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PROTECTANT IN RODENTS AND PRIMATES

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School of Aerospace Medicine
Brooks Air Force Base, Texas

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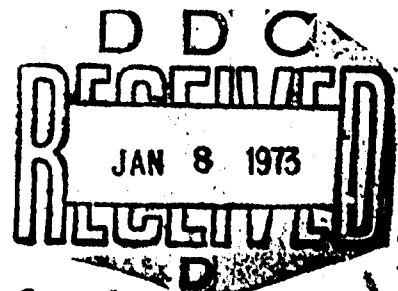
**S-2-(3-AMINOPROPYLAMINO) ETHYL PHOSPHOROTHIOIC
ACID HYDRATE AS A RADIOPROTECTANT
IN RODENTS AND PRIMATES**

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EMMETT J. STORK, B. S.
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13. ABSTRACT S-2-(3-aminopropylamino) ethyl phosphorothioic acid hydrate was studied in rodents and primates to determine its radioprotective capacity. Thirty minutes before being irradiated with 1,200 R cobalt-60, male Sprague-Dawley rats were injected intraperitoneally with sterile water solutions containing 250 mg./kg. body weight of the radioprotective agent. In at least one group of 12 rats, survival at 30 days postirradiation was 100%. All similarly irradiated control rats without medication died. This study was extended to standardized primates, <i>Macaca mulatta</i>. The primates were injected intravenously with the drug in sterile saline solution 30 minutes before exposure to 850 R x-rays. Drug concentrations ranged from 250 to 400 mg./kg. At the two best concentrations (250 and 300 mg./kg.), 12 out of 15 treated primates survived 30 days. All 8 similarly irradiated control primates died between the tenth and fifteenth day postirradiation. Pathology findings are presented for each group.			

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FOREWORD

This report was prepared in the Radiobiology Division under task No. 775703. It covers work performed between September and December 1967. The paper was received for publication on 29 July 1968.

The radioprotective agent (WR 2721) tested in this study was supplied through the courtesy of Dr. David P. Jacobus, Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. A summary of radioprotection data acquired from mice and canine studies was also furnished.

The technical assistance of Horace Hamilton in quantitative analyses and of Major Harold W. Casey and his staff in pathology is gratefully acknowledged.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences-National Research Council.

This report has been reviewed and is approved.

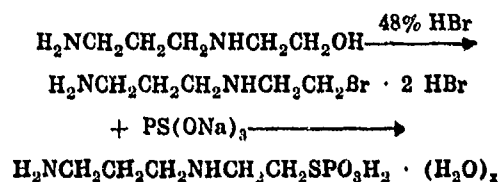


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S-2-(3-AMINOPROPYLAMINO) ETHYL PHOSPHOROTHIOIC ACID HYDRATE AS A RADIOPROTECTANT IN RODENTS AND PRIMATES

I. INTRODUCTION

A newly synthesized compound, S-2-(3-aminopropylamino) ethyl phosphorothioic acid hydrate, has a chemical structure that is expected to afford radioprotection while minimizing the toxicity and undesirable side effects found in many of the older, classical radioprotectants (1). The effectiveness of this compound was studied in rats and in the primate, *Macaca mulatta*. The formula for the synthesis of this radioprotective agent is shown below (3):



II. METHODS

Animal care and maintenance

Male Sprague-Dawley rats were obtained from commercial suppliers. These albino rats averaged approximately 200 gm. in weight at the time of arrival. After ten days of quarantine in the vivarium, the rodents were transferred to wire mesh colony cages in an air-conditioned room. They were fed Purina laboratory chow and allowed water ad libitum. The rats were ear-tagged, weighed, and randomly placed in groups of 6. Each group occupied a separate compartment in the colony cage. The rate of weight gain observed during this regimen was normal according to sales literature furnished by the supplier.

Primates, *Macaca mulatta*, used in this study were Asiatic imports. They were clinically screened and quarantined before delivery to our facility. Upon arrival, they were again quarantined and standardized for our study.

Standardization included fecal examination for ova and parasites, bacterial culturing for pathogenic flora, tuberculosis screening, and a physical examination for gross abnormalities. The monkeys were housed in our vivarium for the duration of the experiment. The environment in the vivarium was one of constant controlled temperature and humidity, individual caging for animals under study, and a constant supply of water. Primate diet consisted mainly of commercially manufactured monkey biscuits fed twice daily and supplemented by fresh apples twice weekly.

Radioprotective agent

The synthetic compound, S-2-(3-aminopropylamino) ethyl phosphorothioic acid hydrate¹ (WR 2721), was supplied to us by the Walter Reed Army Institute of Research. This compound is soluble in either saline or distilled water. Saline solutions were prepared daily and were passed through a sterile Millipore filter before injection. Table I compares the results of chemical analysis of the compound made at the USAF School of Aerospace Medicine with those performed at the Walter Reed Army Institute of Research. An infrared spectra tracing is shown in figure 1.

¹Referred to hereafter as phosphorothioic acid.

TABLE I

Chemistry of S-2-(3-aminopropylamino) phosphorothioic acid

Parameter measured	USAFSAM* results	WRAIR† results
Melting point	133° - 134° C. (with 1.9 moles H ₂ O)	148° - 150° C. (with 1.1 moles H ₂ O)
Nitrogen	11.27%	11.83%
Phosphorus	Qualitatively	—
Primary amine	Qualitatively	—
Secondary amine	Qualitatively	—
Infrared spectrum peaks (μ)	2.92	2.88
	6.28	5.89
	6.65	6.50
	7.10	7.03
	7.82	7.80
	8.43	8.39
	—	8.97
	9.40	9.30
	10.52	10.39

*USAF School of Aerospace Medicine, Radiobiology Division.

†Walter Reed Army Institute of Research.

Radiation

Gamma radiation from a 5,000 curie ⁶⁰Co source (2) was delivered to groups of 6 rats held in six individual compartments within a revolving, horizontal, wire mesh cylinder. Dose rates for different sets of experiments varied from 68 to 800 R/min. and were measured by ion chamber dosimeters. (Victoreen, 100 R). Dose measurements varying less than 3% from nominal dosage were within acceptable limits of error.

X-irradiation was delivered to primates from a Maxitron 300 machine. This therapy machine was operated at a current of 20 ma. Filtration was accomplished with a standard Al-Cu-Sn filter in a configuration which yields a half-value layer of 2 mm. Cu. The target-to-subject distance was 135 cm. to the midline point of the primate, which was held in a vertical, revolving, wire mesh cylinder. X-irradiation was delivered at a rate of 17.5 ± 0.5 R/min. for a total dose of 850 R.

Dosimetry was accomplished with Victoreen 100 R and 25 R ion chamber dosimeters. The x-ray beam was constantly monitored with a Victoreen rate meter, reading directly from a probe adjacent to the midline of the revolving primate.

III. RESULTS

Rat experiments

A modified Dixon and Mood method, as reported by Kimbali et al. (4), was employed to establish the LD₅₀ and the safe maximum dosages of the agent. This statistical method established the LD₅₀ of phosphorothioic acid to be approximately 416 mg./kg. when injected intraperitoneally in rats. This value is subject to a 5% standard error.

The first radioprotection experiment in rats was attempted with a dose range of 375 to 400 mg./kg. given I.P. 30 minutes before exposure to 1,200 R ⁶⁰Co. These doses were too

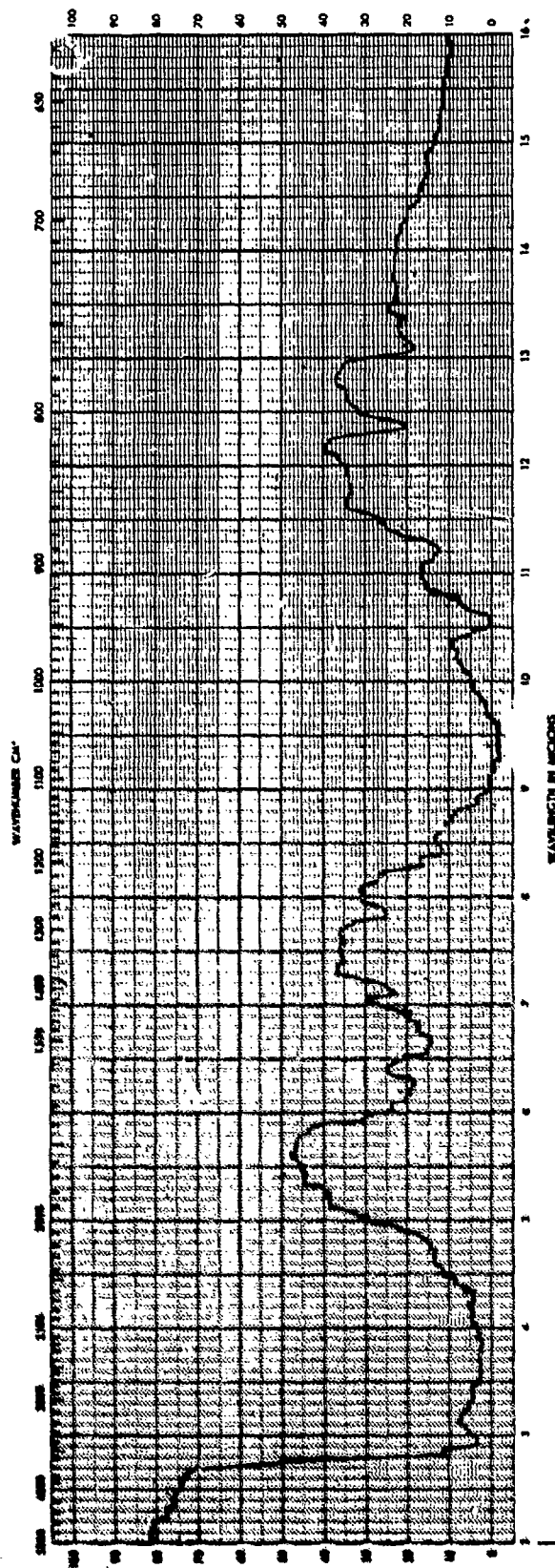


FIGURE 1
Infrared spectrum for phosphorothioic acid.

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TABLE II
Toxicity of phosphorothioic acid in primates

Primate No.	Dosage (mg./kg.)	Result
Administered by stomach tube		
6P7	125	No adverse effect
8P9	250	No adverse effect
3G3	500	No adverse effect
4L9	1,000	Dead 8 days later
Administered intravenously		
P7	50	No adverse effect
8P7	100	No adverse effect
7K9	200	Lightly tranquilized
6G3	400	Deeply tranquilized
4Y7	400	Moderately tranquilized
7Y1	400	Moderately tranquilized, dead 7 days later
9X1	400	Moderately tranquilized, dead 6 days later
7K7	580	Convulsions, extended coma, and complete recovery

toxic and the combined effect of drug toxicity and irradiation proved fatal to all but one rat within 3 days.

The next radioprotection study was done with a dose range of 200 to 250 mg./kg. phosphorothioic acid injected I.P. in rats 30 minutes prior to 1,200 R ⁶⁰Co delivered at 400 R/min. Thirty-day survivors in the 200 mg./kg. group numbered only 5 out of 12, or 42%, with most deaths attributable to irreversible bone marrow damage. These data indicate an insufficient dosage of the radioprotectant. All 12 of the 250 mg./kg. group survived 30 days and were eventually sacrificed. This suggests that 250 mg./kg. is an optimum dose considering both the additive stress of radiation damage and drug toxicity.

Another rat experiment was initiated using two groups of 18 rats each. Both groups were injected I.P. with 250 mg./kg. phosphorothioic acid. One group was exposed to 1,200 R ⁶⁰Co

delivered at 800 R/min. The other group was exposed to 1,200 R ⁶⁰Co delivered at 68 R/min. The first group yielded 67% survival at 30 days postirradiation, while the latter group achieved only 44% survival. These findings suggest that a dose rate effect may be present. All corresponding control rats died from 1,200 R ⁶⁰Co within 9 days. No studies were done in rats to determine whether any other time interval between injection and irradiation was more effective than the 30-minute one. We refer to the work of Storer and Yuhas (5) for additional rodent data on this radioprotective agent.

Primate experiments

Before attempting any radioprotective studies in primates, several monkeys were expended in determining the toxicity of this chemical in primates. Our first study confined itself to administration by stomach tube of doses ranging from 125 to 1,000 mg./kg. Results are shown in table II.

Table II also summarizes results observed when primates were injected intravenously with doses ranging from 50 mg./kg. to approximately 580 mg./kg. Observed symptoms and results are briefly described.

In testing phosphorothioic acid in primates against 850 R x-irradiation, the approximate LD₅₀ radiation dose, injections were made 30 minutes before the start of x-irradiation. Group I (8 primates) received 850 R x-irradiation following a placebo I.V. injection of 5 ml. sterile saline; group II (8 primates) received 850 R x-irradiation following I.V. injection of 400 mg./kg.; group III (9 primates) received 850 R x-irradiation following I.V. injection of 300 mg./kg.; and group IV (6 primates) received 850 R x-irradiation following I.V. injection of 250 mg./kg. Three primates were used as drug controls. They received radioprotectant injections prior to sham-irradiation.

The results of the various groups experiments were as follows:

Group I. Radiation control primates. All 8 primates died between the tenth and fifteenth day post-irradiation. Mean survival time was 12.5 days.

Group 2. *Primates treated with 400 mg./kg. before irradiation.* Between the first and fourth day postirradiation, 5 primates died; 3 survived 30 days. Survival = 3/8.

Group 3. *Primates treated with 300 mg./kg. before irradiation.* During the first day postirradiation, 2 primates died; 7 survived 30 days. Survival = 7/9.

Group 4. *Primates treated with 250 mg./kg. before irradiation.* During the first day postirradiation, 1 primate died; 5 survived 30 days. Survival = 5/6.

Postmortem examinations of group 1 (controls) showed pathology consistent with radiation death. Subcutaneous hemorrhages ranging from petechial to massive coalescing hemorrhages were seen in all primates. Hemorrhages were observed in the gastrointestinal tract, lungs, urinary bladder, kidneys, heart, and peritoneum. In several instances, a large hematoma would cause one or both eyes to be closed, and bleeding from mouth, nose, and rectum was common in the terminal stages.

In group II (400 mg./kg. group), although 3 of 8 primates did survive 30 days, the 5 early deaths showed the effect of drug toxicity plus irradiation stress. Some liver damage had occurred in 30-day survivors as revealed later by necropsies. Pathology reports showed a mild, focal, idiopathic hepatitis. Sections of liver revealed occasional microscopic foci of round cell infiltration surrounding degenerating hepatic parenchymal cells. Bone marrow, spleen, lymph nodes, and gut tissue were unremarkable.

It is of interest to note here that almost simultaneously with the early deaths mentioned above, 2 of the 400 mg./kg. drug controls died evincing similar symptoms. Necropsies were done immediately. The apparent liver damage at 5 to 6 days after injection was evident. Gross examination showed an abnormal liver in both instances. Light tan streaks and areas were found throughout the liver. Histologic examination showed a focal necrotizing hemorrhagic pneumonia of the lung in both instances, and extensive fatty change of the liver was evident. Patchy intralobular necrosis of the liver with microabscess formation was seen. Additionally, there was noted

in one primate a patchy ascending pyelonephritis and an acute nonspecific colitis.

In group III (300 mg./kg. group), 7 out of 9 survived 30 days, but 2 early deaths were seen during the first 24 hours. Histologic examination of one 30-day survivor showed a focal, minimal, interstitial nephritis. Liver, bone marrow, spleen, lymph nodes, and gut tissue were unremarkable.

In group IV (250 mg./kg. group), 5 out of 6 primates survived 30 days, and again one early death was noted during the first 24 hours. No necropsy was performed on the early death, but a necropsy was performed on a sacrificed 30-day survivor. Histologic examination found all tissue mentioned previously to be unremarkable.

IV. DISCUSSION

Our preliminary quantitative and qualitative analysis of S-2-(3-aminopropylamino) ethyl phosphorothioic acid hydrate clearly demonstrated that this compound required constant desiccation at room temperature because of its hygroscopic nature. According to our melting point findings, the desiccated product in our laboratory was a mixture of both dihydrates and monohydrates—the dihydrates being in greater percentage. Other laboratory analyses, including infrared spectra analysis, nitrogen determination, solubility tests, and qualitative tests for phosphorus, primary amine, and secondary amine verified data submitted by Walter Reed Army Institute of Research.

A review of our data on toxicity and pharmacologic effects of phosphorothioic acid in primates indicates that doses which induce only a light tranquilizing effect are safe to use as a radioprotectant. Doses which produce moderate to deep tranquilization are occasionally fatal and uniformly cause death when acute doses of radiation are superimposed.

Our findings of liver and kidney damage are consistent with the findings of other investigators (3). It is possible that our lowest radioprotective dose (250 mg./kg.) is still too

high for optimum results. This is strongly suggested by the fact that 1 out of 6 primates so treated died within 24 hours postirradiation. The ideal radioprotective dose of this agent may be in the range of 200 to 225 mg./kg. body weight.

V. CONCLUSIONS

Further experimentation should be done to elucidate the radioprotective capacity of S-2-(3-aminopropylamino) ethyl phosphorothioic acid hydrate against different kinds of radiation. It does appear that this compound is one

of a few that provides radioprotection to higher mammals (primates) as efficiently as to rodents (mice and rats). If these findings are to be extended to man, effective therapy requires further determination of human toxicity, effective dosage, and optimum routes of medication.

Our findings of 100% survival in at least one group of 12 rats exposed to 1,200 R ^{60}Co , and a survival of 80% (12 out of 15) among primates exposed to 850 R x-rays, are significant when compared with the 100% mortality of primate radiation controls.

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