In Vivo Inorganic Chemistry of Technetium Cations

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Technetium-99m cations are of interest because of their potential use as myocardial perfusion imaging agents. These species can undergo in vivo reactions which markedly affect their biodistribution patterns. Four such cases of reactions are presented and discussed: (1) simple ligand substitution; (2) metal centered redox processes; (3) reactions of coordinated ligands; (4) outer sphere association reactions. New experimental techniques appropriate for investigating these reactions at the $10^{-10}M$ concentration level of technetium encountered in vivo are also presented and discussed. The latter three classes of reactions are illustrated by examples taken from the recent literature and from unpublished data.

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The title of this article was clearly created by a committee (*1*), because the terms "in vivo" and "inorganic" are essentially contradictory: The first refers to chemistry occurring in living systems, whereas, the second refers to the chemistry of materials derived from nonliving systems. Given that "inorganic chemistry in vivo" is at best a rather amorphous concept, the focus of this report is on two subjects that are more easily defined: the possible in vivo reactions of robust cationic technetium complexes; and new experimental techniques appropriate for investigating technetium (Tc) chemistry at the $10^{-10}M$ concentration level.

Following their introduction, these subjects are illustrated by recent examples wherein new experimental techniques are used to elucidate some of the in vivo chemistry exhibited by cationic technetium-99m (^{99m}Tc) complexes that are being investigated as potential myocardial perfusion imaging agents.

REACTIONS

The adjective "robust" is used to describe complexes that are inert to ligand substitution, i.e., they do not lose their ligands under the relatively mild conditions encountered in vivo. This property, of course, is crucial to the functioning of Tc radiopharmaceuticals because it is primarily the ligands themselves that impart biological character to the Tc center. Thus, simple ligand substitution,

$$Tc{-}X + Y \rightarrow Tc{-}Y + X, \qquad (1)$$

the simplest reaction that might possibly occur in vivo, is rarely observed because most Tc radiopharmaceuticals are designed *not* to undergo this process.

Metal centered redox processes, e.g.,

$$TcL_6^+ + e^- \rightarrow TcL_6^\circ, \qquad (2)$$

comprise a much more important category of possible in vivo reactions. Because an electron is being added to (or subtracted from) a metal center, the net formal charge on the whole complex must perforce change as a result of the redox reaction. In the example given as Eq. (2), the addition of an electron to a cationic complex changes it to a neutral species. Clearly, the biodistribution of the neutral species will be different from that of the cationic complex; thus, if this redox reaction takes place in vivo, the observed biodistribution will be that characteristic of neutral molecules and not of cations.

Reactions of coordinated ligands, e.g.,

$$Tc{-}COOCH_3 + H_2O \rightarrow Tc{-}COO^- \qquad (3)$$

comprise another important class of possible in vivo reactions. These processes clearly alter the physical and chemical properties of the complex and, thus, can also

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drastically alter the biodistribution of the complex. Note that in this type of reaction, the metal–ligand bond remains intact—only the coordinated ligand is chemically altered. In the example given as Eq. (3), hydrolysis of a coordinated ester both reduces the lipophilicity and alters the net formal charge of the Tc complex. Although inorganic chemists describe the reaction given in Eq. (3) as the "hydrolysis of a coordinated ligand," a biochemist more likely would refer to it as the "metabolism of a Tc complex." The net effect is the same, only the point of view is different.

Finally, Tc complexes can undergo outer sphere association reactions:

$$TcL_6^+ + P = TcL_6{-}P^+ \quad (4)$$

Again, in this reaction the inner coordination sphere of ligands (L) remains intact—no Tc–L bonds are broken. Rather, the entire complex associates with some other species that, in vivo, is likely to be a protein (P). From the point of view of inorganic chemistry, this is an outer sphere association reaction because the protein is bound outside of the first (or inner) coordination sphere. From the point of view of biochemistry, Eq. (4) describes protein binding. The protein can offer a specific binding site (receptor), or a nonspecific binding site based on physiochemical interactions.

TECHNIQUES

It is now well established that when ^{99m}Tc is eluted from a molybdenum-99 (^{99}Mo)/^{99m}Tc generator, the total Tc content of the eluent (as *both* ^{99}Tc and its daughter ^{99m}Tc) is in the range of 10^{-8} to $10^{-6}M$, with most eluents containing 10^{-8} to $10^{-7}M$ Tc (*2,3*). After formulation as a radiopharmaceutical and injection into a patient, this concentration is reduced by at least 1,000-fold and, thus, a reasonable estimate of the concentration of Tc in biologic samples is 10^{-11} to $10^{-10}M$. Very few techniques are available to the experimentalist for obtaining chemical information on samples that are this dilute in the species of interest, especially when the bulk of the sample is a complicated, variable matrix such as biologic tissue. One such technique is chromatography, and among the various chromatographic techniques that are available, high performance liquid chromatography (HPLC) has proven to be most useful. High performance liquid chromatography can be conducted in a variety of forms, but there are four of these that have proven especially efficacious in probing the in vivo chemistry of Tc cations.

1. Reverse phase chromatography. This form of chromatography separates Tc complexes primarily on the basis of lipophilicity. Because charge is one of the important factors determining lipophilicity, however, the behavior of a complex during reversed phase chromatography can often yield information about its charge.

2. Cation exchange chromatography. This form of chromatography separates complexes on the basis of positive charge density (roughly charge/volume) and is clearly useful in the analysis of cationic complexes.

3. Size exclusion chromatography. This form of chromatography separates large molecules, such as proteins, on the basis of size and shape.

4. Internal surface reverse phase chromatography. This is a new form of chromatography, which allows the rapid removal of large molecules from biologic samples before reversed phase analysis of small molecules (vide infra) (*4,5*).

In addition to HPLC techniques, one can sometimes obtain information about the in vivo chemistry of Tc complexes by indirect, or comparative, techniques. In the following example this is accomplished by comparing the biodistribution of two closely related, but subtly different, coordination complexes.

EXAMPLES

In Vivo Redox Reaction

The first Tc cation to be evaluated as a potential myocardial perfusion imaging agent was the technetium(III) complex $[^{99m}Tc(DMPE)_2Cl_2]^+$ [DMPE is 1,2-bis(dimethylphosphino)ethane] (*6*). These clinical results were disappointing in that the initial heart/liver ratio was unacceptably low and then this ratio decreased with time as activity washed out of the myocardium into the liver. Also, a significant amount of bone (or bone marrow) was visualized. It was hypothesized that this biodistribution pattern might result from in vivo reduction of the Tc(III) cation to yield the corresponding Tc(II) neutral species $[^{99m}Tc[DMPE]_2Cl_2]^0$, which accumulated preferentially in organs of the reticuloendothelial system (*7*).

Indirect approach. This hypothesis was first tested by an indirect technique that utilized the periodic relationship between Tc and rhenium (Re). The Re(III) cation $[Re(DMPE)_2Cl_2]^+$ was prepared and characterized (*8*), and shown, as expected, to exhibit the same size, shape, charge, lipophilicity, etc., as its Tc(III) analog. Thus, to the biochemical milieu (blood, proteins, etc.) the external properties of these two complexes are indistinguishable. However, the crucial difference between these two complexes is that the Tc(III) complex is reduced 190 mV more easily than is the Re(III) analog. Thus, if the Tc(III) cation is reduced in vivo, then the Re(III) cation will not undergo reduction because the 190 mV difference will take its reduction potential out of the range accessible to biologic systems. In this situation the relative biodistributions of coinjected

$[^{99m}Tc(DMPE)_2Cl_2]^+$ and $[^{186}Re(DMPE)_2Cl_2]^+$ will be significantly different. However, because there are other factors that might cause the biodistributions of ^{99m}Tc and ^{186}Re congeners to be different (e.g., ^{99m}Tc is a "no carrier added" isotope, whereas; ^{186}Re contains considerable amounts of ^{185}Re carrier), it is important to also conduct an appropriate control experiment. This can be accomplished by using the analogous Tc(I) and Re(I) cations, $[^{99m}Tc(DMPE)_3]^+$, and $[^{186}Re(DMPE)_3]^+$, because neither undergoes chemical reduction and, thus, when coinjected these two species should exhibit identical biodistributions (*9*).

Figures 1 and 2 (*9*) show the results of the two coinjection experiments just described. When the nonreducible $^{99m}Tc(I)$ and $^{186}Re(I)$ cations are coinjected into rats, the organ uptake ratios $^{99m}Tc/^{186}Re$ essentially are 1.0 for all organs sampled at all sampling times (Fig. 1). However, when the $^{99m}Tc(III)$ and $^{186}Re(III)$ cations are coinjected, the organ uptake

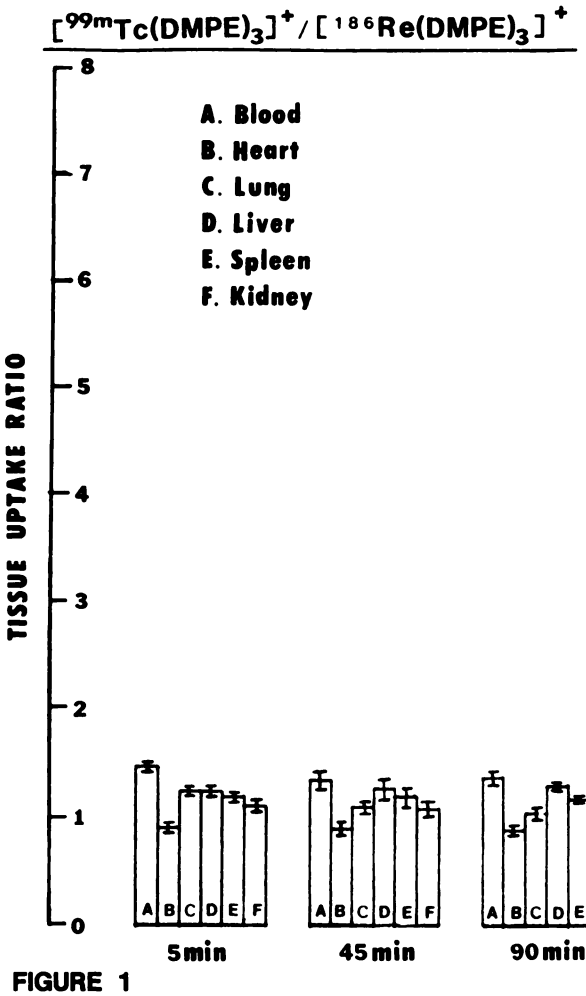

FIGURE 1
Ratios of $[^{99m}Tc(DMPE)_2Cl_2]^+/[^{186}Re(DMPE)_2Cl_2]^+$ tissue uptake in rats (average and standard deviation; n = 5) as a function of tissues sampled and sampling time (post injection). Reproduced from Ref. (*9*) with permission.

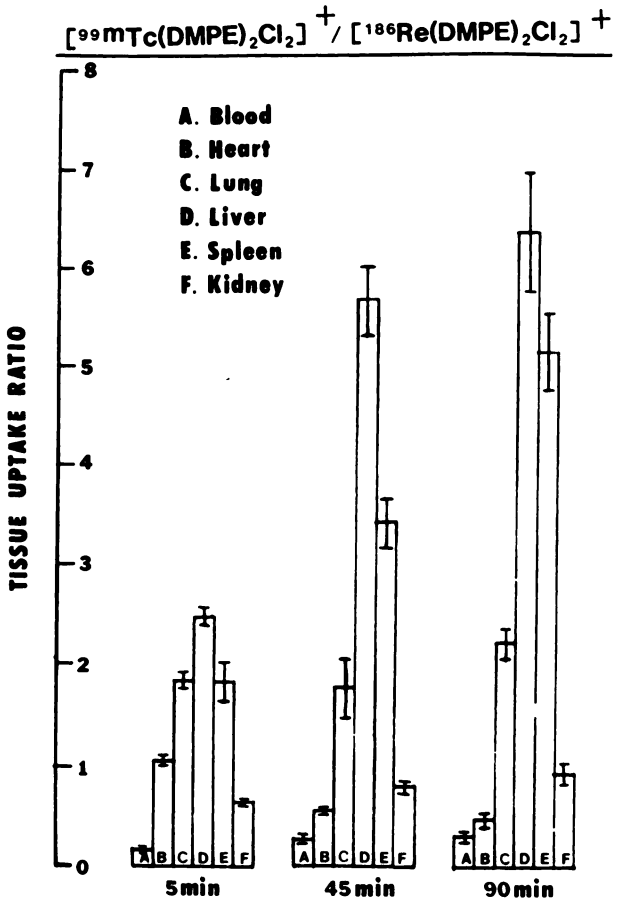

FIGURE 2
Ratios of $[^{99m}Tc(DMPE)_3]^+/[^{186}Re(DMPE)_3]^+$ tissue uptake in rats (average and standard deviation; n = 5) as a function of tissues sampled and sampling time (postinjection). Reproduced from Ref. (*9*) with permission.

ratios vary by over a factor of 60 (Fig. 2), establishing that the $^{99m}Tc(III)$ agent undergoes a significantly different biologic fate than does the $^{186}Re(III)$ congener. This is entirely consistent with the hypothesis that the $[^{99m}Tc(DMPE)_2Cl_2]^+$ cation is reduced in vivo to the neutral $^{99m}Tc(II)$ analog, whereas, the Re(III) cation does not suffer in vivo reduction. From the point of view of myocardial perfusion imaging, this in vivo reduction of the $^{99m}Tc(III)$ cation is undesirable because the resulting neutral species washes out of the heart and accumulates in the liver (presumably in a protein bound form). This leads to very low, and continuously decreasing, heart/liver ratios. The $^{99m}Tc(I)$ cations, and the $^{186}Re(III)$ cation $[Re(DMPE)_2Cl_2]^+$, do not undergo in vivo reduction and, therefore, do not suffer myocardial washout.

High performance liquid chromatography approach. The indirect approach described above in this case yields a definitive result, but it is not an approach that would have been taken if a more direct experiment could have yielded the same information. It would have been much more desirable to directly

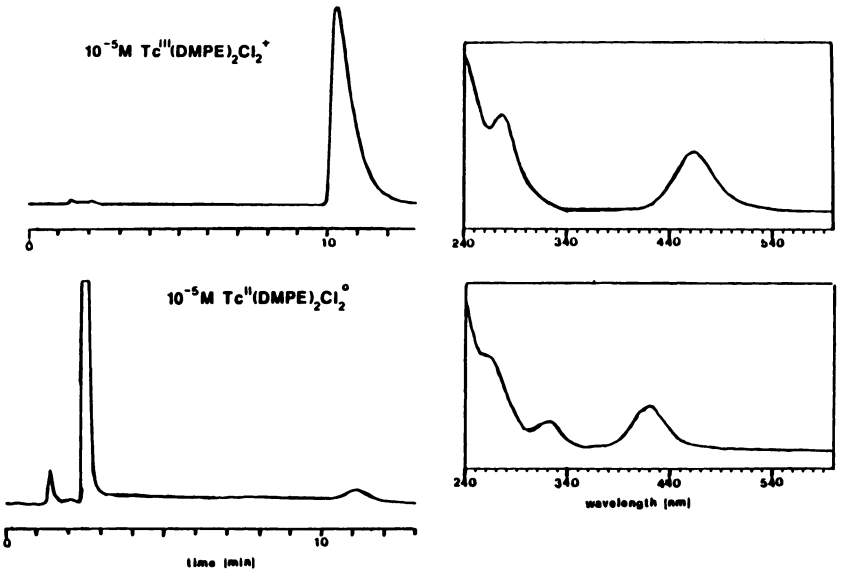

FIGURE 3
Reverse phase HPLC chromatograms of $10^{-5}M$ $[^{99}Tc^{III}(DMPE)_2Cl_2]^+$ and $10^{-5}M$ $[^{99}Tc^{II}(DMPE)_2Cl_2]^0$. Conditions: Alltech C-8 column, 10 micron, 250 × 4.6 mm; 70:30 methanol/(0.015*M* aqueous sodium heptansulfonate) mobile phase; flow rate = 1.5 ml/min; detection with Hewlett-Packard 1040A photodiode array centered at 254 nm; spectrum of eluting peak scanned from 240 to 600 nm.

analyze animal tissues by HPLC for the presence of $[^{99m}Tc(DMPE)_2Cl_2]^+$ and $[^{99m}Tc(DMPE)_2Cl_2]^0$ in order to determine if reduction of $^{99m}Tc(III)$ to $^{99m}Tc(II)$ occurs in vivo. However, HPLC analysis fails in this system for a rather subtle reason related to the very low concentration of Tc present in ^{99m}Tc radiopharmaceuticals.

Figure 3 shows the result of HPLC analysis of $[^{99m}Tc(DMPE)_2Cl_2]^+$ and $[^{99m}Tc(DMPE)_2Cl_2]^0$ when these species are at $10^{-5}M$ concentration. The HPLC apparatus is equipped with a rapid scanning spectrophotometer so that the visible ultraviolet spectrum of the eluting species can be obtained. Figure 3 shows that in this particular reverse phase HPLC analysis at $10^{-5}M$ concentration the cationic $^{99}Tc(III)$ complex elutes at a retention time of about 10 min, whereas, the neutral $^{99}Tc(II)$ species elutes at a retention time of ~2 min. Because the visible-uv spectra of these two complexes are well known from chemical studies and are distinct (*10*), the detector response establishes the chemical form of both complexes upon elution (Fig. 3). Thus, at $10^{-5}M$ concentration, the HPLC separation and detection of the $^{99}Tc(III)$ and $^{99}Tc(II)$ forms is straightforward.

Figure 4 shows what happens in this analysis as the concentration of the injected $^{99}Tc(II)$ species is decreased. The first chromatogram repeats the analysis of $7 \times 10^{-5}M$, and again a single peak with retention time of about 2 min is observed. This is the peak characteristic of Tc(II). When this sample is simply diluted by a factor of five and then reinjected, the resulting chromatogram now exhibits two peaks at retention times about 4 and 6 min. If the original sample is diluted by a factor of ten and then reinjected, the two peaks become better resolved and both exhibit longer retention times. Dilution of the original sample by a factor of 50 yields a solution of $^{99}Tc(II)$ of $1.4 \times 10^{-7}M$, and the HPLC analysis of this solution is shown in the last chromatogram of Figure 4. This chromatogram exhibits a single peak with a retention time characteristic of $^{99}Tc(III)$, even though it was $^{99}Tc(II)$ that was injected onto the column. These successive chromatograms establish that under the conditions of this reverse phase HPLC

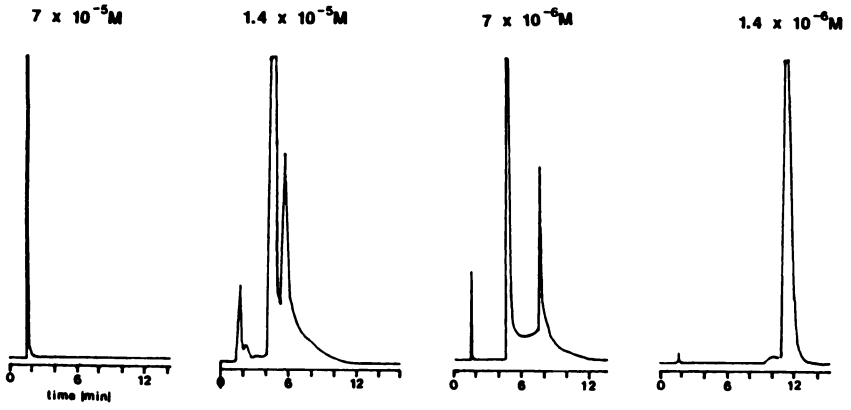

FIGURE 4
Cation exchange HPLC chromatograms of $[^{99m}Tc^{II}(DIARS)_2Br_2]^0$, $[^{99m}Tc^{III}(DIARS)_2Br_2]^+$ and $[^{99m}Tc^{III}(DIARS)_2Br_2]^+$ after incubation in 3 ml human blood at 37°C for 2 hr. Conditions: Vydac cation exchange column, 250 × 4.6 mm; 85:15 methanol/(0.10*M* aqueous tetrabutylammonium phosphate) mobile phase; flow rate = 1.5 ml/min; radiometric detection.

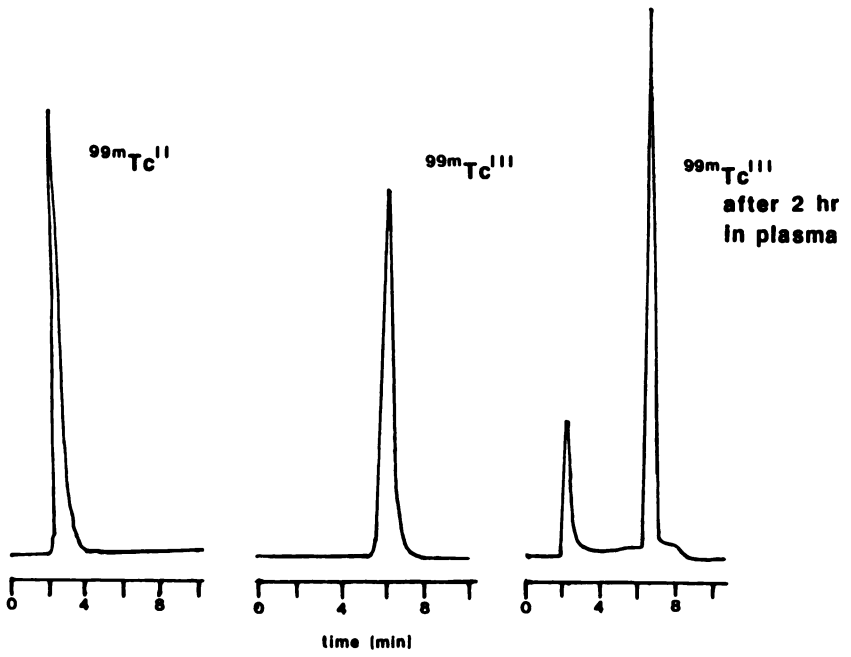

FIGURE 5
Plot of % $[^{99m}Tc^{III}(DIARS)_2Br_2]^+$ remaining in 3 ml human blood versus time of incubation. Analytical conditions as specified in caption of Figure 5.

analysis, $^{99}Tc(II)$ is being oxidized to $^{99}Tc(III)$ *during* the analysis. The extent to which this extraneous oxidation takes place is dependent on the concentration of the originally injected $^{99}Tc(II)$. At $7 \times 10^{-5}M$ no detectable oxidation takes place; however, at $1.4 \times 10^{-7}M$ the $^{99}Tc(II)$ is completely oxidized to $^{99}Tc(III)$. Because $10^{-7}M$ is considerably higher than the $10^{-10}M$ concentration of Tc expected to be encountered within in vivo samples (vide supra), it is clear that $[^{99m}Tc(DMPE)_2Cl_2]^+$ and $[^{99m}Tc(DMPE)_2Cl_2]^0$ could never be distinguished by HPLC analysis of tissue samples. Any $^{99m}Tc(II)$ present at the beginning of the analysis would suffer complete extraneous oxidation during the analysis and would be detected as $^{99m}Tc(III)$.

One of the fundamental reasons that $[^{99m}Tc(DMPE)_2Cl_2]^0$ suffers extraneous oxidation during HPLC analysis is that it is a very good reductant (*10*) and, thus, is relatively unstable in the Tc(II) oxidation state. Even analysis under anaerobic conditions, incorporating antioxidants in the mobile phase, does not alleviate the problem and in our hands extraneous oxidation is always observed at low concentrations. However, other related $^{99m}Tc(II)$ complexes are not such good reductants and, therefore, are more stable in the Tc(II) oxidation state. These $^{99m}Tc(II)$ complexes, indeed, can be analyzed by HPLC without extraneous oxidation and, thus, both the $^{99m}Tc(II)$ and $^{99m}Tc(III)$ forms can be detected in biologic samples. Figure 5 shows the results of such an analysis for the $^{99m}Tc(II)$ complex $[^{99m}Tc(DIARS)_2Br_2]^0$ [DIARS is *o*-phenylenebis(dimethylarsine)], which is relatively stable in the Tc(II) oxidation state (*10,11*). In this cation exchange HPLC analysis, the $^{99m}Tc(II)$ complex is eluted at a retention time of ~2 min, and the $^{99m}Tc(III)$ analog

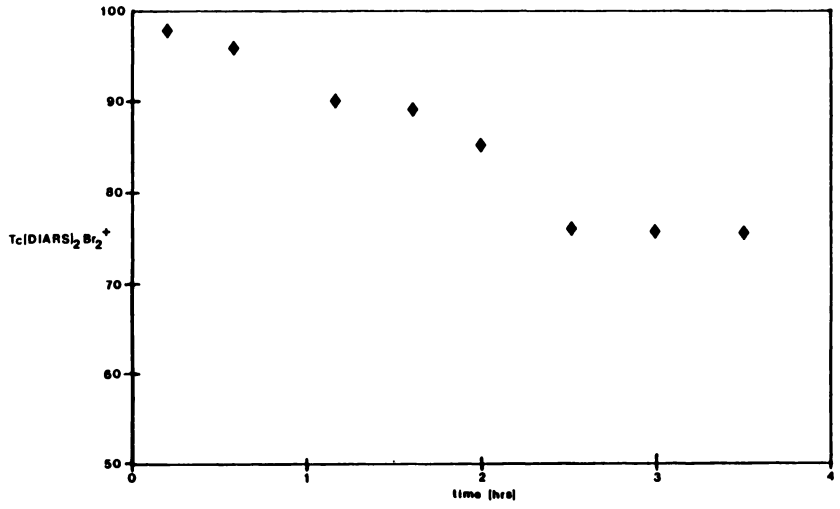

FIGURE 6
Reverse phase HPLC chromatograms of $[^{99}Tc^{II}(DMPE)_2Cl_2]^0$ as a function of concentration. Conditions as specified in caption of Figure 3.

is eluted at a retention time of ~7 min. The ^{99m}Tc(II) does not suffer any apparent oxidation during the analysis, even when it is present at "no carrier added" concentration levels estimated to be $\sim 10^{-7}M$. Thus, this analysis can be used to detect reduction of $[^{99m}Tc(DIARS)_2Br_2]^+$ to $[^{99m}Tc(DIARS)_2Br_2]^0$ in biologically relevant samples. The last chromatogram of Figure 5 shows the results of analysis of a $[^{99m}Tc(DIARS)_2Br_2]^+$ sample, which had been incubated with human plasma for 2 hr. Clearly, during this time a significant fraction of the ^{99m}Tc(III) complex had undergone reduction to ^{99m}Tc(II). Figure 6 shows the results of a series of such human blood plasma analyses, indicating that the extent of reduction in plasma increases with time.

In Vivo Reaction of a Coordinated Ligand

A particularly nice example of this type of reaction has resulted from the joint efforts of the Harvard and MIT groups of A. Jones and A. Davison (*12–14*). These researchers have been developing a series of hexakis(isonitrile)Tc(I) cations as potential ^{99m}Tc myocardial perfusion imaging agents. These cations have the general formula $[^{99m}Tc(CN\text{-}R)_6]^+$, where R can be any one of a wide variety of organic groups. When R is t-butyl, $-C(CH_3)_3$, the ^{99m}Tc complex exhibits high liver and lung uptake, which interfere with myocardial imaging. In an attempt to promote washout from these background organs, R was modified to incorporate an ester functionality: R is $-C(CH_3)_2COOCH_3$. It was anticipated that in vivo hydrolysis of the coordinated ester would occur rapidly in the liver and lung, thus, altering the charge and lipophilicity of the ^{99m}Tc complex (Eq. 3) and promoting washout. Figure 7 shows the sequence of ester hydrolysis reactions that can be expected for $[^{99m}Tc(CN\text{-}C(CH_3)_2COOCH_3]_6)^+$. Successive hydrolysis of the six ester groups is seen to lead to nine possible products (when isomeric forms of the products are considered). Figure 8 shows a reverse phase HPLC analysis of human blood plasma that has been incubated with $[^{99m}Tc(CN\text{-}C(CH_3)_2COOCH_3)_6]^+$. Nine peaks are observed, corresponding to the starting complex and eight of the nine possible products; the peak with the longest retention time corresponds to the unhydrolyzed starting material, whereas, the second (largest) peak corresponds to the neutral product that contains just one hydrolyzed ester group (Fig.

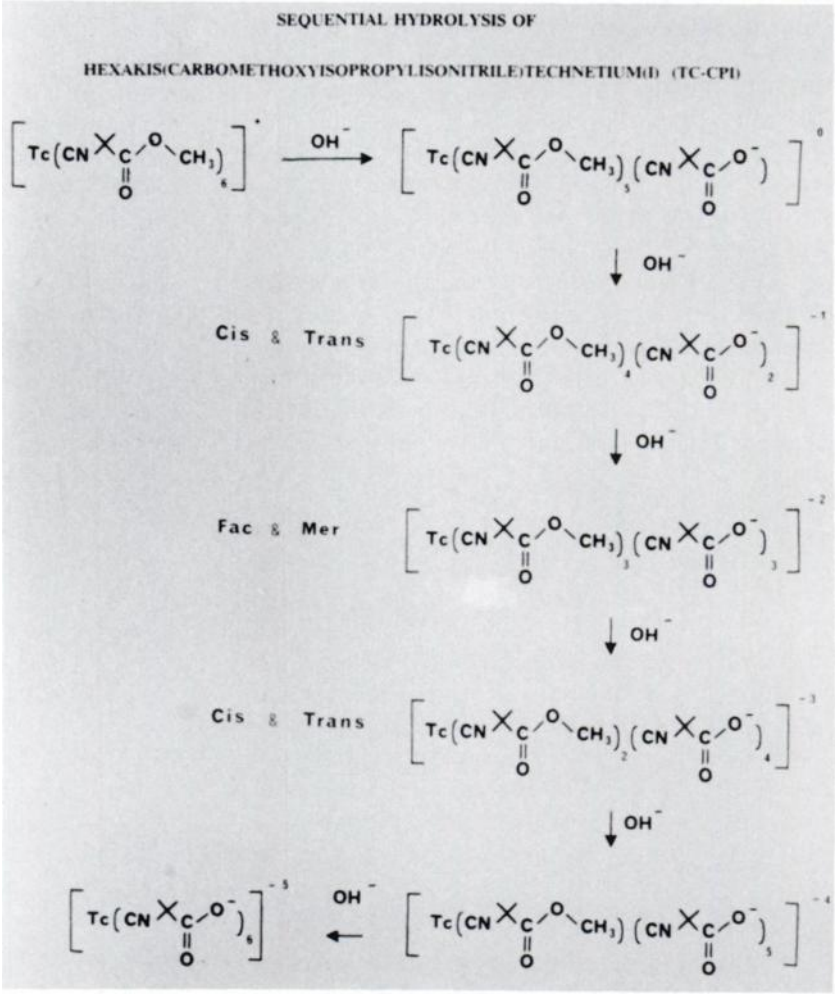

FIGURE 7
Sequence of possible in vivo ester hydrolysis reactions of $[^{99m}Tc(CNC(CH_3)_2COOCH_3)_6]^+$.

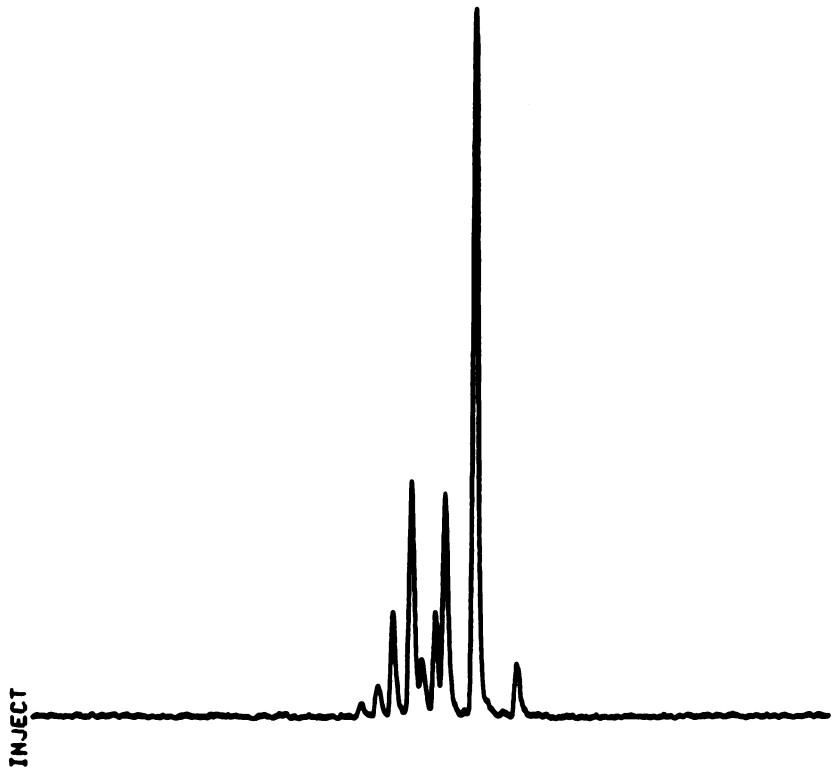

FIGURE 8
Reverse phase HPLC analysis of $[^{99m}Tc(CNC(CH_3)_2COOCH_3)_6]^+$ incubated in human serum.

7). Thus, hydrolysis of the coordinated ester certainly occurs in human plasma, and the results of preliminary clinical trials (*14*) support the conclusion that this hydrolysis also occurs in vivo. The ester complex washes out of the lung and liver much more rapidly than does the parental t-butyl complex, which does not contain a pendant functionality that can undergo reaction in vivo.

In Vivo Outer Sphere Association Reaction

The second potential ^{99m}Tc myocardial perfusion imaging agent to be evaluated in humans was the Tc(I) cation $[^{99m}Tc(DMPE)_3]^+$ (*15*). The results were disappointing because this agent clears from human blood very slowly, although it clears rapidly from dog blood. The same phenomenon was observed for the related Tc(I) cations $[^{99m}Tc(POM\text{-}POM)_3]^+$ and $[^{99m}Tc(TMP)_6]^+$ (*16*). Subsequent studies showed that these three cations bind very tightly to a plasma component that is present in human blood but not present in dog blood. Binding to this component does not alter the complex in any substantive way because the original complex can be released by treatment with organic solvents such as acetonitrile. Thus, this binding phenomenon corresponds to the outer sphere association reaction depicted in Eq. (4). These reactions are conveniently studied by HPLC techniques, which are illustrated below using the $^{99m}Tc(I)$ complex $[^{99m}Tc(DMPE)_3]^+$ as a prototype cation that does bind tightly to human plasma and the $^{99m}Tc(III)$ complex $[^{99m}Tc(DMPE)_2Cl_2]^+$ as a prototype cation that does *not* bind tightly to human plasma.

Size exclusion HPLC. Figure 9 shows size exclusion HPLC analyses of human blood plasma incubated with either the $^{99m}Tc(III)$ complex or the $^{99m}Tc(I)$ complex. Each chromatogram displays a trace resulting from uv detection of the proteins being eluted, as well as a trace resulting from radiometric detection of the ^{99m}Tc being eluted. In size exclusion chromatography the larger molecules are eluted first, whereas, the smaller molecules are retained for longer periods of time.

In the chromatogram resulting from the $[^{99m}Tc(DMPE)_2Cl_2]^+$ sample, all the proteins are eluted in <20 min, whereas, the small $^{99m}Tc(III)$ cation is is not eluted until ~40 min. (Note the change in time scale on the axis of these two chromatograms at 20 min.) No ^{99m}Tc activity is coeluted with any of the proteins, and essentially 100% of the ^{99m}Tc activity is eluted in the single peak at 40 min. These observations establish that under the conditions of this analysis, $[^{99m}Tc(DMPE)_2Cl_2]^+$ does not detectably bind to any component of human plasma.

In the chromatogram resulting from the $[^{99m}Tc(DMPE)_3]^+$ sample, a significant fraction of the ^{99m}Tc activity is coeluted with a plasma component at ~20 min. The rest of the ^{99m}Tc activity is eluted as free $[^{99m}Tc(DMPE)_3]^+$ ~40 min (again, note the change in time scale at 20 min). The arrows in Figure 9 mark on both traces the time at which the bound ^{99m}Tc is eluted (the two traces are slightly offset for clarity). This retention time corresponds to a molecular weight of less

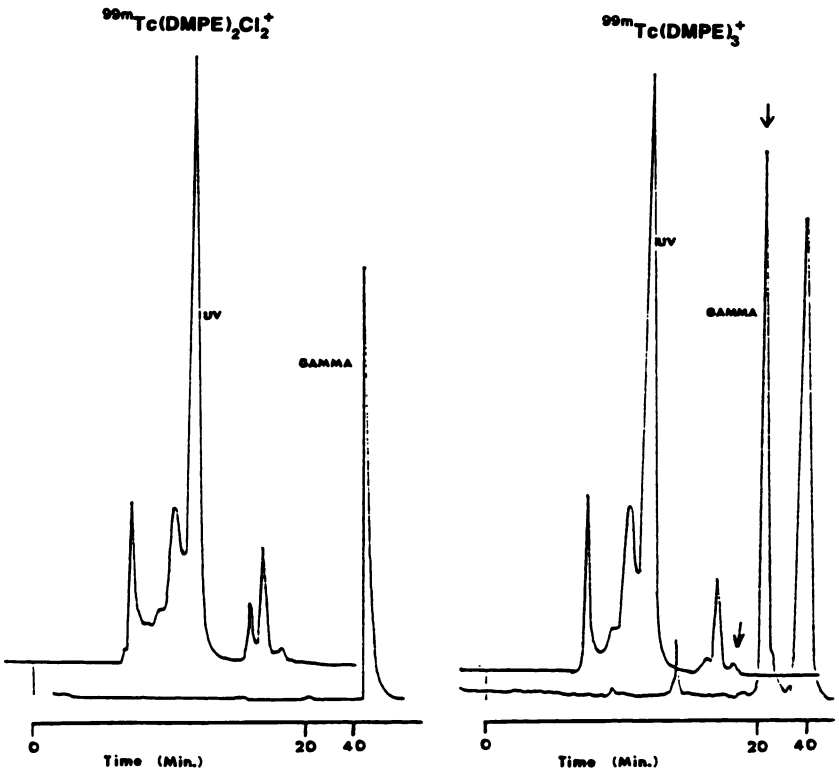

FIGURE 9
Size exclusion HPLC chromatograms of $[^{99m}Tc^{III}(DMPE)_2Cl_2]^+$ and $[^{99m}Tc^{I}(DMPE)_3]^+$ incubated in 2 ml human serum. Conditions: Bio-rad TSK-250 column, 300 × 7.5 mm; aqueous mobile phase containing 0.05*M* Na_2SO_4, 0.10*M* Na_2HPO_4 and 1% w/w tetrabutylammonium phosphate; flow rate = 0.7 ml/min for 20 min, then 1.2 ml/min. Note change of scale on time axis.

than 2,000 daltons. All three of the $^{99m}Tc(I)$ cations $[^{99m}Tc(DMPE)_3]^+$, $[^{99m}Tc(POM\text{-}POM)_3]^+$, and $[^{99m}Tc(TMP)_6]^+$ bind to this same component present in human plasma, but show no equivalent binding to any component in dog plasma. As noted above, these complexes can be recovered intact from their bound form by treating the bound form with organic solvents such as acetonitrile.

Internal surface reverse phase high performance liquid chromatography. A new form of HPLC has been

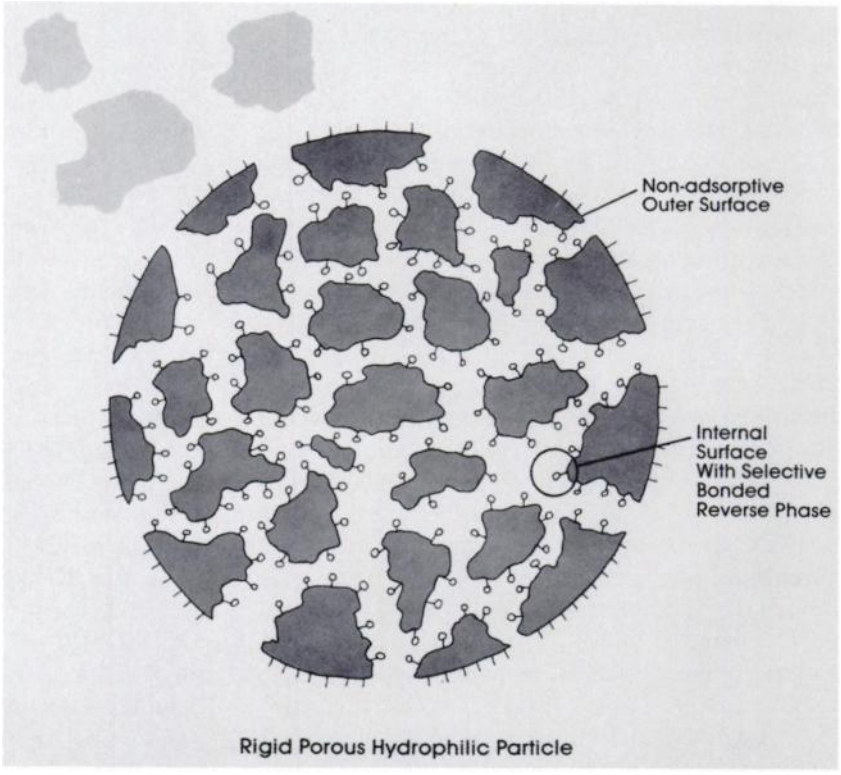

FIGURE 10
Schematic drawing of internal surface reverse phase HPLC substrate particle. Reproduced with permission of Regis Chemical Co.

introduced recently to facilitate the reverse phase analysis of small molecules present in complicated biological samples (*4,5*). Figure 10 shows that in this form of chromatography the internal surfaces of porous silica beads are coated with hydrophobic groups, whereas, the external surfaces are coated with nonabsorptive, hydrophilic groups. Large protein molecules cannot enter the interior portions of the bead, and so they pass by the exterior surfaces without undergoing any sorption or retention. The smaller analyte molecules can penetrate to the interior portions of the bead and become bound to the hydrophobic surface. After the proteins have been eluted, the smaller analyte molecules can be subjected to standard reverse phase chromatographic conditions for separation and identification.

Figure 11 shows internal surface reverse phase (ISRP) HPLC analyses of human blood plasma samples that have been incubated with either $[^{99m}Tc(DMPE)_2Cl_2]^+$ or $[^{99m}Tc(DMPE)_3]^+$. Again, the uv trace monitors elution of the proteins while the radiometric trace monitors elution of ^{99m}Tc activity. (The uv trace also reflects the change in refractive index of the mobile phase as organic modifiers are added in order to conduct the reverse phase chromatography; only the first bundle of peaks in each uv chromatogram corresponds to proteins being eluted.) In the chromatogram resulting from incubation of the $^{99m}Tc(III)$ cation, no ^{99m}Tc is coeluted with the proteins, confirming that $[^{99m}Tc(DMPE)_2Cl_2]^+$ does not detectably bind to components of human plasma. However, in the chromatogram resulting from incubation of the $^{99m}Tc(I)$ cation, a significant fraction of the ^{99m}Tc activity is coeluted with the proteins, confirming that $[^{99m}Tc(DMPE)_3]^+$ binds tightly to a component (or components) of human blood plasma.

This form of chromatography does not yield as much information about the nature of the protein component as does size exclusion chromatography, but it is easier and quicker to conduct. Thus, it provides a useful technique for screening new potential ^{99m}Tc agents for their tendency to bind to human plasma components.

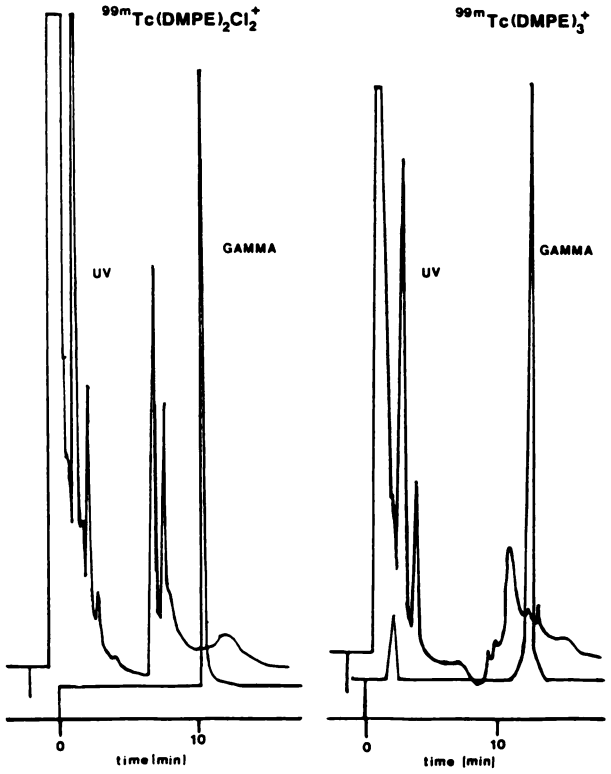

FIGURE 11
ISRP HPLC chromatograms of $[^{99m}Tc^{III}(DMPE)_2Cl_2]^+$ and $[^{99m}Tc^{I}(DMPE)_3]^+$ incubated in 2 ml human serum. Conditions: Regis ISRP column, 150 × 4.6 mm; mobile phase A = 0.05*M* aqueous NH_4OOCCH_3; mobile phase B = 85:15 methanol/(0.05*M* aqueous NH_4OOCCH_3); A is run for 6.5 min at 0.8 ml/min, then a linear gradient over 2.0 min converts the mobile phase to 100% B at a flow rate of 1.2 ml/min, which is then run for 8.0 min.

SUMMARY

Even though the terms "in vivo" and "inorganic" are essentially contradictory, the inorganic chemistry of ^{99m}Tc cations definitely affects the in vivo behavior of these species. The clearest example of this is provided by the in vivo reduction of $[^{99m}Tc(DMPE)_2Cl_2]^+$, which adversely affects it utility as a heart imaging agent. However, the reactions of coordinated ligands and outer sphere association reactions, also occur in vivo for ^{99m}Tc cations and also drastically affect the biodistribution of these species. High performance liquid chromatography techniques are very useful for monitoring this in vivo chemistry, but are complicated by artifacts resulting from the fact that the ^{99m}Tc cations are present in vivo at only $\sim 10^{-10}M$ concentration. Properly designed and executed control experiments are crucial to avoiding the pitfalls generated by these artifacts.

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