require further study of the mechanism of action of metronidazole in presence of reduced NAD-enzymes. It is possible that metronidazole acts as a relatively non-specific electron trap, which can be counteracted by tetrazolium salts. Metronidazole did not act as inhibitor of monoamine-oxidase and diamine oxidase, in reaction systems which were inhibited by other known inhibitors of these 2 enzymes (R. E. RANNEY, Chicago, unpublished)[10].

[10] This research was supported in part by a grant from Ayerst Laboratories (New York), Scientific Advisory Board of the Licensed Beverage Industries (New York), and the Milheim Foundation for Cancer Research (Denver, Colorado). We thank Dr. T. ROBITSCHER of Ayerst Laboratories and Dr. V. DRILL of G. D. Searle & Co. for reagents used, and Dr. DRILL for unpublished information from his laboratories.

Zusammenfassung. Eine unerwartete Möglichkeit, wie Metronidazol zu einer nur scheinbaren Hemmung der Alkoholdehydrogenase führen könnte, wird aufgedeckt.

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Antagonism by Deoxyribosides of the Inhibitory Action of Certain Hydroxamic Acids on Deoxyribonucleic Acid Synthesis

ADAMSON[1] evaluated the activities of a number of congeners of hydroxyurea (HU) against advanced leukemia L1210 in mice and concluded that the essential structural requirement for antitumor activity is the hydroxamic acid group (-CONHOH). From a more recent study of the effects of certain HU analogs on DNA synthesis in HeLa cells in vitro, YOUNG et al.[2] found that a carbonyl group is not a prerequisite in an inhibitory compound in the system under consideration. However, the -NOH group is required for inhibitory activity, and the proton on the hydroxyl group must be unsubstituted.

Reports from this laboratory have revealed additional hydroxamic acids with selectivity of action against DNA synthesis in ascites tumor cell and/or microbial test systems. Salicyl hydroxamic acid (SHA) confers a prompt inhibition of the former system at a 50% inhibitory concentration (IC_{50}) of about $4 \times 10^{-4} M$; inhibition is readily reversible upon removal of the compound[3]. Oxamyl hydroxamic acid (OHA) has an IC_{50} of about $9 \times 10^{-4} M$ in the same system, and its action is also reversible. In addition, this latter compound inhibits growth of, and DNA synthesis by, certain gram-negative bacteria, and induces unbalanced growth characterized by marked elongation of individual cells[4]. Acetoxyoxamide (AOA) shares most of the pharmacological properties of OHA, except that onset of its action is preceded by a latent period which presumably corresponds to the time required for hydrolysis of the O-acetyl group to yield an *N*-hydroxyl group with an unsubstituted proton[5]. 2,3-Dihydroxybenzoyl hydroxamic acid (DHB) has an IC_{50} of about $5 \times 10^{-5} M$ in the ascites tumor system following 1 h exposure of the cells to the compound, and its action is only slowly reversible; i.e. a lag period is evident between removal of the compound from the cells by washing and resumption of the rate of DNA synthesis to near-control values (unpublished data).

It now appears sufficiently documented that a major metabolic defect conferred by HU is an inhibition of the enzymatic conversion of ribonucleotides to deoxyribonucleotides[6-10]. Partial antagonism by a mixture of deoxyadenosine, deoxyguanosine, and deoxycytidine of the action of HU on HeLa cells has been observed[11]; these 3 deoxyribosides plus thymidine virtually completely antagonize the action of HU on mouse fibroblast (L) cells[12]. The observation that HU reverses the orotic aciduria induced in patients by the administration of 6-azauridine, an inhibitor of orotidylic decarboxylase, suggests, in addition, some inhibition by HU of de novo pyrimidine biosynthesis[13]. The following work was consequently initiated to determine if the actions of SHA, OHA, AOA, and DHB on DNA synthesis are likewise antagonized by deoxyribosides. If so, it may be inferred that the mode of action of these agents is similar to that of HU.

Experimental. Determination of the rate of DNA synthesis by Ehrlich ascites tumor cells was substantially as described earlier[3-5]. Eagle's minimum essential medium with Hank's balanced salt solution (MEM) and NCTC-109 medium were from Microbiological Associates. Thymidine-^{3}H was from New England Nuclear Corporation. Deoxyribosides were from Nutritional Biochemicals Corporation or Schwartz Laboratories. The previously reported activity of AOA against DNA synthesis by cells suspended in NCTC-109 medium[5] was found to be reproducible only when this medium was used; no inhibitory action was found when this compound was assessed against the system using MEM. Consequently, all experiments employing AOA were done with NCTC-109 medium, while all others were done with MEM. The MEM reaction mixture consisted of 5.0 ml of a washed 1% cell suspen-

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sion, 0.05 ml of dimethyl sulfoxide containing HU, OHA, SHA, or DHB, and a mixture of deoxyribosides in 0.5 ml of medium to yield final concentrations as follows: deoxyadenosine, $7 \times 10^{-3} M$; deoxyguanosine, $2 \times 10^{-3} M$; and deoxycytidine, $10^{-4} M$. Where indicated in Table II, thymidine-^{1}H was also included at a final concentration of $2 \times 10^{-5} M$. Except for this latter compound, other deoxyribosides were at the same concentrations as were used by ADAMS and LINDSAY[12] in an L cell test system. An appropriate volume of medium alone was substituted for the deoxyriboside mixture in control tubes. In experiments with AOA in NCTC-109 medium, the conditions were the same except 4.5 ml of the 1% cell suspension were used and AOA was added in 0.5 ml of saline. Following incubation at 37 °C with gentle agitation for the intervals indicated in the Tables, 2.0 μc of thymidine-^{3}H (10.0 μc in the AOA experiments) in 0.5 ml of saline were added. After 40 additional min, 4.0 ml aliquots of each tube were removed, added to 4.0 ml of cold 10% trichloroacetic acid, and the insoluble cell material was prepared for liquid scintillation counting as described earlier[3,4] using a Mark I liquid scintillation spectrometer (Nuclear-Chicago Corporation).

Results. Table I shows the effects of the presence of a mixture of deoxyadenosine, deoxyguanosine, and deoxycytidine on inhibition by each of the 4 hydroxamic acids of DNA synthesis in the tumor cell test system. After incubation of the cells with deoxyribosides and each inhibitor for 10 min, inhibition conferred by HU and by OHA was completely overcome and the rate of DNA synthesis actually exceeded that of the control rate. This restoration to greater than the control value was reproducible. Inhibition conferred by SHA and by DHB was reversed to a considerable extent, but 100% of the control value was not attained. Following 1 h preincubation of the cells with deoxyribosides and each inhibitor, a similar pattern was obtained, but following 3 h, reversal, even though still demonstrable, was not complete. AOA was not used in this series of experiments since, of the 2 media used, it was active against DNA synthesis only in the NCTC-109 medium. This latter differs from MEM in several respects, including the presence of thymidine at 10 mg/l; MEM is devoid of thymidine.

Data shown in Table II were obtained under conditions identical to those described for Table I, except that the deoxyriboside mixture was supplemented with thymidine at a final concentration of $2 \times 10^{-5} M$. The inhibitory actions of HU and OHA were again the most readily reversible after 10 min and 1 h incubation. Some restoration of activity occurred in the presence of SHA and DHB, but not to as large an extent as when thymidine was omitted. The gradual increase in inhibitory action of OHA in the presence of deoxyribosides may have been due to some ancillary pharmacological action of this compound; OHA is known to oxidize hemoglobin and to alter the UV-absorption spectrum of pyridoxal phosphate[4].

The data indicate that the 4 hydroxamic acids and a metabolic product[5] of the acetylated derivative interfere, to a greater or lesser degree, with riboside reduction in Ehrlich ascites tumor cells[14,15].

Table I. Effect of a mixture of deoxyadenosine, deoxyguanosine, and deoxycytidine (Deoxyribosides, dR) on the inhibitory action of certain hydroxamic acids on DNA synthesis in Ehrlich ascites tumor cells

Compound	% of control DNA synthesis rate					
	10 min		1 h		3 h	
	Inhibitor alone	Inhibitor + dR	Inhibitor alone	Inhibitor + dR	Inhibitor alone	Inhibitor + dR
HU	9	110	4	126	1	50
OHA	10	124	2	163	9	78
SHA	2	71	1	90	1	33
DHB	3	68	1	63	2	24

All inhibitors were at a final concentration of $10^{-3} M$; dR concentrations are given in text.

Table II. Effect of a mixture of deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine (deoxyribosides, dR) on the inhibitory action of certain hydroxamic acids on DNA synthesis in Ehrlich ascites tumor cells

Compound	% of control DNA synthesis rate					
	10 min		1 h		3 h	
	Inhibitor alone	Inhibitor + dR	Inhibitor alone	Inhibitor + dR	Inhibitor alone	Inhibitor + dR
HU	4	90	22	93	5	115
OHA	2	106	25	70	16	29
SHA	3	70	19	29	4	22
DHB	2	26	18	16	3	13
AOA					13	53

HU, OHA, SHA and DHB were at a final concentration of $10^{-3} M$; AOA was $2 \times 10^{-3} M$; dR concentrations are given in text.

Résumé. L'action inhibitrice de l'hydroxyurée (HU) sur une synthèse de DNA peut être antagonisée par des déoxyribosides (dR). De même, un mélange de déoxyadénosine, de déoxyguanosine, et de déoxycytidine annule complètement l'action de l'acide oxanyl hydroxamique (OHA) et inverse, en partie, les effets de l'acide salicylhydroxamique (SHA) et de l'acide (dihydroxybenzoyl-2.3)-hydroxamique (DHB). Lorsqu'on ajoute de la thymidine à une solution de dR, le schéma général d'antagonisme est similaire. De plus, en présence des quatre dR, l'action de l'acétoxyoxamide (AOA) est en partie antagonisée. Il a été conclu que ces acides hydroxamiques, comme l'HU, interviennent, à un degré plus ou moins grand, avec réduction des ribosides.

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27 July 1967.

[14] The technical skills of A. B. SMITH and G. B. SCHMIDT facilitated the work reported.

[15] Aided by grant No. GM-13958 from the National Institutes of Health, United States Public Health Service.