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8974 P*

Cultivation of the Virus of St. Louis Encephalitis.†

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The virus of St. Louis encephalitis was first cultivated by Syverton and Berry[1] in a living tissue medium composed of minced mouse embryo, rabbit serum and Tyrode's solution. Their results have been confirmed in this laboratory. In pursuing the study further, it has been possible to cultivate this virus in other media[2] and also in the

* P represents a preliminary, C a complete manuscript.

† Conducted under a grant from the Commonwealth Fund of New York.

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

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

developing chick embryo according to the technics adopted for other viruses by Woodruff and Goodpasture[3] and Burnet and Galloway.[4]

Cultures were inoculated with supernatant fluid from 10% suspensions of infected mouse brain. Three strains of the virus were cultivated serially in 4 different media consisting of 0.02 gm. of finely minced mouse or chick embryo suspended in 2.7 cc. of Tyrode's solution or of a mixture of Tyrode's solution and rabbit serum. Transplants were made at 5-day intervals over a period of 4 months. Since no tissue changes attributable to the virus were observed in the cultures, they were tested frequently by intracerebral inoculations of mice. While clinical and pathological findings in mice were identical with those following inoculation with mouse passage virus,[5] culture virus was rarely infective in dilutions higher than 10^{-2}.

Virus inoculated into flasks containing immune serum in place of normal rabbit serum could not be recovered by subculturing in normal serum medium or by inoculation of mice with tissue material washed to remove the antibodies.

After a number of passages *in vitro*, 2 strains of the virus were inoculated into 12- to 16-day chick embryos by placing one or 2 drops of culture upon the chorio-allantoic membrane. Eggs were inoculated in series with tissue from membranes of preceding egg passages and incubated at 38°C. for 2 to 10 days, usually 5 or 6.

The lesion, confined to the chorio-allantoic membrane, appeared first as a cloudy area of proliferation which microscopically involved all 3 layers. In 4 to 7 days the diameter of the lesion reached 1.5 to 2 cm. and its center became necrotic. No pathological changes were found in the body of the embryos although they often died and virus was recovered from brain, liver and spleen in addition to the infected membranes. Mice were infected with tissue from the membranes of each of 7 successive egg cultures of one strain and 10 of the other.

A number of inoculated eggs were allowed to hatch. Most of these embryos died while hatching or within a few hours after emerging from the shell. Sections of the brain of one of these showed extensive perivascular cuffing with mononuclear cells and an occasional small glial nodule.

Of 2 chicks which survived, one was paralyzed in both legs and

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

[4] Burnet, F. M., and Galloway, I. A., *Brit. J. Exp. Path.*, 1934, **15**, 105.

[5] Smadel, J. E., and Moore, E., *Am. J. Path.*, 1934, **10**, 827.

the other was apparently normal. Brain emulsions from these and from one which died soon after hatching were all lethal for mice inoculated intracerebrally. Virus recovered from the latter was carried through young normal chicks and then through mice by intracerebral inoculation. Clinical signs and pathological changes in the mice were typical of encephalitis. Two of the chicks were paralyzed and brain sections from 3 showed characteristic encephalitic changes. Virus was recovered also from one chick which had no clinical or pathological signs of encephalitis.

Summary. Three strains of St. Louis encephalitis virus have been cultivated in 4 different media containing living tissue and in developing chick embryos. The virus was recovered also from chicks which hatched after inoculation and from young chicks inoculated intracerebrally.

8975 P

Experimental Local Bladder Edema Causing Urine Reflux Into Ureters and Kidneys.

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There are no experimental studies recorded, as far as we are aware, of the rôle that a local transitory edema of the ureteral valve plays in the ureteral reflux of urine. Such a local edema we produced by infiltrating the vesical site of the ureter with 25% magnesium sulphate or with physiological salt solution.

We used 14 guinea pigs, 21 rabbits and 17 dogs, narcotized by morphine, or sodium barbital with or without magnesium sulphate, or ether. In dogs, ether only was used. The bladder and ureters were exposed through a median incision; pressure was recorded through a flanged cannula inserted in the bladder apex, and a water or mercury manometer. India ink was injected into the bladder as indicator. A few times the urethra was ligated. Infiltration of the ureter in its vesical course was done through a fine hypo needle; the amount varied between 0.2 and 2.0 cc.

In 14 guinea pigs (9♂,5♀) 10 showed regurgitation (3♂, 7♀). The pressures ranged between 50 and 280 mm. water (4 to 21 mm. Hg.). In 4 non-pregnant females, spontaneous reflux occurred