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* Information available on application to Ministry of Supply, London.

An Experimental Investigation of the 'Ring Hypothesis' of Arsenical Toxicity

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(Received 17 June 1946)

Earlier papers in this series (Stocken & Thompson, 1946*a, b*) described the development between 1940 and 1942 of British Anti-Lewisite (BAL) as an antidote for arsenical poisoning. It is now believed that the most important way in which the toxic arsenicals interfere with the normal life of the cell is by inhibiting an essential stage, that of pyruvate oxidation, in the series of chemical reactions by which it is supplied with energy (Peters, Sinclair & Thompson, 1946). The subsequent discovery (Stocken & Thompson, 1946*a*) that the arsenical protein formed by the interaction of lewisite and kerateine contains over 70% of its arsenic in combination with sulphur in the ratio of 1As:2S, suggested that the vesicant arsenicals act by reacting with two neighbouring essential thiol groups in enzyme proteins (e.g. those of the pyruvate system) with the formation of a relatively stable cyclic thioarsinite. The introduction into this system of a dithiol with which the arsenic can form a cyclic thioarsinite more stable than that which it forms with the protein, causes the arsenic to leave the protein and reverses its toxic effect on the cell. Monothiols, which, in the case of arsenical vesicants, are useless as antidotes, form open-chain thioarsinites which are less stable than the protein thioarsinites and are thus unable to remove the arsenic.

A confirmation of this hypothesis, which may be termed the 'ring hypothesis' of arsenical toxicity, has been sought in a study of the relative effectiveness as arsenical detoxicants of a series of $\alpha\omega$ -dithiols and some monothiols with comparable physical properties. If the formation of a small stable ring is the underlying reason for the ability of

dithiols to detoxicate arsenic *in vivo*, clearly the size of the ring is one of the most important factors in determining their therapeutic effectiveness. Attention was first directed to 1:2 and 1:3-dithiols because the corresponding cyclic thioarsinites are five- and six-membered rings, and by analogy with other types of ring compounds, rings of this size would be expected to be the easiest to form and among the most stable when formed; but it was thought desirable to undertake a study of the variation of antidotal efficiency attendant upon a successive increase in the spacing of thiol groups one from the other in the dithiol molecule in order to investigate the validity of the ring hypothesis still further. The relative effectiveness of the series of $\alpha\omega$ -dithiols was assessed by measuring their ability to reactivate the pyruvate oxidase system after poisoning with lewisite. The results obtained are discussed later in the light of known facts about the stereochemistry of large rings and are considered to support the ring hypothesis.

EXPERIMENTAL

Synthesis of $\alpha\omega$ -dithiols

The starting-point for the synthesis of most of the $\alpha\omega$ -dithiols was the succinic acid series of dicarboxylic acids. These were esterified and reduced by the Bouveault-Blanc method (using Manske's procedure, 1934) to the corresponding diols, which were converted to the dithiols via the dibromides by heating the latter with 6 mol. ethanolic sodium or ammonium hydrosulphide (Stocken, 1947) at 80–90° in a sealed bottle for 24 hr. On cooling, the reaction mixture was acidified (<pH 4), the solvent removed *in vacuo* and the dithiol extracted from the solid residue with chloroform. After drying, the chloroform was removed

and the residual dithiol distilled *in vacuo*. Butanedithiol could not be prepared this way (cf. Schwarzenbach & Epprecht, 1936) and was obtained from the dibromide by a new method via the dithioacetate.

At the time this work was being carried out, only six members of the series of dithiols had been recorded. More recently, Hall & Reid (1943) have described the remaining members of the series up to dodecanedithiol. They, too, had to resort to an indirect method for butanedithiol. In the following account, experimental details are given only of the new compounds and method of preparation. These, like the other dithiols, are colourless oils with mercaptan-like odour, soluble in fat solvents, very sparingly soluble in buffer solutions at pH 7.3, and practically insoluble in water. When pure, they resist oxidation, and cannot be satisfactorily titrated with iodine, unless in very dilute (buffered) solution.

1:4-Butanedithiol. Dibromobutane (10 g.) was refluxed for 6 hr. with an excess of 10% (w/v) ethanolic sodium thioacetate (10 g.). The solution was filtered, evaporated, and 1:4-bis(acetylthio)butane was obtained by ether extraction of the residue (8.6 g.; yield 90%; b.p. 115°/0.8 mm. Found: S, 30.9. $C_8H_{14}O_2S_2$ requires 31.1%). The product (7.9 g.) was kept overnight with ethanolic NaSH (36 ml. of 12% (w/v)), and the reaction completed by warming. The thiol was obtained from the reaction mixture in the usual way (2.3 g.; yield 50%; b.p. 85–87°/15 mm. Found: S, 51.9. Calc. for $C_4H_{10}S_2$: S, 52.5%).

1:6-Hexanedithiol. Diethyl adipate (44 g.) was reduced by Manske's procedure to the glycol (18 g.; yield 80%; b.p. 142°/12 mm.), which was converted to the dibromide (31 g.; yield 81%; b.p. 102°/3 mm.). Treatment with ethanolic NaSH (12% (w/v)) gave the dithiol directly (yield 55%; b.p. 114°/13 mm. Found: S, 42.0. Calc. for $C_6H_{14}S_2$: S, 42.7%). The dibromide was also converted to the thiol indirectly (Found: S, 42.2%) via 1:6-bis(acetylthio)hexane (yield 81%; tablets, m.p. 29.5–30.5°; b.p. 137–138°/0.9 mm.; Found: S, 27.0; $C_{10}H_{18}O_2S_2$ requires S, 27.4%) using the method described above.

1:7-Heptanedithiol. Diethyl pimelate (22 g.) (Müller & Röhl, 1931) was reduced as above, and without isolating the glycol, fuming HBr (145 ml.) was added cautiously with strong cooling to the diluted (120 ml. water) and evaporated reduction mixture. The dibromide (9.8 g.; yield 37%; b.p. 130–132°/10 mm.) was isolated from the reaction mixture after refluxing about 15 hr. and was converted to the thiol (4.5 g.; yield 70%; b.p. 87–90°/1 mm.) with 15% (w/v) methanolic NH_4SH as already described. After extracting from chloroform by $N-NaOH$, and subsequently from the neutralized aqueous phase with fresh chloroform, and redistillation, the dithiol was pure. (Found: S, 39.2. $C_7H_{16}S_2$ requires S, 39.0%.)

1:8-Octanedithiol. Suberic and azelaic acids (Carmichael, 1922) were converted to dibromooctane (b.p. 137–140°/10 mm.) and dibromononane (b.p. 146–147°/13 mm.) as for the heptane homologue. The former yielded octanedithiol (0.9 g.; yield 58%, b.p. 101°/1 mm.) by the ammonium hydrosulphide method. (Found: S, 36.0. $C_8H_{18}S_2$ requires S, 36.0%.)

1:9-Nonanedithiol. This was prepared as for the previous homologue (4.8 g.; yield 74%; b.p. 103°/0.8 mm.). After two further distillations it was pure. (Found: S, 33.1. $C_9H_{20}S_2$ requires S, 33.3%.)

1:10-Decanedithiol. M.p. 20°, b.p. 165–166°/15 mm. (Found: S, 31.1. Calc. for $C_{10}H_{22}S_2$: S, 31.1%.)

1:11-Hendecanedithiol. Ethyl 11-hydroxyhendecanoate (8.5 g.) (Walker & Lumsden, 1901) was reduced by Manske's procedure to the glycol (5.3 g.; yield 81%; prisms from benzene, m.p. 63°) which was converted via the dibromide (6.6 g.; yield 75%; b.p. 175–176°/10 mm.) to the dithiol (2.9 g.; yield 30%; b.p. 173°/13 mm. Found: S, 28.7; $C_{11}H_{24}S_2$ requires S, 29.1%) by heating with 20% methanolic NH_4SH for 3 days.

1:12-Dodecanedithiol. The glycol (3 g.; yield 61%; prisms, m.p. 66–67°, from benzene) from diethyl 1:10-decanedicarboxylic acid (7.3 g.) (Walker & Lumsden, 1901) was converted via the dibromide (3.8 g.; yield 76%; b.p. 186°/10 mm.) to the dithiol (1.5 g.; yield 50%; m.p. 22°, b.p. 155°/2 mm. Found: S, 28.1; $C_{12}H_{26}S_2$ requires S, 27.4%) by the same method.

Monothiols

For purposes of comparison some monothiols possessing similar properties to the dithiols were required.

n-Hexanethiol was prepared from the corresponding bromide using sodium hydrosulphide; b.p. 43.5–44°/14 mm. (Found: S, 26.6. Calc. for $C_6H_{14}S$: S, 27.1%.)

2-Ethyl-n-hexanethiol was prepared in a similar way; b.p. 66°/12 mm. (Found: S, 21.9. Calc. for $C_8H_{18}S$: S, 21.9%.)

Enzyme experiments

The pyruvate oxidase system has been used in this laboratory throughout the work on anti-arsenicals as a convenient test system for assessing antidotal efficiency. In the present work, the pigeon brain *brei* preparation was used (Passmore, Peters & Sinclair, 1933), the respiration of *brei* being measured manometrically at 38° and pH 7.3 by the Warburg technique in the presence of 0.02 M-sodium pyruvate and Ringer phosphate buffer.

Portions of *brei* (50–100 mg.) were transferred to the corked tared bottles containing pyruvate and buffer (final vol. 3 ml. after additions). After reweighing and dividing the tissue with a blunt rod, lewisite (final conc. 16 μM) was added to all bottles (except a pair of controls); 10 min. later, each thiol to be tested (final conc. 24 μM) was added to a pair of lewisite bottles. Normally, one brain was divided between 6 pairs, enabling four thiols to be tested in each experiment. Preliminary experiments showed that the thiols alone, in this concentration and under these conditions, did not themselves inhibit the enzyme (Table 1). NaOH-papers were placed in position and duplicate readings taken at 30 and 60 min.

Solutions. Ringer phosphate buffer: NaCl, 7.2 g., KCl, 0.2 g.; $NaHCO_3$, 0.12 g.; KH_2PO_4 , 13.8 g. in 1 l. glass distilled water with enough 20% NaOH to bring to pH 7.3. Sodium pyruvate (prepared by R. W. Wakelin by method of Peters, 1938): 0.2 ml. of 0.3 M in Ringer phosphate buffer per bottle. Lewisite: solutions of lewisite oxide were freshly prepared in glass distilled water, 0.1 ml. of 480 μM being used per bottle. This concentration gives an approximately 50% inhibition of total respiration very reproducibly (57%, σ 7.7 in 25 experiments).

Thiols: The $\alpha\omega$ -dithiols are practically insoluble in water, and a number of preliminary experiments had to be carried out to devise a satisfactory method of introducing the desired concentrations into the bottles. Two final series of experiments were carried out. In the first, appropriate quantities of freshly prepared saturated solutions of the thiols in Ringer phosphate buffer, made by shaking a few mg. with 50 ml. buffer and filtering to remove oily droplets of undissolved thiol, were added to the bottles after standardization with N/500 iodine. This was unsatisfactory, as thiols above nonanedithiol were too insoluble and there was the possibility of oxidation. A second series was accordingly performed by adding the thiols in 0.025 ml. isopropanol (usually 0.05 ml. of a solution of twice the desired strength diluted with an equal volume of buffer just before addition). Isopropanol has practically the same vapour pressure as water (Dixon & Needham, 1941) and in this concentration inhibits the enzyme to the extent of about 17% (Table 2). This was allowed for by adding equal amounts of the alcohol to the control and lewisite bottles. Solutions of monothiols were made up similarly.

The final concentration of the dithiols was 24 μM (monothiols, 48 μM) giving a thiol/lewisite ratio of 1.5 (monothiols, 3).

Expression of results. If x , y , z are the average oxygen uptakes in $\mu\text{l./g. wet tissue/hr.}$ of the bottles without additions, with lewisite, and with lewisite and thiol respectively, the inhibitions I_0 and I , due to lewisite, alone, and in the presence of thiol, are $I_0 = 100 \frac{x-y}{x}$ and $I = 100 \frac{x-z}{x}$. The percentage reactivation is then defined as $100 \frac{I_0 - I}{I_0} = 100 \frac{z-y}{x-y}$.

RESULTS

Effect of dithiols and isopropanol on enzyme. Table 1 shows that the dithiols themselves, in the very low concentrations used in these experiments are without appreciable toxic effect. Table 2 gives the results of two control experiments with isopropanol.

Relative efficiency of thiols. Table 3 summarizes the results of the two series of experiments. It will be seen, first, that the monothiols are incapable of

Table 1. *Effect of small concentrations of dithiols on pigeon brain pyruvate oxidase*

(Numbers of experiments given in brackets in col. 3.)

Thiol	Concentration (μM)	Inhibition over 60 min. (%)
Ethanedithiol	25	0 (2)
Pentanedithiol	24.5	0 (1)
Heptanedithiol	24	9 (1)
Octanedithiol	30	0 (2)
Nonanedithiol	24	0 (1)

Table 2. *Effect of isopropanol on pigeon brain pyruvate oxidase*

Isopropanol (ml.)	O ₂ uptake in 60 min. ($\mu\text{l./g. tissue}$)		Inhibition (%)
	Control	Alcohol	
0.025	1563	1295	17
0.05	1504	1225	19

reversing, to any significant extent, the toxic action of lewisite (confirming earlier unpublished protection experiments by Sinclair with other monothiols) and second, that there is a definite variation in the antidotal efficiency of the dithiols with the distance apart of the thiol groups in the aliphatic chain. The implications of this result for the ring hypothesis are discussed later.

Table 3. *Reactivation of pigeon brain pyruvate oxidase by thiols 10 min. after poisoning with lewisite*

(Lewisite, 16 μM ; dithiols, 24 μM ; monothiols, 48 μM .)

Thiol	Reactivation over 60 min. (%)		
	Thiols added in buffer	Thiols added in 0.025 ml. isopropanol	Mean of all exp.
Ethanedithiol	100, 93	100, 97	98
Propanedithiol	73, 75	81*, 79	77
Butanedithiol	18	51, 9, 18, 33	26
Pentanedithiol	51, 66, 36	68*, 85, 43, 49	57
Hexanedithiol	84, 98	94, 88	91
Heptanedithiol	83, 76, 95	98, 93	89
Octanedithiol	70, 86, 86	100, 87, 91, 94	88
Nonanedithiol	83, 87, 79	93*, 98	88
Decanedithiol	—	100, 100	100
Henecanedithiol	—	85, 69	72
Dodecanedithiol	—	80, 75	78
Butanethiol	3	7	5
Hexanethiol	4	20	12
Heptanethiol	7	0	4
2-Ethylhexanethiol	—	20	20

* 0.05 ml. isopropanol.

Table 4. *Detoxication of lewisite by thiols in the presence of pigeon brain pyruvate oxidase*

(Lewisite, 16 μM ; dithiols, 24 μM ; monothiols, 48 μM .)

Thiol	Detoxication over 60 min. (%)
Ethanedithiol	99
Propanedithiol	95
Butanedithiol	74
Pentanedithiol	95, 95
Hexanedithiol	91, 100
Heptanedithiol	100, 100
Octanedithiol	100, 100
Nonanedithiol	100
Butanethiol	6
Hexanethiol	4
Heptanethiol	10

Detoxication experiments. In another series of experiments, the thiol and lewisite (added in buffer)

were allowed to react for 30 min. before addition of the brain preparation, and the percentage detoxication (defined by the same equations as the reactivation) measured. Table 4 shows that the difference between the monothiols and dithiols is as marked as before, but that under these much less critical conditions, there is a less marked variation among the various dithiols.

Rate of oxidation of dithiols

A possible objection to any arguments based on the variations in antidotal efficiency of the $\alpha\omega$ -dithiols revealed in Table 3 is that the observed differences are merely due to variations in the ease of oxidation of the dithiols. This is unlikely on theoretical grounds as there would be little tendency for oxidation to occur either (a) in *isopropanol*, or (b) after combination with arsenic. Thus, although oxidation might have occurred in the buffer solution before the experiments in the first series, thereby reducing the effective concentration of the more easily oxidized dithiols, this would be unlikely to occur in the *isopropanol* solutions of the second series; and the good agreement in general tendency between the two series makes it also unlikely that it has in fact occurred in the first. Moreover, if during the experiments the varying efficiency of the different thiols is not due to a genuine variation in ability to remove the arsenic from the protein, but due to a variation in the rate of oxidation after the arsenic has been removed, it would be necessary to assume that thiols are readily oxidized while in combination with arsenic, which is not supported by the properties of such cyclic thioarsenites as have been obtained pure. In order to put the matter beyond doubt, some comparisons were made between the rate of oxidation of butane and pentane dithiol, which have a low antidotal effect, and ethane and hexane dithiol, which are fully effective in the concentrations used.

Appropriate quantities of the dithiols in 0.1 ml. *isopropanol* were added to 200 ml. M/15 phosphate buffer (pH 7.3) to give an initial concentration of 0.5 mm. Of this 25 ml. samples were withdrawn at intervals, acidified and titrated with N/200 iodine. Curve A (Fig. 1) shows the disappearance of ethanedithiol in the absence of aeration; the solution rapidly falls to three-fifths of its original value, and thereafter oxidizes only very slowly, the rate of diffusion of oxygen into the solution probably being the limiting factor. In curve B the results of several experiments with ethane, butane, pentane and hexane dithiols are plotted. Air was drawn through the solutions during the course of the reaction and the curve is now of the ordinary unimolecular form. It will be seen that all the thiols follow essentially the same curve (K approx. 0.02 min.^{-1}) and the

variations in the antidotal efficiency of the dithiols cannot simply be attributed to varying ease of oxidation.

Fig. 1. Oxidation of dithiols. A, $\odot$ ethanedithiol (without aeration); B, $\square$ ethanedithiol, $\bullet$ butanedithiol, $\bullet$ pentanedithiol, $\triangle$ hexanedithiol (with aeration).

DISCUSSION

The most striking of the results recorded above is the superiority of the dithiols as a class over the monothiols, both in the reactivation and in the protection experiments. The most natural explanation of this is that given by the ring hypothesis. On this view, all the $\alpha\omega$ -dithiols form cyclic thioarsenites of a stability greater than, or comparable to, that of the lewisite-enzyme compound, the individual variations in effectiveness being due to the varying stabilities of the thioarsenite rings. The monothiols, on the other hand, form open-chain thioarsenites less stable than the protein thioarsenite, and are thus without effect.

Nevertheless, it is evident that beyond a certain point, the thiol groups will be spaced so far apart in the dithiol molecule that even the cyclic thioarsenite formed by the arsenic with the antidote will be less stable than that formed with the protein. In spite of the unfavourable properties of the dithiols above decanedithiol, which may preclude effective penetration to the active centres of the brain enzymes, it is suggested that such a point may have been reached with hendecane and dodecanedithiol, which are less effective in reactivating the enzyme after poisoning (Table 3). One previous indication has indeed been obtained that stereochemical factors may limit the effectiveness of dithiols as antidotes; thus Thompson found that 2:6-dimercaptopyridine-4-carboxylic acid was unable to protect pyruvate oxidase against lewisite (Stocken & Thompson, 1943). In the writer's opinion, a crucial test would be provided by a comparison of the antidotal effectiveness of *cis*- and *trans*-1:2-cyclopentanedithiol, but so far this has not been attempted.

Table 3 shows that in addition to the falling off in effectiveness at the end of the series, there is a pronounced minimum in the middle of the range, butane and pentane dithiols being the least effective of any. The high dilutions used in these experiments (about 20 μ M) make it very likely that if rings are formed, they will be rings of type 1 and not dimeric or more complex rings (cf. 2):

It is, then, on the stability of these rings in aqueous solution that the effectiveness of the dithiol as an antidote depends. The results show that the least stable ring is that corresponding to butane-dithiol, i.e. the seven-membered ring; the eight-membered ring is intermediate in stability between this and the rings of smaller and greater size, containing 5, 6, 9, 10, 11, 12 and 13 atoms, which are all sufficiently stable to prevent the arsenic acting on the enzyme protein. The rings greater than this again show a decline in their stability. In the absence of any detailed information as to the steric factors involved in the combination of arsenic with the enzyme protein, it is not possible to deduce directly the probable stability of the protein-arsenic compound, or the size of the ring formed, but the reactivation experiments suggest that its stability lies between those of eight- and fourteen-membered thioarsinite rings.

It should perhaps be emphasized that the 80–100 % reactivation obtained with all the dithiols from hexane to decane dithiol does not make it necessary to assume that their cyclic thioarsinites are as stable as those derived from ethane and propane dithiol and containing only five- and six-membered rings. This is illustrated diagrammatically in Fig. 2. Curve *A* shows the expected variation of stability with ring size; the horizontal line *B* represents the stability of the protein thioarsinite. Any dithiol whose thioarsinite has a stability corresponding to a point above *B* will be able to effect maximum, or nearly maximum, reactivation, though its stability may fall very much short of the maximum; conversely, a dithiol corresponding to a point below *B* will give a much lower reactivation, though, from the protection experiments, the curve *A* will at no point lie far below *B*.

Our knowledge of large rings is chiefly due to Ruzicka and his school (Ruzicka, 1926). It is well known that the yields of cyclic ketones and lactones fall off rapidly as the ring size increases beyond six, and then rise again with increasing size. In Ruzicka's work, the improvements in yield are small; but in Zeigler's experiments, in which he used his high dilution technique, the minimum in the curve re-

lating yield and ring size is much more pronounced (cf. Zeigler, 1934).

Stoll & Stoll-Compte (1930) have investigated the stereochemistry of large ring formation and have explained this drop with 7–9-membered rings in terms of a change in the packing of the atoms of the

Fig. 2. Expected relative values for the stability of thioarsinites derived from dithiols and enzyme protein. *A*, cyclic thioarsinites; *B*, protein thioarsinite.

ring as the size increases. This region of minimum yield corresponds to a change from an open ring to a ring large enough to consist essentially of two parallel aliphatic chains lying side by side, joined at the ends, in which the hydrogen atoms are no longer forced to the outside of the ring but can be accommodated in the same positions as they would occupy in an aliphatic chain. This change in the spatial arrangement of the atoms is reflected in a change in molecular density and it is therefore significant that the Stolls were able to correlate changes in density of the ring ketones with changes in yield.

This work offers an explanation of why the ease of formation of rings changes with ring size even in a region where multiplanar strainless rings can exist. The Stolls's arguments are supported by the work of Katz & Selman (1928) who showed by X-ray examination that the larger rings do in fact exist in a collapsed condition.

So far, large rings containing metal or metalloid atoms have not been prepared in a pure state, and until this is done, and a systematic study made of their physical properties, an application of the Stoll-Ruzicka theory of large rings to the present problem can only be tentative. Nevertheless, the same general considerations very probably apply to the cyclic thioarsinites, if account be taken of the fact that S and As atoms are not quite equivalent stereochemically to methylene groups. The difference lies less in their different valency angles

than in the absence of the two hydrogen atoms, which will significantly increase the amount of space available for the packing of the other hydrogen atoms. It is to this that the difference between the extent of the minimum in the antidotal efficiency of the dithiols and the minimum of the carbocyclic rings must be ascribed. Furthermore, in the case of rings like the thioarsinites, which can be opened by hydrolysis and whose stability in aqueous solutions is therefore bound up with their ease of formation, it is evident that the Stoll-Ruzicka theory, though originally applying to ring formation, can here be equally applied to ring stability.

It remains to dispose of a possible alternative explanation of the characteristic form of the results of Table 3. It might be maintained that the antidotal efficiency of the higher dithiols was not due to ring formation at all, but merely to the excessively small solubility which is in all probability a feature

ency due to the increasing insolubility of the thioarsinites.

It would seem that this alternative explanation is excluded by the very poor degree of reactivation (and protection) obtained from the monothiols. It is difficult to see why a dithiol like octane-dithiol should be so efficient and a monothiol like 2-ethyl-*n*-hexanethiol, possessing very similar physical properties, should be so inefficient, unless ring formation is the mechanism involved; and if it is, the rise in antidotal efficiency after butanedithiol must be due to the stereochemical effects outlined above, because physical properties show a steady gradation throughout the series.

It may, therefore, be concluded that the results of Table 3 and in particular the falling off in efficiency with which butane and pentane dithiol reverse the effect of lewisite, is not inconsistent with the ring hypothesis, but is what might be expected from it after a careful consideration of the finer points in the stereochemistry of the larger rings.

SUMMARY

1. A series of $\alpha\omega$ -dithiols from ethane to dodecane dithiol has been synthesized and their relative antidotal efficiency has been measured and compared with that of some monothiols with similar physical properties, using pigeon brain pyruvate oxidase as an *in vitro* test system.

2. The results, when discussed in the light of known facts relating to the stereochemistry of large rings, are found to support the 'ring hypothesis' of arsenical toxicity.

The work described in this paper was undertaken as part of a research programme for the Chemical Defence Research Department of the Ministry of Supply under the direction of Prof. Peters.

I should like to express my gratitude to the Chief Scientific Officer, Ministry of Supply, for permission to publish, and to Dr R. H. S. Thompson, and Dr L. A. Stocken for much help and encouragement. In particular my thanks are due to Dr Stocken for specimens of ethane, propane and pentane dithiols and two monothiols, to Dr Thompson for help in some of the enzyme experiments, and to the N.R.D.C. of the U.S.A. for other thiol samples. I am also grateful to Miss Kempson for technical assistance and to Mr Vincent for various pieces of glass apparatus.

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Fig. 3. Possible factors in reactivation by $\alpha\omega$ -dithiols. A, steric factor; B, solubility factor.

of their thioarsinites. On this view, the minimum is to be explained as a result of two contrary factors, as illustrated in Fig. 3, the first (curve A), the decreasing efficiency of the dithiols due to the successive increase in the distance separating the two thiol groups; the second (curve B), the increasing effi-

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* Information available on application to Ministry of Supply, London.

The Oxidation of Dithiols

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(Received 31 March 1946)

Warburg & Sakuma (1923) demonstrated that cysteine in aqueous solutions was not oxidized by atmospheric oxygen in the absence of heavy metals, and Harrison (1924) showed that this was true also for glutathione. Both SH-containing substances are sluggish oxidation-reduction systems, non-autoxidizable, and oxidized only in the presence of heavy metals or of iron porphyrins. The synthesis of 2:3-dimercaptopropanol (BAL) (see Peters, Stocken & Thompson, 1945) and the preparation of a large number of dithiols by Salzberg, Lazier, Signaigo & Pavlic (personal communication) made it necessary to study the conditions for the oxidation of the SH groups contained in these compounds as an essential step in effecting their stabilization. It will be seen from the experiments reported in this paper that these dithiols behave like the monothiol cysteine and glutathione as regards oxidation, i.e. they are sluggish non-autoxidizable oxidation-reduction systems, easily oxidized by catalytic amounts of copper and iron porphyrins. The spatial arrangement of SH and the addition of different groups to the molecule have made possible a study of the influence of these factors on the rate of oxidation.

EXPERIMENTAL AND RESULTS

Oxidation of 2:3-dimercaptopropanol by copper. The lack of oxidation of 2:3-dimercaptopropanol (dithiol 1 or BAL) in the absence of heavy metals could not be demonstrated conclusively because neither the compound itself nor the salts used in the preparation of the buffers were freed of traces of heavy metals present as impurities. However, the facts that the rate of oxidation was considerably diminished when buffer solutions were prepared with quartz-distilled water and kept in paraffin covered containers and that the rate of this oxidation was still further diminished on addition of cyanide are indications that the dithiol is oxidized only by the catalytic action of heavy metals. In

0.05 M-phosphate buffer pH 7.0 and 7.1 copper was the most powerful oxidizing agent among the heavy metals which act as catalysts for the oxidation of thiol compounds (Table 1). In these experiments,

Table 1. *The oxidation of 2:3-dimercaptopropanol by atmospheric oxygen*

(Metal catalyst, 2×10^{-4} mmol.; dimercaptopropanol 1×10^{-2} mmol.; buffer, 0.05 M-phosphate; pH 7.1; temp. 38°.)

Added substance	Half-oxidation time (min.)
None	70.4
HCN (5×10^{-4} M)	123.0
CuCl ₂	8.8
Cu-glycine (glycine, 0.1 M)	8.0
Cu-albumin (6% (w/v) albumin)	7.1
FeCl ₃	42.5
MnSO ₄	70.4
CoSO ₄	70.4
NiSO ₄	70.4

the amount of oxidizing catalyst was 2×10^{-4} mmol. while that of dithiol was 1×10^{-2} mmol. In the absence of added heavy metal, half-oxidation occurred in 70.4 min., being lengthened to 123 min. on addition of 5×10^{-4} M-HCN. Addition of CuCl₂ increased the rate more than seven-fold so that half-oxidation took place in 8.8 min. and with FeCl₃ in 42.5 min. MnSO₄, CoSO₄ and NiSO₄ had no effect. In the oxidation of ascorbic acid by Cu⁺⁺ as catalyst, non-ionized copper had little catalytic action (Barron, DeMeio & Klemperer, 1936); the state of the copper had no influence on the rate of oxidation of BAL, for the rate remained unchanged on formation of copper complex compounds with alanine or egg albumin. Dimercaptopropanol might, therefore, be oxidized by the copper contained in biological fluids. In all these experiments the rate of oxidation was determined by measuring the O₂ uptake manometrically. The oxidation was complete with an uptake of one oxygen atom/mol. of dithiol, the insoluble disulphide complex being formed. Copper