TELLURIUM-123m-LABELED ISOSTERES OF PALMITOLEIC AND OLEIC ACIDS SHOW HIGH MYOCARDIAL UPTAKE

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ABSTRACT

These studies were directed at determining if the telluro fatty acids prepared by the isosteric replacement of the Δ^9-double bonds of oleic and palmitoleic acids with ^{123m}Te would show heart uptake in rats. The isostere of palmitoleic acid, 9-tellurapentadecanoic acid(II), was prepared by basic hydrolysis of the product formed by the coupling of ^{123m}Te-sodium hexyl tellurol with methyl-8-bromooctadecanoate. Similarly, the isostere of oleic acid, 9-telluraheptadecanoic acid(IV), was prepared by the same route beginning with the reaction of ^{123m}Te-sodium octyl tellurol with methyl-8-bromooctadecanoate. Both ^{123m}Te-(II) and ^{123m}Te-(IV) showed remarkably high heart uptake in rats (2-3% dose/gm) ten minutes after intravenous administration, and the heart/blood ratios were high (20-30/1). Finally, the hearts of rats injected with ^{123m}Te-(IV) have been clearly imaged with a rectilinear scanner.

DISCLAIMER

INTRODUCTION

Since it is well established that the biological properties of many organic compounds are retained when selected double bonds are isosterically substituted with S, Se or Te (1, 2), we wished to determine if this isosteric behavior was also exhibited by aliphatic tellurium isosteres of olefinic compounds. Long-chain unsaturated fatty acids were obvious choices for these investigations since oleic acid (9-octadecenoic acid) is one of the major serum fatty acids and long-chain fatty acids are utilized for energy production by the normal myocardium (3). The preparation and testing of the ^{123m}Te-labeled isosteres of unsaturated fatty acids were therefore prime candidates for determining the potential usefulness of the concept of isosteric replacement of olefinic linkages with a chalcogen radionuclide for the development of new and potentially useful radiopharmaceuticals.

In the present investigation we have prepared ^{123m}Te-labeled 9-tellura-pentadecanoic acid [$CH_3(CH_2)_5$-^{123m}Te-$(CH_2)_7$-COOH] and 9-telluraheptadecanoic acid [$CH_3(CH_2)_7$-^{123m}Te-$(CH_2)_7$-COOH] as isosteres of palmitoleic acid and oleic acid, respectively. Tissue distribution experiments in rats have demonstrated the pronounced heart uptake of radioactivity following intravenous administration of the ^{123m}Te-labeled fatty acids. In addition, rat hearts have been clearly imaged following injection of ^{123m}Te-labeled 9-telluraheptadecanoic acid.

PREPARATION OF TELLURO FATTY ACIDS

The general method that has been developed for the synthesis of telluro fatty acids involves the following transformations:

$$\text{Te} + \text{Na} \xrightarrow[\text{Step 1}]{NH_3} Na_2Te_2 + RX \xrightarrow[\text{Step 2}]{} R_2Te_2 + NaBH_4 \xrightarrow[\text{Step 3}]{MeOH} \text{Na-Te-R} +$$

$$\text{Br-R'-COOMe} \xrightarrow[\text{Step 4}]{MeOH} \text{R-Te-R'COOMe} + NaOH \xrightarrow[\text{Step 5}]{EtOH\text{-}H_2O} \text{R-Te-R'-COOH.}$$

For the preparation of 9-tellurapentadecanoic acid (II), iodohexane was used in Step 2 with subsequent conversion to sodium hexyl tellurol which was coupled with the methyl-8-bromooctanoate in Step 4 followed by basic hydrolysis to give (II). The 8-bromooctanoic acid was obtained commercially and converted to the methyl ester by treatment with diazomethane. Sodium octyl tellurol was prepared in the same manner and coupled with methyl-8-bromooctanoate to give methyl-9-telluraheptadecanoate(III) which was treated with base to yield 9-tellura-heptadecanoic acid (IV). Compounds (I), (II), (III), and (IV) were fully characterized by thin-layer chromatographic, ultraviolet, infrared, mass-spectral

and nuclear magnetic resonance spectral methods and these data are consistent with the proposed structures (Table 1).

PREPARATION OF TELLURIUM-123m-LABELED FATTY ACIDS

Tellurium-123m-labeled dioctyl ditelluride was prepared by iodooctane alkylation of ^{123m}Te-sodium ditelluride (31.3 mCi) by the general procedure described earlier (4). For convenience, these reactions were performed on the 1 mmole scale. Following sodium borohydride reduction (Step 3) of the orange-colored ditelluride solution (19.62 mCi) in benzene-methanol, the resulting colorless solution of ^{123m}Te-sodium octyl tellurol was reacted in Step 4 with methyl-8-bromooctanoate (59 mg, 250 μmoles) by refluxing for 30 minutes. The tellurols in Step 4 were used in excess to insure complete consumption of the haloacid methyl ester. The reaction mixture was cooled, poured into water and the benzene layer washed several times with water, dried over anhydrous Na_2SO_4 and the solvent evaporated *in vacuo* to give a yellow oil which was dissolved in petroleum ether and applied to a 60-200 mesh silicic acid (acidic) column (2 x 20 cm). The column was eluted with petroleum ether (25 ml fractions) which removed a radioactive peak that contained ^{123m}Te-dioctyl ditelluride and ^{123m}Te-octyl ditelluride. Further elution with 2% ether in petroleum ether removed a radioactive component with the same mobility as authentic methyl-9-telluraheptadecanoate (Figure 1). The peak fractions were combined to give ^{123m}Te-methyl-9-telluraheptadecanoate, 5.41 mCi (26% from methyl-8-bromooctanoate). Only one radioactive component was detected upon thin-layer radiochromatographic analyses on silica gel G (solvent, benzene). This material had the same mobility as authentic methyl-9-telluraheptadecanoate (R_f 0.6). The purified material had a specific acticity of ∿ 31 mCi/mmole. For long-term storage the sample was transferred to break-seal tubes, the solvent evaporated under argon and the tubes sealed. Samples were stored in the dark at 8°C.

The ^{123m}Te-labeled 9-telluraheptadecanoic acid (IV) was prepared by basic hydrolysis of the methyl ester (III) in refluxing aqueous ethanol under argon. Aliquots of (III) were dissolved in 20 ml of absolute ethanol and 0.50 ml of a 1 N NaOH solution was added and the reaction system flushed thoroughly with argon. The mixture was then refluxed in the dark for 30 minutes. Following cooling, the mixture was poured into water the pH adjusted to pH 2-3 by addition of 1 N HCl and the resulting cloudy solution extracted with ethyl ether. The organic layer was washed well with water, dried over anhydrous sodium sulfate and the solvent evaporated under argon. The methyl ester (III) was relatively stable and could be stored as a neat oil under argon in the dark at 0°C for several weeks with only marginal decomposition. In contrast, the free acid (IV) was relatively unstable and was formed by hydrolysis of (III) immediately prior to the biological experiments.

The ^{123m}Te-labeled methyl-9-tellurapentadecanoate (I) was prepared exactly in the same manner as described earlier for ^{123m}Te-labeled methyl-9-telluraheptadecanoate (III). The ^{123m}Te metal (24.8 mCi) was converted to ^{123m}Te-

sodium ditelluride (Step 1) and alkylated with iodohexane to give, after work-up, ^{123m}Te-labeled dihexyl ditelluride (11.17 mCi). Following sodium borohydride reduction (Step 3), coupling with methyl-8-bromooctanoate (Step 4) and column purification, ^{123m}Te-labeled methyl-9-tellurapentadecanoate (II) was obtained (1.35 mCi). The methyl ester (II) was hydrolyzed with base to yield the ^{123m}Te-labeled 9-tellurapentadecanoic acid.

BIOLOGICAL STUDIES

Following basic hydrolysis, the ^{123m}Te-labeled fatty acids were dissolved in a small volume of absolute ethanol and added dropwise to a stirred solution of 6% bovine serum albumin at 40°C. Female Fischer strain rats were injected via a tail vein with 1.0 ml. of the bovine serum albumin solution containing 5-10 μCi of the ^{123m}Te-labeled fatty acid. After various time intervals the animals were sacrificed by decapitation after being anesthesized with ether. The various organs were removed, washed with saline, weighed and the radioactive contents determined by counting the organs directly in a autogamma counter. Three animals were used for each time point. For rectilinear scans, the animals were injected with 30-50 μCi of the radiolabeled fatty acid in 1.0 ml. of the 6% bovine serum albumin. The animals were anesthesized by intraperitoneal injection of pentabarbital for the rectilinear scans which were performed with a small animal scanner equipped with a 64-hole gold collimator. The rats were scanned at a rate of 0.25 in/min.

RESULTS AND CONCLUSIONS

The preparation of ^{123m}Te-labeled fatty acids (II) and (IV) is a simple method and the radiolabeled compounds can be synthesized and purified easily in a single day. The use of the ω-halogenated fatty acid methyl ester in Step 4 was chosen as the preferred method after initial attempts to couple the free ω-halogenated fatty acid with the sodium alkyl tellurols resulted in low and non-reproducible yields of the telluro fatty acids. In addition, the methyl esters of the telluro fatty acids have been found to be relatively stable and can be stored in the dark under argon at 0°C for several weeks with very little decomposition. Unfortunately, the free acids (II) and (IV) are relatively unstable and must be generated by basic hydrolysis of the methyl esters immediately before use. Methods to stablize the free acids have not yet been investigated in detail but will be the subject of future studies.

Following intravenous administration of the ^{123m}Te-labeled acids (II) and (IV) complexed in bovine serum albumin very pronounced heart uptake was detected in rats as evidenced by the data illustrated in Figures 2 and 3. It is interesting to note that the % dose/gm tissue values for maximal heart uptake (3-4%) very closely paralleled similar data reported for ^{14}C-labeled oleic acid (5). These data suggest that the radioactivity detected in heart tissue may well represent myocardial uptake of the intact ^{123m}Te-labeled fatty acids. More importantly, these preliminary data may indicate that replacement of the Δ^9-double bonds of palmitoleic acid and oleic acid may be a

true isosteric conversion. The hearts of rats were clearly imaged with a rectilinear scanner after administration of ^{123m}Te-labeled 9-tellurahepta-decanoic acid (Figure 4). The areas of significant radioactivity accumulation were the liver and heart. Subsequent rectilinear scans of the excised, washed heart showed an identical pattern. These results confirm the tissue distribution data and indicate that the radioactivity was present primarily in the heart muscle and not in the blood pooled within the cardiac chambers.

Finally, these studies indicate that the isosteric replacement of selected double bonds with ^{123m}Te or ^{75}Se may be an efficient strategy for the design and development of new radiopharmaceuticals. We feel our results may have some application for the design of new heart imaging agents. Studies are now in progress to determine the effects of both total chain length and the position of the tellurium heteroatom on the heart uptake of a series of ^{123m}Te-labeled fatty acids.

ACKNOWLEDGEMENTS

Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation. The partial support of the investigations at Texas A & M University by the Robert A. Welch Foundation of Houston, Texas is gratefully acknowledged. The technical assistance of L. A. Ferren is gratefully acknowledged, and we thank Dr. D. V. Woo for performing the rectilinear scans, Dr. B. M. Benjamin and L. L. Brown for the nuclear magnetic resonance studies, and C. W. Pritchard and Dr. W. D. Rainey, Jr. for the mass spectral measurements. We also thank C. A. Floyd for typing the manuscript.

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Table 1. Physical Properties of Telluro Fatty Acids and Telluro Fatty Acid Methyl Esters

R_1-Te-$(CH_2)_7COOR_2$

I. Methyl-9-Tellurapentadecanoate; R_1 = n - C_6H_{13}, R_2 = CH_3
II. 9-Tellurapentadecanoic Acid; R_1 = n - C_6H_{13}, R_2 = H
III. Methyl-9-Telluraheptadecanoate; R_1 = n - C_8H_{17}, R_2 = CH_3
IV. 9-Telluraheptadecanoic Acid; R_1 = n - C_8H_{17} R_2 = H

Compound	TLC, R_f	Ultraviolet	Infrared	Mass Spectrum	Nuclear Magnetic Resonance Spectrum
I	0.53*	λ_{max} 234 nm	ν_{max}^{neat} 1750 cm^{-1} (carbonyl)	m/e 372, M m/e 341, M-31	0.91, s, 3H, terminal methyl 2.29, t, 2H, α-CH_2 2.61, t, 4H, CH_2's flanking Te 3.66, s, 3H, -OCH_3
II	0.55**	λ_{max} 235 nm	. . .	. . .	0.76, s, 3H, terminal methyl 1.99, t, 2H, α-CH_2 2.49, t, 4H, CH_2's flanking Te
III	0.53*	λ_{max} 234 nm	ν_{max}^{neat} 1745 cm^{-1} (carbonyl)	m/e 400, M m/e 369, M-31	0.96, s, 3H, terminal methyl 2.31, t, 2H, α-CH_2 2.59, t, 4H, CH_2's flanking Te 3.66, s, 3H, -OCH_3
IV	0.55**	λ_{max} 236 nm	. . .	. . .	0.80, s, 3H, terminal methyl 1.90, t, 2H, α-CH_2 2.40, t, 4H, CH_2's flanking Te

*SiO_2, benzene; **SiO_2, Petroleum ether-ether-acetic acid, 70:30:1

FIGURE 1

Silicic acid column chromatographic profile obtained upon purification of ^{123m}Te-methyl-9-telluraheptadecanoate (III). The radioactive peak for the radiolabeled methyl ester co-chromatographed with authetic unlabeled (III). The peak fractions were combined and aliquots hydrolyzed with base to give ^{123m}Te-9-telluraheptadecanoic acid which was used for the biological experiments described in Figures 3 and 4.

FIGURE 2

The tissue distribution of radioactivity in female Fischer rats following intravenous administration of ^{123m}Te-9-tellurapentadecanoic acid (II). The experimental points represent averaged data (% dose/gm tissue) from three animals. The animals were each injected via a tail vein with 5-10 μCi of radioactive (II) complexed in 1.0 ml of a 6% bovine serum albumin solution.

FIGURE 3

The tissue distribution of radioactivity in female Fischer rats following intravenous administration of ^{123m}Te-9-telluraheptadecanoic acid (IV). The experimental points represent averaged data (% dose/gm tissue) from three animals. The animals were each injected via a tail vein with 5-10 μCi of radioactive (IV) complexed in 1.0 ml of a 6% bovine serum albumin solution.

FIGURE 4

Rectilinear scan of a female Fischer rat following the intravenous administration of 50 μCi of ^{123m}Te-9-telluraheptadecanoic acid (IV). The animal was injected via a tail vein with radiolabeled (IV) complexed in 1.0 ml of a 6% bovine serum albumin solution.

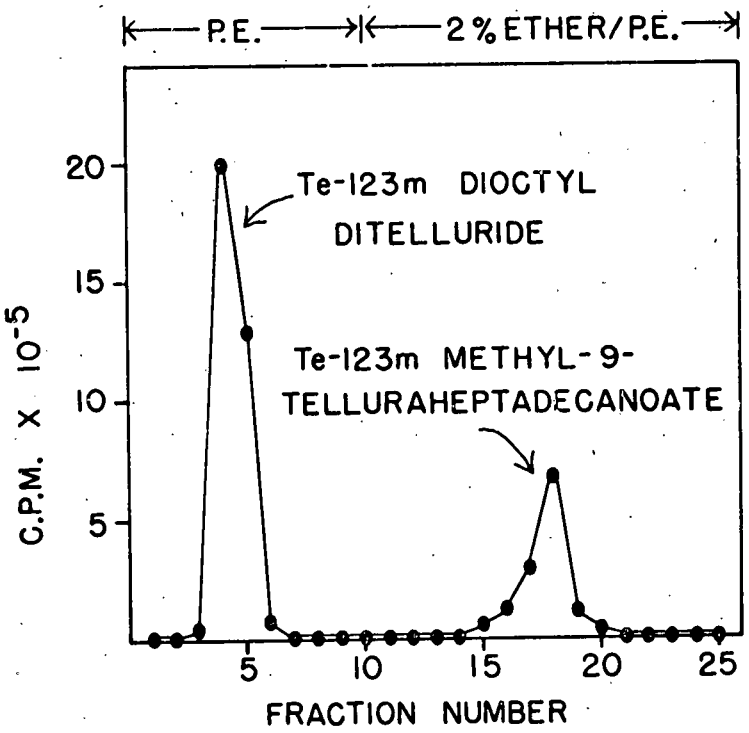

P.E.
2% ETHER/P.E.
Te-123m DIOCTYL DITELLURIDE
Te-123m METHYL-9-TELLURAHEPTADECANOATE
C.P.M. X 10⁻⁵
20
15
10
5
5
10
15
20
25
FRACTION NUMBER

Fig. 1

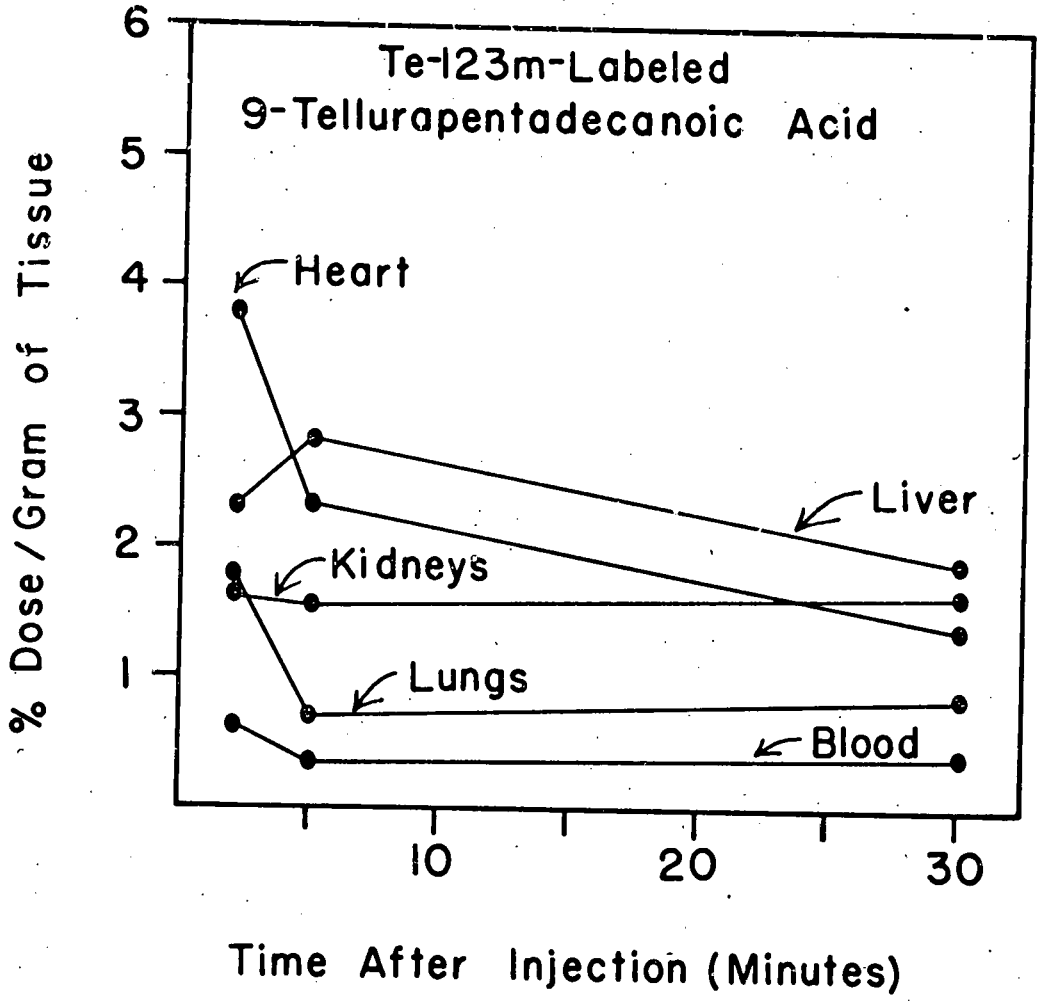

Te-123m-Labeled
9-Telluropentadecanoic Acid
Heart
Liver
Kidneys
Lungs
Blood
% Dose / Gram of Tissue
6
5
4
3
2
1
10
20
30
Time After Injection (Minutes)

Fig. 2

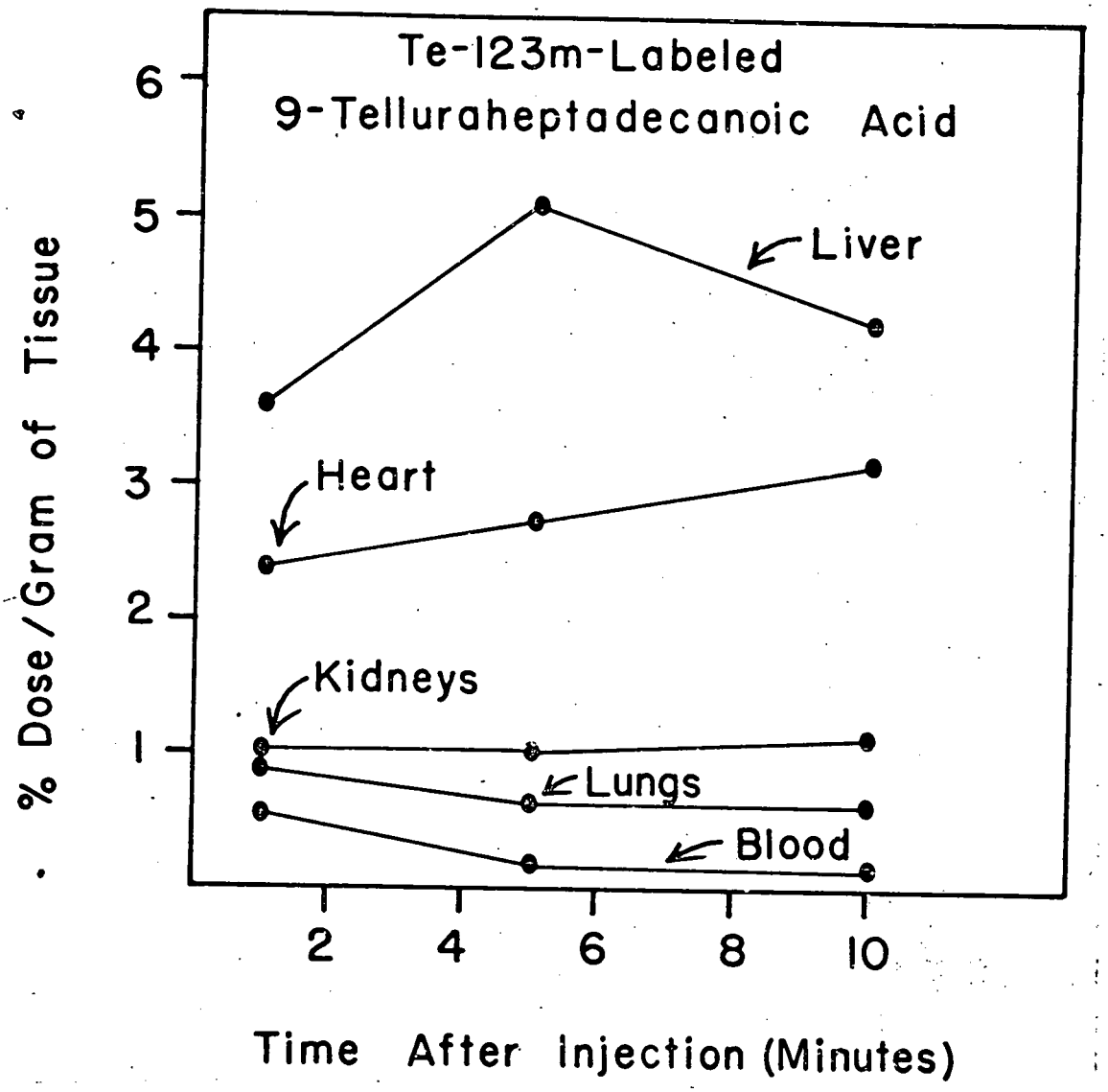
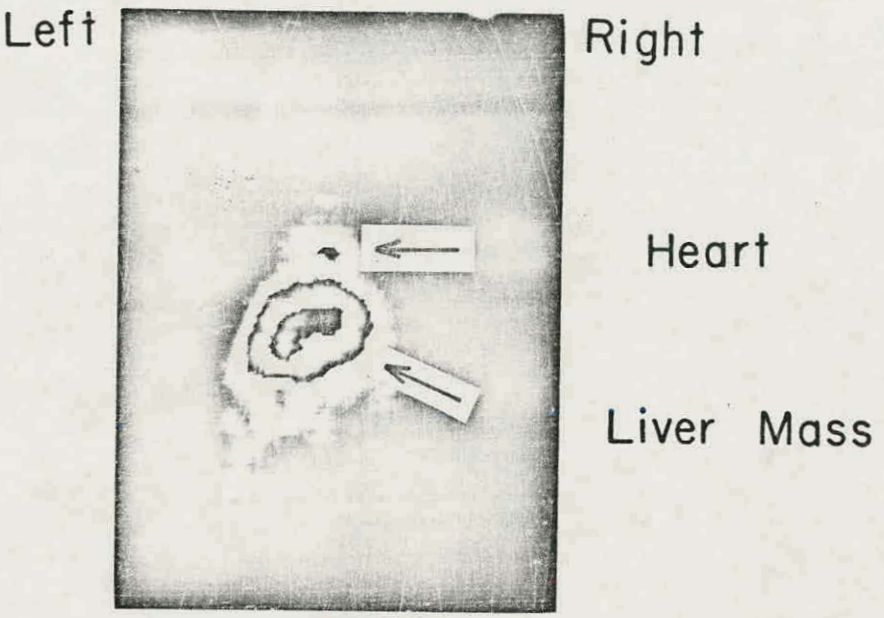

Te-123m-Labeled
9-Telluraheptadecanoic Acid
Liver
Heart
Kidneys
Lungs
Blood
% Dose/Gram of Tissue
Time After Injection (Minutes)
1
2
3
4
5
6
2
4
6
8
10

Fig. 3



Female Rat, Posterior View
Rectilinear Scan

Fig. 4