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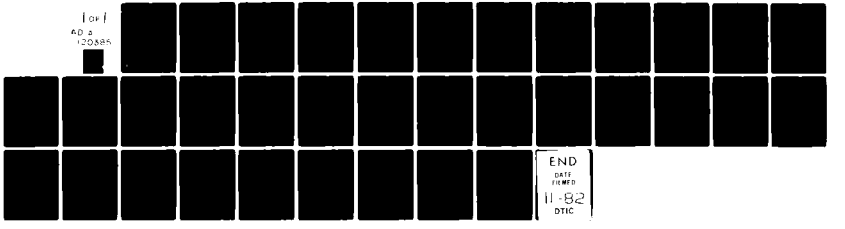

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Prevention and Treatment of Vesication and Poisoning Caused by Arsenicals

Annual Summary Report

H. V. Aposhian

February 1981

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Part I - PROTECTION OF MICE AGAINST THE LETHAL EFFECTS OF SODIUM ARSENITE BY 2,3 DIMERCAPTO-1-PROPANE-SULFONIC ACID AND DIMERCAPTOSUCCINIC ACID (DMSA).

2,3 Dimercapto-1-propane-sulfonic acid (DMPS), used by the Soviets since 1956 and virtually unknown in the United States, is a water soluble analog of British Antilewisite. DMPS and dimercaptosuccinic acid (DMSA) are active orally for the protection of mice against the lethal effects of sodium

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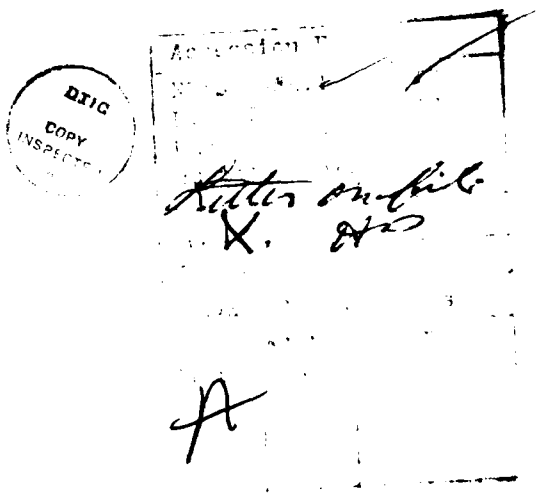
arsenite. They are effective whether given before or after the administration of $NaAsO_2$. Although D-penicillamine and N-acetyl-DL-penicillamine are useful in the treatment of poisoning by other heavy metals, they are devoid of any protective action under these conditions.

Part II. - A QUANTITATIVE COMPARISON OF A NUMBER OF CHELATING AGENTS

The LD50 of $NaAsO_2$ is 0.129 mmol/kg, sc, using white mice. The ip administration of the sodium salt of 2,3 dimercapto-1-propanesulfonic acid (DMPS) or meso-dimercaptosuccinic acid (DMSA) (0.80 mmol/kg) immediately after and 90 min after $NaAsO_2$ increases the LD50 of $NaAsO_2$ about 4.2- and 4.4-fold, respectively. Neither D-penicillamine nor N-acetyl-DL-penicillamine affects the LD50 of $NaAsO_2$ under the same conditions. The LD50 of DMPS and DMSA in mice is 5.22 and 13.58 mmols/kg, ip, respectively. The Effective Dose 50 for treating mice 10 min after receiving an LD100 of $NaAsO_2$ (0.15 mmol/kg) is 0.066 mmol/kg for DMPS and 0.065 mmol/kg for DMSA. The therapeutic index of DMSA against 0.15 mmol/kg $NaAsO_2$ is 209. This is 2.6 times greater than that of DMPS. The explanation for this difference is that although DMSA is as effective as DMPS, it is less toxic. The LD50 of $NaAsO_2$ was not increased by sodium diethyldithiocarbamate, α-mercaptopropionylglycine, DL-N-acetylhomocysteinethiolactone or monomercaptosuccinic acid. A series of polymercapto compounds, some having as many as four mercapto groups per molecule also did not protect against the lethality of $NaAsO_2$. There is extensive experimental and clinical information about DMPS and DMSA available in the Soviet and Chinese literature where these agents are known as Unithiol or Unitiol and succimer, respectively. We have had many of these papers translated for the USAMRDC.

CONCLUSION - It would appear that DMPS and DMSA warrant further experimental studies and eventually clinical trials for the treatment of intoxication by arsenic, especially against lewisite gas. These agents have been used in human therapy in the Soviet Union and China. Soviet investigators and West German investigators have recommended that it replace BAL for treatment of heavy metal poisoning.



SUMMARY

Purpose: to find ways to prevent vesication and poisoning caused by arsenicals.

Major method up to this stage: Protection of mice against the lethal effects of sodium arsenite.

Summary of results: Part I - PROTECTION OF MICE AGAINST THE LETHAL EFFECTS OF SODIUM ARSENITE BY 2,3 DIMERCAPTO-1-PROPANE-SULFONIC ACID AND DIMERCAPTOSUCCINIC ACID

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

A number of metal binding agents have been available in the past for the treatment of heavy metal intoxication. For example, dimercaprol (BAL) (1), polyaminocarboxylic acids (2), D-penicillamine (3), and N-acetyl-DL-penicillamine (4) are used for the treatment of humans intoxicated by arsenic (5), lead (6), copper (3), mercury (7), or other heavy metals. Some of these drugs, however, are far from ideal. Since 1943 in the U.S., BAL has remained the drug of choice for the treatment of arsenic poisoning (5), but it has many disadvantages. It is not effective by mouth; its injection, im, is painful; and toxic reactions to it are not uncommon. In the case of the polyaminocarboxylic acids, most of them are ineffective when given by mouth. D-penicillamine has been life saving in the treatment of the inherited disorder hepatolenticular degeneration (3). After a number of years of clinical use, however, it is now evident that D-penicillamine exhibits serious nephrotoxic signs in some patients (49). Thus, there still remains a need for more specific, less toxic, orally active metal binding agents for use in experimental and clinical situations as well as against arsenic containing chemical warfare agents.

Recently, in Western Europe and the United States, there has been a rediscovery of and an increasing interest in the sodium salt of 2,3 dimercapto-1-propanesulfonic acid (DMPS), a water soluble analog of the lipid soluble BAL. The synthesis and metal binding activity of this compound have been reported by Petrunkin (9) in the Soviet Union. Since then, the activity of DMPS in treating intoxication by a number of different heavy metals has been reported, extensively, in the Soviet, Eastern European, and Chinese literature. It has been shown to be

effective in humans when given either by mouth, injection, or aerosol (10). It has been claimed to be an efficient antidote against mercury (10,11,12), arsenic (13), cobalt (14), organic lead (15), polonium (16), chromium (17), silver (13), and copper (19).

DMPS was unavailable in the West until its recent production by Heyl and Co. Its recent availability has encouraged investigators in West Germany, Norway and the U.S. to study the drug with renewed interest. The studies by Soviet investigators have been confirmed and extended in the case of mercury (20,21,22), arsenic (23), and cadmium (24). A pharmacokinetic study of this dimercapto compound has been reported by Klimova (25) and Gabard (26). The latter paper deals with the absorption and distribution of this water soluble BAL analog. In addition, the extracellular distribution of DMPS is demonstrated.

Another water soluble analog of BAL, meso-dimercaptosuccinic acid (DMSA) was first used in 1954 to increase the uptake of antimony in schistosomiasis therapy (27). The first report of its use to treat occupational intoxication by metals was from Peking and Shanghai in 1965 by Shih-Chun et al. (28). They reported that, in humans, DMSA was as effective as Ca EDTA in the treatment of occupational lead poisoning and as effective as DMPS in the treatment of occupational mercury poisoning, judged by increases in the urinary excretion of the offending metal. Its use in mice for acute and prophylatic treatment of arsenic poisoning was reported from Sverdlovsk by Okonishnikova (29) in 1965. The effectiveness of DMSA for treatment of mercury or lead intoxication has been confirmed and extended by a number of groups (30,31,32) as it has for arsenic (23,33), and for cadmium or zinc (34). Pharmacokinetic studies of ^{35}S-DMSA in the rat have been reported by Okonishnikova and Nirenburg (35) in 1974.

In the present paper, the relative effectiveness of a number of metal binding agents, with particular emphasis on DMPS and DMSA, are evaluated quantitatively by determining their activity in changing the LD50 of $NaAsO_2$ in mice. The therapeutic index of DMPS and DMSA has been determined. In addition, a number of other mercapto compounds, including a series of polymercapto agents, have been tested for their activity in protecting mice against the lethal effects of $NaAsO_2$.

MATERIALS AND METHODS FOR PART I

DMPS, DMSA, BAL, and N-acetyl-DL-penicillamine were purchased from Aldrich Chemical Co. DMPS was obtained also from Heyl & Co., West Berlin. D-penicillamine was a gift of Eli Lilly & Co. MC&B Reagent Grade sodium arsenite was used.

Male albino mice of the TEX: (ICR) strain were purchased from the Timco Breeding Labs, Houston, TX. When used in the experiments, they weighed approximately 25-30 g. Food (Wayne Lab-Blox) and tap water were available ad libitum. However, if the chelating agent was to be given orally, the animals were fasted for the previous 12 hours. The animals were maintained at 22°C with 12 hours of alternating light and dark. The amount of $NaAsO_2$ injected was equal to the approximate LD_{100}. The concentration of the $NaAsO_2$ solution was such that a 25-g mouse received 0.050 ml. The water soluble thiol compounds were dissolved in 0.9% saline immediately before use and the solutions were adjusted to pH 5.5. BAL was dissolved in corn oil. The concentration of the thiol solutions was such that a 25-g mouse received 0.10 ml by the intraperitoneal or oral route. For oral administration, curved 18 gauge oral feeding needles, purchased

from Popper & Sons, New Hyde Park, N.Y., were used. The experiments were performed on different days, with different batches of animals, to confirm and extend the results of previous experiments.

MATERIALS AND METHODS FOR PART II

Animals. Male mice of the Swiss CDL strain (randombred Albino) were obtained from Charles River Mouse Farms, Inc. At the time they were used in the experiments, they weighed approximately 25-30g. Food (Wayne Lab-Blox) and tap water were available ad libitum. The animals were maintained in an air conditioned facility with 12 hrs of alternating light and dark. They were observed and kept for 14 days after the $NaAsO_2$ injection.

Chemicals. DMPS in the form of its Na salt was a gift of Heyl and Co., Berlin. Since each molecule of NaDMPS has a molecule of H_2O associated with it, a molecular weight of 223.2 was used in mol calculations. DMSA and N-acetyl-DL-penicillamine were purchased from Aldrich Chemical Co., Milwaukee, WI. D-penicillamine was a gift of Lilly Research Center, Ltd., Windlesham, Surrey. DL-thioctic acid and monomercaptosuccinic acid were purchased from Cal Biochem. The polymercapto compounds listed in Table 10 were donated by the Evans Chemstries, W. R. Grace & Co., Darien, CT.

Biological studies. The LD50 of $NaAsO_2$ was determined by injecting, sc, various amounts of $NaAsO_2$ dissolved in 0.9% saline. The concentrations of the solutions were prepared so that a 25g animal would receive 0.050 ml. To determine the effectiveness of a compound in protecting against the lethal effects of $NaAsO_2$, the influence of the administration, ip, of that

compound on the LD50 of $NaAsO_2$ was determined. Solutions of the mercapto compounds were prepared immediately before use in 0.9% saline, adjusted to pH 5.5 using NaOH and the concentration adjusted so that a 25g mouse would receive 0.10 ml. DL-thiotic acid was dissolved using a 10% excess of a 5%, freshly prepared solution of $NaHCO_3$. The solution was then brought to volume with 0.9% saline. Injections were made using a 0.25ml glass syringe with a No. 26 needle of 1/2 inch length.

Statistical analysis. Experimental results were analyzed using quantal response methodology. A logistic regression model was used to fit the experimental data and parameters were estimated using the BMDP program package of Dixon and Brown (36) on a CDC Cyber 175 digital computer. Median effective dose and corresponding 95% confidence intervals were estimated following Finney (37).

RESULTS - PART I

None of the mice injected with $NaAsO_2$ and saline survived (Table 1). The deaths occurred within 43 hours after arsenic administration. DMPS and DMSA were found to be potent protective agents against the lethal action of sodium arsenite (Table 1) when either agent is given intraperitoneally immediately after $NaAsO_2$. However, two other well-known, medically useful chelating agents, D-penicillamine and N-acetyl-DL-penicillamine, do not protect (Table 1) under these conditions. The results with these two sulfhydryl compounds are unexpected since there have been three reports of the usefulness of penicillamine in the therapy of arsenic poisoning of humans (38,39,40). However, the clinical reports were based on symptomatic relief. Objective criteria were lacking. None of the metal binding agents

listed in Table 1 is toxic, individually, at these doses, under the conditions of the present experiments (Table 1).

In addition, we have determined that DMPS or DMSA need not be given immediately after $NaAsO_2$. The administration of either one of the compounds can be delayed at least 2 hours and still be effective (Table 2). Of even greater importance for any therapeutic or prophylactic potential is that DMPS or DMSA is effective even when given orally and prior to the administration of the arsenic compound (Table 3). Under the present experimental conditions, they are effective as oral prophylactics against arsenic intoxication.

RESULTS - PART II

DMPS or DMSA increase the LD50 of $NaAsO_2$. The LD50 of subcutaneously administered $NaAsO_2$ was found to be 0.132 and 0.127 mmol/kg in two separate experiments (Table 4). When the data of the two experiments were combined and used to determine the LD50, it was found to be 0.129 mmol/kg. The curve is remarkably steep, having a slope of 40.75, if the proportion survival vs dose model is used. The animals that did not survive usually died within three days after injection. When two ip injections of DMPS (0.30 mmols DMPS/kg/injection) are given, one immediately following and the other 30 min after the $NaAsO_2$, the LD50 of $NaAsO_2$ is increased approximately 4.2-fold to 0.538 mmol/kg (Table 5). Under the same conditions, but using DMSA instead of DMPS, the LD50 of $NaAsO_2$ is increased about 4.4-fold to 0.573 mmol/kg (Table 5). The increase with DMSA is only about 5% more than when DMPS is given. Since the LD50 of $NaAsO_2$ plus DMPS falls within the confidence interval of the LD50 of $NaAsO_2$ plus DMSA, it

appears that the effect of DMPS and DMSA on the LD50 of $NaAsO_2$ is essentially the same under these experimental conditions.

It was also of interest to determine and compare the therapeutic index of DMPS and DMSA. The therapeutic index under these conditions was determined by dividing the LD50 of the dimercapto compound by its ED50. The latter value is defined as the amount of dimercapto compound (mmol/kg) protecting 50% of the animals against the lethal effects of 0.15 mmol $NaAsO_2$ /kg. This dose of $NaAsO_2$, when given, kills 100% of the animals in this laboratory. The LD50 of DMPS, when given ip, was found to be 5.22 mmols/kg (Table 6). For DMSA, the LD50 is 13.53 mmols/kg (Table 7). When mice were given $NaAsO_2$ (0.15 mmol/kg) sc and 10 min later were treated, ip, with different amounts of DMPS, the ED50 was found to be 0.065 mmol/kg (Table 8). The ED50 under these conditions for DMSA was 0.065 mmol/kg. The therapeutic index for DMPS or DMSA under these conditions was 79 and 209, respectively. When the DMPS or DMSA was given 35 min after the $NaAsO_2$, the therapeutic index was found to be 36 and 115, respectively.

Other mercapto compounds. Other metal binding agents were also tested for their activity in protecting against the lethal effects of $NaAsO_2$. Neither D-pen nor N-Ac-DL-Pen changes the LD50 of $NaAsO_2$ significantly at the 95% level of significance (Table 9). Other agents (data not shown) that were also found to be ineffective in this respect are the sodium salt of diethyldithiocarbamate, α-mercaptopropionylglycine, DL-N-acetylhomocysteinethiolactone, and monomercaptosuccinic acid.

In general, it has been accepted that a compound with significant antidotal action for arsenic toxicity should have 2 thiol groups. A series of polymercapto compounds having from 2 to 4 mercapto groups per molecule have been obtained and tested for protecting mice against $NaAsO_2$. None of

the 7 polythiol compounds (Table 10) were active in protecting mice against an approximate LD100 of $NaAsO_2$.

DISCUSSION

The assay of agents that bind and/or mobilize heavy metals can be based on a number of different measurable responses. The basis of one type of assay is the prevention or reversal of the lethal or toxic effects of the particular heavy metal. A second assay is based on the increased excretion of the metal by the putative metal binding agent. There is, however, increasing evidence that supports still another mechanism. Namely, a metal binding agent sometimes forms an insoluble metabolically-inert complex with the metal. The complex, because of its insolubility, is not excreted from the body. It remains in the cell, metabolically-inert and non-toxic. Therefore, it is possible that some metal binding agent might be life saving without increasing the excretion of the metal. This mechanism has been proposed by Catsch and Harnuth-Hoene (41) to explain the effectiveness of N-acetyl-DL-penicillamine. For these reasons we chose, as the basis of the assay used in the present work, the prevention of the lethal action of $NaAsO_2$. Eventually a quantitative comparison will be made of these agents as to their influence on the excretion of ^{74}As.

The results of the experiments reported in this paper clearly show the beneficial effects of DMPS and DMSA in protecting against the lethal effects of $NaAsO_2$. Either compound increases by about fourfold the LD50 of $NaAsO_2$ (Table 2). The ED50 of the two dimercapto compounds is approximately the same when given 10 min after $NaAsO_2$ (Table 8). A definite difference exists, however, between the LD50 of 5.22 mmols/kg for DMPS and

13.53mmols/kg for DMSA (Tables 3 and 4). This is the reason that the therapeutic index for DMSA is about 2.6 times greater than that for DMPS under these conditions (Table 5). The therapeutic index of either DMPS or DMSA, 70 and 200, respectively, under these conditions, however, is not small. It is conceivable that the therapeutic index might be even greater if smaller doses were given more frequently. The ED50 of DMPS given 10 min or 35 min after $NaAsO_2$ are essentially similar to each other as well as being similar to the ED50 of DMSA administered 10 min after $NaAsO_2$. All of these differed from the ED50 of DMSA given 35 min after $NaAsO_2$ indicating that the pharmacokinetic properties of DMSA must differ from those of DMPS.

While an excellent extensive toxicological study of DMPS in rats has been reported by Planas-Bohne et al. (42), we are not aware of published work dealing with the toxicology properties of DMSA. Such work would be of further value in comparing the efficiency of these two useful dimercapto compounds.

We have not studied BAL under these same conditions since it is lipid soluble while DMPS and DMSA are water soluble. However, BAL is approximately 7 times more toxic than DMPS and 19 times more toxic than DMSA, based on the BAL LD50 of 0.726 mmol/kg, ip (43) and the data of this paper (Tables 6 and 7). Also, Hauser and Weger (44) have estimated BAL to be about 15 times more toxic than DMPS when each was given im to mice. They have also studied the influence of BAL and DMPS on the treatment of arsenic poisoning in mice.

It is of interest to note that the LD50 of DMPS, ip, in mice, as reported in this paper (Table 6) is 5.22 mmols/kg and is comparable to the value of 5.57 mmols/kg obtained by Kostygov (45) and 5.02 mmols/kg, ip, in rats, as reported recently by Planas-Bohne et al. (42).

The DMSA LD50, ip, in mice was found to be 13.53 mmols/kg (Table 7) and compares favorably with 12.1 mmols/kg, ip, found in mice by Shih-Chun et al. (23) in Shanghai and Peking and 14.0 mmols/kg determined by Matsuda (46) in Japan. An LD50 in excess of 16.5 mmols/kg has been reported by Friedheim and Corvi (47). It is not clear whether this latter higher value is due to a difference in the mouse strains used or is due to a higher purity of DMSA.

It is pertinent to point out that the meso form of DMSA has been used in the present study and in most of the published reports concerning DMSA. There is, however, one exception. DL-DMSA has been reported to be more active than meso-DMSA in causing an increase in the excretion of ^{203}Hg, ^{115}Cd, and ^{65}Zn when given to male rats challenged by these metals (34).

In conclusion, DMSA and DMPS are very effective agents for protecting mice against the lethal effects of $NaAsO_2$. Limited published reports from the Soviet Union and mainland China have dealt with their effectiveness in humans intoxicated by heavy metals. DMSA has recently been shown by western investigators to be effective in treating lead intoxication of humans (48). Further human studies of these two dimercapto compounds is not unreasonable and should be encouraged.

These agents should be tested experimentally as antidotes for lewisite poisoning which our group is now planning with the Aberdeen BRL.

Table 1. Protection by DMPS and other thiols against the lethal effects of sodium arsenite. The $NaAsO_2$ (0.14 mmoles/kg) was injected subcutaneously in the right rear leg. The chelating agent was administered ip immediately after the $NaAsO_2$. The chelating agents at these doses were not toxic, per se, as shown by the following data. When saline instead of $NaAsO_2$ was given, there was 100% survival of animals receiving the following compounds (mmoles/kg) ip: DMPS (0.80); BAL (0.25); DMSA (0.25); D-Pen (0.80); N-Ac-DL-Pen (0.80). There were at least 12 animals in each group.

Thiol Compound (mmoles/kg) ip	Cumulative 21-day survival No. surviving/No. started				Survival %
	Exp I	Exp II	Exp III	Exp IV	
(Saline)	0/12	0/12	0/12	0/12	0
0.80 DMPS	12/12	8/8	12/12		100
0.40 DMPS	12/12				100
0.25 DMPS	12/12	12/12			100
0.14 DMPS		12/12	9/12		87.5
0.07 DMPS			8/12	11/12	79
0.25 BAL	11/12			11/12	92
0.14 BAL		1/12	1/12		8
0.25 DMSA	12/12			12/12	100
0.14 DMSA		12/12	8/12		83
0.07 DMSA			6/12	10/12	67
0.80 D-Pen			0/12		0
0.25 D-Pen		0/12			0
0.80 N-Ac-DL-Pen			0/12		0
0.25 N-Ac-DL-Pen		0/12			0

Table 2. Experimental therapy with DMPS or DMSA can be delayed after arsenic poisoning. All animals received $NaAsO_2$ (0.14 mmoles/kg) subcutaneously in the right rear leg. DMPS and DMSA were given ip. At the start of the experiment, when $NaAsO_2$ was given, there were 10 animals in each group. However, in three of the experimental groups, one animal died before DMPS or DMSA was administered. Therefore, those groups are listed with 9 instead of 10 started.

Dithiol and time after $NaAsO_2$ it was given	Cumulative 21-day survival No. surviving/No. started		Survival %
	Exp I	Exp II	
(Saline)	0/10	0/10	0
0.25 DMPS			
at 60 min	9/10	7/9	84
at 90 min	9/10	9/9	95
at 120 min	8/10	9/10	85
0.25 DMSA			
at 60 min	7/9	8/10	79
at 90 min	9/10	10/10	95
at 120 min	5/10	6/10	55

Table 3. Prophylactic and oral activity of DMPS or DMSA. The $NaAsO_2$ (0.14 mmoles/kg) was administered subcutaneously in the right rear leg. DMPS or DMSA was given orally fifteen minutes prior to the $NaAsO_2$. The survival of control animals receiving 1.0 mmoles of DMPS per kg and saline, instead of $NaAsO_2$, was 100%.

Thiol Compound (mmoles/kg) oral	Cumulative 21-day survival No. surviving/No. started			Survival %
	Exp 1	Exp 2	Exp 3	
(saline)	0/8	0/10	0/10	0
1.0 DMPS	8/8	8/10		89
0.75 DMPS		8/10		80
0.50 DMPS		6/10	10/10	80
0.25 DMPS		10/10	7/10	85
0.12 DMPS		0/10		0
1.0 DMSA	8/8			100
0.50 DMSA			10/10	100
0.25 DMSA			8/10	80
0.12 DMSA			4/10	40

TABLE 4

LD50 OF SODIUM ARSENITE IN THE MOUSE

NaAsO (mmol/kg,sc)	Exp. 1 Dead / Started	Exp. 2 Dead / Started	Summation Dead / Started
0.08	0/8	---	0/8
0.09	0/8	---	0/8
0.10	0/8	0/12	0/20
0.11	0/8	---	0/8
0.12	1/8	2/12	3/20
0.13	3/8	7/12	10/20
0.14	7/8	12/12	19/20
0.16	---	12/12	12/12
LD50 (mmol/kg)	0.1315	0.1274	0.1290
95% Confidence Interval	(0.122,0.260)	(0.080,0.131)	(0.125,0.139)

2 In subsequent tables of this paper, the data represent the combined results of a number of separate experiments. The combined results were used to calculate the LD50 listed in the tables. This has been done to take advantage of the larger number of animals, resulting from combination of the data, for calculation of median doses and statistical evaluation of data. In addition, it also saved space in this paper. The reason for the number of animals in some groups differing from the number in other groups in the same table is that very often the combined data are the result of from 2-4 separate experiments in which different numbers of animals were used in each experiment. Otherwise, the experiments were performed under identical conditions.

TABLE 5

2,3 DIMERCAPTO-1-PROPANE SULFONATE OR MESO-DIMERCAPTOSUCCINIC ACID INCREASES THE LD50 OF SODIUM ARSENITE[a]

$NaAsO_2$ (mmol/kg,sc)	DMPS	DMSA
	Dead / Started	Dead / Started
0.35	0/12	2/24
0.40	5/24	3/24
0.45	0/12	3/36
0.46	2/12	----
0.50	3/24	5/24
0.55	13/24	11/36
0.60	18/24	15/36
0.65	----	10/12
0.70	23/24	33/36
0.75	----	12/12
LD50 (mmol/kg)	0.538	0.573
95% Confidence Interval	(0.492,0.590)	(0.443,0.703)

[a]DMPS or DMSA, 0.30 mmol/kg, was given, ip, immediately after and 30 mins after $NaAsO_2$.

TABLE 5

LD50 OF DIMERCAPTOPROPANESULFONATE IN MICE

DMPS (mmols/kg,ip)	Dead / Started
3.3	0/3
4.0	0/3
5.0	7/16
5.5	5/3
6.0	7/3
6.6	15/16
7.0	3/3
9.9	3/3
LD50 (mmols/kg)	5.22
95% Confidence Interval	(4.35, 5.51)

TABLE 7

LD50 OF MESO-DIMERCAPTOSUCCINIC ACID IN MICE

DMSA (mmols/kg, ip)	Dead / Started
6.0	0/32
12.0	3/32
13.0	6/12
14.0	9/12
16.0	19/24
18.0	17/20
24.0	32/32
LD50 (mmols/kg)	13.58
95% Confidence Interval	11.35, 15.22

TABLE 8

DETERMINATION OF THE ED50 AND THERAPEUTIC INDEX OF 2,3-DIMERCAPTO-1-PROPANE SULFONIC ACID, NaSALT, AND MESO-DIMERCAPTOSUCCINIC ACID WHEN GIVEN 10 MINUTES OR 35 MINUTES AFTER 0.15 MMOLS $NaAsO_2$/KG

Dimercapto Agent	DMPS +10 min	DMSA +10 min	DMPS +35 min	DMSA +35 min
(mmol/kg, ip)	number surviving/number started			
0.010	----	0/24	----	0/12
0.015	0/36	----	3/36	----
0.030	1/36	5/24	7/36	1/30
0.040	----	6/24	----	----
0.045	6/24	----	8/24	----
0.050	----	10/24	----	----
0.060	6/24	13/24	18/24	6/38
0.0675	15/24	----	----	----
0.070	----	9/23	----	----
0.075	21/24	----	16/24	----
0.080	----	18/24	----	6/12
0.090	20/24	----	15/24	8/10
0.100	----	----	----	16/28
0.105	31/36	----	30/36	----
0.120	35/36	----	34/36	8/12
0.125	----	21/24	----	13/17
0.150	----	----	----	21/30
0.160	----	----	----	6/30
0.200	----	----	----	37/46
0.300	----	----	----	35/38
ED50 (mmol/kg)	0.066	0.065	0.061	0.118
Confidence Interval	(0.059-0.072)	(0.040-0.086)	(0.048-0.072)	(0.071-0.164)
Therapeutic Index	79	209	85	115

TABLE 9

NEITHER D-PENICILLAMINE NOR N-ACETYL-DL-PENICILLAMINE INCREASES THE LD50 OF SODIUM ARSENITE[a]

	none	D-Pen	N-Ac-DL-Pen
$NaAsO_2$ (mmols/kg, sc)	Dead / Started	Dead / Started	Dead / Started
0.10	0/12	0/3	0/3
0.12	2/12	5/3	1/3
0.13	7/12	7/3	5/3
0.14	12/12	3/3	4/3
0.16	12/12	3/3	3/3
0.20	---	3/3	3/3
LD50 (mmol/kg)	0.127	0.119	0.133
95% Confidence Interval	(0.080,0.131)	(0.073,0.191)	(0.054,0.142)
Combined LD50 (mmol/kg)		0.125	
95% Confidence Interval		(0.1171,0.1313)	

[a]D-pen or N-Ac-DL-pen (0.30 mmols/kg) was given, ip, immediately following and at 30 min after the metal binding agent.

TABLE 10

POLYTHIOL COMPOUNDS THAT DO NOT PROTECT AGAINST THE LETHAL EFFECTS OF 0.14 MMOL SODIUM ARSENITE PER KG[a]

Sulfhydryl Compound Tested	Chemical Structure	Dose (mmol/kg)
Pentaerythritol tetrathioglycolate	$C(CH_2OOCCH_2-SH)_4$	0.50
Trimethylol propane trithioglycolate	$CH_3CH_2C(CH_2OOCCH_2-SH)_3$	0.50
Glycol dimercapto-propionate	$H_2COOCCH_2CH_2-SH$ $H_2COOCCH_2CH_2-SH$	0.25
Glycol dimercapto-acetate	$H_2COOCCH_2-SH$ $H_2COOCCH_2-SH$	0.50
Trimethylolethane trithioglycolate	$CH_3C(CH_2OOCCH_2-SH)_3$	0.50
Pentaerythritol tetra-(3-mercaptopropionate)	$C(CH_2OOCCH_2CH_2-SH)_4$	0.50
Trimethylopropane tri-(3-mercaptopropionate)	$CH_3CH_2C(CH_2OOCCH_2CH_2-SH)_3$	0.50

[a]Each polythiol compound was dissolved in peanut oil and administered ip at the dose indicated. $NaAsO_2$ was given sc. The mortality of animals receiving $NaAsO_2$ (0.14 mmol/kg) plus peanut oil or $NaAsO_2$ plus the indicated thiol compound was 100%. There was 0% mortality for animals receiving any one of the sulfhydryl compounds at the dose stipulated but in the absence of $NaAsO_2$. There were 3 mice per group.

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GLOSSARY

Abbreviations:

DMPS	2,3 dimercapto-1-propane-sulfonic acid, Na salt
DMSA	dimercaptosuccinic acid
BAL	British Antilewisite or 2,3-dimercaptopropanol
D-pen	D-penicillamine
N-Ac-DL-Pen	N-acetyl-DL-penicillamine

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