Schwarzenbach, G. & Epprecht, A. (1936). *Helv. chim. Acta*, **19**, 169.
Stocken, L. A. (1947). *J. chem. Soc.* In the Press.
Stocken, L. A. & Thompson, R. H. S. (1943). Report to Ministry of Supply by Peters, no. 64.*
Stocken, L. A. & Thompson, R. H. S. (1946*a*). *Biochem. J.* **40**, 529.
Stocken, L. A. & Thompson, R. H. S. (1946*b*). *Biochem. J.* **40**, 535.
Stoll, M. & Stoll-Compte, G. (1930). *Helv. chim. Acta*, **13**, 1185.
Walker, J. & Lumsden, J. S. (1901). *J. chem. Soc.* pp. 1191, 1197.
Zeigler, K. (1934). *Ber. dtsch. chem. Ges.* **67**, A 139.

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

The Oxidation of Dithiols

By E. S. GUZMAN BARRON, ZELMA BAKER MILLER and G. KALNITSKY
The Chemical Division, Department of Medicine, University of Chicago

(*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 monothiols 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,

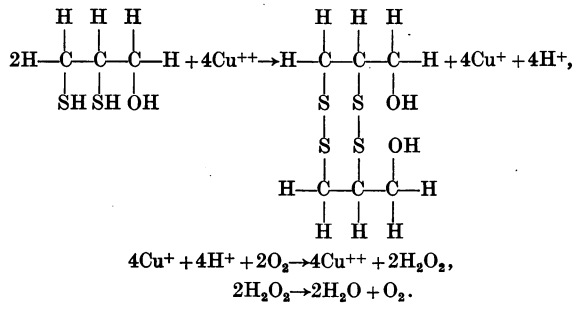
Table 1. *The oxidation of 2:3-dimercaptopropanol by atmospheric oxygen*

(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 $CuCl_2$ increased the rate more than seven-fold so that half-oxidation took place in 8·8 min. and with $FeCl_3$ in 42·5 min. $MnSO_4$, $CoSO_4$ and $NiSO_4$ 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 O_2 uptake manometrically. The oxidation was complete with an uptake of one oxygen atom/mol. of dithiol, the insoluble disulphide complex being formed. Copper

acts as a catalyst by its reduction to Cu^+ by the dithiol and reoxidation to Cu^{++} by oxygen. Whether the oxidation of the dithiol is a direct oxidation or whether there is previous formation of a metal complex, as with cysteine and iron, is not known. However, the lack of oxidation of hexane-1:6-dithiol, where the SH groups are separated by four carbon atoms, speaks in favour of the transitory formation of a cyclic Cu complex. The oxidation by copper may be represented as follows:



$$4Cu^+ + 4H^+ + 2O_2 \rightarrow 4Cu^{++} + 2H_2O_2,$$

$$2H_2O_2 \rightarrow 2H_2O + O_2.$$

Dimercaptopropanol is also oxidized by ferricyanide requiring 2 mol. of ferricyanide/mol. of dithiol. This oxidation was performed manometrically in a solution of 0·1 M-$NaHCO_3$ with N_2 and CO_2 as the gas phase. Under such conditions 2 mol. CO_2/mol. dithiol oxidized were formed. The manometric method can, in fact, be used for the determination of dimercaptopropanol, with the dithiol solution in the main vessel and 0·5 ml. of 10 % (w/v) $K_3Fe(CN)_6$ in the side arm. At 38° the reaction is complete in 3 min. Ferricyanide oxidation can also be used for the colorimetric estimation of dithiols by spectrophotometric measurement at 6900 A., of the ferrocyanide formed as prussian blue. The colorimetric method will measure from 3 to 20 μg. of dithiol. The temperature coefficient for the oxidation of dimercaptopropanol with Cu^{++} as catalyst was $Q_{10} = 1{\cdot}38$. From these data the value of the energy of activation, E, was calculated to be 9000 cal./mol. This low value indicates that the reoxidation of Cu^+ is the reaction governing the rate of oxidation of dimercaptopropanol. That the temperature coefficient was about the same for the 'autoxidation' of dimercaptopropanol further indicates that this oxidation is due to heavy metal catalysis.

Oxidation of dithiols with iron porphyrins. Iron protoporphyrin (blood haemin) is a powerful catalyst for the oxidation of cysteine (Krebs, 1929) and of glutathione (Lyman & Barron, 1937). It has been found to be a more powerful catalyst for the oxidation of the dithiols 1, 2, 3 and 4 (Table 2). (The structural formulae of these dithiols are shown in the Glossary, p. 940.) The first three dithiols were oxidized with great speed. The oxidation of dithiol 4, which was extremely slow with $CuCl_2$ as catalyst, was 30 times as fast with haemin. When dithiols were added to a solution of haemin in phosphate buffer, there was immediate reduction of haemin, which was reoxidized by atmospheric oxygen. At the end of 3 hr., as a result either of the action of H_2O_2 produced during the oxidation of dithiol or of the action of the oxidized dithiol, there was destruction of the haemin molecule with opening of the porphyrin ring and disappearance of the typical Soret band at 3850 A. (Fig. 1). When dithiol was added to a solution of oxyhaemoglobin (dithiol 1, 1×10^{-3} M; oxyhaemoglobin, 2×10^{-5} M), there was also complete destruction of oxyhaemoglobin at the end of 2 hr. with disappearance of the absorption bands at 5415 and 5760 A. and marked decrease of the Soret band at 4150 A. (Fig. 2). The end-product is the so-called pseudohaemoglobin, a precursor of bilirubin. That this destruction of the iron-porphyrin molecule occurs only *during the continuous oxidation of dithiol* was demonstrated by the following two experiments: (1) 1×10^{-3} M-dithiol was added to 5×10^{-5} M-cytochrome *c*. There was immediate reduction of cytochrome, which remained reduced and without further alteration for 5 hr., the duration of the experiment; (2) 1×10^{-3} M-dithiol (saturated with H_2) was added to 2×10^{-5} M-haemoglobin (saturated with H_2). The extinction coefficients measured at 5525, 7550 and 7575 A., remained practically unchanged at the end of 2 hr.

When dithiol 1 (1×10^{-3} M) was added to methaemoglobin (2×10^{-5} M) there was immediate reduction to haemoglobin, which in the presence of

Table 2. *Oxidation of dithiols by atmospheric oxygen with iron protoporphyrin*

(Catalyst, blood haemin (0·0002 mmol.); dithiol (0·01 mmol.); pH 7·1; temp. 38°; vol. 3 ml.)

Dithiol*	Half-oxidation time (min.)
1 (BAL)	5·0
2	6·0
3	3·0
4	13·0

* See Glossary for nomenclature of dithiols.

dissolved oxygen was oxygenated to oxyhaemoglobin. This immediate reduction of methaemoglobin by dimercaptopropanol can be utilized for the treatment of methaemoglobinaemia.

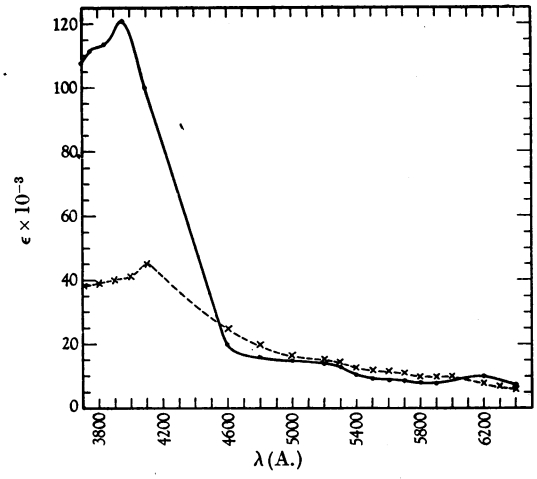


Fig. 1. Effect of BAL (1×10^{-3} M) on haemin ($4{\cdot}61 \times 10^{-6}$ M). Solid line, haemin; broken line, haemin + BAL.

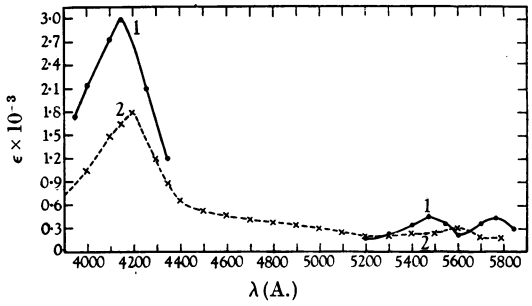


Fig. 2. Effect of BAL on oxyhaemoglobin. Spectrophotometric measurements 2 hr. after addition of BAL. 1, oxyhaemoglobin, 5×10^{-5} M; 2, oxyhaemoglobin + 10^{-3} M-BAL after 2 hr.

Webb & van Heyningen (1943) found that dithiol 1 reduces cytochrome *c* and is oxidized by the system cytochrome *c*-cytochrome oxidase. These findings have been confirmed. The initial rate of oxidation of dithiol did not increase on addition of cytochrome oxidase (prepared according to Haas, 1943); on addition of cytochrome *c* (2×10^{-5} M) and cytochrome oxidase, dithiol was rapidly oxidized (Fig. 3). All these experiments were performed in aqueous solutions with 0·02 M-phosphate buffer pH 7·4.

Dithiol 1 *and ascorbic acid.* It is known that addition of a sluggish oxidation-reduction system of low potential to a system of higher potential protects the latter from oxidation (protection of adrenaline from air oxidation by ascorbic acid, of ascorbic

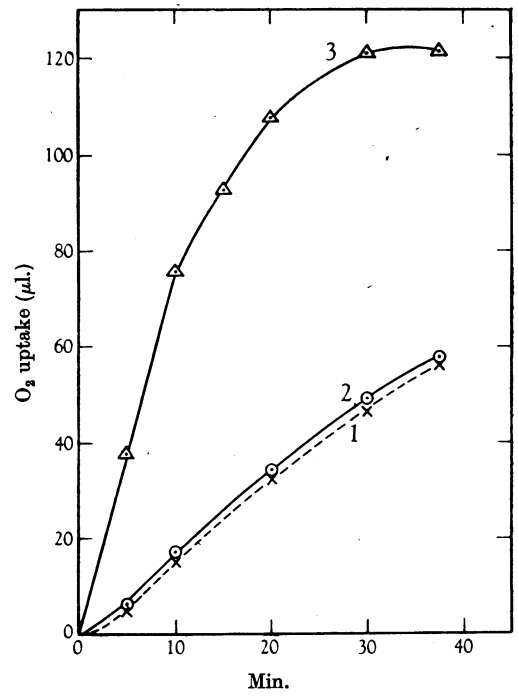


Fig. 3. Oxidation of BAL with cytochrome *c* and cytochrome oxidase. 1, BAL; 2, BAL + enzyme; 3, BAL + enzyme + cytochrome *c*.

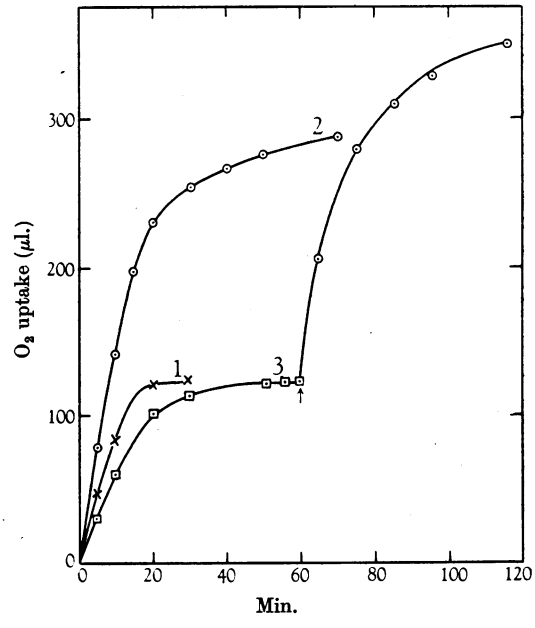


Fig. 4. Protection of ascorbic acid oxidation by BAL. $CuCl_2$, 2×10^{-4} mmol. 1, BAL; 2, ascorbic acid; 3, BAL + ascorbic acid (ascorbic acid oxidase added at arrow).

acid by glutathione). In the same manner dithiol 1 protects ascorbic acid from oxidation. The experiments were performed in 0·02 M-phosphate, pH 7·1 and 38°. The amount of dithiol was 1×10^{-2} mmol.; of ascorbic acid, 2×10^{-2} mmol.; of $CuCl_2$, 2×10^{-4} mmol. (at this concentration of copper and pH value, half-oxidation of ascorbic acid occurs in 10 min. (Barron *et al.* 1936). The oxygen uptake in the vessels containing dithiol alone, and dithiol plus ascorbic acid was the same, showing that ascorbic acid was not oxidized. At the end of 60 min., ascorbic acid oxidase prepared from squash was added to the vessel from the side arm and there was immediate oxidation of ascorbic acid (Fig. 4).

Reaction of 2:3-dimercaptopropanol in aqueous solutions with heavy metals. Dimercaptopropanol, like many other thiol compounds, reacts with heavy metals and gives in many instances almost insoluble coloured compounds. Coloured compounds are formed with Fe^{+++} (blue-green), Fe^{++} (light pink), Pb (green-yellow), Sn (yellow), Bi (green-yellow), Cu^{++} (dark blue), Co (brown), Ni (brown turning to green in NH_4OH), Sb (brown to white precipitate), Se (yellowish white precipitate). Zn, Cd and Hg^{++} give white precipitates. Mg and Mn give neither coloured reaction nor precipitate. The combination of Mg with dimercaptopropanol with formation of a soluble complex seems to be the reason for the inhibition produced by $MgCl_2$ on the rate of oxidation of dithiol. When the rate of oxidation of dithiol with $CuCl_2$ as catalyst was measured in the presence and in the absence of 0·01 M-$HgCl_2$, it was found that Mg inhibited the rate of oxidation by 44%.

Oxidation of other dithiols with copper as catalyst. The position of the SH groups and the nature of the residues attached to the dithiols seem to be an important factor in determining the efficiency of oxidation catalysts like Cu^{++}. To study the relative importance of these factors the rates of oxidation of 20 different dithiols and one trithiol with and without added copper as catalyst were determined. Copper failed to catalyze appreciably the rate of oxidation in four of the compounds tested (dithiols 3, 4, 6 and 7) and had very little effect on the oxidation of 1:2:3-propanetrithiol.

The rate of oxidation of dithiols, having the SH groups contiguous to each other, varied as would be expected according to the nature of the attached residue. Thus while dithiol 1 was half-oxidized in 10·5 min. at 22·4° and pH 7·0, a closely related compound, dithiol 21 with one C atom more in the residue was half-oxidized in 22 min. By replacing the last C groups with an acid group, as in dithiol 10, the rate of oxidation was diminished still further so that half-oxidation occurred in 55 min. Attachment of a methyl ester group to this acid, as in dithiol 11, increased the rate of oxidation sufficiently to eliminate the inhibitory effect produced by the carboxyl group; in fact, half-oxidation occurred in 10·4 min., the same as that of dithiol 1. A progressive increase in the number of C atoms in the molecule, in the form of the ethyl, *n*-propyl and *n*-butyl esters of dithiol 10 (dithiols 8, 18, 19) resulted in an increase in the time necessary for the half-oxidation of each compound. Thus the ethyl ester (dithiol 8) was half-oxidized in 13·5 min.; the propyl ester (dithiol 18), in 23·6 min. and the butyl ester (dithiol 19) in 123 min. The ether derivatives of dithiol 1 behaved like the esters. The methyl ether (dithiol 13) was half-oxidized in 15 min. (much faster than the free acid dithiol 10). Increasing the length of the chain resulted in an increase in the half-oxidation time of each compound: the ethyl ether (dithiol 9) was half-oxidized in 17 min.; and the *iso*propyl ether (dithiol 20) in 25·6 min. The urea and carbamate derivatives of dithiol 1, had a rate of oxidation only slightly slower than that of dithiol 1. Thus dithiol 14 was half-oxidized in 15·5 min. and dithiol 15 in 21 min. The rate of oxidation of phenylethane dithiol (dithiol 17) was six times slower than that of dithiol 1, while the *N*-phenylamine derivative (dithiol 7) was hardly oxidized at all. The only compound which had a rate of oxidation slightly faster than that of dithiol 1 was dithiol 12, which was half-oxidized in 8 min. The addition of another SH by substitution of the OH group of dithiol 1, thus making propanetrithiol (dithiol 5), had the effect of slowing down the rate of oxidation. Propanetrithiol was half-oxidized in 127 min., i.e. 12 times

Table 3. *Oxidation of dithiols by atmospheric oxygen with* $CuCl_2$ *as catalyst*

(Each vessel contained 0·03 M-phosphate buffer pH 7·0; $CuCl_2.2H_2O$ (2×10^{-4} mmol.); dithiol (0·01 mmol.); vol. 3·0 ml.; temp. 22·4°.)

Dithiols*	Half-oxidation time (min.)	
	Copper added	No copper
1	10·5	196
2	25·5	500
3	350	500
4	500	500
5	127	224
6	500	500
7	500	500
8	13·5	500
9	17·0	106
10	55·0	500
11	10·4	80
12	8·0	176
13	15·0	101
14	15·5	176
15	21·0	177
16	27·0	500
17	65·0	312
18	23·6	500
19	123·0	500
20	25·6	116
21	22·0	240

* See Glossary for nomenclature of dithiols.

as slowly as dithiol 1. Dithiol 16, the *bis*-S (*N*-ethylacetamidomethyl) ether of dithiol 1 has been reported to split in solution, and yield dithiol 1 quantitatively (Dr Lazier, personal communication). The resulting dithiol should then be oxidized at a rate similar to that of dithiol 1. This was the case, for dithiol 16 did actually undergo a fairly rapid rate of oxidation in the presence of copper (Table 3).

Separation of SH groups by one C atom and an OH residue between the SH groups (dithiol 2) increased the half-oxidation time to 25·5 min. Substitution of H for OH on the middle carbon atom (dithiol 3) inhibited almost completely the oxidation of the dithiol. Further separation of the SH groups, as in dithiol 4 where the SH groups are separated by 4 carbon atoms, retarded oxidation almost completely.

Effect of pH *on the rate of oxidation.* The pK value of the SH group of glutathione is 9·62 (Pirie & Pinhey, 1929). The rate of oxidation of glutathione would therefore increase as the SH group dissociates (more alkaline pH), and decrease when undissociated (more acid pH). Lyman & Barron (1937) showed, in fact, that the rate of oxidation of glutathione varied markedly with the pH of the medium. There is the same influence of the hydrogen-ion concentration on the rate of oxidation of dithiols. Table 4 shows as an example the effect of pH on the oxidation of dithiol 15. At pH 4·0 there was no oxidation; at pH 6·0 the half-oxidation time was 109 min.; at pH 9·4 it was only 6·5 min.

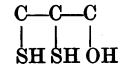
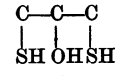
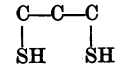
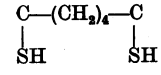
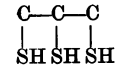
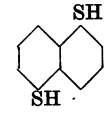
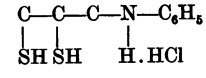
Table 4. *Effect of* pH *on the oxidation of dithiol* 15 *by atmospheric oxygen and* Cu^{++} *as catalyst*

(Each vessel contained 0·03 M-buffer (pH 9·4, borate buffer; pH 6·0, 7·0, 8·0, phosphate buffer; pH 5·4, 4·0, acetate buffer); $CuCl_2.2H_2O$ (2×10^{-4} mmol.); dithiol 15 (0·01 mmol.); total vol. 3·0 ml.; temp., 20°.)

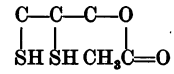
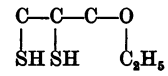
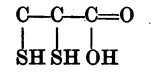
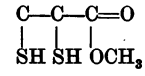
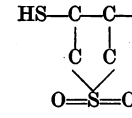
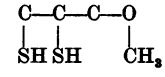
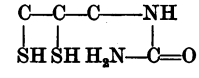
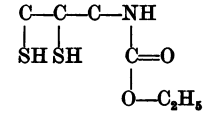
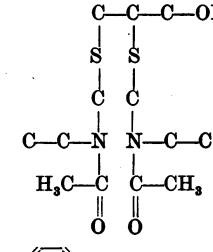
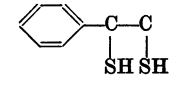
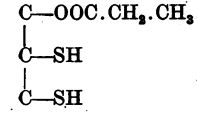
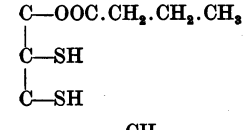
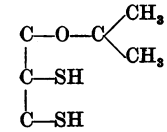
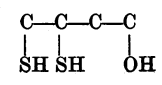
pH	Half-oxidation time (min.)
9·4	6·5
8·0	15·5
7·0	20·5
6·0	109·0
5·4	300·0
4·0	No oxidation

The oxidation-reduction potential of dithiol 1. In electro-active systems there is, under certain conditions, a relation between the oxidation-reduction potential and the rate of oxidation. Whether such a relation exists also in sluggish systems seems doubtful because there must be in these systems a greater difference between the ΔF and the ΔF^* (free energy of formation of the activated state) values of the system. The E_0' of dithiol 1 was determined at pH 7·0 in equimolecular mixtures of the oxidized and reduced component with cresyl violet as the electro-active mediator. It was found to be $-0{\cdot}150$ V. It was not possible to determine the E_0' values of the other dithiols.

GLOSSARY

Compound	Name	Skeleton formula
1	2:3-Dimercaptopropanol (BAL, DTH)	
2	1:3-Dimercaptopropanol	
3	Propane-1:3-dithiol	
4	Hexane-1:6-dithiol	
5	Propane-1:2:3-trithiol	
6	Naphthalene-1:5-dithiol	
7	*N*-Phenyl-aminopropanedithiol-HCl	

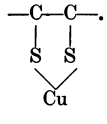
GLOSSARY (*cont.*)

Compound	Name	Skeleton formula
8	2:3-Dimercaptopropyl acetate	
9	2:3-Dimercaptopropyl ethyl ether	
10	2:3-Dimercaptopropionic acid	
11	Methyl 2:3-dimercaptopropionate	
12	3:4-Dimercaptotetrahydrothiophene-1-dioxide	
13	2:3-Dimercaptopropyl methyl ether	
14	2:3-Dimercaptopropyl urea	
15	*N*-(2:3-Dimercaptopropyl) carbamate	
16	bis-*S*(*N*-Ethylacetamidomethyl) ether of BAL	
17	Phenylethanedithiol	
18	2:3-Dimercaptopropyl propionate	
19	2:3-Dimercaptopropyl butyrate	
20	2:3-Dimercaptopropyl *iso*propyl ether	
21	3:4-Dimercaptobutanol-1	

DISCUSSION

Of the 20 dithiol compounds only one, dithiol 12, was oxidized with copper as a catalyst at a rate slightly faster than that of dithiol 1. Dithiol 11 was oxidized at the same rate. The rate of oxidation of all the others was slower. The effect of various groupings on the rate of oxidation of the different dithiols is mainly determined by the electron attracting powers (electronegativity) of the group in question and by the position of the SH groups. For example, in dithiol 10 the COOH group is more electronegative than the OH group in dithiol 1; consequently the hydrogen atoms of the thiol groups may possibly be drawn closer to the molecule, and specifically to the sulphur atom. Therefore, the degree of dissociation of the SH groups in dithiol 10 would be less, compared to that of dithiol 1, and the rate of oxidation slower. Similarly the ester derivatives of dithiol 10, being less electronegative than the acid, should be oxidized at a faster rate. This has been found to be the case. Again, the ether derivatives of dithiol 1, being less electronegative than the acid, have a faster rate of oxidation than dithiol 10. The group C_6H_5 is also a strong electronegative group. The two phenyl derivatives (dithiols 7 and 17) were oxidized at a much slower rate than dithiol 1. It is also apparent that the longer the chain of carbon atoms, the slower the rate of oxidation of the molecule. For example, among the esters the relative rates of oxidation were as follows: methyl (dithiol 11) > ethyl (dithiol 8) > *n*-propyl (dithiol 18) > *n*-butyl (dithiol 19). For the ethers, the methyl ether derivative (dithiol 13) > ethyl (dithiol 9) > *iso*propyl (dithiol 20).

The distance of the SH groups from each other has also an effect on the rate of oxidation. Separation of the SH groups decreased the rate of oxidation so that when they were separated from each other by four C atoms there was practically no oxidation. This was also the case of dithiol 6, where the SH groups are attached at the 1:5 positions of the naphthalene ring. This effect may be explained by assuming that oxidation is preceded by the intermediate formation of the unstable copper cyclic compound



If the rate-determining step in the oxidation process is the formation of this unstable cyclic complex it might help us to understand why the introduction of a CH_2 group between the two thiols (dithiol 3) prevents catalysis by copper. It would also explain the relative ease of oxidation of dithiol 2 and the stability of the trithiol 5. In this case, the two outer SH groups may be competing for the middle SH group to form the cyclic complex. The existence of such unstable metal-thiol complexes during the oxidation of thiols has been observed in the oxidation of cysteine by iron (Michaelis & Barron, 1929).

SUMMARY

1. Dithiols are sluggish oxidation-reduction systems, non-autoxidizable, and rapidly oxidized in the presence of a number of catalysts. Copper and iron-protoporphyrin are the most powerful oxidation catalysts. $FeCl_3$, ferricyanide and cytochrome oxidase (in the presence of cytochrome *c*) also oxidize dithiols.

2. Oxidation by ferricyanide can be used for the estimation of dithiols, either manometrically or colorimetrically.

3. Dithiols combine with a number of heavy metals, forming complex compounds, mostly insoluble. Fe, Pb, Sn, Bi, Cu, Co, Ni, Se and Sb form coloured complexes. Zn, Cd and Hg give white precipitates. Mg combines, forming colourless soluble compounds.

4. In the presence of oxygen dithiols destroy iron-porphyrin compounds (haemin, oxyhaemoglobin) by opening the porphyrin ring. There is disappearance of the Soret band. Dithiols react instantaneously with methaemoglobin and reduce it to haemoglobin.

5. The rate of oxidation of dithiols with copper as catalyst seems to depend on the pH of the solution, on the distance of the SH groups from each other and on the electronegativity of the residual groups.

The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

REFERENCES

Barron, E. S. G., DeMeio, H. R. & Klemperer, F. (1936). *J. biol. Chem.* **112**, 625.
Haas, E. (1943). *J. biol. Chem.* **148**, 481.
Harrison, D. C. (1924). *Biochem. J.* **18**, 1009.
Krebs, H. A. (1929). *Biochem. Z.* **204**, 322.
Lyman, C. M. & Barron, E. S. G. (1937). *J. biol. Chem.* **121**, 275.
Michaelis, L. & Barron, E. S. G. (1929). *J. biol. Chem.* **83**, 191.
Peters, R. A., Stocken, L. A. & Thompson, R. H. S. (1945). *Nature, Lond.*, **156**, 616.
Pirie, N. W. & Pinhey, K. G. (1929). *J. biol. Chem.* **84**, 321.
Salzberg, P. L., Lazier, W. A., Signaigo, F. K. & Pavlic, A. A. Personal communication.
Warburg, O. & Sakuma, S. (1923). *Arch. ges. Physiol.* **200**, 203.
Webb, E. C. & van Heyningen, R. (1943). Report to Ministry of Supply by Dixon, no. 16.*

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