jnm/ RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Development of New Radiopharmaceuticals Based on N-Substitution of Iminodiacetic Acid

Michael D. Loberg, Malcolm Cooper, Elizabeth Harvey, Patrick Callery, and William Faith

University of Maryland, Baltimore, Maryland

A new approach to radiopharmaceutical design is demonstrated, in which small chelating groups capable of binding gamma-emitting radiometals are attached to biologically active molecules, thus producing radiopharmaceuticals based on bifunctional drug and biochemical analogs. The chelating group iminodiacetic acid has been evaluated for this role by examining two N-substituted iminodiacetic acids: methyliminodiacetic acid (MIDA) and N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid (HIDA). Radiochemical and biologic studies showed that both agents were obtained in high radiochemical purity, were stable in vitro and in vivo, and possessed biologic distributions governed almost exclusively by the N-substituted group. These characteristics of ^{99m}Tc-labeled N-substituted iminodiacetic acids, prepared using an "instant kit" method, provide the basis for a valuable new class of radiopharmaceuticals based on bifunctional drug and biochemical analogs.

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Several investigators have synthesized bifunctional radiopharmaceuticals that contain both a drug or biochemical portion as well as a chelating group capable of strongly binding radioactive metals. This paper reports the development and testing of a new chelating group, iminodiacetic acid, for incorporation into drug and biochemical analogs. Sundberg et al (*1*) coupled the chelating group ethylenediaminetetraacetic acid (EDTA) to proteins and labeled these bifunctional macromolecules with ^{111}In. More recently this type of analog formation has been extended to smaller-molecule drugs and biochemicals with the reported synthesis, radiolabeling, and tissue distribution studies of bifunctional analogs of palmitic acid (*2*) and tolbutamide (*3*). The analogs contained either DTPA, EDTA, or diethylene triamine (DTA), chelating agents known to bind ^{99m}Tc strongly but not particularly suited for incorporation into small molecules. We chose to evaluate the chelating agent iminodiacetic acid (IDA) because of its known chelating strength with transition metals (*4,5*), its relatively small size, and the ease with which it can be synthetically incorporated into bifunctional analogs.

Its utility was tested using two new radiopharmaceuticals, ^{99m}Tc-methyliminodiacetic acid (^{99m}Tc-MIDA) and ^{99m}Tc-N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid (^{99m}Tc-HIDA) (Fig. 1). We chose ^{99m}Tc-MIDA for evaluation because MIDA is readily available and represents the simplest N-substituted derivative of iminodiacetic acid. The HIDA was synthesized and labeled with ^{99m}Tc; its tissue distribution properties were expected to be intermediate between those of the antiarrhythmic agents, lidocaine and methyllidocaine (*6*). The rela-

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For reprints contact: M. D. Loberg, Div. of Nuclear Medicine, University of Maryland Hospital, 22 S. Greene St., Baltimore, MD 21201.

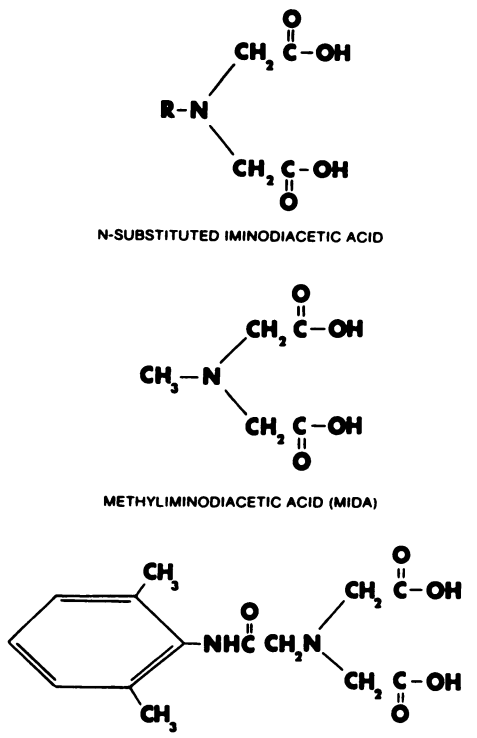

FIG. 1. Radiopharmaceuticals based on N-substituted iminodiacetic acids.

tive chelate strength, the in vivo and in vitro stability, and the effect of N-substitution on target organ specificity are reported for both the new radiopharmaceuticals.

PREPARATION

The MIDA was commercially available (Aldrich) and was used without further purification. The HIDA was synthesized by the reaction of equimolar amounts of ω-chloro-2,6-dimethylacetanilide (7) and IDA (Aldrich) in refluxing EtOH–H_2O solution (3:1). Spectral data and elemental analysis were consistent with the assigned structure for HIDA. Details of the synthesis will be published elsewhere (8).

Both compounds were made into instant kits by adding 0.1 cm^3 of a 2-mg/ml solution of $SnCl_2$ (pH 1.0) to 1.0 cm^3 of physiologic saline (pH 6.0) containing either 15 mg of HIDA or 20 mg of MIDA. The final radiopharmaceutical kits had pH 5–5.5 and were stored under nitrogen until needed. Labeling with ^{99m}Tc was accomplished by adding pertechnetate obtained from a molybdenum generator (HiCon, Squibb, Princeton, N.J.).

Labeling yields and radiochemical purities were studied using paper chromatography (A) in saline; (B) in butanol–acetic acid–water (4:1:1); and (C) in acetonitrile–water (3:1). The R_f values for $^{99m}TcO_4^-$, ^{99m}Tc-MIDA, ^{99m}Tc-HIDA, ^{99m}Tc-DTPA, and ^{99m}Tc-Sn-colloid were recorded for each solvent system using a Packard radiochromatogram scanner. Gel permeation chromatography was used to study radiochemical purity and to assess the stability of the chemical bond between technetium and the N-substituted iminodiacetic acids. A Sephadex G-25 column, 2.54 cm by 35 cm, was eluted with phosphate-buffered saline (pH 7.2). Pertechnetate, ^{99m}Tc-MIDA, and ^{99m}Tc-HIDA exhibited retention volumes of 190, 78, and 99 ml, respectively. The void volume was 40 ml. Sephadex's polysaccharide support material was also employed to make a qualitative differentiation of the stability of the bond between technetium and the N-substituted iminodiacetic acids. The fractions of ^{99m}Tc-HIDA, ^{99m}Tc-MIDA, ^{99m}Tc-DTPA, ^{99m}Tc-pyrophosphate, and ^{99m}Tc-glucoheptonate eluted from the Sephadex column were measured, compared, and used to assess relative chelate stability.

The biodistribution of ^{99m}Tc-HIDA and ^{99m}Tc-MIDA was determined in mice (20–30 gm) by injecting 0.1 ml of the appropriate radiopharmaceutical at a concentration of 15–60 mg/kg body weight and at a specific activity of 10 μCi/cm^3. The mice were killed at times ranging from 1 min to 9 hr after injection. At least two and as many as six mice were used for each data point. At the time of death, the liver, kidneys, heart, intestines, spleen, stomach, and carcass were wet-weighed and counted; blood was collected in 100-λ capillary tubes, which were rinsed with water into the counting tubes. The radioactivity of each was expressed as a percentage of the injected dose per gram of tissue.

The in vivo stability of ^{99m}Tc-HIDA was evaluated in another series of experiments in which five mice were killed 1 hr after injection, the contents of the urinary bladder and gallbladder were withdrawn, and the material was then reinjected for comparison with the findings in the original study.

Kinetic and nuclear imaging studies were performed using fasted mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg). Each dog received a 200-μCi/kg dose of either ^{99m}Tc-HIDA or ^{99m}Tc-MIDA. Blood and urine samples were collected over a 3-hr period. The animals were simultaneously imaged with a Pho/Gamma III camera using a parallel-hole high-sensitivity collimator; 150,000 counts were accumulated for each image.

RESULTS

In vitro studies. Both HIDA and MIDA were labeled with ^{99m}Tc in yields consistently greater than

99%, as judged by paper chromatography in the three solvent systems. The ^{99m}Tc-HIDA, ^{99m}Tc-MIDA, and ^{99m}Tc-DTPA exhibited R_f values of unity in saline, while $^{99m}TcO_4^-$ and ^{99m}Tc-Sn-colloid gave R_f values of 0.75 and 0, respectively. The ^{99m}Tc-HIDA migrated in butanol–acetic acid–water (4:1:1) with an R_f value of 0.34, whereas ^{99m}Tc-MIDA, ^{99m}Tc-DTPA, and ^{99m}Tc-Sn-colloid all remained at the origin. The acetonitrile–water (3:1) solvent system resolved the three chelates, with ^{99m}Tc-HIDA, ^{99m}Tc-MIDA, and ^{99m}Tc-DTPA having R_f values of 0.8, 0.38, and 0.18, respectively. Pertechnetate migrated with an R_f value of 0.9–1.0. Each of the radiopharmaceuticals yielded a single well-defined chromatographic spot in all three solvent systems.

The results of the radiochemical purity studies using Sephadex gel permeation chromatography are shown in Table 1. Ninety-eight percent of ^{99m}Tc-HIDA and 94% of ^{99m}Tc-MIDA were eluted from the column in their respective chelate fractions. Less than 1% of either radiopharmaceutical was found in the $^{99m}TcO_4^-$ fraction (190 ml). No radioactivity was present in the void volume (40 ml). Table 1 also gives the percentage of technetium that was removed from each radiopharmaceutical and bound to the Sephadex G-25 column. Elution of ^{99m}Tc-HIDA and ^{99m}Tc-MIDA from the column was analogous to that obtained with the strong chelating agent DTPA. In contrast, for the weak chelating agents pyrophosphate and glucoheptonate, most of the ^{99m}Tc became bound to the Sephadex G-25 column.

In vivo studies in mice. Figure 2 shows the tissue distribution of ^{99m}Tc-MIDA in mice as a function of time. The ^{99m}Tc-MIDA is cleared rapidly from the blood into urine with only transient concentration in the kidneys. By 30 min, over 60% of the injected ^{99m}Tc-MIDA is cleared through the kidneys, with less than 2.5% and 0.4% localizing in the liver and stomach, respectively.

The tissue distribution of ^{99m}Tc-HIDA in mice (Fig. 3) was found to be radically different from that of ^{99m}Tc-MIDA, with an overall distribution indicative of hepatobiliary clearance. The ^{99m}Tc-HIDA exhibited a very rapid blood clearance: at 5 min only 3% of the injected dose remained in the blood, and at 30 min, less than 1%. Radiopharmaceutical uptake was primarily hepatic: by 3–5 min total activity in the liver and intestines accounted for 70% of the injected dose. This increased to approximately 80% by 30 min and remained at that level until defecation occurred. The uptakes in the kidney, stomach, and spleen were negligible.

The results of the biodistribution data of the reinjected urine and bile substantiated the in vivo stability of ^{99m}Tc-HIDA (Table 2). In addition, prior to reinjection, samples of bile and urine exhibited an R_f of unity when rechromatographed on paper using a saline solvent.

TABLE 1. GEL PERMEATION CHROMATOGRAPHY OF SELECTED RADIOPHARMACEUTICALS

Radiopharmaceutical	Percent in chelate fraction	Percent in TcO_4^- fraction	Percent bound to Sephadex*
^{99m}Tc-MIDA	94	<1	6 ± 4
^{99m}Tc-HIDA	98	<1	2 ± 2
^{99m}Tc-DTPA	97	0.95	2 ± 2
^{99m}Tc-pyrophosphate	9.7	0.3	90
^{99m}Tc-glucoheptonate	<1	<1	99

* This fraction is due to competition for the reduced technetium between the chelating moiety on the radiopharmaceutical and the polysaccharide column material.

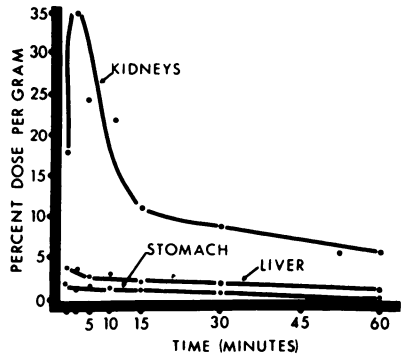

FIG. 2. Tissue distribution of ^{99m}Tc-MIDA in mice.

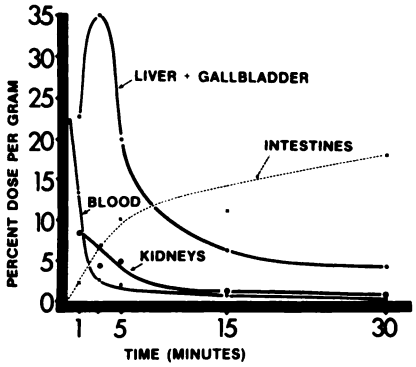

FIG. 3. Tissue distribution of ^{99m}Tc-HIDA in mice.

In vivo studies in dogs. Imaging and kinetic studies in dogs substantiated the biodistribution data obtained in mice. After intravenous administration of ^{99m}Tc-MIDA, 51% of the injected dose was ex-

creted into the urinary bladder by 30 min. The blood clearance was rapid: 21% of the injected dose remained in the blood at 5 min, and 12% at 30 min. Images obtained with ^{99m}Tc-MIDA in dogs showed initial rapid uptake of the radiopharmaceutical by the kidneys, followed by prompt excretion into the urine.

The ^{99m}Tc-HIDA also exhibited rapid blood clearance in dogs, with 3% of the injected dose remaining in the blood at 30 min (Fig. 4). The cumulative urinary clearance at 5 hr after injection accounted for approximately 17% of the injected dose, with 82% of this occurring during the first hour.

Figures 5A and 5B show liver scintigrams obtained in a fasted dog at 5 and 50 min after intravenous injection of 1 mCi of ^{99m}Tc-HIDA. By 50 min, more than 80% of the injected dose was concentrated in the gallbladder. Figures 5C and 5D show the gallbladder activity at 1 and 10 min after administration of cholecystokinin. The gallbladder activity was seen to diminish promptly while the radioactivity concurrently appeared in the small intestine.

TABLE 2. TISSUE DISTRIBUTION OF ^{99m}Tc-HIDA BEFORE AND AFTER EXCRETION INTO THE URINARY BLADDER AND GALLBLADDER*

Organ	^{99m}Tc-HIDA	Contents of urinary bladder	Contents of gallbladder
Liver	1.04 ± 0.08	4.79 ± 3.50	4.56 ± 3.21
Kidney	0.64 ± 0.08	1.80 ± 1.37	0.71 ± 0.40
Intestines	72.60 ± 0.35	53.27 ± 14.66	72.97 ± 4.32
Stomach	0.58 ± 0.17	2.43 ± 3.35	1.81 ± 1.59
Spleen	0.40 ± 0.57	0.18 ± 0.19	0.25 ± 0.18

* Results are expressed as percent of injected dose. In all instances, the animals were killed 1 hr after injection. Error limits represent one standard deviation.

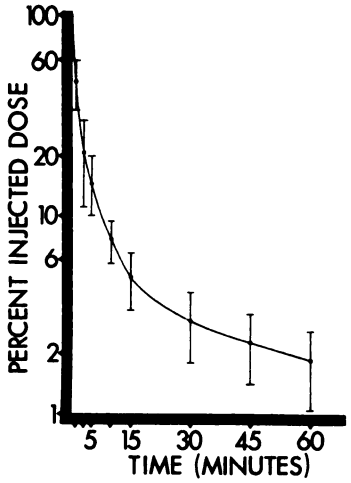

FIG. 4. Blood clearance of ^{99m}Tc-HIDA in nine dogs.

DISCUSSION

This paper represents the first time a small-molecule chelating agent has been evaluated for synthesis into drug and biochemical analogs. Since iminodiacetic acid (IDA) strongly binds transition metals (*4,5*), IDA functional groups can be incorporated into a styrene–divinylbenzene resin and used for the analytic separation of metals (*9*). In addition, compounds similar to, though larger than IDA are known to bind reduced ^{99m}Tc strongly (*10*). In addition, IDA readily undergoes nucleophilic substitution reactions replacing such good chemical leaving groups as Cl^-, Br^-, I^-, and tosylate.

Both MIDA and HIDA formed strong chelates with reduced ^{99m}Tc and yielded radiopharmaceuticals that chromatographed as a single radiochemical in three solvent systems. Paper chromatography in saline confirmed the absence of technetium in both pertechnetate and colloidal form, but it yielded little information about the radiochemical purity of the chelate itself since all chelate-type radiopharmaceuticals exhibited an R_f of unity in saline. In this regard, acetonitrile–water (3:1) proved to be an excellent solvent system since it distinguished ^{99m}Tc-DTPA, ^{99m}Tc-MIDA, and ^{99m}Tc-HIDA from one another. In addition, its radiochromatogram development time was short (less than 10 min), and the paper strips themselves dried quite rapidly. Both ^{99m}Tc-HIDA and ^{99m}Tc-MIDA chromatographed in both organic solvent systems as a single well-defined spot with R_f values between 0 and 1, indicating that the chelate fractions of ^{99m}Tc-MIDA and ^{99m}Tc-HIDA were present in only one radiochemical form. The difference in R_f values between ^{99m}Tc-MIDA and ^{99m}Tc-HIDA further showed that each represents a different N-substituted iminodiacetic acid. The radiochemical purity for both compounds, judged by paper chromatography in all three solvent systems, was sufficient to obviate any need for subsequent purification before administration. This factor, combined with the "instant kit" method of preparation, should make this class of tracers well suited to radiopharmacy practice.

Gel permeation chromatography (Table 1), in addition to confirming the radiochemical purities of ^{99m}Tc-MIDA and ^{99m}Tc-HIDA, revealed that both radiopharmaceuticals were more stable in vitro, when compared in one competitive situation, than are two currently used radiopharmaceuticals, ^{99m}Tc-pyrophosphate and ^{99m}Tc-glucoheptonate. The polysac-

FIG. 5. Liver and gallbladder images in fasted dogs obtained with scintillation camera and parallel-hole collimator 5 min (Frame A) and 50 min (Frame B) after intravenous injection of 1 mCi of ^{99m}Tc-HIDA. Frames C and D show gallbladder emptying and activity entering small intestine 1 min and 10 min after intravenous administration of cholecystokinin.

charide support material of Sephadex G-25 has previously been shown to remove ^{99m}Tc competitively from such weak chelating agents as glucoheptonate and pyrophosphate but to be ineffective against such strong chelating agents as DTPA (*11*). Thus, the percentage eluted from a Sephadex G-25 column can be used to make a qualitative distinction regarding the stability of the bond between reduced ^{99m}Tc and any chelating agent. The results shown in Table 1 indicate that ^{99m}Tc-MIDA and ^{99m}Tc-HIDA are eluted from the column to the same degree as ^{99m}Tc-DTPA. These data do not detract from the diagnostic efficacy of either ^{99m}Tc-pyrophosphate or ^{99m}Tc-glucoheptonate, but they do indicate the in vitro stability of N-substituted iminodiacetic acids. This bond strength between technetium and IDA should be fairly independent of the chemical structure of the N-substituted group, provided that the group was not electron-withdrawing. Delocalization of the electrons on the imino nitrogen is known to reduce the formation constant between N-substituted iminodiacetic acids and various transition metals (*12*).

The animal distribution studies in both mice and dogs (Figs. 2–5) showed striking differences between the two radiopharmaceuticals: ^{99m}Tc-MIDA was rapidly eliminated through the kidneys, while approximately 82–87% of ^{99m}Tc-HIDA was cleared through the hepatobiliary system. This difference in biologic distribution between ^{99m}Tc-MIDA and ^{99m}Tc-HIDA can be taken as indirect evidence for the in vivo stability of both radiopharmaceuticals. If the bond between ^{99m}Tc and the N-substituted iminodiacetic acids were to dissociate and the ^{99m}Tc to transfer to another endogenous ligand, then one would expect the two radiopharmaceuticals to have similar tissue distributions.

More direct evidence for the in vivo stability of ^{99m}Tc-HIDA was obtained when the contents of the urinary bladder and gallbladder were reinjected. The results for that study (Table 2) suggest that ^{99m}Tc-HIDA is excreted in its original radiochemical form, having been neither dissociated nor metabolized. Furthermore, the contents of the urinary bladder and gallbladder appear to be identical, once again indicating that the chelate fraction consists of a single radiochemical. Neither radiopharmaceutical concentrated in the stomach or spleen, the sites of uptake for pertechnetate and radiocolloid, respectively.

While a change in the chemical nature of the N-substituted group did not diminish in vivo stability, it does profoundly alter the biologic distribution of these N-substituted iminodiacetic acids. This consideration is important in the design of new radiolabeled bifunctional analogs. The ability to predict the radiopharmaceutical tissue distribution from that of the parent drug or biochemical would also be of great value. That structural similarities may serve as a useful guide is shown in the case of ^{99m}Tc-MIDA, where renal clearance could be anticipated on the basis of comparison with ^{99m}Tc-EDTA and ^{99m}Tc-DTPA.

The ^{99m}Tc-HIDA was developed as a bifunctional drug analog with structural similarities to both lidocaine and methyllidocaine and with expected lipophilic properties intermediate between the two. Figure 6 includes a schematic representation of ^{99m}Tc-HIDA, showing that the two electrons on the imino nitrogen take part in the chelate formation, which results in a partial positive charge of the nitrogen. (The schematic is not intended to represent the exact radiochemical structure of ^{99m}Tc-HIDA, which is currently under investigation.) The hepatobiliary clearance of ^{99m}Tc-HIDA may be explained by the fact that certain lipophilic chelating groups are eliminated through the bile (*13*). This suggestion is supported by the radiochromatographic data in less polar organic solvents, where ^{99m}Tc-HIDA has a larger R_f value than either ^{99m}Tc-MIDA or ^{99m}Tc-DTPA. A structural comparison of HIDA and MIDA supports the same conclusion since the aromatic ring in HIDA adds substantial lipophilicity to the overall molecule. Lipophilicity is probably not the complete answer, however, because both ^{67}Ga-8-hydroxyquinoline and ^{99m}Tc-8-hydroxyquinoline are

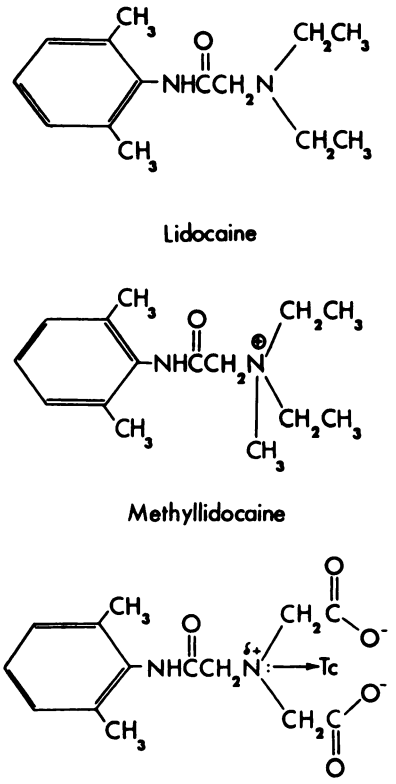

FIG. 6. Structures of lidocaine and methyllidocaine and simplified geometric configuration for ^{99m}Tc-HIDA.

greater than 98% extractable into chloroform, yet exhibit only 43% and 56%, respectively, of the hepatobiliary clearance of ^{99m}Tc-HIDA (*14*).

Perhaps the most important factor in predicting the distribution of bifunctional radiopharmaceuticals is the chemical composition of the chelate. Technetium(IV) is usually surrounded by six ligands in an octahedral environment (*15*), and the quatridentate chelating agent nitrilotriacetic acid, which is structurally similar to ^{99m}Tc-HIDA, is known to form a stable dimer with technetium(IV) (*10*). It would be reasonable, therefore, to consider that ^{99m}Tc-HIDA may exist as a dimer, with two molecules of the tridentate chelating agent [N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid] reacting with each atom of ^{99m}Tc. Clearly, before the distribution of radiolabeled bifunctional analogs can be predicted with any certainty, the chemical structure of the radiolabeled chelate must be known.

In summary, N-substituted iminodiacetic acids have been manufactured into instant kits and labeled with ^{99m}Tc to yield radiopharmaceuticals of high radiochemical purity. The resultant radiopharmaceuticals possess in vitro and in vivo stability and exhibit tissue distributions that are functions of the chemical nature of the N-substituted group. Thus, the N-substituted iminodiacetic acids represent a promising new approach to labeling drug and biochemical analogs with radioactive metals.

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REFERENCES

1. Sundberg MW, Meares CF, Goodwin DA, et al: Selective binding of metal ions to macromolecules using bifunctional analogs of EDTA. *J Med Chem* 17: 1304–1307, 1974

2. Eckelman WC, Karesh SM, Reba RC: New compounds: Fatty acid and long chain hydrocarbon derivatives containing a strong chelating agent. *J Pharm Sci* 64: 704–706, 1975

3. Heindel ND, Risch VR, Burns HD, et al: Syntheses and tissue distribution of ^{99m}Tc-sulfonylureas. *J Pharm Sci* 64: 687–689, 1975

4. Stability Constants of Metal-Ion Complexes. London, Chemical Society, 1964, pp 426–427

5. Martell AE: The relationship of chemical structure to metal-binding action. In *Metal Binding in Medicine*, Seven MJ, ed. Philadelphia, J. B. Lippincott, 1960, pp 1–18

6. Kniffen FJ, Lomas TE, Nobel-Allen NL, et al: The comparative antiarrhythmic actions of lidocaine and its quaternary derivative methyllidocaine. *Circulation* 49: 264–271, 1974

7. Borovansky A, Sekera A, Vrba C: Studies on local anesthetics. XXI: Some derivatives of ω-diethylaminoacetanilide. *J Am Pharm Assoc Sci Ed* 48: 402–404, 1959

8. Callery PS, Faith WF, Loberg MD, et al: Synthesis and tissue distribution of technetium-99m and carbon-14 labeled N-[N′-(2,6-dimethylphenyl)carbamoylmethyl]iminodiacetic acid. *J Med Chem*: to be published

9. Helfferich F: Ligand exchange: A novel separation technique. *Nature* 189: 1001–1002, 1961

10. Gorski B, Koch H: Über die Komplexbildung von Technetium mit chelatbildenden Liganden. II. *J Inorg Nucl Chem* 32: 3831–3836, 1970

11. Valk PE, Dilts CA, McRae J: A possible artifact in gel chromatography of some ^{99m}Tc-chelates. *J Nucl Med* 14: 235–237, 1973

12. Martell AE, Calvin M: *Chemistry of the Metal Chelate Compounds.* New York, Prentice-Hall, 1952, pp 155–158

13. Rubin M, Pachtman E, Aldridge M, et al: The metabolism of parenteral iron chelates. *Biochem Med* 3: 271–288, 1970

14. Levin B, Fields A, Loberg MD: Unpublished results

15. Kotegov KV, Pavlov ON, Shvedov VP: Technetium. In *Advances in Inorganic Chemistry and Radiochemistry*, vol 2, Emelius HJ, Sharpe AG, eds. New York, Academic, 1968