Comparative acyl specificities for transfer and selective uptake of high density lipoprotein cholesteryl esters

Simone R. Green and Ray C. Pittman

Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0613

Abstract This study compares the specificities of selective uptake and transfer mediated by plasma cholesteryl ester transfer protein (CETP) for various species of cholesteryl esters in high density lipoproteins (HDL). [^{3}H]Cholesterol was esterified with a series of variable chain length saturated acids and a series of variably unsaturated 18-carbon acids. These were incorporated into synthetic HDL particles along with ^{125}I-labeled apoA-I as a tracer of HDL particles and [^{14}C]cholesteryl oleate as an internal standard for normalization between preparations. Selective uptake by Y1-BS1 mouse adrenal cortical tumor cells was most extensively studied, but uptake by human HepG2 hepatoma cells and fibroblasts of human, rat, and rabbit origin were also examined. Acyl chain specificities for selective uptake and for CETP-mediated transfer were conversely related; selective uptake by all cell types decreased with increasing acyl chain length and increased with the extent of unsaturation of C_{18} chains. In contrast, CETP-mediated transfer increased with acyl chain length, and decreased with unsaturation of C_{18} chains. The specificities of human and rabbit CETP were also compared, and were found to differ little. Associated experiments showed that HDL-associated triglycerides, traced by [^{3}H]glyceryl trioleyl ether, were selectively taken up but at a lesser rate than cholesteryl esters. The mechanism of this uptake appears to be the same as for selective uptake of cholesteryl esters. —**Green, S. R., and R. C. Pittman.** Comparative acyl specificities for transfer and selective uptake of high density lipoprotein cholesteryl esters. *J. Lipid Res.* 1991. **32:** 457–467.

Supplementary key words cholesteryl ester transfer protein • lipid transfer protein • acyl moieties • triglycerides • adrenal cortical cells • HepG2 hepatoma cells • fibroblasts

Until fairly recently it was widely believed that plasma cholesteryl esters reside immutably in the lipoprotein particles in which they originate, in spite of some early reports to the contrary (1, 2). Of course, cholesteryl esters do transfer between lipoprotein particles in many species, including man, in a process mediated by cholesteryl ester transfer protein (CETP). The cholesteryl ester transfer mechanism and the plasma protein catalyzing that transfer have now been intensively studied and well characterized (3–5).

Similarly, it has been believed that cellular uptake of lipoprotein cholesteryl esters is only by endocytosis, a belief consistent with the paradigm of LDL receptor-mediated uptake (6). It is now clear that some tissues of intact rats (7, 8), a variety of cultured cells of several species (8–13), and some organ culture systems (14, 15) take up cholesteryl esters from HDL without endocytosis of the lipoprotein particle (10) in a process we term "selective uptake." Since selective uptake is observed in cells of human origin (10, 13), the pathway has the potential to play a role in vivo in man. This pathway for the independent movement of cholesteryl esters into cells and the CETP-mediated pathway for the movement cholesteryl esters between lipoproteins must both be taken into account when investigating the fate of plasma cholesteryl esters in humans as well as in other animals.

The specificity for CETP-mediated transfer has been examined for those cholesteryl esters most prevalent in the circulation (16). Almost nothing is known about the acyl specificity of selective uptake of cholesteryl esters. It is important to understand the relative specificities of the two processes. Both processes have been proposed to participate in the return of cholesterol from extrahepatic tissues to the liver (10, 17, 18). CETP mediates the exchange and transfer of HDL cholesteryl esters, and selective uptake also removes cholesteryl esters from this pool. Consequently, the two processes compete. The outcome of this competition depends on a number of factors, such as the intrinsic rates of the processes (e.g., the amount of CETP protein and inhibitors, the capacity of cells for selective uptake) and the quantity of lipoprotein substrates. It may also depend on the types of cholesteryl esters available for transfer and the specificities of the two processes for those esters.

Abbreviations: HDL, high density lipoproteins; CETP, cholesteryl ester transfer protein; LDL, low density lipoproteins; ^{125}I-NMTC-apoA-I, apoA-I derivatized with the radioiodinated N-methyl-tyramine-cellobiose ligand; TLC, thin-layer chromatography.

Aside from the competition of the two processes, there are at least two other reasons to compare the effect of varying cholesteryl ester acyl moieties on their selective uptake and their CETP-mediated transfer. First, it has been suggested that CETP may mediate the direct cellular uptake of cholesteryl esters (19). We have found nothing to support this hypothesis (20), but have not excluded the possibility that CETP contributes to apparent cholesteryl ester selective uptake to some degree in some animals. Differences in substrate specificity between CETP-mediated transfer and selective uptake could provide further evidence on this matter. Second, it would be useful for in vivo studies to have available cholesteryl ester tracers that differ substantially in the relative rates of selective uptake and CETP-mediated transfer. A systematic examination of substrate specificities of the two processes might provide clues for such a tracer.

In this study we have directly compared the specificities of selective uptake and of CETP-mediated transfer. [^{3}H]Cholesterol was esterified with a variety of fatty acids, including a series of variable chain length saturated acids and a series of variably unsaturated 18-carbon acids. Esters of a fish oil fatty acid, docosahexaenoic acid, and several branched acyl chain esters were also studied. The tracer cholesteryl esters were incorporated into synthetic HDL particles (11) which contained cholesteryl oleate as the sole neutral lipid component; tracer [^{14}C]cholesteryl oleate was also included to serve as an internal standard for normalization of the data between particle preparations. Synthetic particles were used because it was unclear that incorporation into native particles using CETP would be effective in all cases. It also seemed desirable to incorporate the tracers into particles of constant and defined composition that contained no plasma enzymes. The most extensive data compared the rate of CETP-mediated transfer, and the rate and kinetics of selective uptake by Y1-BS1 mouse adrenocortical tumor cells. Other studies examined the specificity of selective uptake by human HepG2 heptoma cells and fibroblasts of human, rat, and rabbit origin. Results showed acyl chain specificities that were very different for selective uptake and for CETP-mediated transfer, and this difference in specificities of the two processes was qualitatively maintained for all cell types. The specificities of human and rabbit CETP were also compared, and were found to differ little.

We also report an associated study in which we examined the possibility of selective uptake of triglycerides from HDL using [^{3}H]glyceryl trioleyl ether as a tracer. Selective uptake of cholesteryl esters involves their insertion into a membrane pool from which they are irreversibly internalized (21). There is a small steady-state level of cholesteryl esters in plasma membrane, and a similar pool of triglycerides (22). Thus, it seemed possible that triglycerides might also be taken up by the same process as cholesteryl esters. This proved to be true, although uptake was at a lower rate than for cholesteryl esters.

METHODS

Preparation of lipid and apolipoprotein tracers

Cholesteryl esters with varying acyl moieties were synthesized from [1α,2α(n)-^{3}H]cholesterol (Amersham, sp act 44 Ci/mmol) and the anhydride of the corresponding fatty acid (23). If not commercially available, the anhydride was formed by refluxing the fatty acid with acetic anhydride (24). The purified anhydride was then reacted in 30-fold excess with [^{3}H]cholesterol for 1 h at 50°C in the presence of dimethyl-aminopyridine. The product ester was purified by TLC (hexane–diethyl ether–acetic acid 80:20:1 v:v:v).

[^{14}C]Cholesteryl oleate served as a reference to which the [^{3}H]cholesteryl esters, incorporated into the same particles, were compared. This ester was prepared from [4-^{14}C]cholesterol (Amersham Corp, sp act 60–90 mCi/mmol) in a carrier-free reaction, as previously described (23, 25).

To examine the cellular uptake of triglycerides, an ether analogue of a triglyceride was used as tracer. [^{3}H]Glyceryl-trioleyl ether was synthesized from [1,3-^{3}H]glycerol (1 mCi, 1 mCi/mmol) and a 100-fold molar excess of oleyl methane sulfonate by overnight reaction with potassium metal in toluene at 90°C. The product ether was then purified by TLC on silica gel G developed in hexane–diethyl ether 98:2, and purity was checked by HPLC on a C_{18} column run in isopropanol–methanol–heptane 4:4:1.

In studies of cellular uptake of [^{3}H]glyceryl-trioleyl ether, [^{14}C]cholesteryl-oleyl ether was used as a reference core neutral lipid tracer. This ether was synthesized as previously described (10, 26).

Human apoA-I was purified and labeled with the ^{125}I-N-methyl-tyramine-cellobiose ligand as previously described (10, 27).

Incorporation of tracers into lipoprotein particles

For most of the studies described here, synthetic HDL particles were used and contained three tracers: the test [^{3}H]cholesteryl ester; ^{125}I-NMTC-apoA-I to mark HDL particles and allow determination of selective uptake; and [^{14}C]cholesteryl oleate to act as an internal standard to allow comparison between preparations. All three tracers were present in the particles at negligible mass.

The synthetic HDL particles were prepared essentially as described previously (11), except that in some studies (Tables 1–3) particles were made that resembled HDL_3 rather than HDL_2 as originally described. To prepare the HDL_3 particles, the lipid tracers were mixed with egg yolk phosphatidylcholine (3 mg), and cholesteryl oleate (2 mg)

in a total volume of 10 ml of aqueous buffer and sonicated for 1 h at 50°C. The temperature was then lowered to 40°C, and apoA-I (6.7 mg) and apoA-I tracer in 1 ml of 2.5 M urea were added to the lipid sonicate in small increments over a period of 10 min during continued sonication. Particles were then isolated at d 1.08 < d < 1.25 g/ml by sequential ultracentrifugation (28).

The synthetic HDL particles used in experiments comparing HepG2 cells and fibroblasts of human, rat, and rabbit origin to Y1-BS1 cells were prepared by a variant of the above procedure. One preparation of synthetic HDL containing [^{14}C]cholesteryl oleate and ^{125}I-NMTC-apoA-I tracers was made as just described, but scaled up threefold. After ultracentrifugal purification of the doubly labeled HDL particles, the various test [^{3}H]cholesteryl esters were transferred into separate aliquots of the preparation. Donor liposomal preparations (29) and partially purified human CETP (30) were used to do this, as previously described (10). Donor particles were removed by flotation at d < 1.05 g/ml before dialysis and use.

To examine the cellular uptake of the triglyceride analogue, [^{3}H]glyceryl-trioleyl ether, the tracer and the reference [^{14}C]cholesteryl-oleyl ether tracer were incorporated into synthetic HDL_2 particles as previously reported (11), except that in some preparations varying amounts of glyceryl trioleate were substituted for an equal mass of cholesteryl oleate in the neutral lipid core. In two preparations, cholesteryl oleate was the sole neutral lipid (except tracers); in one preparation equal masses of glyceryl trioleate and cholesteryl oleate were used; and in another the neutral lipid consisted entirely of triolein. The particles were applied to a density gradient (31), and particles in the density range 1.06 < d < 1.10 g/ml were selected for use.

Cellular uptake of HDL tracers

Selective uptake was assayed in Y1-BS1 cells, a stable subclone of the Y1 line (32), generally as described previously (10). Each determination was routinely done in triplicate. Cells were incubated overnight (15–18 h) in serum-free medium containing 10^{-7} ACTH before addition of triply labeled HDL particles in serum-free medium. This was done for two reasons: induction of selective uptake by ACTH (10, 11) increases up to 14 h; and it was thought desirable to maximally deplete the cell of its cholesterol stores. The ACTH-treated cells were then incubated with five concentrations of labeled HDL (5–50 μg HDL protein/ml) in the continued presence of ACTH, but also in the presence of 75 μM aminoglutethimide to block steroidogenesis. After 4 h incubation, the cells were washed extensively, and unlabeled HDL (100 μg protein/ml) was added for a 2-h chase period to remove reversibly cell-associated tracers (10, 21). The amounts of tracers remaining in the cells as well as the amounts reversibly cell-associated were determined. ^{125}I was determined by direct assay of medium or cell suspensions while ^{3}H- and ^{14}C-labeled lipids were assayed after lipid extraction (10, 11); no significant amount of ^{125}I was present in the lipid extract. The amount of irreversibly cell-associated cholesteryl ester tracer in excess of apoA-I tracer is reported as selective uptake. Analogously, the amount of reversibly cell-associated cholesteryl ester tracer in excess of apoA-I tracer is reported as tracer in the reversibly cell-associated cholesteryl ester pool. The uptake of each tracer is shown as apparent HDL particle uptake, and that HDL particle uptake is expressed in terms of HDL protein. This allows direct comparison of the rates of uptake of all tracers.

Selective uptake was also examined in HepG2 human hepatoma cells and in skin fibroblasts from three species: human, rat, and rabbit. These assays were carried out generally according to the protocol described above for Y1-BS1 cells (10), except that ACTH was present only in Y1-BS1 cells, and only one concentration of HDL in the medium (10 μg HDL protein/ml) was studied for each cholesteryl ester examined. The HepG2 cells were cultured as previously described (13), and fibroblasts from all three species were cultured as described for human fibroblasts (10, 11).

Assay of cholesteryl ester transfer

Rates of CETP-mediated transfer of the cholesteryl ester tracers were measured on the same HDL preparations used to measured selective uptake. Again cholesteryl oleate served as a reference by which to normalize different particle preparations. Human lipoprotein-deficient serum served as a source of CETP activity in some experiments (Tables 1–3), while partially purified CETP (30), both human and rabbit, were used in other studies where indicated. Transfer assays were conducted as described previously (25), with acceptor LDL (human) present in large excess of HDL. Initial rates of transfer were calculated and expressed relative to initial rates of transfer of cholesteryl oleate in the same HDL particles.

In studies comparing the specificities of human and rabbit CETP, assays were also as previously described (25) and as outlined above. However, in this case the d > 1.21 g/ml fractions of human plasma and of rabbit plasma served as sources of CETP activity. Transfer by the two activities was compared in parallel using the same synthetic HDL donor particles and human LDL as acceptor.

RESULTS

Tables 1–3 show the rates of CETP-mediated transfer and the rates of selective uptake by Y1-BS1 mouse adrenal cortical tumor cells of HDL-associated cholesteryl esters with varying acyl chains. The data for both processes are normalized to the rates for cholesteryl oleate incorporated into the same particles.

Table 1 shows data for those saturated acyl chains from C_{12} to C_{18} with even carbon number. These esters were transferred by CETP at a fractional rate that progressively increased with chain length. In contrast, the rate of selective uptake progressively decreased with increasing chain length. Consequently, the ratio of selective uptake to CETP-mediated transfer was more than sixfold as great for the C_{12} ester as for the C_{18} ester.

Also shown in Table 1 are values for cholesteryl ester tracers found in the reversibly cell-associated pool (i.e., cholesteryl ester tracer removed from the cells by a post-uptake incubation in the presence of unlabeled HDL in excess of that accounted for by released HDL particles, as described in the Methods section). We have reported that the amount of HDL cholesteryl ester tracer in this pool in Y1-BS1 cells varies almost linearly with irreversible selective uptake of the tracer under a variety of metabolic perturbations, and that cholesteryl ester tracer in this plasma membrane pool can serve as a precursor of irreversibly internalized esters (21). The pool evidently represents an early step in selective uptake. Thus, it was important to know whether other selectively taken-up cholesteryl ester tracers would also reside in the reversibly cell-associated pool and their selective uptake would also be in proportion to their residence in that pool. As shown in Table 1, the reversibly cell-associated pool of each of the various tracers varied in parallel with its irreversible selective uptake; the ratio of irreversible selective uptake to tracer in the reversibly cell-associated pool was in every case nearly the same for both the test ester and the reference cholesteryl oleate in the same particles (i.e., the B/C ratio of Table 1 was in every case near 1).

Table 2 compares selective uptake and CETP-mediated transfer of a series of esters with 18-carbon acyl moieties of varying unsaturation. Experiments were in exact parallel to those of Table 1. The CETP-mediated transfer of oleate and its *cis*-11 isomer, vaccenate, was at a greater rate than transfer of stearate or shorter chain saturated esters. However, the transfer rate progressively decreased with increasing unsaturation beyond a single unsaturated site. In contrast, selective uptake progressively increased with the extent of unsaturation. Thus, in the series of Table 2, as in the series of Table 1, the rates of CETP-mediated transfer and selective uptake were negatively related and thus dissociated. Also in accord with data in Table 1, the ratio of selective uptake to tracer in the reversibly cell-associated pool for the unsaturated series was very similar to that of the reference cholesteryl oleate in every case (Table 2). Thus, again the proportionality of incorporation into the reversibly cell-associated pool and selective uptake was maintained.

Table 3 shows parallel studies of some cholesteryl esters that do not fall into the series of Tables 1 or 2. These are cholesteryl esters of *cis*-4,7,10,13,16,19-docosahexaenoic acid and of short, branched chain fatty acids. Both CETP-mediated transfer and selective uptake of the docosahexaenoyl ester were greater than that of any of the other esters studied. Still, the ratio of selective uptake to tracer in the reversibly cell-associated pool was very near that of the reference cholesteryl oleate. Selective uptake of branched chain esters was also high compared to the series of Tables 1 and 2. However, CETP-mediated transfer was at a much lower rate than for the straight chain esters, and was nil or almost nil in the cases of two

TABLE 1. Relative rates of CETP-mediated transfer and selective uptake of HDL cholesteryl esters with saturated acyl chains of varying length

Cholesteryl Ester	A CETP-Mediated Transfer[a]	B CE Selective Uptake[b,c]	C Reversible Pool[d]	Ratio B/A	Ratio B/C
	relative to [^{14}C]cholesteryl oleate				
Oleate	(1.00)	(1.00)	(1.00)	(1.0)	(1.0)
Laurate	0.26 ± 0.07	1.63 ± 0.06	1.83 ± 0.10	6.3	0.9
Myristate	0.54 ± 0.22	1.09 ± 0.02	1.13 ± 0.02	2.0	1.0
Palmitate	0.74 ± 0.14	1.01 ± 0.01	0.98 ± 0.10	1.4	1.1
Stearate	0.79 ± 0.13	0.87 ± 0.06	0.98 ± 0.05	1.1	0.9

[a]In the experiments of Tables 1, 2, and 3, the rate of transfer of the reference [^{14}C]cholesteryl oleate in the various particle preparations was 0.63 ± 0.19 h^{-1} in the system described under Methods.

[b]The data for selective uptake were collected in triplicate at five concentrations of HDL in the medium (5–50 μg HDL protein/ml). In each case a mean for the uptake ratio of the two tracers at all of those concentrations is shown. This calculation is precise only if the kinetics for uptake of all tracers are alike, which was not true (see Table 4 below). However, the values relative to cholesteryl oleate were not substantially different at any HDL concentration.

[c]In the experiments of Tables 1, 2, and 3, uptake of the reference [^{14}C]cholesteryl oleate was 1461 ± 331 ng HDL protein/mg cell protein per 4 h (at an HDL concentration of 25 μg HDL protein/ml in the medium), and uptake of ^{125}I-NMTC-apoA-I was 186 ± 56 ng/mg per 4 h.

[d]In the experiments of Tables 1, 2, and 3, absolute values for the reference cholesteryl oleate tracer (at an HDL concentration of 25 μg HDL protein/ml in the medium) were: reversibly cell-associated [^{14}C]cholesteryl oleate, 284 ± 67 ng HDL protein/mg cell protein per 4 h; reversibly cell-associated ^{125}I-NMTC-apoA-I, 81 ± 13 ng/mg per 4 h; reversibly cell-associated [^{14}C]cholesteryl oleate in excess of that accounted for by HDL particles, 203 ± 46 ng/mg per 4 h.

TABLE 2. Relative rates of CETP-mediated transfer and selective uptake of HDL cholesteryl esters with 18-carbon acyl chains of varying unsaturation

[^{3}H]Cholesteryl Ester	A CETP-Mediated Transfer[a]	B CE Selective Uptake[b,c]	C Reversible Pool[d]	Ratio B/A	Ratio B/C
	relative to [^{14}C]cholesteryl oleate				
Stearate	0.79 ± 0.13	0.87 ± 0.06	0.98 ± 0.05	1.1	0.9
Oleate	(1.00)	(1.00)	(1.00)	(1.0)	(1.0)
Vaccenate	0.94 ± 0.03	1.03 ± 0.02	1.10 ± 0.04	1.1	0.9
Linoleate	0.80 ± 0.04	1.27 ± 0.03	1.34 ± 0.14	1.6	1.0
Linoleate	0.69 ± 0.03	1.67 ± 0.11	1.75 ± 0.16	2.4	1.0

[a,b,c,d]See the legend of Table 1.

esters with branching in the α position (2-ethyl hexanoate and pentyl-2-cyclohexane carboxylate). In a departure from the pattern seen for the saturated and unsaturated series, the relationship of selectively taken-up tracer and reversibly cell-associated tracer was not always very near that of oleate (represented by the B/C ratio).

Fig. 1 summarizes and expands the above results relating selective uptake and tracer in the reversibly cell-associated cholesteryl ester pool. The data from the experiments of Tables 1 and 2 as well as the data for cholesteryl docosahexaenoate are combined. In Fig. 1A data for these nine esters at the five HDL concentrations examined are shown fitted to a single line (r = 0.966). Fig. 1B shows the same data, except in this case the data at varying concentrations are fitted to an independent line for each of the esters. These lines indicate that there may be small but real differences between the various esters, as was the clear case for some of the branched chain esters which are not plotted in this figure.

The data that are summarized in Tables 1 and 2 and the data for the docosahexaenoyl ester were also used to determine kinetics constants, which are shown in **Table 4**. These values were determined according to the best-fit lines for the Eadie-Hofstee transformations of the data (1/v versus v/[S]). Each ester was examined in an independent experiment; the kinetic constants for the [^{3}H]cholesteryl esters were normalized to the kinetic constants for [^{14}C]cholesteryl oleate in the same particles. This was done because the apparent K_m and V_{max} values for the reference oleate varied significantly between particle preparations (V_{max} = 4298 ± 1549 ng HDL protein/mg cell protein per 4 h; K_m = 47.7 ± 10.3 μg HDL protein/ml); since the correlation coefficients for the Eadie-Hofstee fits of the ^{3}H and ^{14}C data in each particle were themselves highly correlated (r = 0.896), this suggested that major factors accounting for the variability in the [^{14}C]cholesteryl oleate data also applied to the [^{3}H]cholesteryl ester data, thus justifying normalization of the data.

Table 4 shows that the apparent V_{max} values for uptake of the various esters varied in a systematic way. The apparent V_{max} increased with decreasing length of saturated acyl chains, and increased with increasing unsaturation of 18-carbon acyl groups. Apparent K_m values also increased with chain length and generally increased with the degree of unsaturation.

The studies above examined selective uptake by Y1-BS1 cells, our most common in vitro model for studies of selective uptake. These cells offer the advantage of both a high rate of selective uptake and a high rate of receptor-

TABLE 3. Relative rates of CETP-mediated transfer and selective uptake of HDL cholesteryl esters with fish oil or branched chain acyl groups

[^{3}H]Cholesteryl Ester	A CETP-Mediated Transfer[a]	B CE Selective Uptake[b,c]	C Reversible Pool[d]	Ratio B/A	Ratio B/C
	relative to [^{14}C]cholesteryl oleate				
Oleate	(1.00)	(1.00)	(1.00)	(1.0)	(1.0)
Docosahexaenoate	1.46 ± 0.04	2.16 ± 0.14	1.94 ± 0.32	1.5	1.1
2-Ethyl hexanoate	−0.03 ± 0.04	1.69 ± 0.09	2.30 ± 0.12		0.7
Citronellate	0.13 ± 0.03	1.84 ± 0.17	2.33 ± 0.35	14.2	0.8
Pentyl-4-cyclohexane carboxylate	0.06 ± 0.03	1.11 ± 0.02	1.18 ± 0.09	20.7	0.9
4-Cyclohexane butyrate	0.08 ± 0.01	1.66 ± 0.16	2.23 ± 0.10	19.8	0.7

[a,b,c,d]See the legend of Table 1.

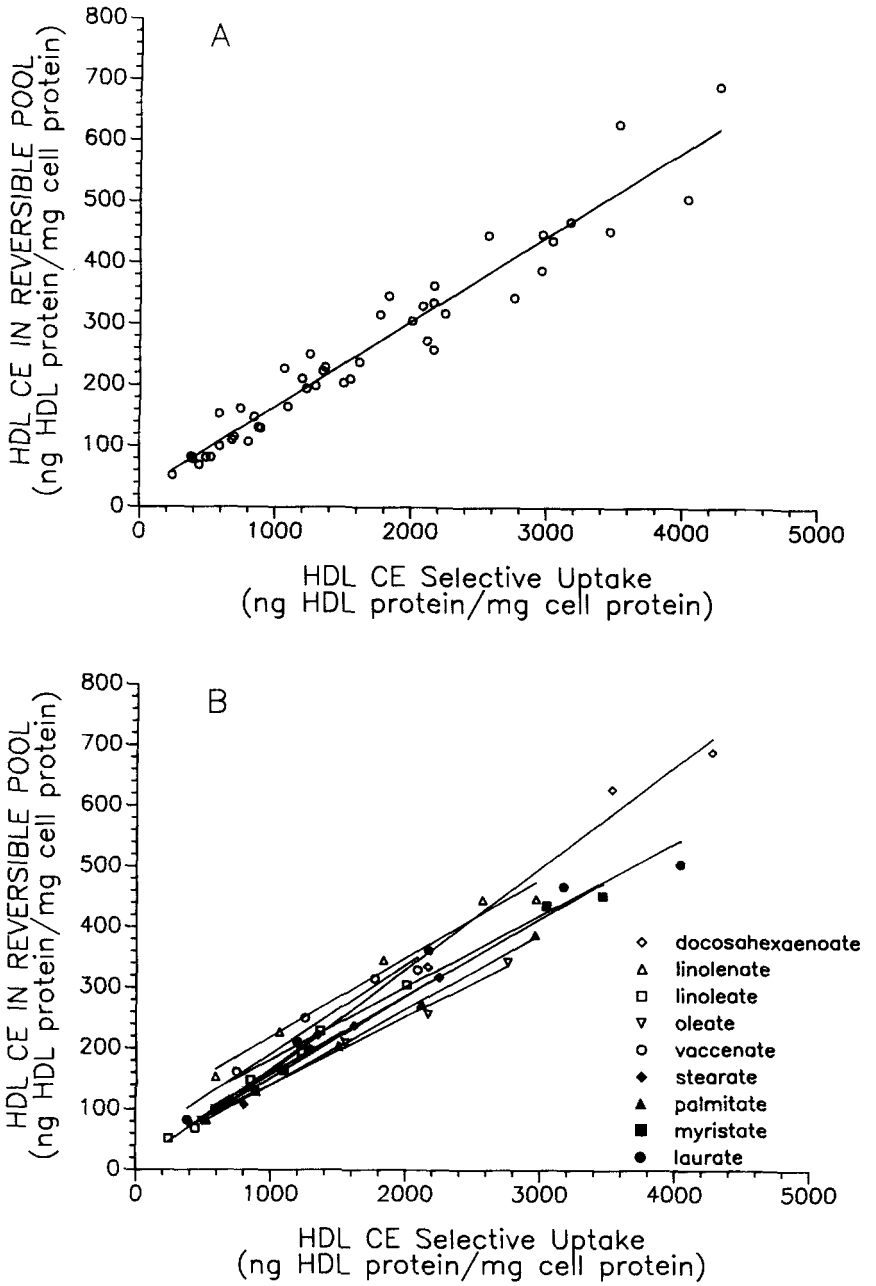

Fig. 1. Relationship of the irreversible selective uptake of cholesteryl ester tracers to the appearance of those tracers in a reversibly cell-associated pool not accounted for by bound HDL particles. Y1-BS1 mouse adrenocortical tumor cells were incubated with the same triply labeled synthetic HDL particles used in Tables 1 and 2. Selective uptake of cholesteryl ester tracers and appearance of the tracers in the reversibly cell-associated pool were measured as outlined under Methods. HDL concentration in the medium was 5, 10, 20, 35, and 50 μg HDL protein/ml. Data for all concentrations of the esters shown in Panel B are plotted in both panels A and B, but in panel A all data are fit to a single line while in panel B they are fit to a separate line for each ester.

mediated LDL uptake, and both processes are regulated by ACTH treatment. However, there was no assurance that all cells would exhibit the same pattern of cholesteryl ester selectivity as Y1-BS1 cells or other steroidogenic cells. Consequently, human hepatoma HepG2 cells were also studied, as were skin fibroblasts of human, rabbit, and rat origin using the same series of saturated esters shown in Table 1 and the same series of variably unsaturated C_{18} esters shown in Table 2. A single low concentration of HDL was studied (10 μg protein/ml); because kinetic parameters were not determined in these studies, conclusions that can be drawn are necessarily

TABLE 4. Kinetic constants for the selective uptake of HDL cholesteryl esters of differing acyl moieties relative to cholesteryl oleate

[^{3}H]Cholesteryl Ester	Relative Apparent V_{max}	Relative Apparent K_m	Correlation Coefficient
	relative to [^{14}C]cholesteryl oleate		
Laurate	1.91	1.24	0.987
Myristate	1.10	1.02	0.973
Palmitate	1.10	0.98	0.939
Stearate	0.77	0.87	0.918
Oleate	(1.00)	(1.00)	0.967
Vaccenate	1.08	1.06	0.987
Linoleate	1.17	0.89	0.946
Linolenate	2.06	1.39	0.995
Docosahexaenoate	2.84	1.47	0.969

limited. Under these assay conditions, the relative specificities of HepG2 cells resemble those of Y1-BS1 cells both qualitatively and quantitatively. The specificity of selective uptake by fibroblasts was qualitatively similar to that of HepG2 and Y1-BS1 cells; selective uptake decreased with increasing acyl chain length and increased with increasing acyl chain unsaturation as was true for Y1-BS1 cells and HepG2 cells. However, the relative rates of selective uptake by fibroblasts were quantitatively different from Y1-BS1 cells and HepG2 cells in that they varied over a wider range, showing higher relative rates of uptake of short chain esters and of polyunsaturated esters. These relative uptake rates were similar for fibroblasts from all three species examined.

The specificities of human and rabbit CETP were also compared (**Table 5**). Labeled HDL preparations were added in tracer quantity to either rabbit or human plasma, and the rates of tracer transfer to apoB-containing particles were determined as described in the Methods section. Table 5 shows that selectivity of the two transfer proteins, expressed relative to cholesteryl oleate. The only significant difference in relative transfer rates from HDL to apoB-containing lipoproteins was a greater relative rate for transfer of cholesteryl laurate by rabbit CETP; a higher apparent relative transfer of cholesteryl myristate by rabbit CETP did not reach statistical significance.

TABLE 5. Comparative selectivities of human and rabbit CETP for transfer of various cholesteryl esters

Cholesteryl Ester	Human CETP	Rabbit CETP	
Laurate	0.092 ± 0.016	0.154 ± 0.027	$P < 0.05$
Myristate	0.124 ± 0.039	0.237 ± 0.099	NS
Palmitate	0.635 ± 0.046	0.472 ± 0.175	NS
Stearate	0.693 ± 0.056	0.593 ± 0.115	NS
Oleate	(1.00)	(1.00)	
Vaccenate	0.753 ± 0.084	0.753 ± 0.032	NS
Linoleate	0.508 ± 0.025	0.494 ± 0.077	NS
Linolenate	0.354 ± 0.017	0.306 ± 0.048	NS

Thus, it appears that selectivities of human and rabbit CETP are similar, except for a somewhat greater transfer of shorter chain esters by the rabbit transfer protein.

As an extension of examining the specificity of selective uptake of cholesteryl esters, we examined the possibility that triglycerides are also selectively taken up from HDL. Triglycerides are transferred by CETP (33, 34), but it has not been known that they are nonendocytotically taken up by cells without prior hydrolysis. Experiments examining triglycerides were of somewhat different design from the above studies of cholesteryl esters. [^{3}H]Glyceryl trioleyl ether rather than an authentic triglyceride was used as a tracer due to the possibility of extracellular hydrolysis and the ready release of labeled metabolic products from the cells. The [^{3}H]glyceryl trioleyl ether tracer was incorporated into synthetic HDL particles that also contained [^{14}C]cholesteryl-oleyl ether and ^{125}I-NMTC-apoA-I tracers. Four preparations of such triply labeled synthetic HDL were studied: two contained cholesteryl oleate as the sole neutral lipid (except for tracers); one contained a 1:1 (w/w) mix of cholesteryl oleate and triolein; one contained only triolein. **Table 6** shows the rates of uptake of the various HDL tracers from these four synthetic HDL preparations by Y1-BS1 cells. Whereas results for the two preparations made with only cholesteryl oleate bracketed the results for the two preparations made with varying amounts of triolein, the results are shown as means for all four preparations. The rate of uptake of both lipids was significantly greater than the rate of uptake of apoA-I.

TABLE 6. Uptake of HDL [^{14}C]cholesteryl-oleyl ether, [^{3}H]glyceryl trioleyl ether and ^{125}I-NMTC-apoA-I tracers by basal and ACTH-stimulated Y1-BS1 cells

Cells	Apparent HDL Particle Uptake		
	[^{14}C]CEt	[^{3}H]TG	^{125}I-ApoA-I
	μg HDL protein/mg cell protein per 4 h		
Basal	0.68 ± 0.14	0.46 ± 0.06	0.15 ± 0.03
ACTH-treated	1.52 ± 0.60	0.67 ± 0.12	0.17 ± 0.03

The following were significant with $P < 0.005$ by two-tailed t test of paired data for tracers in the same particle studied in the same experiment: both CE uptake and TG uptake > apoA-I uptake in both basal and ACTH-stimulated cells; and both CE uptake and TG uptake in ACTH-treated cells > uptake in basal cells.

TABLE 7. Selective uptake of [^{3}H]glyceryl trioleyl ether and its residence in a reversibly cell-associated pool in Y1-BS1 cells

	Selective Uptake			Reversible Pool	
Cells	[^{14}C]CEt	[^{3}H]TG	^{14}C/^{3}H	[^{14}C]CEt	[^{3}H]TG
	μg HDL protein/mg cell protein per 4 h				
Basal	0.53 ± 0.19	0.31 ± 0.06	1.73 ± 0.44	0.18 ± 0.05	0.11 ± 0.02
ACTH	1.35 ± 0.63	0.50 ± 0.14	2.70 ± 0.31	0.28 ± 0.08	0.16 ± 0.03

Data are from the same experiments as Table 5. The following were significant with $P < 0.005$ by two-tailed t test of paired data for tracers in the same particle studied in the same experiment: CE selective uptake > TG selective uptake in both basal and ACTH-treated cells; both CE and TG selective uptake in ACTH-stimulated cells > the respective uptake by basal cells; the ratio of CE selective uptake to TG selective uptake in ACTH-stimulated cells > that ratio in basal cells; reversible pool for both CE and TG in ACTH-treated cells > the corresponding pools in basal cells; and reversible pool for CE in both basal and ACTH-treated cells > the corresponding pool for TG.

Table 7 shows selective uptake, calculated as the difference in the rate of uptake of the lipid tracer and that of ^{125}I-NMTC-apoA-I. The triglyceride tracer was selectively taken up, but at a lower fractional rate than was the cholesteryl ester tracer. ACTH, which stimulates selective uptake of cholesteryl esters from HDL, also stimulated uptake of the glyceryl triether. As observed previously (11), ACTH had little effect on the uptake of HDL particles as assessed by uptake of ^{125}I-NMTC-apoA-I. The ratio of cholesteryl ether selective uptake to glyceryl triether selective uptake was greater in the case of ACTH-stimulated cells than in the case of basal cells, indicating that cholesteryl ester selective uptake was more stimulated by ACTH than was triglyceride uptake.

Table 7 also shows the lipid tracers recovered in the reversibly cell-associated pool (reversibly cell-associated ^{3}H and ^{14}C in excess of that accounted for by HDL particles as measured by ^{125}I). Triglyceride tracer, as well as cholesteryl ester tracer, was found in this reversibly cell-associated pool. Furthermore, selective uptake of both tracers was about in proportion to tracer recovered in the pool. These results indicate that triglycerides are selectively taken up, and suggest that uptake is by the same mechanism as for cholesteryl esters.

DISCUSSION

The data above indicate that CETP-mediated transfer and selective uptake of cholesteryl esters change systematically and conversely with variation in the acyl moiety, at least in the case of cholesteryl esters with straight acyl chains of varying length and unsaturation. Selective uptake by all of the cells examined decreased with increasing acyl chain length and increased with the extent of unsaturation of C_{18} chains. In contrast, CETP-mediated transfer increased with acyl chain length, and decreased unsaturation of C_{18} chains. However, both oleate and linoleate esters were transferred by CETP at a greater rate than stearate and the shorter saturated acyl chain esters. These results for CETP-mediated transfer of the dominant plasma cholesteryl esters are in reasonable agreement with the earlier results of Morton (16).

Since the cholesteryl ester tracers can be hydrolyzed, and since the resulting labeled free cholesterol may efflux from the cells, the possibility should be considered that the rate of hydrolysis may significantly influence the retention of cholesterol tracer within cells; in that case the apparent relative specificities for selective uptake could reflect, in part, and specificity of the cytoplasmic cholesterol esterase that hydrolyzes selectively taken up cholesteryl esters (35). Data within this study indicate that this is not the case; the rate of efflux of cholesterol tracer during a post-uptake chase incubation was linearly related to the amount of selectively taken-up tracer and independent of the ester of origin (Fig. 1). Further evidence against an important influence of the specificity of cytoplasmic cholesterol esterase is that the specificity of the cytoplasmic enzyme is parallel to that of selective uptake, at least for saturated esters (36); this is inconsistent with an important effect of esterase specificity in causing differing rates of cholesterol tracer efflux from the cells. Finally, unpublished studies from this laboratory show that there is very little efflux of free cholesterol from ACTH-stimulated Y1-BS1 cells in the presence of aminoglutethimide, and that nearly all cholesteryl ester tracer taken up from HDL remains associated with the cells during a 4-h incubation; thus, even if there were a differential rate of leakage of cholesterol tracer, the total leakage would not be great enough to make an important impact.

The pattern of specificity for selective uptake by Y1-BS1 cells was closely reproduced in HepG2 human hepatoma cells, at least under the limited conditions studied here. This pattern was only qualitatively maintained in fibroblasts, and the specificities of fibroblasts from three species were quantitatively similar. Thus it may be that

cell type is more important in determining the relative acyl chain specificity for selective uptake than is the species of origin. Both Y1-BS1 cells and HepG2 cells are much more active than fibroblasts in selective uptake. Their similar and apparently less discriminating selective uptake may somehow be a direct consequence of their high rate of selective uptake, or it may, in some other way not directly related to high uptake rate, reflect their special roles in cholesterol metabolism or their transformed nature.

The present study dissociates selective uptake and CETP-mediated transfer on the grounds of substrate specificity. This supplements earlier evidence dissociating the two. For example, selective uptake is very active in rats, which lack plasma CETP activity (37); selective uptake is active in a variety of in vitro systems where CETP is not present (7, 8, 10, 13); addition of exogenous CETP does not enhance selective uptake (10, 20). Thus the present study adds to the evidence that there are no grounds on which to implicate CETP in selective uptake.

Since CETP-mediated transfer and selective uptake draw on the same pool of HDL cholesteryl esters, the two processes must compete. However, the specificities of selective uptake and CETP-mediated transfer are dissimilar, even complementary, and the esters favored by one pathway are generally not favored by the other. The ratio of the rate of selective uptake to CETP-mediated transfer should give an indication of the relative propensity of an ester for selective uptake or for CETP-mediated transfer in vivo. Such consideration leads to the conclusion that selective uptake should be favored by progressive unsaturation (at least of C_{18} chains) and by progressively shorter saturated acyl chains. Conversely, the transfer pathway should be favored by longer, more saturated acyl chains.

The significance of these relative rates in competing processes depend on the absolute rates of the processes in vivo. The rate of CETP-mediated transfer in humans has not been directly quantified, and the rate of selective uptake is unknown. A recent study from this laboratory indicates that selective uptake plays a significant role in HDL cholesteryl ester metabolism in rabbits in vivo, even in the face of about four times the CETP activity of humans (38). This suggests that it may well play an important role in humans also. If so, and if the liver dominates in expression of the pathway as it does in rats, then selective uptake should participate in reverse cholesterol transport in humans. In that case, esters of polyunsaturated acids and esters of short chain acids should favor this pathway over the CETP-mediated transfer pathway; if CETP-mediated transfer of cholesteryl esters to apoB-containing lipoproteins enhances their atherogenecity as proposed (4), then favoring selective uptake should be beneficial.

There may be other consequences of the relative specificities of transfer and uptake shown here. Morton (16) has discussed possible ramifications of the specificity of CETP-mediated transfer in terms of plasma lipoprotein composition and consequential effects on lipoprotein metabolism by endocytotic processes. Selective uptake may also affect uptake of lipoprotein particles by modifying their cholesteryl ester composition. The data above indicate that selective uptake should preferentially remove shorter, more unsaturated esters and so should enrich HDL in the longer chain, more saturated cholesteryl esters. CETP-mediated transfer would have a more complex influence based on exchange as well as transfer reaction, but would tend to enrich HDL in the shorter chain esters and in the more unsaturated cholesteryl esters, and enrich the apoB-containing lipoproteins in the more saturated long chain esters.

This study did not examine the bases for the specificity of CETP-mediated transfer or of selective uptake. Morton (16) concluded that his data did not exclude the possibility that selectivity was determined by the availability of the esters in the lipoprotein coat rather than by the intrinsic selectivity of CETP. Similarly, we cannot conclude that either CETP-mediated transfer or selective uptake is in the order of the availability of cholesteryl esters in the HDL coat (39, 40). However, the rate of CETP-mediated transfer and the rate of selective uptake cannot both be determined by the same process (e.g., availability in the coat) since the relative rates of transfer of the two processes are divergent. In any case, the specificity of either process may represent the product of two or more selective steps (e.g., availability in the coat, and uptake or transfer affinity).

One straightforward explanation for the specificity of selective uptake would be that it reflects the specificity of a protein involved in that process (as may be the case for CETP-mediated transfer). However, no membrane protein is known to be required for selective uptake. Similarly, no specific apolipoprotein is required (11). One need not invoke recognition by a membrane protein to explain the specificity observed here. We have previously shown that the reversible insertion of cholesterol ester into a plasma membrane pool precedes irreversible internalization (21). The relative specificity of selective uptake does not appear inconsistent with the relative amounts of the various cholesteryl esters that can be contained in a bilayer (39, 40). The membrane pool of cholesteryl esters appears to be labeled by HDL in proportion to the cholesteryl ester mass in that pool (21), and in the present study the reversibly cell-associated pool was labeled about in proportion to selective uptake by Y1-BS1 cells. It may be then, that the rate of selective uptake reflects largely the solubility of the cholesteryl ester in the plasma membrane, whether or not a membrane protein mediates in-

corporation into the membrane. Thus, it is possible that the properties of the membrane, or more likely the product of the properties of the membrane and the lipoprotein, determines the relative rates of selective uptake.

We have shown here that Y1-BS1 cells selectively take up a triether analogue of triglyceride by a mechanism that seems to be the same as for selective uptake of cholesteryl esters. Whereas the fractional rate of this uptake is relatively modest, and whereas triglycerides in HDL account for only a small fraction of total plasma triglycerides, it seems highly unlikely that the pathway makes a significant contribution to total clearance of plasma triglycerides. However, it is possible that it plays a significant role in remodelling HDL. Triglycerides associate with HDL, apparently largely by CETP-mediated transfer in exchange for cholesteryl esters (34). Extracellular lipase, particularly hepatic lipase, may then hydrolyze these HDL triglycerides (41). The data here indicate that selective uptake also has the capacity to deplete the HDL pool of triglycerides, and thus may play a role in vivo as well.

One purpose in characterizing the specificity of the two processes for independent movement of HDL cholesteryl esters was to seek clues that might lead to development of a cholesteryl ester tracer that would participate normally in selective uptake but not be transferred by CETP. Such a tracer would be valuable in quantifying the roles of both selective uptake and CETP-mediated processes in animals which express both pathways, an otherwise demanding task heavily dependent on kinetic modelling. The systematic study of esters with saturated acyl chains and esters with C_{18} chains of varying unsaturation indicated that the ratio of selective uptake to CETP-mediated transfer could be substantially altered by altering chain length and unsaturation. However, this difference for the esters examined was not sufficient for a truly selective tracer. A number of other very different types of esters were tried. The most promising of these, the esters of branched chain fatty acids (Table 3), were selectively taken up at a fairly normal rate but transferred very slowly, if at all; α-branched esters were the most discriminating. Development of ether analogues of such a branched chain ester for use as in vivo tracers is now under way.

This work was supported by NIH Grant HL-14197 from the National Heart, Lung, and Blood Institute, by Grant 2207 from the Council for Tobacco Research, and by a gift from Pfizer Central Research.

Manuscript received 16 July 1990, in revised form 19 October 1990, and in revised form 26 November 1990.

REFERENCES

1. Nichols, A. V., and L. Smith. 1965. Effect of very low density lipoproteins on lipid transfer in incubated serum. *J. Lipid Res.* **6:** 206–210.
2. Akanuma, Y., and J. Glomset. 1968. In vitro incorporation of cholesterol-^{14}C into very low density lipoprotein cholesteryl esters. *J. Lipid Res.* **9:** 620–626.
3. Zilversmit, D. B. 1984. Lipid transfer proteins. *J. Lipid Res.* **25:** 1563–1569.
4. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27:** 361–366.
5. Fielding, C. J. 1988. The human plasma cholesteryl ester transfer protein: structure, function and physiology. *Adv. Exp. Med. Biol.* **243:** 219–224.
6. Goldstein, J. L., and M. S. Brown. 1977. The low density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46:** 897–930.
7. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
8. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1985. Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* **260:** 744–750.
9. Leitersdorf, E., A. Israeli, O. Stein, S. Eisenberg, and Y. Stein. 1986. The role of apolipoproteins of HDL in the selective uptake of cholesteryl linoleyl ether by cultured rat and bovine adrenal cells. *Biochim. Biophys. Acta.* **878:** 320–329.
10. Pittman, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor, Jr. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262:** 2443–2450.
11. Pittman, R. C., C. K. Glass, D. Atkinson, and D. M. Small. 1987. Synthetic high density lipoprotein particles: application to studies of the apoprotein specificity for selective uptake of cholesterol esters. *J. Biol. Chem.* **262:** 2435–2442.
12. Rinninger, F., and R. C. Pittman. 1987. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters. *J. Lipid Res.* **28:** 1313–1325.
13. Rinninger, F., and R. C. Pittman. 1988. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters by human fibroblasts and HepG2 hepatoma cells. *J. Lipid Res.* **29:** 1179–1194.
14. Mackinnon, M., J. Savage, R. Wishart, and P. Barter. 1986. Metabolism of high density lipoprotein by the perfused rabbit liver. *J. Biol. Chem.* **261:** 2548–2552.
15. Reaven, E., J. Boyles, M. Spicher, and S. Azhar. 1988. Evidence for surface entrapment of cholesterol-rich lipoproteins in luteinized ovary. *Arteriosclerosis.* **8:** 298–309.
16. Morton, R. E. 1986. Specificity of lipid transfer protein for molecular species of cholesteryl ester. *J. Lipid Res.* **27:** 523–529.
17. Miller, N. E. 1984. Current concepts of the role of HDL in reverse cholesterol transport. *In* Clinical and Metabolic Aspects of High-Density Lipoproteins. N. E. Miller and G. J. Miller, editors. Elsevier Science Publishers B. V., Amsterdam. 187–216.
18. Pittman, R. C., and D. Steinberg. 1984. Sites and mechanisms of uptake and degradation of high density and low density lipoproteins. *J. Lipid Res.* **25:** 1577–1585.
19. Granot, E., I. Tabas, and A. R. Tall. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured HepG2 cells. *J. Biol. Chem.* **262:** 3482–3487.

20. Rinninger, F., and R. C. Pittman. 1989. Mechanism of the cholesteryl ester transfer protein-mediated uptake of high density lipoprotein cholesteryl esters by HepG2 cells. *J. Biol. Chem.* **264:** 6111–6118.
21. Knecht, T. P., and R. C. Pittman. 1989. A plasma membrane pool of cholesteryl esters that may mediate the selective uptake of cholesteryl esters from high-density lipoproteins. *Biochim. Biophys. Acta.* **1002:** 365–375.
22. Upreti, G. C., R. J. deAntueno, and R. Wood. 1983. Membrane lipids of hepatic tissue. I. Neutral lipids from subcellular fractions of liver and hepatoma 7288CTC. *J. Natl. Cancer Inst.* **70:** 559–566.
23. Gupta, C. M., R. Radhakrishnan, and H. G. Khorana. 1977. Glycerophospholipid synthesis: improved general method and new analogs containing photoactivable groups. *Proc. Natl. Acad. Sci. USA.* **74:** 4315–4319.
24. Wallace, J. M., and J. E. Copenhaver. 1941. Anhydrides of normal aliphatic saturated monobasic acids. *J. Am. Chem. Soc.* **63:** 699–700.
25. Green, S. R., W. F. Beltz, D. I. Goldberg, and R. C. Pittman. 1989. Cholesteryl oleyl and linoleyl ethers do not trace their ester counterparts in animals with plasma cholesteryl ester transfer activity. *J. Lipid Res.* **30:** 1405–1410.
26. Pool, G. L., M. E. French, R. A. Edwards, K. Huang, and R. H. Lumb. 1982. Use of radiolabeled hexadecyl cholesteryl ether as a liposome marker. *Lipids.* **17:** 448–452.
27. Pittman, R. C., and C. A. Taylor, Jr. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–627.
28. Schumaker, V. N., and D. L. Puppione. 1986. Sequential flotation ultracentrifugation. *Methods Enzymol.* **128:** 151–155.
29. Hough, J. L., and D. B. Zilversmit. 1984. Comparison of various methods for in vitro cholesteryl ester labeling of lipoproteins from hypercholesterolemic rabbits. *Biochim. Biophys. Acta.* **792:** 338–347.
30. Morton, R. E., and D. B. Zilversmit. 1981. A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256:** 11992–11995.
31. Foreman, J. R., J. B. Karlin, C. Edelstein, D. J. Juhn, A. H. Rubenstein, and A. M. Scanu. 1977. Fractionation of human serum lipoproteins by single-spin gradient ultracentrifugation: quantification of apolipoproteins B and A-I and lipid components. *J. Lipid Res.* **18:** 759–767.
32. Schimmer, B. P. 1981. The adrenocortical tumor cell line, Y1. *In* Functionally Differentiated Cell Lines. G. Sato, editor. Alan R. Liss, New York. 61–92.
33. Chajek, T., and C. J. Fielding. 1978. Isolation and characterization of a human serum cholesteryl ester transfer protein. *Proc. Natl. Acad. Sci. USA.* **57:** 3445–3449.
34. Morton, R. E., and D. B. Zilversmit. 1983. Interrelationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258:** 11751–11757.
35. Sparrow, C. P., and R. C. Pittman. 1990. Cholesterol esters selectively taken up from high density lipoprotein are hydrolyzed extralysosomally. *Biochim. Biophys. Acta.* **1043:** 203–210.
36. Lee, F-T., J. B. Adams, A. J. Garton, and S. J. Yeaman. 1988. Hormone-sensitive lipase is involved in the hydrolysis of lipoidal derivatives of estrogens and other steroid hormones. *Biochim. Biophys. Acta.* **963:** 258–264.
37. Barter, P. J., and J. I. Lally. 1978. The activity of an esterified cholesterol transferring factor in human and rat serum. *Biochim. Biophys. Acta.* **531:** 233–236.
38. Goldberg, D. I., W. B. Beltz, and R. C Pittman. 1991. Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. *J. Clin. Invest.* **87:** 331–346.
39. Smaby, J. M., and H. L. Brockman. 1987. Acyl unsaturation and cholesteryl ester miscibility in surfaces. Formation of lecithin-cholesteryl ester complexes. *J. Lipid Res.* **28:** 1078–1087.
40. Smaby, J. M., and H. L. Brockman. 1981. Novel surface phase containing cholesteryl esters. 2. Nonequivalence of cholesteryl arachidonate and those with 18-carbon, *cis*-unsaturated acyl groups. *Biochemistry.* **20:** 724–730.
41. Goldberg, I. J., N-A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, F. T Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70:** 1184–1192.