Thermodynamic Considerations in Co-ordination. Part XXII.[1] Sequestering Ligands for improving the Treatment of Plumbism and Cadmiumism

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Potentiometrically determined formation constants are reported for cadmium(II), lead(II), and zinc(II) with ethylenediaminetetra-acetic acid, 1,2-di(2-aminoethoxy)ethanetetra-acetic acid, glutathione, cysteine, and D-penicillamine at 25 °C, I = 3.00M($NaClO_4$). Computer-simulated models of blood plasma conditions were used to examine the complexing competition between cadmium(II) and zinc(II), and lead(II) and zinc(II), with these ligands. It was concluded that glutathione is the most promising ligand for future clinical studies.

In previous studies[2,3] we used computer-simulated models of *in vivo* equilibria to show that treatment for poisoning by lead and cadmium using the calcium sodium salt of ethylenediaminetetra-acetate and for lead poisoning with D-penicillaminate were insufficiently selective, thus depleting the body of essential trace elements (especially zinc), and that only the minor fraction of the pollutant metal could be removed by such drugs, the major part remaining complexed to the sulphydryl groups of proteins, *etc.*

We also stated our case for improved cadmium- and lead-sequestering drugs for patients suffering from metal poisoning and we now report the second phase of our investigations—the choice of available ligands based upon the desirable properties as listed in references 2 and 3.

This paper reports data for the ligands formed by proton ionisation from the following acids: ethylenediaminetetra-acetic acid (edta), 1,2-di-(2-aminoethoxy)-ethanetetra-acetic acid (egta), glutathione (L-γ-glutamyl-L-cysteinylglycine) (gsh), cysteine(cys), D-penicillamine (D-pen), mercaptosuccinic acid, and thioglycolic acid. Protonation constants and cadmium, lead, and zinc formation constants were determined potentiometrically at 25 °C, I = 3.00M($NaClO_4$), and then COMPLOT computer models were used to simulate plasma equilibrium concentrations to establish (i) which ligand complexed the largest percentage of cadmium and lead, (ii) the optimum drug : metal ratio, and (iii) the extent to which some of the essential trace elements require replenishing during therapy as a consequence of their being depleted by the new drug.

EXPERIMENTAL

Materials.—The following compounds were used: disodium ethylenediaminetetra-acetate dihydrate (B.D.H. AnalaR) (Found: C, 32.45; H, 5.2; N, 7.55. Calc. for $C_{10}H_{18}N_2Na_2O_{10}$: C, 32.26; H, 4.87; N, 7.53%); 1,2-di-(2-aminoethoxy)ethanetetra-acetic acid (B.D.H.) (Found: C, 43.45; H, 6.65; N, 7.05. Calc. for $C_{14}H_{24}N_2O_{10}$: C, 44.17; H, 6.36; N, 7.36%); glutathione(Sigma) (Found: C, 38.8; H, 5.75; N, 13.5. Calc. for $C_{10}H_{17}N_3O_6S$: C, 39.10; H, 5.58; N, 13.70%); cysteine (E. Merck A.G.) (Found: C, 29.75; H, 5.9; N, 11.4. Calc. for $C_3H_7NO_2S$: C, 29.73; H, 5.82; N, 11.56%); D-penicillamine (Koch–Light) (Found: C, 40.3; H, 7.8; N, 9.15. Calc. for $C_5H_{11}NO_2S$: C, 40.23; H, 7.43; N, 9.42%).

Preparation and standardisation of perchloric acid and sodium perchlorate were as described in ref. 4. Water was purified as in ref. 5. Cadmium(II) perchlorate solution, lead(II) perchlorate solution, and zinc(II) perchlorate solution were prepared and analysed as in refs. 2, 3. and 5 respectively.

Methods.—The potentiometric approach was as described in ref. 6. All studies were carried out at 25 °C, I = 3.00M ($NaClO_4$).

All sets of data for protonation constants gave superimposable Z/pA curves in our RWZPLOT program. However, this was not the case for the metal–ligand systems. The protonation data was then refined using SCOGS[7] but when the more complicated metal–ligand data were examined it was found impossible to refine together all the necessary constants because of exponent overflow. This problem was circumvented by using the MINIQUAD[8] program.

Our PSEUDOPLOT[9] program was then employed to regenerate theoretical formation curves from the 'best' set of constants thus showing how accurately these constants describe the experimental data. Next, these constants were used in COMPLOT[10,11] to give complex species *versus* pH profiles related to those occurring in blood plasma.

RESULTS AND DISCUSSION

Formation curves were established for each ligand and a range of differing total metal and ligand concentrations. The concentration ranges used were dependent upon the solubility of the complexes formed, only low concentrations being attainable with the lead–cysteine and lead–glutathione systems. Cysteine and mercaptosuccinic and thioglycolic acids with cadmium produced insoluble complexes over a wide pH range at a cadmium concentration as low as 1.0mM and so these systems were not studied further.

None of the metal–ligand systems had superimposable formation curves. Thus the presence of either proton-

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[7] I. G. Sayce, *Talanta*, 1968, **15**, 1397.
[8] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.
[9] A. M. Corrie, G. K. R. Makar, M. L. D. Touche, and D. R. Williams, *J.C.S. Dalton*, 1975, 105.
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ated or hydroxo-complexes (or both) is indicated (see Figure 1 for example). In the case of lead, four hydroxo-

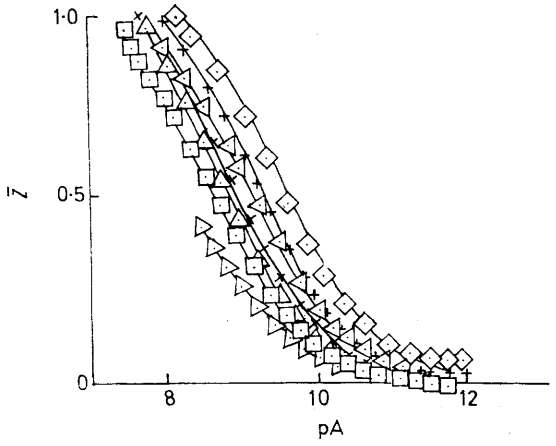

FIGURE 1 PSEUDOPLOT curves for the best set of formation constants for the zinc(II)–glutathione system plotted on the experimental ZPLOT points

lead species [13] had to be included as these had a significant influence upon the sum of the squared residuals. On the other hand, including cadmium and zinc hydroxides made an insignificant least-squares difference and, therefore, these were omitted from the major portion of the study.

Protonation constants are as shown in Table 1. These values are in agreement with those of other workers [14] even though their work was not done at the same temperature and ionic strength as our studies.

Table 2 shows the formation constants obtained for the metal–ligand systems. As a direct result of our 'grid' approach to the potentiometric titrations (*i.e.* working over a range of ligand : metal ratios, 1 : 1 to 8 : 1), a series of non-superimposable formation curves was obtained which was then analysed, using PSEUDOPLOT and MINIQUAD. This demonstrated the presence of several protonated and hydroxo-complexes.

Our constants for the zinc(II)–cysteinate system agree with those of Perrin and Sayce [15] whereas our zinc(II)–penicillaminate constants differ. For the latter system the Canberra researchers found the *110*,* *210*, *211*, and *212* complexes to be present with the *110* being of only minor importance. Our set of constants which excludes the *110* but includes the *21—1*, *430*, and *431* was found to give a significantly lower sum of squared residuals in MINIQUAD and a better PSEUDOPLOT fit.

Our constants for cadmium and zinc with glutathionate are similar to those found by Perrin and Watt,[16] however, we found it impossible, using either SCOGS or MINIQUAD, to obtain either of the *120* complexes. Once again it may be noted that formation constants for a peptide are lower than those of its parent amino-acid anions.[9] The high standard deviations of the hydroxo-species can be attributed to the fact that they

TABLE 1

Log formation constants for ligand protonation at 25 °C, I = 3.00M($NaClO_4$)

	log β_{pqr} [a]				
	101 [a]	*102*	*103*	*104*	n [b]
edta	9.060 ± 0.005	16.100 ± 0.007	18.680 ± 0.017	20.953 ± 0.014	263
egta	9.360 ± 0.014	17.973 ± 0.015	20.970 ± 0.049	23.697 ± 0.039	191
gsh	9.881 ± 0.020	19.043 ± 0.018	22.861 ± 0.019	25.456 ± 0.020	182
cys [12]	10.709 ± 0.030	19.493 ± 0.040	21.933 ± 0.090		
D-pen	11.010 ± 0.008	19.612 ± 0.014	22.044 ± 0.023		160

[a] β_{pqr} refers to the complexes (ligand)$_p$ (metal ion)$_q$ (proton)$_r$ and *101* refers to β_{101}. [b] n = Number of experimental observations.

TABLE 2

Log formation constants for metal–ligand anion complexes at 25 °C, I = 3.00M($NaClO_4$)

	log β_{pqr}									
cadmium(II)	*110*	*111*	*11—1*	*210*	*211*	*212*	*21—1*	*430*	*431*	n
edta	14.677 ± 0.055	17.427 ± 0.032								232
egta	15.020 ± 0.057	18.670 ± 0.055								240
gsh	10.180 ± 0.245	17.024 ± 0.021	0.291 ± 0.631	15.353 ± 0.064	25.086 ± 0.052	33.032 ± 0.040	3.169 ± 5.382			158
D-pen	12.681 ± 0.047	17.152 ± 0.075		20.683 ± 0.057	28.306 ± 0.056	34.533 ± 0.074	9.138 ± 0.079			200
lead(II)										n
edta	15.186 ± 0.078	18.010 ± 0.069								200
gsh	10.567 ± 0.193	17.136 ± 0.034		14.997 ± 0.227	24.664 ± 0.071	32.104 ± 0.111	4.501 ± 1.692			151
cys	12.213 ± 0.016	17.347 ± 0.053		18.571 ± 0.045	27.476 ± 0.043		7.331 ± 0.186			200
D-pen	14.321 ± 0.023	17.723 ± 0.053		19.048 ± 0.050	27.978 ± 0.074	34.035 ± 1.884	7.551 ± 0.087			200
zinc(II)										n
edta	14.873 ± 0.050	17.965 ± 0.034								343
egta	11.485 ± 0.042	17.345 ± 0.032								181
gsh	8.568 ± 0.015	14.762 ± 0.058	−0.074 ± 0.054	13.586 ± 0.098	23.271 ± 0.017	30.616 ± 0.018	3.634 ± 0.267			168
cys				19.394 ± 0.019	25.856 ± 0.058	31.879 ± 0.057		46.247 ± 0.095	52.503 ± 0.102	236
D-pen				20.521 ± 0.019	26.794 ± 0.040	32.724 ± 0.018	8.563 ± 0.057	47.582 ± 0.086	53.826 ± 0.084	198

* Symbols defined in the footnote to Table 1.

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are of only minor importance and only appear at high pH values [Figure 2 (i), (ii), and (iii)]. From our formation constants one might postulate binding sites similar to those suggested by Perrin;[16] however, further information such as enthalpies of complex formation, which are present under investigation, would be useful in confirming these structural suggestions. As is the case with all of the ligands studied, the lead and cadmium glutathione systems are very similar because the divalent cations have similar chemical characteristics.

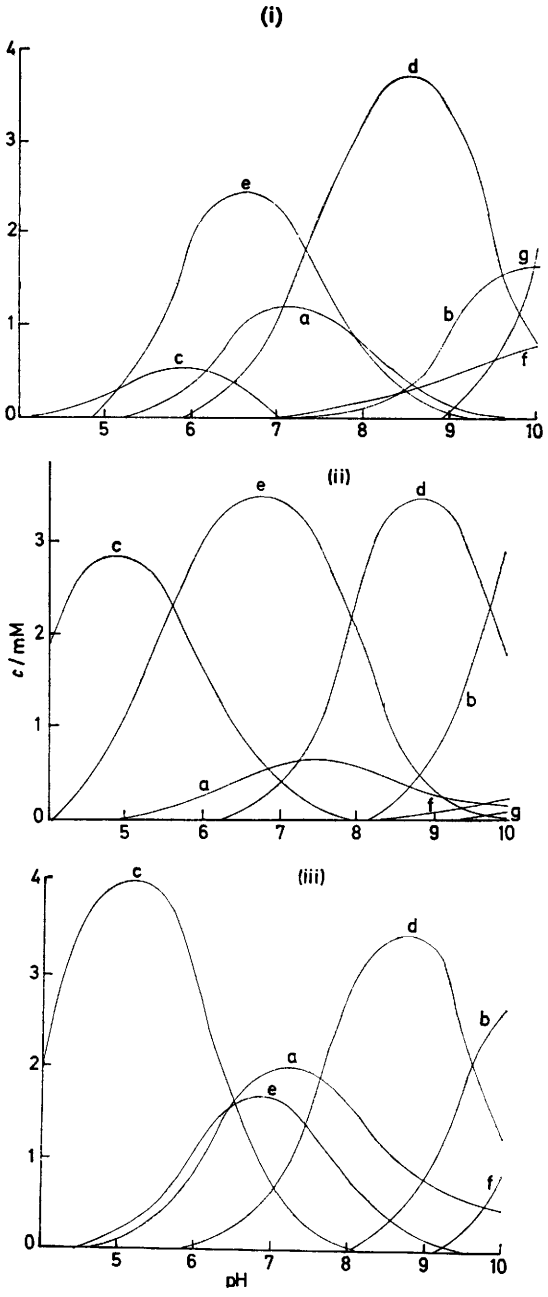

FIGURE 2 (i), (ii), (iii): COMPLOT calculations of the pH dependence for complexes present in (i) the zinc-glutathionate (A)–proton system when A = 10, and Zn = 5 mM; (a), $[ZnA]^-$; (b), $[ZnA_2]^{4-}$; (c), $[Zn(HA)]$; (d), $[ZnA(HA)]^{3-}$; (e), $[Zn(HA)_2]^{2-}$; (f), $[ZnA(OH)]^{2-}$; (g), $[ZnA_2(OH)]^{5-}$: (ii) the cadmium–glutathionate (A)–proton system when A = 10 and Cd = 5 mM; (a), $[CdA]^-$; (b), $[CdA_2]^{4-}$; (c), $[Cd(HA)]$; (d), $[CdA(HA)]^{3-}$; (e), $[Cd(HA)_2]^{2-}$; (f), $[CdA(OH)]^{2-}$; (g), $[CdA_2(OH)]^{5-}$; and (ii) the lead–glutathionate–proton system when A = 10 and Pb = 5 mM; (a), $[PbA]^-$; (b), $[PbA_2]^{4-}$; (c), $[Pb(HA)]$; (d), $[PbA(HA)]^{3-}$; (e), $[Pb(HA)_2]^{2-}$; (f), $[PbA_2(OH)]^{5-}$

The data for the metal complexes of $edta^{4-}$ and $egta^{4-}$ indicate the presence of the *110* and *111* complexes. However, it was found that the PSEUDOPLOTs obtained using these constants are not a very good fit for the experimental curves and this suggested that some other species may be present. For the zinc(II)–$edta^{4-}$ system it was found possible to obtain a string of polynuclear complexes of the form $x(x + 1)0$, but, as these made no difference to the PSEUDOPLOT fits and gave only a marginal improvement in the sum of squared residuals, it was felt that their inclusion was unjustified.

The absence of the *110* complex in the zinc(II)–penicillaminate case accounts for the disparity between the $\Delta\log\beta_{Cd-Zn}$ and $\Delta\log\beta_{Pb-Zn}$ figures in Table 3 and the quantity of cadmium and lead complexed by penicillaminate as shown from our COMPLOT models, some values of which are shown in Table 4.

From the values of $\Delta\log\beta_{Cd-Zn}$ and $\Delta\log\beta_{Pb-Zn}$ in Table 3, it appeared that glutathione would be effective for the complexing of cadmium and lead *in vivo*. COMPLOT models over a range of total cadmium and lead concentrations and at varying glutathione : metal ratios with zinc held constant at its blood plasma concentration (45.88 μM) showed that the most effective ratio for the removal of these polluting metals was 2 : 1 (see Table 5 and Figure 3), higher ratios removing more of the essential zinc. Further models have shown that, for lead, cysteine should be as effective as D-penicillamine and, for cadmium, egta should be much better than edta. The COMPLOTs also show that D-penicillamine ought to be an effective sequestering agent for cadmium but

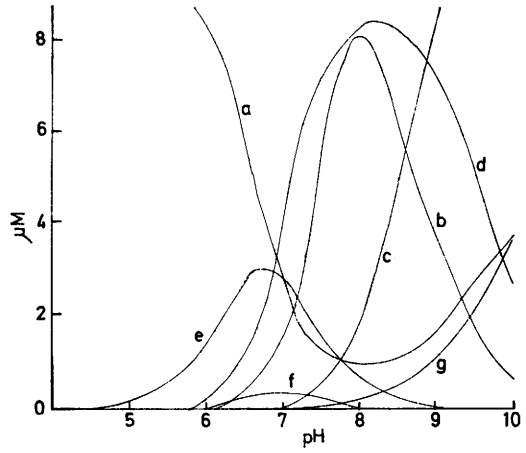

FIGURE 3 COMPLOT calculation of the pH dependence for complexes present in the cadmium–zinc–glutathionate (A)–proton system when Cd = 10, Zn = 45.88, and A = 20.00 μM: (a), Cd^+; (b), $[ZnA]^-$; (c), $[ZnA(OH)]^{2-}$; (d), $[CdA]^-$; (e), $[Cd(HA)]$; (f), $[CdA(OH)]^{2-}$; (g), $[CdA_2(OH)]^{5-}$

clinical trials have shown that it does not promote urinary excretion of cadmium.[17]

Although these computer simulated ' caricatures ' are a gross simplification of the situation *in vivo* there are two important factors encouraging their use: (i) they do narrow the field from random screening down to ' key ' molecules for animal studies, and (ii) many of the complicating factors, for example the presence of sophisticated ligands such as human serum albumin, have been demonstrated to have only a very small effect upon a copper–zinc–amino-acid model.[18]

TABLE 3

$\Delta\log\beta_{Pb-Zn}$ ($\log\beta_{Pb} - \log\beta_{Zn}$) and $\Delta\log\beta_{Cd-Zn}$ for the *110* and *210* complexes of edta, egta, gsh, cys, and D-pen

	edta		egta		gsh		cys		D-pen	
	110	*210*	*110*	*210*	*110*	*210*	*110*	*210*	*110*	*210*
$\Delta\log\beta_{Pb-Zn}$	0.312				1.999	1.411		0.824		−1.472
$\Delta\log\beta_{Cd-Zn}$	−0.062		3.534		1.612	0.591				0.162

TABLE 4

COMPLOT models showing percentages (i) of zinc and lead and (ii) of zinc and cadmium complexed by a range of ligands at pH 7.4

Total concs:
Zn^{2+} = 45.88 μM, Pb^{2+} = 10.00 μM, ligand = 20.00 μM;
Zn^{2+} = 45.88 μM, Cd^{2+} = 10.00 μM, ligand = 20.00 μM

	edta	egta	gsh	cys	D-pen
(i) % Zn complexed	33		14	9	10
% Pb complexed	50		94	100	100
(ii) % Zn complexed	36	20	14		10
% Cd complexed	34	100	89		99

TABLE 5

COMPLOT models showing the percentages (i) of zinc and lead and (ii) of zinc and cadmium complexed by glutathione at pH 7.4 over a range of total polluting metal concentrations and a range of gsh : metal ratios. (with total zinc concentration constant at its blood plasma level of 45.88 μM)

gsh : metal	2 : 1				1 : 1	3 : 1
total polluting metal conc.	3.0 μM	7.0 μM	10.0 μM	15.0 μM	10 μM	10 μM
(i) % Zn complexed	5	10	14	19	3	25
% Pb complexed	83	92	94	96	46	97
(ii) % Zn complexed	5	10	14	19	4	24
% Cd complexed	72	84	89	92	68	94

Whichever of these ligands is used clinically, a small amount of zinc would also be sequestered from the body and it would clearly be preferable to replace it even though the form in which this zinc supplementation is best administered is still under investigation. Computer simulation can easily optimise the quantity of zinc to be introduced.

Thus, our equilibrium study has suggested glutathione and cysteine as possible selective drugs for cadmium or for lead poisoning. Furthermore, there are additional factors favouring the former ligand. (i) Glutathionate fits the criteria (i)—(iv) as listed in ref. 2. (ii) All donor groups of the glutathionate anion cannot be bound to the metal at the same time and so those which are free increase the hydrophilicity and help to keep the complexes in solution. (iii) Studies have been done which show that blood glutathione levels are lowered in lead poisoning.[19]

Conclusions.—From our study it would appear that glutathione and cysteine ought to be effective for the removal of cadmium and lead provided that they can be kept in the reduced form. This may well be achieved by administering the ligands along with a physiologically acceptable reducing agent such as ascorbic acid. Further, since these are naturally occurring ligands, their degradation and excretion should present little problem to the body. (We have recently determined LD_{90} values in mice and our observations support this hypothesis.)

However, as has been noted for the cadmium penicillamine case,[17] one can not *always* extrapolate *in vitro* calculations to the clinical treatment situation. Therefore we feel that clinical trials would be of interest on the glutathione and cysteine systems.

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