Rapid hepatic metabolism of 7-ketocholesterol in vivo: implications for dietary oxysterols

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Abstract 7-Ketocholesterol is a major dietary oxysterol and the predominant non-enzymically formed oxysterol in human atherosclerotic plaque. We tested the hypothesis that 7-ketocholesterol is preferentially retained by tissues relative to cholesterol in vivo. To ensure rapid tissue uptake, acetylated low density lipoprotein, labeled with esters of [^{14}C]-7-ketocholesterol and [^{3}H]cholesterol, was injected into rats via a jugular catheter. At timed intervals (2 min to 24 h) rats (n = 48 total) were exsanguinated and tissues were dissected and assayed for radioactivity. In two experiments the majority of both radiolabels appeared in the liver after 2 min. In all tissues, ^{14}C appeared transiently and did not accumulate. Rather, it was metabolized in the liver and excreted into the intestine mainly as aqueous-soluble metabolites (presumably bile acids). By 9 h, ^{14}C in the liver had decreased to 10% of the injected dose while 36% was present in the intestine. In contrast, at 9 h 38% of ^{3}H was evident in the liver while only 5% was found in the intestine. Unlike [^{3}H]cholesterol, little ^{14}C was found to re-enter the circulation, indicating that enterohepatic recycling of 7-ketocholesterol was negligible. This is the first report of the distribution of an oxysterol relative to cholesterol, administered simultaneously, in a whole animal model. The finding that [^{14}C]-7-ketocholesterol is rapidly metabolized and excreted by the liver suggests that diet may not be a major source of oxysterols in atherosclerotic plaque, and that perhaps dietary oxysterols make little or no contribution to atherogenesis.—Lyons, M. A., S. Samman, L. Gatto, and A. J. Brown. **Rapid hepatic metabolism of 7-ketocholesterol in vivo: implications for dietary oxysterols. *J. Lipid Res.* 1999.** 40: **1846–1857**

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Cholesterol oxidation products (oxysterols) have been the focus of much attention because they are present in human atherosclerotic plaque and display a wide range of atherogenic properties in vitro and to some extent in vivo (reviewed in 1). The major oxysterols present in advanced plaque have been identified as cholest-5-ene-3β,27-diol (27-hydroxycholesterol) followed by cholest-5-ene-3β-ol-7-one (7-ketocholesterol) (2–4). 27-Hydroxycholesterol is the first product of an alternative pathway catalyzed by sterol 27-hydroxylase for hepatic cholesterol catabolism in humans and may be particularly important for the elimination of cholesterol from peripheral tissues (2, 5). On the other hand, the role and source of 7-ketocholesterol in the development of atherosclerosis is still equivocal (6). It is believed to be formed primarily by free radical oxidation of cholesterol in vivo (7, 8) but has been suggested to be derived from dietary absorption (9–11) from processed cholesterol-rich foodstuffs (12).

7-Ketocholesterol appears to be concentrated in human foam cells relative to plaque and plasma (ref. 1 and references therein). These observations, along with the potentially atherogenic array of biological effects in vitro, have implicated 7-ketocholesterol in the development of atherosclerosis. In vitro effects include inhibition of cholesterol release from cells, decreased glucose permeability, disruption of Ca^{2+} flux, altered endothelial monolayer barrier function, down-regulation of low density lipoprotein (LDL) receptor expression, inhibition of NO· release, cytotoxicity, and induction of apoptosis in vascular cells (reviewed in ref. 6).

Of particular interest to our group has been the observation that 7-ketocholesterol can impair the release of cholesterol from cells to acceptor particles. This may indicate some potential to disrupt the first step in reverse cholesterol transport, the process by which cholesterol is transported from peripheral cells to the liver for eventual excretion as bile acids. We have shown in vitro that at high intracellular concentrations, 7-ketocholesterol impairs cholesterol export from murine macrophage-foam cells to apolipoprotein A-I (13). Moreover, 7-ketocholesterol, at levels representative of those found in authentic foam cells, inhibits cholesterol export from human monocyte-derived macrophages (13). Even more striking is the impairment of release of 7-ketocholesterol itself to acceptor particles in these systems. These in vitro data are consis-

Abbreviations: acLDL, acetylated low density lipoprotein; BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoprotein; HPLC, high performance liquid chromatography; PBS, phosphate buffered saline; TLC, thin-layer chromatography.

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tent with one previous in vivo study in which it was found, after intravenous injection of 7-ketocholesterol in rabbits, that the rate of tissue influx was greater than the rate of tissue efflux for 7-ketocholesterol (14). These observations, in light of the concentration of 7-ketocholesterol (with respect to cholesterol) in foam cells relative to plaque and plasma (1), suggest that one mechanism by which 7-ketocholesterol contributes to atherosclerosis is through the inhibition of sterol efflux from cells such as macrophages. However, these findings are unexpected in view of the observation that unesterified oxysterols, including 7-ketocholesterol, can migrate between membranes at rates many-fold greater than cholesterol (15).

This study was designed to test the hypothesis that 7-ketocholesterol is retained by tissues to a greater extent than cholesterol in vivo. We therefore sought to label the reticuloendothelial system of rats by the intravenous administration of acetylated LDL (acLDL) labeled with both [^{14}C]-7-ketocholesteryl oleate and [^{3}H]cholesteryl oleate. The vehicle, acLDL, is a high-uptake modified lipoprotein which is rapidly internalized by cells in vitro (16) and in vivo (17–19). The exchange of label between lipoproteins and tissue prior to uptake was further minimized by using steryl esters (located in the core of the particle) and rats, an animal model which lacks cholesteryl ester transfer protein (20). The appearance of label in high density lipoprotein (HDL) was monitored, as well as that to plasma and erythrocytes. We show in this report that very little 7-ketocholesterol reappeared in the circulation, relative to cholesterol. Moreover, there was no accumulation of 7-ketocholesterol in tissues and, in contrast to cholesterol, 7-ketocholesterol was rapidly cleared from the liver, appearing in a modified form in the intestine. Therefore, having bypassed dietary absorption and delivering directly to the liver we have shown that [^{14}C]-7-ketocholesterol is rapidly metabolized by the liver and subsequently excreted into the intestine. As will be discussed, this casts doubt upon the contribution of diet to oxysterols in atherosclerotic lesions and highlights the significance of in vivo production of 7-ketocholesterol.

METHODS

Materials

Solvents were either high performance liquid chromatography (HPLC) or analytical reagent grade and were purchased from EM Scientific (Merck, Kilsyth, Victoria (Vic), Australia), Mallinckrodt (Selby Biolab, Mulgrave North, Vic), BDH (Kilsyth, Vic), Fluka (Castle Hill, New South Wales (NSW), Australia) or ICN Biomedical (Seven Hills, NSW). Reagents were of analytical reagent grade and were obtained from Sigma-Aldrich (Castle Hill, NSW) or BDH. [4-^{14}C]cholesterol and [1α,2α-^{3}H]cholesteryl oleate were purchased from Amersham (Castle Hill, NSW). The following chemicals were obtained from the companies indicated: oxysterols from Steraloids (Wilton, NH); oleoyl chloride from Nu-Chek Prep (Elysian, MN); chloramphenicol from Boehringer Mannheim (Castle Hill, NSW); sodium pentabarbitone (Nembutal, 60 mg/ml) from Boehringer Ingelheim (Artarmon, NSW); ketamine hydrochloride (Ketamil, 100 mg/ml) from Troy Laboratories (Smithfield, NSW); heparin (10 000 IU/ml) from David Bull Laboratories (Mulgrave, Vic). Protein estimation was by the bicinchoninic acid/Cu^{2+} ion assay supplied by Pierce (Laboratory Supply, Sydney, NSW) with essentially fatty acid-free bovine serum albumin Fraction V as standard. Materials for liquid scintillation counting were purchased from Canberra-Packard (Mount Waverly, Vic).

Synthesis of sterols

[^{14}C]-7-ketocholesteryl oleate. [^{14}C]-7-ketocholesterol was synthesized by a scaled-down version of the method described by Kan, Yan, and Bittman (21) which was based on the methods of Chicoye, Powrie, and Fennema (22) and Parish, Wei, and Livant (23). [^{14}C]-7-ketocholesterol was purified by TLC developed with two different mobile phases designed to separate 7-ketocholesterol from other oxysterols and cholesterol. After purification, both plates showed only one band when visualized by autoradiography. [^{14}C]-7-ketocholesterol was also assessed for purity by normal phase HPLC (4) with radiometric detection and was found to be >99% pure. [^{14}C]-7-ketocholesteryl oleate was synthesized from purified [^{14}C]-7-ketocholesterol (24) and further purified by reversed phase HPLC (25).

[^{14}C]cholest-5-ene-3β,7β-diol and [^{14}C]cholest-5-ene-3β,7α-diol. [^{14}C]cholest-5-ene-3β,7β-diol ([^{14}C]7β-hydroxycholesterol) was synthesized from [^{14}C]-7-ketocholesterol by reduction with lithium aluminium hydride at room temperature for 3 h in ether. Excess lithium aluminium hydride was reacted with water, ether was evaporated, and [^{14}C]-7β-hydroxycholesterol was extracted into hexane. The hexane was evaporated under vacuum and the [^{14}C]-7β-hydroxycholesterol was redissolved in isopropanol–heptane (200 μl) 5:95 (v/v). [^{14}C]cholest-5-ene-3β,7α-diol ([^{14}C]-7α-hydroxycholesterol) is also produced under these conditions.

Isolation of lipoproteins

For production of labeled acLDL, human LDL was isolated from plasma derived from a fasted, healthy donor by single vertical spin density gradient ultracentrifugation (26) followed by a second ultracentrifugation step to remove albumin contamination as described elsewhere (25). LDL (16 mg, 6.0 mg protein/ml) was acetylated by the repeated addition of acetic anhydride (27) and was dialyzed overnight against phosphate-buffered saline (PBS, 10 mmol/ L, 4 × 1 L) containing ethylenediaminetetraacetic acid (EDTA, 270 μmol/L) and chloramphenicol (310 μmol/L). The extent of modification was determined by relative electrophoretic mobility (≥3 compared to native LDL) (25).

Rat HDL was isolated by ultracentrifugation by the method of Sattler, Bone, and Stocker (28) after visualization with Sudan Black B stain (29). An aliquot was taken for saponification and determination of total cholesterol concentration. HDL was also isolated from rat plasma (1.1 ml) by the precipitation of apolipoprotein B-containing lipoproteins by the addition of dextran sulfate (Dextralip 50) and magnesium ions (30).

Incorporation of radiolabeled steryl esters into the core of acetylated LDL

[^{14}C]-7-ketocholesteryl oleate and [^{3}H]cholesteryl oleate were incorporated into the core of acLDL in order to minimize exchange of label prior to tissue uptake. Labeling was achieved by extracting the core lipids and replacing them with the labeled steryl esters and carrier cholesteryl ester followed by reconstitution of the acLDL (31, 32). This acLDL contained 15% of the added radiolabels and 59% of the added protein which compares well with 24% and 56%, respectively, in the published method (31). Three preparations were made, one for Experiment 1 and two for Experiment 2 that was conducted in two parts. The parameters for each experiment are listed in Table 1.

Animals

Two experiments were performed with two strains of male rats. All procedures were in accordance with the Animal Care and

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Ethics Committee, University of Sydney, following guidelines set out by the National Health and Medical Research Council of Australia. All animals were allowed unlimited access to standard rodent chow (Breeders 602 mouse feed, YS Seeds, Young, NSW) and water. They were housed in groups of five or six prior to surgery and individually after surgery. The animal house was maintained with a 12 h light/dark cycle. Eight-week-old inbred Sprague-Dawley rats (n = 22) with body weight 230–270 g were used in Experiment 1. In Experiment 2, 14-week-old outbred Wistar rats (n = 26) with body weight 420–460 g were used. Rats were divided into groups of three per time-point so that groups within each experiment had similar average body weights. They then underwent bilateral cannulation of the jugular vein and administration of dual-labeled acLDL followed by sample collection at timed intervals from 2 min to 24 h.

Cannulation of the jugular veins

Animals underwent bilateral cannulation of the vein according to the procedure described by Waynforth (33). Briefly, the animals were anesthetized by intraperitoneal injection of sodium pentabarbitone (30 mg/kg body weight) and ketamine hydrochloride (50 mg/kg body weight) and the jugular vein was exposed and stripped of connective tissue. The blood flow was halted by an anterior ligature and the vein was partially transected posterior to the ligature with micro-scissors to allow the introduction of a length of silicon tubing. The cannula was fixed with a second ligature and further stabilized with the ends of the first ligature. The cannula was passed subcutaneously to emerge dorsally at the base of the neck and the incision was closed with suture fixed to the cannula. Prior to insertion, the cannula was filled with heparin/saline (100 IU/ml in 150 mmol/L saline) to prevent clotting and stoppered after external exposure. Animals were allowed to recover under supervision on a heat-pad until conscious and then for 48 h prior to their involvement in any experiment.

Administration of acetylated LDL and sample collection

Blood was drawn from the left jugular cannula after heparinized saline was removed. Labeled acLDL was administered and the cannula was flushed with heparinized saline (100 μl). The volumes and quantities of radiolabel injected are as indicated in **Table 1**. At timed intervals (Experiment 1: 2 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h; Experiment 2, part 1: 2 min, 15 min, 1 h, 3 h, 6 h, 9 h; and Experiment 2, part 2: 12 h, 18 h, 24 h) animals were anesthetized with sodium pentabarbitone (24 mg, 400 μl) and exsanguinated via the right cannula. Exsanguination resulted in collection of approximately 10 ml blood. Blood was collected into chilled screw-capped tubes (15 ml, 4°C) containing EDTA (20 μmol, ~2 mmol/L, ~10 ml final volume). The heart was then exposed, an incision was made in the right ventricle and animals were perfused for 5 min via the left ventricle with PBS (10 mmol/L) containing EDTA (0.27 mmol/L) and saturated butylated hydroxytoluene (BHT). Tissues comprising liver, heart, lungs, intestine and stomach, spleen, kidneys, and aorta were excised, blotted dry on lint-free tissues, and placed in sample containers on ice to be stored at −80°C until analysis.

Blood was centrifuged (2000 *g*, 15 min, 10°C), plasma aliquotted (1.1 ml) into microfuge tubes for storage (−80°C), and packed erythrocytes were washed 3 times with an equal volume of isotonic Tris buffer (172 mmol/L) containing glucose (5 mmol/L), EDTA and BHT after removal of the buffy coat.

Sectioning of intestine

Intestines were partially thawed from −80°C and dissected on a plastic tray on ice. The bile duct was identified approximately 40 mm below the stomach. Connective tissue and mesenteric fat were removed using forceps. The stomach was separated from the small intestine by cutting approximately 2 mm above the bile duct. The small intestine, caecum, and large intestine were then isolated by cutting above and below the caecum for measurement of radioactivity (see below).

Homogenization of tissue

Tissues were minced finely with scissors and then homogenized in an equal volume of PBS (10 mmol/L) containing EDTA (2.7 mol/L) and saturated BHT to produce a 50% (w/v) suspension using a Potter-Elvehjem homogenizer or an Ultra-Turrax T8 hand-held disperser (IKA, Crown Scientific, Moorebank, NSW).

Lipid extraction and liquid scintillation counting

Radioactivity was assayed by liquid scintillation counting using a TriCarb 2100TR Liquid Scintillation Analyser (Canberra-Packard) and dual-label protocols. Results (mean ± standard deviation, n = 3 for each time-point) are presented as the quantity of isotope (in disintegrations per minute (dpm)) present in any given sample as a percentage of the total injected.

Total radioactivity. Total radioactivity of tissues (aorta, heart, intestinal sections, kidneys, liver, lungs, spleen) was assayed by solubilization with Soluene 350 according to the manufacturer's instructions (Canberra-Packard) and addition of Hionic Fluor (10 ml). Total radioactivity in plasma and HDL determined by precipitation of apolipoprotein B-containing lipoproteins (500 μl) was assayed by addition of Ultima Gold (10 ml) with shaking until the sample was clear. Hionic Fluor (5 ml) was added to HDL isolated by ultracentrifugation (1.8 ml from 2.0 ml).

TABLE 1. Radiolabel and protein concentrations of the acetylated LDL (acLDL) preparations used in the two experiments

Experiment	Injection Volume per Animal	acLDL Protein	Radiolabel Concentration		Radiolabel Injected	
			[^{3}H]	[^{14}C]	[^{3}H]	[^{14}C]
	μl	*mg protein/ml buffer*	*KBq[a]/mg protein*		*KBq*	
1	100	1.23	56.3	16.3	6.87	2.02
2.1[b]	150	0.675	917	76.0	92.8	7.70
2.2	200	1.07	423	21.5	90.3	4.60

The radiolabel concentrations were markedly increased in Experiment 2, allowing greater sensitivity. Protein was determined by the bicinchoninic acid/Cu^{2+} assay and radioactivity was assayed by direct addition of scintillant to an aliquot of acLDL.

[a] 1 KBq=27.0 nCi.

[b] Experiment 2 was conducted in 2 parts such that part 1 (2.1 above) comprised 2 min, 15 min, 1 h, 3 h, 6 h, and 9 h time-points. Part 2 (2.2 above) comprised 12 h, 18 h, and 24 h time-points.

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Erythrocytes. Packed washed erythrocytes (500 μl) were made up to 1.0 ml with Tris buffer (10 mmol/L) in the presence of EDTA (20 μmol) and BHT (200 nmol). Methanol was added (2.5 ml), followed by hexane (5.0 ml). The sample was vortexed and then centrifuged (2000 *g*, 10°C, 5 min). An aliquot of hexane (4.0 ml) was evaporated under vacuum (Speed Vac Plus SC100A, Savant, Selby-Biolab) and redissolved in solvent (250 μl, isopropanol–acetonitrile 70:30 (v/v). Of this, a portion (150 μl) was taken for liquid scintillation counting with Ultima Gold scintillant (5 ml). The total volume of erythrocytes was calculated by multiplying the mass of the rat (kg) by 62 ml/kg (33, 34) to obtain the total blood volume and then by multiplying this product by 47 (ml/100 ml), the packed cell volume of rats (33). This value was then related to the radioactivity measured in the washed erythrocytes.

Liver and intestine. Liver and intestinal lipids were extracted by the method of Folch, Lees, and Sloane Stanley (35). The sample was redissolved in heptane–isopropanol 3:2 (v/v) (1.0 ml). A portion (800 μl) was assayed for radioactivity (as described above) with Ultima Gold (5 ml) while the remainder of the liver extracts (200 μl) was taken for determination of total cholesterol concentration by reversed phase HPLC (25, 36). Triacylglycerol was removed from liver extracts by solid phase extraction (37) to prevent overloading of the TLC plates with lipid.

Thin-layer chromatography

The metabolic products of [^{14}C]-7-ketocholesterol in the plasma, liver, and small intestine were resolved by TLC analysis of the concentrated lipid portion of lipid extracts. Standards were applied in two lanes: one lane contained [^{14}C]-7-ketocholesterol, [^{14}C]-7-ketocholesteryl oleate, [^{14}C]-7β-hydroxycholesterol, [^{14}C]-7α-hydroxycholesterol and [^{14}C]cholesterol (21 Bq each, 21 Bq [^{14}C]-7β-hydroxycholesterol and [^{14}C]-7α-hydroxycholesterol combined); the other lane contained unlabeled 7-ketocholesterol, 7-ketocholesteryl oleate, cholesterol, cholesteryl oleate, 7β-hydroxycholesterol, and 7α-hydroxycholesterol (20 μg each). The plate was developed in a solvent system of hexane–ethyl acetate 1:1 (v/v). Unlabeled standards were visualized by immersing the plate in copper (II) sulfate in phosphoric acid ($CuSO_4$ 0.4 mol/L dissolved in H_3PO_4 0.87 mol/L, Cu^{2+} 10% w/v dissolved in H_3PO_4 8.5% v/v). ^{14}C-radiolabeled compounds were visualized by exposure to X-ray film at −80°C in the dark for several weeks. Plasma and small intestine TLC plates were cut into bands according to the relative mobility of authentic standards. These were placed into scintillation vials and lipids were eluted by addition of methanol (1.0 ml) followed by diethyl ether (1.0 ml). Ultima Gold (5 ml) was then added and the samples were assayed for radioactivity.

RESULTS

Determination of radiolabels in the circulation

In order to compare tissue release of 7-ketocholesterol and cholesterol into the circulation, esters of [^{14}C]-7-ketocholesterol and [^{3}H]cholesterol were incorporated into a modified lipoprotein, acLDL, to ensure rapid tissue uptake as demonstrated previously (16–19). The [^{14}C]-7-ketocholesteryl oleate, which is not commercially available,

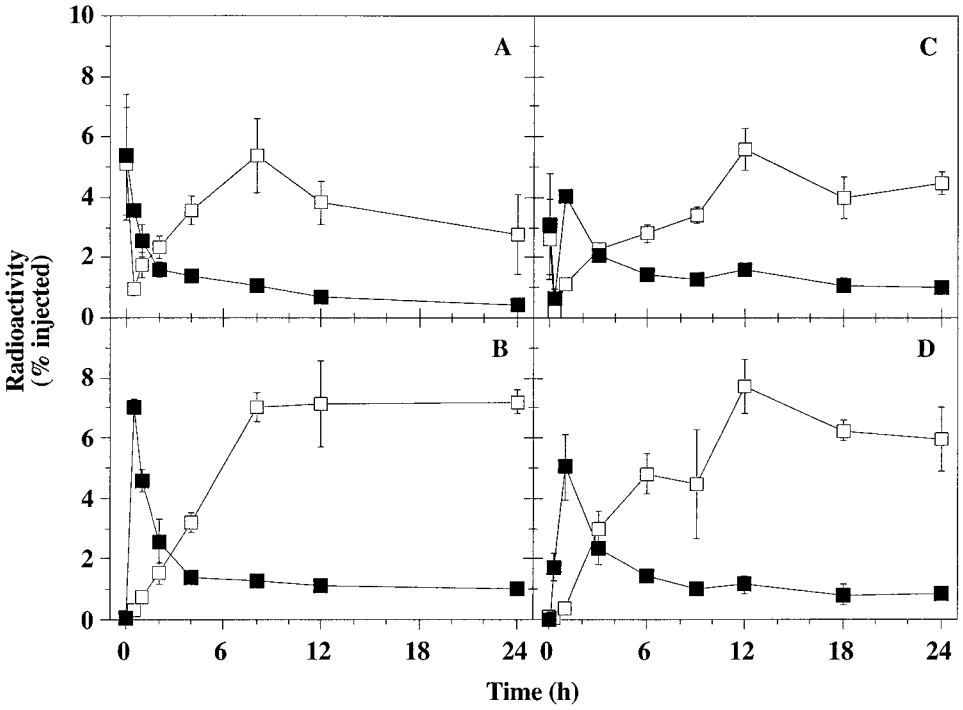

Fig. 1. ^{14}C derived from 7-ketocholesterol disappeared from plasma and erythrocytes much more rapidly than ^{3}H derived from cholesterol. ^{14}C (filled symbols) and ^{3}H (open symbols) in plasma (panels A and C) were determined by the addition of scintillant and assayed with the appropriate protocol by liquid scintillation counting. Erythrocytes (panels B and D) were washed with buffer after removal of the buffy coat. They were then extracted into hexane which was evaporated under vacuum, redissolved in solvent, and assayed for radioactivity (see Methods for details). Data are from Experiment 1 (A and B) and Experiment 2 (C and D). Values are expressed as mean ± SD (3 rats per time-point) except Experiment 1, t = 30 min and t = 2 h and Experiment 2, t = 1 h values expressed as mean ± range (n = 2).

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was synthesized and together with [^{3}H]cholesteryl oleate was incorporated into the core of the lipoprotein particle by the reconstitution method of Krieger et al. (31, 32).

After intravenous administration of the dual-labeled acLDL, ^{3}H was rapidly cleared from the plasma with ⩽1% of the injected dose remaining after 15 to 30 min (**Fig. 1A** and **1C**). ^{14}C was cleared from the plasma to a similar extent in Experiment 2 (Fig. 1C) but the rapid clearance followed by a transient increase was not observed in Experiment 1 (Fig. 1A), probably due to a later sampling time (15 min vs. 30 min, respectively). The clearance of the two labels is consistent with the rapid clearance from plasma of acLDL demonstrated by other workers where <1% remained in the plasma after 1 h (19).

After the clearance of acLDL, ^{3}H and ^{14}C reappeared in plasma in both experiments (Figs. 1A and 1C). ^{3}H increased linearly reaching a maximum of 5.4%–5.6% at 8–12 h and then decreased slightly to 2.8% and 4.5%, respectively, for Experiments 1 and 2, by 24 h. In Experiment 2, ^{14}C reappeared to reach a maximum of 4.1% by 1 h (Fig. 1C) and then decreased initially with a rapid phase (2.1% by 3 h) followed by a gradual phase (1.0% by 24 h). The rates of disappearance and reappearance of the two labels were very different and suggested that while ^{14}C was cleared rapidly from the plasma, ^{3}H was redistributed throughout the plasma pool.

Plasma from Experiment 2 was subjected to lipid extraction and TLC to determine the form of ^{14}C across the time-course (data not shown, n = 23). Of the ^{14}C measured after TLC, unesterified 7-ketocholesterol appeared transiently and predominated in the plasma at 1 h representing 67% of the detectable ^{14}C. Metabolites of 7-ketocholesterol were also detectable in plasma including 7β-hydroxycholesterol and cholesterol (co-eluting with authentic standards), and other more polar species which are presently unidentified. 7β-Hydroxycholesterol reached a maximum of 12% of detectable lipid-soluble ^{14}C at 1 h while cholesterol reached a maximum of 19% at 12 h. More polar compounds eluting closer to the origin reached a maximum of 8% of detectable ^{14}C at 6 h. Over the 24-h time-course, what little ^{14}C was present in the plasma was increasingly esterified such that approximately 82% was esterified at the end of the experiment.

Neither label was present in erythrocytes at the initial (2 min post-injection) time-point but both were detected at subsequent time-points (Figs. 1B and 1D). ^{3}H increased approximately linearly to a maximum of 7.0%–7.7% at 8–12 h and then remained constant or decreased slightly to 24 h (Figs. 1B and 1D). ^{14}C accumulated rapidly within 30 min to 1 h to a maximum of 5.0%–7.0% and decreased with an initial rapid phase (1.4%–1.5% by 4–6 h) and then more gradually (0.8%–1.0% at 24 h) (Figs. 1B and 1D). Both the profiles and the absolute values were similar for both isotopes in each of plasma and erythrocytes (Fig. 1). As erythrocytes are unable to accommodate steryl esters, the detected labels were assumed to be unesterified, indicating that intracellular hydrolysis of steryl esters contained within the acLDL had occurred.

The profile of radioactivity that appeared in HDL isolated by ultracentrifugation (Experiment 2, **Fig. 2A**) mirrored that of plasma (Fig. 1C) and accounted for the major proportion of ^{3}H and a lesser proportion of ^{14}C (56 ± 6% and 21 ± 7% of the label present in the plasma at any given time, respectively, mean ± SD). The two labels appeared at similar levels at 2 min (approximately 0.75%) and both had decreased in a similar fashion by 15 min (0.1% ^{3}H and 0.3% ^{14}C). ^{14}C increased from 15 min to 1 h (1.1%) then decreased rapidly to 3 h (0.3%) and more gradually to 24 h (0.1%). In contrast, ^{3}H increased steadily from 15 min to 12 h (2.8%) and remained constant to 24 h (2.4%).

Determination of radioactivity in HDL isolated by precipitation of apolipoprotein B-containing lipoproteins (whole plasma less apolipoprotein B lipoproteins) is presented in Fig. 2B (Experiment 2). The ^{3}H detected was similar to that found in HDL isolated by ultracentrifugation. However, the ^{14}C was higher than when isolated by ultracentrifugation and followed the profile of ^{14}C in plasma (Fig. 1C) very closely. This difference is suggestive of a pool of ^{14}C not associated with HDL. Such a pool may be

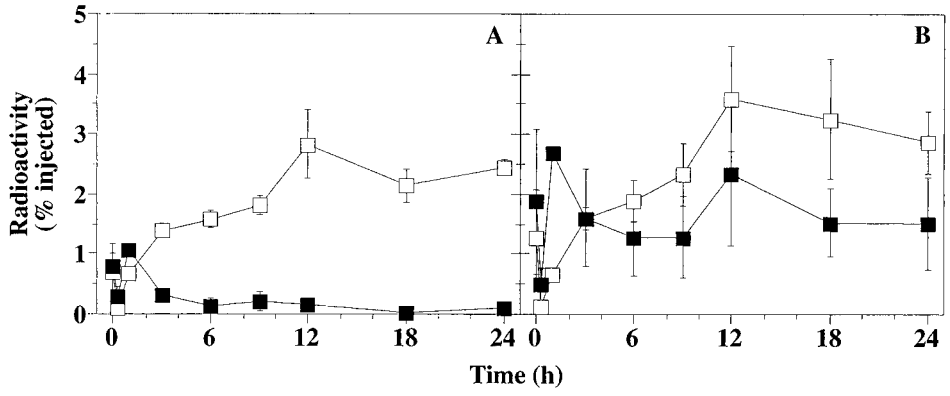

Fig. 2. ^{14}C derived from 7-ketocholesterol disappeared more rapidly than ^{3}H derived from cholesterol from HDL isolated by two different methods. ^{3}H (open symbols) and ^{14}C (filled symbols) in HDL isolated by ultracentrifugation (panel A) and by precipitation of apolipoprotein B-containing lipoproteins (panel B). HDL was isolated as described in Methods. Data are derived from Experiment 2. Values are expressed as mean ± SD (3 rats per time-point) except t = 1 h value expressed as mean ± range (n = 2).

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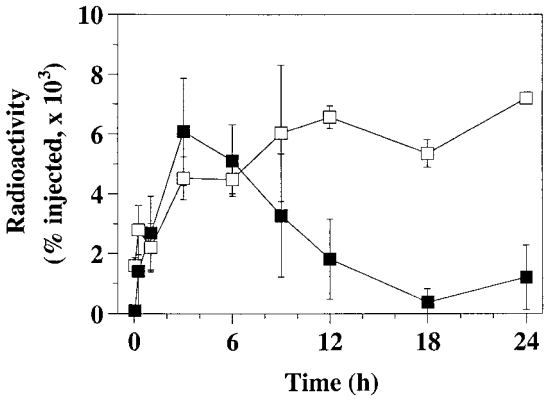

Fig. 3. Measurement of ^{3}H (open symbols) derived from cholesterol and ^{14}C (filled symbols) derived from 7-ketocholesterol in aorta. Whole aorta was solubilized with a strong organic base and then assayed for radioactivity by addition of scintillant and counting with the appropriate protocol (see Methods for details). Data are derived from Experiment 2. Values are expressed as mean ± SD (3 rats per time-point) except t = 1 h value expressed as mean ± range (n = 2).

aqueous soluble (indicative of metabolism) or plasma protein-associated. However, it was found that 99 ± 10% (mean ± SD, n = 10) of ^{14}C was lipid-soluble in plasma over the time-course.

Determination of radiolabels in the aorta

In whole digests of aorta (Experiment 2), ^{3}H increased over 24 h to a maximum of 0.007% of the injected label. ^{14}C increased initially to a maximum of 0.006% at 3 h and decreased to 0.001% by 24 h (**Fig. 3**). Whether or not these labels were esterified was not determined. The rate of loss of ^{14}C from the aorta over this period (Fig. 3) was slower than for its clearance from plasma, erythrocytes (Fig. 1) or HDL (Fig. 2). No accumulation of ^{14}C was evident over this time period.

Determination of radiolabels in the liver

The majority of acLDL administered intravenously to rats is rapidly cleared by the liver within minutes as determined both by labeling the protein moiety (17) and the cholesteryl ester pool (18, 19). The data on the appearance of radiolabeled sterols in the liver presented in **Fig. 4** are consistent with rapid clearance of acLDL by the liver in this study. In Experiment 1, the liver was subjected to a lipid extraction thereby separating aqueous-soluble metabolites from sterols. By this method 43% of both labeled sterols was present at 2 min in the non-polar fraction of liver (Fig. 4A). In Experiment 2, radioactivity was measured in both the non-polar fraction of a lipid extract and also in the whole liver homogenate, thereby measuring the total hepatic radioactivity. In whole liver homogenate, 71% of the administered ^{14}C was detected after 2 min, while 63% of administered ^{3}H was detected (Fig. 4B). Clearance of ^{3}H was approximately linear with 18% remaining after 24 h (Experiment 1 and 2). ^{14}C was cleared much more rapidly than ^{3}H, most within 2 to 3 h. The non-polar fraction of the lipid extraction performed in Experiment 2 accounted for 96% of the total ^{3}H present in the liver at 2 min and from 77% to 83% over the remaining 24 h. In contrast, the non-polar fraction accounted for 98% of ^{14}C at 2 min and then decreased to <30% from 12 to 24 h. The profiles of the clearance of each of the two radiolabels were similar in both experiments as were the final levels detected at 24 h. The total cholesterol concentration in the liver (Experiment 2) was the same for all animals across the time-course (11% coefficient of variation). Hence the specific activity of ^{3}H shows the same profile as the radioactivity expressed as a percentage of the dose injected. As the liver is responsible for disposing of cholesterol, mainly through conversion to bile acids, it seems reasonable to assume that hepatic clearance of both labels in their modified or native forms was most

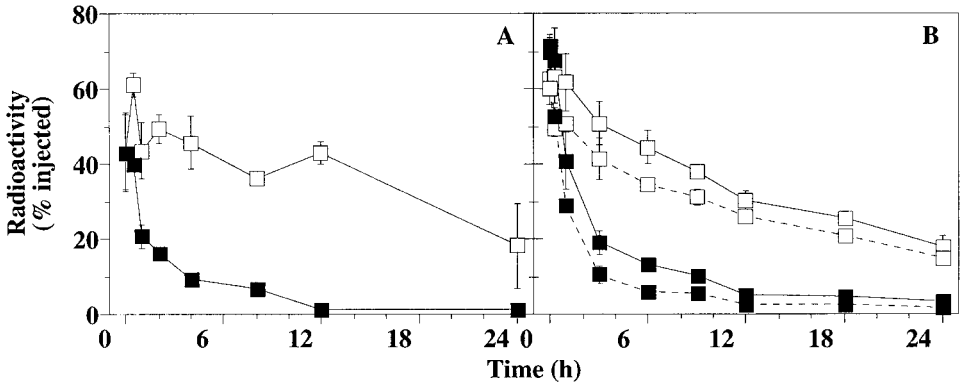

Fig. 4. Clearance of ^{14}C (filled symbols) derived from 7-ketocholesterol and ^{3}H (open symbols) derived from ^{3}H-cholesterol from liver. Whole liver homogenates were extracted (34) and the lipid-soluble phase evaporated under vacuum, redissolved in heptane–isopropanol 3:2 (v/v) and assayed for radioactivity. Total radioactivity (broken line) was also determined in Experiment 2 by solubilization of whole liver homogenate with a strong organic base, addition of scintillant and counting with the appropriate protocol (see Methods for details). Data are derived from Experiment 1 (panel A) and Experiment 2 (panel B). Values are expressed as mean ± SD (3 rats per time-point) except Experiment 1, t = 30 min and t = 2 h and Experiment 2, t = 1 h values expressed as mean ± range (n = 2).

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probably via the bile duct and secretion into the small intestine.

Products of hepatic 7-ketocholesterol metabolism

Analysis of the lipid-soluble fraction of the liver also afforded evidence of rapid hepatic metabolism of [^{14}C]-7-ketocholesterol (**Fig. 5**). At 2 min the ester of [^{14}C]-7-ketocholesterol incorporated into the lipoprotein core is evident at the top of the autoradiograph. Below this unesterified 7-ketocholesterol is observed, indicating that uptake of the particles and hydrolysis of the 7-ketocholesterol ester was extremely rapid. There was no ester remaining by 1 h. In the 2 min and later time-points, a second band is visible eluting ahead of 7-ketocholesterol. This band co-elutes with authentic 7β-hydroxycholesterol by TLC and as such has been tentatively identified as 7β-hydroxycholesterol. Over the time-course an apparent increase followed by a decrease may be seen in [^{14}C]-7-ketocholesterol and both of the metabolites. Such a decrease is probably due to the excretion of the compounds into the intestine.

Determination of radiolabels in other tissues

In order to establish whether 7-ketocholesterol accumulates in extrahepatic cells, various major organs and tissues were analyzed at the initial (2 min), intermediate (9 h), and final (24 h) time-point (**Table 2**) for total (esterified plus unesterified) radioactivity. Apart from liver and spleen, ^{3}H increased over the time-course in both percent injected and per unit mass terms. In contrast, ^{14}C increased from 2 min to 9 h and then decreased to 24 h in all tissues except liver and blood where it decreased continuously over the time-course. Whilst the liver content of both labels decreased, there was a concomitant increase in the intestinal content. Apart from liver, intestine, and blood, very little of either label was present in any of the samples measured, except for the spleen. The spleen was relatively enriched in ^{3}H per gram of tissue over the time-course (although decreasing) and in ^{14}C at the 2 min time-point. These levels reflect the relative amount of each of the labels present in the circulation at each of the time-points and may reflect the role of the spleen in catabolizing erythrocytes. For most tissues examined (aorta, heart, intestine, kidneys, lungs), ^{3}H increased from 2 min to 24 h, whereas ^{14}C displayed only a transient increase.

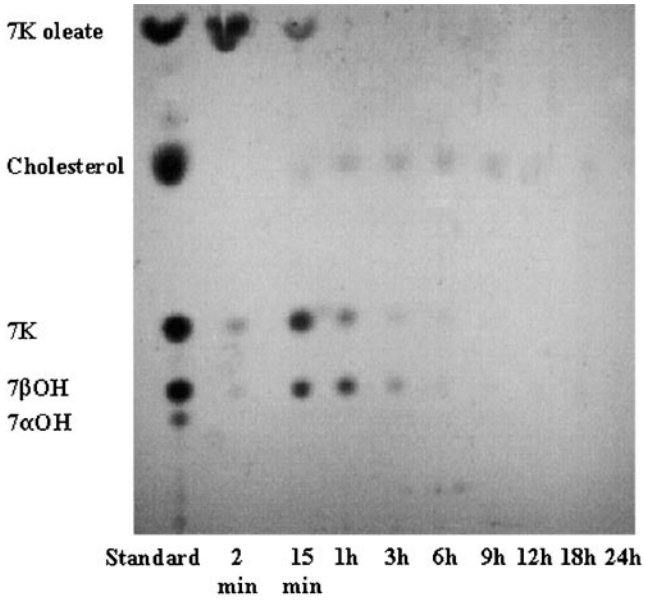

Fig. 5. ^{14}C-labeled metabolites of 7-ketocholesterol (7K) were detected in the liver over the entire time-course as determined by TLC. Whole liver homogenates underwent lipid extraction (34) and the lipid extracts were subjected to TLC and the plates were visualized by autoradiography. The mobile phase comprised hexane–ethyl acetate 1:1 (v/v). The ^{14}C-labeled standards indicate that two of the metabolites are cholesterol and 7β-hydroxycholesterol (7βOH). 7α-Hydroxycholesterol (7αOH) was applied also as a standard. Data are derived from Experiment 2.

Determination of radiolabels in the intestine

As there was no accumulation of ^{14}C in the liver (Fig. 4) or any of the major tissues analyzed (Table 2), the intestine was examined because cholesterol that is excreted ultimately appears in the intestine in one form or another. Results for the detection of the radiolabels in the intestine are presented in **Fig. 6**. Much lower levels of ^{3}H than ^{14}C were present at every time-point, except 2 min. The increase of ^{3}H in each section was approximately linear with the greatest proportion present in the small intestine. The lower levels present in the caecum and large intestine may reflect the relatively efficient reabsorption of cholesterol from the intestine. In contrast, a large proportion of ^{14}C was present in each of the sections. The content increased linearly in the small intestine and reached a maximum at 3 h (19.1%), decreasing linearly to 12 h (9.6%) and then remained constant up to 24 h. This profile was similar for the caecum but was shifted by 6 h so that the peak was at 9 h (18.5%), decreasing linearly to 24 h (7.7%). The increase in the large intestine was not as rapid, increasing to a maximum at 12 h (8.4%) and then decreasing to 24 h (4.3%). The level in the large intestine was not as great, probably because of fecal excretion (not measured) as the gastrointestinal transit time in the rat is 12 to 24 h (37). In contrast, ^{3}H increased slowly in the intestine, which is more consistent with reabsorption than excretion. This is highlighted by the total recovery of radioactivity presented in Table 2. By 24 h, the total ^{14}C recovered was approximately one-third of that present at 2 min while the ^{3}H recovered at 24 h amounted to approximately two-thirds of that at 2 min. These results strongly support the case for rapid transit of ^{14}C through the intestine and fecal excretion.

Metabolic products of 7-ketocholesterol in the intestine

Further examination of the intestine by lipid extraction supplied more evidence of greater metabolism of [^{14}C]-7-ketocholesterol than [^{3}H]cholesterol (**Fig. 7**). The increase of ^{3}H in the intestine was gradual and the proportion of the label in the aqueous phase was variable. In contrast, the level of ^{14}C in the intestine increased to a maximum at 9 h and decreased over the remaining time-course. Moreover, the proportion of ^{14}C in the aqueous

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TABLE 2. Radioactivity in a variety of major tissues and organs at an early (2 min), an intermediate (9 h), and the final (24 h) time-points

Tissue		[^{3}H]Cholesterol-Derived Radioactivity			[^{14}C]-7K-Derived Radioactivity		
		2 min	9 h	24 h	2 min	9 h	24 h
Blood	tissue	2.66 ± 1.32	6.69 ± 0.93	7.41 ± 0.65	3.12 ± 1.66	1.98 ± 0.21	1.44 ± 0.13
	per g	*0.190 ± 0.094*	*0.478 ± 0.067*	*0.529 ± 0.046*	*0.223 ± 0.119*	*0.142 ± 0.015*	*0.103 ± 0.009*
Heart	tissue	0.0760 ± 0.0077	0.0999 ± 0.0145	0.174 ± 0.013	0.0012 ± 0.0017	0.0766 ± 0.0115	0.0199 ± 0.012
	per g	*0.0645 ± 0.0081*	*0.0837 ± 0.0069*	*0.147 ± 0.014*	*0.00096 ± 0.0014*	*0.0642 ± 0.0071*	*0.0169 ± 0.0107*
Intestine	tissue	2.39 ± 0.41	5.46 ± 0.58	13.5 ± 1.5	0.160 ± 0.140	36.2 ± 7.6	20.9 ± 2.8
	per g	*0.147 ± 0.016*	*0.291 ± 0.021*	*0.786 ± 0.025*	*0.00862 ± 0.00806*	*1.79 ± 0.39*	*1.19 ± 0.22*
Kidneys	tissue	0.326 ± 0.154	0.397 ± 0.078	0.535 ± 0.101	0.0787 ± 0.0429	0.569 ± 0.156	0.34 ± 0.077
	per g	*0.115 ± 0.058*	*0.130 ± 0.017*	*0.178 ± 0.023*	*0.0274 ± 0.0156*	*0.183 ± 0.029*	*0.116 ± 0.038*
Liver	tissue	62.7 ± 1.4	38.0 ± 1.1	18.5 ± 2.7	71.4 ± 2.3	10.4 ± 0.6	5.81 ± 1.63
	per g	*4.2 ± 0.36*	*2.56 ± 0.41*	*1.32 ± 0.16*	*4.79 ± 0.48*	*0.701 ± 0.136*	*0.411 ± 0.091*
Lungs	tissue	0.262 ± 0.053	0.760 ± 0.092	1.14 ± 0.07	0.261 ± 0.023	0.518 ± 0.188	0.222 ± 0.058
	per g	*0.136 ± 0.071*	*0.599 ± 0.054*	*0.739 ± 0.131*	*0.131 ± 0.048*	*0.403 ± 0.063*	*0.146 ± 0.054*
Spleen	tissue	1.79 ± 0.48	1.08 ± 0.19	0.977 ± 0.089	1.28 ± 0.41	ND	0.000583 ± 0.000082
	per g	*1.78 ± 0.46*	*1.37 ± 0.16*	*1.18 ± 0.05*	*1.24 ± 0.28*	*ND*	*0.000733 ± 0.000035*
Total	tissue	70.2 ± 2.0	52.5 ± 1.6	42.3 ± 3.2	76.3 ± 2.87	49.8 ± 7.6	28.7 ± 3.26

The results are expressed as a percentage of the total radioactivity injected in the whole tissue or organ (tissue) and as a percentage of the total injected per gram wet weight of tissue (per g). Data are derived from Experiment 2. Values are expressed as mean ± SD (3 rats per time-point) except t = 1 h value expressed as mean ± range (n = 2); ND, not detected

phase was greater than in the lipid-soluble fraction at every time-point (except 6 h). As 7-ketocholesterol itself is lipid-soluble, appearance of ^{14}C in the aqueous phase suggests its metabolism to more polar compounds.

When lipid extracts of the small intestine were analyzed by TLC (data not shown, n = 9) compounds co-eluting with authentic 7β-hydroxycholesterol and cholesterol were observed as well as other unidentified metabolites. Less than 5% of the lipid-soluble ^{14}C detected in the small intestine at any time was in an esterified form. The maximal level of unesterified 7-ketocholesterol was attained at 1 h (27% of the ^{14}C detected). 7β-Hydroxycholesterol appeared transiently reaching a maximum of 73% of the detected ^{14}C at 15 min. [^{14}C]cholesterol reached a maximum of 50% of detected label at 9 h. Other polar metabolites eluting from the origin to below 7β-hydroxycholesterol increased over the time-course so that by 24 h 77% of the ^{14}C detected in the lipid-soluble fraction was in this region. This was the major class of lipid and these data further support the metabolism of 7-ketocholesterol and excretion of polar metabolites (and cholesterol) into the intestine.

DISCUSSION

This study was designed to test the hypothesis that 7-ketocholesterol is preferentially retained by tissues relative to cholesterol and arose from in vitro work which showed that 7-ketocholesterol release to apolipoprotein A-I was impaired from macrophage-foam cells relative to cholesterol (13). The hypothesis was tested by intravenous injection into rats of [^{14}C]-7-ketocholesteryl oleate and [^{3}H]cholesteryl oleate incorporated within acLDL, an ap-

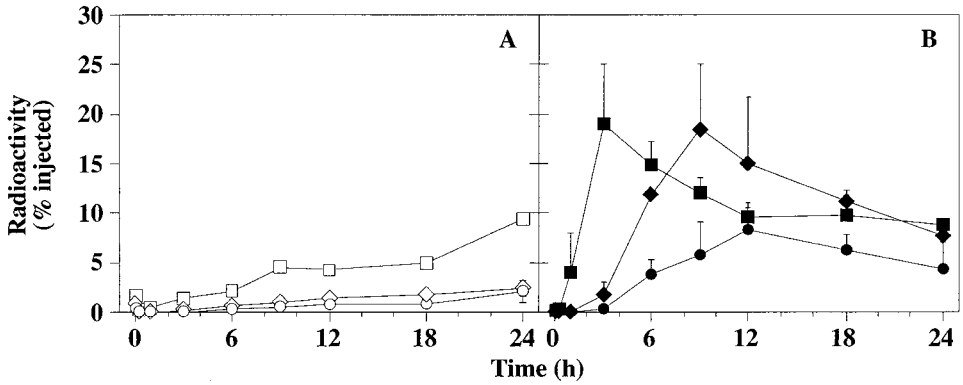

Fig. 6. Measurement of ^{14}C and ^{3}H in intestinal sections. ^{14}C (filled symbols, panel B) was derived from 7-ketocholesterol and ^{3}H (open symbols, panel A) was derived from cholesterol in each section of intestine. Intestines were divided into small intestine (squares), caecum (diamonds), and large intestine (circles) and then whole homogenates of each were solubilized and assayed for radioactivity. Data are derived from Experiment 2. Values are expressed as mean + SD (3 rats per time-point) except t = 1 h value expressed as mean + range (n = 2).

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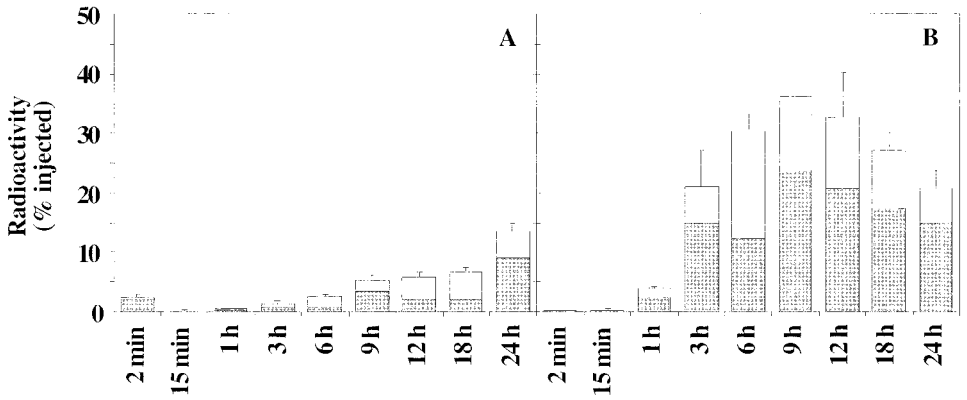

Fig. 7. A greater portion of ^{14}C (panel B) was distributed into the aqueous fraction (filled) of the intestine after lipid extraction than in the lipid-soluble portion (open). This was conducted as a measure of the extent of conversion to bile acids. Data for ^{3}H are presented in panel A. After lipid extraction of intestinal homogenates (34) an aliquot of the lipid-soluble phase was evaporated under vacuum, redissolved in solvent, and assayed for radioactivity (see Methods for details). The radioactivity in the aqueous portion was determined by difference from the total radioactivity detected in the intestine (Fig. 5). Data are derived from Experiment 2. The total value is expressed as mean + SD (3 rats per time-point) except t = 1 h value expressed as mean + range (n = 2).

proach which maximized tissue uptake and minimized exchange of the labels prior to uptake. In this respect, rats have the advantage of lacking cholesteryl ester transfer protein thereby minimizing the transfer of labeled steryl esters to other plasma lipoproteins before tissue uptake. Data from plasma, erythrocytes (Fig. 1) and HDL (Fig. 2) showed that there was little reappearance of [^{14}C]-7-ketocholesterol relative to [^{3}H]cholesterol into the circulation. However, there was no concomitant accumulation of [^{14}C]-7-ketocholesterol in liver (Fig. 4) or extrahepatic tissues (aorta (Fig. 3), heart, lungs, spleen, kidneys (Table 2). In fact, [^{14}C]-7-ketocholesterol was efficiently metabolized by the liver, appearing in the intestine mostly as polar products from where it was eventually excreted. Thus 7-ketocholesterol, a major oxysterol in the human diet (12) and atherosclerotic lesions (reviewed in ref. 1), undergoes rapid hepatic metabolism and elimination in vivo.

Tissue uptake and reappearance of sterols into the circulation

In accordance with others using [^{3}H]cholesteryl ester-labeled acLDL injected into rats (18, 19), the bulk of the radiolabeled steryl esters was cleared from the circulation in a matter of minutes by the liver. These esters were then hydrolyzed as demonstrated by the appearance within minutes of unesterified 7-ketocholesterol and cholesterol in plasma (data not shown) and of labels in the erythrocytes (Figs. 1B and 1D; presumably in the unesterified form, as erythrocytes are unable to accommodate steryl esters). Hydrolysis of 7-ketocholesteryl esters was also observed in the liver (Fig. 5). The initial transient increase of ^{14}C in plasma and erythrocytes is consistent with reports that 7-ketocholesterol (15) and other oxysterols (38) are transferred between lipophilic membranes at much greater rates than that of cholesterol itself. The fact that very little ^{14}C was present in the circulation at 24 h (in contrast to ^{3}H) suggests that the subsequent clearance of 7-ketocholesterol was rapid and that enterohepatic recirculation of 7-ketocholesterol or metabolites was negligible compared with that of cholesterol.

Rapid hepatic metabolism of 7-ketocholesterol

We have shown that, relative to cholesterol, 7-ketocholesterol is rapidly cleared from the liver by metabolism and excretion of metabolites into the intestine (Figs. 4–7), the most probable route being via the bile duct.

Similar findings have been presented from in vivo investigations where it was shown that an intraperitoneal injection in rats of an emulsion containing [^{3}H]-7-ketocholesterol and serum albumin was partially metabolized in 48 h and excreted into the bile (39). Another study showed that 40% of [^{3}H]-7-ketocholesterol was converted to polar compounds and appeared in the bile using a perfused rat liver system (40). Both of these groups demonstrated the presence of a large proportion of unusual bile acids while the latter showed also some similarity with normal rat bile salts (40). It has long been established that various species (including rats (41) and humans (42, 43)) produce 7-keto bile acids although these are detected in the bile in very small amounts (44, 45), suggesting that they are efficiently reduced to 7α- and 7β-derivatives. Therefore, our finding of rapid hepatic metabolism of 7-ketocholesterol is supported by previous (often overlooked) studies demonstrating metabolism of 7-ketocholesterol in vivo and ex vivo.

Other metabolites of 7-ketocholesterol are apparent in the lipid-soluble fraction of the liver when separated by TLC (Fig. 5). Presumably, these products are early intermediates in the pathway for the degradation and disposal of 7-ketocholesterol as they are lipid-soluble and have not yet been excreted from the liver. We have tentatively identified two of these products as 7β-hydroxycholesterol and cholesterol by TLC. It is well documented that 7β-hydroxy-

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cholesterol is produced from 7-ketocholesterol in a variety of models (46). These include perfused rat liver (40), rat liver homogenate (39) or microsomes (47), and human fibroblasts (48). Cholesterol also has been identified as a product of 7-ketocholesterol in a number of these studies (40, 47, 48). However, we are the first to demonstrate rapid hepatic metabolism of 7-ketocholesterol relative to cholesterol with an intact animal model. For instance, we have shown that reduction of 7-ketocholesterol to 7β-hydroxycholesterol occurs within minutes of hepatic uptake (Fig. 5), implying an enzymic rather than a chemical reaction.

Implications for dietary oxysterols

These results have important implications for the relevance of dietary oxysterols that have been proposed to be a source of oxysterols in atherosclerotic plaque. However, there are some aspects of the model used that warrant consideration before these findings can be extrapolated to dietary oxysterols in humans.

Dietary oxysterols, including 7-ketocholesterol, have been shown to be absorbed and incorporated into chylomicrons in rats (11) and humans (9). The bulk of chylomicron remnants are cleared by hepatocytes (49, 50) while acLDL is cleared mainly by hepatic endothelial cells (19, 51). However, radiolabeled cholesterol delivered in acLDL in esterified form is rapidly and efficiently transported from endothelial cells to hepatocytes so that by 2 h the majority of the label was associated with hepatocytes (19). This is consistent with our results for 7-ketocholesterol shown in Fig. 7 where it is clear that appreciable amounts of ^{14}C derived from 7-ketocholesterol appear in the intestine only after 3 h. That is, within 3 h there presumably has been sufficient transfer of 7-ketocholesterol from the endothelial cells to allow metabolism and excretion by the hepatocytes. Furthermore, reports from other studies in rats indicate that radiolabeled cholesterol and cholesteryl esters within chylomicron remnants (49, 50) are processed at a greater rate than their counterparts in acLDL (18, 19) as determined by the appearance of radiolabel in the bile. Thus, 7-ketocholesterol in chylomicron remnants may be metabolized even faster than our results indicate.

We administered a maximum of 1.53 μg radiolabeled 7-ketocholesterol to rats of approximately 440 g which equates to 240 μg in a 70 kg human (or 0.05% of cholesterol for a human consuming 500 mg cholesterol per day). It could be argued that at higher levels of intake, dietary oxysterols could overwhelm hepatic clearance mechanisms. However, at high dietary levels, 7-ketocholesterol feeding to rats lead to a rapid development of tolerance in terms of normalization of two important enzymes involved in cholesterol metabolism. Inhibition of hepatic HMG-CoA reductase (40) and cholesterol 7α-hydroxylase (47) was overcome and this was associated with an increase in total hepatic cytochrome P450 content (40) and 7-ketocholesterol metabolism (40, 47). Therefore it appears that there are mechanisms in place to efficiently deal with any increase in 7-ketocholesterol intake.

This and previous studies in rats indicating rapid hepatic metabolism of 7-ketocholesterol may apply to other species including rabbits and humans. In a recent study in which 70 mg of mixed oxysterols (35% 7-ketocholesterol) was injected intravenously into rabbits over a period of 70 days (52), it was found that there was only a slight accumulation of oxysterols in the livers which accounted for only ~5% of the total injected oxysterols (based on 2.5 kg rabbit and liver weight 30 g per kg body weight (53)). Moreover, we have demonstrated that 7-ketocholesterol is more rapidly metabolized than cholesterol by the human hepatoma cell line, Hep G2, so that by 24 h 20 ± 1% (mean ± SD of 3 cultures) of added [^{14}C]-7-ketocholesterol was metabolized to aqueous-soluble products compared with only 3.0 ± 0.2% (mean ± SD of 3 cultures) of [^{14}C]cholesterol (M. A. Lyons and A. J. Brown, unpublished observations). Thus, the present findings in rats may also be relevant to humans.

Our results showing that 7-ketocholesterol does not accumulate in rat aorta are supported by two recently published studies that investigated the role of oxysterols in atherogenesis in rabbits, a widely used model for atherosclerosis. In both studies (one in which a very sensitive method of detection was used) no accumulation of oxysterols was detected in the aorta but there was a significant increase in aortic cholesterol content (54). In view of the conversion of 7-ketocholesterol to cholesterol we (Fig. 5) and others (40, 47, 48) have demonstrated, it is interesting to speculate whether any of the observed increase in aortic cholesterol content was derived from oxysterols. Both of these studies in rabbits, in which oxysterols were chronically administered, are consistent with the lack of accumulation of 7-ketocholesterol in the rat aorta we have observed acutely, using a more direct radiolabeled approach. This suggests that the diet is not the major source of 7-ketocholesterol in atherosclerotic lesions.

Conclusions

This is the first study to measure the distribution of a radiolabeled oxysterol relative to cholesterol within the circulation and tissues by their simultaneous delivery in a whole animal model. In summary, we have shown that [^{14}C]-7-ketocholesteryl oleate was rapidly taken up by the liver and subjected to hydrolysis thereby releasing unesterified 7-ketocholesterol. Subsequent metabolism of [^{14}C]-7-ketocholesterol led to the production of aqueous-soluble polar metabolites which were then secreted into the intestine presumably via the bile duct for eventual excretion (**Fig. 8**). We are currently in the process of elucidating the primary pathway(s) by which 7-ketocholesterol is metabolized by liver cells.

A re-evaluation of the literature in light of the results herein indicates applicability beyond this rat model in terms of mode of delivery, dose, and duration. We argue that 7-ketocholesterol delivered in chylomicrons will be metabolized at a similar or even greater rate and introduce data that this rapid metabolism also occurs in human hepatocytes. On the basis of our findings we propose that the role of exogenous 7-ketocholesterol (and perhaps dietary oxysterols in general) in the development of atherosclerosis may have been overstated, thereby making the

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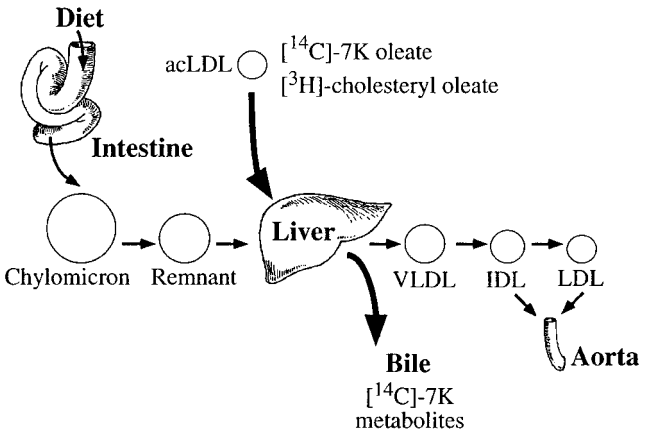

Fig. 8. Acetylated LDL labeled with the oleate ester of [^{14}C]-7-ketocholesterol is taken up by the liver and then excreted from the liver, presumably via the bile duct, into the intestine as bile acids. Therefore it is not available for resynthesis into lower density lipoproteins or accumulation in tissues such as aorta, a prime site for the development of atherosclerosis. The diet has been proposed as a source of oxysterols, including 7-ketocholesterol, found in plaque. However, we hypothesize that 7-ketocholesterol that is incorporated into chylomicrons will be cleared by the liver as chylomicron remnants and rapidly metabolized by the liver for excretion into the intestine. This may indicate that the role of dietary 7-ketocholesterol, and perhaps oxysterols in general, as a source for atherosclerotic plaque may have been overstated.

contribution of 7-ketocholesterol formed in vivo likely to be a more important source of this oxysterol in atherosclerotic plaque.

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