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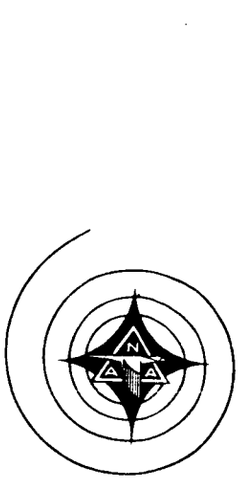

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Second Quarterly Progress Report

INVESTIGATION OF POTENTIAL ANTI-RADIATION AND ANTI-NEOPLASTIC COMPOUNDS RELATED TO PLANT GROWTH REGULATORS

PH43-64-865

1 January 1965

Prepared by

Robert D. Schultz

Robert D. Schultz, Ph.D.
Project Manager, Molecular Biology
Life Sciences Department

David Norman

David Norman, Ph.D.
Assistant Project Manager, Molecular Biology
Life Sciences Department

NORTH AMERICAN AVIATION, INC.
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FOREWORD

This document is submitted in partial fulfillment of Contract PH43-64-865 for the period 1 October 1964 to 1 January 1965. The contract sponsor is the National Cancer Institute, in cooperation with the National Aeronautics and Space Administration, Office of Technology Utilization. The program is being monitored by:

Dr. Hans L. Falk, Chief
Carcinogenesis Studies Branch
National Cancer Institute

Five advance copies of this report were sent on 21 January to:

Contracting Officer
Research Contracts Section
Supply Management Branch
National Institutes of Health
Bethesda, Maryland, 20014

The present document is identical to the advance report except for minor editorial changes. The data, however, are preliminary and are subject to further checking and verification.

The following personnel conducted the experiments discussed in this report: Margaret S. Heimer, B.S., M. Odell Hobbs, B.S., E.S. Meinert, David Norman, Ph.D., R. Gail Otte, Marilyn M. Rawlings, B.S., Robert D. Schultz, Ph.D., Norman Spiere, B.S., C. William Steers, and Richard A. Willis, D.V.M.

NORTH AMERICAN AVIATION, INC. SPACE and INFORMATION SYSTEMS DIVISION

TECHNICAL REPORT INDEX/ABSTRACT

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AUTHOR(S)

Robert D. Schultz and David Norman

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ABSTRACT

This document summarizes contract performance between 1 October 1964 and 1 January 1965, including methodology, working hypotheses, and test results.

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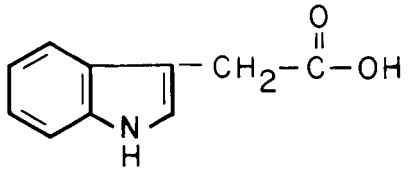
CONTENTS

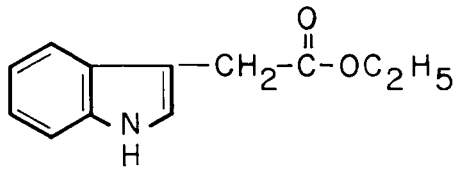
I. CYTOTOXIC EFFECTS OF PLANT GROWTH REGULATORS ON ASCITES TUMORS, IN VITRO

POSSIBLE INTERFERENCE WITH AN ONCOGENIC VIRUS-HOST CELL INTERACTION

We have reported[1,2] that aqueous suspensions of certain alkyl esters of an endogenous plant growth hormone, the auxin indolyl-3-acetic acid (IAA), are lethal to free cells of the Ehrlich ascites carcinoma in vitro. In these early experiments, IAA, by itself, protected these cells from early death; however, a mixture of IAA and its ethyl ester, IAEt, killed the tumor cells

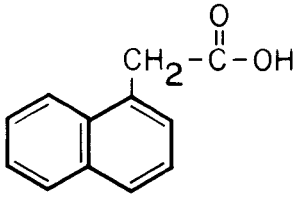


IAA

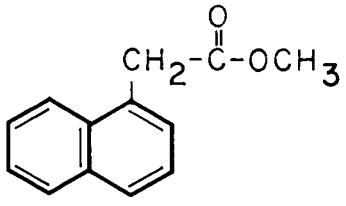


IAEt

more rapidly than did IAEt alone. The synthetic plant growth hormone naphthyl-1-acetic acid, NAA, and its methyl ester, NAMe, affect the Ehrlich cells in a manner analogous to that of IAA and IAEt. These findings suggest



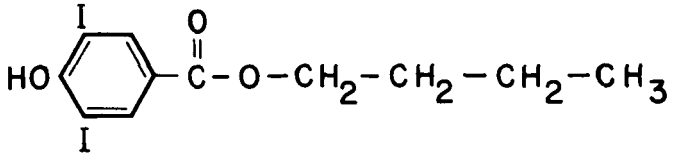
NAA



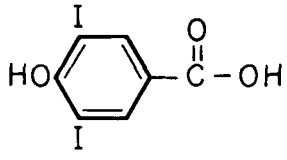
NAMe

that natural or synthetic plant growth hormones interact with a biochemical system in these animal cancer cells that is similar to, if not identical with, a system involved in the growth control of plant cells. To our knowledge, however, normal mature animal cells are relatively insensitive to these plant growth hormones. We therefore speculated that the genetic information that permits a neoplastic animal cell to respond to auxins or auxin analogues is contained within the genome of a masked oncogenic virus (i.e., within a provirus).

Accordingly, we searched the literature for known antiviral agents that are related to known plant growth regulators or selective herbicides. We learned that n-butyl ioxylate, the n-butyl ester of the selective herbicide 4-hydroxy-3,5-diiodobenzoic acid (ioxyl)[3] inhibits the growth of influenza virus in deembryonated eggs or in minced chorioallantoic membrane[4]. The parent compound, ioxyl, has no such antiviral activity when tested in the form of its sodium salt. By analogy with our observations[2] of the cytocidal activity by n-butyl indolyl-3-acetate, IABu(n), and the lack of such



n — Butyl Ioxylate



Ioxyl

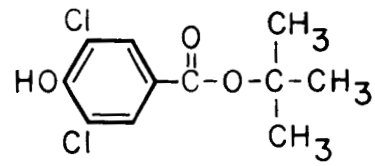
activity by the parent compound, IAA, against the Ehrlich ascites carcinoma, it seemed likely that n-butyl ioxylate would kill free cells of this tumor in vitro and that ioxyl, itself, would have little or no such cytocidal activity. We have confirmed this prediction (Table 1); our experimental techniques are described on page 7.

Table 1. Cytocidal Effect of Antiviral Compounds and Related Auxin Analogues Against Free Cells of the Ehrlich Ascites Carcinoma, In Vitro at 37 C

Test Compound	Solvent*	Drug Concentration (μg/ml)	Experiment No.	Cell Mortality		
				1 hr	2 hr	4 hr
4-Hydroxy-3,5-diiodobenzoate n-butyl ester (n-Butyl ioxylate)	1,2-P	50	M156540		100.0	
		10			15.0	
	DMSO	50	M156540		40.0	
		10			25.0	
4-Hydroxy-3,5-diiodobenzoic acid (Ioxyl)	DMSO	50	M156540		12.0	
		10			3.0	
	1,2-P	50	M156540		2.0	
		10			4.0	
N-methyl isatin-β-thiosemicarbazone (Methisazone)	DMSO	50	M142509	2.0		80.0
		10				53.0
	DMSO	10	M156622		75.0	
Methisazone + L-tryptophan	DMSO	10 + 100	M156622		41.0	
Isatin-β-thiosemicarbazone (Isazone)	DMSO	50	M142509	2.5		5.0
		10				5.5

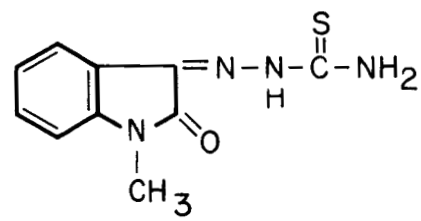
*1,2-P = 1,2-propanediol, DMSO = dimethylsulfoxide

For tumor chemotherapy in mice, n-butyl ioxylate may be too toxic. In the hope of reducing toxicity without diminishing tumorcidal activity, we are arranging for the synthesis and testing of the t-butyl, chlorinated, analogue of n-butyl ioxylate.



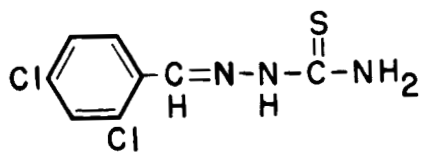
t-Butyl Chloroxylate

Another antiviral drug, methisazone (also known as Marboran), the thiosemicarbazone of n-methyl isatin, has recently received much publicity.

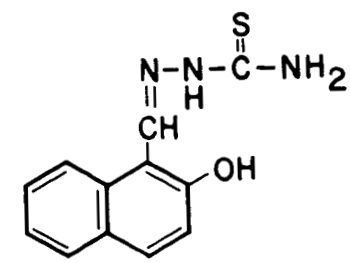


Methisazone

At a conference on antiviral substances, D.J. Bauer[5] reported that methisazone, at 20 μ M, inhibits the multiplication cycle of the vaccinia virus in HeLa cells and, when administered orally to humans, appears to be an effective prophylaxis or treatment for infections by vaccinia and smallpox viruses. Bauer's work is based on the observation, made originally by Hamre[13] and coworkers, of the activity of benzaldehyde thiosemicarbazone against vaccinia infection. Methisazone is structurally related to a large number of thiosemicarbazones that both inhibit the multiplication of Mycobacterium tuberculosis (var. hominis H37Rv) and regulate growth (e.g., stimulate flowering) when tested on canes of Thompson seedless grapes (Vitus vinifera)[6]. Two such compounds are 2,4-dichlorobenzaldehyde thiosemicarbazone (2,4-DBTSC) and 2-hydroxy-1-naphthaldehyde thiosemicarbazone (HNATSC).

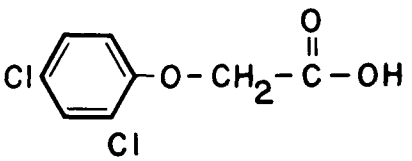


2,4-DBTSC



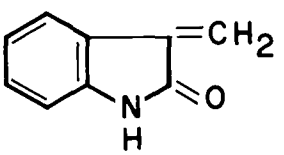
HNATSC

The reader will note the respective relationships of these compounds to the synthetic auxin-type plant growth hormones 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthyl-1-acetic acid (NAA). The reader will also note the



2,4-D

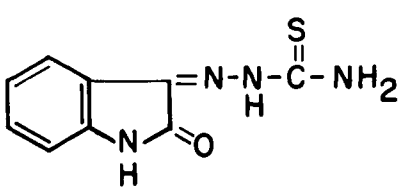
relationship of methisazone, itself, to the endogenous plant growth regulator IAA, to its ester IAEt, and to 3-methylene oxindole, a riboflavin-photo-oxidation product of IAA that inhibits the growth of some bacteria (e.g., S. pombe. and E. coli), the growth of tomato root tips, and the germination of pea seeds[7]. Table 1 indicates that methisazone has a cytocidal effect similar



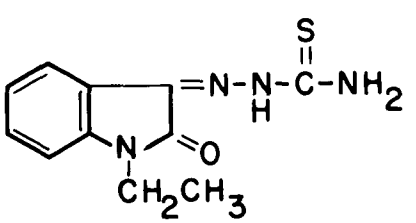
3-Methylene Oxindole

to the alkyl auxin esters against free cells of the Ehrlich ascites carcinoma in vitro. (In Section II the acute in vivo turmorcidal activity of methisazone is reported.)

The antiviral action of an isatin thiosemicarbazone can be influenced by the substituent on its indolylic nitrogen group[8]; activity increases in the homologous series $H < CH_3 < C_2H_5$. By analogy, methisazone should show greater cytocidal activity against free Ehrlich ascites cells than its unmethylated homolog, isatin-β-thiosemicarbazone (isazone), and ethisazone, the ethyl



Isazone



Ethisazone

homolog of methisazone, should show even greater activity. The first prediction is confirmed by data in Table 1; the second prediction will be tested as soon as a supply of ethisazone becomes available.

Tables 1 and 2 demonstrate that L-tryptophan inhibits the cytocidal activity of both methisazone and the alkyl auxin esters against the Ehrlich cells. This phenomenon suggests that a common site is involved in the cytocidal action of these compounds.

Table 2. Synergism or Inhibition by Indolyl Compounds of the Cytocidal Effect of Auxin Esters Against Free Cells of the Ehrlich Ascites Carcinoma, In Vitro at 37 C

Test Compound*	Solvent*	Drug Concentration (µg/ml)	Experiment No.	Cell Mortality		
				1 hr	2 hr	4 hr
IABu(t)	1,2-P	5	M156533	62.0		
IABu(t) + IAA	1,2-P	5 + 50	M156533	60.0		
IABu(t) + L-TP	1,2-P	5 + 100	M156533	22.0		
IABu(t)	1,2-P	3	M156533	15.0		
IABu(t) + IAA	1,2-P	3 + 50	M156533	17.0		
IABu(t) + L-TP	1,2-P	3 + 100	M156533	4.0		
IABu(t)	1,2-P	3	M156529			100.0
IABu(t) + IAA	1,2-P	3 + 50	M156529			100.0
IABu(t) + L-TP	1,2-P	3 + 100	M156529			44.0
IABu(t)	1,2-P	5	M156529		92.0	
IABu(t) + IAA	1,2-P	5 + 50	M156529		100.0	
IABu(t) + L-TP	1,2-P	5 + 100	M156529		29.0	
IABu(t)	DMSO	5	M144854	38.0	93.0	100.0
IABu(t) + IAA	DMSO	5 + 50	M144854	24.0	40.0	100.0
IABu(t) + L-TP	DMSO	5 + 100	M144854	37.0	72.0	82.0
IABu(t)	DMSO	3	M144854	4.5	4.0	78.0
IABu(t) + IAA	DMSO	3 + 50	M144854	9.0	6.0	19.0
IABu(t) + L-TP	DMSO	3 + 100	M144854	3.4		22.0
IABu(t)	DMSO	3	M156529		28.0	
IABu(t) + IAA	DMSO	3 + 50	M156529		95.0	
IABu(t) + L-TP	DMSO	3 + 100	M156629		20.0	
IABu(t)	DMSO	5	M156529			80.0
IABu(t) + IAA	DMSO	5 + 50	M156529			100.0
IABu(t) + L-TP	DMSO	5 + 100	M156529			86.0
IABu(t)	DMSO	3	M156533	5.0		
IABu(t) + IAA	DMSO	3 + 50	M156533	20.0		
IABu(t) + L-TP	DMSO	3 + 100	M156533	2.0		
L-TP	DMSO	100	M156533	2.0		
IABu(t)	DMSO	5	M156533	77.0		
IABu(t) + IAA	DMSO	5 + 50	M156533	32.0		
IABu(t) + L-TP	DMSO	5 + 100	M156533	78.0		
IABu(t)	DMSO	10	M144866	92.0	98.0	100.0
		50	M144866	100.0		

*IABu(t) = t-Butyl indolyl-3-acetate
IAA = Indolyl-3-acetate
L-TP = L-tryptophan
IAEt = Ethyl indolyl-3-acetate
1,2-P = 1,2-Propanediol
DMSO = Dimethylsulfoxide

Table 2. Synergism or Inhibition by Indolyl Compounds of the Cytocidal Effect of Auxin Esters Against Free Cells of the Ehrlich Ascites Carcinoma, In Vitro at 37 C (Cont)

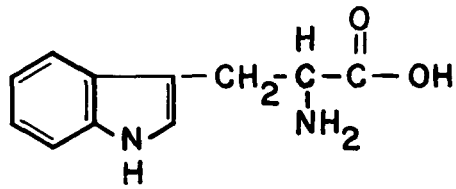
Test Compound*	Solvent*	Drug Concentration (µg/ml)	Experiment No.	Cell Mortality		
				1 hr	2 hr	4 hr
IAEt	DMSO	100	M144854	16.0	20.0	42.0
IAEt + IAA	DMSO	100 + 50	M144854	1.5	12.0	21.0
IAEt + L-TP	DMSO	100 + 100	M144854	10.0	7.0	21.0
IAEt	DMSO	100	M156509			24.0
IAEt + L-TP	DMSO	100 + 100	M156509			19.0
IAEt + IAA	DMSO	100 + 50	M156509			14.0
IAA	DMSO	50	M156509			2.6
IAEt	DMSO	100	M156529			16.0
IAEt + IAA	DMSO	100 + 50	M156529			100.0
IAEt + L-TP	DMSO	100 + 100	M156529			13.0
IAEt	DMSO	100	M156533	27.0		
IAEt + IAA	DMSO	100 + 50	M156533	17.0		
IAEt + L-TP	DMSO	100 + 100	M156533	12.0		
IAA	DMSO	50	M156533	1.0		
IAEt	DMSO	100	M156541	23.0		
IAEt + IAA	DMSO	100 + 50	M156541	13.0		
IAEt	1,2-P	100	M156509			12.0
IAEt + L-TP	1,2-P	100 + 100	M156509			4.0
IAEt + IAA	1,2-P	100 + 50	M156509			3.0
L-TP	1,2-P	100	M156509			1.0
IAA	1,2-P	50	M156509			3.0
IAEt	1,2-P	100	M156529			22.0
IAEt + IAA	1,2-P	100 + 50	M156529			100.0
IAEt + L-TP	1,2-P	100 + 100	M156529			10.0
IAEt	1,2-P	100	M156533	30.0		
IAEt + IAA	1,2-P	100 + 50	M156533	39.0		
IAEt + L-TP	1,2-P	100 + 100	M156533	18.0		
IAA	1,2-P	50	M156533	2.0		
L-TP	1,2-P	100	M156533	2.0		
IAEt	1,2-P	100	M156541	30.0		
IAEt + IAA	1,2-P	100 + 50	M156541	12.0		

*IABu(t) = t-Butyl indolyl-3-acetate
IAA = Indolyl-3-acetate
L-TP = L-tryptophan
IAEt = Ethyl indolyl-3-acetate
1,2-P = 1,2-Propanediol
DMSO = Dimethylsulfoxide

The mechanism by which the isatin thiosemicarbazones inhibit viral multiplication is as yet unknown. Magee and Bach[9] conclude that most known reactions in HeLa cells infected with the vaccinia virus are unhindered by the presence of isazone, except for the final assembly of infectious virus. This situation permits isazone to act chemotherapeutically in an interesting manner, since the drug allows the pox virus to destroy the infected cell but prevents the virus itself from multiplying. According to Appleyard, Hume, and Westwood[10], both DNA-dependent RNA synthesis and protein synthesis are essential to the antiviral action of isazone. Although the synthesis of viral DNA is unaffected, the drug does prevent the formation of at least four soluble viral antigens normally synthesized late in the viral growth cycle. This leads us to speculate that methisazone kills an Ehrlich cell by preventing the formation of certain specific proteins, possibly enzymes, whose synthesis, although vital to the life of the cell, is directed by information coded in the genome of a hidden virus (i.e., the oncogenic provirus). Conceivably, alkyl auxin esters, such as IAEt and IABu(t), destroy Ehrlich cells by a similar mechanism.

SYNERGISM OR INHIBITION BY INDOLYL COMPOUNDS

Table 3, which summarizes the data from Table 2, shows that L-tryptophan



L-Tryptophan

consistently inhibits the cytocidal action of IAEt and IABu(t) against free cells of the Ehrlich ascites carcinoma, in vitro, and that IAA potentiates this cytocidal action in some tests and in others has a protective effect on the tumor cells.

The procedures for all in vitro tests discussed in this report are summarized below:

Tumor cell suspension. Ehrlich ascites carcinoma from Webster white Swiss mice was harvested after 7 to 10 days and diluted 1:1000.

Medium. The medium was prepared from dried Fisher V-614 (Difco Laboratories), to which glutamine, $NaHCO_3$, was added, and the mixture was treated with sufficient CO_2 to establish pH 7.0.

Incubation. A 5-λ aliquot of test compound, dissolved in either dimethylsulfoxide (DMSO) or in 1,2-propanediol (1,2-P), was introduced into a deep well slide; a 0.3-ml aliquot of the tumor cell suspension was added at zero time; and the slide, sealed with Scotch cellophane tape, was immediately placed in an incubator maintained at 37 C.

Cell counts. After a given incubation period, slides were removed, the test compounds were diluted with eosin or nigrosin, and cells were counted with micrometer counting discs. An ability to resist staining was taken as evidence of viability. (The superiority of phase-contrast microscopy in the determination of cell viability is discussed on pages 9 and 10.

Table 3. Summary of Experiments on the Synergism or Inhibition by Indolyl Compounds of the Cytocidal Effect of Auxin Esters Against Free Cells of the Ehrlich Ascites Carcinoma, In Vitro at 37 C

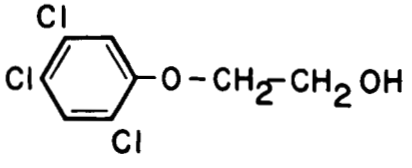
Indolyl Compound	Solvent	Effect on Cytocidal Activity of Auxin Ester								
		IAEt (100 μg/ml)			IABu(t) (3 μg/ml)			IABu(t) (5 μg/ml)		
		1 hr	2 hr	4 hr	1 hr	2 hr	4 hr	1 hr	2 hr	4 hr
IAA (50 μg/ml)	DMSO	RRR	R	RRS	SS	SS	R	RR	R	OS
	1,2-P	SR		RS	O		O	O	S	
L-tryptophan (100 μg/ml)	DMSO	RR	R	RRR	RR	R	R	RO	R	RO
	1,2-P	R		RR	R		R	R	R	

Symbols:

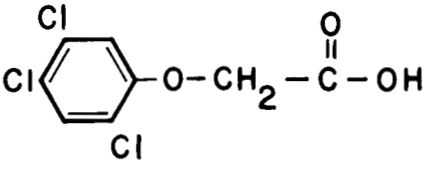
R = Inhibits IAEt = Ethyl indolyl-3-acetate DMSO = Dimethylsulfoxide
S = Synergizes IABu(t) = t-Butyl indolyl-3-acetate 1,2-P = Propanediol
O = No effect

CYTOCIDAL ACTIVITY OF RADIATION-PROTECTIVE AUXIN ANALOGUES AND RELATED COMPOUNDS

We previously reported[11] that 2,4,5-trichlorophenoxyethanol (2,4,5-TOH), the carbinol analogue of the synthetic plant growth hormone and selective herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), protects healthy mice



2,4,5-TOH



2,4,5-T

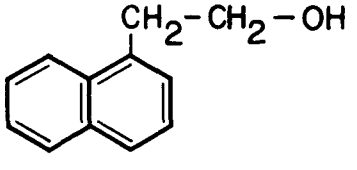
against normally lethal doses of cobalt-60 gamma radiation, and that the effective concentration of 2,4,5-TOH (150 to 200 mg/kg) is lethal in vitro to free cells of the Ehrlich carcinoma. We have recently seen this radiation-

Table 4. Cytocidal Activity of Radiation-Protective Auxin Analogues and Related Compounds Against Free Cells of the Ehrlich Ascites Carcinoma *In Vitro* at 37 C

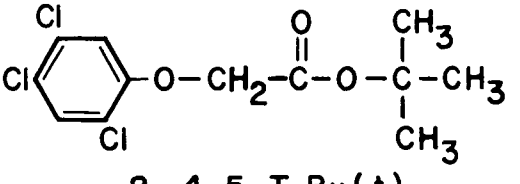
Test Compounds	Drug Concentration (μg/ml)	Cell Mortality (percent) 1 hr	2 hr	4 hr
Naphthyl-1-acetamide	50	3.0	3.6	45.0
	10	3.0	4.0	2.0
2-(1-Naphthyl) ethanol	50	7.5	40.0	85.0
	10	2.5	2.5	5.0
2,4,5-Trichlorophen-oxyethanol (2,4,5-TOH)	50	20.0	10.0	100.0
	10	15.0	6.0	90.0
t-Butyl 2,4,5-trichlorophenoxy-acetate (2,4,5-TBu(t))	50	100.0		
	10	100.0		
Solvent: Dimethylsulfoxide Experiment No.: M144866				

protective agent kill free cells of the following additional ascites tumors *in vitro*: mast cell P815, lymphoma P388, leukemia L-1210, leukemia L-1210AR, (amethopterin resistant), and myeloid leukemia C-1498.

However, cytocidal activity and radiation protection are not directly related. Table 4 shows the significant *in vitro* cytocidal activity against the Ehrlich ascites carcinoma of 2,4,5-TOH; 2,4,5-TBu(t) = t-butyl 2,4,5-trichlorophenoxyacetate; 2-(1-naphthyl) ethanol; and naphthyl-1-acetamide.



2-(1-Naphthyl) Ethanol

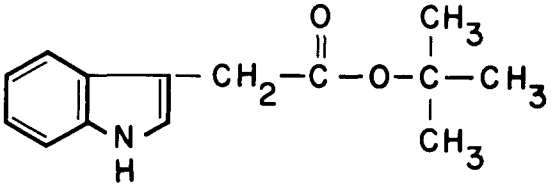
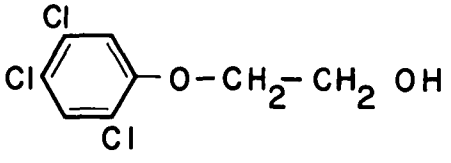
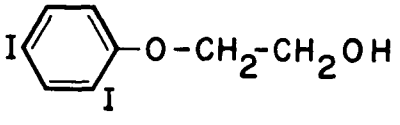
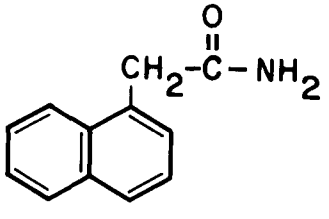


2,4,5-T Bu(t)

Of these compounds, 2,4,5-TOH shows good radiation-protective ability in mice; preliminary data indicate that naphthyl-1-acetamide provides marginal protection and that naphthylethanol and 2,4,5-TBu(t) provide none. Data on these effects will be presented in the next quarterly progress report.

EFFECTS ON CELL MORPHOLOGY

Free Ehrlich ascites carcinoma cells treated *in vitro* with some plant auxin analogues were subjected to phase-contrast microscopy with the aid of an A.O. Spencer Phasestar Microscope L6TG-P4. The morphological changes produced in these cells are summarized as follows:

Drug	Appearance of Cells
t-Butyl indolyl-3-acetate (IABu(t)) 	Live cells have a halo around the inside of the cytoplasmic membrane; dead cells appear grey and have no halo. Inclusions are gathered around the nucleus. The nuclear area is distinct, often appearing to be surrounded by a thick nuclear membrane. Multiple blisters, uniformly dark in color and often containing mitochondria, are present on the cells.
2,4,5-Trichlorophenoxyethanol (2,4,5-TOH) 	Live cells have the halo described above. There seem to be three types of dead cells, in all of which inclusions are gathered around what appears to be the nucleus. The first has no halo and is very dark; it apparently consists of the nuclear area without cytoplasm. In the second the cytoplasm is light gray, and the nuclear area sometimes appears to have a halo, although the cytoplasm does not. The third type, which has blisters containing mitochondria, has no halo; there is seldom more than one blister per cell. A well-defined nuclear area is situated at the side of the cell.
2,4-Diiodophenoxyethanol 	The nuclear area is very brilliant and granular; many vacuoles appear to be present. A few of the cells have blisters, with mitochondria gathered around the outer edge. In nonblistered cells, it is often impossible to distinguish between the nuclear area and the cytoplasm.
Naphthyl-1-acetamide 	The density of damaged cells is apparently decreased since they float to the top layer of the incubation medium. (Normal cells settle to the bottom.) The damaged cells generally appear to be round with hair-like projections, and the cytoplasm appears somewhat more granular than in untreated cells.

In future in vitro studies, counts of cell damage and death will be determined chiefly by this phase-contrast microscopic technique with the aid of A.O. brightline hemocytometers. We have data showing this technique to be more accurate that the dye exclusion method we have been using.

EFFECTS ON CELLULAR RESPIRATION

IABu(t), at 200 μg/ml, markedly increases the rate of oxygen uptake of free Ehrlich ascites cells. In one test, the treated cells consumed an average of 2.5 times as much oxygen as the controls, during the first 60 minutes of exposure to the drug (Figure 1). However, the rates are erratic; note the near-normal consumption rate at 50 minutes. The test was carried out in a conventional Warburg manometer, on an ascites tumor diluted 1:3 with the medium described previously. The drug was added to the Warburg flask as a solution in DMSO. The final concentration of DMSO in the flask was about one percent. Cell mortality for the drug-treated tumor at the end of 60 minutes was only about ten percent, for this relatively undiluted tumor. The data in Figure 1 for both the drug-treated and the control cells were corrected to compensate for the cell mortality at each time of measurement.

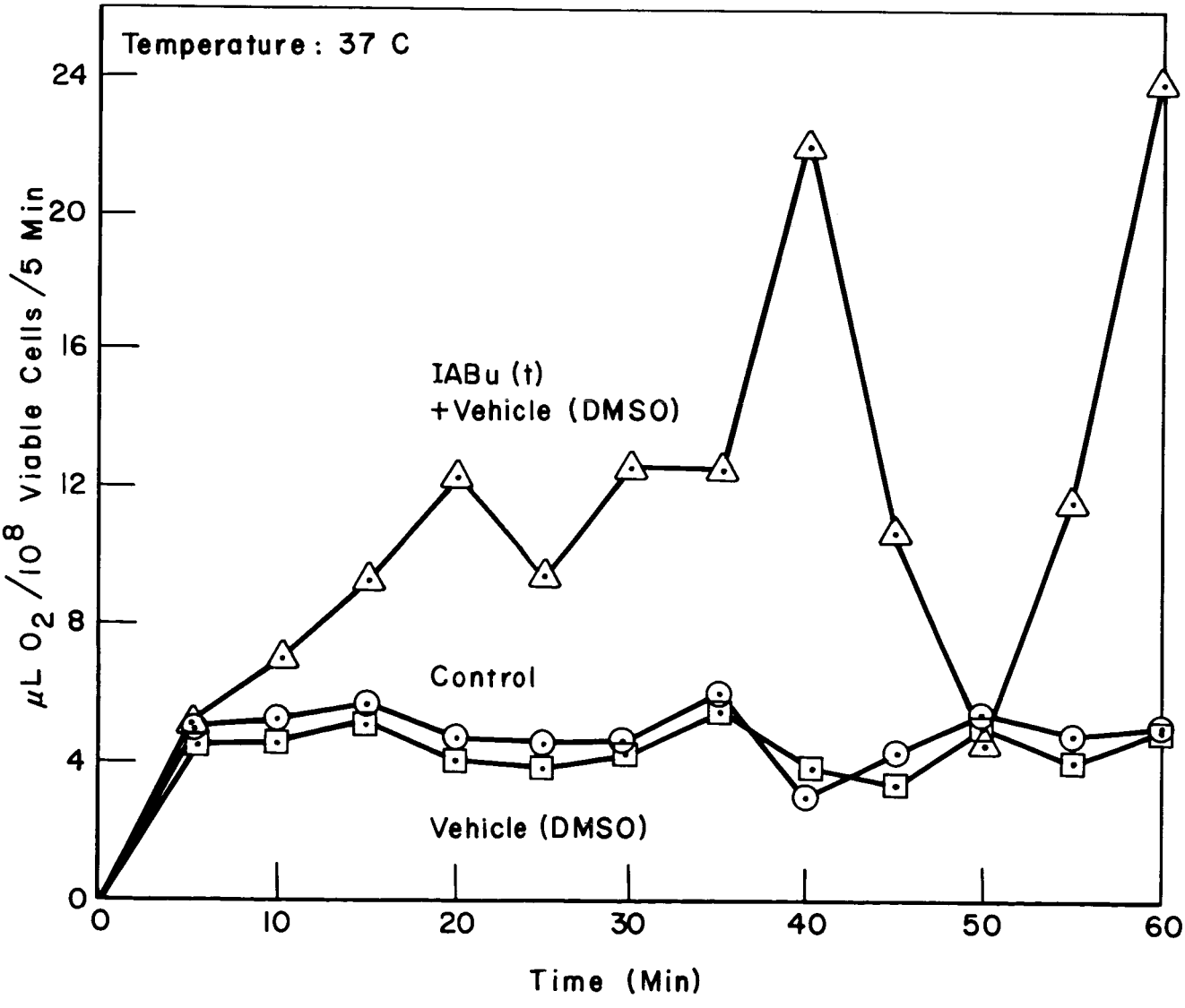


Figure 1. Stimulation by IABu(t) of O_2 Uptake by Ehrlich Ascites Cells

II. CYTOTOXIC EFFECTS OF PLANT AUXIN ANALOGUES ON ASCITES TUMORS, IN VIVO

EFFECT OF INJECTION VEHICLE ON TUMORCIDAL ACTIVITY

The tumorcidal properties of IABu(t), in male Webster white Swiss mice bearing the Ehrlich ascites carcinoma, vary greatly in different injection vehicles. A comparison of four vehicles follows.

Previously, we were able to demonstrate[12] a two- to three-fold increase in survival time of the tumor-bearing mice when a single 0.5 mg/g dose of the drug was injected intraperitoneally, 24 hours after tumor implantation, as a suspension of 10 mg/ml in a vehicle consisting of distilled water containing Tween-20 at 5 µl/ml, Myvacet (i.e., distilled acetylated monoglycerides) at 25 mg/ml, and 1,2-propanediol at 27 µl/ml. However, an i.p. injection of the drug in cottonseed oil was found to have no effect on the tumor.

Recent investigation has indicated that triturated suspensions of IABu(t) in gum arabic are totally devoid of any tumorcidal activity in chronic in vivo experiments. Furthermore, tumor-bearing animals given this gum arabic triturate actually survived a shorter time than noninjected controls. In acute in vivo experiments, this gum arabic triturate of IABu(t) showed no cytocidal activity against a week-old Ehrlich ascites tumor within two hours following an i.p. injection of a 0.5 mg/g dose.

In contrast, an i.p. injection of a similar dose of IABu(t), suspended in our new standard vehicle, completely destroyed tumor cells within the peritoneal cavity two hours after injection. The components of the vehicle, in distilled water, are:

Dimethylsulfoxide (DMSO)	2.5 percent
1,2-Propanediol (1,2-P)	5.0 percent
Tween-20	1.0 percent

Figure 2 shows the gross appearance of mice that received such an injection 24 hours after the i.p. implantation of approximately 5×10^6 free cells of the Ehrlich ascites carcinoma. A considerable difference in weight between the drug-treated and the untreated control group is evident. Each group consisted of 20 mice. At 15 days after tumor implantation, when the photograph was taken, the control animals had an average weight of 42±2 grams; the drug-treated group averaged only 28±2 grams. All mice in this experiment were of the same age, the same strain (male Webster white Swiss), and had an initial body weight of 21±1 grams. The uninjected animals had about 6±2 cc of ascites tumor drainable from the peritoneal cavity; the drug-treated mice had only about 0.5±0.5 cc of the tumor.

Dimethylsulfoxide was chosen as a vehicle ingredient because of its well-known ability to increase cellular permeability. The presence of DMSO in the vehicle had been observed to speed the disappearance of IABu(t) from the peritoneal cavity, a phenomenon which would tend to decrease the effectiveness

of the drug at the primary site of the Ehrlich ascites carcinoma. However, we expected that DMSO would more than compensate for this phenomenon by increasing the rate of uptake of the auxin analogue by the tumor cells. It is probable that DMSO is acting as a drug adjuvant in just this manner. Unexpectedly, however, we found that an injection of 1 ml, per 20 grams of animal weight, of the vehicle (in the proportions described above) has, by itself, an inhibitory effect on the development of the Ehrlich ascites carcinoma. The vehicle was injected, i.p., 24 hours after tumor implanation. At 15 days, the vehicle-injected control mice carried an average of 2±1 cc of the tumor, compared to 6±2 cc in the uninjected controls. The mean weight of the vehicle control animals was 34.3±2 grams; the untreated group weighed an average of 42±2 grams. It is possible that DMSO, by affecting cellular permeability, is interfering with the regular metabolism of the Ehrlich cells.

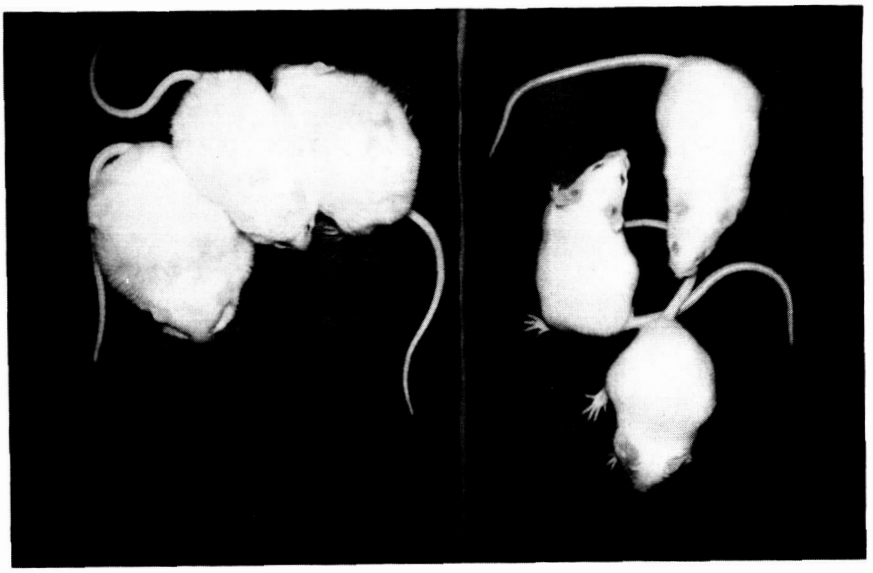


Figure 2. Effect of a single 0.5 mg/g dose of n-butyl indolyl-3-acetate on the development of the Ehrlich ascites carcinoma in male Webster white Swiss mice. The drug was injected intraperitoneally, 24 hours after tumor implantation, as a suspension in 1 cc of a vehicle consisting of 2.5 percent dimethylsulfoxide, 5 percent 1, 2-propanediol, and 1 percent Tween 20 in distilled water. The photograph was taken 15 days after tumor implantation. Drug-treated mice at right have near-normal appearance. Untreated mice at left are swollen with fully developed ascites tumor.

ACUTE CYTOCIDAL EFFECTS AGAINST P815 MAST CELL ASCITES TUMOR

We have studied the acute in vivo cytocidal effects of methisazone and of some plant auxin analogues, suspended in a vehicle containing DMSO, against free cells of nine-day-old mast cell P815 tumor in male CDBA mice. A dose of 0.5 mg/g, in 1 ml of vehicle per 20 grams of animal weight, was injected intraperitoneally. The tumor cell mortality in the peritoneal cavity two hours post-injection is given in Table 5.

Table 5. Acute In Vivo Cytocidal Activity of Some Auxin Analogues Against Free Cells of a Nine-Day-Old P815 Mast Cell Ascites Tumor in Male CDBA Mice

Drug	Vehicle*	Tumor Cell Mortality at Two Hours (percent)
t-Butyl indolyl-3-acetate (IABu(t))	2.5 percent DMSO, 5 percent 1,2-P, 1 percent Tween-20, H_2O	0
t-Butyl 2,4,5-trichlorophenoxyacetate (2,4,5-TBu(t))	2.5 percent DMSO, 5 percent 1,2-P, 1 percent Tween-20, H_2O	42
N-methyl isatin - β - thiosemicarbazone (Methisazone)	5 percent DMSO, 1 percent Tween-20, H_2O	32

*DMSO = Dimethylsulfoxide
1,2-P = 1,2-Propanediol

FUTURE IN VIVO EXPERIMENTATION

The molecular structure of the chains of our auxin analogues will be altered in the hope of improving the rate of uptake of the drug by the tumor cells without increasing overall animal toxicity. It is evident to us that, during chemotherapy, tumorcidal concentrations of these drugs are not reaching tumor cells that have invaded the peritoneum or have otherwise metastasized from the ascites tumor implanted in the peritoneal cavity.

III. RESULTS TO BE REPORTED

We have new data on the radiation-protective effects of auxin analogues that we will present in our next quarterly report. We have also made considerable progress in the two-dimensional chromatography of indolyl compounds (including tryptophan and IAA) that are endogenous to some ascites tumors. Because of the necessity of processing color prints of the chromatographs, we will present these results in a supplementary report we hope to issue prior to the next quarterly progress report.

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