BAL-INTRAV: A New Non-toxic Thiol for Intravenous Injection in Arsenical Poisoning*

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1. BIOLOGICAL OBSERVATIONS

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This paper is based on a report to the Ministry of Supply made in March 1944, and describes investigations culminating in the production of BAL-INTRAV (2:3-dimercaptopropanol glucoside), a non-toxic thiol, affording a high degree of protection against lewisite poisoning, and likely to be of value in other forms of arsenical, metal and metalloid poisoning.

A series of fundamental investigations in the Department of Biochemistry at Oxford (Peters, Stocken & Thompson, 1945), based on the view that the lethal action of lewisite is caused by its combination with chemical groups in enzymes, particularly SH groups, has resulted in the production of BAL (2:3-dimercaptopropanol). This substance, when applied to the skin of a contaminated animal, provides a considerable measure of protection against the vesicant action of lewisite. But as an antidote to systemic poisoning, BAL suffers from the disadvantage of rather high toxicity, and so must be used in relatively small amounts fairly soon after contamination, and cannot be used for intravascular injection in large amounts. The LD_{50} of BAL, when given to rabbits by intramuscular injection, is about 50 mg./kg., i.e. about 10 times the LD_{50} (by weight) of lewisite.

Meanwhile, studies at Porton by Cameron and his colleagues (1940, 1941) had shown that in lewisite poisoning there is, in addition to the local lesion on the skin, a condition of shock with marked haemoconcentration, and a pronounced systemic effect involving damage especially to the liver and lungs. Serum therapy and other anti-shock measures were shown to be useless as a means of prolonging life, and it was concluded that until the arsenical could be removed from the body, or in some way inactivated, little could be done by way of therapy. Further studies at Cambridge showed (*a*) that perfusion of lewisite increases the permeability to protein of the capillaries of the frog (Danielli & Danielli, 1941*a*) and that this will occur with a dosage of as little as 0·2 mg./kg. (Danielli & Danielli, 1941*b*); (*b*) that an increase in rate of loss of plasma appears prior to any apparent haemo-concentration (Cameron, Short, Mitchell & Danielli, 1943); (*c*) that with a dosage of 4 mg. lewisite/kg. (*c.* 1 LD_{50}) given to 2 kg. rabbits, the rate of loss of protein from the blood stream has become marked after 1 hr. and almost maximal 6 hr. after contamination, and (*d*) that of the increased rate of protein loss, 95% was systemic and only 5% was due to the local lesion (Mitchell, Danielli & Short, 1944).

The conclusion was drawn that therapeutic measures must involve two effects; first, the inactivation of the arsenical and preferably its removal from all sites, including intracellular sites, which it has reached, and second, recuperation of the tissues from the abnormalities produced by the arsenical invasion. To achieve the first part of this programme a drug is required with the following properties:

(1) It shall be so non-toxic that no danger exists of complications from an overdose, even when given intravenously. (2) It shall prevent arsenicals penetrating from the blood stream into cells. (3) It shall remove arsenicals from the cells into which they have penetrated. (4) The reaction product between the drug and the arsenical shall be readily excreted.

To meet requirements (1), (2) and (4), use may be made of the principles involved in the normal detoxication processes carried out by the living organism itself, discussed elsewhere (Danielli, 1942).

Use of inability to penetrate the cell membrane is seen in detoxication mechanisms, in which toxic substances are conjugated with amino-acids, with sulphuric acid or with glucuronic acid, so that a toxic, cell-penetrating substance such as bromobenzene or menthol is converted into a new molecule which cannot penetrate into cells, and which, once reaching the blood stream, is bound to be filtered off by the glomerulus of the kidney, and will not be resorbed from the urine in the tubules unless it happens to fit into

* A preliminary account of this work has been given elsewhere [*Nature, Lond.*, **157**, 217 (1946)].

the rather specific secretory mechanism of the tubules. Thus by conjugation with a molecule such as glucuronic acid, two objects are secured simultaneously: (*a*) the toxic substance is prevented from penetrating into cells and (*b*) its excretion into the urine is secured.

Thus, we expected that the toxicity of BAL would be greatly diminished on conversion into its glucuronide or ethereal sulphate, since the ability of the substance to penetrate into cells would then be much reduced. It would at the same time circulate freely in the blood stream, intercellular spaces and lymphatics and would sweep these spaces clear of arsenical since its affinity for arsenic should be almost unchanged; it would also be readily excreted in the urine. From the point of view of synthesis, BAL glucuronide and ethereal sulphate have proved difficult of access, but the required physico-chemical properties connected with permeability may be obtained by use of any molecule containing a number of hydroxyl or carboxyl groups (Danielli, 1943). We have therefore investigated such molecules as BAL glucoside, mercaptosuccinic, and dimercaptoadipic acid.

So far we have dealt with points (1), (2) and (4) above. The remaining point, (3), concerns the removal of arsenic from cells into which it has already penetrated. It might be thought that if the thiol is incapable of penetrating cells, so its ability to remove arsenic from them will be destroyed. This is not so. Most cells contain sufficient reduced glutathione to prevent arsenic being completely localized at a particular intracellular site or becoming completely indiffusible: there must be an equilibrium between the indiffusible arsenic fixed by SH and other groups of proteins, and the diffusible arsenic associated with SH groups of glutathione. In the course of this work we have obtained evidence that glutathione may pass relatively rapidly through some cell membranes. Consequently we may expect that arsenicals will not be completely inaccessible to a thiol drug, even if the drug is completely unable to penetrate into cells, since by virtue of the above equilibrium the arsenic will be slowly removed by the thiol. Thus we anticipated that the compounds conforming to requirements (1), (2) and (4) would also fulfil requirement (3).

EXPERIMENTAL

Treatment of animals. Unless otherwise stated, rabbits of 1·5–2·5 kg. in weight were used. Lewisite (12 mg./kg.) was applied to an area of the back from which hair had been clipped. Subsequently, the animals were given unrestricted access to dry food, green food and water. Sick animals were kept in a warm room until recovery or death.

Preliminary experiments showed that the LD_{50} for the sample of lewisite used, and for the size of rabbit used, was *c.* 4 mg./kg. or less. In choosing the dosage of lewisite to be used we were influenced both by shortage of rabbits and of technical assistance, which jointly precluded the possibility of using large numbers of animals. It was therefore impossible to study small effects which could only be established by statistical analysis. We therefore used a large dosage of lewisite (12 mg./kg.) which killed all animals with certainty in 10–20 hr. if no therapeutic measures were taken. If, after therapy, there ensued marked prolongation of life or survival, we could be quite sure these results were significant and indicated effective therapy.

Immediately before injection the batch of thiol was neutralized to thymol blue, using concentrated HCl or NaOH, and then diluted to be approximately isotonic with plasma. We endeavoured to give a dose of thiol of at least 20 equivalents of the amount of lewisite applied.

No precautions of any kind were observed with regard to sterility.

RESULTS

Toxicity tests

Before use, toxicity tests were made to ensure that the dose of thiol to be used for therapy was not in itself toxic. The rabbits employed for these experiments were given at least twice as much thiol as was to be used for therapy. The dose was split into 5 parts, given intravenously at intervals of 1 hr. Blood samples were taken at intervals over 24 hr. after the first injection and haematocrits determined. Approximate values of LD_{50} for various thiols are given in Table 1. Since only two animals were studied for a given dosage, these are only rough values and should be regarded as significant only as regards order of magnitude. In no case was significant haemo-concentration observed following administration of the thiol.

Table 1. *Toxicity of various thiols*

(2 kg. rabbits; thiol given intravenously*)

Thiol	Approx. LD_{50}/kg. (mg.)
BAL (dimercaptopropanol)	50
Mercaptoacetate	100
Mercaptosuccinate	>1000
Mercaptoadipate	>1000
Acetylmercaptosuccinate	>1000
αα′-Dimercaptoadipate	>1000
Glutathione	>2000
Glucothiose	1000
BAL glucoside (BAL-INTRAV)	>5000

* See Part 2 of this paper for details of synthesis.

The Table shows that the toxicities fall in the order BAL > mercaptoacetate > glucothiose > mercaptosuccinate, mercaptoadipate, acetylmercaptosuccinate, dimercaptoadipate, glutathione and BAL glucoside (BAL-INTRAV). Drs Thompson and Stocken have informed us that trithioglycerol is more toxic than BAL. Thus the toxicities fall into the order required by the theory given above, with the possible exception of glucothiose. With fatal doses of BAL and mercaptoacetate, death occurs

fairly soon after intravenous injection, whereas with glucothiose, only after 4–5 days. We therefore suspect that the former two compounds penetrate rapidly into cells and may cause death by one mechanism, whereas glucothiose penetrates slowly and causes death by a different mechanism, possibly by competition with glucose for some enzyme centres.

Whilst conducting the experiments reported here with mercaptosuccinate, acetylmercaptosuccinate, mercaptoadipate, dimercaptoadipate, glutathione and BAL-INTRAV, we had insufficient material to determine the LD_{50} or even to observe marked symptoms of toxicity. To rabbits, the toxicity of these compounds is so low as to be of no practical importance. Larger supplies of BAL-INTRAV became available later, and a report on its toxicity will be given in another paper (Danielli, Jones & Mitchell, in preparation).

As indicated by the nitroprusside test, mercaptosuccinate and BAL-INTRAV persist in the blood stream for some hours after injection. On the other hand, when 3·6 g. of reduced glutathione were injected intravenously into a 3 kg. rabbit, at least 95% of the glutathione had vanished after 8 min. from the blood stream. This loss was not produced by oxidation to GSSG, or to entry of glutathione into red blood cells. There must be some cellular system in the body which removes glutathione from the blood stream with great rapidity.

Relative therapeutic efficiency of different thiols

Two rabbits were used with each thiol; they were painted with 12 mg. lewisite/kg. Intravenous and subcutaneous injections of thiol were given at 0, 1, 2, 4 and 6 hr. after contamination with lewisite. About half the dose was given by injection into the marginal ear vein, and the remainder by subcutaneous injection made in the vicinity of the contaminated area.

The results are shown in Table 2. Many of the thiols were able to prolong life, and in borderline cases (i.e. with less heavy contamination with lewisite) might perhaps save life. But only the dithiols (dimercaptoadipic acid and BAL-INTRAV) had a very pronounced effect, and of these two compounds, BAL-INTRAV alone prevented death with the dosage used. We therefore made a more intensive study of BAL-INTRAV.

The conditions under which BAL-INTRAV will ensure survival

In their studies Cameron and his colleagues found that profound damage to the liver occurs at an early stage in lewisite poisoning. In our studies on lewisite shock (Mitchell, Danielli & Short, 1944) we found that with a dose of only 4 mg. lewisite/kg., systemic damage to capillary function is significant after 1 hr. and almost maximal after 6 hr. These changes must occur even more rapidly with the dosage used in the present experiments (12 mg./kg.). It is therefore clear that damage to important cellular systems commences at an early stage, and that if this progresses too far, removal of the arsenic by administration of BAL-INTRAV cannot prevent death. We have therefore made an attempt to find how long the administration of BAL-INTRAV may be delayed. The results are given in Table 3. As before, the BAL-INTRAV was given in

Table 3. *The effect of administration of a total of 1·5 g. BAL-INTRAV/kg. at various times after contamination of 2 kg. rabbits with 12 mg. lewisite/kg.*

Time after contamination before first injection of BAL-INTRAV (hr.)	No. of animals	Survival time after dosage (hr.)	Survivors (%)
0	2	None died	100
2	7	,,	100
4	2	,,	100
6·5	8	,,	100
12	4	12, 12, 13, 13	0

Table 2. *Effect of different thiols on rabbits treated with lewisite*

(2 kg. rabbits; 12 mg. lewisite/kg. painted on the skin. Injection of thiol was commenced immediately after contamination.)

Thiol	Dosage (g./kg.)	No. of animals	Survival time after dosage (hr.)	Survivors (%)
None	—	11	10, 12, 15, 18, 13, 13, 14, 13, 14, 14, 15	0
Glutathione	0·7	2	12, 18	0
BAL	0·07	2	10, 15	0
Mercaptosuccinate	0·5	2	48, 70	0
Acetylmercaptosuccinate	0·75	2	20, 40	0
Mercaptoadipate	0·5	2	43, 65	0
Glucothiose	0·5	2	24, 26	0
Dimercaptoadipate	0·75	2	72, 260	0
BAL-INTRAV	1·5	2	None died	100

4–5 doses, half intravenous and half subcutaneous by rapid hypodermic injection.

Animals treated up to 4 hr. after contamination had a moderate lesion on the back, but made an uneventful recovery without loss of appetite at any stage. Animals treated 6·5 hr. after contamination also recovered completely, but were rather ill and of poor appetite for 1–3 days. But animals treated 12 hr. after contamination (i.e. on the point of death) did not appear to be in any way assisted by this late injection of BAL-INTRAV.

We think it probable that after 12 hr. so much cellular damage has been done by this heavy dosage with lewisite that irreversible processes have set in. Our present knowledge of cell physiology is so slight that there can at present be little hope of developing a method of effective therapy once damage has progressed to such a stage that removal of arsenic is inadequate.

Complications and checks

A number of further factors needed checking. These were: (*a*) The reproducibility of therapeutically effective BAL-INTRAV. The possibility existed that repetition of the synthesis might prove impossible. This possibility has been eliminated: the results of Table 3 were obtained with two different batches of BAL-INTRAV, and numerous further batches have since been examined (Danielli, Jones & Mitchell, in preparation). No difference in the effectiveness of the batches was observed.

(*b*) Deterioration on storage might be serious. We have not studied this in detail, but one sample appeared to have unimpaired activity after storage for 3 months at 2°.

(*c*) Different batches of lewisite are of different toxicity. The sample used in the experiments described above appeared to be more toxic than most. Four experiments (with treatment of two rabbits 4 hr. after contamination and of the other two 6·5 hr. after contamination) showed that BAL-INTRAV coped just as readily with a sample of lewisite which (at the same dosage of 12 mg./kg.) produced death in about 24 hr.

(*d*) The size of the animal is likely to be an important variable. For example, young rabbits have very thin skins and so may absorb lewisite more rapidly than older animals; they are also usually less resistant to experimental procedures. We therefore carried out a number of experiments with young rabbits of about 600 g. weight. The results are recorded in Table 4. It is clear that young animals must be treated sooner after contamination than is essential with older animals.

Table 4. *The effect of administration of* 1·5 *g./kg. of BAL-INTRAV to young* (600 *g.*) *rabbits at various times after contamination with* 12 *mg. lewisite/kg.*

Time after contamination before first injection of BAL-INTRAV (hr.)	No. of animals	Survival time after dosage (hr.)	Survivors (%)
4	4	None died	100
6·5	4	14, 12, 12, 80	0

2. CHEMICAL OBSERVATIONS

By J. B. FRASER, L. N. OWEN and G. SHAW

For the reasons already mentioned above, the chemical portion of the investigation was directed towards the preparation of water-soluble thiols containing several carboxyl or hydroxyl groups, and the first experiments were concerned with the former type of compound.

Very few dibasic mercapto-acids have been described in the literature. Mercaptosuccinic acid, and mercaptomethylsuccinic acid are most conveniently prepared by reaction of thioacetic acid (CH_3COSH) with maleic and itaconic acids respectively, followed by removal of the acetyl group by mild alkaline hydrolysis (Holmberg & Schjanberg, 1940). Similarly, by the action of thioacetic acid on allylmalonic acid, Schjanberg (1941) has prepared γ-mercaptopropylmalonic acid. An older method in which the halogenated acid is treated with potassium thioacetate and the resulting compound hydrolyzed to the mercapto-acid was applied by Fredga (1938) to the synthesis of αα′-dimercaptoadipic acid from αα′-dibromoadipic acid.

It has now been found that, as would be anticipated, thioacetic acid reacts readily with αβ-unsaturated esters. With methyl acrylate it gave methyl β-*S*-acetylmercaptopropionate, from which β-mercaptopropionic acid was obtained on saponification, and with ethyl maleate it yielded ethyl *S*-acetylmercaptosuccinate, which on saponification gave mercaptosuccinic acid.

The reaction between potassium thioacetate and halogeno-compounds, to give acetylthiols, which had hitherto been used only for the preparation of neutral thiols (Gehrke & Kohler, 1931; Sjoberg, 1942; D. R.-P., 557, 247), has now been applied to the formation of mercapto-acids through the esters. The reaction proceeds in ethanolic solution and is therefore particularly useful for the treatment of halogeno-esters, which, being practically insoluble in water, do not readily condense with potassium ethyl xanthate. Thus from ethyl α-bromopropionate, ethyl α-*S*-acetylmercaptopropionate was prepared, which on saponification gave α-mercaptopropionic acid. Similarly, ethyl α-bromoglutarate was converted into ethyl α-*S*-acetylmercapto-

glutarate and thence into α-mercaptoglutaric acid; ethyl α-bromoadipate was used for the preparation of α-mercaptoadipic acid. It was hoped to prepare αα′-dimercaptosuccinic acid, but efforts to bring about the addition of thioacetic acid to chloromaleic acid resulted only in the formation of chlorofumaric acid, and although a reaction occurred on treatment of ethyl αα′-dibromosuccinate with potassium thioacetate, the product obtained on saponification contained no sulphur.

In the carbohydrate field, monothiols derived from several sugars have been described. In general, these have been of the glucothiose type, prepared from the acetohalogeno-sugar *via* the disulphide, xanthate, or acetylthioderivative (Wrede, 1922; Schneider, Gille & Eisfeld, 1928; Gehrke & Kohler, 1931; D. R.-P., 557, 247); an exception is the 6-thioglucose of Ohle & Mertens (1935) prepared from a 5:6-anhydro-sugar. Mercaptoalkylglucosides, however, are unknown. Unsuccessful attempts were made to condense acetobromoglucose and BAL, and it was decided to attempt the preparation of BAL glucoside by the following route: Acetobromoglucose → tetra-acetyl β-allylglucoside → tetra-acetyl β-(2′:3′-dibromopropyl) glucoside → hexa-acetyl BAL glucoside → BAL glucoside. The dibromide, previously described by Fischer (1920), reacted rapidly with potassium thioacetate in boiling ethanol, and the syrupy product was deacetylated by the Zemplen catalytic procedure with slightly more than two equivalents of barium methoxide in methanol to yield a barium salt of the thiol. Biological tests on a solution of the free glucoside, obtained by removal of barium as sulphate, were highly satisfactory, and no difficulty was experienced in repeating the operations in the synthesis, but for large-scale work it was obviously desirable to devise an alternative procedure which would avoid the necessity of using acetobromoglucose.

The direct formation of glycosides by the Fischer method (condensation of sugar and alcohol in the presence of hydrogen chloride) proceeds smoothly and in good yield with the lower aliphatic saturated alcohols, in which the sugar is appreciably soluble, but no success was achieved in attempted condensations of glucose with 2:3-dibromopropanol. The method, however (cf. Fischer, 1893), has now been found to be applicable to the preparation of allylglucoside. This section of the work was completed in 1944. More recently, Talley, Vale & Yanovsky (1945) have reported the preparation of the same substance by a similar process, though the yield was inferior to that obtained by the method now to be described. Glucose readily passed into solution when warmed with allyl alcohol containing 3 % of dry HCl; but on continued heating the HCl concentration gradually diminished, owing to the formation of allyl chloride, and for optimal yields it was found necessary to pass in more dry HCl from time to time. After neutralization and evaporation, the mixed α- and β-allylglucosides were, after extraction with boiling acetone, obtained as a syrup which slowly crystallized. Attempts were made to brominate this material in dioxan solution, but the product was very unstable, and the glucosides were therefore acetylated prior to bromination. A portion of the acetylated product, purified by chromatography, was separated by fractional crystallization into tetra-acetyl-α-allylglucoside, and the already known β-isomer. For general purposes, this tedious separation was unnecessary, since there was no reason to suppose that a mixture of the α- and β-glucosides of BAL would be inferior to the β modification originally prepared. The main bulk of the crude tetra-acetate was therefore brominated, treated with potassium thioacetate, and saponified, according to the original procedure. This new specimen of BAL-INTRAV was found to be no less satisfactory than that prepared by the older route. An attempt was also made to convert the dibromide directly into BAL-INTRAV by treatment with sodium hydrosulphide, but the difficulty of separating the product from excess reagent and inorganic salts rendered the modification impractible.

BAL-INTRAV has not so far been prepared in a chemically pure state.* The free dithiol (prepared by neutralization of the sodium or barium salt) resinifies very readily with almost complete loss of thiol reaction when its solutions are evaporated. Chromatographic separation was unsuccessful, probably owing to the insolubility of the compound in organic solvents other than alcohols and dioxan. The mercury salt decomposes rapidly when dried *in vacuo*, and both the lead and silver salts are very soluble in water, and consequently difficult to isolate. In this connexion it is of interest that AgCl is readily soluble in BAL-INTRAV solution, a behaviour which also occurs with many other mono- and dithiols; this is undoubtedly due to the great affinity of thiol groups for the ions of heavy metals. Analyses of the barium and sodium salts of BAL-INTRAV have always given anomalous results, the thiol content, determined by titration with iodine, being less than the total sulphur (see Table 5), but unlike BAL, which on titration with iodine gives a sharp end-point, BAL-INTRAV continues slowly to take up iodine. These results have also been confirmed by American workers (N.D.R.C. Report, 1945). It is possible that crude BAL-INTRAV is present partly in an anhydro-form, derived by loss of water between one of the thiol groups and a hydroxyl group in the glucose moiety, comparable perhaps with the anhydro-glycolglucosides of Helferich & Werner (1942; 1943); in this connexion it may be significant that dithioglycide (anhydro-BAL) reacts with iodine in a similar manner, taking up one equivalent immediately and a further quantity much more slowly. Alternatively the low sulphur

* Mr R. M. Evans, of the Imperial College Laboratories, has recently succeeded in obtaining the hexa-acetate of BAL glucoside in pure crystalline form. Details of this work will be published shortly.

value may be explained by partial formation of a thioanhydro ring by loss of hydrogen sulphide. In either event, the metal content should also be low, and this is indeed observed for the sodium salt, but is not so marked in the case of the barium salt. The higher metal content in the latter case may be due to co-precipitation of barium acetate with barium BAL-INTRAV; this difficulty would not be expected to arise to the same extent with the sodium salt, owing to the much greater solubility of sodium acetate in ethanol.

Table 5. *Analysis of salts of BAL-INTRAV*

	Metal (%)	Sulphur	
		Total S (%)	Thiol-S (%)
Sodium salt (i)	6·9	16·9	8·4
(ii)	7·6	13·9	9·2
(iii)	—	11·9	8·0
$C_9H_{16}O_6Na_2S_2$ requires	13·9	19·4	19·4
Barium salt (i)	33·4	16·8	9·8
(ii)	27·0	8·1	5·7
(iii)	30·4	12·5	7·7
$C_9H_{16}O_6BaS_2$ requires	32·6	15·2	15·2

It was thought that polarographic analysis might be capable of detecting a 1:2-dithiol, and thereby assist in confirming the structure of BAL-INTRAV. Brdicka (1933) reported that very small amounts of cysteine can be estimated polarographically in the presence of the cobaltous hexammine ion. The curves he obtained showed a maximum at $-1{\cdot}2$ to $-1{\cdot}3$ V. due to the cobaltous ion; and also a second maximum at $-1{\cdot}8$ V. characteristic of the thiol group; the height of the latter above the cobaltous maximum being dependent, within certain limits, on the concentration of thiol in solution. This wave maximum at $-1{\cdot}8$ V. was given by mercaptoacetic acid, but not by glutathione.

In order to determine whether this effect was general for substances containing free thiol groups, and in particular if it could be used to distinguish between the two thiol groups in BAL and its analogues, several representative thiols were submitted to polarographic examination. In each case, a varying concentration of thiol was used in M-NH_4OH/NH_4Cl buffer solution (pH 9·8) containing $CoCl_2$ at a concentration of 10^{-3} M. Measurements were made against a standard calomel electrode with a Cambridge Voltamoscope.

All the α-mercapto-acids examined showed thiol peaks at approximately $-1{\cdot}8$ to $-1{\cdot}9$ V., although the heights of these waves were different at any particular concentration; the high value for αα′-dimercaptoadipic acid is especially noteworthy. Each acid was examined over a wide range of concentrations, but, for simplicity, only the curves for a concentration of $2{\cdot}5 \times 10^{-4}$ M are shown in Fig. 1.

β-Mercapto-acids gave very different results. β-Mercaptopropionic and mercaptomethylsuccinic acids showed only a slight inflexion at *c.* $-1{\cdot}8$ V., there being no characteristic peak similar to that observed for α-mercapto-acids. For comparison,

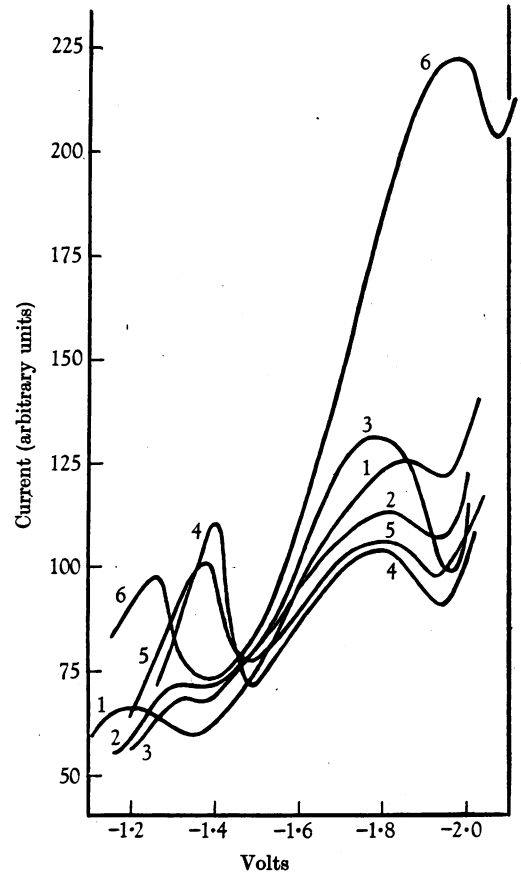

Fig. 1. Polarograms of α-mercapto-acids. 1, Mercaptoacetic acid. 2, α-Mercaptopropionic acid. 3, Mercaptosuccinic acid. 4, α-Mercaptoglutaric acid. 5, α-Mercaptoadipic acid. 6, αα′-Dimercaptoadipic acid.

cysteine was examined at very low concentration, and gave the expected peak at $-1{\cdot}8$ V. (Fig. 2). It has been reported that homocysteine, a γ-mercapto-α-amino acid, gives curves almost identical with those of cysteine, and it appears, therefore, that the presence of the amino group greatly increases the sensitivity of the cobalt-catalyzed polarographic reaction of mercapto-acids.

No thiol inflexion whatsoever was given by BAL, though it did have a marked influence on the general shape of the curve. With thioethyleneglycol, some

indication of an inflexion was observed (Fig. 3), but it is evident that in the absence of a carboxyl group

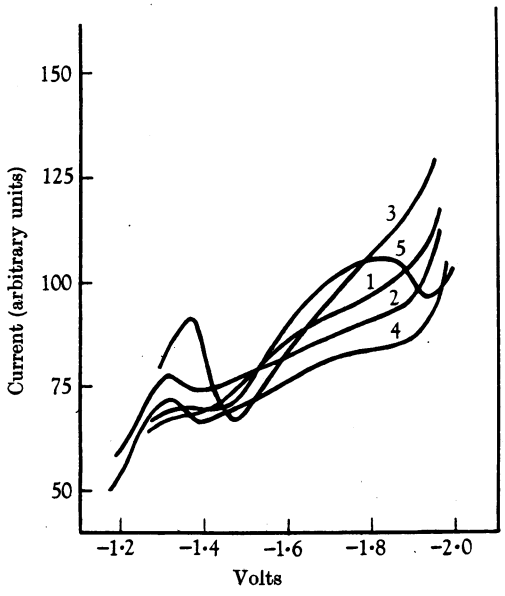

Fig. 2. Polarograms of β-mercapto-acids. 1, β-Mercapto-propionic acid, 10^{-3}M. 2, β-Mercaptopropionic acid, $2{\cdot}5 \times 10^{-4}$M. 3, Mercaptomethylsuccinic acid, 10^{-3}M. 4, Mercaptomethylsuccinic acid, $2{\cdot}3 \times 10^{-4}$M. 5, Cysteine, 10^{-5}M.

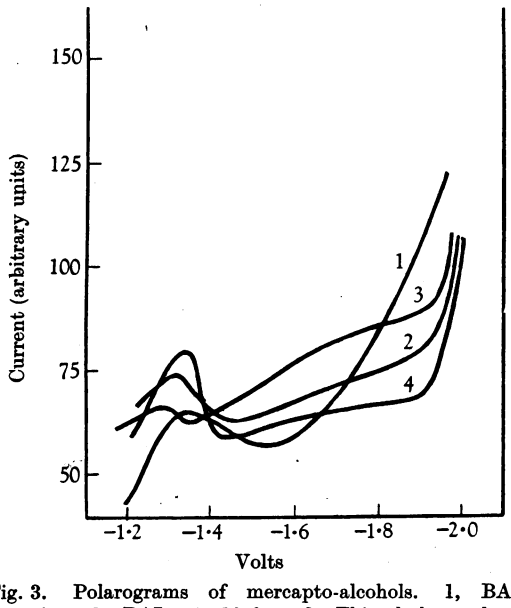

Fig. 3. Polarograms of mercapto-alcohols. 1, BAL, 10^{-4}M. 2, BAL, 5×10^{-6}M. 3, Thioethylene glycol, 10^{-3}M. 4, Thioethylene glycol, $2{\cdot}5 \times 10^{-4}$M.

the polarographic detection of thiols is not possible with the methods at present available.

EXPERIMENTAL

S-Acetylmercaptosuccinic acid, mercaptosuccinic acid, and mercaptomethylsuccinic acid. These were prepared by the method of Holmberg & Schjanberg (1940) and had m.p. 123–124°, 151°, and 106°, respectively. αα′-Dimercaptoadipic acid, m.p. 183°, was obtained by the procedure of Fredga (1938).

Glucothiose. Thioacetic acid (12 g.) in ethanol (50 ml.) was neutralized with 3N-ethanolic KOH and added to a solution of acetobromoglucose (55 g.) in warm ethanol (100 ml.). There was an immediate precipitation of KBr, and after 6 hr. at room temperature, crystals of the penta-acetate had separated from the supernatant liquor. Water was added to dissolve the salt, and the crystals were collected, washed with water, and recrystallized from methanol in colourless needles (45 g.), m.p. 120°, $[\alpha]_D^{19°} + 12{\cdot}4°$ ($c = 5$ in tetrachloroethane). (Found: S, 8·0. Calc. for $C_{16}H_{22}O_{10}S$: S, 7·9%.) Glucothiose solution was prepared from this when required by deacetylation with sodium methoxide, and neutralization with acetic acid, followed by concentration under reduced pressure.

β-Mercaptopropionic acid. A solution of methyl acrylate (6 g.) in thioacetic acid (9 g.) was left at room temperature overnight and then distilled, to yield *methyl β-S-acetylmercaptopropionate* (9·8 g.) b.p. 68°/5 mm., $n_D^{17°}$ 1·4773. (Found: C, 44·55; H, 6·55; S, 20·3. $C_6H_{10}O_3S$ requires C, 44·4; H, 6·2; S, 19·8%.) A portion of this ester (9 g.) on saponification with cold 2N-NaOH overnight gave 3 g. of β-mercaptopropionic acid, b.p. 85°/5 mm., $n_D^{20°}$ 1·4918.

Ethyl mercaptosuccinate. Ethyl maleate (5 g.) was mixed with thioacetic acid (2·4 g.) and heated at 100° for 24 hr. Distillation of the product gave 5·4 g. of a *colourless oil*, b.p. 163°/14 mm. (Found: C, 48·3; H, 6·4. $C_{10}H_{16}O_5S$ requires C, 48·4; H, 6·45%.) Saponification of this ester gave mercaptosuccinic acid, m.p. and mixed m.p. 151°.

α-Mercaptopropionic acid. Ethyl α-bromopropionate (14 g.) was refluxed for 2·5 hr. in ethanol with potassium thioacetate (from 7·5 g. thioacetic acid). The solution was then poured into water and extracted with chloroform. The washed and dried extracts were evaporated and distilled, to give *ethyl α-S-acetylmercaptopropionate* (7·8 g.) b.p. 55°/3 mm., $n_D^{19°}$ 1·4635. (Found: C, 48·0; H, 7·2; S, 18·7. $C_7H_{12}O_3S$ requires C, 47·7; H, 6·9; S, 18·2%.) Saponification of a portion (7 g.) by shaking with cold 2N-NaOH overnight gave 2·3 g. of the acid, b.p. 102°/16 mm., $n_D^{16°}$ 1·4823.

α-Mercaptoglutaric acid. Ethyl α-bromoglutarate (9 g.) in ethanol (20 ml.) was treated with an ethanolic solution of potassium thioacetate prepared by neutralizing a solution of KOH (2 g.) in ethanol (20 ml.) with thioacetic acid. After standing overnight, water (50 ml.) was added, and the oil extracted with ether. Evaporation of the dried extract gave 9 g. of crude ester, a small portion of which was distilled at 178–179°/15 mm., and showed $n_D^{20°}$ 1·4727. The ester (8·5 g.) on saponification with cold aqueous ethanolic 2N-NaOH, gave *α-mercaptoglutaric acid* (2·8 g.) which after recrystallization from ether-benzene had m.p. 97°. (Found: C, 36·9; H, 4·9. $C_5H_8O_4S$ requires C, 36·6; H, 4·9%.) It gave a transient deep blue colour with aqueous ferric chloride and a positive nitroprusside reaction.

α-Mercaptoadipic acid. Similar treatment of ethyl α-bromoadipate (25 g.) gave *ethyl α-S-acetylmercaptoadipate*

(22 g.), b.p. 177°/12 mm., $n_D^{17°}$ 1·4680. (Found: equiv. by saponification, 91. $C_{12}H_{20}O_5S$ requires equiv., 92.) The ester, on saponification with aqueous ethanolic 2N-NaOH, gave *α-mercaptoadipic acid*, m.p. 112–113° after recrystallization from benzene. (Found: C, 40·5; H, 5·7; equiv. by titration with iodine, 180. $C_6H_{10}O_4S$ requires C, 40·4; H, 5·7; equiv., 178.) It gave a transient blue colour with ferric chloride. On standing in alkaline solution, exposed to the air, it readily gave *di(α-adipic acid) disulphide*; it had m.p. 180° after recrystallization from ether-ligroin, and gave no thiol reaction. (Found: C, 40·4; H, 5·3; equiv. by titration with NaOH, 88·5. $C_{12}H_{18}O_8S_2$ requires C, 40·7; H, 5·1%; equiv., 88·5.)

Allylglucoside. Glucose (300 g., anhydrous) was added to a 3% solution of HCl in dry allyl alcohol (1 l.) contained in a 2 l. 3-necked flask fitted with stirrer, inlet-tube and condenser. The mixture was heated to 70° and vigorously stirred for 2–3 hr., after which time only a small amount of glucose remained undissolved. Stirring was discontinued, and the internal temperature was then raised to 95° for a further 12 hr., HCl being passed into the boiling solution from time to time. After cooling, neutralization with barium carbonate, and filtration, the solution was evaporated first at ordinary pressure, and finally under reduced pressure, to a syrup; this was taken up in water and washed twice with ether. The syrup recovered by evaporation of the aqueous solution was then extracted five times with 1 l. portions of boiling acetone. Removal of the solvent from the combined extracts gave a non-reducing syrup (205 g.) which was used as such for the subsequent acetylation. Other preparations of the glucoside, allowed to stand for several weeks, gradually solidified, and recrystallization from acetone gave a mixture of α- and β-allylglucosides, m.p. 75–90°, $[\alpha]_D^{20°}$ +130° (c=1·1 in water). (Found: C, 48·9; H, 7·4. Calc. for $C_9H_{16}O_6$: C, 49·1; H, 7·3%.)

2:3:4:6-*Tetra-acetyl allylglucoside.* The above syrup (205 g.), with fused sodium acetate (100 g.) was dissolved in acetic anhydride (1 l.) and heated on the steam-bath for 1·5 hr. Most of the solvent was then removed, and the residual oil stirred with water to decompose any remaining anhydride. The product was then taken up in chloroform and washed with $NaHCO_3$ solution until neutral. Removal of the solvent from the dried extract gave the crude tetra-acetate (325 g.). In a subsequent preparation, the crude material was dissolved in ether and clarified by filtration through activated alumina. On evaporation of the filtrate, a solid residue was obtained, which was fractionally crystallized from light petroleum (b.p. 40–60°) to give 2:3:4:6-*tetra-acetyl α-allylglucoside*, m.p. 52–53°, $[\alpha]_D^{20°}$ +115° (c=1·1 in methanol). (Found: C, 52·9; H, 6·3. $C_{17}H_{24}O_{10}$ requires C, 52·6; H, 6·2%.) From the mother liquors, the already known β form of m.p. 89° was obtained.

Tetra-acetyl dibromopropylglucoside. The crude tetra-acetate (325 g.), showing $[\alpha]_D^{20°}$ +96° (i.e. mainly the α-form) was dissolved in carbon tetrachloride (1 l.) and treated gradually with a solution of bromine (145 g.) in the same solvent, the temperature being kept below 25°. After washing with $NaHCO_3$ solution, the solvent was removed, to yield the syrupy dibromide (460 g.).

Tetra-acetyl β-(dibromopropyl)glucoside, m.p. 91°, was prepared from acetobromoglucose by the method of Fischer (1920).

BAL-INTRAV. (*First method.*) The crystalline β-dibromide (75 g.) in hot ethanol (200 ml.) was treated with ethanolic potassium thioacetate (from 25 g. thioacetic acid) and refluxed for 6 hr., after which water (1 l.) was added to dissolve the precipitated KBr. The oil, thrown out during this operation, was taken up in chloroform, washed, dried, and evaporated to a syrup (73 g.) which showed $[\alpha]_D^{21°}$ −18° (c=5 in methanol). This crude hexa-acetate, which could not be distilled without decomposition and which failed to crystallize, was dissolved in methanol (80 ml.) cooled to −5°, and well stirred during the addition of 1·13N-barium methoxide in methanol (300 ml.), the temperature being kept below 10°. More methanol (50 ml.) was then added to reduce the viscosity of the semi-solid reaction mixture, which was stirred for a further 15 min. and then filtered. The Ba salt was washed on the filter with methanol, well pressed down, and dried *in vacuo* over $CaCl_2$, and finally over P_2O_5. It showed $[\alpha]_D^{20°}$ −16° (c=10 in water). Other properties of this salt are recorded below.

BAL-INTRAV. (*Second method.*) This differed from the above only in the use of the syrupy dibromide (mainly the α-form) in place of the crystalline β-isomer. From 460 g. of this material, 400 g. of hexa-acetyl BAL-INTRAV were obtained, which was deacetylated as before to give the barium salt, $[\alpha]_D^{20°}$ +45° (c=2 in water). By the use of sodium methoxide for the deacetylation, the sodium salt of BAL-INTRAV was also prepared. Unlike the barium salt, it is soluble in methanol and is therefore precipitated from the reaction mixture by the addition of ethanol. Both salts are exceedingly hygroscopic when first collected on the filter, and in this state are very prone to undergo aerial oxidation. It is therefore important that washing should be carried out as expeditiously as possible. When thoroughly dry, the barium salt is quite stable in the air, but specimens of the sodium salt are usually still somewhat hygroscopic. Analytical results for some of the samples obtained have already been discussed (Table 5). On keeping in a stoppered bottle, the thiol content diminishes somewhat, probably owing to slight oxidation; e.g. a barium salt had an initial SH content of 9·8% which dropped to 8·2% after 14 months and a sodium salt contained 9·2% SH initially and 7·75% after 7 months.

Attempted hydrolysis of BAL-INTRAV. The barium salt (2 g., $[\alpha]_D^{20°}$ −16) was heated at 100° for 3 hr. with 2N-HCl (25 ml.). A viscous oil was gradually precipitated, and the observed rotation of the supernatant liquor changed to +1·30 (1 dm. tube); this solution took up a negligible amount of iodine; there was no evidence for the formation of BAL, but a control experiment, in which BAL (0·75 g.) glucose (1 g.) and 2N-HCl (25 ml.) were heated for 3 hr. at 100°, gave also a viscous oil, and the supernatant liquid contained only a trace of thiol. It is clear, therefore, that BAL itself is not stable under these conditions.

SUMMARY

1. Studies are described which resulted in the production of BAL-INTRAV (BAL glucoside), a thiol which is non-toxic when given by intravenous injection and which affords a high measure of protection against lewisite poisoning.

2. The toxicity of BAL, mercaptoacetate, mercaptosuccinate, mercaptoadipate, acetylmercaptosuccinate, dimercaptoadipate, glutathione, glucothiose and BAL-INTRAV (BAL glucoside) have

been studied when given by intravenous injection. In agreement with theory, increasing polarity of the molecule results in diminished toxicity. Mercaptosuccinate, acetylmercaptosuccinate, mercaptoadipate, dimercaptoadipate and glutathione cause no pathological symptoms in rabbits in dosage of up to 1 g./kg.

3. Many of these thiols when given by intravenous injection were able to prolong the life of rabbits given 3 LD_{50} of lewisite.

4. The most effective therapeutic action was obtained with BAL-INTRAV, which prevented death in 100% of the animals studied, even if not given until 6·5 hr. after administration of 3 LD_{50} of lewisite (which in untreated animals kills in 10 to 20 hr.).

5. Details are given of the preparation of mercaptosuccinic acid, dimercaptoadipic acid, glucothiose, BAL-INTRAV (BAL glucoside), and a number of other thiol acids.

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Changes Undergone by Phenylarsenious Acid and Phenylarsonic Acid in the Animal Body

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It is believed that many of the most important biological properties of the arsonic acids result from their partial reduction in the body to the corresponding arsenious acids. The evidence that this reduction does occur is, however, entirely indirect. Arsonic acids display a negligible parasiticidal effect *in vitro* and are effective *in vivo* only after a lapse of several hours following injection. The corresponding arsenious acids, on the other hand, are effective *in vitro*, and *in vivo* act immediately after injection (Voegtlin & Smith, 1920*a*; Yorke & Murgatroyd, 1930). Serum from animals injected with tryparsamide has been found to be trypanocidal *in vitro*, but the serum shows maximal activity only if it is withdrawn a long time after the injection of the arsonic acid. The arsenic content of the serum when