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GEORGE S. MELVILLE, JR., ET AL

UNCLASSIFIED SCHOOL OF AVIATION MEDICINE, RANDOLPH AFB, TEX.

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DIBROMIDE

SCHOOL OF AVIATION MEDICINE  
RANDOLPH AIR FORCE BASE, TEXAS

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**TOXIC EFFECTS OF S,2-AMINOETHYLISOTHIURONIUM DIBROMIDE**

**GEORGE S. MELVILLE, JR., Captain, USAF  
THOMAS P. LEFFINGWELL, M.A.**

**Department of Radiobiology**

**59-75**

**Air University  
SCHOOL OF AVIATION MEDICINE, USAF  
BROOKS AFB, TEXAS**

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## TOXIC EFFECTS OF S,2-AMINOETHYLISOTHIURONIUM DIBROMIDE

The toxicity and pathologic effects of S,2-aminoethylisothiuronium dibromide (AET-DiBr), a radioprotectant compound, have been studied in rats. The compound is highly toxic to these rodents when given in acute doses, either orally or intraperitoneally. The chronic toxicity and pathology of AET-DiBr has been examined by means of serial intraperitoneal injections. At low doses, large total amounts of the drug can be tolerated. The acute and chronic effects of sodium chloride, sodium bromide, sodium acetate, and sodium nitrate have been tested. Neither the anions nor the cation appears to contribute to the toxicity of isothiuronium salts.

The mercaptoalkylguanidines and related isothiuronium compounds have been shown to afford protection against the lethal effects of x-rays (1,2). Although considerable toxicologic and pharmacologic studies probably have been made with these chemicals leading up to in vivo protection experiments, little data have reached the literature. DiStefano (3) has remarked on the pharmacology of S,2-aminoethylisothiuronium dibromide (AET) in cats. Koch (4) has reported  $LD_{50/30}$  values for several beta-aminoethylthiuronium compounds in mice.

This report summarizes our results on the toxicity of AET in the rat. These data were obtained in the course of radiation protection studies using gamma rays. Since the protective compound cannot be administered without the accompaniment of two moles of anion for each mole of protectant, the contribution of anions to the lethal effects of the protective drug has also been assayed.

### MATERIALS AND METHODS

All animals in these studies were female Sprague-Dawley (Holtzmann) rats weighing between 175 and 250 grams. Rats were caged

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individually and were fed Wayne Lab Blox "R" ad libitum. Animals receiving parenteral treatment were given water ad libitum. Animals receiving oral treatment were given just 25 ml. of water each day. Salts were dissolved in the water.

S,2-aminoethylisothiuronium bromide hydrobromide (AET) was prepared from specially purified reagents according to the method of Shapira (2). Corrected melting points were taken on each preparation and served as the criterion of purity and uniformity.

All salt solutions for parenteral use were prepared with distilled water and C.P. or reagent-grade chemicals.

AET was administered either intraperitoneally or orally (stomach tube) in  $Na_2HPO_4$  - phosphate buffer solution at  $pH 7.3 \pm 0.1$ . For the individually prepared doses, the dry AET-DiBr was weighed, titrated with  $Na_2HPO_4$  solution (0.75 M; pH 8.9) to pH 7.3, and an additional amount of phosphate buffer (pH 7.3) added.

Intraperitoneal injections were limited to 2.5 ml. in size or less; stomach tube doses, to 3 ml. in size or less.

In the chronic AET-DiBr experiments, rats were weighed once a week for the calculation of doses for that week.

## RESULTS

Acute mortality from graded doses of AET given intraperitoneally is shown in table I. No deaths were encountered at or below 0.23 mg./gm. body weight (B.W.), while all rats died from doses of 0.32 mg./gm. B.W. and above.

Acute mortality from graded doses of AET given by stomach tube is shown in table II. No deaths were encountered at or below 0.35 mg./gm. of B.W., while all rats died at doses of 0.50 mg./gm. B.W. and above.

Acute mortality from graded doses of AET given intraperitoneally but not buffered at pH 7.3 is shown in table III. No deaths were encountered at or below 0.30 mg./gm. B.W.,

while all rats died from doses of 0.43 mg./gm. B.W. and above.

Acute mortality from graded doses of bromide ion as sodium bromide is shown in table IV. A dose of 2.74 mg./gm. B.W. or greater is required to cause death or any gross or microscopic pathology.

Acute mortality from graded doses of chloride ion as sodium chloride is shown in table V. A dose of 1.26 mg./gm. B.W. or greater is required to cause death or any gross or microscopic pathology.

In addition, sodium acetate was injected intraperitoneally in rat doses ranging from 100 to 1,000 mg.; 850 mg. was the lowest lethal dose.

TABLE I

*AET-DiBr toxicity in female rats given intraperitoneal injections of compound*

Amount (mg./gm. B.W.)	Number of animals	Alive	Dead (days posttreatment)				
			1	2	3	4	5
0.16	2	2					
0.20	2	2					
0.22	3	3					
0.23	5	5					
0.24	12	10		2			
0.25	5	4			1		
0.26	6	5			1		
0.27	7	6		1			
0.28	8	2	4	1	1		
0.29	7	6		1			
0.30	6	6					
0.31	5	4				1	
0.32	2	0	2				
0.33	2	0	2				
0.35	3	0	3				
0.40	2	1	1				

Sodium nitrate was also injected in rat doses ranging from 100 to 1,000 mg.; 700 mg. was the lowest lethal dose. In these animals, there were no consistent pathologic findings.

Thirty-five rats were used specifically as pathologic controls (5 animals per group) as follows:

A — H <sub>2</sub> O	I.P.	2.25 ml.	
B — Na <sub>2</sub> HPO <sub>4</sub>	I.P.	2.25 ml.	
C — NaBr	I.P.	2.25 ml.	0.219 mg./gm. B.W.
D — AET-DiBr	I.P.	2.50 ml.	0.240 mg./gm. B.W.
E — Na <sub>2</sub> HPO <sub>4</sub>	S.T.	3.00 ml.	
F — NaBr	S.T.	3.00 ml.	0.219 mg./gm. B.W.
G — AET-DiBr	S.T.	3.00 ml.	0.410 mg./gm. B.W.

All animals lived at least 72 hours post-treatment; all deaths were due to sacrifice. Gross and microscopic pathologic studies were performed on the 4th, 6th, 8th, 10th, and 12th

days posttreatment in each group. Neither gross nor microscopic pathology was encountered. It should be noted that since this series was done, an occasional case of hemorrhagic gastritis has been found in animals treated with buffered AET-DiBr by stomach tube.

Chronic mortality from intraperitoneal administration of AET-DiBr at several dose levels is shown in table VI. Chronic oral administration of AET-DiBr is underway but cannot be reported at this time.

Two chronic oral dose levels of sodium bromide (in 25 ml. of drinking water) were given daily for 30 days to two groups of rats — 25 mg./rat and 75 mg./rat. No apparent ill effects or withdrawal symptoms were noted, nor was there any pathology. Sodium acetate and sodium nitrate gave similar results.

TABLE II

*AET-DiBr stomach tube toxicity in female rats*

Amount (mg./gm. B.W.)	Number of animals	Alive	Dead (days posttreatment)				
			1	2	3	4	5
0.25	1	1					
0.30	1	1					
0.35	1	1					
0.36	7	6	1				
0.38	5	5					
0.40	7	5	2				
0.41	11	10	1				
0.42	7	7					
0.425	2	2					
0.45	6	2	1			1	
0.48	5	4	1				
0.50	2	0		2			
0.55	1	0	1				
0.65	1	0	1				
0.75	1	0	1				
0.85	1	0	1				

TABLE III

*AET-DiBr toxicity in female rats given doses of unbuffered compound by intraperitoneal injection*

Amount (mg./gm. B.W.)	Number of animals	Alive	Dead (days posttreatment)				
			1	2	3	4	5
0.12	1	1					
0.25	1	1					
0.30	10	10					
0.35	6	3	3				
0.37	5	1	4				
0.40	2	0	1		1		
0.43	1	0	1				
0.44	1	1					
0.45	2	0	2				
0.48	1	0	1				

TABLE IV

*Br<sup>-</sup> (as NaBr) administration*

Animal No.	Rat dose (mg.)	Mg./gm. B.W.	Dead or sacrificed	Pathology
1	100	0.350	S	None
2	100	0.356	—	—
3	200	0.700	—	—
4	200	0.712	—	—
5	300	1.031	—	—
6	300	1.036	—	—
7	500	1.496	—	—
8	500	1.574	—	—
9	560	2.313	—	—
10	650	2.740	D	None
11	1,000	3.235	S	None
12	1,000	3.235	D	None
13	1,000	3.300	D	None
14	800	3.840	D	None

All doses were given intraperitoneally.

TABLE V  
Cl<sup>-</sup> (as NaCl) administration

Animal No.	Rat dose (mg.)	mg./gm. B.W.	Dead or sacrificed	Pathology
1	30.2	0.124	—	—
2	60.7	0.253	—	—
3	121.2	0.440	—	—
4	121.2	0.462	—	—
5	182.0	0.674	—	—
6	182.0	0.752	—	—
7	242.8	1.000	—	—
8	242.8	1.371	—	—
9	273.2	1.138	—	—
10	273.2	1.264	D	None
11	607.0	2.250	D	None

All doses were given intraperitoneally.

### DISCUSSION

These studies were designed to present necessary basic information for a careful study of the protective effects of several isothiuronium salts in rats and ultimately in primates.

In this light, none of the anions tested would appear to contribute to either the acute or chronic toxicity — which is frequently experienced with the AET-DiBr; neither lethal nor pathologic responses were elicited at dose levels similar to those which might be received as the AET salt in typical protection treatment. When used as a radioprotectant, AET-DiBr is buffered with sodium phosphates or titrated with sodium hydroxide to neutral pH; these data would further indicate, then, that the sodium ion does not contribute appreciably to noted mortality.

It is impressive to note the very limited AET-dibromide concentration gradient which completely encompasses the lethal region. It had been hoped to use the information in tables I and II to calculate the LD<sub>50/30</sub> for acute intra-

peritoneal and oral administration but this was abandoned. Our information also indicates that the material is less toxic when given at an acid pH. This is implied both by table III and by the increased amounts which can be given orally. The amount of the material present as mercaptoethylguanidine is largest at pH values from 7 to 8. Unfortunately, mercaptoethylguanidine appears to be not only the most protective form (2), but also the most toxic.

At lethal dose levels, there appears to be no gross or microscopic pathology; it should be noted that no pathologic studies were made of the central nervous system.

At sublethal dose levels, when AET-DiBr is administered by either the oral or intraperitoneal routes, there appears to be no gross or microscopic pathology of a *systemic* nature. However, there can be local pathology when doses above 0.10 mg./gm. B.W. are administered intraperitoneally (epilation, necrosis of the skin and subcutaneous tissues, tumors, hematomas at site of injection, and some in-

**TABLE VI**  
*Chronic intraperitoneal administration of AET-DiBr*

Group	Number of rats	Daily dose	Number alive after 30 days	Remarks
A	5	0.04 mg./gm. B.W.	5	No pathology.
B	5	0.08 mg./gm. B.W.	5	No pathology.
C	5	0.120 mg./gm. B.W.	3	One animal sacrificed on 10th day, necrotic subcutaneous abdominal abscess; no other pathology. One animal sacrificed on 14th day, same pathologic findings.
C'	5	0.120 mg./gm. B.W.	0 (5 sacrificed)	Two animals sacrificed on 2d day; ulcerative lesions and hematomas due to subcutaneous shots. Three animals sacrificed on 6th day; necrotic lesions due to subcutaneous shots.
D	5	0.200 mg./gm. B.W.	4 (1 sacrificed)	One animal sacrificed on 7th day; this animal had an abdominal tumor attached to left kidney. Several survivors had necrotic areas on abdomen and evidence of subcutaneous tumors.
D'	5	0.200 mg./gm. B.W.	2	Three animals died on 4th day. Two animals sacrificed on 30th day; no significant pathology.
E	5	0.240 mg./gm. B.W.	3	One animal showed calcification on the serosal surface of the bowel. Two animals died on the 4th day.
E'	5	0.240 mg./gm. B.W.	0	Two animals died on 3d day. Three animals died on 4th day.

stances of chemical peritonitis have been observed). Contributory to the local pathology are (a) individual response to the solutions which are of very high ionic strength and (b) inadvertent subcutaneous introduction of minute quantities of the materials.

It has been found that large oral doses of AET-DiBr will cause ataxia, anorexia, gastritis, and hemorrhagic gastritis. The latter condition has not been observed in our rats until 0.42 mg./gm. B. W. is exceeded.

The chronic intraperitoneal experiments summarized in table VI indicate that while AET-DiBr is extremely toxic, very large amounts of the drug can be tolerated when the doses are reduced and given serially.

### SUMMARY

1. The toxicity and pathologic effects of AET-DiBr have been examined in rats. Acute doses have been administered both intraperitoneally and orally. The compound is highly toxic to rats.

2. The chronic effects of AET-DiBr have been examined by means of serial intraperitoneal injections. At low doses, large total amounts of the drug can be tolerated.

3. The acute and chronic effects of sodium chloride, sodium bromide, sodium acetate, and sodium nitrate have been tested. The anions and the cation do not appear to contribute to the toxicity of isothiuronium salts.

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