polyphenol oxidase, carbonic anhydrase, catalase and peroxidase, are known to be metal-containing enzymes. The inhibition of glyoxalase is due to competition with glutathione, the activator of the enzyme. Aldehyde mutase and phosphorylase are also inhibited.

2. The inhibition of mushroom polyphenol oxidase by BAL is reversed by addition of excess of copper salt. This inhibition is due to the formation of an insoluble cupric-BAL compound.

3. The activity of hexokinase in the presence of suboptimal concentrations of Mg^{++} is diminished by BAL, an effect immediately reversed by addition of excess Mg^{++}.

4. BAL can be oxidized by the cytochrome system without any effect on the activity of the latter.

5. BAL may be considered as a potent inhibitor of metal-containing enzymes with the exception of the cytochrome system.

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The Effect of 2:3-Dimercaptopropanol on the Activity of Enzymes and on the Metabolism of Tissues

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The brilliant work of Peters, and co-workers (see Peters, Stocken & Thompson, 1945), on the reversal of lewisite action by dithiol compounds which possess the property of forming stable cyclic arsenical rings has been one of the great achievements in the treatment of certain war-gas casualties. In fact, dithiols have already found a place among the useful drugs for the treatment of a number of intoxications (arsenicals or heavy metals). It was therefore necessary to study carefully the effect of dithiols on the activity of enzyme systems and on the metabolism of tissues in order to understand the toxic effects observed when dithiols in large amounts are injected into animals. Such a reducing agent, with rather negative oxidation-reduction potential, reacting readily with heavy metals, might produce enzyme inhibitions, either by maintaining in the reduced state the electron transfer catalysts of the oxidation enzyme systems, or by combining with the heavy metals which act as the prosthetic groups of the protein moiety of some enzymes. Webb & van Heyningen (1943) have, in fact, shown that 2:3-dimercaptopropanol can combine with the metals acting as prosthetic groups. The experiments presented in this paper demonstrate that the reducing power of dimercaptopropanol is also responsible for some of the toxic effects.

EXPERIMENTAL AND RESULTS

The effect of dimercaptopropanol on the activity of enzymes

Webb & van Heyningen (1943) have investigated the effect of dimercaptopropanol on a number of oxidative and hydrolytic enzymes. Enzymes possessing heavy metals as prosthetic groups were

inhibited (polyphenol oxidase with Cu, carbonic anhydrase with Zn, catalase and peroxidase with Fe^{+++} porphyrin as the prosthetic group; hexokinase and phosphorylase activated by Mg^{++}.) It was suggested that this inhibition might be due to combination of dithiol with the metal of the protein. In fact, inhibition of polyphenol oxidase was counteracted by addition of $CuSO_4$, and inhibition of hexokinase by addition of $MgCl_2$.

It has been stated by Webb & van Heyningen that dimercaptopropanol does not inhibit the system cytochrome-cytochrome oxidase. Since their experiments were performed by measuring manometrically the rate of oxidation of catechol and no precautions were taken to eliminate heavy metal contamination it was decided to reinvestigate this problem. (According to Webb & van Heyningen half-oxidation of 5×10^{-3} M-dimercaptopropanol at 38° occurred in 20 min. In this laboratory half-oxidation took place in 70 min.) The effect of dithiol on the rate of oxidation of cytochrome *c* was determined spectrophotometrically with cytochrome oxidase prepared according to Haas (1943).

Cytochrome *c* (2×10^{-5} M) was reduced with $Na_2S_2O_4$ and the excess of this reagent was oxidized by shaking the solution. To the solution containing varying concentrations

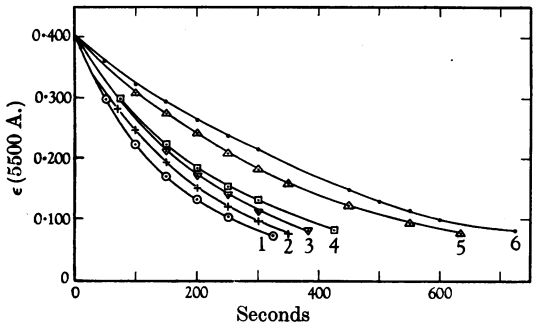

Fig. 1. Effect of BAL on the oxidation of reduced cytochrome *c* by cytochrome oxidase.

Curve no. ...	1	2	3	4	5	6
BAL conc. (M)	0	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}
Time of start of initial reaction (sec.)	9	8	26	106	630	1253

of dithiol plus reduced cytochrome *c* (all dissolved in 0·05 M-phosphate buffer, pH 7·1), there was added with a syringe 0·1 ml. of a dilute solution of cytochrome oxidase, and the rate of oxidation of reduced cytochrome *c* was followed. There occurred in the first place a delay in the initial oxidation of cytochrome *c*, as measured by the time required to diminish the extinction coefficient from the original value of 0·45 to 0·40. While in the absence of dithiol this initial oxidation time was 9 sec., in the presence of 5×10^{-6} M-dithiol it was 26 sec., with 1×10^{-5} M, it was 106 sec., 5×10^{-5} M 630 sec., and with 1×10^{-4} M (the M.L.D. for cats) it was delayed to 1253 sec. (Fig. 1). The rate of oxidation of reduced cytochrome *c* was also inhibited, the inhibition increasing as the concentration of dithiol increased.

The inhibition of cytochrome *c* oxidation must be attributed to the reducing power of dithiol, and is, therefore, quite different from the inhibition produced by HCN, for example, with cytochrome oxidase where there is no delay in the initial rate of oxidation.

According to Webb & van Heyningen, dithiol has no effect on the activity of serum-cholinesterase. The activity of this enzyme in the presence of dithiol was again determined with the purified enzyme from serum and with the enzyme from brain. The inhibition produced by 0·001 M-dithiol was small (Table 1). The other enzyme inhibitions reported by the above authors were also confirmed in this laboratory.

Webb & van Heyningen reported that dithiol had no effect on the activity of succinoxidase. Our experiments, as well as those of Rhoads (personal communication), show that dithiol can inhibit the activity of succinoxidase. When 2:3-dimercaptopropanol was added to pigeon-breast muscle succinoxidase immediately before succinate addition, there was slight inhibition (6·5 %); when dithiol was added to the enzyme 30 min. previous to succinate addition, i.e. when part of the dithiol was in the oxidized state, there was 25 % inhibition (Table 2). The inhibiting effect of oxidized dithiol can best be observed in rat-liver succinoxidase. This liver suspension contains an enzyme which rapidly oxidizes thiol compounds; here addition of dithiol produced complete inhibition of succinoxidase. Other factors responsible for the inhibition of succinoxidase by dithiols are the oxidation-reduction potential of the dithiol and the nature of the residual groups. As shown in Table 3, at a concentration of 1×10^{-3} M, few dithiols had low inhibitory power, these few being dithiols 2, 3 and 10 (the constitution of these and other numbered dithiols is given by Barron, Miller & Kalnitsky (1946)). Of these compounds with low inhibitory power (3, 4·5, 9·8 % inhibition respectively) dithiol 2 is oxidized twice as slowly as 2:3-dimercaptopropanol; dithiol 3 is very little oxidized; and dithiol 10, five times as slowly as dithiol 1. All the other compounds had a definite inhibitory effect. Dithiol 8 with a rate of oxidation about that of dithiol 1 produced half as much inhibition, the difference between these two being that dithiol 8 is an acid while dithiol 1 is an alcohol. The compound with the highest inhibitory power was dithiol 11, which has the fastest rate of oxidation. Of the three least inhibitory compounds (dithiols 2, 3 and 10) dithiols 2 and 10 are more toxic to animals than dithiol 1, while dithiol 3 has the same toxicity. Dithiol 11, the compound which produced the largest inhibition, is reported to be more effective than dithiol 1 in preventing the haemolysis

Table 1. *Effect of BAL on enzyme systems*

Enzyme	Source	Temp. (°)	Substrate	BAL (M)	Inhibition (%)	Reference
Polyphenol oxidase	Mushroom	17	Catechol	$2{\cdot}5 \times 10^{-3}$	95–100	Webb & van Heyningen (1943)
Carbonic anhydrase	Red cells	17	HCO_3^-	$2{\cdot}5 \times 10^{-3}$	60	
Catalase	Beef liver	17	H_2O_2	2×10^{-3}	85	
Peroxidase	Milk	17	H_2O_2-$NaNO_2$	$2{\cdot}5 \times 10^{-3}$	60	
Cytochrome oxidase	Pig heart	37	Cytochrome *c*	5×10^{-3}	0	
Cytochrome oxidase	Pig heart	26	Cytochrome *c*	1×10^{-4}	49	Present authors
Aldehyde mutase	Beef liver	37	Acetaldehyde	4×10^{-3}	90	Webb & van Heyningen (1943)
Phosphorylase	Potato	25	Cori ester	$2{\cdot}5 \times 10^{-3}$	40	
Glyoxalase	Beef liver	37	Methyl glyoxal	3×10^{-3}	80	
Hexokinase	Yeast	37	Glucose + ATP	1×10^{-2}	40	
Pyrophosphatase	Kidney	25	Na pyrophosphate	$2{\cdot}5 \times 10^{-3}$	0	
Succinic oxidase	Heart	37	Na succinate	$1{\cdot}25 \times 10^{-3}$	0	
Succinic oxidase	Pigeon breast	38	Na succinate	1×10^{-3}	65	Present authors
Xanthine oxidase	Milk	38	Hypoxanthine	5×10^{-3}	0	Webb & van Heyningen (1943)
d-Amino-acid oxidase	Kidney	38	*dl*-Alanine	$2{\cdot}5 \times 10^{-3}$	0	
Creatine phosphokinase	Muscle	38	Creatine + ATP	$1{\cdot}25 \times 10^{-2}$	0	
Cholinesterase (serum)	Blood serum	20	Acetylcholine	5×10^{-3}	0	
Cholinesterase (serum)	Blood serum	38	Acetylcholine	1×10^{-3}	16	Present authors
Cholinesterase (brain)	Rat	38	Acetylcholine	1×10^{-3}	7	Present authors
Pepsin		37	Casein	$4{\cdot}16 \times 10^{-3}$	0	Webb & van Heyningen (1943)
Trypsin		37	Casein	$4{\cdot}16 \times 10^{-3}$	0	
Papain		37	Casein	$4{\cdot}16 \times 10^{-3}$	Some activation	
Lactic dehydrogenase	Heart	25	Lactate	1×10^{-3}	0	Present authors

Table 2. *Effect of BAL* (1×10^{-3} M) *on the activity of succinoxidase*

Experimental conditions	O_2 uptake (μl.)	Inhibition (%)
Enzyme from pigeon breast muscle		
A. Enzyme + BAL	0	—
Enzyme + succinate	292·5	—
BAL added before succinate	273·5	6·5
B. Enzyme + succinate	325	—
BAL + enzyme; succinate added 30 min. after BAL	243·8	25
Enzyme from rat liver		
Enzyme + BAL	60·8	—
Enzyme + succinate	311	—
Enzyme + succinate + BAL	41	Complete

Table 3. *Effect of dithiols* on the activity of succinoxidase*

(Each vessel contained 0·6 ml. enzyme; succinate, 0·03 M; 0·05 M-phosphate buffer, pH 7·0; dithiol, 0·001 M. Total vol. 3·0 ml; temp. 38°; duration of experiment, 30 min.)

Dithiol	Inhibition (%)	Dithiol	Inhibition (%)
1	25·0	12	77·0
2	3·0	13	84·6
3	4·5	14	64·6
4	73·0	15	82·5
5	58·3	16	30·4
6	41·0	17	63·0
7	79·5	18	23·5
8	30·1	19	69·6
9	85·4	20	89·6
10	9·8	21	59·5
11	95·3		

* For nomenclature of dithiols see Barron *et al.* (1946).

of red cells by arsine (Rhoads, personal communication).

Webb & van Heyningen reported that dithiol 1 did not inhibit the activity of *d*-amino-acid oxidase, an observation confirmed in this laboratory in studies with enzymes and with tissue slices. Neither the oxidation of *dl*-alanine, nor the oxidation of *l*-glutamate by kidney slices was affected by dithiol 1 (Table 4). The slight increase in the NH_3 formation

Table 4. *Effect of BAL* (10^{-3} M) *on utilization of amino-acids by kidney slices*

	Q_{O_2} 1st hr.	Q_{O_2} 2nd hr.	NH_3 formed (μmol./mg. dry wt. in 2 hr.)
No substrate	16·9	14·5	—
No substrate + BAL	8·4	5·2	—
dl-Alanine	28·6	24·1	0·82
dl-Alanine + BAL	28·9	24·1	1·30
l-Glutamic acid	33·5	33·3	0·66
l-Glutamic acid + BAL	28·0	27·2	0·67

in the presence of dithiol might be due either to reactivation of the enzyme (*d*-amino-acid oxidase is a SH enzyme) or to slight inhibition of NH_3 utilization by the tissues.

Krop (1946) has reported that 1×10^{-3} M-dithiol 1 produced an accumulation of lactate in isolated muscle; Durlacher, Bunting, Harrison, Ordway & Albrink (1946) have also reported an increase in the blood lactate concentration after injection of dithiol 1. The dithiol had no effect on the rate of reduction of diphosphopyridine nucleotide by lactate activated by lactic dehydrogenase.

Effect of 2:3-dimercaptopropanol on tissue metabolism

As reported by Webb & van Heyningen and by Rhoads, dimercaptopropanol inhibits the respiration of tissues. This inhibition increases with time and might be due to three factors: combination of dithiol with heavy metals necessary for enzyme activity; the delay produced by dithiol in the rate of oxidation of cytochrome *c* by cytochrome oxidase; and the inhibition of SH enzymes by oxidized dithiol.

The effect of dimercaptopropanol on the metabolism of pyruvate by tissue slices was not uniform. The oxidation of pyruvate by kidney slices (rat) was inhibited 22 % while the utilization of pyruvate by brain slices was unaffected (Table 5). The effect

Table 5. *Effect of BAL* (1×10^{-3} M) *on the* O_2 *uptake and untilization of pyruvate by rat tissues*

(Utilization of pyruvate is given as μmol./mg. dry wt. in 2 hr.)

	Q_{O_2} 1st hr.	Q_{O_2} 2nd hr.	Pyruvate utilized
Brain:			
No substrate	5·4	2·5	—
No substrate + BAL	5·2	0·88	—
Pyruvate	12·3	11·3	0·836
Pyruvate + BAL	11·4	7·4	0·782
Kidney:			
No substrate	22·1	18·1	—
No substrate + BAL	10·2	3·5	—
Pyruvate	30·3	31·9	2·30
Pyruvate + BAL	31·1	26·5	1·76
Diaphragm:			
No substrate	6·8	12·0	—
No substrate + BAL	3·4	5·5	—
Rectus Abdominalis:			
No substrate	3·0	6·0	—
No substrate + BAL	2·0	3·1	—

of dimercaptopropanol on the utilization of lactate by animal tissues was studied with the diaphragm, brain, and kidney of rats, the diaphragm having been cut into small strips, and the brain and kidney sliced. Diaphragm utilized 0·193 μmol. lactate/mg. dry wt. in 3 hr.; in the presence of dithiol there was no utilization of lactate. There was on the contrary an accumulation of 0·32 μmol./mg. tissue, which is in agreement with the findings of Krop. However, the utilization of lactate by brain and kidney was not altered in spite of the inhibition on the O_2 uptake observed after addition of dithiol (Table 6).

The extensive use of dimercaptopropanol in ointment form for percutaneous administration made necessary a study of its effects on the respiration of skin. BAL was applied to the skin of a 7-day-old rat; ½ hr. later the excess dithiol was wiped off with a gauze and the skin was cleaned with ethanol. The respiration was followed for 3 hr.; the Q_{O_2} values for the control skin and the treated skin were found to be practically identical. The effect of BAL on skin slices was also followed for 3 hr, 1×10^{-3} M-dithiol

Table 6. *Effect of BAL* (1×10^{-3} M) *on the utilization of lactate by tissues*

Tissue	Q_{O_2} 1st hr.	Q_{O_2} 2nd hr.	Lactate utilized (μmol./mg. dry wt. in 2 hr.)
Diaphragm:			
No substrate	5·39	4·61	—
No substrate + BAL	4·62	1·33	—
Lactate	6·45	6·63	0·193
Lactate + BAL	3·39	1·91	None
Brain:			
No substrate	4·83	2·20	—
No substrate + BAL	3·50	0·34	—
Lactate	8·30	6·26	0·54
Lactate + BAL	6·56	2·09	0·58
Kidney:			
No substrate	22·1	18·1	—
No substrate + BAL	10·2	3·5	—
Lactate	30·9	29·5	0·78
Lactate + BAL	25·3	20·1	0·67

being added to the Ringer-phosphate solution. Here, too, dithiol had no effect on the rate of respiration, for the increase observed in the first hour is probably due to the extra O_2 uptake from the oxidation of dithiol (Table 7). This resistance of skin respiration to BAL is remarkably in contrast with the inhibition observed in other tissues.

Table 7. *Effect of BAL on the respiration of young rat skin*

Experimental conditions	Q_{O_2} 1st hr.	Q_{O_2} 2nd hr.	Q_{O_2} 3rd hr.
BAL applied to skin of living rat; wiped off 30 min. later:			
Control	2·66	2·14	1·90
Treated	2·71	2·18	1·87
BAL added to skin slices in the Warburg vessel:			
Control	2·25	2·23	2·18
BAL	3·26	2·57	2·46

In the anaerobic glycolytic process in brain slices there are presumably the following metallo-proteins acting as components of enzyme systems: zymohexase, isomerase, triose mutase, enolase, and phosphopyruvate phosphatase. Since dithiols form complex compounds with a number of heavy metals, dithiol might inhibit glycolysis. In brain glycolysis there was, in the presence of 1×10^{-3} M-dimercaptopropanol a continuous inhibition which at the end of 2 hr. reached 47 %.

Dimercaptopropanol and insulin

It is known that the activity of insulin is destroyed by reduction of the S-S groups of the molecule. Since BAL has a strong reducing power ($E'_0 = -0{\cdot}150$ V. at pH 7·0), it might reduce these

Table 8. *The effect of BAL on insulin hypoglycaemia in rabbits*

Experimental conditions	Blood sugar in mg./100 ml. Insulin	BAL	BAL + insulin	Time after injection (min.)
1. BAL (0·01 M) + 8 units insulin in 30 ml. of 0·154 M-NaCl. 3 ml. injected/kg. wt. to fasting rabbits	111	107	115	Initial, fasting
	50	101	61	60
	44	98	42	120
	50	100	53	180
	56	99	52	240
	84	100	89	300
2. BAL (0·015 M) + 8 units insulin in 30 ml. of 0·154 M-NaCl. 3 ml. injected/kg. wt.	77	93	85	Initial, fasting
	53	87	67	60
	59	87	67	120
	87	91	96	180
	83	89	96	240
3. BAL (0·12 M) + 8 units insulin in 30 ml. of 0·154 M-NaCl. 3 ml. injected/kg. wt.	91	83	90	Initial, fasting
	51	59	114	60
	60	77	102	120
	95	Death	—	180
4. BAL (0·10 M) + 12 units insulin in 30 ml. of 0·04 M-phosphate, 0·1 M-NaCl. 3 ml. injected/kg. wt.	82	114	113	Initial, fasting
	39	103	123	60
	26	—	—	90
	36	84	104	120
	Death	87	106	180
	—	90	105	240

groups and thus destroy the effect of insulin. In fact, Durlacher *et al.* found an initial increase in blood sugar after the injection of dimercaptopropanol. To determine the effect of dimercaptopropanol on insulin the following experiments were performed.

Rabbits were injected intravenously with (*a*) BAL, (*b*) 0·8 unit of insulin/kg. wt., (*c*) 0·8 unit insulin plus BAL. In Exp. I (Table 8) 30 ml. of 0·01 M-BAL in 0·154 M-NaCl were kept for 5 min. at room temperature with 8 units of insulin (Iletin Lilly made from Zn-insulin). Dimercaptopropanol alone (3 ml. 0·01 M-dithiol/kg.) had no effect on the blood sugar; dithiol plus insulin lowered the blood sugar to the same extent as insulin alone. In Exp. 2, 30 ml. 0·015 M-dithiol were used and the contact of insulin with dithiol was increased to 10 min.; dithiol alone had no effect on the blood sugar; dithiol plus insulin produced less hypoglycaemia than insulin alone. In Exp. 3, 30 ml. 0·12 M-dithiol were used, the contact with insulin being 5 min. 3 ml. of this solution/kg. was fatal to the rabbits, the animals dying 3 hr. after the injection. Dithiol alone lowered the blood sugar level 60 min. after the injection, the blood sugar coming back to normal 120 min. later; dithiol plus insulin produced no hypoglycaemia. In Exp. 4, 40 ml. 0·1 M-phosphate, pH 7·4, plus 60 ml. 0·154 M-NaCl were boiled and saturated with N_2. To 30 ml. of this solution were added: (1) 12 units of insulin, (2) 12 units of insulin plus 0·2 ml. dithiol, (3) 0·2 ml. dithiol. Dithiol alone had no effect on the blood sugar; insulin alone produced marked hypoglycaemia.

SUMMARY

1. Dithiols produce inhibition of some enzyme systems by combining with the heavy metals that form the prosthetic group of the protein moiety of the enzyme. During the oxidation of dithiol there is inhibition of enzyme systems containing essential SH groups.

2. Dithiols reduce cytochrome *c* and thus show an apparent inhibition in the rate of oxidation of reduced cytochrome *c* by cytochrome oxidase. This inhibition is quite marked at concentrations which are lethal to animals.

3. Some dithiols produced a slight inhibition of succinoxidase, while others had a powerful inhibiting effect.

4. The reduced respiration of tissue slices brought about by dithiols is probably due to different factors: reduced rate of oxidation of cytochrome *c*; inhibition of metallo-protein enzymes; inhibition of SH enzymes by the oxidation product of dithiols.

5. 2:3-Dimercaptopropanol destroys the physiological activity of insulin. This destruction is probably due to the reduction of the S-S groups of insulin.

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