SCREENING OF NEW CHELATING AGENTS FOR BERYLLIUM

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FOREWORD

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," and Task No. 630202, "Pharmacology and Biochemistry," under Contract No. AF 33(615)-1804 with International Bio-Research, Inc, St Louis, Missouri. Dr med vet Wolfgang Sterner was the principal investigator for International Bio-Research, Inc., and Mr. Leandro Rendon was contract monitor for the Toxic Hazards Branch, Physiology Division. Research was initiated June 1964 and completed March 1965.

The technical assistance of H. Kramer, DVM, and Erika Sterner, Medical Technologist, in the conduct of this work is greatly appreciated.

This technical report has been reviewed and is approved.

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ABSTRACT

Thirteen polyaminopolycarboxylic acid chelating agents were evaluated in vitro and in vivo as potential therapeutic agents for acute beryllium poisoning. In the in vitro test system the ability of the chelating agent to reverse beryllium induced inhibition of alkaline phosphate was measured. In the in vivo system, the chelating agent was evaluated by its ability to prevent mortality of rats that had received an acute lethal dose (LD_{95}) of beryllium sulfate. None of the polyaminopolycarboxylic acids reversed completely the inhibition of alkaline phosphatase at either 35 percent or 65 percent levels of inhibition. All compounds were ineffective in overcoming the toxic dose of beryllium in rats.

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INTRODUCTION

The effectiveness of aurintricarboxylic acid (ATA) in the treatment of acute beryllium poisoning in mice and rats has been described by investigators at Argonne National Laboratory (ref 1, 2). These same investigators demonstrated that ATA reverses the inhibition of rat plasma alkaline phosphatase by beryllium (ref 3).

This study was undertaken to determine (a) whether reversal of the beryllium-induced inhibition of rat plasma alkaline phosphatase might serve as a reliable screening test to disclose compounds that might be useful in the treatment of acute beryllium intoxication and (b) to evaluate a group of polyaminopolycarboxylic acids for effectiveness in counteracting in vitro and in vivo the toxic manifestations of beryllium.

SECTION II

METHODS AND MATERIALS

TEST MATERIALS

Beryllium sulfate tetrahydrate ($BeSO_4 \cdot 4H_2O$) was obtained from Fisher Scientific Company. The in vitro results obtained with this source of beryllium were compared with those using a sample of the same $BeSO_4 \cdot 4H_2O$ (C. P. Eimer & Amend) that was used in the studies of Lindenbaum et al (ref 3). No significant differences could be detected between these two beryllium sources with respect to their effect on alkaline phosphatase activity. Aurintricarboxylic acid (ATA) was obtained from Fisher Chemical Company (A546 Reagent Grade, ammonium salt). Salicylic acid (SA) was obtained from Matheson, Coleman & Bell (CB 748, as sodium salt).

The polyaminopolycarboxylic acids were obtained through the curtesy of Dr. Murray Weiner, Geigy Chemical Corporation, Ardsley, New York. These compounds were available only in small amounts (2 to 5 g each). These acids are coded as follows:

ATA	Aurintricarboxylic acid
SA	Salicylic acid
EDTA	Ethylenediamine-NNN'N'-tetraacetic acid
DAEE TA	β,β-diaminoethylether-NNN'N'-tetraacetic acid
DET PA	Diethylenetriamine-NNN'N'N''-pentaacetic acid
TET HA	Triethylenetetramine-NNN'N'N''N'''-hexaacetic acid
EG(AEE) TA	Ethyleneglycol-bis(β-aminoethylether)-NNN'N'-tetraacetic acid
(HCH)ED TriA	N'-(2-Hydroxycyclohexyl)ethylenediamine-N'NN-triacetic acid
(AE)CHA TA	2-(β-Aminoethoxy)-cyclohexylamine-NNN'N'-tetraacetic acid
(DCMACH)ED TriA	N-[(2-Dicarboxymethylamino)cyclohexyl]-ethylenediamine-NN'N'-triacetic acid
PG(AEE) TA	1,2-Propyleneglycol-bis-(β-aminoethylether)-NNN'N'-tetraacetic acid
2,3-BG(AEE) TA	2,3-Butyleneglycol-bis-(β-aminoethylether)-NNN'N'-tetraacetic acid
1,4-BG(AEE) TA	1,4-Butyleneglycol-bis-(β-aminoethylether)-NNN'N'-tetraacetic acid

TATEA HA . . . . . ββ'-Triaminotriethylamine-NNN'N'N''N'''-hexaacetic acid
TEPA HA . . . . . Tetraethylenepentaamine-NNN'N'N''N'''N''''-heptaacetic acid

TEST ANIMALS

Sprague-Dawley rats were used throughout the study. Female animals weighing approximately 250 g each were used as sources of blood in the in vitro portions of the study. Male rats exclusively were used for the in vivo portions of the study, to avoid complications that might result from interactions between strong chelating agents, inorganic ions, and endocrine functions.

TEST METHODS

Alkaline Phosphatase Determinations

The activity of plasma alkaline phosphatase was measured by the method of Bodansky (ref 4) as modified by DuBois et al (ref 5). This test system involved the incubation of 0.1 ml of plasma from freshly drawn cardiac blood with 1.0 ml of 0.025 M Veronal buffer (pH 8.9) containing 0.015 M sodium β-glycerophosphate and water (or beryllium sulfate and test solutions) to make a final volume of 1.7 ml. After incubation at 38° C for 30 minutes, the reaction was stopped by the addition of 0.3 ml of 50% trichloroacetic acid. After centrifugation, the inorganic phosphate was measured by the method of Fiske and SubbaRow (ref 6).

The inhibition of alkaline phosphatase by beryllium was studied at beryllium concentrations that provided approximately 35 percent and 65 percent inhibition. Reversal of the beryllium-induced inhibition was determined by addition to the in vitro system of a tenfold and twentyfold molar excess of the test compound relative to beryllium. From the measured activity of the enzyme in the presence of both beryllium and the test compound, the percent reversal of the beryllium inhibition was calculated from the formula:

$$100 \left\{ 1 - \frac{\text{\% observed inhibition}}{\text{\% inhibition of Be Control}} \right\}$$

Thus, at a beryllium concentration of 4×10^{-6} M, which produced a 37 percent inhibition of alkaline phosphatase activity, the test chemicals were added to the test system at 4×10^{-5} M and 8×10^{-5} M, a tenfold and twentyfold molar excess, respectively. At beryllium concentrations of 10^{-5} M, which produced a 65 percent inhibition of enzyme activity, test compounds were evaluated at 10^{-4} M and at 2×10^{-4} M (ref 2).

In vivo Toxicity

Animals were individually caged on wood shavings with a standard laboratory ration and water available ad libitum. The animal room was maintained at 23° C and a relative humidity of 50% to 60%. Animals were fasted for 16 hours before administration of the chemicals.

Beryllium sulfate was administered intravenously as a sterile aqueous solution in the tail vein. Injection time in all cases was approximately 15 seconds.

Either aqueous solutions of polyaminopolycarboxylic acids or their solutions after neutralization with equimolar solution of calcium-sodium hydroxide were administered by intraperitoneal injection within 5 minutes after injection of the beryllium sulfate solution.

Two test compounds were administered orally by stomach tube in addition to the intraperitoneal injection.

Data were treated by the method of Litchfield and Wilcoxon to determine the acute toxicities and slopes of mortality curves (ref 7).

SECTION III

EXPERIMENTAL RESULTS

IN VITRO INHIBITION OF ALKALINE PHOSPHATASE ACTIVITY BY BERYLLIUM

The alkaline phosphatase activity of rat plasma was found to be 0.34 units (1 unit = 1 mg P release per gram of tissue or plasma per hour). This value agrees with that observed by DuBois et al (ref 5).

The molar concentration response curve of beryllium on this in vitro system (table I, figure 1) reveals that 6.0×10^{-6} M Be^{++} is required to produce 50 percent inhibition of alkaline phosphatase. This value is approximately 3 times that reported by DuBois (ref 5) and that obtained by extrapolation of the data of Lindenbaum (ref 3). The curve, however, is quite steep, which indicates that relatively small changes in concentration can cause large differences in response. In the test system reported here, a concentration of 3.5×10^{-6} M of Be^{++} appeared to be the 35-percent inhibition concentration while 1.0×10^{-5} M was the 65% inhibition value.

A number of the polyaminopolycarboxylic acids were themselves inhibitors of rat plasma alkaline phosphatase. EDTA, DAEE TA, (DCMACH)ED TriA, PG(AEE) TA, TATEA HA, and TEPA HA all significantly inhibited alkaline phosphatase activity in the concentration range of interest (table II).

The effects of the various chelating agents on beryllium-induced inhibition of alkaline phosphatase are presented in table 3 and summarized in table 4. With the exception of ATA and SA none of the chelating agents was remarkable in its ability to reverse the inhibition by beryllium. DAEE TA, (AE)CHA TA, and TEPA HA were the superior agents in this test. Even in the absence of beryllium each of these agents at the molar concentrations required to provide a tenfold to twentyfold excess of beryllium inhibited the activity of alkaline phosphatase by ten to twenty percent (table II). Since the observed inhibition of enzyme activity in the in vitro system might be an additive, combined inhibition effect of the test chemical and beryllium, it was possible that the in vivo activity of these compounds in reversing beryllium toxicity might be greater than the in vitro data indicated.

TABLE I

EFFECT OF BERYLLIUM ON PLASMA ALKALINE PHOSPHATASE ACTIVITY OF RATS

Molar Concentration of Be^{++}	Percent Inhibition of Alkaline Phosphatase	
	Observed	No. of detm.
1×10^{-6}	9	3
2×10^{-6}	27	6
3×10^{-6}	30	3
4×10^{-6}	37	2
5×10^{-6}	40	8
6×10^{-6}	50	3
1×10^{-5}	64	7
2×10^{-5}	77	6
4×10^{-5}	85	1
5×10^{-5}	87	1
6×10^{-5}	92	1

Observed activity of alkaline phosphatase in fresh rat plasma = 0.34 mg P/ml/hour

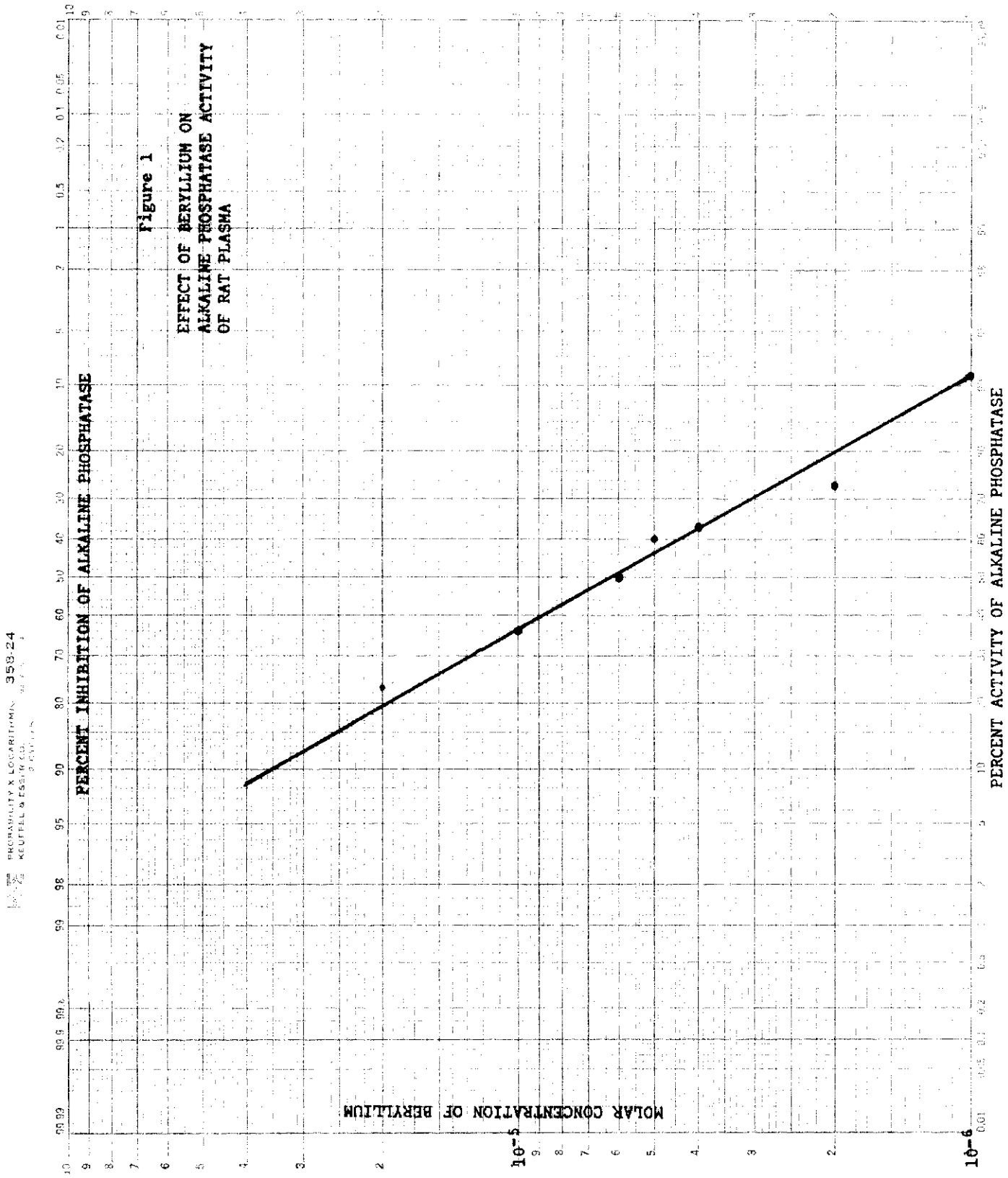

TABLE II

EFFECTS OF VARIOUS CHELATING AGENTS
ON THE IN VITRO ACTIVITY OF RAT PLASMA
ALKALINE PHOSPHATASE

Chelating Agent	Alkaline Phosphatase Activity (% of Control) at		
	1×10^{-4} M	5×10^{-5} M	2×10^{-5} M
ATA	104	108	100
SA	97	100	99
EDTA	82	88	94
DAEE TA	80	101	106
DETPA	90	96	103
TET HA	94	92	100
EG(AEE) TA	104	97	100
(HCH)ED TriA	115	115	112
(AE)CHA TA	90	97	100
(DCMACH)ED TriA	82	97	101
PG(AEE) TA	74	83	90
2,3 - BG(AEE) TA	97	95	101
1,4 - BG(AEE) TA	99	97	100
TATEA HA	81	90	100
TEPA HA	83	88	92

Control: 0.31 units

TABLE III

EFFECTS OF VARIOUS CHELATING AGENTS ON
THE BERYLLIUM INDUCED INHIBITION OF
RAT PLASMA ALKALINE PHOSPHATASE

Chelating Agent	% Reversal of Alkaline Phosphatase Inhibition			
	3×10^{-6} M Be^{++}		1×10^{-5} M Be^{++}	
	Chelate at Molar Excess of		Chelate at Molar Excess of	
	10 x	20 x	10 x	20 x
ATA	72	90	98	113
SA	42	70	76	88
EDTA	26	-4	3	3
DAEE TA	6	37	28	44
DET PA	2	-7	10	6
TET HA	24	-6	18	13
EG(AEE) TA	0	-8	5	4
(HCH)ED TriA	6	-8	14	13
(AE)CHA TA	78	11	19	26
(DCMACH)ED TriA	-6	6	20	36
PG(AEE) TA	13	31	-10	2
2,3 - BG(AEE) TA	9	20	-4	11
1,4 - BG(AEE) TA	22	49	-2	0
TATEA HA	31	22	-6	16
TEPA HA	24	57	28	12

TABLE IV

EFFECT OF POLYAMINOPOLYCARBOXYLIC ACIDS ON
BERYLLIUM INHIBITION OF ALKALINE PHOSPHATASE

	Mean of % Reversal of Inhibition at		% Reversal of Inhibition by Chelate Excess of	
Chelating Agent	3×10^{-6} M Be^{++}	1×10^{-5} M Be^{++}	10 fold	20 fold
ATA	81	106	85	102
SA	56	82	59	79
EDTA	11	3	14	0
DAEE TA	22	36	17	40
DETPA	-2	8	6	0
TET HA	9	16	21	4
EG(AEE) TA	-4	4	2	-2
(HCH)ED TriA	-1	14	10	2
(AE)CHA TA	44	22	48	18
(DCMACH)ED TriA	0	28	7	21
PG(AEE) TA	22*	-4	2	16
2,3-BG(AEE) TA	14*	4	2	16
1,4-BG(AEE) TA	36*	-1	10	24
TATEA HA	26*	5	12	19
TEPA HA	40*	20	26	34

* 4×10^{-6} M Beryllium

ACUTE INTRAVENOUS TOXICITY OF BERYLLIUM

Mortality

The mortality of male rats receiving intravenous injections of beryllium sulfate is presented in table V. Most animals died within 48 hours. Practically all, even at the 0.5 mg of Be^{++}/kg dose died within 14 days.

Clinical Appearance

During injection the animals appeared unduly agitated, as evidenced by squealing and struggling. Some immediately went into deep coma-like depression, resting on side position, with intermittent respiratory arrest and spasmodic breathing. After 5 to 8 minutes, however, most of the animals exhibited normal behavior, although a few were slightly depressed. After 2 days post-treatment the survivors became acutely ill and were in deep depression. Food intake was practically nil. Most of the survivors showed yellowish discoloration of the ears, legs and tails, indicative of jaundice.

Gross Necropsy

Even at the lowest dose (0.35 mg Be^{++}/kg) most of the animals exhibited a yellowish discoloration of the liver. In a number of cases, livers were swollen and spleens were enlarged.

At dosages of 0.51 mg Be^{++}/kg and higher, the same pathologic alterations were noted although jaundice was more pronounced. In addition, there were petechial hemorrhages in various organs: lungs, thymus, intestines and lymph nodes.

In the (10.2 mg Be^{++}/kg) and higher dose groups, additional necropsy findings included pronounced fluid accumulation in the chest cavity (hydrothorax).

LD_{50} Calculations

The mortality data of rats receiving beryllium are plotted in figure 2 and summarized in table V. The 14-day LD_{50} and LD_{95} calculations (ref 7) (see table VI herein) are 0.46 and 1.0 mg Be^{++}/kg, respectively. The LD_{50} value for rats compares with that reported by Aldridge et al (ref 8) (0.53 mg/kg, intravenous) and Cochran et al (ref 9) (0.56 mg/kg intraperitoneal). The LD_{95} value is more than that of 0.7 mg/kg reported by Lindenbaum et al (ref 2).

The 48-hour LD_{50} of beryllium was 2.6 mg/kg (1.5 to 4.6 mg/kg) while the 48-hour LD_{95} was 14.5 mg/kg (6 to 36 mg/kg). Test compounds were evaluated at both the 48-hour LD_{95} of beryllium and at the 0.7 mg/kg dose, reported by Lindenbaum which is, in these experiments, a 14-day LD_{83} acute toxicity.

ACUTE TOXICITY OF POLYAMINOPOLYCARBOXYLIC ACIDS

The available amounts of test chemicals were quite limited (2 to 5 g each). Hence, only a limited number of animals were used to determine the intraperitoneal toxicity of each compound. The results of these experiments are presented in table 7. From these data an estimate was made of the Maximum Tolerated Dose (MTD). In some cases inadequate amounts of the test chemical were available to use the MTD. In those cases doses approaching the maximum

tolerated dose, commensurate with the available amount of chelating agent on hand, were administered. In cases in which all of the rangefinding doses produced some pharmacological effects estimates of the MTD were made. Such estimates were based upon the doses causing mortality and/or other clinical symptoms and frequently represent 0.4 log interval of the lethal dose. In practically all cases, the MTD's for the free acids and the sodium-calcium salts were equivalent.

THERAPEUTIC EFFECTS OF POLYAMINOCARBOXYLIC ACIDS IN BERYLLIUM POISONING

The mortality data presented in table 8 show that none of the compounds was remarkably effective in preventing the toxic effects of beryllium. ATA which reduced the toxicity of Be^{++} from 90% to 55% was the most effective; this effectiveness was achieved only by intraperitoneal administration of the ATA; oral administration of ATA was ineffective.

SA at 200 mg/kg, DET PA at 300 mg/kg, TATEA HA at 200 mg/kg, and TEPA HA at 400 mg/kg, when administered intraperitoneally, reduced the mortality of 0.71 mg/kg of Be^{++} from 90% to 70%. With the limited amount of test chemical and the limited number of animals per group, it could not be determined whether this reduction in mortality was significant.

None of the compounds was effective against 10 mg of Be^{++}/kg. Necropsy of all animals revealed the same gross pathological findings as the "untreated" beryllium control groups.

The results of this study indicate that the polyaminopolycarboxylic acids are rather ineffectual in preventing beryllium-induced mortality. Three compounds, (AE)CHA TA, TATEA HA, and TEPA HA, reduced mortality to about 70% as compared to 90% mortality with no chelating agent. With the exception of (AE)CHA TA in which 20 animals received 400 mg/kg, the reduction of mortality by 2 animals per dose group is of questionable significance. These findings are consistent with the hypothesis of Schubert (ref 10) that compounds, in order to be effective against beryllium, must possess an ortho hydroxy carboxyl structure. ATA and SA both have such structures. The hydroxy and glycol polyaminopolycarboxy acids were ineffective in this study.

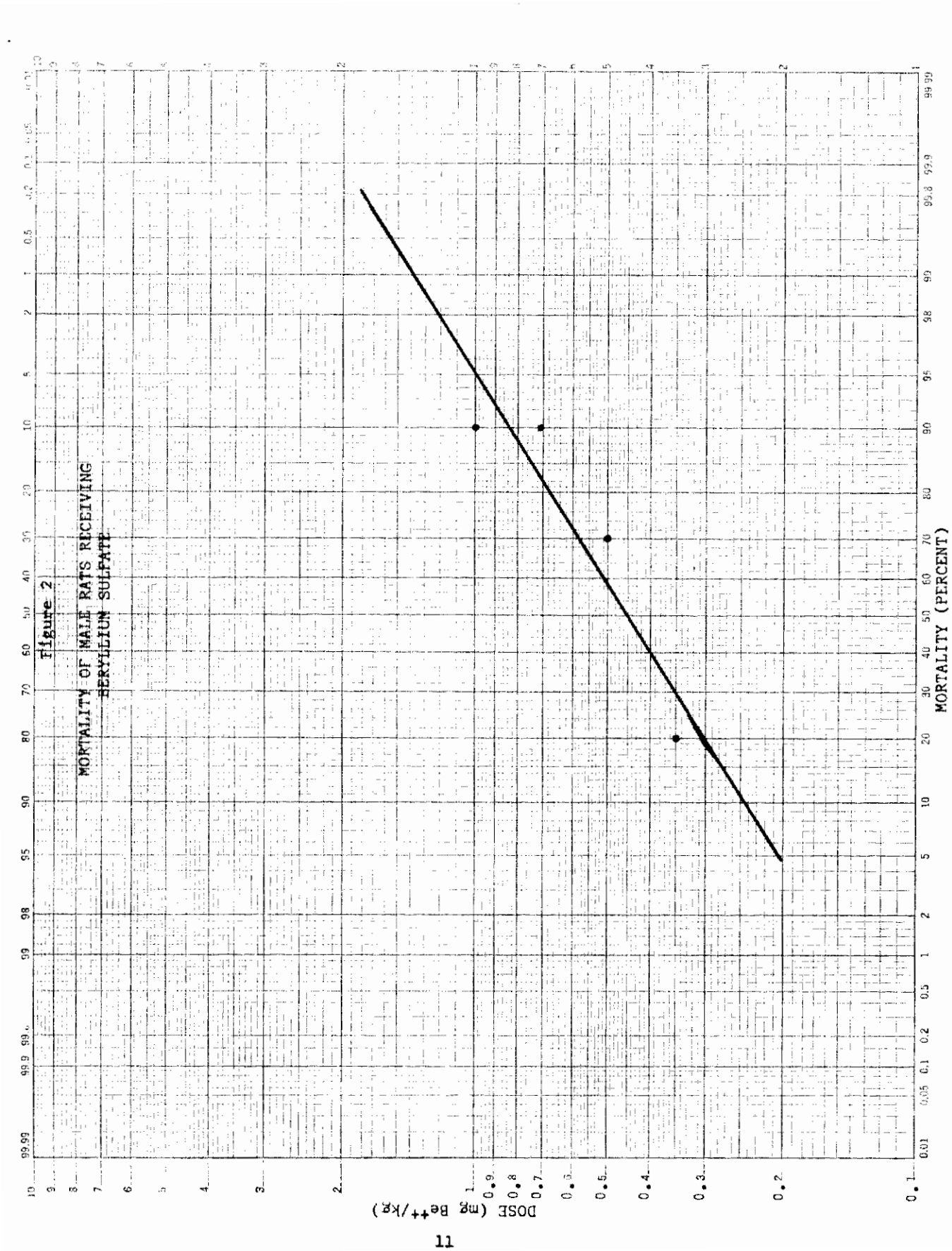

TABLE V

MORTALITY OF MALE RATS RECEIVING AN ACUTE INTRAVENOUS DOSE OF BERYLLIUM SULFATE

Dose (mg/kg)		Range of Body Weights (g)		Mortality at Time (days post injection)								Total	
$BeSO_4 \cdot 4H_2O$	Be^{++}	Initial	Final	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-14	No.	%
6.9	0.35*	116 ± 13	130 ± 12		1					1		2/10	20
9.8	0.50*	118 ± 16	121 ± 4					5		2		7/10	70
14	0.71*	116 ± 15	135				24	18	12			54/60	90
20	1.0 *	114 ± 12	122	1	4	2	2					9/10	90
20	1.0	114 ± 16	128 ± 3	1	4	2	1					8/10	80
40	2.0	116 ± 16	106 ± 4		4	3	1					8/10	80
80	4.1	119 ± 19			7	3						10/10	100
160	8.1	121 ± 12		4	4	2						10/10	100
200	10.2	120 ± 13		4	6							10/10	100
252	12.8	119 ± 14		6	4							10/10	100
318	16.2	120 ± 13		5	5							10/10	100
0	0	123 ± 10	159 ± 11										

* Second Study

TABLE VI

SUMMARY OF LD_{50} ASSAY OF BERYLLIUM

Dosage: 0.35 to 1.0 mg Be^{++}/kg (6.9 to 20 mg $BeSO_4 \cdot 4H_2O$/kg)

Test Chemical: $BeSO_4 \cdot 4H_2O$

Concentration: 1% - 3% in sterile distilled water; approximately 0.1 ml per animal

Animals: Fasted male Sprague-Dawley Rats (100 - 130 g each)

Route of Administration: Intravenous

Symptoms: During injection, animals became unduly agitated. Many animals went into deep depression immediately after injection, with intermittent respiratory arrest and spasmodic breathing; after 5 to 8 minutes, animals acted clinically normal for 24 to 48 hours. Mortalities occurred after 24 to 48 hours in deep depression. Many animals showed signs of jaundice. Necropsy showed enlarged yellow livers, enlarged spleen and jaundice.

	14 days
LD_{50} Be^{++} (mg/kg) + 95% Confidence Limits	0.46 (0.39 - 0.53)
Slope Function-Slope + 95% Confidence Limits	1.64 (1.28 - 2.10)
LD_{95} Be^{++} (mg/kg) + 95% Confidence Limits	1.0 (0.61 - 1.65)

TABLE VII

ACUTE INTRAPERITONEAL TOXICITIES OF VARIOUS CHELATING AGENTS

Test Compound	Dose (mg/kg)	Mortality (No. dead /total)	FINDINGS	Estimated Maximum Tolerated Dose (MTD) (mg/kg)
ATA	207	0/2	Moderate depression for several hours.	150
	261	0/2	Moderate depression for several hours.	
	395	2/2	Deep depression; death within 12 to 24 hours.	
	500	2/2	Deep depression; death after 18 hours, necropsy revealed various amounts of free blood and coagulated blood in the abdominal cavity of most animals.	
SA	50*	0/2	No clinical or pathological symptoms.	200
	100*	0/2	No clinical or pathological symptoms.	
	200*	0/2	No clinical or pathological symptoms.	
	400	2/2	At all levels greater than 200 mg/kg, staggering gait, some animals tonic-clonic convulsions; death after 12 to 16 hours (400 mg/kg), 1 to 2 hours (792 mg/kg), 3 to 6 minutes (1000 mg/kg); necropsy showed whitish discoloration of abdominal cavity content.	
	792	2/2		
	1000	2/2		
EDTA	250	0/2	No clinical or pathological findings.	250
	500	1/1	Depression; tonic-clonic convulsions and death.	
	1000	1/1	Death within 25 minutes; no gross pathological lesions.	
DAEE TA	50*	0/2	No clinical or pathological findings.	125
	100*	0/2	No clinical or pathological findings.	
	125*	0/2	No clinical or pathological findings.	
	250	1/2	With doses of 250 mg/kg a deep depression, sporadic respiration, and death within 20 minutes; no pathological alterations.	
	500	1/1		
	1000	1/1		

TABLE VII (continued)

Test Compound	Dose (mg/kg)	Mortality (No. dead /total)	FINDINGS	Estimated Maximum Tolerated Dose (MTD) (mg/kg)
DET PA	396	0/2	No clinical or pathological findings.	450
	500	0/2	Various degrees of depression; except at the 1000 mg/kg	
	637	0/2	dosage, recovery was complete within 1 to 2 hours, no	
	1000	1/1	pathological alterations.	
TET HA	500	0/2	Depression, sporadic respiration, recovery within 2 hours.	250
	1000	1/1	Agitation, sporadic respiration, collapse; death within 30 minutes.	
	2000	1/1	Agitation; staggering gait; death within 5 minutes.	
EG(AEE) TA	396	0/2	No clinical or pathological findings.	400
	500	1/2	At dosages of 500 mg/kg and higher varying degrees of	
	637	1/2	depression within 1 to 2 hours post treatment; no gross	
	1000	1/1	pathological lesions.	
(HCH)ED TriA	500	0/2	Varying degrees of depression at all doses; at 1000 mg/kg	250
	1000	1/1	dose death within 15 to 20 minutes post treatment; livers	
	2000	1/1	showed white-yellowish discoloration.	
(AE)CHA TA	100*	0/2	No clinical or pathological symptoms.	250
	200*	0/2	No clinical or pathological symptoms.	
	250*	0/2	At all doses, except the 250 mg/kg dose, various	
	500	0/2	degrees of depression immediately after treatment lasted	
	1000	0/1	for 30 to 60 minutes; no gross lesions were found.	
	1260	1/1		
	1587	1/1		
	2000	1/1		

TABLE VII (continued)

Test Compound	Dose (mg/kg)	Mortality (No. dead /total)	FINDINGS	Estimated Maximum Tolerated Dose (MTD) (mg/kg)
(DCMACH)ED TriA	250*	0/2	No clinical or pathological symptoms.	500
	500*	0/2	No clinical or pathological symptoms.	
	1000	1/2	Deep depression immediately, collapse, death; no gross lesions.	
	2000	1/2		
PG(AEE) TA	250	0/2	No clinical or pathological symptoms	250
	500	1/1	Dizziness, depression, circular movements; death after 12 minutes.	
	1000	1/1	Tonic spasms; death within 4 minutes; no gross lesions.	
2,3-BG(AEE) TA	250	0/2	No clinical symptoms.	250
	500	2/2	Depression; intermittent tonic spasms; death within 40 min.	
	637	2/2	Depression; death within 1 hour.	
	793	2/2	Depression; death within 1 hour.	
	1000	2/2	Depression; collapse; death within 20 minutes; no gross pathological lesions.	
1,4-BG(AEE) TA	100*	0/2	No clinical or pathological symptoms.	400
	200*	0/2	No clinical or pathological symptoms.	
	400*	0/2	No clinical or pathological symptoms.	
	500	0/2	Slight depression for 1 to 2 hours.	
	637	2/2	Depression; death within 10 to 14 hours.	
	793	2/2	Depression; collapse; death in 15 to 20 minutes.	
	1000	2/2	Depression; collapse; death within 10 minutes; no gross pathological lesions.	
TATEA HA	2000	0/2	No clinical symptoms; no pathological alterations.	2000

TABLE VII (continued)

Test Compound	Dose (mg/kg)	Mortality (No. dead /total)	FINDINGS	Estimated Maximum Tolerated Dose (MTD) (mg/kg)
	100*	0/2	No clinical symptoms.	
	200*	0/2	No clinical symptoms.	
	400*	0/2	No clinical symptoms.	
TEPA HA	500	0/2	Slight depression; no gross lesions.	400
	637	0/2	Tremors; depression for a few hours.	
	793	0/2	Tremors; depression for a few hours.	
	1000	1/2	Tremors; spastic respiration; depression; death after 12 hours; no gross pathological lesions.	

* Tested with both free acid and calcium-sodium salt.

TABLE VIII

MORTALITY OF MALE RATS RECEIVING CHELATING AGENTS FOLLOWING ACUTELY TOXIC DOSES OF BERYLLIUM

Chelating Agent						Percent Mortality at Day Post-Injection								
Compound	Dose (mg/kg)	Route of Administration	Be^{++} Dose (mg/kg)	Number of Animals	Mean Weight (g)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-14	Total
None	-	-	0.7	60	120 ± 18			10	30	30	20			90
	-	-	10.2	150	115 ± 15	54	46							100
ATA	100	i.p.	0.7	20	116 ± 13	5	15	15	5	5	5		5	55
	400	oral	0.7	10	121 ± 20	10			30	50				90
	150	i.p.	10.2	10	119 ± 8	70	30							100
SA	100	i.p.	0.7	10	121 ± 10	10	10	40			10	10		80
	200	i.p.	0.7	10	119 ± 11			30	40					70
	200	i.p.	10.2	10	117 ± 6	90	10							100
EDTA	200	i.p.	0.7	10	119 ± 10			10	70	20				100
	200	i.p.	10.2	10	119 ± 9	60	40							100
DAEE TA	100	i.p.	0.7	20**	118 ± 7			15	25	40	5			85
	125	i.p.	10.2	10	120 ± 6	90	10							100
DET PA	300	i.p.	0.7	10	115 ± 6			30	40					70
	400	i.p.	10.2	10	119 ± 9	40		50	10					100
TET HA	200	i.p.	0.7	10	118 ± 7			20	50	10	10			90
	250	i.p.	10.2	10	119 ± 5	70		30						100
EG(AEE)TA	300	i.p.	0.7	10	120 ± 8	10		50	30					90
	350	i.p.	10.2	10	119 ± 9	40	50	10						100
HCH(ED) TriA	200	i.p.	0.7	10	115 ± 7		10	50	40					100
	250	i.p.	10.2	10	120 ± 5	70	20	10						100

TABLE VIII (continued)

Chelating Agent Compound	Dose (mg/kg)	Route of Administration	Be^{++} Dose (mg/kg)	Number of Animals	Mean Weight (g)	Percent Mortality at Day Post-Injection 0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-14	Total
(AE)CHA TA	200	i.p.	0.7	10	120 ± 8			20	50	20	10			100
	400	i.p.	0.7	20	118 ± 6		5		25	35				65
	350	oral	0.7	5	123 ± 8			20	20	60				100
	200	i.p.	10.2	10	117 ± 8	40	60							100
(DCMACH)ED TriA	500	i.p.	0.7	10	116 ± 7				90	10				100
	500	i.p.	10.2	10	117 ± 9	70	20	10						100
PG(AEE) TA	250	i.p.	10.2	10	117 ± 5	40	60							100
2,3-BG(AEE) TA	200	i.p.	10.2	10	118 ± 7	10	80	10						100
1,4-BG(AEE) TA	300	i.p.	0.7	10	118 ± 6		30	40	30					100
	400	i.p.	10.2	10	115 ± 9	20	70	10						100
TATEA HA*	200	i.p.	0.7	10	119 ± 6				40	20	10			70
	1000	i.p.	10.2	6	120 ± 3	100								100
TEPA HA	400	i.p.	0.7	10	120 ± 8			10	50	10				70
	400	i.p.	10.2	10	117 ± 5	60	40							100

* insufficient material to administer higher doses.

** Mean of two separate determinations.

TABLE IX

WEIGHT GAIN OF MALE RATS RECEIVING CHELATING AGENTS
FOLLOWING ACUTELY TOXIC DOSES OF BERYLLIUM

Treatment	Number of Animals	2-Week Weight Gain (g/rat)
None	50	35.6
0.71 mg Be^{++} ♦		
100 mg/kg ATA	9	6.8
200 mg/kg SA	3	11.7
400 mg/kg DAEE TA	2	16.0
400 mg/kg AE(CHA) TA	7	10.5
200 mg/kg TATEA HA	3	8.0
400 mg/kg TEPA HA	3	2.0
—	6	3.7

SECTION IV

CONCLUSIONS

None of the polyaminopolycarboxylic compounds were as effective as ATA in preventing the beryllium-induced mortality of male rats. The most effective compound, ATA, was active in both the in vitro system and the in vivo systems. The in vitro reversal of beryllium-induced inhibition of rat serum alkaline phosphatase appears to have merit as a preliminary exclusion screening test for compounds with potential in vivo activity; on the basis of this study, however, a compound which does not cause at least a 50% reversal of alkaline phosphatase inhibition will be of little value in preventing beryllium intoxication in rats.

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13. ABSTRACT

Thirteen polyaminopolycarboxylic acid chelating agents were evaluated in vitro and in vivo as potential therapeutic agents for acute beryllium poisoning. In the in vitro test system the ability of the chelating agent to reverse beryllium induced inhibition of alkaline phosphate was measured. In the in vivo system, the chelating agent was evaluated by its ability to prevent mortality of rats that had received an acute lethal dose (LD_{95}) of beryllium sulfate. None of the polyaminopolycarboxylic acids reversed completely the inhibition of alkaline phosphatase at either 35 percent or 65 percent levels of inhibition. All compounds were ineffective in overcoming the toxic dose of beryllium in rats.

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