Biochemical Pharmacology, Vol. 30, No. 22, pp. 3071–3077, 1981.
Printed in Great Britain.

0006-2952/81/223071–07 $02.00/0

ENZYMATIC PHOSPHORYLATION OF ACYCLIC NUCLEOSIDE ANALOGS AND CORRELATIONS WITH ANTIHERPETIC ACTIVITIES

PAUL M. KELLER,* JAMES A. FYFE, LILIA BEAUCHAMP, CAROL M. LUBBERS, PHILLIP A. FURMAN, HOWARD J. SCHAEFFER and GERTRUDE B. ELION
The Wellcome Research Laboratories, Research Triangle Park, NC 27709, U.S.A.

(*Received* 26 *December* 1980; *accepted* 25 *March* 1981)

Abstract—The inhibitor and substrate specificities of deoxythymidine (dThd) kinase purified from herpes simplex virus (HSV Type 1) were studied. A number of nucleosides and nucleoside analogs were phosphorylated by the virus coded enzyme. These included several compounds structurally related to 9-(2-hydroxyethoxymethyl)guanine (acyclovir), a potent inhibitor of HSV replication. Some contained guanine with 9-substituents differing from that of acyclovir by methylene additions, methylene and thioether substitutions for the ether oxygen, and branching on the distal side of the ether oxygen. Others were various 2-substituted 6-hydroxypurines with the 9-(2-hydroxyethoxymethyl) substituent. A limitation of the specificity of the enzyme with guanine derivatives was the lack of phosphorylation of any derivative with an acyclic moiety branched on the proximal side of the ether oxygen. Many of the compounds that were phosphorylated were subsequently found to inhibit HSV replication. Such compounds apparently inhibited HSV replication via the same route of activation previously described for acyclovir [G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp and H. J. Schaeffer, *Proc. natn. Acad. Sci. U.S.A.* **74**, 5716 (1977)]. Moreover, several compounds not phosphorylated by the enzyme did not inhibit replication. However, some other acyclic nucleoside analogs that were phosphorylated were not good antivirals, indicating that phosphorylation catalyzed by the HSV dThd kinase was not sufficient for inhibition of viral replication to occur. These results emphasize the importance of the specificity of cellular kinases and the HSV DNA polymerase to the mechanism of antiviral activity. The dThd kinase from Vero cells was also purified. With this host cell enzyme, kinetic constants of known antiviral compounds were determined and compared to those of dThd (relative V'_{max}; k'_m): dThd (100; 1.3 μM), 5-iodo-2′-deoxyuridine (87; 1.8 μM), 5-trifluoromethyl-2′-deoxyuridine (91; 1.2 μM), 5-bromo-2′-deoxycytidine (5; 580 μM), and 9-β-D-arabinofuransoylthymine (23; 2300 μM). None of the purine acyclic nucleoside analogs tested (at 1000 μM) was detectably phosphorylated by the Vero cell enzyme, and all had apparent K_i values >300 μM. The phosphorylation catalyzed by host cell dThd kinase correlated with the toxicity of some pyrimidine nucleoside analogs.

The novel acyclic† nucleoside analog, acyclovir, is an effective inhibitor of HSV replication [1, 2]. The mechanism of inhibition appears to require selective phosphorylation catalyzed by the HSV-coded deoxythymidine (dThd) kinase [3]. This kinase preferentially phosphorylates pyrimidine nucleosides [4]. It appears also to catalyze the phosphorylation of pyrimidine nucleoside analogs [e.g. Refs. 3, 5–8] and thymidylate [9]. Substitution of an acyclic moiety for the natural sugar of a pyrimidine resulted in a reduction or loss of substrate activity. However, acyclovir was a better substrate than the natural purine nucleosides Guo or dGuo for this kinase [3]. The antiviral activity of acyclovir has prompted the synthesis of other nucleoside analogs in which the cyclic carbohydrate moieties are replaced by various acyclic chains.‡ These compounds have been evaluated as substrates for the purified HSV dThd kinase and as inhibitors of HSV replication in cell culture. These data are useful for understanding the structural requirements for substrate activity with the enzyme itself, for predicting antiviral activity, and for correlating information from the enzyme studies with the antiviral activities in order to evaluate the role that the HSV dThd kinase plays. In addition, host cell dThd kinase specificity was studied in order to compare substrate activity with toxicity of nucleoside analogs within uninfected cells.

* Author to whom all correspondence should be addressed: Paul M. Keller, The Wellcome Research Laboratories, Burroughs Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, NC 27709, U.S.A.

† Abbreviations: acyclic, any substitution for the carbohydrate moiety of a nucleoside analog which is not a closed ring; acyclovir, 9-(2-hydroxyethoxymethyl)guanine; araT, 9-β-D-arabinofuranosylthymine; araG, 9-β-D-arabinofuranosylguanine; 8-azaguanine, 5-amino-7-oxo-*v*-triazolo[4,5-*d*]pyrimidine; BrdCyd, 5-bromo-2′-deoxycytidine; IdUrd, 5-iodo-2′-deoxyuridine; F_3TdR, 5-trifluoromethyl-2′-deoxyuridine; HSV-1, herpes simplex virus type 1; BHK, baby hamster kidney; BSA, bovine serum albumin, and PEP, phosphoenol pyruvate.

‡ H. J. Schaeffer, L. Beauchamp, P. Collins, and D. J. Bauer. Eighteenth Interscience conference on Antimircrobial Agents and Chemotherapy, Abstr. No. 72 (1978).

EXPERIMENTAL PROCEDURES

Materials

Sources of commercially available compounds, virus stocks and cells were the same as previously

reported [3]. 1-(2-Hydroxyethoxymethyl)-5-bromouracil was synthesized by Dr. John Kelsey. Bart Dolmatch performed the syntheses of 9-[2-(2-hydroxyethoxy)ethyl]guanine, 9-[1-(2-hydroxyethoxy)octyl]guanine, 9-(2-hydroxyethylthiomethyl)guanine, and 9-(2-hydroxyethoxymethyl)-2-dimethylaminohypoxanthine. Robert Lamon synthesized 9-(2-hydroxyethylthiomethyl)adenine. Dr. Lowrie M. Beacham III synthesized 2-amino-1,9-dihydro-9-[2-hydroxy-1-(hydroxymethyl)ethoxy]-(1*R*)-ethyl-6*H*-purin-6-one. Dr. James L. Kelley synthesized 9-(2-hydroxyethoxymethyl)-2-methylthioadenine, 9-(2-hydroxyethoxymethyl)-2-methylthiohypoxanthine, and 9-(2-aminoethoxy)methylguanine. Procedures for the syntheses of novel compounds in this report will be published elesewhere.

Methods

Plaque reduction assays were used to determine the concentration of drug which reduces plaque formation by 50 percent (ED_{50}) [10]. A clinical isolate of HSV-1, strain Cl, was used for these assays unless otherwise noted.

The HSV-1 and host cell (Vero, green African monkey kidney cells) dThd kinases were purified from cytosol fractions as previously reported [3, 11]. A unit of enzyme activity phosphorylates 1 pmole of dThd per min at 37° under standard assay conditions [100 mM Tris–HCl (pH 7.5), 10 mM ATP, 5 mM $MgCl_2$, 1 mM [^{14}C]dThd, 4–6 Ci/mole and 1 mg/ml BSA]. The reaction mixtures were spotted onto DEAE paper and processed as previously described [3].

Reactions to determine inhibition values (percent inhibition of acyclovir phosphorylation by the test compound) contained 150 μM [^{14}C]acyclovir, 300 μM nucleoside or nucleoside analog, 200 μM Tris–HCl (pH 7.5), 2 mM ATP, 2 mM $MgCl_2$, 1 mg/ml BSA, and 500 units/ml of (MacIntyre) HSV-1 dThd kinase. The DEAE paper method was used to measure the reaction rates. The radiochemical nucleoside kinase coupled assay [3] used to determine relative substrate velocities was modified as follows. The reaction mixtures were incubated and spotted on polyethyleneimine-impregnated cellulose plates as before, but the plates were developed immediately in 1 M LiCl, 0.05 M formic acid. The plates were then scanned for radioactivity and cut up for quantitation. The reaction mixture contained 1mM nucleoside or nucleoside analog, 100 mM Tris (pH 7.5), 2 mM ATP 2 mM $MgCl_2$, 50 mM KCl, 0.25 mM phosphoenol [1-^{14}C]pyruvate (50 cpm/pmole), 30 units/ml of pyruvate kinase, 1 mg/ml of BSA, and purified nucleoside kinase. The phosphorylation rates were expressed as a percentage of the rate with dThd as substrate. These rates were initial reaction velocities. Under these conditions, less than 10 per cent of the ATP utilization was from subsequent nucleoside monophosphate phosphorylation [3] catalyzed by the associated dTMP kinase [9]. Reactions to study the kinetics of acyclovir phosphorylation employed the standard conditions, but with 2 mM ATP, 2 mM $MgCl_2$, variable quantities of [^{14}C]acyclovir (57 Ci/mole), and 400 units of enzyme/ml of reaction mixture. These reaction rates were measured by the DEAE paper method. A coupled spectrophotometric assay [12] was used to study the kinetics of other acyclic nucleoside analogs. The reactions contained 100 mM Tris–HCl (pH 7.5), 2 mM ATP, 2 mM $MgCl_2$, 0.5 mg/ml BSA, 0.1 mM NADH, 0.5 mM PEP, 5×10^6 units/ml pyruvate kinase, 10×10^6 units/ml lactate dehydrogenase, variable amounts of nucleoside analog substrate, and 670 units/ml of HSV dThd kinase. All enzyme reactions were at 37°.

Kinetic constants (apparent K_m values, K'_m) were determined for the Vero cell dThd kinase with reactions containing 50 mM Tris–HCl (pH 7.5), 2 mM ATP, 2 mM $MgCl_2$, 1 mg/ml BSA, variable amounts of [^{14}C]dThd (40 Ci/mole), and 60 units of enzyme/ml. Reactions were incubated at 37°, and initial rates were determined by the DEAE paper method. The apparent K_i constants (K'_i) for analogs were determined in a similar manner, but contained 1.3 μM [^{14}C]dThd and variable amounts of nucleoside analogs. Relative substrate efficiency values were calculated from the ratio of V'_{max}/K'_m, assuming that $K'_m = K'_i$ [13] and that $V'_{max} = v[1 + K'_m/S]$.

RESULTS

The binding of each nucleoside analog to the HSV dThd kinase was assessed by using the DEAE paper method to measure its ability to inhibit the phosphorylation of [^{14}C]acyclovir. The analogs were also tested as substrates using the radiochemical coupled

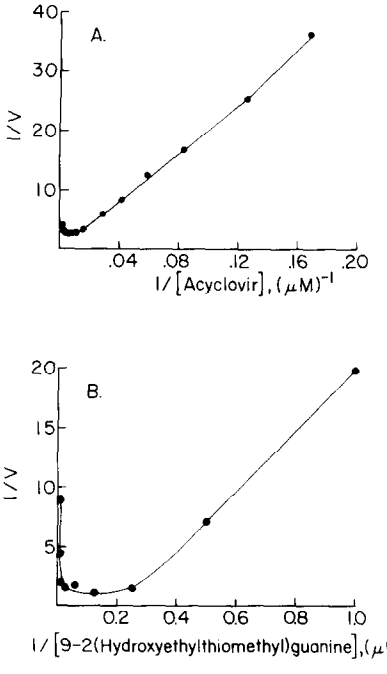
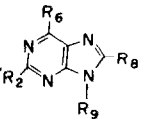
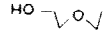
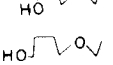
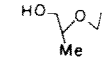
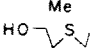
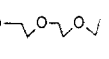
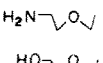


Fig. 1. Effects of acyclic nucleoside analog concentration on the rate of analog monophosphate formation. Activities were measured for acyclovir (panel A) by a radiochemical DEAE paper method, and 9-(2-hydroxyethylthiomethyl)guanine (panel B) by a spectrophotometric assay as described in Methods. Results with acyclovir were similar to those shown here when the spectrophotometric assay was used (P. M. Keller, unpublished data).

Table 1. Enzyme and antiviral activities of nucleosides and nucleoside analogs containing purine bases



Compound Number	R-2	R-6	R-9	R-8	Per Cent Inhibition*	Relative Substrate Velocity*	ED_{50}† (μM)
1	$-NH_2$	-OH		—	69	36	0.1
2	"	"		—	93	66	5.8
3	"	"		—	70	55	138
4	"	"		—	55	18	50
5	"	"		—	86	28	2.3
6	"	"		—	100	20	0.5
7	"	"		—	100	8	2.3
8	"	"		—	29	<3	1.5
9	"	"		—	14	<3	8
10	"	"		—	36	<3	>250
11	"	"		—	94	<3	>250
12	"	"		—	16	<3	>250
13	"	"	Arabinose	—	44	<3	22
14	"	"	Ribose	—	74	12	N.D.‡
15	"	"	Deoxyribose	—	39	5	N.D.
16	-H	$-NH_2$		—	0	<3	33
17	$-SCH_3$	$-NH_2$	"	—	74	35	136
18	$-NH_2$	$-NH_2$	"	—	5	<3	17
19	-H	$-NH_2$		—	10	<3	>250
20	-H	$-NH_2$	Arabinose	—	6	<3	12.3
21	$-NH_2$	$-NH_2$	"	—	0	<3	32
22	$-NH_2$	-OH		-Br	41	14	13
23	$-NHCH_3$	"	"	—	98	55	19.5
24	$-NH_2$	"	"	Aza§	23	7	129
25	$-SCH_3$	"	"	—	97	34	>250
26	$-N(CH_3)_2$	"	"	—	97	38	>250
27	-OH	"	"	—	12	<3	>250

*See "Experimental Procedures"
†Concentration of drug required to inhibit 50% of HSV-1 (Strain Cl) plaque formation in monolayers of Vero cells.
‡Not determined
§N substituted for C-8

assay. The percent inhibition and relative substrate velocity assays reported here were used in lieu of classical kinetic constants. Apparent K_m values were not readily obtainable, as reciprocal plots of initial velocity versus substrate concentration were not linear (Fig. 1). In the examples shown, both plots were concave upward at both high and low concentrations. Neither line fit the equation for substrate inhibition as described by Cleland [14]. Qualitatively similar but quantitatively variable nonclassical kinetic behaviour was also observed with other acyclic nucleoside analogs.*

HSV-1 dThd kinase specificity

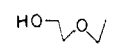
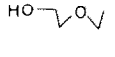
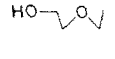
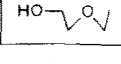
Nucleoside analogs containing purine bases. A number of acyclic nucleoside analogs containing guanine bound to and were substrates for the HSV dThd kinase (No. 1–7, Table I). This group of compounds included acyclovir (No. 1) and several closely related analogs. Several other acyclic nucleoside analogs with guanine did not compete with acyclovir as well as the first group, and were not detectably phosphorylated (No. 8–10 and 12). One compound with a 7-carbon side-chain (No. 11) inhibited well, but was not detectably phosphorylated. Both guanosine and deoxyguanosine (No. 14 and 15) were substrates for the enzyme, but araG (No. 13) was not.

The only compound with a 6-amino substituent which bound to and was a substrate for the HSV dThd kinase was 9-(2-hydroxyethoxymethyl)-2-methylthioadenine (No. 17). A variety of 6-hydroxy-9-(2-hydroxyethoxymethyl)-substituted analogs other than acyclovir were also substrates (No. 22–26). This, however, was not true for the xanthine derivative (No. 27).

Compounds containing pyrimidine bases. For all of the series containing pyrimidine bases, the analogs containing the 1-(2-hydroxyethoxymethyl) substituent competed less well and were less readily phosphorylated than any compounds with pentafuranosyl substituents (Table II). This is in contrast to the parallel series containing guanine as base (Table I). The relative orders of inhibition and substrate velocities within the thymine series were surprisingly much more similar to the cytosine series than those of uracil.

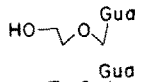
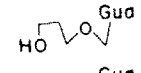
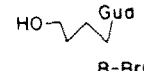
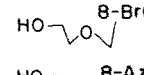
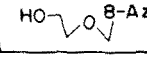
* P. M. Keller, unpublished data.

Table II. Activities* of nucleosides and nucleoside analogs containing pyrimidine bases

No.	Base	1-Substituent	Per Cent Inhibition	Relative Substrate Velocity	ED_{50} (μM)
28	Thymine		45	12	>250
29	"	Arabinose	98	38	1.5
30	"	Ribose	100	14	N.D.
31	"	Deoxyribose	100	100	N.D.
32	Cytosine		9	<3	250
33	"	Arabinose	30	8	1.05†
34	"	Ribose	62	<3	N.D.
35	"	Deoxyribose	84	190	N.D.
36	Uracil		9	<3	>250
37	"	Arabinose	64	140	40‡
38	"	Ribose	49	109	N.D.
39	"	Deoxyribose	100	42	N.D.
40	5-Bromouracil		48	8	>250

*See Table 1 for explanations of values.
†ED_{50} from Peter Collins (personal communication) using HSV-1 strain H29.
‡Data from De Clercq et al.[15].

Table III. Phosphorylation of acyclic purine nucleoside analogs and pyrimidine nucleoside analogs with either HSV or Vero cell dThd kinases.*

No.	Structure	HSV dThd Kinase	Vero Cell dThd Kinase		
		Relative Substrate Velocity	Relative Substrate Velocity	K'_m or $K'_{i(\mu M)}$	Relative Substrate Efficiency
31	dThd	100	100	1.3	3.4
42	F_3TdR	101	91	1.8	2.2
43	5-IdUrd	115	87	1.2	3.2
44	araT	38	23	2,300	15×10^{-4}
45	5-BrdCyd	33	5	580	6×10^{-4}
1		36	<3	20,000	$<0.1 \times 10^{-4}$
2		66	<3	9,000	$<1.5 \times 10^{-4}$
7		8	<3	3,000	$<1.8 \times 10^{-4}$
22		13	<3	300	$<57 \times 10^{-4}$
24		5	<3	4,000	$<1.7 \times 10^{-4}$

*See "Experimental Procedures"

Among the pyrimidine acyclic derivatives with natural bases, only the thymine-containing compound (No. 28) was a substrate. However, the 5-bromouracil analog (No. 40) was also phosphorylated.

Antiviral activity of nucleoside analogs

The activity of each analog was tested for its ability to inhibit HSV-1 replication in Vero cells (Tables I and II, ED_{50} values). No compound was as potent as acyclovir, but several related acyclic analogs and arabinosyl nucleosides were effective inhibitors.

Comparison of substrate activities between HSV and Vero cell dThd kinases

The substrate velocities of some nucleoside analogs were determined with the purified Vero cell dThd kinase (Table III). Kinetic constants were also determined (DEAE paper method). Classical Michaelis–Menten kinetics were observed with this enzyme. The apparent K_m of dThd with this enzyme was found to be 1.3 ± 0.04 μM. Apparent K_i values were determined for several nucleoside analogs. These values should equal the apparent K_m values [13] for the analogs that are substrates (No. 42–45). These four pyrimidine nucleoside analogs, F_3TdR, IdUrd, araT and BrdCyd, were phosphorylated by both the Vero cell* and the HSV dThd kinases. Conversely, five purine acyclic nucleoside analogs (No. 1, 2, 7, 22 and 24) were detectably phosphorylated by only the HSV enzyme. The apparent K_i values of the purine acyclic nucleoside analogs with the Vero cell kinase were generally higher than the K'_m values for the pyrimidine nucleoside analogs.

DISCUSSION

The separation of highly purified HSV dThd kinase from cellular dThd kinase [3] has made possible enzyme specificity studies with acyclic nucleoside analogs. Data generated from these experiments are useful not only for determining structure–activity relationships of nucleoside analogs with the enzyme, but also relationships between enzyme specificity and the antiviral activity.

Initial attempts to obtain K_m constants for acyclic nucleoside analogs resulted in atypical kinetics (Fig. 1). Therefore, the inhibition or relative substrate velocity assays were determined at a single concentration (1 mM) of each analog. For this reason, only semiquantitative statements can be made concerning binding and phosphorylation of the acyclic nucleoside analogs with the enzyme.

These studies have revealed some interesting and unexpected findings about the specificity of the HSV dThd kinase. Since the enzyme phosphorylates thymidine and deoxycytidine, it was anticipated that closely related pyrimidine nucleosides containing a

* Previous workers have not detected host cell phosphorylation of araT [8] or BrdCyd [6] in extracts of uninfected cells. The present results, however, were obtained using purified enzyme, with higher concentrations of nucleoside analog and with dThd kinase from a different cell line (Vero cell vs BHK). More recently, Müller *et al.* [16] reported observations consistent with the data reported here, utilizing BHK cells as the enzyme source.

deoxyribose substituent would be preferred substrates. This is, indeed, the case [3]. Replacement of the sugar moiety of the pyrimidine deoxyribonucleosides by the 2-hydroxyethoxymethyl group leads to a great reduction or complete loss of substrate activity. Some purine derivatives, on the other hand, are substrates for this kinase when the natural sugar is replaced by the acyclic substituent. Data reported here show that derivatives of guanine or closely related 6-hydroxypurine bases were preferred substrates; acyclic derivatives of adenine, 2,6-diaminopurine, or xanthine were very poor substrates. Only one 6-aminopurine analog was found to be phosphorylated (No. 17). Phosphorylation of guanine derivatives was observed using analogs with considerable structural variation in the acyclic substituent, such as methylene and thioether substitutions, and branching on the distal side of the ether oxygen (Table I). Branching of the side chain on the proximal side of the ether oxygen, however, abolished substrate activity (No. 10–12) even though one compound (No. 11) competed quite well for the enzyme.

The phosphorylation of acyclovir by the HSV-specified dThd kinase has been identified as being required for its potent antiviral activity [1, 3]. The monophosphate of acyclovir apparently is converted to the triphosphate by cellular enzymes [3, 17], and this triphosphate acts as a preferential inhibitor of and/or substrate for the viral DNA polymerase [1, 18]. It was of interest to determine which other analogs might inhibit HSV replication by this mechanism. Also, the combined enzyme and antiviral testing data might provide clues about the specificity of other reactions leading to antiviral activity (see below).

For a number of acyclic nucleoside analogs (No. 1–7, 17, and 22–24), phosphorylation was associated with moderate to good inhibition to HSV replication; lack of phosphorylation was associated with poor antiviral activity (No. 10–12, 19, 27, 32 and 36). These results appeared to be consistent with the mechanism of action proposed for acyclovir. However, several analogs were phosphorylated, but had little or no antiviral activity (No. 25, 26, 28 and 40), indicating that some step after monophosphate formation was limiting antiviral activity. First, the inactivity of these analogs could stem from the inability of cellular or virally induced kinases to catalyze the conversion of the analog monophosphates to the di- and triphosphate forms. Second, it is possible that these monophosphates were phosphorylated in the cell, but their respective triphosphates were not inhibitors of viral DNA synthesis.

In contrast to the above, some acyclic nucleoside analogs and arabinosides which inhibited viral replication were not detectably phosphorylated by the HSV dThd kinase (No. 8, 9, 13, 16, 18, 20 and 21). It appeared that phosphorylation catalyzed by this enzyme was not a prerequisite for activity. The most likely explanation was that these compounds are phosphorylated by other enzymes (such as deoxycytidine kinase [19], adenosine kinase [20], or a phosphotransferase), or may be metabolized in the cell by other enzymes before being phosphorylated by the HSV dThd kinase. It is, however, possible that the lack of observed phosphorylation of these compounds by HSV dThd kinase could be due to the substrate inhibition observed at the test concentration of 1 mM analog (Fig. 1). No phosphorylation was observed after retesting several compounds (No. 8–10 and 18–21) at 20 μM.* Another possiblity is that some compounds may act by a yet unknown mechanism of inhibition, as observed with the acyclic nucleoside analogs erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) [21] and (*S*)-9-2,3-dihydroxypropyl)adenine [22].

Phosphorylation of nucleoside analogs by host cell (Vero) dThd kinase could be a first step in the activation of the drug to a toxic substance in host cells. Several pyrimidine nucleoside analogs known to inhibit HSV replication were found to be phosphorylated by the Vero cell dThd kinase (Table III) as well as by the HSV dThd kinase. This was not true for the purine acyclic nucleoside analogs. From the apparent K_m values of F_3TdR and IdUrd it would appear that they could compete favorably with dThd for phosphorylation by dThd kinase in uninfected cells. AraT and BrdCyd appear to compete less well. These findings correlated with observed toxic effects for both F_3TdR and IdUrd [23], and much lower toxic effects observed for araT [8], BrdCyd [6], and acyclovir [1]. None of the active acyclic nucleoside analogs reported here was found to be toxic at concentrations below 100 μM in cultures of human Detroit 98 cells.† In spite of the correlation observed here, one cannot assume that phosphorylation of a compound by the host cell dThd kinase is a prerequisite or sufficient condition for toxicity.

Data from the enzyme assays reported here are useful to explore the limitation of HSV dThd kinase specificity and to provide a rapid test for potential inhibitors of HSV replication. The combination of data from the enzyme and plaque reduction assays indicates other steps in the activation of the nucleoside analogs which may be crucial. Specifically, the steps of phosphorylation subsequent to the reaction catalyzed by HSV dThd kinase, and the interactions of the triphosphates of nucleoside analogs with HSV DNA polymerase, appear to require further evaluation. Finally, assays with host cell dThd kinase show a correlation between toxicity by some nucleoside analogs toward uninfected cells and phosphorylation catalyzed by this particular enzyme.

Acknowledgements—We wish to thank Drs. T. A. Krenitsky, R. L. Miller and T. Spector for helpful discussions during the course of these studies, and F. A. Grubelich for help with the enzyme assays.

* P. M. Keller, unpublished data.

† Naomi K. Cohn, personal communication.

REFERENCES

1. G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp and H. J. Schaeffer, *Proc. natn. Acad. Sci. U.S.A.* **74**, 5716 (1977).
2. H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer and P. Collins, *Nature, Lond.* **272**, 583 (1978).
3. J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller and G. B. Elion, *J. biol. Chem.* **253**, 8721 (1978).

4. A. T. Jamieson, G. A. Gentry and J. H. Subak-Sharpe, *J. gen. Virol.* **24**, 465 (1974).
5. M. S. Chen and W. H. Prusoff, *J. biol. Chem.* **254**, 10, 449 (1979).
6. G. M. Cooper, *Proc. natn. Acad. Sci. U.S.A.* **70**, 3788 (1973).
7. Y-C. Cheng, B. A. Domin, R. A. Sharma and M. Bobek, *Antimicrob. Agents Chemother.* **10**, 119 (1976).
8. J. F. Aswell, G. P. Allen, A. T. Jamieson, D. E. Campbell and G. A. Gentry, *Antimicrob. Agents Chemother.* **12**, 243 (1977).
9. M. S. Chen and W. H. Prusoff, *J. biol. Chem.* **253**, 1325 (1978).
10. P. Collins and D. J. Bauer, *Ann. N.Y. Acad. Sci.* **284**, 49 (1977).
11. Y-C. Cheng and M. Ostrander, *J. biol. Chem.* **251**, 2605 (1976).
12. T. Spector, *Meth. Enzym.* **51**, 219 (1978).
13. I. H. Segel, *Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, p. 810. John Wiley, New York (1975).
14. W. W. Cleland, *Meth. Enzym.* **63**, 103 (1979).
15. E. De Clercq, E. Krajeswka, J. Descamps and P. F. Torrance, *Molec. Pharmac.* **13**, 980 (1977).
16. W. E. G. Müller, R. K. Zahn, J. Arendes and D. Falke, *J. gen. Virol.* **43**, 261 (1979).
17. W. H. Miller and R. L. Miller, *J. biol. Chem.* **255**, 7204 (1980).
18. P. A. Furman, M. H. St. Clair, J. A. Fyfe, J. L. Rideout, P. M. Keller and G. B. Elion, *J. Virol.* **32**, 72 (1979).
19. T. A. Krentisky, J. V. Tuttle, G. W. Koszalka, I. S. Chen, L. M. Beacham III, J. L. Rideout and G. B. Elion, *J. biol. Chem.* **251**, 4055 (1976).
20. R. L. Miller, D. L. Adamczyk, W. H. Miller, G. W. Koszalka, J. L. Rideout, L. M. Beacham III, E. Y. Chao, J. J. Haggerty, T. A. Krenitsky and G. B. Elion, *J. biol. Chem.* **254**, 2346 (1979).
21. T. W. North and S. S. Cohen, *Proc. natn. Acad. Sci., U.S.A.* **75**, 4684 (1978).
22. E. De Clercq, J. Descamps, P. DeSomer and A. Holy, *Science* **200**, 563 (1978).
23. W. H. Prusoff and D. C. Ward, *Biochem. Pharmac.* **25**, 1233 (1976).