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Therapeutic Anti-Viral Activity in Tissue Culture.* (27932)

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5-Iodo and 5-bromo-2′-deoxyuridine have been found to cure corneal infection caused by herpes simplex and vaccinia virus and eradicate culturable virus even when these infections were clinically far advanced. In addition to being therapeutically effective in experimental herpes keratitis in rabbits, these agents appear beneficial in herpetic keratitis in man(1,2).

Although the unique ability to eradicate virus infection of the cornea has made it possible to find several agents active against both herpes simplex and vaccinia, many limitations are inherent in restricting the study of virus chemotherapy to the eye. The need for an *in vitro* correlate of *in vivo* anti-viral activity is apparent. This communication will describe therapeutic arrest of cytopathogenic changes in tissue culture and complete elimination of virus from the culture following herpes simplex and vaccinia infection even after cytopathogenic changes in the culture are obvious.

Methods. Primary rabbit kidney cell cultures were maintained in Hanks' balanced salt solution with 0.5% lactalbumin hydrolysate and 2% calf serum, and primary human amnion cell cultures were maintained in Eagle's minimum essential medium with 10% calf serum. Herpes simplex virus (10^8 infective doses) previously isolated from a patient with dendritic keratitis and vaccinia virus (10^8 infective doses) were used for infection. 5-Iodo-2′-deoxyuridine (IDU) was dissolved in media and the *drug containing media was changed daily.* Control tubes were handled in an identical manner with medium not containing IDU. Membranes of the roller tubes were prepared at intervals by the collodion membrane technic(3) and were stained with hematoxylin and eosin to detect giant cells and cells with virus inclusions. Intracellular virus titers were determined on cells disrupted by ultrasound to yield the highest virus titer and titers so obtained were higher than those found by multiple freezing and thawing(4).

Results. As early as 12 hours after infection cells exhibiting specific cytopathogenic changes could be seen, but these, which consisted of giant cells and inclusion containing cells, were rare. By 24 hours micro-plaques were common and single giant cells as well as cells with both acidophilic and basophilic inclusion bodies were abundant. Shortly thereafter, in untreated cultures degeneration became so extensive that evaluation of the accelerating process of degeneration was difficult, and by 48 hours after infection most cell sheets were completely destroyed. Aliquots of media at this time when diluted 10^5 were all shown to be infectious *in vitro* (Fig. 1).

When IDU was added 24 hours after infection, although many giant cells and inclusion containing cells were visible, by 48 hours (24 hours of treatment) their number had diminished. Following treatment the number of visible giant and refractile cells

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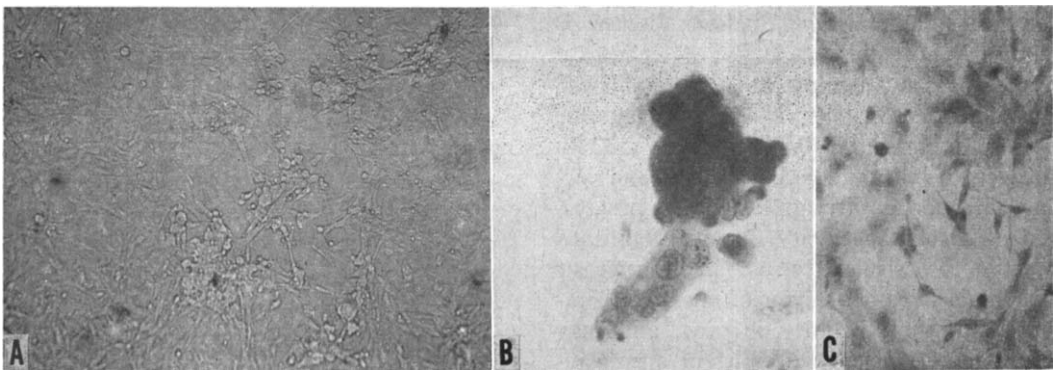

FIG. 1

(A) Rabbit kidney culture 24 hr after herpes simplex infection showing micro-plaque and rounded cells at margin. H & E—60×.

(B) Typical giant cell and viral inclusions in a culture infected for 24 hr. H & E—400×.

(C) Culture treated beginning 24 hr after infection—treatment for 48 hr. The defect in the cell sheet remains but no inclusion containing cells are seen and no detectable virus is present in the media. H & E—160×.

decreased; this was at least in part a function of handling of the tube as the cells with advanced disease appeared to fall off the glass. Some defects initially present in the infected sheet persisted, although the cells appeared healthy and pH of the medium continued to decrease in the manner of uninoculated control preparations. In cultures kept 14 days no progression of cytopathogenic effect was detected. Arrest of the infectious process was observed with concentrations of IDU from 10 μg/ml to 1 mg/ml. When smaller virus inocula were used IDU could be added 48 hours after infection and exert this same effect.

Titrations of virus in supernatant fluids and cells of both rabbit kidney and human amnion cultures infected with herpes simplex virus revealed that after treatment the quantity of infectious virus promptly decreased and virus soon was no longer detectable. A similar effect was observed with the treatment of vaccinia infection, the infectious virus disappearing from the supernatant fluid and cells following IDU therapy even though cytopathic changes were obvious when the drug was added and initial intracellular virus titers were high (Table I). Infected cultures treated with IDU (1 mg/cc) for less than 2 days relapsed when drug was removed, but after 3 days no relapses occurred. By the 4th or 5th day fluorescent antibody staining virus antigen also was eliminated from the treated cultures. In the case of herpes virus only a few agents prevented the progression of CPE (Table II) and these agents were the ones found to be therapeutically active *in vivo*.

In early experiments it was noted that IDU solutions which were not prepared shortly before use appeared to have less anti-viral activity. Since this might have been due to the major breakdown product of IDU, 5-iodouracil, the effect of iodouracil on the anti-viral properties of IDU was tested. Iodouracil prevented the IDU from eliminating virus from the cultures, even when the iodouracil was present in extremely small concentrations (Table III). When IDU was tested in a concentration of 1 mg/ml, iodouracil in concentrations of 0.1 μg/ml permitted visible progression of cytopathogenic change and persistence of virus within cells. Failure to change the drug containing media daily also permitted progression of cytopathogenic changes, presumably due to the IDU degradation products, although not changing media or the addition of iodouracil still allowed IDU to *prevent* cytopathogenic change.

Discussion. The demonstration that virus disease in tissue culture can be "cured" not only confirms previous clinical studies, but also permits far more detailed investigation of the concentrations and biochemical activity of anti-viral agents.

Substances such as specific antibody, interferon, various antimetabolites and even IDU have been shown to prevent or modify infec-

tion when administered before disease is manifest, but this capacity of chemical agents to arrest the progress of established virus infection in culture and eradicate the virus is, to our knowledge, unique(6,7). Only the agents which cure herpes simplex and vaccinia infection *in vivo* appear to halt progression of cytopathogenicity due to established infection by virulent virus. This suggests that the *in vitro* is a mirror of selective therapeutic anti-viral activity *in vivo,* and may permit more efficient screening of agents effective against other viruses as well as a more detailed study of the mechanism of anti-viral action of the agents now known.

Summary. Herpes simplex virus can be eliminated from rabbit kidney tissue culture and human amnion tissue culture and vaccinia virus can be eliminated from rabbit kidney tissue culture by treatment with 5-iodo-2′-deoxyuridine in concentrations from 10 μg/ml to 1 mg/ml if the drug containing media was changed daily. This "cure" of virus infection is possible even when cytopathogenic changes are easily recognizable before treatment is begun, and such treatment results in prevention of progression of such virus induced changes. This property appears to be unique for therapeutically active anti-viral

TABLE I. Treatment of Tissue Cultures with IDU Begun 24 Hours After Infection When Cytopathogenic Changes Were Established.

Days of IDU (1 mg/ml)	Titer/ml (log 10) Cells	Supernate
A. Herpes in rabbit kidney		
0	8	6
1	0	0
2	0	0
3	0	0
4	0	0
5		0
Days of IDU (0.01 mg/ml)	Cells	Supernate
0	8	6
1	4	4
2	5	4
3	1	0
4	0	
5	0	
Control		
0	8	6
1	8	7
2	Cell destroyed	
B. Herpes in primary human amnion		
	Titer/ml (log 10)	
Days of IDU (1 mg/ml)	Supernate	Cells
0	6	8
1	0	2
2	0	1
3	0	0
4	0	0
5		0
C. Vaccinia in rabbit kidney		
	Titer/ml (log 10)	
Days of IDU (1 mg/ml)	Supernate	Cells
0		10
1	1	2
2	0	0
3	0	0
4		0
5		0

TABLE II. Effect of Antimetabolites on Herpes Simplex Infection in Rabbit Kidney Cell Cultures.†

	Conc. mg/ml*	Halts cytopathogenic change
5-fluoro-2′-deoxyuridine	1	—
5-chloro-2′-deoxyuridine	1	+
5-bromo-2′-deoxyuridine	1	+
5-iodo-2′-deoxyuridine	1	+
5-fluorouracil	.001	—
5-iodouracil	.0001	—
5-chlorouracil	.1	—
5-bromouracil	1	—
6-azauridine	1	—
6-azaguanine	.0001	—
Thioguanine	.00001	—
Thioguanosine	1	—
Deoxyuridine	.0001	—
6-choropurine riboside	.001	—
6-mercaptopurine riboside	.1	—
6-mercaptopurine hydrate	.0001	—
5-methylcytosine hemihydrate	1	—
5-methylcytosine hydrochloride	1	—
Aminopterin	1	—
Purin-6-yl-trimethyl ammonium chloride	.1	—

*Concentrations of 1 mg/ml were tested unless this was cytotoxic, in which case the highest, nontoxic concentration was used.

† Since preparation of this paper Underwood (personal communication) has found cytosine arabinoside effective in treating herpes simplex keratitis. In concentrations of 0.1 mg/ml it also halts cytopathogenic change and virus is eliminated from treated cultures.

TABLE III. Herpes in Rabbit Kidney.

IDU (1 mg/ml) & IU (.0001 mg/ml) Days	Titer/ml (log 10) Cells	Supernate
0	8	6
1	4	2
2	2	0
3	4	0
4	7	
5	Cell sheet destroyed	

compounds and may facilitate the screening of agents active against other viruses and the more detailed study of the therapeutically active anti-viral agents now known.

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Further Evidence for a Role of the Renin-Angiotensin System in Regulation of Aldosterone Secretion.* (27933)

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Previous studies(1-7) have shown that saline extracts of dog kidneys, relatively crude preparations of renin, and synthetic valine-5-angiotensin II increase aldosterone secretion in nephrectomized hypophysectomized dogs. Another pressor substance, norepinephrine, had no effect upon aldosterone secretion. Further evidence for the specificity of the renin-angiotensin system in stimulating aldosterone secretion has now been obtained by measuring the effect of purified hog renin preparations, human renin, erythropoietin and a non-pressor synthetic analogue of angiotensin II on aldosterone secretion. The effect of a carboline (1-methyl-6-methoxy 1, 2, 3, 4, tetrahydro-2-carboline), presumably manufactured in the pineal gland(8), has also been measured in nephrectomized hypophysectomized dogs. This carboline has been claimed by Farrell and McIsaac(8) to be a potent stimulator of aldosterone secretion.

Methods. Mongrel dogs were hypophysectomized and/or nephrectomized, the right lumbar adrenal vein cannulated and aldosterone and 17-hydroxycorticoids measured in adrenal venous effluent by technics described previously(3). Hog renin preparations of 2 different purities were injected over one minute. One preparation contained 24 units per mg of protein and the other 3 units per mg. Adrenal venous blood was collected immediately before and from 4 to 14 minutes after start of the injection. Two human kidneys were extracted separately for renin by the method previously described(3) and each was injected as a single injection. Two erythropoietin preparations were injected in the same way as the renin preparation. One hundred and fifteen comparative standard units (9) erythropoietin prepared from human urine (activity 2.3 CS units per mg) and 7.2 comparative standard units erythropoietin prepared from plasma of phenylhydrazine treated sheep (Armour A2-0336) were injected. Angiotensin II diamide (aspartyl-1, valine-5, phenylalanyl-8 diamide) in small volumes of saline was infused for 14 minutes as described previously for angiotensin II(3). Adrenal venous blood was collected immediately before start of the infusion and from 4 to 14 minutes during the infusion. 1 Methyl-6-methoxy 1, 2, 3, 4, tetrahydro-2-carboline was infused for 2 hours in 10% ethanol in saline or ascorbic acid in saline *via* a constant infusion pump. A concentrated solution was made up fresh from crystalline material in the dark at 4°C and at half-hour intervals dilutions were made for infusion. All syringes were covered with aluminum foil to prevent possible destruction by light. From 4 to 10 μg per hour was infused over 2 hours in a total infusate volume of 200-225 ml. Following 2 control adrenal venous samples, 3 to 4 samples of adrenal venous blood were

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