OBSERVATIONS ON DITHIOLS AND THE DISTRIBUTION OF LEAD IN RABBITS

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Dimercaprol (2:3-dimercaptopropanol, B.A.L.) is moderately effective in promoting the excretion of lead in acutely poisoned experimental animals[1,2]. Reports of its clinical efficacy are somewhat variable, and a properly controlled therapeutic trial appears not to have been performed. A fall in the concentration of lead in the blood and an increase in the amount in the urine usually occurs[3,4,5,6,7]. The effect is transient and the amount of symptomatic relief reported is variable. Exacerbation of symptoms is sometimes described[6]. It is possible that other dithiols might be more beneficial, and the effect of some which were available to us has been examined in experimental animals. Those chosen have been the glucoside of dimercaprol, also known as "B.A.L.-Intrav"[8], which is less toxic and more soluble in water; 1:2-dimercaptopropionic acid, which is a simpler derivative of dimercaprol also possessing a hydrophilic polar group; and 1:3-dimercaptopropanol and 1:4-dimercaptoerythritol, in which the two thiol groups are more widely spaced than in dimercaprol and might conceivably be better arranged to form a cyclic compound with the large lead atom than they are in dimercaprol.

Methods

Lead chloride or lead acetate containing some radio-active lead (either ^{210}Pb or ^{212}Pb) as tracer was injected slowly intravenously into rabbits in doses of 0·01 mM./kg. (2·07 mg. Pb/kg.) or 0·1 mM./kg. (20·7 mg. Pb/kg.). Either 1 and 5 hours later or 19 and 23 hours later a dithiol (or saline solution in the control animals) was injected intramuscularly: the doses used are indicated in accounts of particular experiments. 24 hours after the injection of the lead salt, the animals were killed by stunning and bleeding, and samples of their tissues were taken for ashing and the estimation of lead by measurement of their radioactivity in a Geiger counter. Details of technique have been described previously[2,9]. Some of the dithiols used were available as the free thiols and others were prepared from their barium salts and standardised by titration with iodine before use[10]. The individual thiols were similar to or identical with those discussed by Weatherall and Weatherall[11].

Results

The results obtained are shown in Tables I and II. The distribution of lead in the control rabbits was, in general, similar to that which has been reported previously in this species at this stage[2,12,13]. The distribution differs considerably from the classical distribution after chronic absorption,

or a long time after a single dose. Here lead was concentrated particularly in the liver, spleen and bone-marrow, and rather less in the kidneys. Moderate amounts were found in the blood cells, the alimentary canal and bone, and very little occurred in skin and skeletal muscle. The distribution showed no appreciable difference whether the acetate or the chloride of lead was administered. The concentrations of lead in the liver were always lower and those in the kidney were higher than previously

TABLE I

THE MEAN CONCENTRATIONS AND PERCENTAGES OF THE DOSE OF LEAD IN VARIOUS TISSUES 24 HOURS AFTER THE INTRAVENOUS INJECTION OF A LEAD SALT, WITHOUT AND WITH TREATMENT WITH DIMERCAPROL GLUCOSIDE (600 μM./KG.,* IN FIRST DOSE AND 150 μM./KG. IN SECOND DOSE 4 HOURS LATER)

Dose of lead	10 μM./kg.						100 μM./kg.	
Treatment with dimercaprol glucoside	None		At 1 hour and 5 hours		At 19 hours and 23 hours		None	
No. of animals	4		3		3		3	
	μg./g.	per cent.	μg./g.	per cent.	μg./g.	per cent.	μg./g.	per cent.
Plasma	<0·1	<0·1	<0·1	<0·1	<0·1	<0·1	<1·0	<0·3
Blood cells	2·3	3·6	1·0	1·1	0·9	1·2	5·9	0·6
Lungs	2·0	0·5	3·9	0·7	4·6	0·8	40·1	0·7
Small intestine	0·5	0·6	0·5	0·6	1·2	1·1	—	—
Small intestine contents	1·5	0·7	1·8	0·4	1·6	0·2	—	—
Colon	0·7	0·2	0·6	0·1	0·9	0·2	4·4	0·1
Colon contents	3·4	1·4	8·8	1·4	7·8	1·3	—	—
Kidneys	12·4	3·9	11·0	4·1	9·5	3·3	23·5	0·9
Liver	17·9	25·6	13·4	17·9	15·8	21·5	296·9	32·6
Bile	4·4	—	30·8	—	24·1	—	—	—
Spleen	18·3	0·5	11·6	0·1	14·8	0·6	—	—
Skin	<0·1	<0·2	<0·1	<0·2	<0·1	<0·2	—	—
Bone marrow	16·3	17·3	11·5	11·3	10·0	9·6	480·8	41·5
Epiphyses	12·6	15·5	7·4	7·1	11·0	13·3	110·6	6·7
Diaphyses	5·4		2·5		4·6		25·9	
Skeletal muscle	<0·1	<0·3	<0·1	<0·3	<0·1	<0·3	—	—
Excreted urine	—	2·8	—	25·8	—	16·9	—	0·6
Excreted fæces	—	0·5	—	0·7	—	0·5	—	—

* 1 μM. = one millionth of one gramme-molecule.

observed by one of us[2]. Also the urinary excretion was greater in the present experiments, and varied between 1·0 and 4·7 per cent. of the dose instead of between 0·2 and 1·2 per cent. Possibly these differences happened because the lead was injected more slowly in the present experiments, in about 60 instead of 2 to 5 seconds. No other difference of procedure was known to exist consistently between the two sets of experiments.

When dithiols were injected, the urinary excretion was always greater, sometimes substantially so (Table II). Dimercaprol itself, given early, caused about a fivefold increase, but it was less effective than either dimercaprol glucoside or dimercaptopropionic acid, which produced a total excretion in 24 hours of up to 30 or 40 per cent. of the dose. This was particularly striking with dimercaptopropionic acid, of which the doses used were smaller, both absolutely and relatively to the molecular weight, than with dimercaprol. Dimercaptopropionic acid is more toxic than dimercaprol[14] and the dosage used here, both of dimercaprol and of the acid, was about one-third of the median lethal dose. The thiol-acid was therefore more effective even when allowance is made for its

greater toxicity. The dose of dimercaprol glucoside was larger, both absolutely and mole for mole, than that of dimercaprol, but was probably much less than one-third of the median lethal dose, which is remarkably high[8,10,15]. Of the two, dimercaprol glucoside therefore appeared to be the more promising therapeutic agent, and its effect on the distribution of lead in the body was examined. The remaining dithiols were also used in doses about one-third of their median lethal dose, and at this level were not more effective than dimercaprol.

The changes in distribution produced by dimercaprol glucoside were like those already described after dimercaprol[2]. There was in general a levelling out of the differences in concentration in different tissues; but less lead remained in the tissues and so the level approached was lower than after dimercaprol. The quantity of lead was reduced not only in the liver and bone-marrow but also in the kidneys, blood cells and bone.

TABLE II

THE URINARY EXCRETION OF LEAD IN THE FIRST 24 HOURS AFTER INTRAVENOUS INJECTION OF A LEAD SALT AND TREATMENT WITH DITHIOLS

Treatment	None	Dimercaprol	Dimercaprol glucoside		Dimercaptopropionic acid	1:3-dimercaptopropanol	1:4-dimercaptoerythritol
First dose μM./kg.* ..	—	400	600	600	90	100	500
Second dose ,, ..	—	100	150	150	22·5	—	125
Doses at, hours	—	1 and 5	1 and 5	19 and 23	1 and 5	1	1 and 5
		Quantity of lead in urine (μM./kg. body weight)					
Dose of lead:—							
10 μM./kg. Mean ..	0·3 (4)	1·7 (1)	2·6 (3)	1·7 (3)	3·4 (2)	0·6 (2)	1·0 (1)
10 μM./kg. Range ..	0·1–0·4	—	1·8–3·1	1·0–2·5	2·7–4·2	0·6–0·7	
100 μM./kg. Mean ..	0·6 (3)	2·7 (2)	5·6 (2)	3·9 (1)	—	—	—
100 μM./kg. Range ..	0·3–1·4	0·2–5·1	4·7–6·5	—	—	—	—

The figures in brackets indicate the numbers of animals from which the average values have been obtained.

* 1μM. = one millionth of one gramme-molecule.

Counts were not prolonged sufficiently to give estimates of the concentration in plasma and skeletal muscle, and an upward trend in these tissues similar to that observed after dimercaprol was not excluded. In urine and bile considerable increments occurred, accounting for most of the lead removed from the tissues. After 1:3-dimercaptopropanol the changes closely resembled those produced by dimercaprol. Full distributions have not been studied after dimercaptopropionic acid.

Some rabbits were injected with a dose of lead ten times larger than those already discussed. The results are shown in the last column of Table I and the last line of Table II. The quantity of lead given is close to the median lethal dose for rabbits, but no rabbits died during the experimental period. The distribution of lead after this dose was broadly similar to that observed after one-tenth of the dose. However, the greatest concentrations observed (in the liver and bone marrow) were more than ten times those found after the smaller dose, whereas in the organs which took up only moderate amounts of lead, such as the blood cells and the kidneys, the increments were only two to fivefold. Treatment with dimercaprol or dimercaprol glucoside increased the urinary

excretion of lead, and the absolute additional amount of lead excreted was larger than when a small dose of lead had been given, although the dose of thiol was unchanged. The distribution of lead in the tissues was not studied in detail in the rabbits which received a large dose of lead and were treated with dithiols.

Discussion

From these results it is clear that the possible merits of dithiols in lead poisoning have not been fully explored. Two of the four dithiols here examined were more potent than dimercaprol in promoting the urinary excretion of lead, and the reasons for this improvement need to be considered.

It has been suggested that dimercaprol itself is dangerous in lead poisoning because increased mortality has sometimes been observed when dimercaprol has been administered to lead-poisoned animals[1,16,17]. As a similar objection might apply to other dithiols, it is relevant to observe that, as far as we are aware, increased mortality has been observed only when the poisoned animals have received lead subcutaneously or intraperitoneally and have a persistent depot of lead in their soft tissues. When this is so, increased toxicity can readily be accounted for, as can the present results, by the following theoretical considerations.

It may be assumed that lead combines reversibly with various receptors in the soft tissues, or with dithiols, and that in each reaction an equilibrium is reached. The dissociability of the dithiol-lead compound will depend on the particular dithiol. In the case of dimercaprol, the compound is assumed to be moderately dissociable, considerably more so, for example, than the compounds of dimercaprol with arsenicals. Excess of dimercaprol favours formation of the dimercaprol-lead compound: a reduction in the amount of dimercaprol favours its dissociation and increases the amount of free lead available to the tissues. The exact form of this free lead is not important, but in the absence of contrary evidence it may be taken as the lead ion. The actual quantities involved may be so small as to make detection difficult, and this concept does not conflict with the evidence of Jowett[18] that there is no detectable concentration of lead ions, for example, in plasma. Bone evidently takes up lead more slowly than the soft tissues, and, apart from general decalcification[19] the lead so taken up appears to be indissociable, or to be very much less dissociable than soft tissue lead or dimercaprol-lead. The dimercaprol-lead compound presumably does not react directly with the tissues, and is probably distributed evenly in the extracellular fluids or total body water. If an excess of dimercaprol is not present, the complex will dissociate to some extent, and the lead so freed will be fixed by the tissues and so account for its toxicity. Germuth and Eagle's[1] suggestion that the dimercaprol-lead complex is directly toxic is not supported by any explanation of how it acts, and, if a cyclic compound is formed, it is difficult to visualise any mechanism other than dissociation.

On this hypothesis, in the first few days after a single dose of lead salt, most of the lead is concentrated in those soft tissues which contain most

receptors. Some lead remains free, and as this free lead is partly taken up more or less irreversibly by bone and partly lost in the excreta, the soft-tissue lead dissociates and mostly disappears. Increasing the dose of lead will shift the various equilibria between free lead and fixed lead in the direction of more fixed lead. In tissues with few receptors, the proportion of receptors saturated will approach 100 per cent. and little further increase in concentration of fixed lead will be possible: in those with abundant receptors, the increase will be approximately proportional to the increased amount of lead available to the tissue. This has in fact been observed. When the dose of lead was increased tenfold, the concentration of lead rose only two to fourfold in the tissues with moderate affinity, like blood cells, kidneys and bone, but tenfold or more in the liver and bone marrow, the organs with highest affinity for lead. A slight increase is to be expected in the tissues with low affinity, due to the increase in free lead, but measurements in these tissues (e.g., muscle) are too low to detect accurately any such change.

When a dithiol is given, it combines with free lead and favours dissociation of the soft-tissue-lead. When the concentration of tissue lead is high, as in the liver, the net result is a fall in concentration. Where it is low, as in the heart, the addition of lead combined with dithiol to the free lead already present in the tissue fluids produces an overall increase in the concentration of lead in the organ, whether or not the lead fixed by the receptors of that organ is reduced in quantity. Some dithiol remains uncombined, and is metabolised or excreted in the normal way. As this happens and the concentration of dithiol falls, lead dissociates from its combination with thiol and the consequent increase in free lead in turn raises the quantity of lead fixed by the tissues. More lead is excreted while a dithiol is given than if it is not, and the rise in tissue-lead, after the end of treatment, is to a lower level than would have been present without treatment. Similarly the bone lead in due course reaches a lower final value (e.g., Lusky, Braun and Laug[20]).

In the kidney, the dithiol-lead complex is likely to be filtered by the glomeruli, and reach the renal tubules. These cells have some affinity for heavy metals, and any tendency of the dithiol-lead complex to dissociate will be accelerated by the combination of lead with the tubular cell receptors. As the glomerular filtrate is concentrated, the concentration of lead in it will rise, and the cells will be presented with a higher concentration of lead than cells elsewhere in the body, and the amount of lead in the kidney is likely particularly to rise. This has, in fact, been observed to occur sometimes with dimercaprol[2] though not with the glucoside; and a similar but much more striking increase occurs when acute cadmium poisoning is treated with dimercaprol[21].

The small difference between the toxicity of lead acetate and lead-dimercaprol[1] is easily accounted for on the hypothesis that lead-dimercaprol dissociates *in vivo* and that without an excess of dimercaprol a useful amount of lead is not inactivated. This argument resembles that put forward by Peters and Stocken[22] to account for the behaviour of the dimercaprol-oxophenarsine compound, where the toxicity of the

thioarsenite is actually greater than that of the parent arsenical, but dimercaprol is nevertheless therapeutically effective in excess.

The arguments just put forward can also account for the increase in mortality when dimercaprol is administered to rabbits and mice poisoned by parenteral injections of lead salts. Lead in deposits in the subcutaneous tissues or the peritoneal cavity diffuses slowly into the circulation, and if the concentration of free lead is temporarily lowered, the rate of diffusion is likely to increase. If the free lead concentration then rises again, it will retard but not reverse the diffusion and the net result will be an increased absorption of lead from the depots. There is no *a priori* reason why the additional amount so entering the circulation should not be greater than the quantity of lead excreted by the dithiol, and if it is sufficiently greater it will account for the increased toxicity of dimercaprol in these circumstances.

It is interesting to note that dimercaprol glucoside, which promotes much more excretion of lead than dimercaprol, diminished the mortality of mice poisoned by intraperitoneal injection of lead acetate (Weatherall[23]): in this circumstance presumably the additional amount excreted outweighed the probable increased mobilisation of lead. A demonstration is desirable that dimercaprol increases the mortality in any other circumstance than when there is a depot of lead salts liable to be mobilised in the subcutaneous tissues or peritoneum. Otherwise this increase in mortality is not relevant in the majority of practical therapeutic situations.

The greater efficacy of dimercaprol glucoside in the present experiments is partly attributable to the higher dosage used. If dimercaprol glucoside is distributed in the extracellular fluid rather than in the total body water, as Danielli *et al.*[15] suggest, its concentration in plasma and tissue fluids will be higher than after an equimolar dose of dimercaprol. As a rough guess the total thiol concentration in these experiments might be expected to be above five times greater when the glucoside was used, and this could easily account for an effect on urinary excretion, twice as great as that produced by dimercaprol, without postulating any increased affinity of the glucoside for lead. The surprisingly high potency of dimercaptopropionic acid can partly be explained on the same lines, but it would appear also that it must form a more stable compound with lead, since its volume of distribution is not likely to be smaller than that of dimercaprol glucoside, and it was as effective or more effective in doses about one quarter as large, on a molecular basis. It was much more effective than the thiols with the –SH groups more widely spaced, and in any further search for thiols effective against lead the presence of hydrophilic polar groups appears to be in all respects advantageous. Whether dithiols are as effective as or more effective than other chelating agents, such as ethylenediamine tetra-acetate[24,25] remains for direct comparative trials to establish.

Summary

1. The influence of several dithiols on the acute distribution and excretion of lead salts has been studied in rabbits.

2. Dimercaprol glucoside and 1:2-dimercaptopropionic acid were

substantially more effective than dimercaprol in promoting the excretion of lead in the urine.

3. 1:3-dimercaptopropanol and 1:4-dimercaptoerythritol were not more effective than dimercaprol.

4. After dimercaprol glucoside the lead remaining in the body was distributed more uniformly than in the control animals.

5. After a tenfold increase in the dose of lead inequalities in the distribution were exaggerated. Dimercaprol and dimercaprol glucoside caused a bigger absolute increase in the urinary excretion of lead after this dose than after the smaller dose of lead. Relatively to the dose of lead, they were less effective.

6. The mechanism underlying these changes is discussed and a simple theory is put forward to account for them.

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REFERENCES

1. Germuth and Eagle, *J. Pharmacol.*, 1948, **92**, 397.
2. Ginsburg and Weatherall, *Brit. J. Pharmacol.*, 1948, **3**, 223.
3. Ryder, Cholak and Kehoe, *Science*, 1947, **106**, 63.
4. Telfer, *J. Amer. med. Ass.*, 1947, **135**, 835.
5. Bastrup-Madsen, *Lancet*, 1950, **259**, 171.
6. Vigliani and Zurlo, *Brit. J. industr. Med.*, 1951, **8**, 218.
7. Zavanella, *Med. lavoro*, 1951, **42**, 97.
8. Danielli, Danielli, Mitchell, Owen and Shaw, *Nature, Lond.*, 1946, **157**, 217.
9. Adam, Ginsburg and Weatherall, *Brit. J. Pharmacol.*, 1949, **4**, 351.
10. Weatherall, *J. Pharm. Pharmacol.*, 1949, **1**, 576.
11. Weatherall and Weatherall, *Brit. J. Pharmacol.*, 1949, **4**, 260.
12. Weyrauch, *Z. ges exp. Med.*, 1931, **75**, 706.
13. Kehoe and Thamann, *J. Lab. clin. Med.*, 1933, **19**, 178.
14. Fitzhugh, Woodard, Braun, Lusky and Calvery, *J. Pharmacol.*, 1946, **87**, Suppl. 23.
15. Danielli, Danielli, Fraser, Mitchell, Owen and Shaw, *Biochem. J.*, 1947, **41**, 325.
16. Braun, Lusky and Calvery, *J. Pharmacol.*, 1946, **87**, Suppl. 119.
17. Graham and Hood, *Brit. J. Pharmacol.*, 1948, **3**, 84.
18. Jowett, *Biochem. J.*, 1932, **26**, 2108.
19. Brown, *Quart. J. med.*, 1946, **15**, 77.
20. Lusky, Braun and Laug, *J. industr. Hyg.*, 1949, **31**, 301.
21. Tepperman, *J. Pharmacol.*, 1947, **89**, 343.
22. Peters and Stocken, *Biochem. J.*, 1947, **41**, 53.
23. Weatherall, *Brit. J. Pharmacol.*, 1948, **3**, 137.
24. Belknap, *Industr. Med.*, 1952, **21**, 305.
25. Sidbury, Bynum and Fetz, *Proc. Soc. exp. Biol., N.Y.*, 1953, **82**, 226.