

virus from cases in Delaware and Maryland, and one, a Maryland strain designated Md¹, has been compared in preliminary tests with a South Dakota strain of virus designated S. D., which was isolated by the writers¹ in 1932. Neutralization tests, utilizing a hyperimmune horse serum and a hyperimmune rabbit serum, were conducted with the two vira. The horse serum was prepared by Dr. C. M. Haring *et al.*, of California, through the use of California strains of virus, while the rabbit serum was obtained by the writers, who utilized the South Dakota virus. Both sera had previously been repeatedly shown capable of neutralizing California virus as furnished by Dr. Haring, the S. D. virus and a second strain of South Dakota virus² which we recovered from a case occurring during the present 1933 outbreak.

The technique of preparing virus suspensions, mixing and holding serum-virus inocula, was identical to that employed by Howitt³ in neutralization tests of poliomyelitis and equine encephalomyelitis vira.

A series of three tests was conducted, using S. D. and Md¹ vira on the same days, with controls in the form of normal serum-virus mixtures and saline-virus mixtures of the same virus dilution as that in the immune serum-virus mixtures. The guinea-pigs were inoculated intracerebrally after trephination. In each of the tests applied, the serum completely neutralized the S. D. virus as judged by failure of any inoculated animals to show any signs of illness during an observation period of ten days. Normal serum-virus and saline-virus inoculated guinea-pigs died or developed a moribund condition warranting destruction on the fourth to sixth day. No guinea-pig inoculated with mixtures of Md¹ virus and the above immune sera survived for more than four days (some moribund animals were destroyed on the third or fourth days). Likewise animals inoculated with normal serum and saline control mixtures containing the same dilution of virus succumbed in a manner typical of previous passage inoculations of the same virus.

Two further tests using two volumes of immune serum to each volume of Md¹ virus of the same dilution as previously employed failed to demonstrate neutralization of the virus. As an additional check two tests using three volumes of serum to each volume of Md¹ virus likewise gave no indication of virus neutralization or even partial inactivation. Indeed,

in some instances the serum appeared to cause an increased virus activity as evidenced by a shortened incubation period.

With the Md¹ strain of virus well-marked symptoms were often evident on the second day following intracerebral inoculations and death ensued on the third or fourth day after a syndrome indistinguishable from that of the S. D. virus disease.

Of a group of four guinea-pigs which had been shown by at least one intracerebral inoculation to be immune to S. D. virus, two were inoculated intracerebrally with Md¹ virus and two were exposed in the same manner to S. D. virus. The two animals inoculated with the S. D. virus survived without any signs of illness, while those inoculated with Md¹ virus succumbed. Controls inoculated with each virus developed typical encephalomyelitis and succumbed or were destroyed upon reaching a moribund state.

A guinea-pig virus brain (Md¹ strain) was ground in a mortar with sand and saline and centrifuged at 1,000 r.p.m. for 20 minutes. The supernatant fluid was further diluted and guinea-pigs were inoculated intracerebrally with 0.2 cc of dilutions varying from 1:100 to 1:20,000. Those animals which were inoculated with a dilution of 1:7,000 and lower succumbed while those which received dilutions greater than 1:7,000 survived without evidence of illness.

Titration of S. D. virus similarly prepared have disclosed a M. L. D. of 0.2 cc of a 1:2000-1:5000 dilution, depending upon the particular sample tested.

While anti-serum of the Md¹ type was not available at the time these tests were made, our preliminary observations indicate that the Md¹ virus recovered from the current outbreak of encephalomyelitis in the central Atlantic coast states is not identical to the western virus as exemplified by the S. D. strain. The Md¹ virus disease in the guinea-pigs is of a more acute type than the S. D. virus infection and the vira show certain immunological differences.

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BOOKS RECEIVED

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¹ This strain of virus, referred to in SCIENCE, Vol. 78, 2012, pp. 63-64, 1933, was recovered from a specimen submitted by Dr. C. H. Hays, inspector in charge, B. A. I. field station, Pierre, S. Dak., who conducted extensive field studies of the 1932 outbreak in South Dakota.

² Recovered from specimen submitted by Drs. C. H. Hays and C. C. Heacock, collected during the 1933 outbreak in South Dakota.

³ B. Howitt, *Jour. Infect. Dis.*, Vol. 51, No. 3, p. 493, 1932.