ferences in the amount of nuclei per gram of organ or tissue. The unbound concentration of doxorubicin in the cytosol must be ~2.3 times higher than that in plasma according to the pH partition hypothesis.

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Preparation and Biodistribution of ^{99m}Tc-Labeled Tyramine Iminodiacetic Acid

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Abstract □ The synthesis and biodistribution properties of ^{99m}Tc-labeled *N*-substituted tyramine, [*N*-(4-hydroxyphenethyl)iminodiacetic acid] are described. Tissue distribution studies in rats were indicative of high hepatic and kidney extraction, accompanied by rapid plasma and urinary clearance and minimal biliary excretion. These findings were substantiated by organ image analysis. The preliminary data indicate that this labeled material may represent a new class of radiopharmaceuticals for the evaluation of hepatic and renal functions.

Keyphrases □ Biodistribution—^{99m}Tc-labeled tyramine iminodiacetic acid, preparation □ Tyramine iminodiacetic acid—biodistribution and preparation □ Radiopharmaceuticals—preparation and biodistribution of ^{99m}Tc-labeled tyramine iminodiacetic acid

Tyramine (I), a noncatecholic phenethylamine found in a variety of plants and animal tissues (1), is produced by decarboxylation of its parent amino acid tyrosine (2). Normally, the physiological effects of tyramine are minimal because >90% of the tyramine is rendered metabolically inactive by mitochondrial monoamine oxidase during its first passage through the liver after absorption (3, 4). The rapid rate of elimination and its inability to cross the blood–brain barrier (5) protect the body from the adverse peripheral and neurotoxic effects of tyramine.

Abnormal metabolism of tyramine has been implicated in a variety of clinical disorders. Abnormal urinary excretion of the amine occurs in patients suffering from Parkinson's disease (6), schizophrenia, induced hypertensive crisis (7), cystic fibrosis (8), epilepsy (9), hypertyrosinemia (10), depression, and migration (11). We have recently demonstrated that the tyramine concentration is abnormally elevated in the plasma and urine of patients with cirrhosis (12, 13), hepatitis (14), and Reye's syndrome (15), as well as in experimental animals with hepatic insufficiency or portacaval shunt (16).

In view of its clinical importance, we have taken the initiative to develop a radiopharmaceutical from tyramine. In this report, we describe the synthesis, biological distribution, and image characteristics of ^{99m}Tc-labeled *N*-(4-hydroxyphen-

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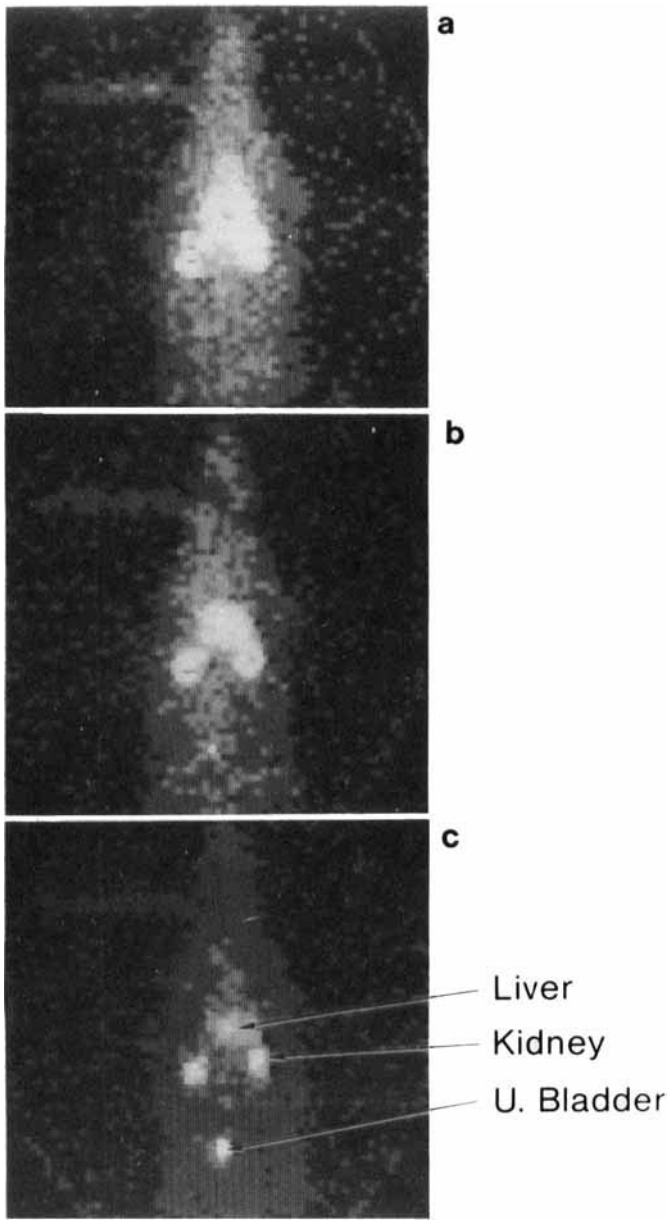
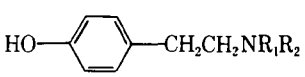


Figure 1—*Scintograms of rats obtained at 2 (a), 5 (b), and 10 (c) min after the intravenous injection of 0.5 mCi/kg of ^{99m}Tc-labeled tyramine diacetic acid.*

ethyl)iminodiacetic acid (II) an iminodiacetic acid-containing radiopharmaceutical which is structurally related to tyramine.

EXPERIMENTAL SECTION

Chemistry—*N*-(4-Hydroxyphenethyl)iminodiacetic acid (II) was prepared by a modified procedure of Callery *et al.* (17). A solution of tyramine[1] (1.37 g, 10 mmol) in 95% ethanol (100 mL) was added dropwise to a stirred solution of chloroacetic acid (1.9 g, 20 mmol) in 95% ethanol (50 mL). During the addition, the mixture was maintained at pH >7 with 5 M NaOH. The resulting mixture was heated under reflux for 48 h, was allowed to cool, and the resulting white precipitate was removed by filtration. This material was



I: $R_1 = R_2 = H$
II: $R_1 = R_2 = -CH_2CO_2H$

washed with 95% ethanol, recrystallized (80% ethanol) and dried under reduced pressure at 50°C, to give 1.0 g (40% yield). The resulting salt was dissolved in water, acidified to pH 3 by the dropwise addition of 6 M HCl, and the resulting precipitate was collected and recrystallized from methanol–water to yield white crystals, mp 284–287°C; IR (Nujol) 3200–2800, 1650, 900, 800, and 700 cm^{-1}; ^{1}H-NMR (D_2O): δ 2.5–3.2 (m, 4, CH_2CH_2), 3.4 (s, 4, CH_2COO), and 6.5–7.0 ppm (m, ArH). TLC on silica gel GF revealed one spot with R_f values of 0 in solvent system A (*tert*-amyl alcohol–benzene–40% methylamine; 24:8:12) and 0.4 in solvent system B (1-butanol–acetic acid–water; 25:4:10). Tyramine had R_f values of 0.75 and 0.65 in solvent systems A and B, respectively.

Anal.—Calc. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.93; N, 5.33. Found: C, 57.50; H, 6.18; N, 5.65.

Labeling with Technetium-99m—The iminodiacetic acid derivative of tyramine was labeled by stannous chloride reduction of sodium [^{99m}Tc]pertechnetate eluate from a molybdenum-99–technetium-99m generator in aqueous solution.

A solution of the derivative (5 mg, 0.043 mmol) in 1 mL of distilled water (pH 5–6) was prepared in an evacuated glass tube. To this was added 0.2 mL (0.25 mg, 0.0011 mmol) of a solution of stannous chloride prepared by the procedure of Mock (18). The mixture was vortex-mixed for 30 s. Labeling with technetium-99m was accomplished over a period of 5 min by the addition of the pertechnetate (5 mCi).

Radiochemical Purity—The radiochemical purity of ^{99m}Tc-labeled material was examined by silica gel (glass fiber support) and cellulose (plastic sheet support) ascending TLC, with normal saline, 1-butanol–acetic acid–water (4:1:7), and acetonitrile–water (3:1) as solvents. The radiochromatograms of imaging agent were compared with those obtained for both pertechnetate anion and for technetium-99m–tin colloid, which was prepared by the method used for the preparation of the ^{99m}Tc-labeled iminodiacetic acid. Gel permeation chromatography was used to assess the stability of the chemical bond between technetium-99m and tyramine diacetic acid. Prepacked (1 × 10-cm) disposable columns containing Sephadex G-25 medium[2] (10-mL volume) were used. The imaging agent (1 μCi) in 0.2 mL of normal saline was applied to the column and eluted with 50 mL of normal saline. Fractions (1 mL each) were collected and counted. The elution pattern of the ^{99m}Tc-labeled iminodiacetic acid was compared with that of pertechnetate and the technetium-99m–tin colloid.

Biodistribution Studies—The biodistribution studies were carried out in male Sprague–Dawley rats (200–300 g) that were fasted overnight. The rats were anesthetized with sodium pentobarbital (25 mg/kg). A catheter[3] (i.d., 0.58 mm; o.d., 0.965 mm) was inserted into the jugular vein for the administration of imaging agent; another one was placed in the carotid artery for blood sampling. A third catheter was inserted into the common bile duct, retrograde to the liver, for collection of bile. The mixture of the radiopharmaceutical preparation was administered intravenously (0.5 mCi/kg) as a bolus injection. Heparinized blood samples (1 mL) were drawn at predetermined intervals. The rats were then sacrificed serially at intervals to 180 min after injection. At the time of death, the wet weights of the liver, kidney, heart, intestine, brain, spleen, stomach, lung, and muscle were determined. The tissue samples were homogenized[4] in a 4:1 water–organ mixture, and 0.5-mL aliquots were counted. The radioactivity in plasma, bile, and tissue samples was expressed as a percentage of the injected dose per gram of sample. The urine (0.1 mL, 1:100 dilution) from the urinary bladder was also counted.

Image Analysis—Ten rats were evaluated. The imaging system consisted of a mobile camera[5] interfaced with a computer[6]. The study began with an anesthetized rat placed supine on a table under the detector of a portable gamma scintillation camera. The detector was positioned so that the field of view included the liver, lung bases, kidneys, and bladder. The imaging agent (0.5 mCi/mg) was injected intravenously into the jugular vein. Data acquisition by the computer started simultaneously with the injection. Serial images of the whole rat were taken and recorded at predetermined intervals over a period of 3 h.

RESULTS

Radiochemical Analysis—Chemical purity of the tyramine diacetic acid was established by elemental, spectral, and chromatographic analyses. The radiochemical purity and labeling yield of the imaging agent were determined by radiochromatographic analysis. The iminodiacetic acid was labeled with technetium-99m in yields >95% as assessed by silica gel and cellulose TLC

[1] Sigma Chemical Co., St. Louis, Mo.

[2] PD-10; Pharmacia Fine Chemicals.
[3] PE-50; Clay Adams, Parsippany, N.J.
[4] Polytron; Brinkman Instruments, Westbury, N.J.
[5] Picker Dynamo.
[6] Muga Cart Medical Data System.

Table I—Average [a] Percent Injected Radioactivity in Tissues and Plasma of Rats after Intravenous Administration of ^{99m}Tc-Labeled Tyramine Diacetic Acid

Tissue	Radioactivity, % of Dose/g of Tissue					
	5 min	10 min	30 min	60 min	120 min	180 min
Liver	5.2	3.2	2.3	1.4	1.0	0.75
Kidney	8.5	5.21	3.0	2.2	1.8	1.0
Brain	0.3	0.01	0.008	0.007	0.007	0.0051
Heart	0.20	0.16	0.13	0.072	0.072	0.060
Intestine	0.30	0.15	0.11	0.067	0.067	0.0051
Plasma	4.1	1.7	0.85	0.65	0.47	0.28

[a] The range was <10% of each value; $n = 6$ rats per group.

in three solvent systems. On silica gel, the ^{99m}Tc-labeled material exhibited an R_f value of 1.0 in each of the solvents [0.9% NaCl, 1-butanol–acetic acid–water (4:1:1), and acetonitrile–water (3:1)], whereas pertechnetate and technetium-99m–tin colloid gave R_f values of 1 and 0, respectively, in these systems. On cellulose, the labeled iminodiacetic acid migrated with R_f values of 0, 0.8, and 0; the colloid remained at the origin and pertechnetate migrated with R_f values of 1.0, 0.4, and 1.0 in these solvent systems. In all cases, ^{99m}Tc-labeled tyramine diacetic acid yielded a well-defined chromatographic spot. The results of Sephadex G-25 chromatographic analysis revealed that >95% of the labeled material was eluted from the column as compared with 1% of labeled colloid. This was an indication that the iminodiacetic acid is a strong chelating agent. Increased dilution and prolonged standing at room temperature had minimal effect on the radiochemical purity of labeled material, providing further evidence of the stability of this ^{99m}Tc-labeled radiopharmaceutical.

***In Vivo* Studies in Rats**—*Tissue Distribution, Plasma, Urine, and Bile Analyses*—The data in Table I represent the activity in various tissues at 5, 10, 30, 60, 120, and 180 min after the intravenous injection of the imaging agent. Data are expressed as the percentage of administered dose per gram of tissue (wet weight). The tissue distribution data indicate a selective accumulation of the ^{99m}Tc-labeled material by the liver and kidney. There was an ~100-fold increase in the concentration of this agent in the liver and kidney as compared with the brain, intestine, and heart at 5 min postinjection. This was followed by a rapid clearance, since 50% of the activity was eliminated from the liver and kidney by 30 min.

The main elimination pathway of radioactivity after administration of the imaging agent was *via* the kidney. At 180 min, ~50–60% of the radioactive dose was accounted for in the urine. Less than 5% of the dose appeared in the bile at 180 min. Radiochromatographic analysis indicated that most of the activity in plasma, urine, and bile was in the form of the injected ^{99m}Tc-labeled material.

Image Analysis—Imaging studies substantiated the tissue distribution data. In Fig. 1 are shown whole rat body scans obtained at 2, 5, and 10 min after intravenous injection of 0.5 mCi/kg of ^{99m}Tc-labeled tyramine diacetic acid. The scans indicate high and selective uptake followed by a rapid wash-out of the radiopharmaceutical from the liver and kidney with concomitant accumulation of the radioactivity in the urinary bladder.

DISCUSSION

In the present study it has been demonstrated that substitution of the terminal amino group of tyramine with the chelating moiety iminodiacetic acid produced a tyramine derivative which was capable of complexing with technetium-99m in quantitative yield. By utilizing silica gel or cellulose TLC in several solvent systems, the labeled material exhibited a single distinct sharp peak and the absence of a radioactive band associated with pertechnetate or technetium-99m–tin colloid. The stability of the chelate in this radiopharmaceutical was indicated when increased dilution, prolonged storage (up to 4 h), and incubation at 37°C for 1 h had a minimal effect on its radiochemical composition.

Biodistribution studies in rats indicated avid extraction of the labeled material by the liver and kidney with minimal accumulation in the brain, heart, and intestine after intravenous administration. The plasma elimination pattern of ^{99m}Tc-labeled tyramine diacetic acid is indicative of the plasma clearance of endogenous and exogenous amines with high first-pass hepatic extraction (19, 20). The main elimination pathway of radioactivity after administration of the imaging agent was *via* the kidneys, which is similar to that of tyramine (3, 21). Biliary excretion was minimal since <5% of the injected dose appeared in the bile at 3 h postinjection.

Furthermore, evidence supporting the fact that extensive dissociation *in vivo* of ^{99m}Tc-labeled material to colloidal technetium or pertechnetate did not occur was obtained when radiochromatographic analysis of plasma, bile, and urine contents produced a chromatogram identical to that of the imaging agent before injection. This suggests that the ^{99m}Tc-labeled iminodiacetic acid chelate remains intact *in vivo* and is eliminated in an unchanged form.

These preliminary results indicate that ^{99m}Tc-labeled tyramine diacetic acid may represent a new class of radiopharmaceuticals for the evaluation of hepatorenal function independent of the hepatobiliary system.

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