Effect of 2-Deoxy-d-Glucose on Cytomegalovirus-induced DNA Synthesis in Human Fibroblasts

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SUMMARY

2-Deoxy-d-glucose (dGlc) was found to selectively inhibit virus DNA synthesis in human embryonic lung cells infected with human cytomegalovirus (HCMV). The effective concentration of dGlc was approx. 10-fold higher in culture medium containing glucose instead of sodium pyruvate. This inhibitory action of dGlc was fully reversible following replacement of the inhibitor medium by fresh medium after a 48 h treatment of infected cells. Virus DNA synthesis could be selectively inhibited by addition of dGlc even after initiation of HCMV DNA replication. In contrast, virus DNA synthesis in herpes simplex virus-infected cells was insensitive to dGlc. The drug was found to deplete HCMV-infected cells of uridine triphosphate and caused a progressive reduction of uridine incorporation into RNA. To substantiate a possible interference by dGlc with the expression and/or function of virus-induced, chromatin-associated factors essential for virus DNA replication, DNA synthesis of chromatin preparations from dGlc-treated, HCMV-infected cells was analysed. In contrast to preparations of untreated or phosphonoacetic acid (PAA)-treated, HCMV-infected cells, those of dGlc-treated cells lacked significant in vitro DNA-synthesizing activity; virus DNA was not synthesized by these preparations. Tunica-mycin in the presence of low concentrations of dimethyl sulphoxide was also found to be effective in abolishing HCMV-induced DNA replication. It is thus suggested that dGlc interferes with the function of an 'early' chromatin-associated glycoprotein essential for virus DNA synthesis.

INTRODUCTION

The sugar analogues 2-deoxy-d-glucose (dGlc) and glucosamine have been shown to interfere with the multiplication of a number of enveloped RNA and DNA viruses (Kaluza et al., 1973; Courtney et al., 1973; Scholtissek, 1975). Virus-induced phenomena, such as cell fusion (Gallaher et al., 1973) and cell agglutinability (Kaluza et al., 1972), are also abolished by dGlc. These effects of dGlc, including those on uninfected fibroblasts (de Groot et al., 1980), have been primarily attributed to an alteration by dGlc of the sugar moiety of cellular and virus glycoproteins (Schwarz & Klenk, 1974; Koch et al., 1979; de Groot et al., 1980), resulting in a loss of the proper function of these macromolecules.

Human cytomegalovirus (HCMV), a member of the herpesvirus group, has been reported to induce several new glycoproteins in permissive cells which were also identified in virions and dense bodies (Stinski, 1977). In addition, two of the 'early' virus-induced polypeptides appear to be glycosylated (Stinski, 1977). During a study which was initiated to establish the subcellular distribution and possible function of the virus-induced 'early' glycoproteins, a novel effect of dGlc became apparent. The sugar analogue was found to selectively abolish virus DNA synthesis in HCMV-infected cells. This report attempts to elucidate some aspects of the inhibitor action.
METHODS

Cells and viruses. Diploid human embryonic lung cells (MRC 5; Flow Laboratories) which are permissive for HCMV were used between the 27th and 32nd passage. Cells were cultivated as monolayers either in plastic flasks (75 cm² or 175 cm²; Lux or Falcon, Seromed, Munich, F.R.G.) or in Roux bottles (250 cm² glass bottles) in Eagle’s minimum essential medium (MEM; Gibco) supplemented with 200 units penicillin/ml and 0.2 mg streptomycin/ml, and 15% foetal calf serum (FCS). All experiments were performed with cultures partially arrested by serum starvation (0.2% FCS for 72 h), beginning on average 3 days after the cultures reached confluence (DeMarchi & Kaplan, 1977; Radsak & Schmitz, 1980). The Towne strain of HCMV (Furukawa et al., 1973) was passaged at weekly intervals on confluent MRC 5 cells at an m.o.i. of 0.1 using 2% FCS. The KOS strain of herpes simplex virus type 1 (HSV-1; Radsak, 1978) was passaged on MRC 5 cells for 3 to 4 days at an m.o.i. of 0.1 and grown in serum-free medium. Virus stocks of HCMV and HSV-1 for experimental infections were prepared according to previously described procedures (Fioretti et al., 1973; Radsak, 1978). Experimental infections were carried out with an m.o.i. of 1 for HCMV and 10 for HSV-1. Under these conditions about 90% of the cells were found to contain virus antigen (Radsak & Schmitz, 1980). Virus titres were determined either by plaque assay or by the endpoint dilution method combined with indirect immunofluorescence for virus antigen (Fioretti et al., 1973; Radsak & Schmitz, 1980). As indicated in results, glucose in the culture medium was replaced by 10 mM-sodium pyruvate (Ludwig & Rott, 1975) in most of the experiments.

Isotopic labelling of cells. For precursor incorporation into DNA, [³H]thymidine (Amersham/Buchler, Braunschweig, F.R.G.; sp. act. 47 Ci/mmol) and/or [¹⁴C]thymidine (Amersham/Buchler; sp. act. 59 mCi/mmol) at the concentrations given in Results were included in the culture medium; for analysis of RNA metabolism, labelling with [5-³H]uridine (Amersham/Buchler; sp. act. 28 Ci/mmol) was used.

Analysis of acid extracts. Acid-soluble phosphorylated derivatives of uridine were separated by thin-layer chromatography as described by Martin et al. (1979). Trichloroacetic acid (TCA) extracts of [³H]uridine pulse-labelled cultures (2 ml/5 × 10⁶ cells) were prepared as described by Scholtissek (1972), precipitated overnight with 6 to 8 vol. acetone at -20 °C, taken up in 10 to 30 µl of distilled water and analysed by thin-layer chromatography on poly(ethyleneimine)-cellulose (Polygram cell 300, Machery & Nagel, Düren, F.R.G.) with 0.25 M-NH₄HCO₃ as solvent and using [³H]uridine, [³H]uridine diphosphate and [³H]uridine triphosphate as markers.

Cell fractionation and incubation of chromatin. Fractionation of mock- and HCMV-infected MRC 5 cells for preparation of chromatin was carried out as described previously (Yamada et al., 1978; Radsak et al., 1980). Following hypotonic swelling in buffer A [10 mM-tris-HCl pH 7.5, 3 mM-MgCl₂, 2 mM-ethylenglycolbis(aminooxyether)tetra-acetic acid (EGTA), 0.5 mM-dithiothreitol (DTT)], a minimum of 1.5 × 10⁷ cells/3 ml were disrupted in a Dounce homogenizer before re-homogenization after addition of one-half the volume of buffer B (0.3 M-tris–HCl pH 8, 2 mM-EGTA, 3 mM-MgCl₂, 0.5 mM-DTT, 0.15 M-glucose and 0.15% Triton X-100). Nuclei were separated from the homogenate by sedimentation at 1000 g for 10 min, resuspended in 2 ml of a mixture of buffers A and B (2:1) and resedimented. Extraction of chromatin from nuclei was achieved by two successive treatments with 1 mM-EDTA pH 7, 1 mM-DTT for 30 min at 4 °C. The final preparation was taken up in 1 mM-tris–HCl pH 8, 0.1 mM-EDTA, 0.5 mM-DTT and 40% glycerol. These preparations could be stored at -80 °C for several weeks without significant loss of endogenous incorporation activity. The preparations contained on average 60 µg DNA/1.5 × 10⁷ cells. The protein : DNA ratio was in the range of 5:1. In vitro incorporation by chromatin preparations of deoxyribonucleoside triphosphates into DNA was performed using the assay
conditions described previously (Radsak et al., 1980). The standard assay consisted of 50 mM-tris–HCl pH 8, 5 mM-MgCl₂, 2.5 mM-DTT, 0.25 mM-ATP, 0.1 mM each of dATP, dGTP, dCTP and 0.01 mM-[³H]thymidine triphosphate ([³H]TTP; sp. act. 800 ct/min/pmol) and chromatin of 10⁷ cells, corresponding to about 40 μg DNA in a total vol. of 1 ml. Incubation was performed for 30 min at 37 °C. The reaction was stopped by addition of 10 mM-EDTA (final concentration) and SDS (final concentration 1%) prior to extraction of DNA (Radsak et al., 1980).

**Extraction and analysis of radioactively labelled DNA.** DNA was extracted from cells or chromatin following lysis with 1% SDS by two cycles of phenol/chloroform/isoamyl alcohol treatment according to established methods (Hirai & Watanabe, 1976; Radsak et al., 1980). Separation of viral and cellular DNA was achieved by isopycnic sedimentation in neutral CsCl where MRC 5 DNA bands at a density of 1.696 g/ml, HCMV DNA at 1.717 g/ml (St. Jeor et al., 1974) and HSV DNA at 1.725 g/ml (Radsak & Schmitz, 1980).

**Determination of radioactivity, protein and DNA content.** Radioactivity of acid-precipitable material was determined by collecting TCA precipitates on nitrocellulose filters (BA 85, Schleicher & Schüll, Dassel, F.R.G.) under suction, followed by washing with 5% TCA, drying and counting in a toluene-based scintillation cocktail (Quickszint, Roth-Karlsruhe, F.R.G.; Radsak, 1978). For analysis of CsCl gradients fractional aliquots were pipetted on to paper discs (3MM, Schleicher & Schüll) which were subsequently subjected to washing in 10% cold TCA, ethyl alcohol and ether prior to counting in Quickszint. Protein content was estimated by the method of Lowry et al. (1951) and DNA content by the procedure of Giles & Myers (1965).

**RESULTS**

**Inhibition of DNA synthesis by dGlc in HCMV-infected MRC 5 cells**

Confluent monolayers of MRC 5 cells (5 × 10⁶ cells) were infected by HCMV at an m.o.i. of approx. 1 and kept in the presence of various concentrations of dGlc after a 2 h adsorption period. The dGlc-treated cultures mock-infected with used medium and untreated HCMV-infected cultures were used as controls. For all experiments, cultures were partially arrested by serum starvation (DeMarchi & Kaplan, 1977; Radsak & Schmitz, 1980). Labelling of the parallel cultures with [³H]thymidine (10 μCi/ml) was performed during a time interval when untreated HCMV-infected cells synthesize significant amounts of virus DNA (Stinski, 1978), i.e. 36 to 72 h post-infection. As shown in Table 1 precursor incorporation into DNA of HCMV-infected cultures was clearly more sensitive to dGlc than that of mock-infected cells, which was essentially unaffected or even increased. In order to identify the type of DNA labelled in infected cells in the presence of dGlc, DNA was extracted and subjected to isopycnic sedimentation in neutral CsCl. Under these conditions virus DNA bands at a significantly higher density (1.717 g/ml) than cellular DNA (1.696 g/ml). Fig. 1(a) C demonstrates that dGlc at a concentration of 1 mM in MEM containing pyruvate not only completely abolished incorporation into virus DNA but also prevented the known HCMV-mediated induction of host cell DNA replication (St. Jeor et al., 1974). At concentrations exceeding 0.5 mM, dGlc has been shown to decrease the cellular uridine triphosphate (UTP) pool in medium containing pyruvate instead of glucose (Scholtissek, 1975; see below). Under our conditions, the effect of dGlc on virus-induced DNA synthesis was, however, still achieved with concentrations as low as 0.2 mM-dGlc and was not prevented by inclusion of 2 mM-uridine into the inhibitor medium (Fig. 1b; Scholtissek, 1975). Furthermore, identical results were obtained using culture medium containing 5 mM-glucose in place of sodium pyruvate and approx. 10-fold higher concentrations of dGlc (Fig. 1c).
Table 1. Effect of dGlc on \(^{3}H\)thymidine incorporation* of mock-infected and HCMV-infected MRC 5 cells

<table>
<thead>
<tr>
<th>dGlc concentration (mM)†</th>
<th>Mock-infected cells</th>
<th>HCMV-infected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7809</td>
<td>18 120</td>
</tr>
<tr>
<td>1</td>
<td>7864</td>
<td>6521</td>
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<td>10</td>
<td>9330</td>
<td>5966</td>
</tr>
<tr>
<td>50</td>
<td>9121</td>
<td>9175</td>
</tr>
</tbody>
</table>

* Cultures were labelled with \(^{3}H\)thymidine (10 μCi/ml) from 36 to 72 h post-infection.
† Concentration of dGlc in medium containing sodium pyruvate and 0.2% FCS; dGlc treatment started immediately after virus adsorption at 2 h post-infection.

Reversibility of the dGlc effect on HCMV-induced DNA synthesis

In order to examine the reversibility of the dGlc inhibition, MRC 5 cells infected with HCMV were kept for 48 h in culture medium containing pyruvate, 1 mM-dGlc and \(^{14}C\)thymidine (0.2 μCi/ml). Subsequently, the inhibitor medium was replaced by fresh medium containing glucose instead of pyruvate. The \(^{3}H\)thymidine (10 μCi/ml) was added for 48 h intervals at various times after dGlc removal prior to extraction of DNA. The radioactivity profiles of the doubly labelled DNAs after banding in CsCl shown in Fig. 1(d) reveal that resumption of host cell induction was observed during the first labelling period, but that of virus DNA synthesis only during the second labelling period, with \(^{3}H\)thymidine. This temporal sequence essentially equals that during an infectious cycle of HCMV in untreated fibroblasts (Huang, 1975; Stinski, 1978).

Effect of dGlc during the late infectious cycle

The following experiments were carried out to determine differences in sensitivity of HCMV-induced DNA synthesis to dGlc during later phases of the infectious cycle which extends over 72 h with virus DNA replication starting at about 36 h post-infection (Hirai & Watanabe, 1976; Huang, 1975). The glucose analogue (1 mM) was added to the culture medium (containing pyruvate) of infected cells at various times up to 60 h post-infection prior to labelling with \(^{3}H\)thymidine (20 μCi/ml) from 60 to 72 h post-infection. Fig. 2 shows that dGlc was apparently effective in abolishing consecutive virus DNA synthesis completely when added until 36 h post-infection before onset of virus DNA synthesis. Addition at later times was followed by labelling of comparatively small amounts of DNA forming a shoulder with low radioactivity at the density of virus DNA. During this late phase the inhibition by dGlc of the host cell induction was also less pronounced. Essentially the same results were observed when the experiment was performed in culture medium containing glucose, using the inhibitor at 10 mM (data now shown).

DNA synthesis in herpes simplex virus-infected cells in the presence of dGlc

DNA synthesis in RK cells of another herpesvirus, HSV-1, has been reported to be resistant to dGlc (Ludwig & Rott, 1975). As this observation is different from our results with HCMV, the effect of dGlc on HSV-1 DNA replication was examined using MRC 5 cells as host cell system, in order to exclude that inherent differences in the host cell system are involved. This was apparently not the case as dGlc added to HSV-1-infected MRC 5 cells at 1 h post-infection exerted essentially no effect on HSV-1 DNA replication (data not shown). Different mechanisms thus appear to control HSV-1 and HCMV DNA replication.
Fig. 1. (a) Isopycnic sedimentation in neutral CsCl (mean density 1.71 g/ml) of ³H-labelled DNA extracted from serum-starved MRC 5 cells: A, DNA of mock-infected culture; B, DNA of HCMV-infected culture; C, DNA of dGlc-treated (1 mM starting at 2 h post-infection), HCMV-infected culture. Labelling was with 10 μCi [³H]thymidine/ml from 36 to 72 h post-infection. Culture medium was MEM with pyruvate and 0.2% FCS. An amount of 4 μg ± 10% DNA was analysed per gradient. The arrow indicates the position of virus DNA (1.717 g/ml). Centrifugation was at 36 000 rev/min for 65 h at 20 °C in a 50 Ti fixed-angle rotor of a Beckman centrifuge. (b) Isopycnic sedimentation in neutral CsCl as in (a): A, DNA of mock-infected culture treated with 1 mM-dGlc and 2 mM-uridine at 2 h post-infection; B, DNA of untreated HCMV-infected culture; C, DNA of HCMV-infected culture treated with 0.2 mM-dGlc at 2 h post-infection; D, DNA of HCMV-infected culture treated with 1 mM-dGlc and 2 mM-uridine starting at 2 h post-infection. Labelling was with 10 μCi [³H]thymidine/ml from 36 to 72 h post-infection. Culture medium was MEM with pyruvate and 0.2% FCS. An amount of 7 μg ± 10% DNA was analysed per gradient. The arrow indicates the position of virus DNA (1.717 g/ml). Centrifugation conditions were as in (a). (c) Isopycnic sedimentation in neutral CsCl as in (a): A, DNA of mock-infected culture treated with 10 mM-dGlc; B, DNA of untreated HCMV-infected culture; C, DNA of HCMV-infected culture treated with 1 mM-dGlc; D, DNA of HCMV-infected culture treated with 10 mM-dGlc. Drug treatment started at 2 h post-infection. Labelling was with 10 μCi [³H]thymidine/ml from 36 to 72 h post-infection. Culture medium was MEM with glucose and 0.2% FCS. An amount of 5 μg ± 10% DNA was analysed per gradient. The arrow indicates the position of virus DNA (1.717 g/ml). Centrifugation conditions were as in (a). (d) Isopycnic sedimentation in neutral CsCl (mean density 1.71 g/ml) of doubly labelled DNA extracted from serum-starved MRC 5 cells exposed to 1 mM-dGlc and [¹⁴C]thymidine (0.2 μCi/ml; A) in MEM with pyruvate and 0.2% FCS from 2 to 48 h post-infection, prior to replacement of the inhibitor medium by MEM plus glucose and 0.2% FCS and labelling with [³H]thymidine (10 μCi/ml; ○) starting at various times post-infection. A, DNA of mock-infected culture labelled with [³H]thymidine from 48 to 96 h post-infection; B, DNA of HCMV-infected culture labelled with [³H]thymidine from 48 to 96 h post-infection; C, DNA of HCMV-infected culture labelled with [³H]thymidine from 72 to 120 h post-infection; D, DNA of HCMV-infected culture labelled with [³H]thymidine from 96 to 144 h post-infection. An amount of 7 μg ± 10% DNA was analysed per gradient. The arrow indicates the position of virus DNA (1.717 g/ml). Centrifugation conditions were as in (a).
Fig. 2. Isopycnic sedimentation in neutral CsCl (mean density 1.71 g/ml) of 3H-labelled DNA extracted from serum-starved HCMV-infected MRC 5 cells exposed to 1 mM-dGlc starting at various times post-infection: (a) DNA of culture without treatment; (b) DNA of culture exposed to dGlc starting at 12 h post-infection; (c) 24 h post-infection; (d) 36 h post-infection; (e) 48 h post-infection; (f) 60 h post-infection. Labelling was with 20 μCi [3H]thymidine from 60 to 72 h post-infection. Culture medium was MEM with pyruvate and 0.2% FCS. An amount of 7 μg ± 10% DNA was analysed per gradient. The arrow indicates the position of virus DNA (1.717 g/ml). Centrifugation conditions were as described in the legend to Fig. 1 (a).

Effect of dGlc on uridine uptake by HCMV-infected cells

Glucose derivatives have been described to deplete the cellular UTP pool (Scholtissek, 1972, 1975) and might thus produce the effect described on HCMV-infected cells by interference with mRNA synthesis essential for virus DNA replication. As shown above, the inhibition of viral DNA replication by dGlc cannot be counteracted by the addition of uridine to the culture medium. In order to measure these dGlc-induced alterations in our experimental system, HCMV-infected cultures were exposed to the drug either immediately after infection or 48 h post-infection and subjected to a 2 h pulse with [3H]uridine (10 μCi/ml) from 50 to 52 h post-infection. Untreated, HCMV-infected cultures were included as controls. Subsequently, acid-precipitable radioactivity was separated from acid-soluble extracts which were further analysed as described in Methods. As Table 2 shows, dGlc causes a drastic reduction of the UTP pool in our experimental system not dependent on the interval of dGlc pretreatment. At the same time there is a relative increase of uridine diphosphate (UDP), probably in part resulting from an accumulation of UDP derivatives which are unstable under our conditions (Schmidt et al., 1974). On the other hand, uridine incorporation during the pulse into RNA is only slightly inhibited following dGlc exposure at 48 h post-infection and approx. by 60% after addition directly post-infection. Considering that the specific activity of
**dGlc effect on HCMV-induced DNA synthesis**

Fig. 3. Isopycnic sedimentation in neutral CsCl (mean density 1.71 g/ml) of doubly labelled DNA extracted from chromatin of serum-starved untreated or inhibitor-treated, HCMV-infected cultures exposed to [14C]thymidine (0.1 μCi/ml; A) from 36 to 72 h post-infection before preparation of chromatin and in vitro labelling with [3H]thymidine triphosphate (●) as described in Methods: (a) DNA of chromatin from untreated culture; (b) DNA of chromatin from PAA-treated culture; (c) DNA of chromatin from dGlc-treated culture. Culture medium was MEM with pyruvate and 0.2% FCS. An amount of 15 μg ± 10% DNA was analysed per gradient. The arrow indicates the position of virus DNA (1.717 g/ml). Centrifugation conditions were as described in the legend to Fig. 1 (a).

### Table 2. Influence of dGlc on labelling* of uridine derivatives in HCMV-infected MRC 5 cells

<table>
<thead>
<tr>
<th>dGlc concn. (mM)</th>
<th>Time post-infection of dGlc addition (h)</th>
<th>Radioactivity (pmol) incorporated/5 × 10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UDP†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UTP‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RNA§</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>2.68</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.42</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>28.56</td>
</tr>
</tbody>
</table>

* HCMV-infected cultures were labelled with [3H]uridine (10 μCi/ml) from 50 to 52 h post-infection.
† Culture medium containing sodium pyruvate and 0.2% FCS.
‡ Acid-soluble radioactivity was analysed as described in Methods.
§ The values represent acid-precipitable radioactivity.

The UTP pool is much higher in the presence of dGlc (Scholtissek, 1975), this observation suggests that RNA synthesis is significantly inhibited also during the short term exposure to dGlc. As this assumption is in line with a 50 to 60% inhibition of 3H-labelled amino acid incorporation in dGlc-treated, HCMV-infected cells (data not shown), it is thus concluded that dGlc significantly decreases mRNA synthesis in infected cultures. Consequently, the selective inhibition of virus DNA replication could be a result of the interference by dGlc with mRNA metabolism.

**In vitro activity of chromatin of HCMV-infected cells**

Chromatin prepared from HCMV-infected cultures according to Yamada et al. (1978), reflects the DNA-synthesizing activity of the intact cells for a limited time interval (Radsak et al., 1980) and should thus contain the essential factors (polypeptides) for virus DNA
replication. Interference by dGlc with expression and/or function of these factors, should result in abolishment of the in vitro activity of the chromatin preparations from dGlc-treated, HCMV-infected cultures. In order to establish this issue for the experimental system described here, chromatin was prepared from dGlc-treated, [14C]thymidine-labelled, HCMV-infected cultures (1.5 × 10^7 cells; 0.1 μCi/ml from 36 to 72 h post-infection) and examined for its in vitro DNA-synthesizing activity by incubation with [3H]TTP under appropriate conditions (Yamada et al., 1978; Radsak et al., 1980). Chromatin preparations from untreated, infected cultures and infected cultures kept in the presence of phosphonoacetic acid (PAA) (100 μg/ml) were included as controls. PAA is known to selectively inhibit HCMV DNA replication and thus allow only the expression of 'early' viral functions (Huang, 1975; Hirai & Watanabe, 1976).

Analysis of the doubly labelled DNA by isopycnic sedimentation in CsCl (Fig. 3) revealed the expected patterns. Both 3H- and 14C-labelled DNA in the positions of viral and cellular DNA were observed for preparations from untreated HCMV-infected cultures, doubly labelled DNA only in the position of cellular DNA for those of PAA-treated cultures and only little radioactivity essentially in the position of cellular DNA for those of dGlc-treated cultures. This absence of viral DNA synthesis is not due to prevention of the induction of HCMV-specific DNA polymerase activity (Huang, 1975) which was found to be diminished in dGlc-treated, infected nuclei by approx. 60%, but not abolished. Furthermore, dGlc does not influence the in vitro activity of the viral DNA polymerase (data not shown).

**Effect of other inhibitors of glycosylation on HCMV-induced DNA synthesis**

Selective inhibition of viral DNA synthesis in serum-starved cultures was also observed when dGlc was replaced in some experiments by glucosamine. The effective concentrations were comparable to those of dGlc (data not shown). In initial experiments with tunicamycin (up to 5 μg/ml), a potent and relatively selective inhibitor of glycosylation (Datema & Schwarz, 1978), no effect on virus DNA synthesis was observed. A significant inhibition of the labelling of viral DNA during a pulse with [3H]thymidine (20 μCi/ml from 60 to 72 h post-infection) was achieved only by addition of the drug directly post-infection in culture medium (0.2% FCS) containing 35 mM-dimethyl sulphoxide (DMSO; von Figura et al., 1979). DMSO alone did not affect virus-induced DNA replication (data not shown).

**DISCUSSION**

Glucose derivatives as used in these experiments are known to have toxic effects if used under inadequate conditions (Scholtissek, 1975). Prerequisite for the induction of specific effects is, therefore, the use of low concentrations of the drug and the inclusion of appropriate controls. The novel effect of dGlc on HCMV DNA replication described here is believed not to be due to toxicity for several reasons as follows. (i) Host cell DNA synthesis in uninfected cultures is unimpaired. The inhibition of viral DNA replication is relatively selective. The virus-mediated induction of host cell DNA replication (St. Jeor et al., 1974; Radsak & Weder, 1981) is inhibited, but host cell DNA synthesis in infected cultures is not abolished by dGlc. (ii) The dGlc at concentrations twofold higher than those used for HCMV-infected cultures essentially does not affect HSV-1 DNA replication in the same cell system.

On the basis of the present knowledge at least two aspects have to be considered in context with this novel effect of dGlc. This compound is known to cause a depletion of the cellular UTP pool (Scholtissek, 1975) and might thus interfere with mRNA synthesis essential for viral DNA replication. Our results on the incorporation of uridine into acid-precipitable material suggest that RNA synthesis is in fact inhibited by dGlc in HCMV-infected cells. Amino acid incorporation by dGlc-treated infected cells is also significantly decreased. Interference by dGlc with the synthesis of virus-induced mRNA might thus be involved,
dGlc effect on HCMV-induced DNA synthesis

resulting in abolishment of, for example, new chromatin-associated polypeptides necessary for viral DNA replication. Chromatin preparations from dGlc-treated, HCMV-infected cells indeed lack in vitro DNA-synthesizing activity. Preliminary data showing that synthesis of several chromatin-associated polypeptides is depressed in dGlc-treated HCMV-infected cells (K. D. Radsak & D. Weder, unpublished results), are in line with this view. This latter effect must possibly be seen in combination with the known inhibition by dGlc of cellular glycosylation activity (Datema & Schwarz, 1978). The action of dGlc in HCMV-infected cells is in this context not concerned with virus envelope glycoprotein (for review, see Scholtissek, 1975) but might involve HCMV-induced glycosylated chromatin-associated polypeptides. Recently, the existence of a major 125 000 dalton glycosylated chromatin-associated polypeptide in rat liver (Rizzo & Bustin, 1977) was reported. In addition, there were minor concanavalin A-binding polypeptides with mol. wt. of 135 000 and 69 000. The biological function of these chromatin-associated glycoproteins remains enigmatic. It is thus open to speculation that dGlc interferes with the synthesis and proper glycosylation of these and new virus-induced polypeptides, and consequently with their function in DNA replication. Tunicamycin, a potent and relatively selective inhibitor of glycosylation (Chatterjee et al., 1979) also inhibited HCMV-induced DNA synthesis under the conditions specified in Results. This observation supports the view that inhibition of cellular glycosylation activity might be the crucial mechanism of this novel dGlc effect. Further experiments are, however, needed to clarify this point.

Mechanisms different in specificity and/or sensitivity have to be involved in DNA replication of HSV-1, a member of the same virus group. A key to the understanding of this difference might be that HCMV induces host cell macromolecular synthesis prior to viral DNA replication, a process which is also abolished by dGlc. HSV-1, on the other hand, switches off host cell synthesis soon after infection (Ludwig & Rott, 1975). The effect of dGlc might thus be a consequence of its interference with synthesis and/or function of a (glycosylated host cell) polypeptide essential for virus-induced DNA replication.

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