tion with 1% HCl solution), thus indicating that the material extracted from the Harderian glands was protoporphyrin. Compared to a standard of coproporphyrin there was approximately 25% more protoporphyrin in the female Harderian gland than in a gland from a male of the same age (75 days) and strain (C_3H).† If a correction for body size be made, this sex difference is even more pronounced.

At 250 days there appeared to be no significant sex difference in the content of protoporphyrins as indicated by the intensity of fluorescence.

Conclusions. 1. The Harderian glands of female mice of C_3H strain at 75 days of life appear to differ in at least two ways from the Harderian glands of male mice of the same age and strain, as follows: (a) They appear to contain under normal light a greater degree of gray pigmentation; (b) they contain approximately 25% more protoporphyrins, in spite of the fact that the glands are smaller and the body weights are also smaller than in males. 2. These differences between sexes are more pronounced at 75 days of age than they are at 250 days.

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Experimental Transmission of St. Louis Encephalitis Virus by *Culex pipiens* Linnaeus.*

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Lumsden[1] and Casey and Broun,[2] on the basis of epidemiological investigations, suggested that the virus of St. Louis encephalitis is mosquito-borne. Mitamura and associates[3] in Japan first reported successful transmission of St. Louis virus by a mosquito, *Culex*

† We are deeply indebted to Doctors C. P. Rhoads and K. Dobriner for their kindness in doing this quantitative determination for us.

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1 Lumsden, L. L., unpublished official report, 1933.

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

3 Mitamura, T., Yamada, S., Hazato, H., Mori, K., Hosoi, T., Kitaoka, M., Watanabe, S., Okubo, K., and Tenjin, S., *Tr. Jap. Path. Soc.*, 1937, **27**, 573.

pipiens var. *pallens* Coq. This has long awaited confirmation and has been regarded with skepticism by many, since various American workers, Leake, Musson and Chope[4] and Fulton, Gruetter, Muether, Hanss and Broun,[5] reported negative results with *Culex pipiens* Linn. Leake and associates[4] and Webster, Clow and Bauer[6] reported negative results with *Anopheles quadrimaculatus* Say; however, the latter found the virus to persist in this mosquito for 42 days, though less than 3 days in *Aedes aegypti* (Linn.). Fulton and associates[5] found it to persist for not more than 10 days in *Culex pipiens*. Hammon, Reeves, Brookman, Izumi and Gjullin[7] reported the isolation of St. Louis virus from naturally infected *Culex tarsalis* Coq. and later confirmed this with 2 other isolations from *C. tarsalis* caught in the same area.[8]

Blattner and Heys[9] have recently transmitted the St. Louis virus under laboratory conditions with *Dermacentor variabilis* (Say), the American dog tick. However, there has been no epidemiological evidence implicating ticks as potential vectors.

Further important evidence supporting transmission by some arthropod is the demonstration of circulating virus in the blood of the monkey (Howitt[10]), the horse (Hammon, Carle and Izumi,[11]), the chicken and the guinea pig (Hammon and Izumi[12]). Thus, virus is available for infection of a blood sucking vector. Serum neutralization tests on animals from the Yakima Valley, Washington (Hammon, Gray, Evans, Izumi and Lundy[13]) showed that a number of domestic and wild birds and mammals had high antibody titers to this virus. It appears probable, therefore, that the potential reservoir for infection of an arthropod vector is large.

A serious difficulty is encountered in any attempt to transmit this

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4 Leake, J. P., Musson, E. K., and Chope, H. D., *J. A. M. A.*, 1934, **103**, 728.

5 Fulton, J. D., Gruetter, J. E., Muether, R. O., Hanss, E. B., and Broun, G. O., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 255.

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

7 Hammon, W. McD., Reeves, W. C., Brookman, B., Izumi, E. M., and Gjullin, C. M., *Science*, 1941, **94**, 328.

8 Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., *J. Infect. Dis.*, in press.

9 Blattner, R. J., and Heys, F. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **48**, 707.

10 Howitt, B. F., *J. Immunol.*, 1941, **42**, 117.

11 Hammon, W. McD., Carle, B. N., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 335.

12 Hammon, W. McD., and Izumi, E. M., unpublished data.

13 Hammon, W. McD., Gray, J. A., Evans, F. C., Izumi, E. M., and Lundy, H. W., *Science*, 1941, **94**, 305.

disease experimentally since the usual laboratory animals fail to develop a fatal encephalitis following subcutaneous inoculation of such a dose of virus as might be expected from a mosquito bite. This probably explains some of the negative results obtained by others. To circumvent this difficulty we have used special methods including isolation of the virus from the brain or blood of the vertebrate host during the course of the inapparent infection, and virus neutralization tests on a preliminary and post-experimental serum specimen.

Culex pipiens, collected as adults, were infected by feeding on a 10% brain suspension of St. Louis virus in 5% sheep serum infusion broth mixed with equal parts of whole defibrinated rabbit blood. A cotton pad was soaked with this suspension, sprinkled with sugar, and the mosquitoes were allowed to engorge upon it. The virus was a strain which had been through relatively few mouse brain passages since isolation from naturally infected mosquitoes.[7] All mosquitoes which had engorged on the infective suspension were held until used at 23° to 27°C.

Four experiments were performed, 3 of which yielded positive results. In each of the first 2, infected mosquitoes were allowed to feed repeatedly on a pigeon whose blood had been demonstrated to be free from antibody. Feedings were begun 5 days after the infective meal. In one experiment there were 111 feedings and in the other, 30. Fifteen days after the last mosquito fed each pigeon was found to have developed a high titer of serum neutralizing antibody. All mice used in the neutralization test survived both dilutions of virus used.[14] A normal pigeon kept in the same cage as the 2 experimental birds, but not subjected to mosquito feeding, did not develop protective antibodies during the experimental period. Neither of the pigeons showed visible clinical signs as a result of infection.

In the next experiment, the results of which are presented in Table I, 100 blood-virus-engorged mosquitoes were employed. They were permitted after various intervals to feed on doves and on baby mice of less than 8 days of age. Virus was isolated from the blood of one dove 24 hours after feeding and from the brain of one mouse. Antibodies were demonstrated to have developed in the serum of 3 doves and not in the control. Several of the doves appeared to be ill.

In a fourth experiment only 30 mosquitoes engorged. Baby mice were used, but a total of only 25 mosquito feedings occurred. No virus was demonstrated in the brains of these mice, or from the mosquitoes tested.

In all experiments samples of mosquitoes were removed at varying

[14] Hammon, W. McD., and Izumi, E. M., *J. Immunol.,* 1942, **43**, 149.

TABLE I.
Results of Third Transmission Experiment with *Culex pipiens.*

Days after infective meal when fed on vertebrate	Virus test on dove's blood*	Virus test on baby mouse's brain†	Neutralization test on dove's blood	Virus test on mosquito suspension
Control dove	—	—	0	—
4	0 (20)‡	+ (10)	++	—
6	0 (21)	0 (15)	0	+ [30]§
8	0 (15)	0 (6)	+	—
10	—	0 (10)	—	—
11	+ (15)	0 (5)	+	—
13	—	0 (5)	+	+ [20]

*Blood tested 24 and 48 hours after mosquitoes fed.
†Baby mice were returned to mother after mosquito feeding, then sacrificed 5 days later and brain tested for virus.
‡( ) Number of mosquitoes which fed.
§[ ] Number of mosquitoes ground up in suspension.

intervals of from 3 to 18 days after the infective meal, and virus was isolated at 3, 6, 7, 9, 12, 13, and 15 days. Only one sample was tested after a longer period of incubation (14 mosquitoes at 18 days) and this was negative. A few samples taken earlier were also negative.

Summary and Conclusions. It appears that certain species of *Culex* mosquitoes may act as vectors of the virus of St. Louis encephalitis. This is supported by the following evidence: 1. In certain areas *Culex* mosquitoes have fitted into the epidemiological picture to the extent that several workers have stressed their probable importance as vectors. 2. Virus is available to mosquitoes in the blood of doves and a number of other species of experimentally infected vertebrates. Evidence of naturally acquired infection has been demonstrated in the serum of many vertebrate hosts. 3. Infected *Culex tarsalis* have been collected in nature. 4. *Culex pipiens* has been shown to act as an experimental vector, transmission having been demonstrated to occur between the 4th and the 11th day after an infective meal. Virus persists in the mosquito for at least 13 days.