Metal Ion Speciation in Blood Plasma: Gallium-67-Citrate and MRI Contrast Agents

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Metal chelate ions are commonly used in medical diagnostic imaging as MRI contrast or imaging agents. The efficacy of these metals depends on their in vivo behavior, which in turn depends on their in vivo speciation. **Methods:** A computer model has been used to simulate the speciation of Ga^{3+} and Gd^{3+} in blood plasma. The model has been tested against known clinical data and then used to investigate Ga^{3+} uptake by tumor cells. The iatrogenic effect of a gadopentetic acid enhanced MRI scan upon the biodistribution of ^{67}Ga citrate has also been calculated. **Results:** The speciation of Ga^{3+} calculated using the computer model is concordant with clinical data. The results support transferrin mediated uptake of Ga^{3+} by tumor cells but also account for Ga(III) biodistribution observed in hypotransferrinemic subjects. In a study of the effect of gadopentetic acid upon ^{67}Ga gallium citrate, neither residual DTPA nor $[Gd(DTPA)]^{2-}$ cause significant changes in the speciation of Ga(III). The calculations show that dissociation of 4% of the administered gadopentetic acid results in the formation of a mixed, Gd(III) and Ga(III), metal transferrin complex and a 100-fold increase in the concentration of $[Ga(OH)_4]^-$. **Conclusion:** Computer simulation is a valuable tool which can be used to explain/understand in vivo behavior of radioactive metal ions.

Key Words: gallium-67; Gd(III); computer simulation; metal speciation

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In recent years, diagnostic patient imaging has rapidly expanded from traditional radiological techniques to a host of new modalities. These include CT scanning, MRI and radionuclide imaging, both two-dimensional and SPECT. Many of the radiopharmaceuticals used as imaging agents in the latter technique are coordination complexes of metal ions (e.g., ^{99m}Tc-DTPA, ^{99m}Tc-DMSA and ^{67}Ga-citrate), as are several contrast media used in MRI are (e.g., Gd-DTPA and Gd-DOTA). Thus, the chemical equilibria in solution of both these types of agents is important as it can affect their behavior in vivo. Most inorganic complexes in solution are labile, undergoing rapid chemical exchange. The possibility therefore exists that, upon injection, the imaging agent or contrast agent may dissociate and interact with ions present in plasma to give rise to a totally different set of chemical species in solution. The behavior of these agents is then a function of this new speciation.

Speciation describes the concentration and composition of every species in a chemical sample including ions and undissociated molecules. It is clearly important in determining the biodistribution, excretion and toxicity of an element. In dynamic systems such as blood plasma, however, it is often very difficult, if not impossible, to determine the speciation of an element. Therefore, powerful computer models have to be used. These permit extrapolation from simple systems to more complex biological fluids. In this article, we describe the chemical speciation of Ga(III) and Gd(III), two metal ions commonly used to prepare imaging agents and MRI contrast agents respectively. We hope to show how a knowledge of chemical speciation can help to explain existing clinical data and how it can be used in the design of new and more effective agents.

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THEORY AND METHODOLOGY

Metal Ion Speciation

Speciation modeling of blood plasma was pioneered by Perrin in a series of papers (*1*). At the same time Sillen (*2*), Morel (*3*), Martell (*4*) and others worked on natural waters and urine. These early models were restricted to one or two metal ions with a limited number of ligands. The development of faster computers with larger memories enabled these early models to be expanded into multicomponent systems. In the blood model developed by May et al. (*5*), 7 metal ions and 40 ligands were considered simultaneously.

An essential process in the development of any speciation model is to define a series of chemical equilibria which represent the system under investigation. In defining the equilibria all the chemical species involved and the equilibrium constants for the reactions have to be specified (Eq. 1). This is the area of greatest difficulty in speciation modelling as accurate equilibrium constants are not always available. Indeed, in some cases, all the possible equilibria have not been identified.

$$S_j = \beta_j \prod_i X_i^{k(i,j)}, \qquad \text{Eq. 1}$$

where S_j is the species concentration, β_j the equilibrium constant, X_i the free component concentration and k(i,j) the component stoichiometric coefficient.

The series of equilibria, together with the total or free component concentrations constitute the computer model or database of the system. From these data, a series of simultaneous, mass-balance equations can be set up for the total component concentrations (Tn) as a sum of all the individual species concentrations:

$$T_i = X_i + \sum_j S_j\, k(i, j). \qquad \text{Eq. 2}$$

The mass-balance equations can then be solved iteratively for the free component concentrations. There are many computer programs that will do this. In this study we have used ECCLES (*6*). Substitution back into Equation 1 yields the individual species concentration.

Metal ions in blood plasma can exist in four distinct forms: the metal ion could be irreversibly bound to protein (e.g., copper bound to ceruloplasmin), reversibly bound to protein (e.g., copper bound to serum albumin), complexed to low-molecular-mass ligands, and bound to water as the aqua ion. The first form is not of interest in speciation modeling as the metal ion is not part of the equilibria being considered unless released from the protein by chemical degradation. The aqua-ion is a low-molar-mass complex but

TABLE 1
Total Ligand and Free Metal-Ion Concentrations Used as Blood Plasma Model

Component	Concentration (*M*)	Component	Concentration (*M*)
Serum albumin	7.2×10^{-4}	Tyrosinate	5.8×10^{-5}
Transferrin	2.5×10^{-5}	Valinate	2.3×10^{-4}
Alanate	3.7×10^{-4}	Carbonate	2.5×10^{-2}
Aminobutyrate	2.4×10^{-5}	Phosphate	1.6×10^{-3}
Arginine	9.5×10^{-5}	Thiocyanate	1.4×10^{-5}
Asparaginate	5.5×10^{-5}	Silicate	1.4×10^{-4}
Aspartate	5.0×10^{-6}	Sulphate	2.1×10^{-4}
Cysteinate	2.3×10^{-5}	Ammonia	2.4×10^{-5}
Cystinate	4.0×10^{-5}	Citrate	1.1×10^{-4}
Citrullinate	2.7×10^{-5}	Lactate	1.8×10^{-3}
Glutamate	4.8×10^{-5}	Malate	3.5×10^{-5}
Glutaminate	5.2×10^{-4}	Oxalate	1.2×10^{-5}
Glycinate	2.4×10^{-4}	Pyruvate	9.5×10^{-5}
Histidinate	8.5×10^{-5}	Salicylate	5.0×10^{-6}
Histamine	1.0×10^{-8}	Succinate	4.2×10^{-5}
Hydroxyprolinate	7.0×10^{-6}	Ascorbate	4.3×10^{-5}
Isoleucinate	6.5×10^{-5}	OH^-	1.2×10^{-6}
Leucinate	1.2×10^{-4}	Ca^{2+}	1.1×10^{-3}
Lysinate	1.8×10^{-4}	Mg^{2+}	5.2×10^{-4}
Methionate	2.9×10^{-5}	Cu^{2+}	1.0×10^{-20}
Ornithinate	5.8×10^{-5}	Fe^{2+}	1.0×10^{-11}
Phenylalanate	6.4×10^{-5}	Fe^{3+}	1.0×10^{-23}
Prolinate	2.1×10^{-4}	Pb^{2+}	1.0×10^{-14}
Serinate	1.2×10^{-4}	Mn^{2+}	1.0×10^{-12}
Threoninate	1.5×10^{-4}	Zn^{2+}	1.0×10^{-9}
Tryptophanate	1.0×10^{-5}		

because of its importance is treated as a separate form of the metal ion. It must be present in all aqueous equilibrium systems and is often referred to as the free (uncomplexed) ion even though it is complexed to solvent.

In blood plasma, the total metal ion concentration is much less than the total protein concentration and the free metal ion concentration is very much less than the concentration of the metal-protein complex. This means that until a substantial portion of the metal is stripped off the protein, the free metal ion concentration remains constant, i.e., is effectively buffered by protein binding. However, if an exogenously administered metal ion is used in high concentrations (e.g., gadolinium(III) or carrier gallium(III)) the protein binding capacity may easily be exceeded. In this case the free metal concentration will not remain constant. Also, metal uptake by proteins is often slow and so a higher than thermodynamically expected free metal ion concentration may exist before equilibrium is established. Notwithstanding these problems, the buffering of the free metal concentration by the protein can be used to simplify the simulation calculations. During the calculation, the free metal ion concentration is fixed rather than the total concentration, but care is taken that the total concentration does not exceed known plasma levels. The free metal concentrations used are given in Table 1.

Because of the slow complexation kinetics of proteins, two extreme simulation conditions exist, one in which protein binding is neglected and one in which protein binding is explicitly included in the calculation. These two simulation conditions serve to illustrate the speciation immediately after injection and at some time later when equilibrium with the protein has been established.

Blood Plasma Database

The number of possible chemical equilibrium reactions that can occur in blood plasma are legion. The medium contains a number of different metal ions and a myriad of low molar mass ligands. May et al. (*5*) have constructed a computer model of blood plasma in an attempt to calculate the concentration of all the possible species at equilibrium. To the original database we have added the appropriate constants for Gd(III) (*7*) and Ga(III). Constants for Ga(III) were, as far as possible, extracted from the literature (*8,9*). In cases where the same system had been studied by several authors, the results were evaluated critically. Where a constant was potentially important but for which no data were available, values were estimated either by using a linear free-energy relationship between Fe(III) and Ga(III) or by chemical analogy. The final set of Ga(III) equilibrium constants used in this study are given in Table 2 and the component concentrations in Table 1.

TABLE 2
Binary Formation Constants Used as Blood Model Database for Gallium(III)

L	*p*	*q*	*r*	log $\beta^\dagger$	L	*p*	*q*	*r*	log β
Phosphate	1	1	1	18.8	Oxalate	1	1	0	6.45
	1	1	0	10.0		1	2	0	12.38
	1	1	2	19.5		1	3	0	17.86
Citrate*	1	1	1	11.6					
	1	1	−1	7.1					
	1	1	0	10.02	Malonate	1	1	0	2.4
	1	2	0	15.3		1	2	0	3.6
Hydroxide	1	0	−1	−3.31	Transferrin	1	1	0	18.1
	1	0	−2	−6.76		2	1	0	35.2
	1	0	−3	−11.16	Salicylate	1	1	0	16.1
	1	0	−4	−17.17	Tartrate	1	1	0	18.5
Cysteinate	1	1	0	16.1	Serinate	1	1	0	9.0
	1	1	1	18.4		1	1	1	10.5
	1	1	2	20.5	DTPA	1	1	0	24.3
DFO	1	1	0	28.17		1	1	1	28.5
	1	1	−1	17.9	EDTA	1	1	0	21.0
						1	1	1	22.8

*Neutral ligand H_3 citrate.
†pGA + qL + rH = GapIq Hr.

Mixed-ligand complex formation occurs widely in biological fluids and has been shown to be important (*10*). Since little data is available on Ga(III) ternary complexes, binary constants were used to estimate the stability of these mixed complexes (*6*).

RESULTS AND DISCUSSION

Validation of Model

Unfortunately the measurement of the in vivo speciation of Ga(III) at n*M* concentrations is beyond the limit of modern analytical chemistry. Hence, it is not possible to test our computer model directly. Instead, the calculated speciation has to be tested, by inference, against clinical observations. This has been done using the normal biodistribution of ^{67}Ga as well as desferrioxamine (Desferal, DFO) and Fe(III) induced changes. The effect of bicarbonate has also been calculated.

Gallium

The chemistry of gallium has been comprehensively reviewed by Moerlein and Welch (*11*) and Hayes and Hubner (*12*). In aqueous solution, the only oxidation state of importance is +3. The binding in Ga(III) is mainly electrostatic and hence size is important. The ionic radius of Ga(III) is 76 pm, which is quite similar to the ionic radius of high-spin Fe(III) (79 pm) (*13*) and results in a good correlation between the stability of high-spin Fe(III) complexes and Ga(III) complexes.

Gallium(III) is extensively hydrolyzed in solution (*14*). This hydrolysis can be represented by Equations 3–6 in which, for convenience, waters of coordination have been omitted.

$$[Ga]^{3+} + H_2O = [Ga(OH)]^{2+} + H^+, \qquad \text{Eq. 3}$$

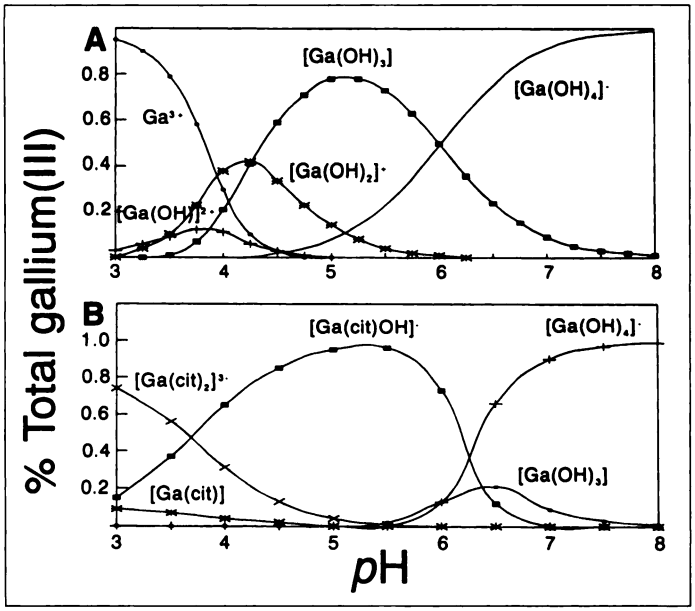

FIGURE 1. (A) Speciation of a 10^{-9} *M* gallium(III) aqueous solution and (B) in the presence of 4×10^{-5} *M* citrate.

$$[Ga(OH)]^{2+} + H_2O = [Ga(OH)_2]^{+} + H^{+}, \quad \text{Eq. 4}$$

$$[Ga(OH)_2]^{+} + H_2O = [Ga(OH)_3] + H^{+}, \quad \text{Eq. 5}$$

$$[Ga(OH)_3] + H_2O = [Ga(OH)_4]^{-} + H^{+}. \quad \text{Eq. 6}$$

The equilibrium constant for each of the steps above is given by:

$$K_n = \frac{[Ga(OH)_n][H^+]}{[Ga(OH)_{n-1}]}. \quad \text{Eq. 7}$$

The hydrolysis constants of Ga(III), together with the total concentration of the metal ion, can be used to calculate the speciation in solution. This is shown in Figure 1A for a 1×10^{-9} *M* solution of $GaCl_3$. This concentration of gallium is typical for a ~5 mCi injection of [^{67}Ga]Ga-citrate. As can be seen, even for this relatively simple system, a number of species exist in equilibrium. As the pH of the solution is raised the speciation changes with the concentration of $[Ga(OH)_3]$ increasing and then decreasing.

To prevent hydrolysis at physiological pH, many radiopharmaceutical preparations of ^{67}Ga(III) include citrate. Figure 1b shows the effect that citrate has upon Ga(III) speciation. At pH values <6.3 the major species present in solution is $[Ga(citrate)OH]^-$. At pH values >6.3, however, the $[Ga(OH)_4]^-$ species predominates. These calculations serve to illustrate the complexity of speciation in equilibrium situations.

Upon intravenous injection of a gallium radiopharmaceutical, the number of possible complexes in solution are numerous. This is because blood plasma is a highly coordinating medium containing many potential ligands. Not only are there a number of low molar mass ligands like phosphate, carbonate, amino acids and carboxylic acids, but there are also a number of proteins which are potential coordinators of gallium. Figure 2 shows the speciation of gallium calculated using our transferrin-free model of blood plasma. At pH 7.4, the major species present is $[Ga(OH)_4]^-$. At blood plasma concentrations, citrate and phosphate complexes are not important.

Transferrin (Tf), which is present in blood plasma, is an avid binder of Ga^{3+} ($\log K_1 = 18.1$, $\log K_2 = 17.1$) (*15*). The protein has two binding sites per molecule and under normal conditions 30% of these sites are occupied by Fe^{3+} (*16*). This

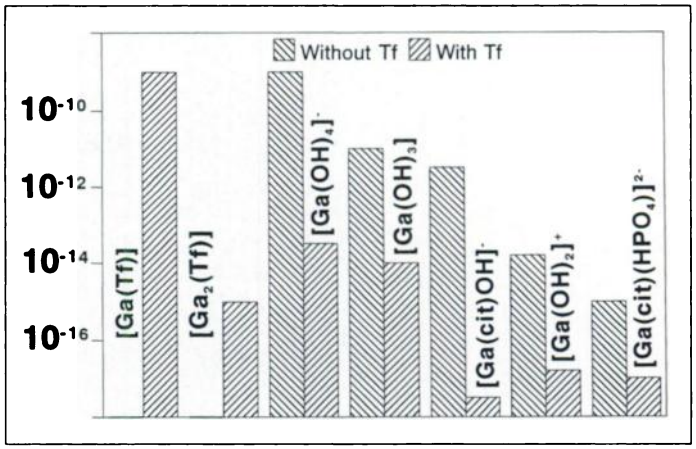

FIGURE 2. Calculated speciation of gallium(III) in blood plasma at pH 7.4 in the presence and absence of Tf. Total Ga(III) = 10^{-9}, Tf = 25 μM. Other component concentrations as given in Table 1.

leaves typically 50 μM of sites available for the coordination of other metal ions. Fe^{3+} binding is too strong ($\log K_1 = 20.7$ and $\log K_2 = 19.4$) (*15*) for it to be displaced by Ga^{3+}. The result of introducing transferrin into our model of blood plasma are shown in Figure 2. Most of the Ga^{3+} is bound to Tf (99.9%), while the remaining metal ion is distributed among the same low molecular mass ligands as before.

The above results, calculated using our model of blood plasma, can be compared with observations in the literature. Clinically, it has been observed that 10%–20% of ^{67}Ga is excreted through the kidneys within the first 24 hr (*19*). Another 10%–20% (*20*) is excreted through the gastrointestinal tract via the bowel wall rather than through the biliary system. The remaining Ga(III) is concentrated in the skeleton (including marrow) (24%) and other soft tissue (34%). The same results are found for the injection of either Ga^{3+} or its citrate complex (*21*). Our simulation results are in accord with these clinical observations in that, irrespective of the presence of citrate, our results show that, the major species present in the injecting solution is $[Ga(OH)_4]^-$. Complexation of metal ions by Tf is relatively slow (*17,18*). Thus, upon injection, Ga(III) is initially distributed in plasma as charged low molar mass complexes but at equilibrium, would become incorporated into Tf, according to our calculations. This change in speciation with time is in accordance with observed changes in tissue distribution with time and the time variation of ^{67}Ga scans.

Desferrioxamine (Desferal, DFO) has been used to improve lesion contrast in humans (*33*) and animals (*34*). Within 12 hr of ^{67}Ga administration, DFO is able to remove the radionuclide that has localized in tissue. After 24 hr, however, it is no longer able to mobilize the metal ion. If DFO is administered prior to ^{67}Ga-citrate, the metal ion is rapidly excreted in the urine and no transferrin binding occurs (*35*). Repeat ^{67}Ga scans after discontinuation of the DFO treatment result in normal diagnostic images. To test our model of blood plasma against these observations, the possibility of Ga(III) binding to lactoferrin (LF) has to be considered. Figure 3 illustrates these results and shows how the amount of Ga^{3+} bound to Tf or Lf changes with the concentration of DFO. At a concentration of 3.2×10^{-6} *M*, DFO can remove 50% of the gallium from Tf but is able to remove the same amount of gallium from Lf only at a concentration of 10^{-3} *M*. This last concentration is unrealistic for clinical use in humans, but it illustrates the result that DFO increases the excretion of Tf-bound gallium but not Lf-bound gallium. Also, the results show that if DFO is administered prior

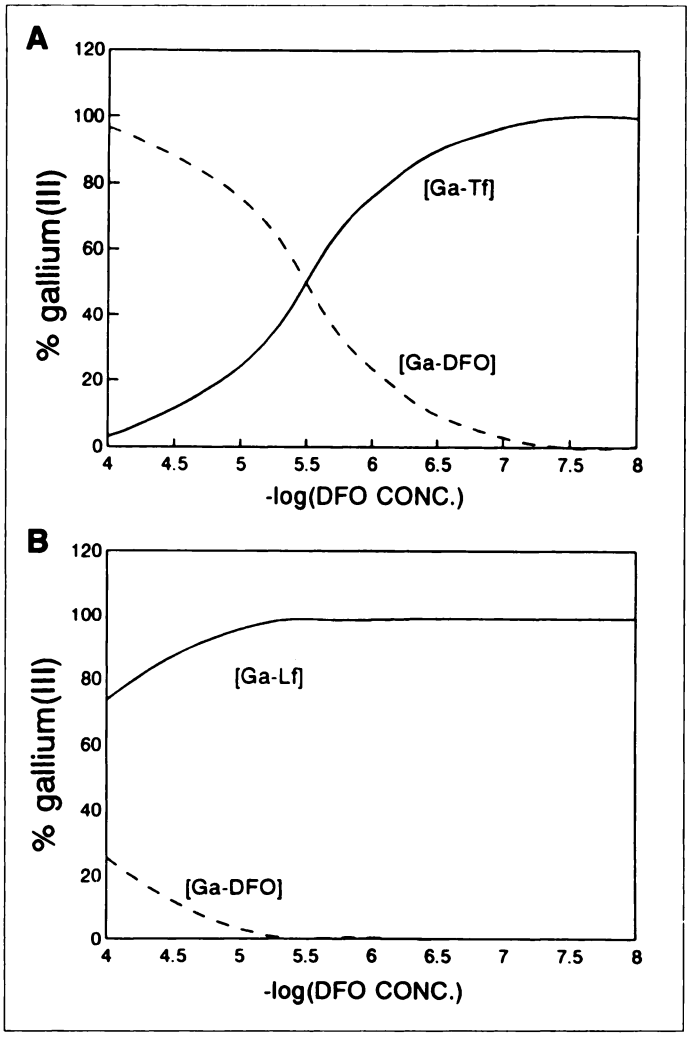

FIGURE 3. Dependence of Ga(III) speciation on DFO concentration in blood plasma at pH 7.4. (A) Total Ga(III) = 10^{-9} *M*, Tf = 25 μ *M*; (B) Total Ga(III) = 10^{-9} *M*, Tf = Lf = 2.5×10^{-5} M. Other component concentrations are as given in Table 1.

to ^{67}Ga-citrate no [Ga(Tf)] is formed and so the metal ion is rapidly excreted.

Metal ions can be used to enhance the contrast of ^{67}Ga images. Thus Fe(III), when administered 24 hr after a tracer injection of ^{67}Ga-citrate, lowers the whole-body retention of ^{67}Ga resulting in lower background activity (*22*).

If, however, Fe(III) or carrier levels of Ga(III) are administered prior to the ^{67}Ga-citrate injection, then the transferrin binding sites are saturated and elevated kidney and spleen and decreased liver activity is seen (*36–38*). A similar picture is seen in iron-overload patients (*39*). When our computer model is tested against these observations the results indicate that the Fe(III) displaces Ga(III) from its transferrin binding site thereby increasing its excretion and lowering the background activity. The [Ga(Tf)] + [Ga(Lf)] already accumulated at the lesion site is less accessible to the Fe(III) and so improved contrast is obtained. The resultant biodistribution is consistent with the computer simulation model excluding Tf binding (Fig. 2).

Finally, the model was tested against the effect of changes in bicarbonate concentration upon the speciation of gallium. Staker et al. (*51*) found that when using gallium citrate, 97%–99% of the gallium is bound to molecules with a MW >10,000 if the bicarbonate concentration is above 13 m*M*. As the bicarbonate concentration decreases, the high molecular weight retention decreases. At a bicarbonate concentration of 7 m*M*, only 83% of the gallium is bound in this way. Changing the bicarbonate concentration in our model gives similar results except that the cut off is ~2 m*M* bicarbonate rather than the 7 m*M* found by Staker et al. (*51*). These figures, however, are dependent on the pH of the solution which is not specified by Staker et al. (*51*).

Application of Model

Having validated the computer model of gallium speciation in blood plasma in a qualitative manner, we then applied it to problems associated with gallium biodistribution.

It has been proposed (*22,23*) that gallium is taken up into cells as low molar mass complexes or localization is mediated by Tf-specific receptors on cell membranes with the gallium being bound to the Tf. Weiner et al. (*24*) showed in man and Sohn et al. (*25*) showed in mice that in hypotransferrinemic (HP) subjects that have severely depressed Tf levels (decreased from 25 to 2.5×10^{-6} *M*), uptake of ^{67}Ga by nonosseous tissue is depressed relative to normal subjects. Increased renal excretion and decreased liver uptake is seen. Computer simulation using the total component concentrations typical of human blood plasma but with the available metal binding site concentration of Tf decreased from 50 to 5×10^{-6} *M* shows (Table 3) that while most of the gallium will still be bound to Tf, the $[Ga(OH)_4]^-$ concentration would increase from 0.03% to 0.3% of the total Ga^{3+}. This 10-fold increase in low molar mass gallium could explain the increased excretion and hence lower tissue uptake of ^{67}Ga which is seen clinically. If the results of Weiner et al. (*24*) and Sohn et al. (*25*) are expressed as %(retained dose)/g then similar tissue distribution of ^{67}Ga in normal and HP subjects is seen (blood-to-liver ratio in normal and HP subjects: man, 1:9 and 1:12; mice 1:14 and 1:12). This

TABLE 3
Calculated Species Distribution of Ga(III) Complexes in Blood Plasma*

Species	NBP†	HP subject‡	NBP + 50 μ *M* Lf	NBP + 0.014 *M* Ca^{2+}	NBP, pH 6.4
[Ga-Tf]*	4.0×10^{-9}	4.0×10^{-9}	4.5×10^{-11}	4.0×10^{-9}	4.0×10^{-9}
$[Ga]^{3+}_{free}$	5.4×10^{-25}	5.4×10^{-24}	6.0×10^{-27}	5.4×10^{-25}	8.8×10^{-22}
$[Ga(OH)_4]^-$	1.4×10^{-12}	1.4×10^{-11}	1.6×10^{-14}	1.4×10^{-12}	2.4×10^{-13}
$[Ga(OH)_3]$	5.8×10^{-14}	5.8×10^{-13}	6.5×10^{-16}	5.8×10^{-14}	9.7×10^{-14}
$[Ga(cit)(OH)]^-$	4.5×10^{-15}	4.5×10^{-14}	5.0×10^{-17}	4.5×10^{-15}	7.1×10^{-13}
[Ga-Lf]	—	—	4.0×10^{-9}	—	—

*Total $[Ga^{3+}] = 4.05 \times 10^{-9}$ *M*.
†NBP = normal blood plasma; Tf = 25 μ*M*.
‡Tf = 2.5 μ*M*.

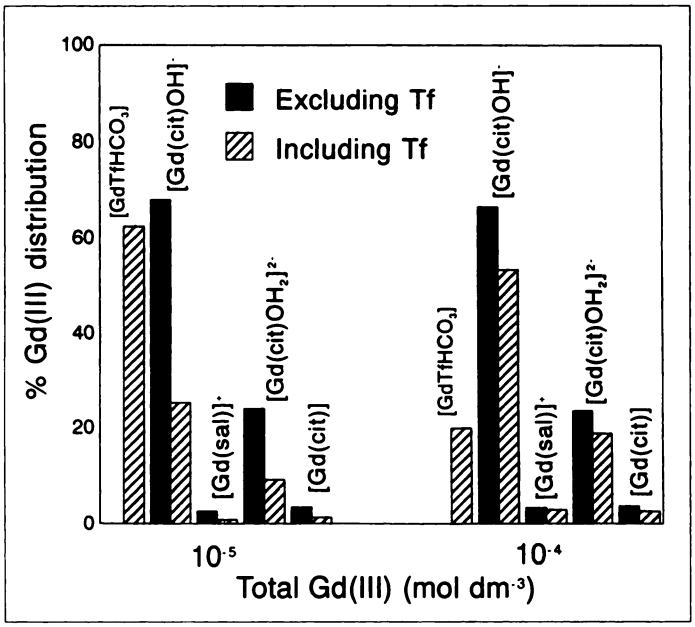

FIGURE 4. Calculated distribution of Gd(III) in blood plasma at pH 7.4, as a function of total metal concentration in the presence and absence of Tf binding. The coordinated ligands and their stoichiometry are as indicated. Tf = transferrin; cit = citrate; sal = salicylate.

is consistent with [Ga(Tf)] being the major gallium complex in both groups.

Sohn et al. (*25*) reported increased uptake of ^{67}Ga by bone in HP mice. The bone-to-liver uptake ratio increases from 1.4 to 8.8, a six-fold increase which is similar to the predicted 10-fold increase in $[Ga(OH)_4]^-$ concentration. The high affinity of Ga(III) for phosphate is consistent with its localization in bone, which is thought to be Tf-independent (*25*).

There is much controversy surrounding the mechanism by which ^{67}Ga accumulates in tumors (*23,26*). That gallium can bind to Tf and enter tumor cells is well documented (*22*). There is a good correlation between gallium uptake and Tf (*23*) and Tf receptor (TfR) (*23,27*) concentration. For some tumors, however, this correlation fails. Kaplan et al. (*28*) and Sturrock et al. (*29*) demonstrated a Tf-independent mechanism in which the rate of iron uptake correlates with the low molar mass concentration of the metal ion. If such a mechanism is postulated for ^{67}Ga, then the increased uptake in XS63 tumor bearing HP mice (*25*) is accounted for by the speciation calculations. The tumor-to-liver ^{67}Ga uptake ratio in normal mice is 1.04 while in HP mice it is 9.04 (*25*). This correlates well with the 10-fold increase in the low molar mass ^{67}Ga concentration of HP subjects (Table 3).

Other explanations have been put forward to account for the varied ^{67}Ga uptake of different tumors. One proposal is that gallium is displaced from Tf by the high concentration of intracellular Ca^{2+} (*30*). The present model cannot be applied to intracellular fluids as the component concentrations are different to blood plasma, but the plasma results (Table 3) show that Tf-mediated uptake of gallium is a viable mechanism as most of the metal ion circulating in the plasma is bound to this protein. On the other hand, the results also show that a 10-fold increase in calcium plasma concentration does not affect gallium speciation.

Another hypothesis is that a local decrease in pH results in dissociation of gallium followed by binding to tumor protein (*31*). In general, equilibrium constants between a metal ion and a protein are measured as conditional constants K_{cond}, which can only be used under the same conditions at which they were determined. The equilibrium is therefore represented by:

$$M + \text{Protein} = [M \cdot \text{Protein}], \qquad \text{Eq. 8}$$

where no account is taken of possible involvement by other ligands. In the case of Tf, the metal is bound by two tyrosine residues, two histidine residues, a water molecule and a bicarbonate anion. At the same time there is a loss of three protons from the protein (*16*). The equilibrium can therefore be represented by the equation:

$$M^{3+} + H_3Tf + HCO_3^- = [M \cdot Tf \cdot HCO_3] + 3H^+, \qquad \text{Eq. 9}$$

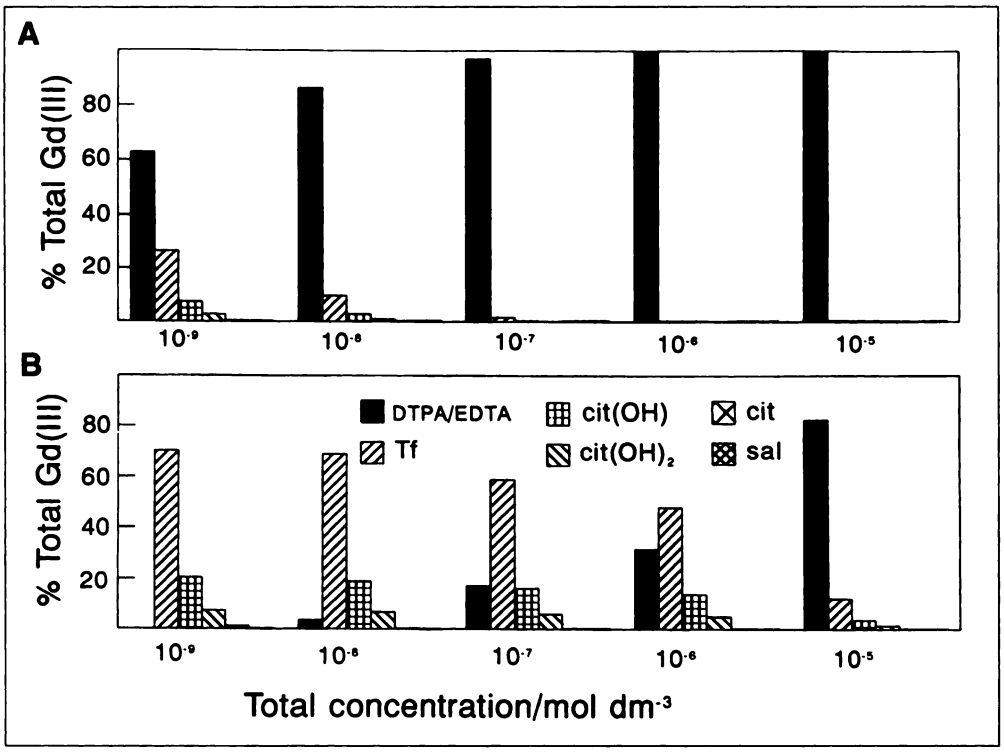

FIGURE 5. Calculated speciation of Gd(III) contrast agents in blood plasma at pH 7.4 as a function of concentration. (A) $[Gd(DTPA)]^{2-}$, (B) $[Gd(EDTA)]^-$. The coordinated ligands and their stoichiometry are as indicated. Tf = transferrin; cit = citrate; sal = salicylate.

TABLE 4
Calculated Species Distribution of Ga(III) Complexes*

Species	Blood plasma, 10 n*M* DTPA, 9.4 n*M* $[Gd(DTPA)]^{2+}$		60 μM Gd(III) 2 n*M* DTPA	
	Conc. (M)	Total Ga^{3+} (%)	Conc. (M)	Total Ga^{3+}(%)
$[Ga(Tf)HCO_3]$	3.0×10^{-9}	99.9	7.6×10^{-10}	25.9
$[GdGa(Tf)(HCO_3)_2]$	—	—	2.2×10^{-9}	73.6
$[Ga(OH)_4]^-$	1.0×10^{-13}	0.1	1.4×10^{-11}	0.5
$[Ga_2(Tf)(HCO3)_2]$	2.5×10^{-14}	0.0	7.0×10^{-14}	0.0
$[Ga(OH)_3]$	4.3×10^{-14}	0.0	5.8×10^{-13}	0.0
$[Ga(citrate)(OH)]^-$	1.0×10^{-14}	0.0	1.2×10^{-13}	0.0
$[Ga(DTPA)]^{2-}$	2.7×10^{-14}	0.0	3.0×10^{-22}	0.0
$[Ga]^{3+}_{free}$	4.0×10^{-25}	0.0	5.3×10^{-24}	0.0

*Total Ga(III) = 3 n*M* and total citrate = 0.34 m*M*. Conc. = concentration.

where the charge of the protein and complex have been omitted. The equilibrium constant, K_l for this reaction, is a true thermodynamic equilibrium constant applicable to different conditions of pH and bicarbonate concentration.

Using this constant it is possible to calculate the speciation of Ga(III) in blood plasma at pH 6.4. The results given in Table 3 show that a decrease in pH from 7.4 to 6.4 affects the low molar mass speciation of gallium but that there is no change in the concentration of [Ga(Tf)]. Hence, from speciation modeling, we conclude that neither a local decrease in pH nor an increase in calcium concentration is likely to result in displacement of gallium from Tf. It is now believed that Ga^{3+} is taken into cells as the Tf complex and that within the cell cytoplasm, Tf loses Ga^{3+} to lactoferrin (Lf), which binds Ga^{3+} ~90 times more strongly than Tf (*32*). Certain tumors, Hodgkin's disease, Burkitt's lymphoma and melanoma, are known to contain Lf. Table 3 shows that in our speciation calculations, Lf is able to remove ~99% of Ga^{3+} from Tf.

Gadolinium

Gadolinium is part of the lanthanide series of elements (Ce-Lu) which, in the neutral atom, have a xenon electronic core structure, partially filled 4f orbitals buried within this core, and three outer electrons in the 5d and 6s orbitals. In proceeding across the lanthanide series of elements, electrons are added to the buried 4f orbitals and so the chemical properties of the elements and ions are so similar that they are largely changes in degree rather than kind (*40*).

The most common oxidation state of gadolinium is +3 in which the three outer electrons are lost. There are therefore no electrons or orbitals available for covalent bonding and so ionic bonding is the norm. This is similar to Ga(III) and in fact the chemistry of the two ions is very similar. However, since the strength of ionic interactions depend on both the charge and interionic distance, Gd^{3+}, which has an ionic radius of 108–125 pm, generally forms much weaker complexes than Ga^{3+} or Fe^{3+}, which have ionic radii of 76 pm and 79 pm, respectively (*13*). There is a good correlation between the stability of Gd(III) complexes and high spin Fe(III) and Ga(III) complexes (*7,11*).

$$\log K_1(Gd^{3+}) = 0.77 \log K_1(Fe^{3+}) - 1.95,$$

$$\log K_1(Gd^{3+}) = 0.92 \log K_1(Ga^{3+}) - 2.0.$$

Unlike Ga^{3+}, Gd^{3+} is only weakly hydrolyzed in aqueous solution and reliable constants only exist for the first hydrolysis step. The value of log K_l for this, −8.4, is some five orders of magnitude lower than the hydrolysis constant of Ga^{3+} (*43*).

The speciation of Gd(III) is more complex in blood plasma than in aqueous solution. The calculated speciation for blood plasma at pH 7.4 is shown for low molar mass ligands in Figure 4. In this we are considering the addition of Gd^{3+} to normal plasma. At low concentrations of Gd(III), the metal ion is bound almost exclusively as the $[Gd(citrate)(OH)]^-$ and $[Gd(citrate)(OH)_2]^{2-}$ complexes. As the concentration of the metal ion is increased, so the amount of available citrate is exceeded and a new species distribution is obtained. While in medical terms this only occurs at high concentrations, it must be remembered that MRI contrast agents are used at millimolar concentrations (*41*).

The above simulation does not take into account the possibility of in vivo protein metal binding. Reuben (*42*) showed that the binding of Gd(III) to bovine serum albumin is weak but the similarity between the coordination chemistry of high spin Fe(III) and Gd(III) suggests that Tf binding should be important. Zak and Aisen (*43*), and more recently Harris and Chen (*44*), reported equilibrium constants for the binding of Gd(III) to Tf. While there is some discrepancy between these two measurements, there is agreement that the binding of Gd(III) is weaker than expected from linear-free energy relationships. Primary binding of the Gd^{3+} ion is at the C-terminal end of Tf. Similar results have been reported for Sm(III) and Nd(III) (*45*) although coordination at both the C- and N-terminal ends was reported. Using the constants of Harris and Chen (*42*) it is possible to calculate the effect of transferrin binding upon the speciation of Gd(III) in blood plasma. A previous report on Gd(III) in vivo speciation is in error as a much higher Gd-Tf binding constant was used in the calculations (*7*).

Figure 4 compares the speciation of simple Gd(III) salts in blood plasma in the presence and absence of Tf binding. At the lower Gd(III) concentration, the metal ion is bound predominantly as the $[Gd.Tf.HCO_3]$ complex, but a significant amount of $[Gd(citrate)OH)]^-$ complex also exists. As the concentration of Gd(III) is increased from 10^{-5} *M* to 10^{-4} *M*, so the binding capacity of Tf is exceeded and $[Gd(citrate)OH)]^-$ predominates. Note, however, that the percent distribution of Gd(III) among the low molar mass ligands does not change.

Clinically, the high toxicity of free Gd(III) precludes its use as an MRI contrast agent. Instead, the metal ion is complexed to powerful chelating ligands like EDTA, DTPA (giving gadopentetic acid) and DOTA. These ligands affect the biodistribution, excretion, toxicity, and hence the efficacy of Gd(III) as a contrast agent. Figure 5 shows the effect of contrast agent concentration upon the calculated Gd(III) species distribution in blood plasma. For DTPA (Fig. 5A), the ligand is able to prevent the loss of the metal ion to Tf, whereas for EDTA (Fig. 5B) a substantially higher concentration of ligand is needed to suppress Tf binding of the metal ion. These results are in accord

with the clinical observation that gadopentetic acid is excreted intact while the Gd(III) is lost from $[Gd(EDTA)]^-$ (*46*).

One of the side effects predicted by our simulation results is that when Gd^{3+} is released from a DTPA or EDTA complex, significant amounts of zinc complex (~48% of plasma zinc) are formed with the liberated ligand. These zinc complexes enhance the excretion of zinc so that animals receiving multiple high doses of contrast agent may show signs of zinc deficiency. Such signs have been reported for rats receiving 5.0 mmole · kg^{-1} of a modified DTPA gadolinium(III) complex, intravenously three times a week for 3 wk (*47*).

Iatrogenic Interactions

Recently, Hattner and White (*48*) reported the interference of $[Gd(DTPA)]^{2-}$ upon the biodistribution of ^{67}Ga-citrate observed in a child at 96 hr. These authors noted that an injection of ^{67}Ga-citrate 4 hr after a $[Gd(DTPA)]^{2-}$-enhanced MRI scan resulted in unexpected renal uptake of the radioisotope together with skeletal uptake emulative of bone perfusion. In addition, little of the expected liver or bowel activity was seen. The authors propose that some dissociation of the $[Gd(DTPA)]^{2-}$ complex occurs in vivo and that the released Gd^{3+} gives rise to a carrier effect on the ^{67}Ga biodistribution. Wiggins et al. (*49*) reject this argument, suggesting instead that the change in biodistribution of ^{67}Ga is due to excess free ligand present in all commercial preparations of MRI contrast agents. Typically, 500 μg free DTPA are administered and Wiggins et al. (*49*) estimate that this would result in a plasma concentration of $\sim 2 \times 10^{-9}$ *M* DTPA at the time of ^{67}Ga administration to the patient described by Hattner and White.

We used a computer model of blood plasma which incorporates both Ga(III) and Gd(III) to calculate what effect a gadopentate-enhanced MRI should have on the speciation of Ga(III). Typically, $[Gd(DTPA)]^{2-}$ is used as a contrast agent in MRI at an administered dose of ~0.1 mmole · kg^{-1}. Within 30 min postinjection, the in vivo plasma concentration falls to 0.3 m*M* and should have dropped to 0.06 m*M* 210 min later (*50*) (this may be an overestimate for an 11-yr-old boy). At the same time, the concentration of DTPA should be 2×10^{-9} *M* (*49*). On the other hand, ^{67}Ga as Ga(III) citrate is used at a concentration of 5.2×10^{-11} *M*. In practice, ^{67}Ga-citrate is typically used at a specific activity of >10 mCi/μg gallium at the reference date. This implies that the maximum carrier levels of Ga(III) are 58.9 times the ^{67}Ga concentration resulting in a maximum Ga(III) concentration in vivo of 3.0×10^{-9} *M*. At the same time, the citrate concentration will be elevated to 0.34 m*M*. We have used these concentrations, together with normal blood plasma component concentrations, to calculate the speciation of Ga(III) and Gd(III).

The results (Table 4) of our calculations show that, contrary to the suggestion of Wiggins et al. (*49*), residual DTPA does not cause a significant change in the speciation of gallium. In support of this claim, we can find no reported instances of DTPA interference of ^{67}Ga scans following the use of ^{99m}Tc-DTPA even though kits of this radiopharmaceutical have a substantial excess of DTPA (4×10^{-5} moles). The reason for DTPA not affecting the gallium speciation is that it preferentially binds to Ca(II) which is present in high concentrations in vivo. Similarly, at the concentrations used in the calculation, $[Gd(DTPA)]^{2-}$ does not significantly affect the speciation of Ga(III).

In the absence of Gd(III), Ga(III) in blood plasma exists mainly as the $[GaTf(HCO_3)]$ complex. In the presence of 0.06 m*M* Gd(III) (the calculated concentration of residual Gd(III) (*50*), the mixed metal $[GdGaTf(HCO_3)_2)]$ predominates (see Table 4). The Gd(III) occupies the C-terminal site of Tf, while the Ga(III) is complexed to the N-terminal site (*44*). At the same time, there is a 100-fold increase in the concentration of $[Ga(OH)_4]^-$. The ^{67}Ga image obtained by Hattner and White (*48*) post-Gd-DTPA appears consistent with an increase in the proportion of low molecular mass complexes of Ga(III) relative to [Ga(Tf)], bearing in mind the changes seen in hypotransferremic subjects. Thus, the calculated increase in $[Ga(OH)_4]^-$ may be sufficient to account for the observed clinical change in the ^{67}Ga image observed 4 days later. Alternatively, the cellular uptake of Ga(III) from $[GdGaTf(HCO_3)_2)]$ may be different to that of [Ga(Tf)] resulting in a different ^{67}Ga image.

CONCLUSION

Our simulation results support the original postulate of Hattner and White (*48*) in that the different radio image obtained with ^{67}Ga-citrate 4 hr after a $[Gd(DTPA)]^{2-}$-enhanced MRI scan is due to dissociation of the $[Gd(DTPA)]^{2-}$ complex. Our calculations show that only 4% of the injected dose of gadopentate need dissociate to cause substantial changes in Ga(III) speciation. On the other hand, our calculations are also clear that neither residual DTPA nor $[Gd(DTPA)]^{2-}$ would affect the biodistribution of ^{67}Ga(III).

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