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THE EFFECT OF DITHIOLS ON SURVIVAL TIME IN RATS AND MICE POISONED WITH ORGANIC ARSENICALS

BY

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The usual method for observing the anti-arsenical activity of dithiols has been to inject two groups of animals with a lethal dose of the arsenical, to treat one group with a dithiol and to compare the mortalities in the two groups (Stocken and Thompson, 1946; *et al.*). As Box and Cullumbine (1947) have pointed out, the use of a quantal response (percentage mortality) for quantitative studies of this sort has considerable disadvantages: the dose range in which there is a suitable mortality is small; large numbers of animals must be used to give accurate estimates of mortality; the accuracy of the estimates depends on the observed mortalities and must be weighted accordingly; and it is difficult or impossible to introduce a number of factors into the experiment and to detect interactions between them. Box and Cullumbine suggested that similarly useful information might be obtained much more efficiently by using the times of survival after a lethal dose of poison instead of the mortalities. Weatherall (1945, 1949) observed that animals poisoned with oxophenarsine and treated with doses of dithiols insufficient to save life lived longer than control animals given no dithiol, and showed that in the controls the log survival time was roughly linearly related to the log dose of oxophenarsine. It was therefore decided to investigate the relation between dose of arsenical and survival time more fully, and to study the effect of dithiols on this relation, in order to see whether a more satisfactory method of measuring anti-arsenical activity could be so obtained.

MATERIALS AND METHODS

Solutions of phenylarsenoxide were prepared by dissolving crystalline phenylarsenoxide (m.p. 159–161°C.) in 3*N* sodium hydroxide, neutralizing with 6*N* hydrochloric acid as nearly as possible without precipitation, and diluting with water until isotonic and then with 0.9 per cent (w/v) sodium chloride solution. Solutions of oxophenarsine (mapharside) were prepared in distilled water from pure material kindly provided by Messrs. Parke Davis and Co. The dithiols used are described in Table I. The preparation and chemical properties have been or will be described elsewhere (Evans and Owen, 1949; Evans, Fraser, and Owen, 1949). Solutions of 1 : 2-dimercaptopentane-3 : 4 : 5-triol, 1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol, dimercaprol glucoside (BAL-Intrav) and 3(2′ : 3′-dimercaptopropyl)-mannitol were prepared by dissolving their barium salts in water, adjusting the *p*H to 6 with 10*N* sulphuric acid, removing any remaining barium ions with saturated sodium sulphate, and removing the barium sulphate by centrifuging for 15 min. at 2,500 r.p.m. The complete removal of barium ions was checked by adding a further trace of saturated sodium sulphate and observing the absence of a precipitate. The concentrations of these solutions were estimated at the time of experiments as follows: sufficient hydrochloric acid was added to make the solutions of normal acidity, and aliquots were then titrated at 0° C. against *N*/10 or *N*/50 iodine in potassium iodide with a few drops of 1 per cent (w/v) starch solution as indicator until a blue colour persisted for 30 sec. 2 : 3-Dimercaptopropionic acid was dissolved in a minimal amount of 3*N* sodium hydroxide before dilution with 0.9 per cent sodium chloride. Solutions of other dithiols in 0.9 per cent sodium chloride or in olive oil were diluted so that the volume of each injection was 5.0 ml./kg. All doses have been expressed in microgramme-molecules (μM) per kg. body weight, in order to facilitate the comparison of chemically equivalent quantities of different substances, and in order to avoid doses numerically smaller than unity and therefore having negative logarithms.

Young female albino rats weighing 55.9±11.9 (S.D.) grammes and adult male mice weighing 20.2±2.9 (S.D.) grammes were used. Experiments were done in a thermostatic room; the temperature varied between 21° C. and 25° C. on different days, but it was checked half-hourly during experiments and rarely varied by more than ±0.5° C. during each experiment. Food was withheld from the animals on the evening before an experiment, and on the following morning each animal was put in a numbered glass jar with a wire mesh top. They were then selected for different treatments by use of a table of random numbers (Fisher and Yates, 1943, p. 90). Each animal was injected intramuscularly with an arsenical in one limb and immediately afterwards with a dithiol or saline in the opposite limb. The time of injection was recorded. For practical convenience the animals in a group receiving the same treatment were

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TABLE I

Substance	Formula	Mol. wt.	Solvent used	LD50 μM/kg.	Species	Route of administration	Reference
				1,220	Rat	Intramuscular	McDonald (1948)
2 : 3-dimercaptopropanol (Dimercaprol, BAL)	$CH_2SH.CHSH.CH_2OH$	124	0.9% NaCl	910	,,	,,	Stocken and Thompson (1946)
				850	,,	,,	Fitzhugh *et al.* (1946)
				1,000	Mouse	,,	Weatherall (1949)
1 : 2-dimercaptobutane-3 : 4-diol (1 : 2-dithiothreitol)	$CH_2SH.CHSH.CHOH.CH_2OH$	154	,,	1,000	,,	,,	Weatherall (1949)
1 : 2-dimercaptopentane-3 : 4 : 5-triol	$CH_2SH.CHSH.(CHOH)_2CH_2OH$	184	,,	1,700–2,500	,,	,,	,, ,,
1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol	$CH_2SH.CHSH.(CHOH)_3CH_2OH$	214	,,	1,700	,,	,,	,, ,,
Dimercaprol glucoside (BAL-Intrav)	$CH_2SH.CHSH.CH_2OC_6H_{11}O_5$	286	,,	3,800–4,700	,,	,,	,, ,,
2 : 3 : 4 : 6-tetramethyl-2′ : 3′-dimercaptopropyl glucoside	$CH_2SH.CHSH.CH_2OC_6H_7O(OCH_3)_4$	342	Olive oil	5,000–8,000	,,	,,	,, ,,
3(2′ : 3′-dimercaptopropyl) mannitol	$CH_2SH.CHSH.CH_2OCH\begin{matrix}/(CHOH)_2CH_2OH \\ \backslash CHOH.CH_2OH\end{matrix}$	288	0.9% NaCl	3,200	,,	,,	,, ,,
1 : 2-dimercaptopropionic acid	$CH_2SH.CHSH.COOH$	138	NaOH and 0.9% NaCl	600	Rat	,,	Fitzhugh *et al.* (1946)
δ-mercapto-γ-valerothiolactone	$CH_2SH.CH.CH_2CH_2CO$ (S bridge)	148	0.9% NaCl	900	Mouse	,,	Weatherall (1949)
1 : 6-hexanedithiol	$CH_2SH.(CH_2)_4CH_2SH$	150	,,	ca.700	Cat	Intravenous	Chenoweth *et al.* (1946)
3-acetoxy-1 : 2-bisacetylthiopropane (triacetyl dimercaprol)	$CH_2(S.CO.CH_3)CH(S.CO.CH_3)CH_2O.CO.CH_3$	250	Olive oil	1,600	Mouse	Intramuscular	Weatherall (1949)

injected consecutively, and the groups in which the largest and smallest survival times were expected were injected first and last respectively. As is indicated later, this concession to practical convenience was perhaps undesirable. The animals were observed about every two minutes for the first few hours and later at longer intervals for forty-eight hours. An animal was deemed dead when it made no respiratory movement while watched for thirty seconds. The time of death was taken as the mean of the time when the animal was last observed alive and the time when it was first observed dead. When the logarithmic or reciprocal transformation of the survival times was used, the mean of the logarithms or reciprocals of the times last seen alive and first seen dead was taken. In order to avoid confusion with means derived from groups of animals, the values for individual animals obtained as just described are referred to hereafter as the survival time, log survival time, and reciprocal survival time, and the word mean is used only in connexion with groups of animals. Any animal which survived longer than forty-eight hours from the time of injection was killed and not counted in the estimation of the mean survival time of its group, except when the reciprocal transformation was used. In fact, only 6 out of 678 animals survived the experimental period. Food and water were withheld during the first ten hours after injection and were then provided *ad libitum*, though the animals seldom showed any desire to eat or drink.

The statistical procedures and symbols used follow the practice of Fisher (1944), unless it is otherwise indicated.

TABLE II

THE EFFECT OF DIMERCAPROL ON THE MEAN LOG SURVIVAL TIME OF PHENYLARSENOXIDE POISONED RATS

Exp.	Dose of dimercaprol		Log survival time in hours (Means and standard errors for groups of four rats)				Mean increment in log survival time† Log hours
			Dose of phenylarsenoxide				
	μM/kg.	Log μM/kg.	μM/kg. 80 Log μM/kg. 1.90	113 2.05	160 2.20	226 2.35	
A	Nil		+0.063±0.0436	+0.003±0.0824	−0.047±0.0515	−0.339±0.0593	—
B			+0.120±0.0738	−0.229±0.0855	−0.380±0.1358	−0.606±0.0305	—
C			+0.094±0.0974	+0.026±0.1713	−0.200±0.0725	−0.519±0.0691	—
D			+0.068±0.0681	−0.262±0.0504	−0.477±0.0661	−0.698±0.0391	—
D	16	1.20	+0.035±0.0336	−0.199±0.0362	−0.437±0.0196	−0.690±0.0293	0.020±0.3555
D	32	1.50	+0.015±0.0148	−0.081±0.0412	−0.394±0.0418	−0 664±0.0642	0.061±0.0372
D	46	1.66	+0.137±0.0570	−0.240±0.2132	+0.048±0.1114	−0.680±0.0380	0.159±0.0687
C	80	1.90	+0.538±0.0940	+0.295±0.0524	+0.229±0.0497	−0.225±0.0735	0.359±0.0653
A	113	2.05	+0.943±0.1292	—	—	—	—
B			+0.676±0.1228	—	—	—	—
C			+0.618±0.0736	+0.506±0.0490	+0.447±0.0756	+0.001±0.0533	0.544±0.0638
A	160	2.20	+1.047±0.0696	+0.832±0.0799	—	—	0.907±0.0499
B			+1.104±0.0528	+0.697±0.0481	—	—	0.955±0.0472
C			+0.964±0.0571	+0.608±0.0351	+0.560±0.0788	+0.135±0.0597	0.717±0.0628
D			+1.027±0.1626	—	+0.410±0.0681	+0.029±0.0918	0.858±0.0645
A	226	2.35	+1.114±0.0340*	+0.914±0.0247	+0.742±0.0542	+0.317±0.0704	0.852±0.0391
B			—	+1.171±0.1091	+0.513±0.0294	+0.327±0.0496	1.072±0.0591
C			—	+0.728±0.0862	+0.727±0.0474	+0.293±0.0366	0.814±0.0648
A	320	2.50	—	+1.015±0.0468	+1.015±0.1156	+0.473±0.0222	0.962±0.0492
B			—	+1.378±0.0013*	+0.770±0.0829	+0.571±0.0614	1.311±0.0567
C			—	—	+0.977±0.0343	+0.494±0.0347	1.095±0.0394
A	452	2.65	—	—	+1.060±0.0529	+0.889±0.0463	1.168±0.0372
B			—	—	+1.081±0.1298	+0.880±0.0822	1.474±0.0733
C			—	—	—	+0.654±0.0959	—

* One survivor in group. † Calculated as described in text.

RESULTS

In general it was found both for oxophe arsine and for phenylarsenoxide that the survival time became shorter as the dose increased, and that for a given dose of poison the survival time of animals treated with dimercaprol became longer as the dose of dimercaprol was increased. The relations were studied first with oxophenarsine, but, as clearer results were obtained with phenylarsenoxide, the latter will be presented first and the data for oxophenarsine thereafter summarized briefly, mainly to indicate the differences observed.

I. Survival time of rats poisoned with phenylarsenoxide with or without treatment with dimercaprol.—No information has been found about the LD50 of phenylarsenoxide in rats, but it is reported to be about 12 μM/kg. intraperitoneally in mice (Eagle, Doak, Hogan, and Steinman, 1940). In the present experiments intramuscular doses of 36 μM/kg. and over killed all rats not treated with dithiols, and doses of 80 μM/kg. and over were nearly always fatal when the amount of dithiol did not exceed twice the equivalent amount of phenylarsenoxide. As survival was not desired, doses of the latter order were used, with the results shown in Table II and Fig. 1.

Sets of sixteen rats were divided into four groups of four, and the groups were injected with 80, 113, 160, and 226 μM/kg. respectively of phenylarsenoxide. Some sets received a dose of dimercaprol which was constant for the set, and one set, the controls, were treated with 0.9 per cent sodium choride solution. Particularly in early experiments the dimercaprol-treated sets were sometimes incomplete, either because groups in which survivors were expected were omitted or because the ranges being explored required more rats than were available or manageable to fill all the groups included in the range. When the mean survival times of the groups in each set were plotted against the dose or the log dose of phenylarsenoxide the points lay on a curve and the variance increased as the mean survival time increased. Two transformations of the data were therefore examined. When the means of the reciprocals of the survival times (cf. Box and Cullumbine, 1947) were plotted against the dose or the log dose of phenylarsenoxide, the points again lay on a curve and the variance still increased as the means increased. But when the means of the logarithms of the survival times were plotted against the log doses of phenylarsenoxide, the points lay roughly on a straight line and the variances showed only slight positive correlation with the means. Moreover, the lines for sets which received a constant dose of dithiol were approximately parallel to those for the set which received no dithiol in the same experiment; and there was no obvious progressive change of slope with increasing doses of dimercaprol. In order to assess the significance of the departures from linearity, analyses of variance were performed on those parts of the data which were symmetrically arranged (Table III). The results of these analyses were not altogether satisfactory. The significance of the regression of log survival time on log dose of phenylarsenoxide and the significance of the effect of dimercaprol, except in the smallest doses (experiment *D*), were indeed beyond question. But in experiments *A* and *C* the mean squared deviations from linearity were very significantly greater than the mean square attributable to random fluctuation, and experiments *B* and *D* showed a less marked but similar tendency. In experiment *B* there was also a greater departure from parallelism between the lines in the presence and absence of dimercaprol than would be expected by chance.

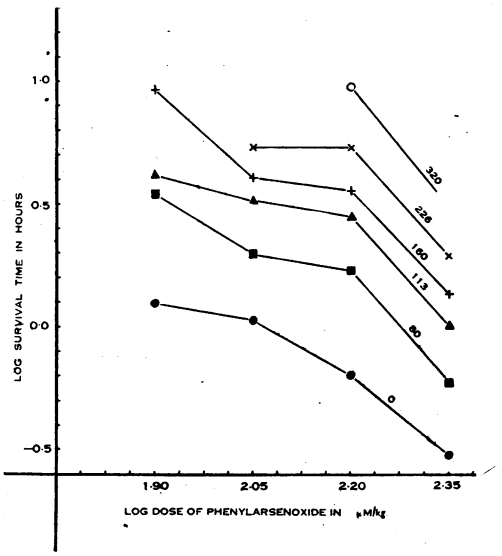

FIG. 1.—The log survival time of rats poisoned with phenylarsenoxide and treated with dimercaprol (experiment *C*). Ordinates: log survival time in hours. Abscissae: log dose of phenylarsenoxide, in μM/kg. ●———● Rats treated with 0.9 per cent sodium chloride. ■———■ Rats treated with dimercaprol, 80 μM/kg. ▲———▲ Rats treated with dimercaprol, 113 μM/kg. +———+ Rats treated with dimercaprol, 160 μM/kg. ×———× Rats treated with dimercaprol, 226 μM/kg. o———o Rats treated with dimercaprol, 320 μM/kg. All points indicate the mean for groups of four rats.

TABLE III

ANALYSIS OF VARIANCE OF THE LOG SURVIVAL TIMES OF RATS POISONED WITH PHENYLARSENOXIDE AND TREATED WITH DITHIOLS

The data analysed here are summarized in Tables II and VI. The values selected are, in experiments A and B, phenylarsenoxide 113, 160, and 226 μM/kg., dimercaprol 0, 226, and 320 μM/kg.; in experiments C, D, E, and F, phenylarsenoxide 80, 113, 160, and 226 μM/kg. and all doses of dithiols for which information was available at the same four doses.

The figures in parentheses are the number of degrees of freedom with which the means are estimated. The mean squares for linear regression and (except in experiment D) for treatment with dithiols are all significantly ($P<0.001$) greater than the mean square for random fluctuation. Other mean squares are printed in italics when they exceed the mean square for random fluctuation by a ratio greater than the 5 per cent point of e^{2z} and in bold type when the ratio is greater than the 1 per cent point.

Variance due to	Mean squares					
	Experiments with dimercaprol				Experiments with other dithiols	
	A	*B*	*C*	*D*	*E*	*F*
Linear regression on log dose of phenylarsenoxide	1.462(1)	2.742(1)	3.929(1)	4.346(1)	6.329(1)	8.534(1)
Deviations from linear regression	**0.239(1)**	*0.143(1)*	**0.159(2)**	*0.078(2)*	0.059(2)	0.037(2)
Treatment with dithiols	3.148(2)	5.855(2)	1.505(3)	0.076(3)	1.593(4)	2.896(4)
Differences of linear regression	0.036(2)	**0.135(2)**	0.016(3)	0.004(3)	*0.102(4)*	**0.199(4)**
Other interaction	0.019(2)	0.061(2)	0.017(6)	**0.099(6)**	0.023(8)	0.025(8)
Random fluctuation	0.017(27)	0.024(27)	0.025(48)	0.022(48)	0.034(60)	0.034(60)

If a sufficiently wide range of doses is examined, the regression of log survival time on log dose of phenylarsenoxide will not be linear. At the lower end of the scale survivals will occur and the log survival time will be practically infinite; and at the upper end a limit is likely to occur depending on the time necessary for the transport of phenylarsenoxide from the site of injection to the site of action and possibly for the accumulation there of toxic substances whose metabolism has been inhibited. If the departures from linearity showed a consistent tendency towards curvature convex towards the zero ordinate, it would not be surprising. But this is not the case: the observed irregularities appear to be distributed more or less fortuitously.

There appears to be a more plausible explanation of the irregularities. As was indicated above, animals in a group receiving the same treatment were injected consecutively, for reasons of practical convenience. The estimate of the random fluctuation, or error, of the experiment is based on the deviations occurring within the individual groups, and therefore does not include any measure of the variation in sensitivity of the animals occurring during the two or three hours in which the injections were performed. If there is any reason why such variation might be appreciable, the estimate of error cannot be regarded as satisfactory and tests of significance based on it will tend to underestimate the probability of observed discrepancies being due to chance. In fact there is present at least one detectable factor which may account for a greater variation between groups treated at different times than is observed within the groups. It was found that the slopes of the regression lines relating log survival time to log dose (in rats treated only with phenylarsenoxide) did not vary greatly from day to day. But the position of the line varied, and it appeared that this was due largely to the temperature of the room, and possibly to the weight of the rats. A multiple regression relating log survival time in hours (Y) to log dose of phenylarsenoxide in μM/kg. (d_P), body weight in grammes (w) and temperature of the room in °C. (t) was therefore calculated for 129 rats. The log survival time was found to be expressed by

$$Y = 5.46 - 1.57d_P - 0.002w - 0.098t$$

The regression for body weight was not significant ($0.2>P>0.1$), but those for dose and for temperature were highly significant ($P<0.001$ in each case). It therefore appeared that apart from the influence of dose, changes of temperature of as little as half a degree altered the log survival time by approximately 0.05 log hours, or 12 per cent. The temperature of the room in which the experiments were performed was not controlled more accurately than this, and the variation due to changes of temperature within these limits is clearly important. It therefore appears legitimate to regard the estimate of random fluctuation as too small and the significance of departures from linearity and parallelism as consequently overestimated.

No very good substitute is available, and probably the least unsatisfactory estimate of overall variation would be given by the mean of the sums of squares attributable to deviations from linear regression, to differences of linear regression, and to other interaction. Even if the largest mean square among these is taken as a basis of comparison, the regression itself and the effect of dimercaprol in sufficient doses are still most significant. Preferable estimates of the error of certain important comparisons are suggested below.

If the lines are parallel, a given dose of dimercaprol produces a constant increment in log survival time irrespective of the dose of phenylarsenoxide. On the assumption that apparent differences in slope were not appreciably more than could be attributed to chance, this increment was estimated by deducting the mean log survival time of a set of rats treated with various doses of phenylarsenoxide from the mean log survival time of a comparable set treated with the same doses of phenylarsenoxide and a constant dose of dimercaprol. (This is equivalent to estimating the mean of the increments produced by dimercaprol at each dose of phenylarsenoxide.) If Z symbolizes the mean increment in log survival time, $y_1, y_2, \ldots y_m$, the log survival times of individual animals treated with doses 1, 2, . . . m, of poison, and $z_1, z_2, \ldots z_m$, the log survival times of individual animals treated with the same doses of poison and a constant dose of antidote, and there are n animals in each group, then

$$Z = \frac{S(z_1) - S(y_1) + S(z_2) - S(y_2) + \ldots + S(z_m) - S(y_m)}{mn}$$

As the mean square for error given by the analysis of variance is likely to be too small, the variance of Z has been estimated from the deviations within the $2m$ groups concerned. If v_z is the variance of Z, then

$$V_z = \frac{S(z_1 - \bar{z}_1)^2 + S(y_1 - \bar{y}_1)^2 + \ldots + S(z_m - \bar{z}_m)^2 + S(y_m - \bar{y}_m)^2}{2m(n-1)}\left(\frac{1}{mn} + \frac{1}{mn}\right)$$

Values for Z and $\sqrt{V_z}$ are given in the last column of Table II.

The smallest doses of dimercaprol used had no significant effect on the survival time of phenylarsenoxide poisoned rats, and it appeared that about 40 μM/kg. was the threshold dose for this effect; 46 μM/kg. produced an appreciable increase in survival time, but this was not significant with the size of group used. With larger doses the relation between the mean increment in log survival time and the log dose of dimercaprol was practically linear, even when points obtained on different days were considered together (Fig. 2). A straight line was therefore fitted to all points where the log survival time was significantly increased (i.e., those obtained with doses of dimercaprol of 80 μM/kg. and over), as shown in Fig. 2. If Z is the mean increment in log survival time for a dose d_A of dimercaprol, this line has the formula

$$Z = 1.18d_A - 1.81$$

when Z is measured in log hours and d_A in log μM/kg.

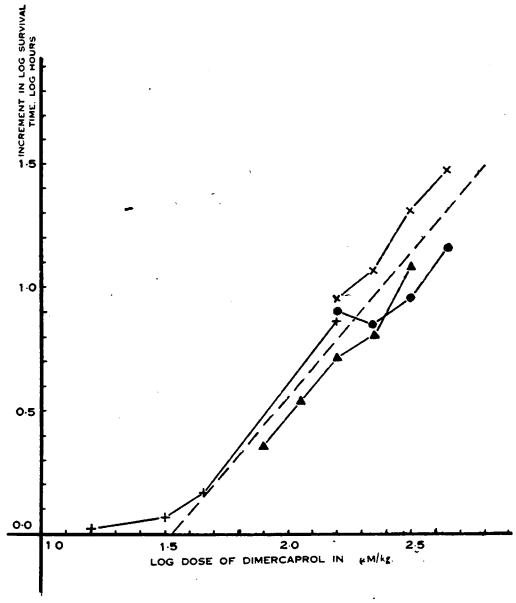

FIG. 2.—The increment in log survival time produced by dimercaprol in rats poisoned with phenylarsenoxide. Ordinates: mean increment in log survival time. Abscissae: log dose of dimercaprol in μM/kg. ●———● Experiment *A*. ×———× Experiment *B*. ▲———▲ Experiment *C*. +———+ Experiment *D*. The line of best fit (– – –) has been calculated from the equation $Z = 1.18d_A - 1.81$.

Examination of the data suggested that within the small range used in the present experiments, environmental temperature had no striking effect on the increment in survival time produced by dimercaprol, and as the data did not give systematic information on this point it was not pursued further.

A way of expressing these findings consists in plotting the dose of phenylarsenoxide against the dose of dimercaprol and drawing lines connecting points where the mean survival time is the same. Such lines may be called isochrons. In order to prepare these lines, the equations given above for the log survival time in the absence of dimercaprol and for the increment produced by dimercaprol have been used. In the first equation, the mean values for

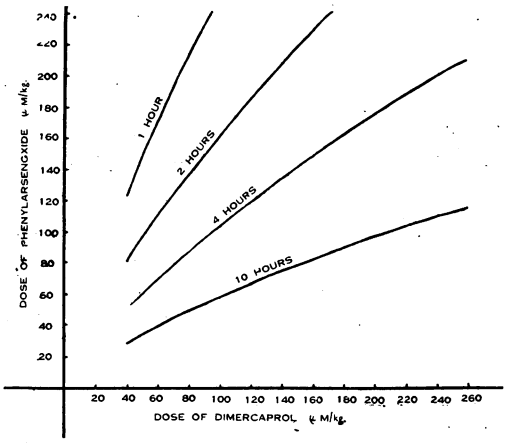

FIG. 3.—Isochronic lines for rats poisoned with phenylarsenoxide and treated with dimercaprol. Ordinates: dose of phenylarsenoxide in μM/kg. Abscissae: dose of dimercaprol in μM/kg. The values for the points have been calculated as described in the text. The lines, from top left to bottom right, connect points for survival times of 1, 2, 4, and 10 hours respectively.

w (56 g.) and t (22°) used in calculating the regression have been substituted, giving the relation between log dose and log survival time as

$$Y = 3.2 - 1.57d_P$$

Isochrons obtained by substitution for d_P and d_A are shown in Fig. 3. It will be seen that, within the present experimental range the isochronic lines are surprisingly nearly straight, even when the doses and not the log doses are plotted against each other. From these lines, or directly from the regression equations, the amount of phenylarsenoxide neutralized by a given dose of dimercaprol can be calculated. This quantity is not constant. Values for it derived from the one hour, four hour and ten hour isochrons at selected dimercaprol dose levels are shown in Table VII. Their significance is discussed later.

II. Survival time of rats and mice poisoned with oxophenarsine, with or without treatment with dimercaprol.—The LD50 of oxophenarsine given by intramuscular injection is reported to be in rats between 85 and 93 μM/kg. (Gruhzit, 1935) and in mice about 150 μM/kg. (Weatherall, 1949). Data on the relation of dose to survival time are presented in Tables IV and V. In both rats and mice the transformation log dose/log survival time gave an approximately linear relation with insignificant positive correlation ($r = +0.51$, $0.1 > P > 0.05$) between the mean and the variance, and with a slope varying between 1.7 ± 0.33 and 2.3 ± 0.43 on different days. Weatherall (1949), with much less accurate estimates of the survival time and with doses which only just caused 100 per cent mortality, found a slope of 2.76 in mice. This confirms the expectation that the line is actually convex downwards and can be regarded as approximately straight only over a limited range. The experiments with dimercaprol were designed at a time when it was expected that the dose of dithiol necessary to produce a constant increment in log survival time would be proportional to the dose of poison used. Consequently data are not available for the effect of the same absolute dose of dimercaprol with different doses of oxophenarsine, and lines comparable to those described above for phenylarsenoxide cannot be fitted. The increments in log survival time produced by different doses of dimercaprol were not inconsistent with the hypothesis of a linear relation between the mean increment in log survival time

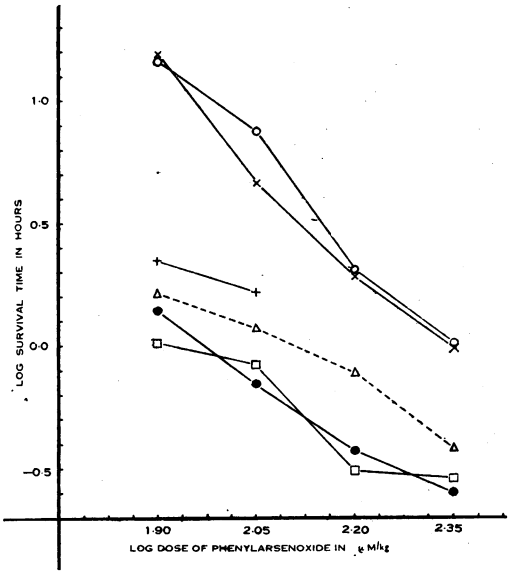

FIG. 4.—The log survival time of rats poisoned with phenylarsenoxide and treated with various dithiols (experiment *F*). Ordinates: log survival time in hours. Abscissae: log dose of phenylarsenoxide in μM/kg. ●———● Rats treated with 0.9 per cent sodium chloride. o———o Rats treated with dimercaprol, 160 μM/kg. ×———× Rats treated with 1:2-dimercaptobutane-3:4-diol. +———+ Rats treated with 1:2-dimercaptopentane-3:4:5-triol. □———□ Rats treated with 1:2-dimercaptohexane 3:4:5:6-tetrol. △------△ Rats treated with δ-dimercapto-γ-valerothiolactone. All points indicate the mean for groups of four rats.

TABLE IV

THE EFFECT OF DIMERCAPROL ON THE LOG SURVIVAL TIME OF OXOPHENARSINE POISONED MICE

Exp.	Dose of dimercaprol		Log survival time (Means and standard errors for groups of ten mice)				
			Dose of oxophenarsine				
	μM/kg.	Log μM/kg.	μM/kg. 250 Log μM/kg. 2.40	320 2.50	400 2.60	500 2.70	600 2.78
a	Nil	—	+0.712±0.0950	+0.391±0.0472	+0.284±0.0576	+0.064±0.0199	—
b			—	+0.357±0.0442	+0.457±0.0824	—	−0.277±0.1039
c			—	+0.437±0.0371	—	—	—
d			—	+0.516±0.0256	—	+0.176±0.0478	—
e			—	+0.460±0.0337	—	+0.029±0.0557	—
c	80	1.90	—	+0.317±0.0390	—	—	—
d			—	+0.477±0.0479	—	—	—
e			—	+0.466±0.0229	—	—	—
d	125	2.10	—	—	—	+0.382±0.0266	—
e			—	—	—	+0.324±0.0219	—
c	160	2.20	—	+0.593±0.0101	—	—	—
d			—	+0.608±0.1152	—	—	—
e			—	+0.424±0.0391	—	—	—
d	250	2.40	—	—	—	+0.388±0.1223	—
e			—	—	—	+0.374±0.0285	—
a	320	2.50	—	+0.814±0.1721†	—	—	—
b			—	+0.650±0.1203*	—	—	—
e			—	+0.774±0.1297*	—	—	—
a	400	2.60	—	—	+0.639±0.0781	—	—
b			—	—	+0.797±0.1772	—	—
a	500	2.70	—	—	—	+0.458±0.0701	—
e			—	—	—	+0.372±0.0504	—

* One survivor in group. † Two survivors in group.

TABLE V

THE EFFECT OF DIMERCAPROL ON THE LOG SURVIVAL TIME OF OXOPHENARSINE POISONED RATS

Dose of dimercaprol		Log survival time in hours (Means and standard errors for groups of ten rats)		
		Dose of oxophenarsine		
μM/kg.	Log μM/kg.	μM/kg. 320 Log μM/kg. 2.50	400 2.60	500 2.70
Nil	—	+0.011±0.0268	−0.072±0.0561	−0.282±0.0689
80	1.90	+0.190±0.0408	—	—
100	2.00	—	+0.028±0.0656	—
125	2.10	—	—	+0.021±0.0546
200	2.30	—	+0.149±0.0746	—
320	2.50	+0.589±0.1263	—	—
400	2.60	—	+0.404±0.1012	—
500	2.70	—	—	+0.647±0.1009

and the log dose of dimercaprol. The slope, however, appeared to be substantially less than with phenylarsenoxide (0.5 for oxophenarsine in mice, 0.6 for oxophenarsine in rats, and 2.2 for phenylarsenoxide in rats) and the threshold dose of dimercaprol was higher (about 80 μM/kg.). Below this dose, at least in mice, actual acceleration of death was observed, although only once (Table IV, exp. *c*) was this large enough to be significant with the size of group used.

III. Survival time of rats poisoned with phenylarsenoxide and treated with other dithiols.—As in the experiments with dimercaprol, sets of sixteen rats were divided into four groups of four and phenylarsenoxide was injected as before. One set received also an intramuscular injection of 0.9 per cent sodium chloride solution, one set the largest dose of dimercaprol which was expected not to save any lives (160 μM/kg.), and the remaining sets the same dose of other dithiols (Table VI and Fig. 4). This dose was in only one case greater than one-quarter of the LD50 of the dithiol (Table I). In certain instances, titration at the end of the experiment indicated that the solutions contained less dithiol than expected. In these cases, the dose was calculated from the results of the titration. Insufficient material was available to complete the lines for certain dithiols. The variance of the symmetrically arranged data was analysed as before (Table III). For no obvious reason, in neither experiments *E* nor *F* were the departures from linear regression of log survival time on log dose of phenylarsenoxide significantly greater than the mean square for error, although the initial fault in the experimental design had not been amended. This suggests that whatever in the dimercaprol experiments increased the variation between groups, whether temperature changes or some other unsuspected factor, was less actively operative; and due weight must therefore be attached to the differences of linear regression, which are significantly greater than can be accounted for by random fluctuation. The lack of parallelism is hardly surprising. Different thiols are likely to be absorbed and excreted at different rates. They will reach a peak concentration in the blood and tissues at different times, and so, for example, a slowly absorbed substance might show no appreciable activity against 226 μM/kg. of phenylarsenoxide, which kills rats in about ten minutes, and yet be quite active against 80 μM/kg. which is lethal in about an hour. Indeed, it can only be fortuitous that dimercaprol happened to be equally active, or nearly so, against all the doses of phenylarsenoxide used. Once lack of parallelism occurs, any estimate of activity based on the increment in survival time becomes an arbitrary measurement depending on the doses of phenylarsenoxide chosen. There is no obvious way of overcoming this difficulty. As the conditions in these experiments have been standardized, with four fixed doses of phenylarsenoxide and one dose of dithiol as nearly constant as was practicable, and as the lack of parallelism is not very great, the mean increments in log survival time still give an approximate indication of the activity of the thiol under these experimental conditions, and they have been calculated as before. Small differences in the mean increments, particularly when there are also differences in slope, are clearly unimportant, but in fact there were considerable differences in the ability of dithiols to increase the survival time. The significance of differences has been estimated as before, using the mean squared deviations of the groups concerned as the basis of comparison in a *t* test. No dithiol was more active than dimercaprol, and all except 1 : 2-dimercaptobutane-3 : 4-diol were significantly less active. Two substances (1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol and 1 : 6-hexanedithiol) accelerated death, the latter significantly. The other substances were intermediate in activity.

To facilitate quantitative comparisons, the activities have been estimated as percentages of the activity of dimercaprol, by assessing from the previously determined dose-response curve how much dimercaprol would have been necessary to produce the observed increments in log survival time. The pooled estimate of the slope of the dose response curve ($b = 1.18$) was used, and the position of the line was fixed by the observed mean increment in log survival time produced by the standard dose of dimercaprol. For example, in experiment *E*, 160 μM/kg. of dimercaprol increased the log survival time by 0.827 log hours, and substituting,

$$0.827 = 1.18 \times 2.20 + a,$$

whence $$a = -1.769$$

and the expected mean increment in log survival time, Z, for any log dose of dimercaprol, d_A, was given by $$Z = 1.18d_A - 1.769$$

By substituting for Z the mean increment in log survival time obtained for an unknown dithiol, e.g., 0.445 log hours for dimercaprol glucoside, the amount of dimercaprol to which the dose used of unknown dithiol was equivalent could be calculated.

Thus $$0.445 = 1.18d_A - 1.769$$

whence $$d_A = 1.875.$$

As the log dose of dimercaprol glucoside in this experiment was 2.146 μM/kg., its activity expressed as a ratio to that of dimercaprol was antilog (1.875–2.146) or 53.7 per cent. Values calculated similarly for other dithiols are shown in the third column from the right of Table VI.

TABLE VI

THE EFFECT OF VARIOUS DITHIOLS AND RELATED SUBSTANCES ON THE LOG SURVIVAL TIME OF PHENYLARSENOXIDE POISONED RATS

Exp.	Dose of dithiol			Log survival time in hours (Means and standard errors for groups of four rats)				Mean increment in log survival time. Log hours	Slope of line relating log dose of phenylarsenoxide to log survival time	Molar anti-arsenical activity as a percentage of that of dimercaprol	Molar toxicity as a percentage of that of dimercaprol	Ratio of anti-arsenical activity and toxicity
				Dose of phenylarsenoxide								
		μM/kg.	Log μM/kg.	μM/kg. 80 Log μM/kg. 1.90	113 2.05	160 2.20	226 2.35					
E	Nil	—	—	−0.010±0.0291	−0.188±0.0754	−0.449±0.0670	−0.491±0.0894	—	−1.15	—	—	—
	Dimercaprol	160	2.20	+1.171±0.0626	+0.583±0.1297	+0.327±0.0243	+0.190±0.0524	0.827±0.0519	−2.04	100	100	1.00
	Dimercaprol-glucoside	140	2.15	+0.732±0.0993	+0.300±0.1115	+0.012±0.0508	−0.302±0.1046	0.445±0.0585	−2.26	54	24	2.25
	3(2′ : 3′-dimercaptopropyl) mannitol	140	2.15	+0.511±0.0695	+0.165±0.1046	+0.083±0.0530	−0.244±0.0479	0.388±0.0499	−1.56	48	31	1.53
	2 : 3 : 4 : 6-tetramethyl-2′ : 3′dimercaptopropyl glucoside	160	2.20	+0.232±0.0460	−0.006±0.1237	−0.187±0.1713	−0.374±0.1273	0.176±0.0716	−1.33	28	15	1.82
F	Nil	—	—	+0.146±0.0880	−0.156±0.0548	−0.434±0.0853	−0.596±0.0405	—	−1.67	—	—	—
	Dimercaprol	160	2.20	+1.161±0.1012	+0.875±0.0495	+0.308±0.0354	+0.010±0.0105	0.848±0.0477	−2.68	100	100	1.00
	1 : 2-dimercaptobutane-3 : 4-diol	160	2.20	+1.187±0.0568	+0.660±0.0513	+0.277±0.0407	−0.008±0.0401	0.789±0.0424	−2.64	89	100	0.89
	1 : 2-dimercaptopentane-3 : 4 : 5-triol	124	2.09	+0.342±0.1367	+0.215±0.0471	—	—	0.289±0.0629	—	43	47	0.90
	1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol	162	2.21	+0.007±0.2083	−0.088±0.1337	−0.507±0.1279	−0.538±0.1087	−0.022±0.0826	−1.36	0	59	0
	δ-mercapto-γ-valerothiolactone	160	2.20	+0.208±0.0500	+0.067±0.0904	−0.112±0.0651	−0.421±0.1611	0.196±0.0615	−1.38	28	111	0.25
G	Nil	—	—	−0.010±0.0479	−0.009±0.0107	−0.336±0.0750	—	—	—	—	—	—
	Dimercaprol	160	2.20	+0.611±0.0686	+0.683±0.0377	+0.366±0.0260	—	0.672±0.0418	—	100	100	1.00
	Triacetyl dimercaprol	160	2.20	+0.623±0.2396	+0.221±0.1252	—	—	0.432±0.0793	—	62	62	1.00
	1 : 2-dimercaptopropionic acid	160	2.20	+0.174±0.0498	+0.185±0.0593	+0.027±0.0238	—	0.247±0.0349	—	43	167	0.26
	1 : 6-hexanedithiol	160	2.20	−0.190±0.0473	−0.317±0.0870	−0.459±0.0482	—	−0.204±0.0410	—	Potentiation	—	—

Discussion

Survival time has not been extensively used in order to measure the effects of drugs in biological work. Bülbring (1937) showed that extracts of adrenal cortex could be assayed by the increase in survival time produced in adrenalectomized drakes. Vogt (1943) showed that this was also true for adrenalectomized rats kept at a low environmental temperature. Both showed that the untransformed survival times of treated animals were approximately linearly related to the logarithm of the dose of cortical extract, but the data of Bülbring do not exclude the possibility that the log survival time is as, or more nearly, linearly related to the log dose of extract. Box and Cullumbine (1947), using mustard gas and phosgene and measuring the dose as the product of the concentration of the gas and the time of exposure, found that the reciprocals of the survival times were normally distributed and linearly related to the dose of gas and that the logarithms of the survival times were not. They discussed the merits of using survival time in assays of antidotes to poisons which produce death but no other easily measured response. Withell (1942) showed that the log survival times of micro-organisms poisoned with a given concentration of bactericides were normally distributed, but apparently did not attempt to relate the mean survival time of the organisms to the concentration of bactericide.

With the two arsenicals and two species used in this work the logarithm of the survival time was within a limited range approximately linearly related to the logarithm of the dose of arsenical; the logarithms of the individual survival times for any one dose of poison were more nearly normally distributed than were the untransformed survival times or the reciprocals of the survival times, and the standard deviations of the individual values were roughly constant and independent of the dose of poison. In animals poisoned with phenylarsenoxide the increment in log survival time produced by dimercaprol was linearly related to the logarithm of the dose of dimercaprol. In view of the widely differing conditions of these various uses of survival time, it is perhaps not surprising that the distributions differ greatly and are not all amenable to the same transformations. Data obtained under other conditions are necessary before generalizations can usefully be attempted.

From the data presented, an attempt can be made to deduce how much poison is inactivated by dimercaprol in the body. Table VII shows what doses of phenylarsenoxide and dimercaprol are required to give certain fixed survival times, according to the regression equations derived from the data. If the survival time of the rats is regarded as depending entirely on the amount of phenylarsenoxide used in the absence of dimercaprol, then the excess necessary to give the same survival time in the presence of dimercaprol can be regarded as the amount neutralized by the dimercaprol. Such amounts are shown in column 4 of Table VII, and are expressed in column 5 as a percentage of the amount of dimercaprol given. It appears that the efficiency of the dimercaprol, as judged by the amount of phenylarsenoxide neutralized per molecule, increases with the dose of dimercaprol to a maximum at about 160 μM per kg. and then remains steady at a level depending on the amount of phenylarsenoxide available. Not much significance need be attached to the values over 100 per cent in the early part of the Table. These values are calculated from two regression equations, in both near or beyond the extremities of the observed values, where the sampling errors of the estimates are largest, the assumption of linearity is most doubtful, and the effect of transforming back from a log dose magnifies discrepancies. In practice, no instance has been observed where the increment in survival time produced by dimercaprol was larger than could be

TABLE VII

CALCULATED DOSES OF PHENYLARSENOXIDE AND DIMERCAPROL NECESSARY TO PRODUCE CONSTANT SURVIVAL TIMES

Survival time. Hours	Dose of phenylarsenoxide, μM/kg.	Dose of dimercaprol. μM/kg.	Phenylarsenoxide neutralized by dimercaprol, μM/kg.	Phenylarsenoxide neutralized, mols per 100 mols dimercaprol
1	108	0	—	—
1	123	40	15	37.5
1	209	80	101	126
1	(359)	(160)	(251)	(157)
1	(481)	(240)	(373)	(155)
4	45	0	—	—
4	51	40	6	15
4	87	80	42	52.5
4	149	160	104	65
4	200	240	155	64.5
4	245	320	200	62.5
4	288	400	243	61
10	(25)	(0)	—	—
10	(28)	(40)	(3)	(7.5)
10	48	80	23	29
10	83	160	63	39.5
10	111	240	86	36

Figures in parentheses are outside the range of experimental observations. The calculation of these figures is described in the text.

accounted for by the inactivation of the equimolar quantity of phenylarsenoxide. Nevertheless, in these conditions (near optimal amounts of dimercaprol and excess of phenylarsenoxide) the efficiency of the antidote does approach very close to 100 per cent of what is chemically possible. Figures similarly calculated for oxophenarsine at optimal dimercaprol levels are about 90 per cent at 1 hour, 40 per cent at 4 hours, and 20 per cent at 10 hours; so that although the total amounts of arsenic in the body are larger, dimercaprol appears to be somewhat less effective against this poison.

Hogan and Eagle (1944) and Chance, Crawford, and Levvy (1945) showed that in rabbits poisoned with phenylarsenoxide or oxophenarsine, phenylarsenoxide was excreted much more slowly than oxophenarsine. Chance and Levvy (1947) showed that dimercaprol given to phenylarsenoxide poisoned rabbits increased the excretion of arsenic tenfold, and in oxophenarsine poisoned rabbits increased the excretion of arsenic only 2.5 times. Peters and Stocken (1947) showed that the compound formed *in vitro* between oxophenarsine and dimercaprol (4-hydroxymethyl-2-(3′-amino-4′-hydroxyphenyl)-1 : 3-dithia-2-arsa*cyclo*pentane) was much more toxic than oxophenarsine, and that treatment with dimercaprol prevented toxic effects of the oxophenarsine-dimercaprol compound. In unpublished experiments (Weatherall, 1949) it was shown that when phenylarsenoxide and dimercaprol were mixed in equivalent amounts in aqueous solution a white precipitate formed: and when a suspension of this precipitate containing 3 × LD50 of phenylarsenoxide was injected intramuscularly animals showed no ill effects.

The toxicity of the oxophenarsine-dimercaprol compound and the small increase in the excretion of arsenic after dimercaprol in oxophenarsine poisoned animals, compared with the apparent harmlessness of the phenylarsenoxide-dimercaprol mixture and the much increased excretion of arsenic after dimercaprol in phenylarsenoxide poisoned animals probably explains why dimercaprol is less efficient in preventing oxophenarsine poisoning than in phenylarsenoxide poisoning.

The other dithiols investigated fall into five chemical classes: 1 : 2-dithiol derivatives of polyhydric alcohols, oxygen ethers of dimercaprol, 1 : 2-dithiols containing a carboxyl group, αω-dithiols and acetylated dithiols. The first group are compounds with the general formula $CH_2SH.CHSH.(CHOH)_nCH_2OH$. In this series (exp. *F*, Table VI) activity was maximal when $n = 0$, i.e., in dimercaprol, and fell off as n increased, until 1 : 2-dimercaptohexane-3 : 4 : 5 : 6-tetrol ($n = 4$) had no activity. This decrease accompanies a decrease in lipoid solubility. The second group were dimercaprol ethers of mannitol, glucose, or tetramethylglucose (exp. *E*, Table VI). The former two were moderately active, but the latter showed little activity. 1 : 2-Dimercaptopropionic acid in the third group was about one and half times as active as δ-mercapto-γ-valerothiolactone. Possibly the lactone ring in this compound did not break *in vivo*, so that the substance contained only one active –SH group. However, Weatherall (1949) showed that a dose of this compound equivalent to 1.54 times the LD99 of oxophenarsine completely prevented mice from dying even when given 80 min. after the LD99 of oxophenarsine. This suggests that the substance does not act *in vivo* as a monothiol, because other monothiols, cysteine and glutathione, are useless in preventing death in oxophenarsine poisoned animals when given only 5 min. after the oxophenarsine (Eagle, Magnuson, and Fleischman, 1946). The only αω-dithiol used in this work potentiated poisoning by phenylarsenoxide. This was surprising because Whittaker (1947) showed that 1 : 6-hexanedithiol reactivated pigeon brain pyruvate oxidase poisoned with lewisite when added ten minutes after the lewisite; Thomson, Savit, and Goldwasser (1947) showed that skin exposed to lewisite was decontaminated by 1 : 6-hexanedithiol, although less efficiently than by dimercaprol, and Kensler, Abels, and Rhoads (1946) showed that it was effective in the treatment of arsine poisoning, again less so than dimercaprol. On the other hand, Weatherall (1949) found that other αω-dithiols (1 : 4-dithiothreitol and 1 : 4-dithioerythritol) accelerated death in oxophenarsine poisoned mice and the former caused a large increase in the mortality from sublethal doses of oxophenarsine. It would be interesting to know more about the changes in survival time produced by other doses of 1 : 6-hexanedithiol in phenylarsenoxide poisoned rats. Possibly larger doses would have increased the survival time. Triacetyldimercaprol, the only acetylated dithiol tested, was about as active as dimercaprol glucoside. This is consistent with the results of mortality experiments (Weatherall, 1949).

For practical purposes the ratio of therapeutic activity to toxicity is more important than the absolute therapeutic activity per gramme or per gramme-molecule. As an increase in LD50 represents a reduction in toxicity, the efficiency of these substances has been expressed as the ratio of the anti-arsenical activity and the toxicity, both measured with unit activity as that of dimercaprol, as shown in the last column of Table VI. Only the sugar ethers of dimercaprol surpass dimercaprol when considered in this way, and of the sugar ethers the glucoside is substantially the best.

No attempt has so far been made to observe the effect of delaying treatment with dithiols on the relation between survival time and the dose of dithiol, nor to extend the method used in these experiments to determine the relation between other poisons and survival time, or the effects of dithiols on poisoning by other substances.

A substance which increases the survival time of experimental animals is not necessarily an effective therapeutic agent in either animals or men. Weatherall (1949) has shown that all the substances discussed here with the exception of 1:2-dimercaptohexane-3:4:5:6-tetrol, 1:2-dimercaptopropionic acid, and 1:6-hexanedithiol, which he did not test, reduced the mortality in oxophenarsine poisoned mice, and the compounds which he found most efficient in preventing death in oxophenarsine poisoned mice are those which in the experiments now reported have been found most efficient in prolonging the survival time of phenylarsenoxide poisoned rats. In the present work the efficiency of the dithiol has been measured only when it has been given immediately after the poison. It is likely that the relative efficiency of different dithiols will differ when they are used in the delayed treatment of acute poisoning or in chronic poisoning. It is, however, unlikely that a dithiol which has no activity when given immediately after the poison, at a time when the poison is circulating freely, will have appreciable effect in chronic poisoning when the poison has been fixed by the tissues. The present procedure, therefore, provides a test for excluding ineffective substances, and a quantitative method for differentiating between substances with almost equal activity and for studying changes in activity produced by changes in chemical structure.

Summary

1. Lethal doses of phenylarsenoxide and oxophenarsine have been injected intramuscularly into rats and mice and the survival times have been measured.

2. Over the range of doses used the log survival time was as a rule linearly related to the log dose and at least with phenylarsenoxide to the temperature of the room also.

3. Some poisoned animals were immediately injected intramuscularly with dimercaprol. Those treated with dimercaprol lived longer than those untreated.

4. For a given dose of dimercaprol, the relation between the log dose of phenylarsenoxide and the log survival time was linear and the slope was the same as in the absence of dimercaprol.

5. The mean increment in log survival time produced by different doses of dimercaprol in phenylarsenoxide poisoned rats was linearly related to the log dose of dimercaprol. This relation was not grossly affected by small differences in environmental temperature.

6. From a consideration of the two regressions, it appeared that the amount of phenylarsenoxide inactivated by dimercaprol increased to a maximum when the dose of dimercaprol was about 160 μM/kg. and was greater with large doses of phenylarsenoxide.

7. Similar data are presented for oxophenarsine and dimercaprol in rats and mice. The results are less comprehensive, but suggest that in very small doses dimercaprol can accelerate death in oxophenarsine poisoned mice, and that with larger doses of dimercaprol the relationship resembles that to phenylarsenoxide.

8. In the presence of dithiols, the relation between the log dose of phenylarsenoxide and the log survival time was linear but not always parallel to the line for phenylarsenoxide alone. The differences were not large and approximate comparisons of the activity of ten dithiols and related substances have been made and are discussed. None of the substances was more active than dimercaprol.

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References

Box, G. E. P., and Cullumbine, H. (1947). *Brit. J. Pharmacol.*, **2**, 27.
Bülbring, E. (1937). *J. Physiol.*, **89**, 64.
Chance, A. C., Crawford, T. B. B., and Levvy, G. A. (1945). *Quart. J. exp. Physiol.*, **33**, 137.
Chance, A. C., and Levvy, G. A. (1947). *Quart. J. exp. Physiol.*, **34**, 79.
Chenoweth, M. B., Modell, W., and Riker, W. F. (1946). *J. Pharmacol.*, **87**, suppl., 6.
Eagle, H., Doak, G. O., Hogan, R. B., and Steinman, H. G. (1940). *J. Pharmacol.*, **70**, 211.
Eagle, H., Magnuson, H. J., and Fleischman, R. (1946). *J. clin. Invest.*, **25**, 451.
Ercoli, N., and Wilson, W. (1948). *J. Pharmacol.*, **92**, 121.

Evans, R. M., Fraser, J. B., and Owen, L. N. (1949). *J. chem. Soc.*, 248.
Evans, R. M., and Owen, L. N. (1949). *J. chem. Soc.*, 244.
Fisher, R. A. (1944). *Statistical Methods for Research Workers.* 9th ed. Edinburgh : Oliver and Boyd.
Fisher, R. A., and Yates, F. (1943). *Statistical Tables for Biological, Agricultural and Medical Research.* 2nd ed. Edinburgh : Oliver and Boyd.
Fitzhugh, O. G., Woodard, G., Braun, H. A., Lusky, L. M., and Calvery, H. O. (1946). *J. Pharmacol.*, **87**, suppl., 23.
Gruhzit, O. M. (1935). *Arch. Derm. Syph., N. Y.*, **32**, 848.
Hogan, R. B., and Eagle, H. (1944). *J. Pharmacol.*, **80**, 93.
Kensler, C. J., Abels, J. C., and Rhoads, C. P. (1946). *J. Pharmacol.*, **88**, 99.
McDonald, F. F. (1948). *Brit. J. Pharmacol.*, **3**, 116.
Peters, R. A., and Stocken, L. A. (1947). *Biochem. J.*, **41**, 53.
Stocken, L. A., and Thomrson, R. H. S. (1946). *Biochem. J.*, **40**, 535.
Thomson, J. F., Savit, J., and Goldwasser, E. (1947). *J. Pharmacol.*, **89**, 1.
Vogt, M. (1943). *J. Physiol.*, **102**, 341.
Weatherall, M. (1945). *Report* No. BC23.
Weatherall, M. (1949). *J. Pharm. Pharmacol.*, in the press.
Whittaker, V. P. (1947). *Biochem. J.*, **41**, 56.
Withell, E. R. (1942). *J. Hyg., Camb.*, **42**, 124.