Recovery of Virus of Eastern Equine Encephalomyelitis from Blood of a Purple Grackle.* (18791)

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(Introduced by Morris Schaeffer.)

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Although investigators have proved numerous avian species to be susceptible to eastern equine encephalomyelitis virus by experimental inoculation(1-6) and have reported its recovery from the brains of naturally infected birds(6-10), attempts to isolate the virus from other tissues of birds infected in nature have failed(10). However, Cox *et al.*(11) reported the isolation of western equine encephalomyelitis virus from the spleen and brain of a prairie chicken, *Tympanuchus cupido americanus* (Reichenbach).

This note reports the isolation of eastern equine encephalomyelitis virus from the blood of an apparently well adult male purple grackle, *Quiscalus quiscula quiscula* (Linnaeus), shot in flight in the Manchac swamp area near Ponchatoula, La., on June 19, 1950. Intracerebral inoculation of its blood into six 3-week-old mice was followed by death in five of the mice on the third day. No growth was observed following culture of the brains from these mice in dextrose or serum broth and on blood agar plates. The agent passed a Seitz EK pad. Infected mouse brain material had a titer of 10^8 LD_{50} upon intracerebral inoculation into mice, as calculated by the method of Reed and Muench(12).

Serum from rabbits immune to eastern equine encephalomyelitis virus gave a neutralization index of 15,000 against this agent when serial 10-fold dilutions of the agent were mixed with equal quantities of undiluted serum, incubated for 18 hours at 4°C, and inoculated intracerebrally in 0.03 ml amounts into 3-week-old mice. Western equine encephalomyelitis and St. Louis encephalitis antisera failed to neutralize in tests conducted simultaneously. Serum from rabbits immunized with this agent demonstrated strong neutralization of eastern equine encephalomyelitis virus. Guinea pigs vaccinated with the new agent resisted intracerebral challenge with 10^7 LD_{50} of eastern equine encephalomyelitis virus. Those immunized with eastern equine encephalomyelitis similarly failed to exhibit signs of infection upon challenge with an equivalent dose of the newly isolated agent.

In limited host range studies, the new virus resembled that of eastern equine encephalomyelitis both as to species susceptibility, and incubation periods. Microscopic examination of brains from infected mice, guinea pigs, and hamsters revealed a pathological picture consistent with that produced by the encephalitis viruses. Presence of the infectious agent in the original material was verified by reinoculation into a second group of mice with production of encephalitic symptoms on the second day, followed by death on the third

* This note constitutes part of an investigation on encephalitis in Louisiana, conducted by the Virus and Rickettsia Section, Montgomery, Ala.

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day. On these bases, the agent was considered to be the virus of eastern equine encephalomyelitis.

To our knowledge, this is the first report of the isolation of the eastern variety of equine encephalomyelitis virus from the blood of a naturally infected wild bird. It is of added interest that the specimen was collected during a period when there were comparatively few cases of the disease reported in horses in the region.

Summary. The virus of eastern equine encephalomyelitis was isolated from the blood of an adult purple grackle collected in Louisiana in June, 1950.

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A Rapid Assay for Xanthurenic Acid in Urine. (18792)

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In man(1) and other mammalian species (2-6), a deficiency of pyridoxine has been shown so to alter the normal metabolism of tryptophan as to result in the urinary excretion of xanthurenic acid (XA). Following the ingestion of tryptophan, the quantitative measurement of XA in urine has been used as an index of pyridoxine deficiency or dysfunction(1). Colorimetric methods available at present for the determination of XA in urine generally involve preliminary time-consuming extraction of XA from urine prior to colorimetric assay(3,7,8).

In view of the increasing significance of pyridoxine in disorders of pregnancy(9-11) and possibly other clinical states(12-14), a rapid, reliable method for the estimation of XA in human urine would be of definite advantage for clinical studies where a large number of routine assays are to be performed. It is the purpose of this paper to present such a method.

Reagents and apparatus. 1. Five % sodium bicarbonate solution, freshly prepared prior to analysis. 2. $FeNH_4(SO_4)_2 \cdot 12H_2O$ solution. 0.85 g of Mallinckrodt analytical reagent grade, dissolved in 50 ml water. 3. Sodium hydrosulfite powder (J. T. Baker Chemical Co.). 4. Standard xanthurenic acid* solution: Stock standard solution, 500 μg per ml of XA, made by dissolving 25 mg of XA in 50 ml of 5% ethyl alcohol which contained 0.1 ml of concentrated ammonium hydroxide.

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* The xanthurenic acid used in these studies was synthesized in our Division of Organic Chemistry by Mr. A. Mebane and Dr. W. Oroshnik. This substance has a melting point of 297-298°C. An elementary analysis revealed the following: $C_{10}H_7NO_4$: Calculated. C, 58.54; H, 3.44; N, 6.83. Found. C. 58.40; H, 3.65; N, 6.66; Ash, 0.