The role of human plasma cholesteryl ester transfer protein (CETP) in the cellular uptake of high density lipoprotein (HDL) cholesteryl ester (CE) was studied in a liver tumor cell line (HepG2). When HepG2 cells were incubated with [3H]cholesteryl ester-labeled HDL, in the presence of increasing concentrations of CETP there was a progressive increase in cell-associated radiolabel to levels that were 2.8 times control. The CETP-dependent uptake of HDL-CE was found to be saturated by increasing concentrations of both CETP and HDL. The CETP-dependent uptake of CE radioactivity increased continuously during an 18-h incubation. In contrast to the effect on cholesteryl ester, CETP failed to enhance HDL protein cell association or degradation. Enhanced uptake of HDL cholesteryl ester was shown for the cells > 1.21 g/ml fraction of human plasma, partially purified CETP, and CETP purified to homogeneity, but not for the cells > 1.21 g/ml fraction of rat plasma which lacks cholesteryl ester transfer activity. HDL cholesteryl ester entering the cell under the influence of CETP was largely degraded to free cholesterol by a process inhibited by chloroquine. CETP enhanced uptake of HDL [3H]CE in cultured smooth muscle cells and to a lesser extent in fibroblasts but did not significantly influence uptake in endothelial cells or J774 macrophages. These experiments show that, in addition to its known role in enhancing the exchange of CE between lipoproteins, plasma CETP can facilitate the in vitro selective transfer of CE from HDL into certain cells.

In several species, including humans, plasma cholesteryl esters are synthesized within high density lipoproteins (HDL) as a result of the activity of the enzyme lecithin-cholesterol acyltransferase (1). HDL-cholesteryl esters may be transferred to less dense triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins) by a cholesteryl ester transfer protein (CETP) (2, 3). Since the remnants of triglyceride-rich lipoproteins are taken up by specific receptors in the liver (4), the CETP potentially provides a mechanism for the transfer of cholesteryl ester from plasma to liver. In contrast to this indirect route of catabolism, HDL-cholesteryl esters are also thought to be taken up directly by certain tissues in a process which is selective for the cholesteryl esters. Thus, a disproportionate uptake of HDL cholesteryl ester compared to apoprotein A-I has been shown in rat ovary, adrenal, and liver, and also in hepatocyte cultures (5, 6).

In the present investigation we have examined whether cholesteryl ester transfer protein can play a role in the direct transfer of HDL-CE into model liver cells, thereby providing an additional potential pathway by which CETP may influence HDL catabolism. A human liver tumor cell line (HepG2) served as a model for studying the effect of cholesteryl ester transfer protein on cellular HDL cholesteryl ester uptake.

**EXPERIMENTAL PROCEDURES**

**Materials**

**Cells**—Human hepatoma cell line (HepG2) cells were kindly provided by Drs. Knowles, Howe, and Aden of the Wistar Institute. The J774 macrophage-like cell line was obtained from Jay Unkeless (Rockefeller University). Porcine aortic endothelial cells (sixth passage) and rabbit aortic smooth muscle cells (third passage) were kindly provided by Dr. Ken Pomerantz, Columbia University. Fibroblasts were from neonatal human foreskin (sixth–eighth passage). All cells were frozen in liquid nitrogen and thawed rapidly prior to use. For each experiment the cells were plated in 18 \times 35-mm plastic Petri dishes in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 \mu g/ml streptomycin, 292 \mu g/ml l-glutamine. Plates were incubated at 37 °C in an atmosphere containing 8% CO2, 92% air. HepG2 cells were routinely split 1:6 every 4–5 days. At the time experiments were performed the cells appeared confluent.

**Lipoproteins and Cholesteryl Ester Transfer Protein**—Human HDLs (1.25–1.21 g/ml) containing radiolabeled cholesteryl esters was prepared as described previously (7). The HDL contained 90% of radioactivity in cholesteryl esters and 1% in cholesterol. Comparative analysis showed that the labeled HDL contained 55% protein, 20% phospholipid, 18% cholesteryl ester, 4% cholesterol, and 3% triglyceride and was therefore similar in composition to HDLs. Also, the radiolabeled preparation co-eluted with HDLs on a 100-cm column of 6% agarose, indicating a similar particle size. In some instances cold HDLs was added to the radiolabeled HDL to achieve the desired specific activity, 2660 cpn/μg of CE. Human HDLs was radiolabeled with [3H] by the iodine monochloride method of MacFarlane (8). Approximately 4% of the radiolabel was associated with lipids. Human plasma cholesteryl ester transfer protein was routinely purified about 500-fold from pooled blood-bank plasma through the carboxymethyl cellulose step (9). In selected experiments this CETP preparation was further purified to homogeneity by incubation with a synthetic lipid emulsion containing egg phosphatidylcholine, triolein, and oleic acid. The mixture was subjected to chromatography on a Sepharose 4B column, and the active CETP was then obtained from the emulsion following delipidation with ethanol/ether. Based on activity, the homogeneous CETP was purified 55,000-fold relative to the plasma d > 1.21 g/ml fraction.

Human and rat lipoprotein-poor plasma fractions were isolated by preparative ultracentrifugation of human and rat plasma at d 1.21 g/
ml for 72 h in a Beckman Ti 50.2 rotor at 45,000 rpm at 10 °C. [3H] Cholesterol ether-labeled HDL was prepared by incubating a sonicated emulsion of 20% egg phosphatidylcholine, 65% cholesteryl linoleate, 15% triolein, and [3H]cholesterol hexadecyl ether (100 μCi) in human plasma (37 °C, 18 h) followed by isolation of the d 1.25-1.21 g/ml fraction by sequential preparative ultracentrifugation.

Dubisco's modified Eagle's medium, penicillin (10,000 units/ml), streptomycin (10,000 μg/ml), glutamine (200 mM), and trypsin were from Gibco. Fetal bovine serum was obtained from MA Bioproducts. Bovine serum albumin (essentially fatty acid-free) and chloroquine were obtained from Sigma. Na2[35S] (in NaOH) (508 mCi/ml), was purchased from ICN Biochemicals Inc. [3H]-Labeled cholesterol (25.7 Ci/mmol) and [3H]-labeled cholesterol hexadecyl ether (46.8 Ci/mmol) were from New England Nuclep.

Methods

Cell Association Assays—At the time of the experiments the cells were incubated at 37 °C with 1 ml of DMEM containing 0.1% BSA (essentially fatty acid-free) and 125I-HDL or [3H]CE-labeled HDLs. Incubations were performed with or without added cholesteryl ester transfer protein. Cholesteryl ester transfer protein was dialyzed against DMEM before being added to the incubation medium. At the end of each incubation the dishes were placed on ice and the medium was removed. The monolayers were then washed as described by Tabas and Tall (11): three rapid (< 1 min) washes in ice-cold 0.05 M Tris-HCL, pH 7.4, containing 0.15 M NaCl and 0.2% BSA (TBS-A), three 10-min washes in TBS-A, and finally three rapid washes in TBS. The monolayer was then dissolved in 1 ml of 0.1% sodium dodecyl sulfate, an aliquot removed for protein assay (Lowry method (12)), and the radioactivity associated with the cells determined. The average protein contents per dish were: HepG2 cells, 0.65 mg; J774 macrophages, 0.5 mg; endothelial cells, 0.22 mg; smooth muscle cells, 0.27 mg; and fibroblasts, 0.26 mg. To ensure that cholesteryl ester transfer activity did not diminish during the incubation period, CE transfer activity was measured in the medium at the end of the 18-h incubations. The activity in medium of incubations performed in the presence of CETP was compared to that in medium from incubations performed without CETP. For all cell systems studied we demonstrated at least constant or slightly increased cholesteryl ester transfer activity in the medium throughout the incubation period. In experiments in which the amount of cell membrane-bound HDL-CE was determined, the cells were treated, at the end of the 18-h incubation, with trypsin at varying concentrations between 0.05 and 1.0% for 5 min. At these trypsin concentrations cell membranes remained intact, as demonstrated by a negative trypsin blue stain at termination of the 5-min incubations. To determine the amount of 125I-labeled HDL bound to the cell surface following the nine sequential washes (as above) monolayers were treated with 0.05% trypsin and incubated for 5 min at 37 °C. After 5 min 1 ml of DMEM with 10% fetal bovine serum was added to inhibit further proteolysis. The cell suspension was then centrifuged (1000 x g) for 10 min at 4 °C. The radioactivity in the supernatant was the trypsin-releasable value. The cell pellet was washed with TBS-A and resuspended at 1000 x g for 10 min. The supernatant was discarded and the pellet counted (tryptic-resistant value). To determine whether the 125I-HDL trypsin-resistant radioactivity represents cellular uptake of 125I-HDL by the cells, displacement experiments were carried out in which excess unlabeled HDL (1 mg of protein/ml) was added to the cells at the end of the 18-h incubation. Cells were washed rapidly three times with TBS and incubated for 3 h at 37 °C with excess unlabeled HDL.

Degradation of 125I-HDL was determined in the medium, at the end of the 18-h incubations, after precipitation of protein with trichloroacetic acid (13).

Measurement of Radiolabeled Free Cholesterol and Cholesteryl Ester in Cells—Following incubations with [3H]CE-labeled HDLs the medium was removed and the cells were washed as described above. After the last wash, cells were scraped with a rubber policeman, suspended in TBS, and spun at 1000 rpm for 10 min, and then the supernatant was discarded. Cellular lipids were extracted by the Folch method (14), and the extracts were applied to thin layer chromatography plates and analyzed in a solvent system of hexane/ether/acetic acid (70:30:1). Free [3H]cholesterol and [3H]cholesterol ester, identified using reference standards, were scraped off the plates, and their radioactivity was determined in a liquid scintillation counter.

RESULTS

To assess the role of CETP in the uptake of HDL cholesterol ester by HepG2 cells, cells were incubated in medium containing [3H]CE-labeled HDL in the presence of increasing concentrations of partially purified CETP (Fig. 1A). With increasing CETP mass, there was a progressive increase in the cellular uptake of HDL-CE up to 2.8 times that of HDL-CE uptake without CETP (Fig. 1A, closed circles). To see whether CETP caused a similar increase in cellular uptake of HDL protein, we incubated [125I]-HDL with HepG2 cells in the presence of increasing concentrations of CETP. Cell uptake of HDL protein was calculated as the amount of [125I]-HDL that remained associated with the cells plus the amount of [125I]-HDL degraded during the incubation period as calculated from the amount of trichloroacetic acid-soluble radioactivity in the medium, i.e. cell association and degradation. As shown in Fig. 1A (open circles), the presence of CETP did not result in a significant enhancement of cellular HDL protein uptake. In six different experiments similar responses were obtained: mean cellular HDL-CE uptake was 1.1 ± 0.16% of the total added radioactivity without CETP and increased progressively to 2.9 ± 0.36% in the presence of CETP (210 μg/ml). Uptake of HDL protein was 1.1 ± 0.09% without CETP and 1.1 ± 0.05% in the presence of CETP. Thus, in the absence of CETP, uptake of HDL protein and HDL-CE were in similar proportions.
proportion to the original HDL. However, in the presence of CETP, there was a selective enhancement of cellular HDL cholesteryl ester uptake compared to HDL protein uptake.

Further experiments were conducted to determine whether the stimulated uptake of HDL-CE was a specific property of CETP. CETP was purified to homogeneity as described under “Methods.” The effect of CETP on cellular uptake of HDL-CE was also shown by the purified homogenous CETP with enhanced cellular HDL-CE uptake at increasing CETP concentrations (Fig. 1B). Based on specific activity (CE transferred/mg of protein in an HDL-LDL exchange assay) the homogenous CETP was purified approximately 350-fold compared to the partially purified CM52 preparation. The fact that a similar increase in specific activity was found for the cellular uptake of HDL-CE (cf. Fig. 1, A and B) suggests that the effect of the partially purified fraction on cellular uptake of CE can be entirely accounted for by its content of CETP. This was confirmed in an experiment where immunoprecipitation of the partially purified fraction with CETP-specific IgG2 completely abolished its ability to stimulate cellular uptake of HDL-CE, whereas non-immune IgG did not.

To prove further that CETP activity was responsible for the selective uptake of HDL-CE, we took advantage of the fact that human d > 1.21 g/ml lipoprotein-poor plasma shows CE transfer activity, whereas rat plasma is devoid of such activity. Therefore, we compared the effect of human and rat lipoprotein-poor plasma on the cellular uptake of HDL cholesteryl ester (Fig. 2). Cellular [3H]CE-labeled HDL uptake increased as a function of increasing concentrations of human lipoprotein-poor plasma (closed circles). By contrast, the rat d > 1.21 g/ml plasma fraction did not enhance HDL-CE uptake by HepG2 cells (open circles).

To characterize further the effect of CETP on the uptake of HDL cholesteryl ester, we studied the time course of the cellular uptake of HDL-CE (Fig. 3). The effect of CETP on HDL-CE uptake was clearly apparent after 6 h of incubation and increased throughout the length of incubations. The effect of increasing HDL-CE mass on the CETP enhancement of cellular HDL-CE uptake was also examined. Incubations were performed with increasing concentrations of [3H]CE-labeled HDL but constant CETP concentrations (Fig. 4). With increasing HDL mass there was an increase in both the basal and CETP-stimulated uptake of HDL cholesteryl ester. However, the increment in CE uptake specifically due to CETP (i.e., the difference of the two curves in Fig. 4) reached a maximum value at a HDL concentration of 45 μg/ml, indicating that, under these conditions, the CETP-specific uptake was saturated at relatively low concentrations of HDL.

Further experiments were conducted to see whether facilitated CE transfer could be observed at physiological levels of HDL and transfer activity. In the earlier experiments (Fig. 2), it was noted that the effect of the human d > 1.21 fraction reached a maximum value at 7.2 mg of protein, at which point the ratio of HDL-CE/d > 1.21 protein approximates the physiological value. In a further experiment the concentrations of both HDL and d > 1.21 fraction were increased, using a fixed, physiological ratio of HDL/d > 1.21 fraction (Fig. 5). There was a continuous increase in both the basal and facilitated transfer of HDL-CE into the cells, with the fold-increase mediated by the d > 1.21 fraction approximately constant. Thus, the enhanced transfer of HDL-CE was observed with physiological concentrations of HDL and d > 1.21 protein in the medium.

HepG2 cells have the capacity to synthesize and secrete apoB-containing lipoproteins (15). Therefore the observed effect of CETP on cellular HDL-CE uptake might actually occur through CETP-mediated transfer of CE from HDL to apoB-containing lipoproteins and subsequent cellular uptake of these lipoproteins. Sodium dodecyl sulfate-gel analysis of the apoproteins isolated from the d < 1.063 fraction of 4 ml of 24-h conditioned medium showed the presence of several micrograms of apoB-100; by contrast, the d 1.063–1.210 g/ml

![Fig. 3. Time course of the uptake of HDL-cholesteryl ester by HepG2 cells, with and without CETP.](image-url)
Facilitated Lipid Transfer into Cells

Cholesteryl ester transfer activity (counts/min transferred) in HepG2 medium

|          | −CETP | +CETP |
|----------|-------|-------|
| Cells    | 406   | 1906  |
| No cells | 0     | 1597  |

Fig. 5. HDL-CE uptake by HepG2 cells at a physiological ratio of HDL, d > 1.21 fraction. Cells were incubated for 20 h at 37°C with 1 ml of DMEM, 0.1% BSA containing the indicated concentrations of [3H]cholesterol ester-labeled HDL 

| MASS (gg/ml) | CE transfer activity (counts/min transferred) in HepG2 medium |
|-------------|------------------------------------------------------------|
| 0.05%       | 480,000 cpm/pg of CE alone | 480,000 cpm/pg of CE alone |
| 0.1%        | 5826 cpm/pg of CE          | 5826 cpm/pg of CE          |

Recent experiments from this laboratory indicate that cholesteryl ester transfer activity accumulates in a time-dependent fashion in the medium of cultured HepG2 cells, suggesting synthesis of CETP by these cells. To compare the amount of CE transfer activity added to cells with that secreted by the cells during an 18-h incubation, the CE transfer activity was measured in conditioned medium with or without added CETP, indicating accumulation of a small amount of endogenous CE transfer activity in the medium. It is possible that this endogenous CE transfer activity contributes to the uptake of HDL cholesteryl ester; however, it is a relatively small amount of activity compared to that added with exogenous CETP.

Further experiments were performed to elucidate the mechanism of cellular HDL-CE uptake. As noted above, CETP had no effect on total cellular uptake of [3H]-HDLC. Furthermore, CETP was also found to have no effect on 125I-HDL cell association (0.13% of total added radioactivity in the presence of CETP as compared to 0.12% without CETP) or on the fraction of the cell-associated radioactivity that was displacable by cold HDL (40.8% in the presence of CETP and 40.3% in control experiments without CETP). Of the cell-associated protein radioactivity a major fraction (about 55%) was trypsin-releasable. By contrast, in another experiment it was found that only 6–9% of CETP-stimulated [3H] cholesteryl ester uptake was trypsin-releasable, indicating internalization of HDL-CE.

Since the major fraction of cell-associated HDL-CE is internalized by the cell, the intracellular fate of cholesteryl ester was studied. Following cellular uptake cell lipids were extracted and separated by thin layer chromatography. In the presence of CETP the amount of cell-associated radioactivity of both free cholesterol and CE was augmented 2–3-fold. Since more than 99% of the label in HDL was in cholesteryl ester and since CETP does not promote transfer of unesterified cholesterol, these results suggest hydrolysis of CE following entry into the cell. This was confirmed by the addition of 25 \( \mu \) M chloroquine, which inhibited the degradation of HDL cholesteryl ester to free cholesterol (Fig. 6). These results indicate that HDL cholesteryl esters entering the cell under...
performed in the absence or presence of CETP (210 pg/ml). At the end of the incubation period cells were washed, lipids extracted, and free cholesterol and cholesteryl ester were separated by thin layer chromatography. Results represent the mean ± S.E. of six experiments. Open bars, free cholesterol; shaded bars, cholesteryl ester.

Table II

| Free cholesterol | Cholesteryl ester |
|------------------|------------------|
| [3H]CE-HDL       | -CETP 543        | +CETP 436 |
| [3H]CE-HDL + chloroquine + acyl-CoA:cholesterol acyltransferase inhibitor | -CETP 674 | +CETP 599 |

The human plasma CETP has been well documented to facilitate both the exchange and net transfer of CE and triglyceride between plasma lipoproteins. Stein et al. (16) have recently shown that CETP can remove cholesteryl esters from lipoproteins bound to cell surface locations and can also remove CE from intracellular locations but only following treatment that permeabilizes the cells. We present evidence for a function of CETP that has not been previously described. Our observations indicate that CETP can promote the transfer of cholesteryl ester from HDL into intact HepG2 and smooth muscle cells. The radiolabeled cholesteryl esters are internalized and undergo lysosomal degradation.

Based on the kinetics of lipid exchange between the lipoproteins, two models for CETP-dependent CE transfer have been suggested. A ping-pong model proposes that CETP acts as a carrier of CE between donor and acceptor lipoproteins (17). Alternatively, CETP may enhance the exchange of lipids during formation of a ternary collision complex consisting of donor and acceptor lipoprotein and CETP (18). Either model could potentially explain the effect of CETP on cellular HDL-CE uptake. However, the failure of CETP to promote cell association or degradation of HDL protein suggests that the influence of CETP are susceptible to lysosomal degradation. To evaluate further the possibility that the accumulating CE reflected re-esterification of [3H]cholesterol by acyl-CoA:cholesterol acyltransferase, we studied HDL cholesteryl ester uptake by HepG2 cells under the influence of CETP (cholesteryl ethers are not susceptible to degradation by hydrolases, and are taken up by cells as intact molecules). HepG2 cells were incubated in DMEM, 0.1% BSA containing [3H]CE-labeled HDL alone or in the presence of CETP. Following an 18-h incubation cell-associated [3H]cholesteryl ether was 14.0-fold higher in the presence of CETP as compared to control. In a simultaneous experiment the cell-associated [3H]cholesteryl ester increased 14.0-fold, at 18 h, in the presence of CETP. Thus, CETP caused stimulation of both cholesteryl ether and cholesteryl ester uptake. The relatively small effect observed was due to the lower specific activity of the partially purified CETP preparation used in this experiment.

In order to determine whether CETP enhancement of HDL cholesteryl ester uptake is specific for HepG2 cells, we evaluated the effect of CETP on HDL-CE uptake by other cell types (Fig. 7). CETP enhanced uptake of HDL-CE by smooth muscle cells; HDL-CE uptake increased to levels that were 2.6 times the control. CETP also enhanced HDL-CE uptake by fibroblasts but to a lesser extent than in smooth muscle cells. In endothelial cells and J774 macrophages there was no significant change in cellular HDL-CE uptake with increasing CETP concentrations, indicating that CETP does not facilitate uptake of HDL-CE in these cell types.

Discussion

The effect of chloroquine on the degradation of HDL cholesterol ester. Cells were incubated for 18 h at 37 °C in 1 ml of DMEM, 0.1% BSA containing [3H]cholesterol ester-labeled HDL, total radioactivity 240,000 cpm/well (specific activity 2660 cpm/μg of CE) alone or plus 25 μM chloroquine. For both sets of conditions incubations were performed in the absence or presence of CETP (210 μl/ml). At the end of the incubation period the cells were washed (as described under "Methods") and lipids extracted by the Folch method (14). Free cholesterol and cholesteryl ester were separated by thin layer chromatography. Results represent the mean ± S.E. of six experiments. Open bars, free cholesterol; shaded bars, cholesteryl ester.

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CETP is not acting by enhancing binding or fusion of HDL with the cell surface. The saturation of CETP-dependent CE uptake by increasing donor (HDL) concentration is typical of the kinetics of carrier-mediated lipid transfer (17). If the transfer into the cell is carrier-mediated, then the findings of the present study imply the existence of a cell surface binding site that can be recognized by CETP. CETP binds readily to phospholipid surfaces in emulsion, lipoproteins, and vesicles, especially in the presence of an increased negative charge (7, 19, 20), suggesting that CETP might also bind to the lipids of the plasma membrane. Another possibility is that CETP binds to a cell surface receptor. However, a specific role of the LDL receptor in mediating the CETP-dependent uptake seems unlikely, since down-regulation of the LDL receptor did not alter the fold stimulation of uptake due to CETP. An analogy to the present results is suggested by the previous descriptions of lipoprotein lipase-enhanced cellular uptake of CE from liposomes or lipoproteins (21). Both the lipase and CETP molecules may have binding sites for neutral lipid which allow them to act as carriers of CE.

CETP increased the uptake of HDL-CE but did not affect the cell association or degradation of HDL protein. Thus, CETP does not enhance binding or internalization of whole HDL particles. These findings are reminiscent of previous studies in which it had been shown that, in selected tissues, there is a disproportionate uptake of HDL-CE compared to HDL protein (5, 6, 22). HDL-CE uptake into rat adrenal cells was not changed during metabolic inhibition of sucrase lipase processes, implying that the disproportionate uptake of HDL-CE in excess of HDL apoA-I probably does not involve whole particle uptake through receptor-mediated endocytosis (23). Although these studies have mostly been performed in the rat, which lacks cholesteryl ester transfer activity, selective uptake of HDL-CE has also been shown in perfused rabbit liver (22), which in other studies has been shown to accumulate cholesteryl ester transfer activity (24).

The CETP promotes neutral lipid transfer between lipoproteins by facilitating a cholesteryl ester-triglyceride heteroexchange process (25). The phenomenon of CETP-dependent cellular uptake of HDL-CE might also involve lipid exchange. Because the amount of CE taken up by HepG2 cells is in the range of 1.5–1.8 μg CE/mg of cell protein, it was not possible to document net mass changes. Currently, there is a paucity of data to show that, upon entry into cells, HDL-derived CE affects cellular metabolism. However, we have shown that internalized HDL-CE undergoes hydrolysis via a lysosomal pathway. The observed increase in cellular free cholesterol suggests that HDL-derived CE has the potential to influence cellular cholesterol metabolism. HDL-CE uptake does promote cellular prostanoid synthesis, in part by transfer of cholesteryl arachidonate from HDL to cellular lipid pools containing arachidonate (10). The CETP has been found to increase markedly the HDL-induced prostanoid release by smooth muscle cells partly as a result of increased incorporation of HDL-CE-derived arachidonate into prostanoids.4 These experiments clearly show that fatty acid derived from CETP-induced CE entry can influence cellular metabolism and strongly imply that the facilitated entry of HDL-CE into cells is not a simple lipid exchange process.

An intriguing aspect of the CETP-dependent uptake of HDL-CE is the cellular specificity. We have observed CETP enhancement of HDL-CE uptake in HepG2 cells, a human tumor cell line, in rabbit smooth muscle cells, and to a lesser extent in human fibroblasts. No effect of CETP on HDL-CE uptake was noted in J774 macrophages or porcine endothelial cells. Specificity is not related to HDL binding, as endothelial cells have been shown to bind ^{125}I-HDL_{S} to a higher degree than either smooth muscle cells or fibroblasts (11) and, similarly, we have also observed, that in the absence of CETP, HDL-CE cell association per mg of cell protein was highest for endothelial cells. CETP augmentation of HDL-CE uptake could be related to cell surface characteristics that enable the cell membrane to bind CETP or to the presence of intracellular lipid pools that provide lipid for CETP-mediated exchange processes.

The physiological significance of the CETP-dependent uptake of HDL-CE is unknown. As these experiments were conducted in a model cell system, definite conclusions relating to in vivo conditions cannot be made. However, the CETP did enhance cellular HDL-CE uptake at a physiological ratio of HDL-CE to human > 1.21 g/ml fraction (Fig. 2) and at physiological concentrations of HDL and d > 1.21 fraction (Fig. 5). Since the d > 1.21 fraction shows similar CE transfer activity to whole plasma,4 this result indicates that the effect was observed at physiological levels of CE transfer activity and HDL. In those species that possess plasma cholesteryl ester transfer activity, the CETP-mediated selective uptake of HDL-CE might constitute a pathway for direct incorporation of HDL-CE, exclusive of other HDL components, into the liver or other tissues.

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