obtained that any significant combination occurs with other groups in the kerateine molecule.

4. At least 75% of the arsenic found in the lewisite-derivative is in combination with two thiol groups, and the hypothesis has consequently been put forward that dithiols may prove more effective antidotes to arsenic than the mono-thiols used in the past.

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British Anti-Lewisite

2. DITHIOL COMPOUNDS AS ANTIDOTES FOR ARSENIC

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In a previous paper the preparation of arsenic derivatives of kerateine has been described (Stocken & Thompson, 1946). In the course of that work it was found that the arsenicals had combined with the SH groups present in the kerateine, and that at least 75% of the arsenic in the lewisite derivative was present in the proportion 1 As : 2 SH. It seemed probable therefore that the arsenic had combined with 2 SH groups closely placed in space on the same molecule to form a ring compound, and it was suggested that efficient protection against trivalent arsenicals would only be afforded by the presence of a dithiol capable of forming a relatively stable ring compound with the arsenical, instead of the much more dissociable 'open chain' compounds formed between arsenic and the monothiols investigated in the past.

Much of the earlier work (Voegtlin, Dyer & Leonard, 1923, 1925; Walker, 1924; Rosenthal & Voegtlin, 1930; Voegtlin, Rosenthal & Johnson, 1931) demonstrating the limited capacity of simple monothiols to protect certain biological systems from the toxic action of the therapeutic arsenicals was also reviewed, but it was pointed out that the protection afforded by compounds of this type was much less complete when exerted against sodium arsenite than against the therapeutic arsenoxides, and that in some cases the protection was inadequate from the physiological standpoint (Walker, 1928; Schmitt & Skow, 1935).

In the case of lewisite, experiments carried out in this laboratory have shown that, of an extensive series of monothiol compounds studied, none of the substances investigated has been able to protect against lewisite. Thus, Sinclair (quoted by Peters, 1940) found that the brain pyruvate oxidase system was not protected against lewisite or arsenite by very large concentrations (a ratio of 1 : 200 mol.) of glutathione or cysteine.

The contrast between these results and those of the earlier workers with aromatic therapeutic arsenoxides probably depends largely on differences in the degree of dissociation of the thioarsinites formed by interaction of these arsenicals with the thiol. In the case of the aromatic arsenoxides Cohen, King & Strangeways (1931) were able to demonstrate that compounds with simple monothiols undergo extensive dissociation in 0·1 N-NaOH, while in sodium bicarbonate solution several of the thioarsinites studied gave a feebly positive nitroprusside reaction, indicating a slight degree of dissociation. It must be remembered also that in the various biological systems studied by Voegtlin a large excess of the thiol was necessary to counteract the toxic effects of the arsenoxide.

More recently, Strangeways (1937) has investigated the toxicity to trypanosomes *in vitro* of three thioarsinites, including the 'arsenoxide'-glutathione compound, and has shown that the lethal activities of equimolar solutions of this thioarsinite and of its parent arsenoxide are identical. Moreover, using the dilution of arsenoxide employed by Voegtlin *et al.* (1923), she confirmed their observation that complete inhibition of toxic action could be obtained with a ratio of 1 : 10 mol. of glutathione, but found that on using more dilute solutions of arsenoxide the protection afforded by 10 mol. of glutathione became less complete, until at high dilutions no protection could be observed at all: in strong solutions the excess of glutathione favours the formation of the thioarsinite, whereas more dilute solutions tend to bring about hydrolysis with reliberation of the toxic arsenoxide.

It is clear, therefore, that the 'arsenic acceptor' in living cells must form a more stable compound with arsenic than do any of the simple monothiols so far investigated. For the reasons already given it was decided therefore to study the protection afforded by simple dithiol compounds.

Two considerations guided us in our choice of dithiols: first, since on physico-chemical grounds it was desirable to form 5- or 6-membered ring compounds between the arsenical and the thiol, investigations were begun with 1:2- and 1:3-dithiol compounds; secondly, since the work was designed primarily to find an antidote to the vesicant action of lewisite, rapid penetrability of human skin was desirable, necessitating the initial choice of simple small molecular dithiols. Philpot (private communication) in a related research in this laboratory had shown that short-chain fatty acids penetrate the skin more rapidly than long-chain compounds.

The first dithiol to be used was toluene-3:4-dithiol ('Dithiol', British Drug Houses Ltd. Spot Test Reagent) and although encouraging results were obtained with an enzyme as the test system, the local irritant action of the compound became apparent when it was applied to the skin of rats contaminated with lethal amounts of lewisite, and though death was prevented it seemed that aromatic thiols, like phenols, are capable of exerting a local necrotizing effect, and hence were unsuitable.

Ethane-1:2-dithiol was next prepared and investigated, but was considered impractical as an anti-lewisite agent under field conditions on account of its high volatility.

Since a water-soluble compound of low volatility, and possibly also of high viscosity, was indicated, 2:3-dimercaptopropanol was next prepared in the hope that its chemical similarity to glycerol might also extend to such properties as volatility and ready skin penetrability. The results of preliminary tests with this compound, later called BAL (or British Anti-Lewisite) by the Americans, suggested that it would be suitable for more extended investigations. *In vivo* tests with animals, and later human tests, were therefore undertaken. In the course of these tests certain other dithiol compounds were prepared and their antidotal activity investigated.

The greater part of the work described here was carried out in 1940 and was reported to the Ministry of Supply in 1941 (Stocken & Thompson). Much of it has since been confirmed and extended both in this country and in the United States. No attempt has been made here to review this later work.

Six monothiols and 12 dithiols have been studied in the course of the work described here. The details of the preparation and properties of these and other thiols are given in a separate communication (Stocken, 1946).

EXPERIMENTAL AND RESULTS

The reaction of trivalent arsenicals with mono- and dithiols

In a previous report (Stocken & Thompson, 1940) it was shown that the addition of lewisite to solutions of kerateine caused a disappearance of the nitroprusside reaction at pH 9·0. This finding was in contrast to the results obtained by Cohen *et al.* (1931), who found that the thioarsinites formed by interaction of arsenoxides with simple monothiols were extensively dissociated in alkaline solution. It

was of interest therefore, in the first place, to determine whether simple dithiols reacted with lewisite to form alkali-stable compounds, similar to the lewisite-kerateine derivative. Briefly, it was found that the addition of equivalent amounts of 'lewisite oxide' (chlorovinylarsenoxide) or of sodium arsenite to solutions of BAL resulted in a complete disappearance of the nitroprusside reaction. The previous observations of Cohen *et al.* (1931) were confirmed by the use of 2-mercaptoethanol.

Experiments were next carried out to discover the relative rates of hydrolysis of thioarsinites prepared by reacting lewisite with monothiols, dithiols and thiolproteins respectively. For this purpose, in addition to kerateine, an acetone-dried rabbit muscle powder and crude crystalline lens protein prepared from ox eyes by a modification of the method of Woods & Burky (1927) were used.

One equivalent of lewisite oxide was added to one equivalent of the thiol in 0·2 M-phosphate buffer, pH 7·3; half an equivalent of porphyrindin was added next and the decolorization times noted. The final concentration of the thioarsinite was the same in all cases. The reactions are:

(1) $R\text{—}S$ $R\text{—}S$ $>AsCH : CHCl + H_2O \rightleftharpoons ClCH : CHAsO + 2RSH$

(2) $2RSH$ + Porphyrindin $\rightarrow R\text{—}S\text{—}S\text{—}R$ + Leucoporphyrindin

In the case of the thiol proteins the SH content was first determined by porphyrindin titration and the end-point confirmed by the nitroprusside reaction. In the absence of lewisite all the thiols included in Table 1 titrated rapidly with porphyrindin, with the exception of monothioethylene glycol which took 1¼ min.; lewisite itself does not decolorize porphyrindin. The results given in Table 1 show that there is a very marked difference in the decolorization times given by thioarsinites derived from monothiols and dithiols, and that those for the protein derivatives are slightly longer than for monothiols. If it be assumed that the rates of formation of the thioarsinites are comparable, these decolorization times should give a measure of the rates of hydrolysis of these compounds. From this it may be concluded that at equilibrium in a system containing equivalent amounts of lewisite oxide and a monothiol, a dithiol and a thiol protein, the amount of lewisite combined with the dithiol will be much greater than that combined with the protein which in turn will be slightly greater than that combined with the monothiol.

Table 1. *Hydrolysis rates of thioarsinites prepared from lewisite plus monothiols, dithiols and thiol proteins*

(Expressed as time taken to decolorize 0·5 equiv. of porphyrindin.)

Thiol	Decolorization time (min.)
Cysteine	0·25
Thiolacetic acid	0·5
Aminothiophenol	0·83
Glutathione	2·5
2-Mercaptoethanol	3·5
Protein from muscle powder	3·0
Lens protein	3·5
Kerateine	6·0
2:3-Dimercaptopropanol (BAL)	>180
Ethane-1:2-dithiol	>180
1:3-Dimercaptopropanol	>180
Propane-1:3-dithiol	>180
$\beta\beta'$-Dimercaptodiethylether	>180

An attempt was also made to measure the decolorization times of ethanethiol, pentane-1:5-dithiol and $\beta\beta'$-dimercaptodiethyl sulphide in the presence and absence of lewisite; it was found, however, that these compounds by themselves decolorized porphyrindin only very slowly, so that no conclusion could be drawn from the reaction of their derived thioarsinites with porphyrindin. This may be due to these compounds having higher redox potentials than the others cited.

Effectiveness of dithiols in protecting the brain pyruvate oxidase system from the toxic effects of arsenic

The principle involved in these tests has been the measurement of the ability of dithiol compounds to abolish, or diminish, the inhibition of pyruvate oxidations in brain produced by the presence of small amounts of lewisite oxide. The pyruvate oxidase system was chosen for these initial biological tests, as it had been previously shown (Krebs, 1933; Peters, 1936; Sinclair & Thompson, 1940) that this enzyme system is highly sensitive to arsenical poisoning, and provides therefore a convenient and rapid biochemical method for determining the effectiveness of water-soluble compounds as antidotes for arsenic. It is interesting to note that the results obtained with this *in vitro* test were strikingly confirmed by the *in vivo* tests described later in this paper.

Pigeon brain *brei* respiring in Ringer-phosphate solution at pH 7·3 and at 38° was used throughout. The earlier experiments were carried out with neutralized lewisite oxide, the final concentrations used (0·016 mM) being sufficient to cause approximately 50 % inhibition of the total respiration. The thiols were dissolved in a small volume of dilute alkali, and were brought to the required concentration with phosphate buffer, so that the pH of the respiring medium was not detectably altered by their addition. The oxygen consumption of the system was measured manometrically over 3 successive half-hour periods.

Table 2 shows the toxicity to the pyruvate oxidase system of the various compounds investigated, the figures representing the percentage inhibition of the total respiration of the brei in the presence of 0·02 M-sodium pyruvate. It will be seen that the aliphatic dithiol compounds investigated are strikingly non-toxic to this enzyme system. The addition of the dithiol in most cases resulted in a small increase (5–15 %) in oxygen uptake. The greater toxicity of toluene-3:4-dithiol may have been due in part to the fact that the sample used had undergone partial oxidation.

Table 2. *Toxicity of dithiol compounds to the brain pyruvate oxidase system*

Compound	Concentration (mM)	Inhibition of O_2 uptake (%) 0–30 (min.)	30–60 (min.)	60–90 (min.)
Toluene-3:4-dithiol	0·21	19	25	26
Ethane-1:2-dithiol	0·36	0	0	0
2:3-Dimercaptopropanol	0·27	0	2	0
1:3-Dimercaptopropanol	0·27	0	0	0
Propane-1:3-dithiol	0·31	0	0	4
$\alpha\beta$-Dimercaptopropionic acid	0·48	6	0	0

The effect of these dithiols in reducing the toxicity of lewisite oxide to the pyruvate oxidase system was next investigated. In each experiment the thiol was added to the system immediately before the addition of the lewisite; flasks were also set up in which the thiol was added to the unpoisoned enzyme system in order to control the small extra oxygen uptake frequently induced by the presence of the thiol. Table 3 gives a comparison of the percentage inhibition of oxygen uptake produced by lewisite oxide in the absence and presence of various thiol compounds.

The above results indicate that all the dithiol compounds here investigated either markedly diminish or completely abolish the inhibition produced by lewisite oxide. Moreover, this effect is present in dilute solutions of the thiols; thus, in the case of BAL a very marked reduction in the toxicity of lewisite is brought about by a ratio of 1 : 4 mol. of dithiol, a ratio of 1 : 8·5 mol. completely abolishing it.

In view of the original *in vitro* work on the reaction between lewisite and kerateine, an experiment was carried out to determine whether any antidotal activity was exhibited by this thiol protein. A solution of kerateine, prepared from hair by the method of Goddard & Michaelis (1934), was therefore made up to give a final concentration of 0·3 % in the Warburg flasks; the toxicity of lewisite was much reduced in the presence of this concentration, thus showing that kerateine also can compete

Table 3. *Antidotal effect of thiol compounds on the toxicity of lewisite oxide to pyruvate oxidation*

Compound	Concentration (mM)	Inhibition of O_2 uptake (%): Lewisite oxide (0·016 mM) 0–30 (min.)	30–60 (min.)	60–90 (min.)	Lewisite oxide (0·016 mM) + thiol 0–30 (min.)	30–60 (min.)	60–90 (min.)
Toluene-3:4-dithiol	0·02	67	59	69	0	1	10
	0·02	63	61	72	3	21	27
	0·21	67	59	69	0	0	0
Ethane-1:2-dithiol	0·07	56	53	50	7	2	4
	0·36	56	53	50	0	0	0
2:3-Dimercaptopropanol (BAL)	0·05	72	46	47	—	18	15
	0·14	47	47	40	0	3	0
	0·27	72	46	47	0	0	0
	0·27	51	46	46	0	3	0
1:3-Dimercaptopropanol	0·05	45	47	42	12	20	23
	0·27	45	47	42	0	2	2
Propane-1:3-dithiol	0·06	49	52	50	4	6	4
	0·31	49	52	50	0	0	0
$\alpha\beta$-Dimercaptopropionic acid	0·10	53	52	54	11	10	9
	0·48	53	52	54	4	6	0
2:3-Dimercaptopropylamine HCl	0·04	61	61	54	68	64	60
	0·27	45	48	49	0	9	17
	0·21	61	61	54	21	20	16
Kerateine (0·3 %)	—	65	52	54	10	8	17
2-Mercaptoethanol	0·09	57	55	—	54	55	—
	0·43	57	55	—	45	64	—
Cysteine hydrochloride	0·14	48	56	38	60	58	40
	0·27	48	56	38	51	56	45

with the pyruvate oxidase system as an 'arsenic receptor'.

The contrast between two monothiols and these dithiols as regards protection of this system against lewisite oxide is also shown; 0·27 mM-cysteine hydrochloride and 0·42 mM-monothioethylene glycol each completely failed to protect against the effects of 0·016 mM-lewisite oxide. The close chemical relationship of 2-mercaptoethanol to ethane-1:2-dithiol and the disparity between these two compounds as antidotes against lewisite oxide is particularly striking.

it seemed desirable to obtain information concerning the stability of the lewisite-dithiol compound. An attempt was made to prepare the compound of lewisite and BAL and to measure its toxicity directly; owing to the extreme insolubility of the compound, however, such a method did not seem easily practicable. It was decided therefore to study this point indirectly by determining the degree of inhibition of pyruvate oxidation produced by adding samples of a brain *brei* to a Ringer-phosphate-pyruvate solution containing equivalent amounts of lewisite and BAL that had been allowed

Table 4. *Effect of* 2:3-*dimercaptopropanol (BAL) on the toxicity of other arsenical compounds to the pyruvate oxidase system*

			Inhibition of O_2 uptake (%)					
	Concentration		Arsenical			Arsenical + BAL		
Arsenical	As (mM)	BAL (mM)	0–30 (min.)	30–60 (min.)	60–90 (min.)	0–30 (min.)	30–60 (min.)	60–90 (min.)
Phenylarsenoxide	0·015	0·05	52	49	50	3	10	7
	0·015	0·27	52	49	50	3	5	0
Sodium arsenite	0·034	0·27	44	41	42	0	0	2
	0·068	0·27	61	56	65	6	13	17
Ethylarsenoxide	0·016	0·14	41	31	—	0	0	—
Methylarsenoxide	0·016	0·14	36	28	—	0	0	—

Phenyl-, ethyl- and methylarsenoxides, obtained by solution and subsequent neutralization of the corresponding dichlorarsines in water, and neutralized sodium arsenite, have also been used to poison the pyruvate system, and the ability of BAL to prevent this poisoning investigated. The experiments were carried out in the same way as those with lewisite described above, and the results are given in Table 4. It will be noticed that in the majority of the above experiments the diminution in toxicity of the arsenical caused by the dithiol remains approximately constant throughout the three half-hour periods studied. In this connexion to stand at room temperature (in the experiments already described the lewisite was added to flasks already containing both thiol and brain).

Lewisite (10 μg. as the oxide) was therefore added to a series of Warburg flasks containing Ringer-phosphate solution; 1 equiv. of BAL (in one experiment 1, 2 and 4 equiv.) was then added to each of a pair of bottles, another pair serving as control; after standing for 20–30 min. at room temperature the brain brei was added, and the rate of pyruvate oxidation measured. It will be seen (Table 5) that whereas 10 μg. of lewisite produced 50 % inhibition of activity, a mixture of 10 μg.

Table 5. *Toxicity of lewisite-thiol mixtures (in equivalent proportions) to the pyruvate oxidase system*

		Inhibition of O_2 uptake (%)					
		Lewisite			Lewisite + thiol		
Thiol	No. equiv. of thiol	0–30 (min.)	30–60 (min.)	60–90 (min.)	0–30 (min.)	30–60 (min.)	60–90 (min.)
2:3-Dimercaptopropanol (BAL)	1	51	51	51	6	4	6
	1	52	50	38	4	2	0
	1	73	70	69	0	0	0
	1	57	54	57	0	0	0
	2	51	51	51	0	0	4
	2	52	50	38	3	5	0
	4	51	51	51	0	2	7
$\beta\beta'$-Dimercaptodiethyl sulphide	1	52	50	38	55	68	60
	2	52	50	38	38	47	49
Pentane-1:5-dithiol	1	57	54	57	23	21	20
n-Heptane thiol	1	73	70	69	61	70	64
	1	57	54	57	44	40	45

lewisite with 1 equiv. of BAL (6 μg.) was almost non-toxic to the enzyme system. Moreover, although the measurement of oxygen uptake was continued for 2 hr., there was no indication of any significant increase in the toxicity of the lewisite-BAL compound.

The activities of two dithiols of a different type (which could form 8- or 16-membered rings with the arsenical) and of a monothiol, heptane thiol, were also studied by this method; this latter compound was prepared and used here in order to determine whether a longer-chain, lipid-soluble monothiol that would form a water-insoluble thioarsinite would show any greater antidotal activity than the other monothiols used in this work. It will be seen that one equivalent of *n*-heptane thiol or of $\beta\beta'$-dimercaptodiethyl sulphide were without significant effect, while one equivalent of pentane-1:5-dithiol showed only moderate activity. From these tests too it would seem therefore that 1:2- (or 1:3-) compounds are the most effective types of dithiol for this purpose.

A few experiments have also been done in order to determine whether it is possible by the addition of a dithiol to reverse the inhibition of a pyruvate oxidase system already poisoned by lewisite oxide. As in the previous experiments 0·016 mM-lewisite oxide was used to poison the system. The dithiol (BAL) was added to the side-bulbs of Warburg flasks, and was tipped into the main compartment 15 or 45 min. after poisoning the system with lewisite oxide. Table 6 summarizes the results of these experiments.

If the BAL is added only 15 min. after the commencement of the poisoning, complete reversal of the enzyme inhibition is ultimately obtained; if the addition of the BAL is delayed until the system has been poisoned for 45 min. only about 50% reactivation is effected at the end of a further 1½ hr. by the concentrations of BAL used. It is possible that a larger excess of BAL might bring about more complete reactivation of the system after poisoning for 45 min.

As indicated previously by the results of earlier workers, enzyme inhibition by arsenic does not, at any rate in the earlier stages, appear to be an irreversible phenomenon, and the addition of a dithiol to the brain pyruvate oxidase system as late as 45 min. after poisoning with lewisite can markedly reverse the toxic effects.

Antidotal activity of thiols against lewisite inhibition of respiration of skin

Since the primary objective was the production of an antidote capable of preventing vesication of skin by lewisite and other chloroarsine derivatives, the effectiveness of BAL as an antidote against the lewisite inhibition of the respiration of skin was also studied.

As in experiments described elsewhere (Thompson 1946) slices of young rat skin were used, and the respiration measured at pH 7·3 and at 38° over periods of 1 hr. in the presence of added sodium pyruvate (0·02 M). The results of 2 experiments are shown in Table 7. Two monothiols, 2-mercaptoethanol and cysteine, were also studied.

Table 6. *Ability of 2:3-dimercaptopropanol (BAL) to reverse the toxic effect of lewisite oxide on the brain pyruvate oxidase system*

Time of contact with poison before BAL tipped in (min.)	Concentration BAL (mM)	Inhibition of O_2 uptake (%): Lewisite 15–45 (min.)	Lewisite 45–75 (min.)	Lewisite 75–105 (min.)	Lewisite 105–135 (min.)	Lewisite + BAL 0–15 (min.)	Lewisite + BAL 15–45 (min.)	Lewisite + BAL 45–75 (min.)	Lewisite + BAL 75–105 (min.)	Lewisite + BAL 105–135 (min.)
15	0·27	51	46	46	—	—↓	1	0	0	—
15	0·27	44	59	43	—	—↓	14	4	0	—
45	0·14	47	47	40	—	—	56↓	36	16	—
45	0·14	64	52	69	60	—	59↓	34	31	28
45	0·14	56	57	53	52	—	54↓	50	23	19
45	0·27	56	57	53	52	—	49↓	48	26	27

(↓ = BAL tipped into system.)

Table 7. *Antidotal effects against lewisite inhibition of respiration of rat skin slices*

(Concentration lewisite = 0·03 mM)

		Thiol (mM)	Inhibition of total O_2 uptake in presence of pyruvate (%): 0–60 (min.)	60–120 (min.)	120–180 (min.)
1	Lewisite	—	42	55	61
2	„	—	58	76	79
1	Lewisite and BAL	0·27	12	13	6
2	„ „	0·27	0	4	1
1	Lewisite and 2-mercaptoethanol	0·54	55	60	22
2	Lewisite and cysteine	0·54	48	71	78

As with pyruvate oxidation in brain it will be seen that under the conditions described above,

BAL causes almost complete protection against the effects of lewisite in skin respiration, whereas cysteine and 2-mercaptoethanol, in equivalent concentrations, completely failed to protect. The significance of this finding is emphasized by the results obtained with BAL and 2-mercaptoethanol in the prevention of vesication of human skin by lewisite described in a later section.

Prevention of poisoning by lewisite in rats by dithiols

It was decided to determine next whether BAL or other dithiols are capable of preventing the death of rats following the application to the skin of lethal doses of lewisite, and to compare the effects of these compounds with those of a monothiol and an alternative treatment with hydrogen peroxide which was under investigation elsewhere at the time of this work.

White rats (80–200 g.) were used. A small area of skin (approx. 10 sq.cm.) on the back of each animal was clipped free from fur with scissors. The animals were not shaved on account of the risk of trauma to the skin. A measured quantity of lewisite, calculated from the Porton figure (LD_{50} dose = 24 mg./kg.) to be between 1 and 2 LD_{50} doses, was then placed on the middle of the shaved area by means of a calibrated capillary pipette. At varying intervals of time after the application of the lewisite a measured amount of BAL was placed on the contaminated site, and was lightly spread over the shaved area with a glass rod, after which the animals were returned to their cages.

In the earlier experiments a large excess of BAL was used in the treatment, approximately 50–70 mg. being applied to a burn caused by 3–8 mg. of lewisite; in Exp. 10 (Table 8), however, the same quantity of BAL was applied to the burn as in the other experiments, but the excess remaining on the skin was rubbed off with cotton-wool at the end of 5 min. In each experiment the animals that survived were kept under observation for at least one month, and in some experiments for 2 months.

These experimental results are summarized in Table 8. The dithiols and the monothiol were all applied in the way described above for BAL; the details of the treatment with hydrogen peroxide are given later.

It will be seen from Table 8 that the local application of BAL is highly effective in saving the lives of rats following contamination with amounts of lewisite that proved uniformly fatal to untreated animals. A survival of 100 % is obtained even when commencement of treatment is delayed for half an hour, after the time of contamination; further, 17 out of 18 animals survived when treatment was delayed for 1 hr., and 14 out of 16 after the elapse of 2 hr.

At the time when this work was reported to the Ministry of Supply, sufficient experiments had not been made with other dithiols to warrant any certain conclusions regarding their particular suitability as therapeutic agents for these lesions.

Table 8. *Summary of inunction treatment of rats contaminated with lethal amounts of lewisite*

Exp.	No. of animals tested	mg. lewisite/ kg. body wt.	Time between contamination and treatment (min.)	Surviving	
				No.	%
1. Untreated	4	27–29	—	0	0
2. ,,	6	28–33	—	0	0
3. ,,	6	29–31	—	0	0
4. ,,	6	31–37	—	0	0
5. ,,	3	31–34	—	0	0
6. ,,	4	35–37	—	0	0
1, 2. 2:3-Dimercaptopropanol	8	28–34	5	8	100
2. ,,	4	27–33	10	4	100
5. ,,	6	32–36	15	6	100
7, 8, 9. ,,	17	30–48	30	17	100
10. ,,	4	34–37	30	4	100
11, 12. ,,	12	30–36	60	11	92
13. ,,	6	31–37	60	6	100
6, 14, 15. ,,	16	29–39	120	14	88
16. 1:3-Dimercaptopropanol	6	32–38	45	4	67
17. Toluene-3:4-dithiol	6	28–35	5	6	100
17. Toluene-3:4-dithiol	6	29–34	15	2	33
18. Propane-1:3-dithiol	5	33–39	60	4	80
18. Ethane-1:2-dithiol	5	34–38	60	1	20
19. 2-Mercaptoethanol	6	30–38	30	0	0
14. Hydrogen peroxide	6	31–38	30	2	33
15. ,, ,,	6	34–38	30	1	17
19. ,, ,,	6	33–39	60	4	67
20. ,, ,,	6	34–39	60	0	0

It may be stated however that the aromatic dithiol, toluene-3:4-dithiol, appeared to be toxic to the rat's skin in the amounts used, a large area of necrosis appearing over the treated area; this has never been seen in the case of the aliphatic dithiols so far investigated.

In the one experiment (19) in which a monothiol was used, all six animals died although treatment was begun 30 min. after contamination.

In order to standardize conditions in the animals treated with hydrogen peroxide each burn was swabbed for exactly 3 min. with pledgets of cotton-wool well soaked in hydrogen peroxide. In all, only 7 out of 24 rats treated in this way survived.

It should be pointed out that in every case marked oedema of the skin and subcutaneous tissues underlying the burn, and in many of the cases diarrhoea (and occasionally lachrymation and salivation) developed within 30 min. of the time of application of the lewisite, so that it would appear that BAL or other suitable dithiols can act not only after the appearance of physical signs at the site of the burn, but also after the development of general signs presumably dependent on the systemic effects of lewisite oxide absorbed from the burn.

In one experiment (13) included in Table 8 the burn was treated at the end of 1 hr. with 10% BAL ointment made up as follows: Hardened palm kernel oil, 8 g.; ethyl phthalate, 8 g.; BAL, 4 g.; talc, 20 g. The ointment was applied in a thin layer over the contaminated site and the surrounding skin. As indicated in Table 8 all six animals so treated survived.

The early treatment of rats following heavy contamination with lewisite was next investigated (Table 9). Groups of animals received applications

Table 9. *Treatment of rats heavily contaminated with lewisite with BAL and hydrogen peroxide*

mg. lewisite/ kg. body wt.	No. of LD_{50} doses applied	No. of animals tested		No. surviving		% surviving	
		BAL	H_2O_2	BAL	H_2O_2	BAL	H_2O_2
173	7	6	6	6	4	100	67
207	9	6	6	6	1	100	17
276	11	6	5	6	0	100	0
345	14	6	12	0	0	0	0

of 7, 9, 11 and 14 LD_{50} doses of lewisite on the clipped skin of the back; 5 min. later they were treated either by the application of BAL to the burn or by swabbing with hydrogen peroxide for 3 min.; in these experiments the excess BAL was not wiped off the contaminated area, but was spread over it with a glass rod.

From the results obtained it appears that BAL can protect completely against the effects of 11 LD_{50} doses of lewisite when treatment is commenced 5 min. after contamination. As in the previous experiments, hydrogen peroxide appears to be less effective than BAL. When 14 LD_{50} doses were applied no survivals were obtained with either form of treatment; this is not altogether surprising since, neglecting the loss of lewisite from the skin by evaporation, in order to protect against such a dose the BAL would have to inactivate well over 90% of the lewisite present, this inactivation commencing 5 min. after the lewisite had begun to penetrate the skin.

It seemed of interest to determine also whether BAL is capable of exerting any prophylactic action when applied to the skin of rats at intervals before the application of lewisite. 100–200 mg. of BAL were therefore spread over the clipped skin in the usual way, and at intervals of from 3 to 23 hr. later measured lethal amounts of lewisite were applied to these areas. It will be seen (Table 10) that in the

Table 10. *Prophylactic action of BAL against lewisite*

Time (hr.)	mg. lewisite/ kg. body wt.	No. of LD_{50} doses applied	No. of animals tested	Survivors	
				No.	%
3	33–38	1·4–1·7	6	6	100
4	34–38	1·5–1·7	6	6	100
8	41–50	1·8–2·2	6	1	17
23	32–37	1·4–1·6	6	5	83

groups of animals tested the inunction of BAL 3 or 4 hr. before the application of $1·5 \times LD_{50}$ of lewisite resulted in the survival of all the animals. But since at the end of 4 hr. small amounts of unabsorbed BAL were still present on the surface of the skin this result was not surprising. In a third group of animals therefore $1·5 \times LD_{50}$ was applied 23 hr. after inunction with BAL. It was found that even at this interval five out of the six animals survived. The prophylactic action of BAL was therefore investigated against larger doses of lewisite; six animals received $2 \times LD_{50}$ 8 hr. after the application of BAL; of these, only one survived.

From these few experiments it would seem therefore that while BAL may exert considerable prophylactic power over short lengths of time, its effectiveness after longer intervals is questionable.

In contrast to the results obtained with guinea-pigs, to be described later, it should be pointed out that in these rat experiments death from lewisite poisoning, irrespective of whatever form of treatment had been attempted, always occurred within 48 hr., and in the great majority of cases within 24 hr.; late deaths after several days were never seen.

Treatment of lewisite contamination in guinea-pigs

Owing to the possible variations in reaction of different animal species it was considered advisable to investigate the antidotal activity of BAL in guinea-pigs as well as in rats.

Guinea-pigs of 300–500 g. weight were used. As in the rat experiments, an area of skin (approx. 16 sq.cm.) was clipped free of fur with scissors. A lethal dose of lewisite, based on the Porton figure for guinea-pigs (LD_{50} = 11 mg./kg. body wt.) was then placed on the skin, and the burn treated with BAL 1 hr. later. This treatment as in the previous experiments, was again compared with the results obtained by swabbing the burn with hydrogen peroxide for 3 min.; in the two experiments involving hydrogen peroxide, treatment was begun 30 min. and 60 min. after the application of the lewisite respectively. The survivors in each of the two experiments were kept under observation for 1 month.

Table 11. *Comparison of BAL and hydrogen peroxide in guinea-pigs following contamination with lethal amounts of lewisite*

	No. of animals tested	Dose of lewisite mg./kg. body wt.	Time (min.)	Survivors No.	Survivors %
Untreated	4	17–19	—	0	0
BAL	5	19–21	60	4	80
BAL	5	30–35	60	5	100
H_2O_2	5	19–21	30	0	0
H_2O_2	4	18–20	60	0	0
H_2O_2	5	29–34	60	0	0

It will be seen (Table 11) that treatment with hydrogen peroxide, begun 30 or 60 min. after contamination with 2 and $3 \times LD_{50}$ of lewisite, failed to cause the survival of any of the animals. Five guinea-pigs, on the other hand, that had received three lethal doses of lewisite all survived following treatment with BAL 1 hr. after contamination, while four out of five survived following treatment after contamination with $2 \times LD_{50}$ 1 hr. previously.

It was observed in these experiments that although guinea-pigs contaminated with lethal amounts of lewisite did not survive following treatment of the burns with hydrogen peroxide the course of the poisoning was much modified by the treatment; thus, the four untreated animals died under 26 hr.; the five animals treated with hydrogen peroxide after 30 min. died after 10, 12, 12, 15 and 16 days respectively; four animals treated after 60 min. died after 2, 10, 11 and 17 days respectively, while of the five animals that received $3 \times LD_{50}$ of lewisite followed by peroxide treatment after 60 min. two died on the first day and the remaining three on the fifth day. Moreover, apart from the fact that the animals were not eating well and were steadily falling in weight, they did not appear to be seriously ill until one or two days before death occurred. At autopsy, however, those that died late showed very marked pathological changes in the liver and gall-bladder accompanied by extensive peritoneal effusion. A possible explanation of the chronicity of the poisoning resulting from treatment with hydrogen peroxide may be that the lewisite in the skin is first oxidized to the less toxic pentavalent form; this subsequently undergoes a gradual reduction leading to a slow liberation of the toxic trivalent form into the circulation. The one death among the animals treated with BAL took place on the 13th day, after a steady fall in body weight. The remaining animals treated in this way showed only a very slight and transient fall in weight, and when they were killed one month after commencing the experiment they were eating well and were all in apparently good condition.

Antidotal activity of BAL administered by injection

In view of the likely possibility of systemic poisoning ensuing from the absorption of arsenic from skin heavily contaminated with lewisite, it was clearly important to determine whether a dithiol given by injection, can prevent death from arsenical intoxication. Although for various reasons BAL is not an ideal compound for injection, it is soluble in water and neutral salt solutions to the extent of about 6 g./100 ml.

Groups of white rats were injected intramuscularly with lethal amounts of neutralized solutions of sodium arsenite. Considerable individual variations in sensitivity were observed among different animals; moreover, if death did not take place within a few hours of injection, the animals recovered and survived; in only one case did a late death (after 4 days) occur. Groups of rats that had received similar doses were treated with BAL after varying intervals of time. The BAL (0·1–0·3 ml. saturated aqueous solution) was given by intraperitoneal injection in order to exclude the possibility of any local detoxication at or near the injection site; most of the animals were seriously sick or even collapsed at the time of the injection. The results of these experiments are shown in Table 12.

Two experiments towards the same end were next carried out in rats that had received approximately $1{\cdot}5 \times LD_{50}$ of lewisite on the clipped skin of the back. In the first experiment two groups of rats were each given subcutaneous injection of a saturated aqueous solution of BAL 1 and 2 hr. after contamination respectively; in the second experiment both groups of animals received a second small dose of BAL 3½ hr. after the first. The results are given in Table 13.

Table 12. *Antidotal activity of injected BAL in acute poisoning with sodium arsenite*

	No. of animals	Dose of poison (mg. As_2O_3/kg.)	Interval (min.) between poisoning and treatment	Survivors No.	Survivors %
Untreated	7	8·5–9·9	—	5	71
	15	10·0–14·6	—	5	33
	6	15·0–17·3	—	0	0
	6	18·1–22·0	—	0	0
Treated	10	14·0–18·0	20	9	90
	6	17·4–18·8	12	6	100
	12	18·1–22·4	7	8	67

Table 13. *Effect of BAL given by subcutaneous injection after lewisite contamination of the skin*

	No. of animals	mg. lewisite/kg.	Survivors No.	Survivors %
(1) Dose: 50–61 mg. BAL/kg.				
BAL after 1 hr.	6	34–38	4	67
BAL after 2 hr.	6	32–38	5	83
(2) Dose: 60–70 mg. BAL/kg., followed after 3½ hr. by a further 30–35 mg./kg.				
1st dose after 1 hr.	6	33–38	6	100
1st dose after 2 hr.	6	32–39	6	100

Human tests

Human tests were first carried out in Oxford on four volunteer subjects. The skin of the forearm was contaminated with measured amounts of undiluted lewisite; after intervals of up to half an hour after contamination the burns were treated with BAL, the results being compared with untreated lesions, or, in the case of one subject, with a lesion treated with 2-mercaptoethanol.

In the cases treated with BAL, the compound (30 mg.) was applied to the contaminated site and then lightly spread over a small surrounding area; after a few minutes the BAL was gently rubbed into the skin. On two of the subjects (A.G.O. and R.B.F.) a drop of olive oil was placed on the 'untreated' control burn and gently rubbed in, in order to control any possible spreading effect on the lewisite resulting from the rubbing of the skin. In one subject (V.P.W.) the two contaminations were made on corresponding spots on the two forearms; in the other cases all the contaminations on each subject were made on the same arm, the untreated control being placed between the two treated burns. The results of these tests are given in Table 14, the readings being taken 24 hr. after contamination. It will be seen that the local application of BAL produces a striking effect on the course of the

Table 14. *BAL treatment of lewisite burns in man*

Subject	mg. lewisite applied	Untreated	BAL after 5 min.	BAL after 15 min.	BAL after 30 min.	2-Mercaptoethanol after 30 min.
R.H.S.T.	0·5	V (1 × 0·3)	Nil	—	—	—
R.H.S.T.	0·5	V (1 × 0·6)	—	Nil	E (0·6 × 0·4)	—
A.G.O.	0·5	V (0·7 × 0·7)	—	S (0·2 × 0·2)	E + (1 × 0·7)	—
R.B.F.	0·5	V (1 × 0·5)	—	E + (0·5 × 1)	E − (0·9 × 0·3)	—
V.P.W.	1·0	—	—	—	Nil	E + (1·2 × 1·2) V (0·9 × 0·7)

S = oedema, no erythema. E − = faint erythema. E = erythema. E + = erythema and oedema. V = vesication.
(Dimensions recorded in cm.)

Table 15. *Human tests carried out in the Physiological Department, Porton*

Subject no.	Contamination	Duration of contamination (min.)	Treatment BAL	Treatment H_2O_2	Treatment Olive oil
			Exp. 1		
1	1·1 mm. drop lewisite	10	Very small E −	Very small V	—
2	,, ,, ,,	15	Very small E −	V (1·8 × 1·0)	—
3	,, ,, ,,	30	Very small E −	E + (1·3 × 1·2) Central V	—
4	,, ,, ,,	15	Nil	—	V (1·8 × 0·8)
			Exp. 2		
1	1·1 mm. drop lewisite	45	E − (1·6 × 1·6)	V (2·2 × 1·1)	—
2	,, ,, ,,	45	Small E −	V (1·3 × 0·7)	—
3	,, ,, ,,	60	Nil	Small E −	—
4	,, ,, ,,	60	Nil	Pinhead V E + (2·0 × 1·5)	—
5	2 mm. drop lewisite	30	E (2·2 × 1·2)	V (3·0 × 1·3)	—
6	,, ,, ,,	30	E (1·9 × 1·3)	E + (2·7 × 2·5) V (2·5 × 2·3)	—

(Symbols as in Table 14.)

lesion, no vesication occurring in any of the four subjects even when treatment was not begun till 30 min. after contamination. Treatment with 2-mercaptoethanol did not prevent the development of a large, well filled vesicle.

Further tests on human volunteers were carried out at Porton by arrangement with the Superintendent of the Physiological Department. The results of these tests are shown in Table 15 for comparison with the tests performed in Oxford. Here again, BAL was compared with olive oil and also with hydrogen peroxide. In these experiments the BAL was lightly rubbed over the contaminated area with a glass rod for 3 min., then with the finger at intervals up to 5 min., when the excess was wiped off; in Exp. 1 hydrogen peroxide was swabbed on the comparison lesions for 30 sec., while in Exp. 2 after swabbing for 30 sec. a cotton-wool swab saturated with hydrogen peroxide was placed over the area for 5 min. and kept well saturated during this period. Both BAL and hydrogen peroxide were also tested alone for intrinsic irritancy to normal skin, but in neither case was there any evidence of irritation at the 24 hr. reading.

Further human tests have since been carried out at Porton against phenyldichloroarsine; it has been found that BAL can also prevent vesication by this arsenical up to intervals of 1 hr. after contamination.

Toxicity of dithiols

When this work was being carried out no pharmacological information was available concerning the actions or toxic effects of dithiols in the body. The lethal dose of BAL to rats was first established by application to the skin and by intraperitoneal injection. In the cutaneous applications the BAL was spread over the clipped backs of rats; the amounts that were necessary to reach the lethal dose were so great that they remained as a thick film of oil, on the surface of the skin, complete absorption not taking place for some hours. A proportion of the amount applied was therefore lost, being rubbed off on to the fur surrounding the clipped skin; further, owing to the animals licking the anointed skin and surrounding fur, small quantities of BAL must also have been swallowed. It follows therefore that the lethal dose by cutaneous application is only an approximate figure, although in good agreement with that subsequently found independently at Porton.

For intraperitoneal injection the BAL was dissolved in freshly distilled thiodiglycol (10 mg./0·1 ml. thiodiglycol). The results of administration of BAL by these routes are shown in Table 16; very roughly, 2–3 g. BAL/kg. body weight are lethal when applied to the skin of rats, and 150 mg./kg. when injected intraperitoneally.

Table 16. *Toxicity of BAL to rats*

Mode of administration	No. animals tested	mg. BAL/kg.	Survivors No.	Survivors %
Cutaneous	11	1000–2000	11	100
	11	2030–3000	6	55
	4	4400–6350	0	0
Intraperitoneal	4	86–98	4	100
	6	100–113	3	50
	10	124–145	4	40
	8	147–167	0	0

A more accurate determination of the LD_{50} dose of a standard preparation of water-purified BAL given by intramuscular injection into white rats was later undertaken. Porton strain white rats, weighing from 120 to 180 g. were used, the animals being starved 24 hr. prior to the test. The BAL was dissolved in an amount of freshly distilled propylene glycol so as to give the required dose in a total volume of 0·1 ml. The injections were made into the hind leg, using a tuberculin syringe. Fifteen rats were used for each dosage level. The animals within any one group were chosen of such weights that in any group the dose (as mg./kg. body weight) never varied by more than ± 5 mg./kg. from the level stated in Table 17. The number of rats dying within

Table 17. *Toxicity to white rats of standard preparation of BAL (intramuscular injection)*

(15 rats in each group)

Dose (mg./kg.)	No. rats dead	% dead
95	1	6·5
105	6	40
115	9	60
125	12	80
135	14	93
175	15	100
205	15	100

72 hr. (in our experience it has been most exceptional for any deaths to occur after this time), together with a summary of the statistical examination of the result (kindly carried out by Dr R. B. Fisher, of this Department) are shown in Table 17. The LD_{50} dose, estimated by Bliss's method, is 113 mg./kg.; more precisely, $\log_{10}(LD_{50}) = 2{\cdot}0527 \pm 0{\cdot}0112$ (the slope of the line connecting log dose (mg./kg.) and probit is $19{\cdot}610 \pm 1{\cdot}353$). This corresponds to a probability of approximately 1 chance in 5 that log (LD_{50}) will be outside the range $2{\cdot}0527 \pm 0{\cdot}0172$, i.e. there is 1 chance in 10 of obtaining a value of LD_{50} below 108·5 or above 117·5 mg./kg. body weight. Therefore the LD_{50} dose = 113 mg./kg. ± 2·6 %. It must be stressed that this estimate applies only to the conditions used here and to this particular strain of rats.

A few rough toxicity determinations of other related dithiols were also made. In all cases except where stated the thiol was dissolved in thiodiglycol, and 0·2 ml. of the solution injected intramuscularly

Table 18. *Toxicities of other dithiols (by intramuscular injection into rats except where stated)*

Compound	No. rats tested	Dose (mg./kg.)	Survivors No.	Survivors %
1:3-Dimercaptopropanol	6[1]	35– 67	0	0
	6	26– 32	2	33
	6	17– 20	4	67
Propane-1:2-dithiol	6	90–112	0	0
	5	48– 53	1	20
	6	25– 33	4	67
Propane-1:3-dithiol	6	94–103	0	0
	6	43– 54	6	100
Pentane-1:5-dithiol	6	77– 90	5	83
	6	45– 48	6	100
2:3-Dimercaptopropylamine hydrochloride[2]	6	77– 89	0	0
	6	36– 38	0	0
	6	20– 23	5	83
Trimercaptopropane	6	76– 85	1	17
	6	24– 25	6	100

[1] By intraperitoneal injection into these 6 rats.
[2] Dissolved in water and adjusted to pH 6·6–7·0.

into the hind leg. Table 18 summarizes these determinations.

It is of interest that the LD_{50} dose of 1:3-dimercaptopropanol, reckoned from the figures given in Table 18, is of the order of only 20–30 mg./kg., so that this compound is about 5 or 6 times as toxic as the 2:3-isomer.

None of the other compounds examined has been found to be less toxic than BAL. In all experiments except those with propane-1:2-dithiol, death took place within 48 hr., and usually within 24 hr. Lethal amounts of these dithiols cause tremors leading to death in convulsions, severe spasm of the muscles of the abdominal wall being noticed shortly before death. Propane-1:2-dithiol is the exception; lethal amounts of this compound cause the animals to pass into a collapsed condition with a profound fall in body temperature (down to 23–29°), the animals remaining in this condition for many hours before death (up to 48 hr. or longer).

Slightly sublethal amounts of BAL will also give rise to convulsions, occurring 1–2 hr. after injection. With smaller amounts still, of the order of $\frac{1}{3}$–$\frac{1}{2}$ lethal dose, it is possible to give repeated doses to rats at 3–4 hr. intervals without the development of any serious or lasting symptoms.

In two experiments, 0·5 and 1·0 ml. respectively of undiluted BAL were applied to the forearm of a volunteer. 0·5 ml. was sufficient to cover the whole of the volar aspect of the forearm, the total area covered being about 300 sq.cm.; in the second experiment 1·0 ml. was spread over the entire forearm from wrist to elbow, the area covered being about 530 sq.cm. A rash, scarlatiniform and slightly oedematous in type, and accompanied by severe tingling, developed rapidly, but disappeared completely within 4 or 5 hr. Slight lachrymation and dilatation of subconjuctival vessels were also observed, but it was impossible to decide whether this was due to the effects of absorbed BAL or to the direct action of the vapour.

In the course of the therapeutic tests carried out on human subjects both in Oxford and Porton, tingling and transient slight erythema were produced in most subjects by the application of the undiluted compound, and in a few cases a localized urticarial response centring round the hair-follicles was observed. These signs of irritancy, however, were all of short duration, usually disappearing in under 4–6 hr.

Toxicity of the lewisite derivatives of thiols

A comparison has been made of the toxicity to rats (by subcutaneous injection) of the lewisite derivatives of 2:3-dimercaptopropanol and of 2-mercaptoethanol.

The lewisite-BAL derivative was prepared as described by Stocken (1946). Samples of the compound were dissolved in thiodiglycol and injected subcutaneously into rats; the LD_{50} dose of lewisite by subcutaneous injection in rats is 5 mg./kg. Table 19 gives the results obtained with the lewisite-BAL compound. The figures in column 3 of Table 19 give the number of LD_{50} doses of lewisite contained in the amount of the derivative injected. It will be seen that on the basis of the arsenic content the LD_{50} dose of this compound is about 5 times that of lewisite, indicating that combination of lewisite with BAL to form the dithiarsenole ring brings about a very substantial decrease in toxicity.

Table 19. *Toxicity of the lewisite derivative of BAL*

No. rats tested	mg./kg. body wt.	Equivalent no. of LD_{50} doses of lewisite	Survivors No.	Survivors %
4	12·5–18·1	2–3	4	100
4	21·0–23·4	3–4	4	100
14	25·6–30·4	4–5	7	50
3	40·5–58	6–9	0	0

We have not so far been able to obtain a pure specimen of the lewisite derivative of 2-mercaptoethanol; from the analytical figures it was calculated that the product contained approximately 11 % of free lewisite oxide. An amount of 2-mercaptoethanol equivalent to the free lewisite oxide was therefore added to the product, and a sample of this mixture, dissolved in thiodiglycol, was injected subcutaneously into rats. The dose of the lewisite compound together with the free lewisite oxide present was equivalent to $1{\cdot}88–2{\cdot}06 \times LD_{50}$ doses of lewisite; the free lewisite oxide present, which was

'neutralized' by the added 2-mercaptoethanol, corresponded to less than one-fifth of an LD_{50} dose. The result of this experiment is shown in Table 20.

Table 20. *Toxicity of the lewisite derivative of 2-mercaptoethanol*

No. of rats tested	mg./kg. body wt.	Equivalent no. of LD_{50} doses of lewisite	Survivors No.	Survivors %
6	13·0–14·6	1·8–2·1	0	0

It will be seen that all six rats died following the injection of an amount of the derivative equivalent to 2 LD_{50} doses of lewisite; in the case of the lewisite-BAL derivative 100% survival was obtained following the injection of an amount equivalent to 3–4 LD_{50} doses.

DISCUSSION

The results presented above indicate clearly that, by contrast with the monothiols so far investigated, a simple aliphatic dithiol, 2:3-dimercaptopropanol (BAL), is capable of exerting a marked antagonistic effect to the toxic action of lewisite on animal cells.

In testing these thiols the results obtained with the brain pyruvate oxidase system, which throughout the work has been used as a rapid and economical initial test system, have been amply confirmed by *in vivo* tests on rats, guinea-pigs and human subjects.

The local treatment of lewisite burns of the skin in man has been tested up to 60 min. after contamination, and the results show that BAL is a highly effective agent in the prevention of vesication. In most of the experiments the BAL was not applied to the lesion until well-marked signs of injury to the skin (erythema and oedema) had developed, and it is of particular interest that successful therapy can be obtained when delayed until this late stage. Since in many of the cases the final erythema 24 hr. after treatment was considerably less in size and intensity than that already developing at the time of treatment, an actual reversal of the underlying pathological change brought about by the lewisite is suggested. Furthermore, the ability of BAL to reverse the poisoning of the pyruvate oxidase system by lewisite oxide indicates that a similar reversal of the combination of lewisite with the arsenic 'acceptor' in living skin is certainly possible.

The systemic effects and ultimate deaths of animals contaminated on the skin by lethal amounts of lewisite can also be prevented by the local application of BAL to the lesion. In these experiments the treatment with BAL was successful when begun after the appearance of physical signs of intoxication (diarrhoea, lachrymation and salivation), presumably dependent on the systemic effects of lewisite oxide absorbed from the damaged skin. BAL, given by injection, has also been found to save animals after either a lethal contamination with lewisite or the injection of a rapidly fatal dose of sodium arsenite, indicating that BAL is not only an effective skin decontaminant, but can also deal with systemic poisoning.

Since no late deaths in either rats or guinea-pigs have been seen following the treatment of lethal lewisite contaminations with BAL it seems unlikely that the lewisite-BAL compound dissociates inside the body to any great extent, with reliberation of a toxic arsenical compound. *In vitro* confirmation of this view is afforded by the enzyme experiments in which a mixture of equivalent amounts of lewisite and BAL proved almost non-toxic to the pyruvate oxidase system and showed no increase in toxicity throughout the 2 hr. of the experiment. As later work has shown (Thompson & Stocken, 1941), the arsenic is in fact rapidly excreted from the system in increased amounts following treatment with BAL.

A brief, preliminary review of the English work on British Anti-Lewisite has already appeared (Peters, Stocken & Thompson, 1945).

SUMMARY

1. The behaviour of monothiols and dithiols in their reactions with lewisite and arsenite has been examined by comparing the abilities of representative compounds of these classes to protect the brain pyruvate oxidase system, to cause survival of animals contaminated with lewisite or injected with arsenite, and to prevent vesication in man.

2. The relative rates of hydrolysis of thioarsinites prepared by reacting lewisite with mono- and dithiols have been compared; the cyclic thioarsinites formed with dithiols are markedly more stable than the non-cyclic compounds formed with monothiols.

3. Low concentrations of 2:3-dimercaptopropanol (BAL) are highly effective in protecting the brain pyruvate oxidase system from the toxic action of chlorovinyl-, phenyl-, ethyl- and methyl-arsenoxides and of sodium arsenite. Other 1:2- and 1:3-dithiols have also been tested and found effective against chlorovinylarsenoxide.

4. A mixture of lewisite oxide and BAL in equivalent proportions, is almost non-toxic to the pyruvate system, when tested at a concentration of lewisite which alone would produce about 50% inhibition.

5. BAL is capable of bringing about a substantial degree of reversal of the lewisite inhibition of the pyruvate system.

6. BAL prevents the inhibition of the respiration of rat skin slices by lewisite; 2-mercaptoethanol and cysteine were ineffective.

7. Survival of rats and guinea-pigs after contamination of the skin with lethal amounts of lewisite has been obtained by the local application of BAL even when treatment was delayed for as long as 2 hr. after contamination.

8. Injections of saturated aqueous solutions of BAL have saved rats after the injection of lethal amounts of sodium arsenite or the contamination of the skin with lethal amounts of lewisite.

9. Vesication in man following the application of small amounts of lewisite to the skin can be prevented by treatment with BAL up to at least 1 hr. after contamination.

10. Rough toxicity tests of a number of dithiols have been carried out and the LD_{50} dose of BAL when given by intramuscular injection to white rats has been accurately assessed.

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British Anti-Lewisite

3. ARSENIC AND THIOL EXCRETION IN ANIMALS AFTER TREATMENT OF LEWISITE BURNS

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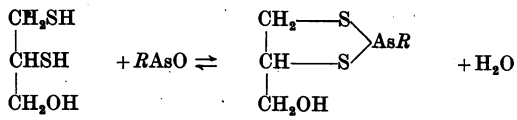
Earlier work in this laboratory (Stocken & Thompson, 1941) has shown that 2:3-dimercaptopropanol (British Anti-lewisite or BAL) reacts with lewisite and other arsenicals of the types *R*AsO and *R*AsCl$_2$ to form stable ring compounds:



the affinity of the dithiol for the arsenical being sufficiently great to bring about combination with the traces of lewisite dissociating from combination with the tissue proteins. Low concentrations of BAL have been shown to protect against and to reverse the toxic action of lewisite on the pyruvate oxidase system of brain, in this respect differing fundamentally from all the monothiols so far investigated. Further, small animals may be caused to survive after contamination with lethal