SUMMARY

1. Reaction between egg albumin and sodium hypochlorite results in degradation of protein and reduction of hypochlorite accompanied by evolution of heat, all of which follow a similar course. There is no significant change after ten minutes.

2. From thermal data it is concluded that three oxidations occur. Oxidation is most rapid at pH 9.8.

3. The pH falls as the reaction proceeds: in alkaline solution there is first a rise, and this rise increases with greater initial alkalinity.

4. Approximately 2.9 molecules of NaOCl are reduced for each amino-acid residue attacked.

5. *N*-Chloro-groups form rapidly and hydrolyze as the oxidation proceeds.

6. *N*-Methylacetamide is not oxidized significantly by sodium hypochlorite under the conditions used.

The author acknowledges with thanks the help given in the preparation of the manuscript by Prof. C. S. Gibson, O.B.E., F.R.S., the technical assistance of Miss B. R. Kirk, and is grateful to Milton Antiseptic Ltd. for permission to publish these results.

REFERENCES

- Block, R. J. (1945). In *Advances in Protein Chemistry*, **2**, 123, ed. Anson, M. L. & Edsall, J. T. New York: Academic Press.
- Briggs, J. F. (1918). *J. Soc. chem. Ind., Lond.*, **37**, 447 R.
- Brigl, P., Held, R. & Hartung, K. (1928). *Hoppe-Seyl. Z.* **173**, 129.
- Cohn, E. J. & Edsall, J. T. (1943). *Proteins, Amino-acids and Peptides*, p. 499. New York: Reinhold.
- Cross, C. F., Bevan, E. J. & Briggs, J. F. (1908). *J. Soc. chem. Ind., Lond.*, **27**, 260.
- Engfeldt, N. O. (1922). *Hoppe-Seyl. Z.* **121**, 18.
- Goldschmidt, S. & Steigerwald, C. (1925). *Ber. dtsh. chem. Ges.* **58 B**, 1346.
- Goldschmidt, S. & Strauss, K. (1929). *Liebig's Ann.* **471**, 1.
- Lieben, F. & Bauminger, B. (1933). *Biochem. Z.* **261**, 387.
- Mauger, R. P. & Soper, F. G. (1946). *J. chem. Soc.* p. 71.
- Miller, L. & Houghton, J. A. (1945). *J. biol. Chem.* **159**, 373.
- Norman, M. F. (1936). *Biochem. J.* **30**, 484.
- Price, T. Slater (1931). *Photogr. J.* (new series), **55**, 59.
- Salkowski, E. (1923). *Biochem. Z.* **136**, 169.
- Trotman, S. R., Trotman, E. R. & Brown, J. (1928). *J. Soc. chem. Ind., Lond.*, **47**, 4 T.
- Vandervelde, A. (1924). *Rec. trav. chim. Pays-Bas*, **43**, 158.
- Wright, N. C. (1926). *Biochem. J.* **20**, 524.
- Wright, N. C. (1936). *Biochem. J.* **30**, 1661.

Antidotal Activity of British Anti-Lewisite against Compounds of Antimony, Gold and Mercury

By R. H. S. THOMPSON AND V. P. WHITTAKER, *Department of Biochemistry, Oxford*

(Received 19 July 1946)

Ever since the discovery of 2:3-dimercaptopropanol (British Anti-Lewisite, or BAL) as an arsenical detoxicant (Stocken & Thompson, 1940, 1941; Peters, Stocken & Thompson, 1945), its application as a possible antidote for compounds of other metalloids and metals had been envisaged in this laboratory, and a few experiments were tried at an early date. For various reasons it was not possible to report this work till 1945 when Whittaker (1945*a*) submitted an account of various aspects of the problem to the Ministry of Supply.

Meanwhile, reports had appeared from America on the effects of BAL on the toxic actions of Bi, Hg, Cd, V and Se (Barron & Kalnitsky, 1944), Cd (Sulzberger & Baer, 1944) and Sb (Calvery, Braun & Lusky, 1944), and in this country on effects obtained with Zn (Bruner, 1945).

From the earlier work with arsenic, theoretical considerations alone rendered it likely that antidotal

effects should be obtained with antimony and also with divalent toxic metals such as copper or mercury. As evidence had been obtained, however, showing that a considerable proportion of cases of post-arsphenamine dermatitis responded clinically to a course of injections of BAL (Carleton, Peters, Stocken, Thompson, Williams, Storey, Levvy & Chance, 1946) it was decided to study first those metals whose compounds are used therapeutically. The work described here therefore deals only with antimony, gold and mercury, and chiefly with the organic compounds of these metals in use in medicine at the present time.

Extension of this work to the treatment of the dermatitis that develops occasionally in patients receiving therapy with compounds of gold has already begun.

As in the earlier work with arsenic, the initial experiments were designed to test the effect of BAL

in counteracting the inhibition of the brain pyruvate oxidase system caused by the presence of compounds of these metals. All the metal compounds described below were found to inhibit this system much less than either lewisite or mapharside; consequently, the 'brain dispersion' preparation, used by Whittaker (1945*b*), in experiments with arsphenamine and nearsphenamine, has been used for most of the experiments. In addition to enzyme work, rat survival experiments are now being carried out with tartar emetic.

EXPERIMENTAL

Two types of pyruvate oxidase preparation have been used: pigeon brain *brei* (Passmore, Peters & Sinclair, 1933) and pigeon brain dispersion (Banga, Ochoa & Peters, 1939). Activity was determined by the Warburg technique in the presence of sodium pyruvate at pH 7.3 and 38°, as described by Stocken, Thompson & Whittaker (1947).

Both protection and reactivation experiments have been carried out; in the former, done with the *brei* preparation, the BAL, freshly dissolved in Ringer-phosphate, was added to the brain sample dispersed in the Ringer-pyruvate mixture, the metal compound being added 2-3 min. later; when the dispersion was used the enzyme was added to the Ringer-phosphate already containing thiol, metal compound and substrate. In the reactivation experiments the BAL was placed in the side-bulbs, and the metal compound added to the unprotected brain in the main compartment of the flask. The manometers were then attached to the thermostat, and 15 min. allowed for temperature equilibration at 38°, after which the side-bulb contents were tipped into the main compartment at measured times, and the effect of this addition on the inhibition was noted over a further measured period of time.

RESULTS

Antimony. Three therapeutic antimonials were selected: potassium antimonyl tartrate (tartar emetic), 'stibophen' (a complex compound of trivalent antimony and sodium catechol disulphonate)

and 'stibacetin' (a compound containing pentavalent antimony, sodium *p*-acetylamino-phenyl-stibonate). These three antimonials differ in their toxicity to the pyruvate oxidase dispersions, pentavalent antimony, like pentavalent arsenic, being very much less toxic than trivalent compounds. Thus, in Table 1, stibacetin is seen to have less than one-tenth of the toxicity of stibophen, and less than a fortieth of the toxicity of tartar emetic. Table 1 also shows that BAL is able to protect the enzyme system from inhibition by tartar emetic or stibophen when present in a ratio of 6 mol. of BAL : 1 of antimonial. Molecular weights for stibophen and stibacetin were calculated from the formulae given by Fischl & Schlossberger (1934). An attempt was made to detoxicate stibacetin, but owing to the relatively high concentrations of this compound required to inhibit the enzyme system, the concentrations of BAL needed to detoxicate were so great as to produce significant inhibitions themselves.

Table 2 shows some reactivation experiments with tartar emetic. In these experiments readings were taken at the end of the first 5 min.; BAL was then tipped in from the side-bulbs and a further reading taken over the period 10-30 min. It will be seen that an appreciable amount of reactivation is obtained even under the very adverse conditions of these experiments in which the enzyme was poisoned for periods of up to 26 min. before the addition of BAL.

Tartar emetic is only about one-fourth as toxic to the *brei* as to the dispersion, a relation which was also found with the arsenicals (Stocken *et al.* 1947). The correspondingly high concentrations of BAL which have thus to be used in experiments with the former preparation are themselves appreciably toxic and therefore render the results of the experiments difficult of interpretation. This is a trouble which was also encountered with the arsphenamines (Stocken *et al.* 1947), and is illustrated in Table 3.

Table 1. *The effect of antimonials and BAL on pigeon brain pyruvate oxidase dispersions*

Concentration Sb (mm)	Mol. ratio BAL/Sb	$\mu\text{l. O}_2/30 \text{ min./2 ml.}$				Inhibition (%)	
		Control	Sb	BAL	Sb + BAL	Sb	Sb + BAL
Tartar emetic							
0.05	6	317	119	468	451	62	4
0.05	6	290	112	460	450	61	2
0.05	6	281	113	495	434	60	12
Stibophen							
0.2	6	456	184	470	470	59	0
0.2	6	334	171	441	452	49	0
0.2	6	468	175	489	488	63	0
Stibacetin							
0.5	—	406	324	—	—	19	—
1.0	—	406	267	—	—	34	—
2.0	—	408	313	—	—	23	—

Table 2. *The reactivation of brain pyruvate oxidase dispersions by BAL after poisoning with 0.05 mM-tartar emetic*(a, O₂ uptake during 0-5 min.; b, during 10-30 min.)

Mol. ratio BAL/Sb	Time of poisoning before addition of BAL (min.)	μ l. O ₂ ml./2 ml.						Inhibition (%)		
		Control		Sb		BAL	Sb + BAL			
		a	b	a	b	b	b	Sb a	Sb b	Sb + BAL b
12	24	115	205	42	46	317	198	64	78	38
12	26	110	206	39	59	236	122	65	71	48
12	17	121	193	44	69	281	215	64	64	24

Table 3. *The effect of 0.2 mM-tartar emetic and BAL on pigeon brain brei pyruvate oxidase*

Mol. ratio BAL/ Sb	0-30 min. inhibition (%)			30-60 min. inhibition (%)		
	BAL	Sb	Sb + BAL	BAL	Sb	Sb + BAL
3	0	57	39	18	70	57
3	2	55	59	24	44	66
6	5	55	11	36	44	35
6	8	48	19	40	63	46

It will be seen that there is considerable (18-40 %) inhibition by the BAL alone during the second half-hour period. Nevertheless, in the first half-hour, which is uncomplicated by serious toxic effects due to BAL, it will be seen that some degree of protection is afforded by 6 molecular proportions of BAL.

A few preliminary experiments have been carried out to determine whether injection of BAL can cause the survival of rats poisoned by intramuscular injection of lethal doses of tartar emetic; although evidence has been obtained that BAL exerts significant antidotal properties against antimony under these *in vivo* conditions, further work along these lines is in progress.

Gold. The gold compounds selected were gold chloride (the double salt $\text{AuCl}_3 \cdot \text{NaCl} \cdot 2\text{H}_2\text{O}$), 'myocrisin' (sodium aurothiomalate, hemihydrate) and 1-aurothioglucose (as the double salt with sodium thioglucose).

The results of enzyme experiments with these compounds using the brain dispersion are shown in Table 4.

Table 4. *The effect of gold and thiols on pigeon brain pyruvate oxidase dispersions*

Concentration gold (mm)	Mol. ratio thiol/gold	μ l. O ₂ /2 ml./30 min.				Inhibition (%)	
		Control	Gold	Thiol	Gold + thiol	Gold	Gold + thiol
Gold chloride							
0.017	—	290	250	—	—	14	—
0.085	—	290	193	—	—	33	—
0.085	—	306	213	—	—	31	—
0.17	—	306	119	—	—	61	—
0.43	—	290	26	—	—	91	—
0.43	—	306	31	—	—	90	—
Gold chloride + BAL							
0.17	3	270	129	378	375	52	1
0.17	3	214	69	395	402	66	0
0.17	3	327	81	455	479	75	0
Gold chloride + glutathione							
0.17	6	264	140	319	203	47	36
0.17	6	225	55	233	161	76	31
0.17	6	279	144	358	291	48	19
Myocrisin + BAL							
1.0	3	310	122	260	414	61	0
1.0	3	416	126	270	420	70	0
1.0	3	333	150	242	423	55	0
Aurothioglucose							
0.5	—	469	388	—	—	17	—
1.0	—	469	371	—	—	21	—
2.0	—	402	273	—	—	32	—
5.0	—	402	247	—	—	39	—
10.0	—	402	186	—	—	54	—

It will be seen that BAL effectively detoxicates both gold chloride and myocrisin. In order to compare a monothiol with the dithiol protection afforded by BAL three experiments have been done using glutathione (Roche Products Ltd.). Although some protection is provided by glutathione it is less active than BAL in this respect.

It will be noted that myocrisin, in which the gold is already attached to a sulphur atom, possesses only approximately one-sixth the toxicity of gold chloride to the pyruvate oxidase system. Aurothio-glucose, which possesses in addition to the sulphur combined with gold a molecule of sodium thio-glucose, is even less toxic, ten times the concentration being required to produce the same enzyme inhibition as is produced by the presence of 1 mM-myocrisin. For this reason detoxication experiments were not carried out, owing to the high concentrations of BAL which would have been required.

Mercury. In view of the existing evidence that mercury combines with essential —SH groups in enzymes (Rapkine, 1931; Hellermann, 1937; Eagle, 1939; Fildes, 1940) it was of interest to investigate the effect of BAL on the toxicity of mercuric chloride by means of the pigeon brain *brei* preparation.

Fig. 1 shows the effect of increasing concentrations of mercuric chloride in this system, while in Table 5 are given the results of the protection experiments.

It will be seen that 6 molecular proportions of BAL are able to protect the enzyme system completely from the effects of 0.18 mM-mercuric chloride, a concentration which produces approximately 50% inhibition of the unprotected enzyme; the presence of only 3 molecular equivalents is able to cause a very significant degree of protection. Since Fildes (1940) had obtained efficient neutralization of the inhibitory effects of mercuric chloride on the growth of *Esch. coli* by the presence of monothiols such as glutathione or cysteine, enzyme experiments were also done with two representative monothiols, glutathione and 2-mercaptoethanol; the former was found to bring about complete protection, while the latter was completely ineffective under our conditions.

DISCUSSION

The ability of BAL to protect the brain pyruvate oxidase system from poisoning by compounds of arsenic has been shown to hold also for the poisoning produced by compounds of antimony, gold and mercury. In the case of these latter metals, however, the difference in effectiveness, as between dithiols and monothiols, that was shown to hold in the case of poisoning by lewisite (Stocken & Thompson, 1946) does not appear to be so striking. On the evidence so far obtained with gold chloride,

glutathione can cause some degree of protection, although it is much less effective than BAL when present in equivalent concentrations. Against mercury, on the other hand, it appears to be an efficient antidote, and Barron (1945) has claimed that it is partially able to reverse the toxic effect of *p*-chloro-mercuribenzoic acid on the pyruvate oxidase system of gonococci.

Fig. 1. The inhibition of pigeon brain *brei* pyruvate oxidase by mercuric chloride (no. of experiments averaged is given in brackets).

Table 5. The effect of thiols on the poisoning of the pigeon brain *brei* pyruvate oxidase system by 0.18 mM-mercuric chloride

Mol. ratio thiol/HgCl ₂	μl. O ₂ /g./hr. over 1 hr.				Inhibition (%)	
	Control	HgCl ₂	Thiol	Thiol + HgCl ₂	Hg	Hg + thiol
BAL						
3	2190	1088	2360	2060	51	13
3	1805	1119	1938	1888	38	3
6	2190	1088	1959	2414	51	0
6	1805	1119	1615	1648	38	0
Glutathione						
12	1979	834	1757	1995	58	0
12	2093	1006	2130	2133	52	0
2-Mercaptoethanol						
12	1979	834	2035	537	58	74
12	2093	1006	2053	665	52	68

Although insufficient experiments have been carried out to explain the remarkable difference in the effectiveness of glutathione and of 2-mercaptoethanol against mercury inhibition, it is certain that there are many factors which influence the effectiveness of thiol antidotes, among them the relative

stability of the 'tissue acceptor' and antidote-mercaptides, and the relative ease of penetration of inhibitor and antidote to the active centres.

The increased oxygen uptake of brain dispersions brought about by the presence of BAL has been observed before, and has been shown to be due to a true stimulation of the enzyme activity (Stocken *et al.* 1947).

SUMMARY

The ability of 2:3-dimercaptopropanol (British Anti-Lewisite or 'BAL') to detoxicate compounds of

antimony, gold and mercury has been demonstrated, using the pyruvate oxidase system of pigeon brain as a test system.

This work was carried out for the Ministry of Supply as part of a larger programme under the direction of Prof. R. A. Peters. Our thanks are due to Dr L. A. Stocken for his helpful suggestions in the course of this work, to the Chief Scientific Officer, Ministry of Supply, for permission to publish, to Messrs May and Baker for a sample of myocrisin, and to the Wellcome Bureau of Scientific Research for samples of stibophen, stibacetin and aurothioglucose.

REFERENCES

- Banga, I., Ochoa, S. & Peters, R. A. (1939). *Biochem. J.* **33**, 1109.
- Barron, E. S. G. (1945). *J. biol. Chem.* **137**, 22.
- Barron, E. S. G. & Kalnitsky, G. (1944). By communication.*
- Bruner, H. D. (1945). Report to Ministry of Supply.*
- Calvery, H. O., Braun, H. A. & Lusky, L. M. (1944). By communication.*
- Carleton, A. B., Peters, R. A., Stocken, L. A., Thompson, R. H. S. & Williams, D. I.; with Appendix by Storey, I. D. E., Levvy, G. A. & Chance, A. C. (1946). *J. clin. Invest.* **25**, 497.
- Eagle, H. (1939). *J. Pharmacol.* **66**, 436.
- Fildes, P. (1940). *Brit. J. exp. Path.* **21**, 67.
- Fischl, V. & Schlossberger, H. (1934). *Handbuch der Chemotherapie*. Leipzig: Georg Thieme.
- Hellermann, L. (1937). *Physiol. Rev.* **17**, 454.
- Passmore, R., Peters, R. A. & Sinclair, H. M. (1933). *Biochem. J.* **27**, 842.
- Peters, R. A., Stocken, L. A. & Thompson, R. H. S. (1945). *Nature, Lond.*, **156**, 616.
- Rapkin, L. (1931). *Ann. Physiol. Physicochim. biol.* **7**, 382.
- Stocken, L. A. & Thompson, R. H. S. (1940). Report to Ministry of Supply by Peters, No. 25.*
- Stocken, L. A. & Thompson, R. H. S. (1941). Report to Ministry of Supply by Peters, no. 33.*
- Stocken, L. A. & Thompson, R. H. S. (1946). *Biochem. J.* **40**, 535.
- Stocken, L. A., Thompson, R. H. S. & Whittaker, V. P. (1947). *Biochem. J.* **41**, 47.
- Sulzberger, M. B. & Baer, R. (1944). By communication.*
- Whittaker, V. P. (1945 a). Report to Ministry of Supply by Peters, no. 91.*
- Whittaker, V. P. (1945 b). Report to Ministry of Supply by Peters, no. 88.*

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

The Inhibition of Succinoxidase by Heavy Metals and its Reactivation with Dithiols*

By E. S. GUZMAN BARRON AND G. KALNITSKY, *The Chemical Division, Department of Medicine of the University of Chicago*

(Received 21 March 1946)

Many heavy metals either combine with —SH groups to form mercaptides or act as oxidizing agents. Their combination with the —SH groups of those enzymes requiring the presence of these groups for activity results in enzyme inhibition. Since a large number of enzymes are sulphydryl enzymes (Barron & Singer, 1945; Singer & Barron, 1945), it is reasonable to assume that the toxicity of these heavy metals must be largely due to inhibition

of —SH enzymes. Mercaptides are in general easily dissociable, i.e. the metals leave the —SH groups to which they were originally attached when in the presence of other —SH groups with which they form more stable compounds. A typical example of such reactions is that given by Hellerman (1937) when he introduced *p*-chloromercuribenzoic acid (Hg-Cl-benzoate) as an inhibitor of —SH enzymes; the inhibitions produced by it were easily reversed on addition of thiols (cysteine or glutathione). In spite, however, of the knowledge that some heavy metals form mercaptides, very little has been done to see if they combine with the —SH groups of proteins. If

* 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.