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# 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU) therapy of cutaneous herpes simplex virus type 1 infection in guinea pigs

Susan Wong  
*Yale University*

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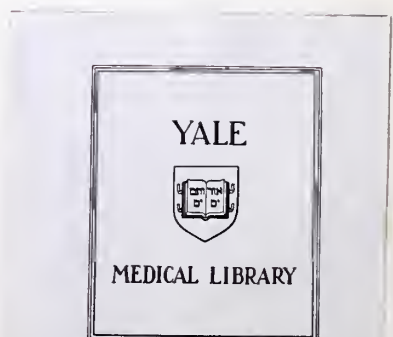
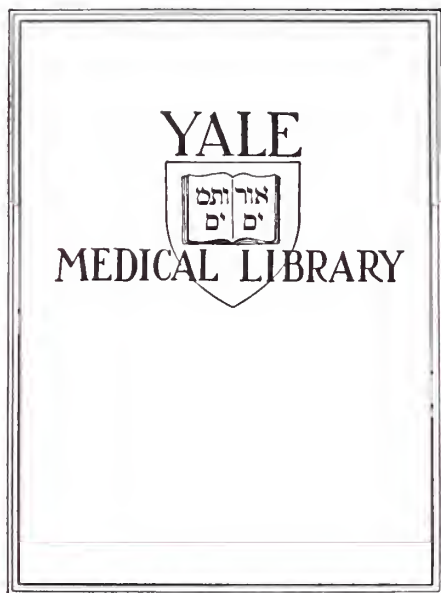
5-iodo-5'-amino-2',5'-dideoxyuridine (AID) THERAPY  
OF CUTANEOUS HERPES SIMPLEX VIRUS TYPE 1  
INFECTION IN GUINEA PIGS

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Susan Wong

1978














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5-iodo-5'-amino-2',5'-dideoxyuridine (AIU) therapy  
of cutaneous herpes simplex virus type 1  
infection in guinea pigs

Susan Wong

B.A. University of California, Los Angeles 1971

M.A. Yale University 1973

A Thesis Submitted in Partial  
Fulfillment of the Requirements for the  
Degree of Doctor of Medicine

Yale University  
School of Medicine  
New Haven, Connecticut

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ABSTRACT:

Cutaneous infection of guinea pigs with herpes simplex-type 1 virus (HSV-1) provides an experimental model for the screening of potential antiviral chemotherapeutic agents in humans. 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU), a novel thymidine analog, is a potentially important antiviral chemotherapeutic agent which has been shown to inhibit the replication of herpes simplex-type 1 virus without causing any detectable host toxicity (1, 2, 3). In contrast, other nucleosides with demonstrated antiviral activity, such as 5-iodo-2'-deoxyuridine (IUdR), 5-trifluoromethyl-2'-deoxyuridine (F<sub>3</sub>dThd), 1-beta-D-arabinofuranosylcytosine (Ara-C), and 9-beta-D-arabinofuranosyladenine (Ara-A), have been shown to induce moderate to severe degrees of cytotoxicity (4, 5, 6, 7, 8).

In the following study, the efficacy of AIU in the treatment of experimentally induced cutaneous HSV-1 infection in guinea pigs was examined and compared with IUdR, Ara-AMP (adenine arabinoside monophosphate), and lactose in a controlled, double-masked experiment.

The results of this experiment demonstrated the effectiveness of AIU in the treatment of cutaneous HSV-1 infection in guinea pigs by a marked reduction in



the development of cutaneous erythema and the number of vesicles at the HSV-1 inoculated sites. In addition, the Ara-AMP and the IUdR treated groups also exhibited a decrease in the severity of the lesions as compared to the lactose treated control group. The relative potency of these antiviral nucleosides, defined in terms of the reduction of cutaneous erythema and the number of vesicles at the inoculated sites, ranked as follows: AIU, IUdR, Ara-AMP, in order of increasing potency.

The statistical analysis of the experimental data documented the significance of the AIU treatment of HSV-1 cutaneous lesions in the guinea pigs by the above mentioned criteria. Similarly, the IUdR and Ara-AMP treatments were also found to be statistically significant.





5-iodo-5'-amino-2',5'-dideoxyuridine (AIU) therapy  
of cutaneous herpes simplex-type 1 virus  
infection in guinea pigs

I. Structure and Composition of the Herpes Simplex Virus:

DNA core

The herpes simplex virus (HSV) is a relatively large virus composed of an inner core of linear double-stranded DNA with a molecular weight of  $(99 \pm 5) \times 10^6$  daltons (9, 10, 11). The structure of the DNA core has been studied by electron microscopy, which showed the HSV DNA to be coiled in the form of a doughnut (12). The base composition of the DNA, in terms of the guanine + cytosine content, is 67 mole percent for HSV-1 and 69 mole percent for HSV-2 (10, 13). Along the DNA strand, there are alkali-labile bonds situated at unique sites of both HSV-1 and HSV-2 DNA (10, 14). Further information regarding the DNA sequences of HSV-1 and HSV-2 has been obtained from the results of reassociation



kinetic studies of the DNA of HSV-1 and HSV-2. The data from these studies indicate that the HSV-1 DNA most likely contains no repetitive sequences (15). On the other hand, 16 percent of the HSV-2 DNA sequences have been found to reassociate more rapidly, thus supporting the theory that repetitive sequences exist in the HSV-2 DNA.

During the productive infection of the HSV within host cells, it is estimated that 50 percent of the DNA is transcribed (16, 17). Hence, if one assumes that transcripts all specify for the synthesis of proteins, then the HSV DNA must code the sequences of approximately 55,000 amino acids.

### Capsid

The DNA core of the HSV is concentrically surrounded by protein layers known as the capsid. The capsid of the HSV is composed of 162 structural subunits called capsomeres which form into an icosahedron (18, 19). In addition, the capsid of the HSV is known to contain spermine in amounts sufficient to neutralize 50 percent of the phosphate in the DNA (20).



## Nucleocapsid

The unit containing the capsid and its enclosed nucleic acid is called the nucleocapsid, which is a rigid structure spanning approximately 95 to 105 nanometers in diameter (11). External to this structure, the capsid incorporates additional proteins to form the tegument. The tegument has been found to bind tenaciously to the capsid, even after detergents have stripped the HSV of its outer envelope (21).

## Envelope

The nucleocapsid is coated by a loose envelope which renders the virion impermeable to negative stain (22). This envelope is composed of lipids, polyamines, and at least 12 glycoproteins (23, 20). It is the qualitative and quantitative differences in the glycoproteins of the HSV envelope which determine the immunologic specificity of the virus (24).

In addition to determining the immunologic specificity, the envelope also confers infectivity to the nucleocapsid. It has been claimed that the naked nucleocapsid does not adsorb well to cell surfaces and is less stable than the enveloped nucleocapsid.





Obtained from either the nuclei or the virion exposed to lipases or lipid solvents which strip the envelope off the nucleocapsid, the resulting naked nucleocapsid are found to be noninfectious. However, an intact envelope may not be required for infectivity, since fragments of the envelope adhering to the nucleocapsid are sufficient to render it infectious (25, 26). De-proteinized HSV-1 DNA, either native or denatured, is also infectious (27). Even portions of the HSV DNA from a deletion mutant lacking  $4 \times 10^6$  daltons in its DNA has been reported to be capable of multiplying (28).

#### Cellular HSV Infection

Cellular infection by HSV can lead to either (1) productive infection in which biosynthesis of infectious progeny and subsequent cell death take place or (2) nonproductive infection in which part or all of the viral genome resides within the host without causing cell death (29, 30). Productive infection of the HSV leads to multiplication of infectious virus progeny, host tissue destruction, and manifestation of the infection clinically. But nonproductive infection is believed to be responsible for recurrent HSV infection and cancer.



## Productive infection and replication of HSV

Productive infection in the host cell begins with the initiation of infection within the host. The mechanism of penetration of HSV is unknown; however, two modes of viral entry into the host cells have been postulated. One method of entry is believed to involve the fusion of the viral envelope with the plasma membrane of the host cell (31), while the other method is believed to be akin to pinocytosis (32).

The entry of virus into cells is influenced by the temperature and the extracellular medium. It is known that polyanionic substances such as heparin and dextran sulfate inhibit HSV infection of cells (33), although HSV-1 is less susceptible to the effects of polyanionic extracellular medium than is HSV-2 (34). It has been postulated that polyanions may compete with HSV for the attachment to the receptors on the cell surface, thus hindering the HSV attachment and entry into the cell (35).

The cell to cell transmission of HSV infection may occur in the absence of detectable infectious particles (36). The transmission is achieved by the fusion of the HSV infected cells with the adjacent



uninfected cells (37). After the entry of the virus into the cell, the outer protein capsid is stripped, resulting in a DNA-protein complex which enters the nucleus. Within the nucleus, the DNA becomes dissociated from the protein, and the viral DNA is transcribed. Forty-four percent of the HSV-1 DNA and twenty-one percent of the HSV-2 DNA is transcribed prior to the onset of DNA synthesis. The transcription of the viral DNA sequences is regulated such that some transcripts are more abundant than others (16, 17).

The virus specified RNA is then processed and transported into the cytoplasm where it directs the synthesis of structural and nonstructural proteins of the virus at the free and membrane-bound polyribosomes (38, 39, 40, 41). These newly synthesized proteins specified by the HSV then migrate to the nucleus. However, a small portion of these proteins remains in the cytoplasm and binds to the cellular membranes (41).

The structural proteins are assumed to regulate the synthesis of HSV DNA. The virus specified enzymes have been identified as thymidine kinase and DNA polymerase. The HSV specified thymidine kinase differs from the host thymidine kinase in its immunologic, electrophoretic, physical, and catalytic activity since HSV-thymidine kinase also phosphorylates deoxycytidine. Differences in these



properties have also been shown to exist between the HSV-1 specified thymidine kinase and the HSV-2 specified thymidine kinase (42).

The synthesis of herpes virus nucleic acid occurs within the Feulgen-positive Cowdry Type A inclusion body found in the nucleus (41). The DNA synthesis is initiated along several sites of the molecule (14). The newly synthesized DNA fragments are not processed beyond the interruptions found at the unique sites. The viral DNA within the intranuclear space has greater number of alkali-labile bonds than those found in the nucleocapsid (14).

The structural proteins of the virus migrate into the nucleus to form the capsid (41). Three types of capsids have been identified from electron microscopic studies. They are as follows: (1) capsids without DNA core, (2) capsids with DNA core, and (3) capsids with tegument adhering to the nuclear membrane. However, the capsids without DNA cores rarely have envelopes surrounding the naked capsid in the HSV-infected cells (43). In addition, the protein compositions of the three types of capsids also differ from each other (21, 43, 44).

The nucleocapsids become enveloped at the inner





lamella of the nuclear membrane at a region of de novo synthesis of nuclear membrane or at a site at which host proteins have been expelled (23, 43, 44). The inner lamella at the site of envelopment thickens due to the acquisition of a layer of protein to form the tegument.

The enveloped virus is sequestered between the inner and the outer lamellae of the nuclear membrane and within the endoplasmic reticulum. The HSV is protected from any direct contact with the cytoplasm. The mode of egress of the HSV from the infected cell is not entirely clear. One theory states that the endoplasmic reticulum containing the HSV forms vesicles which transport the HSV to the extracellular fluid (45). A second theory states that the endoplasmic reticulum connects the extracellular fluid with the perinuclear space containing the HSV and thus provides a pathway for the HSV to migrate from the infected cell (46).

#### Cellular changes due to the HSV productive infection

Productive infection of HSV drastically alters the infected cell's DNA, protein, and RNA synthesis (41, 47, 48). Inhibition of host DNA and protein synthesis begins with the entry of HSV into the cell and is complete within three to five hours. The inhibition of



DNA and protein synthesis is accompanied by the displacement of aggregates of chromatin at the nuclear membrane and the disaggregation of polyribosomes. Host RNA synthesis is altered by the productive infection of HSV. Ribosomal precursor RNA is decreased up to 70 percent. The host RNA is improperly processed, resulting in the degradation of methylated 45S ribosomal RNA rather than the normal cleavage into 18S and 28S segments. The remaining RNA which is processed does not enter the polyribosomal pool to regulate host protein synthesis (47).

Other effects of productive infection by the HSV is manifested by the leakage of macromolecules from the infected cell (49, 50) and the reversal of the transmembrane potential from -20 mV to +10 mV in the infected cell (51).

The HSV infected cells acquired new surface immunologic determinants which are similar to those on the surface of the envelope of the virus (42, 52, 53, 54, 55, 56, 57). These infected cells form loose aggregates or may fuse together, thus creating a direct cell-to-cell contact which may facilitate the dissemination of the HSV infection (58, 59).



## Recurrent HSV Infection

A characteristic of HSV infection in humans, as well as nonhuman species is its ability to persist in the host, leading to recurrent infections localized on specific area of the host's body, such as the cornea, face, or genitals. (29, 30, 60). The origin of the virus causing recurrent infections in the host is not usually apparent, although four theories have been stated to explain these recurrences. They are as follows:

(1) exogenous infection; (2) endogenous infection from another site of the body; (3) chronic, continuous low-level viral multiplication near the site of involvement; (4) persistence of the virus in a non-replicating form at or near the site of recurrent infection.

The data which supports the exogenous reinfection theory is based on occurrences of genital reinfection with exogenous HSV-2 in mice and Cebus monkey. (61). In humans, it is unlikely that exogenous source of virus is responsible for the recurrences of nongenital HSV infection since these recurrences correlate to such precipitating factors as exposure to sunlight, fever, menstruation, hormones, or emotional stress (60, 29, 30).

The theory of endogenous reinfection from another





site of the body is also unlikely to be the primary cause responsible for recurrent infections, even though such instances have been documented in infections of genitals with HSV from a recurrent oral infection (62). This theory cannot account for the fact that only a specific region of the body is involved in recurrent infections.

The most convincing evidence against the theory of chronic, continuous low-level viral multiplication around the site of involvement is the failures of numerous attempts to isolate virus from biopsy specimens taken from the site of recurrences in the interim between recrudescences (29, 30, 60).

The theory that the HSV persists in its non-infectious state at or near the site of recurrence is supported by findings in both animals and humans. HSV have been cultured from the trigeminal ganglion from human cadavers (63, 64). In animal studies, HSV have been found in the sacrosciatic spinal ganglion of mice and in the trigeminal ganglion of rabbits after inoculation in the foot pad and the cornea respectively (65, 66).



## II. The Epidemiologic Patterns of Herpes Simplex Virus Infections

The discovery of two natural variants of HSV (type 1 and type 2) have made the epidemiologic patterns of HSV infections more meaningful. From the epidemiologic studies based on the detection of HSV antibodies (Abs) in the general population, it is evident that the acquisition of HSV Abs is markedly influenced by an individual's age and socioeconomic status. The incidence of finding HSV-1 Abs increases directly with an individual's age, beginning after six months of age when the transplacental Abs from the mother disappear from the newborn's immunologic defense system. On the other hand, the presence of HSV-1 Abs is inversely related to one's socioeconomic status. For example, the adults in the higher socioeconomic group have a 30 to 50 percent chance of having HSV-1 Abs, while the adults in the lower socioeconomic group have an 80 to 100 percent chance of having HSV-1 Abs. Similar trends exist for the incidence of HSV-2



Abs in the general population, although it is modified by the sexual behavior within each population subgroup. A rise of the HSV-2 Abs is found beginning at 14 years of age. The incidence of HSV-2 Abs in the adult population is 10 percent in the higher socioeconomic group, between 20 to 60 percent in the lower socioeconomic group, 3 percent in nuns, and 100 percent in prostitutes (67, 68).

HSV is commonly transmitted by direct contact such as in the cases of herpes gladiatorum (69, 70, 71). The transmission of HSV-1 is predominately via the oral-respiratory route with contaminated secretions from host to host. The transmission of the virus in the saliva is exemplified by the increased incidence of herpetic paronychia in medical and dental personnels exposed to patients with infected oral cavity or contaminated tracheal catheters (72, 73, 74).

HSV-2 is generally transmitted via contaminated genital secretions of symptomatic and even asymptomatic persons infected with HSV-2. In the newborn, the major source of HSV is from the mother who is infected with genital HSV-2 or less frequently HSV-1 (75, 76, 77, 78). The transmission of the virus occurs during the passage of the fetus through the infected birth canal in 50 percent of such cases (77).



### III. Clinicopathologic Aspects of HSV Infections

The incubation period for primary HSV-1 or HSV-2 varies from 2 to 20 days, with an average of 6 days. In patients with primary HSV infections, the HSV Abs can be detected within the first two weeks in the IgM serum fraction, then followed by detection in the IgA and IgG fractions afterwards (79). Delayed hypersensitivity reaction to HSV antigens as exhibited by a positive skin test usually develops (80, 81).

#### Dissemination of Primary HSV Infection

The dissemination of primary HSV infection generally occurs only in the compromised host. The pathologic process of HSV dissemination have been described as follows (75, 82). Initially, primary viremia results from a spillover of the HSV from the infected cells located at the portal of entry. This leads to the spread of HSV infection to the susceptible organs. At this point, histopathologic changes remain minimal





in the infected organs. In the progressive phase of the infection, the viremia disappears at the same time that virus replicates within the target organs. This leads to the cellular damage in these tissues. Following this phase, a secondary viremia develops from the production of virus within the target organs. The secondary viremia leads to further spread of virus to involve other organ systems. This phase is followed by the regression and the recovery of the host from the HSV dissemination.

#### HSV Infection in the Noncompromised Host

HSV viremia is rarely encountered in the non-compromised host who is greater than one month of age (83, 84, 85). In this type of hosts, HSV infection is generally localized in specific target organs without the development of systemic dissemination. The target organs infected by the HSV are derived from the embryonic ectoderm such as the skin, oral cavity, vagina, conjunctiva, and nervous system.

The factor which determines whether HSV-1 or HSV-2 is involved in the infection of a specific organ is the mode by which the virus gains entry into the host. However, one cannot totally disregard the general properties of each virus type in determining the tissue



susceptibility or the route of dissemination within the host.

### HSV Infection of the Oral-respiratory Tract

Oral herpetic infections are generally caused by HSV-type 1, rather than HSV type 2. In children, the mouth is the major site of primary HSV-1 infection. The clinical manifestation of the HSV infection of the mouth in the noncompromised host varies from inapparent, subclinical infection to severe gingivostomatitis with vesicular lesions and ulcers of the mucous membrane, cervical adenopathy, and fever (86). Further direct contact transmission of the contaminated secretions can lead to subsequent infection of other susceptible organs such as the eye, genitalia, fingers, etc. On the other hand, primary HSV-1 infection of the mouth in neonates or in the compromised host can extend to the lungs and the esophagus with a potential for dissemination to other visceral organs or the central nervous system (75, 87, 88, 89).

### HSV Infection of the Lips

Primary HSV infection involving the lips is not common, but it is the site most commonly involved in



recurrent HSV lesions. The site of the lips involved in labial herpes is well correlated with the site of lip cancer (90). Labial herpes generally heals within 5 to 7 days without the development of extensive complications except in those instances involving the compromised hosts of Hodgkin patients (91, 92). In these patients, there is a tendency for the persistence of the lesions or the extension of the infection locally.

### HSV Infections of the Eyes

HSV infection of the eyes is due to HSV-1 in primary or recurrent disease, except in the newborns when HSV ocular lesions are often due to HSV type 2. The severity of the infection is dependent on the depth of the lesion and the recurrent nature of the infectious process. The spectrum of such ocular involvement includes follicular conjunctivitis, superficial or stromal keratitis, cataracts, iridocyclitis, and panuveitis (93, 94, 95, 96). With severe and recurrent HSV ocular infections, the tissue damaged from the infection can markedly impair the vision of the afflicted individual.

### HSV Infections of the Skin

HSV infections of the skin generally occur in



a diseased, broken, or traumatized skin, but not in an intact skin. The skin lesions which develop from primary or recurrent HSV are localized vesicular lesions. The course of these skin vesicular lesions is influenced by the immunologic status of the afflicted individual. In the compromised host, these skin lesions become more extensive and chronic in nature (91, 92). In those individuals having atopic eczema or dermatoses such as Darier's disease, the HSV vesicular lesions become more generalized to form Kaposi's varicelliform eruption or eczema herpeticum (97). Recurrences have been described in such cases, although they are usually less severe and shorter in duration than the initial primary infection.

In traumatic herpes lesion, the HSV enters the skin via a break in the epithelium of the skin from an abrasion, laceration, puncture or burn wound. Such skin lesions often occur in the fingers as herpetic whitlow (72), in wrestlers as herpetic gladiatorum (71, 72), and in burn patients who are more susceptible to systemic herpetic infections (88).

Neonatal herpes can present with skin vesicles, although the skin lesions are sometimes absent (75). Occasionally, a generalized macular erythematous rash precedes the onset of the vesicular lesions. If the infected neonate survives the initial HSV infection, these lesions can recur at the original sites, as well





as new locations.

HSV infections of the skin can be due to either type 1 or type 2, depending on the mode of acquisition of the virus. Hence, neonatal herpes is generally due to HSV-2; skin lesions in adults located above the waist is often due to HSV-1; and those lesions located below the waist of adults is often due to HSV-2.

### HSV Infections of the Urogenital Tract

HSV infection of the urogenital tract is a common venereal disease, predominately due to HSV-2. Only 5 to 10 percent of HSV infections of the urogenital tract are due to HSV-1. HSV-1 urogenital tract infections are mainly due to autoinfection from another site of the body, as seen in those cases involving HSV-1 urogenital tract infection in children (62).

In females, the HSV infection occurs more commonly in the cervix than in the external genitalia (71, 72, 73). In the symptomatic or the asymptomatic males, the virus have been isolated from the penis, urethra, prostate and the seminal vesicles.

The clinical manifestations of HSV genital infection is dependent on the presence of neutralizing Abs to either types of HSV. In the absence of any neutralizing Ab to HSV, the clinical manifestations of



genital HSV infections tend to be more severe. It may be complicated by such symptoms and signs as fever, constitutional signs, regional adenopathy and dysuria. In addition, genital herpetic infections can be accompanied by pleocytosis of the cerebrospinal fluid, even in the absence of meningeal signs, radiculitis, or myelitis (83).

### HSV Infection of the Nervous System

HSV infection of the nervous system can manifest as encephalitis, meningitis, radiculitis, or myelitis (83, 98, 99). HSV infection of the nervous system may be due to a primary infection limited to the brain, or it may occur in disseminated HSV infections, particularly in the cases involving newborns.

Studies indicate that the mode of spread of the HSV may differ according to the type of HSV involved (83). HSV-2 is thought to spread to the nervous tissue via the hematogenous pathway, while HSV-1 is thought to spread to the nervous tissue predominately via the neurogenic pathways.

The type of HSV causing the infection of nervous tissues is dependent upon the age of the host. Except for newborns, these infections are commonly due to HSV-1.



#### IV. The Treatment of HSV Infections

At the present time, there are several approaches available experimentally and some available clinically for the treatment of HSV infection in humans. Anti-metabolites composed of nucleoside analogues have varying degrees of effectiveness against HSV infection. Photodynamic inactivation using heterotricyclic dyes such as neutral red or proflavine is under investigation for the treatment of HSV infection. Immunizing agents such as BCG, smallpox vaccines, and most recently, inactivated HSV vaccines have been examined in this country as well as in Germany and in France. Interferon and interferon inducers are also under investigation for the therapy of HSV infections.

##### Nucleoside Analogs

##### 5-Iodo-2'-deoxyuridine

5-Iodo-2'-deoxyuridine (IUdR), a synthetic nucleoside



analogue discovered in 1959 by W.H. Prusoff, was the first agent used clinically for the treatment of HSV infection (100). The effectiveness of the topical administration of IUdR for the treatment of HSV infection of the corneal epithelium in rabbits and in humans has been demonstrated by several investigators (101, 102, 103, 104, 105). Unfortunately, IUdR has proved to be ineffective for the treatment of herpes labialis in a double-masked study by Kibrick and Katz (106). In addition, IUdR has limited application for the management of cutaneous and genital herpetic infections due to the lack of therapeutic response and the toxicity on the host (98, 107).

The antiviral activity of IUdR is believed to be the result of its incorporation into the viral DNA, in substitution for the thymidine moiety. The incorporation of IUdR into the DNA of animal viruses have been established by several investigators (49, 108, 109, 110, 111, 112). The incorporation of halogenated deoxyribonucleosides have been shown to inhibit the formation of infectious herpesvirus particles to a ratio of  $10^7$  virus particles to one infectious virus (113, 114).

The specific molecular defect responsible for these changes resulting from the incorporation of IUdR into the herpes virus DNA has not been elucidated, but Prusoff





and Goz have reviewed this subject extensively (115, 116).

The use of topical or systemic IUdR is associated with considerable toxic side effects. The toxicity is most likely a consequence of the incorporation of IUdR into the DNA of normal uninfected host cells. In the systemic administration of IUdR in patients at a concentration of 100 mg/kg/day for 5 to 6 days, the toxic effects reported are stomatitis, leukopenia, and alopecia (117). In the topical administration of IUdR in the eyes of patients, ocular toxic effects such as punctate epithelial keratopathy, follicular conjunctivitis, narrowing and occlusion of the puncta, contact dermatitis, lid margin changes and excess lacrimation have been reported (118). During corneal wound healing, toxic changes have been observed in the regenerating epithelium(119, 120) and the stromal regeneration of the cornea (119, 121) .

In experimental models, IUdR has been associated with teratogenic changes in laboratory animals. These teratogenic changes have been demonstrated in pregnant rabbits treated with topical administration of IUdR to the eyes at doses comparable to that used in humans (122). Subcutaneously injected IUdR has also been shown to induce teratogenic abnormalities in neonatal rats and mice, consisting of changes in the granular and



molecular layers of the external cerebellum, impaired nephrogenesis, ocular abnormalities such as posterior subcapsular cataract, retarded retinal maturation, and retinal dysplasia (123, 124).

The clinical use of IUdR is further limited by the existence of IUdR resistant strains of HSV (125, 126). The primary use of IUdR has been in herpes simplex keratitis, using either ophthalmic ointment at 0.5% or ophthalmic solution at 0.1% concentration.

#### 9-Beta-D-arabinofuranosyladenine

9-Beta-D-arabinofuranosyladenine (Ara-A) was originally synthesized in 1960 as an anti-cancer agent (127). Ara-A has antiviral activity against a broad spectrum of DNA viruses including the HSV, as well as some of the of the oncogenic RNA viruses such as the Rous Sarcoma virus and the Gross murine leukemia virus (128, 129, 130, 131).

The current clinical usefulness of Ara-A is predominately in the treatment of ocular HSV infections. It is equally as effective as IUdR in the therapy of acute primary or recurrent dendritic keratitis caused by HSV. In addition, Ara-A can be used to treat those diseases caused by IUdR resistant strains of HSV (119, 132, 133, 134, 135, 136, 137).



The intravenous administration of Ara-A has been successfully used in the treatment of patients with herpes kerato-uveitis (138). Ara-A may be beneficial in the treatment of neonatal herpes if administered early in the course of the infection (139). However, the response of genital herpes simplex infection to Ara-A has been highly variable (140). Ara-A has also been tested in a placebo-controlled study on HSV-1 encephalitis in humans. The results from this study show a reduction of the mortality rate from 70 to 28 percent in patients with HSV-1 encephalitis when they were treated with Ara-A early in the course of the infection (141).

The mechanism of action of Ara-A has not been completely elucidated. The antiviral activity of Ara-A may be attributed to the incorporation into the DNA and/or the inhibition of DNA polymerase (142, 143). The deaminated product of Ara-A, the hypoxanthine derivative of Ara-A, is approximately equal to Ara-A in its antiviral activity (129).

The administration of Ara-A has been associated with the following toxic effects. In the systemic administration of Ara-A to patients with chronic hematologic conditions, a mild to moderate depression in the level of the hemoglobin was observed, without



any accompanying change in the neutrophils and platelet levels (144). Two patients with Hodgkin's disease receiving Ara-A at 20 mg/kg/day have been reported to develop a transient motor aphasia resembling akinetic mutism (144). Chromosomal gaps and breaks have been observed in leukocytes treated with in vitro Ara-A (145). Systemic administration of Ara-A in patients have also been associated with an increase in chromosomal breakage found in the leukocytes (146). Besides its toxicity, the therapeutic usefulness of Ara-A is also limited by its low water solubility.

#### 9-Beta-D-Arabinofuranosyladenine-5'-Monophosphate

9-Beta-D-Arabinofuranosyladenine-5'-monophosphate, or Ara-AMP, is a modified compound of Ara-A by the addition of a phosphate group. Its major advantage over Ara-A is the increased water solubility property, as compared to its parent compound Ara-A (147). Ara-AMP has been shown to have marked in vitro and in vivo activity against a host of DNA viruses (148, 149). Other studies have demonstrated its effectiveness in the treatment of HSV-1 keratitis in rabbits (148, 150), and in herpes virus induced cutaneous lesions in tails of mice (148).





Preliminary toxicity studies have not shown Ara-AMP to be more toxic than Ara-A (151). Because of its greater solubility in water, Ara-AMP can be a potentially important antiviral agent that can be administered via parenteral routes in smaller fluid loads than Ara-A. However, further toxicity studies are needed prior to the initiation of clinical trials of Ara-AMP.

#### 1-Beta-D-Arabinofuranosyl Cytosine

1-Beta-D-Arabinofuranosyl cytosine (Ara-C), originally developed as an antileukemic agent, has a spectrum of antiviral activity similar to Ara-A. The antiviral potential of Ara-C has been reviewed extensively (4, 7, 8, 152, 153). It has been established that Ara-C is active against experimental herpetic and vaccinia keratitis. However, the clinical use of Ara-C in the treatment of herpetic keratitis in humans is restricted, due to its toxicity. Similarly, systemic use of Ara-C for the treatment of other herpes simplex infections are also restricted. The therapeutic to toxic ratio approaches unity for Ara-C, being 10 times more toxic than IUDR (154). The systemic toxicities of Ara-C include teratogenesis, immunosuppression, chromosomal aberration, leukopenia, thrombocytopenia, megaloblastosis, hepatic



toxicity, and gastrointestinal toxicity (155, 156, 157). Reports of toxic changes following the topical use of Ara-C in the eyes include punctate epithelial keratopathy, corneal ulceration, and iritis (158).

In addition to its toxicity, Ara-C's clinical usefulness is further limited by its rapid deamination in the liver and the kidneys to uracil arabinoside, an inactive metabolite of Ara-C. However, the deamination of Ara-C is less of a concern in intrathecal administrations of Ara-C since deaminases are absent in the brain. Thus, the active form of Ara-C will persist much longer when intrathecal injections are given (159).

#### 5-Trifluoromethyl-2'-Deoxyuridine

5-Trifluoromethyl-2'-deoxyuridine ( $F_3TdR$ ) is an antineoplastic agent which is highly active against HSV (160). In contrast to IUDR, the solubility and the potency of  $F_3TdR$  has been reported to be 10-fold greater than that of IUDR in the treatment of herpetic keratitis in rabbits. In clinical trials of  $F_3TdR$  on herpetic keratitis, in which 1% solution of  $F_3TdR$  was tested against 0.1% solution of IUDR,  $F_3TdR$  proved to be more effective than IUDR (161). When compared to Ara-A in the treatment of dendritic keratitis in humans, there was no statistical difference between



the use of F<sub>3</sub>TdR and Ara-A (162).

From the extensive studies performed primarily with vaccinia virus, the mechanism of antiviral action of F<sub>3</sub>TdR is believed to be due to its incorporation into the virus DNA (163, 164) and its subsequent alteration in the transcription of late mRNA (165).

In experimental models, bone marrow toxicity and teratogenic activity have been reported with the use of F<sub>3</sub>TdR (168, 169). F<sub>3</sub>TdR has been found to be mutagenic to bacteriophage T<sub>4</sub> but not mutagenic to Chinese hamster cells in culture (3). F<sub>3</sub>TdR therapy of herpetic keratitis has produced punctate epithelial erosions and epithelial microcysts when administered more frequently than 5 times daily for more than a few days. Further application of F<sub>3</sub>TdR at this point has produced frank epithelial edema with stromal swelling (118). When used topically in the eyes of rabbits, F<sub>3</sub>TdR has not produced teratogenic changes, unlike the teratogenic changes reported with topical use of IUdR (122).

F<sub>3</sub>TdR, as with IUdR, is incorporated into the viral DNA and the uninfected host cell DNA (164). The toxicity of F<sub>3</sub>TdR is thought to be related to its incorporation into the DNA of the uninfected host cell and the irreversible inhibition of thymidylate



synthetase, an enzyme needed in the biosynthesis of dTMP, an essential precursor of DNA (166, 167).

#### 5-Trifluoromethyl-5'-Amino-2',5'-Dideoxyuridine

An analog of  $F_3TdR$  is 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine, which was synthesized by Lin, Chai, and Frusoff in 1976 (170). Although it is four-fold less potent as an antiviral agent against HSV in Vero cells than its parent nucleoside,  $F_3TdR$ , it is also 40 times less toxic than  $F_3TdR$ . Thus, the therapeutic index of  $F_3TdR$  has been improved by a factor of 10 when modified to its 5'-amino analog.

#### 5-Iodo-2'-Deoxycytidine

5-Iodo-2'-deoxycytidine or IdCyd is a precursor analog of IUdR which was initially synthesized in 1961 (171). Studies performed on IdCyd demonstrated its effectiveness against HSV in culture (172) and in herpetic keratitis in rabbits (173). It is known that IdCyd and its bromo analog not only inhibit HSV in culture, but they are also substantially less toxic to the uninfected host cell than their deoxyuridine analog (174).

The selective action of IdCyd on the virus infected cells is dependent on its phosphorylation by only the





virus induced deoxycytidine kinase. This property may also explain why IdCyd is more effective than IUdR in the treatment of experimental deep herpetic keratitis (175). IdCyd is not converted into its active deaminated derivative until it is within the virus infected cells and has been phosphorylated by the virus induced deoxycytidine kinase. Therefore, in deep herpetic keratitis, IdCyd is protected from being metabolized on the surface until it reaches the deep layers of the infected cells. On the other hand, the clinical efficacy of IdCyd is dependent on the quantity of deoxycytidine kinase within the virus infected cells. Although several investigators have found an increase in the quantity of deoxycytidine kinase in the HSV infected cells (176, 177), a contradictory report has shown no increase of deoxycytidine kinase in the HSV infected cells (178). In the latter case, IdCyd is totally ineffective as an antiviral agent.

#### 5-Ethyl-2'-Deoxyuridine

The synthesis of 5-ethyl-2'-deoxyuridine was reported in 1969 (179, 180). It is an inhibitor of the replication of both HSV and vaccinia virus in cell cultures (181, 182) and in experimental deep herpes keratitis in rabbits (184). Clinical investigations



of 5-ethyl-2'-deoxyuridine indicate a positive therapeutic effect in the therapy of herpetic keratitis (185, 186).

5-Ethyl-2'-deoxyuridine is not as effective as IUdR or F<sub>3</sub>TdR, but its potential as an antiviral agent is based on its non-mutagenic effect on the studies in phage and drosophila (187, 188). It also has no effect upon the chromosome morphology of human lymphocytes and fibroblasts in culture (189).

Other 5-alkyl analogs of thymidine such as 5-vinyl-, 5-propyl-, and 5-allyl-2'-deoxyuridine have been synthesized and shown to have antiviral activity against both HSV-1 and HSV-2 in cell cultures (190).

### 6-Azauridine

6-Azauridine is a broad spectrum antiviral agent effective against a variety of RNA and DNA viruses in vitro. The inhibition of HSV replication by 6-azauridine has been reported by several investigators (191, 192). 6-Azauridine is similarly effective against herpes simplex keratitis in rabbits (191) and herpes simplex stromal infection of the eye in humans (193). However, the clinical applicability of 6-azauridine in the treatment of HSV infection is severely limited by its low potency and its toxicity. Reported cases of serious



central nervous system disturbance have been associated with the use of 6-azauridine (194). In addition, this antiviral agent is both immunosuppressive and teratogenic.

#### 1-Beta-D-Ribofuranosyl-1,2,4,-Triazole-3-Carboxamide

1-Beta-D-ribofuranosyl-1,2,4,-triazole-3-carboxamide (Virazole, Ribavirin) is a broad spectrum antiviral agent active against RNA and DNA viruses in vitro and in vivo (195, 196). It has been shown to be effective in the treatment of experimental herpes and vaccinia keratitis in rabbits (197).

The potential of this broad spectrum compound in the treatment of clinical diseases is limited by two factors. Firstly, it must be administered as a prophylactic agent in order to exhibit its effectiveness as an antiviral agent. Secondly, teratogenesis has been observed with this agent (165).

#### Photodynamic Inactivation of HSV Infections

Another investigative approach to the treatment of HSV infections is that of photodynamic inactivation of HSV. The experimental works of Wallis and Melnick revealed the in vitro inactivation of HSV by exposure to heterotricyclic dyes and light (198, 199).



The heterotricycle acridine and phenazine dyes are photoactive compounds capable of absorbing light energy and participating in photo-oxidation reactions. The phenazine compound neutral red was the first dye used in the photodynamic inactivation of herpes simplex skin infections in humans (200). Because of neutral red's potential as a contact allergen (201), it has been replaced by the use of proflavin, an acridine dye, which has greater effectiveness in photosensitizing viruses in vitro (199). The effectiveness of photodynamic inactivation of viruses is dependent upon the dye concentration, temperature, and pH. The progeny virus becomes sensitive to light when grown in cells pre-treated with heterotricyclic dyes (202). The dye is incorporated into the virus DNA (203). The virus is inactivated when the dye-DNA complex absorbs sufficient energy to produce an oxidation reaction, resulting in the loss of guanine, gaps in the base sequence, and the subsequent breaks in the DNA of the virus. (204). In this manner, HSV can be made photosensitive by the incorporation of a heterotricyclic dye into its DNA so that subsequent exposure to visible light renders the HSV noninfectious. (198).

The use of photodynamic dyes has been successfully applied in the treatment of experimental herpes simplex





keratitis in rabbits (205, 206, 207), but it is less effective than that of IUDR (206). Other studies have reported the effectiveness of photodynamic inactivation of eczema herpeticum, orolabial and genital HSV infections in humans (200, 208-216). However, other investigators have reported opposite results which failed to demonstrate antiviral activity in the treatment of genital HSV infection and in recurrent HSV infection. (217, 218, 219).

There are hazards associated with the use of photodynamic dyes, and its clinical application remains rather controversial. The photodynamic inactivated HSV have been shown to induce neoplastic transformation of mammalian cells in vitro (200) which have oncogenic properties in vivo (221).

#### Interferon and Interferon Inducers

Interferon and interferon inducers are of great biologic interest as potential broad spectrum antiviral agents. At the present time, they have not proved to be clinically useful in the prophylaxis or the treatment of viral infections. Studies of the topical application of human interferon in the treatment of herpetic keratitis are rather disappointing. (222).



Studies aimed at the prevention of recurrent ocular herpetic infections in humans with topical leucocyte interferon were also therapeutic failures (223).

Another disadvantage in using induced, systemic, or local interferon is the capability of the host cells to be stimulated only for a limited period of time (6 to 10 weeks), after which they need a rest period of 3 weeks before induction could be started again (224, 225). The use of inducers of interferon such as polymers of double-stranded RNA's (poly I-ploy C) and substituted propanediamines are just as disappointing since they are usually accompanied by considerable toxicity (226, 227, 228, 229).

#### 5-Iodo-5'-Amino-2',5'-Dideoxyuridine

From the previous discussion of antiviral nucleoside analogues, photodynamic dyes, interferon and interferon inducers, it is evident that none of these agents constitute an ideal chemotherapeutic agent against HSV infection in humans. The most worrisome characteristics of the nucleoside analogues are their low solubility and their toxicity to the uninfected host cells. These toxic side effects are presumably due to the incorporation of the drugs into the nucleic acids of the uninfected



cells, resulting in cytotoxicity and the potential of inducing mutagenesis and carcinogenesis. Hence, an antiviral agent which is incorporated into the nucleic acids of only the virus infected cells and not the uninfected cells will provide a major advance toward minimizing the toxicities commonly associated with the current antiviral therapy.

5-Iodo-5'-amino-2',5'-dideoxyuridine or AIU is the 5'-amino analog of IUdR which was originally synthesized in the laboratory of W.H. Prusoff at Yale University in 1974 (230). The initial studies of AIU demonstrated its in vitro capability as a potent inhibitor of HSV-1. AIU was shown to be more potent than Ara-A but less potent than IUdR, F<sub>3</sub>TdR, or Ara-C on a molar basis. The most significant finding was the absence of any cytotoxicity associated with the use of AIU, as opposed to the toxicity observed with similar concentrations of IUdR, F<sub>3</sub>TdR, Ara-A, and Ara-C (2). When compared with Ara-A, a nucleoside analog noted for its high therapeutic index, Ara-A was found to have significant toxicity at concentration that produced less antiviral activity than AIU.

AIU's lack of cytotoxicity was further demonstrated in the following cell lines examined in culture: murine sarcoma 180, Erhlich ascites, BHK-21 (hamster kidney),



HeLa, secondary chicken embryonic fibroblasts, A-9 and L cells (231). In addition, AIU at 200  $\mu$ M produced no detectable morphological changes nor inhibited the normal rate of DNA and RNA synthesis of uninfected Vero cells (2).

In vivo studies with the intraperitoneal administration of AIU in newborn and 8 day old suckling mice also produced no growth retardation nor teratogenic effects. In contrast, IUdR administered intraperitoneally produced growth retardation, as well as histopathological evidence of cataract, retinal dysplasia, cerebellar lesions, cortical lesions, and general retardation of organ development (232).

A study of AIU in the treatment of experimental herpetic keratitis in rabbits established its therapeutic effectiveness on experimental herpetic keratitis in rabbits (233). Although AIU is less potent than IUdR, viral recovery studies showed AIU and IUdR to be equally effective in reducing the titer of HSV-1 in the treated tissues.

Given the experimental data documenting the therapeutic effectiveness of AIU on HSV-1 keratitis in rabbits and the absence of cytotoxicity, AIU appears to be a promising agent which should be tested in the treatment of other forms of HSV-1 infections.





Cutaneous herpes infections are the cause of substantial morbidity in the otherwise healthy population and can sometimes result in the mortality of newborns and patients with diminished immunologic competence. Disseminated or severely localized herpes simplex infections are life-threatening complications in the patients with hematologic malignancies or in patients with immunosuppressive therapy following renal transplantation, severe burns, malnutrition, and immune deficiency syndromes such as the Wiskott-Aldrich syndrome (92, 234, 235, 236). The occurrence of life-threatening HSV infections is expected to increase, due to the current treatment of malignancies with chemotherapeutic agents having significant immunosuppressive action. Hence, there will be an increasing need for an effective antiherpetic agent having minimum cytotoxicity for the topical or systemic treatment and prophylaxis of cutaneous and oral herpes simplex lesions in these patients. Given the preliminary studies of AIU, it appears to be a promising agent which should be investigated for the treatment of cutaneous herpes simplex infection.



## V. Materials and Methods

### I. Drugs.

- A. IUdR was obtained from Sigma Chemical Company, St. Louis, MO.
- B. AIU was synthesized in the laboratory of W.H. Prusoff, Professor of Pharmacology, Yale University, as described by Lin, et al, 1976 (1).
- C. Ara-AMF was provided by Park-Davis Company in ointment form at 10 percent concentration (w/w).

### II. Preparation of the drugs.

The drugs were prepared into ointment form by Al Fiore, Director of the Pharmacy Research and Development Department of the Massachusetts General Hospital, Boston, MA.

- A. 5 gm. IUdR was mixed into petrolatum base to make an IUdR ointment at 0.5% concentration (w/w). The IUdR ointment was then loaded into 70 tuberculin syringes (1 ml.) to yield a total of 70 ml. of 0.5% IUdR ointment.



- B. 9 gm. AIU was mixed into a petrolatum base to make an AIU ointment at 10% concentration (w/w). The AIU ointment was then loaded into 70 tuberculin syringes (1 ml.) to yield a total of 70 ml. of 10% AIU ointment.
- C. 15 tubes of 3.55 gm. of Ara-AMP ointment at 10% concentration (w/w) was provided by Fark-Davis Company. The Ara-AMP ointment was loaded into 50 tuberculin syringes to yield 50 ml. of Ara-AMP ointment.
- D. 70 ml. of 10% (w/w) lactose ointment, as prepared by Al Fiore, was loaded into 70 tuberculin syringes.

The tuberculin syringes containing the drugs were coded by a member of the laboratory who was not involved in the daily examination and the grading of the HSV cutaneous lesions of the guinea pigs. The drugs were labeled as drug A, B, C, and D. The drug code was not known to the investigators until the experiment had been completed.

### III. Guinea pigs.

A total of 21 adult male Hartley strain guinea pigs were used, each weighing between 300 and 500 grams. The guinea pigs within the same treatment group were kept in the same cage. Daily feeding of the



guinea pigs and maintenance care of the cages were provided.

#### IV. Herpes Simplex Virus - Type 1

The HSV-1 was provided by Pravin Bhatt at Yale University. The HSV-1 used in the experiment was NIH strain No. 11124, contained in a solution of  $10^7$  TCID<sub>50</sub>/ml. The virus solution was kept frozen until the time of inoculation.

#### V. Procedures

The hair on the dorsum of the guinea pigs was shaved using electric clippers. A chemical depilatory (Nair, Carter Products, N.Y.) was applied on the moistened, shaved area of the guinea pig dorsum for 10 minutes. The shaved area was then rinsed with warm water to remove the depilatory. The shaved area of the guinea pig dorsum was dried with soft towels. Using indelible ink pens, the shaved dorsum was marked into a grid of six squares, each square measuring 2 cm. x 2 cm.

The guinea pigs were then anesthetized using 0.4 ml. of sodium pentobarbital injected intraperitoneally. While the guinea pigs were under the sodium pentobarbital anesthesia, 0.02 ml. of thawed HSV-1 solution





at  $10^7$  TCID<sub>50</sub>/ml. was applied topically to the surface of the guinea pig at each square of the grid, using a tuberculin syringe to measure the HSV-1 solution. The HSV-1 was then inoculated intradermally with a spring-loaded vaccination instrument (Sterneedle, Fan Ray Division, Ormond Drug, Englewood, New Jersey), 10 times in each square. The spring-loaded vaccination instrument produced a ring of six inoculation site to a depth of 0.75 mm.

After the intradermal inoculation of the HSV-1, the guinea pigs were randomly distributed into four treatment groups as follows:

Drug A-----five guinea pigs

Drug B-----five guinea pigs

Drug C-----six guinea pigs

Drug D-----five guinea pigs

The drug treatment was begun immediately after the inoculation of HSV-1. 0.1 ml. of ointment from the tuberculin syringes was applied topically to each square of the grid and was gently rubbed onto the entire surface of the square. After the drug application, the guinea pigs were returned to their respective cages. The treatment schedule was repeated every 8 hours for 6 consecutive days.

During the morning treatment sessions, two investigators



independently grade the lesions on each guinea pig before the treatment was begun. These two investigators did not know the drug code until the termination of the experiment.

Each guinea pig was examined daily for 11 days by the two investigators, starting 1 day after the intradermal inoculation of HSV-1. The development of the HSV-1 lesions in each square was evaluated by the criteria of:

1. Erythema: graded on a scale of 0 to 4, based on the intensity and the area of erythema.  
0 = no erythema  
1 = slight erythema:involving a small area  
2 = moderate erythema:either greater intensity of erythema than in (1) or extension of the area of erythema found in (1)  
3 = moderately severe erythema:greater intensity of erythema than in (2) and/or extension of the area of erythema found in (2)  
4 = severe erythema:greatest intensity of erythema compared to that found in (1), (2), and (3). The area of erythema greater than in (3)



2. Vesicles : graded on a scale of 0, 1, 2, 3, ...;  
corresponding to the number of vesicles  
found in each square.

The guinea pig hair regrew within 5 days, necessitating another application of chemical depilatory on day 5 on the dorsum of the guinea pigs, as described previously.

#### Histopathologic studies

One guinea pig in each treatment group was selected on day 6 for the biopsy of the skin lesions for histopathologic studies. The lesion was biopsied, by using a disposable 3 mm. diameter Chester Baker Skin Biopsy Punch. The biopsied specimen was then preserved in a formaldehyde solution. The histology slides were prepared by the Pathology Laboratory at the Massachusetts Eye and Ear Infirmary, Boston, MA.



## VI. Results

In this controlled, double-masked experiment, the HSV-1 inoculated guinea pigs were randomly distributed into four treatment groups. Each guinea pig was examined daily for the development of the HSV-1 lesions. The HSV-1 cutaneous lesions were graded for 10 days by two independent investigators, according to the criteria of

(1) erythema

(2) vesicles

in the manner described in the procedures.

Each of the four treatment groups had five guinea pigs within each group (Note: one of the groups had 6 guinea pigs, but one of the guinea pigs died from trauma sustained during one of the procedures on day 5 of the experiment). With 5 guinea pigs per treatment group and 6 HSV-1 inoculation sites per guinea pig, there were 30 lesion sites which were examined daily in each treatment group.

Since each lesion was independently evaluated by the two investigators, 60 lesion scores were recorded





for each criterion per treatment group per day. Given the 4 treatment groups in the experiment, a total of 240 lesion scores were recorded per criterion per day for all 4 treatment groups. For the two criteria to be evaluated, a total of 480 lesion scores were recorded per day for the 4 treatment groups. Over the course of 10 days, there were 4,800 lesion scores recorded for the 4 treatment groups.

In order to evaluate the efficacy of the 4 drug treatments on HSV-1 cutaneous lesions in the guinea pigs, it was necessary to simplify the 4,800 lesion scores by consolidating this data in the form of mean lesion score for each criterion per treatment group per day. The mean scores of the HSV-1 cutaneous lesions of the guinea pigs are tabulated in Table 1 for the mean erythema score and in Table 3 for the mean vesicle score.

In this controlled, double-masked experiment on the efficacy of AIU treatment on cutaneous HSV-1 lesions in guinea pigs, none of the guinea pigs developed zosteriform distribution of the HSV-1 lesions nor manifested any neurologic signs such as limb paralysis or encephalitis. The HSV-1 cutaneous lesions which developed progressed from an area of erythema to vesicle formation, then crusting of the vesicles and finally healing of the lesions by day 11.



From the raw data, as well as from the calculated mean scores of erythema shown in Table 1, it is evident that one day after the HSV-1 inoculation, the inoculated sites became slightly erythematous (mean erythema score  $\leq 1.68$ ). By days 5 and 6, all 4 treatment groups had attained the maximum mean erythema score ( $\leq 2.27$ ). After the 6th day post HSV-1 inoculation, the mean erythema scores progressively declined until the termination of the experiment on the 11th day ( $\leq 0.15$ ). See Plate 1, depicting 3 erythematous lesions at 3 inoculated sites on the 3rd day (erythema score = 1).

Although all 4 treatment groups demonstrated similar trends in the development of erythema at the inoculated sites, the AIU and the Ara-AMF treatment groups differed somewhat in that an initial rise of the mean erythema scores occurred on day 1, which then subsided by days 2 and 3. This was followed by a second rise of the mean erythema scores on days 5 and 6 to produce their maximum scores. See Figure 1 for the graph of the mean erythema score for the 4 treatment groups.

Even though the trends for the development of erythema were similar in all 4 treatment groups, the actual values of the mean erythema scores differed in each of the treatment groups. This is exemplified by the cumulative mean erythema scores of the four treatment



groups (see Table 2). From the graph of the cumulative mean erythema scores shown in Figure 2, it is clear that the lactose group (control) had a greater cumulative mean erythema score than the other 3 treatment groups. In increasing order of magnitude, the cumulative mean erythema scores are : Ara-AMP < IUdR < AIU < Lactose.

The data of the mean vesicular scores, as tabulated in Table 3 and graphed in Figure 3, showed that the development of vesicles began on day 3 in all 4 treatment groups. These vesicles initially developed as small, discrete vesicles, surrounded by an erythematous base (see Plate 2). The vesicles gradually enlarged in size, with some of the juxtaposed vesicles coalescing to form larger, confluent vesicular lesions by the 6th day (see Plate 3). The fully developed lesions, which consisted of discrete and coalescing vesicles on an erythematous base, began to show signs of crusting by day 7 (see Plate 4). The crusting of the lesions progressed from day 7 to day 10 in all 4 treatment groups (see plate 5). By the 11th day, all the vesicular lesions had healed as shown in Plate 6 (mean vesicular score = 0.00).

The trend observed in the mean vesicular scores for the 4 treatment groups differed somewhat among the groups in terms of the time the maximum number of vesicles developed (see Figure 3). That is, the maximum number of vesicles



developed on the 4th day for AIU, on the 6th day for IUdR, on the 7th day for Lactose, and on the 8th day for Ara-AMP. Although the 4 treatment groups had all their vesicles healed by the 11th day, some treatments accelerated and others delayed the onset of the development of the maximum number of vesicles by a few days.

The cumulative mean vesicular scores for the 4 treatment groups are similar to the cumulative mean erythema scores (see Figure 4). Once again, the lactose treated group (control) had the highest cumulative mean vesicular score, as compared to AIU, Ara-AMP, and IUdR. The calculated values of the cumulative mean vesicular scores are tabulated in Table 4. The cumulative mean vesicular scores of the 4 treatment groups are in order of increasing value: Ara-AMP < IUdR < AIU < Lactose.

#### Statistical analysis:

The guinea pigs in the lactose treatment group provided the control group for this experiment. From the data on the mean erythema score and the mean vesicular score, it is evident that the lactose treatment was not as effective as the other 3 treatments for HSV-1 cutaneous lesions in guinea pigs. This is best depicted in Figures 2 and 4 where their respective cumulative scores are graphed, showing the lactose group having the greatest





score in both criteria. The differences between the control group and the other 3 treatment groups are significant:

Mean Erythema Score:

1. Lactose and AIU: minimum modified  $\chi^2$  17.9112 ( $p < 0.001$ )
2. Lactose and IUdR: min. mod.  $\chi^2$  77.0324 ( $p < 0.001$ )
3. Lactose and Ara-AMP: min. mod.  $\chi^2$  109.9078 ( $p < 0.001$ )

Mean Vesicular Score:

1. Lactose and AIU: minimum modified  $\chi^2$  8.4237 ( $p < 0.004$ )
2. Lactose and IUdR: min. mod.  $\chi^2$  69.5720 ( $p < 0.001$ )
3. Lactose and Ara-AMP: min. mod.  $\chi^2$  119.9783 ( $p < 0.001$ )

This statistical analysis demonstrates a significant treatment effect of AIU, IUdR, and Ara-AMP on the development of erythema and vesicles in the experimentally induced cutaneous HSV-1 lesions of guinea pigs.



## VII. Discussion

There is no satisfactory agent available for the clinical treatment of cutaneous herpes simplex infections in humans in the United States at this time. Antiviral agents with therapeutic effectiveness that are available experimentally have thus far proved to be either teratogenic, mutagenic, or too cytotoxic to be used clinically. It is evident that a critical need exists for a clinically effective agent against cutaneous herpes simplex infection which also does not produce intolerable toxic effects.

Cutaneous herpes simplex infections have been studied in the past, using a variety of species and inoculation routes. Studies of cutaneous herpes simplex infections have been reported using the inoculation of HSV into the ear of mice (237), into the skin of guinea pigs (238), into the skin of rabbits (239, 240), into the skin of rabbits with 1% hyaluronidase (241), into the footpad of guinea pigs (242), into the skin of mice (243), into the skin of hairless mice (244, 245, 246, 247), and into the skin of rats (248).



Most of these models have not been totally satisfactory since they failed to produce localized, recurrent lesions of HSV of the skin which simulate the course of the disease in humans. Another problem encountered in these models **has** been the high incidence of generalized herpes simplex infection, leading to herpes encephalitis, paralysis, and death following the intradermal inoculation of HSV. Others have reported the development of segmental, zosteriform distribution of the herpes simplex vesicles, with evidence of virus spread along the peripheral nerves (247).

The inoculation technique of intradermal HSV described by Hubler, et al., in 1974 has none of the above deficiencies (238). This inoculation technique was successfully used in this study to inoculate the guinea pigs with intradermal HSV-1. None of the guinea pigs in this study developed any signs of generalized herpes simplex infection, herpes encephalitis, paralysis, or zosteriform distribution of the cutaneous lesions. The progression of the herpes lesions simulated the course of cutaneous herpes lesions found in humans. Given this experimental model for cutaneous herpes simplex lesions, we were able to test the efficacy of AIU for the therapy of herpes simplex cutaneous infection in guinea pigs.

AIU is a thymidine analogue differing from IUdR by the substitution of an amino group for 5' hydroxyl.



AIU has been shown in previous studies to inhibit the in vitro replication of HSV-1 (2), as well as to be efficacious in the treatment of herpes simplex keratitis in rabbits (240).

The present controlled, double-masked experiment demonstrates a significant treatment effect of AIU in the therapy of cutaneous herpes simplex infection in guinea pigs. The critical difference between AIU and the other antiviral nucleoside analogues is the absence of any detectable teratogenic or cytotoxic effects associated with the use of AIU (231, 232). This finding seems to suggest a virus-specified site of inhibition as the basis of its antiviral action. The supporting evidence for this theory comes from the findings listed below:

1. The critical time of action of AIU appears to be during the replication of HSV within the host cell. The pre-incubation of HSV with AIU prior to in vitro infection or the presence of AIU in the media during the absorption process did not inhibit the production of virus. Only when AIU was added 4 to 6 hours after infection was there a marked reduction in the yield of progeny virus (2).
2. AIU has no inhibitory effect on the rate of RNA and protein synthesis in either the HSV-infected Vero cells or the uninfected Vero cells. More





importantly, AIU does inhibit the uptake of labeled thymidine into the DNA of HSV-infected Vero cells, but not in the uninfected Vero cells (2).

3. AIU is metabolized in vitro by only HSV infected cells. After its conversion to the 5'-triphosphate, it is incorporated into both viral and cellular DNA of the infected cells. AIU appears to be selectively phosphorylated in HSV-infected cells, mediated by the HSV specified thymidine kinase (249).
4. HSV mutants defective in thymidine kinase are totally resistant to AIU.

The selective phosphorylation of AIU and its incorporation into the DNA of the infected cell could certainly account for its specific antiviral activity and its absence of host toxicity. This important characteristic of AIU differentiates it from all the other antiviral agents as a potential drug in the treatment of HSV infections in humans.

This study has shown the effectiveness of AIU on one type of HSV-1 infection, that of cutaneous HSV-1 infection in guinea pigs. Further investigations on the efficacy of AIU on other forms of HSV infections will be worthwhile, particularly those HSV infections presenting as life-threatening disseminated infections of the newborn and the immunosuppressed patients. The possible role of AIU in the treatment of recurrent HSV infection, herpes encephalitis, and genital herpes would also merit further investigation.



### VIII. Summary

1. Significant treatment effect of AIU on experimentally induced herpes simplex virus type 1 cutaneous lesions in guinea pigs was demonstrated.
2. Significant treatment effect of IUdR and Ara-AMP on experimentally induced herpes simplex virus type 1 cutaneous lesions in guinea pigs was also demonstrated.
3. The potency of these three drugs, in order of increasing potency is as follows:  
AIU < IUdR < Ara-AMP.



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TABLE 1

## ERYTHEMA - MEAN LESION SCORE /DAY

Days after Inoculation	AIU	Ara-AMP	IUdR	Lactose
1	1.68	0.85	0.49	0.43
2	0.75	0.50	0.70	0.76
3	1.04	0.33	0.91	1.05
4	1.10	0.86	1.16	1.42
5	2.20	0.95	1.21	2.25
6	1.46	1.76	1.67	2.27
7	0.92	0.84	1.22	2.10
8	0.77	0.72	0.73	1.33
10	0.33	0.43	0.05	0.72
11	0.03	0.15	0.00	0.07



TABLE 2

## ERYTHEMA - CUMULATIVE MEAN LESION SCORE/DAY

Days After Inoculation	AIU	Ara-AMP	IUdR	Lactose
1	1.68	0.85	0.49	0.43
2	2.43	1.35	1.19	1.19
3	3.47	1.68	2.10	2.24
4	4.57	2.53	3.25	3.66
5	6.77	3.48	4.46	5.91
6	8.23	5.24	6.12	8.18
7	9.15	6.08	7.35	10.28
8	9.92	6.80	8.08	11.61
10	10.25	7.23	8.13	12.33
11	10.28	7.38	8.13	12.40



TABLE 3

## VESICLES - MEAN LESION SCORE/DAY

Days After Inoculation	AIU	Ara-AMP	IuDR	Lactose
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	1.33	0.12	1.03	1.12
4	2.28	0.90	1.53	1.97
5	2.23	0.45	1.71	2.92
6	2.03	0.79	1.89	3.56
7	2.33	0.81	1.69	3.88
8	2.35	1.71	1.17	2.26
10	1.34	1.57	0.57	1.72
11	0.00	0.00	0.00	0.00





TABLE 4

## VESICLES - CUMULATIVE MEAN VESICULAR SCORE

Days After Inoculation	AIU	Ara-AMP	IUdR	Lactose
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	1.33	0.12	1.03	1.12
4	3.62	1.02	2.56	3.08
5	5.85	1.47	4.27	6.00
6	7.88	2.25	6.16	9.56
7	10.22	3.06	7.84	13.44
8	12.57	4.78	9.01	15.69
10	13.91	6.35	9.58	17.42
11	13.91	6.35	9.58	17.42



FIGURE 1

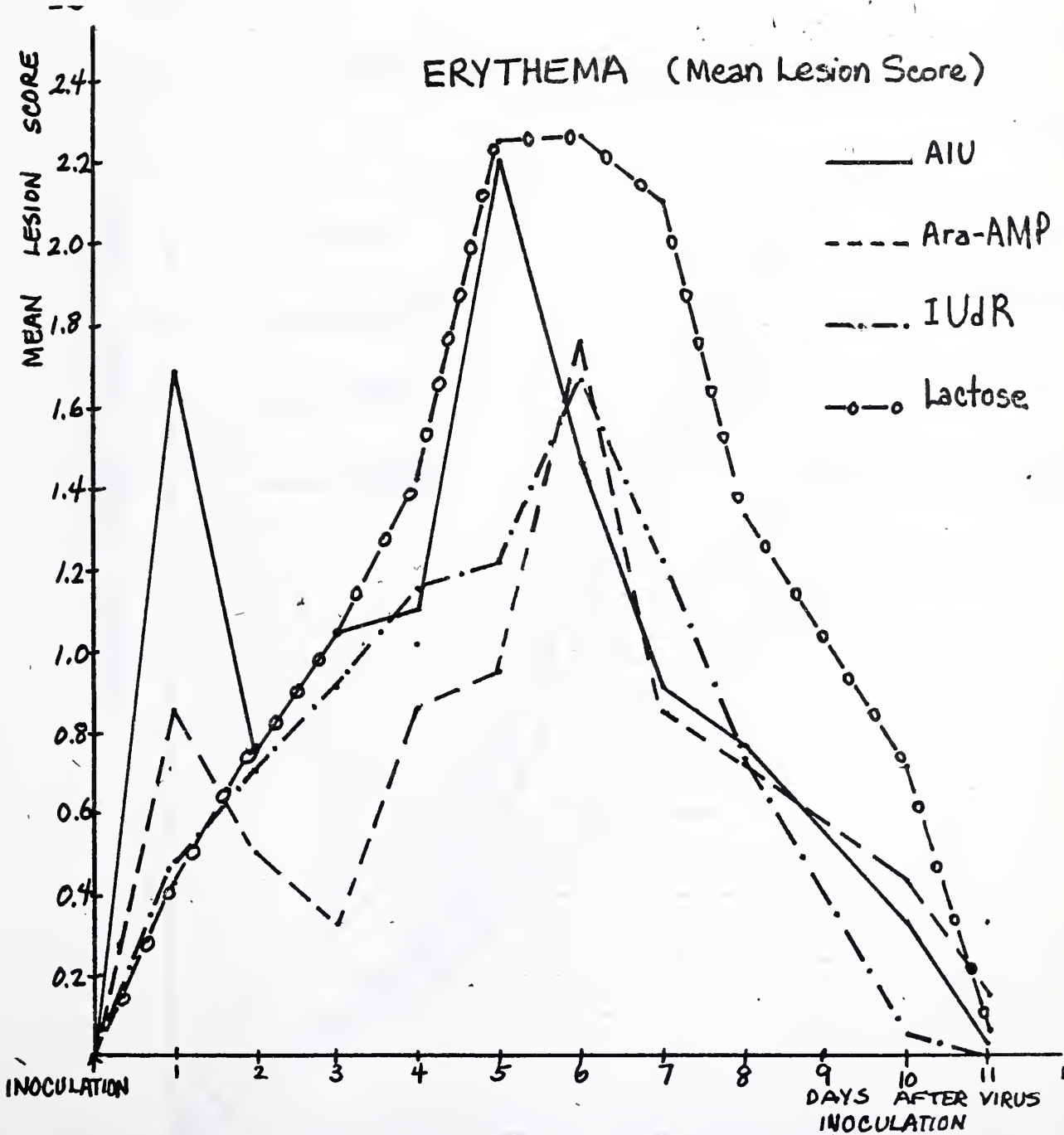
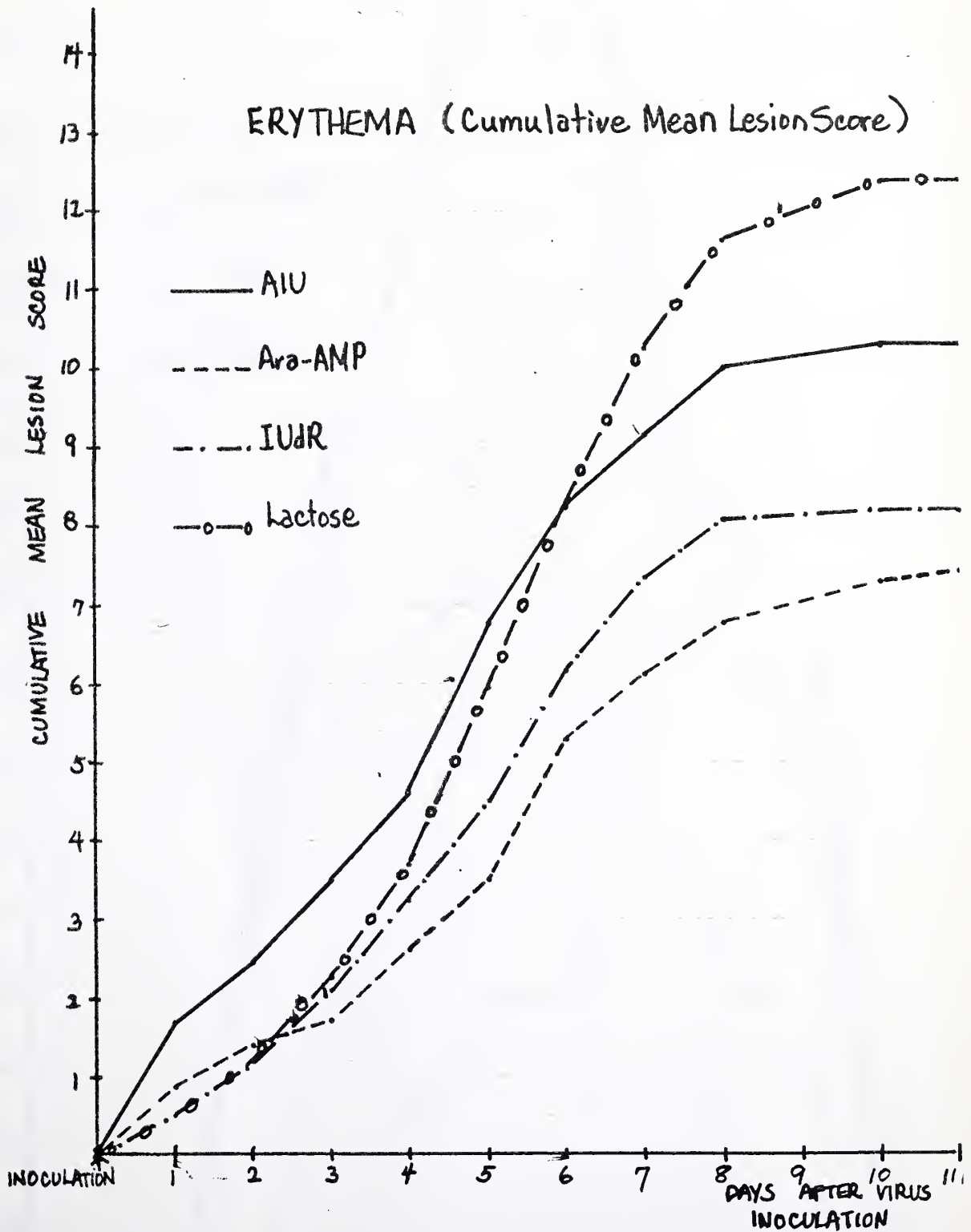




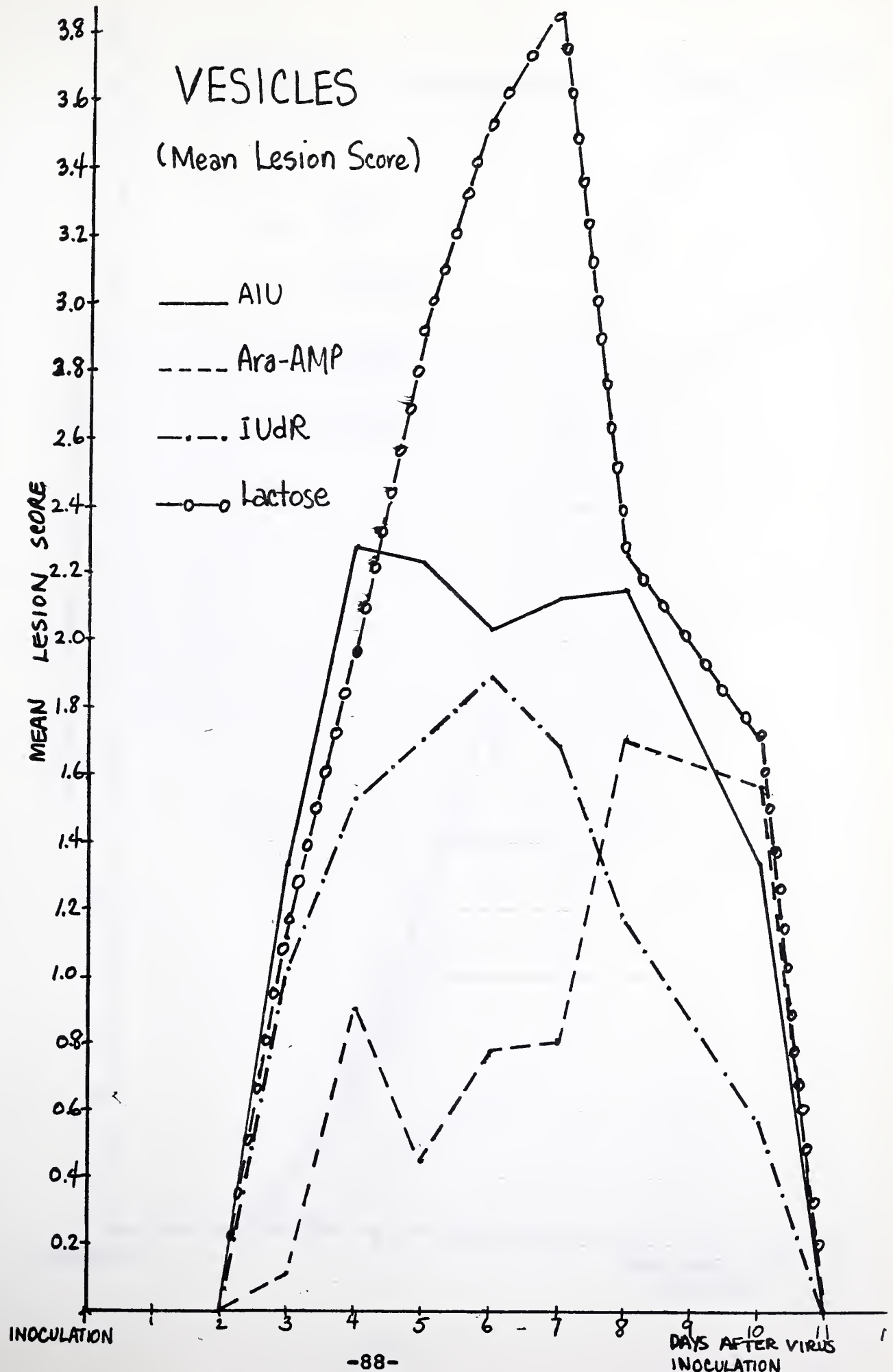
FIGURE 2





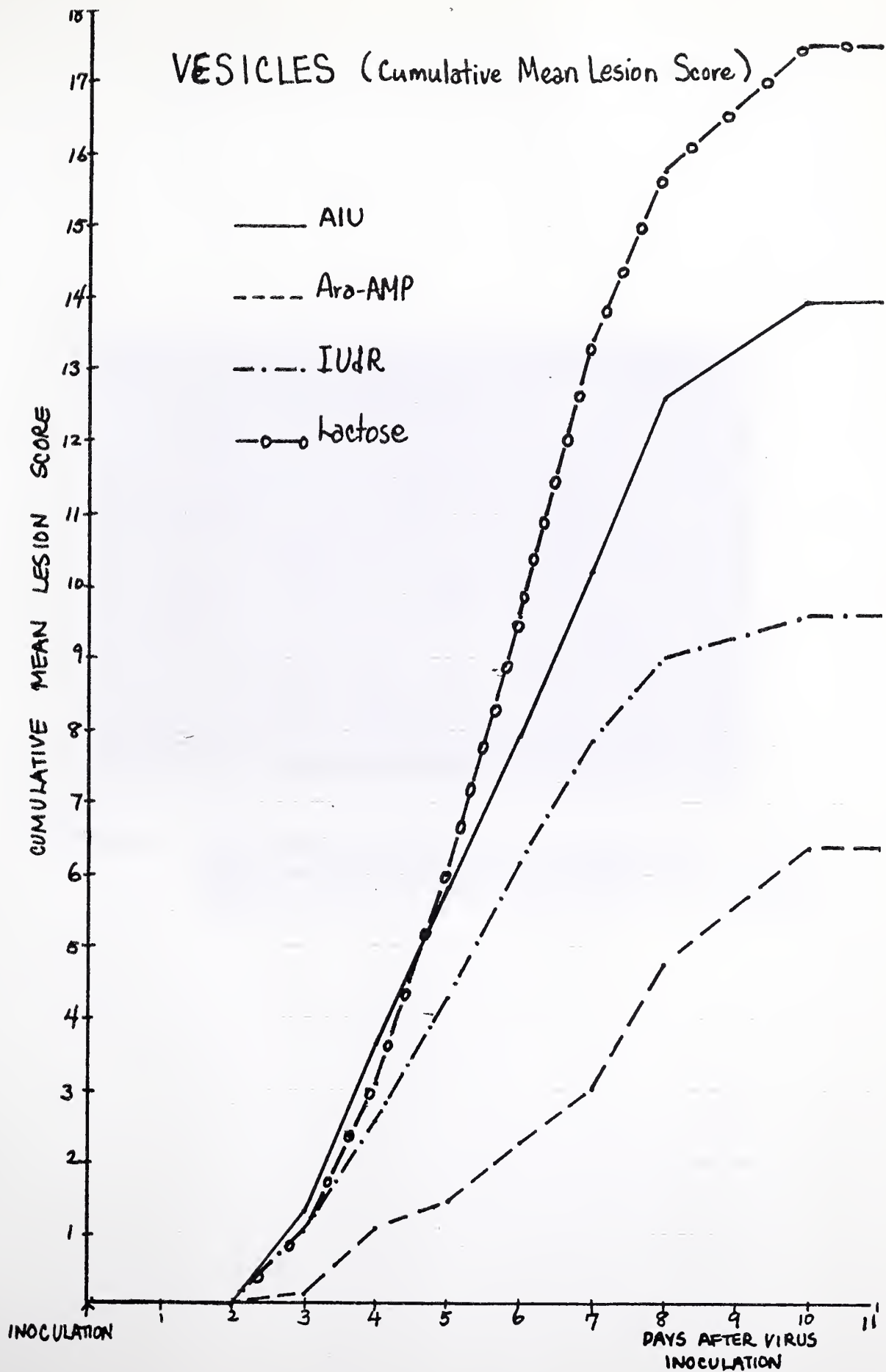
# VESICLES

(Mean Lesion Score)

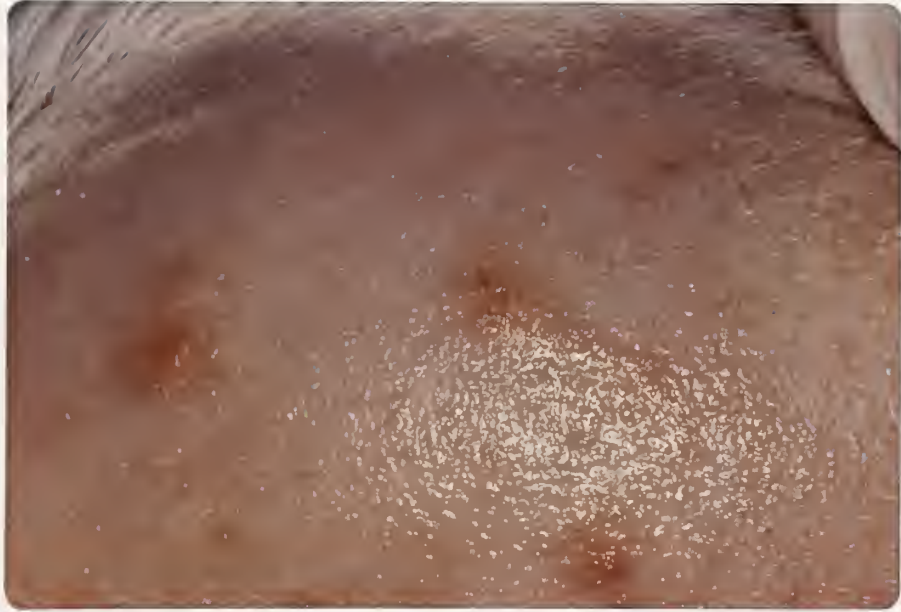












**Plate 1 : Erythematous lesions (erythema score = 1)  
at 3 inoculated sites of guinea pig  
skin on the 3rd day after HSV-1 inoculation.**





**Plate 2 : Small, discrete vesicles surrounded by an erythematous base found at the inoculated site of guinea pig skin 4 days post HSV-1 inoculation.**



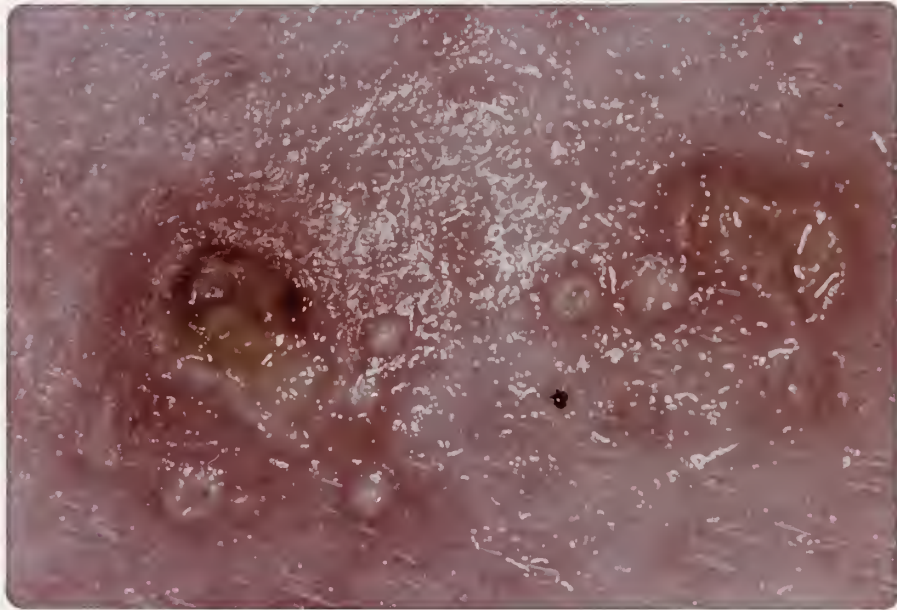


Plate 3 : Juxtaped vesicles have coalesced to form larger, confluent vesicular lesions at 2 inoculation sites of guinea pig skin at 6 days post HSV-1 inoculation.







**Plate 4 :** The vesicular lesions of guinea pig skin demonstrate the initial signs of crusting, as shown here at 7 days post HSV-1 inoculation.





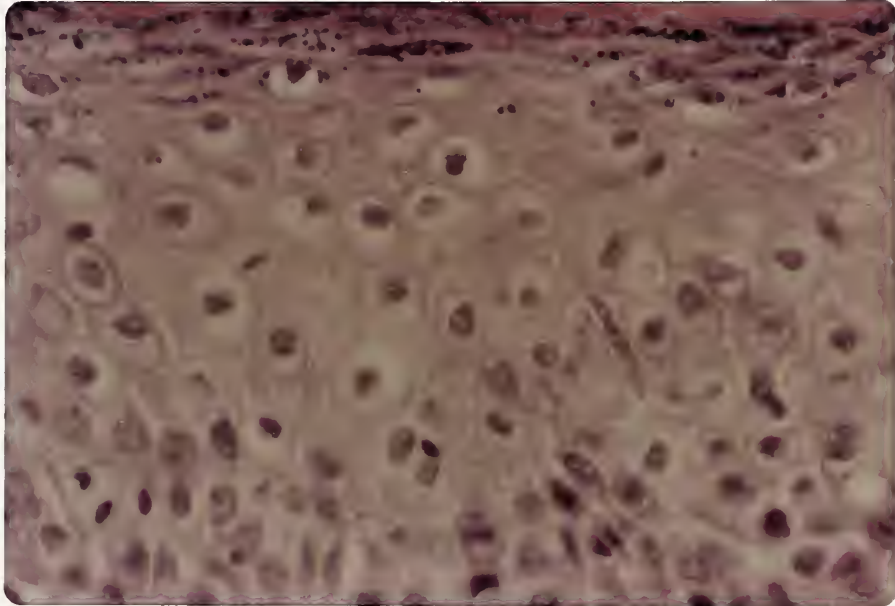
Plate 5 : Crusting of the HSV-1 lesions found in the guinea pig skin 10 days after HSV-1 inoculation.





Plate 6 : On the 11th day post HSV-1 inoculation, the lesions have completely healed at the HSV-1 inoculated sites of the guinea pig skin.

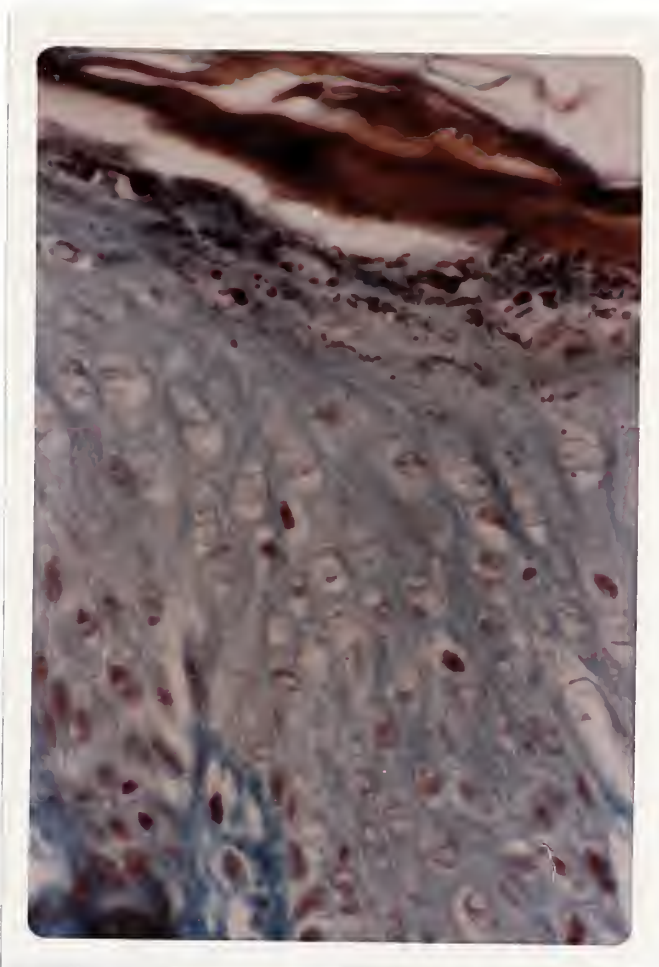




**Plate 7 :** Histology of guinea pig skin infected with herpes simplex virus type 1 at 6 days after virus inoculation, demonstrating the ballooning degeneration of epidermal cells and eosinophilic viral inclusion bodies (H.E.Stain).







**Plate 8 :** Histology of guinea pig skin infected with herpes simplex virus type 1 at 6 days after virus inoculation, demonstrating the ballooning degeneration of epidermal cells and the red viral inclusion bodies (Page-Green Stain).







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