PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 1–7 (1982)

5′-Amino-5′-deoxythymidine: Topical Therapeutic Efficacy in Ocular Herpes and Systemic Teratogenic and Toxicity Studies (41386)

D. PAVAN-LANGSTON,*,† N. H. PARK,* J. LASS,‡ J. PAPALE,†
D. M. ALBERT,*,† T. LIN,§ W. H. PRUSOFF,§ AND D. H. PERCY‖

Eye Research Institute of Retina Foundation, 20 Staniford Street, Boston, Massachusetts 02114; ‡Case Western Reserve University, 2065 Adelbert Road, Cleveland, Ohio 44106; †Massachusetts Eye & Ear Infirmary, 243 Charles Street, Boston, Massachusetts 02114; §Yale University, New Haven, Connecticut 06520; and ‖Health Science Center, University of Western Ontario, London 72, Ontario, Canada

Abstract. AdThd (5′-amino-5′-deoxythymidine) is a highly soluble new antiviral drug specifically activated by herpes simplex viral thymidine kinase. Ten and fifteen percent AdThd eye drops were significantly better than placebo in therapy of herpetic keratouveitis in rabbits and the slope of the therapeutic curve of 15% AdThd was similar to that of idoxuridine (IdUrd). Systemic administration of these drugs in the neonatal mouse model revealed no adverse effect *in vivo* or by histopathologic exam in AdThd or saline-treated animals but that IdUrd was extremely toxic and teratogenic. AdThd is a new, effective and nontoxic specific antiherpes agent.

Efforts to improve the selectivity of anti-Herpes simplex virus (HSV) agents with consequent reduction in cellular toxicity have resulted in the development and evaluation of new classes of antimetabolites which include acyclic derivatives of purine nucleosides (1, 2) and analogs of pyrimidine nucleosides (3–6). In the latter class, the key substitution of an amino group in place of the hydroxyl group at the 5′ carbon position renders these drugs incapable of phosphorylation by enzymes in uninfected cells. The drugs are not incorporated into normal cellular DNA thereby obviating, in theory, the toxicity and mutagenicity seen with nonspecific antivirals such as idoxuridine (IdUrd), vidarabine (Ara-A), and trifluridine (F_3dThd) (5). The 5′-amino analogs, however, are good competitive inhibitors of the phosphorylation of thymidine by cellular thymidine kinase (7), modest inhibitors of cellular thymidylate kinase (8), and good substrates for the herpesvirus-encoded thymidine kinase (9, 10). These nucleosides have significant antiviral action *in vitro* and *in vivo* (4, 11, 12).

AIdUrd, the 5′-amino analog of 5′-iodo-2′-deoxyuridine (IdUrd), approaches IdUrd in efficacy against Herpes simplex in tissue culture, and in therapy of herpetic keratitis whether given in aqueous solution (8 mg/ml) or at 10% ointment. The mechanism of selective antiviral action is phosphorylation of AIdUrd by virus-encoded thymidine kinase thereby allowing DNA incorporation of the analog in HSV-infected but not in uninfected cells (13). Defective viral progeny are believed to be the end product of this action. Some *in vivo* studies indicate that AIdUrd is nontoxic (14). Neonatal mice given AIdUrd (450 mg/kg/day) and IdUrd (250 mg/kg/day) subcutaneously for 5 days were examined at 25 days of age. IdUrd-treated mice had a high mortality rate. Survivors were stunted and had histologic evidence of cerebellar, renal cortical and retinal hypoplasia, and cataracts. All AIdUrd-treated mice developed normally without evidence of abnormality on histopathologic examination. In another study on systemic AIdUrd treatment of herpetic encephalitis in mice, no therapeutic efficacy was found but drug-treated control animals lost weight significantly indicating toxicity at 1000 mg/kg/day. This may, however, have been a function of the poor solubility of the crystals (15).

AdThd (5′-amino-5′-deoxythymidine) shares many of the above properties with AIdUrd, but is 20 times more soluble in

0037-9727/82/050001-07$01.00/0

water. Studies by Cheng *et al.* (8), using mice ascites sarcoma 180 cells, confirmed earlier reports by Neenan and Rhode (16) that AdThd is a potent inhibitor of cellular thymidine kinase. Such inhibition would have little or no effect on normal uninfected cells since thymidylate required for DNA biosynthesis is supplied by the *de novo* pathway thereby bypassing the inhibition of thymidine kinase. However, the 5'-amino analog is a *substrate* for the herpesvirus thymidine kinase and this property is essential for the subsequent inhibitory effect on herpesvirus replication. Thus AdThd is an effective inhibitor of HSV-1 *in vitro* (17). At concentrations of 400 μM there is 97% inhibition of virus yield from infected Vero cells with no evidence of growth inhibition of these cells in uninfected cultures treated with the same levels of drug.

In this paper we report the *in vivo* therapeutic efficacy of AdThd in HSV-1 keratitis in rabbits and its failure to produce a teratogenic effect or toxicity in the neonatal mouse model. IdUrd was used as a positive control and saline as a negative control.

Materials and Methods. *Animals and materials.* Two-kilogram albino male rabbits were used for the keratitis model. Neonatal Balb/C mice with a mean birth weight of 1.5 g were used for the teratogenic and toxicity study.

Drugs. AdThd was synthesized using a modification of the method of Horwitz *et al.* (5, 18). Commercially available IdUrd was used for ophthalmic therapy. IdUrd powder for teratology and toxicity studies was obtained from Smith Kline and French Labs, Philadelphia, Pennsylvania. Both AdThd and IdUrd were prepared as suspensions in isotonic phosphate-buffered saline (PBS).

Histopathology. In the teratology–toxicity study tissues were immersed in Bouin's fixative, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS). The central nervous system, kidneys, liver, and eyes were examined.

Keratitis model. The unscarified eyes of rabbits were inoculated with one drop of McKrae strain HSV-1 (2.6×10^7 PFU/ml) and the closed lids massaged gently for 30 sec. Three days postinoculation (p.i.) slit lamp examination revealed 100% of the eyes had dendritic or geographic keratitis and the eyes were put into one of the following treatment groups: (A) AdThd 10%; (B) AdThd 15%; (C) IdUrd 0.1%; (D) PBS. Fifteen percent AdThd was deliberately used in a group of animals with a significantly higher mean starting score of epithelial disease to test its efficacy under more challenging circumstances. One drop of drug or PBS was given hourly, 8 hr daily for 6 days. Eyes were examined single blind daily on the slit lamp biomicroscope scoring 0–4 of increasing severity of ulcerative keratitis, stromal edema, conjunctivitis, and iritis. Viral cultures were taken from the conjunctival fornices at the beginning and end of the treatment period. The 10%

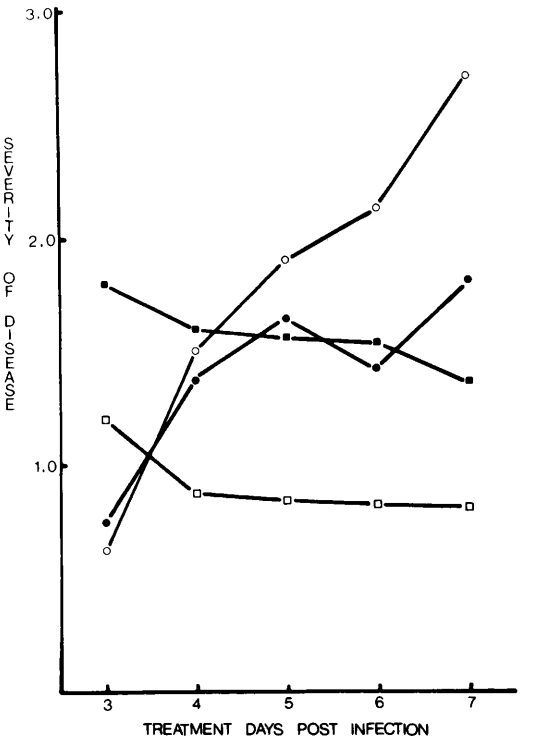

FIG. 1. Treatment response of Herpes simplex epithelial keratitis. ○, Saline control; ●, 10% AdThd, ■, 15% AdThd; □, IdUrd.

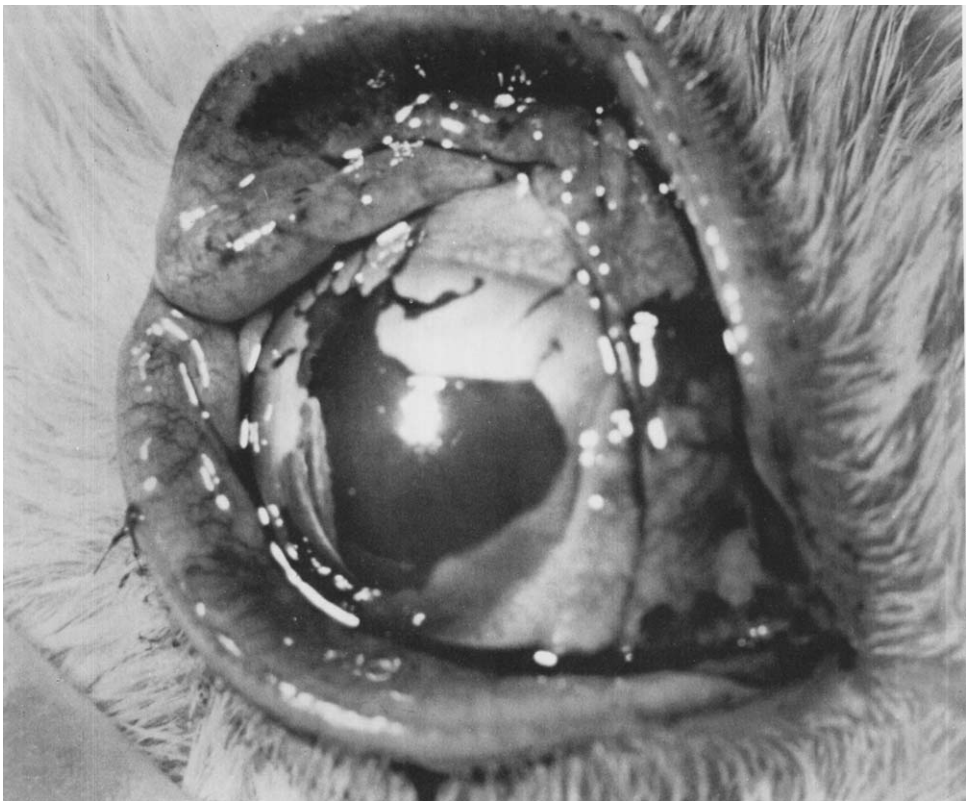

FIG. 2. Placebo-treated herpes simplex keratitis on Day 5 of treatment showing extensive epithelial ulceration.

AdThd post-treatment cultures were not done in the present experiment.

Neonatal mouse model. One hundred sixty-two mice were injected subcutaneously daily for 5 days beginning one day after birth after being placed in a treatment group: (A) AdThd, 225 mg/kg (30 mice); (B) AdThd, 450 mg/kg (33 mice); (C) IdUrd, 125 mg/kg (31 mice); (D) IdUrd, 250 mg/kg (35 mice); (E) PBS (33 mice). Animals were weighed daily during treatment and at autopsy. Mice were sacrificed at 13 to 30 days.

Statistical analysis. Statistical analysis of the keratitis model was by Student's t test comparing each group against the others. Analysis of the weights of mice was by one-way analysis of variance, Duncan's multiple range test to detect differences between groups.

Results. *Keratitis.* Epithelial ulceration daily mean scores are shown in Fig. 1. All controls (8 eyes) had increasing severity of disease throughout the treatment period. A comparison of the drug-treated eyes showed two distinct groupings. Both AdThd groups (14 eyes, 10% AdThd; 10 eyes, 15% AdThd) were significantly better than the controls ($P < 0.01$) on postinoculation days 6 and 7, but never significantly different from each other ($P > 0.1$). IdUrd was significantly better than either control and the AdThd groups from postinoculation days 4 through 7 ($P < 0.001$ for controls, $P < 0.05$ for AdThd). The iritis, stromal, and conjunctivitis mean group scores fell into groups similar to but less obvious than those seen with epithelial disease and were felt to reflect the severity of disease rather than therapeutic effect on those structures per se (data not shown). Figures 2 and 3 show a control and AdThd-treated eye on treatment day 5.

Viral cultures were 100% positive in all

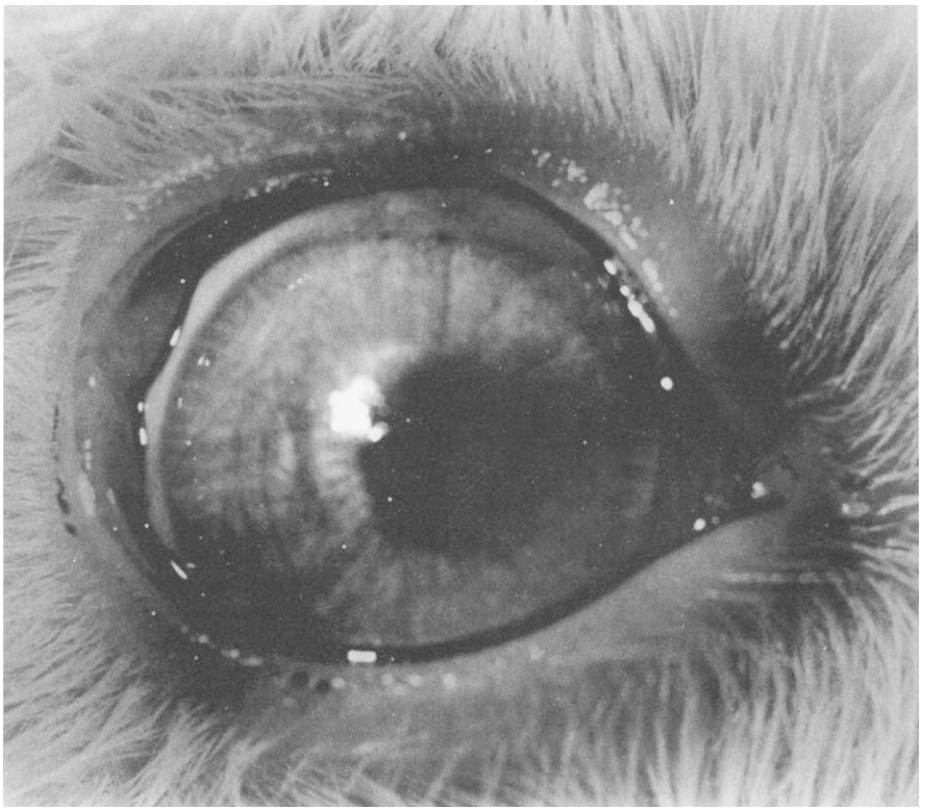

FIG. 3. AdThd-treated herpes simplex keratitis on Day 5 of treatment showing minimal epithelial ulceration.

pretreatment groups. On postinfection Day 8, 9/10 of the 15% AdThd eyes (90%), 15/15 of the IdUrd eyes (100%), and 7/8 of the control eyes (88%) were positive for virus. There was no statistically significant difference among the groups ($P > 0.1$).

Toxicity. There were no detectable differences between control and AdThd-treated mice in somatotype or hair growth. In contrast, all IdUrd-treated mice appeared emaciated, partially or totally hairless, and stunted. Table 1 shows the weight gain of animals during the treatment period. There was no significant difference between control or AdThd groups ($P > 0.05$). However, significant differences between these groups and the IdUrd-treated mice began to appear at 3 days of age ($P < 0.05$) and the IdUrd group weighed, on the average, 60% less than control or AdThd-treated mice at autopsy. There was no significant difference between the two IdUrd-treated groups ($P > 0.05$).

One animal in the control group died and two were cannibalized. No other animals died spontaneously but eight of the highest dose IdUrd-treated mice were also cannibalized, about two to four times as many as in the remaining treatment groups. This may have reflected the fact that these neonatal mice were quite sick.

Teratogenesis. Table 2 shows the eye teratology data. There was no significant difference among the controls, AdThd and IdUrd treatment groups with reference to cataract formation or retinal dysplasia. No other abnormalities were found in the eyes with the exception of ciliary body dysplasia and primary hyperplastic vitreous in one of the lower-dose IdUrd-treated animals.

Table 3 shows the teratology studies on the cerebellum, kidney, and liver. There

TABLE I. GROWTH AND SURVIVAL OF MICE TREATED WITH AdThd AND IdUrd

Drug dosage (mg/kg/day)	Number initially treated	Number surviving to necropsy	Mean age at necropsy (days)	Mean wt. at onset (g)	Mean wt. at Day 5 (g)	Mean wt. at necropsy (g)
AdThd, 250	30	28	22	1.89	3.93	11.31
AdThd, 500	33	30	22	1.82	3.94	11.78
IdUrd, 125	31	27	22	1.85	2.84	6.82
IdUrd, 250	35	27	23	1.83	2.63	7.01
PBS	33	31	22	1.72	3.67	11.37

were no differences between the AdThd-treated animals and the control animals. Conversely, the mice treated with IdUrd did show lesions, most noticeable in the kidney, where there were focal nests of poorly differentiated cells confined to the superficial cortex. The cerebellar lesions consisted of a slight reduction in the number of granule cells in the internal granule layer. Minimal lesions were also noted in the liver. The histopathology of the lesions in these organs was identical to those previously described (12).

Discussion. Primary HSV infection usually occurs in childhood and is commonly associated with a disseminating viremia. Ninety percent of the adult population has HSV antibodies indicative of this infection by age 35. Of these, approximately 33% suffer recurrent herpetic infections of the eye, skin, mouth, or genitalia (19).

Recent work on locating the site of persistent viral reservoirs which serve as the source of recurrent infections indicates that the sensory and sympathetic ganglia and possibly the brain itself are the harborers of latent virus (20). Although the antiviral drugs trifluridine, idoxuridine, and vidarabine are all efficacious as topical agents in therapy of ocular disease, no topical drug, be it an antimetabolite or interferon, will eliminate the deep-seated neuronal reservoirs for any manifestations of herpetic disease in the body. To this end, nontoxic, effective antiviral agents with potential for systemic use are being developed and evaluated. In previous publications we have shown that acyclovir and vidarabine are each significantly effective for topical therapy of ocular and oral herpetic disease and for systemic therapy to reduce, albeit transiently, latent virus in the trigeminal ganglia (19–24).

In the present paper we have reported a third drug which holds potential for topical or, more importantly, systemic therapy of herpetic disease. The ocular keratitis work is the first *in vivo* analysis of AdThd as a therapeutic agent. It has significant antiviral activity in the eye. While 10% AdThd appears not quite as potent as IdUrd, 15% AdThd, used in more severe disease, resulted in a therapeutic efficacy slope similar to IdUrd. In addition, the toxicity and teratology studies indicate a far greater safety factor with AdThd than with IdUrd when the drugs are used systemically. No adverse effects of AdThd could be demon-

TABLE II. EYE HISTOPATHOLOGY

Drug and dosage (mg/kg/day)	Number of eyes examined	Cataracts	Retinal dysplasia	Ciliary body dysplasia	PHPV	RPE dysplasia
AdThd, 250	18	4	3	0	0	1
AdThd, 500	23	4	4	0	0	0
IdUrd, 125	23	5	4	1	1	0
IdUrd, 250	17	5	3	0	0	0
PBS	24	3	4	0	0	0

Note. One eye was examined from each animal.

TABLE III. LESIONS IN MICE TREATED POSTNATALLY WITH 5'-AMINO-5'-DEOXYTHYMIDINE (AdThd) AND 5-IODODEOXYURIDINE (IdUrd)

Dosage (mg/kg/day)	Number examined at necropsy	Age at necropsy (days)	Cerebellum	Kidney	Liver
		5'-Amino-5'-deoxythymidine			
250	7	13–17	—	—	—
250	6	19–21	—	—	—
250	16	23–25	—	—	—
500	6	13–17	—	—	—
500	5	19–21	—	—	—
500	18	23–25	—	—	—
		5-Iododeoxyuridine (IdUrd)			
125	6	13–17	+	+++	±
125	5	19–21	+	++	±
125	16	23–25	±	++	—
250	3	13–17	+	+++	+
250	5	19–21	+	++	±
250	19	23–25	±	++	—

Note. Extent of lesions: histologically normal —; minimal ±; moderate +; marked ++; extensive +++. All PBS controls were negative in the development of lesions in cerebellum, kidney, and liver.

strated in the neonatal mouse model, while in the IdUrd-treated animals there was histologic evidence of cerebellar hypoplasia and focal renal cortical dysplasia. These results are similar to those previously reported, although no differences could be found in the ocular histopathology (14). Some of the mice in the control group had histopathological evidence of cataracts and retinal dysplasia. This is not uncommon in highly inbred strains, and may result from mutations, intrauterine infections, or placental–maternal circulatory disturbances. As with AIdUrd, the safety of AdThd may be related to its relatively selective activation by herpesvirus thymidine kinase.

Because of its significant therapeutic effect, greater solubility (up to 15% by weight in phosphate-buffered saline), and lack of significant toxicity by topical or systemic routes, AdThd has the potential for use at higher levels than currently available antimetabolites without concomitant increase in toxicity.

1. Elion GB, Furman PA, Fyfe JA, DeMiranda P, Beauchamp L, Schaeffer HJ. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc Nat Acad Sci USA **74**: 5716–5720, 1977.
2. Schaeffer HJ, Beauchamp L, DeMiranda P, Elion GB. 9-(2-Hydroxyethoxymethyl)guanine activity against viruses of the herpes group. Nature (London) **272**:583–585, 1978.
3. DeClercq E, Descamps J, DeSomer P, Barr PJ, Jones AS, Walker RT. (E)-5-2-Bromovinyl)-2'-deoxyuridine: A potent and selective anti-herpes agent. Proc Nat Acad Sci USA **76**:2947–2951, 1979.
4. Cheng YC, Goz B, Neenan JP, Ward DC, Prusoff WH. Selective inhibition of herpes simplex virus by 5'-amino-2',5'-dideoxy-5-iodouridine. J Virol **15**:1284–1285, 1975.
5. Lin T-S, Prusoff WH. Synthesis and biological activity of several amino analogues of thymidine. Amer Chem Soc **21**:109–112, 1978.
6. Prusoff WH, Fischer PH. Basis for the selective antiviral and antitumor activity of pyrimidine nucleoside analogues. In: Nucleoside Analogues: Chemistry, Biology and Medical Applications. Vol 286:pp 281–318, 1979.
7. Cheng Y-C, Prusoff, WH. Mouse ascites sarcoma 180 deoxythymidine kinase. General properties and inhibition studies. Biochemistry **13**:1179–1185, 1974.
8. Cheng Y-C, Prusoff WH. Mouse ascites sarcoma 180 thymidylate kinase. General properties, kinetic analysis, and inhibition studies. Biochemistry **12**:2612–2619, 1973.
9. Chen MS, Prusoff, WH. Phosphorylation of 5-iodo-5'-amino-2',5'-dideoxyuridine by herpes simplex virus type 1 encoded thymidine kinase. J Biol Chem **254**:10449–10452, 1979.
10. Chen MS, Shiau GT, Prusoff WH. 5'-Amino-5'-deoxythymidine: Synthesis, specific phosphory-

lation by herpes virus thymidine kinase, and stability to pH of the enzymically formed diphosphate derivative. Antimicrob Agents Chemother **18**:433–436, 1980.

11. Albert DM, Lehav M, Bhatt PN, Reid TW, Ward RE, CyKiert RC, Lin T-S, Ward DC, Prusoff WH. Successful therapy of herpes hominis keratitis in rabbits by 5-iodo-5′-amino-2′-5′-dideoxyuridine (AIU): A novel analog of thymidine. Invest Ophthalmol Vis Sci **15**:470–478, 1976.
12. Puliafito CA, Robinson NL, Albert DM, Pavan-Langston D, Lin T-S, Ward DC, Prusoff WH. Therapy of experimental herpes simplex keratitis in rabbits with 5-iodo-5′-amino-2′-5′-dideoxyuridine. Proc Soc Exp Biol Med **156**:92–96, 1977.
13. Chen MS, Ward DC, Prusoff WH. Specific herpes simplex virus-induced incorporation of 5-iodo-5′-amino-2′,5′-dideoxyuridine into deoxyribonucleic acid. J Biol Chem **251**:4833–4838, 1976.
14. Albert DM, Percy DH, Puliafito CA, Fritsch E, Pavan-Langston D, Lin T-S, Ward DC, Prusoff WH. AIU: An antiherpetic drug with low neonatal toxicity in mice. In: Gauri KK, ed. Anti-herpesvirus Chemotherapy: Experimental and Clinical Aspects. (Advances in Ophthalmology Series) Basel, Karger. Vol 38:p89, 1979.
15. Park NH, Pavan-Langston D. Unpublished data.
16. Neenan JP, Rhode W. Inhibition of thymidine kinase from Walker 256 carcinoma by thymidine analogs. J Med Chem **16**:580–581, 1973.
17. Lin T-S, Neenan JP, Cheng YC, Prusoff WH, Ward DC. Synthesis and antiviral activiral activity of 5- and 5′-substituted thymidine analogs. J Med Chem **19**:495–498, 1976.
18. Horowitz J, Tomson A, Urbanski R. Nucleosides. I. 5′-Amino-5′-deoxyuridine and 5′-amino-5′-deoxythymidine. J Org Chem **27**:3045–3048, 1962.
19. National Institutes of Health. Workshop on the treatment and prevention of herpes simplex virus infection. J Infect Dis **127**:117–119, 1973.
20. Nesburn AB, Green MT, Radnoti M, Walker B. Reliable in vivo model for latent herpes simplex virus reactivation with peripheral virus shedding. Infect Immun **15**:772–775, 1977.
21. Pavan-Langston D, Campbell R, Lass J. Acyclic antimetabolite therapy of experimental herpes simplex keratitis. Amer J Ophthalmol **86**:618–623, 1978.
22. Park NH, Pavan-Langston D, McLean S, Albert DM. Acyclovir in oral and ganglionic herpes simplex. J Infect Dis **140**:802–806, 1979.
23. Pavan-Langston D, Park NH, Lass J. Herpetic ganglionic latency: Acyclovir and vidarabine therapy. Arch Ophthalmol **97**:1508–1510, 1979.
24. Pavan-Langston D, Park NH, Hettinger M. Ganglionic herpes simplex and systemic acyclovir. Arch Ophthalmol **99**:1417–1419, 1981.

Received May 12, 1981. P.S.E.B.M. 1982, Vol. 170.