# Metal-ion Speciation in Blood Plasma as a Tool for elucidating the *in vivo* Behaviour of Radiopharmaceuticals containing $^{153}Sm$ and $^{166}Ho$

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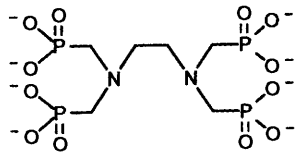
In order to elucidate the *in vivo* behaviour of $Sm^{III}$ and $Ho^{III}$, complex-formation constants for a range of blood plasma metal ions as well as these with the octaanion ethylenediaminetetramethylenephosphonate (edtmp) have been measured at 37 °C and $I$ = 150 mmol $dm^{-3}$. The speciation in the absence and presence of edtmp and/or transferrin was calculated by the program ECCLES and is used to explain the apparently anomalous discrepancies in the uptake of the two metals by bone in a baboon model. The computer results predicted the mobilisation of $Zn^{II}$ and, when tested clinically, it was found that edtmp indeed removes this ion from blood plasma.

During clinical trials in South Africa by a multi-disciplinary task group of which the Atomic Energy Corporation is a member,[1] gratifying results have been achieved in pain palliation therapy in patients having metastatic bone cancer using $^{153}Sm$ and the bone-seeking octaanion ethylenediaminetetramethylenephosphonate (ethylenedinitrilotetramethylenetetraphosphonate, edtmp). Samarium-153 has a half-life of 46.75 h and emits β-particles with maximum energies of $1.1 \times 10^{-13}$ and $1.3 \times 10^{-13}$ J. Selective uptake of $^{153}Sm$ by tumours was achieved. Patients were divided into three groups and received either 30, 60 or 120 MBq $kg^{-1}$. Considerable pain relief was achieved with the average visual pain level (on a scale from one to ten) being reduced from eight to three. Pain palliation lasts an average of 9 weeks.



edtmp

In order to achieve longer periods of pain relief, it was thought that other lanthanide isotopes which emit more energetic β-particles, such as $^{166}Ho$ (half-life = 26.8 h, maximum β-particle energy = $3.0 \times 10^{-13}$ J) could be used in conjunction with edtmp. Accordingly, biodistribution studies on baboons were performed at the H. A. Grové Research Centre in Pretoria. Baboon physiology remarkably resembles that of humans.[2] It was found that the uptake of $^{166}Ho$ by bone was poor in comparison with $^{153}Sm$.[3] A possible reason is that edtmp cannot compete as efficiently for $Ho^{III}$ with respect to other compounds present in blood plasma. We measured the complex-formation constants of edtmp with a number of trivalent lanthanide metal ions[4] and found a maximum at $Gd^{III}$. Thereafter, as the ionic radius decreased the complexes became less stable. This phenomenon was attributed to steric problems caused by the large number of negatively charged oxygens brought into close proximity with one another by the complexation of edtmp with smaller metal ions.

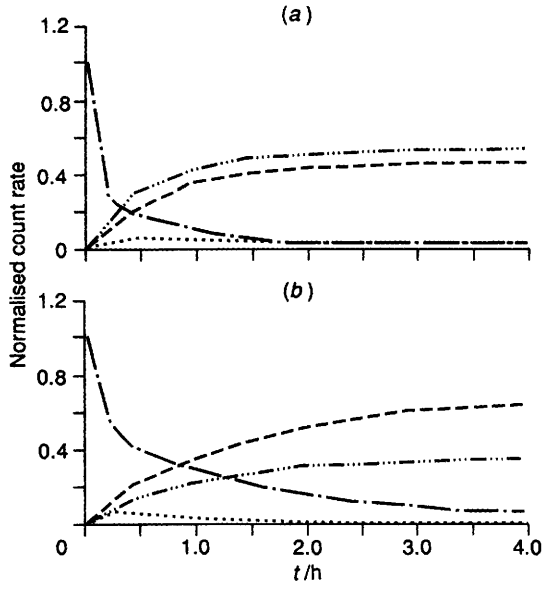



**Fig. 1** Biodistribution *versus* time for $^{153}Sm$ (*a*) and $^{166}Ho$ (*b*) and edtmp in baboons. Data taken from ref. 3: — · —, blood; · · ·, kidney; — · · · —, bone; – – –, urine

Compartmental models based on biodistribution studies in the baboon work have been established for $^{153}Sm$ and $^{166}Ho$.[3] Biodistribution data are illustrated in Fig. 1. The plots for $^{153}Sm$–edtmp in Fig. 1 closely resemble data, shown in Fig. 2, collected on rats.[5] In order to understand better the *in vivo* behaviour of the lanthanides complexed to bone-seeking ligands, we decided to employ the blood-plasma model of May[6] which is contained in the computer program ECCLES. The results reported in this paper show that the predictions of ECCLES may be reconciled with clinical data. This allows insight into ligand design for $Ho^{III}$ as well as predictions to be made about the *in vivo* behaviour of the metal ions with new ligands. The obvious advantage is that animal tests may be

**Table 1** Protonation and formation constants for edtmp (L) determined in this study at 37 °C and $I$ = 150 mmol dm$^{-3}$ NaCl

| Equilibrium * | log $K$ | Number of data points | Hamilton $R$ factor | Equilibrium * | log $K$ | Number of data points | Hamilton $R$ factor |
|---|---|---|---|---|---|---|---|
| $H + L \rightleftharpoons HL$ | 10.67 ± 0.01 | 697 | 0.0071 | $Co + L \rightleftharpoons CoL$ | 13.95 ± 0.01 | 320 | 0.015 |
| $H + HL \rightleftharpoons H_2L$ | 9.47 ± 0.01 | | | $CoL + H \rightleftharpoons Co(HL)$ | 8.18 ± 0.01 | | |
| $H + H_2L \rightleftharpoons H_3L$ | 7.63 ± 0.01 | | | $Co(HL) + H \rightleftharpoons Co(H_2L)$ | 6.20 ± 0.01 | | |
| $H + H_3L \rightleftharpoons H_4L$ | 6.31 ± 0.01 | | | $Co(H_2L) + H \rightleftharpoons Co(H_3L)$ | 5.29 ± 0.01 | | |
| $H + H_4L \rightleftharpoons H_5L$ | 5.08 ± 0.01 | | | $Co(H_3L) + H \rightleftharpoons Co(H_4L)$ | 3.97 ± 0.03 | | |
| $H + H_5L \rightleftharpoons H_6L$ | 2.83 ± 0.01 | | | $Cu + L \rightleftharpoons CuL$ | 16.20 ± 0.01 | 445 | 0.010 |
| $H + H_6L \rightleftharpoons H_7L$ | 1.24 ± 0.04 | | | $CuL + H \rightleftharpoons Cu(HL)$ | 7.91 ± 0.01 | | |
| $Ni + L \rightleftharpoons NiL$ | 11.76 ± 0.02 | 405 | 0.017 | $Cu(HL) + H \rightleftharpoons Cu(H_2L)$ | 6.34 ± 0.01 | | |
| $NiL + H \rightleftharpoons Ni(HL)$ | 8.80 ± 0.02 | | | $Cu(H_2L) + H \rightleftharpoons Cu(H_3L)$ | 4.89 ± 0.01 | | |
| $Ni(HL) + H \rightleftharpoons Ni(H_2L)$ | 7.77 ± 0.02 | | | $Cu(H_3L) + H \rightleftharpoons Cu(H_4L)$ | 4.15 ± 0.01 | | |
| $Ni(H_2L) + H \rightleftharpoons Ni(H_3L)$ | 5.68 ± 0.02 | | | $Mg + L \rightleftharpoons MgL$ | 5.49 ± 0.01 | 348 | 0.0080 |
| $Ni(H_3L) + H \rightleftharpoons Ni(H_4L)$ | 4.09 ± 0.03 | | | $MgL + H \rightleftharpoons Mg(HL)$ | 9.21 ± 0.01 | | |
| $Zn + L \rightleftharpoons ZnL$ | 13.16 ± 0.02 | 311 | 0.018 | $Mg(HL) + H \rightleftharpoons Mg(H_2L)$ | 8.63 ± 0.01 | | |
| $ZnL + H \rightleftharpoons Zn(HL)$ | 9.09 ± 0.02 | | | $Mg(H_2L) + H \rightleftharpoons Mg(H_3L)$ | 7.06 ± 0.02 | | |
| $Zn(HL) + H \rightleftharpoons Zn(H_2L)$ | 6.76 ± 0.02 | | | $MgL + OH \rightleftharpoons MgL(OH)$ | 2.34 ± 0.02 | | |
| $Zn(H_2L) + H \rightleftharpoons Zn(H_3L)$ | 5.17 ± 0.02 | | | $Sm + L \rightleftharpoons SmL$ | 14.44 ± 0.02 | 155 | 0.012 |
| $Zn(H_3L) + H \rightleftharpoons Zn(H_4L)$ | 4.30 ± 0.02 | | | $SmL + H \rightleftharpoons Sm(HL)$ | 7.13 ± 0.02 | | |
| $Ca + L \rightleftharpoons CaL$ | 6.41 ± 0.01 | 184 | 0.011 | $Sm(HL) + H \rightleftharpoons Sm(H_2L)$ | 6.00 ± 0.02 | | |
| $CaL + H \rightleftharpoons Ca(HL)$ | 8.94 ± 0.01 | | | $Ho + L \rightleftharpoons HoL$ | 13.32 ± 0.02 | 138 | 0.0076 |
| $Ca(HL) + H \rightleftharpoons Ca(H_2L)$ | 8.06 ± 0.02 | | | $HoL + H \rightleftharpoons Ho(HL)$ | 7.60 ± 0.02 | | |
| $CaL + OH \rightleftharpoons CaL(OH)$ | 3.33 ± 0.01 | | | $Ho(HL) + H \rightleftharpoons Ho(H_2L)$ | 6.33 ± 0.02 | | |
| | | | | $Ho(H_2L) + H \rightleftharpoons Ho(H_3L)$ | 5.17 ± 0.03 | | |

* Charges on metal ions, edtmp and complexes have been omitted for simplicity.

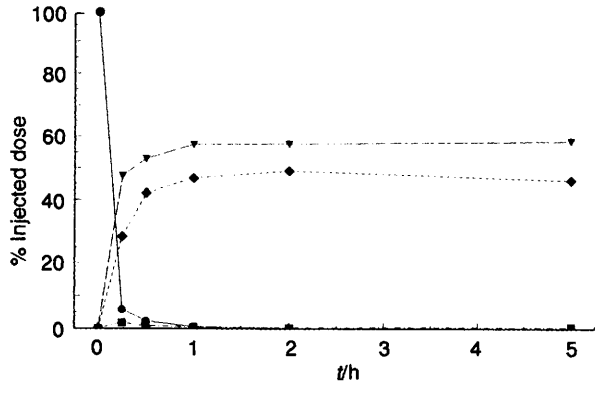



**Fig. 2** Biodistribution *versus* time for $^{153}$Sm, injected as $^{153}$Sm–edtmp, in rats. Data taken from ref. 5: ●, blood; ■, kidney; ◆, urine; ▼, bone

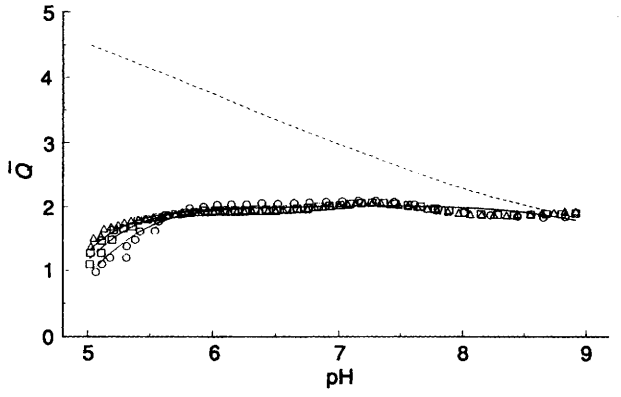



**Fig. 3** Experimental (points) and modelled (lines) deprotonation curves for complexation of Ho$^{III}$ by edtmp; pH is the negative logarithm of the free hydrogen-ion concentration. The dashed line is the $\bar{n}$ curve, where $\bar{n}$ is the protonation state of the tetraphosphonate in the absence of the metal ion. Three separate titrations were performed of 0.000 520 mol dm$^{-3}$ Ho$^{III}$ and (○) 0.001 00 mol dm$^{-3}$ edtmp in 0.0134 mol dm$^{-3}$ HCl, (△) 0.001 50 mol dm$^{-3}$ edtmp in 0.0135 mol dm$^{-3}$ HCl and (□) 0.002 00 mol dm$^{-3}$ edtmp in 0.0134 mol dm$^{-3}$ HCl *versus* 0.0500 mol dm$^{-3}$ NaOH in 0.10 mol dm$^{-3}$ NaCl. All solutions were at 37 °C and 0.15 mol dm$^{-3}$ NaCl or 0.15 mol dm$^{-3}$ total ionic strength

avoided on new ligands which are predicted not to work well. Side effects such as the mobilisation of other metal ions from blood plasma may also be predicted.

## Results and Discussion

*Potentiometry.*—The results of modelling using the computer program ESTA[7] are given in Table 1. Good fits, indicated by low Hamilton $R$ factors and standard deviations in log β values, were obtained for all the systems studied. A typical example is shown in Fig. 3 where the calculated and experimental deprotonation functions, $\bar{Q}$ (the average number of protons released on complexation per metal ion), for the Ho$^{III}$–edtmp system are shown. The dashed curve is $\bar{n}$, the protonation state of the tetraphosphonate in the absence of the metal ion. Interpretation of these curves assists greatly in model selection. In the case shown, if we assume the complexes contain one metal ion and one ligand molecule, we can predict, for example, that between pH 5 and 6 an $M(H_2L)$ (L = totally deprotonated ligand) species is present. This is because $\bar{n} = 4$ while $\bar{Q} = 2$. Therefore the ligand has lost a total of six of its eight dissociable protons. Similarly, between pH 6 and 7, an M(HL) species is present while at pH 9, where $\bar{Q}$ and $\bar{n}$ intersect, an ML species occurs. These species were all found in the best-fitting model. Species distribution curves (Fig. 4), confirm the occurrence of these complexes in the different pH regions.

Formation constants for edtmp with some of the metal ions used here have previously been measured at 25 °C and 0.1 mol dm$^{-3}$ KCl by Motekaitis *et al.*[8] With the exception of Cu$^{II}$, constants were generally found to be lower than the corresponding metal ion–ethylenediaminetetraacetate (edta) equilibrium constants. In this work we find a similar trend with the constant for Cu$^{II}$ also lower than the corresponding edta value. The differences in the constants measured here and those of Motekaitis *et al.*[8] may be attributed to the different experimental conditions, especially temperature, used.

Inspection of the protonation constants of the complexes gives some insight into the site of protonation and structure of the various complexes. Comparison of these results with literature results for structurally related ligands allows one to

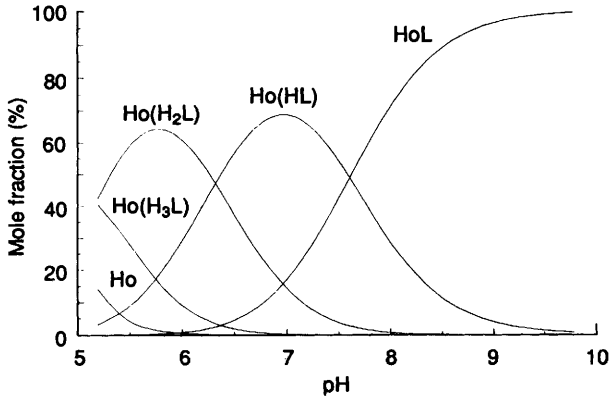
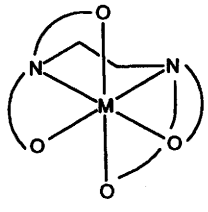



**Fig. 4** Species distribution curves for the complexation of $Ho^{III}$ by edtmp at 37 °C and 0.15 mol $dm^{-3}$ NaCl, as calculated from the formation constants in Table 1. Initial concentrations were 0.000 520 mol $dm^{-3}$ $Ho^{III}$ and 0.002 00 mol $dm^{-3}$ edtmp

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postulate the structure **I** for octahedral co-ordination. Thus log $\beta_1$ for $Ca^{II}$ and $^{2-}O_3PCH_2CH_2CH_2PO_3^{2-}$ and $^{2-}O_3PCH_2NHCH_2PO_3^{2-}$ are 2.6 and 3.8 respectively,[9] suggesting that both amino groups of edtmp are co-ordinated. Similarly, the value for $Co^{II}$ binding $^{2-}O_3PCH_2NHCH_2$-$CH_2NHCH_2PO_3^{2-}$ is $\approx$4 log units less than for edtmp suggesting that all four phosphonate groups are co-ordinated to the metal ion. Note also that $Mg^{II}$ binding to edtmp is less strong than that to $Ca^{II}$, a phenomenon which has been noted for edta and attributed to steric strain induced by simultaneous co-ordination of six donor groups to such a small metal ion. A similar solution structure was postulated by Motekaitis *et al.*[8] using potentiometry and by Oakes and Smith[10] using NMR evidence.

For $Ni^{II}$, $Zn^{II}$, $Mg^{II}$ and $Ca^{II}$ the $pK_a$ of the complex is close to $pK_{a2}$ of edtmp. This is evidence for protonation taking place at one of the nitrogen centres. On the other hand, for $Cu^{II}$, $Sm^{III}$ and $Ho^{III}$ the $pK_a$ of the complex is close to $pK_{a3}$ of edtmp, indicating that protonation is occurring at one of the equivalent phosphonate groups. The situation with $Co^{II}$ appears to be intermediate. Generally, the trend is that the less-stable complexes are protonated at a nitrogen which must entail metal–nitrogen bond rupture. Rizkalla and Choppin[11] have presented NMR evidence suggesting a similar protonation scheme for the different complexes.

In vivo *Speciation of* $Sm^{III}$.—Fig. 5 shows the speciation of $Sm^{III}$ amongst the low-molecular-weight compounds present in normal blood plasma. It is dominated by citrate complexation. The speciation in the absence of transferrin can be assumed to be the situation shortly after the introduction of $^{153}Sm$ into blood plasma. Complexation by transferrin is kinetically slow and so the speciation in the presence of transferrin reflects the situation after a number of hours once equilibrium has been established. It can be seen that transferrin eventually complexes 95% of the $Sm^{III}$ with the relative percentages of the low-molecular-weight complexes of $Sm^{III}$ remaining unchanged.

In vivo *Speciation of* $Sm^{III}$ *and* $Ho^{III}$ *in the Presence of edtmp*.—Since the uptake of $^{153}Sm$ and $^{166}Ho$ by bone is rapid

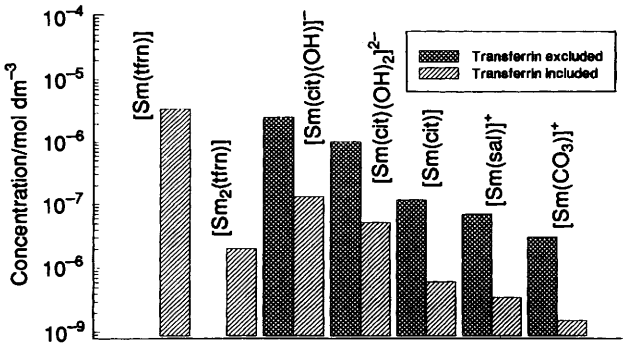


**Fig. 5** Speciation of $Sm^{III}$ in normal blood plasma. tfrn = transferrin; $H_3cit$ = citrate and $H_2sal$ = salicylic acid (3-carboxy-3-hydroxy-pentane-1,5-dioic acid)

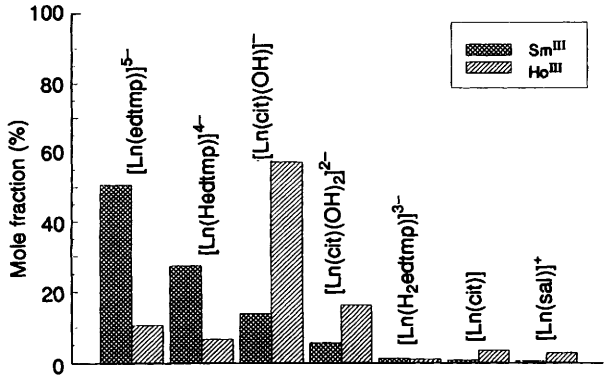


**Fig. 6** Speciation of $Sm^{III}$ and $Ho^{III}$ in blood plasma with edtmp added

and essentially complete within 2 h of injection of $^{153}Sm$– or $^{166}Ho$–edtmp (see Fig. 1), it was decided to neglect transferrin in this modelling. The results are shown in Fig. 6. The complexes involving edtmp show a significant difference between *in vivo* speciation of $Sm^{III}$ and $Ho^{III}$. Only 18% of the $Ho^{III}$ is involved in complexation with edtmp as opposed to 79% for $Sm^{III}$. The observed increase in uptake by bone for $Sm^{III}$ relative to $Ho^{III}$ in the baboon model can therefore be explained by this result.

The program ECCLES further predicts that $Zn^{II}$ will be significantly mobilised from blood plasma by edtmp. This is reflected in the plasma mobilisation index (p.m.i.) curve in Fig. 7, equation (1). In order to test this prediction, 24 h urine samples

$$\text{p.m.i.} = \frac{\text{(total concentration of low-molecular-weight metal complex species in the presence of the drug)}}{\text{(total concentration of low-molecular-weight metal complex species in normal plasma)}} \quad (1)$$

before and after treatment with $^{153}Sm$–edtmp were collected from three patients participating in clinical trials and analysed for Zn. The results, contained in Table 2, show significant increases of Zn in patients' urine after treatment. Zinc topping-up therapy may have to be considered should treatment be repeated regularly, *e.g.* if smaller doses of $^{153}Sm$ were given over a period of a few days instead of one relatively large dose given once. The results obtained here are gratifying and highlight the efficiency of the ECCLES program to predict *in vivo* behaviour of metal ions.

Fig. 7 also shows that $Ca^{II}$ is not significantly mobilised by edtmp at the concentrations used clinically. Some clinical trials have been carried out in the United States wherein the calcium complex of edtmp is used in the injection mixture for fear of depleting patients of calcium. From the results obtained here it appears that this procedure is unnecessary.

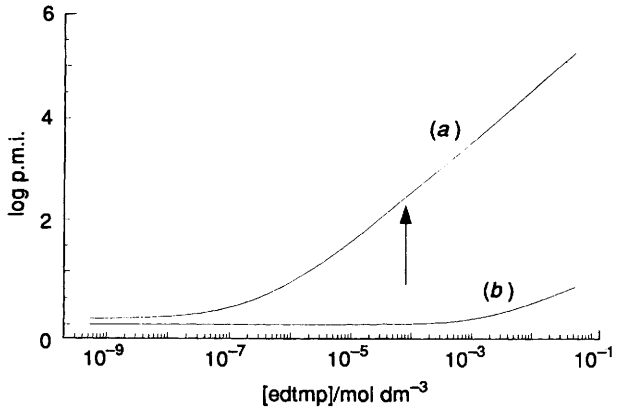


**Fig. 7** Plasma mobilisation index curves for (*a*) $Zn^{II}$ and (*b*) $Ca^{II}$ *versus* edtmp concentration. The arrow indicates the edtmp concentration used in clinical trials

**Table 2** Urine analysis results for $Zn^{II}$ before and after treatment with $^{153}Sm$–edtmp. The edtmp concentration used is reflected in Fig. 7

| | ppm Zn | |
|---|---|---|
| Patient | Before | After |
| 1 | 0.8 | 6.0 |
| 2 | 0.9 | 9.5 |
| 3 | 0.6 | 6.8 |

*Mechanism of Samarium*(III) *Uptake into Tumours.*—This mechanism is, as yet, not fully understood. Two apparently opposing mechanisms have been suggested with the point of contention being whether the $^{153}Sm$–edtmp complex remains intact or dissociates. A study by Chirby *et al.*[12] showed that the adsorption of edtmp on calcium hydroxyapatite (hydroxide phosphate) is independent of the concentration of $Sm^{III}$. This observation suggests that the $^{153}Sm$–edtmp dissociates with both the edtmp and $^{153}Sm$ being adsorbed independently. However, greater uptake of $^{153}Sm$ is observed in the presence of higher concentrations of edtmp. Further experiments on the elution of hydroxyapatite columns with diethylenetriaminepentaacetate (dtpa) seemed to indicate that $^{153}Sm$ is indeed adsorbed as part of the $^{153}Sm$–edtmp complex and not as the free metal ion following dissociation. Clinical studies on rats[4,5] showed that for a series of aminomethylenephosphonate and aminocarboxylate complexes of samarium an inverse relationship exists between skeletal uptake and complex stability. This is evidence for uptake of $^{153}Sm$ by bone occurring after dissociation of the complexes.

Speciation modelling provides some insight into the mechanism of uptake. The blood-plasma model with the total calcium concentration raised by an order of magnitude (to simulate the high concentration of intracellular $Ca^{II}$ in tumours) was re-run and the speciation of $Sm^{III}$ calculated. Fig. 8 indicates the somewhat unexpected result that a greater percentage of the $Sm^{III}$ is involved in edtmp complexation. This is probably due to competition by $Ca^{II}$ for other plasma ligands such as citrate which strongly complex $Sm^{III}$. Owing to this, the free concentration of $Sm^{III}$ is trebled from $2.2 \times 10^{-10}$ to $7.7 \times 10^{-10}$ mol dm$^{-3}$. The increase in $Sm^{III}$ in bone-seeking complexes as well as increased vascularity of the tumour contributes to the preferential uptake of the $Sm^{III}$ into the tumour.

In the light of the above, we propose a dual mechanism whereby the $^{153}Sm$–edtmp complex is adsorbed onto bone followed by its dissociation allowing uptake of $Sm^{III}$ into tumours. Samarium(III) may also be complexed by proteins such as lactoferrin which are found in high concentrations within tumour cells. This dual mechanism has important

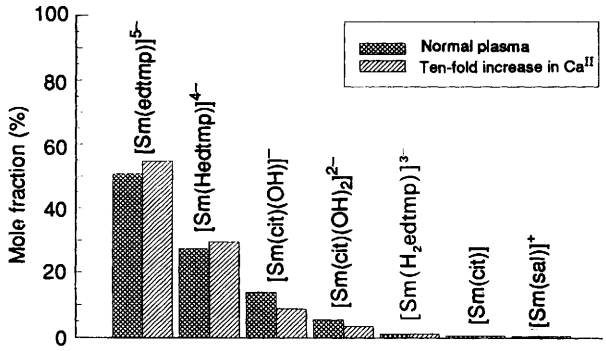


**Fig. 8** Comparison of speciations of $Sm^{III}$ in blood plasma with edtmp added to normal plasma and with a ten-fold increase in calcium(II) concentration

implications for future ligand design. It is important to achieve a formation constant for the ligand–lanthanide complex which ensures that enough lanthanide is in the complex to give appreciable bone uptake. Other blood-plasma metal ions competing for the ligand as well as the protonation of the ligand must also be considered; this is essentially what is being done with ECCLES. A narrow range of formation constants, ensuring that enough lanthanide is adsorbed onto the bone in the complex followed by the break-up of the complex, is required. In this type of work ECCLES becomes indispensible.

## Experimental

*Database.*—Formation constants for all reactions involving $Sm^{III}$ and $Ho^{III}$ with blood-plasma ligands are required for the ECCLES modelling. These were, as far as possible, extracted from the literature.[9] The rest were calculated by comparison with data collected by Jackson and du Toit[13,14] for modelling the *in vivo* speciation of $Gd^{III}$. Linear free-energy plots were obtained for $Sm^{III}$ *versus* $Gd^{III}$ and for $Ho^{III}$ *versus* $Gd^{III}$ using literature data.[9] The straight lines obtained were as in equations (2) and (3). The first carbonate constant was estimated

$$\log K(\mathrm{Sm}) = 0.994 \log K(\mathrm{Gd}) - 0.163 \quad r = 1.000 \quad (2)$$

$$\log K(\mathrm{Ho}) = 1.011 \log K(\mathrm{Gd}) + 0.216 \quad r = 1.000 \quad (3)$$

from a linear free-energy plot of $\log K_1(OH^-)$ *versus* $\log K_1(CO_3^{2-})$ which gave rise to the linear relationship (4).[9] The

$$\log K_1(CO_3^{2-}) = 0.851 \log K_1(OH^-) + 1.66 \quad r = 0.934 \quad (4)$$

samarium(III)–transferrin constant used was that of Harris[15] converted into a form suitable for input into ECCLES.

The total concentrations of $Sm^{III}$ and $Ho^{III}$ employed in the modelling were $4.17 \times 10^{-6}$ and $1.70 \times 10^{-6}$ mol dm$^{-3}$ respectively. The edtmp concentration was $8.5 \times 10^{-5}$ mol dm$^{-3}$. These values were calculated from the actual amounts used in baboon tests.[2]

The formation constants of other metal ions occurring in blood plasma with edtmp were measured by potentiometric titration. Where potentiometry did not lend itself to formation-constant determination, these constants were estimated by comparison. A listing of the database is available from the authors.

*Potentiometry.*—Potentiometric titrations were performed using a Metrohm Titroprocessor 670 with a Metrohm 665 dosimat and a combination glass electrode (Ag–AgCl reference). The electrode was calibrated regularly using strong acid–base titration data. All titrations were performed under an inert atmosphere and solutions were held at a constant ionic strength of 0.15 mol dm$^{-3}$ NaCl and a temperature of

37.0 ± 0.1 °C. The titrations were performed beginning at low and ending at high pH, adding 0.10 $cm^3$ aliquots of 0.050 mol $dm^{-3}$ NaOH (carbonate-free) in 0.10 mol $dm^{-3}$ NaCl. Protonation constants were calculated from data obtained from titrations of the ligand in the presence of various hydrochloric acid concentrations. Metal–edtmp formation constants were calculated from titration data at three different metal:edtmp ratios. Data were analysed by the ESTA [7] library of programs. During the analysis the previously determined protonation constants were held constant. Hydrolysis constants and $pK_w$ were taken from the literature [9] and held constant during optimisation procedures. The models were tested for plausibility by comparing experimental and calculated formation and deprotonation curves.

*Reagents.*—The tetraphosphonate edtmp was synthesised as previously described [16] and found to be pure by microanalysis. Fresh metal-ion solutions were employed in the titrations. These were made by dissolving reagent-grade chloride salts of the metal ions in distilled water and standardised by inductively coupled plasma atomic emission spectroscopy. Where necessary, solutions were acidified to prevent hydrolysis.

## Acknowledgements

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