Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 4684–4688, October 1978
Biochemistry

Erythro-9-(2-hydroxy-3-nonyl)adenine as a specific inhibitor of herpes simplex virus replication in the presence and absence of adenosine analogues

(adenosine deaminase/cordycepin/adenine arabinonucleoside)

THOMAS W. NORTH AND SEYMOUR S. COHEN

Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794

Contributed by S. S. Cohen, June 26, 1978

ABSTRACT *Erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA; *erythro*-9-[3-(hydroxynonyl)]adenine), a reversible inhibitor of adenosine deaminase, significantly inhibits replication of herpes simplex virus (HSV), whereas the more active inhibitor of the deaminase, 2′-deoxycoformycin, does not. At 10 μM EHNA, which does not affect viability, growth, or DNA synthesis of uninfected HeLa cells, production of HSV and HSV-specific DNA is inhibited 75–90% and 60%, respectively. HSV multiplies normally in cells pretreated with EHNA and washed to remove this inhibitor. EHNA (10 μM) also markedly potentiates the toxicity of adenine arabinonucleoside and of cordycepin (3′-deoxyadenosine) against HeLa cells and against the production of HSV in those cells. Cordycepin alone (10 μM) does not inhibit HSV replication whereas in combination with 10 μM EHNA there is a greater than 99% inhibition of virus production. Under these conditions, RNA synthesis is inhibited by more than 80% whereas protein and DNA synthesis are inhibited to a lesser extent; in this system, virtually all of the DNA synthesis in infected cells is that of host DNA. Thus, EHNA appears to affect the synthesis of HSV DNA specifically in two different ways, depending on whether it is used alone or in the presence of cordycepin.

9-β-D-Arabinofuranosyladenine (ara-A) and cordycepin (3′-deoxyadenosine) are rapidly deaminated to relatively nontoxic products by adenosine deaminase. Inhibitors of this enzyme greatly potentiate the toxicity of these adenine nucleosides, an effect first shown in this laboratory with *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA)* (1). This result was also obtained later with deoxycoformycin (2, 3), a compound that inactivates adenosine deaminase essentially irreversibly. In this paper we report on the ability of EHNA to potentiate the antiherpes activity of araA and cordycepin. Surprisingly, we have found an antiherpes activity of EHNA alone at a concentration nontoxic to the host cell.

As expected, EHNA does increase the antiherpes activity of adenosine analogues against herpes replication. However, it was also observed that, at certain concentrations of EHNA and cordycepin in combination, the mixture markedly and specifically affected the synthesis of viral DNA but not of host DNA in the infected cells.

MATERIALS AND METHODS

Materials. EHNA was provided by H. Schaeffer and G. B. Elion (Wellcome Research Laboratories). Deoxycoformycin was obtained from the National Cancer Institute. Cordycepin was provided by R. J. Suhadolnik (Temple University) and J. R. Tata (National Institute of Medical Research). Ara-A was purchased from Pfanstiehl Laboratories, Inc. (Waukegen, IL). [*methyl*-^{3}H]Thymidine, [5-^{3}H]uridine, and [4,5-^{3}H]leucine were obtained from New England Nuclear Corp. Optical grade CsCl was obtained from Hanshaw Chemical Corp.

Cells and Virus. HeLa F cells and the Miyama strain of herpes simplex virus type 1 were kindly provided by Arthur Weissbach (Roche Institute, Nutley, NJ). HeLa cells were grown at 37° in monolayer cultures in Joklik-modified minimal essential medium supplemented with 10% fetal calf serum (both from Grand Island Biological Co., Grand Island, NY). Prior to use all sera were heated to 56° for 20 hr (4). All cultures were examined for mycoplasma every 2 weeks (5) and were found to be negative.

Virus stocks were prepared by a modification of the Weissbach procedure (6). Confluent monolayers of HeLa cells were infected with herpes simplex virus (HSV) at an input of 0.1–0.2 plaque-forming units (PFU) per cell in 2.5 ml of medium. Virus was allowed to adsorb for 1 hr at 37°, 10 ml of fresh medium was added, and incubation was continued at 37° for 2–3 days, by which time infected cells had formed polykaryocytes and had come off the surface. Infected cells were collected at 1000 × *g* for 10 min and resuspended in 5 ml of fresh medium. Cells were disrupted by sonication in a model B-220 ultrasonic cleaner (Branson Ultrasonics Corp.). Debris and undisrupted cells were removed at 1000 × *g* for 5 min and the supernatant was used as virus stock with a virus titer of 1–2 × 10^9 PFU per ml. Plaque assays were performed on monolayers of HeLa cells. Virus in 0.5 ml of medium was allowed to adsorb for 1 hr at 37°; then monolayers were washed with 2.0 ml of phosphate-buffered saline (7) and overlaid with medium containing 1.3% methylcellulose. After 3 days of incubation at 37°, plaques were observed by staining cells with Giemsa stain.

Virus Yield Experiments. Subconfluent HeLa cells (4–6 × 10^5 per 35-mm petri dish) were infected with HSV at 10 PFU per cell in 0.3 ml of medium and adsorption was allowed for 1 hr at 37°. Cells were washed twice with 2.0 ml of phosphate-buffered saline, and then 1.0 ml of fresh medium was added to each petri dish. For experiments using inhibitors, 0.5 ml of medium with or without EHNA was added; 15 min later, medium with or without EHNA containing 2 times the final concentration of the analogue was added. Thus, adenosine deaminase was blocked prior to addition of the analogue. Time zero is taken as the end of the adsorption period.

At the indicated times, cells were scraped from the petri dishes, and the cells from the combined suspensions were pelleted at 1000 × *g* for 5 min. They were then washed with 2.0 ml of phosphate-buffered saline and resuspended in 2.0 ml of fresh medium. This treatment removed any drugs present in the experiment. Up to 24 hr after infection, more than 99% of the virus detectable by plaque assay remained intracellular. Virus was released from infected cells by sonication and titrated by plaque assay.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. § 1734 solely to indicate this fact.

Abbreviations: EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine (more accurately named *erythro*-9-[3-(2-hydroxynonyl)]adenine); ara-A, 9-β-D-arabinofuranosyladenine; HSV, herpes simplex virus-1; PFU, plaque-forming units.

* A more accurate name is *erythro*-9-[3-(2-hydroxynonyl)]adenine.

Cell Growth and Viability. HeLa cells were seeded onto 35-mm petri dishes at 0.8–1.0 × 10^5 cells per plate. After the cells had attached to the surface of the plates and 12 hr of subsequent growth, 1.0 ml of fresh medium (containing cordycepin with or without EHNA as indicated) was added at time zero. Cells were removed by scraping from plates with a rubber policeman, followed by a wash of the cells with another 1.0 ml of medium. The suspension and wash were combined, and cell number was determined by counting an aliquot in a model B Coulter Counter. Cell viability was measured by plating for colony formers as described (1). Plating efficiencies of untreated cells were 55–65%.

Measurement of DNA, RNA, and Protein Synthesis. DNA, RNA, and protein synthesis were measured by incorporation of ^{3}H-labeled thymidine, uridine, and leucine, respectively. Cells were infected and treated with cordycepin with or without EHNA. Uninfected cells were treated identically except that they were mock-infected with 0.3 ml of fresh medium. Radioactive compounds were then added as follows: [5-^{3}H]-uridine (final concentration, 1 μM) or [4,5-^{3}H]leucine (final concentration, 0.4 mM) was added, 1 μCi/ml, immediately after addition of cordycepin (time 0); or [*methyl*-^{3}H]thymidine (final concentration, 1 μM) was added, 1 μCi/ml, at 2 hr. Cells were scraped from the plates, and the plates were washed. The cell suspension and wash were combined and the cells were pelleted at 1000 × *g* for 10 min, washed with 2.0 ml of phosphate-buffered saline, and then resuspended in 1.0 ml of cold 10% (wt/ml) trichloroacetic acid. The precipitates were collected on Whatman GF/C filters and the filters were washed four times with 5 ml of cold (4°) trichloroacetic acid 5% and twice with 5 ml of ethanol. The filters were dried under a lamp and assayed for radioactivity in 10 ml of Liquifluor (New England Nuclear Corp.) in a Packard model 3390 liquid scintillation spectrometer.

CsCl Density Gradient Analysis. CsCl gradients of DNA were analyzed (8). HeLa cells on 60-mm petri dishes (2–3 × 10^6 cells per dish) were infected with HSV at a multiplicity of 10 PFU per cell in 0.5 ml of medium. Virus was allowed to adsorb at 37° for 1 hr; then, the monolayers were washed twice with 2.5 ml of phosphate-buffered saline and covered with 2.0 ml of fresh medium. Cordycepin or EHNA was administered as described above. Two hours after adsorption, [^{3}H]thymidine was added (5 μCi/ml; final concentration, 0.25 μM). Incorporation of labeled thymidine into DNA was linear for at least 14 hr. At 12 hr, the cells were scraped from the plates. The plates were washed with another 1 ml of medium and this was combined with the above suspension. Cells were then centrifuged at 1000 × *g* for 10 min and washed once with PBS phosphate-buffered saline; the pellet was resuspended in 4 ml of 0.15 M NaCl/0.1 M EDTA, pH 8.2. Samples in 2% sodium dodecyl sulfate were heated to 60° for 10 min and then rapidly chilled to 4°. CsCl was added (1.29 g/ml) and the mixtures were centrifuged at 12,000 × *g* for 5 min. The solution was then separated with a pasteur pipette from the white material floating on top and containing sodium dodecyl sulfate. The refractive index of each solution was adjusted to 1.4015 at 25°. Centrifugation was in a Ti 50 rotor at 36,000 rpm for 72 hr at 25°. Gradients were fractionated with a peristaltic pump through a stainless steel tube that had been carefully lowered to the bottom of the centrifuge tube. Six-drop fractions were collected. A 0.1-ml aliquot of each fraction was added to 1.0 ml of 10% trichloroacetic acid, the samples were chilled, and the precipitated material was collected onto Whatman GF/C filters and assayed for radioactivity.

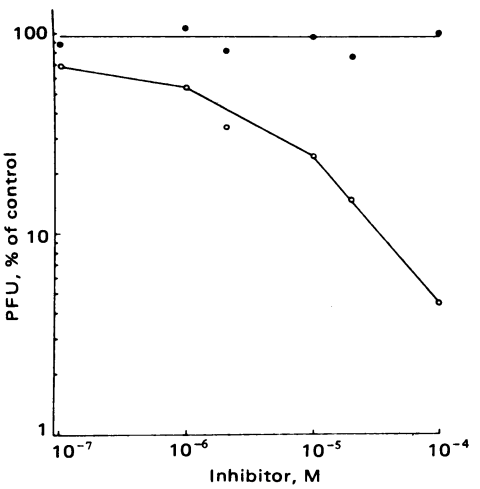

FIG. 1. Effects of EHNA (○) and deoxycoformycin (●) on production of infectious HSV.

RESULTS

Effects of EHNA on Cell Growth and Viability. The growth and viability of HeLa cells are inhibited less than 10% by 10 μM EHNA (1). At 50 μM EHNA, the increase in cell number was only slightly decreased in 12 hr but was about 50% that of the control after 48 hr, when only about 5% of the cells are viable. Deoxycoformycin did not affect cell growth at either concentration.

Inhibition of HSV Replication by EHNA. Fig. 1 shows the effect of EHNA concentration on production of HSV. In this experiment, HSV production was inhibited 75% by 10 μM EHNA, a concentration that did not significantly affect growth or viability of HeLa cells. In several other experiments, virus production was inhibited as much as 90% and was always inhibited more than 50%.

Deoxycoformycin is a more potent inhibitor of adenosine deaminase than EHNA, having a K_i of 10^{-11} M (9) compared to a K_i of 10^{-8} M for EHNA (10). As shown in Fig. 1, deoxycoformycin has no effect on HSV replication (11). Nevertheless, at 10 μM this material increased the antiherpes activity of 10 μM cordycepin by more than 100-fold. Thus, the antiherpes activity of EHNA does not appear to result from inhibition of adenosine deaminase.

Virus growth curves were determined in the presence or absence of 10 μM EHNA. In both instances, after adsorption there was a latent period of 5 hr, with progeny detected shortly thereafter and increasing until 20–21 hr. However, EHNA produced a decrease in final yield rather than a delay in the reproductive cycle. To determine if EHNA was metabolized to a difficultly removable inhibitor or irreversibly inhibited by

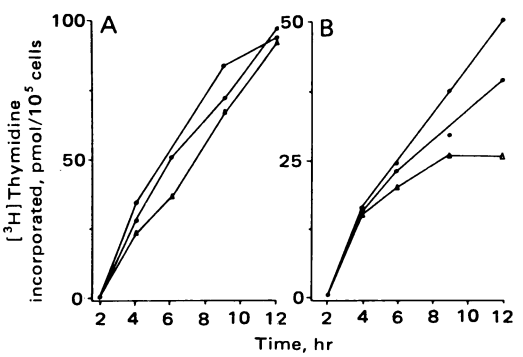

FIG. 2. Effect of EHNA on DNA synthesis in uninfected (*A*) and HSV-infected (*B*) HeLa cells. ●, Control; ○, 10 μM; △, 50 μM EHNA.

a host component essential for HSV multiplication, cells were treated for 24 hr with EHNA and washed to eliminate external inhibitor. They were then infected with HSV in the presence or absence of 10 μM EHNA. Cells pretreated with EHNA supported HSV replication in the absence of EHNA as well as cells that were not pretreated. In addition, pretreatment did not change the amount of virus produced when 10 μM EHNA was present during infection. These data show that the antiherpes activity of EHNA is reversible (12).

Effect of EHNA on DNA Synthesis. EHNA caused a significant inhibition of DNA synthesis in HSV-infected but not in uninfected cells (Fig. 2). The incorporation of thymidine into DNA between 2 and 12 hr after infection was inhibited significantly by both 10 and 50 μM EHNA after 4 hr (Fig. 2*B*). This corresponds to the time when virus DNA synthesis begins, suggesting that EHNA specifically inhibits HSV DNA synthesis.

Because of its high G-C content, HSV DNA can be separated from cellular DNA in CsCl gradients (8), as shown in Fig. 3*A*. The peak of higher density (1.74 g/cm^3), that of HSV DNA, was absent in DNA from uninfected cells labeled under identical conditions. Treatment with 10 μM EHNA decreased the area under the peak by 60% whereas the area under the peak of host DNA remained unchanged (Fig. 3*B*). Treatment with 50 μM EHNA decreased HSV DNA by 90% and host DNA by 55%. Thus, EHNA selectively inhibits HSV DNA synthesis relative to host DNA synthesis. Also, the extent to which HSV DNA synthesis is inhibited corresponds closely with the extent to which HSV replication is inhibited. There was little or no effect of EHNA, even at 50 μM, on RNA or protein synthesis in infected or uninfected cells.

Potentiation of Anti-HSV Effect of ara-A by EHNA. These results are presented in Fig. 4. In the presence of 1 μM EHNA, which alone has an effect of less than 30% on HSV production (Fig. 1), 10 μM ara-A decreased HSV production more than 99%. This inhibition was 60-fold greater than in the absence of EHNA.

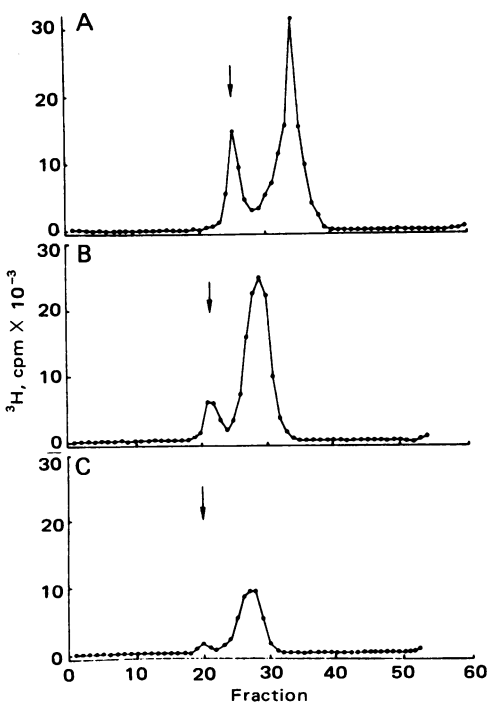

FIG. 3. CsCl density gradient analysis of DNA from HSV-infected cells labeled with [^{3}H]thymidine. (*A*) Control; (*B*) 10 μM EHNA; (*C*) 50 μM EHNA. Arrow, position of HSV DNA (1.74 g/cm^3) as measured by refractive index.

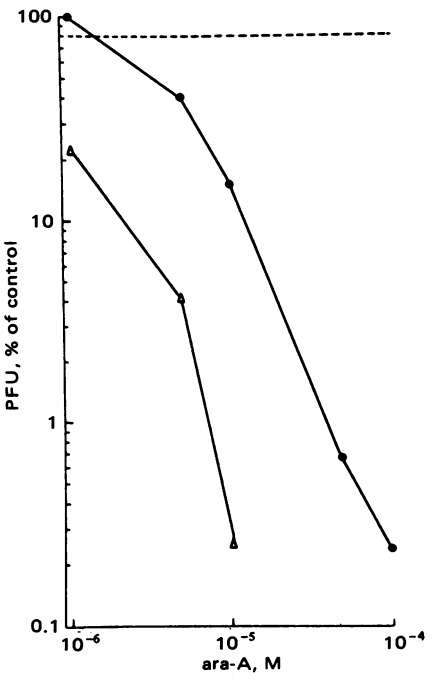

FIG. 4. Effect of ara-A on HSV production in the presence (Δ) and absence (●) of 1 μM EHNA. The dashed line represents HSV production in the presence of 1 μM EHNA alone (no ara-A).

Potentiation of Anti-HSV Effect of Cordycepin by EHNA. Cordycepin is a terminator of RNA chains (13), and it was of interest to explore its effects on the replication of a DNA virus. At 10 μM, cordycepin alone had no effect, and only a slight inhibition was evident at 100 μM (Fig. 5). In the presence of 10 μM EHNA, 10 μM cordycepin produced greater than 99% inhibition. Virus growth curves showed that this decrease in virus production represents a decrease in final yield rather than a prolongation of the normal replication cycle.

At 100 μM cordycepin alone completely inhibited growth of uninfected HeLa cells for 48 hr but the cells remained viable. In the presence of 10 μM EHNA, cordycepin at a concentration of 10 μM or higher rapidly killed the cells. At 10 μM cordycepin with 10 μM EHNA, only 10^{-4} survivors were found by 12 hr. This mixture also decreased virus production to 10^{-4}–10^{-3}. Under these conditions, then, the combination appeared to

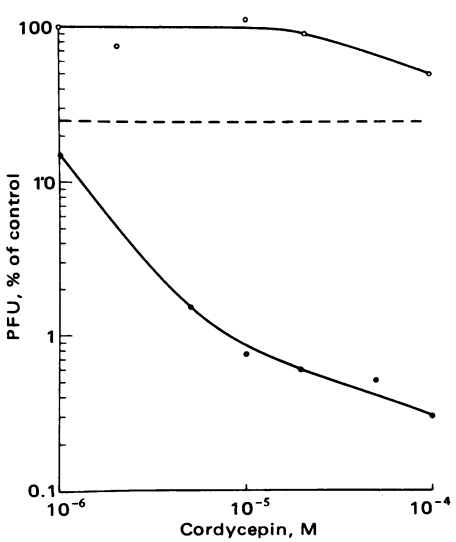

FIG. 5. Effect of cordycepin on production of HSV in the presence (●) and absence (○) of 10 μM EHNA. The dashed line represents HSV production in the presence of 10 μM EHNA alone.

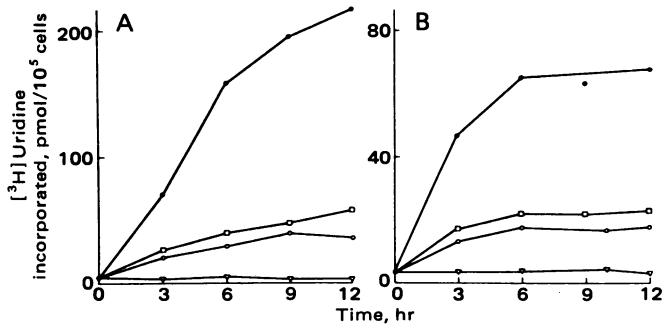

FIG. 6. RNA synthesis in uninfected (*A*) and HSV-infected (*B*) HeLa cells. ●, Control; □, 10 μM cordycepin; ○, 10 μM cordycepin plus 10 μM EHNA; 100 μM cordycepin plus EHNA.

possess little specificity toward virus or cells. However, evidence for specificity was obtained in studies of macromolecular synthesis.

Effects of Cordycepin on RNA Synthesis. The incorporation of uridine into trichloroacetic acid-precipitable material in uninfected and HSV-infected HeLa cells is shown in Fig. 6. In the absence of inhibitors, the rate of uridine incorporation during the first 3 hr in HSV-infected cells was only 70% of that in uninfected cells. This rate then slowly decreased until about 6 hr, after which there was little net accumulation of labeled RNA (14). Inhibition of RNA synthesis was increased by cordycepin and this inhibition was markedly augmented by EHNA. Inhibition occurred to the same extent in HSV-infected and uninfected cells.

Effects of Cordycepin on DNA Synthesis. DNA synthesis in uninfected and HSV-infected HeLa cells is shown in Fig. 7. The rate of thymidine incorporation into DNA of uninfected cells was about double that in HSV-infected cells. In infected cells the thymidine triphosphate pool expands after HSV-infection (15) and a new HSV-specific thymidine kinase is induced (16), thereby altering the specific activity of the radioactive label in the thymidine triphosphate pool and hence in the newly made DNA. Moreover, thymidine labeling would be expected to underestimate the amount of HSV DNA synthesized relative to that of HeLa cells by a factor of 2 because HSV DNA is only 33% A+T whereas HeLa DNA is 60% A+T.

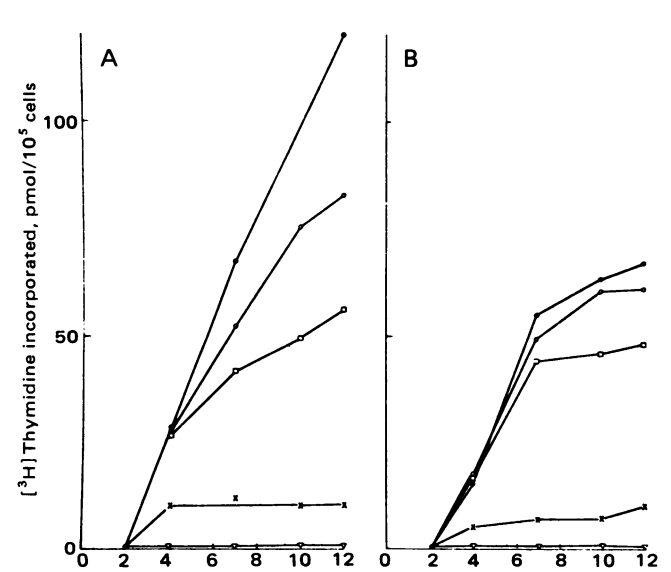

FIG. 7. DNA synthesis in uninfected (*A*) and HSV-infected (*B*) HeLa cells. ●, Control; □, 100 μM cordycepin; 10 μM cordycepin plus 10 μM EHNA; ×, 50 μM cordycepin plus μM EHNA; ▽, 100 μM cordycepin plus 10 μM EHNA.

Fig. 7 also shows the effect of cordycepin on DNA synthesis in uninfected and HSV-infected HeLa cells. Cordycepin at 100 μM decreased DNA synthesis 50% in uninfected and 25% in HSV-infected cells. When deamination of cordycepin is prevented by 10 μM EHNA, this same level of cordycepin (100 μM) completely inhibits DNA synthesis in both infected and uninfected cells (1). This effect of the combination on growing cells is attributed mainly to the prevention of deamination of cordycepin. Although EHNA alone (10 μM) does cause a decrease in DNA synthesis in HSV-infected cells, as shown in Fig. 2, the effect of cordycepin plus EHNA on DNA synthesis in infected cells is greater than the sum of the individual effects of each. Nevertheless, DNA synthesis is not the most sensitive site of inhibition by cordycepin, because RNA synthesis was inhibited by 80% under these conditions in both uninfected and infected cells.

The most significant result is that, in the presence of 10 μM EHNA, 10 μM cordycepin had only a slight effect on the amount of DNA synthesis in HSV-infected cells (Fig. 7*B*), whereas virus production was inhibited by 99% (Fig. 5). Fig. 8*C* shows a density gradient analysis of DNA from HSV-infected cells that were treated with 10 μm cordycepin and 10 μM EHNA. The DNA synthesized under these conditions was almost entirely cellular DNA, with only a trace of radioactivity detectable at the position of HSV DNA. Thus, HSV DNA synthesis is almost completely and specifically blocked.

This inhibition of HSV-specific DNA synthesis under conditions such that cellular DNA synthesis is decreased by only 25% (Fig. 7*A*) could be due either to a specific inhibition of HSV DNA synthesis or to an inability to synthesize the enzymes required for HSV DNA synthesis. This latter effect would most likely result from the inhibition of RNA synthesis under these conditions (Fig. 6*B*). To distinguish these possibilities, we analyzed the DNA made in HSV-infected cells that were treated with 10 μM cordycepin and 10 μM EHNA at 8 hr after infection and labeled with [3H]thymidine from 8¼ to 12 hr after infection. By 8 hr, HSV DNA synthesis was well under way and production of infectious HSV particles was occurring. HSV DNA was made essentially normally after the addition of 10 μM cordycepin and 10 μM EHNA at 8 hr.

In the presence of 100 μM cordycepin (without EHNA) there

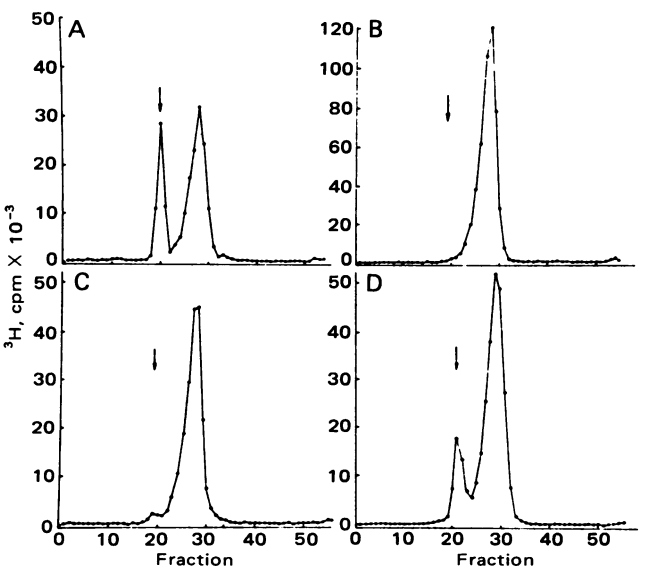

FIG. 8. CsCl density gradient analysis of [3H]thymidine-labeled DNA from uninfected (*B*) and HSV-infected (*A*, *C*, and *D*) HeLa cells. (*A* and *B*) Untreated control; (*C*) 10 μM cordycepin plus 10 μM EHNA (C); (*D*) 100 μM cordycepin. Arrow, position of HSV DNA (1.74 g/cm^3) as determined by refractive index.

was a 50% reduction in virus production. This level of cordycepin also caused a 50% reduction in the synthesis of HSV-specific DNA (Fig. 8*D*).

Effect of Cordycepin on Protein Synthesis. Protein synthesis was determined in uninfected and HSV-infected HeLa cells. The rate of leucine incorporation slowed down after HSV infection, with a pattern similar to that of uridine incorporation (Fig. 6*B*). Protein synthesis was inhibited by cordycepin. Cordycepin alone (100 μM) inhibited the initial rate of protein synthesis by 20% in uninfected cells and by 35% in HSV-infected cells. When this concentration of cordycepin was used together with 10 μM EHNA, protein synthesis was more than 90% inhibited in both infected and uninfected cells. Incorporation of [^{3}H]leucine was decreased more than 80% in the first 3 hr, and there was no further accumulation beyond that time. A combination of 10 μM cordycepin and 10 μM EHNA inhibited the rate of protein synthesis by about 40% in both uninfected and HSV-infected HeLa cells. As discussed above, this treatment had little effect on total DNA synthesis, inhibited RNA synthesis by 80%, and completely blocked HSV-specific DNA synthesis.

DISCUSSION

EHNA, an active inhibitor of adenosine deaminase, markedly potentiates both the toxicity of ara-A and cordycepin to animal cells (1) and their inhibition of herpes virus multiplication. In each instance the protection against deamination leads to an increased formation of the triphosphate. These effects may lead to an increased toxicity for the animal treated for cancer or virus infection. Therefore, the use of EHNA in combination with adenosine analogues must be tested for such adverse consequences. Nevertheless, such undesirable effects cannot be predicted in advance.

In this paper we have demonstrated that EHNA alone has a surprising effect: it inhibits HSV production significantly at a concentration of drug that has little or no effect on the growth or viability of HeLa cells. Because deoxycoformycin does not produce this effect, this result cannot be due simply to the inhibition of adenosine deaminase. Similar results with EHNA were obtained with the McIntyre strain of HSV type 1 growing in mouse L cells. Vaccinia virus is also inhibited by EHNA to a lesser extent (T. W. North and D. Hruby, unpublished results).

EHNA at 10 μM specifically inhibits synthesis of HSV DNA but not that of RNA or protein; this correlates well with the inhibition of virus production. Synthesis of DNA, RNA, and protein in uninfected cells was not inhibited by EHNA. Thus, the antiherpes action of EHNA seems to be due to a relatively specific inhibition of HSV DNA synthesis. However, the mechanism of this action is unknown. In addition to the specificity of inhibition of viral DNA synthesis produced by use of EHNA alone, additional evidence of specific inhibition of this function was obtained when EHNA was used in combination with cordycepin.

Increasing evidence, such as that presented in this paper, suggests that EHNA may be preferable to deoxycoformycin in the treatment of HSV infections in conjunction with araA or other adenine nucleosides. EHNA alone is inhibitory to HSV multiplication whereas deoxycoformycin is not. The irreversibility of inhibition of adenosine deaminase by deoxycoformycin leaves cells susceptible to killing by the naturally occurring nucleosides adenosine and deoxyadenosine (12). Deoxycoformycin is also toxic to lymphocytes and may thereby affect immune processes more adversely than does EHNA.

Subsequent to the preparation of this paper, it was reported (17) that another inhibitor of adenosine deaminase, similar in structure to EHNA, (*S*)-9-(2,3-dihydroxypropyl)adenine (18), also inhibits the replication of a number of DNA and RNA viruses at concentrations at which cellular DNA and RNA synthesis was not affected. The compound is active in tissue culture systems, although possibly less active than EHNA, and in experimental infections in mice. It was also found that this analogue potentiated the inhibitory action of araA on HSV infections. It will be of interest to compare EHNA and the new inhibitor in the same systems.

Although it has been known for many years that cordycepin is an inhibitor and terminator of RNA synthesis, under previous conditions of use the compound was found to be only partially inhibitory. This result appears to relate to the limited synthesis of cordycepin triphosphate under conditions of extensive deamination. When this deamination is prevented, as with EHNA, far more complete inhibitions of RNA synthesis were obtained, as we have shown, as well as the expected effects on protein and DNA synthesis. The use of cordycepin with deaminase inhibitors should help to clarify the study of many aspects of RNA synthesis in cellular systems.

We thank Ms. Elaine Champey for technical assistance and Drs. F. McCormick and R. P. Klett for helpful discussion. This work was supported by Grant NIAID 7-R01-AI14005 from the National Institutes of Health, and by American Cancer Society Postdoctoral Fellowship PF-1246 awarded to T.W.N.

1. Plunkett, W. & Cohen, S. S. (1975) *Cancer Res.* **35**, 1547–1554.
2. LePage, G. A., Worth, L. S. & Kimball, A. P. (1976) *Cancer Res.* **36**, 1481–1485.
3. Johns, D. G. & Adamson, R. H. (1976) *Biochem. Pharmacol.* **25**, 1441–1444.
4. Plunkett, W. & Cohen, S. S. (1975) *Cancer Res.* **35**, 415–422.
5. Hayflick, L. (1965) *Texas Rep. Biol. Med.* **23**, 284–303.
6. Weissbach, A., Hong, S., Anker, J. & Muller, R. (1973) *J. Biol. Chem.* **248**, 6270–6277.
7. Plunkett, W., Lapi, L., Ortiz, P. J. & Cohen, S. S. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 73–77.
8. Halliburton, I. W. & Timbury, M. C. (1976) *J. Gen. Virol.* **30**, 207–221.
9. Agarwal, R. P., Spector, T. & Park, R. E. (1977) *Biochem. Pharmacol.* **26**, 359–367.
10. Schaeffer, H. J. & Schwender, D. F. (1974) *J. Med. Chem.* **17**, 6–8.
11. Sloan, B. J., Kielty, J. K. & Miller, F. A. (1977) *Ann. N. Y. Acad. Sci.* **284**, 60–80.
12. Lapi, L. & Cohen, S. S. (1977) *Biochem. Pharmacol.* **26**, 71–76.
13. Shigeura, H. & Boxer, G. W. (1974) *Biochem. Biophys. Res. Commun.* **17**, 758–763.
14. Roizman, B., Borman, G. S. & Kamali-Rousta, M. (1965) *Nature (London)* **206**, 1374–1375.
15. Cheng, Y., Goz, B. & Prusoff, W. H. (1975) *Biochim. Biophys. Acta* **390**, 253–263.
16. Kit, S. & Dubbs, D. R. (1963) *Biochem. Biophys. Res. Commun.* **11**, 55–59.
17. DeClercq, E., Descamps, J., DeSomer, P. & Holy, A. (1978) *Science* **200**, 563–565.
18. Schaeffer, H. J., Vogel, D. & Vince, R. (1965) *J. Med. Chem.* **8**, 502–506.