RESEARCH PAPERS

THE ANTIDOTAL ACTIVITY OF SOME DITHIOLS AND ACETYLDITHIOLS IN MICE POISONED WITH OXOPHENARSINE

By M. Weatherall

*From the Department of Pharmacology, University of Edinburgh**

Received June 30, 1949

It is well known that dimercaprol (2:3-dimercaptopropanol, BAL) and other dithiols reduce the toxicity of compounds of arsenic to animals and enzyme systems (Peters, Stocken and Thompson[1]; Stocken and Thompson[2]; Fitzhugh, Woodard, Braun, Lusky and Calvery[3]; *et al.*). A number of 1:2-dithiols and three $\alpha:\omega$-dithiols not previously studied have been examined for toxicity and anti-arsenical activity, and the results are reported here. Some of the dithiols were prepared from the corresponding acetylated dithiols, and as it seemed possible that deacetylation might occur in the body, the activity of the acetylated dithiols was also examined. In order to have an adequate basis for this study, the activity of dimercaprol and dimercaprol glucoside (BAL-Intrav) was first explored in some detail.

The compound of dimercaprol and oxophenarsine is more toxic than oxophenarsine alone (Peters and Stocken[4]; Friedheim and Vogel[5]), and the toxicity of this compound has been examined further. Its high toxicity suggests that circumstances may exist in which dimercaprol potentiates oxophenarsine poisoning. This possibility has been examined, as has the possibility of potentiation by other dithiols.

Methods

Mice weighing 17 to 25 g. were used. They were kept at a temperature between 20° and 24°C. during and after experiments. They were fed on a standard diet of cubes of the following composition:—wheat bran, 19·2 per cent.; wheat ground middlings, 19·2 per cent.; Sussex ground oats, 19·2 per cent.; ground maize, 9·5 per cent.; ground barley, 9·5 per cent.; meat and bone meal, 9·5 per cent.; skim milk powder, 7·0 per cent.; fish meal, 4·8 per cent.; dried yeast, 1·3 per cent.; cod-liver oil 0·4 per cent.; salt mixture, 0·4 per cent. (manufacturer's figures). Food was withdrawn on the evening before experiments, and was restored immediately after treatment. Except where it is otherwise indicated, all poisons were injected into the muscles of the right hind leg, and all thiols into the muscles of the left hind leg. Doses were adjusted according to body-weight, and have been expressed in mg.-molecules/kg. (mM/kg.) to facilitate comparison between the amounts of poison and antidote used. The mice were observed at least 1, 2, 4, 8, 20 to 21, 28 to 32

* Present address: Department of Pharmacology, London Hospital Medical College, London, E.1.

and 48 hours after injection in most experiments, and approximate estimates of the survival time were made from these observations. Deaths were rare after 48 hours, and were not included in estimates of the mean survival time or of the mortality. The mice were watched until four weeks after the day of injection. With one exception discussed below, late deaths occurred seldom and erratically, and have not been reported as their significance is doubtful. Post-mortem examinations and weighing of organs, when performed, were carried out within an hour of death. The preparation and chemical properties of the dithiols and acetylated dithiols used (Table V) have been or will be reported elsewhere (Evans and Owen[6]; Evans, Fraser and Owen[7]). Some of the free dithiols (dimercaprol glucoside, 0:16, 0:17, 0:19, 0:20 and 0:24) were prepared from their barium salts, and the solutions so obtained were standardised by titration in acid solutions at 0°C. with iodine (Weatherall and Weatherall[8], and *infra*). The other substances were received pure or nearly pure and were generally used freshly dissolved or suspended in olive oil or peanut oil. Dithiodulcitol was dissolved in aqueous sodium hydroxide, and the solutions were neutralised with boric acid. Certain acetylated dithiols (0:11, 0:14) which were difficult to dissolve in oil or in propylene glycol were dissolved in diethylene dioxide and the volumes injected were kept below 1·0 ml./kg. Otherwise the volumes injected were 10·0 ml./kg. in toxicity tests and 5·0 ml./kg. each of dithiol and arsenical in antidotal tests. The doses reported have been corrected for purity. The organic solvents in the volumes used were not lethal and had no detectable anti-arsenical activity.

RESULTS

I. The toxicity of oxophenarsine.

The LD50 of oxophenarsine by subcutaneous injection in mice is given as 0·164 mM/kg. by Ercoli and Wilson[9]. Cranston, Clark and Strakosch[10] give the value 0·17 mM/kg. for intraperitoneal injection and cite the manufacturer's figures of 0·1 mM/kg. for intravenous and about 0·12 to 0·13 mM/kg. for subcutaneous injection. Eagle, Hogan, Doak and Steinman[11] found an LD50 of 0·165 mM/kg. intraperitoneally. Data obtained in the present series of experiments are collected in Table I and show that the mortality after intramuscular injection varied over the range 0·08 to 0·20 mM/kg. with an LD50 of about 0·14 mM/kg. In two experiments in which oxophenarsine was injected intraperitoneally in some mice and intramuscularly in others there was no significant difference in the mortality on any dose.

Death occurred more rapidly when the doses of oxophenarsine were large, and even with the rather crude measurements of survival time, in the range studied the means of the logarithms of the survival times were approximately linearly related to the logarithm of the dose in groups in which there were no survivors (Fig. 1 and Table I). When some animals survived, the mean survival time tended to be less than the value expected for a mortality of 100 per cent. An arbitrary correction for survivors

might be used, but it is doubtful whether such a procedure would be helpful in interpreting the results.

The weight of the lungs was measured in some of these mice. The lungs were substantially heavier than normal in mice poisoned with a small dose of oxophenarsine, and only slightly heavier when the dose was large (Table I). As the mice poisoned with a small dose lived longer, it seems probable that the development of heavy lungs depended

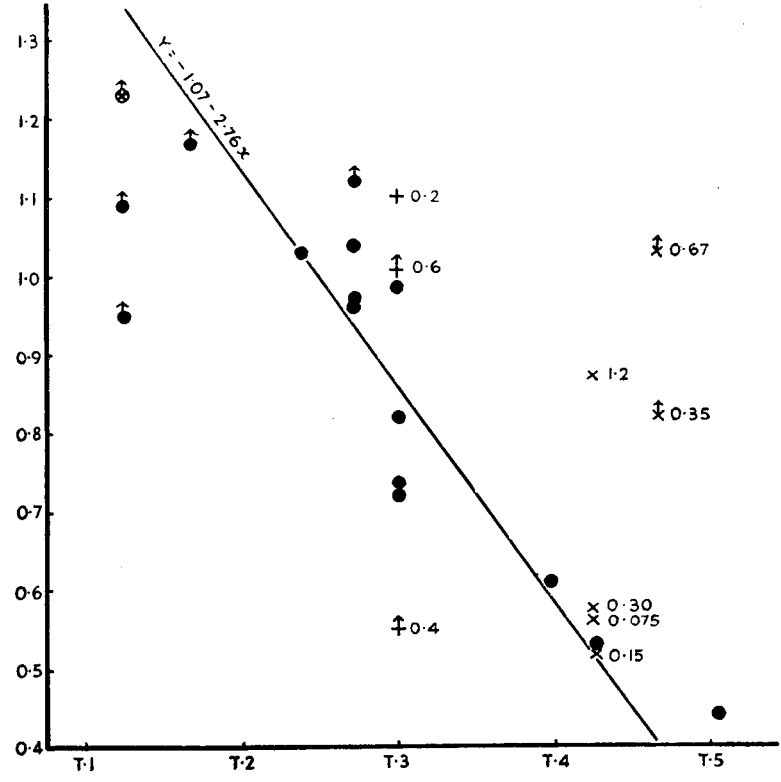


FIG. 1. The relation between log dose and log survival time in mice poisoned with oxophenarsine.

Ordinates—Survival time in log. hours.
Abscissae—Dose of oxophenarsine in log. mM/kg.

Each point represents a group of 5 to 10 mice, all of which died. Each arrow represents a group of 5 to 10 mice of which at least half died. No allowance has been made for survivors in calculating the mean survival time.

● No treatment
\+ Treated with dimercaprol
× Treated with dimercaprol glucoside
} The figure against the point gives the dose of dithiol in mM/kg.
⊗ Injected with an approximately equimolar mixture of oxophenarsine and dimercaprol glucoside

The regression line is that which fits best the points for oxophenarsine alone ($Y = -1{\cdot}07 - 2{\cdot}76x$).

The doses of dimercaprol glucoside are approximate.

TABLE I

THE TOXICITY OF OXOPHENARSINE GIVEN BY INTRAMUSCULAR INJECTION IN MICE

Dose of oxophenarsine	Total mortality No. dying/total No.		Log survival time of mice dying		Lung weight of mice dying	
			Mortality	Log Hours M±S.E.	Number of Mice	mg./g. body wt. M±S.E.
mM/kg.	Normal	per cent. Mice*				
0			—	—	10	6·6±0·32
0·067	0/15	0	—	—	—	—
0·080	4/9	44	4/9	1·25±0·19	2	12·2±2·46
0·093	1/5	20	—	—	—	—
0·107	2/8	25	—	—	—	—
0·113	4/19	21	3/10	0·94—	—	—
0·120	6/21	29	5/15	1·05±0·15	4	10·0
0·133	26/48	54	7/10	1·09±0·10	—	—
			16/20	0·95±0·07	—	—
			2/8	1·32	—	—
0·147	15/30	50	4/10	0·94±0·10	4	8·2±0·66
			8/10	1·17±0·10	—	—
0·153	8/20	40	5/10	1·23±0·13	—	—
0·160	15/18	83	—	—	—	—
0·173	15/17	88	10/10	1·03±0·08	—	—
0·178	6/15	40	5/10	0·95±0·11	—	—
0·187	38/43	89	6/6	0·96±0·10	—	—
			9/9	0·97±0·16	—	—
			10/10	1·04±0·09	10	6·6±0·42
			8/10	1·12±0·10	—	—
0·200	80/82	98	10/10	0·82±0·12	9	7·9±0·39
			10/10	1·00±0·09	8	7·1±0·37
			8/8	0·72±0·18	5	7·9±0·41
			10/10	0·73±0·16	—	—
0·250	34/34	100	10/10	0·61±0·10	—	—
0·267	16/16	100	10/10	0·53±0·03	—	—
0·320	10/10	100	10/10	0·44±0·01	—	—

* Killed by breaking neck.

on the length of life, but conclusive evidence on this point was not obtained.

II. The toxicity of the oxophenarsine-dimercaprol compound.

As was shown by Peters and Stocken[4], when oxophenarsine and dimercaprol are mixed in equimolar amounts before injection or given as the previously crystallised compound, the toxicity is substantially enhanced. This has been confirmed for the pure compound given by intramuscular injection (Table II), but it is interesting to note that in rats and mice it is not true when the compound is given intraperitoneally. In these circumstances the compound is actually less toxic than oxophenarsine, the toxicity of which is about the same by either route. After intramuscular injection of just lethal amounts of the compound, the mice died if anything more rapidly than mice poisoned with a (rather larger) just lethal amount of oxophenarsine. Their viscera were unusually congested and their lungs hæmorrhagic and substantially heavier than normal. After intraperitoneal injection some mice had convulsions and died in a few minutes: others survived for a few hours and at death tended to show less pulmonary congestion. Rats showed the same series of symptoms and difference in toxicity by intramuscular and intraperitoneal injection, and again the toxicity of oxophenarsine by either route was intermediate. Deaths and survivals observed after different dosages, in all in a dozen rats, do not suggest that the toxicity in rats is greatly different from that in mice.

III. The effect of dimercaprol in oxophenarsine poisoning.

The mortalities when various doses of dimercaprol have been given at various times after various doses of oxophenarsine are shown in Table III. In Figure 2 the dose of oxophenarsine has been plotted against the

TABLE II

THE TOXICITY OF THE OXOPHENARSINE-DIMERCAPROL COMPOUND AND OF MIXTURES OF OXOPHENARSINE AND DIMERCAPROL GLUCOSIDE IN MICE

Substance	Dose mM/kg.	Route of administration	Mortality No. dying/total No.	Log survival time of mice dying Log hours M±S.E.	Lung weight of mice dying No. of Mice	Lung weight of mice dying mg./g. M±S.E.
Oxophenarsine dimercaprol compound	0·013	Intra-muscular	0/5	—	—	—
	0·027		0/5	—	—	—
	0·040	,,	2/20	—	2	14·2
	0·053	,,	2/5	—	—	—
	0·067	,,	16/20	0·87±0·049	14	14·0±0·80
	0·107	,,	4/5	—	—	—
	LD50=0·055 mM/kg.					
	0·013	Intra-peritoneal	0/5	—	—	—
	0·027		0/5	—	—	—
	0·053	,,	0/5	—	—	—
	0·107	,,	0/5	—	—	—
	0·120	,,	1/5	—	1	11·6
	0·213	,,	0/5	—	—	—
	0·233	,,	2/9	—	2	14·7
	0·300	,,	5/9	0·50±0·186	5	12·0
	LD50=0·295 mM/kg.					
Oxophenarsine D.G. Mixture approx 3 : 1	0·133*	Intra-muscular	16/20	1·15±0·08	7	8·1±0·59
Oxophenarsine D.G. Mixture approx. 3 : 4	0·133*	,,	11/20	1·23±0·09	7	8·5±0·31
Oxophenarsine alone	0·133	,,	16/20	0·95±0·07	6	7·7±0·53

* mM/kg. of oxophenarsine in mixture.

dose of dimercaprol with different symbols for mortalities below and above 50 per cent. A line has been drawn to separate the two sets of points, and therefore gives approximately the LD50 of combinations of the two substances. As the LD50 of dimercaprol alone is of the order of 1·0 mM/kg., 0·60 mM/kg. approaches the largest dose which can be used therapeutically without itself causing death. This dose is quite effective against 0·30 mM/kg. of oxophenarsine, and still has some effect even when it is given 80 minutes after a smaller dose of the poison; but these are about the limits of its activity. These findings are in reasonable agreement with those of Ercoli and Wilson[9] under slightly different conditions. Mice which were treated with dimercaprol but did not survive generally lived longer than untreated poisoned mice. This point has been explored further, as reported elsewhere (Weatherall and Weatherall[8]), and slight but just significant acceleration of death has been observed when very small doses of dimercaprol were given to mice

poisoned with very large doses of oxophenarsine. With equivalent quantities of poison and antidote, or an excess of antidote, only prolongation of life has been observed.

Observations have not been made on the mortality from sublethal doses of oxophenarsine treated with less than one equivalent of dimercaprol, but no potentiation was observed by one equivalent (Table III). Poisoned

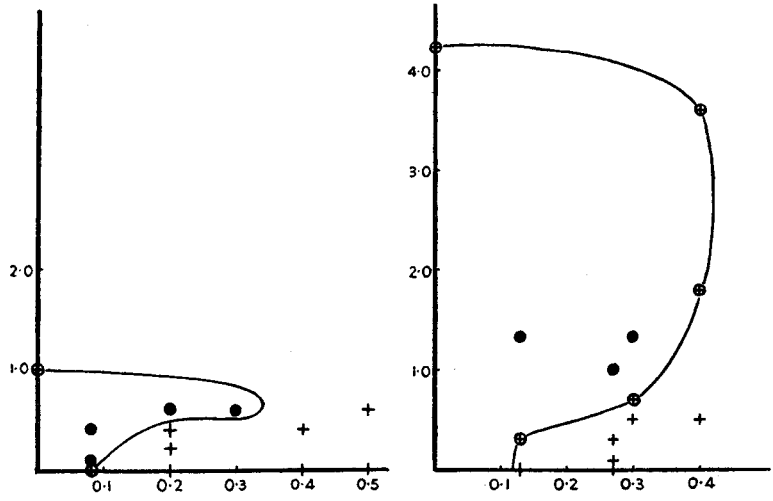


FIG. 2. Isobols for oxophenarsine and dimercaprol (left-hand graph) and for oxophenarsine and dimercaprol glucoside (right-hand graph).
Ordinates—Dose of dithiol in mM/kg.
Abscissae—Dose of oxophenarsine in mM/kg.
Each point represents the mortality for a group of mice.

● Less than 50 per cent. mortality.
⊕ Approximately 50 per cent. mortality.
\+ More than 50 per cent. mortality.
—— Line separating points where the mortality is less than and more than 50 per cent.

mice treated with dimercaprol had heavier lungs than had untreated mice poisoned with the same dose of oxophenarsine. Dimercaprol itself increases the lung weight slightly, and this increase may be an additive effect. The lungs were not heavier than those of untreated mice poisoned with less oxophenarsine and living about as long as the mice treated with dimercaprol: so if the increase in lung weight depends on the time of exposure rather than directly on the dose of oxophenarsine, the increase is not appreciably affected by dimercaprol. In any case, the great increase in lung weight observed in poisoning with oxophenarsine-dimercaprol was not observed when the two substances were given separately, and attempts to imitate its toxicity in this way have been practically unsuccessful.

IV. The toxicity of dimercaprol glucoside and its effects in oxophenarsine poisoning.

As dimercaprol glucoside has not been purified and isolated and the only materials available for biological work have been solutions pre-

TABLE III

THE MORTALITY OF MICE POISONED WITH OXOPHENARSINE AND TREATED WITH DIMERCAPROL OR DIMERCAPROL GLUCOSIDE

Treatment, minutes after oxophenarsine	Immediate							Im-med.	20 minutes	30 minutes	40 minutes	80 minutes	160 minutes
Dose of oxophenarsine mM/kg.	0·08	0·13	0·20	0·27	0·30	0·40	0·50			0·18—0·20			
No treatment	4/9	—	8/8	—	—	10/10	10/10			5/5			
Dimercaprol :—													
0·08 mM/kg.	2/9	—	—	—	—	—	—	—	—	—	—	—	—
0·20 „	—	—	8/8	—	—	—	—	—	—	—	—	—	—
0·40 „	0/9	—	6/9	—	—	10/10	—	—	—	—	—	—	—
0·60 „	—	—	1/8	—	2/9	—	10/10	3/5	2/5	—	2/5	4/5	5/5
No treatment	—	24/30	—	24/24	—	—	—			10/10			
Dimercaprol glucoside :—													
ca 0·08 mM/kg.	—	—	—	10/10	—	—	—	—	—	—	—	—	—
ca 0·30 „	—	10/20	—	10/10	—	—	—	—	—	—	—	—	—
ca 0·50 „	—	—	—	—	9/10	10/10	—	—	—	—	—	—	—
ca 0·70 „	—	—	—	—	4/9	—	—	—	—	—	—	—	—
ca 1·00 „	—	—	—	11/24	—	—	—	—	0/10	—	4/5	—	—
ca 1·33 „	—	7/20	—	—	0/10	—	—	0/10	2/10	6/9*	10/10	10/10	10/10
ca 1·80 „	—	—	—	—	—	5/10	—	—	—	—	—	—	—
ca 3·60 „	—	—	—	—	—	5/10	—	—	—	—	—	—	—

* Dose of oxophenarsine 0·33 mM/kg.

pared from the barium salt, itself of uncertain purity and always with less than the theoretical thiol content, figures for toxicity are necessarily approximations and depend on what criterion is used in calculating the concentration of the solution used. Danielli, Danielli, Mitchell, Owen and Shaw[12] and Danielli *et al.*[13] give figures based on the weight of the barium salt used per 100 ml. of solution prepared, and allow only for the replacement of the barium atom by two hydrogen atoms. In the present series of experiments it has been found that the toxicity of different batches, measured in these units, varies very widely, and that more constant values are obtained when the solutions are standardised by iodine titration at the time the toxicity is determined and the doses are measured in accordance with the titres. Iodine titration at room temperature gives a rapidly fading and rather arbitrary end-point, and better results are obtained in N hydrochloric acid at 0°C. This was not at first appreciated, and in early experiments solutions were standardised by a modification of Sampey and Reid's method[14] for monothiols, by adding excess of iodine, leaving overnight, and estimating the excess with sodium thiosulphate. This procedure gave a better defined end-point than direct titration at room temperature, but it subsequently appeared that with dithiols in these conditions the reaction proceeded beyond the disulphide stage, to a variable extent according to the amount of iodine present. In most of the indirect titrations, the excess of iodine was within the limits of 50 to 100 per cent. excess, and when further titrations were carried out under these conditions in parallel with direct titrations in N hydrochloric acid at 0°C., the indirect titre varied only between 2·5 and 3·5 times the direct titre. When only an indirect titration was performed and the excess of iodine was not outside the above limits, the strengths of solutions have been calculated on the assumption that the direct titre would have been one-third of the indirect, and are regarded as rough approximations. It has been assumed throughout that, in the direct titration, one gramme-molecule of iodine is equivalent to one gramme-molecule of dimercaprol glucoside.

Data about various batches of dimercaprol glucoside are given in Table IV, where the doses are expressed (A) as derived from the amount of barium salt used in making the solution and (B) from iodine titration. The dose-mortality curve was determined in one experiment with a large number of mice, and as the estimated slope of the line relating log dose to probit mortality was in good agreement with less accurate estimates obtained in other experiments, the LD50 was sometimes deduced from the mortality on a single dose by the use of this slope.

It will be seen that, whereas the most toxic solution (OB1) was about seven or eight times as lethal as the least toxic (142) when the concentrations were calculated from the amount of barium salt used, the difference was not more than two to threefold in terms of the iodine titre. If solutions prepared from specimen SO.1443 are not considered, the toxicity in terms of the iodine titre is reasonably constant. Specimens SO.1422 and SO.1443 were both somewhat unsatisfactory and de-

teriorated rapidly, possibly because they had not been freed sufficiently from traces of organic solvents (Owen, personal communication), and it seems reasonable to attribute the consistently higher toxicity of solutions prepared from SO.1443 to the presence of impurities. In the other solutions, toxicity is evidently attributable to the free thiol present, as might be expected; and the material which does not react with iodine, presumably chiefly products of oxidation, does not contribute much to the toxicity. The sulphydryl content may also be assumed to regulate the anti-arsenical activity, and so all doses in the present work have been

TABLE IV

THE TOXICITY OF SOLUTIONS OF DIMERCAPROL GLUCOSIDE

Specimen of barium salt used in preparation	Soln. No.	Dose mM/kg. A	Mortality No. dying/total No.	Slope of line relating log dose and probit	LD50 mM/kg. A	LD50 mM/kg. B
—	3	17·5 14·0 11·2	7/10 3/10 0/10	11·0	15·7	3·8
S0·1001	40	35·2 31·2 28·9	4/5 1/5 3/5	—	31·0	*ca* 4·7
S0·1001	48	29·5	10/10	—	<29·5	<*ca* 3·5
S0·1229	108	21·0 18·6 16·6 14·7 13·1 11·8 10·5 9·5	20/20 20/20 20/20 15/20 7/20 5/20 3/20 3/20	10·7	12·8	*ca* 4·4
S0·1422	142	46·7	8/15	—	45·0	4·5
S0·1443	0B1	10·5 7·9 5·2	8/8 8/8 2/16	—	5·9	2·1
S0·1443	143	10·1	8/15	—	9·7	2·6
S0·1443	144	23·8	5/15	—	25·6	2·1
9374A	241	14·0 12·6	14/15 9/15	12·5	11·9	—

(A) Calculated from the amount of barium salt used in preparing the solution.
(B) Calculated from the iodine titre of the solution (see text).

calculated from the results of iodine titration. As a result of doing so, a number of apparent irregularities in the behaviour of dimercaprol glucoside have disappeared. Another result of doing so is that the toxicity appears to be greater than the figures of Danielli *et al.*[14] suggest. As their doses include in the weight material which does not behave as a free thiol, they give an exaggerated suggestion of the harmlessness of the substance. Nevertheless its toxicity is undoubtedly much less than that of dimercaprol.

The activity of dimercaprol glucoside against oxophenarsine is shown in Table III and Figures 1 and 2. The activity is on the whole similar to that of dimercaprol itself. If anything the glucoside is a little less

active when given at the same time as the oxophenarsine. About 0·8 mM/kg. of the glucoside saved 5 of 9 mice poisoned with 0·30 mM/kg. of oxophenarsine, while 0·6 mM/kg. of dimercaprol was sufficient to save 7 of 9: but the difference is not significant. As the glucoside was less toxic, more could be given, and it was consequently superior against very large doses of oxophenarsine. Its much greater activity in these conditions is strikingly illustrated by the much larger area enclosed by its isobol than by that of dimercaprol (Figure 2). On the other hand, once poisoning was established, the glucoside was less effective. Dimercaprol still saved an occasional animal 80 minutes after oxophenarsine had been given, whereas no reduction in mortality was obtained with the glucoside after 40 minutes, even with large doses and in rather more extensive trials. As Danielli *et al.* suggest, the water-soluble thiol probably enters cells less readily, and so has less access to arsenic once the arsenic has been fixed by the tissues.

Mixtures of oxophenarsine and dimercaprol glucoside in proportions about a 1 to 1 molar ratio have been injected intramuscularly in mice, and no enhancement of toxicity of the arsenical has been found (Table II). Deaths were neither increased nor accelerated, and the lungs were, as usual in mice poisoned with oxophenarsine and treated with a dithiol, only moderately heavier than normal. Solutions probably containing less than one molecule of dimercaprol glucoside per molecule of oxophenarsine gave a negative nitroprusside reaction, whereas the reaction was positive when the dithiol was in excess; so it may be assumed that the two substances had combined. The evidence does not suggest that the compound has any enhanced toxicity.

IV. The toxicity of other dithiols and their effects in oxophenarsine poisoning.

The results of toxicity tests are summarised in Table V. The figures are sometimes very approximate, either because the amount of the material available was limited and the toxicity was low, or because preliminary tests indicated that the toxicity was too high for the substance, however active as an antidote, to be likely to be of any therapeutic use. The LD50s have been obtained either by injecting into one group of 15 to 20 mice a dose slightly larger than the LD50 and into another group a dose slightly smaller and estimating the LD50 by linear interpolation after transformation to log dose and probit mortality; or by the method of Kärber[15]. The efficacy against oxophenarsine was tested (a) by giving the antidote immediately after an LD95 to 99 (0·16 to 0·20 mM/kg.) to show whether any protection at all was obtained; (b) by giving the antidote 40 minutes, and sometimes at other times, after the oxophenarsine, to show whether protection could be obtained late in acute poisoning; (c) by giving a 50 per cent. larger dose of oxophenarsine and the thiol immediately afterwards, to show whether the thiol was effective against massive poisoning. The dose of thiol chosen was about one-third of its LD50, and tests (b) and (c) show roughly the maximal activity of dimercaprol and dimercaprol glucoside respectively at this level of

TABLE V

THE TOXICITY AND ANTI-ARSENICAL ACTIVITY OF SOME THIOLS AND ACETYLDITHIOLS

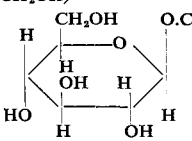
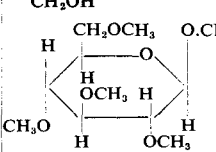
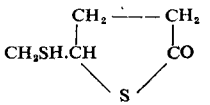
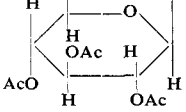
No.	Name	Formula	Mol. Wt.	Purity per cent.	LD50 mM/kg.	No. of mice in estimate of LD50	Activity against oxophenarsine	
							Immediate	After 40 minutes
—	Dimercaprol (BAL)	$CH_2SH.CHSH.CH_2OH$	124	100	1·0	99	†††	† †
0 : 13	1 : 2-dimercaptobutane-3 : 4-diol ...	$CH_2SH.CHSH.CHOH.CH_2OH$	154	80	1·0	20	†††	†††
0 : 17	1 : 2-dimercaptopentane-3 : 4 : 5-triol	$CH_2SH.CHSH.(CHOH)_2.CH_2OH$ ...	184	†	1·7–2·5	28	†††	†
0 : 24	1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol	$CH_2SH.CHSH.(CHOH)_3.CH_2OH$ ...	214	†	1·7	29	Not done	
0 : 8	2 : 3-dihydroxypropyl 2 : 3-dimercaptopropyl ether	$CH_2OH.CHOH.CH_2.O.CH_2.CHSH.CH_2SH$	198	78	1·3	55	†††	†
0 : 9	1 : 3-dihydroxy-2-propyl 2 : 3-dimercaptopropyl ether	CH_2OH / CH_2OH $>CH.O.CH_2.CHSH.CH_2SH$ ...	198	92	1·3	50	† †	†
—	(2 : 3-dimercaptopropyl) glucoside (dimercaprol glucoside, BAL-Intrav)		286	†	3·8–4·7	230	††††	†
0 : 19	3(2′ : 3′-dimercaptopropyl) mannitol...	$CH_2OH.CHOH.CH<$ $O.CH_2.CHSH.CH_2SH$ / $CHOH.CHOH.CH_2OH$	288	†	3·2	40	††††	0
0 : 16	6(2′ : 3′-dimercaptopropyl) mannitol...	$CH_2SH.CHSH.CH_2.O.CH_2.(CHOH)_4.$ CH_2OH	288	†	2·6	40	†††	† †
0 : 20	6(2′ : 3′-dimercaptopropyl)sorbitol ...	$CH_2SH.CHSH.CH_2.O.CH_2.(CHOH)_4.$ CH_2OH	288	†	2·6	29	†††	†††
0 : 23	2 : 3 : 4 : 6-tetramethyl 2′ : 3′-dimercaptopropyl glucoside		342	94	5·0–8·0	9	†††	†

TABLE V—continued

No.	Name	Formula	Mol. Wt.	Purity per cent.	LD50 mM/kg.	No. of mice in estimate of LD50	Activity against oxophenarsine	
							Immediate	After 40 minutes
0 : 15	δ-mercapto-γ-valerothiolactone ...		148	100	0·9	40	† †	† †
0 : 2	1 : 4-dithiothreitol	$CH_2SH.(CHOH)_2.CH_2SH$	154	100	0·7	25	—	Not done
0 : 22	1 : 4-dithioerythritol	$CH_2SH.(CHOH)_2.CH_2SH$	154	97	2·0–2·5	30	–	–
0 : 1	1 : 6-dithiodulcitol	$CH_2SH.(CHOH)_4.CH_2SH$	214	90	3·4, 2·2 *	55	† †	0
0 : 21	Diacetyl dimercaprol	(Probably) $CH_2SAc.CHSH.CH_2OAc$ ...	208	100	<2·5	18	–	–
0 : 12	3-acetoxy-1 : 2-bisacetylthiopropane (triacetyl dimercaprol)	$CH_2SAc.CHSAc.CH_2OAc.$	250	100	1·6	44	† †	† †
0 : 14	3 : 4-diacetoxy-1 : 2-bisacetylthiobutane	$CH_2SAc.CHSAc.CHOAc.CH_2OAc.$ ...	322	100	*ca* 3·0	12	† †	†
0 : 18	3 : 4 : 5-triacetoxy-1 : 2-bisacetylthiopentane	$CH_2SAc.CHSAc.(CHOAc)_2.CH_2OAc.$ ...	394	100	>10·0	16	0	Not done
0 : 5	2 : 3-diacetoxypropyl 2 : 3-bisacetylthiopropyl ether	$CH_2SAc.CHSAc.CH_2.O.CH_2.CHOAc.$ $CH_2OAc.$	366	76	2·9	40	†††	0
0 : 6	1 : 3-diacetoxy-2-propyl 2 : 3-bisacetylthiopropyl ether	CH_2OAc CH_2OAc $CH.O.CH_2.CHSAc.CH_2SAc.$ ... CH_2OAc $O.CH_2.CHSAc.CH_2SAc$	366	95	3·2	24	†††	Not done
0 : 11	Hexa-acetyl β-(2 : 3-dimercaptopropyl) glucoside		538	100	>5·0	10	†	0
0 : 10	Diethyl 3 : 4-bisacetylthiobutane-1 : 1-dicarboxylate	$CH_2SAc.CHSAc.CH_2.CH(CO.O.C_2H_5)(CO.O.C_2H_5)$	302	70	*ca* 10·0	9	0	0
0 : 7	Ethyl 2 : 3-bisacetylthiopropoxyacetate	$CH_2SAc.CHSAc.CH_2.O.CH_2.CO.O.C_2H_5$	294	77	2·8	15	†	Not done
0 : 4	Tetra-acetyl 1 : 4-dithiothreitol ...	$CH_2SAc.(CHOAc)_2.CH_2SAc.$	322	100	>1·3	10	–	Not done
—	Cysteine	$CH_2SH.CH(NH_2).COOH$	121	100	–	–	†	0

Ac –CO.CH_3.
* From mortality at 28 days.
† Prepared from barium salt : doses derived from iodine titre.

† Delayed death.
† † Saved some lives against *ca.* 1·5 LD50
††† Saved all lives against *ca.* 1·5 LD50
†††† Saved all lives against *ca.* 2·2 LD50

– Accelerated death.
— Killed all mice on *ca* 0·67 LD50 of oxophenarsine.

dosage. The quantitative method of assessing anti-arsenical activity by the increment produced in survival time (Weatherall and Weatherall[8]) had not been devised when the majority of these dithiols were tested, and so the foregoing procedure was used. Some of the substances were later evaluated by the more precise method, with results as reported in a separate paper and briefly indicated below.

Brief comments can be made on the individual substances.

A. 1:2-dithiols derived from polyhydric alcohols. The higher analogues of dimercaprol tended to be less toxic and less active against oxophenarsine. 1:2-dimercaptobutane-3:4-diol (0:13) was pharmacologically indistinguishable from dimercaprol. 1:2-dimercaptopentane-3:4:5-triol (0:17) was more water-soluble, less toxic, and less efficient when given late in oxophenarsine poisoning. 1:2-dimercaptohexane-3:4:5:6-tetrol (0:24) was about as toxic as the pentane derivative. It was not tested against oxophenarsine, but Weatherall and Weatherall[8] found that it had no activity at all against phenylarsenoxide.

B. O-Ethers of dimercaprol. As with the dithiols derived from polyhydric alcohols, the more water-soluble substances with a longer carbon chain and more hydroxyl groups were less toxic, but here there was more loss of toxicity and less loss of therapeutic activity. The glucoside appeared to be the best of this group, but the sorbitol ether (0:20) was surprisingly active in established oxophenarsine poisoning and still gave 100 per cent. protection after 40 minutes. 1:3-dihydroxy-2-propyl 2:3-dimercaptopropyl ether (0:8) and the sugar ethers contain the grouping $-O-CH_2-CROH-CR_2OH$, to which Bradley and Berger[16] attribute the ability of causing paralysis as does myanesin. Berger and Bradley[17] describe the paralysis as not accompanied by excitement, tremors, twitchings or convulsions at any time. None of these ethers produced exactly such a paralysis. An apparent flaccid paralysis was observed after nearly lethal doses of 6(2′:3′-dimercaptopropyl) mannitol (0:16), but when disturbed the mice made quite powerful movements, and lethal doses of this substance, like most of the other dithiols, produced convulsions.

C. Other 1:2-dithiols. The γ-lactone of γ:δ-dimercaptovaleric acid (0·15) was a little more toxic than dimercaprol. Deaths occurred more quickly, usually within 90 minutes of injection, and were preceded by very vigorous convulsions. Congestion of the lungs was more conspicuous post-mortem than with most dithiols. Anti-arsenical activity was a little less than that of dimercaprol.

D. α:ω-Dithiols. 1:4-dithiothreitol (0:2) behaved quite differently from its 1:2-isomer, 0:13. It was about 30 per cent. more toxic, and caused violent convulsions and death within 5 or 10 minutes of intramuscular injection. Post-mortem examination of mice killed by the drug and of survivors killed 28 days later, showed nothing macroscopically abnormal. When injected in doses by themselves innocuous, 0:2 greatly accelerated death after oxophenarsine and increased the mortality from sublethal doses. Death in animals so treated was preceded by convulsions, and at post-mortem examination gross pulmonary congestion

and hæmorrhages were observed, with a highly significant increase in lung weight (11·0±0·53 mg/g. compared with 7·9±0·20 mg./g. in mice poisoned with the same dose of oxophenarsine alone). In the occurrence of convulsions, the rapidity of death and the increase in lung weight, poisoning with oxophenarsine and 1:4-dithiothreitol resembles poisoning with the oxophenarsine-dimercaprol compound, and it seems possible that a similar mechanism may underlie both phenomena. 1:4-dithioerythritol (0:22) was considerably less toxic than its stereo-isomer, and caused death less rapidly: but it also accelerated death in oxophenarsine poisoning, though less dramatically. 1:6-dithiodulcitol (0:1) had a particularly low acute toxicity, of about 3·4 mM/kg. But mice receiving doses over about 2 mM/kg. developed a remarkable disease at some time between 3 and 20 days after injection. The hind legs became paralysed and the posterior part of the body slowly wasted until the body weight fell sometimes by as much as 50 per cent. Diarrhoea was common, and a crust of dirt and excreta was usually present at the base of the tail. There was often dermatitis of the tail, and a number of mice developed periorbital abscesses. Death occurred at any time up to at least 28 days after injection and 14 days after the onset of paralysis, but a proportion of mice receiving doses of 2·5 mM/kg. or less recovered and appeared normal 28 days after injection. The latter were killed and were found to have paler and firmer lungs than normal, but no other gross abnormality. Two paralysed mice, killed at the same time, showed gross wasting and contractures, generalised loss of size and weight of the viscera proportional to the body-weight, and no macroscopic lesions of the central nervous system. This dithiol gave slight protection against oxophenarsine poisoning, but some protected mice subsequently developed the paralytic syndrome. The dithiodulcitol was dissolved in a solution containing sodium borate, but no such syndrome developed in mice treated with sodium borate in amounts corresponding to the amounts used in dissolving the dithiodulcitol and no other mice under experiment at the same time developed the same syndrome. Sodium borate gave no protection against oxophenarsine poisoning. A rabbit which received 2 mM/kg. of dithiodulcitol intraperitoneally had much diarrhoea and died within 24 hours with gross lung œdema and congestion. Another rabbit into which 0·2 mM/kg. was injected showed no ill-effects in the next three weeks.

E. Acetyldithiols. The acetyldithiols were all less toxic, on a molecular basis, than the corresponding free dithiols. Some, particularly triacetyl dimercaprol (0:12), had considerable activity against oxophenarsine. Both toxicity and anti-arsenical activity diminished as the molecular weight increased. The lethal doses of 3:4:5-triacetoxy-1:2-bisacetylthiopentane (0:18) and hexa-acetyl β-(2:3-dimercaptopropyl) glucoside (0:11) were too large to be dissolved in a harmless quantity of any solvent tried, and 0:11 only increased slightly the survival time of oxophenarsine-poisoned mice without saving any lives, while 0:18 was inactive. Triacetyl dimercaprol (0:3) behaved very similarly to

dimercaprol itself. Diacetyl dimercaprol (0:21) was anomalous. The method of synthesis was directed to producing the di-S-acetyl compound, but by iodine titration it appeared to be a monothiol, and one acetyl group had therefore presumably wandered to the oxygen atom. Its toxicity was low, though large doses produced symptoms like dimercaprol; but unlike either dimercaprol or triacetyl dimercaprol, it saved no lives and considerably accelerated death in mice poisoned with oxophenarsine. Tetra-acetyl 1:4-dithiothreitol (0:4) appeared to be less toxic than the free $\alpha:\omega$-dithiol and, like it, potentiated oxophenarsine poisoning.

Discussion

The main object of the work reported here was to examine the possibility that other dithiols might be more satisfactory therapeutic agents than dimercaprol. In order to do so, the toxicity of various dithiols was estimated and their anti-arsenical activity was compared with that of dimercaprol. The quantitative method of assessing anti-arsenical activity described by Weatherall and Weatherall[8] was not devised until most of the substances described here had been tested, and only approximate comparisons are afforded by the present data. Danielli *et al.*[13] found that the toxicity of dithiols was decreased by the introduction of hydrophilic groups into the molecule, and this is borne out by the present findings. Unfortunately, the diminution in toxicity has been accompanied by a loss of anti-arsenical activity, at least when oxophenarsine was used as the arsenical. Which loss preponderated depended on how the hydrophilic groups were introduced. Simple lengthening of the chain of a hydroxythiol by the introduction of -CHOH- groups resulted in more loss of activity than of toxicity. On the other hand, conjugation by means of an ether linkage with, for example, a sugar produced dithiols which were substantially less toxic than dimercaprol, but retained quite good activity against oxophenarsine. The most marked defect of the sugar ethers was their inability to save lives when they were given some time after the arsenical poison. Dimercaprol glucoside was clearly less efficient than dimercaprol in this respect, although the difference was less conspicuous with 6(2′:3′-dimercaptopropyl) sorbitol. Dimercaprol glucoside was the least toxic of all the dithiols tested, and had otherwise good anti-arsenical activity, and seemed clearly to deserve further investigation from the point of view of possible therapeutic value.

An alternative method of diminishing the toxicity of dithiols lay in masking the -SH groups by some combination which might be readily broken down in the body. As the acetyl derivatives of several of the dithiols studied were readily available, some were tried, and were found to possess moderately good activity against oxophenarsine. The acetylated dithiols of low molecular weight were more effective than larger molecules. The smaller molecules tend to be more soluble in water and may be expected to hydrolyse more readily to the free dithiols, so this difference was not surprising. It is possible that by slow hydrolysis suitable acetylated dithiols would liberate a moderate sustained concentration of

dithiol, which would be therapeutically more useful than the rather short action of dimercaprol. Experiments in which hexa-acetyl β-(2:3-dimercaptopropyl) glucoside (0:11) was given three hours before a dose of oxophenarsine indicated that no protective concentration of thiol had been liberated. Possibly some other acetyl dithiol would have been more effective.

Separation of the -SH groups of dithiols has been found in the three instances studied here to be undesirable. 1:6-dithiodulcitol was only weakly active against oxophenarsine and produced a peculiar wasting disease by some mechanism which has not been examined. Both 1:4-dithiols potentiated oxophenarsine poisoning, in a manner which resembled the toxic effects of the oxophenarsine-dimercaprol compound. The similarity suggested that a common mechanism might be involved. Peters and Stocken[4] suggested that the compound of oxophenarsine and dimercaprol possibly penetrated cells and there dissociated with the intracellular liberation of toxic arsenic. The 1:4-dithiols are likely to form a less stable ring with arsenic than the 1:2-compounds (Whittaker[18]), and possibly form such compounds *in vivo* which penetrate cells and then dissociate again with fatal results. Some support is lent to Peters and Stocken's concept by the harmlessness of the compound of oxophenarsine and dimercaprol glucoside, which is presumably more soluble in water and consequently less liable to enter cells. As indicated above, no evidence at all could be found of any enhancement of toxicity of oxophenarsine by such combination.

Summary

1. The toxicity in mice has been studied of oxophenarsine, the oxophenarsine-dimercaprol compound, 15 dithiols and 10 acetyl dithiols.

2. The LD50 of oxophenarsine given intramuscularly was about 0·14 mM/kg. Significant differences in mortality were not observed when oxophenarsine was given intraperitoneally.

3. Death occurred more rapidly after large than after small doses of oxophenarsine. The lungs of mice dying of oxophenarsine poisoning were heavier than normal, particularly when the doses were small and the time of survival long.

4. The LD50 of the oxophenarsine-dimercaprol compound given intramuscularly was about 0·06 mM/kg. and given intraperitoneally was about 0·30 mM/kg. Death occurred more rapidly than in oxophenarsine poisoning and the lungs were much heavier than either normally or in oxophenarsine poisoning.

5. The LD50 of dimercaprol glucoside was found to depend on the iodine titre of the solution used. Estimated on this basis, the least toxic samples examined had an LD50 of about 4·5 mM/kg.

6. Dimercaprol glucoside was effective against larger doses of oxophenarsine than was dimercaprol when the thiols were given immediately after oxophenarsine. The glucoside was less effective than dimercaprol when the thiols were given more than about 20 minutes after oxophenarsine.

7. Approximately equimolar mixtures of oxophenarsine and dimercaprol glucoside were not more toxic than oxophenarsine alone.

8. No other dithiols examined had as good a combination of low toxicity and high activity against oxophenarsine as dimercaprol glucoside. The nearest approach was made by other dimercaprol sugar ethers.

9. Acetylated dithiols of low molecular weight had quite good antiarsenical activity, but were not more active relative to their toxicity than free dithiols.

10. Acetylated dithiols with a molecular weight greater than about 300 had very low toxicity and little or no activity against oxophenarsine.

11. Three substances (diacetyl dimercaprol, 1:4-dithioerythritol and particularly 1:4-dithiothreitol) accelerated death and increased the mortality in mice poisoned with oxophenarsine.

The dimercaprol used in these experiments was a sample of water-purified BAL kindly presented by Professor R. A. Peters. Some of the dimercaprol glucoside was prepared by Dr. L. N. Owen, some was provided by the Ministry of Supply, and one sample was provided by Boots Pure Drug Co., Ltd. The other dithiols and all the acetylated dithiols were prepared by Dr. L. N. Owen and his associates. Oxophenarsine (mapharside, not diluted with sucrose as in commercial preparations) was generously presented by Dr. J. S. White, of Parke Davis and Co., Ltd. The oxophenarsine-dimercaprol compound was synthesised and kindly presented by Dr. L. A. Stocken. I am most grateful for all these gifts. I am much indebted also to Dr. L. N. Owen for numerous helpful discussions, to Mr. Leslie Angus, Miss Jean Tulloch and Miss Irene Munro for technical assistance, to Mrs. J. A. C. Weatherall for performing certain of the toxicity tests, and particularly to Professor J. H. Gaddum for his advice and criticism. The work was initiated during the tenure of a personal grant from the Medical Research Council and was supported by a grant for expenses from the Council.

REFERENCES

1. Peters, Stocken and Thompson, *Nature,* 1945, **156,** 616.
2. Stocken and Thompson, *Biochem. J.,* 1946, **40,** 535.
3. Fitzhugh, Woodard, Braun, Lusky and Calvery, *J. Pharmacol.,* 1946, **87,** Suppl., 23.
4. Peters and Stocken, *Biochem. J.,* 1947, **41,** 53.
5. Friedheim and Vogel, *Proc. Soc. exp. Biol., N.Y.,* 1947, **64,** 418.
6. Evans and Owen, *J. chem. Soc.,* 1949, 244.
7. Evans, Fraser and Owen, *J. chem. Soc.,* 1949, 248.
8. Weatherall and Weatherall, *Brit. J. Pharmacol.,* 1949, **4,** (in the press).
9. Ercoli and Wilson, *J. Pharmacol.,* 1948, **92,** 121.
10. Cranston, Clark and Strakosch, *J. Pharmacol.,* 1944, **81,** 284.
11. Eagle, Hogan, Doak and Steinman, *J. Pharmacol.,* 1942, **74,** 210.
12. Danielli, Danielli, Mitchell, Owen and Shaw, *Nature,* 1946, **157,** 217.
13. Danielli, Danielii, Fraser, Mitchell, Owen and Shaw, *Biochem. J.,* 1947, **41,** 325.
14. Sampey and Reid, *J. Amer. chem. Soc.,* 1932, **84,** 3404.
15. Kärber, *Arch. exp. Path. Pharmak.,* 1931, **162,** 480.
16. Bradley and Berger, *Nature,* 1947, **159,** 813.
17. Berger and Bradley, *Brit. J. Pharmacol.,* 1946, **1,** 265.
18. Whittaker, *Biochem. J.,* 1947, **41,** 56.