of reducing agents, and 0.001 M diisopropylphosphofluoridate reduced proteolysis partially. The enzymes from both strains were inactivated fairly quickly at 70°C.

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Anti-herpetic Activity of 5-Iodo-2'-deoxyuridine in Presence of Its Degradation Products. (28825)

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Kaufman and Maloney(1) noted that, after storage, 5-iodo-2'-deoxyuridine (IUDR) solutions appeared to lose antiviral activity in tissue culture. More recently these investigators(2) reported that an 'aged' solution of IUDR not only lost its activity against herpes simplex virus in tissue culture, but also interfered with the anti-herpetic activity of freshly prepared IUDR. They attributed this biological instability to the presence of the degradation products of IUDR, namely, 5-iodouracil (IU) and possibly deoxyuridine (DU). They concluded that the presence of minute amounts of IU inhibited the ability of IUDR to eliminate virus from herpes simplex-infected rabbit kidney tissue culture. The antagonism of IUDR by its degradation products, which was apparently observed in tissue culture, was not found *in vivo* in experimental keratitis in the rabbit (2). Thymidine has been previously reported (3) to antagonize the anti-herpetic activity of IUDR in tissue culture. This observation also has been confirmed in our laboratory. The purpose of this investigation is 2-fold: (a) to study further the effect of the degradation products, IU and DU, on anti-herpetic activity of IUDR and (b) to show the effect of prolonged storage of solutions of IUDR on anti-herpetic activity as determined in tissue culture.

Material and methods. Plaque inhibition tests using chick embryo cell cultures were carried out as described previously(4) using the HF strain of herpes simplex virus. Filter paper discs, impregnated with 0.1 ml of solutions of either 'fresh' IUDR, 'aged' IUDR, IU, DU, thymidine, and IUDR-IU combinations, were air-dried prior to use. The zone of inhibition of virus growth, evidenced by the absence of plaques surrounding each active disc, was measured in millimeters.

The chemotherapeutic activity of IUDR preparations also was determined in tube cultures of rabbit kidney. Tubes containing approximately 10^5 cells were infected with 100 $TCID_{50}$ of the Virtue strain of herpes simplex virus and treated simultaneously with varying concentrations of test material. Treatment was administered again 4 days after infection. Microscopic examination of the tubes and scoring of cytopathogenic effect (CPE) were done on the 4th and 6th days after infection; the experiment was terminated on the 6th day. The cytopathogenic changes were scored from 0 to 4+, where 0 represents a complete absence of viral CPE and 4+ represents total destruction of the cells. The minimal effective dose represents that concentration of test material that protects at least 50% of the cell layer from viral destruction.

Experimental results. Freshly prepared IUDR at a concentration of 50 μg/disc consistently produced considerable anti-herpetic activity, with zones of inhibition averaging

TABLE I. Anti-Herpetic Activity of IUDR Alone and in Combination with IU.

IUDR, μg/disc	IU, μg/disc	Zones of inhibition, diameter in mm	Avg
—	10	0 (inactive)	0
50	—	70, 75, 70, 68, 60, 62, 70, 70, 75, 72, 70, 68, 60	68
50	10	71, 70, 73	71
50	1	68, 70, 72, 72, 69	70
50	.1	71, 70, 74, 74, 72	72

TABLE II. Anti-Herpetic Activity of 'Fresh' *vs* 'Aged' Solutions of 'Stoxil' in Chick Embryo Fibroblasts.

'Stoxil' dilution	Storage	Chemical analysis of solution mg % IUDR	mg % IU	Zone of inhibition, diameter in mm
1:4	6 mo @ R.T.	94	.4	48, 46, 44
1:4	6 mo @ 37°C	82	12	46, 44
1:4	'fresh'	102		46

68 mm in diameter, whereas thymidine (10 to 1,000 μg/disc), DU (1 to 10 μg/disc), or IU (10 to 100 μg/disc), produced no apparent zones of inhibition or cellular toxicity. However, when a disc with 1000 μg of thymidine was placed in close proximity to one with 50 μg of IUDR (approximately 30 mm apart), the formation of plaques was not inhibited. The reversal of plaque inhibition was not observed when IUDR discs were similarly placed in close proximity to discs containing either 10 μg of IU or 10 μg of DU. Further study showed that antagonism also was not observed when concentration of IU was increased to 100 μg/disc, whereas thymidine at a concentration as low as 10 μg/disc effectively produced this antagonistic effect.

As seen in Table I, when known concentrations of IU ranging from 1 to 100 μg/ml were added to 500 μg of IUDR/ml and 0.1 ml of this preparation was added to each disc, all combinations of IUDR with IU produced zones of inhibition averaging close to 68 mm, corresponding to those produced by IUDR alone.

The effect of prolonged storage on the efficacy of 'Stoxil,' a 0.1% aqueous solution of IUDR containing 0.002% thimerosal prepared by SK&F Laboratories, was studied. Two aliquots were drawn from a 'Stoxil' preparation; one was stored at room temperature for 6 months and a second was stored at 37°C for 6 months. These were diluted 1:4 in distilled water for the preparation of discs. The anti-herpetic activity of these samples, whether stored at room temperature or 37°C, was comparable to that of a freshly prepared lot of 'Stoxil,' as measured by the zones of inhibition surrounding each disc (Table II).

Solutions of IUDR containing known amounts of degradation products also were tested against herpes simplex virus in rabbit kidney tissue culture tubes; the experimental results are given in Table III. Freshly prepared solutions containing 70 or 90 mg of IUDR per 100 ml and free of degradation products were comparable in activity. The

TABLE III. Solutions of IUDR Containing Known Amounts of Degradation Products *vs* Herpes Simplex in Rabbit Kidney Tissue Culture.

IUDR conc, mg/100 ml	Degradation products conc, mg/100 ml IU	DU	Potassium iodide	Minimum effective dose,* μg/ml	Remarks
90	—	—	—	1	
90	—	6.4	—	1	
90	—	—	4.7	1	
98.7	.8	1	—	1	'fresh' 'Stoxil'
70	—	—	—	1	
70	17.0	—	—	4	
72	17.3	2	—	1	'Stoxil' incubated at 37°C for 1 yr
50	48.0	N.D.	—	2	autoclaved 'Stoxil'
—	—	—	—	None†	Thimerosal control

N.D. = Not determined.

* Two-fold (± one tube dilution) variation in experimental results considered insignificant.

† Placebo diluted 1:62; this dilution represents that concentration of thimerosal contained in a 0.1% 'Stoxil' preparation diluted to 16 μg.

addition of IU, DU, or potassium iodide did not change appreciably the minimal effective dose within the confines of test variation. Three 'Stoxil' preparations were analyzed chemically and tested in this system. A preparation stored one year at 37°C, in which 17.3% of the IUDR had been converted to IU, was comparable in activity to a fresh preparation in which 0.8% of the IUDR had been converted to IU. It is interesting to note that an autoclaved sample, 50% degraded and containing 48% of the IUDR as IU, demonstrated significant anti-herpetic activity in this test system.

Discussion. The major degradation product of IUDR is IU, while DU is formed in lesser amounts(2). The question of whether or not the presence of IU causes a biological lability of solutions of IUDR has been raised. Maloney and Kaufman have reported that 0.1 μg of IU per ml (0.001%) antagonizes the anti-herpetic activity of 1000 μg of IUDR per ml in rabbit kidney tissue culture(2), a ratio of 1:10,000. They also noted this antagonism when a solution of IUDR, 100 μg/ml, which had been stored at room temperature for 2 months, was added to a freshly prepared solution of IUDR, 10 μg/ml.

Experiments were designed, using either chick embryo fibroblasts or rabbit kidney cells, to elucidate this question concerning the effects of degradation products of IUDR. No antagonism was noted in the chick embryo fibroblast system when discs of DU or IU were placed in close proximity to IUDR, even when the degradation product was present at a ratio of 1:5 to the parent compound, a concentration far in excess of that previously reported to have caused interference (2). The location of the discs was predetermined from previous experiments, so that the disc containing IU or DU was placed at the outermost limits of the diffusion gradient of IUDR, thus assuring maximum concentration of the degradation product in contact with minimal inhibitory amounts of the parent compound.

IUDR solutions containing from 12 to 20% of the IUDR in the form of IU produced zones of inhibition comparable to those produced by IUDR alone in the chick embryo test system. It is interesting to note that 'Stoxil' stored for 6 months at 37°C contained IU at a level of only 12% that of the initial concentration of IUDR; this concentration of IU did not cause antagonism.

Data obtained in the chick embryo fibroblast test system were confirmed by more extensive studies done in rabbit kidney tissue culture. A 'Stoxil' preparation stored for one year at 37°C, that contained 17.3% of the IUDR as IU, and less than 2% as DU, by chemical analysis, was comparable in activity to that of a fresh preparation of IUDR containing no degradation products; this indicated that the efficacy of the 'Stoxil' preparation was not affected by prolonged storage.

In addition to the 2 tissue culture systems described, the anti-herpetic activity of the 'aged' 'Stoxil' preparation was tested against experimental herpetic keratitis in the rabbit eye. Data obtained using this *in vivo* system indicate no interference with the efficacy of these 'aged' preparations, thus confirming the tissue culture findings.

Summary. Antagonism of the antiviral activity of 5-iodo-2'deoxyuridine (IUDR) by its degradation products, 5-iodouracil and deoxyuridine, was not demonstrable either in chick embryo fibroblasts or in rabbit kidney cells grown in culture and infected with a strain of herpes simplex virus. The degradation products were tested in concentrations far in excess of those previously reported to be antagonistic by Maloney and Kaufman. Prolonged storage of 'Stoxil,' a 0.1% solution of IUDR containing 0.002% thimerosal, did not affect the antiviral activity of these preparations.

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