Protein Synthesis in Semliki Forest Virus-infected Cells is Not Controlled by Permeability Changes

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SUMMARY

The uptake of the GTP analogue guanylyl(β,γ-methylene)diphosphonate (GppCH2p) is the same in Semliki Forest virus (SFV)-infected BHK cells as in mock-infected cells, in spite of the fact that protein synthesis is inhibited by GppCH2p more markedly in SFV-infected cells than in control cells. A possible explanation for this difference is that infected cells have a lower concentration of GTP and a lower ratio of GTP:GDP than uninfected cells, and the analogue may thus be a more effective competitive inhibitor of translation. We conclude that in this system, the difference between infected and uninfected cells lies not at the plasma membrane but within the cytoplasm.

INTRODUCTION

The mechanisms by which virus-infected cells switch from synthesis of host proteins to synthesis of virus proteins are poorly understood. It has been postulated that permeability changes occur early in infection and that these changes result in alterations of the intracellular ionic environment to favour the translation of virus over host cell polypeptides (Carrasco & Smith, 1976; Carrasco, 1977; Garry et al., 1979a, b, 1982). The evidence for the proposed permeability changes in infected cells rests largely on the demonstration that normally impermeant inhibitors of protein synthesis, such as the GTP analogue guanylyl(β,γ-methylene)diphosphonate (GppCH2p), depress protein synthesis in infected, but not in uninfected cells (Carrasco, 1978; Contreras & Carrasco, 1979; Benedetto et al., 1980; Lacal et al., 1980; Carrasco, 1981). In the work presented here we have examined the uptake of [3H]GppCH2p in uninfected and Semliki Forest virus (SFV)-infected cells.

METHODS

Cells and viruses. BHK (clone 13) cells in monolayer were grown to confluency (1.5 x 10⁶ to 2.0 x 10⁶ cells) on 35 mm plates in Dulbecco's minimal essential medium (MEM) containing 10% newborn calf serum and infected with SFV or mock-infected, washed and maintained as described by Gray et al. (1983a). Haemolytic ('3-day') Sendai virus was prepared as described by Impraim et al. (1980). At various times after infection or exposure to Sendai virus, cells were washed and uptake of labelled compounds, guanine nucleotide content and protein synthesis in intact cells or cell extracts were measured.

Uptake of labelled compounds. Washed cells in 0.5 ml methionine-free MEM were exposed to 2 mM-[3H]GppCH2p (0.35 μCi/μmol) purified by chromatography on a DE52 DEAE-cellulose column (see Fig. 2) and 2 mM-[14C]β-methylglucoside (0.15 μCi/μmol), or to [3H]-labelled guanine nucleotide (80 μCi/μmol or 0.8 μCi/μmol), all from Amersham International, for varying periods of time at 37 °C. Cells were washed three times in 10 mM-HEPES-buffered isocotic choline chloride and extracted with 0.3 ml 0.1% Triton X-100. Cells and extract were scraped off the plates, centrifuged and the supernatant assayed for radioactivity. The pellet contained < 30% of the radioactivity after exposure to [3H]GppCH2p. Cells were also extracted with trichloroacetic acid (TCA), as described below.

Protein synthesis in intact cells. Washed cells in the same medium as above were exposed to L-[35S]methionine (0.01 to 0.3 μCi/plate) for varying times at 37 °C. Cells were washed three times in 10 mM-HEPES-buffered...
isotonic saline and extracted with 0-3 ml ice-cold 5% TCA. The residue on the plates was washed three times with ice-cold 5% TCA, extracted with 0-3 ml 1 m-NaOH and assayed for 35S and protein (Lowry et al., 1951). Incorporation into TCA-insoluble material was linear for up to 40 min under all conditions. The uptake into TCA-soluble material was not decreased either by infection with SFV or by addition of Sendai virus. The uptake of [35S]methionine in full MEM was less than that in methionine-free MEM, but the effects of infection by SFV, exposure to Sendai virus, or addition of GppCH2p, on incorporation into protein were essentially the same under the two conditions; the effects were also the same when [3H]leucine, in leucine-free MEM or in full MEM, was used. The values quoted are the means of three or more determinations made between 5 and 40 min after exposure to [35S]methionine.

Analysis of guanine nucleotides. Washed cells were extracted with 0-3 ml of an ice-cold aqueous solution containing 10% TCA and 20% methanol; 20 nmol CMP was added to each plate as an internal standard. The extract was washed with water-saturated ether in order to remove TCA and the pH was adjusted to 7 with Tris base. The extract was centrifuged, and a sample of the supernatant analysed with a Waters high pressure liquid chromatography system using a 10 cm × 5 μm Hypersil-APS column and eluted with a gradient of 3 to 100 g/l KH2PO4 adjusted to pH 2-5. The cell pellet was used to assay protein as described above.

Cell-free protein synthesis. The maintenance medium, 4-5 h after infection of cells with SFV, or mock-infection, was replaced with ice-cold Dulbecco's phosphate-buffered saline lacking Ca2+ and Mg2+. Cells were scraped off the plate with a rubber policeman, spun (800 g for 10 min at 4°C) and extracts of the pellets prepared by methods previously described for Ehrlich ascites tumour cells (Pain et al., 1980), except that cell lysis was achieved using 0-25% Nonidet P40 (NP40) rather than homogenization. An energy regeneration system (1 mM-ATP, 5 mM-creatine phosphate, and 180 μg/ml creatine phosphokinase) was added to the undialysed cell extracts. The extracts were then incubated at 30°C for 30 min at final concentrations of 49-3 A260/ml (mock-infected) or 35-1 A260/ml (infected) with or without exogenous GTP (250 μM) under the conditions described by Pain et al. (1980); GppCH2p was added as indicated. Incorporation of radioactivity from [35S]methionine (50 μCi/ml) into protein was assayed in 20 μl samples as described by Pain et al. (1980).

RESULTS AND DISCUSSION

The proposals for non-specific increases in membrane permeability following virus infection of mammalian cells were based partly on the observation that certain paramyxoviruses cause just such a change (Pasternak & Micklem, 1973, 1974; Poste & Pasternak, 1978; Impraim et al., 1980) when fusing (Knutton, 1978) with susceptible cells. By exposing BHK cells to haemolytic Sendai virus (which, although haemolytic is not cytolytic: Pasternak & Micklem, 1973; Knutton et al., 1976; Poste & Pasternak, 1978; Wyke et al., 1980), we have confirmed that 'permeabilized' cells indeed take up more [3H]GppCH2p (Fig. 1), and as a result show an inhibition of methionine incorporation into protein (Table 1); similar results have been obtained with Lettre cells (Gray et al., 1983b). On the other hand, BHK cells infected with SFV for 4 h take up no more [3H]GppCH2p than uninfected cells (Fig. 1), even though methionine incorporation in infected cells is inhibited more strongly by GppCH2p at this time (Table 1). The lack of any difference in the uptake of [3H]GppCH2p cannot be accounted for by greater leakage from the infected cells during the washing procedure since cells treated with haemolytic Sendai virus which are leaky to nucleotides and other phosphorylated compounds (Pasternak & Micklem, 1973, 1974; Impraim et al., 1980), nevertheless retain more [3H]GppCH2p than untreated cells (Fig. 1). It might be argued that [3H]GppCH2p associated with cells is not inside cells but bound at the cell surface. This is also unlikely since in the presence of 2 mM-Ca2+, which is known to block membrane permeability changes (Pasternak & Micklem, 1974; Impraim et al., 1979, 1980), the uptake of GppCH2p (Fig. 1) and its action on Sendai virus-treated cells is inhibited (data not shown). In addition, α-methyl[14C]glucoside, which is not bound by animal cells but which enters by simple diffusion, follows a time-course of uptake similar to that of [3H]GppCH2p (Fig. 1). It is important to note that when cells are truly permeabilized, as by exposure to Sendai virus, uptake of [3H]GppCH2p is rapid, whereas in uninfected and SFV-infected cells it is slow (Fig. 1). Likewise, the sensitivity of SFV-infected cells to GppCH2p develops only over several hours post-infection (data not shown), whereas sensitivity to GppCH2p in Sendai virus-treated cells is induced within 5 min (Table 1). As observed by Carrasco (1981), protein synthesis in uninfected cells is somewhat inhibited by GppCH2p, especially at higher concentrations (Table 1).
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The above data show that the total amount of $^3$H taken up by cells exposed to [$^3$H]GppCH$_2$p is the same in infected as in uninfected cells. Because of the possibility of extracellular and intracellular hydrolysis of [$^3$H]GppCH$_2$p to [$^3$H]GMP and [$^3$H]guanosine, and because the uptake and incorporation of such hydrolysis products might complicate the interpretation of our results, this point was investigated.

First, extracellular hydrolysis of GppCH$_2$p is not appreciable during the time-course of the experiment. Fig. 2 shows that approximately 1% of added $^3$H is present as [$^3$H]GMP in the extracellular medium after 60 min incubation with uninfected or SFV-infected cells, which is not significantly different from the amount of [$^3$H]GMP present at the start of incubation; 99% of the radioactivity is as [$^3$H]GppCH$_2$p, with no detectable [$^3$H]guanosine under any condition. The data of Table 2 show that a 1% contamination with [$^3$H]GMP could at best account for 20% of the $^3$H actually taken up by cells (0.8 and 0.95 ct/min per 10$^4$ ct/min added per $\mu$g protein taken up by uninfected and infected cells respectively, during 60 min incubation in the presence of 20 $\mu$M-$^3$H]GMP; 4.4 and 4.8 ct/min per 10$^6$ ct/min added per $\mu$g protein taken up by uninfected and infected cells respectively, during 60 min incubation in the presence of 2 mM-$^3$H]GppCH$_2$p). Hence, > 80% of radioactivity that enters uninfected or infected cells does so in the form of [$^3$H]GppCH$_2$p. This stability of GppCH$_2$p in the incubation medium confirms a previously published report on encephalomyocarditis virus-infected L cells (Dawson et al., 1979); these authors also concluded that any increased uptake of GppCH$_2$p that might have taken place occurred too late for significant inhibition of virus production to be manifest, which probably accounted for the inefficacy of in vivo administered GppCH$_2$p on the survival of EMC-infected mice (Dawson et al., 1979). The data of Table 2 also show that the uptake of [$^3$H]GMP and [$^3$H]GTP and the subsequent intracellular distribution of $^3$H are the same in uninfected as in infected cells. Table 2 also shows that [$^3$H]GMP and [$^3$H]GTP enter cells (by whatever mechanism) 4 to 20 times more effectively than [$^3$H]GppCH$_2$p. 
Table 1. Effect of GppCH₂p* on [³⁵S]methionine incorporation† by uninfected and SFV-infected BHK cells

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Time (min)</th>
<th>Preincubation</th>
<th>Uninfected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Compounds present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>-</td>
<td>73</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2 mM-GppCH₂p</td>
<td>65</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>-</td>
<td>149</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Sendai virus</td>
<td>148</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.2 mM-GppCH₂p</td>
<td>151</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+ Sendai virus</td>
<td>125</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2 mM-GppCH₂p</td>
<td>142</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+ Sendai virus</td>
<td>45</td>
<td>14</td>
</tr>
<tr>
<td>3‡</td>
<td>5</td>
<td>-</td>
<td>177</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Sendai virus</td>
<td>117</td>
<td>ND§</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2 mM-GppCH₂p</td>
<td>152</td>
<td>42</td>
</tr>
<tr>
<td></td>
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<td>+ Sendai virus</td>
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</tr>
<tr>
<td></td>
<td>90</td>
<td>-; then 5 min with Sendai virus</td>
<td>178</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2 mM-GppCH₂p</td>
<td>114</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>+ Sendai virus</td>
<td>46</td>
<td>ND</td>
</tr>
</tbody>
</table>

* At 4 h post-infection cells were washed and exposed to GppCH₂p with or without Sendai virus (10⁻⁴ HAU/cell) at 37 °C for the times indicated.
† L-[³⁵S]Methionine was added as described in Methods and incubation continued for various times up to 40 min (additional GppCH₂p was added to maintain the concentrations shown). Cells were sampled as described in Methods.
‡ In experiment 3 some plates were incubated for 90 min with or without GppCH₂p and then exposed to Sendai virus for 5 min.
§ ND, Not determined.

Second, although intracellular hydrolysis of [³H]GppCH₂p does occur, it is approximately the same in uninfected and infected cells, and as can be seen from Fig. 2 some 14 to 18% of ³H in extracts of uninfected and infected cells is still present as GppCH₂p even after 60 min incubation.

Since GppCH₂p appears to enter infected and uninfected cells equally (Fig. 1 and Table 2), the question arises as to why GppCH₂p is a more effective inhibitor of protein synthesis in infected cells. Several possibilities exist. An increased Na⁺ concentration in infected cells has been postulated to act synergistically with inhibitors of protein synthesis (Alonso & Carrasco, 1980, 1981). This is unlikely in the case of GppCH₂p since nigericin-treated BHK cells, in which intracellular Na⁺ is increased sixfold, are no more susceptible to the action of the nucleotide analogue than are untreated cells (Gray et al., 1983b); in any case, an increase in intracellular Na⁺ does not appear to be responsible for the decreased host protein synthesis in virus-infected cells (Norrie et al., 1982; Pasternak et al., 1982; Schaeffer et al., 1982). On the other hand, there does appear to be a correlation between the onset of GppCH₂p sensitivity and the inhibition of host protein synthesis in infected cells (Lacal et al., 1980; M. A. Gray et al., unpublished observations).

The data in Table 3 provide a more likely explanation for the differential effects of GppCH₂p. We found that the levels of GTP and the GTP:GDP ratio, although somewhat variable between experiments, fall consistently and significantly in SFV-infected BHK cells, as observed by Whitehead et al. (1981). Either of these changes could be responsible for the overall inhibition of translation during infection, since GTP hydrolysis is an essential requirement for initiation,
Permeability of SFV-infected cells

Table 2. Uptake of \(^{3}H\)-labelled guanine nucleotides by uninfected and SFV-infected BHK cells*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concen.</th>
<th>Uninfected</th>
<th>Infected</th>
<th>Uninfected</th>
<th>Infected</th>
<th>Uninfected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{3}H)GppCH(_{2})p</td>
<td>2 mM</td>
<td>1.8</td>
<td>2.1</td>
<td>2.6</td>
<td>2.7</td>
<td>4.4</td>
<td>4.8</td>
</tr>
<tr>
<td>(^{3}H)GMP</td>
<td>20 (\mu)M</td>
<td>36.1</td>
<td>36.1</td>
<td>43.5</td>
<td>59.4</td>
<td>79.6</td>
<td>95.5</td>
</tr>
<tr>
<td>(^{3}H)GTP</td>
<td>20 (\mu)M</td>
<td>9.3</td>
<td>9.0</td>
<td>11.9</td>
<td>13.0</td>
<td>21.2</td>
<td>22.0</td>
</tr>
<tr>
<td>(^{3}H)GTP</td>
<td>2 mM</td>
<td>9.8</td>
<td>10.0</td>
<td>9.5</td>
<td>10.4</td>
<td>19.3</td>
<td>20.4</td>
</tr>
</tbody>
</table>

* At 4 h post-infection cells were washed and exposed to labelled nucleotide at 37 °C for 1 h, washed and sampled as described in Methods. Protein synthesis (in the absence of GppCH\(_{2}\)p) in infected cells, assessed by \(^{35}\)S methionine incorporation, was 30% of that in uninfected cells.

† Note that an uptake of 4 ct/min per 10\(^{6}\) ct/min of 2 mM-GppCH\(_{2}\)p added corresponds to an uptake of 4 pmol, an uptake of 80 ct/min per 10\(^{6}\) ct/min of 20 \(\mu\)M-GMP added corresponds to an uptake of 0.8 pmol, an uptake of 20 ct/min per 10\(^{6}\) ct/min of 20 \(\mu\)M-GTP added corresponds to an uptake of 0.2 pmol and an uptake of 20 ct/min per 10\(^{6}\) ct/min of 2 mM-GTP added corresponds to an uptake of 20 pmol.

Table 3. Guanine nucleotide content of BHK cells*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Time of sampling (h post-infection)</th>
<th>Uninfected cells</th>
<th>Infected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GDP (nmol/mg protein)</td>
<td>GTP (nmol/mg protein)</td>
</tr>
<tr>
<td>1</td>
<td>6.5</td>
<td>1.1</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>0.45</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>2.3</td>
<td>0.64</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.29</td>
<td>2.6</td>
</tr>
<tr>
<td>4</td>
<td>4.7</td>
<td>0.40</td>
<td>2.7</td>
</tr>
<tr>
<td>6</td>
<td>6.0</td>
<td>0.34</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* At various times post-infection cells were washed, extracted and nucleotides analysed as described in Methods. The values shown are means of duplicate analyses and were derived by integrating the area under the respective peaks and calculating the amount relative to the CMP standard.

elongation and termination of polypeptide chain synthesis (Pain & Clemens, 1980). Furthermore, the rate of initiation is particularly sensitive to the GTP : GDP ratio, through modulation of the activity of initiation factor eIF-2 (Walton & Gill, 1976). Since GppCH\(_{2}\)p is an analogue of GTP it is not unreasonable to suppose that the GppCH\(_{2}\)p : GDP ratio in cells would influence the rate of protein synthesis. That it does so in vitro is shown in Fig. 3. When protein synthesis is assayed in extracts from uninfected and SFV-infected BHK cells, in the presence or absence of exogenous GTP, GppCH\(_{2}\)p inhibits the completion of polysomal nascent polypeptide chains in a dose-dependent fashion. The concentrations which give 50% inhibition of \(^{35}\)S methionine incorporation are 6 \(\mu\)M and 4.5 \(\mu\)M in mock-infected and infected extracts respectively, when translation is dependent on endogenous GTP. The corresponding concentrations for 50% inhibition are 45 \(\mu\)M and 18.5 \(\mu\)M respectively, when 250 \(\mu\)M-GTP is added (Fig. 3). Clearly the amount of GTP present can determine the extent of inhibition by the analogue, although whether the two- to threefold difference in sensitivity between the two types of extract with added GTP reflects the difference in sensitivity of the intact mock-infected and SFV-infected cells to GppCH\(_{2}\)p cannot be decided on these data alone. It may be noted that an intracellular GTP content of 2 to 4 nmol/mg protein (mock-infected) or 1 to 2 nmol/mg protein (SFV-infected) (Table 3) corresponds to a concentration of approx. 0.4 to 0.8 mM and 0.2 to 0.4 mM respectively; an uptake of 2 to 4 nmol/mg protein of \(^{3}H\)GppCH\(_{2}\)p (2 to 4 ct/min per 10\(^{6}\) ct/min of 2 mM-GppCH\(_{2}\)p added per \(\mu\)g protein in 60 min; Fig. 1 and Table 2) would correspond to an intracellular concentration of approx. 0.4 to 0.8 mM. This is clearly an upper value, since much of the intracellular \(^{3}H\)GppCH\(_{2}\)p is metabolized to \(^{3}H\)GMP, which is then
For legend, see opposite.
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Fig. 2. Analysis of hydrolysis products of [3H]GppCH2p. Samples of [3H]GppCH2p after an initial purification and before dilution with unlabelled GppCH2p, or of 2 mM-[3H]GppCH2p-containing medium after incubating for 60 min at 37 °C with BHK cells which had either been infected with SFV or mock-infected 4 h previously, or of Triton X-100 extracts prepared from similar cells at the end of the 60 min incubations, were diluted and passed down a DE52 DEAE-cellulose column in the presence of unlabelled guanosine (G, 0.18 µmol), GMP (0.28 µmol), GDP (0.23 µmol) and GTP (0.19 µmol) as markers, then washed and eluted with a linear gradient of 0 to 0.3 M-NaCl in 10 mM-Tris, pH 8.4. ▲, Absorbance at 254 nm; ●, 3H content; ■, conductivity. (a) [3H]GppCH2p; (b) 3H in medium after incubation with uninfected cells; (c) 3H in medium after incubation with infected cells; (d) 3H in cell extract after incubation with uninfected cells; (e) 3H in cell extract after incubation with infected cells. (b, c) Note the 'shoulder' of u.v.-absorbing material between GDP and GTP due to GppCH2p. The areas under the [3H]GMP and [3H]GppCH2p peaks respectively, expressed as a percentage of the total radioactivity eluted from the column, were approx. as follows: (a to c) 1% and 99%; (d) 72% and 14%; (e) 72% and 18%.
Fig. 3. Inhibition of cell-free protein synthesis by GppCH₂p. Cell-free extracts were prepared from BHK cells at 4-5 h post-infection and protein synthesis in the presence (---) or absence (----) of added GTP (250 μM) measured as described in Methods; GppCH₂p was present at the concentrations indicated. The point at which protein synthesis is inhibited half maximally by GppCH₂p is indicated by an arrow on each concentration curve. (a) Mock-infected cell extract; (b) SFV-infected cell extract.

incorporated into TCA-insoluble material, presumably via conversion to [³H]GTP. At the end of 60 min the intracellular concentration of intact GppCH₂p is probably around 40 to 80 μM, which is in the range at which protein synthesis in vitro is very sensitive to variations in GTP concentration (Fig. 3). Such calculations should, of course, be taken as no more than an indication that the amount of GppCH₂p in cells is, within an order of magnitude, compatible with its effect on protein synthesis in uninfected and infected cells. There is, moreover, the possibility that methylene diphosphate (pCH₂p), formed by hydrolysis of GppCH₂p to GMP, might itself inhibit protein synthesis.

While these arguments account reasonably well for the effect of GppCH₂p on virus-infected versus uninfected cells, they are clearly not applicable to the differential actions of compounds such as hygromycin B and other antibiotics (Contreras & Carrasco, 1979; Lacal et al., 1980). Yet an alteration of GTP:GDP ratio is not the only parameter relevant to protein synthesis that changes in virus-infected cells. Several alterations in regulation of the translational machinery can occur following infection. These may result from: (i) competition between viral and cellular messenger RNAs (Lodish & Porter, 1980); (ii) inactivation of initiation factors required for translation of host but not virus mRNAs (Centrella & Lucas-Lenard, 1982; Lee & Sonenberg,
unpublished observations). We therefore question the validity of the hypothesis (Carrasco & Pasternak, 1976; Lacal et al., 1980) by definition inhibitors of some stage of protein synthesis, it seems not unreasonable that these compounds, like GppCH₂P, may be more inhibitory to infected cells because of qualitative and quantitative changes in the protein synthesis machinery itself, and not because of any alteration in surface membrane permeability. In confirmation of this supposition, experiments with cells infected with non-haemolytic Sendai virus, measles virus, influenza virus, vesicular stomatitis virus or herpes virus have likewise failed to reveal any changes in membrane permeability to normally impermeant compounds at times when synthesis of viral proteins is fully developed and synthesis of host proteins diminished (Pasternak et al., 1982; Gray et al., 1983a; Foster et al., 1983; M. James & C. A. Pasternak, unpublished observations). We therefore question the validity of the hypothesis (Carrasco & Smith, 1976; Carrasco, 1977, 1978) that changes in the permeability of the plasma membrane of virus-infected cells have a major role to play in the regulation of protein synthesis.

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REFERENCES


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