Characterization of Ribonucleotide Reductase Induction in BHK-21/C13 Syrian Hamster Cell Line Upon Infection by Herpes Simplex Virus (HSV)

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SUMMARY

Ribonucleotide reductase is an essential enzyme in mammalian DNA replication. In quiescent BHK-21/C13 cells exhibiting a low level of ribonucleotide reductase activity, infection with herpes simplex virus (HSV) resulted in the early induction of an altered ribonucleotide reductase. The extent of the induction was dependent upon the m.o.i. and could be diminished or prevented by u.v. treatment of the viral stock, or by inhibitors of mRNA synthesis or protein synthesis. The induction followed the same course of synthesis as viral thymidine kinase and DNA polymerase, and could thus be classified with them as a β polypeptide. These results suggested that the new activity was produced as a consequence of the virus genome expression. Comparisons of the properties of ribonucleotide reductase extracted from exponentially growing BHK-21/C13 cells showed that the HSV-induced enzyme differed from the cellular isozyme by its insensitivity to inhibition by dTTP, dATP or araATP and its resistance to high salt concentrations. On the other hand, the virus-induced enzyme and the cellular isozyme exhibited a similar sensitivity to hydroxyurea. Therefore, the reported inhibition of HSV DNA replication by hydroxyurea could be the result of inhibition of both HSV-induced and cellular reductase activities.

INTRODUCTION

The process of DNA synthesis in vivo and in vitro requires a continuous and balanced supply of the four deoxyribonucleoside triphosphates. The ribonucleotide diphosphate reductase (EC 1.17.4.1) which catalyses the reduction of ribonucleoside diphosphates is thought to be a rate-limiting enzyme in the production of these DNA precursors. It seems that the mammalian enzyme, like the bacterial one, can utilize the four substrates, ADP, CDP, GDP and UDP (Reichard, 1972). This enzymic activity is subject to complex allosteric control by a variety of positive and negative nucleotide effectors (Larsson & Reichard, 1966a, b; Moore & Hurlbert, 1966). Of concern in this report, the lethal effects of high concentrations of thymidine or deoxyadenosine to cells in culture have been explained by a starvation for dCTP produced by the feedback-inhibition of the CDP reduction by elevated intracellular concentration of dTTP or dATP (Reichard, 1972; Bjursell & Reichard, 1973). Furthermore, inhibitors of mammalian DNA synthesis such as hydroxyurea (HU) and guanazole, act primarily through inhibition of ribonucleotide reductase as demonstrated in in vitro assays (Kraffoff et al., 1968), and further substantiated by the finding that mammalian

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cell lines resistant to HU and guanazole exhibit a ribonucleotide reductase activity less sensitive to inhibition by these drugs (Lewis & Wright, 1974, 1978).

Evidence is accumulating that a ribonucleotide reductase activity with altered properties is induced in cells infected by herpes simplex virus (HSV) (Cohen, 1972; Langelier et al., 1978), equine herpes virus (EHV) (Cohen et al., 1977; Allen et al., 1978) and Epstein–Barr virus (EBV) (Henry et al., 1978). The HSV-induced ribonucleotide reductase differed from the cellular isozyme on the basis of its insensitivity to dTTP and dATP inhibition (Cohen, 1972; Langelier et al., 1978) and of its non-requirement for exogenous magnesium (Ponce de Leon et al., 1977). The simplest hypothesis to explain this induction is that the virus genome codes for a new enzyme which differs entirely or at least in part (e.g. a new regulatory subunit) from the cellular isozyme. This will only be demonstrated by genetic and biochemical studies correlating genetic mutation with structural modification of the enzymic polypeptide.

In the present work, we further studied the HSV-induced ribonucleotide reductase activity after infection of exponential or quiescent BHK-21/C13 cells, in order to obtain a better understanding of the role of the HSV genome expression in this induction and to search for inhibitors which might allow the selection of virus mutants. We found that the expression of the HSV genome was essential for the appearance of the new ribonucleotide reductase activity which could be distinguished from the cellular activity by including dTTP or dATP, or ammonium sulphate in the assay. However, this altered enzyme exhibited the same degree of sensitivity to HU as the cellular enzyme and, therefore, this drug could be useful in the selection of virus mutants.

METHODS

Virus strains. The F and B-12 strains of HSV-1 and the MS strain of HSV-2 were obtained from P. Sheldrick (Villejuif); the HG-52 strain of HSV-2 was provided by J. Subak-Sharpe (Glasgow). The procedures for virus production and titration have been previously described (Langelier et al., 1978). Suspensions of virus stock were u.v.-irradiated according to Munyon et al. (1971). Samples (4 ml) of suspension in 90 mm plastic Petri dishes were exposed for various intervals to u.v. irradiation (20 ergs/mm²/s) produced by a GE germicidal lamp (G8T5).

Cell culture, cell infection and cellular extract preparation. Conditions for growth of BHK-21/C13 cells and procedures for preparation of confluent or quiescent cells by culture in an Eagle's reinforced (ER) medium supplemented with 0.5% foetal calf serum (ER₀.₅) were the same as described in a previous report (Langelier et al., 1978). Cells were normally infected at an input multiplicity of 20 p.f.u./cell in 10 ml of ER medium containing 2% foetal calf serum (ER₂.₀) and the virus was allowed to adsorb at 37 °C for 1 h. The medium containing unattached viruses was then replaced by ER₂.₀ (or ER₀.₅ for quiescent cultures) and the incubation was continued at 37 °C as indicated. When it was necessary to block mRNA or protein synthesis, actinomycin D or cycloheximide were added at the appropriate time to cell culture medium to yield final concentrations of 10 and 50 μg/ml respectively (Honess & Roizman, 1974). Mock-infected or infected cells were washed and collected for enzyme extraction as previously described (Langelier et al., 1978).

Ribonucleotide reductase assay. The cell extract was passed through a small column of cation exchange resin (AGI-X8, Bio-Rad) previously equilibrated with 0.05 M tris-HCl pH 7.8, containing 1 mM-dithiothreitol (DTT) to remove nucleotides. The standard reaction mixture contained in a total 92.5 μl vol. was 5.4 mM-tris-HCl pH 7, 0.054 mM-FeCl₃, 4.3 mM-NaF, 6.5 mM-magnesium acetate, 2.7 mM-ATP, 5.4 mM-dithioerythritol (DTE), 0.054 mM-CDP plus 1 × 10⁵ ct/min [5-³H]CDP and enzyme extract (200 to 800 μg protein). After incubation for 60 min at 37 °C, the reaction was stopped by the addition of 46 μl of 4 M-perchloric acid. Nucleotides in the supernatant were converted to their monophosphate
form by boiling at 100 °C for 10 min. The mixture was neutralized with KOH and the precipitated material was eliminated by centrifugation. A 30 μl sample of supernatant, to which CMP and dCMP were added as markers (final concn. 2.5 × 10^{-3} M each), was applied to a polyethyleneimine-cellulose (PEI-cellulose) sheet. Then, the plate was washed by ascending irrigation with distilled water for 40 min. After drying of the layer, the front was cut off and the plate developed in the other direction overnight in a solution of 20:40:100:0:5 (v/v) 5 M-ammonium acetate pH 9.8: saturated sodium tetraborate: 95% ethanol:0-25 M-EDTA. After chromatography the CMP and dCMP spots were localized by u.v. illumination, cut out and counted in a PPO-POPOP-toluene mixture. No radioactivity was found outside these spots. In some experiments, the identity of [3H]dCMP detected as a product of viral or cellular ribonucleotide reductase activity was confirmed by rechromatography on PEI-cellulose using a solution of 2:1 (v/v) 2 M-LiCl and 2% boric acid. Moreover, the yields of the reaction were identical when separation of PEI-cellulose chromatography was compared to the previously used separation by column chromatography (Langelier et al., 1978).

Chemicals and radiochemicals. CMP, CDP, ATP, dATP and HU were obtained from Sigma; dCMP and dTTP from P.L. Biochemicals, Milwaukee, Wis., U.S.A.; araATP from Terra Marine, La Jolla, Ca., U.S.A.; [5-3H]CDP (13 Ci/mmol), obtained from Amersham, Arlington Heights, Ill., U.S.A. was repurified by ionic exchange chromatography. Polygram CEL 300 PEI/UV254 plastic sheets for thin-layer chromatography were purchased from Macherey-Nagel, Düren, F.R.G.

RESULTS

Ribonucleotide reductase induction in uninfected or HSV-infected quiescent BHK-21/C13 cells

The activity of ribonucleotide reductase in uninfected confluent BHK-21/C13 cells is high but, like other enzymes involved in deoxynucleotide metabolism, is present at low level in quiescent cells obtained by serum starvation (Noronha et al., 1972; Meuth & Green, 1974; Langelier et al., 1978). After 4 or after 5 days of culture in low serum concentration (0-5%), the reductase activity was respectively, 5 to 10% and 0-5 to 2-0% of the value found for exponentially growing cells. Addition of 10% foetal calf serum to quiescent cells resulted in a 10-fold increase in reductase activity which started around 10 h after the addition.

Effect of virus strain differences and input multiplicity

As shown in Table 1, infection of quiescent BHK-21/C13 cells with all strains of HSV tested (F and B-12 strains of type 1 or MS and HG-52 strains of type 2) produced strong stimulation or ribonucleotide reductase activity. The relationship between the enzyme activity and input multiplicity was studied for strain F and the results indicated that the induction was roughly proportional to the input multiplicity between 1 and 10 p.f.u./cell, where a plateau was attained. All the subsequent experiments have been performed with strain F using multiplicities varying between 10 and 30 p.f.u./cell.

Time-course

The virus-induced activity appeared in infected ‘quiescent’ BHK-21/C13 cells between 2 and 4 h after infection (Fig. 1). The plateau level (15-fold stimulated as compared to mock-infected cells) was obtained between 6 and 7 h and thereafter the activity declined. The induced activity was insensitive to inhibition by 1 mM-dTTP throughout the course of the experiment.
Fig. 1. Time course of ribonucleotide reductase induction by HSV-1 (F) in quiescent BHK-21/C13 cells (4 days in ER_{0.5}). Activity expressed as nmol dCMP formed/h/mg protein was measured in extracts of mock-infected cells (●) and of infected cells in absence (▲) or presence of 1 mM-dTTP (■).

Table 1. Ribonucleotide reductase induction in quiescent BHK-21/C13 cells infected with different strains of virus at different input multiplicities*

<table>
<thead>
<tr>
<th>HSV strain</th>
<th>Multiplicity of infection</th>
<th>Specific activity (nmol/h/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock-infected</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>F (HSV-1)</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>F (HSV-1)</td>
<td>6</td>
<td>0.80</td>
</tr>
<tr>
<td>F (HSV-1)</td>
<td>12</td>
<td>1.21</td>
</tr>
<tr>
<td>F (HSV-1)</td>
<td>40</td>
<td>0.83</td>
</tr>
<tr>
<td>F (HSV-1)</td>
<td>100</td>
<td>1.01</td>
</tr>
<tr>
<td>B-12 (HSV-1)</td>
<td>25</td>
<td>1.74</td>
</tr>
<tr>
<td>MS (HSV-2)</td>
<td>1</td>
<td>0.42</td>
</tr>
<tr>
<td>HG-52 (HSV-2)</td>
<td>10</td>
<td>2.18</td>
</tr>
</tbody>
</table>

* Ribonucleotide reductase activity was measured 8 h after infection of quiescent culture (5 days in ER_{0.5}) with HSV.

Table 2. Ribonucleotide reductase induction in quiescent BHK-21/C13 cells infected with u.v.-irradiated HSV*

<table>
<thead>
<tr>
<th>Time of u.v. irradiation (min)</th>
<th>Infectivity after u.v. irradiation (p.f.u./inoculum)</th>
<th>Specific activity (nmol/h/mg)</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock-infected</td>
<td>0</td>
<td>0.02</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2 × 10^9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.5 × 10^3</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* After u.v. irradiation or not, identical volumes of HSV-1 (F) stock were used to infect quiescent cells (5 days in ER_{0.5}) for 8 h.
† 1 mM-dTTP was added to the assay.

Ribonucleotide reductase induction as a consequence of HSV genome expression

u.v. irradiation of the HSV stock before infection

As shown in Table 2, extracts of cells infected with virus stock u.v.-irradiated for 10 min exhibited only 16% of the ribonucleotide reductase activity found in extracts of cells infected
Ribonucleotide reductase induced by HSV

Fig. 2. Effect of actinomycin D or cycloheximide addition upon ribonucleotide reductase induction by HSV-1 (F) in quiescent BHK-21/C13 cells (4 days in ERo.5). Actinomycin D or cycloheximide were added at 1, 2, 4 or 7 h post-infection and after harvesting the cells at 7 h post-infection ribonucleotide reductase was measured (a) in the absence of dTTP or (b) in the presence of 1 mM-dTTP. The results are expressed as percentages of the value obtained for the 7 h infected culture without drug (sp. act. 1.35 nmol/h/mg. ○, Mock-infected cells without inhibitor; △, infected cells with actinomycin D; ▲, infected cells with cycloheximide.

with non-irradiated virus and this enzymic activity became undetectable in extracts of cells infected with 20 min u.v.-irradiated virus stock. These results make two other possibilities which could explain the observed induction unlikely: (i) the presence of a stimulatory factor for the cellular enzyme in the unpurified virus stock; (ii) changes in the cellular regulation of ribonucleotide reductase activity due to the penetration per se of viral particles.

Effects of inhibitors of mRNA or protein synthesis

In the experiments presented in Fig. 2(a, b), the ribonucleotide reductase activity was measured at 7 h post-infection for cultures which had been exposed to actinomycin D or cycloheximide during various intervals (between 1 and 7 h post-infection). The two inhibitors completely prevented the ribonucleotide reductase induction if they were added between 0 and 1 h post-infection. When actinomycin D was introduced into the infected culture 1 h later (2 h post-infection) the amount of enzyme activity at 7 h post-infection was 70% of that present in the culture infected without addition of the drug. In the culture treated in parallel with cycloheximide, the HSV-induced activity was detectable only when the assay was performed in the presence of 1 mM-dTTP which reduced the level of activity in the mock-infected control (Fig. 2b). These results suggested that the mRNA transcription required for the reductase induction started and increased rapidly between 1 h and 2 h post-infection, and that a small amount of enzyme was present in infected cells as soon as 2 h post-infection. This last point has been confirmed in other experiments where the activity was measured shortly after the infection (results not shown). When actinomycin D was added at 4 h post-infection, the amount of enzyme activity at the harvest was reduced by only 10% as compared to the untreated control, suggesting that the maximum rate of synthesis of reductase mRNA preceded this time. In the cycloheximide-treated cultures, the amount of activity was 40% of that present in the control. Addition of the drugs just before the harvest did not inhibit the enzyme induction at all.

Biochemical differences between the HSV-induced and the cellular ribonucleotide reductase activity

In order to analyse such differences, it was important to measure the HSV-induced activity in preparations containing a very low amount of cellular isozyme. For this purpose, cells were kept for 5 days in ERo.5 before infection with HSV-1 (F) and the activity was measured at 8 h
post-infection. Uninfected, exponentially growing cells were used as the source of cellular activity.

**Inhibition by dATP or dTTP**

Fig. 3 (a) shows the degrees of inhibition exerted by dATP or dTTP on the cellular and virus-induced enzymes. The cellular activity was inhibited by 50% at 0.025 mM concentration of either nucleoside triphosphate and the maximum inhibition (90 to 95%) was obtained at 0.2 mM. In contrast, the virus-induced enzyme retained between 95 and 100% of activity at 2 mM-dATP or dTTP. When equal amounts of enzyme activity from the two types of extracts were mixed together and the activity measured in presence or absence of 2 mM-dATP or dTTP, nearly 50% of the activity measured in the absence of nucleosides was retained. This suggests that none of the extracts contained compounds (inhibitors or activators) which would affect the reductase activity or modify this property in a different extract.
Ribonucleotide reductase induced by HSV

Inhibition by araATP

AraA has been described as a good inhibitor of HSV replication (Sidwell et al., 1968) and it is believed that the phosphorylated derivative of this nucleotide analogue blocks the HSV DNA polymerase (Muller et al., 1977). However, it has been also reported that araATP could interfere with ribonucleotide reductase activity (Moore & Cohen, 1967); therefore, a high sensitivity of HSV-induced ribonucleotide reductase could contribute to the toxicity of araA for HSV. Reductase activities in extracts of either uninfected exponential or HSV-infected quiescent BHK-21/C13 cells were measured in presence of increasing concentrations of araATP and the results are presented in Fig. 3 (b); the HSV-induced enzyme was completely insensitive to araATP, whereas the cellular isozyme was sensitive to the presence of the analogue. Comparison of Fig. 3 (a), (b) shows that, as reported by Moore & Cohen (1967), araATP was less active on the cellular activity than dATP on a concentration basis. These results suggest that the inhibition of HSV DNA replication by araA is probably not related to an inhibitory effect of araATP on the ribonucleotide reductase.

Inhibition by HU

Since the block of DNA synthesis by HU in mammalian cells is believed to be produced by inhibition of ribonucleotide reductase (Lewis & Wright, 1974) it was important to test the effects of this product on the HSV-induced activity. As shown in Fig. 3 (c), the ribonucleotide reductase activity in HSV-infected cells was slightly more sensitive to HU than the activity present in uninfected, exponentially growing cells. In the two types of extract, 20 to 25% of the activity was still present at HU concentrations of 3 mM. Therefore, it is possible that the reported inhibition of HSV DNA replication by HU could be the result of inhibition of both HSV-induced and cellular reductase activities.

Inhibition by ammonium sulphate

Ammonium sulphate has already been used to distinguish the cellular from the HSV DNA polymerase, since the viral enzyme is much less sensitive to this salt than the cellular isozyme (Keir et al., 1966). As can be seen in Fig. 3 (d), the HSV-induced ribonucleotide reductase activity was also less sensitive to high concentrations of the salt in the reaction mixture: 50% inhibition of the cellular enzyme was obtained with 10 mM-(NH₄)₂SO₄ concentration whereas a sixfold higher concentration was necessary to obtain the same level of inhibition for the viral isozyme. A concentration of 50 mM would be useful for tests on extracts containing the two activities since only 5% of cellular activity remained, whereas 60% of the virus-induced activity was retained. The HSV-induced enzyme was still active at 100 mM-(NH₄)₂SO₄, whereas the cellular isozyme was completely inhibited.

Induction of ribonucleotide reductase in confluent BHK-21/C13 cells

As the cellular reductase activity was inhibited with dTTP or (NH₄)₂SO₄, it was possible to study the HSV-induced activity in confluent cells normally used for HSV production (Fig. 4). In infected cells a threefold increase of the total reductase activity was obtained between 5 h and 7 h post-infection. Both dTTP and (NH₄)₂SO₄ resistant activity was detectable around 2 h post-infection and attained a maximum at 5 to 7 h post-infection. At zero time, nearly all the activity was inhibited by dTTP. During the course of the infection, this sensitive activity disappeared gradually and at 7 h post-infection 90% of the total activity was resistant to dTTP. Since the reductase activity in mock-infected cells did not vary throughout the same period (results not shown), this result indicates that the cellular reductase could be inhibited early during infection. The curve obtained for the measurements in the presence of
Fig. 4. Time course of ribonucleotide reductase induction by HSV-1 (F) in confluent BHK-21/C13 cells. Activity expressed as nmol dCMP formed/h/mg protein was measured without any addition (●) or in presence of 1 mM-dTTP (▲) or 50 mM-(NH₄)₂SO₄ (■).

(NH₄)₂SO₄ reflects the incomplete inhibition of the cellular activity and the partial resistance of HSV-induced activity at this salt concentration (see Fig. 3d). At the beginning of the experiment 10% of the activity was resistant to (NH₄)₂SO₄ and at the maximum of the induction a level of 70% resistance was obtained.

**Discussion**

Following our previous observations that, in quiescent BHK-21/C13 cells, infection with HSV-1 or HSV-2 produced the induction of an altered ribonucleotide reductase completely insensitive to inhibition by dTTP (Langelier et al., 1978), we present here results which strongly suggest, but do not prove, that the new reductase activity is encoded by a virus gene. This we conclude from three observations. First, the extent of production was dependent upon the multiplicity of infection; second, the production was diminished or prevented by u.v. treatment of the virus stock prior to infection or by the presence of inhibitors of mRNA synthesis or protein synthesis; and third, the activity appeared early after infection and the time of appearance was independent of the level of reductase present prior to infection.

The time course of the induction was similar in confluent and in quiescent cells and was roughly comparable to the kinetics of induction described for other HSV-specified enzymes involved in DNA metabolism, namely thymidine kinase, DNA polymerase and DNase (Klemperer et al., 1967; Jamieson & Bjursell, 1976a, b; Weissbach et al., 1973; Rolton & Keir, 1974). Therefore, ribonucleotide reductase can be classified like them as a β polypeptide (Honess & Roizman, 1974; Leung, 1978).

The ribonucleotide reductase induced in HSV-infected quiescent BHK-21/C13 cells differed from that present in uninfected, exponentially growing cells by its insensitivity to the nucleoside triphosphates dATP, dTTP and araATP, and also by its higher resistance to ammonium sulphate. The differences that we report here for crude extracts have been also observed with partially purified preparations by Ponce de Leon et al. (1977) for dTTP and recently in our laboratory for dTTP, dATP and araATP (J. Charron & Y. Langelier,
unpublished results). This insensitivity to feedback-inhibition by nucleoside triphosphates could serve as a basis for selecting stable acquisition of this activity by mammalian cells.

Our finding that araATP also was not an inhibitor of the HSV-induced reductase supports the hypothesis that the primary site of action for araA in the inhibition of HSV DNA synthesis is located at the level of the HSV DNA polymerase activity (Muller et al., 1977).

Interestingly, the purified bacteriophage T4 enzyme induced in Escherichia coli exhibits an insensitivity to inhibition by dTTP and dATP (Berglund et al., 1969; Berglund, 1972) similar to that observed for the HSV-induced enzyme. Moreover, the two virus-induced enzymes do not have the absolute requirement for Mg$^{2+}$ which exist for the E. coli or mammalian isozymes (Berglund, 1972; Ponce de Leon et al., 1977). The sensitivity to HU of the HSV-induced reductase that we have observed here has also been recently described for the T4 enzyme (Yeh & Tessman, 1978). On the other hand, the HSV-induced reductase differed from the EBV- or EHV-induced enzyme which appears to be resistant to HU (Cohen et al., 1977; Henry et al., 1978); this dissimilarity seems to explain why the replication of EBV and EHV is resistant to HU (Hampar et al., 1972; Mele et al., 1974; Cohen et al., 1975), whereas the replication of HSV is blocked by the drug (Wagner et al., 1972; Shlomai & Becker, 1977; S. Qualizza & Y. Langelier, unpublished results). Therefore, selection of a HU-resistant HSV mutant inducing a reductase activity refractory to the action of HU might be possible and should give genetic evidence for a virus-coded product.

The biological role of the HSV-induced ribonucleotide reductase in the infectious process is still unclear. It is known that in mammalian cells the deoxyribonucleoside triphosphate pools are low (e.g. calculations on the size of dGTP pool show that it suffices for about only 15 s of DNA synthesis, Thelander & Reichard, 1979) and that these DNA building-blocks are derived via the reductase pathway, as strongly suggested by isotope experiments with regenerating rat liver (Larsson & Neilands, 1966). Therefore, the continuous activity of ribonucleotide reductase is essential for cellular DNA synthesis and we can postulate that the synthesis of a new reductase in HSV-infected cells would free the virus from need of the cellular enzyme. This synthesis could be essential in natural infection for virus DNA replication in cells which have shut down their own machinery for DNA synthesis as has been suggested for thymidine kinase (TK) activity (Jamieson et al., 1974).

On the other hand, as demonstrated for the HSV DNA polymerase (Aron et al., 1975; Hay et al. 1976), the virus reductase could also be essential in actively growing cells. Two observations suggest this last hypothesis. First, the cellular reductase seems to be shut off in HSV-infected BHK-21/C13 cells; a similar inhibition has been observed in EHV-infected cells (Cohen et al., 1977). Second, both Cheng et al. (1975) and Jamieson & Bjursell (1976a) reported a rapid and large increase in dTTP levels in HSV-1-infected mammalian cells, resulting probably for the most part from the HSV TK activity (Jamieson & Bjursell, 1976b). Such an increase would rapidly block the CDP reduction by the cellular enzyme producing a deficiency in dCTP which might be overcome by the synthesis of the virus enzyme resistant to dTTP. Large increases of dGTP, dATP and dTTP pools have also been observed in cells infected by HSV (Cheng et al., 1975; Roller & Cohen, 1976) and we can predict that the virus reductase will also catalyse the reduction of the other ribonucleoside diphosphate substrates: ADP, GDP and UDP.

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Y. LANGELIER AND G. BUTTIN
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