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Acyclovir inhibition of Epstein–Barr virus replication

(antiviral chemotherapy/acyclovir triphosphate/viral DNA polymerase/competitive inhibition/reversal of viral genome number)

ALOK K. DATTA*, BRENDA M. COLBY†, JAMES E. SHAW†, AND JOSEPH S. PAGANO†‡

Cancer Research Center, Departments of ‡Medicine, *Biochemistry and †Bacteriology, and Immunology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

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ABSTRACT **Acyclovir [9-(2-hydroxyethoxymethyl)guanine] triphosphate inhibits Epstein–Barr virus (EBV)-associated DNA polymerase (DNA nucleotidyltransferase; EC 2.7.7.7) to a greater extent than it inhibits host α and β DNA polymerases. The affinity of the compound for viral polymerase is 100-fold higher than for α-polymerase. The extent of inhibition is dependent upon the base composition of the template-primer. The inhibition is prevented by increasing concentrations of deoxyguanosine triphosphate. The EBV-associated DNA polymerase reaction in the presence of the inhibitor, although depressed, proceeds at a linear rate over a long period of time. In contrast, the reaction of *Escherichia coli* DNA polymerase I in the presence of 2′,3′-dideoxythymidine 5′-triphosphate, a DNA chain terminator, levels off after initial linearity. Preincubation of acyclovir triphosphate with DNA and enzyme does not increase its inhibitory activity. The virus-producing cell line P3HR-1 consistently shows reduced viral genome numbers and viral capsid antigen on prolonged exposure to acyclovir. The number of EBV genomes returns to the control level when the cells are grown in drug-free medium. The results suggest that a competitive mechanism is the major mode of acyclovir inhibition of EBV replication.**

Selective prevention of virus replication can be attained by compounds that inactivate the novel enzymes necessary for virus growth. Virus-specific DNA polymerase (deoxynucleoside triphosphate:DNA nucleotidyltransferase, EC 2.7.7.7) is one of the target enzymes most suitable for this purpose (1–3). Several nucleoside analogs inhibit virus replication by affecting this enzyme (4–9). Acyclovir [ACV; 9-(2-hydroxyethoxymethyl)guanine] is one of a class of antiviral compounds recently shown to be effective in a variety of herpesvirus infections *in vitro* and in animal models (10). In both herpes simplex virus (HSV) type I and type II systems the drug is highly effective with an ED_{50} (effective dose required for 50% inhibition) of 0.1–0.2 μM without adverse effect on the host cells. Studies on the mechanism of action of this compound in HSV systems have shown that the drug is phosphorylated by the virus-specified thymidine kinase to its monophosphate form, which is subsequently converted to triphosphate by the host enzyme systems (11, 12). The triphosphorylated form of this compound inhibits viral DNA synthesis by inhibiting viral DNA polymerase by competing with dGTP (13). Results have been presented to suggest that the drug is incorporated into HSV DNA chains and thus terminates viral DNA synthesis (13). Recent genetic experiments also suggest that at least two virally coded functions, namely, virus-specific thymidine kinase and DNA polymerase, are responsible for the action of acyclovir in HSV-infected cells (14, 15).

The compound is also effective in inhibiting Epstein–Barr virus (EBV) replication at drug-dosage levels (6–7 μM) that are essentially nontoxic to host cells (16). In contrast, there is little if any effect on human cytomegalovirus at drug concentrations up to 50 μM (17). This intermediate sensitivity of EBV to this drug with respect to HSV and human cytomegalovirus prompted us to look into its effect on EBV DNA replication.

The present studies show that acyclovir triphosphate inhibits EBV-associated DNA polymerase to a much greater extent than it inhibits host α- and β-polymerases. The triphosphorylated form of the drug behaves as a classical competitive inhibitor of EBV DNA polymerase with respect to dGTP. The concept that the action of this drug is reversible is further supported by the fact that the virus-producing cells (P3HR-1) exposed to the drug over a long period of time show lowered levels of viral capsid antigen (VCA) and viral genome numbers that return to control levels with the removal of the drug. These observations, coupled with the apparent inefficient phosphorylation of ACV in EBV-infected cells (unpublished results), in contrast to the striking and selective phosphorylation in HSV-infected cells (11, 12), lead us to propose that the high sensitivity of EBV-associated DNA polymerase towards ACV triphosphate accounts for the inhibition of EBV replication. In this case a small amount of phosphorylated drug, phosphorylated perhaps by host thymidine or purine nucleoside kinases, is sufficient to inhibit EBV.

MATERIALS AND METHODS

Cells. The Burkitt lymphoma-derived cell line P3HR-1 (gift of E. Kieff) (18), was maintained between 5×10^5 and 10^6 cells per ml in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum supplemented with penicillin at 100 international units/ml and streptomycin at 100 μg/ml.

Chemicals. 12-*O*-Tetradecanoylphorbol 13-acetate was from Sigma. Synthetic deoxyhomopolymer-oligomer templates, unlabeled deoxynucleoside triphosphates and 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP) were obtained from P-L Biochemicals. ^{3}H-Labeled deoxynucleoside triphosphates were the products of New England Nuclear. ACV and its triphosphorylated derivative were generously provided by G. Elion of the Burroughs Wellcome (Research Triangle Park, NC). *Escherichia coli* DNA polymerase I purified by the method of Jovin *et al.* (19) was the gift of Sankar Mitra of Oak Ridge National Laboratory, Oak Ridge, TN.

Purification of DNA Polymerases. EBV-associated DNA polymerase and other host polymerases were purified according to the method of Datta *et al.* (20). Nuclear extract from P3HR-1 cells treated with tetradecanoylphorbol acetate was used for enzyme purification. The purified virus-associated enzyme could be differentiated from cellular polymerases by its activation with salt and its degree of sensitivity to *N*-ethylmaleimide and phosphonoacetic acid. The enzyme shows maximal

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Abbreviations: EBV, Epstein–Barr virus; HSV, herpes simplex virus; ddTTP, 2′,3′-dideoxythymidine 5′-triphosphate; EA, early antigen; VCA, viral capsid antigen; ACV, acyclovir [9-(2-hydroxyethoxymethyl)guanine].

activity for copying deoxyoligomer-homopolymer templates, but fails to copy poly(rA)·$(dT)_{10}$ and $(dT)_{10}$, which shows absence of γ DNA polymerase, reverse transcriptase, and terminal deoxynucleotidyltransferase (20–23).

Immunofluorescence Assay. EBV early antigen (EA) and VCA antigen were determined from cell smears according to Henle and Henle (24). EA^-/VCA^+ (Kampala) and EA^+/VCA^+ (Ghana) sera were used for these determinations. Sera were the generous gift of W. Henle.

DNA Polymerase Assay. DNA polymerase was assayed as follows: The reaction mixture (100 μl) contained 25 mM Hepes (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, 0.2 mM EDTA, dialyzed bovine serum albumin at 20 μg/ml, 100 mM ammonium sulfate, 10 μg of activated calf thymus DNA, 20 μM ^{3}H-labeled deoxynucleoside triphosphates (either dTTP or dGTP as mentioned, specific activity 1000 cpm/pmol), 20 μM dATP, dGTP (or dTTP), and dCTP, and the amount of enzyme required to keep the reaction in the linear range. For α, β, and *E. coli* DNA polymerase I assays salt was omitted from the reaction mixture. The reaction was carried out at 37°C for 30 min or as indicated, after which it was stopped with 20 μl of 0.1 M EDTA. Aliquots (60 μl) were taken and soaked into a DE-81 filter paper (Whatman). The papers were washed with 5% Na_2HPO_4 and alcohol as described by Lindell *et al.* (25), and the radioactivity was determined in toluene-based solvent. At 37°C the reaction is linear for at least 1 hr.

Determination of Viral Genome Number by cRNA·DNA Hybridization. Cellular DNAs from mock-treated and drug-treated P3HR-1 cells were prepared as described (26). cRNA·DNA hybridization, with virus-specific [^{3}H]RNA as a probe, was conducted as described (16).

RESULTS

Effect of ACV Triphosphate on Viral and Cellular DNA Polymerases. Results presented in Fig. 1 show the effect of ACV triphosphate on viral and cellular DNA polymerases. With activated calf thymus DNA as template (Fig. 1*A*) the EBV-associated polymerase is strongly inhibited by increasing concentrations of ACV triphosphate. At 100 μM little residual activity is detected, whereas α and β polymerases retained 50% and 90% of the residual activity, respectively, at the same concentration. With the omission of dGTP as one of the substrates, the extent of inhibition is reduced at comparable concentrations of the drug (Fig. 1*B*). To decide whether the extent of inhibition is dependent on the template-primer composition,

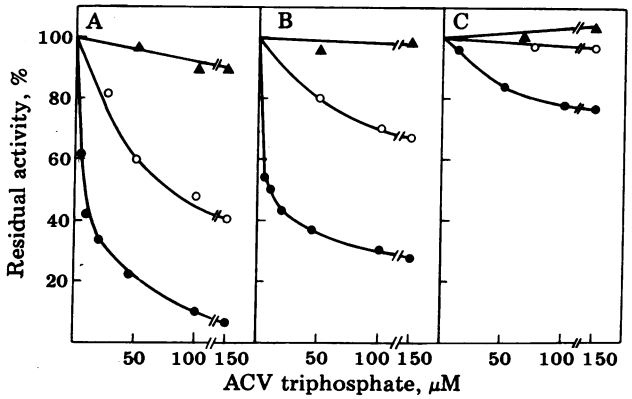

FIG. 1. Effect of ACV triphosphate on different DNA polymerase activities: EBV-associated DNA polymerase (●), α DNA polymerase (○), and β DNA polymerase (▲). The methods of assay are as described in *Materials and Methods* except that in experiments shown in *B* dGTP was omitted from the reaction, and in *C* poly(dA)·$(dT)_{12-18}$ was used as template-primer and [^{3}H]dTTP as the only substrate.

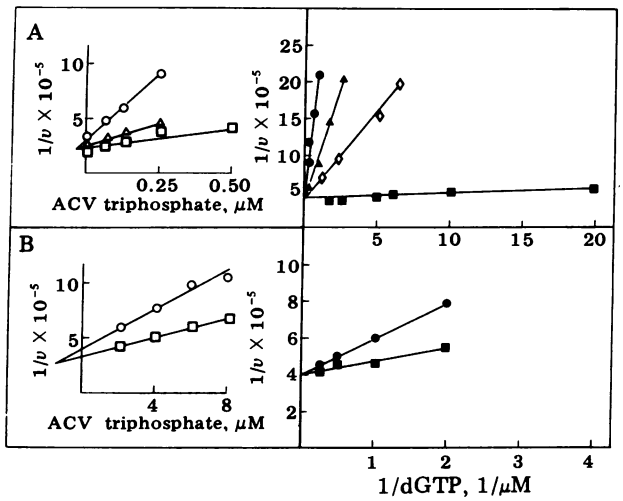

FIG. 2. Inhibition of EBV and α-DNA polymerase activities by ACV triphosphate as a function of dGTP concentration. The results are displayed in Lineweaver–Burk (*Right*) and Dixon (*Left*) plots. Reaction velocities v are expressed as counts of dTMP (specific activity, 1000 cpm/pmol) incorporated per 30 min per mg of protein. (*A*) EBV polymerase. For Lineweaver–Burk plots, concentrations of ACV triphosphate were 0 (■), 1 (◇), 2.5 (▲), and 6 (●) μM. In the Dixon plots, concentrations of dGTP were 2 (○), 4 (△), and 8 (□) μM. (*B*) α-Polymerase. For Lineweaver–Burk plots, concentrations of ACV triphosphate were 0 (■) and 2.5 (●) μM. In the Dixon plots, concentrations of dGTP were 2 (○) and 4 (□) μM.

we also studied the effect of this triphosphorylated compound on synthetic poly(dA)·$(dT)_{12-18}$ template-primer. The extent of inhibition is much less with this template. Our results agree with the findings of Furman *et al.* (13) with HSV-I-specified DNA polymerase and point to the fact that ACV triphosphate competes more efficiently with dGTP than with other deoxynucleoside triphosphates.

Mode of Inhibition by ACV Triphosphate and Determination of Inhibition Constant(s). Although the observations above indicated differential sensitivity of different polymerases towards ACV triphosphate, the results disclosed nothing of the nature of the inhibition. The results in Fig. 2 in the form of Lineweaver–Burk and Dixon plots and Table 1 demonstrate that the analog behaves as a classical competitive inhibitor of dGTP with both EBV-specified and α-polymerases. The affinity of EBV-associated polymerase (Fig. 2*A*) for the competitive inhibitor ($K_i = 0.015 \pm 0.002$ μM) is almost 100 times greater than that of α-polymerase ($K_i = 1.5 \pm 0.05$ μM) (Table 1). Moreover, the differential inhibitory potency of the analog for the two polymerases is further enhanced by the fact that their values of apparent K_m for the competitive substrate, dGTP, differ in a precisely reciprocal manner (Table 1).

Kinetics of DNA Polymerase Reaction in the Presence of Inhibitors: Competition Versus Chain Termination. Furman *et al.* (13) have evidence that ACV triphosphate is not only a competitive inhibitor of HSV-I-specified DNA polymerase but also a substrate for this enzyme. Incorporation of ACV mono-

Table 1. Apparent kinetic constants for different DNA polymerases

DNA polymerase	Apparent K_m for dGTP, μM	Apparent K_i for ACV triphosphate, μM
Lymphoblastoid cell α	0.18 ± 0.02	1.5 ± 0.05
EBV	0.025 ± 0.005	0.015 ± 0.002

± indicates variation in five experiments.

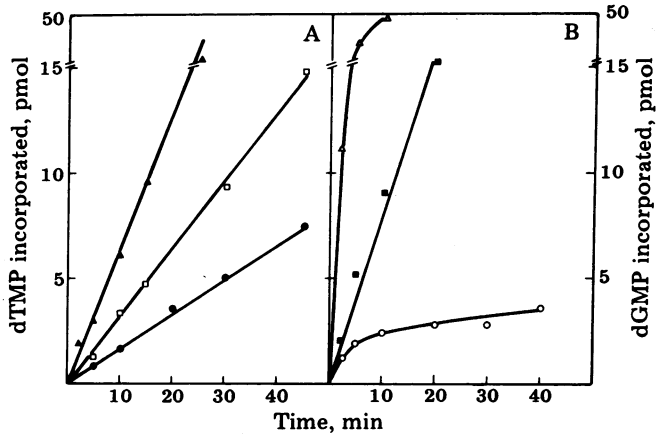

FIG. 3. Kinetics of EBV DNA polymerase (*A*) and *E. coli* DNA polymerase I (*B*) reactions in presence of ACV triphosphate and ddTTP, respectively. The reactions were carried out as described in *Materials and Methods* except that the following were used as substrates. In *A*, EBV DNA polymerase: four dNTPs (▲), three dNTPs without dGTP (□), and three dNTPs plus ACV triphosphate (10 μM) instead of dGTP (●); in *B*, for *E. coli* DNA polymerase I: four dNTPs (△), three dNTPs but without dTTP (■), and three dNTPs plus ddTTP (200 μM) instead of dTTP (○).

phosphate resulted in reduction in the rate of HSV-I-specified DNA polymerase reaction *in vitro* with activated calf thymus DNA as template-primer. This result led to the conclusion that incorporation of ACV monophosphate into the 3′ termini of DNA leads to chain termination, a basis for inhibition of virus replication (10). In order to determine whether the same mechanism is operative in EBV DNA polymerase-mediated reactions, we measured the kinetics of reaction in the presence and absence of ACV triphosphate.

The results in Fig. 3*A* show that the EBV-specified DNA polymerase reaction, although inhibited, proceeded linearly over a long period of time when ACV triphosphate was substituted for dGTP as one of the substrates. Such kinetics of reaction are possible only if the effect of the drug is competitive with respect to the substrate. In contrast, the velocity of the reaction of *E. coli* DNA polymerase I in the presence of ddTTP, a well-characterized chain terminator (27), rapidly fell after the first few minutes of linearity (Fig. 3*B*). Our unpublished results also show that when the kinetics of reaction is followed with EBV DNA polymerase in the presence of ddTTP, a linear but depressed reaction is obtained over time. Thus, the differences in the kinetics of reaction between EBV-specified DNA polymerase and *E. coli* DNA polymerase I in the presence of ACV triphosphate and ddTTP, respectively, point to a different mode of inhibition of DNA synthesis by the two inhibitors with the respective enzymes.

Table 2. Effect of preincubation of EBV-associated DNA polymerase and *E. coli* DNA polymerase I with ACV triphosphate and ddTTP

DNA polymerase	Experiment	Inhibitor	Conc., μM	Residual activity, %
E. coli I	Control	None	—	100
	No preincubation	ddTTP	200	83 ± 5
	Preincubation	ddTTP	200	33 ± 3
EBV	Control	None	—	100
	No preincubation	ACV triphosphate	10	40 ± 3
	Preincubation	ACV triphosphate	10	42 ± 2

The assay conditions were the same as described in *Materials and Methods*. For the EBV DNA polymerase preincubation experiment, DNA, dATP, dCTP, dTTP (20 μM each), ACV triphosphate (10 μM), and enzyme were preincubated for 30 min at 37°C. The reaction was then followed for another 30 min after addition of [^{3}H]dGTP (1000 cpm/pmol) at a final concentration of 20 μM. For the *E. coli* polymerase I preincubation experiment, DNA, dATP, dCTP, dGTP (20 μM each), ddTTP (200 μM), and enzyme were preincubated for 30 min at 37°C. The reaction was then followed for another 30 min after addition of [^{3}H]dTTP (1000 cpm/pmol) at a final concentration of 20 μM. For the rest of the experiments the same sequence of additions was followed but without preincubation. ± indicates variation in five experiments.

Table 3. Determination of EBV genome number

Culture*	Days	EBV genome equivalents per cell†	VCA, %
Mock-treated P3HR-1	—	240	10
ACV-treated P3HR-1	7	14	<0.05
ACV-treated P3HR-1	154	18	ND
ACV-removed P3HR-1	154 + 40	323	10

ND, not determined.

* P3HR-1 cells were mock-treated or treated for various lengths of time with 100 μM ACV. ACV was removed from one culture after 154 days; cells were pelleted and suspended in fresh medium lacking ACV and maintained for 40 days.

† cRNA·DNA hybridization used to quantitate EBV DNA was conducted as described in *Materials and Methods*. Average hybridization values were determined for duplicate DNA filters, each standardized to 50 μg of DNA per filter. Counts per min bound by calf thymus and HEP-2 human carcinoma cell DNA were subtracted as background. Genome number is based on Raji = 50–60 EBV genome equivalents per cell (28).

Effect of Preincubation. Atkinson *et al.* (27) demonstrated that the chain terminator ddTTP causes nondissociable binding of *E. coli* DNA polymerase I to the primer end blocked with 2′,3′-dideoxythymidine 5′-monophosphate (ddTMP). To ascertain whether such binding also occurs in the case of ACV triphosphate inhibition of EBV DNA polymerase, the effect of a prior incubation of polymerase with activated calf thymus DNA, dATP, dCTP, dTTP, and ACV triphosphate on the rate of subsequent polymerization was studied. As shown in Table 2, such a prior incubation does not enhance the inhibitory effect of ACV triphosphate. In contrast, the identical preincubation experiment with *E. coli* DNA polymerase I, calf thymus DNA, dATP, dCTP, dGTP, and ddTTP shows an enhanced inhibitory activity.

Determination of Viral Genome Number in the Presence and After the Removal of the Drug. Table 3 shows the effect of ACV on viral DNA synthesis in the virus-producing P3HR-1 cell line. As determined by EBV cRNA·DNA membrane hybridization, the number of EBV genomes per cell in the producer cell line decreased drastically with exposure to ACV. The reduction in viral genome number reached a steady level on exposure of the cells to ACV for 7 days, after which further exposure did not lead to any more reduction. This result was confirmed by exposing the cells to the drug for 154 days. Concomitant with the reduction of viral genome number, VCA also decreased proportionally in the cells. However, when the ACV-exposed cells were grown again in drug-free medium, the viral genome number and VCA returned to control levels.

DISCUSSION

In this communication it is shown that EBV DNA polymerase is extremely sensitive to ACV triphosphate and that the sensitivity of this enzyme to the drug is greater than that of the host polymerase (Fig. 1). The inhibition by ACV triphosphate of both viral and cellular polymerases is dependent on the base composition of the template-primer. Greater inhibition is observed with activated calf thymus DNA, whereas use of

poly(dA)·$(dT)_{12-18}$ as template-primer produces much less inhibition. Thus the template specificity suggests that ACV triphosphate competes with dGTP (13). The competitive nature of the inhibition is also evident from the Lineweaver–Burk and Dixon plots (Fig. 2). The specific inhibition of viral DNA synthesis without much effect on host DNA synthesis (16) can be explained by the fact that the apparent K_i (0.015 ± 0.002 μM) of ACV triphosphate for EBV polymerase is approximately $1/100$ that of host α-polymerase (1.5 ± 0.05 μM) (Table 1). In addition, unpublished results from this laboratory show that the concentration of ACV triphosphate formed within Raji cells superinfected with EBV is 2–5 times higher than the apparent K_i of ACV triphosphate for EBV polymerase, but much lower than the K_i for host polymerases. Thus, a higher affinity of ACV triphosphate towards EBV polymerase in comparison with HSV-I-specified polymerases (13) accounts for the fact that, in spite of inability to detect EBV-specific thymidine kinase (unpublished results; Y.-C. Cheng, personal communication) and apparent inefficient phosphorylation of ACV, the drug is effective in EBV system.

The inhibition of the activity of DNA polymerase by ACV triphosphate can be explained by two possible mechanisms. First, ACV triphosphate causes a nondissociable binding of EBV DNA polymerase to the primer end blocked with ACV monophosphate, and the chain cannot be extended further because of unavailability of the free 3′-OH group of the sugar moiety. This mechanism of inhibition has been suggested in the inhibition of HSV-I-specified DNA polymerase by the drug (13). This mode of inhibition is identical to the proposed mechanism by Atkinson *et al.* (27) for ddTTP and *E. coli* DNA polymerase I. Second, the viral DNA polymerase is inhibited competitively by a lower concentration of ACV triphosphate compared with cellular polymerases so that the drug inhibits EBV DNA synthesis much more strongly than host DNA synthesis. However, of the two possible mechanisms of inhibition our results are consistent with the latter one because of the following experimental facts: (*i*) The inhibition could be reversed *in vitro* by the addition of excess dGTP; (*ii*) the extent of inhibition is less when dGTP is omitted from the DNA polymerase reaction mixture; (*iii*) the linear inhibition kinetics of DNA polymerase reaction in the presence of the inhibitor over a long period of time; (*iv*) the irreversible binding of EBV DNA polymerase to DNA chains terminated with ACV monophosphate residue is ruled out by the observation that preincubation of enzyme, deoxynucleoside triphosphates, ACV triphosphate, and DNA did not lead to an increase in the inhibitory activity; and lastly (*v*) virus-producing cell lines when exposed to ACV show marked reduction of viral genome numbers, which recover to control levels with the removal of the drug from the growth medium. Therefore, inhibition of EBV DNA polymerase activity seems to be the major cause of inhibition of EBV replication, and chain termination after incorporation, if it occurs, must take place at extremely low frequency. The phenomenon of chain termination by a particular nucleoside analog is not universal. ddTTP, which is a well-characterized chain terminator in reactions mediated by *E. coli* DNA polymerase I (27) and reverse transcriptase (29, 30), acts as a purely competitive inhibitor in the case of eukaryotic α and β DNA polymerases (31). So we infer that, apart from structural features of a particular nucleoside analog, the active sites of the enzymes might play some role in determining the mode of action.

The findings described in this paper will allow further use of ACV triphosphate as a tool for identification of different DNA polymerases and also for studies of the mechanism of DNA synthesis *in vivo* in virus-infected cells. In addition, the drug might prove to be useful in treatment of EBV-associated diseases and other herpetic infections.

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1. Gentry, G. A. & Aswell, J. R. (1975) *Virology* **65**, 294–296.
2. Müller, W. E. G., Zahn, R. K., Arendes, J. & Falke, D. (1979) *J. Gen. Virol.* **43**, 261–271.
3. Cheng, Y. C., Ostrander, M., Derse, D. & Chen, J. Y. (1979) in *Nucleoside Analogues*, eds. Walker, R. T., Clercq, E. D. & Eckstein, F. (Plenum, New York), pp. 319–335.
4. Cheng, Y. C., Hoffman, P. J., Ostrander, M., Grill, S., Caradonna, S., Tsou, J., Chen, J. Y., Gallagher, M. R. & Flanagan, T. D. (1979) *Adv. Ophthalmol.* **38**, 173–186.
5. Cheng, Y. C., Domin, B. A., Sharma, R. A. & Bobek, M. (1976) *Antimicrob. Agents Chemother.* **10**, 119–122.
6. Cheng, Y. C., Goz, B., Neenan, J. P., Ward, D. C. & Prusoff, W. H. (1975) *J. Virol.* **15**, 1284–1285.
7. North, T. W. & Cohen, S. S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4684–4688.
8. Schildkraut, I., Cooper, G. M. & Greer, S. (1975) *Mol. Pharmacol.* **11**, 153–158.
9. Collins, P. & Bauer, D. J. (1977) *Ann. N.Y. Acad. Sci.* **2**, 49–59.
10. Schaeffer, J. J., Beauchamp, L., deMiranda, P., Elion, G. B., Bauer, D. J. & Collins, P. (1978) *Nature (London)* **272**, 583–585.
11. Elion, G. B., Furman, P., Fyfe, J. A., deMiranda, P., Beauchamp, L. & Schaeffer, J. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5716–5720.
12. Fyfe, J. A., Keller, P. M., Furman, P. A., Miller, R. L. & Elion, G. B. (1978) *J. Biol. Chem.* **253**, 8721–8727.
13. Furman, P. A., St. Clair, M. H., Fyfe, J. A., Rideout, J. L., Keller, P. M. & Elion, G. B. (1979) *J. Virol.* **32**, 72–77.
14. Coen, D. M. & Schaffer, P. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2265–2269.
15. Schnipper, L. E. & Crumpacker, C. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2270–2273.
16. Colby, B. M., Shaw, J. E., Elion, G. B. & Pagano, J. S. (1980) *J. Virol.* **34**, 560–568.
17. Crumpacker, C. S., Schnipper, L. E., Zaia, J. A. & Levin, M. J. (1979) *Antimicrob. Agents Chemother.* **15**, 642–645.
18. Hinuma, Y., Kohn, M., Yamaguchi, U., Wudovski, D. J., Blakeslee, J. R. & Grace, J. T. (1967) *J. Virol.* **1**, 1045–1051.
19. Jovin, T. M., Englund, P. T. & Bertsch, L. L. (1969) *J. Biol. Chem.* **244**, 2996–3008.
20. Datta, A. K., Feighny, R. J. & Pagano, J. S. (1980) *J. Biol. Chem.* **255**, 5120–5125.
21. Bolden, A., Noy, G. P. & Weissbach, A. (1977) *J. Biol. Chem.* **252**, 3351–3356.
22. Baltimore, D. (1970) *Nature (London)* **226**, 1209–1211.
23. Chang, L. M. S. & Bollum, F. J. (1970) *Proc. Natl. Acad. Sci. USA* **65**, 1041–1048.
24. Henle, G. & Henle, W. (1968) *J. Bacteriol.* **91**, 1248–1256.
25. Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G. & Rutter, W. J. (1970) *Science* **170**, 447–449.
26. Pagano, J. S. & Huang, E.-S. (1974) in *Viral Immunodiagnosis*, eds. Kurstak, E. & Morrisset, R. (Academic, New York), pp. 279–299.
27. Atkinson, M. R., Deutscher, M. P., Kornberg, A., Russel, R. & Moffatt, J. G. (1969) *Biochemistry* **8**, 4897–4904.
28. Huang, E.-S. & Pagano, J. S. (1977) in *Methods in Virology*, eds. Maramorosch, K. & Koprowski, H. (Academic, New York), Vol. 6, pp. 457–497.
29. Faras, A. H., Taylor, J. M., Levinson, W. E., Goodman, H. M. & Bishop, J. M. (1973) *J. Mol. Biol.* **79**, 163–183.
30. Smoler, E., Molineux, I. & Baltimore, D. (1971) *J. Biol. Chem.* **246**, 7697–7700.
31. Fisher, P. A., Wang, T. S.-F. & Korn, D. (1979) *J. Biol. Chem.* **254**, 6128–6137.