10817

Cultivation of *Herpes febrilis* Virus on Agar Slant Tissue Cultures.

F. S. CHEEVER. (Introduced by H. Zinsser.)

From the Department of Bacteriology and Immunology, Harvard University Medical School.

Since Löwenstein[1] reported the transmission of *Herpes febrilis* lesions from rabbit cornea to rabbit cornea in 1919 several methods of propagating the virus have been developed. Those most widely used today are the intracerebral inoculation of white mice (Andervont[2]) and inoculation of the chorio-allantoic membrane of a developing chick embryo (Saddington[3]). Tissue cultures methods as developed by Parker and Nye,[4] and Andrewes[5] while successful are more cumbersome than the two mentioned above. Recently Zinsser and his associates[6, 7] devised a solid medium consisting of Tyrode's solution, horse or beef serum and agar which used in conjunction with mouse or chick embryonic tissue gave large yields of different Rickettsiæ. It was suggested that this medium might be satisfactory for the cultivation of the herpes virus, particularly since FitzPatrick[8] and Kurotchkin[9] in this laboratory have reported success with the Eastern strain of equine encephalitis and with vaccinia respectively. This paper reports the successful propagation of the *Herpes febrilis* virus on this medium.

The virus used was one furnished through the kindness of Dr. F. P. Gay of New York City, neurotropic for both mice and rabbits, causing typical lesions on the chorio-allantoic membranes of embryo chicks, and propagated for some months in this laboratory by mouse brain passage. Infective material was secured by sacrificing a moribund mouse, removing the brain aseptically and grinding it in a mortar with 2 cc of broth and a small quantity of pyrex glass. The resulting suspension was centrifuged at 2500 rpm for 10 minutes and the clear supernatant fluid pipetted off. An intracerebral inoculation

1 Löwenstein, A., *Münch. Med. Wochenschr.*, 1919, **66**, 769.

2 Andervont, H. B., *J. Infect. Dis.*, 1929, **44**, 383.

3 Saddington, R. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1012.

4 Parker, F., Jr., and Nye, R. N., *Am. J. Path.*, 1925, **1**, 337.

5 Andrewes, C. H., *J. Path. and Bact.*, 1930, **33**, 301.

6 Zinsser, H., Wei, H., and FitzPatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

7 Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

8 FitzPatrick, F., personal communication.

9 Kurotchkin, T. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 407.

of 0.03 cc of this material invariably caused typical signs followed by death within 36-60 hours. Titrations carried out by making dilutions in broth averaged close to 10^{-8} as the final end-point. Finely minced chick embryo tissue was deposited upon agar slants prepared according to Zinsser's method. One 7-day chick embryo generally served to inoculate 4 tubes. Five drops of the virus suspension from a standardized Pasteur pipette (approximately 0.1 cc) were allowed to run over the tissue, and the tube incubated for 4 (rarely 3 or 5) days after being tightly stoppered. Transfers were effected by scraping off the tissue fragments of one tube and mixing them with a freshly minced chick embryo, which was subsequently inoculated upon 4 tubes—*i. e.*, each transfer represented at least a fourfold dilution of the original virus. To demonstrate the presence of virus the tissue fragments were ground in 2 cc of broth plus a small amount of pyrex glass, centrifuged, and the resulting supernatant fluid injected intracerebrally into mice in 0.03 cc amounts. In the course of several experiments during the past 2 months the presence of virulent virus has been demonstrated after 11 passages, covering over 6 weeks in the incubator. The virus originally titrated to 10^{-8}; after the eighth passage the end-point was $10^{-8.5}$, at which time the original virus would have been diluted over 10,000 times if no multiplication had taken place. The only difference noted between mouse brain passage and tissue culture passage was that in the latter case the incubation period in about one-third of the mice tended to be about 24 hours longer.

As a comparative experiment tubes containing tissue killed by heating to 60°C for 30 minutes immediately prior to transfer on to the agar slants and parallel tubes containing live tissue were inoculated with equal amounts of the same virus suspension and incubated for 4 days before being injected into mice. Absence of tissue metabolism in the "heated tissue" culture tubes was evidenced by failure of the medium to assume an acid reaction, as always occurs in the presence of living tissue. Suspensions made from non-heated tissue fragments were invariably fatal for mice, those made from heated tissue fragments never caused demonstrable signs, the mice remaining healthy and not immune to subsequent intracerebral inoculations with a potent virus suspension. A series of "blind passages"—first generation on "heated tissue" tubes, second and subsequent ones on "non-heated tissue" tubes, have, after 3 generations, failed to show any evidence of the presence of the virus.

Summary. The virus of *Herpes febrilis* apparently persists and multiplies on agar slant tissue cultures prepared according to the method of Zinsser.