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Susceptibility of Non-Immune, Hyperimmunized Horses and Goats to Eastern, Western and Argentine Virus of Equine Encephalomyelitis.

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Through the studies of TenBroeck and Merrill[1] and Giltner and Shahan[2] it is known that the Eastern viruses of equine encephalitis isolated during the epidemic of 1933 are serologically distinct from those responsible for the disease in California, Nevada, Colorado, Utah and South Dakota. Rosenbusch[3] studied a similar if not identical malady in Argentine. He found the South American virus immunologically identical with the California virus. A comparative study of the 3 viruses, on horses, suggested itself. The senior author is familiar with the clinical and pathological findings observed in horses during the 1912 epidemic in Delaware and during the outbreaks in California (1930 to 1933), Colorado and Texas. The results of these studies may be summarized as follows:

(1) Two Eastern viruses (Delaware isolated from a brain sent to California in glycerine, New Jersey courteously furnished by Dr. Carl TenBroeck) infected horses when injected intracerebrally, intravenously (filtrates) and intracutaneously. In one series 1 cc. of a 20% suspension of guinea pig passage virus fatally infected 4 of 14 horses injected intracutaneously on the neck (distribution of cervical nerves). The clinical picture (fever, stupor and motor disturbances) differed in no way from that recorded in the many horses infected with the Western virus, although the course of the disease was greatly accelerated. Several animals died in from 62 to 163 hours after the administration of the virus or they were unable to rise between the 80th and 96th hour and were consequently sacrificed. Irrespective of the mode of infection, the virus was demonstrable in the blood serum from the 12th to 65th hours. The spinal fluid was invariably increased and turbid with a cell count of from 600 to 25,000 cells (5 to 20% granulocytes), and a positive Rivolta test and occasional web formation. The gross

[1] TenBroeck and Merrill, PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 217.

[2] Giltner and Shahan, *Science*, 1933, **78**, 587.

[3] Rosenbusch, *Anales d. l. Sociedad Rural Argentina*, 1934, and personal communication to senior author.

anatomical lesions were slight but the histologic examination revealed very extensive and pronounced degenerations with inflammatory alterations in the central and peripheral nervous system.

(2) A virus obtained from a fatal infection in Utah and the Argentine virus infect by the intracerebral route only. Filtrates injected intravenously or suspensions administered intracutaneously fail to incite the disease.

(3) Thirty horses injected subcutaneously with 5 cc. of a 20% suspension of California guinea pig passage virus had slight febrile reactions. Subsequently they tolerated large doses of Western virus.

(4) Of 3 horses previously hyperimmunized for 2 years with California, Nevada and South Dakota virus and injected intracerebrally with Delaware and New Jersey virus, 2 reacted severely and were sacrificed for humane reasons. One horse had a sharp febrile reaction and transitory symptoms of encephalitis but recovered. It had been previously recognized that the intracerebral mode of infection is an exceedingly drastic method to determine the immunity of horses against encephalitis (Meyer, Haring and Howitt, Records and Vawter), and doubtless not well suited for cross-immunity tests.

(5) Twelve horses highly hyperimmunized for several months with Western viruses yielding a serum with antiviral substances injected intracutaneously with 1 or 2 cc. and subsequently with 4 cc. of a 20% suspension of Eastern virus survived. Several of the animals had slight transitory febrile reactions (39 to 40° C.). Four control horses injected simultaneously by the same route developed typical encephalomyelitis, and either succumbed to the disease or were sacrificed.

(6) Two horses hyperimmune to the Western virus and injected intracerebrally with Argentine virus showed no signs of illness. The control horse developed encephalitis and finally succumbed. During the febrile reaction the virus was demonstrated for from 12 to 60 hours in the blood serum. Simultaneously, horses hyperimmune to the California and Nevada virus were tested intracerebrally with the Utah virus. They failed to react.

(7) One horse, which had survived an intracerebral injection of Argentine virus, tolerated the intracutaneous injection of the Eastern virus (4 cc.).

(8) Two horses injected at weekly intervals with Eastern virus (1, 2 and 5 cc. of a 20% virus suspension) failed to react to an intracerebral injection (5 cc.) of a potent California virus, which fatally infected (with 2 cc.) a normal animal.

(9) The Eastern, Western and Argentine viruses injected intracerebrally may produce in goats a transitory or a rapidly fatal encephalitis.

These observations indicate a very close relationship between the Western and Argentine virus not only with respect to infectivity but also with regard to cross protection. By contrast the Eastern virus of 1933 exhibits a greater virulence and thus may break the immunity established against the Western virus. On the other hand, the Eastern virus apparently protects against the Western virus. Further, these experiments lend considerable support to the conception of an insect transmission of the encephalitis virus as demonstrated by Kelser. The Eastern virus infects readily by the cutaneous route and the infective agent circulates for many hours in the blood of the horse.

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Propagation of Virus of Equine Encephalomyelitis after Intranasal Instillation in the Guinea Pig.

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In an attempt to determine whether or not the virus of equine encephalomyelitis reported by Meyer, Haring and Howitt[1] spreads by way of an initial blood stream invasion and secondary penetration of the meningo-choroid plexus or by axonal propagation as in poliomyelitis,[2–5] the distribution of the infective agent in the tissues of guinea pigs following the intranasal instillation of the virus was studied.

Several series of small guinea pigs were each given 2 cc. of a 20% saline suspension of California virus dropped into the nares. Three animals were killed by bleeding from the heart at each of the different periods of time as shown in Table I. No blood was removed at the twelfth hour, however. The tissues were removed

[1] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227.

[2] Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **57**, 933.

[3] Flexner, S., *Science*, 1933, **77**, 413.

[4] Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235.

[5] Schulze, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.