Free Cholesterol Exchange *in Vitro*: A Comparison of Endogenous and Exogenous Cholesterol[1] (34612)

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There have been relatively few studies directed toward assessment of the comparative physiological behavior of exogenous and endogenous cholesterol. Hellman *et al.* (1) reported that, in man, dietary cholesterol is mixed indistinguishably with cholesterol of biosynthetic origin. Our own studies in the baboon (2) have demonstrated that the serum α- and β-lipoprotein show no preference in the transport of exogenous or endogenous cholesterol. In the course of the same work it was found that the half-life ($t_{1/2}$) of disappearance of cholesterol from the serum was the same for cholesterol of dietary or biosynthetic origin. On the other hand, Sodhi (3) has suggested that, in the rat, the biological half-lives of endogenous and exogenous cholesterol may differ.

To further evaluate this problem we have investigated free cholesterol exchange in two systems *in vitro*. The two parameters studied were plasma lecithin–cholesterol acyltransferase (LCAT) (4) and the exchange of plasma free cholesterol with red cell free cholesterol (5). These two systems could yield information on the behavior of endogenous and exogenous cholesterol in an enzymically catalyzed exchange reaction between plasma free cholesterol and phospholipid, as well as on the exchange of free cholesterol between plasma and the lipoprotein of the red cell membrane.

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Materials and Methods. All labeled substrates were purchased from the New England Nuclear Corp., Boston, Mass. Mevalonic acid (2-^{14}C-DL-mevalonic acid) was obtained as the dibenzylethyldiamine (DBED) salt and was prepared as the free acid according to the method of Purcell *et al.* (6). Tritium-labeled cholesterol (7α-^{3}H or 1,2-^{3}H) was at least 97% radiopure.

Male Wistar rats were used in all experiments. The rats were starved for 24 hr prior to isotope administration but were permitted free access to water. The labeled substrates were administered by gavage. The 2-^{14}C-DL-mevalonic acid and the sodium 1-^{14}C-acetate were dissolved in distilled water. The tritiated cholesterol (1.0 mCi corresponding to 15–30 μg) was dissolved in propylene glycol (0.2 ml).

The rats were given laboratory chow 8 hr after administration of the isotopically labeled materials. At 24 or 72 hr after administration of the ^{3}H-cholesterol and the carbon-labeled mevalonate or acetate the rats were sedated with sodium pentobarbital and blood was drawn from the abdominal aorta into a syringe containing an anticoagulant (acid citrate dextrose, ACD). The blood:ACD ratio was maintained at 5:1. An equal number of control rats were treated in a similar fashion. The blood was centrifuged at 350*g* for 15 min in a refrigerated centrifuge. The red blood cells (RBC) were resuspended in an equal volume of 0.9% saline containing 0.2% sodium EDTA and centrifuged at 250*g* for 10 min. This procedure was repeated twice.

An aliquot of the plasma and red blood cells obtained was set aside as the zero-time control. To study the LCAT enzyme, plasma was incubated with shaking for 24 hr at 37°. For studies of plasma–RBC cholesterol ex-

TABLE I. Exchange of Endogenous (^{14}C) and Exogenous (^{3}H) Free Cholesterol between Plasma and Red Blood Cells (RBC) and in Lecithin Cholesterol Acyltransferase (LCAT) Reaction.

Exp.	Substrate	Sampling time (hr after feeding)	Length of incubation (hr)	$^{3}H/^{14}C$ (dpm)[a] Plasma[b] free cholesterol (range)[d]	$^{3}H/^{14}C$ (dpm)[a] RBC[c] free cholesterol (range)
1	2-^{14}C-Mevalonic acid (36 μCi)	24	0	1.29 (1.25–1.33)	1.08 (1.07–1.09)
	7α-^{3}H-Cholesterol (70 μCi)		24	1.39 (1.36–1.42)	1.11 (1.07–1.16)
			LCAT (24)	1.39 (1.33–1.44)	—
2	2-^{14}C-Mevalonic acid (32 μCi)	24	0	0.29 (0.26–0.35)	0.23 (0.22–0.25)
	7α-^{3}H-Cholesterol (100 μCi)		6	0.48 (0.45–0.48)	0.24 (0.21–0.26)
			LCAT (24)	0.24 (0.21–0.25)	—
3	2-^{14}C-Mevalonic acid (36 μCi)	72	0	0.19 (0.14–0.24)	0.23 (0.20–0.27)
	7α-^{3}H-Cholesterol (143 μCi)		6	0.21 (0.20–0.23)	0.23 (0.20–0.29)
			24	0.20 (0.18–0.23)	—
			LCAT (24)	0.22 (0.18–0.29)	—
4	Sodium-1-^{14}C-acetate (60 μCl)	72	0	2.04 (1.94–2.19)	2.20 (2.17–2.22)
	1,2-^{3}H-Cholesterol (100 μCi)		6	—	2.07 (1.97–2.10)
			LCAT (24)	1.98 (1.94–2.01)	—

[a] Average of two determinations.
[b] Incubation of labeled plasma and unlabeled RBC.
[c] Incubation of labeled RBC and unlabeled plasma.
[d] Range of values includes all incubations.

change, either labeled plasma and control RBC or control plasma and labeled RBC were reconstituted to a plasma:RBC ratio similar to that of the original pool. These plasma–RBC suspensions were incubated at 37° for either 6 or 24 hr. In this series of experiments sodium *p*-hydroxymercuribenzoate (0.001 *M*) was added to all the incubation mixtures to inhibit LCAT activity. At the end of the incubation period the plasma and RBC were separated and the RBC washed with cold 0.9% saline. The incubation component which had originally been labeled was taken for determination of the cholesterol $^{3}H/^{14}C$ ratio. The free cholesterol was isolated as the digitonide, following the procedure of Sperry and Webb (7), and the digitonide assayed directly (8) in a Packard liquid scintillation spectrometer, Model 314X. The tritium and carbon-14 activity in each sample was calculated using the screening method of Okita *et al.* (9). Two separate incubations were carried out for each determination. The reaction mixtures were extracted and several aliquots of each extract were used for determination of $^{3}H/^{14}C$ ratios.

Results and Discussion. The results of four different experiments are summarized in Table I. The DPM data are presented in Table II. In the first experiment, in which ten 200-g rats were used, the $^{3}H/^{14}C$ ratio was 1.29 in the plasma free cholesterol and 1.08 in the RBC free cholesterol. After 24 hr of incubation of labeled plasma with unlabeled RBC or vice versa, the free cholesterol $^{3}H/^{14}C$ ratio was virtually unchanged in either plasma or RBC. The radioactivity ratio in the plasma free cholesterol after the LCAT enzyme had been permitted to act for 24 hr was also unchanged. In the second experiment using nine 230- to 260-g rats the starting free cholesterol $^{3}H/^{14}C$ ratios of plasma and RBC were similar, and incubation did not affect the ratios in the RBC after 6 hr nor after LCAT action of 24 hr. In the labeled plasma–control RBC incubation, a rise in the free cholesterol $^{3}H/^{14}C$ ratio was observed, but in light of the other data

this is thought to be an anomalous result. In Expt. 3 the seven rats (400 g) were not killed until 72 hr after isotope administration. Here again, the free cholesterol $^{3}H/^{14}C$ ratios in plasma and RBC were similar. The ratio of hydrogen to carbon radioactivity in the plasma free cholesterol did not change after 6 or 24 hr of incubation with unlabeled RBC. The action of LCAT did not alter the serum free cholesterol $^{3}H/^{14}C$ ratio. The results observed on incubation of labeled RBC with control plasma were similar to those

TABLE II. DPM Present in Endogenous (^{14}C) and Exogenous (^{3}H) Free Cholesterol in Study of Exchange between Plasma and Red Blood Cells (RBC) and in Lecithin Cholesterol Acyltransferase (LCAT) Reaction.

			$^{3}H/^{14}C$ (dpm)			
Exp.	Substrate	Length of incubation (hr)	No. aliquots counted	Plasma[a] free cholesterol	No. aliquots counted	RBC[b] free cholesterol
1	2-^{14}C-Mevalonic acid	0	6	2826 ± 87[c]	4	10666 ± 71
				2192 ± 71		9898 ± 60
	7α-^{3}H-Cholesterol	24	3	811 ± 95	6	5238 ±175
				585 ± 74		4734 ±152
		LCAT (24)	4	2198 ±104		—
				1575 ± 50		
2	2-^{14}C-Mevalonic acid	0	6	1309 ± 95	3	5842 ±290
				4482 ±252		24981 ± 62
	7α-^{3}H-Cholesterol	6	4	445 ± 23	4	5236 ±387
				930 ± 33		23865 ±136
		LCAT (24)	8	7223 ±136		—
				30438 ±280		
3	2-^{14}C-Mevalonic acid	0	3	404 ± 57	8	920 ± 35
				2117 ± 3		4012 ± 53
	7α-^{3}H-Cholesterol	6	4	114 ± 10	7	1405 ± 84
				552 ± 45		6268 ± 52
		24	4	176 ± 23		—
				885 ± 32		
		LCAT (24)	4	70 ± 9		—
				316 ± 7		
4	Sodium-1-^{14}C-acetate	0	8	586 ± 11	7	1072 ± 36
				288 ± 2		519 ± 17
	1,2-^{3}H-Cholesterol	6		—	8	2462 ± 15
						1122 ± 7
		LCAT (24)	4	688 ± 53		—
				348 ± 25		

[a] Incubation of labeled plasma and unlabeled RBC.
[b] Incubation of labeled RBC and unlabeled plasma.
[c] Standard error.

seen in the two previous experiments. In the fourth experiment we used different labeled substrates (sodium 1-^{14}C acetate and 1,2-^{3}H-cholesterol) but results for the LCAT experiment were similar to those of the other experiments. In this experiment seven 350-g rats were used, and they were killed 72 hr after the labeled substrates had been administered.

The ratio of free cholesterol $^{3}H/^{14}C$ found in the plasma and RBC of the animals used in the four experiments is variable (0.19–2.04). This variability might be attributed to the fact that pooled blood was used and individual variabilities or impaired absorption in one or two rats per group could affect the overall average. It is of interest, however, that when the ratio of administered $^{3}H/^{14}C$ was relatively low (1.67 in Expts. 1 and 4), the final plasma or RBC free cholesterol $^{3}H/^{14}C$ was high. The substrate $^{3}H/^{14}C$ ratios in Expts. 2 and 3 were 3.13 and 4.00, respectively, and the final plasma and RBC free cholesterol $^{3}H/^{14}C$ ratios were low. We have no explanation for this observation which suggests that at a high ratio of cholesterol to cholesterol substrate more of the latter appears in plasma and RBC.

These experiments demonstrate that in the rat, as in man and the baboon, there is no discrimination in the plasma or RBC membrane lipoprotein between endogenous and exogenous free cholesterol. This is true both for plasma–RBC exchange and for esterification of plasma free cholesterol by the LCAT enzyme. Our experiments on the incorporation of free and ester cholesterol into baboon serum α- and β-lipoprotein (2, 10) suggest that if there is a differential utilization of exogenous or endogenous cholesterol, it probably occurs in the serum cholesterol esters in the very early stages of cholesterol synthesis or absorption.

It would be of interest to determine the influence of hypercholesteremia upon the plasma–RBC free cholesterol exchange. Cholesteremia due to cholesterol feeding (which inhibits cholesterol biosynthesis (11) or Triton WR-1339 injection [which enhances cholesterogenesis (12)] have been found to inhibit the LCAT reaction (13).

Summary. The exchange of free cholesterol of endogenous or exogenous origin between plasma and red blood cells has been studied. Using tritiated cholesterol and ^{14}C-mevalonic acid or sodium-1-^{14}C-acetate, it has been shown that after incubation of labeled plasma with control RBC or of labeled RBC with control plasma, the free cholesterol $^{3}H/^{14}C$ ratio in plasma or RBC is unchanged. This is true for incubation of 6 or 24 hr. In a parallel series of experiments it was observed that the plasma $^{3}H/^{14}C$ free cholesterol ratio was unchanged after 24 hr of incubation in the presence of native lecithin cholesterol acyl transferase. The data confirm the findings in man and baboon which suggest that it is not possible to distinguish between plasma free cholesterol of endogenous or exogenous origin.

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