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EFFECTS OF BAL AND BAL GLUCOSIDE IN ACUTE LEAD ACETATE POISONING

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2:3-Dimercaptopropanol (BAL) was described by Peters, Stocken, and Thompson (1945) as an antidote to lewisite and other forms of arsenical poisoning. It has also been found to protect animals poisoned by several other heavy metals, but so far as is known the only data on its effect in lead poisoning are those of Braun, Lusky, and Calvery (1946), who found that after single or repeated intraperitoneal injections of lead nitrate into rabbits courses of BAL increased the mortality above that of control groups of animals. The experiments reported in the present paper are part of a study of the action of BAL and other dithiols in lead poisoning.

MATERIALS AND METHODS

Materials

The BAL used in this investigation was a water-purified sample kindly presented by Prof. R. A. Peters. It was stored in a refrigerator and solutions were prepared freshly as required. Solutions of BAL glucoside (Danielli, Danielli, Mitchell, Owen, and Shaw, 1946) were prepared from its barium salt by liberating the free dithiol with sulphuric acid, precipitating any residual barium with sodium sulphate and removing the precipitate by centrifuging. The strength of the solutions was estimated by iodine titration before use; this gave somewhat variable results, but was more satisfactory than computing the strength from the amount of barium salt used, as the latter varied considerably in its thiol content. The doses quoted must be regarded as only approximate.

Red cell fragility experiments

Freshly prepared, heparinized, and washed rabbit red blood cells were allowed to react with lead acetate or BAL or plasma or mixtures of these, usually for one hour at room temperature. Their fragility was then determined by adding aliquots to a series of sodium chloride solutions ranging from 0.28 to 0.64 per cent (w/v) at intervals of 0.04 per cent, centrifuging and estimating the haemoglobin concentration in the supernatant fluid by visual comparison with standards at 20 per cent intervals prepared from a water-laked suspension of the same red cells. The percentage lysis plotted against the salt concentration gave a sigmoid curve from which the 50 per cent lytic concentration (LyC50) was obtained graphically. The probit of the percentage lysis plotted against the logarithm of the salt concentration allowed a straight line to be fitted fairly closely; such lines did not give a substantially different estimate of the LyC50 and were used only when a quantitative estimate of the slope was required. For measurements *in vivo* the same procedure was followed, except that aliquots of the washed cell suspensions were added immediately and without other manipulation to the series of sodium chloride dilutions.

Blood estimations

Reticulocyte and red cell counts were performed by the usual techniques. Haematocrits were determined in capillary tubes of 0.1 ml. capacity. Haemoglobin was estimated in early experiments as carboxyhaemoglobin by means of a dilution comparator and later as cyanmethaemoglobin (King, Gilchrist, and Delory, 1944) by means of a Hilger Spekker absorptiometer calibrated with rabbit blood of known oxygen capacity. Calculations based on duplicate estimations made in the course of these experiments gave the standard error of the red cell counts as $\pm 0.22 \times 10^6$ cells per cu. mm. and of the haemoglobin determinations by the cyanmethaemoglobin method as ± 0.28 g. haemoglobin per 100 ml.

Rabbit metabolism experiments

Adult rabbits of both sexes and various breeds were kept in metabolism cages for 22 hours a days and fed in the remaining two hours on bran, oats, greens, and turnips. Water was allowed *ad libitum*. Lead acetate was given by stomach tube and dithiols by injection into the muscles beside the vertebral column. Three to five rabbits were handled in a single experiment. Of these, one was treated with lead acetate and either olive oil as used to dissolve the BAL or glucose equivalent to the amount of BAL glucoside given to the other rabbits; sometimes one was treated with a dithiol and no lead; and the rest received lead and a dithiol. The rabbits were allotted to different treat-

ments by a random procedure. Variation due to external conditions therefore affected all groups as far as possible equally.

Coproporphyrin estimations

Coproporphyrin was estimated, usually in duplicate, on toluol-preserved three-day samples of urine and on three-day samples of faeces ground with anhydrous sodium sulphate, as described by O'Brien (1946). The urine and faeces were acidified with glacial acetic acid and extracted with ether. The ether was washed with 2 per cent (w/v) sodium acetate and extracted with 5 per cent HCl. The HCl was neutralized with solid sodium acetate and extracted with ether; this ether was extracted with 0.5 per cent HCl. The last three steps were repeated once or twice if necessary to give a bright red fluorescent solution. The faecal extracts were taken into 0.2 per cent HCl and washed with chloroform before estimation. Completeness of extraction was checked at each stage by observing the absence of fluorescence in ultra-violet light. The coproporphyrin in the extracts was estimated fluorimetrically in 0.5 per cent HCl against a standard solution of coproporphyrin I, kindly provided by Mr. J. R. P. O'Brien, in a Rimington-Schuster comparator (Rimington, 1943). The standard error, calculated from duplicate estimates, in these experiments was ±8.2 per cent for urines containing 3 to 100 μg. per 100 ml. The porphyrin was not further identified, but in view of the technique of extraction used and the well-established excretion of coproporphyrin in lead poisoning (Fischer and Duesberg, 1932; Watson, 1936; etc.) there is little doubt as to the substance estimated.

Results

I. Preliminary experiments

Preliminary experiments were carried out in mice. Promising results were obtained (Table I), but it was difficult to produce lead poisoning suitable for experimental study in these animals. They were very resistant to lead salts given by stomach tube or in the diet, and they suffered severely from the local necrotic action of solutions injected into the peritoneum or intramuscularly. Larger and less resistant animals—viz., rabbits, were therefore used, in which haematological changes could conveniently be taken as an index of lead poisoning (Flury, 1934, for review). The experiments were based particularly on those of Aub, Reznikoff, and Smith (1924a and b) *in vitro* and of Key (1923) *in vivo*, as they appeared to provide means whereby toxic effects of lead could be produced rapidly and reproducibly.

TABLE I

The effect of BAL and BAL glucoside injected intramuscularly in mice poisoned by lead acetate injected intraperitoneally

Dose of lead acetate mg./kg./day for 5 days	Dithiol	Dose of dithiol mg./kg./day for 5 days	Mortality at 14 days from start of expt.	Significance of difference, control and treated χ^2	P
50	None	—	10/10	—	—
50	BAL	20	7/10	1.57	about 0.2
50	BAL glucoside	1000	3/10	7.90	<0.01

II. In vitro experiments. Fragility of normal rabbit washed red cells

The sigmoid curve relating the percentage lysis to the logarithm of the salt concentration was found to be fairly constant in shape but to vary somewhat in position. Variation occurred from day to day in the same rabbit and between the mean values for different rabbits. The former factor was the more conspicuous. From determinations on 22 rabbits, the mean LyC50 was found to be 0.51 per cent with a standard deviation of the individual values of ± 0.042 per cent NaCl. When the probit of the percentage lysis was plotted against the logarithm of the salt concentration, lines with a slope (*b*) of −20 to −30 were obtained.

Fragility of red cells after exposure for one hour to lead acetate (9 μg.Pb per ml.)

After exposure to lead acetate, the cells were more resistant to lysis by hypotonic saline. As was found by Aub, Reznikoff, and Smith (1924a), the change apparently affected mainly those cells which were in any case most resistant, as there was a much greater difference in the salt concentration necessary to effect over 50 per cent lysis than in that to effect 10 or 20 per cent lysis. Hence the fragility curves were flatter than normal, and the slopes of the transformed curves were in the region of −10. The reduction in the LyC50 varied (Table II) and was apparently not related to the initial value. The effect was smaller if the fragility was estimated half an hour instead of one hour after exposure to lead acetate.

Fragility of red cells after exposure for one hour to BAL

After exposure to 22.7 μg. BAL per ml., the cells were slightly more resistant to hypotonic haemolysis than normal. In one or two experiments in which 113 μg. BAL per ml. were used,

TABLE II

The mean fragility of rabbit red blood cells exposed to lead acetate and BAL

Observation	No. of observations	Mean ± S.E. % NaCl	S.D. of individual values	P
Normal LyC50 ..	*	0.51 ±0.009	±0.042	—
LyC50 after lead acetate 9 μg.Pb/ml...	51	0.35 ±0.010	±0.068	—
LyC50 after BAL 22.7 μg./ml. ..	5	0.48 ±0.015	±0.034	—
LyC50 after lead acetate 9 μg.Pb/ml. + BAL 22.7 μg./ml.	5	0.48 ±0.017	±0.038	—
Mean difference, normal and lead treated cells ..	51	0.15 ±0.011	±0.079	<0.001
Mean difference, normal and BAL treated cells ..	5	0.03 ±0.009	±0.020	<0.05
Mean difference, normal and BAL + lead treated cells	5	0.03 ±0.011	±0.024	<0.05

The 50 per cent lytic concentrations (LyC50) were determined graphically from sigmoid fragility curves.

*Weighted mean of 54 observations on 22 rabbits.

the change was more obvious. It was not accompanied by any appreciable change in the slope of the fragility curve.

Fragility of red cells after exposure to lead acetate and BAL

When BAL was added immediately after lead acetate (9 μg.Pb per ml.) the effects of lead on the shape and position of the fragility curve were more or less prevented (Table II): 1.13 μg. of BAL per ml. (0.2 mols per mol of lead acetate) had no effect; 5.7 μg./ml. (1 mol per mol) prevented about 80 per cent of the effect; and 22.7 μg./ml. (4 mols per mol) prevented it almost or quite completely. The effect of lead acetate took some time to develop fully. It was incomplete at half an hour, and if BAL was added at this time, further development was prevented but there was very little reversal of the established change; this was so even if BAL was allowed to act for three hours or if BAL concentrations up to 20 mols per mol of lead acetate were used.

Fragility of red cells after exposure to lead acetate and BAL glucoside

Like BAL, solutions of BAL glucoside added immediately after lead acetate abolished or reduced the lead effect. Mol for mol the activity of the glucoside appeared to be of the same order as that of free BAL.

Influence of normal rabbit plasma on the lead acetate-red cell system

The results described above suggested that inhibition of the effect of lead acetate on red cells might provide a much needed method for assaying fairly small quantities of BAL in plasma. The fragility of normal red cells showed only small and variable changes when up to 18 per cent (v/v) of plasma was present in the reaction mixture, whether the plasma came from the same or a different rabbit. As was found by Aub, Reznikoff, and Smith (1924a) plasma inhibited the effect of lead, but this inhibition was fairly variable. With 9, 13.5 or 18 per cent (v/v) of plasma, usually about two-thirds of the effect of lead acetate was prevented, but values from 7 to 100 per cent were sometimes obtained. There was no obvious difference between the responses to plasma from the rabbit which had supplied the cells and those to plasma from other rabbits.

Influence of normal rabbit plasma and BAL on the lead acetate-red cell system

When BAL and plasma were both added to the reaction mixture immediately after the lead acetate, there was always less inhibition of the lead effect than was produced by the similar amount of BAL alone. Sometimes there was also less inhibition than that produced by plasma alone, particularly with the higher concentration of plasma (18 per cent (v/v)) and smaller concentrations of BAL (5.7 μg./ml.). This smaller inhibition occurred also if the BAL and plasma were added an hour after the lead acetate, or if the BAL and plasma were mixed half an hour before addition to the reaction mixture of red cells and lead acetate.

Influence of plasma from BAL treated rabbits on the lead acetate-red cell system

Two rabbits were used in each experiment. The first rabbit was bled (1 ml.) and injected intramuscularly with 50 mg./kg. BAL in nut oil or in 66 per cent (v/v) propylene glycol in water, or, in control experiments, with solvent alone. Further 1 ml. samples of blood were taken half, two and four and a half hours after injection. The samples were heparinized and centrifuged and the plasma was separated at once. The anti-lead activity of the plasma so obtained was assayed on lead acetate-red cell systems prepared about the same time with cells from the second rabbit. Sometimes the samples were re-assayed using other cells next day. The results are given in Table III. There was, in general, not an increase but a decrease in

TABLE III

ANTI-LEAD ACTIVITY OF PLASMA FROM RABBITS BEFORE AND AFTER INJECTION OF BAL

The 50 per cent lytic concentrations were determined graphically from sigmoid fragility curves. Figures in the four final columns are the percentages of the lead effect prevented by adding 0.1 ml. plasma to 1.0 ml. of cell suspension treated with lead acetate,

$$\text{i.e. } \frac{\text{(Plasma + lead) LyC50–Lead LyC50}}{\text{Normal LyC50–Lead LyC50}} \times 100.$$

Duplicate estimates on separate fragility systems are shown independently. The final averages for BAL-treated and untreated rabbits are based on the mean values for each rabbit.

Experiment			50% Lytic concentrations						% of lead effect prevented by plasma			
Material injected	Rabbit No.	Exp. No.	Normal	+ $PbAc_2$	+ $PbAc_2$ + Plasma							
					Before injec.	Hrs. after injec. ½	2	4½	Before injec.	Hrs. after injec. ½	2	4½
BAL 50 mg./kg. in nut oil	43	4*a*	0.63	0.29	0.52	0.30	—	—	70	3	—	—
,, ,,	43	4*b*	0.48	0.39	0.46	0.46	0.43	—	78	78	45	—
			0.48	0.35	0.46	0.46	0.42	0.46	85	85	54	85
,, ,,	48	4*d*	0.56	0.25	0.44	0.28	0.26	—	61	10	3	—
			0.55	0.28	0.41	0.28	—	0.49	48	0	—	74
,, ,,	52	4*g*	0.49	0.35	0.46	0.44	—	—	79	64	—	—
			0.51	0.30	—	—	0.40	0.45	—	—	48	71
BAL 50 mg./kg. in propylene glycol	49	4*h*	0.48	0.29	0.43	0.36	—	—	70	40	—	—
			0.47	0.34	—	—	0.44	0.41	—	—	77	54
Average values for BAL-treated rabbits			..	..	..	..	..	..	70	40	45	71
Nut oil	48	4*f*	0.55	0.30	0.52	0.52	—	—	92	92	—	—
			0.55	0.42	—	—	0.50	0.47	—	—	69	38
			0.47	0.37	0.46	—	—	0.43	90	—	—	50
,, ,, ...	51	4*g*	0.49	0.35	0.46	0.46	—	—	79	79	—	—
			0.51	0.30	—	—	0.39	0.44	—	—	43	66
Propylene glycol ..	51	4*h*	0.48	0.29	0.42	0.40	—	—	70	60	—	—
			0.47	0.34	—	—	0.45	0.40	—	—	85	46
Average values for control rabbits			..	..	..	..	..	..	80	70	66	52

the anti-lead activity of plasma after BAL. Much weight cannot be attached to this finding, because some fall occurred also in the later samples from the control rabbits: however, large changes were observed only in the post-BAL plasmas (e.g., exp. 4*a*, 4*d*) and especially in the half-hour sample. Such a change is consistent with the purely *in vitro* finding that small amounts of BAL can diminish the anti-lead activity of plasma. In experiment 4*d*, the effect of the plasma samples on the fragility of cells without lead was examined. Neither the normal plasma nor the samples after BAL had any effect on the normal fragility. The effect of the plasma therefore seems to be on the lead rather than directly on the cells.

Aub, Reznikoff, and Smith (1924b) claimed that the extent to which plasma or serum prevented the effect of lead on red cells was parallel to the phosphate concentration of the plasma or serum. The phosphate concentration of four sets of plasma, two from control and two from BAL-treated rabbits, were therefore estimated by a small scale modification of the Briggs-Bell-Doisy method, using a Hilger Spekker absorptiometer for the colour intensity determinations. A difficulty arose in that it was generally difficult to bleed rabbits by a single venepuncture when they had been treated with BAL, and that in samples obtained by allowing blood to drip from the ear some haemolysis occurred and the plasmas were pink or red. These plasmas gave a high phosphate content, presumably owing to phosphate liberated from damaged cells. Excluding samples with visible lysis, no change exceeding ± 5 per cent in phosphate concentration was found in control or BAL plasmas, although their anti-lead activity varied considerably. No correlation, positive or negative, was observed between the presence of haemoglobin in plasma samples and the anti-lead activity.

III. In vivo experiments. Changes in untreated lead acetate poisoned rabbits

The normal blood picture and coproporphyrin excretion of stock rabbits in this laboratory are summarized in Table IV. After a single dose of

TABLE IV

Blood picture and coproporphyrin excretion of normal laboratory rabbits

	♂	♀	Combined
R.B.C. × 10^{-6}/cu.mm. ..	6.1 ± 0.9	5.9 ± 0.4	6.0 ± 0.7
Haemoglobin g./100 ml.	14.3 ± 1.7	12.6 ± 1.7	13.5 ± 1.8
Haematocrit %	41.5 ± 0.3	38.4 ± 1.5	39.8 ± 1.2
Reticulocytes per 100 R.B.C.	1.3 ± 0.9	1.7 ± 1.3	1.4 ± 1.0
Mean corpuscular haemoglobin concentration, μμg./cell	23.6 ± 3.3	21.5 ± 3.2	22.7 ± 3.2
Mean corpuscular volume, cu.μ.	66.7 ± 2.9	65.3 ± 1.7	65.9 ± 2.3
Coproporphyrin excretion μg./day, urine	4.1 ± 2.6	5.7 ± 3.6	4.8 ± 3.1
,, faeces ..	5.1 ± 4.7	4.6 ± 2.8	4.8 ± 3.9

The values given are the mean and standard deviation of individual values for nine male and seven female rabbits.

300 mg./kg. (0.8. mM./kg.) of lead acetate by stomach tube, anaemia, reticulocytosis, punctate basophilia, slight albuminuria, haemoglobinuria, and increased coproporphyrin excretion were observed (Table V and Fig. 1). The anaemia varied in severity. It was greatest about the third to sixth day, and recovery took three to six weeks. The mean corpuscular haemoglobin concentration did not vary significantly, though apparent high values tended to occur at the onset and low values appeared in the second week. The reticulocyte count rose rather slowly and reached its peak after about a week. Punctate basophils were found roughly in proportion to the number of reticulocytes present. The red cell fragility did not change as strikingly as in the rather similar experiments described by Aub, Reznikoff, and Smith (1924a). In the present experiments changes of fragility were observed mainly in rabbits which developed the worst anaemia. In these the fragility curve became flatter, with slopes of about −12, and the position of the LyC50 showed little change. If anything it increased, though by not more than 0.10 per cent NaCl, indicating that the cells were less resistant to hypotonic haemolysis. The change in LyC50 did not exceed normal limits: the flattening was similar to that observed *in vitro*, though less definite. Haemoglobinuria, sometimes sufficient to produce a dark brown urine, occurred in the first two or three days, but no red cells were found in the urine. The excretion of coproporphyrin was greatly increased, mainly in the urine, and continued for at least nine days: in one rabbit quantities above normal were still found after three weeks. Of the eight rabbits treated with lead acetate and no dithiol, three died. In

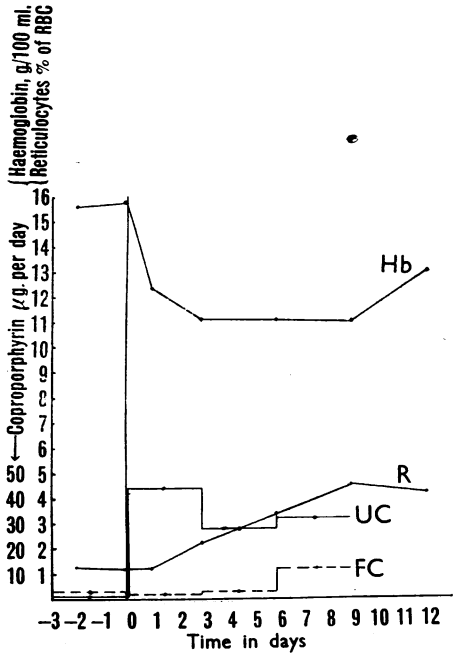

FIG. 1.—Acute lead acetate poisoning in rabbits. Ordinates: haemoglobin (Hb) g./100 ml.; reticulocyte count (R) per 100 R.B.C.; coproporphyrin excretion μg./day; UC, urinary; FC, faecal. Abscissae; time in days from administration of lead acetate, 300 mg./kg., by stomach tube. Values for haemoglobin and reticulocytes based on eight rabbits and for coproporphyrin excretion on three rabbits.

one of these the haemoglobin fell by over half in the first day and its death on the second day was probably due to anaemia. The other two died on the third and sixteenth days respectively, without gross anaemia or other obvious cause.

Changes in rabbits receiving only dithiols

The actions of dithiols alone are being studied further and will be reported in detail separately. The amount of BAL used in the present experiments caused transient haemoconcentration and occasionally a rise in reticulocyte count. Otherwise the blood picture and red cell fragility were unaltered. Increased excretion of coproporphyrin, or of a red fluorescent pigment which behaves

TABLE V

INFLUENCE OF DITHIOLS ON THE EFFECTS OF LEAD ACETATE IN RABBITS

The fall in R.B.C., haemoglobin and haematocrit are calculated from the mean difference between two control values before treatment and values on the first and third days after administering lead acetate. The values given are the mean and standard error for all the rabbits in each group. The coproporphyrin excretion figures are those for the first three days after administration of lead acetate, and are based on 2 or 3 rabbits in each group.

Lead acetate 300 mg./kg. orally and dithiols intramuscularly	No. of rabbits	No. died	Fall in R.B.C. $\times 10^{-6}$	Fall in Haemoglobin g./100 ml.	Fall in h'crit %	Haemoglobinuria	Coproporphyrin excretion μg./day Ur.	Fc.	Significance of difference from untreated R.B.C. fall t	P<
No dithiol	8	3	1.64±0.41	4.23±1.07	12.0	++	45	1.3	—	—
BAL 50 mg./kg. at 1 hr. + 25 mg./kg. 8-hrly. for 7 doses	4	2	0.12±0.19	0.19±0.63	2.2	Trace	92	9.4	13.55	0.01
BAL glucoside 250 mg./kg. at 1 hr. + 125 mg./kg. 8-hrly. for 7 doses ..	3	0	1.23±0.44	2.25±0.46	11.5	0 to +	50	14.0	2.16	0.1
BAL glucoside 250 mg./kg. at 1 and 5 hours	4	0	0.47±0.22	1.02±0.37	4.0	0 to +	—	—	9.85	0.01
BAL 25 mg./kg. + BAL glucoside 125 mg./kg. at 1 hr. + BAL 12.5 mg./kg. + BAL glucoside 62.5 mg./kg. 8-hrly. for 7 doses ..	2	0	0.52	1.30	—	0 to +	42	9.8	—	—
BAL glucoside 250 mg./kg. at 21 hrs. + 125 mg./kg. 8-hrly. for 4 doses ..	1	0	2.60	6.85	17.5	++	—	—	—	—

similarly, occurred consistently. Assuming the pigment to be coproporphyrin, the excretion after doses of BAL such as were used here was of the order of 12 to 20 μg./day. BAL glucoside alone had no appreciable effect on the blood picture. Its effect on porphyrin excretion has not been studied.

Effect of dithiols in lead acetate poisoned rabbits

Lead poisoned rabbits treated with BAL in a dosage of 50 mg./kg. (0.4 mM./kg.) intramuscularly one hour after the lead acetate followed by 25 mg./kg. every eight hours for two days—i.e., to a total dosage of 200 mg./kg. (1.6 mM./kg.) developed very little anaemia as long as BAL was being administered. Later the anaemia increased, but the fall was less than in untreated animals (Fig. 2). Reticulocytosis was smaller, and occurred earlier than in rabbits receiving lead acetate but no BAL. This response was somewhat variable and the difference may be due to chance. The coproporphyrin excretion was greater than in rabbits receiving no dithiol, but subsequently fell off more quickly. Although there was good protection against the anaemia, the mortality was not appreciably altered, as two rabbits of the four in

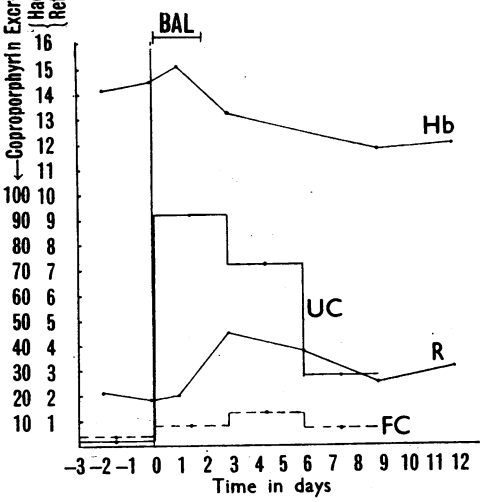

FIG. 2.—Acute lead acetate poisoning treated with BAL. Ordinates and abscissae as in Fig. 1. BAL, 200 mg./kg. in all, given during first two days after lead acetate. Values for haemoglobin and reticulocytes based on four rabbits and for coproporphyrin excretion on three rabbits.

this group died, on the fifth and twelfth days respectively.

Treatment with BAL glucoside (Fig. 3) on a similar dosage system but with a total dosage of about 1,000 mg./kg. (3.5 mM./kg.) had only a slight and not significant effect on the anaemia. However, when the BAL glucoside was given in two 250 mg./kg. doses one and five hours after the lead acetate, it was about as effective as BAL

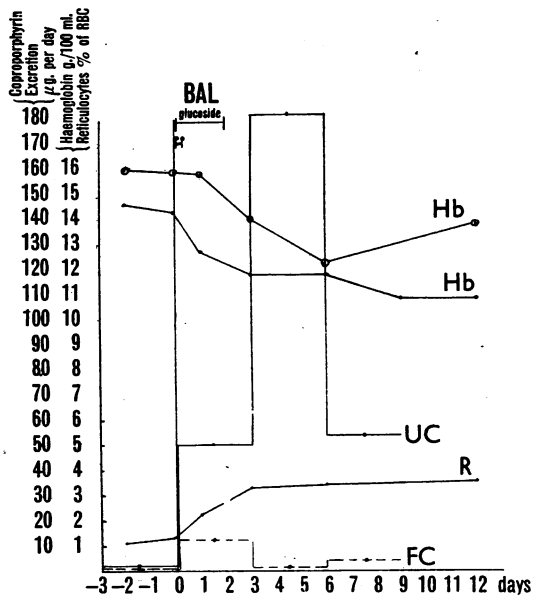

FIG. 3.—Acute lead acetate poisoning treated with BAL glucoside. Ordinates and abscissae as in Fig. 1. BAL glucoside, 1,000 mg./kg. in all, given during first two days after lead acetate. Values for haemoglobin and reticulocytes based on three rabbits and for coproporphyrin excretion on two rabbits. The line joining points enclosed by circles is for a group of four rabbits treated with BAL glucoside, 500 mg./kg. in all, given during the first six hours after lead acetate.

in delaying the onset of anaemia. Porphyrin excretion and reticulocytes were not followed in the latter group. In the former, as in BAL treated rabbits, the coproporphyrinuria was greater and the reticulocytosis less than in those receiving lead acetate alone. Of a pair of rabbits treated with BAL and BAL glucoside, as suggested by Danielli *et al.* (1946), one developed very little and one considerable anaemia. Both produced large amounts of coproporphyrin. One rabbit treated with BAL glucoside twenty-one hours after lead acetate, by which time anaemia was well developed, did not become more anaemic, but did not recover more rapidly than those of the control group. In all the dithiol treated rabbits, red cell fragility changes occurred as in the controls—i.e., only when there was fairly severe anaemia. None of the eight rabbits treated with BAL glucoside alone died, nor did the two treated with BAL and BAL glucoside together.

DISCUSSION

In suitable concentrations BAL prevented the action of lead acetate on rabbit red blood cells *in vitro*. If BAL was added half or one hour after the lead acetate, the effect of BAL was small and consisted chiefly in preventing the full development of the effect of the lead, not of significantly reversing an established change in fragility. The rate of uptake of lead ions by red cells *in vitro* is fairly slow (Behrens and Pachur, 1927; Mortensen and Kellogg, 1944) in concentrations of the order of those used here, and, to judge from Mortensen and Kellogg's data, corresponds quite closely with the development of the fragility change. The action of BAL therefore appears to be one of inactivating lead ions not yet taken up by cells, rather than of actual de-leading cells or altering the cell lead so as to prevent its fragility effect. There is certainly no reversal of poisoning comparable to that which occurs with arsenicals —e.g., of pyruvate oxidase by lewisite (Stocken and Thompson, 1946). Further experiments are being conducted to see what effect BAL has on the uptake of lead by red cells *in vitro* and *in vivo*.

Plasma, like BAL, prevented the effect of lead acetate *in vitro*. However, the combination of BAL and plasma, particularly in the proportion of 30–60 μg. BAL per ml. of plasma, was less effective than either alone, and a similar reduction in plasma anti-lead activity occurred *in vivo* after the injection of large doses of BAL. How this happens is not known. It has been suggested that lead is taken up by plasma as inorganic phosphate (Aub and Reznikoff, 1924; Brooks, 1927), or as a double phosphate of lead and calcium (Bischoff and Maxwell, 1928; Maxwell and Bischoff, 1929; Jowett, 1932), or as an organic complex, probably with albumin (Teisinger, 1935) or citrate (Kety, 1941). In the present experiments the altered plasma anti-lead activity, which presumably reflects the capacity of the plasma to combine with lead, was not related to changes in inorganic phosphate content. Beyond that the mechanism has not been studied.

Interpretation of how BAL acts *in vivo* depends on the view taken of the mechanism of the lead anaemia (Cantarow and Trumper, 1944; Flury, 1934). In the present experiments the initial

anaemia was clearly haemolytic, because of the rapidity of the fall and the appearance of haemoglobin in the urine. The tendency of the mean corpuscular haemoglobin concentration towards high values at this stage was probably an artefact due to the presence of haemoglobin in the plasma. The present observation that changes in fragility were most definite in rabbits with the severest anaemia is consistent with Aub, Fairhall, Minot, and Reznikoff's hypothesis (1925) that the primary change is in the red cell. It does not follow that the anaemia of chronic plumbism has the same origin, and, in fact, the known changes in the bone marrow and the increased porphyrin excretion cannot be accounted for on this basis (Duesberg, 1931; Flury, 1934) particularly as there is no evidence of abnormal conversion of circulating haemoglobin to coproporphyrin (Björkmann, 1941; Kark and Meiklejohn, 1941). As increased coproporphyrin excretion occurred in the present experiments disturbances of blood formation as well as blood destruction were probably involved.

In these circumstances the effects of BAL are unlikely to be simple. Clearly BAL prevents the acute haemolytic anaemia as long as BAL is available in the circulation. This is comparable with its action *in vitro* and may be similarly attributed to prevention of the uptake of lead ions by red cells. Experiments now in progress on the effect of BAL on lead distribution *in vivo* have lent support to this hypothesis. On the other hand, certain features of lead poisoning are enhanced, notably the coproporphyrin excretion and possibly the speed of the reticulocyte response. Both these effects can be produced by BAL alone, but the coproporphyrin output is considerably more than can be accounted for by simple addition of BAL and lead effects.

The actions of BAL glucoside are on the whole similar, though the inefficacy of the glucoside given in a course comparable with that used in the BAL experiments is not immediately explicable. Possibly this very water-soluble substance is excreted very rapidly. As the *in vitro* experiments with BAL suggest that the protection of red cells is mainly prophylactic, a sustained high concentration of thiol at an early stage would be important, and the shorter and more intensive course would be more likely to be effective.

The number of rabbits used was too small for the mortality figures to be significant, though it is worth noting that all the BAL glucoside treated rabbits survived. BAL treatment apparently did not save life, but there was no evidence that it increased the mortality as might have been expected, since the doses used were at the upper limit of tolerance and those of lead acetate were within lethal limits. These experiments differed from those of Braun *et al.* (1946) in that BAL was given in a shorter and more intensive course at an earlier stage of poisoning. The only observation made here which might relate to the increased mortality described by Braun *et al.* is that of the diminished plasma anti-lead activity. Assuming that this reflects a diminished affinity of the plasma for lead, it may account for larger amounts of lead being free to act at some more susceptible site. But there is no evidence as to what this site may be, or whether the effect has any relevance at all to the combined actions of lead and BAL. Unfortunately it is not known whether BAL glucoside has a similar action on the plasma. Experiments on this question have been prevented by lack of active preparations of the glucoside. The action of BAL in preventing the effects of acute intoxication with lead, and perhaps in producing some other fatal effect, and the prevention of poisoning by BAL glucoside are strongly reminiscent of their respective actions in cadmium poisoning (Gilman, Philips, Allen, and Koelle, 1946) and suggest further work on such lines. The actions of BAL in chronic lead poisoning and with reference to the distribution of lead in the organism require further study. The available evidence does not warrant the use of BAL in clinical plumbism, but it would perhaps be premature to reject all dithiols as useless or dangerous. It is also possible that they will throw further light on the still poorly understood mechanisms of lead poisoning.

Summary

1. In mice poisoned by repeated intraperitoneal injections of lead acetate the mortality was reduced slightly by BAL and significantly by BAL glucoside.

2. *In vitro* BAL and BAL glucoside prevented the decrease in the fragility of washed erythrocytes due to lead acetate. If the lead was added much before the thiol, the effect of the lead was scarcely at all reversed.

3. Mixtures of BAL and plasma in certain proportions, and plasma from rabbits injected with BAL, protected washed erythrocytes from the effect of lead acetate less than did equal amounts of BAL or plasma alone.

4. In rabbits poisoned by a single dose of lead acetate given by stomach tube BAL and BAL glucoside each significantly decreased the subsequent anaemia and increased the coproporphyrinuria. The mortality was apparently unaffected by BAL, but was reduced by BAL glucoside: the

number of rabbits was too small for this difference in mortality to be significant.

5. The significance of these findings is discussed.

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