Synthesis of Cytomegalovirus DNA Is an Antiviral Target Late in Virus Growth

By A. S. TYMS,* J. M. DAVIS, J. R. CLARKE AND D. J. JEFFRIES
Division of Virology, Department of Medical Microbiology, St Mary's Hospital Medical School, Paddington, London W2 1PG, U.K.

(Accepted 10 February 1987)

SUMMARY

The mechanism of action of 9-(1,3-dihydroxypropoxymethyl)guanine (DHPG) and phosphonoformic acid (PFA) but not 5-fluorouridinedeoxyribose (FUdR), provides selective action against cytomegalovirus (CMV)-coded events and this was used to demonstrate that the synthesis of viral DNA was continuous during the extended phase of virus growth. The synthesis de novo of viral DNA was measured by restriction enzyme analysis after exposure to $[^{32}P]$orthophosphate and its interruption by DHPG or PFA resulted in a cessation in the extrusion of infective virus from treated cells. The rate of decline in infectivity appeared to correspond to the failure of cells to maintain the synthesis of late proteins once DNA synthesis was blocked. Thus, regulation of late protein synthesis appeared to be linked to synthesis de novo of viral DNA even at late stages in CMV growth. The synthesis of the polyamines spermidine and spermine, considered obligatory for CMV growth, was unaffected by early or late inhibition of viral DNA and this showed that some virus-induced events were unaffected by the restriction on virus growth by DHPG. This provided evidence that polyamine biosynthesis was a target independent of viral DNA synthesis per se, which may be important in future considerations of combined drug therapies.

INTRODUCTION

Herpesviruses are a major cause of disease in man. Although palliative antiviral chemotherapy is now available for herpes simplex virus (HSV) (Fiddian et al., 1984), infection with these viruses is complicated by their ability to induce latent or persistent infections (Stevens, 1975). Human cytomegalovirus (CMV) is a major cause of congenital or post-natal infections (Stagno et al., 1975) and is also an important opportunistic pathogen in the immunocompromised patient (Meyers et al., 1982). After intrauterine or neonatal infection, virus is excreted in the urine of infected babies to high titre for months or years of early life. Between 10 and 20% of babies who excrete virus at birth are symptomatic and some of the remainder, who are initially asymptomatic may develop disabilities in later life, such as sensorineural deafness (Peckham et al., 1983). If appropriate drugs can be developed to interrupt this persistent virus infection, it may be possible to modify or prevent disease.

Little is known about the nature of persistent infections with CMV, although there is evidence that the virus is harboured in various tissues such as kidney, liver, salivary glands and cervix and probably in cells of leukocyte lineage (Jordan, 1983). Virus is continuously excreted in the presence of circulating antibody (Reynolds et al., 1973) but cell-mediated immunity may be specifically impaired (Gehrz et al., 1977). Infected cells could, therefore, persist in organs and tissues protected from the host immune system, the long-term viability and productivity of such cells being ensured by the ability of CMV to induce host cell macromolecular synthesis. In cells infected in culture, this is reflected by increased levels of ornithine decarboxylase activity (Isom, 1979) with a marked increase in the synthesis of the aliphatic amines putrescine, spermidine and spermine (Tyms & Williamson, 1980; Tyms et al., 1983; Clarke & Tyms, 1986). The synthesis of

0000-7430 © 1987 SGM
these polyamines appears to be obligatory for CMV replication (Tyms & Williamson, 1982; Gibson et al., 1984).

The response of persistent infections with CMV to antiviral action has been difficult to study due to a lack of effective drugs of sufficiently low toxicity. Recently it has been shown that 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) has potent activity against CMV in vitro (Smee et al., 1983; Mar et al., 1983; Tyms et al., 1984a) with low toxicity (Cheng et al., 1983; Tyms et al., 1984a). Most important, this drug was effective in treating CMV infection in vivo (Shepp et al., 1985; Felsenstein et al., 1985; Bach et al., 1985; Masur et al., 1986; Collaborative DHPG Treatment Study Group, 1986).

In the present study, we have investigated the antiviral activity of DHPG, 5-fluorodeoxyuridine (FUdR) and phosphonoformic acid (PFA), during long-term CMV infection in a cell culture model. FUdR and PFA also have antiviral activity against CMV (Goodheart et al., 1963; Wahren & Oberg, 1980) but with a different mode of action to DHPG and both have been clinically evaluated (Cangir et al., 1967; Feigin et al., 1971; Apperley et al., 1985; Ringden et al., 1985).

Evidence is presented here that the synthesis of viral DNA is continuous during virus growth and is essential to maintain long-term production of infective virus. The results suggest that this may provide a constant target for antiviral drugs and may be of importance in treating persistent CMV infections in man.

METHODS

Cells. Human embryo fibroblast cells (HEF) used were either MRC-5 cells or the WF strain of skin/muscle embryonic fibroblasts generated in this laboratory. Growth and maintenance of cells were as described previously (Tyms et al., 1984b).

Viruses. Most experiments were carried out with the prototype strain of human cytomegalovirus, AD169. A laboratory-adapted strain RA and low-passage clinical isolate CX1-Air were used in some experiments. Virus suspensions were prepared and titrated as previously described (Tyms & Williamson, 1980). Cell monolayers were infected at an m.o.i. of 5 to 10 p.f.u./cell by adsorption for 2 h at 20°C.

Drugs and radiochemicals. The acyclic nucleoside DHPG was provided by Wellcome and Syntex Corporation, Palo Alto, Ca., U.S.A. FUdR and PFA were purchased from Sigma. Sterile stocks were prepared and stored at -20°C. [%P]Orthophosphate (carrier-free), L-[35S]methionine (1170 Ci/mmol), [1,4-14C]putrescine (109 mCi/mmol) and restriction enzymes were purchased from Amersham.

DNA analysis. Infected cells were maintained in low-phosphate medium (10−5 M-NaHPO4) and exposed to [%P]orthophosphate (15 μCi/ml) at various times post-infection (p.i.) for 24 h periods. Cells were harvested into 0·1 × SSC, lysed in 1% sodium laurylsarcosinate, deproteinized and the total DNA was precipitated with ethanol. For restriction enzyme digestion, equal amounts of total DNA were used in each digestion which was carried out in accordance with manufacturers' instructions. Full details of this protocol are described elsewhere (Tyms et al., 1984b).

Polypeptide analysis. Infected and uninfected cells were exposed to [35S]methionine (10 μCi/ml) for 2 h at selected times, harvested and stored frozen before analysis by discontinuous SDS-PAGE (Tyms et al., 1984b). Polypeptides were separated in 10% acrylamide gels and transferred electrophoretically to nitrocellulose (Hybond, Amersham), stained with 0·1% amido black and subjected to autoradiography (Fuji RX film).

Polyamine analysis. Infected and uninfected cells were harvested at various times after pre-exposure to [14C]putrescine (0·2 μ Ci/ml) for 24 h. The levels of putrescine, spermidine and spermine were determined by HPLC (Clarke & Tyms, 1986). Briefly, polyamines were extracted by precipitation with TCA, reacted with benzoyl chloride and the derivatives separated using a reverse phase C8 column (Spher RP 8, Chrompak) and a Waters HPLC system. 1,6-Diamine hexane was used as the internal standard. Peak fractions were collected and the radioactivity was determined by liquid scintillation counting.

RESULTS

Virus growth and DNA synthesis

To monitor the growth characteristics of CMV in cell culture, HEF cells were infected at high multiplicity and cell-associated or cell-free infectivity was determined at intervals of 24 h. A daily change of maintenance medium resulted in a rate determination for cell-free virus. At corresponding times, cell cultures were exposed to [%P]orthophosphate (15 μCi/ml) for 24 h and the total DNA content of cells was extracted. Radiolabelled viral DNA was quantified by
Late synthesis of CMV DNA and DHPG treatment

Fig. 1. HEF cells, infected at a multiplicity of 5 p.f.u./cell with AD169 virus, received a daily medium change. (a) Cell-associated (●) and cell-free (○) infectivity was determined by plaque assay. (b) Beginning 4 days p.i. parallel cultures were radiolabelled with 15μCi/ml ³²Porthophosphate (carrier-free) for 24 h periods on successive days (lanes 1 to 4 respectively). After each pulse period, cells were harvested, DNA was extracted and viral DNA resolved by restriction enzyme analysis and agarose gel electrophoresis. On separation of the DNA fragments after cleavage with EcoRI, the gel was dried and an autoradiographic image formed at -70°C using intensifying screens and Fuji X-ray film.

Table 1. Effect of FUdR (100μM) treatment on cell-associated and cell-free infectivity titres

<table>
<thead>
<tr>
<th>Time post-infection (days)</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) Cell-associated virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ra virus control</td>
<td>6.61 x 10⁴</td>
<td>4.17 x 10⁵</td>
<td>1.0 x 10⁶</td>
<td>1.2 x 10⁶</td>
<td>–</td>
<td>1.32 x 10⁶</td>
</tr>
<tr>
<td>Treated 72 h p.i.</td>
<td>–</td>
<td>1.74 x 10⁵</td>
<td>1.74 x 10⁵</td>
<td>2.09 x 10⁵</td>
<td>8.7 x 10⁴</td>
<td>–</td>
</tr>
<tr>
<td>Treated 120 h p.i.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.4 x 10⁶</td>
<td>9.12 x 10⁵</td>
<td>5.5 x 10⁵</td>
</tr>
<tr>
<td><strong>(b) Cell-free virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ra virus control</td>
<td>2.1 x 10⁴ (100%)</td>
<td>2.3 x 10⁵ (100%)</td>
<td>2.1 x 10⁶ (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated 120 h p.i.</td>
<td>2.0 x 10⁴ (95%)</td>
<td>6.9 x 10⁵ (30%)</td>
<td>8.3 x 10⁵ (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD169 virus control</td>
<td>4.5 x 10⁴ (100%)</td>
<td>7.5 x 10⁵ (100%)</td>
<td>1.5 x 10⁶ (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated 120 h p.i.</td>
<td>5.0 x 10⁵ (111%)</td>
<td>2.4 x 10⁶ (32%)</td>
<td>5.5 x 10⁵ (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Maintenance medium with or without drug was changed 24 h prior to infectivity titres being measured.

restriction enzyme analysis (Fig. 1). The overall growth characteristics of CMV were as previously described with consistently high levels of cell-associated and cell-free infectivity. The extracellular levels were due to the constant release of virus, as deduced from the rate determination of infectivity in the extracellular medium. The continued synthesis of viral DNA during the extended period of virus growth was indicated by the ability to resolve newly labelled viral DNA late in the infection. Virus growth and de novo synthesis of viral DNA were measurable for up to 12 days p.i. (see below, and unpublished data) and overall this represented the dynamic state of CMV-infected cells in vitro.

Non-selective inhibition of DNA synthesis

In initial experiments, the nucleoside analogue FUdR was used to study the effect of DNA inhibitors on CMV replication. Concentrations of this compound between 10μM and 100μM
prevented virus growth entering the exponential phase if treatment was started at the time of infection. The inhibitory effect was readily reversed by the simultaneous addition of equimolar thymidine (results not shown).

To study the introduction of FUdR during the productive stage of CMV growth, infected cells were exposed to the drug (100 μM) during the exponential (3 days p.i.) or plateau (5 days p.i.) phases of growth and cell-associated infectivity was determined. Treatment at the later time produced about a twofold reduction in the level of infectivity whereas addition of the drug during the exponential phase caused a cessation in virus growth, although the infectivity extant within the cells appeared to be retained (Table 1a). This suggested a virostatic effect in the treatment with FUdR. The rate determination of infectivity used to monitor virus in the extracellular medium would depend on the continued extrusion of virus from infected cells. When FUdR was added to cells infected with either the Ra or AD169 strain of CMV at a time when the rate of extracellular virus levels was approaching maximum, virus infectivity was reduced 25-fold within 48 h (Table 1b). The drug had no effect in the presence of thymidine. These results indicate that the growth characteristics of CMV were altered after inhibition of DNA synthesis.

Selective inhibition of viral DNA synthesis

DHPG and PFA were examined for an inhibitory effect late in CMV replication. Cells infected with AD169 virus were exposed to DHPG (50 μM) or PFA (300 μM) when extracellular infectivity was approaching maximum levels. Infected cultures received fresh medium with or without drug at daily intervals and the extracellular infectivity was determined. Changes in the rate of extrusion of infectious virus in the presence of either of the two inhibitors was used to monitor the response to treatment (Fig. 2). The rate of decline in the extracellular infectivity after treatment with either drug was similar to that described above for FUdR (Table 1). The presence of DHPG, PFA or FUdR in the medium did not alter the stability of AD169 virus after overnight incubation in cell-free conditions (unpublished observation). The inhibitory effect of DHPG or PFA was an indication of a specific requirement for the synthesis of viral DNA late in virus growth.

Synthesis of viral DNA

To verify the inhibitory effect of DHPG (50 μM) or PFA (300 μM) on the synthesis of viral DNA, AD169-infected cells were exposed to \(^{32}\)Porthophosphate (15 μCi/ml) and the DNA was characterized by restriction enzyme analysis. Infected cells were treated with inhibitor beginning 5 days p.i. and radiolabelled for periods of 24 h before DNA was extracted and analysed for viral DNA (Fig. 3). The enzyme cleavage products were separated by gel electrophoresis; the accumulated viral DNA was resolved by ethidium bromide staining and newly synthesized DNA by autoradiography. Ethidium bromide fluorescence resolved DNA fragments in all lanes but autoradiographic images of restriction fragments were identified only

---

**Fig. 2.** At a time approaching maximum levels of extracellular infectivity, HEF cells previously infected with AD169 virus had a daily change of fresh medium containing (a) 50 μM-DHPG (6 days p.i.) or (b) 300 μM-PFA (7 days p.i.) or left drug-free. The rate of production of extracellular infectivity was determined by virus titration. Open circles represent drug-free controls and solid circles the drug-treated cells.
Late synthesis of CMV DNA and DHPG treatment

Fig. 3. HEF cells infected with AD169 and treated with DHPG (50 μM, lanes 2) or PFA (300 μM, lanes 3) at 5 days p.i. or left untreated (lanes 1) were exposed to 15 μCi/ml [32P]orthophosphate (carrier-free) for 24 h periods starting at (a) 0, (b), 1, (c) 2 and (d) 3 days after treatment. Cells were harvested, DNA was extracted and restriction enzyme cleavage products were separated by agarose gel electrophoresis. DNA fragments were stained by ethidium bromide (top panel) the gels were then dried and autoradiographic images formed at −70 °C with intensifying screens using X-ray film (bottom panel).

in those lanes which represented DNA from untreated, control cells. Similar observations were made when treatment began at 8 days p.i. or if FUdR was substituted for DHPG or PFA.

The results shown in Fig. 3 were confirmation that DHPG or PFA inhibited viral DNA synthesis and supported the view that this was necessary to maintain productive CMV infection.

Synthesis of viral protein

The results presented so far show that de novo synthesis of CMV DNA was essential to maintain the high rate of virus production seen late in virus growth. It was, therefore, of interest to determine the effects of DNA inhibitors, in particular DHPG, on the synthesis of late viral proteins which are normally in low abundance in the absence of viral DNA synthesis. Resolution of virus-coded, cell-associated proteins is complicated by the ability of CMV to stimulate the synthesis of host proteins although three viral proteins of mol. wt. 153K, 69K and 53K can be readily resolved (Gibson, 1983; Gibson & Irmiere, 1984). The synthesis of these abundant late proteins were used to monitor the effects of DHPG treatment.

The failure of infected cells to synthesize late proteins in the absence of viral DNA synthesis
Fig. 4 (a) HEF cells were either infected with AD169 virus or mock-infected and treated with 50 μM-DHPG (lanes 2) at the time of infection or left untreated (lanes 1). At 8 days p.i. cells were exposed to [35S]methionine for 2 h at 37 °C and polypeptides analysed by SDS-PAGE in a 10% running gel. The presence of the major capsid protein (153K), matrix protein (69K) and DNA-binding protein (53K) was detected by autoradiography. (b) HEF cells were either infected with AD169 virus or mock-infected and treated with 50 μM-DHPG (lanes 1) or left untreated (lanes 2) beginning 5 days p.i. On days 2 and 3 post-treatment, cells were exposed to [35S]methionine for 2 h at 37 °C and polypeptides analysed by SDS–PAGE and autoradiography. In both experiments, the amount of sample loaded onto the gel was similar for each track as determined by amido black staining after electrophoretic transfer to nitrocellulose.

was evident after treatment of infected cells with DHPG from the time of infection. Drug-treated cells along with infected and uninfected control cells were exposed to [35S]methionine (10 μCi/ml) for 2 h at 8 days p.i. In the virus control cells, separation by electrophoresis and the use of autoradiography identified the major capsid protein (153K), the matrix protein (69K) and the DNA-binding protein (53K), although in some gels the 53K protein was less abundant (Fig. 4a). The profile of polypeptides after treatment with DHPG from the time of infection resembled uninfected control cells by the absence of identifiable viral proteins (Fig. 4a).
Late synthesis of CMV DNA and DHPG treatment

Table 2. The effect of early treatment with DHPG (50 μM) on the synthesis of spermidine and spermine induced by infection with AD169 virus

<table>
<thead>
<tr>
<th>Cell</th>
<th>Polyamine production (nmol/10^6 cells)</th>
<th>Spermidine : spermine</th>
<th>D.p.m.*/ 10^6 cells</th>
<th>Specific activity (mCi/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected Spermidine</td>
<td>2-32</td>
<td>1-23</td>
<td>3432</td>
<td>1.33</td>
</tr>
<tr>
<td>Uninfected Spermine</td>
<td>1-88</td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Uninfected with DHPG Spermidine</td>
<td>2-38</td>
<td>1-36</td>
<td>3732</td>
<td>1.40</td>
</tr>
<tr>
<td>Uninfected with DHPG Spermine</td>
<td>1-75</td>
<td></td>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td>Infected Spermidine</td>
<td>4-21</td>
<td>0-82</td>
<td>16964</td>
<td>3.63</td>
</tr>
<tr>
<td>Infected Spermine</td>
<td>5-16</td>
<td></td>
<td>5364</td>
<td>0.94</td>
</tr>
<tr>
<td>Infected with DHPG† Spermidine</td>
<td>4-84</td>
<td>0-92</td>
<td>19156</td>
<td>3.57</td>
</tr>
<tr>
<td>Infected with DHPG† Spermine</td>
<td>5-28</td>
<td></td>
<td>3736</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* Polyamines synthesized from [14C]putrescine (0.2 μCi/ml) added 24 h prior to harvesting on day 4 p.i.
† Virus growth was completely inhibited at 4 days p.i.

clear from the untreated, control cells that the synthesis of late viral proteins was continuous during virus production.

Experiments to determine the effect of treating productively infected cells with DHPG involved the addition of inhibitor at the plateau stage in virus growth. At 5 days p.i., treated and untreated cells were pulse-labelled with [35S]methionine for 2 h at 24 h intervals and prepared for gel electrophoresis. Results showed little change in the rate of protein synthesis after exposure to DHPG for 24 h. However, as treatment continued, a progressive drop in the synthesis of all major late proteins was evident, with only the 69K matrix protein being synthesized in abundance after 72 h exposure to DHPG (Fig. 4b). It was apparent from three independent experiments that the rate of incorporation of [35S]methionine into host cell proteins in the infected cells was also significantly reduced after 72 h of treatment. Amido black staining of total proteins after electrophoresis confirmed that similar quantities of cell extract had been loaded into each track. In overall terms the constant rate of synthesis of late viral proteins in the virus controls matched the constant rate of infectious virus production whereas the apparent rate of decline in viral protein synthesis after treatment correlated with the loss of infectivity in the extracellular state (see Fig. 2). It was concluded that late treatment of CMV-infected cells with DHPG resulted in a major restriction on the synthesis of virus-coded and virus-induced host-coded proteins.

DHPG treatment and virus-induced polyamine biosynthesis

Infected and uninfected control cells were exposed to [14C]putrescine (0.2 μCi/ml) by overnight incubation in the presence or absence of the drug, before harvesting at 4 days p.i. The total polyamine content of the cells was determined as the benzoylated derivatives and the radiolabelled spermidine and spermine was collected from respective samples and quantified by liquid scintillation counting. Increased polyamine biosynthesis was observed in the infected series with or without drug (Table 2). As previously reported (Tyms et al., 1983; Clarke & Tyms, 1986), the high spermine content was evident by the characteristic reduction in the spermidine to spermine ratio when compared with uninfected controls. The rate of polyamine biosynthesis was indicated by the 2-5-fold or greater increase in the specific activities of spermidine and spermine. This stimulation of polyamines by CMV infection appears to explain the finding of raised levels of spermine in the urine of babies infected with this virus (J.R. Clarke & A.S. Tyms, unpublished observations).

If the synthesis of polyamines in CMV-infected cells continued after late as well as early addition of DHPG this would provide evidence for a metabolically active host cell in conditions where virus-specific events were significantly affected.

HEF cells infected with a low-passage CMV isolate (CX1-Air) were exposed to DHPG (50 μM) at 5 days p.i. or left untreated and polyamine biosynthesis was measured on consecutive days after overnight exposure to 0.2 μCi/ml [14C]putrescine. In the control cells, the rate of
polyamine biosynthesis, as deduced from the specific activities of spermidine and spermine measured on successive days, was progressively lowered over the 3 day study period (Fig. 5). The overall pattern of polyamine biosynthesis was unchanged by treatment with DHPG even though the rate of extracellular virus production was reduced by more than 100-fold during 3 days of drug treatment. In quiescent, uninfected cells the level of polyamine biosynthesis remained constant as previously described (Tyms et al., 1983) and this is illustrated in Fig. 5. Cells infected with strain CX1-Air and exposed to DHPG (50 µM) at the time of infection showed no signs of virus growth at 8 days p.i. but, as expected from the results reported above, both spermidine and spermine synthesis was unaffected when measured between 7 and 8 days p.i. Similar results were recorded in separate experiments using cells infected with AD169 virus instead of CX1-Air.

DISCUSSION

Previous clinical experience with non-discriminating drugs, such as idoxuridine (Conchie et al., 1968), cytosine arabinoside (Plotkin & Stettler, 1969) and adenine arabinoside (Baublis et al., 1975), suggested that CMV infection could be modulated by an inhibitory action on DNA synthesis. Once phosphorylated, nucleotide analogues tend to interfere with DNA synthesis as competitive inhibitors or allosteric effectors. FdUrd is also non-selective in its action but is of interest in view of its ability to bind irreversibly to thymidylate synthetase (Santi et al., 1974), a key enzyme in the endogenous pathway for nucleotide biosynthesis. The marked sensitivity of CMV replication to this inhibitor in cells unable to scavenge thymidine, shown here and in previous studies (Goodheart et al., 1963), implied that virus growth depended heavily on de novo synthesis of nucleotides and corresponds to the highly energetic state of host cells during infection (see Isom, 1979). It may be related to this that CMV appears to be less destructive than other herpesviruses, which would facilitate the persistence of CMV-infected cells in vivo and might explain the long-term virus excretion seen in infected babies (Stagno et al., 1975). Most important, such a strategy for virus replication appears to offer targets late in virus growth for antiviral action.

The catalytically activated, irreversible mode of action described for FdUrd is an important concept in antimicrobial chemotherapy but the action of selective inhibitors such as PFA and
DHPG against CMV is of major interest. The structural similarity of PFA to pyrophosphate suggests the compound may function competitively during nucleotide polymerization with selectivity for the virus-coded DNA polymerase (Wahren et al., 1985). Likewise, DHPG triphosphate was a more potent inhibitor of the viral DNA polymerase than of cellular a-polymerase (Mar et al., 1985) although the nature of the enzyme that catalyses the formation of the monophosphate, an important factor in the selective action of the drug against HSV infections (Cheng et al., 1983), is not yet clearly defined.

The selective action of DHPG and PFA provide evidence that de novo synthesis of viral DNA is essential to maintain the production of infectious virus late in virus growth. In contrast, the replication of HSV became insensitive to treatment with DHPG (Cheng et al., 1983) before maximum virus titre was reached and similar observations were made with vaccinia virus (Salzman, 1960) using FUdR as the inhibitor. In both cases, the phase of viral DNA synthesis appeared to be completed before the major period of virion maturation. In the assembly of adenovirus temperature-sensitive mutants, only newly synthesized genomes were incorporated into virions in conditions favouring DNA degradation (Weber et al., 1985) and this may be the case during CMV maturation. Degradation or modification of CMV DNA is known to give rise to non-genomic length molecules (Stinski et al., 1979; Ramirez et al., 1979) and defective non-infectious particles (Ramirez et al., 1979). Aberrant DNA molecules were indicated by altered restriction enzyme profiles (Stinski et al., 1979) but this was not the case in our studies when total viral DNA was analysed after late treatment with DHPG or PFA (Fig. 3). This suggests that genome length molecules are in abundance in CMV-infected cells during such treatment.

If degradation of viral DNA due to DNase activity is involved in the continuous requirement for newly synthesized genomes then control of such enzyme activity may be important. Polyamines are positively charged molecules that readily associate with nucleic acid (Pegg & McCann, 1982) and this appears to influence DNase activity. Both the exonuclease and endonuclease activities induced by HSV infection were inhibited by spermine and spermidine with the endonuclease activity sensitive to a 10-fold lower concentration of spermine (Hoffmann & Cheng, 1979; Cheng et al., 1979). Likewise, chromosomal damage in adenovirus-infected cells was accounted for by virus-induced nuclease activity in conditions of low abundance of spermine (Cheetham et al., 1982) which was reversed by exogenous provision of the polyamine. Cells infected with either adenovirus or HSV showed an inhibition of polyamine biosynthesis (Cheetham & Bellett; 1982; Isom, 1979) but CMV infection caused a marked stimulation of this pathway (Tyms & Williamson, 1980; Tyms et al., 1983; Clarke & Tyms, 1986). The rate of polyamine biosynthesis appeared unaffected by DHPG treatment and infected cells were abundant in polyamines, particularly spermine, which was evidence that CMV was not exposed to conditions that appear to favour DNase activity. In fact, the polyamine pathway may offer distinct targets for antiviral chemotherapy (for review, see Tyms & Williamson, 1986), which are independent of viral DNA synthesis per se and may be applicable to regimens for combined drug therapies.

Assuming genome stability, then late inhibition of virus growth by DHPG may have been mediated through changes in the expression of genes coding for viral structural proteins. Transcription of the CMV genome is a sequential process through immediate early, early and late times (Stinski, 1978). The synthesis of late proteins was not detected when initiation of viral DNA synthesis was blocked in non-permissive cells (Stinski, 1978; Mocarski & Stinski, 1979) or by DNA inhibitors (Fig. 4; Stinski, 1978; Mar et al., 1983). This regulation of late protein synthesis appeared to be at a post-transcriptional level (Geballe et al., 1986). The genes coding for the 53K DNA-binding protein and 69K matrix protein were transcriptionally active to the same extent at early (4 h p.i.) and late (48 h p.i.) times but only low levels of both proteins were made at the early time. It is apparent from the results of the present study that the post-transcriptional events that control late protein synthesis are subject to down-regulation in cells unable to synthesize viral DNA late in virus infection. The mechanism involved in this novel regulation is being investigated further. A fuller understanding of the response of CMV infections to treatment may help to optimize drug regimens, which could be important if prophylaxis is difficult or expensive to maintain.
We wish to thank Mrs Helen Brown for her expertise in preparing the manuscript. We also thank Wellcome Research Laboratories, Beckenham, U.K. and Syntex Corporation, Palo Alto, U.S.A. for provision of DHPG. The work was supported by grants from Action Research for the Crippled Child, Syntex Corporation and an anonymous donor.

REFERENCES


Late synthesis of CMV DNA and DHPG treatment


(Received 28 August 1986)