Suppression of the Synthesis of Cellular Macromolecules by Herpes Simplex Virus

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(Accepted 2 May 1978)

SUMMARY

Synthesis of cellular protein was substantially inhibited within 1 h of infection with herpes simplex virus, type 2, strain G (HSV-2). The inhibition also occurred, although no virus-specific protein synthesis was detected, after infection with u.v. irradiated virus and in cytoplasts that had been enucleated before infection. The inhibitory activity could not be distinguished from infectivity by dilution, sedimentation or reaction with γ-globulin. HSV-2 also suppressed the synthesis of Sendai virus proteins, but not those specified by HSV-1.

Host protein synthesis was no more sensitive than virus protein synthesis to an increased concentration of NaCl in the medium, nor could the suppression of host synthesis be prevented by adding excess MgCl₂ to the medium or by omitting CaCl₂ or NaCl. It was accompanied by the breakdown of polyribosomes, which also occurred in the presence of cycloheximide but not at 4 °C. The breakdown yielded ribosomes that were sensitive to a high salt concentration, unlike those produced by treatment of polyribosomes with RNase. The synthesis of cellular DNA and RNA was also inhibited following infection with u.v.-inactivated virus.

It is concluded that the suppression of host protein synthesis (and probably also of host DNA and RNA synthesis) is caused by a constituent of the infecting virus particles. The mechanism is obscure but probably does not depend on the leakage out of the cell of Mg²⁺ or into the cell of Ca²⁺ or Na⁺ ions, nor on the specific inhibition of initiation of host polypeptide chains, nor on RNase-like attack on host polyribosomes.

INTRODUCTION

During the first 2 h after infection with herpes simplex virus, type 1 (HSV-1), the polyribosomes of the host cell are dispersed and host protein synthesis declines (Sydiskis & Roizman, 1966) and is superseded by the synthesis of virus proteins. The breakdown of polyribosomes did not occur if the virus was heavily irradiated with u.v. light before infecting cells (Sydiskis & Roizman, 1967), suggesting that a virus-specified inhibitor had to be made after infection. The polyribosomes of non-permissive cells were also disaggregated after infection. If actinomycin D or p-fluorophenylalanine was added before and during infection, polyribosome breakdown was prevented in non-permissive but not in permissive cells. It was surmised that in permissive cells a small amount of a very efficient inhibitor was probably made even in the presence of the drugs. It has also been reported (Ben-Porat et al. 1971) that the breakdown of host polyribosomes following infection with pseudorabies virus requires the synthesis of new proteins.
Host cell protein synthesis is even more rapidly suppressed after infection with certain strains of type 2 herpes virus (HSV-2; Powell & Courtney, 1975; Pereira et al. 1977). For this reason we have used HSV-2 in investigating the effect and have come to the conclusion that it is probably caused by the infecting virus particles themselves.

The rates of synthesis of cellular DNA (Kaplan & Ben-Porat, 1963) and RNA (Roizman et al. 1965) also decline during the first hours after infection with herpes virus. Our experiments suggest that these effects, similarly, may be caused by some constituent of the infecting virus particles.

**METHODS**

**Cells.** Monolayers of African green monkey kidney (Vero) cells were grown in 25 cm² tissue culture flasks (Nunc, Denmark) in Dulbecco’s modified Eagle’s medium with 10% calf serum.

**Virus.** The ‘F’ strain of HSV-1 and the ‘G’ strain of HSV-2 (Ejercito et al. 1968) were grown by low multiplicity passage in Vero cells. Unfractionated cell lysates with a titre of 5 to 20 × 10⁸ p.f.u./ml were stored at −70 °C. Plaque assays were done with Vero cell monolayers in the presence of 0.2% human γ-globulin.

Sendai virus grown in embryonated hens’ eggs was collected from the allantoic fluid by centrifugation and suspended at a concentration of 10⁴ H.A.U./ml in Hank’s saline. It was kindly provided by Mrs Mary Williams. Earlier estimates have indicated a ratio of approx. 10⁶ p.f.u./H.A.U.

**Infection and labelling.** Confluent cultures of cells were infected with 1.5 ml of growth medium containing 10 to 20 p.f.u. of HSV-2/cell unless otherwise stated. After 20 min at room temperature the inoculum was replaced with 2 ml of growth medium and placed in an incubator at 37 °C (at zero time).

For labelling of polypeptides the medium was replaced by 1 ml of medium lacking amino acids with 1% dialysed foetal calf serum and 1 μCi of U-14C-protein hydrolysate (Radiochemical Centre, Amersham, 56 mCi/mAtom) per ml.

**Electrophoresis.** Samples of whole cell lysates were subjected to electrophoresis in polyacrylamide gradient gels and autoradiograms prepared as described before (Fenwick et al. 1978).

**Irradiation.** Stock virus was diluted 1/30 in PBS containing 1% glucose and exposed (2 ml in a 9 cm diam Petri dish) to a 15 W germicidal lamp (Sylvania, G15T8). The intensity of the radiation at 254 nm was measured with a Latarjet u.v.-meter. The irradiated virus was used to infect a flask of cells at a multiplicity of 10 original p.f.u./cell. A radiation dose of 200 ergs/mm² reduced the infectivity of the virus by 90%.

**Enucleation.** Vero cells growing in plastic Leighton tubes (Nunc) were centrifuged in the presence of cytochalasin B as described earlier (Fenwick & Roizman, 1977). It was estimated from fixed and stained preparations that 2.5% of the surviving cells contained nuclei.

**Gradient centrifugation.** Polyribosomes were analysed by preparing lysates of 4 × 10⁶ cells in 4 mM-Mg acetate, 40 mM-Na phosphate (pH 7.2), 0.2% Na deoxycholate, and centrifuging on gradients of 30 to 8% (w/v) sucrose in 4 mM-Mg acetate, 40 mM-Na phosphate for 30 min at 45000 rev/min in a Beckman rotor SW 50.1 at 10 °C. Absorbance at 254 nm was monitored with an ISCO density gradient fractionator.

RNA was analysed by lysing 2 × 10⁶ cells in 0.1 M-NaCl, 5 mM-EDTA, 1% SDS and centrifuging on sucrose gradients containing the same concentrations of NaCl, EDTA and SDS for 100 min at 45000 rev/min in the SW 50.1 rotor at 20 °C. Fractions were collected on filter papers and washed with trichloroacetic acid solution and ethanol and dried before counting.
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Mol. wt. 17

Fig. 1. Effect of u.v.-irradiated virus on cellular protein synthesis. A series of cell cultures were infected with HSV-2 that had been previously irradiated with the doses indicated (ergs/mm²) of u.v. light. UN, Uninfected. ¹⁴C-amino acids (1 μCi/ml) were added at 2 h after infection and the cells were lysed at 3 h. Lysates were subjected to electrophoresis and an autoradiogram prepared. Approximate mol. wt. (× 10⁸) are shown on the left (Fenwick et al. 1978). ICP, Infected cell polypeptide (Pereira et al. 1977).

(Fenwick, 1971). The positions of the large and small ribosomal RNA were determined by monitoring absorbance at 254 nm during fractionation.

DNA was extracted from 2 × 10⁶ cells by shaking with phenol in the presence of 2 % SDS, 0·1 M-NaCl, 5 mM-EDTA. After precipitation from the aqueous phase with an equal volume of ethanol the DNA was washed with 50 % ethanol and dissolved in 0·3 ml of 0·1 M-NaCl, 5 mM-EDTA. It was then layered on 2 ml of 60 %, w/w, CsCl solution and centrifuged for 24 h at 33000 rev/min in the SW 50.1 rotor at 20 °C. Two-drop fractions were collected from the DNA-containing region of the gradient and acid-precipitable radioactivity measured as above.

Materials. U-¹⁴C-protein hydrolysate, 5-³H-uridine and methyl-³H-thymidine were obtained from the Radiochemical Centre, Amersham; cycloheximide (actidione) from Calbiochem, actinomycin D from B.D.H. and human γ-globulin from Sigma.
RESULTS

Infection with u.v.-irradiated virus

In a preliminary experiment the incorporation of $^{14}$C-amino acids into acid-insoluble material was measured in HSV-2-infected and uninfected cultures of Vero cells incubated from zero time in the presence of actinomycin D (1 μg/ml). The rate of protein synthesis began to decline in the infected cells at 30 min and fell to 20% of the control rate by 60 min after infection.

Samples of virus were exposed to u.v. light and then used to infect monolayers of cells. Polypeptides were labelled from 2 to 3 h later and separated by electrophoresis. The autoradiogram prepared from the dried polyacrylamide gel (Fig. 1) shows, first, the range of polypeptides that were made in uninfected cells and in cells infected with unirradiated virus. Most of the host polypeptides that were being made (channel 1) were no longer made 2 to 3 h after infection and production of a number of new polypeptides had started (channel 2). If the virus was irradiated with 500 ergs/mm$^2$ of u.v. light no virus-specific polypeptide synthesis was detectable at 2 to 3 h but the inhibition of host synthesis was as great as with intact virus (channel 3). After higher levels of irradiation, between 2000 and 8000 ergs/mm$^2$ (channels 5 to 7), the ability of the virus to inhibit host protein synthesis progressively declined.
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Infection of enucleated cells

Anucleate cytoplasts continue to make proteins for several hours after removal of the nucleus and can be infected by a number of cytoplasmic viruses (Follett et al. 1975). Herpesvirus does not grow in cytoplasts because the nucleus is the site of production of virus RNA and DNA as well as assembly of the nucleocapsids. We examined the proteins made in cytoplasts and the effect of infection on their synthesis. Polypeptides labelled from 1 to 1.75 or 2 to 2.75 h after infection or mock-infection were analysed by electrophoresis and autoradiography. The autoradiogram (Fig. 2) shows that the rate of protein synthesis remained steady in uninfected cytoplasts over the period of the experiment but declined rapidly after infection with HSV-2 although no labelled virus-specific polypeptides (such as those in Fig. 1, channel 2) could be detected.

Correlation between infectivity and inhibition of host protein synthesis

The above experiments suggested that the suppression of host protein synthesis was caused by an inhibitor present in the inoculum, which was a crude lysate of infected cells. Three further experiments were done to see whether the inhibitor could be distinguished from infectious virus particles. In the first, the multiplicity of infection (m.o.i.) was reduced progressively by diluting the inoculum. Actinomycin D (1 µg/ml) was present during the adsorption and throughout to inhibit the synthesis of virus mRNA. 14C-amino acids were added for 30 min starting at 1, 2 or 3 h after infection. Analysis of the lysates (Fig. 3) shows
Table 1. Sedimentation of infectivity and inhibitory activity

<table>
<thead>
<tr>
<th>Centrifugation (g for 15 min)</th>
<th>p.f.u./ml in supernatant</th>
<th>p.f.u./cell</th>
<th>Uninfected cells (%)*</th>
<th>Rate of protein synthesis (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.4 \times 10^7$</td>
<td>6</td>
<td>0.2</td>
<td>11</td>
</tr>
<tr>
<td>600</td>
<td>$9.1 \times 10^6$</td>
<td>4</td>
<td>2</td>
<td>12</td>
</tr>
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<td>$3.4 \times 10^6$</td>
<td>1.4</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>10000</td>
<td>$1.3 \times 10^6$</td>
<td>0.5</td>
<td>61</td>
<td>43</td>
</tr>
<tr>
<td>55000</td>
<td>$5.3 \times 10^5$</td>
<td>0.2</td>
<td>82</td>
<td>92</td>
</tr>
</tbody>
</table>

* Assuming Poisson distribution of all of the p.f.u. remaining in the supernatant fluid after centrifugation.
† Acid-precipitable radioactivity expressed as % of that of a lysate of mock-infected cells.

that with a m.o.i. of 20 p.f.u./cell, host protein synthesis was strongly inhibited within 1 h and no virus protein synthesis was detected up to 3·5 h (channels 2 to 4). Comparison of the two mock-infected samples (channels 1 and 10) shows that actinomycin did not inhibit host protein synthesis. As the multiplicity (M) was reduced below 4 p.f.u./cell the inhibition was less marked. If all of the virus particles were adsorbed with a Poisson distribution, the following proportions of the cells would escape infection: at $M = 4$, 2% would escape; at $M = 1$, 37%; at $M = 0.5$, 61%; and at $M = 0.2$, 82%. The relative rates of protein synthesis were estimated from the total area under the curve obtained by scanning each channel of the autoradiogram with a densitometer. The values obtained were: at $M = 4$, 13% of the rate in the uninfected control; at $M = 1$, 36%; at $M = 0.5$, 56%; and at $M = 0.2$, 74%. These are very similar to the proportions of the cells that would have remained uninfected at each multiplicity, indicating that the inhibitor of cellular protein synthesis is initiated either by the infectious virus particles or by some other factor whose minimum effective level is reached at approximately the same dilution of the inoculum.

In the second experiment samples of crude virus were centrifuged at different speeds and the supernatant fluid was assayed for infectivity and for ability to suppress cellular protein synthesis in the presence of actinomycin D, as before. The results (Table 1) show a fair correlation between these two activities. The overall rates of host protein synthesis were determined by labelling with $^{14}$C-amino acids from 2 to 3 h after infection and measuring acid-precipitable radioactivity in each lysate. It is clear that the suppression was not mediated by a soluble factor that would have remained in the supernatant after centrifuging at 55000 g for 15 min.

Thirdly, virus was incubated for 10 min at 37 °C with a series of increasing concentrations of human γ-globulin and then used to infect monolayers of cells. Proteins were labelled between 2 and 3 h after infection and subjected to electrophoresis. With 0·5% γ-globulin, no synthesis of virus proteins was detectable and host protein synthesis was not inhibited. Lower concentrations permitted intermediate levels of both processes. Other evidence (Morgan et al. 1968) indicates that antiviral antibody interferes with attachment and penetration of herpes virus particles.

**Mixed infection**

Recombinants between HSV-1 and HSV-2 have been isolated and at least some mRNA of each type must be translated in a single cell infected by such a virus particle. However, as HSV-2 is more efficient than HSV-1 in suppressing host protein synthesis, cells were infected with a mixture of both types in order to see whether HSV-2 affected the production of HSV-1 polypeptides. Cultures of cells were labelled with $^{14}$C-amino acids from 3 to 4 h after infection
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Fig. 4. Autoradiogram of polypeptides labelled after mixed infection with HSV-1 and HSV-2. Cultures of cells were mock-infected (UN) or infected with 10 p.f.u./cell of HSV-2 (channel 2) or HSV-1 (channel 3) or both (channel 4). Polypeptides were labelled from 3 to 4 h after infection and separated by electrophoresis. ICP, Infected cell polypeptide.

with 10 p.f.u./cell of HSV-1 or HSV-2 or both types together. The autoradiogram of electrophoretically separated polypeptides (Fig. 4) shows that HSV-1 or HSV-2 alone reduced host protein synthesis and induced the synthesis of type-specific virus polypeptides (channels 2 and 3). In cells infected with both types there was no reduction in the rate of synthesis of HSV-1 polypeptides. In another experiment cells were infected with u.v.-irradiated HSV-2 and superinfected at 1 h with HSV-1. HSV-1 polypeptides were made normally from 3 to 4 h. It can be concluded that the suppression mechanism of HSV-2 affects host protein synthesis only and not that of another (related) virus.

In order to discover the effect of HSV-2 on the synthesis of proteins of an unrelated virus, cells were infected with Sendai virus, incubated at 37 °C and superinfected 7 h later with HSV-2 or with u.v. inactivated HSV-2. The autoradiogram in Fig. 5 shows (a) that a number of virus-specific polypeptides were made in cells infected with Sendai virus (channel 4), (b) that Sendai did not inhibit cellular protein synthesis, as reported by Lamb et al. (1976), (c) that HSV-2-specific polypeptides can be made in cells pre-infected with Sendai virus (channel 5) and (d) that HSV-2 or u.v.-inactivated HSV-2 is able to suppress substantially the synthesis of Sendai polypeptides (channels 5 and 6). Evidently the suppression mechanism discriminates between herpes virus and paramyxovirus protein synthesis.
Suppression of Sendai virus protein synthesis by superinfecting with HSV-2. Cells were mock-infected or infected with Sendai virus (4 × 10⁴ HAU/cell) and superinfected 7 h later with HSV-2 (channels 2 and 5) or u.v.-irradiated HSV-2 (1000 ergs/mm²; channels 3 and 6). ¹⁴C-amino acids were added after a further 2 h for 1 h and labelled polypeptides were detected by autoradiography after electrophoresis. Identification of Sendai-specific polypeptides is based on the report of Lamb et al. (1976).

Effect of hypertonic medium

When cells are incubated in hypertonic media, protein synthesis quickly stops and polyribosomes are disaggregated. The synthesis of virus proteins in cells infected with poliovirus, however, is appreciably less sensitive to such treatment than is host protein synthesis (Nuss et al. 1975). In an experiment to compare the sensitivities of cell and virus protein synthesis in cells infected with herpesvirus, we incubated infected or mock-infected cells in media to which increasing amounts of NaCl had been added. Polypeptides were labelled and separated by electrophoresis. The autoradiogram (Fig. 6) shows that host and virus protein synthesis were similarly affected. Virus protein synthesis was not less sensitive than host and may have been slightly more sensitive to 0·15 M-added NaCl. Both were substantially inhibited in the presence of 0·2 M-extra NaCl.

It has been reported (Robbins et al. 1970) that the effect on polyribosomes of incubating cells in hypertonic medium can be counteracted if the concentration of Mg²⁺ ions in the medium is also raised, to more than ten times the normal level. We tested the effect of a high Mg²⁺ concentration on the suppression of host protein synthesis, measuring incorporation...
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Fig. 6. Inhibition of host and virus protein synthesis by increasing the concentration of NaCl. Cells were mock-infected (channels 1 to 6) or infected with HSV-2 (channels 7 to 12). At 3 h after infection the medium was replaced by labelling medium containing additional NaCl at the concentrations indicated. Ten min later $^{14}$C-amino acids were added (1 µCi/ml) and incubation continued for 1 h. Lysates were analysed as before.

of $^{14}$C-amino acids between 2 and 2.5 h after infection or mock-infection in the presence of actinomycin D. With the normal concentration (0.8 mM) of Mg$^{2+}$ in the medium, 84% inhibition of incorporation of radioactive amino acids was observed after infection. With additional 10 mM- or 20 mM-Mg$^{2+}$, 82 and 77% inhibition occurred. In another experiment cells were pre-incubated for 1 h in abnormal ionic environments before infection and subsequent labelling (from 2 to 4 h post infection in the presence of actinomycin) in the same media. In normal medium 95% inhibition of protein synthesis was observed and with 40 mM-Mg$^{2+}$, 90%. In medium from which NaCl was omitted and replaced by 6% (w/v) sucrose 90% inhibition occurred. In a further experiment, cells were washed, infected and incubated in medium lacking Ca$^{2+}$ and containing 1% dialysed serum. The rate of protein synthesis between 2 and 3 h after infection was 10% of that in uninfected cells in the same medium. Thus it was not found possible, by raising the Mg$^{2+}$ or by lowering the Na$^+$ or Ca$^{2+}$ concentration in the medium, to prevent host protein synthesis being turned off in infected cells.

Effects of cycloheximide and low temperature

Polyribosomes are degraded if initiation of protein synthesis is prevented but elongation and termination of polypeptide chains are permitted. Single ribosomes are then released and accumulate. This mechanism has been proposed to explain the action of hypertonic medium and of sodium fluoride in causing the breakdown of polyribosomes in intact cells (Colombo et al. 1968; Saborio et al. 1974). Cycloheximide inhibits the formation of peptide bonds and thus prevents the breakdown of polyribosomes in the presence of inhibitors of initiation,
such as sodium fluoride (Felicitti et al. 1966), and we have confirmed this protective effect of cycloheximide using Vero cells treated with NaF (0·015 M for 45 min at 37 °C) in the presence or absence of cycloheximide (50 #g/ml added 5 min before NaF).

The effects of cycloheximide and of low temperature on the breakdown of polyribosomes by HSV-2 were next examined. Monolayers of cells were infected or mock-infected at 20 °C and then incubated at 37 °C or at 4 °C for 2 h in the presence or absence of cycloheximide. Lysates were then centrifuged in sucrose gradients and ribosomes and polyribosomes detected by monitoring and recording absorbance at 254 nm. The resulting tracings are shown in Fig. 7. In uninfected cells treated with cycloheximide or kept at 4 °C for 2 h (Fig. 7a, c) there was some loss of the larger polyribosomes, but in infected cycloheximide-treated cells all polyribosomes disappeared (Fig. 7b). On the contrary, if cells were transferred to a refrigerator at 4 °C immediately after infection at 20 °C the pattern of polyribosomes (Fig. 7d) was the same as in similarly treated mock-infected cells (Fig. 7e), whereas in infected cells incubated at 37 °C, of course, no polyribosomes remained (Fig. 7e). Thus the breakdown of polyribosomes following infection with HSV-2 is a temperature-dependent process that does not require continuing protein synthesis and is therefore probably not caused by inhibition of the initiation of host polypeptides.

**Effect of RNase**

Polyribosomes may be disaggregated by treating them *in vitro* with RNase, which probably breaks the mRNA linking them and yields single ribosomes bearing fragments of mRNA and possibly nascent polypeptide chains. Such breakdown products are more stable than the single ribosomes normally found in cells or those released by treating cells with puromycin. The latter can be dissociated into subunits by exposure to a high ionic strength (0·5 M-Na+, 0·05 M-Mg²⁺ (Zylber & Penman, 1970)). Fig. 8 shows the distribution of ribosomes and subunits after centrifugation in sucrose gradients containing 0·5 M-Na⁺ and
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Fig. 8. Dissociation of ribosomes at high ionic strength. Lysates of cells were centrifuged through sucrose gradients containing 0.05 M-NaCl, 0.05 M-Mg acetate, 0.01 M-tris (pH 7) for 1.25 h at 45,000 rev/min at 10 °C before monitoring absorbance at 254 nm. (a) Untreated cells. (b) Cells incubated with puromycin (20 µg/ml) for 5 min at 37 °C. (c) Lysate of untreated cells exposed to RNase (5 µg/ml, 2 min at 20 °C). (d) Cells infected 2 h previously with HSV-2. S, L, Small, large ribosomal subunits. R, Single ribosomes.

0.05 M-Mg²⁺. Gradients a to c are controls confirming the observations of Zylber & Penman (1970). The single ribosomes from untreated cells dissociated into small and large subunits (Fig. 8a). When the cells were treated with puromycin (20 µg/ml for 5 min) single ribosomes were released from polyribosomes and these also dissociated into subunits (Fig. 8b). In contrast, the ribosomes released by the action of RNase on polyribosomes in vitro were stable at high ionic strength (Fig. 8c). The ribosomes that accumulate in HSV-2-infected cells were found to be of the dissociable type (Fig. 8d), i.e. they resembled ribosomes that had been released by puromycin rather than by RNase.

Inhibition of host DNA synthesis

In order to determine whether the capacity of the virus to suppress host DNA synthesis (Kaplan & Ben-Porat, 1963) was sensitive to u.v. light, samples of virus were irradiated for different times and used to infect cultures of Vero cells. ³H-thymidine was added at 4 h and DNA was extracted and centrifuged in CsCl in order to separate cellular from virus DNA. The distribution of tritium in the gradients is shown in Fig. 9. In cells infected with untreated virus host DNA synthesis was inhibited by about 75% and a distinct peak of labelled virus DNA can be seen (Fig. 9b). Irradiated virus caused an even greater inhibition of cellular DNA synthesis although no new virus DNA was made (Fig. 9c, d, e). The effect was somewhat less after 8000 ergs/mm² (Fig. 9e) than after lower doses of irradiation. This is a similar result to that obtained in the experiment of Fig. 1 in which protein synthesis was measured.

Inhibition of host RNA synthesis

The rate of RNA synthesis also declines after infection with herpesvirus (Roizman et al. 1965). The experiment illustrated in Fig. 10 shows that u.v.-irradiated HSV-2 has the same capacity as infectious virus to inhibit host RNA synthesis. Sucrose gradient analysis of labelled RNA shows that the rate of ribosomal RNA synthesis in infected cells (Fig. 10b) was less than 50% of normal. In cells infected with irradiated virus no labelled small (18S) ribosomal RNA was detected (Fig. 10c), suggesting that the radioactivity in the 18S region
Fig. 9. Inhibition of cellular DNA synthesis by u.v.-irradiated virus. Cells were (a) mock-infected or (b) infected with HSV-2, or infected with virus that had been exposed to u.v. light of (c) 500 ergs/mm², (d) 2000 ergs/mm², or (e) 8000 ergs/mm². They were labelled with ³H-thymidine (4 µCi/ml) from 4 to 5 h after infection. DNA was extracted and centrifuged in CsCl solution. Fractions of the gradients were collected and the acid-precipitable radioactivity of each measured.

Fig. 10. Effect of u.v.-irradiated virus on cellular RNA synthesis. Cells were (a) mock-infected or (b) infected with HSV-2 or (c) with virus that had been irradiated with 1000 ergs/mm². They were labelled with ³H-uridine (1 µCi/ml) from 3 to 4·5 h after infection. Lysates of the cells were layered on sucrose gradients and centrifuged for 100 min at 45000 rev/min at 20 °C. Fractions were collected and acid-precipitable radioactivity measured. S, L, Small (18S), large (30S) ribosomal RNA.

of the gradient (and possibly elsewhere) in Fig. 10(b) may be due to virus mRNA. In both cases synthesis of the larger (45S) precursor RNA was about 70% of normal.

DISCUSSION

The experiments described here strongly suggest that the decline of cellular protein, DNA and RNA synthesis that follows infection with HSV-2 is caused by the infecting particles
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themselves and does not require the synthesis of new RNA or protein. Earlier reports that the suppression of host protein synthesis does depend on newly synthesized protein may possibly indicate that the inhibitory constituents of the virions of HSV-1 (Sydiskis & Roizman, 1967) and pseudorabies virus (Ben-Porat et al. 1971) are less active than those of HSV-2. Others have concluded that in cells infected with vaccinia (Esteban & Metz, 1973), vesicular stomatitis (Baxt & Bablanian, 1976) or Semliki Forest (Wengler & Wengler, 1976) viruses the synthesis of host protein is suppressed by a constituent of the infecting virus particles.

The breakdown of polyribosomes by HSV-2 occurred at 37 °C (but not at 4 °C) in the presence of cycloheximide and therefore does not require the formation of peptide bonds. In this respect it differs from the breakdown caused by inhibitors of initiation, such as NaF. It is not caused by RNase attack on host mRNA because the single ribosomes produced were dissociated into subunits at high salt concentration.

Varying degrees of suppression of host macromolecular synthesis are frequently observed after infection by viruses and it has been suggested (Carrasco, 1977) that there is a general mechanism involving increased permeability of the cell membrane with a consequent disturbance of the intracellular concentrations of Na⁺ or Mg²⁺ or Ca²⁺ ions (Durham, 1977). However, in cells infected with HSV-1 the synthesis of HSV-specific proteins can be inhibited by superinfecting several hours later with poliovirus (Saxton & Stevens, 1972). In this case it seems that poliovirus must employ a suppression mechanism that is different from that of herpesvirus.

Whatever the mechanism employed by HSV-2, it discriminates against the synthesis of Sendai virus proteins as well as those of the host cell. We have been unable to obtain evidence to support the increased permeability mechanism. The breakdown of host cell polyribosomes is probably not caused by increased intracellular Na⁺ concentration because it occurs in medium lacking NaCl. Furthermore hypertonic NaCl concentrations in the medium reduce both host and virus protein synthesis similarly, while the virus-mediated effect is of course specific for cell protein synthesis; and the virus effect, unlike the hypertonic NaCl effect, is not counteracted by raising the Mg²⁺ ion concentration, nor was it prevented by omitting Ca²⁺ from the medium. Nevertheless, the possibility that some ionic imbalance is involved remains attractive, particularly since such diverse cellular functions as protein, DNA and RNA synthesis are all progressively inhibited during the first hours after infection, although to different extents.

Other conceivable mechanisms for which we have no evidence involve an interaction between a virion component and cellular mRNA or ribosomes. Based on the absorbance of extracted ribosomal RNA we estimate that the number of ribosomes in a HeLa or Vero cell is of the order of 10⁶ (Maclean, 1965, estimated that there were about 5 × 10⁵ ribosomes in a yeast cell). Particle counts (S. Foote, unpublished data) have indicated a ratio of about 20 particles per p.f.u. in our HSV-2 preparations. Even if all of these are able to enter the cell, which is unlikely as many particles lack envelopes, the number of particles entering during infection with 5 p.f.u./cell would be about 100. Thus at least 10⁴ inhibitor molecules per particle would be needed to react with 10⁶ ribosomes. Several virion polypeptides occur in quantities of about 1000 molecules per particle (Heine et al. 1974). A direct permanent attachment to all ribosomes therefore seems improbable. Spermine and spermidine are present in relatively large amounts (about 40000 and 70000 molecules per virion (Gibson & Roizman, 1973)) but are probably derived from cellular pools that existed before infection (Gibson & Roizman, 1971) and are therefore unlikely to be effective specific cellular inhibitors, unless they are modified in some way during virus growth.
Halliburton & Timbury (1976) found that heavily irradiated HSV-2 was unable to suppress cellular DNA synthesis. However, with the lower doses of u.v. light used here, suppression of host protein, DNA and RNA synthesis occurred although no new virus proteins or DNA were made, suggesting that all three inhibitions are caused by virion components. Certain temperature-sensitive mutants of HSV-2 cannot suppress cellular DNA synthesis at the restrictive temperature (Halliburton & Timbury, 1976) although a few virus-specific antigens are made. It would be interesting to know whether they have a temperature-sensitive virion protein, and whether they are able to inhibit host protein synthesis at the restrictive temperature.

The work was supported in part by grants to Professor H. Harris from the Cancer Research Campaign.

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(Received 8 February 1978)