CLINICAL USES OF 2,3-DIMERCAPTOPROPANOL (BAL). I. THE SYSTEMIC TREATMENT OF EXPERIMENTAL ARSENIC POISONING (MAPHARSEN, LEWISITE, PHENYL ARSENOXIDE) WITH BAL[1]

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INTRODUCTION

There is now a convincing body of evidence that the toxic effects of arsenicals are referable primarily to the fact that they combine with -SH groups in the tissues, and thus block one or more physiologic sytems vital to the cellular economy. The toxic action of 3-amino-4-hydroxyphenyl arsenoxide against both trypanosomes and rats has been shown to be diminished or abolished by cysteine or glutathione (1 to 3). Cysteine also delayed the toxic action of sodium arsenite in mice (4); and a similar inhibition of the neurotoxic action of sodium arsenite was observed *in vitro* (5). It was further shown (6, 7, 7a) that the toxic effects of arsenicals were not only prevented, but could actually be reversed by -SH compounds. Protozoa (*Colpidium*) already immobilized by diphenyldichloroarsine, and spirochetes (*S. pallida*) immobilized by phenyl arsenoxides, were resuscitated on the addition of monothioethyleneglycol and cysteine respectively.

The foregoing observations were indicative of the strong affinity of arsenicals for -SH groups, as already noted by Ehrlich (8), but did not necessarily prove that arsenicals owed their toxic effects to the fact that they combined with -SH groups in tissue. The missing link in this chain of evidence was provided by the observation that when arsenicals combined with tissue proteins, the reactive -SH groups of the latter simultaneously disappeared (9 to 11). The widely varying systemic toxicity of a series of phenyl arsenoxides was shown to be correlated with, and probably determined by, the varying degree to which they were bound by the host tissues (12); and a high degree of correlation was noted (13) between the trypanocidal action of phenyl arsenoxides and the extent to which they were bound by the trypanosomes. Finally, a series of enzyme proteins containing free -SH groups were shown to be reversibly inactivated by arsenicals *in vitro,* with the disappearance of the titratable -SH groups (14, 15). The implication that the toxic action of arsenicals is referable to the inactivation of similar -SH-containing enzyme proteins in living cells is clear.

Although monothiol compounds had been found to protect against the toxic action of some arsenicals, and in isolated cases actually to reverse that toxic effect, the antidotal action was not regular. Cysteine and glutathione delayed, but did not prevent, the toxic action of sodium arsenite on the medullated nerves of frogs, measured either by their respiration or by their action potential (5). Moreover, once the action current had been abolished, even a large excess of sulfhydryl compound failed to induce recovery. Similarly, monothiols in general failed to prevent the inhibition by sodium arsenite or Lewisite of the pyruvate oxidase system in a pigeon brain "brei" (15, 16), or the respiration of rat skin slices (16), and failed also to protect human or rat skin from the vesicant action of Lewisite (17, 18). Even with the trivalent aromatic arsenicals studied by Voegtlin and his co-workers and by Eagle, a 10- to 40-fold excess of cysteine or glutathione was required to prevent toxic effects *in vitro;* the latter compound was most effective *in vivo* if administered immediately before the arsenical (2); and if even a few minutes were allowed to elapse between the injection into rabbits of a lethal dose of the 3-NH_2-4-OH-phenyl arsenoxide and a following injection of cysteine or glutathione, the latter had no protective action (Table I).

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TABLE I

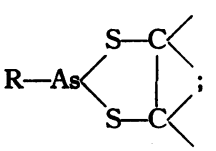
The protection of rabbits against an acute lethal dose of mapharsen by a single intravenous injection of BAL, and the absence of such protection with cysteine or glutathione

—SH compound used to detoxify mapharsen		Outcome		Protective dose of —SH compound		
		Dead	Survived	$PD_{>95}$	PD_{50}	
	mgm. per kgm.			*mgm. per kgm.*	*mgm. per kgm.*	
Cysteine	800	2	0	>800	>800	No protection even with doses approaching the lethal level, and representing a 15- to 60-fold molar excess relative to the arsenical.
	400	6	0			
	200	4	0			
Glutathione	400	2	0	>400	>400	
	300	1	0			
	200	3	0			
BAL	36	0	4	25 (0.20 millimols per kgm.)	7.5 (0.06 millimols per kgm.)	A lethal dose of mapharsen was neutralized *in vivo* by 1 to 3 molar equivalents of BAL.
	24	0	3			
	12	1	2			
	6	2	1			
	2.4	2	1			
0 (Controls: No —SH compound)*	0	5	0			

Rabbits received 20 mgm. per kgm. (0.008 millimols per kgm.) of mapharsen intravenously (LD_{50} = 13 mgm. per kgm.; $LD_{>95}$ = 17 mgm. per kgm.). Five minutes later, the indicated amount of BAL (NDR133-11), cysteine or glutathione in 0.85 per cent NaCl at pH 7.4 was also injected intravenously. The LD_{50} value of this particular lot of BAL on intravenous injection in rabbits was 40 to 50 mgm. per kgm.

* Control animals, receiving no —SH compound, died in 30 to 60 minutes. In animals receiving cysteine and glutathione, death was not significantly delayed. In the case of animals receiving BAL, those listed as "survived" were alive and well 30 days after treatment. In most of those which died despite BAL, death was significantly delayed, the time varying between 20 minutes and 8 days after treatment.

An important and fruitful advance was made with the discovery (11) that when Lewisite reacted with keratin, approximately 75 per cent of the bound arsenic was in combination with two thiol groups. This suggested the formation of a relatively stable ring structure such as



and it seemed possible that "the high toxicity of trivalent arsenicals might be due to their ability to combine with essential -SH groups in certain tissue proteins to form stable arsenical rings" (15). Conversely, in a search for compounds capable of acting as decontaminants or antidotes for toxic arsenicals, Stocken and Thompson reasoned that "simple dithiol compounds might form relatively stable ring compounds with Lewisite or other trivalent arsenicals, and might consequently compete effectively with the dithiol proteins in the tissues" (15). Accordingly, a large series of dithiol compounds was prepared and tested with respect to (*a*) reactivity with arsenicals and the stability of the formed thioarsenites (16), (*b*) ability to protect enzyme systems from the toxic effects of Lewisite and other toxic arsenicals (17, 18), and (*c*) protective action on local application to a skin area contaminated with vesicant or even lethal amounts of Lewisite (18, 19). These extensive studies, summarized in part in two recent reviews (20, 21) brilliantly confirmed the original thesis. The cyclic thioarsenites formed by the interaction of simple dithiols and trivalent arsenicals proved far more stable than those formed by monothiols or by the interaction of tissue proteins and dithiols; and presumably in consequence of that stable ring structure, these dithiols could successfully compete with tissue proteins for such toxic arsenicals as Lewisite or phenyldichloroarsine. Enzyme systems were protected *in vitro* even 1 hour after the introduction of an arsenical vesicant; and local decontamination of the skin or eye with a dithiol prevented or minimized the appearance of local toxic manifestations.

Of the numerous dithiol compounds prepared and tested by Stocken and Thompson and their coworkers, one in particular, the 2,3-dimercaptopropanol,

$$\begin{array}{l} CH_2CHCH_2OH \\ \quad|\quad| \\ \quad SH\ SH \end{array},$$

recommended itself as a local decontaminant in combating the arsenical blister gases. This compound, generally known as "BAL" (British anti-Lewisite) was compounded in ointment form for use on the skin and eyes, and the value of such preparations was abundantly demonstrated in numerous experimental studies.

THE EFFICACY OF BAL (IN AQUEOUS OR PROPYLENE GLYCOL SOLUTION) IN THE SYSTEMIC TREATMENT OF EXPERIMENTAL ARSENIC POISONING

The early use of BAL was limited to its local application in the treatment of experimental Lewisite burns, or of the arsenical dermatitis resulting from antisyphilitic treatment. It seemed clear, however, that the drug so applied had a systemic as well as local action. Thus the application of BAL to a skin area of rats 15 minutes after its contamination with Lewisite resulted in a 4-fold increase in the urinary excretion of arsenic (22). Similarly, the urinary excretion of arsenic increased in human cases of exfoliative dermatitis caused by mapharsen, and treated by the local application of BAL ointment (23). Moreover, BAL ointment was therapeutically effective in such cases when applied to normal skin distant from the lesion. These results clearly indicated that BAL was absorbed through the skin in sufficient quantity to have a systemic effect; and it seemed likely that BAL given by injection might have a therapeutic effect in the treatment of arsenic poisoning.

This was borne out by the finding (19) that aqueous solutions of BAL (50 to 60 mgm. per kgm.) injected intraperitoneally into rats 1 to 2 hours after the skin application of a lethal dose of Lewisite saved 67 to 83 per cent of the animals, and that all were saved by an initial dose of 60 to 70 mgm. per kgm., followed by a second smaller dose 3½ hours later.

Our initial experiments on this point (24) dealt with animals poisoned by the systemic administration of mapharsen. As shown in Table I, rabbits injected intravenously with a single large dose of mapharsen (20 mgm. per kgm., or 0.08 millimols per kgm.), and which otherwise died in 30 to 60 minutes, were saved if treated with BAL 5 minutes after the arsenical. Approximately 25 mgm. per kgm. BAL, or 0.20 millimols per kgm., protected all the animals, and 7.5 mgm. per kgm. (0.06 millimols per kgm.) protected half. The revivifying effect in the animals, many of which were already in acute distress at the time of administration of the BAL, was striking. Under the conditions of this acute experiment, the arsenical was therefore neutralized *in vivo* by 1 to 3 moles of BAL. In marked contrast, neither cysteine nor glutathione, similarly injected in amounts approaching the lethal level (800 and 400 mgm. per kgm., respectively), had a demonstrable protective action. These results were qualitatively confirmed in cats (25).

A more rigorous test of the detoxifying action of BAL was provided by rabbits which had received 8 mgm. per kgm. mapharsen at hourly intervals, for a total of 4 injections. This procedure gave the arsenical time to be fixed by the tissues, and corresponded more closely to the serious treatment reactions encountered in the course of antisyphilitic treatment, as well as the systemic arsenic poisoning observed after skin burns with Lewisite. With this drastic form of cumulative arsenic poisoning, 15 per cent of the animals (34 of 223) had died before treatment with BAL was begun, and many of the remainder were moribund. The detoxifying action of BAL in these animals was not as striking as that observed after the acute injection of a single massive dose, but was none the less definite. As shown in Table II, on all the treatment schedules used, and at total BAL dosages varying between 10 and 80 mgm. per kgm., approximately 40 per cent of the animals were saved, and in an additional 35 per cent death was appreciably delayed. As shown in the right-hand section of the Table, BAL in propylene glycol administered by inunction at similar intervals, in individual doses of 10 to 80 mgm. per kgm., and total doses of 40 to 320 mgm. per kgm., also saved some of the rabbits, but was somewhat less effective in this respect than much smaller doses given by injection. Similar results were obtained with aqueous solutions of BAL, in mice poisoned

TABLE II

The efficacy of BAL (1 to 10 per cent solution in propylene glycol) in the treatment of rabbits poisoned with multiple doses of mapharsen

Frequency and number of BAL injections	Rabbits injected intravenously, subcutaneously or intramuscularly				Rabbits treated by inunction of BAL solution			
		Died		Survived		Died		Survived
		<5 days	>5 days			<5 days	>5 days	
	mgm./kgm. at each injection				*mgm./kgm. at each inunction*			
Single injection 1 hour after last mapharsen	40	4	1	4	160*	2	1	0
	20	3	3	3	80	1	0	2
	10	1	3	5	40	1	0	2
	5	2	5	2	20	1	1	1
	Totals	11	12	15		5	2	5
4 injections, at 2 hourly intervals, beginning 1 hour after last mapharsen	20	5	2	1	80	2	1	0
	10	2	4	4	40	0	2	1
	5	2	3	4	20	2	1	0
	2	1	3	2	10	2	1	0
	Totals	10	12	11		6	5	1
4 injections, at 24-hour intervals beginning 1 hour after last mapharsen	20	3	2	4	80	0	3	0
	10	1	5	3	40	0	2	1
	5	1	4	4	20	1	1	2
	Totals	5	11	11		1	6	3
GRAND TOTAL on all schedules of injection, and all dosages		26 27 per cent	35 36 per cent	37 38 per cent		12 35 per cent	13 38 per cent	9 26 per cent

Mapharsen (8 mgm. per kgm.) was injected intravenously 4 times at hourly intervals. One hour after the last injection, BAL was injected at the dosage and frequency indicated in the Table. There was no significant difference between rabbits injected intravenously, intramuscularly or subcutaneously, and they are not distinguished in the Table.

with skin applications of Lewisite (26) and in dogs exposed to Lewisite vapor (27). In both groups, animals could be saved by systemic treatment which were not protected by skin application alone.

Although BAL in aqueous or propylene glycol solution given systemically was thus definitely effective in the treatment of arsenic poisoning, the practical difficulty lay in its rapid deterioration in such solutions. It seemed clear that if BAL could be compounded in a stable preparation suitable for injection, one might be able to avoid the application of an irritating ointment or solution to an already inflamed or exfoliating skin area, and to control the dosage accurately. More important, such an injectable preparation might prove effective in the treatment, not only of arsenical dermatitis, but also of the systemic arsenic poisoning which sometimes follows exposure to the arsenical blister gases, as well as the serious toxic reactions other than dermatitis often resulting from the intensified treatment of early syphilis.

A STABLE SOLUTION OF BAL SUITABLE FOR SYSTEMIC ADMINISTRATION

1. Stability of BAL in peanut oil and benzyl benzoate. The development of preparations suitable for parenteral use presented difficulties chiefly because of the instability of the BAL itself. Although saturated aqueous solutions of BAL had been administered intravenously, intraperitoneally, intramuscularly and subcutaneously in experimental animals, such solutions could not be sterilized, and had to be freshly prepared, seriously limiting their usefulness. Propylene glycol was a desirable solvent in many respects, but the solutions were locally irritating, and the BAL in such solutions slowly deteriorated (28). A satisfactory vehicle was provided with the finding that BAL was soluble in peanut oil to approximately 5 per cent

(weight/volume), and that with the addition of 2 parts of benzyl benzoate for each part of BAL, it was miscible with peanut oil (*Oleum arachis*) in all proportions (24). Such solutions were homogeneous at ordinary room temperatures, became cloudy when stored at ice-box temperatures, but regained their normal appearance on warming. The solutions could be ampuled and sterilized by autoclaving at 120° C. for 20 minutes, or by oven sterilization at 160° C. for 1 hour. In our early experiments such treatment of 5 per cent solutions caused a loss of titratable sulfhydryl groups of up to 11 per cent. This was not increased by further heating, as by daily autoclaving for 20 minutes on 4 successive days, or by storage at 63° C. for 6 weeks. In lots of BAL subsequently processed in this laboratory, using different batches of peanut oil and benzyl benzoate, the loss in -SH groups on the similar sterilization of solutions in peanut oil containing 10 per cent BAL and 20 per cent benzyl benzoate varied between 0.8 to 5.5 per cent. The pH of the peanut oil-benzyl benzoate solution has been reported to be an important factor in the loss of titratable -SH groups on heating, and the minimum loss of 1.5 to 2.5 per cent was observed at pH 6.0 and 6.6 respectively (29). In our hands, however, when there was no admixture with water or alcohol, only minor differences in the degree of loss were observed in the apparent pH range 4.3 to 7.7 (Table III).

TABLE III

The stability of a solution of BAL in peanut oil and benzyl benzoate at varying pH

5 per cent BAL and 10 per cent benzyl benzoate in peanut oil			10 per cent BAL and 20 per cent benzyl benzoate in peanut oil		
pH of aqueous extract		Percentage loss of titratable –SH groups	pH of aqueous extract		Percentage loss of titratable –SH groups
before heating	after heating		before heating	after heating	
2.47	3.40	9.0	2.05	3.48	6.1
2.66	3.61	8.4	2.30	3.86	5.7
*4.30	4.50	5.5	4.36	4.98	4.6
6.04	5.50	3.5	6.17	6.17	3.7
6.52	5.75	3.5	6.62	6.48	4.5
6.66	6.48	4.0	7.04	6.89	4.0
7.38	7.12	3.0	7.36	7.18	6.0
7.74	7.58	4.0	7.74	7.43	9.5

HCl (or NH_3) gas passed through a peanut oil solution containing 10 grams BAL and 20 grams benzyl benzoate per 100 ml. These solutions were then mixed with the stock untreated solution in varying proportions, and each dilution was further mixed with an equal volume of peanut oil to give solutions containing 5 per cent BAL and 10 per cent benzyl benzoate. The pH of aqueous extracts of each preparation was determined (29) before and after sterilization of the oil in glass-sealed tubes under air at 170° C. (1 hour). Two ml. of each mixture were shaken vigorously with 2 ml. H_2O for 2 minutes. The H_2O layer was removed after centrifugation, and its pH determined with a glass electrode (Beckman pH meter). The degree to which the BAL had deteriorated on sterilization was determined by direct iodometric titration of a suspension of the oil in a large excess of water.

* Stock solution, not treated with HCl or NH_3.

As of February 5, 1945, 102 different lots of BAL solution had been commercially processed for the armed forces. Each lot consisted of 10,000 ampules, each containing 4.6 to 4.7 ml. of a peanut oil solution of BAL (10 grams BAL and 20 grams benzyl benzoate per 100 ml. solution). The glass-sealed ampules, with an average of approximately 2 ml. air space, were sterilized at 160 to 170° C. for 1 to 1¼ hours. The percentage deterioration in these 102 lots, measured by the assay of free -SH groups, varied between 0 and 8.43 per cent, averaging 2.53 (30). No attempt was made to control the acidity of the solution, and in 8 representative lots, in which the degree of inactivation varied between 0 and 7.4 per cent, the pH of aqueous extracts was found in this laboratory to vary between 4.1 and 4.7, without apparent relation to the degree of inactivation.

This slight decomposition of BAL was probably due in part to oxidizing substances in the peanut oil, in part to the presence of traces of water, and to a lesser extent, to the small amount of oxygen in the free air space of the tube in which the solution was sterilized. Once these substances had been consumed, there was no further loss on additional heating or aging. While this degree of loss is slight, it should nevertheless be measured in the preparation and processing of every lot of BAL solution.

2. Toxicity of peanut oil-benzyl benzoate solution of BAL in rabbits. The toxicity of the BAL-benzylbenzoate-peanut oil mixture in rabbits is summarized in Tables IV and V. The toxicity was a function of the total amount of BAL administered, and was independent of its concentration. In studying the tissue damage produced by such solutions at the site of injection, 5 and 10 per cent solutions in peanut oil, containing 10 and 20 per cent respectively of benzyl benzoate, did not cause undue local damage when injected intra-

TABLE IV

*The toxicity in rabbits of a solution of BAL in peanut oil and benzyl benzoate**

Frequency of injection	Method of administration	BAL each injection	Died	Survived**	Maximal tolerated dose per injection†	LD_{50} per injection†
		mgm. per kgm.			*mgm. per kgm.*	*mgm. per kgm.*
Single injection	Intramuscular	80	3	0	40	60
		60	3	2		
		40	0	6		
	Subcutaneous	80	3	0	40	60 to 80
		60	1	4		
		40	0	5		
Multiple injections (repeated at 4-hour intervals for total of 4 injections)	Intramuscular	40	4	1	25	35
		30	1	4		
		25	0	5		
Multiple injections (repeated at 2-hour intervals for total of 4 injections)	Intramuscular‡	40	3	0	15 to 20	25
		30	4	1		
		25	2	3		
		20	1	4		
	Subcutaneous	30	3	2	20	25 to 30
		25	1	4		
		20	0	5		

* 5 grams BAL and 10 grams benzyl benzoate up to 100 ml. with peanut oil.
** Alive and well after thirty days.
† Because of small number of animals, these values are approximations only.
‡ Followed by daily intramuscular injections at same dosage level for 6 days. This probably had only a slight effect on toxicity.

TABLE V

The effect of the concentration of BAL on its acute and cumulative toxicity on intramuscular injection into rabbits

Solution injected			Schedule of injections							
			Single injection		4 injections at 2-hour intervals		4 injections at 4-hour intervals		4 injections at 8-hour intervals	
BAL	Benzyl benzoate	Peanut oil	LD_{50}	MTD	LD_{50}	MTD	LD_{50}	MTD	LD_{50}	MTD
per cent	*per cent*									
20	40	to volume	85	70			140	100		
5	10	to volume	80	60	100	75	145	110	185	140
5	to volume	none					120			
					(All doses are expressed as total mgm. BAL per kgm. For toxic levels per injection, these should be divided by 4.)					

CONCLUSION: The systemic toxicity of BAL on intramuscular injection in rabbits was independent of the concentration of BAL or benzyl benzoate in the range studied. There was definite cumulative toxicity if injections were repeated at 8 hour intervals or less.

Concentrations of BAL and benzyl benzoate are expressed as grams per 100 ml. solution. LD_{50} = dose of BAL which killed half of animals, and MTD = "maximal tolerated dose" (less than 10 per cent mortality), in both cases expressed as total mgm. BAL per kgm.

muscularly in dosages comparable with those recommended for use in man (31). In general, the tissue reaction to such doses at the site of injection was not appreciably greater than that produced by the intramuscular injection of the suspensions of bismuth subsalicylate in oil as used in the treatment of syphilis. BAL dissolved in benzyl benzoate alone caused somewhat greater local reaction than the solutions in peanut oil.

It should be noted in Tables IV and V that, although BAL is known to be rapidly excreted (22), there was definite cumulative toxicity when injections were repeated at short intervals. In rabbits, the maximum tolerated dose of a given

preparation on single intramuscular injection was 60 to 70 mgm. per kgm. This was decreased to 35 mgm. kgm. per injection when 4 doses were given at 8-hour intervals, and was further decreased to 28 to 20 mgm. per kgm. when the injections were repeated at 4- and 2-hour intervals respectively. In determining the optimum dosage schedule for man, it was therefore necessary to strike a balance between the desirability of prompt and intensive early treatment with BAL, and the cumulative toxicity of injections repeated at short intervals (32, 24).

THE EFFICACY OF BAL IN PEANUT OIL AND BENZYL BENZOATE IN THE SYSTEMIC TREATMENT OF EXPERIMENTAL ARSENIC POISONING

1. Mapharsen poisoning. Solutions of BAL in peanut oil injected intramuscularly were as active as the solutions in propylene glycol in protecting rabbits poisoned with repeated large doses of mapharsen (24) (Table VI). Of the various schedules tried for the administration of the BAL, one of the most effective consisted of 4 injections at 2-hour intervals, followed by single daily injections for 6 days. On that schedule, BAL in doses of 1 to 10 mgm. per kgm. per injection saved 55 per cent of the animals, and death was significantly delayed in an additional 22 per cent.

2. Lewisite and phenylarsenoxide poisoning. In rabbits injected subcutaneously with a solution of Lewisite in propylene glycol at twice the LD_{50} level, treatment with BAL in peanut oil and benzyl benzoate was effective if begun 2 hours after the injection of the arsenical (24) (Table VII). In contrast to the results obtained in

TABLE VI

The efficacy of a solution of BAL in peanut oil and benzyl benzoate in the treatment of massive arsenic poisoning in rabbits

Method of treatment with BAL solution	BAL per injection	Died		Survived*
		<5 days	>5 days	
Single injection	*mgm. per kgm.*			
	20	2	2	4
	10	4	0	4
	5	1	4	2
	2.5	2	4	1
	Total	9 30 per cent	10 33 per cent	11 37 per cent
Four injections, at 2-hour intervals	10	1	6	3
	5	1	5	4
	2.5	3	3	4
	1	2	4	4
	Total	7 18 per cent	18 45 per cent	15 37 per cent
Four injections, at 24-hour intervals	10	4	0	6
	5	2	4	4
	2.5	3	4	2
	Total	9 31 per cent	8 28 per cent	12 41 per cent
Four injections on first day, at 2-hour intervals, followed by single daily injections for 6 days	10	3	1	5
	5	2	2	7
	2.5	2	3	5
	1	2	3	5
	Total	9 22.5 per cent	9 22.5 per cent	22 55 per cent
Grand Totals (All schedules of injections, all dosages)		34 24 per cent	45 33 per cent	60 43 per cent

Rabbits were injected intravenously with 8 mgm. per kgm. mapharsen, repeated at hourly intervals for a total of four doses. One hour after the last injection, intramuscular treatment with BAL was begun as indicated in the Table.

* Alive and well after 30 days. Control rabbits, receiving no BAL, died regularly within 1 to 72 hours after the mapharsen injections.

TABLE VII

The efficacy of intramuscular BAL in the treatment of systemic Lewisite poisoning in rabbits

Total BAL administered	Method of administration of BAL					
	Single injection	4 equal injections at 4-hour intervals	4 injections at 4-hour intervals (first injection large and next 3 small)*	10 equal injections at 4-hour intervals	10 injections at 4-hour intervals (first 2 injections large and next 8 small)**	8 equal injections at 2-hour intervals
	Proportion of survivors to total treated					
mgm. per kgm.						
80			3/6	2/8	8/10	2/6
40	4/8	2/8	5/8	2/10	2/6	3/4
20	5/8	6/8	2/8	3/9		2/3
10	0/6	3/8	1/8	2/8		2/4
5	1/4	0/3	1/5			
PD_{50} (approximate dose which saved half of animals)	*mgm. per kgm.* 20	*mgm. per kgm.* 15	*mgm. per kgm.* 25±	*mgm. per kgm.* >80	*mgm. per kgm.* 50±	
Individual doses at PD_{50} level, mgm. per kgm.	20	4, 4, 4, 4	14, 3.5, 3.5, 3.5		2×14 8×3.5	
Estimated margin of safety between the maximal tolerated dose† and the PD_{50} level	3	7	4	<3	3	

Treatment with BAL in peanut oil was begun 2 hours after the subcutaneous injection of 4.5 mgm. per kgm. Lewisite ($2\times LD_{50}$, and approximately $1.3\times LD_{100}$ level of the particular lot used). Figures in the body of Table (*e.g.* 3/8) represent proportion of survivors to total treated.

* First dose 4 times larger than following 3: total amount of BAL injected in this series 10 per cent less than indicated in first column.

** One-fourth of total at each of first 2 injections, and 1/16th at each of following 8: first 2 injections 4 times larger than following 8.

† cf. Tables IV and V.

rabbits poisoned with multiple injections of mapharsen (Tables I and VI), the efficacy of treatment in these animals varied markedly with the total dosage of BAL. Of the various schedules tried, 4 equal injections at 4-hour intervals proved at least as effective, and provided a somewhat wider margin of safety (Table VII) than more condensed or more prolonged schedules of treatment, or schedules in which 1 or 2 large doses were followed by smaller doses. With that schedule of treatment, half the animals could be saved by a total of approximately 15 mgm. per kgm. of BAL, or 4 mgm. per kgm. per injection. This was 1/7 of the maximal tolerated dose of BAL on that schedule of injections, and suggested the feasibility of using BAL systemically in the treatment of severe Lewisite poisoning in man. The satisfactory results listed in Table VII were, however, limited to animals treated 2 hours after the Lewisite injection. When treatment with BAL was begun as late as 6 hours after the injections of Lewisite, no protection was afforded either by a single large injection of BAL, or by 4 injections repeated at 4-hour intervals.

Similar results were obtained in the treatment of animals poisoned with slightly more than the LD_{95} dose of phenyl arsenoxide (hydrolyzed form of the arsenical blister gas phenyldichloroarsine), injected subcutaneously (Table VIII). Treatment with relatively small doses of BAL in peanut oil (1.25 to 5 mgm. per kgm.), begun 2 hours later, and repeated 4 times at 4-hour intervals, followed by single daily injections for 6 days, saved approximately half the animals.

Comparable results with a solution of BAL in peanut oil and benzyl benzoate were obtained in dogs (33). After the application of Lewisite or phenyldichloroarsine to the skin in LD_{100} doses, local treatment with BAL ointment alone, begun 30 or 60 minutes later, was relatively ineffective;

TARLE VIII

The efficacy of intramuscular BAL in the treatment of systemic phenyl arsenoxide (hydrolyzed phenyldichloroarsine) poisoning in rabbits

Phenyl arsenoxide	BAL dosage		Number of rabbits	Died	Survived	Percentage of animals surviving*
	Per injection	Total in first 24 hours				
mgm. per kgm.	*mgm. per kgm.*	*mgm. per kgm.*				
2.2 (1.1 times the LD_{95} dose)	5	20	6	3	3	50
	2.5	10	6	2	4	67
	1.25	5	6	4	2	33
	Controls (No BAL)		7	7	0	0

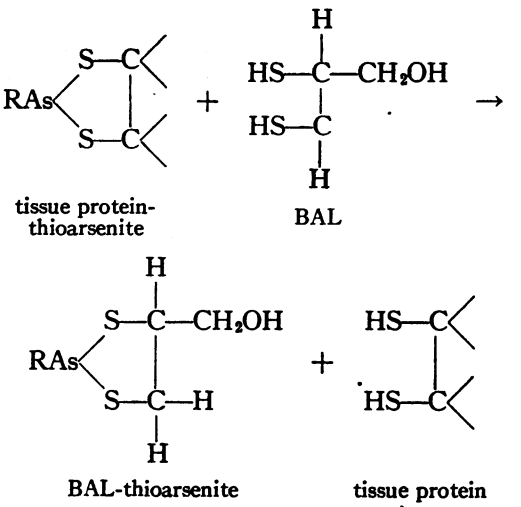
Treatment with BAL (in peanut oil) was begun 2 hours after the injection of the arsenical, and given in 4 equal doses at 4-hour intervals, followed by single daily injections at the same dosage level, for 3 days.

* Alive and well after 30 days. Untreated controls died in 14 hours to 6 days.

but such local treatment, combined with the intramuscular injection of BAL in peanut oil and benzyl benzoate, saved the majority of the animals. The effective dose (total of 10 to 30 mgm. per kgm.) thus agreed with that found in rabbits poisoned with Lewisite or phenyl arsenoxide. In dogs poisoned by the inhalation of Lewisite at LD_{80} levels, the mortality was not reduced by a single intramuscular injection of BAL in a dose of 20 mgm. per kgm., but the survival time was prolonged. Mortality in dogs similarly poisoned with phenyldichloroarsine was significantly reduced by 2 such doses, administered ½ and 2½ hours after gassing.

MECHANISM OF SYSTEMIC ACTION OF BAL: EFFECT ON ARSENIC EXCRETION

As discussed in the introduction, the antidotal action of BAL has been ascribed (15, 20, 21) to the fact that it reacts with arsenicals to form a stable ring compound, and can thus effectively compete for the arsenical with the thiol groups of tissue proteins. This competition involves two distinct processes: (*a*) combination with the toxic arsenical before it combines with the tissues, and (*b*) removal of arsenic from the tissues after it has already combined, with the formation of BAL-thioarsenite from the tissue protein-thioarsenite, and the release of the tissue thiol groups:



In the systemic use of BAL for the treatment of arsenic poisoning, the latter is perhaps the more important reaction. Highly toxic arsenicals combine with the tissues rapidly (12); and in the case of arsenic compounds used therapeutically, a toxic complication which indicates the use of BAL is presumably the result of a similar combination of arsenic with cellular thiol groups.

That sulfhydryl compounds in general can dissociate arsenic from its combination with cells was indicated by the observation (6) that protozoa (*Colpidium*) immobilized by dichloroarsine could be resuscitated by the addition of monothioethylene glycol, and the similar observation (7, 7a) that *S. pallida* immobilized by a series of phenyl arsenoxides could be resuscitated by cysteine. The even more striking effect of BAL in reviving trypanosomes immobilized by aromatic arsenicals (24) is illustrated in Table IX. Suspensions of trypanosomes (*T. equiperdum*) were exposed to a final concentration of phenyl arsenoxide (1.3×10^{-5} molar = 1:460,000) which caused their complete immobilization within less than 1 minute. Within 5 minutes obvious degenerative changes had become evident in most of the organisms, which tended to increase in size, to assume a globular shape, and to become vacuolated; and in the following 30 minutes a significant proportion had completely lysed. At varying intervals after the admixture of arsenical and organisms, aliquot portions were withdrawn and added to varying concentrations of BAL and cysteine. As little as 3 molar equivalents of BAL added to the poisoned

TABLE IX

The resuscitation by BAL and by cysteine of trypanosomes immobilized by phenyl arsenoxide

Time between addition of arsenical and of —SH compound to trypanosomes	Amount of —SH compound added, relative to arsenical (molar)											
	BAL						Cysteine					
	12	6	3	1½	¾	⅜	2500	1250	625	312	156	78
	Percentage of motile organisms 60 minutes after addition of —SH compound											
(—SH compound added to arsenical 10 min. before trypanosomes)			>95	>95	0	0		>95	>95	75	0	0
(—SH compound added immediately after arsenical)			>95	>95	0	0	>95	>95	84	0	0	0
5 min.	>95	>95	92	0			79	77	41	0		
15 min.	73	50	40±	0			20±	20±	6	0		
30 min.	12	15	4	0			0	0	0	0		

Reagents: 4×10^{-5} M phenyl arsenoxide (1 volume); suspension of *T. equiperdum* (5×10^{7} per ml.) in 20 per cent rabbit serum and 0.04 M phosphate buffer at pH 7.4 (2 volumes); solution of —SH compound (1 volume). The arsenical used completely immobilized all the organisms within 1 minute, and was 20 times the amount which immobilized half the organisms in 60 minutes.

suspension 5 minutes after the arsenical caused the prompt resuscitation of the trypanosomes. Within a few minutes, more than 90 per cent of the organisms were actively motile, and 92 per cent were still fully motile 60 minutes later. The longer the poisoned suspension was allowed to stand before the addition of BAL, the smaller the proportion which could be so revived. Thus, only 12 to 15 per cent could be resuscitated when BAL was added 30 minutes after the arsenical; and the proportion was actually smaller than indicated, since a significant number had by that time undergone complete autolysis. Although cysteine had qualitatively the same effect, from 200 to 400 times as much was required as in the case of BAL, and the maximum proportion of organisms which could be resuscitated was regularly lower.

The effect of BAL in resuscitating organisms immobilized and poisoned by arsenicals is related to the fact that it abstracts the arsenic from the cells. As is indicated in Table X, actively trypanocidal compounds may be concentrated by the organisms more than 200-fold. On the addition of BAL, the arsenical is rapidly removed, and the concentration in the cells is reduced to a nonlethal level. Since the widely varying trypanocidal activity of a series of trivalent arsenicals is determined by the degree to which these compounds are bound by the organisms (13), one may conclude that the demonstrated ability of BAL to remove arsenic from its combination with cell components is the basis of its efficacy in reversing the toxic action of arsenicals.

In the animal body also, the protective action of BAL is associated with an increased excretion of arsenic. Stocken and Thompson (22) demonstrated a 4-fold increase in rate after the local application of BAL to a skin area burned with Lewisite. In rabbits poisoned with phenyl arsenoxide or Lewisite, and to a lesser extent, with mapharsen, we have found a striking increase in the rate of urinary arsenic excretion on systemic treatment with BAL. It had been previously shown in this laboratory (12) that the rate of excretion of arsenicals was related to their toxicity: the more toxic the compound, the slower it was excreted, presumably because of its firmer and more extensive combination with the host tissues. Thus, in the first 24 to 72 hours after the injection of phenyl arsenoxide (0.56 mgm. per kgm. = 0.0033 millimols per kgm.) into rabbits, the hourly urinary excretion was only 0.12 to 0.35 per cent of

the amount injected (Table XI). The administration of BAL to such rabbits, whether intravenously in aqueous solution (Figure 1) or subcutaneously in peanut oil (Figure 2), caused a striking increase in the rate of urinary excretion. The greatest effect was observed when BAL was given immediately after the arsenical, in which case the hourly excretion reached as high as 21.9 per cent, or approximately a *hundredfold* increase over control rabbits.

It is to be noted that the effect of the BAL was temporary. After both intravenous and intramuscular injection, with both aqueous and oil solutions respectively, its action was largely completed within 2 to 4 hours. By that time all the BAL had presumably been either excreted, or metabolized to an inactive form. Further, with multiple injections of BAL, each injection was followed by a definite spurt in urinary excretion. These observations suggested the advisability of administering BAL in repeated small doses, rather than a single massive dose, a procedure already indicated on the basis of minimum toxicity and maximum efficacy (Tables IV to VII).

A single experiment with Lewisite is summarized in Figure 3. BAL in peanut oil (10 mgm. per kgm.) administered intramuscularly 24 hours after the intravenous injection of 0.0033 moles per kgm. Lewisite caused a definite increase in the rate of urinary arsenic excretion, sustained for a period of 2 to 4 hours.

One of many similar experiments with mapharsen is summarized in Figure 4. As is there illustrated, with this compound the effect of BAL on urinary excretion, although definite, was less pronounced than in the case of either phenyl arsenoxide or Lewisite. This probably reflects the several facts (*a*) that much larger doses of

TABLE X

The removal of arsenic from trypanosomes by BAL and by cysteine

Arsenical		Total number trypanosomes	−SH compound added	Time between addition of As and centrifugation	Percentage of motile organisms at time of centrifugation	As content, γ		Ratio of trypanosome As concn.** / supernatant As concn.
Compound used*	Total As added, γ					Trypanosomes	Supernatant	
				min.				
4-$CONH_2$ phenyl arsenoxide	16.7	1.9×10^9	0	10	0	12.7	6.5	257
			BAL: 1 ml. of 0.02 M solution added 3′ after arsenical	10	>95	2.5	13	28
Phenyl arsenoxide	16.7	2.3×10^9	0	10	0	9.5	6.0	189
			0	60	0	9.0	7.0	154
			Cysteine: 1 ml. of 0.1 M solution added 10′ after arsenical	60	43	3.5	12.5	33
			BAL: 1 ml. of 0.01 M solution added 10′ after arsenical	60	81	1.17	—	9
3-NH_2-4-OH phenyl arsenoxide (Mapharsen)	13.5	3×10^9	0	15	0	7.6	5.9†	118
			BAL: 1 ml. of 0.002 M solution added 5′ after arsenical	15	>95	1.9	12‡	15

(Nine ml. trypanosome suspension +1 ml. arsenical solution +1 ml. −SHR or NaCl solution)

* ϕ = phenyl.

** 3×10^9 trypanosomes = 0.12 ml. Thus, in the experiment with 4-$CONH_2$ phenyl arsenoxide, 1.9×10^9 trypanosomes, measuring 0.076 ml., contained 12.7 γ As, or 167 γ per ml. The supernatant fluid contained 0.65 γ per ml., giving a concentration ratio of 257.

† 9.8 ml. of supernatant contained 5.3 micrograms arsenic.

‡ 9.6 ml. of supernatant contained 10.5 micrograms arsenic.

TABLE XI

The effect of BAL on the urinary arsenic excretion in rabbits after the intravenous injection of phenyl arsenoxide

BAL administration (10 mgm. per kgm.)		Rabbit number	Pre-BAL		Post-BAL			Percentage of increase in urinary As excretion caused by BAL
Vehicle for BAL	Route of injection		Time in hours between injections of arsenical and BAL	Hourly As excretion in urine*	Time in hours after BAL injection: Hourly excretion of As in urine* 0 to 2	2 to 4	4 to 24	
Aqueous solution	Intravenous	7634	0		7	3	0.36	3900**
		8347	24	0.35	2		0.6	570
		8391	24	0.13	4.8		0.43	3700
		8846	24	0.125	5.1		0.16	4100
Peanut oil and benzyl benzoate	Subcutaneous	9145	24	0.22	8.4	2.1	0.33	3800
		9168	0		21.4	1.4	0.38	11900**
	Intramuscular	9666	0		21.9	11.8	0.33	12200**
		9678	24	0.29	5	5.5	0.25	1700
Controls	No BAL	N.B.	Time in hours after injection of arsenical					
			0 to 24	0.1				
			24 to 48	0.23				
			48 to 72	0.17				
		6274	0 to 4	0.2				
			4 to 24	0.29				
			24 to 48	0.08				
		6460	0 to 48	0.07				
			48 to 72	0.017				

All animals received 0.56 mgm. per kgm. phenyl arsenoxide = 0.25 mgm. per kgm. As = 0.0033 millimols per kgm. All BAL injections were at 10 mgm. per kgm. = 0.08 millimols per kgm.

* Percentage of total amount injected. Method used for As analysis was that of Magnuson and Watson (34).

** Average hourly excretion in absence of BAL taken as 0.18 per cent of amount injected (cf. column 5).

mapharsen were used than in the case of the other more toxic arsenoxides, and (*b*) that mapharsen is normally excreted at a faster rate than is either Lewisite or phenyl arsenoxide (12). In consequence, the increased excretion resulting from the administration of BAL was partially obscured by the significant amounts excreted without reference to BAL.

As in the case of microorganisms, the increased rate of excretion of arsenic caused by BAL, and its therapeutic efficacy, are presumably due to the fact that it abstracts the arsenical from an otherwise firm combination with the tissue cells, with the release of vital tissue thiol groups and the excretion of the BAL-thioarsenite. The nature of the chemical grouping in the cells with which the arsenic combines to exert its toxic action has been discussed in a preceding section.

It should be emphasized that thiol compounds, including BAL, successfully reversed the toxic action of arsenicals on spirochetes or trypanosomes only if applied soon after the organisms are immobilized. Beyond that period secondary reactions apparently occur, so that the mere removal of the arsenical does not then suffice to restore cell function (6, 7a). In the animal also, the efficacy of BAL in preventing or reversing the systemic toxic action of arsenicals depended to a large extent on the time elapsed between the application or injection of the arsenical and the administration of BAL. When BAL was injected 5 minutes after a single massive dose of mapharsen all the animals could be saved by a single dose; but when it was administered 30, 60 and 120 minutes after lethal doses of mapharsen, Lewisite, or phenyldichloroarsine, the protective action of the BAL became progressively less striking, and a smaller proportion of the animals could be saved. Six hours after the injection of a lethal dose of Lewisite in rabbits, even intensive and prolonged

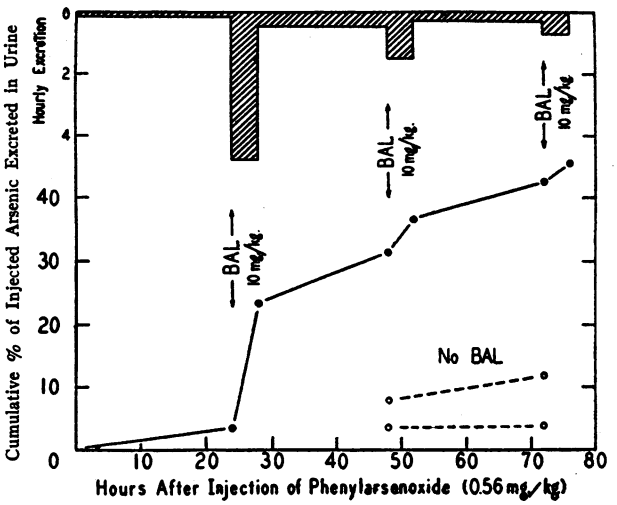


FIG. 1. THE EFFECT OF BAL (INJECTED INTRAVENOUSLY IN SALINE SOLUTION) ON THE URINARY EXCRETION OF PHENYLARSENOXIDE IN RABBITS

A rabbit was injected intravenously with 0.56 mgm. per kgm. phenyl arsenoxide. Twenty-four, 48 and 72 hours later the rabbit was given an intravenous injection of 10 mgm. per kgm. BAL in saline solution. Urine specimens were collected by catheterization just before and 4 hours after each BAL injection. The open circles at the bottom of the figure refer to 2 control rabbits receiving no BAL. The striking effect of BAL on the hourly excretion of arsenic is indicated in the cross-hatched blocks at the top of the figure.

treatment with BAL, at doses approaching the toxic range of the BAL itself, was ineffective (24).

The implication is clear that for optimum results, BAL should be administered as soon as possible after exposure to toxic arsenicals, or after the development of toxic manifestations as a complication of arsenical chemotherapy. In patients heavily exposed to arsenical blister gases, local decontamination with BAL to remove the surface material should be supplemented by its prompt systemic administration to counteract the effects of the material already absorbed.

The effect of BAL on the urinary excretion of arsenic in normal human volunteers, in subjects exposed to an arsenical smoke, and in human cases of arsenic poisoning are discussed in following papers of this series (35, 23). The results obtained with BAL in the treatment of 227 cases of arsenic poisoning (encephalitis, dermatitis, blood dyscrasias, jaundice, fever) will be described elsewhere (32, 35).

SUMMARY

1. 2,3-Dimercaptopropanol ("BAL") injected subcutaneously, intramuscularly, or intravenously in aqueous or propylene glycol solution, proved effective in the treatment of acute and subacute mapharsen poisoning in rabbits.

2. The antidotal action of BAL was referable

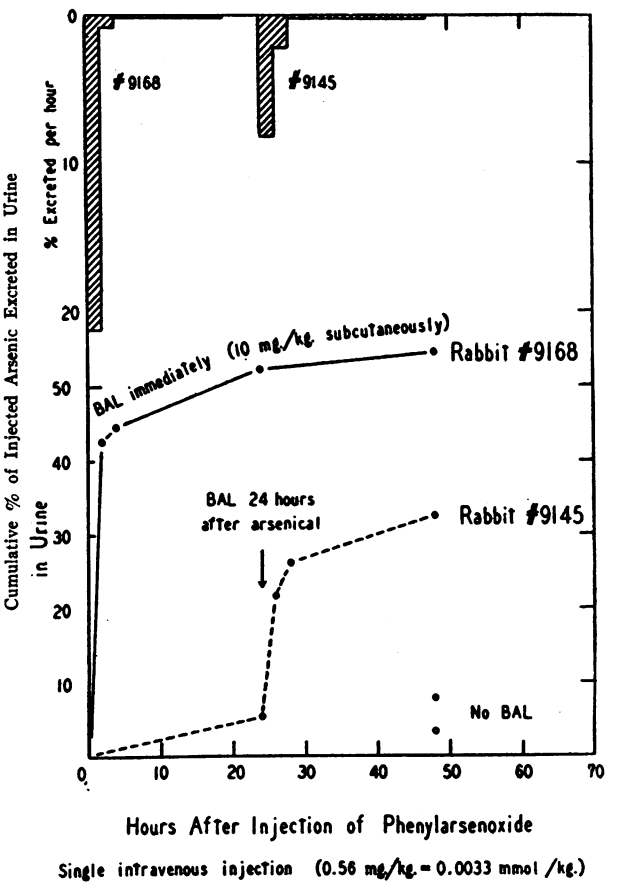


FIG. 2. EFFECT OF BAL (INJECTED INTRAMUSCULARLY IN PEANUT OIL-BENZYL BENZOATE SOLUTION) ON THE URINARY EXCRETION OF PHENYLARSENOXIDE IN RABBITS

Four rabbits were injected intravenously with 0.56 mgm. per kgm. phenyl arsenoxide. One was immediately injected subcutaneously with 10 mgm. per kgm. BAL (5 per cent solution in peanut oil with 10 per cent benzyl benzoate), while the second rabbit received a similar single injection of BAL 24 hours after the arsenical. Urine specimens were obtained by catheterization just before the injection of BAL and 2, 4 and 24 hours later. The open circles at the bottom of the figure indicate the urinary arsenic excretion in the 2 control rabbits receiving no BAL.

to its ability to remove the arsenical from its combination with cells, with the excretion of the stable and relatively non-toxic thioarsenite so formed. Trypanosomes rapidly immobilized and apparently killed by arsenicals were resuscitated on the addition of BAL, due to the removal of the bound arsenic from the cell. Similarly, in rabbits injected with mapharsen, Lewisite or phenyl arsenoxide, the administration of BAL caused a striking increase in the rate of urinary arsenic excretion, in some cases exceeding a hundred-fold.

3. Although BAL was unstable in aqueous or propylene glycol solution, solutions in peanut oil could be sterilized by heat with only slight loss in activity. With the addition of 2 grams of benzyl benzoate for each gram of BAL, the latter was miscible with peanut oil in all proportions.

4. The toxicity of such solutions in peanut oil and benzyl benzoate was determined in relation to the frequency and number of injections, the route of administration, and the concentration of the solution.

5. BAL dissolved in peanut oil and benzyl benzoate injected intramuscularly proved effective in the treatment of mapharsen, Lewisite and phenyl arsenoxide poisoning in rabbits.

6. The widest margin of safety between the effective and toxic levels of BAL so administered was provided by a schedule involving 4 injections at 2- to 4-hour intervals, followed in some cases by daily injections for 6 days. On this schedule, individual BAL doses of 1 to 10 mgm. per kgm. saved approximately half the animals injected with lethal doses of mapharsen, Lewisite or phenyl arsenoxide. Since the maximum tolerated dose of

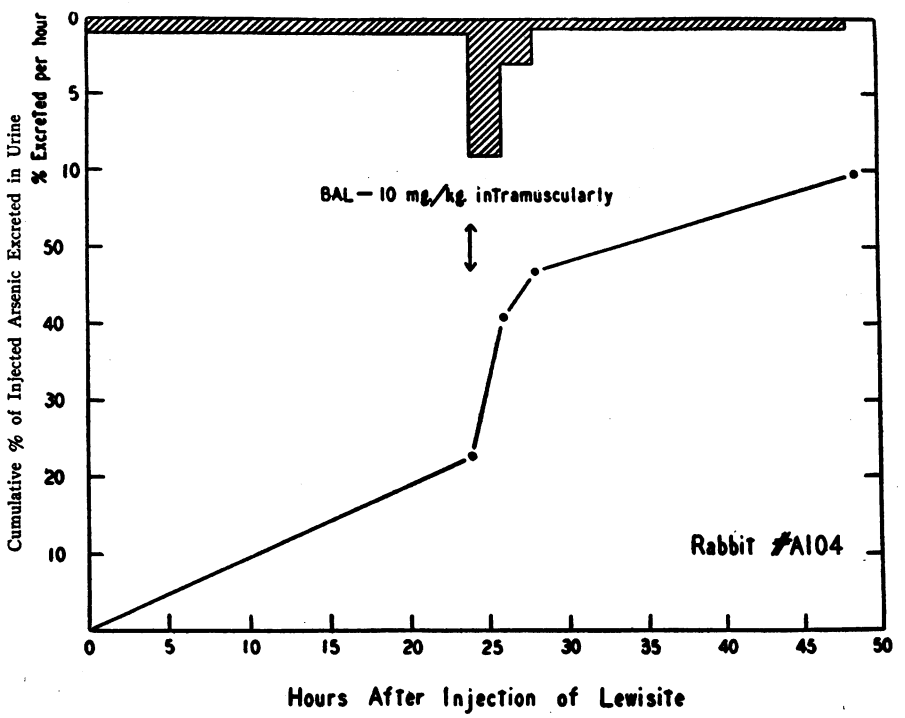


FIG. 3. THE EFFECT OF BAL (INJECTED INTRAMUSCULARLY IN PEANUT OIL-BENZYL BENZOATE SOLUTION) ON THE URINARY EXCRETION OF LEWISITE IN RABBITS

Lewisite was injected intravenously in propylene glycol solution (0.69 mgm. per kgm.). Twenty-four hours later the rabbit was given a single intramuscular injection of 10 mgm. per kgm. BAL (5 per cent solution in peanut oil with 10 per cent benzyl benzoate). Urine specimens were obtained by catheterization just before the injection of BAL, and 4, 8 and 24 hours later.

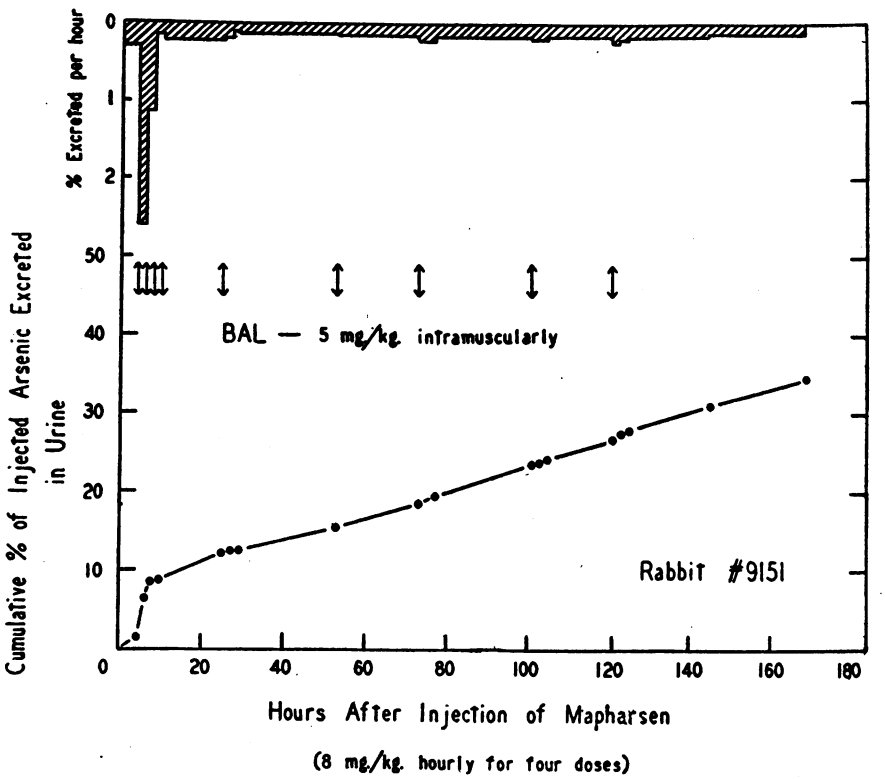


FIG. 4. THE EFFECT OF BAL (INJECTED INTRAMUSCULARLY IN PEANUT OIL-BENZYL BENZOATE SOLUTION) ON THE URINARY EXCRETION OF MAPHARSEN IN RABBITS

Rabbits were injected intravenously with 8 mgm. per kgm. mapharsen, repeated 4 times at hourly intervals. One hour after the last injection of mapharsen the animals received 5 mgm. per kgm. BAL intramuscularly (1 per cent solution in peanut oil with 2 per cent benzyl benzoate). This was repeated 4 times on the first day, and daily thereafter. Urine specimens were obtained by catheterization. The effect of BAL on the hourly urinary excretion of arsenic is indicated in the cross-hatched portion of the figure.

BAL so injected was 35 mgm. per kgm., the margin of safety provided was sufficiently large to indicate the feasibility of its human use.

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