Effect of Respiratory Syncytial Virus Infection of HeLa-Cell Macromolecular Synthesis

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SUMMARY

Cells infected with respiratory syncytial (RS) virus eventually die but there appears to be no specific mechanism for shutting off cellular synthesis of macromolecules. DNA and RNA synthesis, as measured by the incorporation of labelled thymidine or uridine, do not begin to shut down until some time between 11 and 18 h after infection. By 18 h their rates of synthesis are reduced to approx. 50% for DNA and 35% for RNA.

Protein synthesis continues throughout the course of infection at approximately the same rate. Synthesis of most of the cellular polypeptides also continues, but the distribution of polypeptides of high and low mol. wt. shifts. The increase in the proportion of those of high mol. wt. includes a peak that represents one of the seven previously identified virion polypeptides.

Another consequence of RS virus infection is an increase in glucosamine incorporation, beginning near the end of the virus eclipse period (12 h after infection), which may be associated with virion glycoprotein synthesis. Polyacrylamide-gel electrophoresis of glucosamine-labelled cells reveals that at 18 h after infection two of the three previously identified virion glycoproteins are present.

INTRODUCTION

Respiratory syncytial (RS) virus has been classified with the paramyxoviruses, which it resembles in overall morphology and general appearance, and in maturing by a process that involves budding from the host cell's surface enclosed in an envelope derived from plasma membrane. However, RS virus differs from other paramyxoviruses in particle size, nucleocapsid diameter, general appearance of the intracytoplasmic inclusions caused by infection, and in not agglutinating red blood-cells (Berthiaume, Joncas & Pavilanis, 1964; Norrby, Marusyk & Orvell, 1970).

The long growth cycle of RS virus, 20 to 30 h, includes an eclipse period of 10 to 12 h. Virus RNA synthesis begins approx. 5 h after infection and levels off at 15 to 18 h. Throughout the cycle, over 90% of the produced virus remains associated with plasma membrane and very little is ever released into the medium. In cells infected with a multiplicity of 3 to 5 p.f.u./cell, syncytia formation starts 16 to 18 h after infection and by 24 h syncytia are numerous (Levine & Hamilton, 1969).

Relatively little is known about the effect of the virus on cellular biosynthesis. Nothing
has been reported on the effect of infection on cell survival, apart from syncytia formation, or on the synthesis of such components of the host-cell as DNA, RNA or protein, but there have been reports on the synthesis of what may be virion-specified RNA. According to some investigators, cells infected with RS virus and treated with actinomycin D contain 10S and 28S single-stranded RNA species (Hodes, Schauf & Chanock, 1974; Baldridge & Senterfit, 1976). Other investigators found a major RNA component of 16S and a minor one of 50S (Wunner, Faulkner & Pringle, 1975).

We report here on the effects of RS-virus infection on host-cell biosynthesis, specifically on the viability of HeLa cells, as judged by their ability to form colonies and to synthesize DNA, RNA and protein. In addition, we demonstrate that RS-virus infection stimulates the uptake of glucosamine.

METHODS

Cells used in all experiments were HeLa, maintained in continuous cultures, and grown for virus assay and virus stocks as monolayers in plastic Petri dishes (60 or 100 mm), for the earlier experiments in a medium of Hank's balanced salt solution and 0.5% lactalbumin hydrolysate, supplemented with 20% calf serum (HL20), and more recently in MEM with 10% foetal calf serum.

Virus used in all experiments was the Long strain of RS, grown and assayed as described elsewhere (Levine & Hamilton, 1969; Levine, Buthala & Hamilton, 1971). Virus was labelled with 3H-reconstituted protein hydrolysate, and purified, as described elsewhere (Levine, 1977). For polyacrylamide-gel electrophoresis, purified, radiolabelled virus was dissociated in 0.005 M-phosphate buffer (pH 7.2) containing 1% sodium dodecyl sulphate (SDS) and 1% β-mercaptoethanol by immersion in boiling water for 2 min.

Cell killing experiments. HeLa monolayers in 60 mm Petri dishes were infected with 0.3 ml of either undiluted virus stock or virus diluted in Hank's balanced salt solution (HBSS), and incubated at 37 °C for 1 or 2 h. Then the inoculum was removed, and the monolayers were washed three times with HBSS, overlaid with virus growth medium and re-incubated at 37 °C. The multiplicity of infection (m.o.i.) was determined by assaying the inoculum for unadsorbed virus.

At approximately 5 h after infection the medium was removed, and the monolayers were washed with HBSS and treated at 37 °C with 0.25% trypsin (2 ml/monolayer) for 10 to 15 min. Then the cells were suspended in virus growth medium (5 ml/plate) and centrifuged at 1500 rev/min for 5 min. The pellet was resuspended in virus growth medium, and the cells were counted, diluted in that medium, and added to HeLa cell monolayers (0.2 ml/monolayer and at least 2 monolayers per dilution). These monolayers were incubated at 37 °C for 1 h and then overlaid with agar plaque-medium in 2 steps: first with 1 ml/plate and after 20 min at room temperature, with an additional 5 ml/plate. To assay for cell killing, trypsinized cells, infected and non-infected, were diluted in HL20 medium and plated in duplicate, 100 or 500 cells/plate, in 4 ml of HL20 containing 1% ferret RS-virus antiserum, to prevent virus spread in the cultures of infected cells. As a control for the effectiveness of the antiserum, virus was added to a set of non-infected cells (10^5 p.f.u./plate, representing the virus yield from approx. 1000 infected cells). All plates were incubated at 37 °C in an atmosphere of 5% CO_2 in air for 12 days. Then the colonies were fixed, stained and counted.

Experiments to determine the effect of infection on cellular DNA, RNA and protein synthesis. Monolayers were infected with approx. 4 p.f.u. of virus/cell. Infected and non-infected monolayers were pulsed with 0.1 to 0.4 µCi/ml of 3H-thymidine, 3H-uridine or 14C-phenylalanine in virus growth medium for 4 h, starting at 2, 11 and 18 h after infection. At the end
of the labelling period, the monolayers were chilled on crushed ice, the radiolabelled medium was removed, and the monolayers were washed three times with cold HBSS, three times with cold 10% trichloroacetic acid (TCA) and three times with cold 95% ethanol. Then the monolayers were dissolved in 1 N-NaOH for scintillation counting.

Experiments to compare the polypeptides synthesized in infected and non-infected cells. At 18 h after infection with approx. 4 p.f.u./cell, monolayers were trypsinized, washed and replated in virus growth medium containing 5 μCi/ml of 14C-reconstituted protein hydrolysate, 5 μCi/ml of 14C-glucosamine or 16 μCi/ml of 3H-glucosamine. Then they were incubated at 37°C in a CO2 incubator for 6 h, frozen at -80°C and thawed. Duplicate plates were pooled and precipitated by adding an equal vol. of cold 20% TCA. The precipitates were washed with cold TCA and acetone and dissociated for polyacrylamide-gel electrophoresis in 0.005 M-phosphate buffer (pH 7.2) containing 1% SDS and 1% β-mercaptoethanol, by heating for 2 min in boiling water. Non-infected monolayers were trypsinized at the same time, radiolabelled with either 5 μCi/ml of 3H-reconstituted protein hydrolysate or 5 μCi/ml of 14C-glucosamine and treated in the same way as the infected monolayers.

Experiments to determine the effect of infection on glucosamine incorporation. Samples (0.05 or 0.1 ml) of the dissociated infected and non-infected cells labelled with glucosamine at 18 h after infection, described in the last section, were hydrolysed in 0.1 ml of 1 N-NaOH for scintillation counting. In the first experiment, the infected cells were pulsed with 16 μCi/ml of 3H-glucosamine, the non-infected with 5 μCi/ml of 14C-glucosamine. In the second experiment the labels were reversed, and in addition a second set of identical plates was pulsed with radiolabelled amino acids; infected cells were pulsed with 5 μCi/ml of either 14C-glucosamine or 14C-reconstituted protein hydrolysate, non-infected cells with 16 μCi/ml of 3H-glucosamine or 5 μCi/ml of 3H-reconstituted protein hydrolysate. To determine when, in the virus growth cycle, glucosamine incorporation is increased, infected and non-infected monolayers were pulsed with 5 μCi/ml of 3H-glucosamine in virus growth medium for 2 h, at different times after infection. Then the monolayers were washed three times with cold HBSS, three times with cold 10% TCA and three times with cold 95% ethanol and dissolved in 1 N-NaOH for scintillation counting.

Polyacrylamide-gel electrophoresis. Polypeptides were separated by the procedure of Weber & Osborn (1969) in 10 cm gels of 10% polyacrylamide containing 0.1% SDS, overlaid with a 0.5 cm stacking gel of 2.5% polyacrylamide. The electrode buffer was 0.1 M-phosphate (pH 7.2) containing 0.1% SDS. Gels were pre-run at 4 mA/gel for 2 h. Dissociated samples of 50 or 100 μl containing 5000 to 10000 cpm/min were run at 2 mA/gel for 16 to 18 h, then at 8 mA/gel until the tracking dye reached the bottom of the gel (2 to 3 h).

Scintillation counting. Liquid samples, 0.05 or 0.1 ml, were mixed with 0.1 ml of 1 N-NaOH and hydrolysed at 50°C for 2 h. Then 0.8 ml of 0.1 N-HCl and 10 ml of a toluene-Triton X-100 scintillation cocktail were added to each vial. Polyacrylamide gels were frozen at -20°C, cut into 2 mm slices with stacked razor blades, and placed in counting vials along with 0.2 ml of 1 N-NaOH. The vials were incubated at 50°C for 2 h and stored at room temperature overnight. Then 0.6 ml of 0.2 N-HCl and 10 ml of the toluene-Triton X-100 cocktail were added to each vial. Before counting in a Packard Model 2420 Tri-Carb liquid scintillation counter, all vials were stored in the dark at room temperature until counts had stabilized.

Protein concentrations in the samples were determined by the method of Lowry et al. (1951).

Isotopes. The 14C-D-glucosamine and 6-3H-D-glucosamine were obtained from ICN, the reconstituted 3H- and 14C-protein hydrolysate from Schwarz/Mann, and the 3H-uridine and 14C-phenylalanine (uniformly labelled) from New England Nuclear.
Table I. *HeLa* cell killing by RS virus infection

<table>
<thead>
<tr>
<th>M.o.i. (p.f.u./ml)</th>
<th>Infectious centres</th>
<th>% cells infected</th>
<th>Treatment</th>
<th>PE† (%)</th>
<th>Average % survivors</th>
<th>Cells not forming colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found</td>
<td>Expected*</td>
<td>Infected</td>
<td>13-5</td>
<td>31</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-infected</td>
<td>43</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-infected</td>
<td>58</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ 10⁵ p.f.u.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.72</td>
<td>48</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Infected</td>
<td>23</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-infected</td>
<td>80</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-infected</td>
<td>82</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ 10⁵ p.f.u.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>73</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Expected value = (1 − e⁻m₀°) 100. † PE = plating efficiency.

RESULTS

Cell killing

It has long been known that RS virus infection eventually kills cells following their massive fusion, but the direct effect of the infection on cell viability remained unknown. To study this effect in the absence of syncytia formation we followed the standard procedure for cell killing. This involves trypsinizing representative monolayers to determine the number of cells per dish, and assaying the virus inoculum before and after virus attachment to determine the m.o.i. After infection, the monolayers were trypsinized and the proportion of infected cells determined directly by plating a sample on sensitive monolayers. The proportion of viable cells was determined by their ability to form colonies when plated in cell growth medium containing antiserum to the virus (Table I).

When cells were infected with a multiplicity of 0.72 p.f.u./cell, where the expected proportion of infected cells was 50 %, approx. 50 % of the cells initiated plaques and 69 % failed to form colonies. The colony count for the controls that received an additional 10⁵ p.f.u. of virus (135 %) demonstrates that the antiserum in the medium prevented the spread of virus from infected to non-infected cells.

When cells were infected with 4.5 p.f.u./cell, where the expected proportion of infected cells was 99 %, only 73 % initiated plaques, but that proportion was essentially the same as the 72 % that failed to form colonies.

These results suggest that RS virus kills infected cells even under conditions where syncytia cannot form (when small numbers of single cells are plated 5 h after infection).

Effect of infection on DNA, RNA and protein synthesis

Rates of macromolecular synthesis were determined for three periods: 2 to 6 h after infection, which is early in the eclipse period and overlaps the time when virus RNA synthesis starts; 11 to 15 h, the end of the eclipse period to the time when RNA synthesis begins to level off; and 18 to 22 h, near the end of the growth cycle, when RNA synthesis has stopped and syncytia formation has started. During both of the earlier periods, thymidine and uridine incorporation were virtually the same in infected and non-infected cells.
RS virus infection and host-cell synthesis

Table 2. DNA, RNA and protein synthesis by non-infected and infected HeLa cells at different times after infection with RS virus*

<table>
<thead>
<tr>
<th>Time after infection</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-infected</td>
<td>Infected</td>
<td>Non-infected</td>
</tr>
<tr>
<td>2 h</td>
<td>ct/min/ml</td>
<td>5040</td>
<td>5784</td>
</tr>
<tr>
<td></td>
<td>%†</td>
<td>100</td>
<td>115</td>
</tr>
<tr>
<td>11 h</td>
<td>ct/min/mg of protein</td>
<td>12102</td>
<td>11867</td>
</tr>
<tr>
<td></td>
<td>%†</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>18 h</td>
<td>ct/min/mg of protein</td>
<td>9412</td>
<td>4135</td>
</tr>
<tr>
<td></td>
<td>%†</td>
<td>100</td>
<td>44</td>
</tr>
</tbody>
</table>

* Starting at the indicated times, monolayers were pulsed for 4 h with medium containing 0·2 μCi/ml of 3H-thymidine for DNA synthesis or 0·4 μCi/ml of 3H-uridine for RNA or 0·1 μCi/ml of 14C-phenylalanine for protein. Protein concentrations of representative infected and non-infected plates were determined only at 11 and 18 h after infection.

† Percentage incorporated into TCA-insoluble products.

However, by 18 h, the infected cells incorporated only 44% as much thymidine and 33% as much uridine as the non-infected. At no time was the rate of amino-acid incorporation reduced (Table 2).

These results suggest that RS virus infection does not affect cellular DNA and RNA synthesis until late in the virus growth cycle. The delayed effects of infection on DNA and RNA synthesis, along with the lack of any apparent effect on protein synthesis, further suggest that RS virus has no specific mechanism for shutting off cellular macromolecular synthesis.

Effect of infection on polypeptide synthesis by HeLa cells

In the preceding experiment, although protein synthesis continued at the same rate throughout the virus growth cycle, the possibility remained that the synthesis of cellular proteins had been shut down and replaced by the synthesis of virus proteins. To determine what proteins were being synthesized, infected and non-infected cells were pulsed with radiolabelled protein hydrolysate for 6 h, starting 18 h after infection, and then processed for electrophoresis as described in Methods. Samples of the dissociated infected and non-infected cells were then mixed and co-electrophoresed on 10% polyacrylamide gels.

In the electropherogram of this mixture (Fig. 1), the polypeptides are not well resolved, but it is evident that infection did not shut off the synthesis of cellular protein. However, in the infected cells the distribution of polypeptides of high and low mol. wt. appears to have shifted, resulting in a greater proportion of those of high mol. wt. A peak in the pattern (slice 7) seems to correspond with one of the virion glycoproteins, proof for which is given below.
Fig. 1. Co-electrophoresis of a mixture of non-infected HeLa cells labelled for 6 h with 5 μCi/ml of ³H-reconstituted protein hydrolysate, and HeLa cells infected with RS virus and labelled for 6 h with 5 μCi/ml of ¹⁴C-reconstituted protein hydrolysate. Labelling started 18 h after infection.

Table 3. Effect of RS virus infection on HeLa cell incorporation of glucosamine into TCA-insoluble products*

<table>
<thead>
<tr>
<th>Radiolabel</th>
<th>Experiment 1 (ct/min/plate)</th>
<th>Experiment 2 (ct/min/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>Non-infected</td>
<td>Infected</td>
</tr>
<tr>
<td>Amino acids</td>
<td>52,670</td>
<td>438,320</td>
</tr>
</tbody>
</table>

* All monolayers were trypsinized 18 h after infection, replated in virus growth medium containing radiolabelled glucosamine or radiolabelled reconstituted protein hydrolysate and incubated for 6 h at 37 °C. In Expt. 1, all cells were replated at 1 × 10⁶ cells/plate and the medium contained 16 μCi/ml of ³H-glucosamine for infected cells and 5 μCi/ml of ¹⁴C-glucosamine for non-infected. In Expt. 2, infected cells were replated at 2.2 × 10⁶/plate and non-infected cells at 3.5 × 10⁶/plate. The glucosamine labels were reversed, and infected cells were pulsed with 5 μCi/ml of ¹⁴C-glucosamine and non-infected with 16 μCi/ml of ³H-glucosamine. A second set of replated cells was pulsed with 5 μCi/ml of ¹⁴C-reconstituted protein hydrolysate/infected plate or 5 μCi/ml of ³H-reconstituted protein hydrolysate/non-infected plate.

**Effect of infection on glucosamine incorporation**

Monolayers of HeLa cells, non-infected and infected with 4 p.f.u. of RS virus per cell were trypsinized at 18 h after infection, re-plated in virus growth medium containing radiolabelled glucosamine and incubated for 6 h at 37 °C in a CO₂ incubator. From two such experiments it is evident that infection stimulates the incorporation of radiolabelled glucosamine into TCA-insoluble products (Table 3). Because the serum in the virus growth medium invalidates the use of an assay for protein, amino-acid incorporation served as a control in Expt. 2. In contrast to the glucosamine incorporation, amino-acid incorporation by the infected cells increased only slightly.

To determine when in the virus growth cycle the stimulation of glucosamine incorporation begins, infected and non-infected monolayers were pulsed with ³H-glucosamine for
RS virus infection and host cell synthesis

Table 4. Effect of RS virus infection on HeLa cell incorporation of glucosamine into TCA-insoluble products at different times after infection*

<table>
<thead>
<tr>
<th>Hours after infection when glucosamine was added</th>
<th>Experiment 1 (ct/min/mg of protein)</th>
<th>Experiment 2 (ct/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-infected</td>
<td>Infected</td>
</tr>
<tr>
<td>2</td>
<td>2296 (100%)</td>
<td>2525 (111%)</td>
</tr>
<tr>
<td>3</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>4814 (100%)</td>
<td>5102 (106%)</td>
</tr>
<tr>
<td>8</td>
<td>4996 (100%)</td>
<td>7028 (141%)</td>
</tr>
<tr>
<td>9</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>12</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

* Following infection with approx. 2 to 4 p.f.u. of virus/cell, monolayers were pulsed for 2 h with medium containing 5 μCi/ml of 3H-glucosamine, starting at the indicated time after infection.

Fig. 2. Co-electrophoresis of a mixture of non-infected HeLa cells labelled for 6 h with 5 μCi/ml of 14C-glucosamine, and HeLa cells infected with RS virus and labelled for 6 h with 16 μCi/ml of 3H-glucosamine. Labelling started 18 h after infection. • --- ●, 3H; ○ --- ○, 14C.
Fig. 3. Co-electrophoresis of a mixture of HeLa cells infected with RS virus and labelled for 6 h, starting 18 h after infection, with 5 μCi/ml of 14C-glucosamine, and purified RS virions labelled with 3H-amino acids. Numbers over curves mark virion polypeptides. •—•, 3H; •—•, 14C.

Fig. 4. Co-electrophoresis of a mixture of HeLa cells infected with RS virus and labelled for 6 h with 16 μCi/ml of 3H-glucosamine, and HeLa cells infected with RS virus and labelled for 6 h with 5 μCi/ml of 14C-reconstituted protein hydrolysate. Labelling started 18 h after infection. •—•, 3H; •—•, 14C.
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2 h, at different times after infection (Table 4). Results of two experiments suggest that no persistently increasing stimulation occurs before 12 h after infection, which is around the end of the eclipse period.

Presence of virus glycoprotein in whole-cell lysates of cells radiolabelled 18 h after infection

Purified RS virus has previously been resolved into seven polypeptides whose approximate mol. wt. ranges from 20000 to 80000. When purified virus labelled with either $^{14}$C-glucosamine or with $^3$H-amino acids was co-electrophoresed, three of the virus polypeptides (numbers 1, 2 and 7) were found to be glycoproteins (Levine, 1977). To look for the virus glycoproteins in infected cells, they were labelled with $^3$H-glucosamine and non-infected cells were labelled with $^{14}$C-glucosamine over a period of 6 h, starting 18 h after infection, and then prepared for electrophoresis as described in Methods.

In an electropherogram on a 10% polyacrylamide gel of a mixture of the infected and non-infected cells, the infected cells have two peaks, slices no. 7 and 32, that are absent from the non-infected cells (Fig. 2). That these peaks correspond to virion proteins no. 1 and 7 is suggested by an electropherogram of a mixture of infected cells labelled with $^{14}$C-glucosamine 18 h after infection and purified virus labelled with $^3$H-amino acids (Fig. 3). The large polypeptide found in cells labelled with $^{14}$C-amino acids 18 h after infection is probably also a virion protein (see Fig. 1), because it co-electrophoreses with the $^3$H-labelled glucosamine peak found in these cells which migrates with virion glycoprotein no. 1 (Fig. 4).

DISCUSSION

The effect of paramyxoviruses on host cells differs according to the type of cell, as well as the infecting virus (Choppin & Compans, 1975). Of the systems studied, that of SV5 in BHK 21-F cells seems most like the RS virus-HeLa cell system. In that system the eclipse period is 6 to 7 h, the yield of virus is low, and 12 to 24 h after infection there is extensive cell fusion with accumulations of nucleocapsid in the cytoplasm (Compans et al. 1966; Holmes & Choppin, 1966). Holmes and Choppin concluded that in cells infected with SV5, the inhibition of cellular macromolecular synthesis and subsequent cell death are a consequence of the massive cell fusion. In the case of RS virus, the inability of single infected cells to form colonies suggests that cell fusion per se is not a prerequisite for cell death.

Although infection with RS virus eventually kills cells, the virus does not seem to have a specific mechanism for shutting off cellular macromolecular synthesis. Tests by pulse labelling from 11 to 15 h after infection, a period including the end of the eclipse period and the time when virus RNA synthesis reaches a plateau, indicate that no change occurs in the levels of DNA, RNA or protein synthesis in infected cells. Not until 18 h after infection, when the growth cycle is almost completed and syncytia formation has started, is there a reduction in the rates of DNA and RNA synthesis. These rates were measured by the incorporation of radiolabelled thymidine and uridine, so the reduction could have resulted from lower rates of transport of those precursors, rather than lower rates of de novo synthesis of DNA and RNA. Another possibility is that the rates of DNA and RNA synthesis remain unchanged at 2 and 11 h after infection only because the synthesis of virus components has replaced the synthesis of cellular components, but this is unlikely. In the first place, DNA is not generally considered to be a component of RS virus. In addition, treatment with actinomycin D at 2 and 11 h was found to depress RNA synthesis to the same level in infected and non-infected cells (S. Levine, unpublished data).
Not even at 18 h after infection with RS virus is there a reduction in the rate of protein synthesis by cells. Polyacrylamide-gel electrophoresis of a mixture of infected and non-infected cells has demonstrated that in the infected cells there is no dramatic shift in the polypeptide pattern, such as occurs with viruses that shut off cellular protein synthesis, and also that the infected cells do indeed continue to synthesize cellular proteins as late as 18 h after infection. However, polypeptide synthesis does shift to include the synthesis of virion polypeptides. To demonstrate all the virus polypeptides, other procedures such as cell fractionation or immune precipitation may be required.

Apart from synthesis of the virus itself, the major change effected by RS virus infection in the pattern of HeLa cell macromolecular synthesis appears to be an increase in glycosylation. Infection stimulates glucosamine incorporation into TCA-insoluble products. This could simply represent increased glucosamine uptake, resulting in a pool with a greater proportion of radiolabelled precursor. However, no persistent increase in the rate of glucosamine incorporation occurs until about 12 h after infection. Since this is approximately the end of the eclipse period, the higher rate of glucosamine incorporation may be related to the synthesis of virion glycoprotein. Most glucosamine in the virion is associated with virion protein no. 1 (Levine, 1977). In addition, by 16 to 18 h after infection a large fraction of the glucosamine incorporated by infected cells co-electrophoreses with virion protein.

If cells infected with RS virus do indeed die even where there is no cell fusion, as the single-cell experiments suggest, what remains as the cause of cell death seems to be the slow shutting off of DNA and RNA synthesis. A possible explanation for that shut off is some alteration in plasma membrane function. After budding, Long strain RS virus releases so poorly that over 90% remains attached to plasma membrane. Perhaps this piling up of virions, along with the increased demand for activated sugars required for glycosylation of the virion glycoprotein, reduces the quantities of precursors available for DNA and RNA synthesis, either by depressing transport of the precursors or by depleting cellular pools of some of them.

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REFERENCES


RS virus infection and host-cell synthesis


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