Site-Specific/Stable Radioiodination of 1,2-Pal-3-IPPA: An Agent for the Potential Clinical Evaluation of Pancreatic Insufficiency by Urine Analysis

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To measure pancreatic lipase activity, we synthesized a triglyceride containing a radioiodinated fatty acid. The urinary excretion of radioactivity was measured in five rats following administration of the agent by feeding tube. We attached 15-phenylpentadecanoic acid (PPA) to position-3 of 1,2-dipalmitoyl-rac-glycerol (1,2-Pal) to form 1,2-Pal-3-PPA. The 1,2-Pal-3-IPPA (expected lipase substrate) was prepared by the thallation-iodide displacement method. In a dual-label study, the ^{125}I-1,2-Pal-3-IPPA triglyceride was administered with the ^{131}I-IPPA free acid to rats (n = 5) by oral gavage. Urine and feces were collected daily and the tissue distribution of both tracers was evaluated over a five-day period. A significant portion of the administered activity was excreted in 24 hr in the urine (^{125}I, 30.31% + 4.32%; ^{131}I, 35.0% + 7.29%), which cochromatographed with hippuric acid by thin layer chromatography. Release of the acidic components from the conjugated excretory products by acid hydrolysis of the urine provided the radioactive acidic metabolites. Analysis of the Folch extracts of fat samples demonstrated that the radioactive components cochromatographed in the triglyceride region. This agent appears useful for the evaluation of various gastrointestinal diseases.

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An accurate evaluation of fat adsorption can be an indirect measure of pancreatic lipase activity which is an important indicator for the diagnosis of many types of gastrointestinal disease. Traditional techniques to evaluate steatorrhea are often unpleasant or impractical and involve chemical fecal fat analysis (*1,2*) and the evaluation of blood and fecal levels of radioactivity after administration of radioiodinated fats (e.g., "triolein"). (The terms ^{131}I-labeled "triolein" and "oleic acid" are misnomers, although widely used in the early literature. They refer to the products obtained by [^{131}I]iodine monochloride (ICl) addition to the double bond of oleic acid and would be expected to consist of mixtures of the 9-chloro-10-iodo- and 9-iodo-10-chlorohexadecanoic acid isomers, as shown in Figure 1.) Iodine-131-labeled olive oil was first used to evaluate intestinal absorption of fat (*1–5*) and ^{131}I-labeled "triolein" was then introduced for the evaluation of aberrations in fat absorptions (*6–12*) to evaluate the availability of pancreatic lipases and thus to diagnose pancreatic insufficiency. Since absorption of radioactivity from dietary ^{131}I-labeled triglycerides depends not only on the availability of hydrolytic enzymes but also upon both the availability of bile acids for emulsification and the patency of the small intestine for absorption (Fig. 2), ^{131}I-oleic acid was later introduced to evaluate the absorption stage (*13,14*). However, continued use of this substance was not further pursued because of the apparent purity and instability problems (*1,2,15*) and the inconsistency in relating either blood or fecal levels of radioactivity with pancreatic lipase activity (*16,17*).

We have prepared a triglyceride agent containing radioiodinated IPPA, in which the iodide is stabilized by attachment to the para-position of the terminal phenyl ring. Our approach is based on the expected urinary excretion of radioactive short-chain acid catabolites formed by beta-oxidation of the radioiodinated IPPA released from the triglyceride by tissue lipase hydrolysis. Use of a triglyceride containing radioiodinated IPPA would thus be expected to overcome the problems encountered with the triolein approach. With an IPPA-labeled triglyceride, free radioiodide would not be expected to be formed, the agent would be stable for long-term storage and the expected metabolite IPPA released via hydrolysis would have a well defined metabolic fate.

The goals of the present studies were thus to synthesize a site-specific triglyceride analog containing the radioiodinated IPPA moiety attached to the 3(β)-position of the triglyceride and to measure the levels and kinetics of urinary excretion of radioactivity in rats. Rapid urinary ex-

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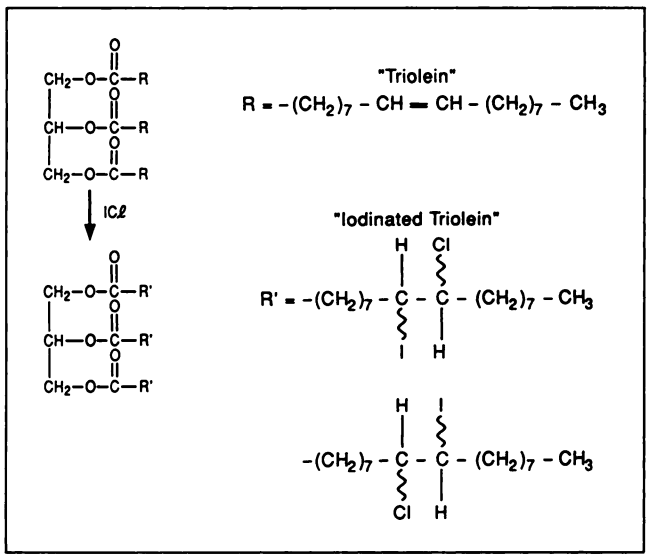

FIGURE 1. Possible structures of "radioiodinated triolein" obtained by [^{131}I]iodine monochloride (ICl) addition to triolein.

cretion of significant levels of radioactivity would suggest that this agent may be used as an index of the activity of pancreatic lipase.

MATERIALS AND METHODS

The 1,2-palmitoyl-rac-glycerol (1) substrate was purchased from Sigma Chemicals, Inc., and 15-phenylpentadecanoic acid (PPA) was obtained from EMKA-Chemie (7145 Markgroeningen-Taulhausen, Germany). The 15-(p-iodophenyl)pentadecanoic acid (IPPA) was prepared as described earlier, MP 91-92°C; Lit. MP, 92–93°C (*18*). All other chemicals and reagents were analytical grade. Melting points are uncorrected and were determined with a Thomas Hoover melting point apparatus. Both proton (^{1}H) and ^{13}C (carbon) nuclear magnetic resonance (NMR) spectra were obtained with a Gemini 200 instrument in the solvents indicated with the resonances reported downfield (delta) from tetramethylsilane in the ^{1}H spectra and from $CHCl_3$ in the ^{13}C spectra. The thin-layer chromatographic (TLC) analyses were performed using precoated 250-nm thick layers of SiO_2-PF-254 adsorbent on aluminum sheets obtained from Merck, Inc. The mass spectral analyses (MS) were determined by the Oak Ridge National Laboratory (Oak Ridge, TN) Organic Spectroscopy Group by laser ionization FT mass spectrometry. Elemental analyses were determined by Galbraith Laboratories, Inc. (Knoxville, TN). Details of the syntheses and properties of the synthetic products are summarized in the Appendix. Iodine-125 and ^{131}I were obtained from Du Pont New England Nuclear (N. Billerica, MA). The ^{131}I-IPPA was prepared in the usual manner by the thallation-iodide displacement method. Radioisotopic analyses were conducted using a high purity germanium detector. The urine, feces and rat tissues were analyzed with an Packard "Minaxi" Autogamma Counter. A window was set to analyze the ^{125}I (25–35 keV) and ^{131}I (360 keV) photopeaks and calibrated for efficiency. Standards from the injected dose were kept and counted with the samples for correction of decay.

Preparation and Purification of ^{125}I-1,2-Pal-3-IPPA and ^{131}I-IPPA

The detailed labeling procedure is described in the Appendix (from 1,2-Pal-3-PPA). Radioiodinations were conducted on the 0.005 mmole scale: 1,2-Pal-3-PPA substrate (4.8 mg, 0.005 mmole), thallium(III)trifluoroacetate (5.4 mg, 0.010 mmole), trifluoroacetic acid (3 ml), ^{125}I (5–10 mCi, <200 μl in NaOH solution) and 0.5 ml of KI solution (0.16 mg/ml 0.0025 mmole). TLC (SiO_2-G, solvent = benzene, R_f 0.60) indicated greater than 99.5% purity. The ^{131}I-IPPA was also prepared in the same manner by thallation-iodination of 15-phenylpentadecanoic acid.

In Vivo Biodistribution Studies in Rats

All animal care procedures were in accordance with federal regulations, and all animal use procedures were reviewed and approved by the institutional animal care and use committee.

The female Fischer strain rats were fasted overnight. The radiolabeled triglyceride and IPPA were each dissolved in warm ethanol and an appropriate aliquot was added to 0.5 ml of Tween 20. The solution was warmed and the ethanol removed under a gentle argon stream. The Tween 20 solution was then diluted with 10 ml of saline to give a solution containing 2.1 μCi ^{125}I and 6.0 μCi ^{131}I/1.0 ml. The ^{125}I-1,2-Pal-3-IPPA/^{131}I-IPPA solution was then administered to metofane-anesthetized rats by oral gavage with a feeding needle. One group of four rats was housed in metabolism cages (Nalgene) and allowed food and water ad libitum. The separated urine and feces were collected daily, aliquots counted, and the daily and cumulative levels of ^{125}I and ^{131}I in the urine and feces then calculated (Table 1). Animals were killed at

TABLE 1
Distribution of Radioactivity in Urine and Feces from Rats Following Oral Administration of a ^{125}I-1,2-Pal-3-IPPA/^{131}I-IPPA Mixture

	^{125}I (from 1,2-Pal-3-IPPA) and ^{131}I (from ^{131}I-IPPA)					
	Urine*		Feces*		Total†	
Day	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
1	30.31 ± 4.32	35.0 ± 7.29	8.00 ± 1.69	4.54 ± 1.27	38.31	39.54
2	4.97 ± 0.56	4.87 ± 0.68	1.34 ± 0.23	0.59 ± 0.16	44.62	45.00
3	2.97 ± 0.48	3.16 ± 0.60	0.45 ± 0.11	0.17 ± 0.06	48.04	48.33
4	2.14 ± 0.36	2.40 ± 0.46	0.31 ± 0.10	0.13 ± 0.07	50.49	50.86
5	1.22 ± 0.06	1.42 ± 0.08	0.16 ± 0.04	0.09 ± 0.04	51.87	52.37

*Mean values ±s.d. for five female Fisher rats. Each rat received a mixture of 1.1 μCi ^{125}I-1,2-Pal-3-IPPA and 2.4 μCi ^{131}I-IPPA in 0.5 ml of the formulation mixture by oral gavage with a feeding needle.

†Mean cumulative values.

TABLE 2

Results of Thin-Layer Chromatographic Analysis of Folch Extracts of Fat Samples, Urine and Chloroform Extracts of Urine Acid Hydrolysates Following Oral Administration of a Mixture of ^{125}I-1,2-Pal-3-IPPA and ^{131}I-IPPA to Rats*

Sample	Major component		Mean % ± s.d. of total on plate	
Fat extract	Mobility, R_f	Solvent system	^{125}I	^{131}I
4 hr	0.55†	$CHCl_3$	74.73 ± 4.44	76.48 ± 4.85
24 hr	0.55†	$CHCl_3$	92.21 ± 2.46	90.67 ± 2.64
24 urine	0.45‡	$CHCl_3$:HOAc, 2:1	63.92 ± 3.48	77.56 ± 3.46
24 urine hydrolysate	0.55§	MeOH:$CHCl_3$, 6:94	66.43 ± 4.98	79.93 ± 2.92

*Data represent the mean ± s.d. of the total applied to the TLC plates from samples from four rats. (Same rats described in Table 1.)
†Cochromatographs with triglyceride standard.
‡Cochromatographs with hippuric acid standard.
§Chromatographs in region of short-chain free acids.

various time periods (n = 5 rats/group) and the distribution of the two radioisotopes then determined in the major organs, blood and thyroid and a sample of fat taken from the abdominal cavity (Tables 2 and 3).

Chromatographic Analysis of Metabolites

Fat samples (0.98–2.27 g) were homogenized in 10 ml of 2:1 $CHCl_3$:MeOH with a loose-fitting ground glass homogenizer. After addition of 0.5 ml of 10% H_2SO_4 and 2 ml of 0.29% saline solution, the samples were centrifuged, the lower organic layer separated, dried over anhydrous Na_2SO_4 and the solvent removed under a stream of argon. Samples were dissolved in $CHCl_3$ and aliquots applied to SiO_2 plates which were developed in $CHCl_3$. Aliquots of 24-hr urine samples were analyzed by TLC using a $CHCl_3$:HOAc (2:1) solvent system. Urine aliquots (0.1 ml) were also combined in a reaction vial with 2 ml H_2SO_4, sealed and heated at 100°C for 30 min and cooled and extracted with $CHCl_3$. The upper organic layer was dried and aliquots analyzed by TLC using 6% MeOH in $CHCl_3$.

RESULTS

Chemical Syntheses

The 1,2-Pal-3-IPPA triglyceride was prepared in 55%–70% yield (Fig. 3) by the modified Hassner esterification (*19,20*) as described earlier (*21,22*). The substrate for preparation of 1,2-palmitoyl-3-[15-(p-iodophenyl)pentadecan-1-oyl]-rac-glycerol (**1**, 1,2-Pal-3-IPPA; Fig. 3) was prepared by esterification of PPA with 1,2-palmitoyl-rac-glycerol.

TABLE 3

Distribution of Radioactivity in Rat Tissues Following Administration of a ^{125}I-1,2-Pal-3-IPPA/^{131}I-IPPA Mixture by Oral Gavage*

Tissue	Radioisotope	Time after administration (mean %ID/g)				
		4 hr	1 day	2 days	3 days	5 days
Blood	^{125}I	0.81 ± 0.35	0.15 ± 0.02	0.07 ± 0.01	0.03 ± 0.01	0.02 ± 0.00
	^{131}I	0.86 ± 0.39	0.08 ± 0.02	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
Heart	^{125}I	0.89 ± 0.37	0.30 ± 0.05	0.20 ± 0.09	0.21 ± 0.13	0.06 ± 0.01
	^{131}I	0.93 ± 0.39	0.29 ± 0.05	0.22 ± 0.12	0.23 ± 0.13	0.06 ± 0.01
Kidneys	^{125}I	0.54 ± 0.20	0.55 ± 0.09	0.48 ± 0.10	0.37 ± 0.10	0.25 ± 0.06
	^{131}I	0.51 ± 0.20	0.53 ± 0.11	0.49 ± 0.11	0.40 ± 0.12	0.28 ± 0.08
Liver	^{125}I	1.34 ± 0.61	0.48 ± 0.05	0.13 ± 0.02	0.06 ± 0.00	0.04 ± 0.01
	^{131}I	1.56 ± 0.74	0.54 ± 0.06	0.12 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
Stomach	^{125}I	4.74 ± 1.10	0.21 ± 0.09	0.15 ± 0.05	0.08 ± 0.02	0.08 ± 0.04
	^{131}I	5.72 ± 1.63	0.21 ± 0.09	0.16 ± 0.05	0.08 ± 0.03	0.09 ± 0.05
Thyroid†	^{125}I	0.24 ± 0.11	0.36 ± 0.11	0.36 ± 0.05	0.38 ± 0.11	0.23 ± 0.02
	^{131}I	0.17 ± 0.09	0.15 ± 0.05	0.17 ± 0.03	0.15 ± 0.03	0.09 ± 0.01
Fat‡	^{125}I	0.60 ± 0.32	2.24 ± 0.77	2.35 ± 0.52	2.35 ± 0.58	1.84 ± 0.41
	^{131}I	0.66 ± 0.36	2.51 ± 0.85	2.62 ± 0.55	2.68 ± 0.63	2.17 ± 0.51

*Mean and ±s.d. for five female Fisher rats. Same rats described in Table 1.
†Thyroid data expressed as % dose/organ values.
‡White fat samples were taken from the abdominal cavity.

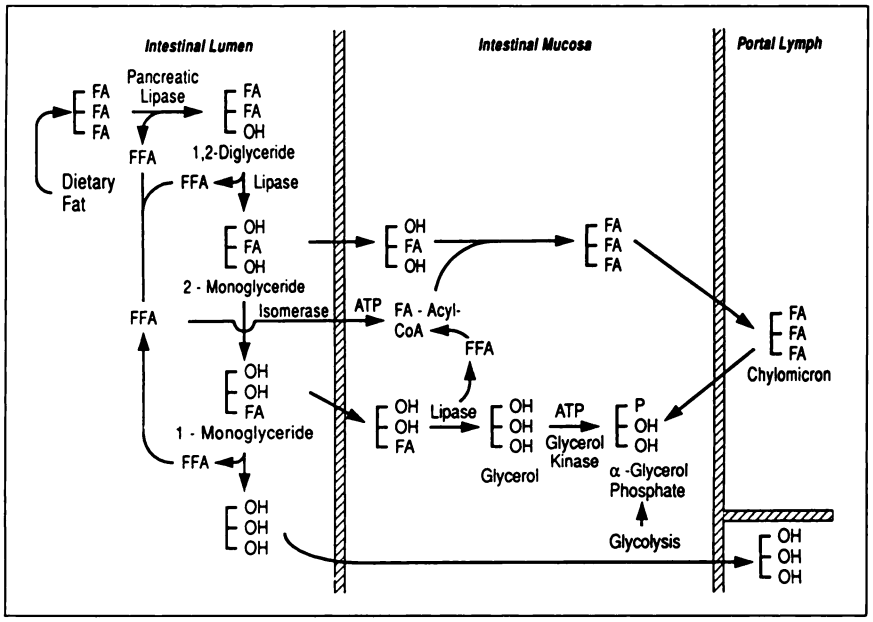

FIGURE 2. Pathway for intestinal enzymatic cleavage and absorption of ingested triglycerides.

Iodine was introduced by reaction of the 1,2-Pal-3-PPA substrate with thallium(III) trifluoroacetate (TTFA) followed by treatment with potassium iodide (Fig. 3). The triglyceride substrate was insoluble in the usual TTFA/aqueous solution (ratio: 3 ml/0.5 ml) (*23,24*) and also in acetonitrile as an alternative (*25*). Successful iodination was accomplished by minimizing the amount of the aqueous KI (0.10–0.20 ml) in TFAA (3 ml). Hydrolysis was minimized (<5%) by careful control of the temperature (98–102°C) and timing of the iodination step (15 min). The 1,2-Pal-3-IPPA prepared in this manner (Fig. 3) was identical to authentic iodinated 1,2-Pal-3-IPPA prepared by esterification of IPPA. The analyses of the structures of the synthetic products are described in the Appendix.

Preparation of Radioiodinated 1,2-Pal-3-IPPA Triglyceride

The final yield of the radiolabeling procedure for 1,2-Pal-3-IPPA was 50%–65% with a purity greater than 99.5%. Storage conditions for the radioiodinated 1,2-Pal-3-IPPA were studied in detail and samples could be stored in the cold (8°C) either as solids or as a precipitate in ethanol for at least 4 wk with no decomposition.

Rat Tissue Distribution and Excretion Studies

For the dual-label studies, [^{125}I]1,2-Pal-3-IPPA and ^{131}I-IPPA were combined and orally administered to rats. It was expected that the free ^{131}I-IPPA would be directly absorbed with subsequent reesterification and further metabolism, whereas ^{125}I-IPPA must first be released from the triglyceride by hydrolysis prior to absorption and reesterification. Since the metabolism of both tracers is the same after adsorption, excretion results should thus be similar for both tracers. Rapid and high urinary excretion of both tracers was observed in the first two days (Table 1), followed by low excretion of radioactivity. The mean cumulative values are summarized in Table 1. Significantly more radioactivity was excreted in the urine when compared to the feces. Urine samples and the chloroform extracts of aliquots hydrolyzed with H_2SO_4 were also analyzed by TLC (Table 2).

Rats were also killed at various time points to determine the distribution of radioactivity in fat samples and various tissues. These data are expressed as percent injected dose/gram (%ID/g) of tissue for the two tracers in Table 3. Fat samples were homogenized and extracted by the Folch technique and analyzed by TLC (Table 2).

The results demonstrate no significant accumulation of radioactivity in any of the tissues evaluated and low thyroid uptake, indicating low deiodination of both tracers. TLC analysis of the 24-hr urine demonstrated the presence of a major radioactive component with the mobility of authentic hippuric acid (R_f 0.45, $CHCl_3$:HOAc, 2:1; Table 2). The remainder of radioactivity on the TLC plates consisted of polar activity at the origin. The organic extracts of acid hydrolyzed urine samples were also analyzed by TLC and showed the presence of a major radioactive component chromatographing in the region of short-chain free acids (R_f 0.55, 6% MeOH in $CHCl_3$). Fat samples were extracted by the Folch technique and the organic soluble products analyzed by TLC, demonstrating the presence of a radioactive component (Table 2) with the mobility of the triglyceride standard (R_f 0.55, 6% MeOH in $CHCl_3$). The remainder of the radioactivity on the plate was found at the origin and is expected to represent phospholipids.

DISCUSSION

Iodine-131-labeled triolein, which became commercially available in the 1960s, held great promise as a diagnostic tool and was used by many investigators. Interpretation of the results varied between investigators, however, and questions arose concerning the radiochemical purity and stability of these preparations. Some investigators found that only 30%–60% of the radioactivity in commercial ^{131}I-

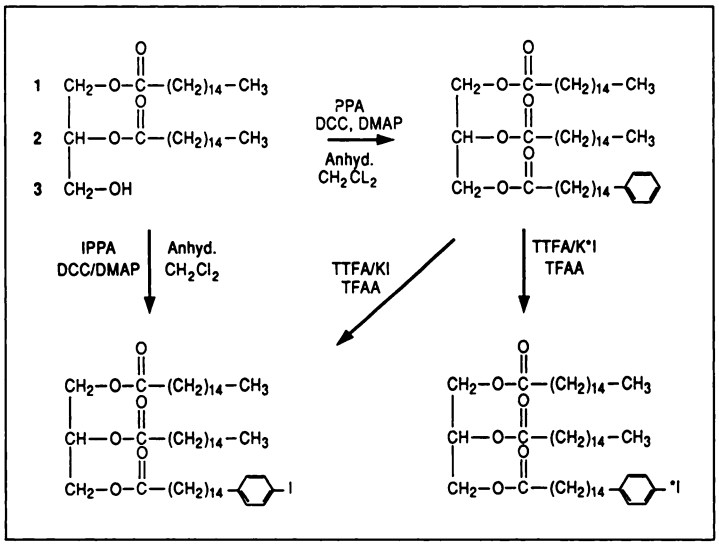

FIGURE 3. Chemical synthesis of 1,2-palmitoyl-3-[15-(p-iodophenyl)pentadecan-1-oyl]-rac-glycerol (1,2-Pal-3-IPPA, 3).

"triolein" preparations was associated with the "triolein" fraction and suggested the use of purified preparations (*26,27*), but other investigators found no benefit in the use of "purified ^{131}I-triolein" (*28*). In addition, investigators disagreed on the utility of blood analysis versus fecal analysis (*1*). Evaluation of radioactivity in the stool was found to be of no value because of the wide overlap between data from patients with proven steatorrhea and controls (*29*). In addition to being very unpleasant, accurate stool analysis is also difficult because of incomplete fecal recovery and possible contamination with urine. On the other hand, the results of blood analysis were evidently difficult to interpret since radioactivity in the blood represents the presence of not only ^{131}I-labeled triglycerides but also radioiodinated free "oleic acid" and free iodide (*2*).

Another approach which was developed involved analysis of either stool ^{14}C content or expired $^{14}CO_2$ after ingestion of triglyceride substrates radiolabeled in the carboxyl groups of the fatty acid moieties (*30,31*). Many investigators felt this method was superior to the use of ^{131}I-triolein, but analysis of the beta-emitting ^{14}C requires special sample preparation and counting equipment, and this approach can also be inconvenient for fecal analysis. For the evaluation of expired $^{14}CO_2$, a special apparatus for collection of the expirate is also required.

We have thus reassessed the use of radioiodinated triglycerides using a simple urine analysis by the "site-specific," stable attachment of radioiodide to a new triglyceride agent. Since pancreatic lipase (LPS, triacylglycerol lipase, EC 3.1.1.3) is specific for hydrolysis of the 1- and 3-acyl positions, the fatty acid released would be absorbed, reesterified and transported to the liver for storage and other tissues to be available to action of peripheral tissue lipase. The metabolism of the IPPA is well understood (*32*). The final catabolic products from beta-oxidation of IPPA are short-chain acids such as p-iodobenzoic acid (IBA) and (p-iodophenyl)propionic- and propenoic acids.

In contrast with the oxidation of "iodooleic acid," radioiodide may be expected to be readily lost from the intermediary products (3-iodo-4-chlorododecanoic acid, 3-chloro-4-iodododecanoic acid isomers). The catabolism of the so-called "iodooleic acid," presumably a mixture of the 9-iodo-10-chloro- and 9-chloro-10-iodo- isomers of octadodecanoic (stearic) acid (Fig. 1), thus results in the formation of free radioiodide (*1*).

It would also be expected that iodine monochloride (I-Cl) addition to triolein would result in a heterogeneously radiolabeled product; that is, the fatty acid moieties attached to the 1 and 3 positions, for instance, might well have a different specific activity than the 2 position. Thus, different preparations would probably be expected to have different degrees of inhomogeneous radiolabeling and this might explain to some extent the inconsistent results comparing data from different patients using different batches of ^{131}I-labeled triolein. The use of a site-specific radiolabeled triglyceride, where radioiodine is stable to facile deiodination, would overcome these problems. The 1,2-Pal-3-IPPA agent described here also has a well-defined urinary excretion of the final catabolite and may be useful for human testing.

In conclusion, these studies have clearly demonstrated that a significant level of radioactivity is rapidly excreted in the urine after oral administration of the ^{131}I-labeled 1,2-Pal-3-IPPA triglyceride. Future studies will evaluate the relative urinary excretion from normal controls and patients with established pancreatic disease to determine if this agent will be useful for broader human trials.

APPENDIX

1,2-Palmitoyl-3-[15-(p-iodophenyl)pentadecan-3-oyl]-rac-glycerol (1,2-Pal-3-PPA, *2*)

Preparation of triglycerides was conducted under a dry, nitrogen atmosphere as described earlier (*18,19*). Dicyclohexylcarbodiimide (DDC, 41 mg, 0.2 mmole) was added to a dry dichloromethane solution (3 ml) containing 1,2-palmitoyl-rac-glycerol (1, 45 mg, 0.08 mmole), dimethylaminopyridine (DMAP, 10 mg, 0.08 mmole) and 15-phenylpen-

tadecanoic acid (PPA, 32 mg, 0.10 mmole). The solution was stirred in the dark overnight at room temperature. The insoluble urea byproduct was filtered through a glass wool plug and the filtrate diluted with dichloromethane (50 ml) and washed with 10% HCl, saturated $NaHCO_3$ solution and water. After drying over Na_2SO_4 and evaporation of the solvent, the solid residue was dissolved in 10% ethyl ether in petroleum ether and applied to a silicic acid column (15 g, 1.5 cm diameter, Sigma SIL-A-200) prepared in the same solvent. Fractions (8 ml) were eluted with 10% ether/PE. Aliquots of each fraction were monitored by TLC (SiO_2-G, solvent benzene; 1,2-Pal-3-IPPA, R_f = 0.55–0.65; PPA, R_f = 0.20–0.25). Fractions 6–10 contained the product and were combined and evaporated to dryness. Crystallization from ethyl ether at 10°C provided pure 1,2-pal-3-IPPA (*2*), 36.3 mg (52%), MP 57°C; ^1H NMR ($CDCl_3$) δ 0.875 (t, $-CH_3$, 6H), 1.25 (bs, CH_2, 86H), 1.61 (m, 16H), 2.31 (t, 8H), 2.59 (t, 2H), 4.18 (m, 5H), 5.26 (m, 1 H) and 7.19 (t, 3H); ^{13}C NMR consistent with proposed structure; MS ion at m/z 868 (M^+); analysis: calculated for $C_{56}H_{100}O_6$: C, 77.36%; H, 11.59%; found: C, 77.26%; H, 11.37%.

1,2-Palmitoyl-3-[15-(p-iodophenyl)pentadecan-1-oyl]-rac-glycerol (1,2-Pal-3-IPPA, (*3*)

From 1,2-Pal-rac-Glycerol. As described above for the preparation of 1,2-Pal-3-PPA, authentic 15-(p-iodophenyl-)pentadecanoic acid (IPPA, 44 mg, 0.10 mmole) was reacted with 1,2-Pal-rac-glycerol (*1*, 45 mg, 0.08 mmole) and the product purified by column chromatography. Crystallization from ether at 10°C provided pure (*3*), 57.3 mg (72%), MP 57°C; ^1H NMR ($CDCl_3$) δ 0.879 (t, 6H), 1.25 (bs, 90H), 1.58 (bs, 6H), 2.31 (t, 6H), 2.53 (t, 2H), 4.22 (o, 5H), 5.27 (m, 1H) and 7.25 (A_2B_2, 4H); ^{13}C NMR consistent with the proposed structure; MS ion at m/z 994 (M^+); analysis: calculated for $C_{56}H_{99}O_6I$: C, 67.58%; H, 10.03; found: C, 67.77%; H, 9.88%.

From 1,2-Pal-3-PPA. The 1,2-Pal-3-PPA substrate (*2*, 34.7 mg, 0.04 mmole) was combined with thallium(III)trifluoroacetate (43.2 mg, 0.08 mmole) in trifluoroacetic acid (TFFA, 3 ml) under red lights. After storing overnight, an aqueous KI solution (6.64 mg, 0.04 mmole, 0.65 ml, 10 mg/ml) was added and heated in the dark for 15 min at 98–102°C. After cooling, the solution was poured into a dilute solution (10%) of sodium bisulfite and extracted with ethyl ether. The organic layer was washed with bisulfite solution and water, dried over Na_2SO_4 and the solvent evaporated under argon or nitrogen. The product was dissolved in 5 ml of 10% ethyl ether in petroleum ether and applied to a silicic acid column (15 g, 1.5 cm diameter, Sigma SILA-200) prepared in the same solvent. Fractions (10–15 ml) were eluted with the 10% ether/PE and Fractions 3–6 were combined and evaporated to dryness. The product was crystallized from ethyl ether in the cold to provide 12.3 mg of (*3*), identical by TLC, to the product prepared by reaction of IPPA with 1,2-pal-rac-glycerol (vide ante).

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(continued from page 907)
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SELF-STUDY TEST
Skeletal Nuclear Medicine

QUESTIONS *(continued)*

Figures 4, 5 and 6 illustrate radiographs of the lumbar spine and bone mineral images obtained with a dual-photon bone mineral analyzer. For each figure (items 8-10), select the best description or interpretation of the findings (options A-E).

A. Machine-produced artifact is apparent in this study.

B. Aortic calcification, hypertrophic changes in the facet joints and wedging of L2 result in mildly inhomogeneous bone mineral distribution. A smaller than usual region of interest should be used.

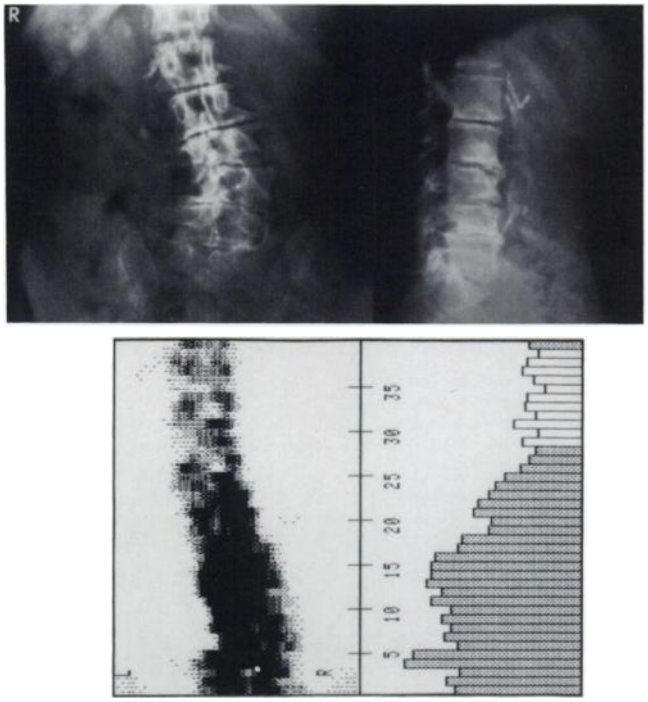

Figure 4

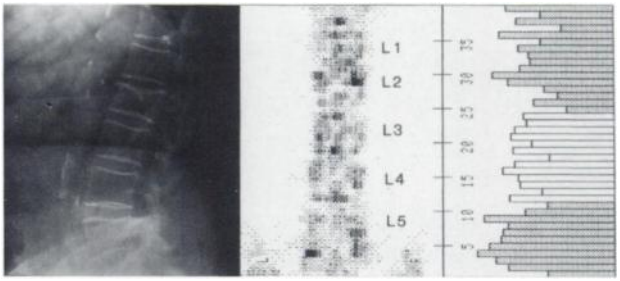

Figure 5

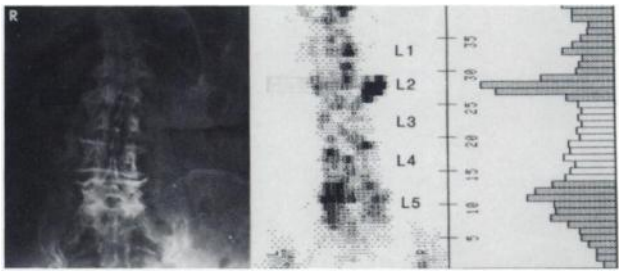

Figure 6

C. Significant scoliosis and post-traumatic changes are present in the lumbar spine. If bone mass assessment is necessary, it should be performed at the hip or a peripheral site, such as the radius or calcaneus.

D. Major degenerative changes are present in the lumbar spine. A region of interest smaller than the standard L2 to L4 region should be used.

E. There is evidence for reduced bone mineral density in the lumbar spine. The findings are more likely due to osteomalacia than to osteoporosis.

8. Figure 4
9. Figure 5
10. Figure 6

SELF-STUDY TEST
Skeletal Nuclear Medicine

ANSWERS

Items 1-4: Bone-Seeking Radiopharmaceuticals
Answers: 1, B; 2, D; 3, B; 4, A

The phosphate moieties in the condensed polyphosphates (including pyrophosphate) and the diphosphonates provide oxygen atoms, which allow binding to calcium atoms in hydroxyapatite. The exact nature of their chemical binding (and that of the associated technetium) to bone crystal has not been elucidated. The fluoride ion exchanges for hydroxyl groups in hydroxyapatite because of similarities in charge and size of this monovalent anion with those of the hydroxyl ion. Strontium is in Group II of the Periodic Table, along with calcium, and radionuclides of strontium, as well as those of barium and radium, are capable of substituting for the divalent calcium cation in hydroxyapatite crystals.

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(continued on page 967)