THE EFFECT OF I.D.U. ON EXPERIMENTAL AND CLINICAL HERPES SIMPLEX INFECTIONS

BY *Richard C. Ey*, M.D. (BY INVITATION), *William F. Hughes*, M.D., AND (BY INVITATION) *A. W. Holmes*, M.D., AND (BY INVITATION) *Friedrich Deinhardt*, M.D.*

THE EFFECTS OF 5-iodo-2-deoxyuridine (I.D.U.) on herpes simplex keratitis have been reported by several authors.[1-8] These have dealt primarily with the course of the disease in patients and, to a lesser extent, in rabbits. Tamm,[9] in 1960, reported that inhibitors of DNA synthesis could suppress the production of herpes simplex virus in tissue culture. Kaufmann,[8,10] however, was the first to report the beneficial effects of I.D.U. in experimental and clinical herpes keratitis. Our studies to be reported in this paper include: (1) the effect of I.D.U. on herpes keratitis in rabbits and patients, correlated with the presence or absence of demonstrable virus; (2) the influence of immunity in rabbits on the clinical and virological course of the disease; and (3) the influence of I.D.U. on virus and host cells in a tissue culture system.

In our rabbit experiments, the corneas were abraded by cross-hatching with a needle, and two drops of a herpes simplex strain of low neuropathogenicity, obtained from Dr. Kaufmann,* were instilled. Corneal lesions developed in all eyes within 24 to 48 hours after inoculation. Treatment consisted of 0.1 per cent (3×10^{-3} *M*) I.D.U. drops in a pH 7.4 phosphate buffer every hour during the day and every two hours at night for six days. Specimens for virus cultures were obtained with a wet cotton swab applied over most of the cornea anesthetized with Ophthaine®, and virus titers were assayed in primary rabbit kidney tissue culture. The results of a representative experiment in non-im-

*From the Departments of Ophthalmology, Medicine, and Microbiology, Presbyterian-St. Luke's Hospital, Chicago 12, Illinois. This research project was supported in part by the Louise C. Norton and Harry J. Williams Ocular Research Funds in Ophthalmology, the Liver Research Fund, and the Joseph and Helen Regenstein Foundation.

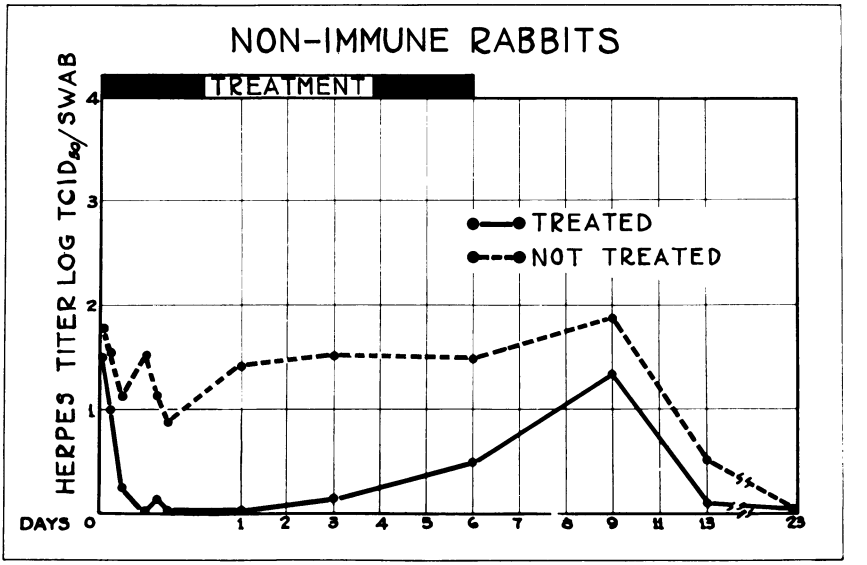

FIGURE 1. SERIAL VIRUS TITERS IN EYES OF NON-IMMUNE RABBITS

mune animals (12 treated, 6 untreated eyes) are shown in Figure 1. Forty-eight hours after inoculation, all eyes showed typical dendritic ulcers, and treatment was initiated. Specimens for virus cultures were obtained immediately before treatment, every two hours during the first 12 hours of treatment, and every 24 hours thereafter. In the untreated animals, the amount of recoverable virus remained high for the first nine days and then slowly disappeared during the next two weeks. In the animals treated for six days the virus titers fell rapidly and remained low for three days, but then gradually rose while on treatment until the ninth day when they became essentially parallel to the titers of the untreated animals. The untreated animals developed a severe and persistent keratitis as well as severe conjunctivitis and iritis. The treated animals initially showed improvement in both corneal and extracorneal disease, but the reappearance of virus was associated with deterioration of the clinical condition even in the face of continued therapy. At the end of the experiment, little clinical difference was noted between the two groups.

A similar experiment was performed in immune animals (Figure 2) to more closely approximate the disease in humans who usually have an acquired immunity. These animals (12 treated, 4 untreated eyes) were immunized by corneal abrasion and instillation of a low titer

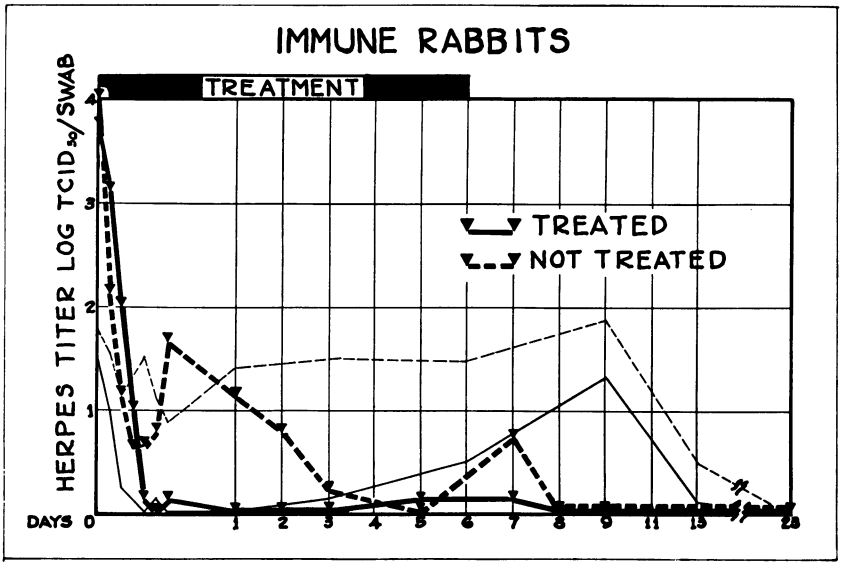

FIGURE 2. SERIAL VIRUS TITERS IN EYES OF IMMUNE RABBITS
(Data from Figure 1 of non-immune animals are represented by light lines.)

virus preparation so that no clinical disease resulted although the animals developed neutralizing antibody titers of 1:32 to 1:256. Two months later these rabbits were then re-inoculated, cultured, and treated according to the same protocol as the non-immune animals. In the untreated immune animals, the virus titer dropped rapidly but immediately rose again and then slowly dropped to undetectable levels between the first and fifth days. In the treated eyes, virus could not be detected after the first 12 hours, and remained so throughout the experiment. In general, the disease in immune animals was less pronounced than in the non-immune animals, particularly with regard to conjunctivitis and iritis. However, treatment of immune animals had little perceptible effect on the keratitis. Thus, it seems that the virological and clinical course of the disease is more dependent on the immune status of the animal than on the presence or absence of I.D.U. therapy.

The reported results on the treatment of human herpetic keratitis with I.D.U. alone have generally been favorable in dendritic keratitis. In our series of 36 patients (Table 1), treatment consisted of 0.1 per cent I.D.U. drops every hour during the day and every two hours during the night. I.D.U. was continued four days after apparent healing, but the interval between instillations was increased to every

TABLE 1. DENDRITIC KERATITIS TREATED WITH I.D.U.

Number of patients	*Result*
26	cured average days staining—7 (3–20)
4	initial cure, recurrence, later cured with I.D.U.
2	no improvement after 6 days rx: iodine-curettage with cure
4	developed stromal herpes

two to four hours during the day and one time at night. Twenty-six patients had an initial cure with no recurrence with an average staining time of seven days (varying between three to twenty days). Four showed an initial cure with recurrence of the disease at four days, two weeks, one month, and two months. These patients were subsequently re-treated with I.D.U. with favorable results. Two patients showed no improvement of their epithelial disease after six days of therapy and were successfully treated with iodine cautery. Four patients showed initial epithelial improvement, subsequent breakdown while on I.D.U., and the development of persistent stromal

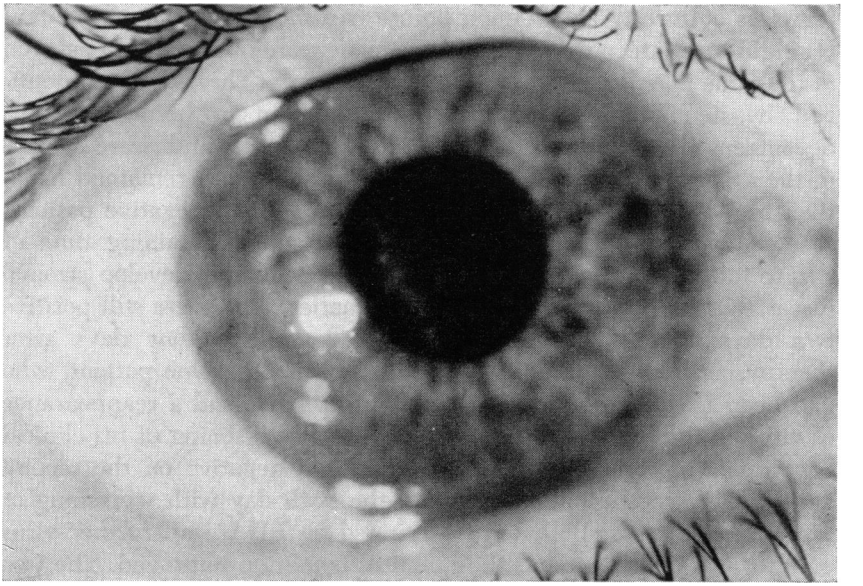

FIGURE 3. HEALED DENDRITIC ULCER SIX WEEKS AFTER I.D.U. THERAPY, SHOWING RESIDUAL STROMAL OPACITIES

disease. As noted by others, we have also observed the almost constant nebular haze of Bowman's membrane and superficial stroma beneath the previous dendritic ulcer following treatment with I.D.U. (Figure 3). These opacities gradually became less dense, but complete clearing seemed to be the exception rather than the rule in the follow-up of our cases for as long as one year. Opacities over the pupil reduced vision one to two lines in two patients, whereas in several others a subjective sensation of blurring was noted if the opacities were near the pupillary area.

TABLE 2. TREATMENT OF DENDRITIC KERATITIS WITH I.D.U.

		Days							
		0	2	4	6	8	10	14	18
Virus	pos.	17	6	4	3	0	0	0	0
	neg.	7	7	7	7	7	7	7	7
Epithelial disease	pos.	17	17	15	10	8	3	3	3
	neg.	7	5	2	0	0	1	1	1
Stromal disease	pos.	0	0	0	1	2	2	3	3
	neg.	0	0	0	0	0	1	1	1

Twenty-four of these patients with a clinical diagnosis of dendritic keratitis were followed with serial virus cultures (Table 2). Specimens for virus culture were obtained before treatment and every two days for eight to eighteen days. These specimens were obtained and assayed in the same manner as in the rabbit experiments. The virus was identified by neutralization with specific antiserum to herpes simplex. Seventeen patients were positive when first seen and were placed in the first group. Seven were originally negative and remained negative throughout the course of our observations. The negative patients seemed to have a milder disease with an average staining time of two to four days. However, one of these patients did develop stromal disease. Of the original 17 virus positive patients, six were still positive two days after therapy, three remained positive four days after therapy, and two were still positive after six days. One patient, who had been negative on the second day of therapy, had a reappearance of virus on the fourth day with a concomitant worsening of his clinical condition. One other patient, who had been negative on the second and fourth days, became positive on the sixth day with worsening of the clinical disease. Both were continued on I.D.U.; all further virus cultures were negative and their clinical condition improved. The two originally positive patients, who remained positive while on therapy for six days, were cauterized with iodine, became virus negative, and

improved clinically. In general, those patients who were virus positive showed a completely typical dendritic figure and had a staining period of six to ten days. The course of the seven patients with a history of previous disease showed no significant difference from the ten patients with an initial attack of herpes keratitis. Three of these initially virus positive patients developed stromal disease. In no cases have we been able to isolate virus in the follow-up examination (up to one year) of the entire group of 24 patients.

In our series of patients with stromal herpes (Table 3), two of

TABLE 3. STROMAL HERPES KERATITIS TREATED WITH I.D.U.

Number of patients	*Result*
2	improved
4	improved after addition of local steroids
2	no change
3	progression with vascularization
1	perforation

twelve patients improved on I.D.U. alone after 14 to 16 days of therapy. Four other patients improved after the addition of steroids, but three of these patients had a total of four recurrences while attempting to withdraw steroids. Two other patients showed essentially no change on I.D.U. alone for 25 days. In three patients we noted progression of the disease with vascularization of the cornea. One patient perforated after 16 days of I.D.U. therapy. In this patient, I.D.U. therapy was started 60 days after the onset of the disease at which time he had a rather deep central ulcer. To our knowledge, he had not been on steroids. Histologic examination of the corneal button showed no evidence of bacterial or mycotic infection.

For a better understanding of the action of I.D.U. on herpes simplex virus multiplication, we studied its influence on virus and host cells in a tissue culture system. Details of these experiments will be published elsewhere.[11] First of all, we found that even when 0.1 percent (3×10^{-3} *M*) I.D.U. and herpes simplex virus are incubated together for 18 hours, the I.D.U. has no effect on the infectivity of the virus preparation. This shows that there is no direct "antiviral" action of I.D.U., but rather that whatever inhibitory mechanism is present must be mediated through the cell. This could be expected from theoretical considerations since the virus of herpes simplex, like most other viruses, has no synthetic enzymes of its own and is, in its role as an ultimate parasite, totally dependent on the host cell for the nucleic

acid and protein synthesis necessary for virus replication. It is, therefore, unreasonable to expect that an antimetabolic agent should have a direct effect on a micro-organism which has no metabolism of its own.

The possibility exists, however, that cellular synthesis of viral nucleic acid and protein may follow other pathways than those used by the cell in manufacturing its own components. Such a selective pathway for viral nucleic acid synthesis has been suggested in the case of one of the RNA viruses,[12] so that this possibility must be considered. If this were the case, however, one would expect that it would be possible to suppress production of an infectious virus without interfering with cell multiplication. In Figure 4, we see the effects of I.D.U. in concentrations varying from 10^{-3} to 10^{-6} *M* on multiplication of mammalian cells in tissue culture and on production of infectious virus by those cells. One can suppress virus multiplication with a 10^{-4} *M* concentration of I.D.U., but only at the expense of a loss in cell multiplication as well. Concentrations of I.D.U. which permit cell multiplication at a normal or near normal rate also permit production of infectious virus. It is not correct, therefore, to speak of a specific antiviral action of I.D.U. We have only been able to find evidence of suppression of virus multiplication in the presence of simultaneous suppression of cell multiplication.

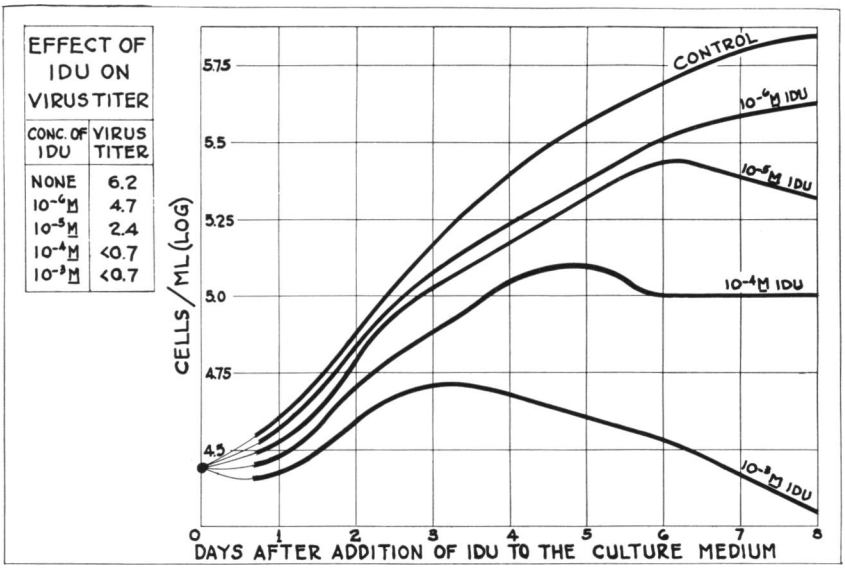

FIGURE 4. EFFECT OF I.D.U. ON CELL MULTIPLICATION AND PRODUCTION OF INFECTIOUS VIRUS IN TISSUE CULTURE

The summary of our results indicates that I.D.U.: (1) has no effect on herpes virus itself; (2) inhibits viruses and cells to the same extent; (3) has little beneficial effects in rabbits, whereas the immune status governs to a large extent the fate of the inoculated virus as well as the course of the disease; (4) and it has little or no effect on established stromal disease in humans.

Although many dendritic ulcers eventually healed on I.D.U. therapy, complications were not infrequent and residual nebular opacities usually resulted. For a final evaluation of I.D.U. therapy, cases must be compared to a control series and to other methods of treatment.

REFERENCES

1. Corrigan, M. J., M. B. Gilkes, and D. St. Clair Roberts, Treatment of dendritic corneal ulceration, Brit. M.J., 5300:304, 1962.
2. Corwin, M. E., M. Okumoto, P. Thygeson, and E. Jawetz, IDU in experimental herpes keratitis, Am. J. Ophth., 55:225, 1963.
3. Gordon, D. M., and D. A. Karnofsky, Chemotherapy of herpes keratitis, Am. J. Ophth., 55:229, 1963.
4. Havener, W. H., and J. Wachtel, IDU therapy of herpetic keratitis, Am. J. Ophth., 55:234, 1963.
5. Maxwell, E., IDU in corneal disease, Am. J. Ophth., 55:237, 1963.
6. Perkins, E. S., R. M. Wood, M. L. Sears, W. H. Prusoff, and A. D. Welch, Anti-viral activities of several iodinated pyrimidine deoxyribonucleosides, Nature, 194:985, 1962.
7. Furguiele, F. P., G. F. Pentecost, M. Klein, and J. S. McGavic, The effectiveness of 5-iodo-2-deoxyuridine in the treatment of herpes simplex keratitis, Tr. Am. Ophth. Soc., 60:243, 1962.
8. Kaufman, H. E., A. B. Nesburn, and E. D. Maloney, IDU therapy of herpes simplex, A.M.A. Arch. Ophth., 67:583, 1962.
9. Tamm, I., Symposium on the experimental pharmacology and clinical use of antimetabolites. III. Metabolic antagonists and selective virus inhibition, Clin. Pharmacol. & Therap. 1:777, 1960.
10. Kaufman, H. E., E. L. Martola, and C. Dohlman, Use of 5-iodo-2′-deoxyuridine (IDU) in treatment of herpes simplex keratitis, A.M.A. Arch. Ophth., 68:235, 1962.
11. Holmes, A. W., R. C. Ey, R. E. Dedmon, and F. W. Deinhardt, The influence of IDU on virus and host cell multiplication. In preparation.
12. Baltimore, D., and R. M. Franklin, Preliminary data on a virus-specific enzyme system responsible for the synthesis of viral RNA, Biochem. Biophys. Res. Comm., 9:388, 1962.

DISCUSSION

DR. TRYGVE GUNDERSEN. I feel particularly honoured to have been asked to discuss this fine paper of Dr. Ey and others.

Coming from the birthplace of I.D.U., I regret to say that my clinical experience with I.D.U. supports the experience of Dr. Ey and co-workers. The number of patients I have seen with acute epithelial herpes is not great. Perhaps not more than eight have been carefully followed by weekly

visits, slit-lamp examinations, photographs, and so forth. I have yet to see one dendritic ulcer which has shown a dramatic response to the use of the drug even though it has been used according to the standard set forth by Kaufman. I know that this is not in accord with the observations of some of my colleagues. The best result I have seen is a cure in twelve days, that is, a cure judged by absence of staining and congestion. In the other cases, there has been no variation from the normal course of the disease.

This brings up the question, what is the normal course of epithelial herpes?

In an article I wrote on herpes simplex corneae, in 1936, I described my experiences with two hundred and twenty-one patients who had this disease. Fifty-three had had no treatment and were used as a control series. Twenty-eight patients, 53 percent, were well in six weeks. Thirty-four patients, 64 per cent, were well in nine weeks. Each patient was considered well not only when there was no further staining, but when the eye was white and asymptomatic.

It is more important for me to point out the possible dangers of I.D.U. It is a potent anti-metabolite and especially when used in conjunction with a steroid can be a dangerous form of treatment. This is best exemplified by the following case report.

A plasterer, J.N., aged sixty-one, consulted Dr. Donald Kaplan of Groton, Connecticut, on December 6, 1961. He stated that on November 29, 1961, while at work he was struck in the left eye by some foreign material, presumably gypsum. He treated himself with Metimyd® ointment four or five times during the following three or four days, but since the eye remained sore, he consulted Dr. Kaplan who found that he had dendritic keratitis. There were three centrally placed dendritic figures: "Each figure was not of the usual small variety, but each figure was two or three millimeters in extent and the clubbing and branching were much larger than usual. There was apparent stromal extension." He was treated with Aureomycin solution until December 12, when the dendritic figures were no longer evident. By December 22, there was marked stromal involvement with bullous keratopathy. The epithelium was then completely abraded. In three days, the epithelium had regrown over the cornea, but there was no change in the stromal involvement, or the corneal edema. Marked hypesthesia was evident. I first examined the patient on January 2, 1962. The left eye was moderately congested but not painful. Vision, 2/200.

[Slide] The cornea showed marked bullous keratopathy with one to two millimeter bullas scattered over the lower central pupillary area. The cornea was greatly thickened and infiltrated especially in its lower two-thirds. The pupil was dilated, except between 3:00 and 6:00 o'clock where there were broad posterior synechias. There were numerous, approximately fifteen, K.P.'s. The aqueous was obviously turbid though poorly seen. The ocular pressure measured: right, 26 and left, 33, Schiotz.

The patient was sent to Massachusetts Eye and Ear Infirmary where

treatment was continued by Dr. H. Kaufman and myself. I.D.U. was prescribed every hour during the day, every two hours during the night. Four percent atropine solution was instilled every six hours. He was given two hundred and fifty milligrams of Diamox® every six hours. There seemed to be some clearing of the cornea and he went home after four days.

[Slide] The same treatment was continued at home, but he was given 0.2 percent prednisolone every four hours in addition to I.D.U. and Diamox. The patient made regular visits to the Corneal Clinic at the Massachusetts Eye and Ear Infirmary, but there was no significant change in his condition until he returned on April 2, 1962. For two days the eye had been increasingly painful and red.

[A series of slides were shown.]

Examination then showed the eye to be intensely red and stony-hard to palpation. Light perception was present. There was mixed hyphema and hypopyon in the anterior chamber forming a fluid level four millimeters above the lower limbus. Of special interest was the appearance of approximately 50 separate and occasionally conglomerate gray lesions scattered over the corneal surface from one-fourth to two millimeters in diameter. The lesions were most unusual and bore a faint similarity to Saltzman's dystrophy. A fungus infection was suspected. Dr. H. F. Allen was asked to see the patient and, after taking direct smears, found each lesion to be composed of pure colonies of staphylococcus aureus. The corneal surface had, in fact, become a culture plate and was studded with colonies of these organisms.

The patient was again admitted to the hospital and during the afternoon was given 200,000 units of penicillin under the conjunctiva near the lower limbus. I saw the patient at 8:00 P.M. when the cornea had obviously perforated. The course of the disease from this time onward was much as one would expect. The entire cornea became a slough and the eye was irretrievably lost. [Slide] A cuff evisceration was done on May 11, 1962.

In this instance, it appeared that I.D.U. had no effect on the stromal infection. It only inhibited cellular metabolism. Prednisolone blocked the inflammatory response. In other words, the cornea was completely susceptible to secondary infection with no resistance. I have never seen a more fulminating infection of a cornea. If both I.D.U. and steroids are to be used together, it seems imperative that the conjunctival sac be kept sterile by the continuous use of a broad spectrum antibiotic.

In this connection it might be well to mention the possible influence of interferon on herpes simplex. Since Isaac and Lindemann (1957) first named and described "interferon," the soluble factor produced by the interaction of inactivated influenza virus with chick chorioallantoric tissue, much has been written about this antiviral factor. It is a by-product of cell-virus interaction. It increases resistance of cells by inhibiting intercellular virus replication. How important interferon is in our natural body defense against a virus such as herpes simplex remains to be proved, but it may be most important. In any event, it is apparent that steroids suppress the

formation of interferon. Perhaps this is the reason for the adverse effect of steroids on herpes corneae.

DR. A. GERARD DEVOE. I would like to very briefly report our experience at the Columbia Presbyterian Medical Center with I.D.U. At the present time, we have treated about 150 cases. About 75 of these have been acute epithelial disease, the rest stromal and non-herpetic disease. In acute epithelial disease, we have had a cure rate of about 75 percent with an average time interval of about 8 to 10 days. In stromal disease, our cure rate has been about 7 percent. In non-herpetic disease, our cure rate is again about 7 percent. In other words, the cure rate of stromal and non-herpetic disease is the same.

That brings up again the point which Dr. Gundersen raised. The prime issue here, one we do not thoroughly understand, is the normal life history of epithelial herpes. I think this has varied throughout the years. Initially, as I recall, twenty-odd years ago, we were told the normal cure rate was somewhere around 10 percent. Subsequent observers have been steadily raising that figure, and 25 or 30, even 40, percent has now been stated by some people as the normal cure rate of epithelial disease. I think we will not know until we have a large, thoroughly studied, controlled series of clinically treated cases.

DR. MICHAEL J. HOGAN. I would like to make just one statement which Dr. Thygeson has made, and I am sure he would get up and say it if he was here. The normal cure rate of corneal herpes is 100 percent.

DR. BENNETT Y. ALVIS. I wish to report one case very briefly and ask a question concerning another that we have had recently. The first case was treated for about two weeks before beginning I.D.U. This gentleman was treated first by his ophthalmologist in New York, for some ten days. He was then transferred to a well-trained ophthalmologist near his plantation in Georgia, where he was treated for another week. Mr. Smith, of Smith, Kline and French, suggested I.D.U. When he came to us, the I.D.U. came by air express at the same time. We started using I.D.U. every hour in the day and every two hours at night. In three days, his eyes were quiet and the ulcer was healed. When he first had arrived, he had had a typical dendritic form of ulcer.

The second case was treated for about three or four weeks. He had a typical dendritic pattern, and after the advent of I.D.U., the ulcer gradually healed, although the pattern remained, and we added steroids to our treatment. This resulted in a quiet eye with a vision of 20/20. Although the eye was quiet, without redness, there was considerable photophobia. The subepithelial dendritic pattern remained. We decided to denude the cornea with iodine, and following the denuding, within twenty-four hours, his vision had dropped to practically 20/200. The cornea was hazy with folds in Descemet's. Treatment with I.D.U. was resumed and in a few days, the

ulcer was healed, but the corneal haze remained, which gradually cleared. About one week later, his vision had returned to 20/20 minus.

DR. HUGHES, JR. We thank Dr. Gundersen and the other discussers for their important contributions about the problem of herpes simplex infection. Because of time limitation, I will only summarize briefly our present impressions of the clinical value of I.D.U. therapy considered also in the light of their work and other work published in the literature.

In view of the dramatic effect of I.D.U. in some cases of an early and primary attack of dendritic ulcer, strictly limited to the epithelium, it may be justifiable to treat such cases for two days. If no dramatic improvement occurs after that time, especially in staining, we believe a relatively large area should be cauterized for one minute with 2 percent iodine, then 4 percent cocaine, followed by clean mechanical removal of all cauterized epithelium with a number 15 blade Bard-Parker knife. Early elimination of the infected cells and virus antigen may reduce the amount of residual nebular haze in the anterior corneal stroma which is so regularly present after the slower response to I.D.U. therapy.

The crucial question, as to whether I.D.U. represents a specific antiviral agent, depends on its ability to prevent the incorporation of the cell's supply of thymidine into virus D.N.A. without destroying cell multiplication itself. Otherwise I.D.U. would represent only another mild cauterizing agent. The answer to this question must come from tissue culture studies, which, at Presbyterian-St. Luke's Hospital, Chicago, have been carried on by Dr. Deinhardt, Head of the Department of Micro-biology, his associate, Dr. Holmes, and Dr. Ey. I would like one of them to comment on this problem of specific antiviral action of I.D.U.

DR. HOLMES. First of all, I should like to thank the Society for the invitation to attend the meeting, and the opportunity to say a few words. I think there are two points which have been elucidated in the laboratory, which are worthy of consideration by the clinicians who use this agent to treat patients who have herpes keratitis.

First of all, if I may explain briefly, following the penetration of virus into a cell (which is the initial process of infection), the virus disappears and one cannot find it either with fluorescent antibodies or by attempts to isolate it. This is called the eclipse phase. The eclipse phase for herpes simplex starts within the first two hours after the virus has been put in contact with the cells and lasts for perhaps six to eight hours.

It has been shown in a number of laboratories that, if I.D.U. is added to a culture during this eclipse period, the process of virus multiplication stops. If one then neutralizes the I.D.U. by the addition of thymidine—and one can do this very easily—virus multiplication picks up again and continues without any detriment.

We have demonstrated that one can hold this eclipse phase in abeyance for a period of six days with I.D.U. At the end of these six days, we then

add the thymidine, and the virus again starts to multiply. In other words, you cannot eliminate the virus with I.D.U. You may hold the process of multiplication in abeyance, but you cannot get rid of it altogether.

Secondly, it has been shown recently by Green using adenovirus which is also a D.N.A. virus, that although you can suppress the production of viral nucleic acid—and incidentally, cells—that certain other viral products are produced without any diminution in rate. That is, the virus itself consists of a nucleic acid core and a protein coat on the outside. You can stop the production of the nucleic acid core, but protein coat material is produced anyhow. I think this is worth keeping in mind because this protein coat material is antigenic. Dr. Hogan in his paper and Dr. Braley in his discussion pointed out the thought that the stromal disease may be an antigen antibody response. Therefore, if we are permitting antigen to be produced, it may be we can have some effect on the superficial disease, but we may not really interfere with the stromal disease which is after all, the main form of the disease which bothers us most.