

intracerebrally with a 10 per cent. suspension of the brain of a guinea pig infected with the "Culex 116 virus." The normal guinea pig succumbed in the course of a typical encephalitic infection, but the immune animal remained normal. In the next experiment two normals and two hyperimmune controls were used and similar results obtained. It appears therefore that the virus obtained from this pool of *C. tarsalis* is that of western equine encephalomyelitis.

Experiments are now in progress to test the ability of *C. tarsalis* to serve as a host to, and to transmit these viruses. Already *C. tarsalis* has been fed on a guinea pig infected with the western equine virus and the virus readily demonstrated after 5 days incubation. Until actual transmission of one or both of these viruses has been demonstrated the role of this mosquito as a vector is not proven. These findings have, however, increased the evidence incriminating mosquitoes as vectors of these encephalitic virus diseases.

#### ADDENDA

*Culex tarsalis* is a North American species distributed throughout the states west of the Mississippi River. In the Yakima Valley it is the most common mosquito, and its larvae are found in many types of water: permanent ponds, irrigation seepage, barnyard drainage and sewage. Adults were taken in all areas where light traps were run, and were collected in large numbers in shelters such as barns and houses. In all areas of the Valley where encephalitis occurred in man or horses it was collected in significant numbers. It is more abundant than *Culex pipiens*, the other common Culecine of this area. In temperate regions adult females are reported to hibernate in sheltered places, emerging in the spring to commence egg laying (Hearle.)<sup>10</sup>

The feeding habits of *C. tarsalis* have not been extensively studied. Direct observations made in the Yakima Valley indicate that it feeds on man, horses, mules, cows and mallard ducks. Other workers have indicated that it feeds on avian blood (Freeborn)<sup>11</sup> and on man (Hearle)<sup>10</sup>.

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<sup>10</sup> E. Hearle, *Nat. Res. Council Rept.* 17, Ottawa, Canada, 1926.

<sup>11</sup> S. B. Freeborn; *Univ. of Calif. Publ. Tech. Bull. Entom.*, Bull. III, 5: 333, 1926.

#### DIAGNOSIS OF EPIDEMIC ENCEPHALITIS BY COMPLEMENT-FIXATION TEST

DURING the months of August and September, 1941, encephalitis in man occurred in epidemic form in Manitoba, Canada, and central United States. Blood sera from patients were dispatched to the Rockefeller Institute for diagnosis.<sup>1</sup>

Complement-fixation tests were carried out on each serum against the antigens of Western equine encephalomyelitis, Eastern equine encephalomyelitis, lymphocytic choriomeningitis and St. Louis encephalitis. The sera were inactivated at 60° or 65° C. and tested both undiluted and in twofold dilutions through 1:16 according to the method we have previously described.<sup>2</sup>

Of thirty-six sera from Manitoba, twenty-two gave a strong and completely specific reaction with the Western equine encephalomyelitis antigen; of eight sera from Colorado, two exhibited a similarly strong specific reaction. The titres of the positive sera, as determined by the highest dilution of serum giving a 2+ or better reaction, were 1:4 in four cases, 1:8 in three cases and 1:16 or 1:16+ in seventeen cases.

Of nine sera drawn from patients within 10 days or less after onset of illness, none was positive; whereas of thirty-five sera drawn from patients 11 to 30 days after onset, twenty-four or 69 per cent. proved positive.

Two samples of serum were obtained from each of two cases in Colorado, and they are of special interest. The first samples from each patient, taken a few days after onset of illness, were negative, whereas the second samples, taken during the second week of illness, gave a strongly specific reaction with Western equine encephalomyelitis antigen.<sup>3</sup>

The above tests indicate that the present epidemic of encephalitis in Manitoba and central United States is caused by the Western equine encephalomyelitis virus and demonstrate the value of the complement-fixation test as a practical and speedy diagnostic tool.

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<sup>1</sup> Sera and brain tissue specimens were kindly sent us by Dr. Daniel Nicholson, Winnipeg, Canada; Captain C. E. G. Gould, Camp Shilo, Manitoba; Lieutenant S. Young, Brandon, Manitoba, and Dr. J. E. Smadel, New York.

<sup>2</sup> J. Casals and R. Palacios, *SCIENCE*, 93: 162, 1941; *Jour. Exp. Med.* (in press), October, 1941.

<sup>3</sup> From the brain tissue of one fatal case of encephalitis in Winnipeg a virus was obtained by inoculation into W-Swiss mice. An antigen was prepared from this virus-containing brain tissue, which fixed complement specifically with known Western equine encephalomyelitis immune serum. Neutralization and protection tests confirmed this result.