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In vitro and in vivo Cultivation of the Virus of Rift Valley Fever.

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That viruses may be cultivated successfully through many generations is being increasingly found as new ones are being submitted to the process of cultivation. The method now widely employed for securing cultures *in vitro* is that devised by Li and Rivers[1] for vaccine virus. A similar device introduced by Goodpasture,[2] of the chorio-allantoic membrane of the developing chick, may be regarded as an example of cultivation *in vivo* quite distinct from the ordinary multiplication which occurs in the course of usual infection in developed, susceptible animal species. Just as it had previously been shown that herpes virus grown in tissue culture and in chick embryo may be titrated in the brain of mice,[3] it has now been shown that it is possible to cultivate and titrate, in the ways indicated, the virus of Rift Valley fever of sheep.

Dr. G. M. Findlay kindly supplied the material with which the cultivations were begun. It consisted of the liver of a mouse in the 86th passage. We owe indeed to Dr. Daubney and Dr. Findlay[4] the discovery of the virus nature of Rift Valley fever.

In vitro Cultures. The medium employed consisted of Tyrode solution to which minced 10-day-old chick embryo was added. The Rivers' flasks contained 4.5 cc. of this medium, and to each was added 0.2 cc. of a Berkefeld filtrate of a 10% phosphate buffer emulsion of infected mouse liver. The titration was made by inoculating 0.2 cc. of a dilution of a culture intraperitoneally into mice. Two series of cultures, I and II, were run. Series I was carried through 11 and Series II through 12 generations.

The results of the inoculations indicate (a) that the optimum incubation period for the cultures was from 4 to 5 days; (b) that

[1] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465; Rivers, T. M., *idem.*, 1931, **54**, 453.

[2] Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209; Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Science*, 1931, **74**, 371; Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1932, **55**, 911.

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

[4] Daubney, R., Hudson, J. R., and Garnham, P. C., *J. Path. and Bact.*, 1931, **34**, 543; Findlay, G. M., and Daubney, R., *Lancet*, 1931, **2**, 1350; Findlay, G. M., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1932, **25**, 229.

intraperitoneal injection of the cultures gave more uniform results than intracerebral, intramuscular, intratesticular, or intradermal inoculations; and (c) that both Seitz and Berkefeld N filters keep back large amounts of the virus.

In vivo Cultures. The usual technique was employed. The inoculum consisted of 2 drops of a Berkefeld filtrate of a 10% saline suspension of mouse liver. The inoculation was made into the chorio-allantoic membrane of 9 to 10-day-old chick embryos. Three series were run, the largest consisting of 5, and the smaller of 3 and 2 passages respectively. Examination of the embryos was made after 5 days of incubation following the inoculation, and the subsequent inoculations were made with (a) membrane, (b) liver, (c) amniotic fluid of the embryos. Titrations were carried out in mice.

The inoculated chick embryos showed greyish discoloration or a gelatinous swelling of the embryo, and yellow or yellow-white mottling or stippling of the liver. The liver, membrane, and amniotic fluid all contained virus, although not constantly. The liver was more regularly infectious for mice than the other materials employed for inoculation.

Pathological Histology. Chorio-allantoic chick membranes. Certain areas of epithelial cells show evidences of hyperplasia, while other areas consist of necrotic cells. The adjacent connective tissues are undergoing inflammatory reactive changes; while the livers of embryos exhibit focal or widespread necrosis, the latter involving most of the organ. Nuclear inclusions were not found in the membranes or organs of the embryos.

Chick embryo tissue. Fragments of the tissues used in the *in vitro* cultures were sectioned and stained. Necrotic and living cells lie side by side. No nuclear inclusions are seen.

Mouse livers. The livers of the inoculated mice succumbing to infection show extensive degenerative lesions similar to those resulting from mouse to mouse passages of the virus. The lesions are so extensive that cords of hepatic cells are reduced to fragments or wholly destroyed, their places being taken by dilated blood spaces. Acidophilic inclusion bodies are present in the liver cells.

In view of the amount of dilution produced by the successive subcultures, there is little doubt that it has been shown that the virus of Rift Valley fever of sheep is subject to cultivation outside the body of infected sheep and mice.