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The Propagation of St. Louis Encephalitis Virus in Mouse Testicle.

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The virus of St. Louis encephalitis displays so marked an affinity for nervous tissue that inoculation by any route other than the nasal or cerebral leads to infection only when relatively large amounts of virus are used. That this neurotropic tendency may be limited, however, was suggested by Webster and Clow,[1] who found that the virus apparently multiplied to some extent in the spleen.

With a view to modifying the tissue affinities of the virus, we resorted to testicular passage in the Swiss mouse. To initiate the testicular series, we used the Hubbard strain of virus, isolated in 1937.[2] A 10% suspension in broth of a mouse brain from the 68th intracerebral passage was injected in 0.03 cc amounts into the testes of 4 mice. After 5 days, 3 animals were sacrificed and the testes removed with sterile precautions by the abdominal route. Two testicles were frozen and preserved (in each passage) in order to avert loss of the testicular virus in the event of bacterial contamination or other accident. The remaining testes were weighed, ground without abrasive and suspended in sufficient broth to make a 10% emulsion. The supernatant fluid obtained after allowing gross particles to settle out by gravity constituted the inoculum, which was always cultured in broth and on blood agar plates.

The above procedure was adopted as a routine, passage being made at 5-day intervals, with groups of 4 mice, from 0.02-0.03 cc being injected into each testicle. By means of a 0.25 cc tuberculin syringe and a 27 gauge needle, this amount can be readily introduced into the testicle, although care must be exercised to avoid rupturing the organ. At each passage, 0.03 cc of the testicular suspension was injected intracerebrally into mice as a check on the presence of virus. As a precaution against possible loss of the virus, the experiments were carried on in 2 parallel series of mice.

Up to the present time, the virus has undergone 12 passages

1 Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 433.

2 McCordock, H. A., Smith, M. G., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 288.

through the testicle, with no evidence of diminution in virulence as tested by the cerebral route.

We have found that the virus multiplies in the testicle with facility and in 3 days reaches a titer of 10^8 M.I.D. per testicle. The titer is approximately the same as that reached in the brain after intracerebral inoculation. This high virus content of the testicle persists for at least 2 weeks, since dilutions of testicular tissue of 10^{-6} are still infectious for mice at the end of this time; titrations at intervals longer than this have not been done as yet.

Levaditi and Lépine,[3] who compared the effects of various routes of inoculation of the St. Louis encephalitis virus in mice state that an occasional animal succumbs to encephalitis following testicular inoculation, but the majority remain unaffected and become resistant to cerebral introduction of virus. Most of our animals showed no ill effects during our observation period of 3 weeks; the occasional deaths were preceded by listlessness and ruffling of fur, and at no time did we note the occurrence of definite cerebral symptoms. Bacteriologic cultures of brain material from these mice were sterile and cerebral passage to test mice did not result in encephalitis.

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*The Assay of Vitamins K_1 and K_2.

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To explore more thoroughly the potencies of Vitamins K_1 and K_2, we have assayed the two pure compounds by 3 different procedures and a modification of one of them. The methods are: 1. The procedure previously described by us.[1] 2. A procedure suggested by Ansbacher's[2] work but differing from the method[3] which he finally

[3] Levaditi, C., and Lépine, P., *Les ultravirus des maladies humaines*, 1938, Librairie Maloine, Paris, V. 1, 513.

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[1] Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 478.

[2] Ansbacher, S., *Science*, 1938, **88**, 221.

[3] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.