

32), 256 ($M^+ - C_8H_{16}I - OH$, 10). Anal. ($C_{16}H_{31}O_2ITe$) C, H. Method B

The ditelluride **21** (2.84 mg, 0.5 mmol) and 1,8-diiodooctane (1.5 g, 4 mmol) were allowed to react according to the procedure for **29**. The crude product was crystallized from petroleum ether (30–60 °C) to afford 71 mg (20%) of **26**, mp 60–62 °C.

17- $[^{131}I]$ Iodo-9-telluraheptadecanoic Acid. Sodium $[^{131}I]$ -iodide (4.87 mCi, 15 mg, 0.1 mmol) and **29** (46 mg, 0.1 mmol) were refluxed in 15 mL of acetone as described for **25**. The dried ether extracts were concentrated by a stream of argon to give ^{131}I -labeled **26** (1.52 mCi, 30%). The radiochemical and chemical purity were confirmed by TLC (SiO_2 -GF) in MeOH- $CHCl_3$ (8:92), R_f 0.50.

Bis(methoxyoctanoyl) $[^{123m}Te]$ Ditelluride (21**).** Tellurium-123m metal (4.5 mCi, 63 mg, 0.5 mmol), sodium hydride (22 mg, 0.55 mmol), and methyl 9-bromooctanoate (130 mg, 0.55 mmol) were allowed to react as described for **21**. The $CHCl_3$ fractions were concentrated by a stream of argon to give ^{123m}Te -labeled **21** (400 μ Ci, 9%). The radiochemical and chemical purity were confirmed by TLC (SiO_2 -GF) in $CHCl_3$ (R_f 0.56).

17-Iodo-9- $[^{123m}Te]$ telluraheptadecanoic Acid. A mixture of ^{123m}Te -labeled **21** (400 μ Ci, 13 mg, 0.025 mmol) and 1,8-diiodooctane (1.0 g, 3.33 mmol) were allowed to react according to the procedure for **29**. The dried ether extracts were concentrated by a stream of argon to give ^{123m}Te -labeled **26** (165 μ Ci, 41%).

The radiochemical and chemical purity were confirmed by TLC (SiO_2 -GF) in MeOH- $CHCl_3$ (8:92), R_f 0.50.

16- $[^{131}I]$ Iodoheptadecanoic Acid.¹⁶ Sodium $[^{131}I]$ iodide (3.5 mCi, 15 mg, 0.1 mmol) and 18-bromohexadecanoic acid (35 mg, 0.1 mmol) were refluxed in 15 mL of acetone according to the procedure for **25**. The dried ether extracts were concentrated by a stream of argon to give 16- $[^{131}I]$ iodohexadecanoic acid (2.46 mCi, 70%). The chemical and radiochemical purity were confirmed by TLC (SiO_2 -GF) in MeOH- $CHCl_3$ (8:92), R_f (0.50).

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Potential Organ- or Tumor-Imaging Agents. 22. Acyl-Labeled Cholesterol Esters¹

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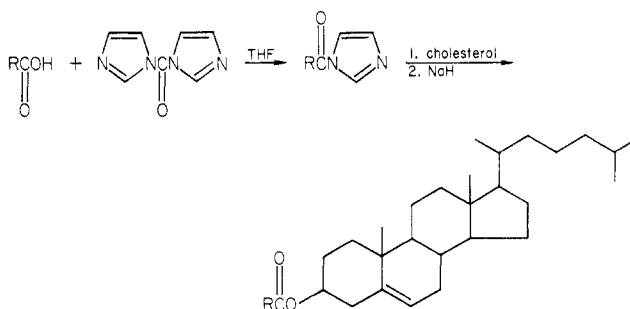
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A series of cholesteryl phenylalkanoic esters was synthesized in which the acyl moiety served as the carrier for radioiodine. Tissue distribution studies in rats revealed that several of these radioiodinated esters selectively accumulated in steroid-secreting tissues, such as the adrenal cortex and ovary. Furthermore, this selective uptake was shown to correlate with the stability of these esters to *in vivo* hydrolysis. An unexpected finding was the unusually high propensity of some of these esters to localize in the ovary and thus afford a possible approach to ovarian imaging agents.

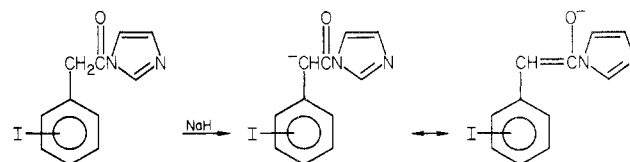
Cholesterol is the essential precursor for the biosynthesis of the steroid hormones. In the steroid-secreting endocrine glands, such as the adrenals and gonads, the three major sources of cholesterol are (1) the circulation, (2) intracellularly stored cholesterol ester, and (3) intracellular *de novo* synthesis from acetate (Figure 1). Moreover, recent studies have demonstrated that under normal circumstances lipoprotein-carried cholesterol derived from the plasma represents the major substrate for steroidogenesis in the adrenal^{2,3} and ovaries.^{4,5}

Radioiodinated derivatives of cholesterol⁶⁻⁸ have been

Scheme I. Synthesis of Cholesteryl Iodophenylalkanoates



Scheme II. Action of NaH on Iodophenylacetates



widely used as imaging agents for the diagnosis of a variety of human adrenal disorders.⁹ Animal studies have revealed that 19-radioiodinated cholesterol rapidly becomes associated with plasma lipoproteins following intravenous

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Table I. Iodophenylalkanoic Esters of Cholesterol

acyl group	no.	recrystn solvent	mp, °C	formula ^a	yield, ^b %
2,3,5-triiodobenzoyl	1	acetone	199–200.5	C ₃₄ H ₄₉ I ₃ O ₂	66.9
3,4,5-triiodobenzoyl	2	acetone	179–181	C ₃₄ H ₄₇ I ₃ O ₂	69.3
2-iodobenzoyl	3	EtOH/H ₂ O	92–94	C ₃₄ H ₄₉ IO ₂	72.5
3-iodobenzoyl	4	EtOH/H ₂ O	125–127.5	C ₃₄ H ₄₉ IO ₂	72.9
4-iodobenzoyl	5	EtOH/H ₂ O	181–182	C ₃₄ H ₄₉ IO ₂	68.6
2-iodophenylacetyl	6	EtOH/H ₂ O	124.5–125	C ₃₅ H ₅₁ IO ₂	36.6
3-iodophenylacetyl	7	EtOH/H ₂ O	91.5–92.5	C ₃₅ H ₅₁ IO ₂	25.7
4-iodophenylacetyl	8	EtOH/H ₂ O	124.5–125	C ₃₅ H ₅₁ IO ₂	32.5
4-iodophenylpropanoyl	9	EtOH/H ₂ O	130–131	C ₃₆ H ₅₃ IO ₂	54.1
4-iodophenylbutanoyl	10	EtOH/H ₂ O	113–114	C ₃₇ H ₅₅ IO ₂	73.9

^a Satisfactory C, H, and I analyses ($\pm 0.4\%$) were obtained for all compounds. ^b Yield of recrystallized material based on starting acid.

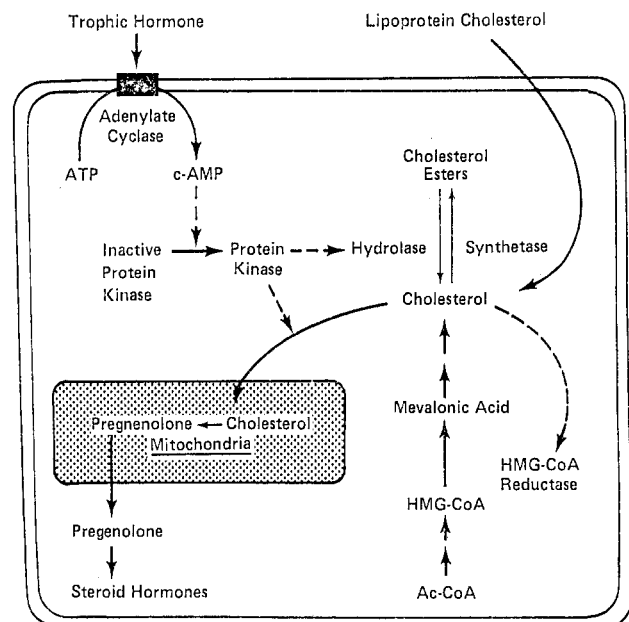


Figure 1. Sources of cholesterol for hormone synthesis in steroid-secreting tissues.

administration and that uptake by steroid-secreting tissues can be related to a lipoprotein receptor-mediated process similar to that shown for cholesterol itself.¹⁰ Since lipoprotein cholesterol is predominantly in an esterified form, it became of interest to investigate radioiodinated cholesterol esters and especially those esters in which the label was associated with the acyl moiety.

In a recent communication,¹¹ we reported that cholesteryl 2,3,5-tri[¹²⁵I]iodobenzoate (1) not only localized in adrenal cortical tissue but also showed an even greater propensity to accumulate in the ovary. The unavailability of a satisfactory diagnostic agent for the noninvasive visualization of the ovary prompted continuation of these studies in order to ascertain the structural features important for the site-specific delivery of such agents to the ovary. In this paper, we report our findings for a series of radioiodinated phenylalkanoic esters of cholesterol.

The iodophenylalkanoic esters were prepared by means of the imidazolidine as shown in Scheme I. As indicated in Table I, the use of 1,1'-carbonyldiimidazole¹² as the activating agent for acylation gave very satisfactory results.

An exception was the synthesis of the iodophenylacetates 6–8. A possible explanation for the poor yields obtained for these esters is offered in Scheme II. The benzylic protons of the iodophenylacetates are activated to removal by strong base by both the carbonyl group and the aromatic ring. The resulting resonance-stabilized anion effectively removes the imidazolidine for acylating purposes.

All of the cholesteryl phenylalkanoates were radiolabeled with iodine-125 for in vivo tissue distribution studies. This was accomplished by isotopic exchange with Na¹²⁵I under a variety of conditions. For compounds 5 and 7, the exchange was performed in a melt of the compound itself in the absence of any solvent.¹³ While this procedure afforded sufficient radiolabeled material for animal studies, it proved to be unsatisfactory in terms of percent incorporation of radioiodine. Better incorporation was achieved when acetamide was used as the exchange medium.¹⁴ This latter method replaced the more tedious and time-consuming procedure originally employed for the radioiodination of 1, which involved radiolabeling of the acid followed by acylation of cholesterol.¹¹ All radioiodinated products were purified by chromatography, and the purity was established by TLC in at least two different solvent systems.

Female Sprague-Dawley rats were employed for the in vivo tissue distribution studies. The results of these studies are summarized in Table II. As anticipated, a number of the radioiodinated esters were found to concentrate in the adrenal cortex and ovary. This was especially true for compounds 1–4 and 6. Moreover, except for 6, the concentration of radioactivity in the ovary exceeded that for the adrenal cortex. In general, the greatest propensity to localize in the steroid-secreting tissues was shown by the benzoate esters (e.g., 3 vs. 6 and 4 vs. 7) and when the iodine atom was ortho to the carboxyl (e.g., 6 vs. 7 and 8). Polyiodination of the aromatic ring did not appear to hamper the ability of the benzoate esters (e.g., 1 and 2) to concentrate in steroid-secreting tissues. Extension of the alkanoate chain resulted in a decline in ovarian uptake (e.g., 5 vs. 8, 9, and 10).

These results suggested that the ability of these sterol esters to achieve selective localization in steroid-secreting tissues may be related to their in vivo stability. Lipid extraction of the 24-h plasma samples and TLC analysis of the extract revealed the amount of radioactivity extractable into CHCl₃/CH₃OH and the percent remaining as cholesterol ester. Table III summarizes these results. With the possible exception of 7, these results indicate a possible correlation between in vivo ester stability and capacity to localize in steroid-secreting target tissues. At

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Table II. Distribution of Radioactivity 24 h After Intravenous Administration of ^{125}I -Labeled Cholesteryl Esters to Rats

no.	n	% dose/g of tissue ^a								
		adrenal cortex	blood	heart	liver	lung	ovary	plasma	spleen	thyroid
1	4	9.03 (1.85)	0.57 (0.06)		5.11 (0.22)	0.70 (0.04)	16.19 (0.78)	1.02 (0.16)	1.68 (0.09)	2.63 (0.47)
2	5	7.48 (0.91)	0.54 (0.05)	0.20 (0.02)	4.80 (0.19)	0.97 (0.07)	12.53 (0.85)	1.01 (0.09)	2.98 (0.22)	6.06 (0.57)
3	3	14.67 (1.47)	0.44 (0.05)	0.21 (0.02)	5.18 (0.20)	0.94 (0.14)	13.68 (1.77)	0.80 (0.09)	1.89 (0.14)	1.96 (0.17)
4	4	12.76 (0.71)	0.30 (0.02)	0.16 (0.01)	5.03 (0.43)	0.46 (0.01)	15.70 (0.86)	0.55 (0.05)	1.86 (0.08)	7.30 (0.58)
5	4	3.86 (0.18)	0.20 (0.01)	0.11 (0.01)	3.28 (0.08)	0.94 (0.10)	6.21 (0.34)	0.36 (0.01)	1.49 (0.06)	6.92 (0.76)
6	4	9.19 (0.72)	0.21 (0.01)	0.15 (0.01)	3.99 (0.21)	0.43 (0.04)	5.97 (0.55)	0.29 (0.02)	1.31 (0.06)	46.31 (5.70)
7	4	3.40 (0.22)	0.14 (0.01)	0.11 (0.01)	2.11 (0.12)	0.27 (0.01)	5.05 (0.07)	0.24 (0.01)	0.77 (0.02)	32.62 (5.67)
8	4	3.29 (0.25)	1.02 (0.08)	0.33 (0.02)	1.25 (0.15)	0.65 (0.07)	2.32 (0.40)	1.88 (0.14)	0.50 (0.04)	27.09 (5.49)
9	4	3.56 (0.30)	0.13 (0.01)	0.08 (0.01)	0.42 (0.07)	0.50 (0.07)	1.35 (0.17)	0.16 (0.01)	0.51 (0.16)	67.52 (11.20)
10	5	1.94 (0.13)	0.87 (0.05)	0.25 (0.02)	0.36 (0.03)	0.40 (0.02)	3.23 (0.21)	1.51 (0.10)	0.16 (0.01)	30.09 (3.32)

^a Numbers in parentheses are standard errors of the means.**Table III.** Results of TLC Analysis of Lipid-Soluble Radioactivity from Plasma of Rats 24 h After Intravenous Administration of Acyl-Labeled Cholesteryl Phenylalkanoates

no.	% $\text{CHCl}_3/\text{CH}_3\text{OH}$ extractable	% ester ^a
1	81.8 (1.9) ^b	81.8 (2.9) ^b
2	89.7 (1.6)	97.2 (0.3)
3	87.8 (0.3)	94.2 (0.8)
4	82.8 (3.5)	97.2 (1.0)
5	54.0 (2.8)	93.4 (1.0)
6	72.7 (5.3)	73.0 (3.5)
7	72.6 (1.4)	82.4 (3.0)
8	23.1 (7.7)	46.1 (3.7)
9	57.1 (1.6)	62.3 (1.4)
10	9.3 (1.0)	60.9 (2.6)

^a TLC analysis in petroleum ether/ethyl ether (7:2, v/v). ^b Number in parentheses are standard errors of the means for at least three animals.

this time, it is not known whether this stability is related to resistance to deiodination or to ester hydrolysis.

The important finding of this study is that certain cholesteryl esters localize in ovarian tissue without prior hydrolysis. This now offers the possibility of affixing the diagnostic nuclide to the acyl moiety rather than the more chemically inaccessible steroid nucleus. This propensity for ovarian uptake was observed earlier with 19-radioiodinated cholesteryl oleate¹⁵ but is a distinction from 19-radioiodinated cholesterol, which shows a more than twofold greater concentration of radioactivity in the adrenal cortex than the ovary of rats at 24 h. These findings suggest that these cholesterol esters may be participating in some carrier-mediated process which is somewhat specific for ovarian localization. We are continuing to examine this possibility in our laboratory.

Experimental Section

Infrared spectra (IR) were determined on a Perkin-Elmer 281 spectrophotometer. Compounds were analyzed as KBr pellets or in CHCl_3 solution. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian EM360A spectrophotometer on com-

pounds dissolved in CDCl_3 , using tetramethylsilane as the internal standard. Melting points were done on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, or Midwest Microlab, Ltd., Indianapolis, IN. Thin-layer chromatography (TLC) was done with Eastman polyethylene-backed silica gel plates with fluorescent indicator. TLCs of radiolabeled compounds were scanned with a Vanguard 930 autoscanner. Column chromatography was done on silica gel from Grace, Davison Chemical, Baltimore, MD. THF was dried over LiAlH_4 and freshly distilled before use. Cholesterol and other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. Tween 20 was obtained from Sigma Chemical Co., St. Louis, MO. Rats were obtained from Spartan Research Animals Inc., Haslett, MI.

General Procedure for Esterification with 1,1'-Carbonyldiimidazole.¹² The acid (1.0×10^{-3} mol, 1.0 equiv) and 1,1'-carbonyldiimidazole (0.1703 g, 1.05×10^{-3} mol, 1.05 equiv) were dissolved in THF (5 mL) and heated at reflux for 15 min. The sterol (1.1×10^{-3} mol; 1.1 equiv) was added, followed by a spatula-tip full of NaH, and the reaction was stirred at room temperature for 1 h. Workup consisted of removing the solvent with a rotary evaporator, then careful addition of H_2O (10 mL), and extraction with benzene. The benzene extracts were combined, the solvent was removed, and the residue was chromatographed on a silica gel column with benzene or a hexane/ethyl acetate gradient as the eluent. Yields, recrystallization solvents, and melting points are given in Table I.

Cholesteryl 2,3,5-triiodobenzoate (1): IR (KBr) 1726, 1701 cm^{-1} (ca. equal); IR (CHCl_3) 1723 cm^{-1} ; NMR δ 4.85 (m, 3 H), 5.45 (m, C-6 vinyl H), 7.73 (d, $J = 3$ Hz, Ar 4-H), 8.32 (d, Ar 5-H).

Cholesteryl 3,4,5-triiodobenzoate (2): IR (KBr) 1722, 1710 cm^{-1} ; NMR δ 4.85 (m, 3 H), 5.45 (m, C-6 vinyl H), 8.50 (s, Ar H).

Cholesteryl 2-iodobenzoate (3): IR (KBr) 1733, 1718 cm^{-1} ; IR (CHCl_3) 1725 cm^{-1} ; NMR δ 4.85 (m, 3 H), 5.45 (m, C-6 vinyl H), 7.95–8.05 (m, Ar H).

Cholesteryl 3-iodobenzoate (4): IR (KBr) 1725 cm^{-1} ; NMR δ 4.85 (m, 3 H), 5.45 (m, C-6 vinyl H), 7.2 (t, $J = 7.5$ Hz, Ar 5-H), 7.95 (t, Ar 4,6- H_2), 8.4 (m, Ar 2-H).

Cholesteryl 4-iodobenzoate (5): IR (KBr) 1712 cm^{-1} ; NMR δ 4.9 (m, 3 H), 5.6 (m, C-6 vinyl H), 7.7 (s, Ar H).

Cholesteryl 2-(2-iodophenyl)acetate (6): IR (KBr) 1740 cm^{-1} ; NMR δ 3.55 (s, CH_2), 4.85 (m, 3 H), 5.35 (m, C-6 vinyl H), 6.9 (m, Ar 6-H), 7.25 (d, $J = 8$ Hz, Ar 4,5- H_2), 7.8 (d, Ar 3-H).

Cholesteryl 2-(3-iodophenyl)acetate (7): IR (KBr) 1735 cm^{-1} ; NMR δ 3.55 (s, CH_2), 4.6 (m, 3 H), 5.45 (m, C-6 vinyl H), 7.0 (m, Ar H).

Cholesteryl 2-(4-iodophenyl)acetate (8): IR (KBr) 1730 cm^{-1} ; NMR δ 3.55 (s, CH_2), 4.55 (m, 3 H), 5.35 (m, C-6 vinyl H), 7.05

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(d, $J = 9$ Hz, Ar 2,6- H_2), 7.7 (d, Ar 3,5- H_2).

Cholesteryl 3-(4-iodophenyl)propanoate (9): IR (KBr) 1730 cm^{-1} ; NMR δ 4.6 (m, 3 H), 5.35 (m, C-6 vinyl H), 6.95 (d, $J = 7$ Hz, Ar 2,6- H_2), 7.6 (d, Ar 3,5- H_2).

Cholesteryl 4-(4-iodophenyl)butanoate (10): IR (KBr) 1730 cm^{-1} ; NMR δ 4.55 (m, 3 H), 5.35 (m, C-6 vinyl H), 6.9 (d, $J = 7$ Hz, Ar 2,6- H_2), 7.6 (d, Ar 3,5- H_2).

Compounds Exchanged Without Solvent.¹³ The ester (0.005–0.01 g) was placed in acetone (1 mL). Na^{125}I (1–5 mCi) was added. The solvent was removed with a gentle stream of nitrogen, and then the reaction flask was heated to 180 °C (7) or 200 °C (5). After the reaction flask cooled, the residue was dissolved in CCl_4 , washed with H_2O , and chromatographed on a silica gel column with CCl_4 as the eluent. Radiochemical purity was established by TLC with unlabeled ester as the standard.

Cholesteryl 4-[^{125}I]iodobenzoate (5'): reaction time 1 h; radiochemical yield 6.8%; specific activity 280.3 $\mu\text{Ci}/\text{mg}$; TLC, R_f 0.66 (benzene), 0.30 (CCl_4).

Cholesteryl 2-(3-[^{125}I]iodophenyl)acetate (7'): reaction time 1.5 h; radiochemical yield 3.5%; specific activity 59.8 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.56 (benzene), 0.34 (CCl_4).

Compounds Exchanged in Acetamide Melt.¹⁴ The ester (0.005 g) and acetamide (0.02 g) were placed in a flask containing 10^{-9} M NH_4Cl (1.0 equiv based on the amount of pH 9 Na^{125}I solution to be added) and 10^{-7} M $\text{Na}_2\text{S}_2\text{O}_7$ (1.3–1.5 equiv based on $^{125}\text{I}^-$ with a specific activity of 2.5×10^9 mCi/mol). Na^{125}I (1.0 to 20 mCi) was added and rinsed in with acetone (0.15 mL). The flask was heated to the exchange temperature under a slight positive nitrogen pressure for approximately 1–1.5 h to evaporate the acetone and H_2O present. After the desired time at the exchange temperature, the flask was allowed to cool. The solid residue was dissolved in benzene, washed with H_2O , and chromatographed on silica gel with CCl_4 , benzene, or a hexane/ethyl acetate gradient. Radiochemical purity was established by TLC with unlabeled ester as the standard. Reaction times, temperatures, and yields are given for each compound as follows.

Cholesteryl 2,3,5-tri[^{125}I]iodobenzoate (1'): 1 h at 195 °C; radiochemical yield 34.7%; specific activity 30.8 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.65 (benzene), 0.43 (CCl_4).

Cholesteryl 3,4,5-tri[^{125}I]iodobenzoate (2'): 1 h at 195 °C; radiochemical yield 16.5%; specific activity 54.0 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.67 (benzene), 0.47 (CCl_4).

Cholesteryl 2-[^{125}I]iodobenzoate (3'): 2 h at 200 °C; radiochemical yield 10.0%; specific activity 75.2 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.69 (benzene), 0.23 (CCl_4).

Cholesteryl 3-[^{125}I]iodobenzoate (4'): 7 h at 200 °C; radiochemical yield 37.2%; specific activity 20.8 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.62 (benzene), 0.34 (CCl_4).

Cholesteryl 2-(2-[^{125}I]iodophenyl)acetate (6'): 1 h at 170 °C; radiochemical yield 10.0%; specific activity 28.0 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.54 (benzene), 0.20 (CCl_4).

Cholesteryl 2-(4-[^{125}I]iodophenyl)acetate (8'): 1 h at 180 °C; radiochemical yield 10.3%; specific activity 95.5 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.57 (benzene), 0.23 (CCl_4).

Cholesteryl 3-(4-[^{125}I]iodophenyl)propanoate (9'): 1 h at 195 °C; radiochemical yield 61.1%; specific activity 436.3 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.63 (benzene), 0.27 (CCl_4).

Cholesteryl 4-(4-[^{125}I]iodophenyl)butanoate (10'): 1 h at 195 °C; radiochemical yield 29.2%; specific activity 65.3 $\mu\text{Ci}/\text{mg}$; TLC R_f 0.56 (benzene), 0.25 (CCl_4).

Tissue Distribution Studies. The radiolabeled compounds were dissolved in benzene. To this solution was added Tween 20 (0.1 mL/mg of compound). The benzene was evaporated under

a stream of nitrogen, and physiological saline was added. Any remaining benzene was removed by a stream of nitrogen until a clear solution (2–3% in Tween) resulted. The radiolabeled compound, thus solubilized, was administered intravenously to adult female Sprague–Dawley rats weighing 200–300 g. Three to five rats were used for each compound, and the dose ranged between 5 and 50 μCi per animal. The rats were killed by exsanguination under ether anesthesia at 24 h, and the major organs were removed and blotted free of excess blood. Large organs were minced with scissors. Weighed samples of tissue were placed in cellulose acetate capsules and counted (84% efficiency) in a well scintillation counter (Searle 1185). The concentration of radioactivity in each tissue was expressed as the percentage of administered dose per gram of tissue, which was calculated as follows:

$$\frac{\text{cpm} - \text{background}/\text{mg of tissue} \times 1000 \times 100}{\text{efficiency} \times 2.2 \times 10^6 \text{ dpm}/\mu\text{Ci} \times \mu\text{Ci dose}}$$

The results are summarized in Table II.

Plasma Extraction. Radioactivity was extracted from plasma samples using a variation of the procedure described by Folch et al.¹⁶ Aliquots of plasma (0.1 mL) were brought up to 0.5 mL with distilled water, and 3 mL of chloroform/methanol (2:1) was added in a ground glass homogenizer. The samples were homogenized and filtered through Whatman No. 1 filter paper. The homogenizers were rinsed twice with 3 mL of $\text{CHCl}_3/\text{CH}_3\text{OH}$, and these rinses were filtered. The combined filtrate from homogenizing and rinsing was brought up to 10 mL with $\text{CHCl}_3/\text{CH}_3\text{OH}$. The filter paper was air-dried, placed in a polystyrene gamma tube, and assayed for radioactivity to ascertain the radioactivity associated with precipitable material (84% efficiency). Each filtrate was placed in a 15-mL conical tube. Distilled water (2.0 mL) was added, and the tube was shaken. The tubes were then centrifuged at low speed for 10 min to separate the phases. The upper phase of each sample was removed and adjusted to 5 mL by the addition of upper phase solvent ($\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}_2\text{O}$, 3:48:47). A 1.0-mL aliquot of the upper phase was placed in a glass culture tube, which was then placed in a polystyrene counting tube and assayed for radioactivity (75% efficiency). Each lower phase was evaporated to dryness under nitrogen at 20–30 °C, and the residues were redissolved in 1.0 mL of benzene. An aliquot (0.1 mL) of each benzene fraction was placed in a glass culture tube, which was then placed in a polystyrene counting tube and assayed for radioactivity (75% efficiency). The remainder of the benzene solution from each sample was stored overnight in a capped vial at 4 °C. The benzene was then evaporated, and the residues were redissolved in diethyl ether (20–25 drops) and spotted on TLC plates. The plates were then developed in petroleum ether/diethyl ether (7:2) for 14.5 cm and air-dried. The plates were cut into 1-cm strips starting 0.5 cm below the origin and continuing to the solvent front. Each strip was placed in a counting tube and assayed for radioactivity. Each unlabeled cholesteryl ester and the free acid of the ester were cochromatographed with the radioactive samples and visualized with iodine vapor to serve as reference standards. The results are summarized in Table III.

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