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Cultivation of Vaccinia Virus by Rivers' Method.

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There are at present two reliable methods of growing vaccinia virus in avian tissues under bacteriologically sterile conditions, namely, those of Rivers and his associates[1] and of Goodpasture and his coworkers.[2] Our experience, which was confined to the former technique, has convinced us of its extreme practicability for the cultivation of vaccinia virus. Previous reports of the successful application of this means of propagation of vaccinia virus, for use in Jennerian prophylaxis in man, have been made by Rivers,[1] Rivers and Ward,[3] Herzberg,[4] and Coffey.[5] With the exception of those of Rivers and his colleagues, none of these communications deal with prolonged cultivation of the virus.

Two strains of vaccinia virus have been grown during our cultivation experiments, one known as neurolapine (Reichgesundheitsamt) given to us by Haagen[6] after 24 mouse-brain passages; the other, Rivers' second revived cultivated strain of dermal vaccine virus after 88 subcultures in minced chicken embryo tissue and Tyrode solution.[3]

The neurolapine virus was cultivated for 30 passages in a medium of whole chicken embryonic tissue and normal monkey serum—Tyrode solution. The first series of cultures were inoculated with the supernatant fluid after centrifugalization of a 2% suspension of virus infected mouse brain in 0.9% sodium chloride solution. Each subsequent transfer was made at 4- to 7-day intervals. The infectivity of the supernatant fluid from each series of subcultures was routinely tested by the intracerebral inoculation of a group of 6-12 mice, of which number nearly all died of vaccinal meningitis 4 to 6 days following inoculation.

Rivers' second revived strain of cultivated vaccinia virus was grown for 94 subcultures during a period of 15 months without in-

1 Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

2 Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Am. J. Path.*, 1932, **8**, 271.

3 Rivers, T. M., and Ward, S. M., *J. Exp. Med.*, 1933, **58**, 635.

4 Herzberg, K., *Klin. Wchnschr.*, 1932, **11**, 2064.

5 Coffey, J. M., *Am. J. Pub. Health*, 1934, **24**, 473.

6 Haagen, E., *Zentralb. f. Bakt., Parasitenkunde u. Infektionskrankheiten*, I Orig., 1934, **131**, 420.

tercurrent animal passage in a medium of minced whole chicken embryo tissue and Tyrode solution. Subcultures were made at 3 or 4 daily intervals. Our serial subcultures commenced with the 88th and ended with the 182d passage. Subinoculations of culture fluid were made from culture to culture of corresponding number. This procedure rarely led to loss of the virus and ensured greatly against widespread bacterial contamination.

The potency of the virus in representative series of subcultures was determined by the intracutaneous reactions induced in rabbits from 5 to 7 days after inoculation with 0.2 cc. amounts of the decimal dilutions of the supernatant culture fluid in Tyrode solution. Although the titre varied at different times from 10^{-3} to 10^{-6}, there was no demonstrable trend to a falling titre with later passages. Such variations in apparent virus concentration as were observed from one series of subcultures to another were caused in small part by technical irregularities, but were principally due to variation in the susceptibility of individual rabbits. This deficiency was occasionally met by titrating the virus content of the culture fluid in more than one rabbit. Throughout the period of cultivation the virulence of the strain as judged by its pathogenesis for rabbit skin was well maintained and compared not unfavorably with the parent dermal strain of calf lymph origin. In all susceptible animals the intradermal inoculation of 0.2 cc. of undiluted culture fluid was followed in 2 to 5 days by the development of raised rounded lesions at the site of inoculation, with erythema of the skin, pronounced vascular congestion, slight to marked induration and peripheral dermal and subcutaneous edema. Frequently this lesion, 1.5-3 cm. in diameter, showed central hemorrhagic necrosis in dilutions of 10^{-1} and 10^{-2} and occasionally in dilutions of 10^{-3}, 10^{-4}, and 10^{-5}.

At frequent intervals during the period of cultivation, supernatant fluid from the various subcultures was diluted with an equal quantity of normal human serum and desiccated in vacuum in the frozen state. This procedure provided for the preservation of the virus at various stages of the cultivation experiment; it also offered a practical means of ensuring against loss of strain through bacterial contamination.

After dilution with an equal quantity of normal horse serum, various pools of culture fluid were desiccated in vacuum in the frozen state. Such samples when stored at 4°C. for 69 days showed an undiminished virulence for rabbits in which intradermal inoculation produced lesions in dilutions as great as 10^{-5}. The degree of preservation for a longer interval was not tested. The same lot of dried

virus when subjected to a temperature of 28°C. for 28 days showed a diminution in titre for rabbit skin from 10^{-5} to 10^{-4}. Subsequent storage at 28°C. for a period of 69 days did not produce, however, a further fall in titre. The virus still produced lesions in a dilution of 10^{-4}. It has not been possible to keep the desiccated virus at undiminished titre for longer than 14 days at 37°C.

Although it was not our original purpose to test the potency of this cultivated strain of vaccinia virus in man, it is worthy of note that one previously vaccinated man inoculated intradermally with 0.2 cc. of the culture fluid from the 162d subculture developed a typical vaccinal lesion (accelerated reaction) with vesiculation, rupture of the epidermis and subcutaneous edema, induration, and ecchymosis. The lesion healed by cicatrization without secondary bacterial infection.

The possibility of the application to Jennerian prophylaxis of such a simple, practicable, bacteriologically sterile, and mammalian virus-free method as that perfected by Rivers and his coworkers is worthy of wide trial.

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Red Cell Size and Resistance to Osmotic Hemolysis.

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In explanation of the fact that the red cells of different mammals show different "fragilities", *i. e.*, different resistances to osmotic hemolysis, it has been pointed out that the extent dA to which the least resistant cell membrane can be stretched without there being a loss of pigment depends on the mean initial cell volume V, so that the ratio dA/V is substantially constant. This purely experimental result can be interpreted as meaning that molecules adjacent to the thin (perhaps bi-molecular) layer upon which semi-permeability depends can enter it when it is stretched, thereby allowing of a certain amount of stretching before lysis occurs (Ponder[1]).* Another well known fact is that different red cells of the *same* animal show different "fragilities", so that a resistance distribution of roughly

[1] Ponder, E., *J. Physiol.*, 1935, **83**, 352.

* It is to be borne in mind that in these experiments the cells were in their spherical form. We have no information as to how the area of the red cell in its discoidal form behaves during swelling.