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Antiviral Activity of Arabinosyladenine and Arabinosylhypoxanthine in Herpes Simplex Virus-Infected KB Cells: Selective Inhibition of Viral Deoxyribonucleic Acid Synthesis in Synchronized Suspension Cultures

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The drug 9-β-D-arabinofuranosyladenine (ara-A) significantly suppressed the formation of herpes simplex virus type 1-induced syncytia in BHK-21/4 cells at concentrations as low as 0.1 μg/ml. Optimal activity was noted when the drug was added before initiation of viral deoxyribonucleic acid (DNA) synthesis (3.5 h postinfection). The deaminated derivative of ara-A, 9-β-D-arabinofuranosylhypoxanthine (ara-H), was at least 10 times less effective in suppressing the development of herpes simplex virus-induced syncytia. The replication of herpes simplex virus was measured by assaying fluids and cells from infected drug-treated cultures by using a plaque production technique. Ara-A at drug levels of $>10 < 32$ μg/ml completely blocked the replication of infectious virus particles. Ara-H was less effective than ara-A in reducing the replication of virions. Rates of host and viral DNA synthesis were monitored by pulse labeling herpes simplex virus-infected synchronized KB cells with [^{3}H]thymidine and subsequently separating viral from cellular DNA in CsCl density gradients. During synthetic (S) phase, ara-A or ara-H at concentrations ranging from 3.2 to 32 μg/ml selectively inhibited viral DNA synthesis. At 3.2 μg of ara-A per ml, viral DNA synthesis was reduced 74% although total cellular DNA synthesis was unaffected. Increasing concentrations of ara-A produced increasing temporal delays in the maximal rate of host DNA synthesis. This time shift was not observed in cells treated with ara-H.

The nucleoside antibiotic 9-β-D-arabinofuranosyladenine (ara-A) was first synthesized in 1960 as a potential anticancer agent (16). The antiviral activity of the drug was announced in 1964 by Privat de Garilhe and de Rudder, who found that ara-A had marked antiviral activity toward herpes simplex virus (HSV) and vaccinia virus (22) and that 333 μM ara-A completely suppressed viral replication but not the associated virus-induced cytopathogenic effect (7). Independently, investigators at Parke, Davis and Co. observed antiviral activity directed against HSV in an antibiotic concentrate derived from a fermentation beer of *Streptomyces antibioticus*. In 1967 the active component was identified as ara-A, and in 1968 the in vitro antiviral activities of this naturally occurring substance and its deaminated catabolite 9-β-D-arabinofuranosylhypoxanthine (ara-H) were reported (18). Subsequent studies have shown ara-A to possess broad-spectrum activity against deoxyribonucleic acid (DNA) viruses in cell culture (24).

It has been reported (17) that HSV type 1 was more susceptible to ara-A than was HSV type 2. Other workers, however, have found that the observed variation was explainable in terms of the cell culture system used and the passage history of the virus (21) and the HSV types 1 and 2 cannot be reliably separated by type using in vitro drug susceptibility patterns (S. R. Jones, P. J. Luby, and D. McElligott, Clin. Res. 21:603, 1973).

Hubert-Habart and Cohen noted in 1962 that ara-A inhibited DNA synthesis in a purine auxotroph of *Escherichia coli* (15). Subsequent to this observation it has been suggested that ara-A or its corresponding nucleotide may inhibit DNA synthesis in eukaryotic or prokaryotic cells by (i) acting as a chain terminator (30), (ii) inhibiting the enzyme ribonucleotide reductase (19, 31), or (iii) inhibiting the action of DNA polymerase (10, 19). Although biochemical studies of this nature have not been extended to virus-infected cells, it is presumed that the antiviral activity of ara-A is due to the

inhibition of viral DNA synthesis.

We have previously shown (28) that ara-A selectively inhibits chromosomal but not circular DNA synthesis in a line of virus-transformed embryonic rat cells. In this communication we present experimental evidence for the antiviral activity of ara-A and its catabolite ara-H. Our findings suggest that both drugs selectively inhibit the synthesis of viral DNA.

MATERIALS AND METHODS

Ara-A was provided through the courtesy of Harold E. Machamer and Robert Hodges of Parke, Davis and Co., Detroit and Ann Arbor, Mich. Ara-H was purchased from Pfanstiehl Laboratories, Inc., Waukegan, Ill. The purity of ara-H was examined chromatographically by gas-liquid and high-pressure liquid techniques. A 1.2 to 1.5% contaminant was detected which chromatographed as ara-A in both systems. [*methyl*-^{3}H]thymidine was obtained from New England Nuclear Corp., Boston, Mass. HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and Pronase were purchased from Calbiochem, La Jolla, Calif.

Serum. Extensively heated (12 h at 56 C) calf serum (Flow Laboratories, Inc., Rockville, Md.) was used in experiments involving ara-A in order to inhibit the serum-associated deamination of ara-A to ara-H. We have previously shown (26) that prolonged heating of calf serum at 56 C eliminates serum-associated adenosine deaminase activity without significantly affecting the nutritive properties of the serum.

Cell culture. BHK-21/4 cells (see ref. 27) were grown in Ham nutrient mixture F-12 supplemented with 5% unheated fetal bovine serum.

KB cells were the gift of Gary H. Cohen, The University of Pennsylvania, Philadelphia, Pa.

Monolayer cultures of KB cells were grown in Eagle minimal essential medium (MEM) with either Hanks salts or Earle salts supplemented with 10% unheated calf serum. The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cells were passaged according to conventional procedures by using 0.05% trypsin plus 0.02% ethylenediaminetetraacetic acid in a HEPES-buffered balanced salt solution (see ref. 27).

Suspension cultures of KB cells were grown at 37 C in MEM with spinner salts ($NaHCO_3$ omitted) supplemented with 10% extensively heated calf serum. The pH was maintained at 7.0 by using 10 mM HEPES. Suspension-grown KB cells had an average population doubling time of 30 h. Cells were maintained at a density of 2×10^5 to 4×10^5 cells/ml at a volume of 300 ml in hanging bar-type 500-ml spinner flasks (Bellco Glass Co., Inc., Vineland, N.J.).

To increase the likelihood of detecting bacterial and/or mixed bacterial and mycoplasmal contamination, antibiotics were never included in any tissue culture media (14). All cell lines were screened periodically (tests performed by Microbiological Associates, Bethesda, Md.) and were found to be mycoplasma free.

Cell counts. Total cell counts were made with a Coulter counter equipped with a 100-μm orifice. Viable counts were determined by means of trypan blue dye exclusion.

Virus. The HF strain of HSV was obtained from Gary H. Cohen. High-titered virus stock preparations were prepared as follows. Nearly confluent monolayer cultures of KB cells grown in 16- or 32-oz (about 480- or 960-ml) glass bottles (Brockway Glass Co., Inc., Brockway, Pa.) containing MEM plus Hanks salts supplemented with 10% unheated calf serum and 2× arginine were infected at an input multiplicity of 5 to 10 plaque-forming units (PFU) per cell. After 24 h the cells were harvested by vigorous shaking, washed with fresh medium, and concentrated by centrifugation (5 min at $300 \times g$). The cell pellet was resuspended in 1/40 of the original volume of medium and sonicated for 60 s at 20 kHz in a Bronwill Sonicator (Bronwill Scientific Inc., Rochester, N.Y.) set to deliver 70 W of acoustical power through a needle probe. The preparations were clarified by centrifugation at $500 \times g$ for 10 min to remove cell debris. The resulting supernatant fluids, which exhibited titers ranging from 3×10^8 to 1×10^9 PFU/ml, were stored at −76 C until retrieved.

Titration of virus. HSV was assayed by using monolayer cultures of BHK-21/4 cells grown in Integrid tissue culture dishes (60 by 15 mm; Falcon Plastics, Oxnard, Calif.). The cells were planted at 10^6 cells/dish in Ham nutrient mixture F-12 supplemented with 5% unheated fetal bovine serum. After 22 to 24 h the cells were 90% confluent and were inoculated with 0.2 ml of the virus suspension to be assayed and incubated in a humidified 3% CO_2-97% air atmosphere for 1 h to permit viral adsorption.

For the syncytial assay, the medium was replaced with 5 ml of F-12 medium and incubated at 37 C for an additional 22 to 24 h. After incubation, the cells were fixed in situ, stained with alum hematoxylin, and rinsed with a HEPES-buffered balanced salt solution, and syncytia were enumerated.

For plaque assays, the cells were overlaid after adsorption with 5 ml of F-12 medium containing 0.5% methocel (4,000 centipoise/s) (Dow Chemical Co., Midland, Mich.) and incubated an additional 4 days. After fixation with neutral formalin, the cells were stained with Giemsa tissue stain, Wolbach modification (Paragon C. and C. Co., Inc., Bronx, N.Y.), and macroscopic plaques were enumerated by using a Digimatic colony counter (Lab Line Instruments, Inc., Melrose Park, Ill).

Cell synchronization. Suspension cultures of KB cells were synchronized by the double-thymidine block method (5) as modified by Bello (3). In brief, KB cells in suspension culture were treated with 2 mM thymidine for 18 to 20 h, centrifuged at $300 \times g$ for 5 min, resuspended in fresh warm medium at 37 C to reverse the thymidine block, and then allowed to grow an additional 9 to 12 h. At this time, 2 mM thymidine was again added for 18 to 20 h. Experiments were started after reversal of the second thymidine block.

Rate of cellular DNA synthesis in synchronized cell cultures. Replicate 100-ml spinner cultures of

synchronized KB cells were prepared containing 0, 3.2, 10, and 32 μg of drug per ml, respectively. At selected times, 5-ml samples were removed and placed in screw-cap tubes (25 by 160 mm) containing sufficient [^{3}H]thymidine to result in a final concentration of 2 μCi/ml. The resulting cell suspension was maintained in a reciprocating water bath shaker (200 excursions/min) at 37 C for the duration of the pulse. Pulse durations were either 30 or 60 min. At the end of the pulse period, 100 μl of the cell suspension was spotted in triplicate on numbered 1-inch (about 2.5-cm) square pieces of Whatman no. 1 filter paper and dried rapidly under an infrared lamp. After drying, the squares of filter paper were washed thrice with ice-cold 5% trichloroacetic acid, twice with 95% ethanol, and once with diethyl ether. The dried squares of filter paper were then immersed in 10 ml of toluene-2,5-diphenyloxazole, and the samples were counted in a Beckman model LS-250 liquid scintillation spectrometer.

Rate of viral DNA synthesis in infected synchronized cells. Tritium-labeled cellular and viral DNA was obtained from infected synchronized KB cells grown in suspension by incubating 5 ml of the culture with 2 μCi of [^{3}H]thymidine per ml (final concentration) for 1 h at 37 C in a reciprocating water bath shaker (200 excursions/min). The reaction was stopped by the addition of 5 ml of ice-cold MEM plus spinner salts supplemented with 10% extensively heated calf serum and 10 mM HEPES (pH 7.0). The cells were pelleted by centrifugation at 600 × *g* for 5 min, and the pellet was stored at −76 C for subsequent analysis. After resuspending the pellet in TES [30 mM tris(hydroxymethyl)aminomethane, 5 mM ethylenediaminetetraacetic acid, 50 mM NaCl, pH 8.0], an equal amount of a solution containing 2 mg of Pronase per ml (autodigested for 15 min at 37 C) and 1% Sarkosyl in TES was added and incubated for 15 min at 37 C. The DNA was partially sheared by pipetting before ultracentrifugation.

Isopycnic density gradient centrifugation. CsCl density gradient ultracentrifugation was used to separate viral from cellular DNA. One milliliter of the DNA-containing preparation was added to 9.9 g of CsCl in 7 ml of TES. (Technical-grade CsCl [Kawecki Berylco Industries, Inc., Boyertown, Pa.] was dissolved in a minimal amount of water and recrystallized after ultrafiltration through a fiberglass prefilter and a 0.22-μm filter [Millipore Corp., Bedford, Mass].) After centrifugation for 65 h at 100,000 × *g* and 15 C using a Ti-50 rotor in a Beckman model L3-50 preparative ultracentrifuge, 8-drop fractions were collected from the bottom of the tube by using a Buchler polystaltic pump and an LKB Ultrorac fraction collector. Fifty-microliter samples were spotted on Whatman no. 1 chromatography paper squares and then processed and counted as described above. About 40 fractions were collected from each tube, and every third fraction was used for determination of refractive index and buoyant density.

RESULTS

Inhibition of HSV-induced syncytia by ara-A and ara-H. Table 1 compares and contrasts the effects of ara-A and ara-H on the ability of the HF strain of HSV type 1 to induce syncytia in monolayers of BHK-21/4 cells. Ara-A, when added within 6 h of the viral adsorption period, was a potent inhibitor of syncytia formation. Ara-H was approximately one-tenth as effective as ara-A, reducing syncytia formation 85% at the highest drug level tested.

Interference by ara-A with the replication of HSV. The effect of ara-A on the replication of HSV in synchronized KB cells is shown in Fig. 1A. The titer of HSV in the cells and supernatant fluid was inversely proportional to the amount of drug present. Although 10 μg of ara-A per ml reduced the titer at 12 h by 70%, 32 μg/ml was required to block de novo replication of HSV virions.

Interference by ara-H with the replication of

TABLE 1. *Effect of ara-A and ara-H on syncytia formation by herpes simplex virus*[a]

Time of drug addition (h) after infection		Percent reduction of HSV-induced SFU when compared with control (no drug)								
ara-A	ara-H	0.1[b]	1	2	5	10	20	40	80	160
1		20[c]	26	29	40	71	97	99	>99	100
3						87	97	>99	>99	100
6						79	95	96	98	>99
9						0	ND[d]	78	89	86
12						10	14	32	ND[d]	>99
	1					4	2	35	65	85

[a] 200 syncytium-forming units (SFU) of herpes simplex virus were added to nearly confluent monolayers of BHK-21/4 cells in Integrid plastic dishes (60 by 15 mm). Adsorption was allowed to occur at 37 C for 1 h, after which time Ham nutrient mixture F-12 supplemented with 5% fetal bovine serum was added. Varying concentrations of drug were added at the times indicated above.

[b] Concentration of drug (in micrograms per milliliter).

[c] Significant at $P = 0.04$.

[d] ND, Not done.

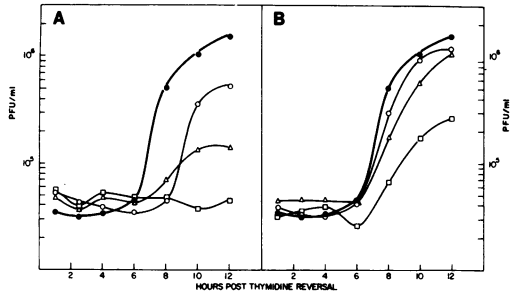

FIG. 1. *Effect of ara-A (A) and ara-H (B) on the replication of HSV in synchronized cultures of KB cells. A suspension culture of KB cells was synchronized by the double-thymidine block method. The cells were divided into four centrifuge tubes and pelleted at 700 × g for 5 min. The cells then were resuspended in a high-titer virus suspension, producing a slurry containing 12 × 10⁶ cells/ml infected at an input multiplicity of 15 PFU/cell. After a 1-h adsorption period the cells were brought to initial volume with fresh medium and transferred to 100-ml spinner flasks. The replicate cultures contained no drug (●), 3.2 μg of drug per ml (○), 10 μg of drug per ml (△), and 32 μg of drug per ml (□). Aliquots were aseptically removed from the infected cultures at time points indicated above, subjected to freezing and thawing, and assayed for plaque-forming ability on BHK-21/4 cells.*

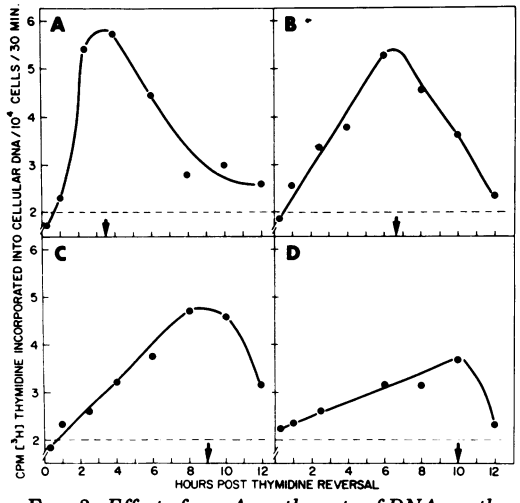

FIG. 2. *Effect of ara-A on the rate of DNA synthesis in uninfected synchronized cultures of KB cells. A suspension culture of KB cells was synchronized by the double-thymidine block method. The cells were then divided into four replicate cultures in fresh medium at a concentration of 3.0 × 10⁵ cells/ml. The replicate cultures contained no drug (A), 3.2 μg of ara-A per ml (B), 10 μg of ara-A per ml (C), and 32 μg of ara-A per ml (D). The rate of DNA synthesis was determined by pulse labeling 5.0-ml samples with [³H]thymidine for 30 min. The time at which the maximal rate of DNA synthesis occurred is shown in each panel with an inverted arrow.*

HSV. Figure 1B shows the effect of ara-H on virus production in drug-treated suspension cultures of synchronized KB cells. Ara-H was less efficient than ara-A as an inhibitor of viral replication. Low levels of ara-H had a minimal effect on the replication of HSV virions. The highest dose tested, 32 μg/ml, did not completely block synthesis of virus as had an equivalent concentration of ara-A, although the titer of virus at 12 h was reduced by 84%.

Inhibition of DNA synthesis by ara-A in uninfected cells. The effect of ara-A on the rate of DNA synthesis in uninfected cultures of KB cells synchronized by the double-thymidine block method is illustrated in Fig. 2. A typical burst of DNA synthetic activity in the absence of drug is shown in panel A. The maximal rate of DNA synthesis occurred at 3.5 h after thymidine reversal. Increasing concentrations of ara-A (Fig. 1 B–D) produced increasing temporal delays in the maximal rate of DNA synthesis. The net synthesis of cellular DNA in the presence of 3.2 μg of ara-A per ml (Fig. 1B) was virtually unchanged (102% of control) compared with the amount of DNA synthesis that occurred in the absence of drug. Ten and 32 μg of ara-A per ml reduced net DNA synthesis 10 and 44%, respectively.

Inhibition of viral DNA synthesis by ara-A. To determine the effect of ara-A on the rate of viral DNA synthesis, replicate suspension cultures of synchronized KB cells were infected with HSV. Pulse labeling with [³H]thymidine and subsequent separation of viral from cellular DNA by means of isopycnic centrifugation in CsCl enabled us to measure rates of viral DNA synthesis. This method has been used by Cohen et al. (6) and others to give clear separation of viral and host DNA. Each point in Fig. 3 represents the net amount of trichloroacetic acid-insoluble radioactivity recovered from the peak representing viral DNA in a CsCl density gradient. In the absence of drug, the rate of viral DNA synthesis increased rapidly, with a maximum at approximately 2.5 h. Little or no synthesis of viral DNA occurred after 10 h. In the presence of 3.2 μg of ara-A per ml (Fig. 3B), net synthesis of viral DNA was reduced 74%. Higher levels of ara-A (Fig. 3C,D) virtually abolished viral DNA synthesis. The selectivity of ara-A as an inhibitor of viral DNA synthesis can be seen by comparing Fig. 2B and 3B.

Inhibition of DNA synthesis by ara-H in uninfected cells. Ara-H was evaluated by a method analogous to that used to determine the efficacy of ara-A in reducing the rate of host

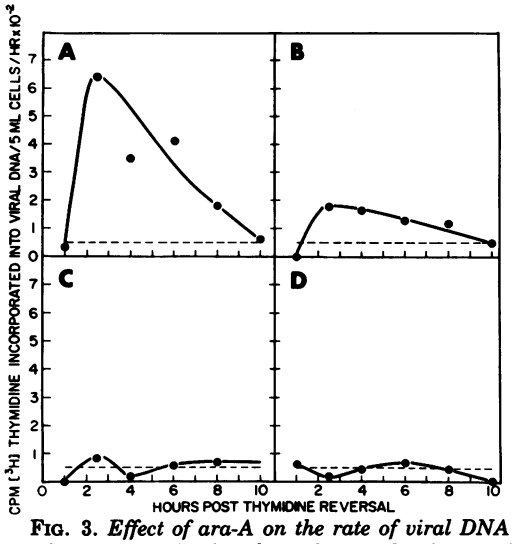

FIG. 3. *Effect of ara-A on the rate of viral DNA synthesis in HSV-infected synchronized cultures of KB cells. A suspension culture of KB cells was synchronized by the double-thymidine block method. The cells were then divided into four centrifuge tubes and pelleted at 1,000 × g for 5 min. The cells were resuspended in the virus suspension, producing a slurry containing 12 × 10⁶ cells/ml infected at an input multiplicity of 15 PFU/cell. After a 1-h adsorption period the cells were brought to initial volume with fresh medium and transferred to 100-ml spinner flasks. The replicate cultures contained no drug (A), 3.2 μg of ara-A per ml (B), 10 μg of ara-A per ml (C), and 32 μg of ara-A per ml (D). The rate of DNA synthesis was determined by pulse labeling 5.0-ml samples with [³H]thymidine for 1 h. Viral DNA was separated from cellular DNA by isopycnic centrifugation in CsCl.*

DNA synthesis. In contrast to the results seen with ara-A (Fig. 2), ara-H did not induce a temporal delay in the maximal rate of DNA synthesis (Fig. 4). Net host DNA synthesis in the presence of the highest dose of ara-H used, 32 μg/ml, was approximately 60% of the control value. Thus ara-H inhibited DNA synthesis to a slightly lesser extent than ara-A. Data from other experiments (results not shown) have confirmed that ara-H inhibited cellular DNA synthesis to a lesser but significantly different degree than ara-A.

Inhibition of viral DNA synthesis by ara-H. Figure 5 illustrates the inhibition by ara-H of HSV DNA synthesis in infected KB cells. Whereas doses of >10 μg of ara-A per ml virtually abolished viral DNA synthesis, ara-H was less effective as an inhibitor of viral DNA synthesis, producing a 49% inhibition at 3.2 μg/ml, a 69% inhibition at 10 μg/ml, and a 91% inhibition at 32 μg/ml. Thus ara-H can be seen to selectively inhibit viral DNA but to a lesser extent than ara-A.

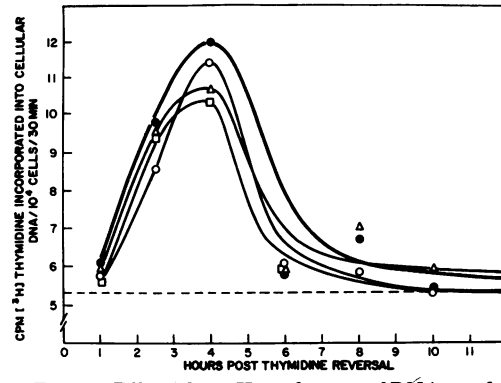

FIG. 4. *Effect of ara-H on the rate of DNA synthesis in uninfected synchronized cultures of KB cells. The experimental procedures used were the same as in Fig. 2. Symbols: (●) No drug; (○) 3.2 μg of ara-H per ml; (△) 10 μg of ara-H per ml; (□), 32 μg of ara-H per ml.*

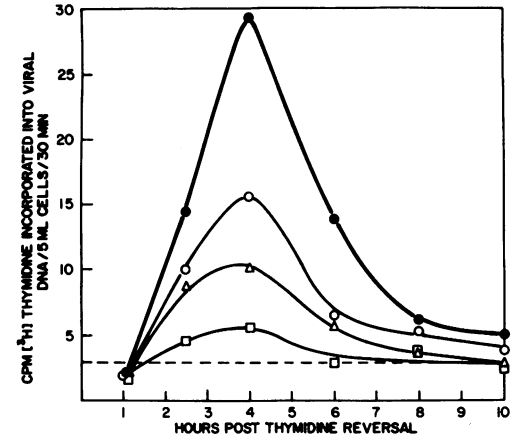

FIG. 5. *Effect of ara-H on the rate of viral DNA synthesis in HSV-infected synchronized cultures of KB cells. The experimental procedures used were the same as in Fig. 3. Symbols: (●) No drug; (○) 3.2 μg of ara-H per ml; (△), 10 μg of ara-H per ml; (□) 32 μg of ara-H per ml.*

DISCUSSION

When compared in their ability to reduce syncytia formation by HSV, ara-A was approximately 10 times more active than its deaminated catabolite ara-H. These results are consistent with those published earlier by Miller and co-workers (18). The inhibitory action of ara-A was optimal when added before or during viral DNA synthesis. The drug was markedly less active when added at times greater than 6 h postinfection.

There is a preponderance of evidence indicating cell fusion as the principal means by which macroscopic syncytia are formed (23). Inhibition of DNA-dependent ribonucleic acid synthesis by

actinomycin C (9) or inhibition of protein synthesis by cycloheximide (8) prevents HSV-induced polykarocyte formation. In contrast, inhibition of viral DNA synthesis by halogenated pyrimidines (see, for example, ref. 29) does not affect syncytium formation although the production of infectious virions may be completely suppressed. Thus, one may infer that cell fusion is dependent upon early transcription and translation but not upon reduplication of the viral genome of HSV. Arabinosyladenine and arabinosylhypoxanthine appear to function in reducing the number of apparent plaques in an assay not by blocking initial polykaryocyte formation but rather by limiting the size of the resulting syncytium. Observations in our laboratory by means of phase microscopy of drug-treated HSV-infected cells support this hypothesis.

As a means of further studying the antiviral activity of ara-A and ara-H, the synchronized KB cell suspension system of Bello (3) and of Cohen et al. (6) was modified for use. It was anticipated that this system would not only offer the convenience of working with suspension cultures but would also enable us to study the inhibitory effects of the drugs on DNA synthesis during the synthetic (S) phase of the cell cycle.

Virus replication was measured by assaying cell-associated and free virus from drug-treated, infected, synchronized cultures. As reflected in Fig. 1, ara-A was seen to depress peak virus production at an efficiency some 8 to 10 times greater than that observed with ara-H. Preliminary observations in the electron microscope (results not shown) indicate that although there is a dose-dependent decrease in the number of PFU, the total number of apparently normal virions remains relatively constant when the drug-treated infected cells are compared with the nontreated infected cells. This would suggest that the physical particle/PFU ratio is increasing with drug treatment. This hypothesis is currently being explored in our laboratory and will be the subject of a future communication.

Rates of both cellular and viral DNA synthesis were studied in infected and noninfected drug-treated synchronized cells. Unexpectedly, we observed that ara-A induced a temporal shift in the maximal rates of DNA synthesis in control cells. In contrast, ara-H produced only a small reduction in the net amount of DNA synthesized. A careful examination of Fig. 2 reveals that although there is indeed a temporal shift in the maximal rate of DNA synthesis, S phase begins at precisely the same time after thymidine reversal. Thus, whereas the acceleration in the rate of DNA synthesis is 2.5 counts/min of [^{3}H]thymidine incorporated into cellular DNA/10^4 cells (in a 30-min pulse) per h in the control population, the acceleration in the rate of DNA synthesis in cells treated with 3.2, 10, or 32 μg/ml is approximately 0.7, 0.4, or 0.1 counts/min of [^{3}H]thymidine incorporated into cellular DNA/10^4 cells (in a 30-min pulse) per h, respectively. The acceleration in the rate of DNA synthesis of ara-H-treated cells, in contrast, remains virtually unchanged although increasing concentrations of drug decrease the maximum rate reached.

The selective inhibition of herpesvirus DNA synthesis compared with cellular DNA synthesis has been noted with other inhibitors of DNA synthesis. Becker and Weinberg (2) reported in 1972 that the oligopeptide distamycin A at a concentration of 50 μg/ml completely inhibited the synthesis of Epstein-Barr virus DNA while allowing cellular DNA synthesis to continue, albeit at a lower rate. Phosphonoacetic acid has similarly been shown to selectively inhibit the synthesis of HSV DNA (20) and herpesvirus of türkeys (L. F. Lee and J. A. Boezi, Abstr. Annu. Meet. Am. Soc. Microbiol., S 137, p. 239, 1975). The mode of action of this interesting drug appears to be a selective inhibition of the DNA-dependent DNA polymerase coded for by the virus and expressed in the infected cell. Schildkraut et al. (25) have recently reported the selective inhibition of HSV replication by 5-bromodeoxycytidine and 5-iododeoxycytidine. Again the selective action appears to be dependent upon a virus-induced enzyme that catalyzes the conversion of the halogenated analogues of deoxycytidine to the corresponding nucleotides. A selective inhibition of *cellular* DNA synthesis in HSV-infected and Epstein-Barr virus-infected cells has been reported with cordycepin (1) and with hydroxyurea (12).

The above studies suggest that the selective inhibition of the synthesis of HSV DNA by ara-A and ara-H is most probably due to the preferential inhibition of a step in nucleotide metabolism catalyzed by a virus-specified enzyme. The need for these enzymes is clear (see, for example, ref. 13). The DNA polymerase of the mammalian cell, for instance, functions to make one copy of the cell genome in each cell generation. Viral DNA replication involves the formation of a large number of copies of viral DNA in one permissive cycle. Total dependence on cellular enzymes would impose such restrictions on the replication of the viral genome that reduplication of a virion would have little likelihood of being accomplished. Although attempts to demonstrate the selective inhibition of the HSV-induced polymerase by ara-ATP

have not been encouraging in crude systems (4; C. Reinke and C. Shipman, unpublished observations), we feel confident that highly purified preparations of this or other virus-specified enzymes will support the hypothesis that arabinosyl nucleosides owe their selective action to the differential inhibition of virus-induced enzymes.

A somewhat similar phenomenon was reported by Yoshikura (32). Treatment of C3H2K cells arrested in G1 phase with arabinosylcytosine for 2 h caused a delay in DNA synthesis when the cells were subsequently induced to divide by medium change. In this experiment, however, it was the onset of the synthetic phase that was delayed. Once DNA synthesis was initiated, the rate appeared consistent with that observed in the control cells.

Both York and LePage (31) and Moore and Cohen (19) have shown that the triphosphate of ara-A (ara-ATP) inhibits ribonucleoside diphosphate reductase in ascitic TA3 and Novikoff hepatoma cells. The K_i of ara-ATP for inhibition of this enzyme was found to be 30 to 110 μM. Although this K_i is considerably greater than the 1 μM K_i of ara-ATP for DNA polymerase (11), it is possible that even low concentrations of ara-A may inhibit DNA synthesis in cells that are synchronized by means of a thymidine-induced blockade of ribonucleotide reductase. This hypothesis is directly amenable to experimental exploration by studying the effects of ara-A on DNA synthesis in cells synchronized by means other than a blockage of the reductase enzyme system.

The mechanism by which ara-H acts in inhibiting host DNA synthesis may be very different from that of ara-A. Unfortunately, studies dealing with the inhibition of DNA polymerase or ribonucleoside diphosphate reductase by the triphosphate of ara-H are lacking in the published literature.

The process by which arabinosyladenine and arabinosylhypoxanthine selectively inhibit the synthesis of viral DNA is currently unknown. Studies with asynchronous suspension and monolayer cultures of KB cells (P. M. Schwartz, C. Shipman, Jr., and J. C. Drach, submitted for publication) confirm the selective effect of these nucleoside antibiotics, and the trivial explanation that the selectivity is dependent upon the cell system used can be rejected.

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