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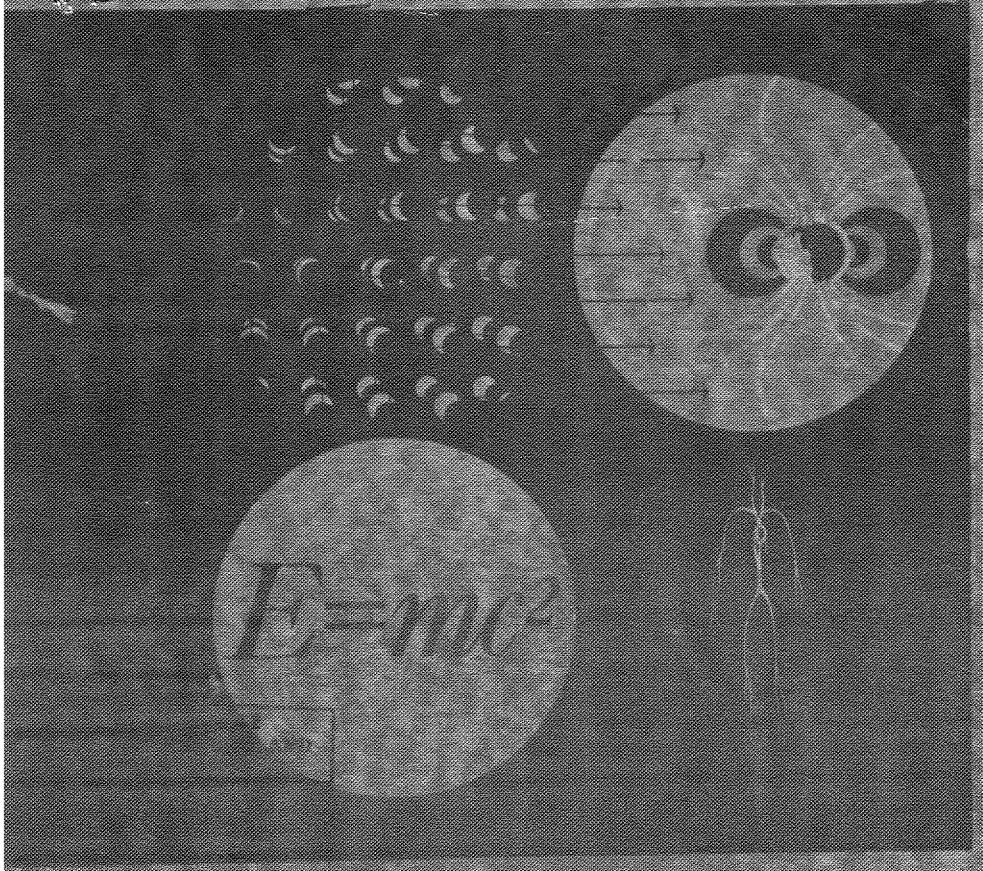

RADIATION PROTECTION BY AUXIN ANALOGUES AND ANTIVIRAL AGENTS

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DOUGLAS CORPORATE OFFICES — ADVANCED RESEARCH LABORATORY

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ABSTRACT

Although plant growth modifiers were found to be totally devoid of radiation-protective activity, their structural analogues were found to protect male Wobbrel white Swiss mice against lethal doses of ^{60}Co radiation. A broad-spectrum antiviral agent, statolon, which alone exhibited no activity, was found to synergize the protective action of certain growth modifying analogs. The possibility of latent virus involvement in radiation damage is examined, similar to bacterial lysogeny is also discussed.

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Section 1

BACKGROUND OF THE STUDY

This study, to determine whether auxin analogues or plant growth modifiers would influence the rate or magnitude of the response of mice to ionizing radiation, was suggested by earlier investigations by the authors which demonstrated the ability of the auxin analogue 2-2,4,5-trichloro-phenoxyethanol to protect mice against lethal doses of ^{60}Co γ-radiation. Experiments were accordingly conducted to test the ability of auxin analogues and antiviral agents to delay or prevent the death of male Webster white mice subjected to normally lethal doses of ^{60}Co γ-radiation. Certain auxin analogues were found to afford a significant degree of protection from radiation; others did not demonstrate this activity.

Section 2

DISCUSSION

Figure 1 shows the structures of some of these plant growth modifiers and their radiation-protective analogues. (Abbreviations are defined in the glossary at the end of this paper.) The first, 2,4,5-T -- 2,4,5-trichlorophenoxyacetic acid (Figure 1, line 1) -- is a well-known auxin-type selective herbicide (i.e., a weed killer). At low concentrations, 2,4,5-T stimulates plant cell elongation in the same manner as the natural plant growth hormone (auxin) IAA -- indolyl-3-acetic acid (line 2). Although neither of these compounds shows radioprotective activity in mice (Table 1), their carbinol analogues, 2,4,5-TOH and IOH, protected 40 and 70 percent, respectively, of groups of mice against 1000 r ^{60}Co γ-radiation, a dose that killed 97 percent of the untreated controls. Ioxyl -- 3-hydroxy-4-iodobenzoic acid (line 3) -- is also a selective herbicide, possibly of the auxin type. Ioxyl itself does not reduce radiation mortality; however, its tributyl-tin derivative does. Ioxynil has been reported to inhibit the growth of influenza virus in de-embryonated eggs and in tissue culture. TIBA -- 2,3,5-triiodobenzoic acid (line 4) -- is a synthetic plant growth modifier, which shows auxin, auxin-synergist, or auxin-antagonist activity, depending upon concentration and type of plant tissue. This plant growth modifier does not protect mice against radiation either, but its structural analogue, PIBA (p-iodobenzoic acid), does. Kasein (line 5), an indole oxidation product occurring naturally in plants and animals, has recently been shown by Calistro and Chan to

Figure 1. Relationship between activity of some plant growth modifiers and their structural analogues.

Table 1

RADIATION MORTALITY OF HALE WEBSTER MOUSE MICE TREATED WITH VARIOUS AUXETIVE COMPOUNDS RELATED TO RADIATION-PROTECTIVE AUXIN ANALOGUES

Compound	Concentration (mM/gm)	1000 r Mortality	No. of Mice
2,4,5-T	0.2	100/14*	20
Ioxyl	0.3	100/13	15
	0.4	100/13	15
TIBA	0.2	100/20	10
	0.3	60/29	10
Ioxin	0.5	92/13	12
IAA	0.6	100/12	12
Benzoic acid	0.25	100/13	5
	1.0	100/13	10
PABA	0.35	100/27	20
DCBA	0.5	0/30	10
4-Ribazine	0.25	100/14	20
	0.3	100/13	20
Ioxbu(sec)	0.1	80/19	20
Station	0.2	100/13	5
	0.5	100/12	5

*The first number is the percentage of treated mice that died within 30 days post-irradiation; the second number is the day post-irradiation after which no deaths occurred within the 30 days.

stimulate plant growth in the same manner as IAA. Our preliminary tests in this area seem to bear out this prediction, as we have found that IUdR is capable of stimulating the growth of tobacco callus tissue in culture. IUdR (also known as merifluridine, Stoxosan, and Compound 31258) has recently received much publicity as an antiviral agent whose activity in vitro and in vivo seems limited to the pox group of viruses.

Each of our test compounds (except IUdR, which was dissolved in dimethylsulfoxide) was suspended with an equal weight of gum arabic in physiological saline in a concentration that permitted the desired drug dose to be administered in a volume of 0.2 ml/20 gm of animal body weight. White male mice of the Swiss strain, weighing 25 to 30 gm, were injected intraperitoneally with the compound 24 hours before irradiation. Non-injected and vehicle-injected mice were irradiated as controls. Irradiation in the radiation chamber, operated with one X-ray tube at 250 kvp and 15 ma, was used to restrain the mice within the chamber. A series of homogeneous resins, each containing a different number of hundred-#g Co-60 foils, surrounded the chamber to provide radiation scatter. Measurements of the delivered radiation dose were made by triplicate ferrous sulfate dosimetry. Irradiation of animals in groups of ten was carried out in the chamber, with a total number of drug-treated and control mice per experiment. Mice were placed in the irradiation chamber individually, at 45-to-60-second intervals, for 10 to 15 minutes before the 2.4-second irradiation period. Mice were obtained from Carworth, Inc., New City, New York, and Flow Laboratories, New York, and Vitamin Corporation, Guayalan, Chile. Pseudomonas aeruginosa was obtained from Berkeley Pasteur Laboratories.

Prior to these tests, all our mice were screened for Pseudomonas aeruginosa according to the methods of Flynn and Marshall, et al.,[3] in which gizzard broth is used as the bacterial growth medium. We eliminated all mice from cages in which an active P. aeruginosa infection was detected. In addition, we took heart-blood samples from all animals that died after radiation and streaked them onto Pseudomonas agar F plates (Difco Laboratories). A 95-percent incidence of active P. aeruginosa was thus found post-irradiation in both drug-treated and control groups. Subcultures made from the growth on these plates showed the following incidence of gram-negative bacteria:

Bacteria	%*
Escherichia	50
Aerobacter	3
Salmonella	8
Shigella	10
Proteus	10

*It is apparent, therefore, that our radiation-protection experiments were carried out with mice that harbored a variety of potentially pathogenic bacteria. Despite these latent infections in our colonies, 2-[4,5-TOBr, toxBain], PBA, XRP, and 2OH provided absolute protection for about 90 to 95 percent of the mice exposed to 1000 r 60Co radiation. (In Figures 2 and 3, each curve represents at least two experiments and at least 40 mice.)

[illegible]

[illegible]

[illegible]

[illegible]

of 10 percent statolon; a mixture of $NaHCO_3$ plus glucose made up the remaining 90 percent. The ineffective lot, also in powder form, consisted of 14 percent statolon; a mixture of $(NH_4)_2CO_3$ plus glucose made up the remaining 86 percent. There was also a difference in the physical appearance of the statolon-IoxBu(n) triturated preparations from the two lots. Statolon by itself provided no radiation protection in preliminary tests (Table 1).

To learn if any tissues are permanently injured by protective doses of these auxin analogues, we examined representative tissues from drug-treated mice. Irradiated mice and their non-irradiated controls (4 to 18 mice per test condition) were sacrificed 60 or 90 days after drug administration or irradiation, this period being considered sufficient for most tissue repair processes. Following incisions to expose the viscera and major cavities to fixative, the bodies were totally immersed in 10-percent formalin long enough for tissue fixation. Samples were then taken and embedded in paraffin for sectioning, and the sections were stained with hematoxylin and eosin. The tissues included mesenteric lymph node, femoral bone marrow, mesentery, omentum, genital omentum, pancreas, spleen, liver, heart, lung, thyroid, thymus, stomach, large and small intestines, kidney, adrenal and both testes.

Microscopic examination of the tissues from drug-treated, non-irradiated mice failed to reveal any detectable histopathologic changes. Of the tissues examined from drug-treated, irradiated mice exposed to 700 r, only the testis [illegible] showed such histopathological changes: moderate to severe arrest of spermatogenesis and/or replacement of spermatogonia by Sertoli cells.

The drug-concentration range over which these compounds are radiation-protective is very narrow: a 30-percent increase from the optimum radiation-protective dose can markedly sensitize the animal to radiation, although it gives no evidence of being toxic. For example, IoxBu(n) at 0.1 mg/gm is optimal for radiation protection, but a dose of 0.15 mg/gm can sensitize to radiation. In most instances, the protective dose is approximately 1/2 LD_{50}. Table 2, which gives toxicity data for auxin analogues in the absence of radiation, shows the relatively narrow therapeutic index of these drugs.

Our data on structure-activity relationships for these radiation-protective compounds are limited. Substitution of an amino group (PABA) or a chlorine atom (PCBA) for the iodine atom in PIBA reduces its effectiveness against γ-radiation. Similarly, the secondary butyl ester analogue of IoxBu(n) is essentially without protective activity (Table 1).

Radiation-protective doses of both 2,4,5-TOH and IOH act as hypnotic agents in mice for periods up to an hour. The other auxin analogues do not produce this effect; IoxBu(n) and PIBA produce only a slight temporary depression.

Knowing that hypnotic and anaesthetic agents decrease oxygen uptake, we checked for a correlation between this decrease and the radiation protection afforded by 2,4,5-TOH (Table 3). This compound reduces the oxygen uptake of the animal to a little less than that during normal sleep. However, a dose of sodium pentobarbital that reduced oxygen uptake by a similar amount afforded no protection against 1000 r ^{60}Co γ-radiation. This finding suggests that general (but not necessarily localized) hypoxia may not be the mechanism of radiation protection by 2,4,5-TOH.

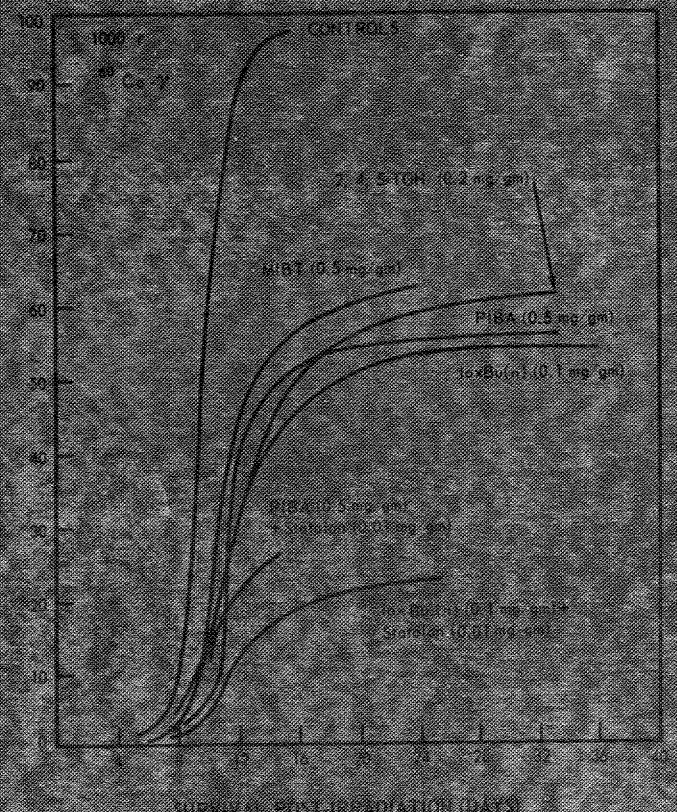

Figure 2. Effect of intraperitoneal injections of some indole analogues and antiviral agents upon the radiosensitivity of mature female white mice after whole body exposure to a 1800 r dose of ^{60}Co γ-radiation.

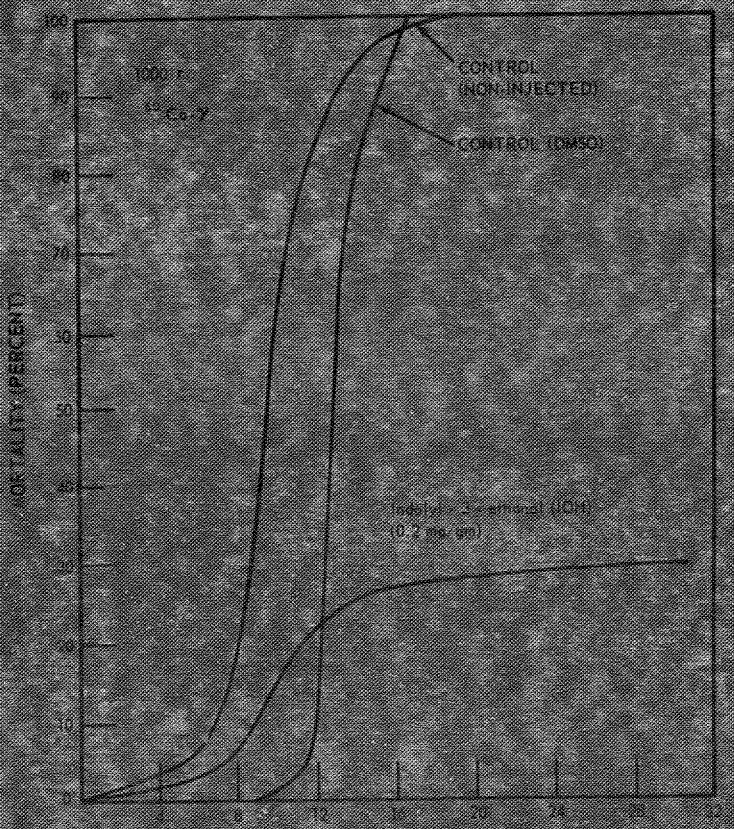

Figure 3. Effect of an intraperitoneal injection of indole-3-carbinol in reducing the mortality of mature male white Swiss mice exposed to a 1800 r dose of ^{60}Co γ-radiation.

Table 2

TOXICITY DATA FOR RADIATION-PROTECTIVE AUXIN ANALOGUES

Compound	Dose (mg/gm)	Vehicle*	Mortality (%)
[illegible]	0.25	GA + S	0
	0.35		60
	0.40		>99
IOH	0.3	PD + S	0
	0.4		40
	0.5		>99
2,4,5-TOH	0.3	PD + S	0
	0.35		20
	0.4		[illegible]
NAA	[illegible]	GA + S	[illegible]
	[illegible]		[illegible]
[illegible]	1.0	GA + S	[illegible]
	1.5		50
	3.0		>99
[illegible]	2.0	GA + S	0
[illegible]	[illegible]	GA + S	0
	0.15		

*GA = gum acacia [illegible]

Table 3

EFFECT OF 2,4,5-TOH ON OXYGEN UPTAKE BY WEBSTER WHITE SWISS MICE

Treatment*	Oxygen Uptake, ml O_2/gm/hr (±SE)				1000 r Mortality
	Before Injection	15 min	70 min	120 min	
No injection	4.5 ± 0.2 (14)**	4.1 ± 0.3 (15)	4.22 ± 0.1 (15)	3.4 ± 0.3 (16)	95/14
Saline	4.4 ± 0.1 (12)	4.1 ± 0.1 (14)	4.2 ± 0.1 (13)	4.1 ± 0.1 (14)	95/14
Pentobarbital 0.05 mg/gm	4.6 ± 0.1 (16)	2.4 ± 0.2 (22)	4.4 ± 0.2 (17)	3.9 ± 0.3 (17)	95/14
2,4,5-TOH 0.2 mg/gm	5.0 ± 0.2 (12)	2.7 ± 0.2 (16)	2.5 ± 0.1 (20)	2.9 ± 0.2 (18)	62/33

*Four or five mice per condition.

**( ) readings.

Saline vs. pentobarbital treatment: at 15 minutes post-injection, $p < 0.001$; not significant at other time points.

Saline vs. 2,4,5-TOH treatment: at 15, 70, and 120 minutes post-injection, $p < 0.001$.

Pentobarbital vs. 2,4,5-TOH treatment: at 70 and 120 minutes post-injection, $p < 0.001$ and $p < 0.02$, respectively; not significant at other time points.

The fact that the radiation-protective auxin analogues IoxBu(n) and MIBT and the synergist statolon have antiviral activity suggests that radiation sickness in mammals from 1000 r or less may be a phenomenon somewhat similar to the radiation induction of infectious virus in lysogenic bacteria. Monod, Changeux, and Jacob[1] have suggested that a hormone of small molecular weight is an allosteric effector that inactivates a specific genetic repressor. This allosteric transition removes or peels off the repressor from the operator gene of the operon and permits its cistrons to transcribe m-RNA. If this concept is correct, a hormone analogue of suitable structure could function as an antihormone to block this allosteric transition and prevent the operon from functioning. We suspect that our radiation-protective auxin analogues act in this manner to inhibit or repress the vegetative multiplication of initially latent but perceptibly cytopathic viruses that were activated by the radiation. This hypothesis is discussed in some detail in another paper.

Section 3

GLOSSARY

Abbreviation	Compound
DMSO	dimethylsulfoxide
4-HBPr(n)	n-propyl 4-hydroxybenzoate
IAA	indolyl-3-acetic acid
IOH	indolyl-3-ethanol
IoxBu(n)	n-butyl 4-hydroxy-3,5-diiodobenzoate
IoxBu(sec)	sec-butyl 4-hydroxy-3,5-diiodobenzoate
MIBT	N-methyl isatin-β-thiosemicarbazone
pABA	4-aminobenzoic acid
pClBA	4-chlorobenzoic acid
pIBA	4-iodobenzoic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TIBA	2,3,5-triiodobenzoic acid
2,4,5-TOH	2,4,5-trichlorophenoxyethanol
SE	standard error of the mean
P	probability level of t according to R. A. Fisher[2]

10. Monod, J., Changeux, J. P., and Jacob, F., J. Mol. Biol., 6, 306 (1963).

11. Fisher, R. A., Statistical Methods for Research Workers (Oliver and Boyd, Edinburgh, 1958).

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