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Intragastric and Intraintestinal Inoculations with St. Louis Encephalitis Virus.

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While mice can readily be infected with the virus of St. Louis encephalitis by intranasal and intracerebral inoculation, neither subcutaneous, intraperitoneal nor oral administration of the virus ordinarily results in infection. The possible importance of the oral method of infection has again been raised by the work of Harford, Sulkin and Bronfenbrenner.[1] These investigators noted that adult animals which ate infected new born mice not infrequently succumbed to encephalitis.

We have seen occasional instances of infection following oral administration of virus emulsions. This procedure is open to the criticism that in the process of feeding upon the virus emulsion, the animal's nasal mucosa may become contaminated with the virus and hence infection may actually take place through the nasal route.

Direct intragastric and intraintestinal inoculation would effectively obviate the danger of infecting the nasal mucosa during the process of feeding. Brodie[2] reported that he obtained no encephalitic infection either after oral feeding or after intragastric inoculation. Since he carried out gastric injections on only 3 animals, we felt repetition of intragastric and intraintestinal inoculations on a larger scale should be attempted. Each of 28 adult mice was anaesthetized and an opening made into the peritoneal cavity. Under direct observation the stomach was located and .06 cc of a 1/10 dilution of the brain of a mouse killed when moribund from St. Louis encephalitis was injected into the lumen of the stomach with a 26 gauge hypodermic needle. The abdomen was then closed and the animal allowed to recover. Nine animals died within 10 days after inoculation. In each instance the brain, liver, and spleen were removed under sterile conditions, emulsified and inoculated unfiltered intracerebrally into mice. Pieces of stomach and intestines were likewise removed, emulsified in broth and after filtration, inoculated

1 Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 332.

2 Brodie, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1647.

intracerebrally into mice. None of these transplants caused any evidence of encephalitis in the inoculated animals. The cause of death of the animals subjected to intragastric inoculation remains uncertain.

Eighteen animals survived the intragastric inoculation. Between the nineteenth and thirtieth day after the intragastric inoculation, each of these animals was reinoculated intracerebrally with 100 lethal doses of St. Louis encephalitis virus. Only one animal out of the 18 survived this procedure. Hence, no strong degree of protection developed as a result of the intragastric virus inoculation.

The pooled serum of one group of 5 animals bled 15 days after intragastric inoculation was tested for its protective power against St. Louis encephalitis virus by the virus neutralization technique employed in our laboratory.[3] Protective power against 10 lethal doses of virus was found. This is a rather low titer of antibodies compared to that usually found in convalescent human patients.

Another group of 27 mice were anaesthetized and the abdominal cavity opened. Under direct observation the upper part of the small intestine was located and .06 cc of 1/10 dilution of the brain of a mouse dying of St. Louis encephalitis was injected into the lumen of the intestine. The abdomen was then closed and the animal allowed to recover.

Five animals died within 10 days after operation. The brain, liver, and spleen were removed under sterile conditions, emulsified and inoculated into mice intracerebrally. Pieces of intestine were likewise removed, emulsified in broth and after filtration inoculated intracerebrally into mice.

The transplants from 4 of these animals failed to show any indication of the presence of virus. Presumably death was due to operative trauma. The brain of the fifth animal when emulsified and inoculated intracerebrally into 3 mice gave rise to evidences of encephalitis and caused the death of all 3 animals within 4 to 6 days. The brains of these animals were bacteriologically sterile. Virus neutralization tests carried out, making use of this material showed evidence of the presence of St. Louis encephalitis virus.

Fourteen of the animals which survived the intraintestinal inoculation were reinoculated intracerebrally with 100 lethal doses of St. Louis encephalitis virus between the nineteenth and twenty-third day after the intraintestinal injection. One animal out of 14 survived. This inoculation showed that the vast majority of the mice

[3] Broun, G. O., and Ruskin, J., *J. Mo. State Med. Assn.*, 1936, **33**, 19.

had developed no immunity as a result of the intraintestinal injection of virus.

The pooled serum of one group of animals bled 23 days after intraintestinal inoculation showed no neutralizing antibodies when tested by the virus neutralization test. However, another group of animals bled 21 days after intraintestinal inoculation showed protective power against 200 lethal doses, which is a strong degree of protection. There were 4 animals in this group whose sera were pooled. It was in this group that one animal survived the subsequent intracerebral inoculation of 100 lethal doses of virus.

Summary. Our studies indicate that it is difficult to secure cerebral infection when the virus of St. Louis encephalitis is introduced into the intestinal tract. Nevertheless one definite infection did occur after intraintestinal inoculation. We feel the experimental procedure ruled out the nasal route of infection. Some degree of humoral immunity was produced in mice both by intragastric and intraintestinal inoculation. Only two animals survived the subsequent intracerebral injection of 100 lethal doses of virus, showing that only rarely is adequate protection against infection provided by intragastric or intraintestinal virus inoculations.

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Influence of Certain Bacteriophages on the H Antigens of *Salmonella poona* and *E. typhi.*

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There is a large body of evidence indicating that the usual site of action on a bacterial cell by a bacteriophage is the soma. However, reports have appeared which call attention to the fact that in the phenomenon of bacteriophagy some other portion of the bacterial cell may be affected. Sertic and Boulgakov[1] described a phage whose action on *E. typhi* was solely on the flagella. Craigie and his associates[2] have shown that in order for certain typhoid phages to

1 Sertic, V., and Boulgakov, N. A., *Compt. rend. Soc. de Biol.*, 1936, **123**, 887.

2 Craigie, J., *J. Bact.*, 1936, **31**, 56; Craigie, J., and Brandon, K. F., (a) *Canadian Publ. Health J.*, 1936, **27**, 165; (b) *J. Path. and Bact.*, 1936, **43**, 233; (c) *Ibid.*, 1936, **43**, 249.