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Replication of Herpes-Type Virus in a Burkitt Lymphoma Cell Line

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Replication of the herpes-type virus in the P3HR-1 Burkitt lymphoma cell line was studied. The cell cultures with 10^6 viable cells/ml were incubated at 33 C for 15 days. The amount of virus in both the cell and fluid portions of the cultures was determined by the loop-drop particle-counting procedure with electron microscopy. An apparent growth curve of the virus was constructed. The maximal cell-associated virus, 10^{10} virus particles in an 80-ml culture, was observed after 9 days of incubation. The maximal extracellular virus, 2.5×10^9 particles per culture, was observed at the 12th day. About 10% of the released virus particles were enveloped. Under these conditions, there was little or no cell multiplication, but the percentage of immunofluorescent cells reactive to a selected human serum (probably indicating the presence of virus in the cells) increased to a maximum of 50% at the 9th day.

There have been numerous reports of detection by electron microscopy of a herpes-type virus in certain Burkitt lymphoma cell lines (1, 2, 5, 8, 14). So far as we can determine, this is the first report concerning studies of virus replication in Burkitt cells.

We recently derived a number of sublines of the P3 (Jijoye) Burkitt cell line by a modification of a soft-agar technique which had been used for cloning polyoma-transformed hamster cells (4, 6; Y. Hinuma and J. T. Grace, Jr., Cancer, *in press*). One of these sublines, P3HR-1, under certain cultural conditions regularly showed 50 to 80% virus-containing cells (3*a*). The relatively large virus production of this cell line, combined with suitable methods of virus extraction and virus particle counting, permitted construction of an apparent virus growth curve. This paper concerns these studies.

MATERIALS AND METHODS

Burkitt lymphoma cell line and medium. The P3HR-1 cell line in phase II was used. The properties of this subline and its two phases were fully described (3*a*). The medium was Eagle's minimum essential medium with the addition of 20% unheated fetal calf serum, 20 μg/ml of L-serine, 100 μg/ml of sodium pyruvate, 100 μg/ml of streptomycin, and 100 units/ml of penicillin. Total and viable cells were counted in a hemocytometer after addition of trypan blue.

Immunofluorescence. The indirect method of immunofluorescence as described by Henle and Henle (3) was used. This involved use of a human serum (VO-7) known to be positive for virus-containing Burkitt cells and goat anti-human Ig G globulin conjugated with fluorescein isothiocyanate. This technique was described in a previous publication (3*a*).

Procedure for extraction of virus particles. The cell culture to be studied was sedimented at $3,000 \times g$ for 20 min to separate the cells from the fluid. The cells were then resuspended in 5 ml of distilled water and disrupted by sonic oscillation for 1 min (Sonifier Model S 125, Branson Sonic Power, Danbury, Conn.). After clarification at $3,000 \times g$ for 20 min, the supernatant fluid was centrifuged at $53,000 \times g$ for 60 min. The pellet was resuspended in 0.2 or 0.4 ml of distilled water. The culture medium was centrifuged at $53,000 \times g$ for 60 min and the pellet was resuspended in 0.2 or 0.4 ml of distilled water. The viral concentrates from both the cell and medium fractions were dispersed with a Vortex Jr. Mixer (Scientific Instruments Inc., Queens Village, N.Y.) before electron microscopy.

Virus particle enumeration. The loop-drop method of Watson and co-workers was used for counting the virus particles (9, 12). Equal volumes of virus suspension, a polystyrene latex particle suspension, and 0.5% phosphotungstic acid solution containing 0.2% sucrose, *p*H 7.0, were thoroughly mixed. The latex particle (diameter, $0.088\ \mu \pm 0.0080\ \mu$; Dow Chemical Co., Midland, Mich.) suspension contained 10^{10} particles/ml in distilled water. The latex particles were calibrated by the method of Williams and Backus (13).

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A platinum loop of the complete mixture was placed on a carbon-coated collodion-covered grid, dried, and scanned with a Hitachi HU-11A electron microscope at a magnification of 30,000 × with the aid of a 7 × viewing binocular. The virus particles were clearly recognizable by their capsid structure. The enveloped virus particles were readily distinguished from naked particles. The detailed morphology of these particles was described in a previous paper (14). This technique produced well-dispersed virus suspensions with negligible clumping of the particles. Each sample usually contained 100 latex particles, and the number of virus particles in the fields was also determined. The virus concentration of the entire preparation was then calculated. Figure 1 shows an electron microscopic field with the virus and latex particles.

Procedure. Earlier studies showed that the virus yield of the P3HR-1 cells could be markedly enhanced by incubating the cells at 32 to 33 C without refeeding for 8 to 12 days (3*a*). In addition, these studies also indicated that the immunofluorescence detected cells which contained virus. Based on these observations, the growth-curve studies were performed as follows. A culture of P3HR-1 cells in phase II containing 21% immunofluorescent-positive cells and 72% viability was used. The cells were washed once with fresh medium, and 80-ml cultures containing 10^6 viable cells/ml in 16-oz (0.473 liter) prescription

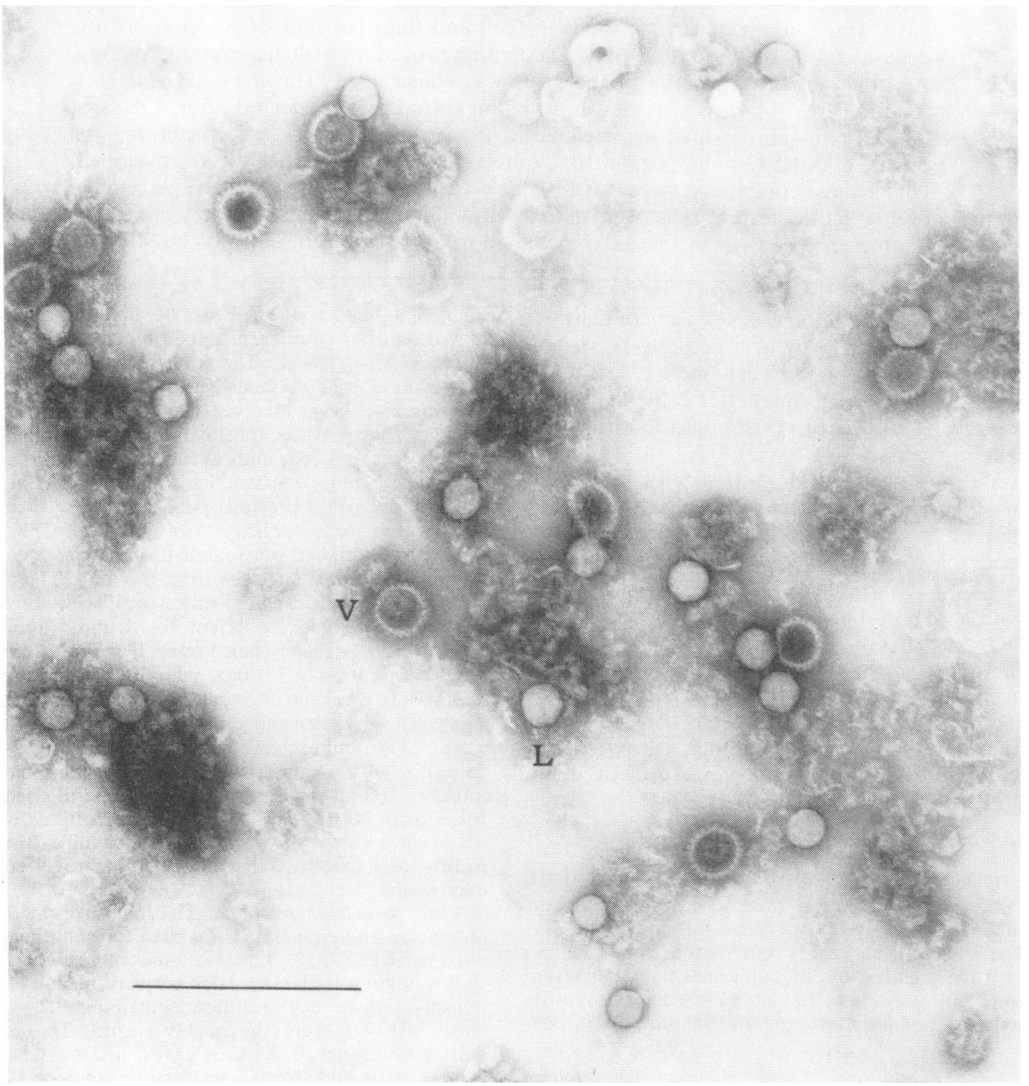

FIG. 1. *Electron micrograph of virus and latex particles in a field for counting. V, virus particle; L, polystyrene latex particle. The bar represents 0.5 μ.*

bottles were prepared. The cultures were incubated at 33 C and held for 15 days without medium change. Every 3 days, one bottle culture was removed and the number of virus particles, the number of immunofluorescent cells, and the number of total and viable cells were determined.

RESULTS

The results of a typical experiment are summarized in Fig. 2 and 3. There was no significant multiplication of the cells during the 15 days of incubation. However, there was a near exponential decrease of viable cells between the 3rd and the 15th day. This is shown in Fig. 3. When the cultures were started, the cell-associated viruses numbered 6×10^8 particles per culture. With incubation, the titer progressively increased, reaching the maximum of 9×10^9 particles per culture at 9 days, and then decreased. Cell-associated enveloped particles were found in the initial cells and on the 3rd day of incubation, but they were not seen later.

After 3 days of incubation, virus particles were detected in the culture medium at a concentration of about 6×10^7 particles per culture. This number increased progressively, reaching a maximum of 2.5×10^9 particles per culture at 12 days. There was also a progressive increase in the number of enveloped particles in the culture supernatant fluid, with the maximal number observed at 12 days.

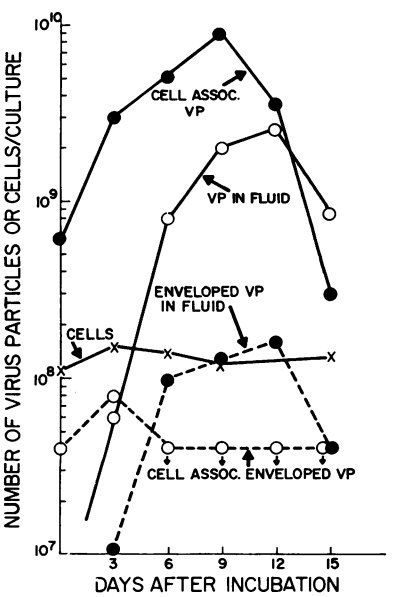

FIG. 2. *Growth of herpes-type virus particles in the P3HR-1 Burkitt lymphoma cells at 33 C. VP indicates virus particles.*

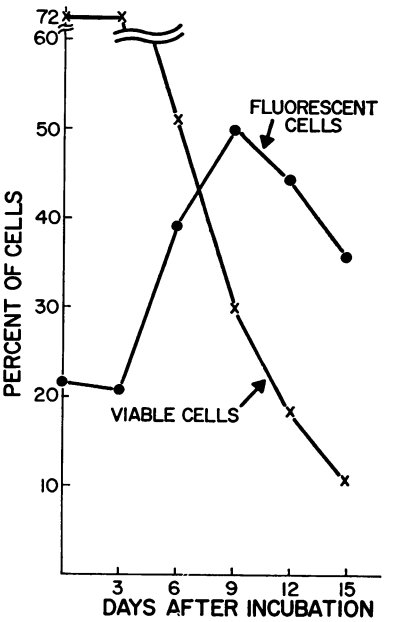

FIG. 3. *Immunofluorescent-positive and viable cells during the period shown in Fig. 2.*

As shown in Fig. 3, there was also a significant increase in the percentage of immunofluorescent-positive cells from the 3rd day to the 9th day. The maximal percentage of immunofluorescent cells occurred at the 9th day, and this coincided with the time at which maximal cell-associated virus was found.

DISCUSSION

The results of these studies indicated that, under the cultural conditions employed, there was little or no cell multiplication, but viral replication and release did occur. Virus replication increased steadily for 9 days and then decreased sharply. The correlation that was observed between the number of immunofluorescent cells and the amount of cell-associated virus is further evidence that the immunofluorescence detects virus-containing cells.

If one assumes that only the immunofluorescent cells produced virus, it can be calculated that a maximum of about 200 virus particles per cell were formed at the time of peak viral replication. This is based on the 9th day observations that 50% of the total of 1×10^8 cells were immunofluorescent-positive and 9×10^9 virus particles were extracted from the cells.

We found that only about 10% of the herpes-type virus particles which were released into the medium were enveloped. This contrasts with the studies of Herpes hominis (simplex), propagated

in HEp-2 cells, in which about 90% of the extracellular virus particles possessed envelopes (10).

This difference in the number of enveloped particles may be due to the cell systems employed. Propagation of the Burkitt-associated herpes-type virus in another cell line might well result in the release of a higher percentage of enveloped particles. The importance of the envelope in determining infectivity of the herpesviruses has been somewhat controversial. Smith (7) reported data indicating that the envelope was responsible for infectivity. However, Watson et al. (11) concluded that both the enveloped and naked particles were capable of initiating infection.

These studies have defined the conditions for achieving maximal virus production in our system. This information should be helpful in future attempts to demonstrate biological activity of this virus.

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