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Cultivation of the Virus of St. Louis Encephalitis.*

E. W. SCHULTZ, G. F. WILLIAMS AND A. HETHERINGTON.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Syverton and Berry[1] reported the successful cultivation of the virus of St. Louis encephalitis (epidemic encephalitis, Type B) in a medium containing minced living embryonic mouse-brains. Later, Harrison and Moore,[2] confirmed this work and in addition reported success in propagating the virus on the chorioallantoic membranes of developing chicks. This paper is a confirmation of both these reports.

The methods followed in our studies were in all essential respects the same as those reported by our predecessors. The mouse-embryo method was the same as that employed by Syverton and Berry, with the exception that 0.4 cc of material was transferred serially to culture-wells containing 2.5 cc of Tyrode's solution, 0.5 cc of normal rabbit serum and 0.5 cc of finely minced embryonic brains in Tyrode's solution (five, 14-day embryo-brains in 10 cc Tyrode's). Both the Li and Rivers[3] collar-flasks and 1 ounce flat-sided screw-cap bottles were used as receptacles for the medium. In propagating the virus on chorioallantoic membranes we initially followed the procedure (work carried out in 1936) described by Woodruff and Goodpasture,[4] and later a modification of this technic introduced by Burnet.[5] One-tenth cubic centimeter of a 10% suspension of the freshly harvested membrane in Tyrode's was inoculated in making the transfers. Ten- to 14-day eggs were employed. Webster's virus, strain No. 3, was used throughout.

The embryonic mouse-brain cultures of the virus were carried through 16 passages, with a final titer of 10^{-8}. From the 6th passage on, the virus was also carried on 2 additional media: (a) minced adult brain in place of embryonic brain, (b) minced adult brain without serum; the difference in volume was made up with

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1 Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

2 Harrison, R. W., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 359; *Am. J. Path.*, 1937, **13**, 361.

3 Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

4 Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

5 Burnet, F. M., Med. Research Council, Special Rept. Series, No. 220, 1936.

Tyrode's solution. In both cases the virus was carried to the 12th passage (6 passages on adult brain), but in both it disappeared by the 14th passage.

A 7th-passage culture in embryonic mouse-brain after storage at 37.5°C for 35 days, caused fatal infection in mice.

On chorioallantoic membranes the virus was carried in one series through 15 passages; in another, through 16 passages, and in a third series through 22 passages. The first series was run by the shell-flap technic of Goodpasture, *et al.*,[4] the last 2 series by the artificial air-sac technic of Burnet.[5] In the first series transfers were made with 0.1 cc of a 1:5 suspension of the previous membrane; in the second and third 0.1 cc of a 1:10 suspension was inoculated. In the first 2 series the inoculated membranes at no time proved infectious in dilutions above 10^{-1}. In the third series it was 10^{-1} at the 5th passage, 10^{-2} at the 11th passage, 2×10^{-3} at the 17th passage and 2×10^{-4} in the 22d passage. This is considerably above that previously reported. Titration of the virus in brains of infected embryos (age 13 to 16 days) in the 17th passage was 2×10^{-4}. This suggests that the virus proliferates more actively in the brain than in the chorioallantoic membrane.

Changes similar to those previously described[2] were observed in chorioallantoic membranes. In the gross these ranged from a faint whitish stippling to a diffuse thickening with a certain amount of opacity, hyperemia, and hemorrhage. These changes were most evident after the third day, at which time foci of necrosis began to appear in the thickened areas. These increased in extent and death of the embryo occurred between the sixth and seventh day. Microscopically the membranes showed foci of ectodermal (sometimes endodermal) hyperplasia, vacuolation and necrosis of epithelial cells, and a purulent surface-exudate. The mesoderm often showed edema, polymorphonuclear-cell infiltration, and occasionally some increase in fibroblasts. The cytoplasm of some of the ectodermal cells presented small, round basophilic (sometimes eosinophilic) bodies of varying size. As D'Aunoy and Evans[6] have recently pointed out, certain of these changes, including the inclusion-bodies, may appear in chorioallantoic membranes under natural conditions. These changes, however, tend to be much more prominent in virus-inoculated membranes than in normal membranes and are supplemented by ectodermal necrosis and polymorphonuclear-cell infiltration.

[6] D'Aunoy, R., and Evans, F. L., *J. Path. and Bact.*, 1937, **44**, 1937.