

LATENT VIRAL INFECTION OF CELLS IN TISSUE CULTURE

IV. LATENT INFECTION OF L CELLS WITH PSITTACOSIS VIRUS*

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In previous studies (1) it was demonstrated that chick embryo cells maintained in a nutritionally deficient medium (inorganic salts and glucose) and then infected with psittacosis virus lose their capacity to support the growth of psittacosis virus, and that the virus can later be stimulated to multiply upon addition of embryo extracts or a combination of amino acids and water-soluble vitamins (2). Subsequent investigations of this latent infection showed that it could be maintained up to 15 days, and that during this period the virus was present in a non-infectious phase (3). However, there are certain disadvantages in using chick embryo tissue fragments for such studies, since the pieces of tissue conserve nutrient substances which slowly diffuse into the medium used to deplete the cells nutritionally, and the system is cellularly inhomogeneous since a variety of tissue cell types are present though the cellular outgrowth from the fragments is made up almost entirely of fibroblasts (1). Since it was found that psittacosis virus would grow well (4) in the L strain of mouse fibroblasts (5), studies were initiated of latent infections in this homogeneous cell line which can be propagated in a single layer on a glass surface.

Materials and Methods

Virus.—The 6BC strain of psittacosis virus, maintained in this laboratory by yolk sac passage in chick embryos, was used. Virus suspensions prepared as previously described (3), stored at -40°C ., and diluted in Earle's (6) balanced salt solution (BSS) to give a final concentration of $10^{2.0}$ to $10^{4.0}$ LD₅₀ per ml. were used to infect the cultures.

L Cell Tissue Cultures.—Stock cultures of L cells, kindly furnished by Dr. W. R. Earle, were maintained by growing cells on the flat glass surface of a T-60 flask (7) in a medium (CM) consisting of Earle's BSS with 0.2 per cent lactalbumin hydrolysate,¹ 0.06 per cent yeast extract,² and 25 per cent horse serum. After a homogeneous layer of cells was established, the cells were shaken or scraped off the glass surface with a flattened glass rod into fresh nutrient medium and, after agitation to secure a homogeneous suspension, they were counted in a blood

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¹ Obtained from Nutritional Biochemicals Corp., Cleveland.

² Obtained from Difco Laboratories, Detroit.

counting chamber. The cell suspension was diluted to contain 200,000 to 400,000 cells per ml. 1.8 ml. of this suspension was inoculated into a series of T-15 flasks which were then incubated at 36°C. After 3 or 4 days, one-half of the culture medium was removed and replaced with fresh nutrient medium. After 5 to 8 days' incubation when it was observed microscopically that a uniform sheet of cells covered the bottom of the flask, the medium was removed and replaced with Earle's BSS. All subsequent fluid changes were made at daily intervals and the medium was completely removed before fresh medium was added. The BSS was replaced with fresh BSS 24 hours later and again 48 hours later, and this latter BSS contained the virus inoculum. After virus inoculation, the virus content of the fluids removed from the tissue cultures was determined daily. Subsequent to virus inoculation, BSS was used as replacement medium for 1 or more days, depending on the microscopic appearance of the cells, and then the CM medium was again added. Three cultures were treated identically for each experimental group.

At certain intervals after infection, the virus content of the tissue cells was determined by removing the culture fluid, washing the cells three times with BSS, and then scraping the cells from the glass surface into BSS. The cell suspensions were disrupted by repeated freezing and thawing until no intact cells were visible on microscopic examination and the viral content of the cell lysates determined.

Virus Titration.—The single dilution method of Golub (8) was used as previously described (3). Titers of less than $LD_{50} 10^{0.1}$ cannot be determined and so are all listed as < 0.1 in the figures, which indicates that none of the injected embryos died. In certain experiments in which none of the injected embryos died as a result of virus infection, to insure that even small amounts of virus were not present in such embryos, the yolk sacs of the injected eggs were harvested, homogenized, and injected into additional eggs to exclude the presence of virus.

EXPERIMENTAL

Period of Exhaustion of L Cells Required to Render Them Incapable of Supporting Growth of Psittacosis Virus.—In a series of experiments it was found that maintenance of these cells for 2 days or more in BSS before infection rendered the cells incapable of supporting virus multiplication and that the addition of enriched medium (CM) resulted in virus multiplication after a lag period of 24 to 48 hours (Fig. 1), when cells had been cultivated in BSS for up to 4 days after infection for a total period of BSS of 6 days. Longer periods of maintenance of L cells in BSS resulted in rapid death of the cells as observed by direct microscopic examination. Thus, a latent infection with psittacosis virus can be established in a homogeneous culture of a pure line of cells grown on glass. These L cells can be rendered incapable of supporting the growth of psittacosis virus by treatment with BSS for a much shorter period of time than fragments of chick embryo tissue (1), probably due to the fact that each L cell is subject directly to the leaching action of the BSS.

Nature of Virus in Latent Infection of Cells.—Since the previous work with latent infections of chick embryo tissues revealed that the psittacosis virus in such cells was in a non-infectious phase, L cells with such a latent infection were examined for the presence of psittacosis virus. It was found that within 24 hours the virus added to the depleted L cells had disappeared from the culture fluids, but that it could not be recovered from the cells for up to 3 days after infection while the cells were maintained in BSS (Fig. 2), even when the

very sensitive test of passaging the yolk sacs of the chick embryos injected with tissue culture fluids or homogenates was used. However, when the enriched medium (CM) was added to the cells, virus was detectable in the cells in minute

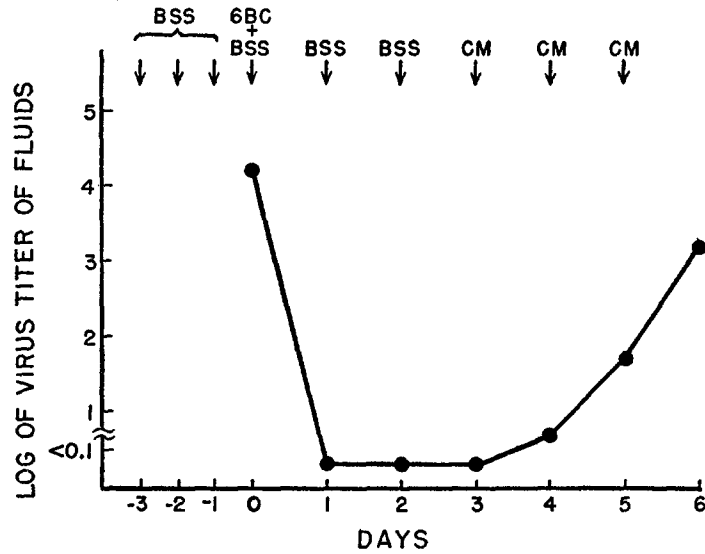


FIG. 1. Stimulation of growth of psittacosis virus in L cells previously maintained in BSS.

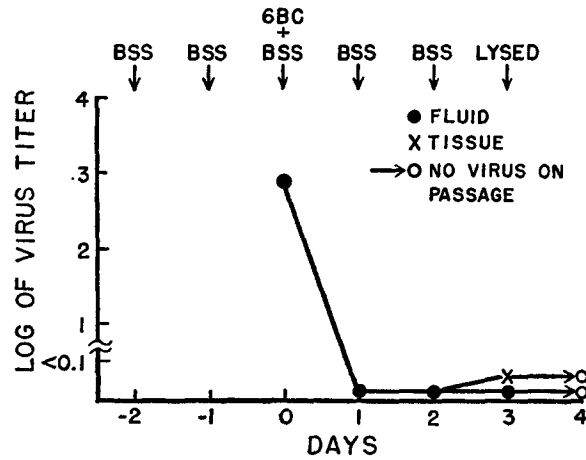


FIG. 2. Viral content of L cells maintained in BSS and infected with psittacosis virus in a latent state.

amounts within 24 hours (Fig. 3) and after 48 hours had reached high titers in the cells and was also detectable in the extracellular fluids (Fig. 4).

Thus, it appears that psittacosis virus in the latent infection of L cells re-

mains in a non-infectious phase until an enriched medium (CM) is added to the cultures, following which virus first appears within the cells in the infectious phase and then is released into the medium following its multiplication.

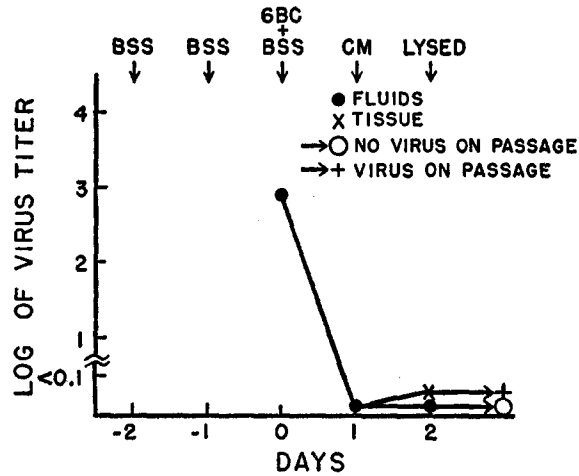


FIG. 3. Activation of latent infection of L cells.

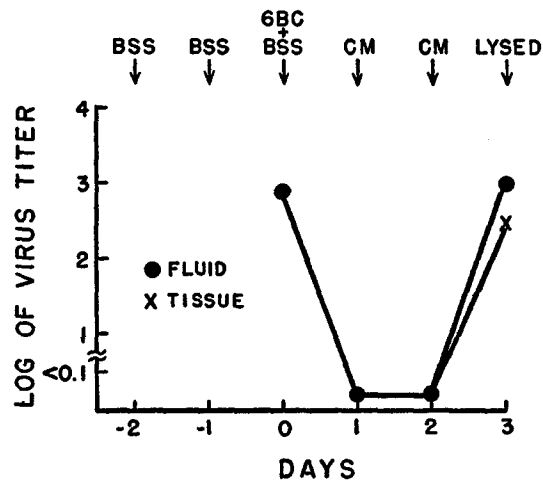


FIG. 4. Activation of latent infection with psittacosis virus in L cells.

DISCUSSION

A latent infection with psittacosis virus can be established in the pure cell line L cultivated directly on glass and nutritionally depleted in which the virus exists in a non-infectious phase. This latent infection can be converted at will

to an active infection by supplying the cells with added nutrients. Thus, the disadvantages of the use of chick embryo tissue fragments, which require a much longer period for depletion and consist of an inhomogeneous population of cell types, have been overcome. These cells are uniformly susceptible to virus infection and respond in an identical manner to nutritional depletion (9). Furthermore, cultivation of the L cells directly on glass permits the regular evaluation of the number and quality of the host cells by direct microscopic observation.

Since psittacosis virus multiplies rapidly in such cells, significant quantities of virus accumulate quickly in the extracellular fluids making frequent determinations of the virus yield possible. This fact, plus the rapid nutritional exhaustion of L cells in BSS, has made it possible to conduct studies on the nutritional factors responsible for reactivation of the latent infection in a few days rather than over a period of weeks. Thus, a rapid assessment of the significance of the individual water-soluble vitamins and amino acids contained in the mixture, found earlier (2, 10) to represent the essential nutrients for stimulation of virus growth, is being carried out and will be reported subsequently.

The fact that a latent infection with the non-infectious phase of a well studied virus has been established in a cell which has been shown to possess malignant properties (11) may have implications in the host-virus relationships of certain virus-induced tumors in which silent infections occur (12).

SUMMARY

By maintaining L cells in a balanced salt solution of inorganic salts and glucose (BSS) for 2 days or more, they are rendered incapable of supporting the growth of psittacosis virus (6BC), though it infects such cells and is present intracellularly for as long as 3 days in a non-infectious phase. The addition of an enriched medium to such a culture of cells at any time up to 4 days after infection results in the appearance of infectious virus within these cells, which multiplies and is released from the cells, providing the entire period of exposure of such cells to the BSS does not exceed 6 days, following which the cells die. A latent infection with psittacosis virus in a non-infectious phase has been established in a pure line of cells which possess properties of malignancy.

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