Thermodynamic Considerations in Co-ordination. Part XIV.[1] Formation Constants for Lead(II)–Amino-acid Complexes and their Use in Computing the Complexing Competition between Lead(II) and *in vivo* Essential Metal Ions, and in Computer Evaluation of Ligands currently employed as Lead(II) Chelating Therapeuticals

By **Anna M. Corrie, Murray L. D. Touche,** and **David R. Williams,*** Department of Chemistry, University of St Andrews, Fife, Scotland KY16 9ST

Potentiometrically determined formation constants are reported for the lead(II) asparaginate, aspartate, cysteinate, glutaminate, histidinate, phenylalanate, serinate, and tryptophanate systems at 25°, I = 3·00M $(Na^+)ClO_4^-$. Computer simulated models of blood plasma conditions were used to examine (a) the complexing competition between lead(II) and manganese(II), iron(II), cobalt(II), copper(II), and zinc(II)–amino-acid anion complexes, and (b) to assess the selectivities of EDTA and D-penicillamine for lead(II) and the *in vivo* essential amino-acids listed. The more important conclusions under (a) were that the lead(II) complexes the cysteinate ligand at the expense of the zinc(II)–cysteinate system and then produces increased concentrations of other zinc complexes, and under (b) that more specific drugs may be synthesized as peptides derived from L-cysteine, histidine, and aspartic acid.

MAN is becoming increasingly exposed to lead poisoning and so the average concentration of lead in blood plasma is slowly approaching the clinical behavioural threshold concentration. Cases of accidental lead poisoning in industrial workers, or in children, have usually had several months of lead uptake before the first symptoms are noticed. Thus, in comparison to the low concentrations of *in vivo* essential transition metals in plasma, relatively large quantities of lead must sometimes be removed. Illustrative figures for an average individual are daily intakes of 300 μg (intestinal) and 5—50 μg (respiratory). Poisoned individuals take in far more, it being possible to diagnose plumbism at 2·9 μM and above in blood plasma. Under these latter conditions, amino-acids are lost from the body.

It is highly relevant to measure the formation constants between lead(II) and representative amino-acid anions and to compare the metal ion's binding potential with those for the *in vivo* essential transition metal ions. Previous parts of this series have reported the formation constants for manganese(II), iron(II), cobalt(II), copper(II), and zinc(II) with asparaginate, aspartate, cysteinate, glutaminate, histidinate, phenylalanate, serinate, and tryptophanate at 25·0° and I = 3·00 M $Na^+ClO_4^-$;[1-6] these latter conditions being chosen since it has been shown that they effectively hold activity coefficients constant in spite of large changes in the concentrations of charges present. For reasons of direct comparison, we have measured the lead constants under the same experimental conditions.

The accepted treatment of plumbism employs 'calcium disodium edetate' ($CaNa_2EDTA$) and D-penicillamine, $[(CH_3)_2{\cdot}C(SH){\cdot}CH(NH_2)]{\cdot}COOH$, 'Cuprimine' or 'Distamine'). Adequate formation constants

[1] Part XIII, D. R. Williams, *J.C.S. Dalton*, 1973, 1064.
[2] D. R. Williams, *J. Chem. Soc.* (*A*), 1970, 1550.
[3] D. R. Williams, *J.C.S. Dalton*, 1972, 790.
[4] R. D. Graham, D. R. Williams, and P. A. Yeo, *J.C.S. Perkin II*, 1972, 1876.
[5] D. R. Williams and P. A. Yeo, *J.C.S. Dalton*, 1972, 1988.
[6] A. C. Baxter, unpublished results.

for their lead and other metal complexes are available from the literature.[7]

This research used Sillén's HALTAFALL program [8] to compute models of the equilibria involved in blood plasma. First, we established which amino-acids were most strongly complexed to lead. Secondly, we determined the extent of the interference caused by lead to the essential metal ion–amino-acid complexes; and finally, the biological effectiveness of penicillamine and EDTA was examined.

EXPERIMENTAL

The suppliers, purification, and analyses of the L-amino-acids used have been previously reported.[1-5]

Perchloric acid, sodium perchlorate, and sodium hydroxide were prepared as in ref. 9. Lead(II) perchlorate was prepared by dissolving lead oxide in perchloric acid (60%) and filtering. Analysis for lead(II) was by titration against EDTA (Xylenol orange) and gravimetrically as molybdate. The mineral acid content of the stock solution was determined by Gran titrations. Water was purified as in ref. 5.

Formation Constants.—Potentiometric data were obtained using our usual glass electrode approach,[2,9] and when combined with our previously reported protonation constants for the amino-acid anions gave superimposable formation curves as plotter output from our RWZPLOT program (with the exception of the aspartate system which formed a protonated metal complex). This data was then used in the SCOGS program [10] and the 'best' fits were produced by the formation constants given in Table 1. These constants and those for manganese, iron, cobalt copper, and zinc(II)–amino-acid anion complexes were then used in the HALTAFALL computed models for blood plasma.

RESULTS AND DISCUSSION

The anions of asparagine, glutamine, phenylalanine, serine, and tryptophan form mono-, bis-, and tris-complexes with lead(II). Formation curves were established for each ligand for a range of differing total metal and ligand concentrations. With the exception of aspartic acid, each ligand had superimposable curves (*i.e.* protonated and hydroxy-complexes could be assumed to be absent). The relative positions of these formation curves are shown in Figure 1. In general, these curves often tended to level off at $\bar{Z} = 1$ and to straddle the formation curves of the comparable manganese, iron, cobalt, and zinc systems.

Aspartate first gave a mono complex, Pb·asp, and then a protonated mono-complex Pb·asp·H^+, in preference to forming bis- and tris-complexes. The cysteinate and histidinate mono-complexes of lead were also reluctant to form bis-complexes. A possible explanation of the persistence of 1 : 1 complexes and of the curve straddling is that lead(II) may be either four- or eight-co-ordinate in solution; tridentate ligands such as aspartate, cysteinate, or histidinate arrest the complexing when three of the four tetrahedral bonds are occupied whereas the other amino-acids studied are bidentate and, presumably, cause lead to exhibit its higher co-ordination number of eight (cubic, square antiprism, or triangulated dodecahedron). Although four- and eight-co-ordinate lead(II) has previously been reported for hydroxy-complexes [$Pb_4(OH)_4^{4+}$ and $Pb_6(OH)_8^{4+}$ from Raman spectra and *X*-ray scattering work [11]] our work is the first to suggest that both types of complexes occur for amino-acids and is also the first report of tris amino-acid

TABLE 1

Log formation constants for lead(II)–amino-acid anion complexes at 25°, I = 3·00M $NaClO_4$

	log β_{pqr} [a]					
	110	210	310		n [b]	Other workers, I, T log β values
Asn	4·914 ± 0·018	7·815 ± 0·030	8·815 ± 0·318		91	β_{110} = 4·36, β_{210} = 6·23, 1·0M-KNO_3, 30° [c]
Asp	6·668 ± 0·044	(9·433 ± 0·394)		β_{111} = 12·277 ± 0·019	136	β_{110} = 5·88, β_{210} = 7·38 1·0M-KNO_3, 30° [d] **β_{110} = 6·02, β_{210} = 8·18,** 0·30M-$NaClO_4$, 25° [e]
Cys	13·163 ± 0·120	19·203 ± 0·152	22·470 ± 0·379		72	β_{110} = 11·39, 0·1M-KNO_3, 25° [f] β_{110} = 12·75, 0·15M-KNO_3 25° [g]
Gln	4·697 ± 0·080	8·364 ± 0·144	10·123 ± 0·854		78	
His	6·903 ± 0·004	9·806 ± 0·098			192	β_{110} = 5·96, 0·15M-KNO_3, 37° [h] β_{110} = 6·84, 0·15M-KNO_3, 25° [g]
Phe	4·628 ± 0·042	8·353 ± 0·056			86	
Ser	5·054 ± 0·026	8·265 ± 0·060	9·957 ± 0·145		104	
Trp	4·888 ± 0·245	10·271 ± 0·097			62	
EDTA	16·8 [i]					0·1M-KNO_3, 30° [j]
D-penicillamine	13·0 [i]					0·15M-KNO_3, 25° [k]

[a] β_{pqr} refers to the complexes (ligand)$_p$(metal ion)$_q$(proton)$_r$. [b] n = number of experimental observations. [c] G. N. Rao and R. S. Subrahmanya, *Proc. Indian Acad. Sci.*, 1964, **60**, 165, 185. [d] G. N. Rao and R. S. Subrahmanya, *Current Sci.*, 1962, **31**, 55. [e] M. Kodama and S. Takahashi, *Bull. Chem. Soc. Japan*, 1971, **44**, 697. [f] G. R. Lenz and A. E. Martell, *Biochemistry*, 1964, **3**, 745. [g] Ref. 12. [h] D. D. Perrin and V. S. Sharma, *J. Chem. Soc.* (*A*), 1967, 724. [i] Constants selected from literature. [j] V. L. Hughes and A. E. Martell, *J. Phys. Chem.*, 1953, **57**, 694. [k] E. J. Kuchinos and Y. Rosen, *Arch. Biochem. Biophys.*, 1960, **97**, 370.

[7] 'Stability Constants of Metal-ion Complexes,' eds. L. G. Sillén and A. E. Martell, Chem. Soc. Special Publ., Nos. 17(1964) and 25(1971).

[8] N. Ingri, W. Kakołowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261.

[9] A. D. Jones and D. R. Williams, *J. Chem. Soc.* (*A*), 1970, 3138.

[10] I. G. Sayce, *Talanta*, 1968, **15**, 1397.

[11] V. A. Maroni and T. G. Spiro, *J. Amer. Chem. Soc.*, 1967, **89**, 45; *Inorg. Chem.*, 1968, **7**, 188.

lead(II) complexes in aqueous solution (Pb^{II}–Asn, Cys, Gln, Phe, Ser, Trp systems).

Evidence for some ligands being tridentate to lead(II) and an estimate of the magnitude of the increment in bond strengths between bi- and tri-dentate ligands may be seen (*a*) in a comparison of log β_{110} values for asparaginate and aspartate (**4·91** and **6·67** respectively), and (*b*) in the relative positions of the formation curves for cysteinate, histidinate and aspartate in Figure 1 (*i.e.* Pb binds $RS^- \gg \backslash N/ > -COO^-$).

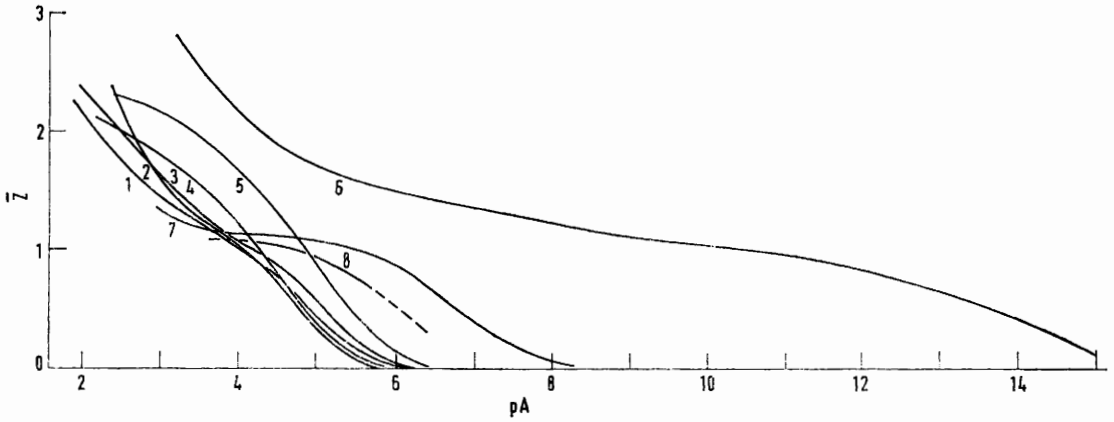

FIGURE 1 Relative positions and shapes of lead(II)–amino-acid anion formation curves for the eight systems studied. These curves are calculated from the constants given in Table 1. Key: 1, asparagine; 2, glutamine; 3, serine; 4, phenylalanine; 5, tryptophan; 6, cysteine· 7. histidine; 8, aspartic acid

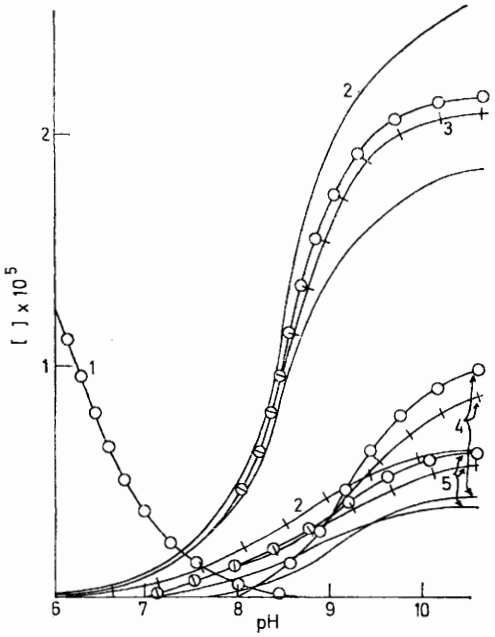

FIGURE 2 HALTAFALL model (i). Total lead(II) = 0 (——○——), 10 (—|—), and 50 (———) μM. The Mn^{II} and Co^{II} complexes are present in the concentration range 10^{-7}—10^{-9}M and so are not plotted. Key: 1, Cu–serinate; 2, Pb–serinate; 3, Zn–serinate; 4, $Zn(serinate)_2$; 5, Fe–serinate

However, the exceptionally high value of log β_{110} for Pb^{II}–cysteinate cannot be explained solely as the difference in bond strengths between bi- and tri-dentate ligands. Li and Manning [12] have also investigated this system and pointed out that the complexing order $Pb^{II} > Cd^{II} > Zn^{II}$ for cysteinate parallels that for metal ion sulphydryl group bonding in bovine serum albumin complexes.[13] (Other amino-acid systems follow the order $Zn^{II} > Pb^{II} > Cd^{II}$.) It is noteworthy that, in both instances, the sulphur ligands could be described by Jorgensen as non-innocent, *i.e.* likely to alter the oxidation state of the metal ions, and hence to increase the Pb–RS^- bond strengths.[14]

Computer Evaluation of the Biological Effectiveness of Ligands used in Treating Plumbism.—A large value of β does not necessarily mean that a chelating drug will be effective under biological competition conditions. For example, Perrin and Agarwal have noted that although $HgEDTA^{2-}$ has a larger β than $ZnEDTA^{2-}$, administering EDTA removes the essential zinc from the body rather than the polluting Hg^{2+}.[15] Fortunately, computer programs can assist us by calculating models of the *in vivo* complexing competition between essential metal ions and ligands and indicating the influence of the polluting metal ion (Pb^{II}) and the effect of a variety of ligands suggested as therapeuticals.

Current ideas concerning the mechanism of lead poisoning are that Pb^{II} displaces essential transition metal ions from biologically important groups (predominantly the sulphydryl groups of enzymes) thus causing a biochemical lesion. (Such a lead lesion is known to inhibit the biosynthesis of haem.) Complexing drugs must be carefully selected to remove the Pb^{II} from the site it is blocking and yet not complex the essential metal ions required by that site, *i.e.* they must be very lead-specific.

Three models were set up using the HALTAFALL

[12] N. C. Li and R. A. Manning, *J. Amer. Chem. Soc.*, 1955, **77**, 5225.

[13] I. M. Klotz, J. M. Urquhart, and H. A. Feiss, *J. Amer. Chem. Soc.*, 1952, **74**, 5537.

[14] C. K. Jorgensen, ' Structure and Bonding,' Springer, Berlin, 1966, **1**, 234.

[15] D. D. Perrin and R. P. Agarwal, ' An Introduction to Bioinorganic Chemistry,' ed., D. R. Williams, Chapman and Hall, 1974, at press.

program: (i) A representative amino-acid (serine) complexing with Mn^{II}, Fe^{II}, Co^{II}, Cu^{II}, and Zn^{II} of total concentration conditions as in Table 2. The concentration

TABLE 2

Total concentrations for blood plasma models [a]

Component	μM
Asn	43·9
Asp	2·2
Cys	94·7—121·3
Gln	62·3
His	74·1—88·9
Phe	50·8—59·9
Ser	106·5
Trp	54·3—62·2
Mn	0·73
Fe	23·3
Co	0·63—2·82
Cu	18·25
Zn	45·88
Pb	2·89 [b]

[a] Figures from 'Biochemists' Handbook,' ed., C. Long, Spon, London, 1961. [b] Lower limit, 2·89 μM is the diagnostic threshold.

of each complex present at a given pH was computed with total $Pb^{II} = 0$, 1×10^{-5}, and 5×10^{-5}M, and the results plotted in Figure 2 show quite clearly that the Pb^{II} complexes predominantly at the expense of the Zn^{II} complexes.

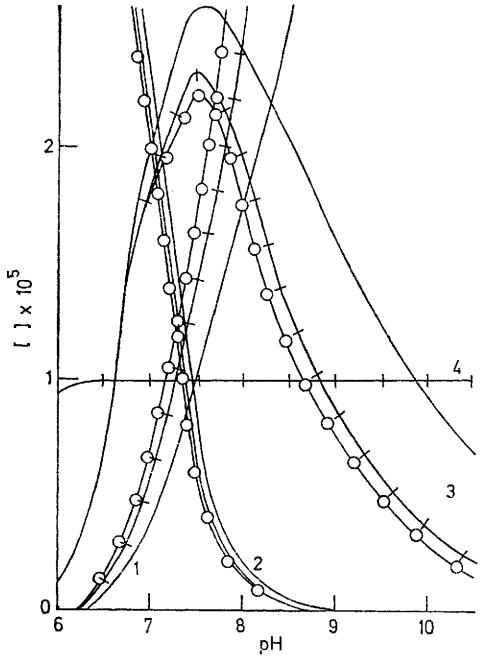

FIGURE 3 HALTFALL model (ii). Concentrations of aspartate, cysteinate, histidinate, and serinate–Zn^{II} and Pb^{II} complexes for total $Pb^{II} = 0$ (—○—), 10 (—|—), and 50 (———) μM. Only those complexes present in concentrations greater than 10^{-6}M are shown. (Pb–cysteinate at 50 μM is off scale of graph). Key: 1, Zn–cysteinate; 2, Zn^{II}; 3, Zn–histidinate; 4, Pb–cysteinate

(ii) Several amino-acids complexing with Zn^{II} and total Pb^{II} as in (i). In the light of the positions of the formation curves in Figure 1 and our previous remarks concerning the tridentate nature of the cysteinate to Pb^{II} it was not surprising that the predominant zinc complex robbed by the lead was zinc cysteinate (Figure 3). It was remarkable that the zinc, when relieved of its role of complexing cysteinate, then complexed the

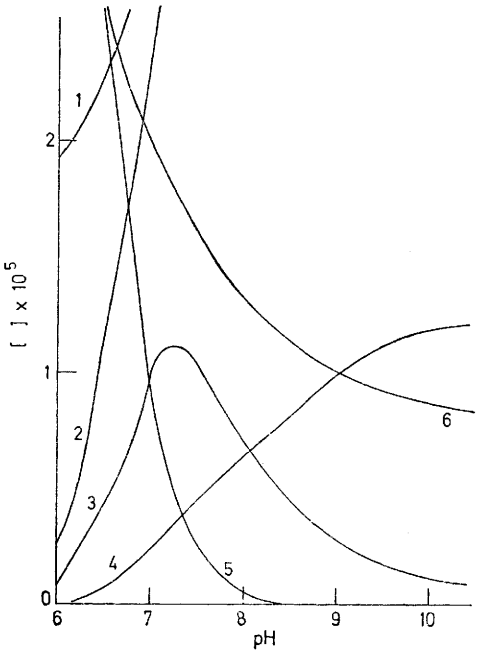

FIGURE 4 HALTAFALL model (iii) Zn^{II}, Pb^{II}–cysteinate, histidinate, and penicillaminate complexes present for concentrations as in Table 2 and total $Pb^{II} = 50$ μM, total penicillamine = 50 μM. Key: 1, Pb–cysteinate; 2, Zn–penicillaminate; 3, Zn–histidinate; 4, Zn–cysteinate; 5, Zn^{II}; 6, Pb–penicillaminate

other amino-acids more extensively and thus increased their equilibrium concentrations.

(iii) Cysteinate–histidinate–Zn^{II}–Pb^{II}–penicillamine (or EDTA). Total Pb^{II} as in (i); total drug 0, 5×10^{-5}, and 1×10^{-4}M. Representative results are plotted in Figures 3—5. Salient features are as follows. (a) In the absence of the drug (Figure 3) as the extent of lead pollution increased the lead is progressively complexed by cysteinate and the displaced zinc produces higher concentrations of other complexes. (b) When penicillamine is present (Figure 4) the zinc penicillamine complex occurs at a high concentration and so the zinc cysteinate and histidinate complexes are correspondingly lower than they are before the drug is administered. (c) The lead penicillamine complex occurs as a high concentration but decreases as the pH is raised since the lead now becomes complexed by cysteinate. (d) EDTA distribution plots (Figure 5) display approximately the same pattern as the zinc–lead penicillamine, *i.e.* the therapeutic system is seriously challenged by cysteinate (or its equivalent in *in vivo* sulphydryl groups).

CONCLUSIONS

Our findings that lead(II) can form tris-complexes with bidentate ligands suggests that a multidentate drug superior in selectivity to penicillamine might be found. Although mixed ligand complexes (ternary complexes)

have not been discussed in this paper it is an accepted fact that the effect of such complexes would be to increase the quantity of lead complexed. Thus, it

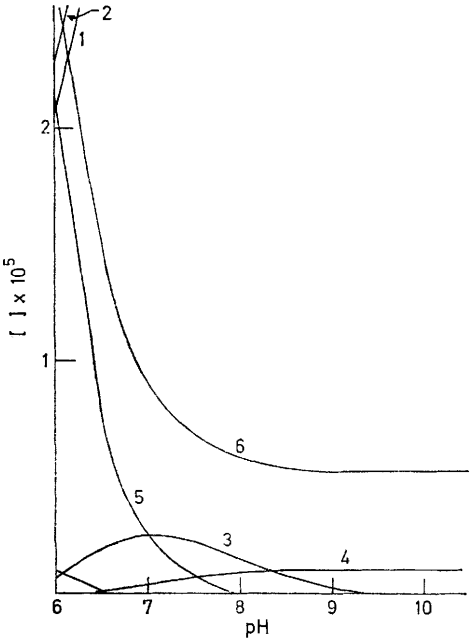

FIGURE 5 HALTAFALL model (iii) having penicillamine replaced by EDTA (10 μM). Key: as Figure 4 but for penicillamine read EDTA

appears that a ligand might be designed such that it could successfully compete with the *in vivo* ligands already sequestering Pb^{II}; the ligand being designed to have the following properties: (i) The ligand is to be a polypeptide being multidentate. (ii) Its amino-acid residues being mixed and selected from cysteine, histidine, and aspartic acid. (iii) These amino-acid components are of the L configuration so that, when degraded, they are non-toxic to the patient.[15,16] (iv) Such a polypeptide might sequester Pb^{II} in a fairly large cavity, the component amino-acid residues of which are sufficiently flexible to permit the ligand to approach and extract the lead in its sequestered position. It is noteworthy that factors (i)—(iv) can be investigated further *in vitro* without recourse to animal experiments. (Although these latter are very necessary to establish the toxicity, specificity, and dose–response relationships when a peptide is eventually selected).

Computer based models are only successful in so far as they suggest new experimental directions for research. This study has suggested a need for (i) β values for cysteine–histidine–aspartic acid peptide–Pb^{II} interactions, (ii) crystallographic studies on Pb^{II} amino-acid and peptide systems, (iii) more computer model studies to quantify the necessity to increase, or reduce, the body contents of the essential metal ions whose complex concentrations are affected by ligand therapy, and (iv) a keener awareness that high concentrations of essential metal complexes in plasma may actually be symptomatic of metal pollution.

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[3/647 *Received*, 28*th March*, 1973]

[16] D. R. Williams, *Inorg. Chim. Acta Rev.*, 1972, **6**, 123.