Interferon Production and Inhibition of Host Synthesis in Cells Infected with Vesicular Stomatitis Virus

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Infection of L cells with wild-type (L_1) vesicular stomatitis virus at high or low multiplicities does not result in the production of interferon; however, infection of L cells with low multiplicities of a small-plaque mutant (S_2) results in the synthesis of large amounts of interferon. In chick embryo (CE) cells, both viruses induce synthesis of interferon; there is no significant multiplicity effect in CE cells. The rate and efficiency of shutoff of macromolecular synthesis in the different host cells is a critical factor in determining the ability of the viruses to induce interferon synthesis. If host ribonucleic acid or protein synthesis is shut off by the virus before the required new ribonucleic acid is transcribed or translated, interferon production does not occur. The relative yield of the two viruses in CE and L cells is not related to the effects of interferon produced during the course of infection.

The ability of a virus to affect host cell function is an important aspect of the infection process. The influence of the virus on the host can be inhibitory and result in the shutoff of host macromolecular synthesis or it can be stimulatory and induce the synthesis of certain host cell products, for example, interferon.

In the case of infection of cell cultures with vesicular stomatitis virus (VSV), Huang and Wagner (3) reported that the cytotoxicity of this virus for Krebs-2 carcinoma cells of mice seemed to be related to the rapid inhibition of cellular ribonucleic acid (RNA) synthesis. In addition, they showed (6) that VSV was unable to stimulate interferon synthesis, but could inhibit the production of interferon induced in Krebs-2 cells previously infected with Newcastle disease virus (NDV). They pointed out that VSV acted much like actinomycin D in its effect on RNA and interferon synthesis and concluded that the inhibition of host RNA synthesis was responsible for (i) the failure of VSV to induce interferon and (ii) the ability of VSV to inhibit interferon synthesis in cells previously infected with NDV.

We previously reported the isolation of large- and small-plaque clones of VSV and we described some of the properties of these viruses (9). In mice, both viruses elicited equivalent titers of circulating interferon at 12 hr, although the wild-

type virus was approximately 1,000 times more lethal for mice than the small-plaque mutant.

The present report deals with the replication of the wild-type VSV (L_1) and a small-plaque mutant (S_2) in mouse L cells and in primary cultures of chick embryo (CE) cells. The two viruses were found to differ in ability to shut off host RNA and protein synthesis and in ability to induce interferon synthesis. The relations of these phenomena are considered in detail.

MATERIALS AND METHODS

Cell cultures. Primary cultures of CE cells and a continuous line of L cells (clone 929) were propagated and maintained as previously described (8).

Media and solutions. Eagle's minimal essential medium supplemented with 4% heat-inactivated calf serum was used for growth and maintenance of CE- and L-cell cultures. Hanks' balanced salt solution (BSS) was used as a diluent for tritiated compounds. Phosphate-buffered saline (PBS; 0.01 M, pH 7.4) was used to wash cells and to dilute virus. Nutrient agar (1%), used as an overlay for CE and L cells, contained the following ingredients: 25 ml of heat-inactivated calf serum, 80 ml of 3× Eagle's medium, 7.5 ml of 5% sodium bicarbonate, 1 ml of 100,000 units each of penicillin and streptomycin, 1 ml of 15 mg of diethylaminoethyl (DEAE)-dextran and 200 ml of 1.5% agar (Difco) containing 1:20,000 parts neutral red.

Virus. The Indiana strain of VSV was propagated in CE cell monolayers. Cell cultures in 32-oz (960-ml) bottles were inoculated with virus at a low

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multiplicity of infection (MOI; 0.001) to avoid the formation of incomplete, autointerfering particles. After an adsorption period of 60 min at 37 C, the inoculum was removed, the cells were washed with PBS, 20 ml of medium was added, and the cultures were incubated for 20 hr at 37 C. Infected fluids were harvested, pooled, and centrifuged at $1,000 \times g$ for 15 min to remove cellular debris. The clarified fluids were centrifuged at $54,450 \times g$ for 90 min. Virus pellets were suspended in ethylenediaminetetraacetic acid (EDTA) to 1/30 the original volume and re-centrifuged at $54,450 \times g$. The pellets were resuspended in EDTA or medium to 1/100 the volume of the starting material. To disaggregate clumps of virus, the resuspended pellets were treated for 30 sec in a 250-w, 10-kc Raytheon sonic oscillator. Virus concentrates were stored at -60 C. There was no significant loss of infectivity during the concentration process.

Concentrated virus suspensions were further purified by treatment with fluorocarbon by the method of Hackett et al. (2). The partially purified virus concentrates were layered on 15 to 45% linear sucrose density gradients and centrifuged at 20,000 rev/min for 105 min in a model L-2 Spinco (SW 25.1 rotor). Fractions (1 ml) were collected from the bottom of the gradient with monitoring at 280 nm in a continuous-flow cell of a Gilford recording spectrophotometer.

All incorporation experiments dealing with the effect of virus on host macromolecular synthesis were performed with virus purified either by differential centrifugation or by differential centrifugation and then by sucrose gradient centrifugation. The results obtained were identical with both virus preparations. All input MOI were based on the number of plaque-forming units (PFU). By assuming a particle to PFU ratio of 1 for L_1 VSV [based on the data of Galasso (1) for the Indiana strain of VSV] and by correlating optical density units at 280 nm (OD_{280} units) of peak fractions of sucrose gradient purified virus with PFU, it was determined that there may be as many as six physical particles of the S_2 VSV mutant for every PFU.

Chemicals. Cycloheximide (actidione), purchased from the Upjohn Co., was diluted in medium and used at a concentration of 100 μ g/ml. Actinomycin D, made available through the courtesy of H. B. Woodruff of Merck & Co., Inc., Rahway, N.J., was used at a concentration of 2 μ g/ml. Uridine-5- 3H (specific activity, 20 Ci/mmol) and L-leucine-4,4- 3H (specific activity, 40 Ci/mmol) were purchased from Schwarz BioResearch Inc., Orangeburg, N.Y.

Interferon production. CE- or L-cell monolayers containing 3×10^6 cells per 60-mm petri dish were inoculated with 0.5 ml of appropriate virus dilutions. Three cultures were used for each sample. After an adsorption period of 30 min at 37 C, the inoculum was drawn off, the cultures were washed twice with PBS, and 3 ml of medium was added. The cultures were incubated at 37 C for designated periods, after which the culture fluids were harvested and pooled. Infective virus in the harvested fluids was destroyed by dialysis for 24 hr against glycine-HCl buffer (pH 2). The pH

level was returned to 7 by dialysis against PBS, and the fluids were stored at -20 C.

Interferon assay. Interferon was assayed in CE- or L-cell monolayer cultures by using the plaque reduction technique (8).

Incorporation of labeled precursors. RNA and protein synthesis were studied in monolayer cultures containing 2×10^6 CE or L cells per dish. Growth medium was removed from the cells and replaced with 1 μ Ci of 3H -uridine or 3H -leucine in 1 ml of BSS. The cultures were incubated at 37 C in a CO_2 -gassed incubator for the following labeling times: L cells, 10-min exposure to 3H -uridine and 20-min exposure to 3H -leucine; CE cells, 20-min exposure to 3H -uridine and 10-min exposure to 3H -leucine. After the labeling period, the fluid was discarded, and the cells were washed twice with cold BSS. The cells were removed from the glass by scraping, resuspended in cold BSS to a concentration of 10^6 cells/ml, and disrupted by sonic oscillation for 5 min. Samples were prepared for assay of isotope incorporation by the following procedure. Duplicate 1-ml samples were treated with 1 ml of 10% trichloroacetic acid containing filter aid (4 g/100 ml). The trichloroacetic acid-precipitable RNA or protein fractions were collected by suction filtration on Whatman no. 1 filter paper. The precipitates were washed to remove unincorporated label: twice with cold 5% trichloroacetic acid, once with 95% ethanol, and once with ethyl ether. The dry samples were removed from the filters by scraping, placed in glass scintillation vials, and solubilized with 0.5 ml of 10N hydroxide of Hyamine for 20 hr at room temperature. To each of the solubilized samples was added 10 ml of a toluene-based scintillation mixture. The samples were counted in a Packard model 3375 liquid scintillation spectrometer, and radioactivity was expressed as counts per minute per 10^6 cells.

RESULTS

Replication of L_1 and S_2 VSV in L and CE cells. Monolayer cultures containing 2×10^6 CE or L cells were infected at a MOI of 10. Virus was allowed to adsorb for 30 min at 37 C, the inoculum was removed, the cells were washed with medium, and the cultures were incubated at 37 C. At intervals, released and cell-associated virus were measured. In L or CE cells infected with L_1 or S_2 VSV, a 2-hr latent period was followed by a period of exponential increase of infective virus which lasted for 8 hr in cells infected with S_2 VSV and for 10 hr in cells infected with L_1 VSV. The total yield of virus per cell, i.e., cell-associated plus released virus, was calculated at the time of maximal yield. In L cells, L_1 VSV produced an average yield of 147 PFU per cell, whereas S_2 VSV yielded an average of 6 PFU per cell. Both viruses replicated more efficiently in CE cells; the average yield of L_1 VSV was 850 PFU per cell, whereas the average yield of S_2 VSV was 129 PFU per cell.

Effect of MOI on interferon production in L

cells infected with L_1 or S_2 VSV. Monolayer cultures of L cells were infected with S_2 or L_1 VSV at MOI of 100, 10, 1, 0.1, and 0.01. At 24 hr after infection, the medium from three replicate cultures was assayed for interferon. The MOI had a marked effect on the ability of the S_2 mutant to stimulate the production of interferon in L cells (Table 1). Cells infected with high multiplicities of S_2 VSV (100 and 10) did not produce interferon, whereas cells infected at low multiplicities (0.1 and 0.01) produced high titers of interferon. The identity of this inhibitor as interferon was established by its stability at pH 2, its inactivation by trypsin, and its ineffectiveness when tested in CE cells.

To determine the time of appearance of interferon in L cells infected with S_2 VSV, the following experiments were performed. Monolayer cultures of L cells were infected at a MOI of 0.1. At intervals after a 30-min adsorption period, the medium was harvested and assayed for interferon. No interferon was detected in the medium until 8 hr after infection. The interferon titer then increased with time and reached peak titer at 24 hr (see control curve, Fig. 1).

The interferon content of the cells from infected cultures was also determined. After the medium was harvested from infected cultures, the cell monolayers were washed, suspended in 3 ml of medium, and disrupted by sonic oscillation; the cell lysate was tested for interferon content. No significant accumulation of interferon in the cells was detected at any time after infection.

In contrast to the findings with S_2 VSV in L cells, interferon was not detected in L-cell cultures infected with any multiplicity of L_1 VSV (Table 1). In fact, if L-cell cultures were infected with S_2 VSV at a MOI of 0.1 and 30 min later were infected with L_1 VSV at a MOI of 100, 10, or 1, the production of interferon was completely inhibited.

Effect of actinomycin D and cycloheximide on interferon production in L cells infected with S_2

TABLE 1. Influence of multiplicity of infection on production of interferon in L cell cultures by S_2 VSV or L_1 VSV

Multiplicity of infection	Interferon titer of medium (units/3 ml) 24 hr after infection with	
	S_2 VSV	L_1 VSV
100	0	0
10	0	0
1.0	20	0
0.1	1,200	0
0.01	610	0

FIG. 1. Effect of actinomycin D on interferon synthesis in L cells infected with S_2 VSV. L-cell monolayers were infected with S_2 VSV at an MOI of 0.1. At intervals from time zero, medium from infected cultures was harvested and tested for interferon (●). At the times indicated by arrows, medium was removed from infected cultures and replaced by medium containing actinomycin D (2 μ g/ml). Medium harvested at 24 hr was tested for interferon (○).

VSV. L-cell monolayers were infected with S_2 VSV at a MOI of 0.1. At intervals starting at the end of the adsorption period, the medium was harvested (to be assayed for interferon) from three replicate cultures and replaced by 3 ml of fresh medium containing actinomycin D (2 μ g/ml). This concentration of actinomycin D inhibited 96 to 98% of cellular RNA synthesis within 30 min. The medium from the actinomycin D-treated cultures was harvested 24-hr after infection and assayed for interferon. Figure 1 shows the effect of actinomycin D on interferon yield at 24 hr. In infected cultures without actinomycin D, a small amount of interferon was demonstrable in the medium at 8 hr, and the titer of the inhibitor reached a peak at 24 hr. If actinomycin D was added to the cultures at any time before 8 hr, interferon synthesis was completely inhibited. If the antibiotic was added at 8, 10, or 12 hr after infection, interferon was synthesized but the yield at 24 hr was always less than the yield in the absence of the antibiotic. Similar results were obtained with cells infected at a MOI of 1. These findings confirm the report by Wagner (5) that induction of interferon in cell cultures requires the production of a new actinomycin D-sensitive cell product, presumably messenger RNA. The data in Fig. 1 imply that the new RNA required for the production of interferon was synthesized beginning approximately 8 hr after infection of

L cells with S₂VSV. These findings differ from the observation of Wagner and Huang (6) that the RNA required for interferon production was synthesized 4 hours after infection of Krebs-2 cells with NDV.

Since the addition of actinomycin D at times after 8 hr reduced the interferon yield at 24 hr, experiments were performed to determine how long the interferon titer in the medium continued to increase after actinomycin D was added to cultures already synthesizing interferon. L-cell cultures were infected with S₂VSV at a MOI of 0.1 as described above. At 12 hr after infection, when interferon synthesis was underway, the medium was removed and replaced with 3 ml of fresh medium with or without added actinomycin D (2 µg/ml). At intervals after the medium change, the fluids from three replicate cultures were pooled and assayed for interferon. The results showed the interferon titer continued to increase for 3 hr after the addition of actinomycin D; after 3 hr there was no further increase in interferon content of the medium. This finding indicates that continued cellular RNA synthesis is required for prolonged synthesis of interferon. Wagner and Huang (6) obtained different results by using Krebs-2 cells infected with NDV. They found that actinomycin D added 6 hr after infection, when interferon synthesis was in progress, did not decrease the amount of interferon produced subsequently. On the basis of this finding, they concluded that the messenger RNA required for the synthesis of interferon in Krebs-2 cells was quite stable.

The effect of cycloheximide (100 µg/ml) on interferon synthesis was also investigated. No interferon was produced when cycloheximide was added to L-cell cultures up to 6 hr after infection with S₂VSV. Addition of cycloheximide to infected cultures at any time after 6 hr resulted in reduced yields of interferon at 24 hr.

Effect of MOI on interferon production in CE cells infected with L₁ or S₂VSV. CE-cell cultures were infected with S₂ or L₁VSV at MOI of 100, 10, 1, 0.1, and 0.01. At 24 and 48 hr after infection, the culture fluids were harvested and assayed for interferon. Very little interferon was produced in CE cells infected with S₂VSV (Table 2). The only significant titer (20 units) was produced in CE cells infected with S₂VSV at a MOI of 1. There was no striking effect of MOI on interferon production in CE cells infected with L₁VSV; significant amounts of interferon were produced at MOI of 100, 10, 1, and 0.1. In contrast to the findings in L cells, the interferon produced in CE cells did not appear until 12 hr after infection, and the peak titer was reached between 24 and 48 hr after infection. The production of interferon

TABLE 2. *Influence of multiplicity of infection on production of interferon in chick embryo cell cultures infected with S₂VSV or L₁VSV*

Multiplicity of infection	Interferon titer of medium (units/3 ml) 48 hr after infection with	
	S ₂ VSV	L ₁ VSV
100	0	60
10	5	100
1	20	30
0.1	2	24
0.01	0	15

in CE cells infected with L₁VSV required new cellular RNA and protein synthesis. Synthesis of interferon was completely inhibited when either cycloheximide (100 µg/ml) or actinomycin D (2 µg/ml) was added to infected cultures before 8 to 10 hr after infection. Addition of either inhibitor after interferon synthesis was initiated resulted in reduced yields at 48 hr.

Influence of infection with L₁ and S₂VSV on RNA synthesis in L cells. RNA synthesis was measured in L cells infected with S₂VSV at MOI of 10, 1, and 0.1 or with L₁VSV at MOI of 100, 10, and 1. At intervals starting at the end of a 30-min adsorption period, the medium was removed, the cells were washed and exposed to ³H-uridine, and trichloroacetic acid-precipitable counts were determined as described above.

There was a rapid initial inhibition of RNA synthesis in cells infected with S₂VSV at high MOI (Fig. 2A). By the end of the adsorption period, RNA synthesis in cells infected at a MOI of 10 and 1 was 65 and 85%, respectively, of control levels. RNA synthesis was unchanged at time zero in cells infected at a MOI of 0.1. After this initial inhibition, the decline of RNA synthesis with time was essentially the same at all MOI. By 8 hr, RNA synthesis in cells infected at MOI of 10 or 1 was only 20% of the rate in control cells; and RNA synthesis in cells infected at a MOI of 0.1 was 30% of the control rate.

Inhibition of RNA synthesis in L cells infected with L₁VSV (Fig. 2B) was similar to that described for S₂VSV. In cells infected at high MOI, there was significant inhibition by the end of the adsorption period. After this initial inhibition, the decline of RNA synthesis was progressive with time and was essentially the same at all MOI. By 8 hr after infection, RNA synthesis in cells infected at all multiplicities had declined to 20 to 27% of the level in control cells. The mechanism of the rapid initial shut-off of RNA synthesis will be the subject of a separate report.

These findings indicated that the rates of shut-

FIG. 2. RNA synthesis in L cells infected with S₂VSV or L₁VSV: effect of MOI. L-cell monolayers were infected with designated MOI of S₂ or L₁VSV. At intervals from time zero, infected cultures and uninfected control cultures were exposed for 10 min to 1 μ Ci of ³H-uridine. The label was removed, the cultures were washed twice with BSS, and the cells were suspended in BSS (10⁶ cells/ml). Trichloroacetic acid-precipitable radioactivity per 10⁶ cells in infected cultures was expressed as per cent of counts incorporated into uninfected controls. Control values (counts per min per 10⁶ cells) ranged from 10,027 to 17,959 during the 8-hr observation period.

off of RNA synthesis in L cells starting 30 min after infection with various multiplicities of L₁ or S₂ VSV were not significantly different. However, the method employed did not differentiate residual cellular RNA synthesis in infected cells from new viral RNA synthesis. By using actinomycin D (2 μ g/ml) to inhibit cellular RNA synthesis, it was possible to measure the fraction of RNA synthesis that was viral; a correct estimate of host cell RNA synthesis was obtained by subtracting viral RNA synthesis from total RNA synthesis. Figure 3 shows total RNA synthesis, host cell RNA synthesis (total corrected for viral), and viral RNA synthesis. In L cells infected with S₂VSV at a MOI of 10 (Fig. 3A), host cell RNA synthesis was approximately 20% of the control rate at 8 hr. Viral RNA synthesis reached a peak rate at 2 hr after infection and was so low by 8 hr that it did not significantly affect total RNA synthesis. In cells infected with L₁VSV at a MOI of 100 (Fig. 3B), there was no significant synthesis of host cell RNA after 8 hr; only viral RNA was being synthesized at this time. Similar results were obtained at lower multiplicities (10 and 1) due to rapid reinfection of cells; between 2 and 4 hr after infection of L cells with L₁VSV, there was a 100-fold increase in the amount of released PFU.

In summary, in L cells infected with L₁VSV at high MOI, host cell RNA synthesis was completely inhibited by 8 hr, whereas L cells infected

with high MOI of S₂VSV were still able to synthesize RNA at approximately 20% of the normal rate.

Effect of infection with L₁ and S₂VSV on protein synthesis in L cells. Protein synthesis was measured in monolayer cultures of L cells infected with S₂VSV at MOI of 10, 1, 0.1, and 0.01 or with L₁VSV at MOI of 100, 1, or 0.1. At intervals after the adsorption period, the cells were labeled with ³H-leucine and trichloroacetic acid-precipitable counts were determined as described above.

In L cells infected with S₂VSV at MOI of 10 and 1, there was a rapid inhibition of protein synthesis (Fig. 4A). By 2 hr after infection at a MOI of 10, protein synthesis was reduced to 9% of the control rate. In cells infected at a MOI of 1, protein synthesis declined to 9% of the rate in control cells by 6 hr after infection. Inhibition of protein synthesis proceeded more slowly at multiplicities of 0.1 and 0.01. In cells infected at a MOI of 0.1, protein synthesis was 70% of the control rate by 3 hr; no further decline occurred until between 6 and 8 hr when protein synthesis fell from 70 to 30% of the control rate. Cells infected at a MOI of 0.01 were still able to synthesize proteins at 80% of the normal rate at 8 hr after infection.

The ability of the S₂VSV mutant to shut off protein synthesis at low MOI is explainable by reinfection; cells infected at a MOI of 0.1 showed a 100-fold increase in released virus between 4 and 6 hr postinfection. Therefore, in cells originally infected at a MOI of 0.1, the actual MOI is approximately 10 by 6 hr. After 6 hr, the rate of shutoff of protein synthesis in cells infected at a

FIG. 3. Differentiation of host and viral RNA synthesis in L cells infected with VSV. Panel A: S₂VSV, MOI = 10; panel B: L₁VSV, MOI = 100. Rate of RNA synthesis in infected cultures is expressed as per cent of rate in uninfected control cultures at each interval. Total RNA synthesis in infected cells (○, broken line); viral RNA synthesis in actinomycin D-treated cultures (●); host RNA synthesis (total RNA synthesis minus viral RNA synthesis; ○, solid line). For counts per minute incorporated into control cultures, see legend to Fig. 2.

FIG. 4. Protein synthesis in L cells infected with S_2 VSV or L_1 VSV: effect of multiplicity of infection. L cell monolayers were infected with designated MOI of S_2 or L_1 VSV. At intervals from time zero, infected cultures and uninfected control cultures were exposed for 20 min to $1 \mu\text{Ci}$ of ^3H -leucine. The label was removed, the cultures were washed twice with BSS, and the cells were suspended in BSS (10^6 cells/ml). Trichloroacetic acid-precipitable radioactivity per 10^6 cells in infected cultures was expressed as per cent of counts incorporated into uninfected controls. Control values (counts per min per 10^6 cells) ranged from 15,140 to 22,989 during the 8-hr observation period.

MOI of 0.1 is identical to that of cells originally infected at a MOI of 10 between 0 and 2 hr. Preliminary experiments to find a diffusible inhibitor of protein synthesis have been unsuccessful.

Inhibition of protein synthesis in L cells infected with L_1 VSV was also rapid (Fig. 4B). In cells infected at a MOI of 100, there was a 40% inhibition by the end of the adsorption period; by 8 hr, protein synthesis was 7% of the control rate. The initial inhibition of protein synthesis was less severe at MOI of 10 or 1, protein synthesis was reduced to 9 and 24%, respectively, of the control rate. Protein synthesis was unaffected for 4 hr in cells infected at a MOI of 0.1; synthesis declined to 64% of the control rate at 8 hr.

Effect of infection with L_1 and S_2 VSV on RNA synthesis in CE cells. CE cells were infected with S_2 VSV at MOI of 10, 1, 0.1, and 0.01, or with L_1 VSV at MOI of 100, 10, 1, and 0.1, and the effects on RNA synthesis were determined as described in the legend to Fig. 5.

In CE cells infected with S_2 VSV (Fig. 5A) at a MOI of 10, inhibition of RNA synthesis began immediately after infection; after virus adsorption, RNA synthesis was already only 75% of the control rate. Even with an MOI of 1, there was significant inhibition (15%) of RNA synthesis by 1 hr. The rate of decline of RNA synthesis was progressive with time and essentially the same at MOI of 10 or 1. In cells infected at MOI of 0.1 and 0.01, RNA synthesis continued unchanged

for 3 hr and then declined progressively with time. By 8 hr, RNA synthesis in cells infected with MOI of 10, 1, 0.1, and 0.01 had declined to 15, 30, 50, and 90%, respectively, of the control rate.

In CE cells infected with L_1 VSV (Fig. 5B), the rapid initial inhibition of RNA synthesis seen in infected L cells (Fig. 2B) did not occur. Inhibition of RNA synthesis in cells infected at a MOI of 100 and 10 began soon after adsorption and was progressive with time. At 8 hr after infection at MOI of 100 or 10, RNA synthesis was about 20% of the control rate. With low MOI (1 or 0.1), RNA synthesis was unchanged for 4 hr; by 8 hr after infection at a MOI of 1 or 0.1, RNA synthesis was 40 and 60%, respectively, of the control rate.

Total RNA synthesis in infected CE cells was corrected for viral RNA synthesis by using actinomycin D, as described above. With both mutants, total RNA synthesis was essentially the same as host cell RNA synthesis by 8 hr because, by this time, viral RNA synthesis had already reached a maximum and declined (Fig. 6). The greatest difference between host cell and total RNA synthesis occurred 3 to 4 hr after infection when the rate of viral RNA synthesis was maximum.

In summary, in CE cells, viral RNA synthesis reached a maximum 3 to 4 hr after infection and

FIG. 5. RNA synthesis in CE cells infected with S_2 VSV or L_1 VSV: effect of multiplicity of infection. CE cell monolayers were infected with designated MOI of S_2 or L_1 VSV. At intervals from time zero, infected cultures and uninfected control cultures were exposed for 20 min to $1 \mu\text{Ci}$ of ^3H -uridine. The label was removed, the cultures were washed twice with BSS, and the cells were suspended in BSS (10^6 cells/ml). Trichloroacetic acid-precipitable radioactivity per 10^6 cells in infected cultures was expressed as per cent of counts incorporated into uninfected controls. Control values (counts per min per 10^6 cells) ranged from 3,809 to 4,994.

declined rapidly. At 8 hr after infection, CE cells were able to synthesize RNA at approximately 10% of the control rate.

Effect of infection with S_2 and L_1 VSV on protein synthesis in CE cells. Inhibition of protein synthesis in CE cells infected with S_2 VSV at a MOI of 10 began soon after infection and progressed with time (Fig. 7A); by 8 hr, protein synthesis

was 17% of the control rate. In cells infected at a MOI of 1, there was no significant inhibition of protein synthesis for 3 hr after infection; inhibition was progressive with time after 3 hr, and by 8 hr protein synthesis was 30% of the control rate. There was little change in protein synthesis in CE cells infected with S_2 VSV at MOI of 0.1 or 0.01; 8 hr after infection with these low MOI protein synthesis was only slightly below the control rate.

Inhibition of protein synthesis was much less efficient in CE cells infected with L_1 VSV (Fig. 7B). For example, in cells infected at a MOI of 100 and 10, after an inhibition at 1 hr to approximately 80% of the rate in control cells, there was no significant change until between 6 and 8 hr when protein synthesis decreased from 70% to about 40% of the control rate. Inhibition of protein synthesis at MOI of 1 or 0.1 was not severe; at 8 hr, protein synthesis in cells infected at these multiplicities continued at 70 and 88%, respectively, of the control rate.

DISCUSSION

The data we have presented show that the rate and efficiency of shutoff of host macromolecular synthesis by the infecting virions determine whether interferon can be produced by cells infected with wild-type VSV (L_1) or a small-plaque mutant (S_2). In L cells infected with L_1 VSV, cell RNA synthesis is completely inhibited by 8 hr after infection; this precludes synthesis of the RNA required for interferon production and no interferon synthesis takes place. In L cells infected with S_2 VSV, the virus does not completely inhibit cell RNA synthesis and the required RNA is transcribed. In this case, the determinant of whether interferon will be produced is the effect of the mutant on host protein synthesis. At high MOI values, S_2 VSV shuts off host protein synthesis so rapidly and so completely that interferon cannot be made. At low MOI values with S_2 VSV, the shutoff of protein synthesis is slow enough to allow interferon to be synthesized. However, the interferon synthesized is produced under restrictive conditions of protein synthesis. If induction of interferon synthesis took place under conditions in which shutoff of protein synthesis did not occur, interferon titers would probably be greater, and synthesis might be sustained for longer periods of time. From these observations, it appears that in L cells infected with L_1 or S_2 VSV there is a race between the cell and the virus; if the virus inhibits cell RNA or protein synthesis before the RNA required for interferon production can be transcribed or translated, the cell cannot produce interferon.

FIG. 6. Differentiation of host and viral RNA synthesis in CE cells infected with VSV. Panel A: S_2 VSV, MOI = 10; panel B: L_1 VSV, MOI = 100. Rate of RNA synthesis in infected cultures is expressed as per cent of rate in uninfected control cultures at each interval. Total RNA synthesis in infected cells ($\circ$, broken line); viral RNA synthesis in actinomycin D-treated cultures ($\bullet$); host RNA synthesis (total RNA synthesis minus viral RNA synthesis) ($\circ$, solid line). For counts per minute incorporated into control cultures, see legend to Fig. 5.

FIG. 7. Protein synthesis in CE cells infected with S_2 VSV or L_1 VSV: effect of multiplicity of infection. CE cell monolayers were infected with designated MOI of S_2 or L_1 VSV. At intervals from time zero, infected cultures and uninfected control cultures were exposed for 10 min to 1 μ Ci of 3 H-leucine. The label was removed, the cultures were washed twice in BSS, and the cells were suspended in BSS (10^6 cells/ml). Trichloroacetic acid-precipitable radioactivity per 10^6 cells in infected cultures was expressed as per cent of counts incorporated into uninfected controls. Control values ranged from 16,077 to 26,253.

The idea of a contest between virus and host is in agreement with the findings of Wagner and Huang (6). They reported that VSV, which was incapable of stimulating production of interferon in Krebs-2 cells, inhibited cell RNA synthesis very effectively. Furthermore, they found that VSV inhibited interferon synthesis in Krebs-2 cells previously infected with NDV, which is an efficient interferon stimulus in this system. They concluded that the inhibition of cell RNA synthesis by VSV was responsible for the lack of interferon production and compared the action of VSV to the effects of actinomycin D. In the L cell-VSV system we found that interferon production by cells infected with S₂VSV was prevented by superinfection with L₁VSV. Again, this demonstrates the similarity of action of actinomycin D and VSV on RNA synthesis and on interferon production. The findings of Wagner and Huang (6) that the interferon messenger RNA was produced 4 hr after infection in the Krebs-2-NDV system differs from our findings in L cells infected with S₂VSV. In the latter case, the RNA required for interferon production is not synthesized until approximately 8 hr after infection.

In contrast to the findings in L cells, CE cells infected with L₁ or S₂VSV produce interferon. In CE cells, neither RNA nor protein synthesis is completely inhibited by 8 hr after infection with either virus, and, for this reason, the cell is able to synthesize interferon after infection. These findings differ from those of Wagner et al. (7), who could not demonstrate interferon production in CE cells by their large- and small-plaque VSV populations.

The finding that both L₁ and S₂VSV stimulate the production of interferon in CE cells emphasizes the importance of the host cell in determining the ability of a virus to induce the synthesis of interferon. L₁VSV cannot stimulate synthesis of interferon in L cells because it effectively shuts off cell RNA synthesis. In contrast, CE cells infected with L₁VSV are capable of synthesizing interferon because the virus does not shut off RNA synthesis as rapidly or efficiently as it does in L cells. The titers of interferon produced in CE cells in response to infection with S₂ or L₁VSV are low compared to the interferon titers produced by L cells infected with S₂VSV under similar conditions of RNA and protein synthesis inhibition. Thacore and Youngner (4) reported similar findings in L and CE cells infected with mutants of NDV. It is possible that CE cells are not as inducible for interferon synthesis as are L cells.

In summary, these data show that the effect of the virus on host RNA and protein synthesis is a

prime factor in determining the ability of a host cell to synthesize interferon when infected by a virus. However, the relationship between host and virus functions is not as uncomplicated as this conclusion makes it appear. The effect of a virus on host macromolecular synthesis is critically dependent on the MOI and on the type of cell infected. A virus that is capable of stimulating interferon production in one type of cell at a given multiplicity may fail to do so in another cell, or at another multiplicity, because of differences in rate or efficiency of inhibition of cell macromolecular synthesis. It is apparent that control of interferon synthesis in cell cultures is complex and involves at least three variables: the virus, the MOI, and the type of host cell.

The possibility that virus replication is controlled by interferon synthesized by the infected cell was suggested by Wagner and his co-workers (7). They postulated that the poor replication and limited cytopathic effect of their small-plaque VSV mutant in L cells was due to resistance caused by feedback in infected cells of interferon produced during the course of infection. The hypothesis that endogenous interferon can inhibit the replication of the inducing virus does not seem to apply to the systems in which interferon synthesis is induced by L₁ or S₂VSV. S₂VSV replicates less efficiently than L₁VSV in both CE and L cells. Since S₂VSV also stimulates production of interferon in both types of cells, it is tempting to accept the theory that the interferon synthesized may feedback in infected cells and inhibit the replication of the virus. The fact that the replicative cycle of the small-plaque mutant has passed the period of exponential replication and has reached a peak titer before interferon can be detected makes feedback inhibition an unlikely possibility to explain the low yields of S₂VSV. Additional evidence that feedback inhibition is not responsible for low yields of virus is provided by the observation that growth of S₂VSV at a high MOI, or in actinomycin D-treated cells, does not result in increased yields of virus although both of these conditions prohibit the production of interferon. It is unlikely that autointerference is responsible for the reduced virus yields at high multiplicities since the S₂ mutant does not produce detectable auto-interfering particles (Wertz and Youngner, *unpublished data*). The fact that L₁VSV replicates more efficiently in CE cells (in which interferon is synthesized) than in L cells (in which no interferon is produced) provides further evidence that endogenous interferon does not inhibit the growth of the inducing virus.

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