JOURNAL OF VIROLOGY, Aug. 1969, p. 133-140
Vol. 4, No. 2
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Effect of Puromycin and Actinomycin D on a Persistent Mumps Virus Infection In Vitro

R. L. NORTHROP

Department of Microbiology, Presbyterian-St. Luke's Hospital, and Department of Microbiology, University of Illinois College of Medicine and Graduate College at the Medical Center, Chicago, Illinois 60612

Received for publication 7 May 1969

Puromycin and actinomycin D were used to treat a line of human conjunctiva cells persistently infected with mumps virus (C-M cells) in order to determine where virus synthesis is inhibited. Although 90% of the cells in C-M cultures are infected, little or no infectious virus is produced by most cells in a growing culture. Adding puromycin to inhibit protein synthesis resulted in the production of infectious virus. Thus, all the viral proteins needed for virus completion were made in the growing cells. When actinomycin D was added to growing cells, infectious virus was again produced. Since mumps virus synthesis is actinomycin D-insensitive, this suggested a host control of the virus. Interferon was not detected. The possible mechanisms of host control are discussed.

The establishment and the general characteristics of human conjunctiva cells persistently infected with mumps virus, the C-M system, were first described by Walker and Hinze (12). They showed in fluorescent antibody studies that over 90% of the cells in growing cultures were infected. In spite of the extensive infection of these cultures, the growth rate, the general morphology as seen by light microscopy, and the cloning efficiency of these cells were nearly indistinguishable from an uninfected parent line of cells (13). A small amount of infectious virus was always found in the medium of these cultures. Apparently this virus was produced by the 1 to 3% of the cells that adsorb chicken erythrocytes. The majority of infected cells were hemadsorption-negative and they produced no infectious or incomplete virus particles. The virus in the medium was not essential to maintain this persistent infection. Adding mumps virus antisera to these cultures for several subcultivations did not "cure" the infection, nor did it alter the general characteristics of the system, suggesting that the infection was maintained by passage of viral components from parent to daughter cells at mitosis. All these features were unchanged after 150 subcultivations, indicating the stability of the virus-cell relationship.

In spite of the fact that this infection had only subtle effects on the actively growing cells, whenever cell growth diminished, virus was released by most if not all of the infected cells, and cell death resulted. It can therefore be concluded that the virus genome was faithfully and completely copied in each growing infected cell in order for the cells to retain the capacity to produce infectious virus for hundreds of cell generations.

Currently little is known of the basis for this persistent infection. No antiviral substances have been added exogenously to the cultures to account for the maintenance of the infection. Interferon is presumably not the controlling factor, since C-M cells can support the growth of several viruses antigenically unrelated to mumps virus, but interferon has not been tested for specifically. One possible mechanism of control that was recently examined was whether the virus genome existed in a "provirus state" with the host genome (14). If the virus genome was integrated into the cellular genome, then the rate of virus replication would be expected to be synchronized with the cell growth rate. However, it was shown that, when C-M cells were cultivated under conditions which stimulate cell growth maximally, the number of infected cells was markedly reduced. In fact, the persistent infection of one C-M culture was completely eliminated by promoting cell growth. This suggested that the growth rates of the genomes of the virus and of the cell are independent and separable and that the two genomes are probably not integrated. Other mechanisms that could account for the basis of this persistent infection have not been examined.

The present paper describes experiments utilizing puromycin and actinomycin D to treat growing C-M cells in an attempt to determine the

extent of virus development in these cells and thereby elucidate where virus formation is blocked. The information obtained from these experiments permits speculation on the role of the host cell in controlling this infection.

MATERIALS AND METHODS

Virus. The Dunai strain of mumps virus was propagated in human conjunctiva cells as described previously by Walker and Hinze (12). This strain of virus is cytopathic for human conjunctiva cells when the infected cultures are incubated in maintenance medium at 35 C. Cytopathic effects and virus production are maximal 4 to 6 days after inoculation.

Cell cultures. The methods used to establish and maintain cultures of C-M cells were described in detail by Walker and Hinze (12). Growth medium for C-M cells consisted of Eagle basal medium in Earle's salt solution containing 20% horse serum, and 100 units of penicillin and 100 μg of streptomycin per ml. Maintenance medium for these cultures was the same medium, containing only 3% serum. Growth and maintenance of Vero and human embryonic fibroblast cells were described previously (10, 18).

Infectivity titrations. The indirect hemadsorption plaque assay used for most of these experiments has been described previously (14). In some experiments titrations of mumps virus were performed in Vero cells using the hemadsorption procedure to determine infectivity.

Chemicals. Actinomycin D was a gift from Merck Sharp and Dohme Research Laboratories, Rahway, N.J. Puromycin dihydrochloride was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Radioactive ^{14}C-algal protein hydrolysate (specific activity, 200 to 300 μc/mg), ^{14}C-amino acid mixture, and ^{3}H-5-uridine (specific activity, 5 to 20 c/mmole) were obtained from New England Nuclear Corp., Boston, Mass. Hyamine 10×, 2,5-diphenyloxazole (PPO), and 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) were obtained from Packard Instrument Co., Inc., Downers Grove, Ill.

Biochemical procedures. Ribonucleic acid (RNA) of C-M cells was labeled by adding medium containing 5 μc of ^{3}H-5-uridine per ml for the times indicated. Protein synthesis was measured by adding ^{14}C-algal protein hydrolysate or ^{14}C-amino acid mixture to the medium of cultures in the amounts and for the times indicated in separate experiments. When the cultures were harvested, the labeling medium was discarded, and the cell sheets were washed twice with cold saline containing 0.0005 M $MgCl_2$ and 0.001 M $CaCl_2$. One milliliter of 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (*p*H 7.4), containing 0.05 M KCl and 0.0015 M $MgCl_2$, was added to each culture, and the cultures were frozen rapidly. During thawing, the ice served to scrape the cells from the surface of the flask. The cell suspension was transferred to a centrifuge tube containing an equal volume of 1 M trichloroacetic acid. The precipitate that formed overnight at 4 C was collected by centrifugation and washed two times with 0.5 M trichloroacetic acid, twice with ether-alcohol (1:1, v/v), and once with ether. The final precipitate was suspended in 0.5 ml of Hyamine 10× and mixed with 15 ml of scintillation fluid (4 g of PPO and 0.3 g of POPOP/liter of toluene). Radioactivity was counted in a Tri-Carb spectrometer (Packard Instrument Co., Inc.).

RESULTS

Virus release from nongrowing C-M cells. When cultures of actively growing C-M cells in growth medium developed into confluent monolayers, the medium was changed to maintenance medium and the incubation was continued. At indicated times (Fig. 1), 1 ml of the 3 ml of medium was removed and was stored at −70 C for later virus assay. One milliliter of fresh maintenance medium was added to each culture to maintain a constant volume of medium in the culture throughout the experimental period. At days 0, 3, and 11, the cells of representative cultures were counted. Each

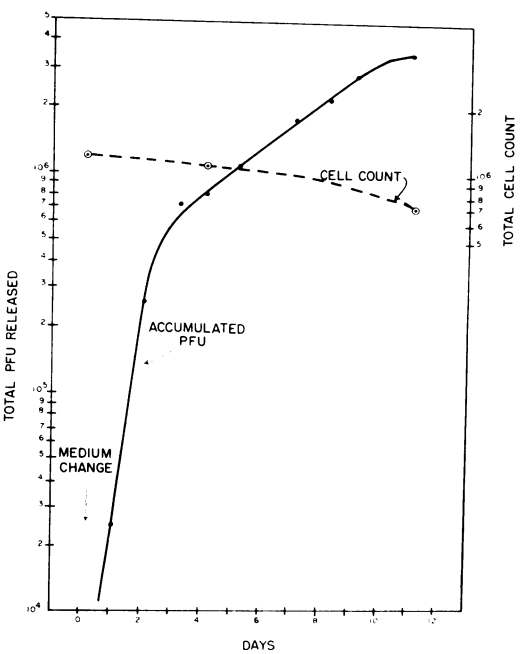

FIG. 1. *Virus release from nongrowing C-M cells. Actively growing C-M cells in 1-oz (30-ml) bottles were allowed to grow to confluent monolayers at 37 C. The growth medium (Eagle medium and 20% horse serum) was replaced with 3 ml of maintenance medium (Eagle medium and 3% horse serum), and the cultures were incubated at 35 C. At daily intervals, 1 ml of medium was taken from each culture and stored at −70 C for later PFU assay. One milliliter of fresh maintenance medium was added to each culture to retain a 3-ml volume. At days 0, 3, and 11, representative cultures were taken for cell counts. Average accumulated PFU titer per culture (●); average number of cells per culture (⊙).*

point in Fig. 1 representing plaque-forming units (PFU) produced is the average titer of five cultures.

In the 24-hr period prior to beginning this experiment, an average of 0.0008 PFU/cell was released by these cultures while they were in growth medium. In the first 24 hr after being changed to maintenance medium, 0.023 PFU/cell was released. This rate of release of virus continued for the next 48 hr. Thereafter, the amount of virus in the medium continued to increase but at a slower rate. The experiment was terminated on the 11th day, when the majority of cells became detached from the surface of the culture flask. Based on these data, it was calculated that each cell in these cultures had produced 3 to 4 PFU in 11 days. Theoretically, the virus yield per infected cell may have been greater, considering that (i) approximately 5% of the cells in these cultures were not infected and (ii) the half-life of C-M virus in maintenance medium was 9 hr (*unpublished data*). Accordingly, it was estimated that each C-M cell had maximally produced 10 PFU in the entire experimental period of 11 days.

Attempts to detect interferon in cultures of growing C-M cells. In one series of experiments, approximately 10^6 C-M cells were seeded into flasks containing 8 ml of medium and, after 3 days of incubation, the medium was collected. Each culture contained 3 to 4 million cells at that time. The medium was clarified by low-speed centrifugation, and 1 ml of the supernatant fluid was added to each of five tube cultures of human embryonic fibroblast cells. After an overnight incubation at 37 C, the medium was discarded and the human embryonic fibroblast cells were inoculated with 100 $TCID_{50}$ of vesicular stomatitis virus. The cell sheets were examined daily for 3 days for cytopathic effect. Seven such tests failed to reveal any interference with vesicular stomatitis virus infection. In further tests, 10^7 growing C-M cells were homogenized in 5 ml of medium. After centrifugation at 78,000 $\times$ *g* for 2 hr to remove cell debris and virus, 1 ml of the supernatant fluid was added to human embryonic fibroblast cell cultures as described above. After an overnight incubation, the medium was discarded and the cells were inoculated with vesicular stomatitis virus. A complete destruction of the cells, due to the vesicular stomatitis virus infection, occurred in both treated and untreated cultures. These findings are consistent with the idea that interferon, or other interfering factors, are not present in detectable amounts in the C-M system.

The effect of puromycin on a primary mumps virus infection. Each of 21 monolayer cultures of human conjunctiva cells was inoculated with $10^{4.5}$ $TCID_{50}$ of mumps virus adapted to human conjunctiva cells. After 1 hr of incubation at 35 C, the residual inoculum was discarded, and the cell sheets were washed twice with Earle's balanced salt solution. The cultures were next treated with antiserum specific for mumps virus for 15 min. The cell sheets were again washed twice with Earle's balanced salt solution, and 3 ml of maintenance medium containing 50 μg of puromycin per ml was added to 3 of the 21 flasks. The remaining 18 cultures received normal medium. All of the cultures were incubated at 37 C. The media of the 3 treated and of the 18 untreated cultures were collected separately 7 hr after treatment was begun, and the respective fluids were pooled and were stored at -70 C. At each time indicated in Fig. 2, three previously untreated infected cultures were treated with puromycin, and the remaining cultures received normal medium. The respective media were

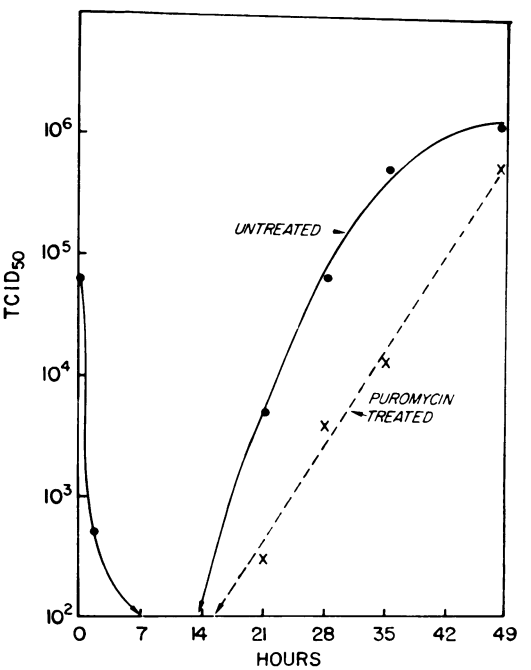

FIG. 2. *Effect of puromycin on primary mumps virus infection. Cultures of human conjunctiva cells were infected with the Dunai strain of mumps virus. Maintenance medium containing 50 μg of puromycin per ml was added to groups of three cultures at 7-hr intervals after infection. After 7 hr of incubation at 35 C, the media of the three treated and of the untreated cultures were collected separately. Accumulated virus released from infected, untreated cultures (●); accumulated virus released from infected puromycin-treated cultures (×).*

collected and pooled as described above. These procedures were necessary to avoid cell toxicity due to puromycin which developed in 10 hr. Infectivity titrations of all the samples were performed in Vero cell cultures.

The data presented in Fig. 2 show that new virus was first detected 21 hr after infection. The amount of virus released in the puromycin-treated cultures, when compared to that produced in the untreated cultures, was inhibited more than 93% during the period of logarithmic growth of the virus. Late in the infectious cycle, i.e., 48 hr after infection, the yield of virus was inhibited 43% by puromycin. These findings indicated that mumps virus growth early in a primary infection is more sensitive to puromycin than late in the infection.

Effect of puromycin on C-M cells. Actively growing C-M cells were treated with puromycin to determine whether infectious virus could be completed and released while protein synthesis was inhibited.

The growth medium was discarded from 21 cultures of C-M cells grown in 1-oz (30-ml) plastic flasks. Each culture contained approximately 1.3×10^6 cells. One milliliter of growth medium containing 0, 6, 12, 25, 50, 100, or 200 μg of puromycin was added to each of three cultures. The cultures were incubated for 0.5 hr at 37 C, and 0.1 ml of medium containing 0.5 μc of ^{14}C-amino acid mixture was added to each flask. After 4 hr of incubation, the fluids were collected for infectivity assay, the cell sheets were washed twice with cold saline, and the cells were processed to determine the ^{14}C-amino acid incorporated into trichloroacetic acid-insoluble material.

The normal rate of incorporation of ^{14}C-labeled amino acids into protein was inhibited by puromycin (Fig. 3). The amount of radioactivity incorporated into cells declined as the concentration of puromycin in the medium increased. At the same time, the amount of virus recovered from the medium increased as the concentration of puromycin increased.

The hemadsorption test was used to determine whether the virus found in the medium during puromycin treatment was produced by a few or most of the cells in C-M cultures. The monolayers of 16 actively growing C-M cultures were exposed to chicken erythrocytes under aseptic conditions. Approximately 7.3% of the cells were hemadsorption-positive. Eight of these cultures were treated with 50 μg of puromycin per ml of the culture medium and eight cultures were not treated. After 8 hr of incubation, the hemadsorption test was repeated, and 47.8% of the cells in the puromycin-treated cultures were

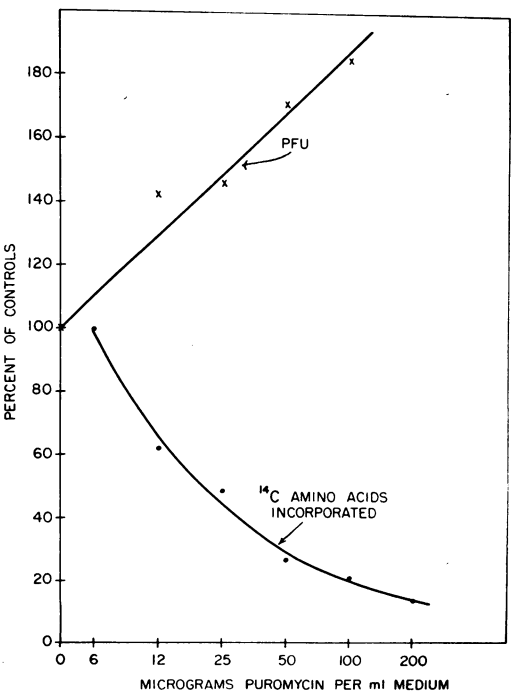

FIG. 3. *Effect of different concentrations of puromycin on virus release* (X) *and protein synthesis* (●) *in growing C-M cells. The growth media of cultures, each containing* 1.3×10^6 *C-M cells, were discarded. One milliliter of growth medium containing 0, 6, 12, 25, 50, 100, or 200 μg of puromycin was added to each of three cultures. At 0.5 hr later, each culture received 0.1 ml of medium containing 0.5 μc of ^{14}C-amino acid mixture. After an incubation period of 4 hr, the fluids were collected for infectivity assay. The cell sheets were washed twice with saline at 4 C, and the cells were processed to determine ^{14}C incorporation into the trichloroacetic acid-insoluble fraction.*

hemadsorption-positive, whereas 8.9% were positive in the untreated cultures. This indicated that even in this short time nearly half of the cells in the treated cultures were the source of the virus recovered in the medium.

Puromycin inhibition of protein synthesis is reversible (15). If release of virus during puromycin treatment is specifically related to inhibited protein synthesis, then virus release would be expected to decrease after removing puromycin. This possibility was tested. Cultures of growing C-M cells were treated with 50 μg of puromycin per ml of growth medium. At 2 and 4 hr after beginning treatment, the medium of four cultures containing puromycin was replaced with normal growth medium, and the incubation of each group of cultures was continued for 4 and 2 hr, respectively. The titer of virus in the puromycin-

treated cultures increased 1 log for each 10^6 cells present in the culture in the 6-hr test period (Fig. 4). In comparison, less than 0.3 log of virus was released in the same time by the untreated cultures. Removing puromycin at 2 and 4 hr resulted in a reduction of the amount of virus subsequently released. These findings suggested that a protein or some component of the protein-synthesizing machinery is involved in the blockade of infectious virus production.

Effect of actinomycin D on a primary mumps virus infection. The effect of actinomycin D on primary mumps virus infection has not been previously reported. It was important to establish this point for mumps virus in order to evaluate the effect of actinomycin D on the persistent mumps virus infection in C-M cells.

Sixteen cultures of human conjunctiva cells were inoculated with the Dunai strain of mumps virus at a multiplicity of 0.1. At the time of the inoculation, eight cultures were concurrently treated with 10 μg of actinomycin D per ml. After 1 hr at 35 C, the inoculum and the inhibitor (if present) were removed and maintenance medium was added to each culture. It was determined in separate experiments that removing the actinomycin D at this time did not change the inhibitory effect of actinomycin D on RNA synthesis. At 4, 8, 12, and 24 hr after inoculation, the media of two treated and two untreated cultures were collected and the amount of virus in the respective fluids was measured. Virus production was similar in both treated and untreated cultures (Fig. 5). The first increase in virus was detected 10 to 12 hr after infection, thereafter the titers in both groups of cultures increased equally and in parallel. These experiments indicated that mumps virus replication in

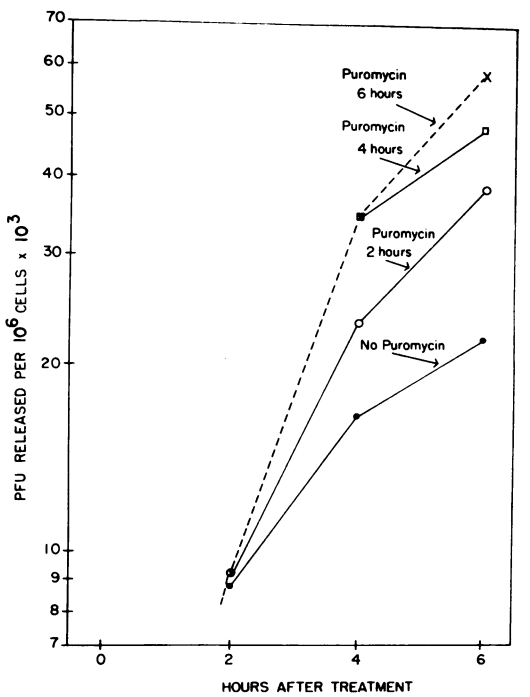

FIG. 4. *Effect of removing puromycin on virus production from treated cultures of C-M cells. Six cultures of actively growing C-M cells were treated with 50 μg of puromycin per ml of growth medium and incubated at 37 C. Two cultures on normal growth medium served as controls. Accumulated virus released from untreated growing cultures of C-M cells (●); accumulated virus released from cultures treated 2 hr with puromycin and subsequently cultured in growth medium for 4 hr (○); accumulated virus released in cultures treated with puromycin for 4 hr followed by incubation in growth medium for 2 hr (□); accumulated virus released by cultures treated with puromycin for entire 6-hr period (×).*

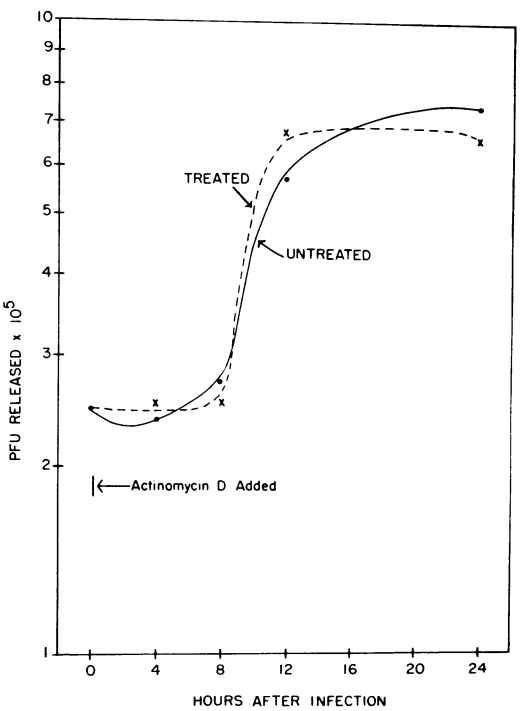

FIG. 5. *Effect of actinomycin D on a primary infection of human conjunctiva cells with mumps virus. Eight cultures of human conjunctiva cells were inoculated with $10^{4.3}$ PFU of the Dunai strain of mumps virus. Four of the cultures were concurrently treated with 10 μg of actinomycin D per ml of maintenance medium (×). The other four cultures served as infected controls (●). At 2 hr after infection, the media of both groups were changed to maintenance medium and samples of media were collected for PFU assay at the times indicated.*

human conjunctiva cells is actinomycin D-insensitive.

Effect of actinomycin D on C-M cells. When cultures of growing C-M cells were treated with increasing concentrations of actinomycin D (from 0.001 to 20 μg/ml of medium), the incorporation of ^{3}H-5-uridine into trichloroacetic acid-insoluble fraction of the cells decreased proportionally (Fig. 6). The amount of virus released into the medium was not appreciably affected by low levels (0.001 to 0.1 μg/ml) of actinomycin D. When ^{3}H-5-uridine incorporation was inhibited more than 75% by 1, 10, or 20 μg of actinomycin D per ml, the amount of virus released increased measurably. These results suggested that the completion of mumps virus in growing C-M cells is insensitive to actinomycin D as is mumps virus production in a primary infection. However, from the fact that infectious virus was produced after treatment with actinomycin D suggested that the control mechanism which usually suppresses virus completion in growing C-M cells was actinomycin D-sensitive.

Effect of combined actinomycin D and puromycin treatment on C-M cells. The effects of actinomycin D alone and puromycin alone were compared with the combined actions of these antibiotics on virus production by C-M cells (Fig. 7). Treating growing cultures of C-M cells with actinomycin D alone for 2 hr resulted in higher titers of infectious virus appearing in the medium than was released by untreated growing cultures. When only puromycin was added to companion cultures for 2 hr, a transient, stimulating effect on virus release occurred which was similar to the results shown in Fig. 4. Adding both anti-

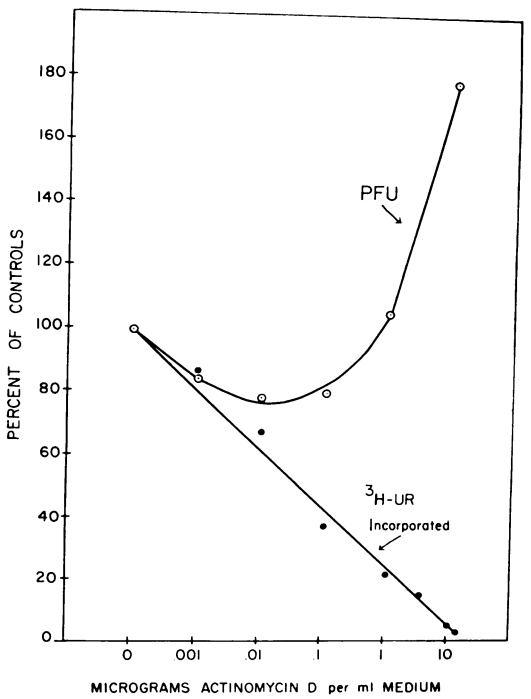

FIG. 6. *Effect of actinomycin D on virus release (⊙) and ^{3}H-uridine incorporation (●) by cultures of C-M cells. Actinomycin D ranging in concentrations from 0.001 to 20 μg per ml of growth medium was added to separate cultures of actively growing C-M cells. After 0.5 hr, the medium was changed to normal growth medium containing 5 μc of ^{3}H-uridine per ml of medium. The cultures were incubated for 3 hr at 37 C. The radioactivity incorporated into trichloroacetic acid-insoluble fraction of the cells was determined. The media of the treated and control cultures were collected separately for PFU assay.*

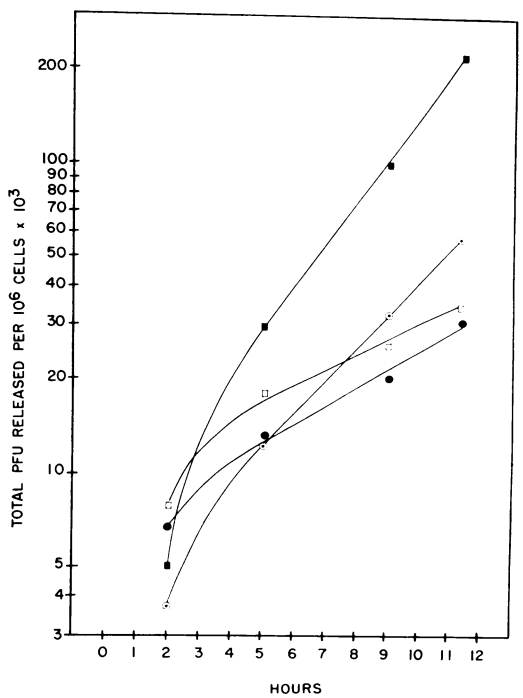

FIG. 7. *Virus released from cultures of C-M cells treated separately or simultaneously with puromycin and actinomycin D. Puromycin, 50 μg/ml (□); actinomycin D, 7 μg/ml (⊙); or both drugs at these concentrations (■) were added to cultures of growing C-M cells. One group of cultures served to measure virus released from untreated (●) growing C-M cells. After incubating the cultures for 1.5 hr at 37 C, the media of all cultures were changed to growth medium. The fluids of respective cultures were harvested 1.5, 5, 8, and 11 hr later, and they were stored at −70 C for PFU assay. Representative cultures of the control and each treated group were taken at 0 and 11 hr for cell count.*

biotics simultaneously to growing C-M cells for 2 hr resulted in a marked enhancement in virus release not attainable by either antibiotic alone.

DISCUSSION

The data presented here pertain to the 90% of cells of actively growing cultures that contain viral antigens, are hemadsorption-negative, and do not actively produce infectious virus. Even though these cells are not producing infectious virus, they retain the capacity to do so even after they have been in culture for hundreds of cell generations. When the growing cultures were placed on a maintenance medium of low serum concentration, virus was released continuously over a 11-day period. Since virus was not released in a burst, it was possible that not all the viral components had been synthesized in the cells and the missing component(s) had to be made before infectious virions could be assembled and released. Walker and Hinze (12) previously showed that infectious virus does not accumulate intracellularly in C-M cells. To examine the completeness of viral protein synthesis in growing C-M cells, puromycin was added to the cultures to inhibit protein synthesis. Relatively high titers of infectious virus were recovered in the medium of the treated cultures compared to the virus titers in the control cultures. This new virus was produced by a large percentage of the cells which had become hemadsorption-positive during the treatment and not by a few very productive cells. Thus the viral proteins required for complete virus formation were synthesized and had accumulated in these cells to account for the production of infectious virus in the absence of continuous protein synthesis. The accumulation of viral proteins in infected cells is not a unique characteristic of C-M cells. Wheelock (16) showed that viral proteins were made early in HeLa cells infected with Newcastle disease virus in excess of what was used for virus particle formation, and the excess proteins could be assembled into complete virus later when protein synthesis was inhibited by puromycin. Similarly in a primary mumps virus infection, viral proteins accumulated in the cells. Particularly in the early stages of the primary infection, virus production was sensitive to puromycin, whereas later it was only moderately inhibited. These data and the fact that this infection persists for hundreds of cell generations with the cells having the potential to produce infectious virus strongly indicated that the viral RNA was copied faithfully and completely in each generation and its role as messenger for coding viral proteins was also complete.

In the absence of recognizable intrinsic errors in the virus genome, the failure in infectious virus formation could be due to viral or cell products which block an event in virus completion. The finding that infectious virus was released from C-M cells during puromycin treatment indicated that active protein synthesis was necessary to maintain the suppressed state of the virus. This suggestion was further supported by the finding that after puromycin was removed from C-M cell cultures production of infectious virus was again reduced, which is in accordance with puromycin being a reversible inhibitor of protein synthesis (15). Since puromycin inhibits active synthesis of both viral and host proteins, it could not be determined from these data whether the puromycin-sensitive inhibitor of virus completion was virus- or host-specific.

The actinomycin D experiments presented here indicated that the control of this persistent infection is deoxyribonucleic acid-dependent. Since mumps virus in a primary infection was found to be actinomycin D-insensitive, the host genome in C-M cells probably codes for the factor which interferes with virus completion. The factor in question could be normal ribosomal components in the cytoplasm of the cell for which host and viral messenger RNA compete. It has been found in several myxovirus infections that virus production can be increased by inhibiting RNA and protein synthesis of the host cell (2, 4, 8, 17). The enhancement has been suggested to be due to a reduced competition between host and viral messenger RNA for ribosomes. Such a mechanism of control seems unlikely in the C-M system, assuming viral protein synthesis is complete in growing C-M cells.

If a host protein is proposed to be the regulator of infectious virus formation based on the puromycin and actinomycin D data presented here, it probably is not interferon or the interferon-induced virus inhibitor (1). Interferon was not detected in either the fluids or the cellular extracts of C-M cultures. Also, C-M cells are not resistant to infection by unrelated viruses (12). Furthermore, as shown here, the suppressed state of the virus in growing C-M cells was actinomycin D-insensitive, whereas interferon-mediated interference is not rapidly effected by actinomycin D after its induction (7, 11). This latter finding also would exclude a consideration of the intrinsic type of interference described by Marcus and Carver (9).

When Walker et al. (14) subjected cultures of C-M cells to an environment that promoted cell growth maximally, the eventual result was that the number of cells in culture capable of producing infectious virus was reduced. In that study one culture was cured of the infection in this way. Choppin and Holmes (3) later observed

that less SV5 virus was produced by rapidly dividing cells than when the infected cells were maintained in stationary culture. In accordance with these findings, the latter investigators suggested that a cellular substance which would suppress virus activity may be made only when cells prepare for or enter into mitosis.

The results of the data discussed here suggest the host-controlled protein affects a phase of viral RNA synthesis. However, the nature of the inhibition and the site of the inhibitory action in the C-M system may be more complicated than these interpretations indicate. For example, when growing C-M cells were treated with actinomycin D and puromycin concurrently, the amount and rate of infectious virus produced was greater than could be accounted for by a simple additive effect of either inhibitor alone. It is difficult at present to evaluate these findings. It is possible that, in addition to either drug blocking the synthesis of the proposed inhibitor protein, other secondary, or associated, changes may occur in the treated cells which could facilitate virus completion. The alteration brought about by these drugs may be on cell membranes. Compans et al. (5) and Holmes and Choppin (6) proposed, as a result of their studies of the SV5 infections of primary monkey kidney and BHK-21-F cells, that the plasma membrane of the infected cell may act as a barrier to virus assembly and release, and only when membrane alterations develop will virus be assembled and released successfully. Along these lines, C-M cells, owing to the treatment with both actinomycin D and puromycin, may have the altered membranes which would allow virus assembly and release to proceed more rapidly and more extensively.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants AI06334 and AI06652 from the National Institute of Allergy and Infectious Diseases.

I thank Duard L. Walker for support and useful advice in the early stages of these experiments. I also thank Friedrich Deinhardt for his helpful discussions and critical review of the manuscript.

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