Biological Distribution of Chemical Analogs of Fatty Acids and Long Chain Hydrocarbons Containing a Strong Chelating Agent

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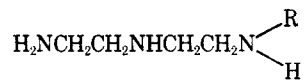
Abstract □ The pharmaceutical preparation, chromatography, and biological distribution of a series of new chemical analogs of palmitic acid and diethylenetriaminepentaacetic acid, ethylenediaminetetraacetic acid, or diethylenetriamine are described. The biological distribution in rabbits 30 min after intravenous administration of these ^{99m}Tc-labeled and ^{57}Co-labeled derivatives was compared to the biological distribution of the parent compound, ^{3}H-palmitic acid. The average myocardial uptake for these compounds was 0.04%/g, compared to 0.15%/g for palmitic acid. The heart to blood ratio at 30 min reached a maximum of 3:1 for the best physiological analog of palmitic acid, compared to an average of 30:1 for palmitic acid. Although none of these analogs appears to be clinically useful, their production methods might be applicable to the synthesis of new compounds that might increase the specificity of radiopharmaceuticals.

Keyphrases □ Fatty acid analogs, ^{99m}Tc- and ^{57}Co-labeled—containing chelating agents, biological distribution, compared to ^{3}H-palmitic acid, rabbits □ Distribution, biological—^{99m}Tc- and ^{57}Co-labeled fatty acid analogs containing chelating agents, compared to ^{3}H-palmitic acid, rabbits □ Radiochemistry—technetium Tc 99m as label for fatty acid analogs containing chelating agents, biological distribution, compared to ^{3}H-palmitic acid, rabbits □ Technetium Tc 99m—label for fatty acids containing chelating agents, biological distribution, compared to ^{3}H-palmitic acid, rabbits

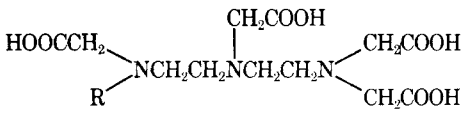
Of the conveniently available radionuclides, technetium Tc 99m has the best physical characteristics for external imaging; however, poor chemical properties have limited its use as a label for biological molecules. The weak chelate ion characteristics of technetium Tc 99m in aqueous solution are such that many organic compounds have been only nominally labeled with it. For example, for such molecules as tetracycline (1) and the carbohydrates (2), the chelating strength of the functional groups on the molecule itself is apparently too weak to assure the integrity of the chelate *in vivo*.

The preparation of derivatives may be a means of producing radiopharmaceuticals with better metal binding properties but similar biological properties. Specifically, a biologic molecule covalently bound to a strong chelating group should then bind technetium Tc 99m more strongly than the biologic molecule itself. Simple polyaminopolycarboxylic acids like diethylenetriaminepentaacetic acid (I) have been shown to bind technetium Tc 99m so that it is distributed and excreted in vertebrates in a manner similar to that of ^{14}C-labeled I (3). Compound I, therefore, seems to be a useful chelating agent to bind to interesting biologic molecules.

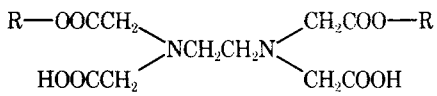
The fatty acids are a class of compounds known to localize in the myocardium. By combining long chain fatty acids with I-like compounds (strong chelating agents to carry the ^{99m}Tc-label for other cations), new compounds were obtained (4). This paper describes the pharmaceutical preparation, chromatography, and relative distribution at 30 min of this series of compounds designed as myocardial imaging agents.



II: R = $C_{12}H_{25}$
III: R = $C_{16}H_{33}$
IV: R = $C_{18}H_{37}$
V: R = $(CH_2)_6CH{=}CH(CH_2)_7COOH$



VI: R = $C_{12}H_{25}$
VII: R = $C_{16}H_{33}$
VIII: R = $C_{18}H_{37}$
IX: R = $(CH_2)_6CH{=}CH(CH_2)_7COOH$



X: R = $C_{16}H_{33}$
XII: R = $C_{18}H_{37}$
XIII: R = $C_{14}H_{28}COOH$
XIV: R = $C_{15}H_{30}COOH$

EXPERIMENTAL

Synthesis, Purification, and Characterization of New Compounds—The synthesis, purification, and physical characterization of II–X and XII–XIV were published previously (4).

Preparation of Radioactive Compound—The tin chelates of VII, IX, X, and XIV were prepared in a nitrogen atmosphere. Twenty-five milligrams of stannous chloride dihydrate was placed in a 100-ml serum vial, and the vial was nitrogen purged and sealed. Three milliliters of nitrogen-purged absolute ethanol was then added by syringe, and the vial was shaken. To the vial was added 1.25 ml of a hot nitrogen-purged ethanol solution of the chelating agent (150 mg/ml, pH 4), and the mixture was shaken vigorosly. Then a nitrogen-purged ethanol–water solution was added so that the final volume was 100 ml and the resulting ethanol concentration was 20% by volume.

After the solution cooled to room temperature, it was filtered through a 0.22-μm filter. Then 2 ml of ^{99m}Tc-labeled sodium pertechnetate was added to 2 ml of the tin chelate solution. In every case, the reduced technetium bound to the chelating agent. Chromatography data showed no evidence of either $^{99m}TcO_4^-$ or ^{99m}Tc-labeled technetium stannous oxide for all of the compounds (Table I). Potentiometric titration of an aliquot of the tin chelate solution was performed for each preparation using a glass electrode[1] and standard iodine solution prior to addition of the $^{99m}TcO_4^-$ to the tin chelate solution.

The cobalt-57 chelates of IV, VII, IX, X, and XIV were prepared by heating alcoholic solutions of ^{57}Co-labeled cobalt chloride and the chelating agent in sealed serum vials for 1 hr at 100°. Labeling of each compound with cobalt-57 was verified by paper chromatography or TLC, using ^{57}Co-labeled cobalt chloride as a reference standard (Table I). Cobalt chelates were preferred for preliminary studies because of the stable oxidation state of cobalt(III).

[1] Corning platinum combination electrode.

Table I—Summary of Chromatography Data for Radiolabeled Chelates

Compound	Chromatography System	R_f Values: Cobalt-57 Chelates	R_f Values: Cobalt-57 Control
	Cobalt-57 Chelates		
IV	Cellulose, chloroform–methanol (5:1, v/v)	0.89	0.12
IV	Cellulose, 0.9% NaCl	0.00	0.92
IV	Cellulose, ethanol	0.75	0.00
VII	Cellulose, 0.9% NaCl	0.00	0.92
VII	Cellulose, ethanol	0.92	0.00
IX	Cellulose, 0.9% NaCl	0.42	0.92
IX	Cellulose, ethanol	0.86	0.00
X	Cellulose, 0.9% NaCl	0.00	0.92
X	Cellulose, ethanol	0.86	0.00
XIV	Cellulose, 0.9% NaCl	0.00	0.92
XIV	Cellulose, ethanol	0.85	0.00
	^{3}H-Labeled Chelating Agents	**R_f Values**	
V	Silica gel G, 50% 2-propanol	0.77	
V	Silica gel G, 10% ammonium acetate–ethanol (1:1, v/v)	0.46	
VII	Silica gel G, 0.1 *N* NaOH–methanol (1:1, v/v)	0.77	
IX	Silica gel G, 0.1 *N* NaOH–methanol (1:1, v/v)	0.60	
XIV	Silica gel G, 0.1 *N* NaOH–methanol (1:1, v/v)	0.83	
XIV	Cellulose, ethanol	0.91	
		R_f Values or Elution Volume	
	Technetium Tc 99m Chelates	Technetium Tc 99m Chelate	TcO_4^- Control
VII	Sephadex LH-20, methanol	34 ml	58 ml
VII	Sephadex C-25, 0.9% NaCl	V.V.[a]	34 ml
VII	Paper, methanol–0.1 *N* NaOH (1:1, v/v)	0.63	0.75
VII	Sephadex G-75, 0.9% NaCl	V.V.	54 ml
VII	Sephadex G-100, 0.9% NaCl	V.V., 36 ml	55 ml
VII	Sephadex G-150, 0.9% NaCl	V.V., 35 ml	58 ml
VII	Sephadex G-200, 0.9% NaCl	V.V.	60 ml
IX	Sephadex LH-20, methanol	V.V.	38 ml
X	Paper, 0.9% NaCl	0.50	0.75
XIV	Sephadex LH-20, methanol	V.V.	37 ml

[a] Void volume.

Table II—Biodistribution (Percent Dose per Gram) of Radiolabeled Compounds in Rabbits[a]

Compound	IV	V	VII	VII	VIII	IX
Chelating agent, μg	7	10	125	10	100	10
Isotope	^{57}Co	^{3}H	^{99m}Tc	^{3}H	^{57}Co	^{57}Co
Solvent	50% ethanol	Ammonium acetate–ethanol[b]	25% HSA[c]	Ethanol	Ethanol	0.9% NaCl
Heart	0.03 ± 0.01	0.05 ± 0.007	0.06 ± 0.01	0.03 ± 0.00	0.04 ± 0.005	0.05 ± 0.01
Lungs	0.08 ± 0.03	0.03 ± 0.007	0.10 ± 0.01	0.15 ± 0.06	0.45 ± 0.23	0.11 ± 0.03
Liver	0.12 ± 0.007	0.16 ± 0.08	0.75 ± 0.46	0.15 ± 0.02	0.38 ± 0.05	0.54 ± 0.16
Muscle	—	0.02 ± 0.007	—	0.03 ± 0.006	0.02 ± 0.005	0.03 ± 0.006
Blood	0.07 ± 0.03	0.08 ± 0.04	0.24 ± 0.11	0.03 ± 0.01	0.03 ± 0.009	0.03 ± 0.01

Compound	IX	IX	IX	IX
Chelating agent, μg	800	10	10	10
Isotope	^{3}H	^{3}H	^{57}Co	^{57}Co
Solvent	0.9% NaCl	Ethanol	Ethanol	HSA
Heart	0.12 ± 0.04	0.03 ± 0.01	0.03 ± 0.006	0.03 ± 0.006
Lungs	0.09 ± 0.08	0.07 ± 0.01	0.27 ± 0.007	0.14 ± 0.01
Liver	0.20 ± 0.11	0.06 ± 0.02	0.56 ± 0.23	0.19 ± 0.04
Muscle	0.04 ± 0.03	0.03 ± 0.01	0.01 ± 0.002	0.02 ± 0.006
Blood	0.07 ± 0.006	0.02 ± 0.006	0.04 ± 0.02	0.01 ± 0.01

Compound	X	X	XIV	XIV
Chelating agent, μg	100	100	500	100
Isotope	^{57}Co	^{99m}Tc	^{99m}Tc	^{3}H
Solvent	Ethanol	50% Ethanol	50% Ethanol	Ethanol
Heart	0.04 ± 0.009	0.03 ± 0.01	0.03 ± 0.007	0.05 ± 0.04
Lungs	1.52 ± 0.30	0.32 ± 0.20	0.12 ± 0.0002	0.18 ± 0.07
Liver	0.23 ± 0.04	0.27 ± 0.007	0.28 ± 0.09	0.09 ± 0.02
Muscle	0.01 ± 0.0009	0.003 ± 0.002	0.01 ± 0.00	0.03 ± 0.006
Blood	0.06 ± 0.01	0.08 ± 0.02	0.08 ± 0.03	0.01 ± 0.005

[a] Blanks in table indicate that particular organ was not assayed. Each value in Table II represents the average for three rabbits. [b] Ten percent aqueous ammonium acetate and absolute ethanol (1:1, v/v). [c] Fraction V human serum albumin.

Table III—Biodistribution (Percent Dose per Gram) of ^{3}H-Palmitic Acid in Rabbits[a]

Diet	Normal	Normal	Dextrose[b]	Dextrose	16-hr Fast	16-hr Fast	Normal	Normal	Normal
Palmitic acid, μg	7	7	7	700	7	700	7	7	7
Sacrifice time, hr	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	2.0
Solvent for injection	HSA[c]	PBS[d]	Absolute ethanol	Absolute ethanol	Absolute ethanol	Absolute ethanol	Absolute ethanol	Absolute ethanol	Absolute ethanol
Heart	0.15 ± 0.05	0.03 ± 0.007	0.21 ± 0.06	0.18 ± 0.00	0.11 ± 0.07	0.08 ± 0.03	0.24 ± 0.13	0.19 ± 0.10	0.05 ± 0.02
Lungs	0.13 ± 0.08	1.64 ± 0.71	0.20 ± 0.11	0.16 ± 0.18	0.28 ± 0.05	0.35 ± 0.03	0.29 ± 0.17	0.05 ± 0.04	0.17 ± 0.03
Liver	0.14 ± 0.04	0.33 ± 0.11	0.26 ± 0.15	0.18 ± 0.13	0.30 ± 0.12	0.23 ± 0.06	0.28 ± 0.17	0.12 ± 0.07	0.16 ± 0.007
Spleen	0.26 ± 0.04	0.62 ± 0.05	0.18 ± 0.18	0.19 ± 0.04	0.08 ± 0.05	0.07 ± 0.01	—	—	—
Skeletal muscle	0.02 ± 0.01	0.00	0.04 ± 0.00	0.01 ± 0.008	0.01 ± 0.006	0.01 ± 0.006	0.01 ± 0.00	0.02 ± 0.06	0.02 ± 0.01
Blood	0.005 ± 0.05	0.02 ± 0.006	0.01 ± 0.007	0.02 ± 0.00	0.003 ± 0.001	0.01 ± 0.007	0.005 ± 0.001	0.05 ± 0.00	0.006 ± 0.03

[a] Female New Zealand White rabbits, 2.5–3.5 kg, were used. Each value in Table III represents the average for three rabbits. Blanks in table indicate that particular organ was not assayed. [b] With 250 ml of 10% dextrose in place of drinking water the night before and 1 ml iv of 50% dextrose 10 min prior to injection. [c] Fraction V human serum albumin, fatty acid free (Miles Laboratories). [d] Phosphate-buffered saline, pH 7.4.

Compounds V and IX were labeled with tritium by catalytic reduction, using tritium gas[2] and palladium-on-charcoal catalyst[2]. Compounds VII and XIV were labeled with tritium by Wilzbach bombardment with tritium gas over 2 weeks. All tritiated compounds were purified by suitable preparative thin-layer methods and were redissolved in absolute ethanol. 9,10-^{3}H-Palmitic acid[2] was used as a reference standard for biodistribution studies, since this simple long chain fatty acid is known (5, 6) to localize in, and be metabolized by, normal myocardium in humans.

Chromatography of Radiolabeled Chelates and Chelating Agents—Paper chromatography[3], TLC on silica gel glass plates[4] or cellulose plates[5], and column chromatography on 1 × 30-cm cross-linked polysaccharide columns[6] were performed (Table I). Tritium counting of scraped sections from radiochromatograms was performed using a liquid scintillation counter; the channels-ratio method was used for quench correction. All strips containing γ-emitting isotopes were cut and counted in a sodium iodide crystal scintillation counter. In most cases, two systems are presented for verification of the degree of labeling.

Thirty-Minute Biodistribution Studies—Male New Zealand White rabbits, 2–4 kg, were used for each distribution study. After purification by preparative chromatography, 0.10 ml (~10 μCi) was injected into the marginal ear vein of the rabbit. The animal was sacrificed 30 min postinjection, unless otherwise specified. A midline incision was made, and the heart, lungs, liver, spleen, and kidneys were removed, as well as samples of skeletal muscle, perirenal fat, urine, and blood.

For each whole organ or tissue sample containing a tritium-labeled compound, one 25–50-mg sample of each type of tissue from each animal was removed, weighed (±0.01 mg), digested in 0.5 ml of 5 *N* NaOH overnight, bleached with 2 drops of hydrogen peroxide, and finally suspended in 10 ml of scintillation fluid[2]. After 4-day dark-adaption and cooling, each sample was counted for 40 min (at ≥5000 counts/sample) in a liquid scintillation counter. All data are reported in terms of percent administered dose per organ and percent administered dose per gram of organ.

Table II summarizes the tissue concentrations for the various labeled compounds and specifies the experimental conditions of the biodistribution study. Ethanol, saline, and dilute human serum albumin were used as solvents to determine if the insolubility of the fatty acid derivatives is a factor in the distribution. The dose of the chelating agent was varied to study the possibility of the saturation of the transport mechanism. Fasted animals received water *ad libitum*. The dextrose diet consisted of the normal pellet diet plus 10% dextrose solution instead of water and 1 ml of 50% dextrose injected intravenously 10 min before injection of the compound. The distribution with time was studied for ^{3}H-palmitic acid.

In a preliminary experiment, the cobalt-57 chelate of IX was studied in two 18–20-kg mongrel dogs with experimentally induced infarcts. The infarcts were induced by ligation of the anterior descending branch of the left coronary artery just distal to the origin of the circumflex branch. This procedure produced an area of ischemia in the apical region of the left ventricle, which was evident by ECG. The radiopharmaceutical was injected intravenously 30 min after sudden and complete ligation. The animal was killed 30 min after injection, and the heart was immediately excised and divided into the left and right ventricles and the interventricular septum. Each part was then further subdivided, and the radioactivity was counted.

RESULTS AND CONCLUSIONS

Chromatographic studies of the radiolabeled derivatives in the appropriate system (Table I) indicate that the technetium Tc 99m or cobalt-57 was quantitatively bound to the new derivative. However, the results presented in Tables II and III show a significantly lower ($p \leq 0.05$) concentration in the myocardium for all chelates tested than for palmitic acid, the reference standard. Approximately 0.04%/g of the administered dose localized in the entire heart, compared to 0.15%/g for ^{3}H-labeled palmitic acid. The generally high degree of localization of these compounds in the liver (~20–30%) was approximately the same as that for palmitic acid. There was no evidence of particle formation in the chromatographic studies of the original chelate.

A significant percentage of the injected dose of most of these synthetic compounds remained in the intravascular space (15–25% of the admin-

[2] New England Nuclear, Boston, Mass.
[3] Whatman No. 1 paper, Whatman Inc., Clifton, N.J.
[4] E. M. Laboratories, Elmsford, N.Y.
[5] J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Sephadex, Pharmacia Fine Chemicals, Piscataway, N.J.

istered dose) at the 30-min observation point. This finding compares with an average of about 3% remaining in the blood at 30 min for palmitic acid. Heart–blood ratios for palmitic acid on a per gram basis were in the range of from a 30:1 to 40:1; for the metal chelates the concentration was always less than 3:1, thus making it impossible to differentiate myocardium from blood.

The high blood concentration may be due to the fact that these compounds are more tightly bound to serum albumin so that the transport of fatty acid derivatives across the cell membrane into the intracellular fluids is reduced. The greater difference in polarity between a simple fatty acid, *e.g.*, palmitic acid, and fatty acid derivatives (*i.e.*, IX) could affect the localization of the labeled compound. Compounds containing a diethylenetriamine chelating group also showed slow blood clearance.

The 30-min distribution was chosen to screen this series of compounds rapidly. Because the palmitic acid does not concentrate in an infarct but does concentrate in the normal myocardia, the 30-min myocardia to blood ratio is the important factor and should indicate if the compound may be useful.

The results of the distribution of the labeled IX in dogs with experimentally induced infarcts revealed that an average accumulation of 0.94% of the injected dose was localized in the heart, 16.6% was in the liver, and 2.7% was in the lungs and that 3.8% remained in the intravascular compartment at 30 min. The infarct contained less than half of the radioactivity found in adjacent normal myocardium.

The chemical analogs described here are not sufficient biological analogs to act as tracers for fatty acid metabolism in the myocardium. However, this approach of preparing drug derivatives should lead to more specific radiopharmaceuticals and will be pursued using other biologic molecules.

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Chemical Ionization Mass Spectra of Phenothiazine Derivatives and Their Oxygenated Analogs

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Abstract □ Twenty-nine derivatives of phenothiazine formed relatively stable ions by proton capture under conditions of chemical ionization, using methane or isobutane as reagent gas. Fragments of generally low abundance formed by simple bond cleavage in the trimethylene portion of the side chain and by loss of water or hydrogen halide. Mass spectra obtained from pyrolyzates of amine salts and from the corresponding free bases were essentially identical.

Keyphrases □ Chemical ionization mass spectrometry—various phenothiazines and oxygenated analogs □ Phenothiazines, various—and oxygenated analogs, chemical ionization mass spectra □ Mass spectrometry, chemical ionization—various phenothiazines and oxygenated analogs

Phenothiazines have dominated the interest of researchers in many diverse areas since the discovery that they exert profound effects upon the central nervous system, and a wealth of information pertaining to pharmacological action, therapeutic applications, and biotransformation pathways has been accumulated (1, 2). Some of the vicissitudes encountered in assaying phenothiazine compounds and their metabolites in biological fluids were summarized by Usdin (3).

Elucidation of the mechanisms of action and metabolic conversion and evaluation of the relationship of plasma concentrations of these drugs to their clinical efficacy became possible only after analytical procedures possessing adequate sensitivity and specificity had been developed. A recent monograph outlining many aspects of current research efforts in the area of phenothiazine derivatives describes three approaches to mass spectrometric identification of promazine, chlorpromazine, and several oxygenated homologs (4–6). Whereas electron-impact mass spectrometry has made significant contributions to the biomedical and pharmaceutical sciences over the years, application of chemical ionization mass spectrometry to these areas represents a fairly recent development (7). Finkle *et al.* (8) reported chemical ionization (methane) mass spectra of pharmaceutical agents, including some phenothiazines, and their metabolites as part of a broad program for rapid identification of the contents of body fluids in patients suspected to be intoxicated by a drug overdose.

As part of a continuing program of developing applications of chemical ionization GC–mass spectrometry in neurochemistry (9, 10), the chemical ionization mass spectra for 29 derivatives of phenothiazine were investigated including the tranquilizing agents chlorpromazine (VII), promazine (XV), promethazine (XVI), prochlorperazine (XVIII), trifluoperazine (XXII), perphenazine