that the present agent is not identical with the vaccine virus with which we have worked.

Information on the host range of the virus is still incomplete. We have not succeeded in inoculating rats but in one instance the virus has been recovered from the testicle of a guinea pig inoculated intratesticularly. The inoculation of mice has also been accomplished by means of the intracerebral route. With this procedure death occurs in 4 to 6 days and the brain contains active virus. It is now in the fifth serial mouse to mouse passage. Other animal species and the calf in particular, are being tested for susceptibility to the virus.

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Cultivation of Vesicular Stomatitis Virus.

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Carrel, Olitsky, and Long[1] reported the successful cultivation of the virus of vesicular stomatitis of horses in 1928. In their cultivation experiments, guinea pig tissues were used throughout. The method they employed consisted in placing embryonic tissue or adult bone marrow in contact with the virus which had been suspended in Tyrode's solution for 1½ to 24 hours and then transferring to Carrel flasks containing coagulated plasma to which serum was added when marrow was used, or embryonic juice when embryonic tissue was used. The flasks were incubated for 7 to 10 days at 39°C.

No further reports of successful *in vitro* cultivation of the virus of vesicular stomatitis have been made.

This paper describes a much simplified method, similar to that employed by Li and Rivers[2, 3] in the cutlivation of vaccine virus, for the cultivation of the virus of vesicular stomatitis. A satisfactory and readily available technique for the titration of the virus is given. Through the kindness of Dr. W. E. Cotton, samples were sent us of Indiana and New Jersey strains of virus which had

[1] Carrel, A., Olitsky, P. K., and Long, P. H., *Compt. rend. Soc. biol.*, 1928, **98**, 827.

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

[3] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

been propagated in guinea pig pads for several years. The respective strains were then carried through 25 or more pad passages, filtered through Seitz filters, and this filtrate, or the Seitz filtrate of infected mouse brain tissue,[4] was used to initiate the cultures. The tissue employed was that of a 9 to 12 day chicken embryo which, after removal of the head and limbs, was minced in 3.5 cc. of Tyrode's solution. The Tyrode's solution was freshly prepared every 10 days according to the following formula: NaCl, 8.0 gm.; KCl, 0.2 gm.; $CaCl_2$, 0.2 gm.; $MgCl_2$, 0.1 gm.; NaH_2PO_4, 0.05 gm.; $NaHCO_3$, 0.5 gm.; glucose, 1.0 gm.; double distilled water q.s.ad. 1000.0 cc.; pH adjusted to 7.7 and sterilized by filtration through a Seitz or a tested Berkefeld N filter. Rivers' flasks[2] were used throughout.

The cultures were prepared by diluting one part of the titrated Seitz filtrate with 9 parts of Tyrode's solution. To 3.5 cc. of the diluted suspension contained in a Rivers' flask were added 3 drops of the minced embryonic tissue suspension. New cultures were subsequently prepared by directly diluting the old culture 1:10 with Tyrode's solution and to 3.5 cc. of the resultant diluted material 3 drops of a freshly prepared suspension of minced chick embryonic tissue were added. All cultures were incubated at 37.5°C. for 3 days and bacteriological controls were made for each successive generation. This simple method suffices for the propagation of an indefinite number of culture generations.

In a preceding report,[4] it was shown that the virus of vesicular stomatitis was uniformly lethal for white mice 36 to 72 hours after the intracerebral inoculation* of 0.03 cc. of an active virus suspension. This neurotropism for the white mouse provides a readily available, satisfactory, and inexpensive means of titration. Titrations were carried out in dilutions of 10^3 to 10^8 with every third generation.

Both strains of virus were used in the initiation of the 5 series of cultures made.

Series A. A Seitz filtrate of the Indiana strain derived from mouse brain tissue, lethal for mice in a dilution of 1-10,000 or 10^4, was used. This was carried through 18 generations. Titration of the third generation was lethal in a dilution of 10^6; of the sixth 10^6; of the ninth 10^6; of the twelfth 10^5; of the fifteenth 10^5; and of the eighteenth 10^5.

[4] Cox, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 654.

* Ether anesthesia was used in all operative procedures.

Series B. A Seitz filtrate of the New Jersey strain derived from guinea pig pad tissue, lethal in a dilution of 10^3, was used. This has been carried through 12 generations to the present. Titration of the third generation was lethal in a dilution of 10^5; of the sixth 10^4; of the ninth 10^5; and of the twelfth 10^4.

Series C. A Seitz filtrate of the New Jersey strain derived from guinea pig pad tissue, lethal in a dilution of 10^2, was used. This has been carried through 15 generations to the present. Titration of the third generation was lethal in a dilution of 10^5; of the sixth 10^4; of the ninth 10^5; of the twelfth 10^5; and of the fifteenth 10^6.

Series D. A Seitz filtrate of the Indiana strain derived from mouse brain tissue, lethal in a dilution of 10^3, was used. This has been carried through 15 generations to the present. Titration of the third generation was lethal in a dilution of 10^6; of the sixth 10^6; of the ninth 10^5; of the twelfth 10^5; and of the fifteenth 10^5.

Series E. A Seitz filtrate of the Indiana strain derived from guinea pig pad tissue, lethal in a dilution of 10^1, was used. This was carried through 15 generations. Titration of the third generation was lethal in a dilution of 10^3; of the sixth 10^5; of the ninth 10^5; of the twelfth 10^6; and of the fifteenth 10^6.

The final generation in each passage series gave rise to typical lesions in the pads of guinea pigs.

In conclusion, it appears that the virus of vesicular stomatitis is capable of multiplication *in vitro* by the simple method of cultivation described, and that the white mouse provides a satisfactory means of titration.

The close generic relationship of the virus of vesicular stomatitis to the incitant of foot-and-mouth disease suggests that the virus of foot-and-mouth disease may be cultivated with equal facility.

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Isolation of Treponema Pallidum from a Subcutaneous Sarcoid.

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Various hypotheses have been advanced in regard to the etiology of the several types of cutaneous and subcutaneous tumors passing under the name of sarcoids.[1, 2, 3, 4] Upon the basis of histologic