Tissue Distribution of High-Density Lipoprotein Labeled with Radioiodinated Cholesterol

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This preliminary study was undertaken in order to explore possible methods for enhancing the target organ specificity of radioiodinated cholesterol. 19-[^{125}I]cholesterol (I*C) was rapidly incorporated and transported in high-density lipoproteins (HDL) following i.v. administration to rats, and thus behaved like cholesterol in this regard. Incorporation of I*C into HDL (I*C-HDL) before administration produced little change in the tissue distribution profile except for a reduction in the amount of radioactivity appearing in the thyroid. This suggested that the lipoprotein carrier may have afforded protection from metabolic dehalogenation of I*C. When rats were pretreated with 4-aminopyrazolo-(3,4d)-pyrimidine to drastically reduce the circulating lipoprotein levels, a fourfold enhancement in adrenal uptake was observed following administration of I*C-HDL. This finding was consistent with the current view that the rat adrenal contains high-affinity and saturable receptors for HDL.

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For the past decade radioiodinated derivatives of cholesterol (*1–3*) have proven to be useful imaging agents for the diagnosis of a variety of adrenal disorders in humans (*4,5*). Despite the widespread use of radioiodinated cholesterols as radiodiagnostics, little is known about the metabolic fate of these substances. The purpose of this study, therefore, was to analyze the ability of radioiodinated cholesterol to become incorporated into lipoproteins and to explore the feasibility of using lipoproteins as carriers of radioiodinated cholesterol.

Mainly as a result of the efforts of several groups (*6–8*), our understanding of the mechanisms whereby steroid-secreting tissues obtain and metabolize cholesterol has advanced considerably. According to current concepts, tissues responsible for the biosynthesis of steroid hormones derive their cholesterol from three possible sources, namely: (a) circulating plasma; (b) hydrolysis of intracellular cholesterol esters; and (c) intracellular biosynthesis (Fig. 1). Most animal studies to date indicate that the adrenal cortex relies mainly upon the circulating plasma pool as its source of cholesterol (*9,10*). Once this source becomes depleted, as occurs upon treatment of animals with 4-aminopyrazolo-(3,4d)-pyrimidine (4-APP), the other two pathways are called upon to meet the metabolic needs of the cell for cholesterol.

When 19-radioiodinated cholesterol is administered intravenously to female rats, over 85% of the radioactivity present in the adrenals and ovaries is associated with the cholesterol ester fraction at 96 hr (*11*). Thus, in the rat, 19-radioiodinated cholesterol mimics cholesterol by accumulating in steroid-secreting tissues and becoming esterified. Further studies have revealed, however, that these esterified forms of 19-radioiodinated cholesterol are very poor substrates for the rat's adrenal cholesterol esterase (*12*). Thus the administered radioiodinated cholesterol appears to reach a metabolic dead-end in the rat adrenal by being converted to esters that are only slowly hydrolyzed by the tissue esterases. This feature very likely accounts, at least in part, for the retention of 19-radioiodinated cholesterol by the adrenals. These findings have led us to examine other aspects

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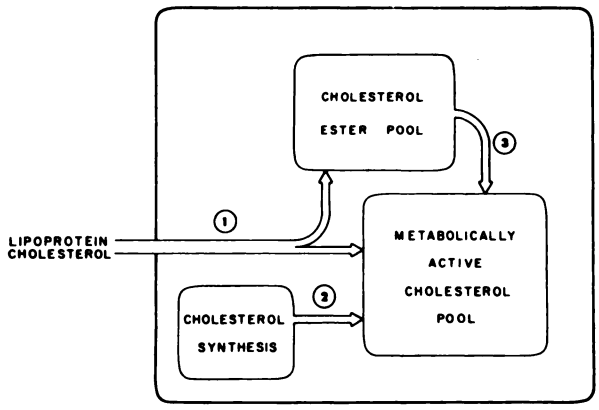

FIG. 1. Cholesterol balance in adrenal and gonadal tissues.

of the metabolic fate of 19-radioiodinated cholesterol in relation to what is currently known about cholesterol metabolism. In particular, we were interested in determining the manner whereby 19-radioiodinated cholesterol is delivered to the tissues secreting steroid hormone, such as the adrenal cortex and ovary.

In animals and man, cholesterol is carried in the blood stream as a complex with plasma lipoproteins. In man, those known as low-density lipoproteins (LDL) carry the major portion of the total blood cholesterol and high-density lipoproteins (HDL) carry most of the remainder. Detailed studies by Brown and Goldstein (*6,9*) have shown that human fibroblasts, as well as a variety of other cell types, obtain their cholesterol by binding LDL to a specific high-affinity receptor site (LDL receptor) on the cell membrane; this is followed by internalization of the intact lipoprotein by adsorptive endocytosis. The uptake of cholesterol by this process is followed by a prompt inhibition of intracellular cholesterol biosynthesis and an enhancement in the conversion of cholesterol to cholesterol esters.

The relevance of these studies to the uptake of cholesterol by steroid-secreting tissues has only recently come under scrutiny. Gwynne et al. (*8*) showed that the uptake of cholesterol into rat adrenal slices was two to three times greater from HDL than from LDL, and that this uptake was stimulated by the administration of ACTH. Andersen and Dietschy (*10*) subsequently demonstrated that infusion of HDL, but not LDL, into 4-APP-treated rats suppressed intracellular cholesterol synthesis, and increased cholesterol-ester content of the adrenals, ovaries, and testes. Conversely, Kovanen et al. (*13*) demonstrated that membranes from bovine adrenal cortex and ovarian corpus luteum have binding sites for LDL, but not HDL. Mouse adrenal gland, on the other hand, appears to have selective uptake processes for both HDL and LDL (*14*).

In light of these observations, it became of interest to examine the role of lipoproteins in the tissue distribution of 19-radioiodinated cholesterol in the rat. This report describes the time course of incorporation of 19-radioiodinated cholesterol into the rat's plasma lipoproteins following i.v. administration, as well as preliminary observation on the tissue distribution of HDL labeled in vitro with 19-radioiodinated cholesterol.

METHODS AND MATERIALS

Radioiodinated cholesterol. 19-[^{125}I]iodocholesterol (I*C) was prepared as previously described (*1*) and stored in benzene at 4°C until used. Radiochemical purity was ascertained by thin layer chromatography (TLC) using silica gel plates† developed in benzene:ethyl acetate (9:1). The single radioactive peak was coincident with the single spot visualized with iodine vapor. For administration to animals, I*C was formulated with the aid of polyoxyethylene sorbitan mono-oleate-Tween-80 (I*C-Tween) or by incorporation into rat HDL (I*C-HDL).

The I*C-Tween solutions were prepared immediately before administration. An aliquot of I*C dissolved in benzene was placed in a preweighed vial. The benzene was evaporated under N_2 and the vial reweighed. The residue was then redissolved in 2 ml of isotonic saline containing 10% ethanol and 1.6% Tween-80.

Incorporation of radioiodinated cholesterol into HDL (I*C-HDL). Lipoprotein fractions were obtained by layering rat plasma under a solution of NaCl and KBr until the final density (d) of the solution was adjusted to d. 1.070 by the method of Havel et al. (*15*). After ultracentrifugation, the very-low-density lipoproteins (VLDL) and LDL were removed by the method of Lindgren (*16*). The residual HDL solution was stored at 4°C under N_2 until needed, but was always used within 24–48 hr after isolation.

I*C was dispersed onto Celite 545 (diatomaceous earth) in a ratio of 1:50 (w/w) using the procedure of Jonas et al. (*17*). A total of 3.213 mCi of I*C with a specific activity of 791.0 μCi/mg was adsorbed onto Celite and the dispersion incubated with the HDL solution for 24 hr in a ratio of 50 mg Celite/ml HDL. Experiments were carried out in 10-ml Erlenmeyer flasks in a Dubnoff shaker incubator at 25°C. I*C-HDL was then centrifuged and separated from any remaining Celite with a Millipore filter (0.45 μm pore size), as described previously (*17*). Aliquots of the I*C-HDL solution were analyzed by polyacrylamide gel electrophoresis (PAGE) and Sepharose-4B column chromatography.

PAGE analysis involved preparation of gels following the methods described by Narayan et al. (*18,19*). The gels consisted of a 3.75% main gel and a 2.5% large-pore stacking gel. An aliquot of 0.025 ml of sample was applied to the gels, which were then run at 2.5 mA/tube until a distance of 1.7 cm into the main gel had been achieved. Gels were run after ultracentrifugation for

qualitative determination of HDL isolation, and also after incubation but before injection, in order to ascertain if any decomposition had taken place. Gels containing radioactivity were sliced in the regions corresponding to HDL, LDL, VLDL, and chylomicron as diagramed by Narayan (*18*). Slices were then placed in polystyrene gamma-counting tubes and assayed for radioactivity in a well scintillation counter, being counted for either 10 min or 1 million counts. Counting efficiency for I-125 was 84%.

Gel filtration columns (1.5 × 30 cm) were packed with Sepharose 4B to a bed height of 27 cm. Aliquots of I*C-HDL were applied and eluted with Tris-HCl buffer, 0.1 *M*, pH 8.0, under a hydrostatic head pressure of 15 cm. Fractions were collected in samples of 1.5 ml and assayed directly for I-125 activity. The peak containing I-125 activity corresponded with a similar peak obtained for HDL assayed for protein content at 280 nm.

The I*C-HDL solution was then dialyzed for 12 hr against 0.02 *M* phosphate buffer, pH 7.4 (containing 0.9% NaCl) 2 × 250 ml, in order to bring the solution closer to isotonicity for injection purposes and to remove any unbound or non-HDL-associated radioactivity. Aliquots of the dialyzed solution were once again analyzed by PAGE and Sepharose-4B chromatography. An aliquot of the I*C-HDL solution was then extracted (*20*) with $CHCl_3$:CH_3OH (2:1 v/v) and the extract was analyzed by TLC in order to determine if any alteration of the I*C occurred as a result of the incorporation procedure.

Tissue distribution studies. Female Sprague-Dawley rats weighing 175–224 g were housed in temperature- and light-controlled quarters with free access to food[‖] and water.

The rats were divided into two groups. One group was untreated and each rat in the other group received a daily i.p. injection of 4-APP[§] (5 mg/ml) in 10 m*M* sodium phosphate at pH 2.5. The dose was 40 mg/kg body weight. At 24 hr after the third injection, the rats were used for tissue distribution studies. To confirm the absence of lipoproteins in the 4-APP-treated rats, blood samples were obtained, before the injection, by puncture of the orbital sinus.

Thirty-five untreated rats were divided into seven groups. Each rat received an i.v. injection of I*C-Tween (23.8–27.1 μCi) in 0.15–0.25 ml of vehicle. The specific activities of the solutions used were 248–600 μCi/mg. One group of rats was killed at each of the following time periods: 0.25, 0.5, 1, 3, 6, 24, and 96 hr after injection.

Six 4-APP-treated rats were divided into two groups. One group received i.v. injections of 10.4 μCi of I*C-HDL. A group of five untreated rats also received the same dose of I*C-HDL. The other group of 4-APP-treated rats received i.v. injections of 31.3 μCi of I*C-Tween in 0.1 ml of vehicle. The specific activity of the solution was 231 μCi/mg. All of these rats were killed at 0.5 hr after injection.

The rats were killed by exsanguination from the heart while under ether anesthesia. The following tissues were removed: adrenal cortex, blood, plasma, liver, ovary, and thyroid. Samples of tissues were placed in preweighed cellulose acetate capsules and weighed. These samples were assayed for radioactivity in a well scintillation counter for 10 min or until 40,000 counts had accumu-

TABLE 1. DISTRIBUTION OF RADIOACTIVITY FOLLOWING I.V. ADMINISTRATION OF I*C-TWEEN TO RATS

Time (hr)	Mean % administered dose/g tissue (range)[†]					
	Adrenal cortex	Blood	Liver	Ovary	Plasma	Thyroid
0.25[‡]	12.604 (11.477–14.859)	3.634 (3.287–3.876)	3.514 (3.279–3.795)	3.272 (2.388–4.159)	5.406 (4.960–5.713)	2.264 (1.294–3.136)
0.5	15.830 (12.216–18.272)	2.710 (2.430–3.080)	5.002 (4.571–5.476)	3.996 (3.193–4.912)	3.643 (2.852–4.744)	5.587 (2.732–9.825)
1	14.785 (11.351–16.163)	2.016 (1.792–2.217)	4.821 (4.450–5.082)	5.008 (2.190–7.069)	1.959 (1.754–2.236)	6.167 (3.745–8.667)
3	11.320 (9.947–12.561)	1.697 (1.578–1.778)	3.568 (2.850–4.642)	7.275 (5.502–9.415)	1.304 (1.251–1.384)	25.180 (17.544–38.958)
6	10.388 (9.201–11.318)	1.385 (1.219–1.558)	2.548 (2.480–2.619)	8.488 (6.509–11.594)	1.136 (0.944–1.319)	49.025 (40.057–63.302)
24	10.195 (9.502–10.958)	0.252 (0.225–0.274)	0.431 (0.366–0.518)	5.929 (4.706–6.832)	—	199.096 (137.238–244.180)
96	9.572 (8.378–10.551)	0.021 (0.015–0.026)	0.059 (0.054–0.069)	2.736 (1.883–3.816)	—	152.042 (116.216–181.420)

[†] Five rats per time period unless noted.

[‡] Four rats.

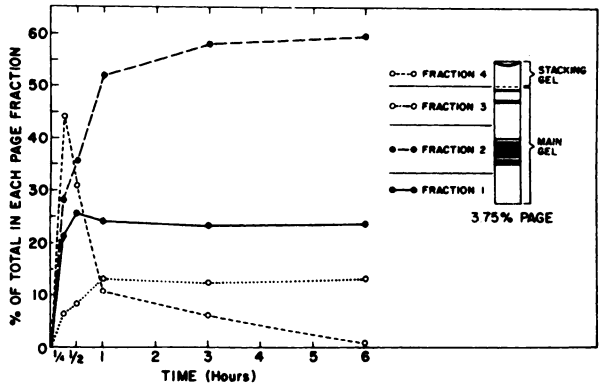

FIG. 2. Distribution of radioactivity in plasma fractions from three rats/time period following i.v. administration of I*C-Tween. Fraction 1, main gel region below albumin; Fraction 2, main gel albumin and HDL region; Fraction 3, main gel LDL and VLDL region; Fraction, 4, stacking gel.

lated. Counting efficiency for I-125 was 84%.

Plasma samples taken after exsanguination were analyzed for lipoprotein radioactivity by method of PAGE. A 0.2-ml sample was mixed with 0.1 ml sudan black (a specific lipoprotein prestain) and let stand for 1 hr, after which 25 μl were applied to 3.75% polyacrylamide gels as described previously. Gels were sliced into four fractions corresponding to the migration and appearance of the various lipoproteins. The four fractions contained the following components (see Fig. 2): Fraction 1, region below albumin (main gel); Fraction 2, albumin, HDL_2, HDL_3, (main gel); Fraction 3, LDL, VLDL (main gel); Fraction 4, chylomicron, unbound I*C (stacking gel). Each fraction was assayed for radioactivity as described above.

RESULTS AND DISCUSSION

Distribution of radioactivity following administration of I*C. Initial studies (Table 1) were performed in untreated rats using I*C-Tween to determine the time interval to use in future studies and also to follow the incorporation of radioactivity into HDL in vivo (Fig. 2).

There was rapid accumulation of radioactivity in the adrenal cortex, with peak levels appearing at 0.5 hr. This was followed by a decline in activity to plateau levels by 6 hr, where it remained for as long as 96 hr after injection. In the ovary, levels of activity were not as high as in the adrenal cortex, and the peak level was not seen until 6 hr.

Tissues such as blood, plasma, and liver initially showed high levels of activity, but they declined more rapidly than those in the target tissues, so that by 24 hr after injection the ratio of adrenal cortex to liver was 24 ± 1 (mean ± s.e.m.), which increased to 164 ± 8 at 96 hr. Adrenal cortex to blood ratio at 24 hr was 41 ± 2, and increased to 479 ± 51 at 96 hr after injection.

Levels of activity in the thyroid were initially the lowest of all tissues sampled, but they increased rapidly so that by 3 hr after injection the thyroid displayed the highest activity. Levels in this tissue continued to rise and only began to show a decline at 96 hr.

Figure 2 illustrates the distribution of radioactivity in the various plasma fractions over the same time course. No attempts were made to resolve Fraction 3 into LDL and VLDL because of the closeness in their migration upon electrophoresis. HDL, Fraction 2, is the major lipoprotein in the rat, and this group was readily separated from the other lipoproteins under the conditions employed.

The levels of activity seen in Fraction 1 (below albumin) remained relatively constant (21–25% of the total in plasma) at all time periods studied. This fraction did not bind sudan black and had no lipoprotein-like properties. Radioactivity was observed within Fraction 2 (HDL) as early as 0.25 hr following administration. The activity in this fraction increased with time, so that by the 1-hr interval it accounted for over 50% of the total. This level was maintained throughout the 6-hr period. A similar profile was noted for Fraction 3 (VLDL and LDL) except that activity in this fraction never exceeded 15% of the total. Although the activity present in Fraction 4 (stacking gel) at 0.25 hr was greater than that in the other three fractions (1.5–7.0 times), activity decreased rapidly such that by 6 hr less than 1% of the plasma activity was associated with this fraction. This rapid drop in activity of Fraction 4 (more than 30% decrease between the 0.25- and 1-hr intervals) was accompanied by a corresponding increase in the activity of Fraction 2 (HDL). The fact that the appearance and disappearance of radioactivity in Fractions 2 and 4 mirror one another suggests that the activity present in Fraction 4 represents injected I*C that becomes incorporated into lipoprotein. When I*C-Tween solution itself was subjected to PAGE analysis, radioactivity appeared predominantly in Fraction 3 (LDL and VLDL)—53.5%, and Fraction 4 (stacking gel)—38.9% with only 7.7% noted in the other two fractions.

Thus, in this study, 19-radioiodinated cholesterol not only mimicked cholesterol with regard to selective concentration in the adrenal, as has been reported previously (*1*), but also in its incorporation into and transport by HDL in the rat (*21*). This differs from the results of Fukushi et al. (*22*) who found, in the Wistar rat, that halogenated cholesterol analogs were bound mainly to LDL and VLDL as opposed to HDL. We are now conducting studies in Wistar rats to determine whether species difference can account for this discrepancy.¶

Incorporation of radioiodinated cholesterol into HDL (in vitro). Based on the above results, studies were undertaken to incorporate 19-radioiodinated cholesterol into HDL. This was accomplished by adopting a procedure used by Jonas et al. (*17*) for the incorporation of cholesterol into HDL. 19-Radioiodinated cholesterol was

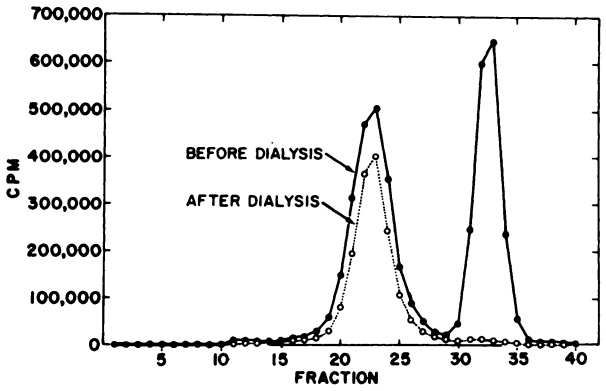

FIG. 3. Sepharose-4B gel filtration analysis of I*C-HDL prepared in vitro.

dispersed onto Celite 545 in a ratio of 1:50 (w/w) sterol:Celite. This ratio was approximately half that recommended by Jonas but twice that used originally by Avigan (*23*).

The HDL employed for the in vitro experiments was isolated from rat plasma according to the method of Havel et al. (*15*). Previous studies have shown that chylomicrons and VLDL are removed at d. 1.006; LDL at d. 1.006–1.063; HDL_2 at d. 1.063–1.120, and HDL_3 from d. 1.120–1.21 (*24*). LDL, however, has been shown to be contaminated by HDL d. 1.040–1.063 (*25,26*). Dudacek and Narayan (*27*) suggest that no lipoprotein at all is present in the range d. 1.040–1.050. Lasser (*21*) has indicated that no contamination with HDL is found below d. 1.050. For these reasons d. 1.070 was chosen to avoid any possible contamination by LDL while sacrificing only small amounts of HDL d. 1.050–1.070.

Incubation of the HDL solution with the I*C-Celite dispersion for 24 hr, followed by filtration, resulted in a net transfer of 7.22% of the total radioactivity to the HDL filtrate. Figure 3 shows the elution profile of this filtrate following gel filtration, before and after dialysis. Before dialysis, radioactivity was essentially equally distributed between two fractions; the first was shown by PAGE to be HDL, whereas the second was shown to be nonlipoprotein in character on the basis of both migration and staining characteristics upon PAGE. As shown in Fig. 2, radioactivity associated with this second peak was completely removed upon dialysis. Moreover, i.v. administration of this second fraction into a rat showed most of the radioactivity to accumulate in the thyroid (75% dose/g) after 0.5 hr. On the basis of these results, it was clear that dialysis of the HDL filtrate was essential in order to remove radioactivity not associated with HDL before administration to animals.

It now became important to characterize the nature of the radioactivity in the HDL solution after dialysis. Lipid extraction (*20*) of this solution revealed that 82% of the radioactivity was extractable into the chloroform phase and the remaining radioactivity was associated with the precipitated protein isolated by filtration. Further analysis of the chloroform layer by TLC, using the system previously described, showed the presence of two radioactive bands, which were coincident with those seen for free and esterified I*C in a ratio of 4.3:1.

Tissue distribution of I*C-HDL in normal and 4-APP-treated rats. Using HDL and LDL preparations radioiodinated with iodine-125 (I-125 HDL and I-125 LDL), Kovanen and coworkers (*14*) recently demonstrated a selective and saturable uptake for these lipoproteins in mouse adrenal. Because of the saturable nature of these adrenal binding sites, it is necessary either to employ high-specific-activity lipoproteins or in some manner to lower the endogenous unlabeled lipoproteins so that they do not dilute and thereby compete with exogenous labeled lipoproteins. For our initial experiments, we chose the latter course, since endogenous lipoprotein levels are rapidly and efficiently reduced by 4-APP. This purine analog blocks lipoprotein synthesis by the liver and causes plasma cholesterol levels to fall by more than 90% within 48 hr after i.p. administration (*9*).

Rats treated with 4-APP lost 8–18% of their body weight during the course of treatment. The adrenal medulla could not be separated from the cortex, so samples of the whole adrenal were taken.

The distribution of radioactivity at 30 min following administration of I*C-HDL to rats is summarized in Table 2. I*C-HDL administration to untreated rats resulted in a tissue distribution profile remarkably similar to that seen when 19-[^{125}I]iodocholesterol is administered in the usual manner (Table 1). One notable difference was a decrease in thyroid activity when the HDL

TABLE 2. DISTRIBUTION OF RADIOACTIVITY 0.5 HR AFTER I.V. ADMINISTRATION OF I*C-HDL TO UNTREATED RATS, AND TO RATS TREATED WITH 4-APP

	Mean % administered dose/g tissue (range)	
Tissues	Untreated rats†	4-APP-treated rats‡
Adrenal	14.414 (11.798–16.480)	60.033 (52.746–67.229)
Blood	2.047 (1.864–2.260)	1.865 (1.685–2.042)
Liver	6.372 (5.880–6.902)	3.605 (2.903–4.558)
Ovary	3.258 (1.867–4.344)	6.128 (2.825–8.754)
Plasma	2.959 (2.601–3.355)	1.095 (0.845–1.357)
Thyroid	1.844 (1.491–2.557)	1.355 (1.215–1.445)

† Five rats.
‡ Three rats.

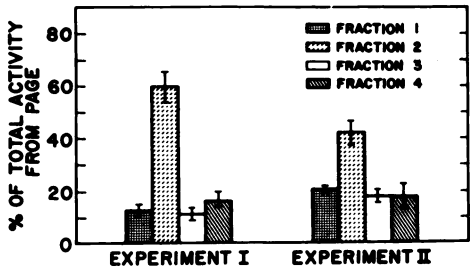

FIG. 4. PAGE analysis of plasma following i.v. administration of I*C-HDL in normal (experiment I) and 4-APP-treated rats (experiment II). Fraction 1, main gel region below albumin; Fraction 2, main gel albumin and HDL region; Fraction 3, main gel LDL and VLDL region; Fraction 4, stacking gel. Values represent mean and range for five rats (experiment I) and three rats (experiment II).

carrier was used. When I*C-HDL was administered to rats pretreated with 4-APP, however, there was increased radioactivity in the adrenal (×4) and ovary (×2), with a concomitant reduction in radioactivity present in other tissues when compared with that seen in the untreated rats receiving either I*C-HDL or I*C-Tween. When I*C-Tween was injected into rats treated with 4-APP, (data not shown) the mean activity in the adrenal was 19.11 (range = 16.18–22.21), which was not as great as that in 4-APP-treated rats receiving I-125 HDL, and only slightly greater than that seen in untreated rats receiving either I*C-HDL or I*C-Tween.

Electrophoretic analysis of serum samples following administration of I*C-HDL. Figure 4 shows the distribution of radioactivity among the various serum fractions following PAGE analysis. In normal rats, radioactivity was still primarily associated with the HDL fraction (60%) at 0.5 hr. In the 4-APP-treated rats, the HDL fraction still represented the major carrier of radioactivity (42%), but its contribution was lower than that found for the untreated controls. This lower value in the treated animals is difficult to explain without additional studies, but may be associated with the greater clearance of I*C-HDL from the circulation by adrenal, ovary, and possibly other tissues not examined.

CONCLUSION

This study in rats reveals that within 1 hr after i.v. administration of I*C in the usual manner (I*C-Tween), approximately half of the radioactivity in the plasma is associated with the HDL fraction. Earlier studies by Andersen and Dietschy (*10*) have shown that HDL is the major lipoprotein carrier for cholesterol substrate to the rat adrenal. Such appears to be the case for radioiodinated cholesterol as well.

When radioiodinated cholesterol is administered to female rats in the form of I*C-HDL, little difference in the tissue distribution profile is observed from that normally seen with I*C-Tween. Under these conditions, the low specific activity of the I*C-HDL is further diluted by the circulating plasma HDL and, as a consequence, fails to show enhanced tissue selectivity upon administration. When the animals are pretreated with 4-APP to lower the endogenous level of circulating HDL, however, a greater than fourfold increase in adrenal radioactivity is observed. This finding supports the current view that the adrenal of the rat contains high-affinity and saturable receptors for HDL.

Although 4-APP could not be used clinically in a manner employed in this study, our preliminary results lend credence to the possibility that suitably radiolabeled lipoproteins of sufficient specific activity may afford an approach to superior adrenal-imaging agents. In addition, the recent finding that adrenal-gland imaging with ^{131}IC can sometimes be accompanied by a greater than anticipated radiation dose to the thyroid (*28*) would seem to warrant further studies aimed at enhancing the target-organ uptake of this radiopharmaceutical with a concomitant reduction in metabolic dehalogenation. Studies are now in progress to assess the feasibility of using lipoproteins as a carrier for radioiodinated cholesterol in order to achieve these goals.

FOOTNOTES

† Eastman Kodak, Rochester, NY.
‡ John Manville Co.
‖ Teklad 4% Rat and Mouse Diet.
§ Aldrich Chemical Company, Inc., Milwaukee, WI.
¶ Since submission of this manuscript 19-radioiodinated cholesterol has been found to become incorporated into plasma HDL of Wistar rats to essentially the same extent as that observed for Sprague-Dawley rats in this manuscript (unpublished data).

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