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Western Encephalitis Virus in Massachusetts.*† (27074)

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(Introduced by Geoffrey Edsall)

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This report describes the first recorded recovery of western encephalitis (WE) virus from mosquitoes and birds in Massachusetts and serological evidence indicating that a considerable amount of enzootic activity of the virus has occurred there.

The initial isolation of WE virus east of the Appalachian Mountains was obtained from an immature house sparrow *(Passer domesticus domesticus)* collected in New Jersey during Sept. 1953(1). In 1952 the virus was isolated in Louisiana(2) from a loggerhead shrike *(Lanius ludovicianus)*, a Carolina chickadee *(Parus carolinensis)*, a cardinal *(Richmondea cardinalis)*, and from 2 mosquito species *(Culiseta melanura* and *Aedes infirmatus)*. Subsequent isolations of WE virus from mosquitoes, *Culex pipiens* in North Carolina and *C. melanura* in New Jersey, during 1955 and 1956 led to the hypothesis that "———— *C. melanura* may be an endemic vector of WE in the eastern part of the country"(3).

Materials and Methods. In southeastern Massachusetts, mosquitoes were collected alive in New Jersey-type light traps and bird-baited traps, anesthetized with chloroform and identified, grouped by species in lots numbering up to 50, and stored in an electric freezer at −65°C in corked Wassermann tubes until tested. Samples of avian blood were obtained by cardiac or wing vein puncture, diluted 1:5 in a physiological saline solution containing 0.01% heparin, and placed on wet ice for transport to the virus laboratory for testing. Pheasant brain specimens were obtained through the courtesy of Dr. George P. Faddoul‡. Chick embryo tissue cultures(4) were used in testing the mosquitoes and avian specimens for virus.

Suspensions of the mosquitoes were prepared for inoculation into the tissue cultures. To prepare the suspensions, a few drops of diluent were added to each pool of mosquitoes in its Wassermann storage tube. The diluent used was Hanks' balanced salt solution buf-

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fered at pH 7.8 with .04 M hydroxymethyl aminomethane, 0.3% bovine albumin Fraction V, and 2% stock antibiotics (5,000 units mycostatin and 10,000 μg each of penicillin and streptomycin per ml sterile distilled water). After this addition, the mosquitoes in the tube were ground with a sterile wood applicator, and enough diluent was added to bring the volume to 2 ml.

The suspensions were centrifuged 20 minutes in a refrigerated centrifuge at 4000 RPM; Seitz filtration with a Boerner centrifugal filter was occasionally needed to remove bacteria or fungus contaminants. A tissue culture was inoculated with 0.1 ml of the supernatant fluid from each suspension, and the remaining portions were stored at −65°C. Each day for 3 days the tissue cultures were examined for cytopathogenic effect (CPE), and when this was observed a subculture was made. On the third day, all cultures that had shown no change were "blind passed," and these were regarded as negative if no CPE appeared by the end of 3 more days.

The pheasant brain material suspension was tested for virus in chick embryo tissue culture after being ground with alundum in a mortar and pestle.

Transmissible agents isolated in the tissue cultures were identified by complement-fixation techniques on the tissue culture supernatant. If anticomplementary or cross reactions occurred, a chick embryo passage was made and the allantoic fluid was tested by complement-fixation. Eight antibody units of eastern encephalitis (EE)-immune and WE-immune serum were used in the tests, and controls served to exclude cross reactions and nonspecific fixation of complement.

Tests of the avian sera for presence of WE and EE antibody were made by the hemagglutination inhibition (HI) technique of Clarke and Casals(5) and using antigens consisting of crude tissue culture supernatants, together with pyrophosphate buffer. Neutralizing antibody tests by a plaque reduction method previously described(6) were done on selected sera for confirmation.

Results. WE virus was isolated from 26 pools of *Culiseta melanura* and from one pool of *Aedes canadensis* collected during the period August 31-Oct. 6, 1959 in southeastern Massachusetts. Of these positives, 3 were collected at Middleboro and Lakeville, and the remainder were obtained from 2 locations in Raynham, Mass.

Strong complement-fixing (CF) antigen in allantoic fluid was demonstrable by specific hyperimmune sera under controlled conditions for all the WE isolates obtained from mosquitoes.

Unengorged female *C. melanura,* without visible ingested blood in the abdomen, comprised 15 of the pools which were positive for WE virus. The number of specimens in these positive pools ranged from 10 to 50. Three of these pools were collected in light traps, and the other 12 were collected in bird-baited traps.

Engorged female mosquitoes, with recently ingested blood in the abdomen, comprised the remaining 11 positive pools of *C. melanura.* The number of specimens in these positive pools ranged from 15 to 50. All of the positive pools of engorged mosquitoes, including the positive pool of 33 engorged *A. canadensis,* were collected in bird-baited traps.

WE virus was isolated from the brain of a 3-year-old Impeyan pheasant stricken during the summer of 1959 at a wild-animal farm in Middleboro, and the initial isolation was confirmed by reisolation of the virus from a suspension of the original brain material. Tissue culture supernate contained a weak (2+) CF antigen which reacted with WE hyperimmune guinea pig serum but not with EE hyperimmune guinea pig serum. Allantoic fluid passage material contained a strong (4+) CF antigen. Tissue culture hemagglutinin behaved like WE, since it was inhibited by WE convalescent rabbit serum and not by EE convalescent rabbit serum, and its maximum hemagglutination titer was at pH 6.0. One of two 1-week-old chicks inoculated in the breast muscle with tissue-culture-passage material survived and produced WE antibody. Blood collected 12 days after inoculation neutralized stock WE virus, Fleming strain, by reducing the average number of plaque forming units from 120 to 4. An uninoculated control chick bled at the same time did not have WE antibody. Thus, the isolate from the pheasant brain was proved identical to WE virus by reciprocal serological tests.

TABLE I. Hemagglutination Inhibition Antibody Among Massachusetts Domestic Birds Hatched During 1959.

Species	Age in Mo	No. Tested WE	No. Tested EE	No. Positive WE	No. Positive EE	No. Positive Both	% Positive WE	% Positive EE	% Positive Both
Chicken	4-6	181	231	5	15	1	3	6	.5
Turkey	4-6	202	202	54	13	7	27	6	3
Pheasant	4-9	46	96	0	0	0	0	0	0
Totals		429	529	59	28	8	14	5	2

WE virus also was isolated from the blood obtained from a 3-week-old chicken from Bridgewater, Mass. on Aug. 17, 1959, and from 3 pools of unengorged *C. melanura* collected from Raynham, Mass. during Aug. 1960.§

In HI tests on 529 domestic birds hatched during 1959, 5 chickens and 54 turkeys were positive for WE antibody; 15 chickens and 13 turkeys were positive for EE antibody, but no antibody was detected among the 96 pheasants tested (Table I). A nonspecific or cross-reaction occurred between WE and EE virus in one of the chicken sera and in 7 of the turkey sera tested. It is not known whether or not other group A viruses occur in Massachusetts; if so, they may contribute to the broad reactivity expected with HI(5).

Discussion. The described isolations of WE virus from mosquitoes, a chick, and a pheasant are the first clear-cut demonstrations of active infection with the virus in Massachusetts. Serologic evidence obtained in 1953 that the virus was present then was questioned(7), but subsequent results (unpublished data) of neutralization tests by the plaque reduction technique(6) on sera obtained from chickens and turkeys in Massachusetts during 1958, 1959, and 1960 substantiated the earlier findings.

The 15 isolations of WE virus from pools of unengorged *C. melanura* constitute the majority of isolations reported from the area east of the Appalachian mountains.

The number of mosquito pools is too small for conclusive statistical determination of the probability that blood from birds used as bait on given nights might have been the source of virus in the 11 positive pools of engorged *C. melanura.* However, there was no difference (chi-square, 5% probability level) between the total numbers of positive and negative pools of unfed and engorged specimens. Since the distribution was random, it would seem that the birds were not a source of virus.

The mosquitoes and the birds from which WE virus was isolated were obtained from the adjoining towns of Bridgewater, Lakeville, Middleboro, and Raynham. The high WE antibody rate obtained in turkeys was due primarily to detection of 34 positive birds among 39 tested, and to 19 positive among another 50 tested, at farms located in East Weymouth and Middleboro, respectively.

The virus of WE is responsible for the majority of human and equine cases of arthropod-borne encephalitis in North America, but it has not been associated with known human disease along the eastern seaboard. The unimportance of WE as a disease-producing agent in man and horse in the eastern United States was suggested as being due to the absence there of a vector as efficient as *Culex tarsalis* is in the West(8). Evidence is accumulating, however, that *Culiseta melanura* is an enzootic vector of WE as well as EE in the eastern United States. In laboratory studies, several eastern mosquito species (e.g., *Aedes triseriatus, A. sollicitans,* and *A. vexans*) have been found to be potential vectors of WE virus(9).

Three elements (virus, vectors, and susceptible hosts) essential for a WE outbreak now have been reported in Massachusetts, New Jersey, and Louisiana. Therefore, it is important to realize the existence of the potential for an outbreak of WE in the eastern United States.

Summary. The first recognized active infections of western encephalitis (WE) in Massachusetts are described. The virus was isolated from 15 pools of unengorged and 11 pools of engorged *Culiseta melanura* and from 1

§ Virus isolated in 2-week-old mice and chick embryos at Encephalitis Section Virus Lab., Communicable Disease Center, Greeley, Colo.

pool of engorged *Aedes canadensis* collected in southeastern Massachusetts during 1959. and from 3 pools of unengorged *C. melanura* collected in 1960. The virus also was isolated from the brain of an infected 3-year-old Impeyan pheasant and from the blood of a 3-week-old chicken in 1959. WE and EE (eastern encephalitis) antibodies were detected in domestic fowl hatched and reared in widely separated areas in eastern Massachusetts during 1959. Although no human or equine outbreaks of WE have occurred in the eastern United States, the potential for such an outbreak apparently exists.

Addendum. Since this paper was submitted for publication, there have been three isolations of western virus from 1961 collections—two from pools of *C. melanura* and one from the blood of a catbird during the nesting season.

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Effect of Local X-Irradiation on Growth Capacity of Mouse Kidney.* (27075)

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It is well known that cells may continue to enlarge after dosages of X-rays sufficient to block cell division. Renal compensation after unilateral nephrectomy is generally attributed to hypertrophy(1). This system, therefore, lends itself to the study of radiation effects on a growth process characterized mainly by enlargement of existing cells(2).

In the present investigation the following experiments were performed: 1) effect of uninephrectomy on size and DNA content of the remaining kidney in mice of various ages, 2) effect of immediate or delayed local X-irradiation on renal compensatory response. We have confirmed the fact that mitosis plays a relatively minor role in the kidney enlargement following uninephrectomy in the young adult mouse. We have also found this compensatory phenomenon to be radioresistant.

* This work was performed under the auspices of U.S. Atomic Energy Commission.

Materials and Methods. CF no. 1 female mice of various age groups received sodium pentothal intravenously and the right kidney was exposed by a dorsal surgical incision, ligated and removed. Care was taken to maintain the integrity of the adrenal gland. Unilaterally nephrectomized mice were sacrificed 5 to 6 days post-operatively and the kidneys taken for DNA analysis by the spectrophotometric method of Schneider(3) as modified by Barton(4). These were compared with the normal kidneys removed from the same animals at time of operation. The values reported for the 2-month-old animals are based on individual microdeterminations; those of mice in other age groups represent pooled samples of several kidneys. In the radiation studies, the left kidney was freed from the adrenal and ovary and exteriorized after removal of the right kidney. Local irradiation of the organ was accomplished through a one centi-