In vitro incorporation of cholesterol-^{14}C into very low density lipoprotein cholesteryl esters

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ABSTRACT The cholesteryl esters of very low density lipoproteins become labeled when human plasma is incubated with cholesterol-^{14}C. The relative order of magnitude of the specific activity of the cholesteryl esters of the major lipoprotein fractions is: high density lipoproteins $\gg$ very low density lipoproteins $>$ low density lipoproteins. This pattern of labeling is similar to that found by others in experiments performed in vivo. Very low density lipoprotein cholesteryl esters are probably not formed by direct action of the plasma lecithin:cholesteryl acyltransferase, since significant esterification of cholesterol does not occur when very low density lipoproteins are incubated separately with the enzyme. Instead, labeled cholesteryl esters formed in the other lipoprotein fractions transfer to the very low density lipoproteins, the relative amount of monounsaturated esters transferred being slightly greater than that of saturated and polyunsaturated esters. The results support the possibility that the acyltransferase indirectly increases the concentration of very low density lipoprotein cholesteryl esters in vivo.

KEY WORDS lipoproteins · very low density · low density · high density · lecithin:cholesterol acyltransferase · cholesteryl esters · transfer · human plasma

EVIDENCE IS ACCUMULATING that the lecithin:cholesterol acyltransferase reaction is an important source of human plasma cholesteryl esters (1). The reaction has been most strongly implicated (2) in the formation of cholesteryl esters of high density lipoproteins (HDL), whereas the importance of the reaction is less clear for the low and very low density lipoproteins (LDL and VLDL). In fact, studies in the rat have suggested that VLDL cholesteryl esters are mainly formed in the liver (3, 4). Nevertheless, the metabolism of VLDL cholesteryl esters appears to differ in rats and humans (5, 6), and the possibility remains that the acyltransferase reaction is a major source of VLDL cholesteryl esters in man. Our purpose in the present investigation was to explore this possibility by studying the incorporation of labeled cholesterol into human VLDL cholesteryl esters in vitro.

MATERIALS AND METHODS

Plasma was prepared from the blood of fasting human males, with 0.005 μmole of EDTA per ml of whole blood as an anticoagulant.

Cholesterol-4-^{14}C (New England Nuclear Corp., Boston, Mass.) was purified by thin-layer chromatography (2) until radioactive contaminants having the mobility of cholesteryl esters comprised less than 0.02% of the total. However, when the purified tracer was chromatographed with mixtures of neutral lipids, a higher proportion of the radioactivity always accompanied the cholesteryl esters. Albumin-stabilized emulsions of labeled cholesterol were prepared (7), stored overnight at 4°C, and used in incubation experiments on the following day. In most experiments, approximately 1.16×10^5 dpm of the labeled emulsion was added per ml of incubation mixture. The incubations were performed in plastic bottles in a Dubnoff shaker at 37°C. Each experiment included a control in which labled, emulsified cholesterol was incubated with appropriate lipoproteins and acyltransferase that had been blocked by the addition of 2 μmoles of PCMPS per ml of incubation mixture. This was done to allow correction for labeled contaminants in the cholesteryl ester fractions.

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein(s) ($d < 1.006$); LDL, low density lipoprotein(s) ($1.006 < d < 1.063$); HDL, high density lipoprotein(s) ($1.063 < d < 1.21$, sometimes $d > 1.063$); PCMPS, *p*-chloromercuriphenylsulfonate.

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Albumin used in the experiments with purified enzyme was pretreated with charcoal according to Chen (8). This procedure reduced the lysolecithin content of the albumin (human plasma albumin, Cohn fraction V, Calbiochem, Los Angeles, Calif.) from 0.53 to 0.01 μmole/g.

Ultracentrifugation

VLDL were prepared by centrifugation in a No. 40 rotor of a Spinco model L ultracentrifuge. The plasma was layered under 1 volume of a solution of NaCl in 0.01 M EDTA that had pH 7.4 and density 1.006 g/ml, and the tubes were centrifuged for 22 hr at 40,000 rpm. When VLDL was prepared from the various incubation media, the densities of the latter were adjusted to that of plasma by the addition of solid KBr, and the media were layered under saline–EDTA and centrifuged as in the case of plasma. After centrifugation, the tubes were sliced 1.5 cm from the top, and the lipoproteins in the supernatant fraction were washed by recentrifugation. A fraction containing predominantly LDL was prepared from the VLDL infranatant fraction by addition of solid KBr to a final density of 1.080 g/ml and centrifugation for 22 hr at 40,000 rpm. Again the tubes were sliced 1.5 cm from the top and the supernatant lipoproteins were washed by recentrifugation at a density of 1.063 except in the experiments shown in Tables 1 and 4, where the density of the washing solution was 1.080. Finally, HDL were prepared by the addition of solid KBr to the LDL infranate to a final density of 1.21 g/ml and centrifugation for 48 hr at 40,000 rpm. In some experiments LDL and HDL were separated by chromatography on hydroxylapatite as reported earlier (2), except that after elution of the HDL with 0.25 M potassium phosphate buffer, LDL lipids were directly eluted from the columns with 10 volumes of 1:1 chloroform–methanol per column volume.

Preparation of Acyltransferase

Lecithin:cholesterol acyltransferase (LCAT) was prepared from human plasma by a slight modification of the procedure described earlier (9). The plasma was chromatographed on DEAE-cellulose and precipitated by the addition of ammonium sulfate as before. However, the LCAT was then further purified by the addition of solid KBr to a final density of 1.25 g/ml and centrifugation for 48 hr at 38,000 rpm in a No. 40.3 rotor of a Spinco model L ultracentrifuge. The tubes were sliced 1.5 cm from the top and the infranatant solution was dialyzed against 0.02 M potassium phosphate buffer, pH 7.4, and chromatographed on hydroxylapatite in the same buffer (approximately 200 ml of hydroxylapatite was used for the infranatant fraction prepared from 1 liter of original plasma). The eluate from the hydroxylapatite column contained 15–20% of the LCAT activity of the original plasma, but only traces of cholesterol or lecithin. The procedure resulted in a 40- to 200-fold purification.

Gel Filtration

Pearl-condensed agarose was prepared by the method of Bengtsson and Philipson (10), except that the Agar Noble (Difco Laboratories, Detroit, Mich.), was first purified as follows. 1 liter of a 3% agar solution in water was prepared by autoclaving at 120°C and 20 atm for 10 min. The solution was allowed to cool to 75°C, 140 g of Dowex 1 × 2 was added, and the mixture was stirred for 15 min on a hot plate. Subsequently, the mixture was filtered through a warmed glass filter and applied to a 140 g column of Dowex 1×2 equipped with a water jacket at 65°C. The agarose was washed through the column with hot water, the gelled effluent was frozen and thawed to facilitate the removal of excess water by decantation, and the residue was lyophilized. This procedure reduced the sulfur content of the agar from approximately 0.3% to 0.08–0.1% as measured by the method of Gustafsson (11, 12). 3.5% agarose pearls were made (10), fine particles were removed by flotation, and particles of approximately 50–200 μ diameter were collected by passage through a U.S. standard sieve, size 140. The particles were then stored in Tris–EDTA–NaCl (2) at 4°C.

Gel filtration experiments were performed at 6°C in plastic columns: 132 × 4.4 cm for the whole plasma experiments, and 50 × 2.4 cm for the VLDL subfractionation experiments. The sample sizes applied to the columns were 33 and 2.7 ml, respectively. Tris–EDTA–NaCl buffer (2) was used for the filtration experiments. Void and total buffer volumes were determined by using large particles of india ink and of N-ethylmaleimide, respectively. The elution volumes and yields of VLDL and LDL were determined for lipoprotein samples that had been prepared by ultracentrifugation. Recoveries of VLDL cholesterol varied from 56 to 75% when the total amounts of VLDL cholesterol applied to 50 × 2.4 cm columns of agarose were 3.4–32.6 μmoles. Recoveries of LDL cholesterol varied from 77 to 80% when the total amounts of LDL cholesterol applied to columns of the same size were 6.2–27.5 μmoles. The low recoveries of cholesterol were probably caused by adsorption of LDL and especially VLDL to residual sulfated polysaccharide present in the agarose. Attempts to reduce this adsorption by removing the residual sulfur were unsuccessful. Although agarose that contained 0.01–0.02% sulfur could be prepared by further treatment with Dowex 1, this agarose was not suitable for filtration experiments because it no longer formed rigid gels.

Analytical Methods

The following analytical procedures were used. Protein was determined by measurement of the absorbance at 280

mμ. Lipids were extracted, fractionated, and analyzed as described previously (2). Cholesteryl esters were subfractionated on thin-layer plates of $AgNO_3$-coated silica gel by a method similar to that of Morris (13) except that the samples were applied to the plates and the latter developed under N_2. Areas of silica gel containing saturated and mono-, di-, and tetraunsaturated cholesteryl esters were saponified in 1 N ethanolic KOH and analyzed by the same procedure used to determine total esterified cholesterol (2). Triglyceride glycerol was analyzed as follows: areas of silica gel containing triglyceride were scraped from the plates, eluted with chloroform, saponified in 1 N KOH in ethanol (70°C, 30 min), and analyzed for glycerol by the enzymic method of Wieland (14).

Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer with Liquifluor (Pilot Chemicals, Inc., Watertown, Mass.) as the counting solution.

RESULTS

Experiments with Whole Plasma

Three experiments were performed in which samples of fresh human plasma were incubated for 1 hr at 37°C with albumin-stabilized emulsions of cholesterol-^{14}C. The VLDL, LDL, and HDL were subsequently fractionated by ultracentrifugation and chromatography on hydroxylapatite. The purpose of these experiments was to determine the relative extent of incorporation of labeled cholesterol into VLDL cholesteryl esters in vitro. The results are shown in Table 1. The proportions of the total radioactive cholesteryl ester in the VLDL, LDL, and HDL were 1–5%, 22–32%, and 67–75%, respectively. On the other hand, the specific activity of the VLDL cholesteryl esters was intermediate between those of the HDL and the LDL. This pattern of incorporation is very similar to that reported to occur after the in vivo injection of labeled mevalonate in normal humans (5).

Fig. 1 shows the results of another type of experiment in which plasma was incubated with cholesterol-^{14}C for 1 hr at 37°C, and the plasma lipoproteins were fractionated by gel filtration on 3.5% agarose. The upper part of the figure shows the amount of VLDL (first peak) and LDL triglyceride in the effluent from the agarose column (HDL triglyceride was not measured in this experiment). The lower part of the figure shows the cholesteryl ester specific activity in the fractions corresponding to VLDL, LDL, and HDL (LDL and HDL were separated from each other by chromatography on hydroxylapatite). Again, the specific activity of the VLDL cholesteryl esters appeared to be intermediate between those of the HDL and LDL cholesteryl esters; moreover, it varied as a function of elution volume. To decrease contamination of the VLDL subfractions by LDL, we performed a second agarose experiment in which the VLDL were isolated by ultracentrifugation before being filtered through agarose. The results of this experiment are shown in Fig. 2 and Table 2. The relation between cholesteryl ester specific activity and elution volume of VLDL is similar to that shown in Fig. 1. The triglyceride and cholesterol contents of the VLDL subfractions are presented in Table 2. The ratios of cholesteryl esters to unesterified cholesterol and cholesterol esters to triglyceride increase (after the initial peaks) with elution volume. This suggests that the relation noted above between cholesteryl ester specific activity and elution volume may be due to the presence of larger amounts of unlabeled cholesteryl esters in the more slowly eluted VLDL.

TABLE 1 INCORPORATION OF CHOLESTEROL-^{14}C INTO LIPOPROTEIN CHOLESTERYL ESTERS DURING INCUBATION OF WHOLE PLASMA

Expt. No.	Lipoprotein Fraction	Amount	Cholesteryl esters: Specific Activity*	Cholesteryl esters: Relative Specific Activity†
		μmoles/fraction	*dpm/μmole*	
1	HDL	8.9	4070	1.0
	LDL	25.4	440	0.11
	VLDL	2.3	1080	0.26
2	HDL	4.9	3970	1.0
	LDL	11.9	470	0.12
	VLDL	1.0	940	0.24
3	HDL	6.2	4190	1.0
	LDL	18.1	690	0.16
	VLDL	0.4	1260	0.30

In experiments 1 and 2, 11.6×10^5 dpm of emulsified cholesterol-4-^{14}C was incubated with 10 ml plasma for 1 hr at 37°C. In experiment 3, 8.1×10^5 dpm of labeled cholesterol was incubated with 7 ml plasma. The acyltransferase reaction was blocked by the addition of 2 μmoles of PCMPS per ml of plasma. VLDL were prepared by ultracentrifugation; HDL and LDL were separated by chromatography of the VLDL infranatant solution on hydroxylapatite. Control samples of plasma were incubated with identical amounts of labeled cholesterol in the presence of PCMPS, and then fractionated by the same procedure used for the test samples. This facilitated correction for contamination of the test samples by the emulsified, labeled cholesterol, 12%, 62%, and 26% of which was in the VLDL, LDL, and HDL fractions, respectively.

* Calculated after subtraction of control values (mean control dpm for HDL, LDL, and VLDL were 1.5, 6.1, and 1.8% of test samples).

† (Specific activity of lipoprotein cholesteryl esters)/(specific activity of HDL cholesteryl esters).

Incubation of VLDL with Partially Purified Lecithin:Cholesterol Acyltransferase

To determine whether VLDL cholesterol can be esterified through direct action of LCAT on the lipoprotein, VLDL were prepared by ultracentrifugation and incubated for 24 hr with partially purified enzyme. For comparison

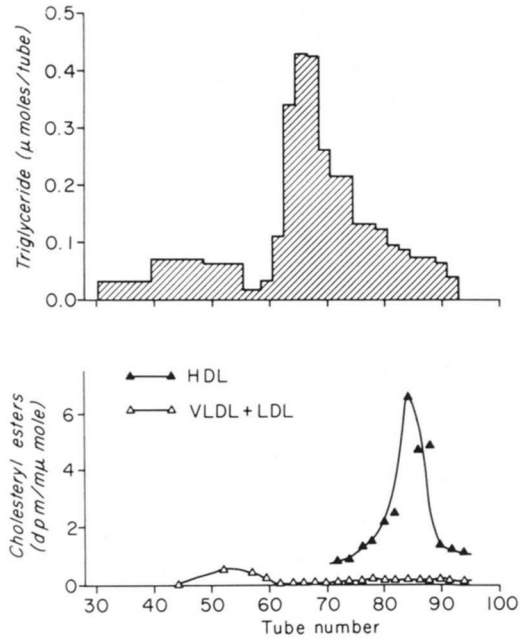

FIG. 1. Gel filtration of incubated whole plasma on 3.5% agarose. 33 ml of fresh plasma was incubated with 3.84 × 10^6 dpm of emulsified cholesterol-4-^{14}C for 1 hr at 37°C. Subsequently, the acyltransferase reaction was blocked by the addition of PCMPS, and the plasma was filtered through a column of agarose. The flow rate was about 30 ml/hr, and each fraction collected contained 21 ml. The labeled, emulsified cholesterol (not shown) essentially emerged with the void volume (735 ml; tube No. 35). The total buffer volume was 2016 ml (tube No. 96). After the filtration each effluent fraction was further chromatographed on a column of hydroxylapatite (30–50 ml total volume in column) to separate the HDL from VLDL and LDL. The upper part of the figure shows the triglyceride content of the VLDL (mainly in tubes 30–58) and LDL (tubes 59–92). The lower part of the figure shows the specific activity of the cholesteryl esters in the VLDL, LDL, and HDL.

parallel incubations with LDL and HDL were also performed. The results of two experiments of this type are shown in Table 3. The enzyme markedly affects the HDL and definitely affects the LDL but has no significant effect on the VLDL. Although these experiments are not strictly comparable with those shown in Table 1, they strongly suggest that little, if any, of the labeled cholesteryl esters of the VLDL were formed by direct action of the acyltransferase on these lipoproteins.

Transfer of Labeled Cholesteryl Esters from HDL to VLDL and LDL

Because of the failure of LCAT to esterify VLDL cholesterol, the possibility that labeled cholesteryl esters were transferring from HDL to VLDL and LDL was explored. In these experiments d > 1.063 plasma proteins were prepared by ultracentrifugation, dialyzed against Tris–EDTA–NaCl buffer, and incubated for 1 hr at 37°C with cholesterol-^{14}C–albumin. Subsequently, the acyltransferase reaction was blocked by the addition of PCMPS, VLDL or LDL were added, and the incubation was continued for an additional hour. Finally, the VLDL and LDL were separated from the HDL by ultracentrifugation and chromatography on hydroxylapatite, respectively. The results of two experiments of this type are shown in Table 4. Clearly, labeled cholesteryl esters are transferred from the HDL to VLDL and LDL. Furthermore, the specific activity of the VLDL cholesteryl esters exceeds that of the LDL cholesteryl esters, as in the experiments with incubation of whole plasma shown in Table 1. These results confirm and extend those of

TABLE 2 LIPIDS IN VLDL SUBFRACTIONS OBTAINED BY GEL FILTRATION ON 3.5% AGAROSE

Tubes Combined	UC*	CE*	TG*	CE/UC	CE/TG
		μmoles/tube			
18–21	12	20	50	1.6	0.40
23–28	44	53	246	1.2	0.21
29–31	260	280	656	1.1	0.38
32–35	370	429	700	1.2	0.61
36–39	350	471	651	1.4	0.72
40–45	171	290	378	1.7	0.77
46–53	40	89	74	2.2	1.21

Experimental details as for Fig. 2. The effluent in selected tubes was pooled as indicated before extraction and analysis.

* UC, CE, TG refer to unesterified cholesterol, cholesteryl ester, and triglyceride, respectively. Recovery of these lipids from the column was 65, 82, and 65%, respectively.

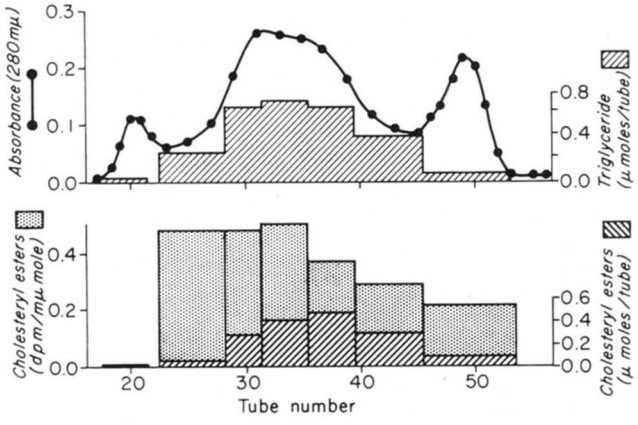

FIG. 2. Gel filtration of VLDL from incubated plasma on 3.5% agarose. 1.4 × 10^7 dpm of emulsified cholesterol-4-^{14}C was incubated with 120 ml of plasma for 1 hr at 37°C. The acyltransferase reaction was subsequently blocked by the addition of PCMPS, and VLDL were prepared by ultracentrifugation. The final volume of VLDL was 5.0 ml; 2.7 ml of this was applied to an agarose column. The void volume was 120 ml (tube No. 20) and the total buffer volume was 290 ml (tube No. 48). The flow rate was 5 ml/hr and 6.0 ml of effluent was collected in each tube. The upper part of the figure shows the absorbance of the effluent at 280 mμ as well as the triglyceride content of the effluent. The significance of the first and third absorbance peaks is not known. The lower part of the figure shows the content and specific activity of the cholesteryl esters of the same effluent fractions. Note that the bars representing the top of both the hatched and dotted columns should be read from the abscissa.

TABLE 3 INCUBATION OF UNLABELED LIPOPROTEINS WITH LCAT

Expt. No.	Lipoproteins Incubated	Unesterified Cholesterol 0 hr	Unesterified Cholesterol 24 hr	P*
		μmoles ± SEM (n = 6)		
1	HDL† + Tris–EDTA–NaCl	0.242 ± 0.009	0.247 ± 0.006	NS
	HDL† + LCAT	0.260 ± 0.013	0.065 ± 0.014	<0.001
	LDL + Tris–EDTA–NaCl	0.349 ± 0.007	0.348 ± 0.007	NS
	LDL + LCAT	0.365 ± 0.014	0.320 ± 0.008	<0.05
	VLDL + Tris–EDTA–NaCl	0.757 ± 0.014	0.745 ± 0.010	NS
	VLDL + LCAT	0.758 ± 0.012	0.747 ± 0.012	NS
2	HDL + LCAT + albumin	0.392 ± 0.006	0.078 ± 0.006	<0.001
	LDL + LCAT + albumin	1.21 ± 0.022	1.07 ± 0.024	<0.01
	VLDL + LCAT + albumin	0.354 ± 0.007	0.355 ± 0.008	NS

VLDL, LDL, and HDL were prepared by ultracentrifugation and dialyzed against Tris–EDTA–NaCl buffer. In experiment 1, HDL refers to d 1.063 infranate. In experiment 2, HDL refers to fraction of d = 1.063–1.21 washed once by recentrifugation. The individual lipoprotein fractions were incubated for 24 hr at 37°C. The incubation volumes were 1.0 ml each. The LCAT activity per ml was approximately equal to that of fresh plasma as judged by assay with heated substrate (8).

* The difference of the means was analyzed by Student's "t" test.

† LCAT contaminating the HDL was heat inactivated (56°C, 30 min) before the incubation.

TABLE 4 TRANSFER OF HDL CHOLESTERYL ESTERS TO VLDL AND LDL

Expt. No.	Lipoproteins Incubated	Lipoproteins Analyzed	Cholesteryl Esters Amount	Cholesteryl Esters Specific Activity*	Cholesteryl Esters Relative Specific Activity†
			μmoles/fraction	*dpm/μmole*	
1	HDL + buffer	HDL	1.23	8600	1.0
	HDL + LDL	HDL	1.58	8150	0.94
		LDL	7.06	380	0.04
	HDL + VLDL	HDL	7.76	8490	0.99
		VLDL	2.34	1590	0.19
2	HDL + buffer	HDL	1.97	14100	1.0
	HDL + LDL	HDL	1.57	12500	0.89
		LDL	4.16	1000	0.07
	HDL + VLDL	HDL	5.03	11400	0.81
		VLDL	1.72	6100	0.43

VLDL, LDL, and HDL (d > 1.063 g/ml) were prepared by ultracentrifugation, and dialyzed against Tris–EDTA–NaCl. The HDL was incubated separately for 1 hr at 37°C with 2.32 × 10^5 dpm of emulsified cholesterol-4-^{14}C per ml of original plasma. Subsequently, the acyltransferase reaction was blocked by the addition of PCMPS; LDL or VLDL were added in such a way as to simulate as closely as possible the concentrations present in the original plasma; and the incubation was continued for an additional hr (Expt. 1) or 2 hr (Expt. 2). Incubation volumes for HDL + VLDL were 25 and 12 ml, respectively, in experiments 1 and 2. Corresponding values for HDL + buffer and HDL + LDL were 5 ml in experiment 1 and 4 ml in experiment 2. After the incubation, the lipoproteins were refractionated by ultracentrifugation. Control samples were treated similarly except that PCMPS was added to the HDL before the incubation with labeled cholesterol.

* Calculated after subtraction of control values (mean control dpm for HDL, LDL, and VLDL were 11.4, 10.3, and 10.8% of test samples, respectively).

† (Specific activity of lipoprotein cholesteryl ester)/(specific activity of HDL cholesteryl ester).

Nichols and his colleagues (15, 16), which demonstrated a sizable exchange of unlabeled, HDL cholesteryl esters for VLDL triglycerides in plasma incubated for 16 hr.

Because the fatty acid composition of VLDL cholesteryl esters differs somewhat from that of HDL cholesteryl esters (17), it was of interest to determine whether the transfer of cholesteryl esters from HDL to VLDL was selective. We performed a transfer experiment similar to that described above, except that after the incubation the labeled cholesteryl esters were subfractionated by chromatography on plates of $AgNO_3$-impregnated silica gel (Table 5). Compared to what was present in the HDL, a slightly higher proportion of monounsaturated and a slightly lower proportion of saturated and tetra-unsaturated cholesteryl-^{14}C esters were transferred. Since the relative specific activity of the monounsaturated esters of the HDL incubated with buffer alone was no higher than that of the other esters (compare the radioactivity data with the mole distribution data given in parentheses), the disproportionate transfer of radioactivity also must have been accompanied by a disproportionate transfer of mass.

DISCUSSION

Whether the cholesteryl esters of human plasma VLDL are mainly formed intracellularly (in the liver and intestine) or extracellularly (in the plasma) remains to be established. However, the data obtained in the present investigation support the latter possibility. Thus, the results of the incubation experiments presented in Table 1 are qualitatively similar to the results of the in vivo labeling experiments of Goodman (5). In our three experiments in which whole plasma was incubated for 1 hr with cholesterol-^{14}C the ratio of the mean specific activities of the HDL, VLDL, and LDL cholesteryl esters was 1.00:0.27:0.13. The corresponding ratio from the earliest sample obtained by Goodman (withdrawn from subject E.H. 1.83 hr after the injection of mevalonate-^{14}C) was 1.00:0.24:0.13. This similarity suggests that the LCAT

TABLE 5 TRANSFER OF INDIVIDUAL CHOLESTERYL ESTERS FROM HDL TO VLDL

Lipoproteins		Number of	Composition of Cholesteryl-^{14}C Esters*			
Incubated	Analyzed	Incubations	Saturated	Δ^1	Δ^2	Δ^4
			% ± SEM			
HDL + buffer	HDL	4	13.3 ± 0.40† (13.3 ± 0.37)	19.4 ± 0.19‡ (21.8 ± 0.25)	56.8 ± 0.28 (55.0 ± 0.27)	10.6 ± 0.23§ (9.9 ± 0.25)
HDL + VLDL	HDL	5	12.4 ± 0.26 (13.1 ± 0.21)	20.1 ± 0.66 (21.5 ± 0.37)	57.4 ± 0.82 (54.3 ± 0.65)	10.1 ± 0.25 (10.1 ± 0.30)
	VLDL	5	10.3 ± 0.49† (12.1 ± 0.30)	23.3 ± 0.27‡ (23.4 ± 0.34)	56.6 ± 0.55 (54.4 ± 0.57)	9.8 ± 0.15§ (10.1 ± 0.16)

VLDL and HDL (d > 1.063) were prepared by ultracentrifugation. HDL obtained from 150 ml of plasma were incubated for 1 hr at 37°C with 3.5 × 10^7 dpm of emulsified cholesterol-4-^{14}C (same experiment as Expt. 2, Table 4). PCMPS was added to block the acyltransferase reaction, VLDL or buffer was added, and the incubation was continued for 2 hr. Each incubation flask contained lipoproteins from 15 ml of original plasma and was made up to a final volume of 20 ml by the addition of Tris–EDTA–NaCl. After the incubation, the lipoproteins were reisolated by ultracentrifugation. Control experiments were performed in which HDL were incubated with labeled cholesterol in the presence of PCMPS and subsequently with buffer or VLDL. The control samples were then analyzed in parallel with the test samples to permit correction of the radioactivity in the cholesteryl ester fractions due to contaminating, unesterified cholesterol-^{14}C.

* Calculated after subtraction of control values (mean dpm for saturated and mono-, di-, and tetraunsaturated cholesteryl esters were 15.5, 9.5, 9, and 14.5% of test samples, respectively). Numbers in parentheses represent the molar distribution of cholesteryl esters determined colorimetrically.

Degree of significance (by Student's t test) for difference between HDL incubated above and in presence of VLDL:
† $P < 0.05$,
‡ $P < 0.01$,
§ $P < 0.05$.

reaction was a significant source of VLDL and LDL cholesteryl esters in Goodman's experiments. In view of the relation between the specific activity of VLDL cholesteryl esters and the elution volume of VLDL subfractions from agarose columns (Figs. 1 and 2), it might be of value to test this possibility further by seeking a similar relation in human plasma VLDL labeled in vivo.

The data from the transfer experiment presented in Table 5 also support the importance of LCAT in forming VLDL cholesteryl esters. They confirm the finding of Goodman and Shiratori (17) that the cholesteryl esters of human VLDL are similar to those of human HDL, and suggest that even the small difference in fatty acid composition which does exist (a higher percentage of monounsaturated esters in VLDL) may be caused by a disproportionate transfer of cholesteryl esters from HDL to VLDL.

Unfortunately, our results provide only a little information about a crucial point, namely the molar rate of transfer of HDL cholesteryl esters. If the assumptions are made that the rates of transfer were constant during the incubation, that the specific activity of the cholesteryl esters transferred was essentially equal to that of the total HDL cholesteryl esters, and that little back transfer of labeled cholesteryl ester occurred, the rates of transfer from HDL to VLDL would be 0.017 and 0.031 μmole/ml per hr for experiments 1 and 2 in Table 4. These rates would correspond to turnover times of 5.5 and 4.6 hr for the VLDL cholesteryl ester if the following calculation is used.

Rate of transfer = VLDL cholesteryl ester (dpm/ml per hr) ÷ HDL cholesteryl ester (dpm/μmole).

Turnover time = VLDL cholesteryl ester (μmoles/ml) ÷ rate of transfer (μmoles/ml per hr).

Similarly calculated rates of transfer of cholesteryl esters from HDL to LDL would be 0.062 and 0.037 μmole/ml per hr. These calculations suggest that the total amount of *labeled* cholesteryl esters transferred to LDL is equal to or greater than that transferred to VLDL. The mechanism and significance of this transfer of labeled cholesteryl esters from HDL to LDL is currently under study.

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