Stimulation of Cellular Thymidine Kinases by Human Cytomegalovirus

JOHN E. ESTES* AND ENG-SHANG HUANG

Department of Medicine and The Cancer Research Center, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

Received for publication 20 May 1977

Thymidine kinase (TK) activity in WI-38 and MRC-5 human fibroblasts was analyzed by discontinuous polyacrylamide gel electrophoresis (disc-PAGE) and discontinuous glycerol gradient electrophoresis (disc-GEP) after subculture or human cytomegalovirus (HCMV) infection. Two peaks of TK activity with different relative fraction-of-migration (R_f) values were resolved by disc-PAGE or disc-GEP in extracts from log-phase and infected cells. Growing WI-38 cells expressed a slowly migrating ($R_f = 0.14$ PAGE, $R_f = 0.4$ GEP) peak of TK activity, which was partially inhibited by 1.0 mM dCTP, but which retained little activity at pH 4.5. Growing MRC cells also displayed a slowly migrating peak ($R_f = 0.10$ PAGE) with similar properties. Both cell types expressed a faster-migrating TK activity ($R_f = 0.45$ PAGE, $R_f = 0.7$ GEP) in the growing and resting state that was strongly inhibited by 1 mM dCTP but retained 50% activity at pH 4.5. When either cell type was infected with HCMV, there was a rapid and high-level stimulation of the slowly migrating form of TK and a slight stimulation of the faster-migrating form. Two strains of HCMV (AD169 and Town) failed to produce an electrophoretically distinct virus TK in either cell type after infection. TK enzymes were partially purified by disc-GEP from extracts of log-phase WI-38 or AD169-infected WI-38 cells. Characterization of these enzymes with respect to phosphate donor specificity, pH optima, thermostability, and salt inhibition showed the HCMV-stimulated TKs to be of cellular origin.

The process of DNA polymerization in vivo and in vitro depends upon adequate deoxynucleoside 5'-triphosphate concentrations for maximal rates of DNA synthesis (5). This dependence holds for procaryote, eucaryote, and virus-induced polymerases, including herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV) (3, 26, 27). Several new enzyme activities involved in DNA metabolism are found in HSV-1-infected cells within hours after infection. DNA polymerase (15, 26), DNase (J. M. Morrison and H. M. Keir, *Biochem. J.* 103:70P, 1967), thymidine kinase (TK), deoxycytosine kinase (14, 16), and ribonucleotide reductase (22) activities appear to be coded by the viral genome and represent a considerable investment of the nonstructural viral information in the HSV-1 system. Although the importance of HSV-1 TK has been questioned because mutant HSV-1 strains that do not produce virus TK grow to high titer in cells with very low TK activity, the HSV-1 DNase is capable of providing thymidylate and other 5'-deoxynucleotides through exonucleolytic degradation of host cell DNA as an alternate path-

way (Morrison and Keir, *Biochem. J.* 103:70P, 1967). It appears, then, that HSV-1 has evolved several overlapping pathways in order to insure nucleoside triphosphate precursors for the viral DNA polymerase. Studies of HCMV-infected cells have shown the presence of a virus-specific DNA polymerase, as well as a stimulation of all three host cell DNA polymerase activities (12). Our preliminary results (presented at the Third Meeting on Herpesviruses, Cold Spring Harbor, N.Y., 3 September 1976) and published data (29) indicate that HCMV strain AD169 does not induce a virus-specific TK, but that cellular TK is stimulated by AD169 infection.

MATERIALS AND METHODS

Cells and virus. AD169 (passages 290 to 295 [23]) and Town (passages 30 to 35 [9]) strains of HCMV were adapted and propagated in WI-38 (ATCC CCL75) and MRC-5 (ATCC CCL171) human fibroblasts. Cells were cultivated in Eagle minimal essential medium (MEM) with 15 mM *N*-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES)-NaOH (pH 7.2), 100 U of penicillin per ml, 100 μ g of streptomycin per ml, and 2 mM glutamine. Heat-inactivated fetal calf serum (Kansas City Biological

was used in MEM at concentrations of 10% for growth and 3% for maintenance. WI-38 cells (passages 24 to 28) and MRC-5 cells (passages 22 to 26) were grown in 75-cm² plastic flasks (Falcon Plastics) or 840-cm² disposable glass roller bottles (Wheaton).

Virus infection. (i) Confluent monolayers of WI-38 or MRC-5 cells in flasks or roller bottles were washed once with Hanks balanced salt solution containing 10 mM HEPES (pH 7.2) and then inoculated with 2 to 10 PFU of HCMV per cell and adsorbed for 60 min at 37°C. Monolayers were washed with balanced salt solution containing 10 mM HEPES (pH 7.2), fed with maintenance MEM, and incubated at 37°C. The end of virus adsorption was taken as time zero of infection. Sham-infected cells were inoculated with a volume of maintenance MEM equal to the volume of the virus inoculum.

(ii) Three volumes of the stock Town inoculum (Fig. 3) were centrifuged in an SW50.1 rotor at 39,000 rpm for 60 min at 4°C to pellet virus. The top 1/3 volume was used as virus-cleared supernatant, the middle 1/3 was discarded, and the pellet was suspended in the bottom 1/3 volume for use as 3 $\times$ -concentrated virus. WI-38 cells were infected as above with either the cleared or the 3 $\times$ -concentrated virus.

TK extraction. At various times after infection or sham infection, duplicate 75-cm² flasks or 840-cm² roller bottles of cells were harvested by washing twice with Hanks solution without Ca²⁺ and Mg²⁺ and incubated at 25°C with 0.08% trypsin, 2 ml per flask or 8 ml per roller bottle. Cells were dislodged after 10 to 15 min by gentle tapping and were suspended in 8 ml of growth MEM containing 0.05 M epsilon amino caproic acid on ice. Cells were pelleted at 200 $\times$ g for 10 min at 4°C; the pellet was then suspended in 0.5 ml of extraction buffer containing 0.01 M Tris-hydrochloride (pH 8.0), 0.15 M KCl, 0.0014 M β -mercaptoethanol, 0.01 M ATP, 0.0002 M thymidine (TdR), and 1% Nonidet P-40. After 15 min at 0°C and several cycles of high-speed Vortex mixing, the cell lysate was centrifuged at 150,000 $\times$ g for 1 h at 2°C. The supernatant fluid was decanted, divided into equal portions, and stored over liquid nitrogen for up to 1 week before discontinuous polyacrylamide gel electrophoresis (disc-PAGE). Protein concentration was determined by the method of Lowry et al., with bovine serum albumin as standard (20).

Disc-PAGE. The system used was that of Kit et al. (17), except that the separating gels were 5 by 100 mm and the samples (50 μ l containing 150 μ g of protein) were applied in stacking-gel buffer with 10% glycerol and 0.001% bromophenol blue. Electrophoresis was terminated when the marker dye was within approximately 1 mm of the bottom of the tube, and the gel was cut into 2.5-mm slices (about 40 slices per gel) for enzyme assay.

Preparative glycerol electrophoresis (GEP). The buffers used were identical to the disc-PAGE system above, but a gradient (15 to 60%) of glycerol was substituted for the 5% acrylamide gel. The gradient column (100 by 6 mm) was poured in six steps of 0.5 ml each. Samples containing from 0.25 to 1.0 mg of protein in 200 μ l of stacking buffer with 0.001%

bromophenol blue and 7.5% glycerol were layered onto the gradient and electrophoresed at 3 mA/tube at 4°C until the marker dye reached the bottom of the gradient. Thirty-five 100- μ l fractions were collected from the bottom of the tube, and 5- or 10- μ l portions were used for assay.

TK assay. Each gel slice or 10- μ l portion of the GEP fractions was incubated with 100 μ l of TK reaction mixture {0.1 M Tris-maleate (pH 7.0), 0.025 M KCl, 0.01 M MgCl₂, 0.0014 M β -mercaptoethanol, 0.015 M ATP, 0.004 M NaF, and 0.024 mCi of [³H]thymidine per ml (specific activity, 60 Ci/mmol [4×10^{-7} M])}. Cold TdR was in the reaction at a concentration of 0.02 mM (2×10^{-5} M) in the GEP assay and 0.05 mM (5×10^{-5} M) for gel slices. Reactions were carried out in multiwell plastic plates (Linbro MVC-96) at 36°C. GEP fractions were incubated for 30 min, and gel slices were incubated for 60 min; the reaction was stopped by cooling to 0°C on ice. The entire reaction mixture from each well was soaked up by 25-mm DEAE-cellulose disks (DE-81, Whatman), and the disks were washed four times with 0.001 M ammonium formate buffer (pH 3.6) by filtration and twice with 95% ethanol. After drying, radioactivity bound to each filter was determined in a liquid scintillation counter at a ³H efficiency of about 6%. Radioactivity of 10³ cpm/h equaled 1.66 nM/h for gel and 0.65 nM/h for GEP fractions ([³H]TdR $\rightarrow$ [³H]TMP). Enzyme pH optima, phosphate donor specificity, and feedback inhibition were determined by pooling the two highest activity fractions of each GEP peak, dialyzing against extraction buffer without ATP, and incubating 5- μ l portions of the pooled enzyme with 100 μ l of the standard reaction mixture containing 0.1 M Tris-maleate or Tris-hydrochloride and nucleoside phosphates in place of ATP, as listed in Table 1, for 2 h at 36°C. Heat inactivation and salt inhibition studies were also performed on these dialyzed, pooled peak fractions.

Chemicals and radiochemicals. All nucleosides and nucleoside 5'-triphosphates were obtained from Sigma Chemical Co., St. Louis, Mo. [methyl-³H]thymidine in a sterile aqueous solution (specific activity, 60 Ci/mmol) and β -mercaptoethanol were obtained from Schwarz/Mann, Orangeburg, N.Y. Nonidet P-40 (1%) was obtained from BDH Chemicals Ltd., Poole, England. All other chemicals were reagent grade.

RESULTS

Cellular TK activity. As previously reported (17), mammalian cells contain two major TK activities separable by disc-PAGE: a rapidly migrating enzyme with low specific activity in dividing and resting cells associated with mitochondria, and a slowly migrating enzyme with high specific activity from the cytoplasm of dividing cells. Other investigators have shown the cell cycle dependence of the cytoplasmic enzyme, which shows maximal activity in fetal tissue and after cells are stimulated to divide *in vitro* (1, 25). Figure 1 shows time-course studies of the PAGE profile of TK activity in freshly

passed (1:2) WI-38 and MRC-5 cells compared with resting cells. The slowly migrating or cytosol enzyme has a different relative fraction-of-migration (R_f) value, depending on cell type. The cytosol enzyme of WI-38 migrates with an R_f of 0.14, whereas the corresponding activity

in MRC-5 cells migrates slightly slower, with an R_f of 0.10. This difference in migration of the cytosol enzymes was repeatable, whereas the mitochondrial activities showed the same R_f value of 0.45 regardless of cell type. In WI-38, both the cytosol and mitochondrial enzyme ac-

FIG. 1. Disc-PAGE electropherograms of TK extracts of 1:2-passaged WI-38 (left column) and MRC-5 (right column) cells run and assayed for TK per Materials and Methods. Rows from top to bottom represent resting cells, 12, 24, 48, 72, and 96 h after passage. The mean of lowest sample counts from each gel (400 cpm/sample) was subtracted as background. Protein (150 μ g) was loaded onto each gel.

tivities rose after cell passage to maximal values of 10.5 and 1.5 times their resting levels, respectively, 48 h after trypsinization. MRC-5 cells showed a 12.0-fold increase of the R_f 0.10 cytosol enzyme at 48 h and a 5.6-fold increase in the R_f 0.45 mitochondrial enzyme at 72 h. As the cells approached confluence (96 h), the activities of cytoplasmic and mitochondrial enzymes decreased toward resting levels. The enhancement and decay of the mitochondrial enzyme appeared to lag behind that of the cytoplasmic enzyme, especially in the MRC-5 cells.

Virus-infected cells. (i) Infection with either the AD169 or Town strain of HCMV produced a marked stimulation of TK in both MRC-5 and WI-38 cells. Assays performed on portions (5 μ l) of crude enzyme extracts of infected cells demonstrated a rapid increase in the specific activity of TK, beginning at 12 h and plateauing at 72 h after infection (data not shown). Sham-infected cells showed no such increase. PAGE separation of the crude extract resolved two peaks of TK activity. Figure 2 shows a maximal 38-fold increase in the R_f 0.10 activity in MRC-5 cells infected with the Town strain at 48 h postinfection. Enzyme enhancement was seen at 12 h and extended through 72 h after infection. The R_f 0.45 enzyme was also stimulated, reaching a peak of 6.8 times the sham-infected levels 48 h after infection. Similar results were obtained with AD169-infected MRC-5 cells (data not shown), in that the R_f 0.10 enzyme activity increased to a maximum of 16 times the sham levels, and the R_f 0.45 enzyme activity increased to a maximum of 10 times the sham level.

When WI-38 cells were infected (Fig. 3) with AD169, the R_f 0.14 TK activity increased 28.7-fold and the R_f 0.45 peak increased 3.6-fold over sham-infected levels at 48 h postinfection. Town strain infection of WI-38 showed a 40-fold stimulation of the R_f 0.14 and a 3.6-fold stimulation of the R_f 0.45 enzyme activity at 48 h postinfection. The stimulation of cellular TK activity by HCMV resulted in higher TK specific activities than 1:2 split cells and a prolonged elevation of these higher enzyme levels, especially evident in the Town strain-infected WI-38 cells.

(ii) Twenty-four-hour postinfection extracts of WI-38 cells that were inoculated with virus-cleared supernatant or 3 $\times$ -concentrated virus were compared by PAGE. With concentrated virus, both cellular TK forms were highly stimulated, exhibiting a positive dose-response effect to particles present in the virus inoculum. The virus-free supernatant fluid showed no stimulation of either host enzyme at 24 h after inoculation, whereas the 3 $\times$ -concentrated virus

inoculum stimulated both cell enzymes faster and to higher levels (R_f 0.14 = 2.4-fold, R_f 0.45 = 1.5-fold) than 1 $\times$ -concentrated virus at the same time after infection (data not shown).

Although significant viral DNA synthesis occurs as early as 23 h after infection (13) under these conditions, the PAGE system showed no evidence of any new (with unique electrophoretic mobility) TK activity up to 72 h after infection, a time beyond the peak of viral DNA synthesis (data not shown). MRC-5 cells infected at a low multiplicity (0.1) with AD169 virus also showed no evidence of a new enzyme activity at 12 days after infection, when 4+ cytopathic effect was present; however, there was a 16-fold increase in the R_f 0.10 activity and a 10-fold increase in the R_f 0.45 peak activity. To determine if a viral TK activity was superimposed over one of the cell enzyme peaks, we subjected samples of AD169-infected WI-38 and uninfected, passaged WI-38 extracts showing maximum enzyme activity (usually 48 h postinfection or postpassage) to preparative electrophoresis in glycerol gradients. Although disc-GEP gave broader peaks and different R_f values than PAGE, there was clear separation of the two cellular enzyme peaks (Fig. 4). Different R_f values for the same enzyme illustrate the fact that disc-GEP resolved a protein mixture more on the basis of charge than molecular size when compared with the disc-PAGE system, which uses a molecular-sieving gel (21). As in the PAGE system, the pattern of enzyme activity in HCMV-infected cells subjected to disc-GEP closely resembled the pattern for 1:2-passaged cells, and there were no new peaks resolved even though this technique separated proteins by a slightly different mechanism than PAGE. The top two fractions from each peak (Fig. 4) were pooled for characterization of the enzymes. Table 1 shows that the phosphate donor requirements of the rapidly migrating R_f 0.70 enzymes from infected or passaged cells were very similar to each other and to those of the mitochondrial enzyme (1, 17, 25), which utilizes ribonucleoside triphosphates more efficiently than does the cytosol enzyme. The slowly migrating R_f 0.40 enzyme peaks from infected and passaged cells exhibited phosphate donor specificities similar to those published for the cytosol enzyme (1, 17, 25) and similar to each other, in that ribonucleoside triphosphates were less well suited as phosphate donors. The R_f 0.7 (mitochondrial) peaks from both infected and passaged cells also showed more feedback inhibition by dCTP in the presence of ATP than the two R_f 0.40 (cytosol) peaks, as previously observed (1, 25).

We also determined pH optima and thermo-

stability curves (Fig. 5 and 6) for the four partially purified enzymes and found them to be similar according to their respective R_f values. The two R_f 0.7 (mitochondrial) peaks demonstrate the broad pH range of these enzymes, especially the reported (25) preservation of activity at pH 4.5. The R_f 0.40 cytosol enzyme activities fell off sharply below pH 6.5, as described previously (25).

Inhibition of activity by ammonium sulfate

has also been used to distinguish the mitochondrial and cytosol enzymes (19), and Figure 7 illustrates the effect of ammonium sulfate on the R_f 0.4 and R_f 0.7 peaks. Again, the enzymes were similar according to their respective R_f values, and the cytoplasmic R_f 0.4 enzymes were more sensitive to inhibition by this salt.

DISCUSSION

The data presented in this paper and those of

FIG. 2. Disc-PAGE electropherograms of TK extracts of sham-infected (left column) or Town-infected (right column) MRC-5 cells run and assayed per Materials and Methods. Rows from top to bottom represent 12, 24, 48, and 72 h after infection. The mean of lowest sample counts from each gel (450 cpm/sample) was subtracted as background. Protein (150 μ g) was loaded onto each gel.

FIG. 3. Disc-PAGE electropherograms of TK extracts of AD169-infected (left column), sham-infected (center column), and Town-infected (right column) WI-38 cells run and assayed for TK per Materials and Methods. Rows from top to bottom represent 12, 24, 48, and 72 h after infection. The mean of lowest sample counts from each gel (450 cpm/sample) was subtracted as background. Protein (150 μ g) was loaded onto each gel.

Závada et al. (29) indicate that these laboratory strains of HCMV do not produce a virus-specific, electrophoretically separable TK enzyme activity *in vitro*. They do, however, stimulate both cellular TK enzymes to high, sustained levels. The slight differences in virus-infected versus growing-cell disc-GEP pool reactivities in the characterization studies are probably due to the fact that the protein concentration of the pools were not controlled. However, HCMV may produce some modification of the host enzymes; we are investigating this possibility.

Indeed, the stimulation of cellular TK activities could result from either induced *de novo* synthesis or some modification of preexisting inactive forms of the enzymes.

HSV-1 has a broad spectrum of pathogenicity and productive cytolitic activity *in vivo* and *in vitro* (6). In contrast, HCMV infection is limited to man, and the *in vitro* spectrum includes only human diploid fibroblasts (28) for productive infection. Also, HSV-1 demonstrates a short eclipse period (4 to 6 h) and then rapidly kills the infected cell (6). The eclipse period for

FIG. 4. Disc-GEP electropherogram of 48-h TK extract of AD169-infected WI-38 cells run and assayed per Materials and Methods. Results with a TK extract of 1:2-passaged WI-38 cells were identical except for lower peak activities.

TABLE 1. Comparison of the effects of various phosphate donors on TK activity of disc-GEP peaks: WI-38 and AD169

Phosphate donor (10 mM)	WI-38 (passaged) ^a		WI-38 (infected) ^b	
	R _f 0.70	R _f 0.40	R _f 0.70	R _f 0.40
ATP	100 ^c	100	100	100
dATP	62	55	58	65
CTP	88	32	89	65
GTP	87	39	83	10
UTP	86	25	90	7
TTP	5	2	6	0
ATP + 1 mM dCTP	20	51	25	58

^a Passaged 1:2 in growth MEM; harvested 48 h later.

^b Infected with AD169 per Materials and Methods; harvested 48 h after infection.

^c Activity expressed as percentage of ATP.

HCMV is at least 24 to 48 h, and infected cells survive for many days (9, 28). On a molecular level, HSV-1 inhibits host cell protein, RNA, and host DNA synthesis (3) early in infection, whereas HCMV stimulates host protein (M. Stinsky, personal communication), RNA (25), and DNA synthesis (8) early in infection and causes high levels of host DNA polymerase enzymes to accumulate (12). This stimulatory effect of HCMV, especially the stimulation of host enzymes associated with the S-phase of the cell cycle (TK and DNA polymerases), represents a fundamental difference in the mode of replication of HSV-1 and HCMV, even though both viruses induce virus-specific DNA polymerases (12, 15, 26).

A preliminary model of the HSV-1 lytic cycle would stress viral independence from the cellu-

lar DNA synthetic apparatus, demonstrated by virus-specific enzyme functions to the point of redundancy for the assured production of essential precursors such as deoxynucleoside triphosphates and the suppression of cellular DNA-synthesizing functions. In contrast, a preliminary HCMV lytic-cycle model would stress viral dependence on specifically stimulated host cell functions for supply of essential precursors (in this case, deoxynucleoside triphosphates).

In light of these different models, the restricted in vitro spectrum of HCMV may represent the inability of HCMV to stimulate the appropriate host enzyme pathways for essential precursors in cells other than human diploid

FIG. 5. Plot of pH versus percentage of activity at pH 7, assayed per Materials and Methods (mean of three assays; standard deviation, $\pm 15\%$). (A) GEP R_f 0.7 pool from AD169-infected (■) and 1:2-passaged (□) WI-38. (B) GEP R_f 0.4 pool from AD169-infected (●) and 1:2-passaged (○) WI-38.

FIG. 6. GEP peak fractions were heated at 45°C; 5- μ l portions were removed to 100 μ l of TK reaction mixture on ice at 10-min intervals and simultaneously assayed for TK per Materials and Methods (mean of three assays; standard deviation, $\pm 15\%$). R_f 0.4 pools of AD169-infected (□) or 1:2-passaged (○) WI-38. GEP R_f 0.70 pools of AD169-infected (■) or 1:2-passaged (●) WI-38.

FIG. 7. Plot of percentage of TK activity versus ammonium sulfate concentration under standard reaction conditions (mean of three assays; standard deviation, $\pm 15\%$). GEP R, 0.4 pools of AD169-infected ($\square$) or 1:2-passaged ($\circ$) WI-38. GEP R, 0.7 pools of AD169-infected ($\blacksquare$) or 1:2-passaged ($\bullet$) WI-38.

fibroblasts in vitro. The prolonged eclipse period of HCMV would represent the time after infection necessary for the stimulation of appropriate host synthetic pathways in order to provide essential precursors for later viral replication. Enhancement of host RNA synthesis by HCMV is dependent on early protein synthesis (2 to 6 h postinfection) (24) and may be mediated by a recently described HCMV-specific early nuclear antigen detectable within 4 h after infection (10). Delayed cytolysis would insure prolonged availability of host-supplied components for maximal virus production and would also represent the greater tendency of HCMV to produce subchronic and latent infections in vivo. Admittedly, these models are sketchy at present; however, they explain some of the biological differences between HSV and HCMV. The dependence of a certain herpes-group virus, equine abortion virus, on host cell functions (S-phase) has been previously reported (18), and it is interesting that equine abortion virus seems to lack TK (14), as does HCMV. However, specific induction of host cell enzymes by herpesviruses is not well documented at present, and cellular enzymes may not be required for, or linked to, productive infection. Arguing against this point is our unpublished data showing that 5-fluorodeoxyuridine (dTTP synthetase)-blocked WI-38 cells cannot synthesize HCMV DNA unless TdR is

present in the media. This clearly indicates that TK is important to HCMV replication in the absence of de novo dTMP synthesis.

Transformation of cells by HCMV in vitro is known (2, 11). The ability of HCMV to stimulate the activity of host enzymes associated with the S-phase as well as host DNA and RNA synthesis as a regular function of infection provides mechanistic evidence that HCMV may produce transformation under conditions that favor expression of the early (host-stimulatory) viral functions. Such conditions include infection by virions with genomes defective in the lytic functions, or host cell restriction of lytic viral functions. Many characteristics of HCMV infection are analogous to the polyoma virus, simian virus 40 systems: appearance of an early nuclear antigen; expression of host cell S-phase enzymes; permissive and nonpermissive cell types; and rescue of virus from nonpermissive cells by cocultivation (10, 28).

HCMV produces significant morbidity and mortality in the human population (28). Chemotherapeutic agents (thymidine analog) specific for HSV-1-induced TK activities are being investigated by use of TdR affinity column-purified virus-specific and host cell TK enzymes (Y. C. Cheng, personal communication). This rational approach to herpesvirus chemotherapy offers great promise for HSV treatment; however, TdR analogs are unlikely to be effective in the HCMV system, because there is no virus-specific TK enzyme in affinity column-purified extracts of HCMV-infected cells (Y. C. Cheng, personal communication). In fact, recent experiments using thymidine arabinoside have shown that although thymidine arabinoside is a potent inhibitor of HSV and varicella-zoster virus in tissue culture, it is much less effective against HCMV (R. L. Miller, personal communication). The most likely virus-specific target for chemotherapeutic agents in the HCMV system appears to be the HCMV-induced DNA polymerase (12).

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Susan Estes and Mohammed Fattah.

This work was supported by Public Health Service fellowship grants F22CA03113-02 and T32CA09156 (J.E.) and Public Health Service grant AI12717 from the National Institute of Allergy and Infectious Diseases (E.S.H.).

LITERATURE CITED

- Adler, R., and B. R. McAuslan. 1974. Expression of thymidine kinase variants is a function of the replicative state of cells. *Cell* 2:113-117.
- Albrecht, T., and F. Rapp. 1973. Malignant transformation of hamster embryo fibroblasts following exposure to ultraviolet-irradiated human cytomegalovirus. *Virology* 55:53-61.

3. Ben-Porat, T., and A. S. Kaplan. 1973. Replication—biochemical aspects, p. 163–220. *In* A. S. Kaplan (ed.), *The herpesviruses*. Academic Press Inc., New York.
4. Bessman, M. J., I. R. Lehman, E. S. Simms, and A. Kornberg. 1958. Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction. *J. Biol. Chem.* 233:171–177.
5. Bjursell, G., and P. Reichard. 1973. Effects of thymidine on deoxyribonucleoside triphosphate pools and deoxyribonucleic acid synthesis in Chinese hamster ovary cells. *J. Biol. Chem.* 238:3904–3909.
6. Darlington, R. W., and A. Granoff. 1973. Replication—biological aspects, p. 93–132. *In* A. S. Kaplan (ed.), *The herpesviruses*. Academic Press Inc., New York.
7. Davis, D. B., W. Munyon, R. Bushbaum, and R. Chawda. 1974. Virus type-specific thymidine kinase in cells biochemically transformed by herpes simplex virus types 1 and 2. *J. Virol.* 13:140–145.
8. DeMarchi, J. M., and A. S. Kaplan. 1975. Replication of human cytomegalovirus DNA: lack of dependence on cell DNA synthesis. *J. Virol.* 18:1063–1070.
9. Furukawa, T., A. Fioretti, and S. Plotkin. 1973. Growth characteristics of cytomegalovirus in human fibroblasts with demonstration of protein synthesis early in viral replication. *J. Virol.* 11:991–997.
10. Geder, L. 1976. Evidence for early nuclear antigens in cytomegalovirus-infected cells. *J. Gen. Virol.* 32:315–319.
11. Geder, L., R. Lausch, F. Rapp, and F. O'Neill. 1976. Oncogenic transformation of human embryo lung cells by human cytomegalovirus. *Science* 192:1134–1137.
12. Huang, E. S. 1975. Human cytomegalovirus. III. Virus-induced DNA polymerase. *J. Virol.* 16:298–310.
13. Huang, E. S., S. T. Chen, and J. S. Pagano. 1973. Human cytomegalovirus. I. Purification and characterization of viral DNA. *J. Virol.* 12:1471–1481.
14. Jamieson, A. T., G. A. Gentry, and J. H. Subak-Sharpe. 1974. Induction of both thymidine and deoxycytidine kinase activity by herpes viruses. *J. Gen. Virol.* 24:465–480.
15. Keir, H. M., H. Subak-Sharpe, W. I. H. Shedden, D. H. Watson, and P. Wildy. 1966. Immunological evidence for a specific DNA polymerase produced after infection by herpes simplex virus. *Virology* 30:154–157.
16. Kit, S., D. R. Dubbs, and M. Anken. 1967. Altered properties of thymidine kinase after infection of mouse fibroblast cells with herpes simplex virus. *J. Virol.* 1:238–240.
17. Kit, S., W. C. Leung, and D. Trkula. 1973. Properties of mitochondrial thymidine kinase in parental and enzyme-deficient Hela cells. *Arch. Biochem. Biophys.* 158:503–513.
18. Lawrence, W. C. 1971. Evidence for a relationship between equine abortion (herpes) virus deoxyribonucleic acid synthesis and the S-phase of the KB cell mitotic cycle. *J. Virol.* 7:736–748.
19. Lee, L. S., and Y. C. Cheng. 1976. Human deoxythymidine kinase. I. Purification and general properties of the cytoplasmic and mitochondrial isozymes derived from blast cells of acute myelocytic leukemia. *J. Biol. Chem.* 251:2600–2604.
20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
21. Raymond, S. 1963. Acrylamide gel electrophoresis. *Ann. N. Y. Acad. Sci.* 121:350–365.
22. Roller, B., and G. H. Cohen. 1976. Deoxyribonucleoside triphosphate pools in synchronized human cells infected with herpes simplex virus types 1 and 2. *J. Virol.* 18:58–64.
23. Rowe, W. P., J. W. Hartley, S. Waterman, H. C. Turner, and R. J. Huebner. 1956. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. *Proc. Soc. Exp. Biol. Med.* 92:418–424.
24. Tanaka, S., T. Furukawa, and S. Plotkin. 1975. Human cytomegalovirus stimulates host cell RNA synthesis. *J. Virol.* 15:297–304.
25. Taylor, A. T., M. A. Stafford, and O. W. Jones. 1972. Properties of thymidine kinase partially purified from human fetal and adult tissue. *J. Biol. Chem.* 247:1930–1935.
26. Weissback, A., S.-C., Hong, J. Aucker, and R. Muller. 1973. Characterization of herpes simplex virus-induced deoxyribonucleic acid polymerase. *J. Biol. Chem.* 248:6270–6277.
27. Weissback, A., A. Schlaback, B. Fridlender, and A. Bolden. 1971. DNA polymerases from human cells. *Nature (London) New Biol.* 231:167–170.
28. Wright, H. T. 1973. Cytomegaloviruses, p. 353–388. *In* A. S. Kaplan (ed.), *The herpesviruses*. Academic Press Inc., New York.
29. Závada, V., V. Erban, D. Rezáčová, and V. Vonka. 1976. Thymidine kinase in cytomegalovirus infected cells. *Arch. Virol.* 52:333–339.