Latent Viral Infection of Cells. V. Reappearance of Psittacosis Virus in Chick Embryo Tissues.* (23565)

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It has been demonstrated previously that cultures of minced chick embryo tissues, when kept in Hanks' balanced salt solution for more than 13 days, can be infected with psittacosis virus, but this tissue is not able to support the multiplication of the virus until the cells are stimulated by enriching the culture medium through the addition of embryo extracts or a synthetic tissue culture medium(1,2). This latent infection can be maintained for 11 to 12 days, *i.e.*, as long as the cells survive and infectious virus is not detectable during this period in the tissue culture fluid or in the tissue and thus is present as a noninfectious, latent infection(2). After stimulation of the cultures with nutrients, infectious virus reappears in the fluid, an effect which can be brought about whenever desired within the above-mentioned period. The present investigations were undertaken to see how rapidly the virus reappeared in such cultures when the nutrition of the cells was improved and to obtain further information about the nature of the latent infection and the growth cycle of the reactivated virus.

Material and methods. The psittacosis virus used was the 6BC strain which was passed in embryonated eggs via the yolk-sac route. The preparation of the stock virus has been described earlier(2). To infect the depleted tissue, the stock virus was diluted 1:1000 in Hanks' balanced salt solution (BSS), since it was found that a dilution of the stock virus 1:10 or 1:100 contained enough yolk material from the inoculum to stimulate the tissue, so that no latent infection could be obtained. The inoculum usually contained $10^{3.0}$ to $10^{4.0}$ LD_{50} per ml. The tissue culture technic has been described previously in detail(1,2), the only difference here being the use of Pyrex glass wool as a substrate for tissue growth instead of cellophane discs. The Pyrex glass wool was carefully washed in absolute alcohol and then rinsed several times with distilled water and once with triple-distilled water. It was then cut in circular discs to fit the bottom of a 10-ml Erlenmeyer flask and autoclaved. Minced chick embryo tissue was transferred to the Erlenmeyer flasks and to each was added 1.8 ml of BSS containing 0.025 ml of 1.4% sodium bicarbonate and 40 μg of streptomycin/ml. The cultures were incubated at 37°C and the BSS was changed completely after 24 hours and every 4 days thereafter. On the 13th day the cultures were inoculated with psittacosis virus and 24 hours later the fluids were replaced with a complete medium, *i.e.*, 10% beef embryo extract (BEE) in Hanks' BSS. Before changing to BEE and at varying intervals thereafter, 3 flasks were removed and were treated separately. The fluid was removed, diluted with an equal amount of sterile infusion broth and then tested in eggs for the presence of virus. The glass wool disc with the tissue was washed twice with 2 ml of infusion broth and then transferred to a mortar and ground, a procedure which has been shown not to inactivate the virus(2). 4 ml of infusion broth was added and the tissue suspension was titrated in eggs. Virus titers in culture fluids and tissues were calculated as the LD_{50} for 7-day embryonated eggs, according to the single-dilution method of Golub(3). Over a period of several years it has been noticed that the above-mentioned tissue culture technic does not work with chick embryo tissues during the hot summer months because the viability of the tissue is so decreased that after 13 days' depletion in BSS almost all of the cells are dead and there is

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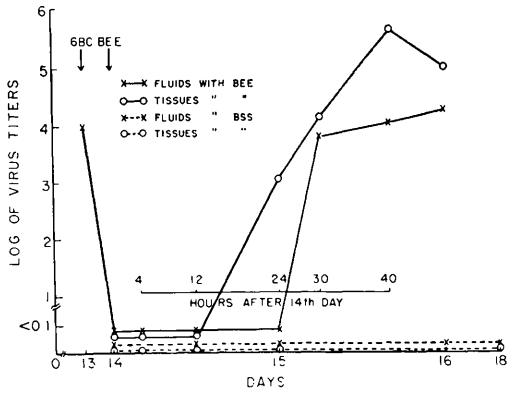

FIG. 1. Reactivation of latent infection with psittacosis virus following addition of BEE to tissue cultures.

no stimulating effect of BEE.

Results. Results of a representative experiment are shown in Fig. 1. In the present experiments it was found that no virus could be detected either in fluids or in tissue at 4 or 12 hours after stimulation of the cultures with BEE, though it had disappeared from the extracellular fluids. At 24 hours, infectious virus could be found in the tissue and sometimes in small amounts in the fluids also. At 30 hours, the virus was present in large quantities in the tissues and the fluids. After 40 hours, the virus could always be found in the fluids and tissues, although no virus was detectable in the fluids or tissues of cultures to which no BEE had been added. Virus appears earlier in the tissues and the titers remain higher than those of the corresponding fluids.

Discussion. In an earlier paper(2) the possibility that psittacosis virus was only attached to the deficient cells but had not entered the cells in the absence of factors present in BEE was discussed and data presented to refute it. Another possibility remained, namely, that the stimulation with BEE merely caused a liberation of absorbed virus from the depleted cells, but this was unlikely as no infectious virus could be detected even though the cells were homogenized to release any virus present(2). A prompt reappearance of virus would have supported such a theory, but, as the present investigations show, the reappearance of virus follows a normal growth curve. Girardi, Allen and Sigel(4) found that in tissue culture the titer of intracellular meningopneumonitis virus, which is closely related to psittacosis virus, starts to increase 20 to 30 hours after inoculation. Similar results were obtained by Buckley, Whitney and Rapp(5) using the fluorescent antibody technic, since inoculation of moderate amounts of psittacosis virus in tissue cultures resulted in intracellular appearance of psittacosis antigen in significant amounts in most of the cells within 20 to 24 hours. On the basis of these experiments it can be assumed that psittacosis virus in this experimental latent infection is present in the cells in the eclipse phase at a stage where the process of multiplication can easily be restored by the addition of nutrients such as amino acids(6), though the virus has lost its property of infectivity(2). The virus then readily resumes its normal growth cycle, even though this can be interrupted for as long as 12 days(2). The virus appears to exist in this latent infection in a ready though suspended state of animation.

Summary. Studies on the reappearance of psittacosis virus kept in a latent phase under experimental conditions in tissue culture indicate that the virus is in the eclipse phase and that it readily resumes a normal growth cycle following the addition of nutrients such as beef embryo extract.

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