

benzoate, 2,4-, 2,5- and other dihydroxybenzoates = 4-aminosalicylate (PAS) = 5-sulphosalicylate (all inactive) and in the parallel series: ortho-vanillin, salicylaldehyde, 2,4- and 2,5-dihydroxybenzaldehydes, 2,4,6-trihydroxybenzaldehyde, 2,4- and 2,5-dihydroxybenzaldehydes, 2,4,6-trihydroxybenzaldehyde, pyridoxal, pyridoxal-5-phosphate. Potency increased in the series: salicylamide, salicylate, salicyldoxime, salicylanilide, 3,5-dihydroxy-naphth-2-oate, salicylaldehyde, *o*-monohydroxynaphthoates, *o*-monohydroxynaphthaldehydes — the latter were strong inhibitors at 2×10^{-5} M and approximately 100 times as potent as salicylate. Saligenin, pyridoxine, *o*-methoxybenzoate, *o*-hydroxyacetophenone, methyl salicylate, *m*- and *p*-hydroxybenzaldehydes, 2,4-dimethoxybenzaldehyde, *m*- and *p*-hydroxybenzoates and other hydroxynaphthoates were almost devoid of *in vitro* activity and formed only weak, or no, complexes with ferric ions in ethanol.

Phenylbutazone (butazolidine) exists at physiological pH predominantly as the enol form (about 90 per cent, calculated from the optical density at 264 mμ) and will form complexes with metal ions in ethanol. The methyl enol-ether will not and is inactive as a drug *in vitro* and *in vivo*³. Among phenylbutazone (PB) analogues, potency decreased with increasing water solubility in the series: PB, 4-*n*-propyl analogue, oxy-PB, mono-*p*-carboxy(phenyl)-PB, di-*p*-carboxy(phenyl)-PB. This last compound is inactive *in vitro*, only feebly active *in vivo*⁴. Some pyrazol-5-ones exhibited activity *in vitro*, which diminished in the series: 1,3-diphenyl-pyrazol-5-one, pterolonie acid, 1-phenyl-3-methyl-pyrazol-5-one, 1-phenyl-2,3-dimethyl-pyrazol-5-one (antipyrine, and inactive). These pyrazolones formed highly coloured complexes with ferric chloride in ethanol. With the possible exception of antipyrine, they can exist as enols in aqueous solution.

Among analogues of cinchophene (2-phenyl-quinoline-4-carboxylic acid), activity decreased in the series: cinchophene, 2-hydroxyquinoline-4-carboxylic acid, isonicotinic acid (inactive). Hydroxychloroquine (plaquenil) was less active than chloroquine (resochin), in turn less potent than mepacrine (quinacrine, atebirin) and santoquin (3-methylchloroquine). These antimalarials only inhibited polysaccharide biosynthesis after a measurable time lag (1–2 hr.), reminiscent of their delayed therapeutic action *in vivo*⁵. Their drug action was not reversible. The action of all other drugs tested was reversed by washing the drug-incubated tissues and transferring them to a drug-free medium.

8-Hydroxyquinoline (oxine) was a potent inhibitor *in vitro* and has been shown to exhibit anti-inflammatory properties *in vivo*⁶. 2-Hydroxyquinoline was inactive *in vitro*. Oxine, salicylic acid, salicylaldehyde⁷ and a number of anti-inflammatory corticosteroids⁸ will chelate cuprous and ferrous ions in non-aqueous media. These collected observations and the inhibitory activity of lipophilic thiols able to form chelates (but not cysteine and glutathione) suggest that ability to interact with metal ions in (or across) a lipid phase may be the chief determinant in conferring anti-inflammatory properties on a compound, rather than ability to uncouple oxidative phosphorylation *per se*⁹. Extension of this hypothesis to the corticosteroids may explain the greater anti-inflammatory activity of methyl derivatives (dexamethasone, medrol, paramethasone)^{10,11} and enol ethers¹² than the parent steroids, and the striking increase in anti-inflammatory activity of steroid 16α-, 17α-diols

upon transformation to the corresponding acet-onides^{11,13}.

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OPHTHALMOLOGY

Anti-viral Activities of Several Iodinated Pyrimidine Deoxyribonucleosides

KAUFMAN¹ has reported that 5-iodo-2'-deoxyuridine (IUDR or IDU), the deoxyribonucleoside of 5-iodouracil, is effective in controlling herpes simplex keratitis in rabbit and human eyes. IUDR², 5-iodo-2'-deoxycytidine (ICDR), that is, the deoxyribonucleoside of 5-iodocytosine³, as well as their 3',5'-diacetyl derivatives, were synthesized with the view of examination of them as potential chemotherapeutic agents in cancer. Both IUDR and ICDR have been shown to inhibit the biosynthesis of deoxyribonucleic acid (DNA) and, in addition, to be incorporated as IUDR 5'-phosphate into DNA, in place of thymidine 5'-phosphate⁴⁻⁶. This communication confirms the efficacy of IUDR in the treatment of an infection of rabbit eyes with a DNA-virus, namely, herpes simplex, and describes a similar anti-viral activity for ICDR and 3',5'-diacetyl-5-iodo-2'-deoxyuridine (Ac₂IUDR); in addition, a preliminary test of 3',5'-diacetyl-5-iodo-2'-deoxycytidine (Ac₂ICDR) has indicated that this derivative is also active.

Both eyes of 14 and 17 rabbits in the first and second experiment, respectively (Table 1), were infected with the MP strain of herpes simplex after scratching the cornea; in all cases, marked lesions developed within 3 days. In the first experiment, the left eyes were treated with the test compounds dissolved in Hanks's balanced salt solution supplemented with penicillin (500 u./ml.) and streptomycin (100 μgm./ml.), while the right eyes were treated with only the antibiotic agents in the saline solution. Two drops of the solution were placed into each conjunctival sac at hourly intervals for 10 hr. and at intervals of 2 hr. for 14 hr.; this regimen of dosage was repeated during a second 24-hr. period. The animals were divided into 2 groups (Table 1).

After treatment for 48 hr., all the right eyes, to which antibiotic agents only were administered, showed severe corneal lesions, while the left eyes showed marked regression of the lesions from the pre-

Table 1. COMPARISON OF VARIOUS IODINATED PYRIMIDINE NUCLEOSIDES IN THE TREATMENT OF HERPES SIMPLEX KERATITIS

Exp. No.	Compound administered	No. of eyes treated	Concentration of compound		Clinical effectiveness
			mgm./ml.	Relative molar concentrations	
1	IUDR	7	1.24	1.0	++++
	Ac ₂ IUDR	7	1.7	1.1	++++
	None	14	—	—	0
2	IUDR	6	1.0	1.0	++++
	IUDR	6	4.0	4.0	++++
	ICDR	6	1.0	1.0	++++
	ICDR	6	4.0	4.0	++++
	IUR	5	4.0	4.0	0
	None	5	—	—	0

treatment condition. The right eyes had large areas of epithelial ulceration with circumcorneal congestion and in many cases an anterior uveal reaction. Ulceration was minimal or absent in the left eyes and none of these eyes showed anterior uveal reaction. In the concentrations used, IUDR and its diacetyl derivative were both effective in controlling infection.

In the second experiment, a comparison was made of IUDR and ICDR, each at two equivalent concentrations (Table 1). In addition, it was shown that 5-iodo-uridine (IUR), the ribonucleoside of 5-iodouracil, a compound which undergoes metabolic degradation in a manner similar to that of IUDR, but exerts no inhibitory effect on the biosynthesis of DNA, is without anti-viral activity. The compounds were dissolved in the manner described above and 2 drops of the appropriate solution were placed into each conjunctival sac at intervals of 1 min. for 5 min. and thereafter at intervals of 0.5 hr., but for only 8 hr.; this regimen was repeated during the subsequent 2 days. All the control eyes, as well as those treated with IUR, developed severe corneal lesions, whereas in those eyes which received IUDR or ICDR in each of the concentrations used, the lesions regressed strikingly, in a manner similar to that observed in the first experiment.

This more intensive but abbreviated regimen of treatment appears to be as effective as that used in the first experiment and no evidence of corneal irritation was observed. In the concentrations administered, IUDR and ICDR appeared to be equally efficacious.

In a preliminary experiment, in which the anti-viral activity of ICDR at 5.1 mgm./ml. was shown, its diacetyl derivative also was active. Further experiments with these four compounds will be required in order to determine whether there are significant qualitative or quantitative differences between these iodinated pyrimidine deoxyribonucleosides. It is clear, however, that each compound has marked activity in prevention of spread and in causing the regression of the severe corneal lesions caused by this pathogenic DNA-virus.

The mechanism of action of IUDR as an inhibitor of the biosynthesis of DNA has been discussed in several papers^{4,5,7-9} and has been attributed to the intracellular formation of phosphorylated derivatives of IUDR. The concept of obtaining a metabolically more stable compound than is the case with 5-bromo- and 5-iodo-2'-deoxyuridine, through the formation of the corresponding derivatives of 2'-deoxycytidine, has been discussed in some detail elsewhere⁹. Suffice it to say here that ICDR, which is also much more thermostable and soluble than IUDR, acts as a reservoir-form of IUDR and of its 5'-phosphorylated derivatives, and that these compounds inhibit the utilization of thymidine and its 5'-phosphorylated derivatives, normal precursors of DNA; in addition, as has been shown elsewhere^{5,6}, 5-iodo-2'-deoxyuridine

5'-phosphate, formed metabolically from either IUDR or ICDR, can enter DNA in place of thymidine 5'-phosphate. It is proposed that, in this manner, the replication of the DNA-virus either is inhibited or a 'fraudulent' viral DNA is formed that fails to replicate, or both. The corneal epithelium appears to offer an unusual opportunity for selective chemotherapeutic inhibition of the replication of a pathogenic DNA-virus, in circumstances that avoid the toxicity of these agents for reproducing mammalian cells.

The 3',5'-diacetyl derivatives of IUDR and ICDR were designed to achieve greater lipid-solubility than is exhibited by the parent compounds, with the view of two possible applications: one, oral administration in systemic chemotherapy⁸, and two, the local treatment of viral lesions of the mucous membranes and skin. The results of the experiments described herein show that the diacetyl derivatives are converted, presumably by the corneal epithelial cells, to the active metabolites. Although the relative activities of these and the parent compounds have not yet been determined, the clinical importance of the application of these agents in the chemotherapy of herpes simplex lesions of the cornea justifies this report of the confirmation and extension of the findings of Kaufman¹.

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Effect of Fluorinated Pyrimidines on Herpes Simplex Virus

SEVERAL reports have appeared recently regarding the effects of 5-fluoropyrimidines on nucleic acid metabolism¹⁻¹⁰. One of these compounds, 5-fluorouracil, has been shown to exert a profound effect on the quantity of thymine component of nucleic acids, perhaps by interfering with the methylation of thymine^{1,2,8}. Other investigators have shown that this fluoropyrimidine can be incorporated