# Electrophoretic Determination of Charge on Carrier-Free $^{99m}$Tc-Labeled Complexes

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Received July 18, 1979, from the *Division of Nuclear Medicine and Department of Chemistry, Johns Hopkins University, Balitmore, MD 21205.* Accepted for publication August 6, 1980.

**Abstract** □ A new method for determining the charge on carrier-free $^{99m}$Tc-labeled complexes is described. This method and mixed-ligand experiments were used to determine if the charge on the technetium 99m complex of *N*-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid (I) is −1 and if the ligand to technetium ratio in the complex is 2:1. The preparation of an iodinated analog (II) of I and its $^{99m}$Tc-labeled complex is described, as is the biodistribution of the $^{99m}$Tc-labeled complex in mice. The syntheses of the $^{51}$Cr(III)- and $^{57}$Co(III)-labeled complexes also are described. The net biliary excretion in mice of both the $^{99m}$Tc- and $^{51}$Cr-labeled complexes of II was significantly greater than that of the $^{99m}$Tc-labeled complex of I.

**Keyphrases** □ *N*-(2,6-Dimethylphenylcarbamoylmethyl)iminodiacetic acid—complexation with technetium 99m, electrophoretic determination of charge on carrier-free $^{99m}$Tc-labeled complexes, synthesis and evaluation of net biliary excretion □ Radiopharmaceuticals—$^{99m}$Tc-labeled iminodiacetic acid derivatives, electrophoretic determination of charge on carrier-free $^{99m}$Tc-labeled complexes, synthesis, evaluation of net biliary excretion □ Complexes—technetium 99m with iminodiacetic acid derivatives, electrophoretic determination of charge on carrier-free $^{99m}$Tc-labeled complexes, evaluation of net biliary excretion

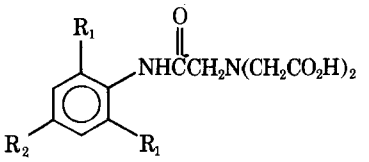
Since the first reported use of a technetium 99m-labeled compound as a diagnostic radiopharmaceutical in 1962 (1), numerous attempts have been made to design $^{99m}$Tc-labeled radiopharmaceuticals. Of the many compounds that have been labeled, a large number have one common feature: their biodistributions depend to some extent on their interaction with structurally specific receptors located in various tissues in the body (2, 3).

## BACKGROUND

In most cases, the structural requirements for receptor binding are rigid so that the structure of the compound must be controlled carefully. There has been little success in the design of $^{99m}$Tc-labeled radiopharmaceuticals of this type, in part due to the lack of a well-defined chemistry of technetium. The technetium concentration utilized in $^{99m}$Tc-labeled radiopharmaceuticals is $10^{-10}$ *M*, which is too low to permit the study of their chemistry by standard techniques (4).

To facilitate the design of $^{99m}$Tc-labeled radiopharmaceuticals, an investigation was aimed toward developing new techniques for obtaining structural information. A mixed-ligand experiment was described previously and can be used to determine ligand–technetium stoichiometry in $^{99m}$Tc-labeled complexes at concentrations as low as $10^{-13}$ *M* (4). This paper reports an extension of the previous study; the net charge on $^{99m}$Tc-labeled complexes at concentrations as low as $10^{-13}$ *M* can be determined. This approach is illustrated with work on a new hepatobiliary radiopharmaceutical, the $^{99m}$Tc-labeled complex of *N*-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid[1] (I).

To prepare a $^{99m}$Tc-labeled lidocaine analog, I was synthesized containing an iminodiacetic acid functional group (5). This functional group can form stable complexes with transition metals such as technetium. Harvey *et al.* (5) showed that I forms a stable complex with technetium 99m, which is excreted unchanged after intravenous injection into mice and dogs. Excretion occurs mainly *via* the hepatobiliary system with minimal urinary excretion. The extensive biliary excretion of the $^{99m}$Tc-labeled complex of I led to considerable interest in this complex as a radiopharmaceutical for the evaluation of hepatobiliary function.




I: $R_1 = CH_3$, $R_2 = H$
II: $R_1 = CH_3$, $R_2 = I$
III: $R_1 = H$, $R_2 = CO_2H$
IV: $R_1 = H$, $R_2 = NH_2$

It was recently reported that the ratio of ligand to technetium in $^{99m}$Tc-labeled I is 2:1 and that the oxidation state of technetium is +3 (6). This finding and results of stoichiometry and charge studies led to the study of the $^{51}$Cr(III)- and $^{57}$Co(III)-labeled complexes of a similar compound, *N*-(2,6-dimethyl-4-iodophenylcarbamoylmethyl)-iminodiacetic acid (II). The iminodiacetic acid and methyliminodiacetic acid complexes of Cr(III) and Co(III) were prepared previously and shown to be mononegative bis-complexes. This paper reports the results of experiments with I that have established the ligand–technetium stoichiometry and the overall charge of the $^{99m}$Tc-labeled complexes of I–IV.

The mixed-ligand and charge determination experiments required (in addition to I) analogs of I that would complex technetium similarly but that differed in charge. Two such ligands are *N*-(*p*-carboxyphenylcarbamoylmethyl)iminodiacetic acid (III) and *N*-(*p*-aminophenylcarbamoylmethyl)iminodiacetic acid (IV). The iminodiacetic acid functional groups of these compounds form essentially identical complexes with technetium 99m as I. In addition, the arylcarboxyl group of III imparts additional negative charge to the complex at pH > 5, and the amino functional group of IV makes the $^{99m}$Tc-labeled complex more positive than that of I at pH < 5.

## EXPERIMENTAL

Compounds I and III were synthesized as reported previously (7). The amino-substituted (IV) and iodinated (II) analogs were prepared as described later. PMR spectra were obtained on a 60-MHz spectrometer[2], with chemical shifts reported relative to tetramethylsilane. IR spectra were obtained on a grating IR spectrometer[3]. Melting points[4] were determined and are uncorrected. Elemental analyses were performed for carbon, hydrogen, and nitrogen[5].

All chemicals used were at least reagent grade. Sodium [$^{99m}$Tc]pertechnetate was eluted from a molybdenum 99–technetium 99m generator[6] with normal saline at a specific concentration of 10–20 mCi/ml. [$^{51}$Cr]Chromic chloride and [$^{57}$Co]cobaltous chloride were obtained commercially in 0.5 *M* HCl[7].

***N*-(4-Aminophenylcarbamoylmethyl)iminodiacetic Acid (IV)**—A solution of *N*-(4-nitrophenylcarbamoylmethyl)iminodiacetic acid (7) (2.0 g, 0.0064 mole) in 250 ml of water was prepared, and sufficient ammonium hydroxide was added to raise the pH to 8. A catalytic amount of 10% palladium-on-charcoal was added, and the solution was hydrogenated for 16 hr at 40 psi[8]. Removal of the catalyst by filtration and evaporation to dryness *in vacuo* yielded a yellow oil, which solidified upon addition of ether and anhydrous ethanol. Recrystallization from 95%

[1] HIDA.

[2] Perkin-Elmer Hitachi R-12A.
[3] Pye-Unicam Sp-1000.
[4] Mel-Temp apparatus.
[5] Robertson Microanalytical Laboratory, Florham Park, N.J.
[6] Squibb, Minitech.
[7] New England Nuclear Corp.
[8] Parr apparatus.



*0022-3549/81/0400-0436$01.00/0*

ethanol yielded 1.47 g (78.3%) of the monoammonium salt of IV as white needles, mp 298° dec.; IR (mineral oil): 3200–3400 (broad, NH) and 1680–1700 (broad, C=O) $cm^{-1}$; PMR ($D_2O$): δ 3.34 (s, 4H, $NCH_2CO_2^-$), 3.55 (s, 2H, $NCH_2CON$), 6.62 (d, 2H, aromatic H), and 6.98 (d, 2H, aromatic H).

*Anal.*—Calc. for $C_{12}H_{15}N_3O_5 \cdot NH_3$: C, 48.32; H, 6.09; N, 18.78. Found: C, 48.09; H, 6.12; N, 18.48.

**N-(2,6-Dimethyl - 4 - iodophenylcarbamoylmethyl)iminodiacetic Acid (II)**—A suspension of nitrilotriacetic acid (3.87 g, 0.02 mole) in 50 ml of anhydrous pyridine was placed in a three-necked, round-bottom flask equipped with a nitrogen inlet tube, a condenser, a calcium sulfate drying tube, and a thermometer. After flushing with nitrogen, the suspension was heated at 50° for 10 min, at which time most of the nitrilotriacetic acid had dissolved. Acetic anhydride (3.09 g, 0.03 mole) was added, and the solution was heated at 100° for 60 min. After cooling to 50°, *p*-iodoxylidene (5.0 g, 0.02 mole) was added, and the solution was heated to 100° for 2 hr.

Evaporation of the pyridine *in vacuo* left a purple oil, which then was dissolved in a minimal amount of aqueous sodium hydroxide (pH 10–11). After extraction with equal portions of ether and treatment with decolorizing carbon, the almost colorless aqueous layer was acidified to pH 4, resulting in the formation of a white syrupy solid. Trituration with ethanol–ether yielded a white powder. This powder was recrystallized from 85% ethanol–water as white needles (3.5 g, 40%), mp 205° dec.; IR (mineral oil): 3300 (NH) and 1660–1710 (broad, acid and amide C=O) $cm^{-1}$; PMR (dimethyl sulfoxide-$d_6$): δ 2.14 (s, 6H, aromatic $CH_3$), 3.4 (s, 6H, $COCH_2N$), and 7.48 (s, 2H, aromatic H).

*Anal.*—Calc. for $C_{14}H_{17}IN_2O_5$: C, 40.02; H, 4.08; N, 6.67. Found: C, 40.15; H, 3.93; N, 6.66.

**Preparation of $^{99m}$Tc-Labeled Complexes of I, III, and IV**—A solution of 10 mg (0.02–0.034 mmole) of the appropriate compound (I, III, or IV) in 0.5 ml of 0.1 *N* NaOH was prepared, and the pH was adjusted to 5.0–5.5 with 0.5 *N* HCl. After addition of 0.3 ml of the generator eluate (obtained by saline elution of a 400-mCi molybdenum 99–technetium 99m generator at a specific concentration of 10–20 mCi/ml), the solution was purged with nitrogen for 5 min. Then 0.1 ml of freshly prepared stannous chloride dihydrate solution (250 μg/ml in 0.001 *N* HCl) was added. The solution was kept at room temperature for 20 min prior to use.

**Preparation of $^{99m}$Tc-Labeled Complex of I *via* Sodium Borohydride**—A solution of 10 mg (0.034 mmole) of I in 0.5 ml of 0.1 *N* NaOH was prepared, and the pH was adjusted to 5.5 with 0.5 *N* HCl. After addition of 0.3 ml of the generator eluate, the solution was purged with nitrogen for 5 min. Then 0.1 ml of freshly prepared 0.5 *N* sodium borohydride solution (18.0 mg/ml of water) was added. After 20 min at room temperature, the solution was filtered[9], and an aliquot of the filtrate was analyzed by electrophoresis.

**Mixed-Ligand Experiment**—A solution containing 5 mg (0.016 mmole) of I and 5 mg (0.016 mmole) of III in 0.5 ml of 0.1 *N* NaOH was prepared, and the pH was adjusted to 5.0–5.5 with 0.01 *N* HCl. After addition of 0.3 ml of the generator eluate, the solution was purged with nitrogen for 5 min. Then 0.1 ml of freshly prepared solution of stannous chloride dihydrate (250 μg/ml in 0.001 *N* HCl) was added. This solution was left at room temperature for 20 min prior to use.

**Preparation of Co(III)–II Complex**—To a solution of II (10 mg, 0.034 mmole) dissolved in 0.95 ml of 0.05 *M* aqueous NaOH was added 2 mg (0.005 mmole) of cobaltous chloride hexahydrate. After the salt dissolved, 0.05 ml of [$^{57}$Co]cobaltous chloride in 0.5 *M* HCl and 0.05 ml of 0.5 *M* NaOH were added simultaneously to the stirred solution from syringes. Hydrogen peroxide (1.5 ml, 30%) was added to the clear pink solution. The oxidation of Co(II) to Co(III) required 20–30 min, at which time the solution became deep purple.

The solution then was absorbed into a 15 × 0.7-cm diameter cation-exchange column[10] in the $Na^+$ form. [This column reserves any remaining unoxidized Co(II).] The column was eluted with a solution of 0.15 *M* NaCl and 0.01 *M* $NaHCO_3$. The desired product eluted with the bed volume and was identified easily by the purple color characteristic of Co(III) complexes chelated by two iminodiacetic acid moieties.

Evidently, the presence of the methylcarbamoyl group reduces the ligand field strength of the iminodiacetic acid group sufficiently to destabilize the Co(III) state relative to the Co(II) state. Consequently, if the solution was to be stored, 1 ml of 30% $H_2O_2$ was added to maintain the Co(III) oxidation state. The complex showed a band at 552 nm (ε 269). The absorptivity was determined by using a cobalt concentration determined by atomic absorption spectroscopy.

**Preparation of Cr(III)–II Complex**—Water (0.2 ml) and dimethylformamide (0.3 ml) were added to a 2-ml vial containing II (150 mg, 0.5 mmole). After the addition of sodium hydroxide (15 mg, 0.32 mmole), the mixture was stirred at ~70° until all solids were dissolved. The pH at this point was ~4.8. To this hot solution was added [$^{51}$Cr]chromic chloride (25 μl in 0.5 *M* HCl), and the solution was maintained at 70° for 15 hr. Then, the hot solution was placed on a 15 × 0.7-cm column of fine grain sand[11].

On cooling, the reaction mixture solidified and formed a pellet in the top of the sand. Elution with 95% ethanol yielded the desired product in the sixth 0.3-ml fraction. (A neutral complex was obtained in the 19th 0.3-ml fraction.) The ethanol was evaporated from the sixth fraction, and the $[Cr-(II)_2]^-$ was redissolved in saline.

**Preparation of $[Tc-(II)_2]^-$**—To a 2-ml vial containing 18 mg (0.043 mmole) of II and 0.3 ml of 0.2 *N* NaOH was added 0.2–0.4 ml of a sodium [$^{99m}$Tc]pertechnetate solution in saline. Product formation was complete within 30 min of the addition of a stannous chloride solution (0.1 ml) containing 250 μg of $SnCl_2 \cdot 2H_2O$/ml of ethanol. The final solution was filtered[12].

**Electrophoresis**—Low-voltage electrophoresis was performed on paper[13] at 300 v for 30 min or at 600 v for 15 min in a 0.01 *M* $NaHCO_3$ buffer (pH 7). Under these conditions, the [$^{99m}$Tc]pertechnetate anion migrated ~13 cm. A pertechnetate standard was utilized with all samples. High-voltage electrophoresis was performed on paper[14] using 0.01 *M* $NaHCO_3$ buffer at 3000 v at 17° for 25 min.

Silical gel[15] chromatography was performed with 7.5-cm strips and dimethylformamide–*n*-butanol (1:1 v/v) saturated with 3 *M* $NH_4OH$. The distance each complex migrated was determined by scanning the dried paper with a chromatogram scanner equipped with a 2.54-cm sodium iodide detector and a collimator, which consisted of a 0.635-cm thick sheet of lead with a 2.54-cm × 3-mm slit. The results obtained on any given electrophoragram were expressed as:

$$R_s = \frac{\text{distance migrated by complex}}{\text{distance migrated by pertechnetate ion}} \qquad \text{(Eq. 1)}$$

These $R_s$ values were essentially independent of the distance migrated by the pertechnetate ion over at least 11–15 cm.

Low-voltage electrophoresis at pH 3 was performed as described except that a mixed citrate–phosphate buffer was used in place of the bicarbonate buffer. The citrate–phosphate buffer was prepared by mixing 39.8 ml of 0.1 *M* citric acid (19.21 g/1000 ml) and 10.2 ml of 0.2 *M* dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$, 53.65 g/1000 ml), followed by dilution to 1000 ml.

Chromatography was performed on silica gel[15] with acetone as the elution solvent.

Biodistributions were performed in 25–35-g ICR albino mice[16]. A solution of the complex in 0.1 ml of saline was injected into the tail vein. Six mice were used for each data point. The animals were sacrificed by ether asphyxiation 60 min postinjection. A blood sample (100 μl) was obtained immediately before death by cardiac puncture. The lungs, heart, spleen, liver, stomach, intestines, kidneys, and bladder were removed and weighed. Care was taken to prevent urine from contaminating the carcass when the bladder was removed. The organs and carcasses were counted, and the percent of the injected dose per organ and carcass was calculated by comparison to a standard prepared from 0.10 ml of the injection solution at the time of injection.

## RESULTS AND DISCUSSION

Preparation of the $^{99m}$Tc-labeled complex of I using stannous chloride reduction yielded a complex that migrated as a single discrete spot on electrophoresis with an $R_s$ of 0.68. Use of sodium borohydride instead of stannous chloride as a reducing agent for the preparation of this complex also gave a complex that migrated as a single spot with an $R_s$ of 0.68.

This result, coupled with the fact that both procedures yielded complexes with identical biodistributions in mice (Table I), indicates that tin is not incorporated into the complex. This interpretation is supported

[9] Millipore.
[10] Chelex 100, 50–100 mesh.
[11] Fisher.
[12] Millipore, 0.45 or 0.2 μm.
[13] Whatman 3 mm.
[14] Whatman No. 1.
[15] Bakerflex 113-P.
[16] Camm Research Laboratories.

**Table I—Biodistribution of Technetium 99m–$(I)_2$ Complex in Mice 60 min Postinjection**

| Sample | Percent of Dose per Organ[a] | |
|---|---|---|
| | Stannous Chloride Preparation | Sodium Borohydride Preparation |
| Blood[b] | 0.05 ± 0.02 | 0.07 ± 0.03 |
| Kidneys | 0.60 ± 0.13 | 0.57 ± 0.23 |
| Lungs | 0.08 ± 0.03 | 0.10 ± 0.07 |
| Heart | 0.02 ± 0.01 | 0.03 ± 0.01 |
| Spleen | 0.02 ± 0.01 | 0.03 ± 0.01 |
| Liver | 1.52 ± 0.96 | 1.53 ± 1.05 |
| Stomach | 0.63 ± 0.11 | 1.40 ± 0.90 |
| Intestine | 68.6 ± 4.2 | 66.7 ± 3.7 |
| Carcass | 3.44 ± 1.17 | 3.79 ± 1.10 |
| Excreted | 24.1 | 24.5 |

[a] Each point represents the mean of six mice ± 1 *SD*. [b] Per 0.1 ml.

by recent work where tin 113 was not incorporated into the complex (6).

Electrophoresis of the $^{99m}$Tc-labeled complex of III showed only one complex in solution; it migrated with an $R_s$ of 0.97. Since it was impossible to distinguish between this complex and $^{99m}$Tc-labeled pertechnetate using this electrophoretic procedure, chromatography was performed for all samples on silica gel eluted with acetone. Chromatography easily established the absence of pertechnetate in the sample since the complex remained at the origin while pertechnetate migrated with the solvent front. The finding that the $^{99m}$Tc-labeled complex of III migrated farther than the $^{99m}$Tc-labeled complex of I on electrophoresis was expected since III has an additional carboxyl group, which imparts a greater overall charge to the complex.

When the mixed-ligand experiment was performed with I and III, electrophoresis showed three complexes. One migrated with an $R_s$ of 0.68 and corresponded to the $^{99m}$Tc-labeled complex of I. A second complex migrated with an $R_s$ of 0.97, identical to that of the $^{99m}$Tc-labeled complex of III. The third complex migrated with an $R_s$ of 0.83 and represented a new, mixed-ligand complex containing one molecule of I and one molecule of III. As discussed previously (4), this result in a mixed-ligand experiment indicates that the complexes formed are bis-complexes with two molecules of ligand bound per molecule of technetium.

Electrophoresis of the $^{99m}$Tc-labeled complex of I at pH 3 showed that the complex migrated the same distance as it did at pH 7, indicating that no functional groups can be protonated over the pH 7–3 range. This finding implies that all of the iminodiacetic acid carboxyl groups are bound to technetium 99m in these complexes. This finding also permitted the determination of the net charge on the complex. From the mixed-ligand experiment, it was shown that these iminodiacetic acid ligands form bis-complexes with technetium 99m, as is illustrated in Fig. 1 for the $^{99m}$Tc-labeled complex of IV. The overall charge on this complex at pH 7 was identical to that of the $^{99m}$Tc-labeled complex of I since both were approximately the same size and both migrated the same distance when electrophoresis was performed at pH 7. When electrophoresis was performed at pH 3, the $^{99m}$Tc-labeled complex of I still migrated toward the anode with an $R_s$ of 0.68; however, the $^{99m}$Tc-labeled complex of IV migrated toward the cathode, indicating that the overall charge on the complex was positive at this pH.

Since the $^{99m}$Tc-labeled complex of IV contains only two amino groups that are protonated at pH 3, protonation can, at most, add two units of positive charge to the complex. The fact that addition of only two units

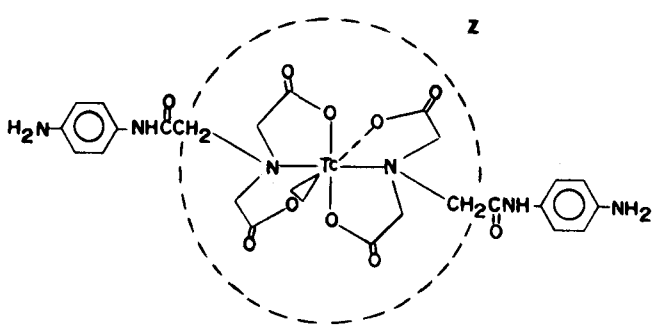



**Figure 1**—*Suggested structure of iminodiacetic acid complexes of technetium illustrating the presence of two iminodiacetic acid molecules in the complex.*

**Table II—Chromatographic and Electrophoretic Behaviors of $[M–(II)_2]^-$[a]**

| | Technetium | Cobalt | Chromium |
|---|---|---|---|
| Chromatography[b] | 0.65 | 0.58 | 0.8 |
| Low-voltage electrophoresis[c] | 8 | 8 | 7.5 |
| High-voltage electrophoresis[c] | 11.5 | 11.5 | 11.5 |

[a] M = transition metals technetium, cobalt, and chromium. [b] $R_f$ values. [c] Distance from origin in centimeters.

**Table III—Biodistribution of $^{99m}$Tc–$(II)_2$ and $^{51}$Cr–$(II)_2$ in Mice 60 min Postinjection**

| Sample | Percent of Dose per Organ[a] | |
|---|---|---|
| | $^{99m}$Tc–$(II)_2$ | $^{51}$Cr–$(II)_2$ |
| Blood[b] | 0.01 ± 0.001 | 0.07 ± 0.01 |
| Kidneys | 0.21 ± 0.12 | 1.05 ± 0.43 |
| Lungs | 0.02 ± 0.01 | 0.09 ± 0.02 |
| Heart | 0.01 ± 0.01 | 0.02 ± 0.01 |
| Spleen | 0.01 ± 0.01 | 0.02 ± 0.01 |
| Liver | 1.04 ± 0.89 | 8.2 ± 3.7 |
| Stomach | 0.05 ± 0.04 | 0.22 ± 0.08 |
| Intestine | 82.3 ± 4.5 | 80.6 ± 6.5 |
| Carcass | 0.79 ± 0.08 | 4.8 ± 1.5 |

[a] Each point represents the mean of six mice ± 1 *SD*. [b] Per 0.1 ml.

of positive charge to the $^{99m}$Tc-labeled complex of IV yielded a cationic complex indicates that, at pH 7, the negative charge on the $^{99m}$Tc-labeled complex of IV can be no more than 1. The fact that the $^{99m}$Tc-labeled complex of IV migrated toward the anode at pH 7 indicates that the negative charge can be no less than 1. These findings, taken together, establish that the net charge on this complex at pH 7 (and, thus, the net charge on the $^{99m}$Tc-labeled complex of I) is −1.

The results of chromatographic and electrophoretic experiments comparing the $^{99m}$Tc-, $^{57}$Co-, and $^{51}$Cr-labeled complexes of II are shown in Table II. The finding that each complex behaved in essentially the same manner on chromatography and electrophoresis, coupled with the previous report that Cr(III) and Co(III) formed mononegative bis-complexes with iminodiacetic acid ligands, supports our conclusion that the charge on the $^{99m}$Tc-labeled complex of I is −1. These findings also suggest that the extensive known chemistry of Cr(III) and Co(III) may be useful in predicting the types of complexes formed by Tc(III), at least for amino acid-type ligands.

The biodistributions of the $^{99m}$Tc- and $^{51}$Cr-labeled complexes of II in mice 60 min postinjection are shown in Table III. The net biliary excretion of these complexes was higher than that of the $^{99m}$Tc-labeled complex of I, with 83% of the complex excreted in the bile at 60 min compared to 67–68% for the $^{99m}$Tc-labeled complex of I. Furthermore, the distributions of the $^{99m}$Tc- and $^{51}$Cr-labeled complexes of II were essentially identical. The biodistribution of the $^{57}$Co-labeled complex of II could not be obtained since the Co(III) center was reduced too readily to Co(II).

The recent report by Loberg and Fields (6) that the oxidation state of technetium 99m in the $^{99m}$Tc-labeled complex of I is +3, coupled with the present finding that the net charge on this complex is −1, suggests that, in this case, technetium is hexacoordinate with only the iminodiacetic acid nitrogens and oxygens bound to technetium (Fig. 1).

Although the possibility for *cis*- and *trans*-isomers exists, only the *trans*-isomer probably actually forms. Iminodiacetic acid complexes of Cr(III) form only the *trans*-isomer when the iminodiacetic acid nitrogen is substituted by methyl or larger groups due to steric hindrance between the *N*-substituents in the *cis*-isomer (8).

The possibility that the technetium is in a $Tc(V)O^{3+}$ unit cannot be excluded, but the similar biodistribution of the technetium and chromium compounds and the reported (6) determination of the oxidation state as +3 make such an explanation unlikely.

Although this report demonstrates the utility of this type of experiment for determining structural information on carrier-free $^{99m}$Tc-labeled complexes, these methods can be applied to other $^{99m}$Tc-labeled radiopharmaceuticals, provided appropriate ligands can be designed and synthesized. A particularly attractive feature of this approach is that the isolation of chemically pure technetium complexes is not required. The difficulties involved in the isolation and characterization of $^{99m}$Tc-labeled complexes that are functionalized to be radiopharmaceuticals are reflected by the fact that no $^{99m}$Tc-labeled complex identical to a clinically used $^{99m}$Tc-labeled complex in solution has been characterized. Of par-

ticular note was the failure of many attempts to obtain crystalline products of I or analogs of I when they were attached to Co(III) or Cr(III), although the iminodiacetic acid and methyl iminodiacetic acid complexes are prepared and isolated easily. It is hoped that the approach outlined here for gaining information on the structure of $^{99m}$Tc-labeled radiopharmaceuticals will be useful for understanding the mechanisms of localization of currently used radiopharmaceuticals and in the design of new radiopharmaceuticals.

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## ACKNOWLEDGMENTS


Supported by U.S. Public Health Service Grants GM10548 and HL17655.


# Radiolabeled Benzoylcholine Derivatives as Possible Myocardial-Imaging Agents


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Received February 22, 1980, from the *Division of Nuclear Medicine and the Department of Chemistry, Johns Hopkins University, Baltimore, MD 21205.* Accepted for publication September 25, 1980.



**Abstract** □ Two radioiodinated analogs of benzoylcholine were investigated as possible myocardial-imaging agents. *O*-([2-$^{125}$I]Iodobenzoyl)-choline and *N*-([2-$^{125}$I]iodobenzoyl)cholamine were prepared by nucleophilic substitution of sodium [$^{125}$I]iodide for stable iodine in *O*-(*N,N*-dimethylaminoethyl)-2-iodobenzoate and *N*-(*N',N'*-dimethylaminoethyl)-2-iodobenzamide, respectively, and by methylation with methyl iodide. The *in vivo* distribution of each compound in mice was determined as a function of time. Favorable heart-to-blood and heart-to-lung ratios were obtained with *N*-([2-$^{125}$I]iodobenzoyl)cholamine.



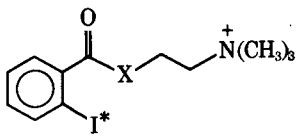
**Keyphrases** □ Benzoylcholine, radioiodinated analogs—synthesized and evaluated as possible myocardial-imaging agents, biodistribution studies in mice □ Radionuclide imaging, myocardial—radioiodinated benzoylcholine analogs synthesized and evaluated for use as myocardial-imaging agents, mice □ Radiopharmaceuticals, iodinated—benzoylcholine analogs, synthesis and evaluation for use as myocardial-imaging agents, mice □ Biodistribution—radioiodinated benzoylcholine analogs synthesized and evaluated for use as myocardial-imaging agents, mice


The use of radiolabeled enzyme inhibitors as potential organ-imaging agents has been suggested by several investigators. Wieland *et al.* (1) studied the distribution of several radiolabeled inhibitors of the adrenal cortex enzymes, 20$\alpha$-hydroxylase, 11$\beta$-hydroxylase, and 17$\alpha$-hydroxylase. The finding that some of these inhibitors localized in the adrenal cortex suggested that radiolabeled enzyme inhibitors might be useful in the design of new diagnostic radiopharmaceuticals.

## BACKGROUND

Recently, Burns *et al.* (2) studied the distribution of a simple, positively charged inhibitor of acetylcholinesterase. Their results suggested that labeled inhibitors of acetylcholinesterase might be useful as myocardial-imaging agents due to relatively high levels of acetylcholinesterase activity in the heart of some species.

Another study demonstrated that the cholinesterases of erythrocytes and plasma were different (3). The work of several investigators (4–8) clearly established the distinguishing characteristics between acetylcholinesterase (true cholinesterase or acetylcholine hydrolase) and pseudocholinesterase (butyrylcholinesterase or acylcholine acylhydrolase). Acetylcholinesterase predominates in erythrocytes, the central nervous system, and the motor endplates of skeletal muscle. Pseudocholinesterase predominates in the liver, plasma, and many types of smooth muscle. Both enzymes occur in high concentrations in autonomic ganglia (9). In some species, the distribution of pseudocholinesterase activity (10) indicates that an appropriate radiolabeled substrate or inhibitor might be a useful imaging agent for the myocardium.




V: X = O
VI: X = NH

Many compounds are hydrolyzed by pseudocholinesterase including butyrylcholine, acetylcholine, and benzoylcholine. The fact that benzoylcholine is a substrate for butyrylcholinesterase, coupled with the observation that iodoaryl compounds are generally resistant to *in vivo* deiodination (11), prompted the investigation of two radioiodinated analogs of benzoylcholine as potential myocardial-imaging agents. This report details the syntheses and preliminary biological studies of *O*-([2-$^{125}$I]iodobenzoyl)choline (V) and *N*-([2-$^{125}$I]iodobenzoyl)cholamine (VI).

## EXPERIMENTAL[1]

***O*-(*N,N*-Dimethylaminoethyl)-2-iodobenzoate (I)**—2-Iodobenzoic acid (19.7 g, 0.077 mole), thionyl chloride (19.2 g, 0.162 mole), and a catalytic amount of dimethylformamide (50 $\mu$l) were warmed gently in a water bath. The evolution of hydrogen chloride and sulfur dioxide ceased after 60 min, and the solution was evaporated under reduced pressure to remove excess thionyl chloride. Benzene (100 ml) was added to the

[1] PMR spectra were obtained on a Varian T-60 spectrometer, with chemical shifts reported relative to tetramethylsilane or 3-(trimethylsilyl)propionic acid sodium salt as noted. IR spectra were recorded on a Pye-Unicam SP-1000 spectrometer. Melting points were determined in a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Robertson Microanalytical Laboratory, Florham Park, N.J. Radioactive iodine was obtained from New England Nuclear Corp. as a carrier-free solution of sodium [$^{125}$I]iodide in a pH 8–10 aqueous solution (reductant free) at a specific activity of ~350 mCi/ml. The solution was diluted with normal saline to a specific activity of 10 mCi/ml.


0022-3549/81/0400-0439$01.00/0