Isolation of Eastern Equine Encephalomyelitis Virus from *Aedes vexans* in Connecticut. (25551)

R. C. WALLIS,* R. M. TAYLOR† AND J. R. HENDERSON† (Introduced by R. H. Green)
Section of Epidemiology and Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

Aedes vexans has been a mosquito suspected of transmission of eastern equine encephalomyelitis (EEE) since 1935, when Ten Broeck and Merrill(1) demonstrated in laboratory tests that this species was capable of transmitting the virus. It was further implicated in 1939, when Feemster and Getting(2) studied distribution of mosquitoes in Massachusetts following the 1938 epidemic and concluded that *A. vexans* was the most probable vector since it occurred in all areas where the disease appeared and was relatively more abundant than other *Aedes* mosquitoes during the fall season. However, EEE virus had never been isolated from collections of this species, and the purpose of this communication is to add *A. vexans* to the list of mosquitoes from which the virus has been obtained.

Methods. Epidemics of encephalitis among pheasants raised in Connecticut have occurred irregularly during the late summer and early fall for the last 2 decades. Each year since 1953, when EEE developed among penned pheasants at the Shade Swamp Fish and Game Wild Life Refuge in Farmington, Conn., this site has been used as a study area for investigation of the natural history of EEE. The study has included regular samplings of the mosquito population by taking biting collections, capturing adult mosquitoes in diurnal resting places, and examining larval breeding sites. In 1959, every week between March 1 and Nov. 1, adult female mosquitoes from these collections were transported in cardboard cartons to the laboratory where they were stupefied with cigarette smoke, sorted into pools by species (Table I), placed in small vials and stored frozen until processed in the virus laboratory.

The mosquito pools were triturated, either in a porcelain mortar with pestle or in a Ten Broeck grinder, then suspended in the proportion of one mosquito to 0.2 ml of Hanks' balanced salt solution. This solution contained antibiotics and was enriched by addition of 0.5% lactalbumin hydrolysate and 2% calf serum(3). The suspension was centrifuged at 10,000 rpm for 20 minutes and the supernatant inoculated into chick embryo tissue culture tubes as previously described(4).

Results. More than one-half of the 3194 mosquitoes collected were *Aedes cinereus* which, combined with *Aedes canadensis,* the next most numerous species, comprised 70% of the season's catch at this collecting site (Table I). Although *Aedes vexans* accounted for less than 5% of the specimens and only 10 of the 80 pools tested, the only virus isolation was made from a pool of 13 female *A. vexans.* The mosquitoes in this pool were hand-caught on Sept. 1, 1959, and contained no visible blood.

TABLE I. Mosquitoes Collected at Farmington, Conn., 1959, Number of Pools, Number of Specimens, and Number of Pools Positive for EEE Virus.

Species	No. pools	No. specimens	No. pools positive
Aedes cinereus	20	1675	0
canadensis	12	553	0
vexans	10	148	1
triseriatus	4	137	0
stimulans	1	18	0
grossbecki	1	3	0
Anopheles punctipennis	4	78	0
Culex pipiens	3	53	0
salinarius	2	39	0
territans	4	31	0
restuans	1	10	0
species	4	201	0
Culiseta morsitans	6	147	0
melanura	8	101	0
Totals	80	3194	1

* Medical Entomologist, The Conn. Agri. Exp. Station, New Haven, Conn.

† This study was conducted under auspices of Commission on Viral Infections of Armed Forces Epidemiological Board and supported by Office of Surgeon General, Dept. of Army, and U.S.P.H.S.

On the third day following seeding of a suspension of this pool on tissue culture, definite cytopathogenic effects (CPE) suggesting the presence of a virus were noted. Subpassage produced CPE on the second day, and embryonated hen's eggs inoculated into the yolk sac were killed within 48 hours. The virus was identified as EEE by both hemagglutinating-inhibition and neutralization methods by titrating against a known EEE immune serum. The isolation was validated by reisolation of the virus from the original mosquito suspension.

Discussion. The sporadic incidence of EEE infections, lack of direct contact except among pheasants in the same pen, association of outbreaks with swampland areas and occurrence of infections following the peak of the mosquito season has long led to the suggestion that the virus was transmitted by mosquitoes. This theory has been supported by an accumulation of circumstantial evidence in recent years, despite the fact that virus has been isolated from several other arthropods. Howitt *et al.*(5) isolated it from mites (*Oermanyssus gallinae*) and chicken lice (*Eumenacanthus stramineus*) collected in Tennessee. The same workers isolated the virus from a mosquito (*Mansonia pertubans*) in Georgia(6). Chamberlain and co-workers(7) obtained the virus from the mosquito, *Culiseta melanura,* in Louisiana, and later from another mosquito, *Anopheles crucians,* in the same area(8). Holden *et al.*(9), in New Jersey, recovered virus from 3 pools of *C. melanura.* Recently, Karstad and co-workers(10) reported isolations from pools of *Anopheles crucians* and *Aedes mitchellae,* and from *Culicoides* species in Georgia. In 1956, during an encephalitis epidemic in pheasants in N. J., 4 isolations of EEE virus were obtained from pools of *C. melanura*(11), and during the same year 5 isolations were obtained from the same species in Massachusetts(12). This was strong evidence that *C. melanura* was a significant vector of transmission among birds. However, it was suggested in 1959 (13) that other species must still be considered, because in studies of the mosquito populations at farms in Connecticut where virus activity occurred between 1938 and 1956, including 20 farms at the time of pheasant and horse deaths, *C. melanura* could be found on only 3 of 25 farms. This species was considered rare in Connecticut, and the correlation of its distribution with that of the incidence of the disease could not be confirmed.

During these studies, however, *Aedes vexans* was found repeatedly, and although not always in great numbers, its distribution extended to all areas where virus activity occurred. This important epidemiological consideration, as well as the bionomics of the mosquito, led Feemster and Getting(2) to suggest it as a probable vector. They pointed out that *A. vexans* is a vicious biting mosquito. It has a wide variety in its host range and feeds readily on man, horses, cattle and birds. It commonly enters houses and barns, and the population peak occurs late in the summer just prior to the EEE epidemic season in the New England states. Now, with the isolation of the virus from *A. vexans,* this mosquito becomes more significant from the epidemiological standpoint.

It is interesting to note that the mosquitoes in the pool of *A. vexans* which yielded EEE virus were collected at Farmington, Conn., more than 3 miles from the nearest site where an epidemic of EEE occurred in penned pheasants during the current season. It is unlikely, therefore, that the mosquitoes had recently fed on infected domestic pheasants. It is more probable that they became infected by feeding on wild birds in the Shade Swamp Fish and Game Wildlife Refuge.

Summary. The virus of eastern equine encephalomyelitis was isolated from one pool of mosquitoes limited to 13 specimens of *Aedes vexans* collected Sept. 1, 1959, at Farmington, Conn. Distribution, seasonal incidence and bionomics of this mosquito fit epidemiological qualifications necessary for implication as a vector of eastern equine encephalomyelitis in this area.

The technical assistance of Jean Rawson and Paul Blodinger is gratefully acknowledged.

1. Ten Broeck, C., Merrill, M. H., *Am. J. Path.,* 1935, v11, 847.

2. Feemster, R. F., Getting, V. A., *Am. J. Pub. Health,* 1941, v31, 791.

3. Melnick, J. L., *Ann. N. Y. Acad. Sci.,* 1955, v61, 754.

4. Wallis, R. C., Taylor, R. M., McCollum, R. W., Riordan, J. T., *Mosq. News,* 1958, v18, 1.

5. Howitt, B. F., Dodge, H. R., Bishop, L. K., Gorrie, R. H., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 622.

6. Howitt, B. F., Dodge, H. R., Bishop, L. K., Gorrie, R. H., *Science,* 1949, v110, 141.

7. Chamberlain, R. W., Rubin, H., Kissling, R. E., Edison, M. E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 396.

8. Chamberlain, R. W., Sikes, R. K., Nelson, D. B., Sudia, W. D., *Am. J. Hyg.,* 1954, v60, 278.

9. Holden, P., Miller, B. J., Jobbins, D. M., PROC. SOC. EXP. BIOL. AND MED., 1953, v87, 457.

10. Karstad, L. H., Fletcher, O. K., Spalatin, J., Roberts, R., Hanson, R. P., *Science,* 1957, v125, 395.

11. Chamberlain, R. W., Sudia, W. D., Barbutis, D. P., Bogue, M. D., *Mosq. News,* 1958, v18, 305.

12. Feemster, R. F., Wheeler, R. E., Daniels, J. B., Rose, H. D., Schaeffer, M., Kissling, R. E., Hayes, R. O., Alexander, E. R., Murray, W. A., *New England J. Med.,* 1958, v259, 107.

13. Wallis, R. C., *Mosq. News,* 1959, v19, 157.

Received December 15, 1959. P.S.E.B.M., 1960, v103.

Prevention by Stress and Cortisol of Gastric Ulcers Normally Produced by 48 80.* (25552)

HANS SELYE, PIERRE JEAN AND MARC CANTIN

Inst. de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

48/80, a product obtained by condensation of p-methoxyphenyl-methylamine with formaldehyde, is a particularly potent histamine liberator(1). Its most conspicuous toxic actions in the rat are anaphylactoid inflammation (with edema and cyanosis of the paws, snout, ears, and genitals) and acute hemorrhagic stomach ulcers with intense gastric edema(2). It is known that stress and glucocorticoids can also produce acute gastric ulcers, although they actually prevent anaphylactoid inflammation(3). The experiments reported here indicate that various stressors and cortisol inhibit all the just-mentioned toxic actions of 48/80, including the gastric lesions.

Methods. Eighty female Sprague-Dawley rats, with a mean initial body weight of 145 g (range: 140-154 g), were subdivided into 8 equal groups and treated as indicated in Table I. 48/80 was given intraperitoneally at the dose of 300 μg in 0.2 ml of water at 10 a.m. and 5 p.m. on one day only (Groups 1-7). The stressors employed were: Groups 3 and 8, *restraint* (animals strapped to board by adhesive tape in prone position 24 hours); Group 4, *cold* (rats were shaved and kept at 2°C for 3 hours in morning and 2 hours in evening); and Group 5, *motor denervation of extremities* (both cervical plexuses, as well as femoral and sciatic nerves, were severed under ether anesthesia). All these stressors were applied on day of 48/80 treatment, but one additional group (Group 2) was restrained on preceding day only, to show the effects of *pretreatment with stress.* In Group 6, *cortisol* was administered as suspension of microcrys-

TABLE I. Prevention by Stress and Cortisol of 48/80 Intoxication.

Group	Treatment	Shock + anaphylactoid inflammation	Gastric Ulcers	Gastric Edema
1	48/80	4+	4+	4+
2	48/80 + restraint (pretreatment)	0	Trace	Trace
3	48/80 + restraint	0	2+	0
4	48/80 + cold	0	Trace	0
5	48/80 + denervation of extremities	0	0	0
6	48/80 + cortisol (10 mg once)	0	Trace	0
7	48/80 + cortisol (1 mg/day, 10 days)	Trace	1+	Trace
8	Restraint	0	2+	0

* Supported by grant from Nat. Inst. of Arthr. and Metab. Dis., U.S.P.H.S., Consol. Grant Nat. Res. Council of Canada, and by Gustavus and Louise Pfeiffer Res. Found.