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Persistence of Vaccinia Virus and Chick-Embryonic Cells in Suspended Cell Tissue Cultures.

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Introduction. It has recently been shown that in roller tube cultures various tissues would actively multiply and support the growth of viruses for unexpectedly long periods of time.[1, 2, 3] For purposes of comparison, it became of interest, therefore, to determine more exactly than had been done hitherto the period of survival of both virus and tissue cells in suspended cell cultures (Maitland,[4] Rivers,[5] Zinsser and Schoenbach[6]) which for several years have been routinely used by many investigators. As pointed out by Feller, Enders and Weller,[1] there is no evidence in the literature for the survival of vaccinia virus in such cultures beyond the 12th day.

Method. Seventy cultures were set up essentially according to the technic described by Rivers.[5] One-tenth gm of minced 10-day chick embryo was placed in a 50 cc Erlenmeyer flask containing 5 cc of Tyrode's solution and 0.005% phenol red. To 35 such flasks 0.25 cc amounts of a freshly prepared emulsion in infusion broth of vaccinia-infected chick chorio-allantoic membrane were added. The virus was a dermal strain originally obtained from the Massachusetts Antitoxin Laboratory.[7] Part of the inoculum was stored at −17°C for subsequent titration. All of the flasks were closed tightly with rubber stoppers and placed in the incubator at 37°C. At regular intervals thereafter some of the tissue fragments were withdrawn and 10 plasma clot transplants were made from each to test for viability of cells. These transplants were made by transferring ten small bits of tissue to a Petri dish, removing the excess fluid and then covering each with a drop of chicken plasma and a

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1 Feller, A. E., Enders, J. F., and Weller, T. H., *J. Exp. Med.*, 1940, **72**, 367.

2 Gey, G. O., and Bang, F. B., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 393.

3 Pearson, H. E., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 140.

4 Maitland, H. B., Laing, A. W., and Lyth, R., *Brit. J. Exp. Path.*, 1932, **13**, 90.

5 Rivers, T., *J. Exp. Med.*, 1933, **58**, 635.

6 Zinsser, H., and Schoenbach, E. B., *J. Exp. Med.*, 1937, **66**, 207.

7 Robinson, E., *Virus and Rickettsial Diseases*, Harvard School of Public Health Symposium Volume, Harvard University Press, Cambridge, Mass., 1940.

drop of embryonic extract. The plates were sealed with a mixture of equal parts of paraffin and petroleum jelly. All plates were incubated at 37°C and observed for 4-5 days. The appearance of any typical newly developed fibroblasts at the margin of the fragment was taken as a criterion for the presence of viable cells. The remaining material in each of the flasks inoculated with virus was stored at −17°C and, after several had accumulated, a titration of the contents on the skin of the rabbit was carried out in the usual manner.

Results. As indicated on the chart, cells from both inoculated and uninoculated cultures were viable for as long as 30 days but not after this time. After 20 days there was uniformly more evidence of viability in the control cultures.

Virus was detected in the material removed from all the infected cultures up to 20 days and from one even after 27 days, but not thereafter.

Discussion. That at least some cells survived for over 4 weeks in a medium consisting of Tyrode's solution was somewhat surprising because of the findings of Maitland, Laing and Lyth[4] and Zinsser and Schoenbach,[6] who were unable to demonstrate in this type of medium cellular respiration by the Warburg technic after 3 to 6 days. Possibly this discrepancy may be due to the incapacity of the respirometer to register the respiration of the very few viable

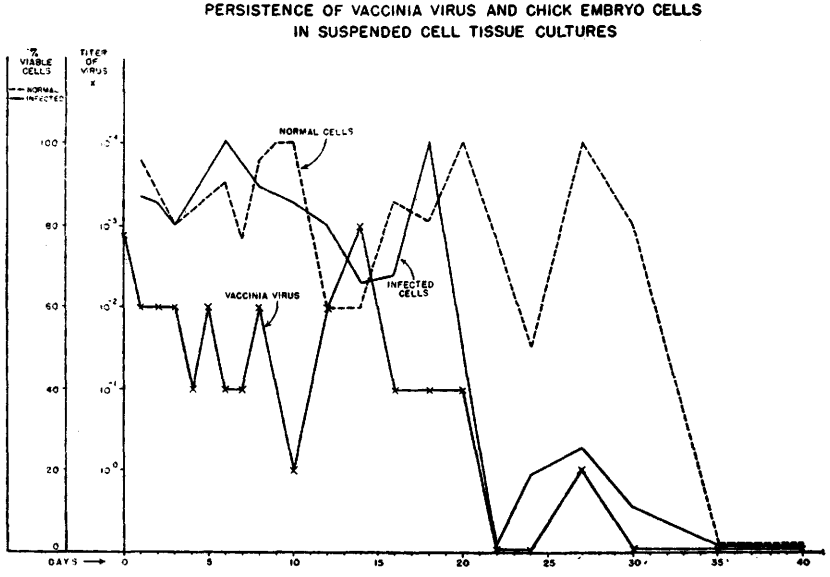

FIG. 1.

cells which may remain after incubation has been continued for several days, although Zinsser and Schoenbach failed to cultivate tissue fragments removed from the flask at a time when respiration had apparently ceased. However this may be, our experimental results left no doubt that living cells remained in the cultures for about 30 days. Not only did direct cultivation of the tissue fragments in plasma clots indicate this, but the fact that in cultures containing vaccinia virus, death of the tissue and disappearance of the virus occurred at approximately the same time.

The percentage of fragments of viable tissue removed from cultures containing virus was definitely less after the 22nd day of cultivation in comparison to uninoculated cultures. This fact suggests either that the virus may exert a more injurious effect on the cells as their vital processes become impaired or it may be possibly accounted for by differences due to random sampling. In roller tube cultures[1] where more suitable conditions for cell growth and metabolism are provided, no definite evidence was obtained of injury to the cells by the virus of vaccinia even after contact for 9 weeks.

Although in the suspended tissue medium the cells and virus both remained active longer than was expected on the basis of observations by other workers, in contrast to the roller tube method, increase of the virus was only moderate in degree and ceased after a relatively short time with resultant disappearance of viral activity.

In regard to the curve presented in this paper, it is of interest to note that in another experiment not recorded here but similar in all respects except that about 1 g of tissue was employed instead of about 0.1 g neither virus nor living cells could be demonstrated after 15 days. Once more this emphasizes the importance of adjusting the quantity of tissue in cultures of suspended tissue fragments and may account for the fact that Plotz and Ephrussi[8] were unable to observe viable cells in similar cultures after 12 to 13 days.

Conclusions. At 37°C viable cells and vaccinia virus can be demonstrated in cultures composed of Tyrode's solution and chick embryonic tissue during about 4 weeks following inoculation. These results are contrasted with those previously obtained in roller tube cultures where survival of both cells and virus persists much longer and the multiplication of the virus is greatly increased.

[8] Plotz, H., and Ephrussi, B., *Compt. rend. Soc. Biol.*, 1933, **112**, 525.