Inhibition of Pseudorabies Virus Replication by Vesicular Stomatitis Virus

II. Activity of Defective Interfering Particles

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Received for publication 5 December 1975

Purified defective interfering (DI) particles of vesicular stomatitis virus (VSV) inhibit the replication of a heterologous virus, pseudorabies virus (PSR), in hamster (BHK-21) and rabbit (RC-60) cell lines. In contrast to infectious B particles of VSV, UV irradiation of DI particles does not reduce their ability to inhibit PSR replication. However, UV irradiation progressively reduces the ability of DI particles to cause homologous interference with B particle replication. Pretreatment with interferon does not affect the ability of DI particles to inhibit PSR replication in a rabbit cell line (RC-60) in which RNA, but not DNA, viruses are sensitive to the action of interferon. Under similar conditions of interferon pretreatment, the inhibition of PSR by B particles is blocked. These data suggest that de novo VSV RNA or protein synthesis is not required for the inhibition of PSR replication by DI particles. DI particles that inhibit PSR replication also inhibit host RNA and protein synthesis in BHK-21 and RC-60 cells. Based on the results described and data in the literature, it is proposed that the same component of VSV B and DI particles is responsible for most, if not all, of the inhibitory activities of VSV, except homologous interference.

One of the characteristics of the inhibition of virus growth by defective interfering (DI) particles is specificity for the homologous virus (14). When DI particles of vesicular stomatitis virus (VSV) were tested against encephalomyocarditis virus (17) or foot-and-mouth disease virus (5), no inhibition was demonstrated. Dubovi and Youngner (7) showed that B particles of VSV rendered noninfectious by UV irradiation inhibited the replication of a heterologous virus, pseudorabies virus (PSR). Since heavily irradiated VSV possesses little if any ability to direct the synthesis of viral RNA or proteins, these data suggested that a virion component was responsible for the inhibition of PSR replication. DI particles of VSV contain all of the proteins of the infectious particle (19, 33) and therefore should inhibit the replication of PSR. This possibility was tested along with the ability of DI particles to inhibit host RNA and protein synthesis.

MATERIALS AND METHODS

Cells. Primary chicken embryo, BHK-21, RK-13, and RC-60 cells were propagated as previously described (7).

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Chemicals. [3H]Juridine (specific activity of >25 Ci/mmol) and [3H]leucine (specific activity of >40 Ci/mmol) were purchased from New England Nuclear Corp. Cycloheximide was purchased from Upjohn Co., and actinomycin D was obtained through the courtesy of H. B. Woodruff of Merck, Sharpe and Dohme.

Viruses. The conditions for the growth of the large-plaque mutant of VSVND(L1 VSV) and PSR were described previously (7).

Production and purification of DI particles of VSV. DI particles were produced by serial undiluted passage of L1 VSV in BHK-21 cells. Fluid from 80 to 100 32-ounce (oz) culture bottles of the third undiluted passage of VSV was the starting material for DI particle isolation. Virus was concentrated as previously described (7), and DI particles were isolated using 10 to 40% (wt/wt) sucrose gradients. One milliliter of concentrated virus material was layered on top of 11-m1 gradients that were centrifuged in an SW41 rotor at 25,000 rpm for 95 min. Virus bands were collected and diluted with NTE buffer (0.1 M NaCl, 0.01 M Tris, 0.001 M EDTA, pH 7.4). The particles were pelleted in a no. 40 rotor at 36,000 rpm for 75 min. The pellet was resuspended in NTE buffer and sonicated for 2 min in a Raytheon sonic oscillator at a setting of 1.2. One or two additional sucrose gradient centrifugations were used to reduce the concentration of infectious B particles.

Quantitation of DI particles. The procedure described by Bellett and Cooper (1) was used to quantitate DI particles. Serial twofold dilutions of a prepa-
ration containing DI particles were added to monolayers of BHK-21 cells simultaneously with infectious VSV (multiplicity of infection \( \text{MOI} = 1 \)). The virus inoculum was adsorbed for 1 h at 37 C, and unadsorbed virus was removed by three washes with Eagle minimal essential medium. After overnight incubation, culture fluids were harvested and assayed for VSV infectivity. As reported by Bellett and Cooper (1) and Marcus and Sekellick (24), the interference by DI particles with infectious VSV synthesis followed a one-particle-per-cell dose response. The titer of DI particles was calculated as described for PSR-inhibiting particles (7).

**Double infections with DI particles and PSR.** Unless otherwise stated, DI particles and PSR were added simultaneously to cell cultures in 35- or 60-mm petri dishes at an input MOI of 5 to 10 for PSR and 1,000 for DI particles. The conditions of incubation and infectivity assays were those described previously (7).

In vivo assay of primary transcription by the virion-bound polymerase of VSV. The in vivo assay of primary transcription was done according to Manders et al. (23) as previously described (7).

**Inactivation of DI particles by UV.** The conditions for irradiation of DI particles were those described for infectious VSV (7).

Labeling of host RNA and proteins. Host RNA or proteins were labeled with either \(^{3}H\)uridine or \(^{3}H\)leucine in Hanks balanced salt solution (HBSS). Cells in 35-mm petri dishes were exposed to the labeled precursors for 30 min at 37 C. Cell monolayers were washed three times with cold HBSS and dissolved by the addition of 1% sodium dodecyl sulfate in 0.1 M NaCl-0.01 M EDTA. The solubilized cells were mixed with an equal volume of 20% trichloroacetic acid, and acid-precipitable material was collected on glass-fiber filters (Whatman GF/A or GF/C). The filters were treated with 0.5 ml of NCS tissue solubilizer (Amerham/Searle) and counted in a toluene-based scintillation cocktail. The data were plotted at the beginning of the labeling time.

**RESULTS**

Isolation and characterization of DI particles of L, VSV. Three serial undiluted passages of L, VSV in BHK-21 cells resulted in a 3-log\(_{10}\) reduction in infectivity and the production of two sizes of DI particles that were isolated by sucrose gradient centrifugation and examined with the electron microscope. The larger particle (designated DI-40) was approximately 40% of the length of the B particle, and the smaller particle (designated DI-30) was 30% of the length of the B particle. Using the biological assay of Bellett and Cooper (1) described in Materials and Methods, the third undiluted passage of L, VSV was found to contain 5.2 \(\times\) 10\(^{10}\) DI particles/ml and 1.5 \(\times\) 10\(^{10}\) PFU/ml.

DI particles concentrated by the polyethylene glycol 6000 procedure of McSharry and Benzinger (26) were purified using two or three successive sucrose gradient (10 to 40%) centrifugations. DI particle bands from the second sucrose gradient contained 6 \(\times\) 10\(^{11}\) DI particles/ml and less than 10\(^{6}\) PFU/ml. However, Doyle and Holland (6) suggested that plaque assays in the presence of large numbers of DI particles may be inaccurate. To circumvent this problem, they devised a cell destruction assay for the detection of residual B particle infectivity in DI particle preparations. Using their procedure, less than 1 infectious particle per 10\(^{6}\) DI particles was detected in the DI particle preparation from a second sucrose gradient centrifugation. As a further check for the presence of significant quantities of B particles, an in vivo assay for primary transcription at an MOI of 1,000 was done using DI particles from a second sucrose gradient. This preparation did not show any in vivo primary transcription, nor did it prevent the detection of primary transcription when cells were co-infected with DI and B particles. ("B particle" will be used synonymously with "infectious VSV" or "PFU," especially when comparisons are made with DI particles.) These data, together with other findings to be presented, rule out the possibility that B particles are responsible for the inhibitory activities of the DI particle preparations that are described below.

**Inhibition of PSR replication by DI-40 particles.** Serial 10-fold dilutions of a DI-40 particle preparation purified by two successive sucrose gradients and a constant amount of PSR (MOI = 5) were added simultaneously to monolayers of BHK-21 cells. Cultures were harvested after 18 h at 37 C and assayed as previously described. High multiplicities of DI-40 particles (3,400 and 340) inhibited the replication of PSR, whereas lower multiplicities (34 and 3.4) did not (Table 1). The inoculum of DI-40 particles that inhibited the yield of PSR by nearly 3 logs (MOI = 340) had been diluted by a factor of 5 \(\times\) 10\(^{-4}\) from the concentrated preparation of DI-40 particles that contained 1.2 \(\times\) 10\(^{10}\) DI-40 particles per ml and less than 10\(^{6}\) B particles per ml. A dilution of 5 \(\times\) 10\(^{-4}\) reduced the B particle content to less than 10\(^{5}\) per ml, suggesting that B particles played no role in the inhibition of PSR replication by the DI-40 particle preparation.

<table>
<thead>
<tr>
<th>MOI of DI-40 particles</th>
<th>Yield of PSR (PFU/ml)</th>
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<tbody>
<tr>
<td>3,400</td>
<td>6.3 (\times) 10(^{4})</td>
</tr>
<tr>
<td>340</td>
<td>5.9 (\times) 10(^{5})</td>
</tr>
<tr>
<td>34</td>
<td>2.0 (\times) 10(^{8})</td>
</tr>
<tr>
<td>3.4</td>
<td>3.0 (\times) 10(^{9})</td>
</tr>
<tr>
<td>PSR control</td>
<td>2.7 (\times) 10(^{9})</td>
</tr>
</tbody>
</table>
tion. In addition, it would require approximately 10 B particles per cell to reduce the PSR yield by 3 logs (unpublished observation). Considering the number of cells and the dilution factor, the undiluted DI-40 particle preparation would have also had to contain $5 \times 10^{10}$ B particles/ml, a quantity which would have been easily detectable in the assays previously described.

The inhibition of PSR replication by DI particles was not due to an interference with PSR adsorption. Adsorption of PSR for 1 h at 4°C before the addition of DI particles did not increase the yield of PSR compared with the yields in cultures that received PSR and DI particles simultaneously.

Comparison of the ability of DI-40 particles to interfere with the replication of B particles and PSR. Serial twofold dilutions of DI-40 particles and a constant amount of PSR (MOI = 5) were added simultaneously to monolayers of BHK-21 cells. Infected cells and fluids were harvested and assayed as previously described. Unlike DI-40 particle interference with the replication of B particles, DI-40 particle interference with the replication of PSR exhibited a multi-particle-per-cell dose response. Since the inhibition of PSR replication by DI-40 particles was not a one-particle-per-cell dose response, the procedure for quantitating PSR-inhibiting particles (7) could not be used to estimate the PSR-inhibiting activity in DI-40 particle preparations. However, a comparison was made by testing the PSR- and B particle-inhibiting activity of different concentrations of DI-40 particles. Figure 1 shows that the PSR-inhibiting activity of the DI-40 particle preparation was significantly less than the B particle-inhibiting activity. A comparison of the dilutions of DI-40 particles that produced similar levels of interference with PSR and B particles revealed that the B particle-inhibiting activity was at least 10 times greater than PSR-inhibiting activity.

If the PSR-inhibiting activity of the DI-40 particle preparation was due to B particles, additional purifications on sucrose gradients would reduce the PSR-inhibiting activity of the DI-40 particle preparation. The data in Fig. 1 were obtained using a DI-40 particle preparation purified by two successive sucrose gradient centrifugations. An additional sucrose gradient centrifugation of this material produced no change in the concentration of PSR-inhibiting activity, again suggesting that B particles play no role in the inhibition of PSR replication by DI-40 particle preparations. In addition, DI-30 particle preparations purified by two successive gradient centrifugations showed the same relationship between PSR-inhibiting activity and B particle-inhibiting activity as did DI-40 particle preparations.

Effect of UV irradiation on the ability of DI-40 particles to inhibit the replication of PSR and B particles of VSV. Huang and Wagner (17) showed that the ability of DI particles to inhibit the replication of B particles was abolished by UV irradiation. An experiment was done to determine whether the ability of DI-40 particles to inhibit the replication of PSR was also sensitive to UV irradiation. A DI-40 particle preparation purified by two successive sucrose gradient centrifugations was diluted 1:400 in phosphate-buffered saline and irradiated with UV light as previously described (7); the 1:400 dilution of the DI-40 particles was the lowest concentration of DI-40 particles that produced maximum inhibition of PSR replication. DI-40 particles to be tested for their ability to inhibit the replication of B particles were diluted 1:3,200 (1:400 before irradiation; 1:8 after irradiation) to achieve the minimum concentration of DI-40 particles that produced maximum inhibition of B particle synthesis. These mini-

![Graph](image-url)
mum concentrations were used to prevent the masking of the effect of UV irradiation by excessively high concentrations of DI-40 particles. The irradiated samples were tested separately against PSR and VSV in BHK-21 cells. The results confirmed the report of Huang and Wagner (17); the ability of DI particles to inhibit the replication of B particles was abolished by UV irradiation (Fig. 2). However, UV irradiation did not affect the inhibition of PSR replication by DI-40 particles. These results suggest that the mechanism of the inhibition of PSR replication by DI-40 particles is different from the homologous interference with B particle replication. The failure of UV irradiation to affect the PSR-inhibiting activity of DI-40 particles indicates a lack of involvement of VSV-directed RNA or protein synthesis in this inhibition. In addition, the findings reemphasize that B particles are not responsible for the inhibition of the replication of PSR by DI particle preparations; UV irradiation significantly diminished the ability of B particles to inhibit PSR replication (7).

Inability of interferon to prevent DI particle interference with the replication of PSR. Since rabbit cells are defective in their ability to express interferon-mediated interference with the replication of DNA viruses (38), it was possible to test the effect of interferon pretreatment on the ability of DI particles to inhibit PSR replication. RC-60 cells were pretreated for 24 h with 500 U of rabbit interferon, as described elsewhere (38). Monolayers with and without interferon pretreatment were infected simultaneously with PSR (MOI = 10) and either DI-40 particles (MOI = 1,000) or B particles (MOI = 15). All cultures were harvested after 20 h at 37 C and assayed as previously described. As expected (38), interferon did not diminish the yield of PSR in RC-60 cells pretreated with 500 U of interferon (Table 2, groups 1 and 2). Interferon pretreatment blocked the inhibition of PSR replication by the infectious B particle (Table 2, groups 3 and 4) but did not diminish the ability of DI-40 particles to inhibit PSR replication (Table 2, groups 5 and 6). Interestingly, the level of inhibition seen with interferon pretreatment and B particles was similar to the levels of inhibition produced by DI-40 particles with and without interferon pretreatment. These data may be a result of the addition of a similar number of VSV particles to each culture. At an MOI of 15 PFU per cell, each cell would be infected with 480 to 960 PSR-inhibiting particles (7) com-

![Figure 2. Effect of UV irradiation on the ability of DI-40 particles to inhibit replication of VSV and PSR in BHK-21 cells.](http://jvi.asm.org/)

### Table 2. Effect of interferon pretreatment on the ability of B and DI-40 particles to inhibit the replication of PSR in RC-60 cells

<table>
<thead>
<tr>
<th>Expt group</th>
<th>Interferon pretreatment (500 U)</th>
<th>Virus infection</th>
<th>PSR yield (PFU/ml)</th>
<th>Fold decrease compared with PSR control (group 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B particles (MOI = 15)</td>
<td>DI-40 particles (MOI = 1,000)</td>
<td>PSR (MOI = 10)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.4 × 10⁸</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3.4 × 10⁸</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>2.6 × 10⁷</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4.1 × 10⁸</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2.8 × 10⁸</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>2.8 × 10⁸</td>
</tr>
</tbody>
</table>
pared with 1,000 DI particles per cell. Similar results were obtained using RK-13 cells. These data again suggest that B particles were not involved in the inhibition of PSR replication by DI-40 particle preparations since interferon failed to diminish the inhibition of PSR replication by the defective virus particles.

Inhibition of host RNA synthesis by DI particles of VSV. The inability of UV irradiation and interferon to diminish the PSR-inhibiting activity of DI particles suggested that a virion component might be responsible for the inhibition of PSR replication. A virion component of VSV has also been implicated in the inhibition of host RNA synthesis (16, 37). This possibility was examined with the same DI-40 particle preparation that inhibited PSR replication. Monolayers of BHK-21 and RC-60 cells in 35-mm petri dishes (2.5 × 10⁶ cells) were infected with DI-40 particles (MOI = 1,000). After adsorption for 1 h at 4°C, the inoculum was removed, and 1 ml of Eagle minimal essential medium was added. At different times after infection, the cells were exposed to 1 μCi of [³H]uridine in 1 ml of HBSS for 30 min. Cells were processed for radioactivity as previously described.

DI-40 particles effectively shut off RNA synthesis in both BHK-21 and RC-60 cells (Fig. 3). This effect of DI particles on host RNA synthesis has been reported by Huang et al. (15). The rapid inhibition of host RNA synthesis reported by Wertz and Youngner (35) was seen in both cell lines. In the earliest labeling interval (immediately after removal of the virus inoculum), host RNA synthesis was reduced by nearly 20%. These data strongly support the concept of an inhibitory component in the virion of VSV. The observed inhibition of host RNA synthesis by DI-40 particles was not a result of an impairment of uridine uptake as has been suggested by Genty (9); [³H]uridine was not prevented from entering the soluble pools of nucleotide precursors in cells infected with DI-40 particles (unpublished observation).

A comparison of the dose response for the inhibition of host RNA synthesis and PSR replication by DI particles revealed a striking similarity. The lowest concentration of DI particles (16/cell) that could produce detectable inhibition of host RNA synthesis was approximately the same as the lowest concentration (16 to 32/cell) that could produce detectable inhibition of PSR replication. Also, the lowest concentration of DI particles (210/cell) that could produce maximum inhibition of RNA synthesis was the lowest concentration (200 to 300/cell) that could produce maximum inhibition of PSR replication. These data suggest that inhibition by DI particles of PSR replication and host RNA synthesis may share a common mechanism.

Inhibition of host protein synthesis by DI-40 particles. Monolayers of BHK-21 and RC-60 cells (2.5 × 10⁶ cells) were infected with DI-40 particles (MOI = 1,000). Cellular proteins were labeled for 30 min at different times after infections with [³H]leucine (1 μCi in 1 ml of HBSS), and the cells were processed as previously described. The results support the idea of an inhibitory component in the virion of VSV (Fig. 4). As with B particles in L cells (36), inhibition of protein synthesis in BHK-21 cells was evident at the earliest sampling time. However, in RC-60 cells inhibition of protein synthesis was not detected until 3 h elapsed. At 3 h, the degree of inhibition was the same in both cell lines, and by 6 h RC-60 showed a greater inhibition. The pattern of inhibition of RC-60 cell protein synthesis by B particles is very similar.
to that seen with DI-40 particles (John La Montagne, personal communication).

**DISCUSSION**

The data presented elsewhere on the inhibition of PSR replication by B particles (7) and the data in this study, along with numerous reports in the literature, have led us to conclude that many, if not all, of the inhibitory activities of VSV can be explained by the action of an inhibitory component of the virus that is present in infected cells, in B particles (infectious or inactivated), and in DI particles. The inhibitory activities of VSV are summarized in Table 3.

The infectious B particle can express all of the inhibitory activities listed in Table 3. A crucial difference between infectious B particles and inactivated B particles or DI particles is the ability of the infectious particles to amplify the components of the infecting virion. It is possible that the amplification process itself is inhibitory, but, since all the viral proteins made in the infected cells are found in the virion of VSV (31), it is also possible that the de novo synthesized viral proteins that are destined to become virions are inhibitory. (The role of viral RNA will be discussed below.) If the virion of VSV contains an inhibitory component, then the inhibitory activities of VSV should be expressed under conditions where amplification is not necessary, i.e., infection at high MOI. This is indeed the case. UV-irradiated B particles at high MOI can kill cells (2), can inhibit host RNA (16, 37) and protein synthesis (36), and can inhibit the replication of PSR (7). DI particles of VSV are similar to UV-irradiated B particles in that DI particles cannot amplify the components of the infecting virion. DI particles can express at high MOI the following activities, which also are shown by UV-irradiated B particles: cell killing (6), inhibition of host RNA synthesis (15; Fig. 3), infection of host protein synthesis (6, Fig. 4), and inhibition of PSR replication (Table 1). In addition, complementation group I temperature-sensitive mutants of VSV, which are defective in

![Graph showing the effect of infection with DI-40 particles on host protein synthesis.](http://jvi.asm.org/)

**FIG. 4.** Effect of infection with DI-40 particles on host protein synthesis. Monolayers of BHK-21 cells and RC-60 cells were infected with DI-40 particles (MOI = 1,000). At different times after infection, proteins were labeled by incubation for 30 min with [3H]leucine (1 μCi/ml) in HBSS. Labeled cells were solubilized with 1% sodium dodecyl sulfate and processed for radioactivity determinations as described in Materials and Methods. Average of uninfected controls (counts/min per 10^6 cells): BHK-21 cells = 122,600; RC-60 cells = 60,800.

**Table 3. Summary of inhibitory activities of VSV based on data in this paper and in literature references**

<table>
<thead>
<tr>
<th>VSV activity</th>
<th>B particles (PFU, d-CKP, PSRIP)</th>
<th>DI particles ts mutants at nonpermissive temperature (Low MOI) in complementation group:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonirradiated</td>
<td>UV irradiated (high MOI)</td>
</tr>
<tr>
<td>Cell killing</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(2)</td>
</tr>
<tr>
<td>Inhibition of host RNA synthesis</td>
<td>(32, 35)</td>
<td>(16, 37)</td>
</tr>
<tr>
<td>Inhibition of host protein synthesis</td>
<td>(10, 36)</td>
<td>(36)</td>
</tr>
<tr>
<td>Inhibition of PSR replication</td>
<td>(7)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

* = Mutant ts-G-41 was used as the representative of complementation group IV.
* +, Yes; -, no; ±, partial inhibition; NR, no report. Numbers in parentheses are reference numbers.
* Unpublished observations.
primary transcription (30) and therefore unable to amplify the infecting virion, can neither kill cells at low MOI (25), nor inhibit host RNA or protein synthesis (unpublished observation), nor inhibit the replication of PSR (7). However, at high MOI, group I mutants can kill cells (25), inhibit host RNA synthesis (13), and inhibit the replication of PSR (unpublished observation). These findings are all consistent with the hypothesis that the same component of VSV may be responsible for all the inhibitory activities of VSV except homologous interference.

In the case of VSV, there are several possible candidates for the inhibitory component: (i) viral RNA; (ii) one of the five viral proteins; (iii) viral protein(s) plus RNA; and (iv) combinations of viral proteins. Since B and DI particles contain RNA with common base sequences, albeit DI particle RNA is lower in molecular weight (22), and since B and DI particles contain the same viral proteins (19, 33), all possible inhibitory components are found in both particles. With the data presently available, none of these possibilities can be eliminated, but some appear more likely than others.

The role of viral RNA in the inhibition of host metabolism has been most extensively studied using poliovirus. Ehrenfeld and Hunt (8) showed that double-stranded poliovirus RNA could inhibit the initiation of protein synthesis. They suggested that the accumulation of double-stranded RNA molecules within the infected cell might account for the cessation of host protein synthesis. Although this work has been confirmed (4, 18), others have questioned the significance of these data because of the low concentration of double-stranded RNA found in infected cells (3, 21). It appears unlikely that viral RNA is the inhibitory component of VSV. To produce double-stranded RNA within the infected cell, primary transcription would be necessary. DI particles do not demonstrate this activity and the inhibitory process is insensitive to UV irradiation, an agent that affects primary transcription. In addition, the RNA of VSV probably does not exist in a double-stranded configuration at any time during the replication cycle (29). There are no reports dealing with inhibition of host RNA or protein synthesis by any complex of viral RNA and protein.

Very little information is available concerning the inhibitory activity of specific VSV proteins. However, several studies on the inhibition of protein synthesis in HeLa cells (27) and rabbit reticulocytes (12) have suggested that interactions at the cell membrane can influence cellular protein synthesis. In their study on the inhibition of host protein synthesis by VSV, Wertz and Youngner (36) presented data that suggested the interaction of the VSV virion with the cell membrane partially inhibited host protein synthesis. Heine and Schnaitman (11) demonstrated that VSV enters the cell through a process of membrane fusion and that viral proteins become associated with the host cell membrane. During the attachment and fusion process, it is possible that viral proteins interfere with regulatory processes associated with the cell membrane.

If interactions on the cell membrane influence host metabolism, then two proteins of VSV, G and M, are likely candidates for the inhibitory component, since they are always closely associated with the cell membrane (20, 34). McSharry and Choppin (Abstr. Annu. Meet. Am. Soc. Microbiol., 1974) reported that G protein of VSV inhibited host RNA and DNA synthesis in BHK-21F cells. The M protein of VSV has also been implicated in the inhibition of host RNA synthesis (9). VSV mutant tsO-23 produces a defective M protein (20, 28) and is unable to cause a progressive inhibition of host RNA synthesis. However, the possibility cannot be excluded that a combination of both G and M may be necessary for the inhibition process. Although the cell membrane has been discussed as the site of action of the inhibitory component, there is no evidence available to rule out a cytoplasmic site for the inhibitory process.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-06264 from the National Institute of Allergy and Infectious Diseases.

The collaboration of John J. Cardamone, Jr., with electron microscopy and DI particle measurements is gratefully acknowledged.

LITERATURE CITED


