

Susceptibility of the Hamster to St. Louis and Japanese Encephalitis Viruses by Feeding.*

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It was reported previously¹ that hamsters, when allowed to feed upon St. Louis encephalitis virus, either die with encephalitis, or develop an immunity as evidenced by appearance of serum antibody and resistance to intracerebral inoculation. Subsequent tests have indicated that hamsters, fed Japanese encephalitis virus react in much the same way. The present report describes the extent of the infection in hamsters following feeding with St. Louis (Hubbard strain) and with Japanese (Nakayama strain) encephalitis viruses, as determined by subinoculation of mice with various tissues.

The possibility of infection by way of the alimentary tract with neurotropic viruses, other than those of poliomyelitis and encephalomyelitis of mice, has received some attention. Infection of mice by introduction of St. Louis virus into the alimentary tract has been described by 2 groups of investigators,^{2,3} and both report development of immunity in survivors. The latter group also fed 2 hamsters with St. Louis virus and found neutralizing antibody some days later.⁴

In our experiments hamsters were induced to eat virus by starving them overnight, and offering them the heads of infected mice. Tissues to be tested for virus were carefully

removed from exsanguinated animals with separate sets of instruments, emulsified in a minimum quantity of broth to which 10% normal sheep serum had been added, centrifuged, and the supernatant inoculated into 3 or 4 mice each. In the case of bacterially contaminated tissues such as the intestine, the specimens were washed repeatedly in sterile physiological saline and were thereby rendered sufficiently free of bacteria to allow intracerebral inoculation.

In most of the experiments a second intracerebral passage to mice was made with the brain tissue of any mouse found ill or dead, in which there was doubt concerning the cause. A simple technic, as described elsewhere,⁵ was used. Blood specimens were taken by cardiac puncture and immediately injected intracerebrally into mice.

Preliminary tests for St. Louis virus in hamster tissues included the large and small gut, the lymphoid follicles present in the wall of the latter, and the mesenteric lymph node. Virus was found on occasion in one or more of these tissues from the first to the ninth day after the hamster had fed upon virus but the incidence was greatest in the 2 lymphoid tissues on the fourth or fifth day. Three different strains of virus recovered in these experiments from intestine or associated lymphoid tissue of hamsters were identified as St. Louis virus in neutralization tests with immune rabbit serum.

Ten attempts to recover virus from intestinal contents of hamsters fed upon St. Louis virus included both filtered and unfiltered samples, but were unsuccessful.

The results of more extensive experiments are presented in Table I. It is evident that both viruses become widespread in the ham-

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¹ Gordon, F. B., *J. Bact.*, 1944, **47**, 465 (abstract).

² Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 332; Harford, C. G., and Bronfenbrenner, J., *J. Inf. Dis.*, 1942, **70**, 62.

³ Mezera, R. A., Broun, G. O., Muether, R. O., and LeGier, M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 357.

⁴ Broun, G. O., LeGier, M., Mezera, R. A., and Muether, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 310.

⁵ Schabel, F. M., Jr., and Gordon, F. B., *Science*, 1947, **106**, 549.

TABLE I.
Distribution of St. Louis and Japanese Encephalitis Viruses in the Hamster After Feeding.

Tissue tested	St. Louis virus	Japanese virus
(3rd to 8th day)		
Buccal mucosa	3/10	2/10
Tongue	7/10	6/10
Larynx	5/10	5/10
Cervical lymph nodes	8/10	7/10
Esophagus	3/10	3/10
Stomach	0/10	0/10
Small gut	7/10	1/10
Lymphoid follicles of small gut	6/10	4/10
Large gut	6/10	3/10
Mesenteric lymph node	8/10	4/10
Inguinal lymph node	5/10	5/10
Nasal mucosa	4/10	8/10
Olfactory bulbs	3/10	8/10
Cerebrum	5/10	7/10
Brain stem	4/10	6/10
Spinal cord	4/10	6/10
Spleen	6/10	
Striated muscle	0/10	
(1st to 5th day)		
Blood	10/46	30/38

Denominator indicates number of tests made; numerator indicates number positive.

ster after feeding, being recoverable from many tissues, including the blood, a fact which obscures both the site or sites of multiplication of virus, and the sequence of events in the pathogenesis of the infection.

Viremia has been present as early as the second day after feeding with St. Louis virus, and the first day in the case of Japanese, but it is intermittent in the former case and with neither virus has it been demonstrable after the fifth day. The presence of virus in the blood and certain other tissues before it is recoverable in the central nervous system (3rd to 8th day) suggests that multiplication may occur outside the central nervous system.

Both of these viruses have been recovered by swabbing the mouths of hamsters on the sixth to ninth days after feeding, when clinical signs were present. The cotton swabs

were washed out in serum-broth, penicillin was added (2000 units per cc) and the supernate after centrifugation was injected intracerebrally into mice. By this technic St. Louis virus was recovered in 3 of 5 attempts in 3 hamsters, and Japanese virus in 12 of 13 tests on 12 hamsters. The latter virus has also been found in the mouth 7 times in 6 hamsters on the first to fifth day after feeding, before any clinical signs of infection were apparent. St. Louis virus was isolated from the mouths of hamsters (2 of 3 on third day; one of 2 on fourth day) that had been injected intracerebrally.

We are unaware of any direct evidence at present that transfer of virus to or from the alimentary canal is significant in the epidemiology of these encephalitides. It is of interest to note, however, that evidence has been submitted⁶ for the presence of St. Louis virus in the nasopharyngeal washings of patients and has been recovered from nasal washings of an intracerebrally inoculated horse.⁷ Venezuelan equine encephalomyelitis virus has been recovered from human nose and throat washings.^{8,9,†} It appears that the possibility of natural hosts of these viruses acquiring infection through the alimentary tract, and transmitting either by droplets from the mouth or by the feces, deserves careful consideration.

⁶ Sulkin, S. E., Harford, C. G., and Bronfenbrenner, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 327.

⁷ Cox, H. R., Phillip, C. B., and Kilpatrick, J. W., *Pub. Health Rep., U. S. P. H. S.*, 1941, **56**, 1391.

⁸ Casals, J., Curnen, E. C., and Thomas, L., *J. Exp. Med.*, 1943, **77**, 521.

⁹ Lennette, E. H., and Koprowski, H., *J. A. M. A.*, 1943, **123**, 1088.

† There are also several pertinent references in the Japanese literature, the originals of which we have been unable to obtain.