Science*, 1938, **88**, 450.

² Kelser, R. A., Jr., *A. V. M. A.*, 1933, **35**, 767.

³ Simmons, J. S., Reynolds, F. H., and Cornell, V. H., *Am. J. Trop. Med.*, 1936, **16**, 289.

⁴ Merrill, M. H., Lacaillade, C. W., and Ten Broeck, Carl, *Science*, 1934, **80**, 251.

⁵ Webster, L. T., Clow, A. D., and Bauer, J. H., *J. Exp. Med.*, 1935, **61**, 479.

⁶ Report on the St. Louis Outbreak of Encephalitis, Public Health Bulletin, No. 214, United States Public Health Service.

At the end of this time the engorged mosquitoes were separated from the unengorged and placed in another cage. Small culture dishes of semi-stagnant water were placed on the floor of the cage to provide a place for the deposition of any eggs that might be laid and as a moisture source for the mosquitoes.

A number of engorged mosquitoes were selected at random and killed in ether fumes. Usually 3 mosquitoes were ground in a mortar and diluted with 10 cc of salt free broth, and then passed through a Berkefeld filter. Serial dilutions of 1:5, 1:10, 1:100 were made of the filtrates and each injected intracerebrally into 3 Swiss mice in 0.03 cc amounts. The injected animals were observed for 15 days after injection.

In 29 series of experiments in which the mosquitoes which previously had engorged on infected mice were macerated and injected into normal mice, some of the injected mice died in 10 instances. In only 3 cases, however, were we able to carry the virus in serial transfers and to prove conclusively by neutralization tests that the virus of St. Louis encephalitis had been the infecting agent.

In the successful experiments we have so far shown survival of the virus in the body of the mosquito no longer than 10 days. The concentration of the virus within the body of the mosquito has not been shown to be greater than 100 intracerebral lethal mouse doses. This is a very much smaller concentration than was shown by Webster to be present in the body of the *Anopheles* mosquito.⁵

Two series of experiments were conducted in which larvæ of about the first or second instar were placed in water containing a macerated infected brain suspension. The dilution of the brain suspension in water was 1:10. When these larvæ transformed into the adult form, the mosquitoes were macerated in broth, filtered and injected into normal mice. None of the mice showed any evidence of infection after such injections.

Nine series of experiments were conducted in which eggs laid by mosquitoes which had previously been allowed to feed on infected mice were macerated in broth, filtered and injected into normal mice.⁴ In all cases the mice remained normal and healthy.

In 15 experiments normal mice were exposed in the biting cages to 50 to 100 mosquitoes which had previously been allowed to feed on an infected mouse and then allowed to stand until they became empty. They were then observed until they had been allowed to feed on the normal mice. The mice were allowed to remain in the cage for 12 hours and then removed. In all cases the mice were healthy and normal after 15 days.