# Cholesterol Transport Function of Pancreatic Cholesterol Esterase: Directed Sterol Uptake and Esterification in Enterocytes

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ABSTRACT: We have recently hypothesized that neutral lipids can, in part, move across biological membranes via a mechanism involving enzymes anchored to membrane proteoglycans such as those found in the brush border of the enterocyte [Bosner, M. S., Gulick, T., Riley, D. J. S., Spilburg, C. A., & Lange, L. G. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85,* 7438–7442]. Present results now show a subsequent, essential protein-mediated sorting of neutral lipids for further intracellular metabolism. Thus, in the absence of enzyme, 0.002 pmol of cellular ester appeared after 2 h, and its level increased only 3.5-fold after 12 h. However, in the presence of cholesterol esterase, the level of cholesterol ester increased 39-fold in the same time period, indicating that the enzyme-mediated uptake accounts for 10-fold greater ester synthesis than that from basal absorption. Kinetic analysis reveals that both enzyme-mediated and background absorption depend on taurocholate concentration and are second-order reactions more likely dependent on collision than diffusion. Other lipid-recognizing proteins such as pancreatic triglyceride lipase and the intestinal fatty acid binding protein are not stimulatory to intracellular cholesterol processing. Taken together, these data suggest that pancreatic cholesterol esterase and possibly other proteoglycan-binding extracellular enzymes of neutral lipid metabolism may facilitate movement of neutral lipids into the plasma membrane and direct them into functional intracellular sites.

In the absence of receptor-mediated endocytosis, the molecular basis for the movement of lipids at physiologic rates is poorly understood. Unesterified cholesterol is virtually insoluble in water so its transport in the small intestine depends critically on its solubility in micelles composed of bile salts and other amphiphiles. Similarly, cholesterol can be "dissolved" in the intestinal membrane, presumably through hydrophobic interactions or by the presence of sterol binding proteins (Scallen et al., 1985; Teerlink et al., 1984). Since cholesterol does not enter the small intestinal cell by the uptake of micellar aggregates (Thomson & Dietschy, 1981), the mechanism of transport of sterol from one hydrophobic environment to another remains unclear.

We have suggested that anchoring mechanisms may exist to juxtapose neutral lipid binding proteins or enzymes to the plasma membrane to facilitate absorption. Indeed, this appears to be the case for movement of cholesterol and free fatty acid across the brush border membrane via anchored pancreatic cholesterol esterase (CEase)[1] and triglyceride lipase (Bosner et al., 1988, 1989). In these examples, as well as for lipoprotein lipase, immobilization occurs by a high-affinity, specific interaction with membrane proteoglycans.

Similarly, in the absence of other factors, such as proteins, hydrophobic lipids are unlikely to leave the plasma membrane for the aqueous cytosol. For cholesterol, this next transport process is especially important because this sterol must be converted to cholesteryl ester for packaging in the chylomicron for subsequent transport to the liver. Previous investigations using cultured intestinal cells demonstrated that the intracellular pool of newly synthesized or preformed cholesterol was not affected by incubation of cells with sources of free cholesterol (Stange et al., 1983; Herold et al., 1984). All these considerations now suggest the existence of protein-directed sorting of neutral lipids for intracellular metabolism. Therefore, in this work, we have examined the role that exogenously secreted proteins such as pancreatic CEase may play in directing lipid for intracellular processing.

## MATERIALS AND METHODS

*Cholesterol Esterase and Other Proteins.* Bovine pancreatic CEase was isolated and purified to homogeneity following the procedure described by Cox et al. (1990) and assayed with [$^{14}$C]cholesteryl oleate (Kyger et al., 1990). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (7.5%) revealed a single band at 72 kDa. Protein concentration was determined by Lowry assay. Liver fatty acid binding protein was provided by Dr. D. Cistola (Washington University, St. Louis, MO) and porcine pancreatic lipase was from Sigma Chemical Co.

*Labeled Vesicle Preparation.* [1,2-$^{3}H_2$]Cholesterol (48.1 Ci/mmol) was purified by thin-layer chromatography on silica gel with hexane–diethyl ether (97:52). Sterol-containing vesicles were prepared by combining 10 μL of labeled cholesterol (1.0 mCi/mL) with 500 μL of egg phosphatidylcholine (1.0 mM) and drying the mixture under nitrogen. Ten milliliters of 100 mM Tris buffer, pH 7.2, was added, and the suspension was dispersed by sonication at 250–300 W for 30 min with a Branson sonifier.

*Caco-2 Cell Uptake Studies.* Colonic adenocarcinoma cells (Caco-2 cells; American Type Culture Collection) were grown in Eagle's minimum essential medium and fetal bovine serum (20%), supplemented with nonessential amino acids (1%) and 100 mg/mL penicillin–streptomycin. Confluent cells were

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[1] Abbreviations: CEase, cholesterol esterase; PBS, phosphate-buffered saline; ACAT, acylcoenzyme A:cholesterol acyltransferase.

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Table I: Absorbed Cholesterol as a Function of CEase Concentration[a]

| CEase (nM) | absorbed cholesterol [pmol ($2 \times 10^6$ cells)$^{-1}$] |
|---|---|
| 0 | 0.11 ± 0.03 |
| 5 | 0.16 ± 0.02 |
| 25 | 0.25 ± 0.02 |
| 50 | 0.33 ± 0.04 |
| 100 | 0.36 ± 0.03 |

[a] Caco-2 cells ($2 \times 10^6$) were incubated in PBS containing 8.0 mM taurocholate, 1.0% bovine serum albumin, 1.0 nM [$^3$H]cholesterol, and CEase at the given concentration. The experiment was stopped after 4 h and cellular cholesterol determined as described in Materials and Methods.

treated with fresh trypsin (0.25%), seeded into plastic wells (22.6 mm; 4 cm$^2$) and grown to confluence ($2.0 \times 10^6$ cells/well). For cholesterol uptake studies, cells were incubated overnight in Eagle's minimum essential medium and 10% lipoprotein-deficient serum. The cells were rinsed with PBS, and 500 μL of PBS containing 8 mM taurocholate, 1% bovine serum albumin, and 0.5 pmol of [$^3$H]cholesterol phosphatidylcholine vesicles were added. Experiments were initiated with the addition of 100 nM bovine pancreatic CEase. At various times, the reaction was stopped by removing the incubation medium and rinsing the cell monolayers with cold PBS. The cells were detached from the well with 0.10% sodium dodecyl sulfate solution (200 μL) and added to 7.5 mL of Aquasol (Dupont), and the radioactivity in the cellular debris was counted to determine the amount of cholesterol associated with the cells.

To determine the amount of cholesteryl ester in the cellular debris, 20 μL of 1 mM cholesteryl oleate containing [$^{14}$C]-cholesteryl oleate (1000 cpm) were added as carrier and efficiency standard, respectively. The lipids were extracted by adding 1.8 mL of chloroform–methanol (2:1) followed by 0.5 mL of water and 0.5 mL of chloroform. The chloroform layer was removed and evaporated under a nitrogen stream, and the residue was redissolved in 150 μL of petroleum ether–methanol–chloroform (1:1:1). The lipids were chromatographed on silica OF plates using petroleum ether–diethyl ether–acetic acid (75:5:1). After visualization with iodine vapor, the cholesteryl ester spot was scraped into scintillation fluid, and the amount of synthesized [$^3$H]cholesteryl ester was calculated after correction for spillover from the [$^{14}$C]-cholesteryl oleate efficiency standard. The efficiency of extraction was always between 60% and 75%.

## RESULTS

*Intestinal Cell Uptake of Cholesterol Is Stimulated by Pancreatic CEase.* To assess the CEase-stimulated uptake of cholesterol into intestinal cells, experiments were first performed to delineate critical experimental parameters. For example, the amount of cell-associated cholesterol was measured at a single incubation time, 4 h, and a fixed cholesterol concentration, 1.0 nM, but with enzyme varying in concentration from 0 to 100 nM, the intestinal concentration of CEase. As shown in Table I, in the absence of enzyme, cultured intestinal cells become associated with 0.11 pmol of cholesterol ($2 \times 10^6$ cells)$^{-1}$. As enzyme is added, the amount of cell-associated cholesterol increases to 0.33 pmol ($2 \times 10^6$ cells)$^{-1}$ and then plateaus above 50 nM CEase. This experiment demonstrates that cholesterol can bind to intestinal cells by two different pathways, an enzyme-independent process and a CEase-mediated process.

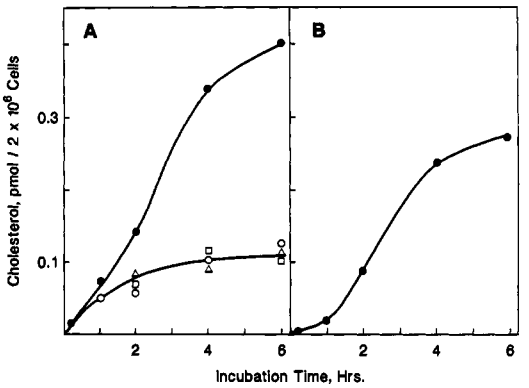


FIGURE 1: Time dependence of cholesterol uptake. (A) Caco-2 cells ($2 \times 10^6$) were incubated with 1.0 nM [$^3$H]cholesterol in the absence of enzyme (○) or in the presence of 100.0 nM bovine CEase (●) or 100.0 nM pancreatic triglyceride lipase (□) or 100.0 nM fatty acid binding protein (△) in PBS containing 8.0 mM taurocholate and 1% bovine serum albumin. As a function of time, cellular [$^3$H]cholesterol content was determined. (B) Cellular [$^3$H]cholesterol content for the CEase-stimulated uptake was determined by subtracting values for curve (○) from those for curve (●).

*Specificity and Time Dependence of Cholesterol Uptake.* The time dependence of cholesterol uptake at a fixed cholesterol concentration (1.0 nM) and in the presence of a physiological concentration of bile salt (8 mM) was next examined either in the absence of enzyme or in the presence of 100 nM bovine CEase. In the absence of enzyme (Figure 1A, lower curve), there was an initial burst of sterol uptake which occurred during the first 30 min of incubation; thereafter, cellular cholesterol content increased slowly at a rate of 0.017 pmol h$^{-1}$ ($2 \times 10^6$ cells)$^{-1}$. This kinetic complexity has been observed in other systems, such as in the transfer of cholesterol from red blood cells to sealed ghosts, and presumably reflects initial saturation of available sterol binding sites in the outer leaflet of the bilayer (Steck et al., 1988).

CEase-mediated cholesterol uptake into Caco-2 cells occurs at a markedly enhanced rate (Figure 1A). To determine the true time dependence of the enzyme-mediated process, the enzyme-independent values were subtracted to give the derived values shown in Figure 1B. These data demonstrate the presence of a 30-min lag period in the enzyme-stimulated uptake, followed by uptake at a rate of 0.105 pmol h$^{-1}$ ($2 \times 10^6$ cells)$^{-1}$ or 6 times greater than that found in the absence of enzyme. Other lipid binding proteins, such as 100 nM pancreatic triglyceride lipase or 100 nM liver fatty acid binding protein (Teerlink et al., 1984), have no effect on cholesterol uptake (Figure 1A). Importantly, triglyceride lipase also binds to heparin (Bosner et al., 1989) so the enhanced cholesterol absorption seen with CEase is not due to a modification of the thickness/structure of the unstirred water layer. Therefore, these results show that, under physiological conditions, pancreatic CEase specifically and significantly stimulates cholesterol uptake into Caco-2 cells.

*Mechanism of Uptake.* Kinetic analyses of this process were performed following the method of Thurnhofer and Hauser (1990), who showed that with brush border membranes the initial rate of cholesterol uptake, $v_0$, is characterized by the equation

$$v_0 = k_2 c_A{}^a c_D{}^d$$

where $k_2$ is the second-order rate constant and $c_A$ and $c_D$ are the lipid concentration in the acceptor and donor, respectively. At a fixed concentration of lipid in the acceptor, the slope of a plot of log $v_0$ as a function of log donor lipid concentration

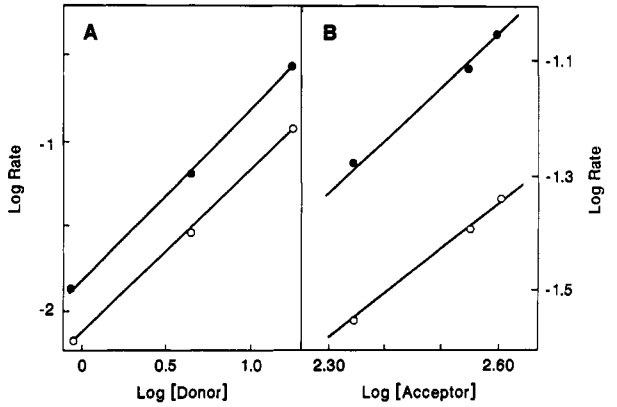


FIGURE 2: Reaction order of cholesterol uptake. (A) At a fixed cell density ($2 \times 10^6$), the rate of uptake was measured as a function of donor cholesterol in the presence (●) or absence (○) of 100.0 nM CEase. The donor cholesterol was tested at 0.86, 4.30, and 17.2 pmol. (B) The same experiment as in (A) but this time the acceptor concentration was varied while the donor cholesterol remained fixed at 17.2 pmol. Since cholesterol content could not be measured in the cell, the cell number was varied by using cells at different degrees of confluency. One group of cells was used for uptake studies and another group was used for protein determination. The concentration shown on the abscissa is the measured protein and it is assumed that the acceptor cholesterol concentration is proportional to the protein concentration. This proportionality factor will shift the position of the line, but it will not change the slope (order of the reaction).

gives the order of the reaction, *d*. Using the experimental conditions described here, the rate of sterol uptake into a fixed number of Caco-2 cells (acceptor) was found to be first order with respect to the donor cholesterol concentration for both the enzyme-mediated process and the enzyme-independent process (Figure 2A). Similar experiments were also performed with fixed donor cholesterol concentration in the vesicle, but in this case, the number of cells was changed as a means to vary the acceptor concentration. While varying the cell number over a wide range is experimentally difficult, for the attainable, 2–3-fold range used here, the rate of uptake was also a first-order reaction (Figure 2B). Therefore, cholesterol uptake by Caco-2 cells either in the presence or in the absence of enzyme is a second-order reaction overall. Under the experimental conditions used here, these data favor a mechanism involving collisional exchange or transfer rather than an aqueous diffusion model.

*Dependence of Uptake on Cholesterol Concentration and Solvent Composition.* The preceding experiments delineate the conditions which must be used to obtain reproducible results for the kinetics of CEase-stimulated cholesterol uptake. Therefore, in all subsequent studies, the following protocol was used. First, the enzyme and cells were preincubated for 30 min before addition of cholesterol to eliminate the burst kinetics and the time lag which were described in Figure 1. Second, for all kinetic experiments, a fixed acceptor concentration ($2 \times 10^6$ cells) was used and the enzyme-independent rate was subtracted from the CEase-stimulated uptake. Finally, since these cells have a limited capacity for sterol uptake (Table I), cellular [$^3$H]cholesterol was measured at several time points to ensure linearity. With these constraints, the kinetics of cholesterol uptake were determined as a function of sterol and taurocholate concentration.

The CEase-mediated uptake of cholesterol by Caco-2 cells depends critically on the taurocholate concentration in the medium. Kinetic experiments were performed at 0, 2.0, 4.0, 8.0, 10.0, and 20.0 mM taurocholate, and the net rate of cholesterol uptake was the difference between the enzyme-catalyzed rate and the background uptake, both measured at the same bile salt concentration. As shown in Table II, a saturable stimulation of the CEase-dependent rate of uptake occurs between 2.0 and 8.0 mM, demonstrating that free cholesterol transport is enhanced by CEase over and above the basal rates observed in the presence of bile salt. This same bile salt dependence is seen for the hydrolysis of cholesteryl esters, indicating that hydrolytic activity may be a key feature of cholesterol absorption. This finding also bears importantly on previous work which found that CEase did not stimulate uptake of unesterified cholesterol (Huang & Hui, 1990). These studies were performed in the presence of only 0.0625 mM taurocholate, 100-fold less than the physiological concentration found in the small intestine and, as shown here, a concentration that is insufficient to stimulate enzyme-mediated uptake. Using this optimum bile salt concentration, uptake studies were performed as a function of sterol concentration varying from 0.1 to 10 nM cholesterol (Figure 3, inset). Lineweaver–Burk analysis reveals a $K_m$ of 0.3 nM and a $V_{max}$ of 30 pmol h$^{-1}$ ($2 \times 10^6$ cells)$^{-1}$ (mg of CEase)$^{-1}$ (Figure 3).

Table II: Taurocholate Dependence of the CEase-Stimulated Uptake of Cholesterol[a]

| taurocholate (mM) | net cellular cholesterol uptake[b] [pmol h$^{-1}$ ($2 \times 10^6$ cells)$^{-1}$ (mg of CEase)$^{-1}$] |
|---|---|
| 0 | 0 |
| 2 | 0.7 |
| 4 | 8.6 |
| 8 | 13.6 |
| 10 | 13.0 |
| 20 | 6.2 |

[a] All assays were performed in PBS, pH 7.2. [b] At each taurocholate concentration, net cellular cholesterol was measured at 2, 4, and 6 h in the presence or absence of 100 nM CEase. The rate of cellular cholesterol uptake shown here is the difference between the enzyme-catalyzed rate and the background uptake, both measured at the indicated taurocholate concentration. The cholesterol concentration in the medium was 1.0 nM.

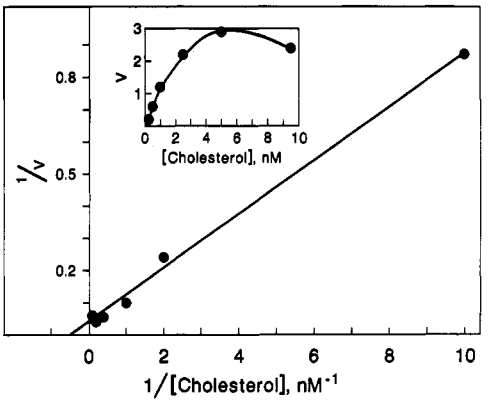


FIGURE 3: Lineweaver–Burk plot for the CEase-stimulated uptake of cholesterol by Caco-2 cells. As a function of time, [$^3$H]cholesterol was measured in Caco-2 cells ($2 \times 10^6$) in the presence of PBS, pH 7.2, 1% bovine serum albumin, 100.0 nM bovine CEase, and 8.0 mM taurocholate. The velocity of uptake is expressed as picomoles per hour per $2 \times 10^6$ cells per milligram of CEase. The linear rates of uptake are plotted as a function of cholesterol concentration (inset) and show saturation kinetics. The corresponding Lineweaver–Burk plot yields a $K_M$ of 0.30 nM and $V_{max}$ of 30 pmol h$^{-1}$ ($2 \times 10^6$ cells)$^{-1}$ (mg of CEase).$^{-1}$

*Metabolic Fate of CEase-Enhanced Cholesterol Uptake.* We observed that CEase preferentially directs new cell-associated sterol into intracellular metabolism pathways. After cells were incubated for various times with cholesterol and 8 mM taurocholate in the presence or absence of 100 nM CEase,

Table III: Effect of CEase on the Conversion of Absorbed Cholesterol into Cholesteryl Ester[a]

| | cholesteryl ester [pmol ($2 \times 10^6$ cells)$^{-1}$] | |
|---|---|---|
| time (h) | no enzyme | enzyme |
| 0 | 0.0 | 0.0 |
| 2 | 0.002 | 0.002 |
| 4 | 0.004 | 0.007 |
| 8 | 0.005 | 0.022 |
| 12 | 0.008 | 0.078 |

[a] Caco-2 cells were incubated with 0.5 pmol of cholesterol and 8.0 mM taurocholate in the presence or absence of 100 mM CEase. At the indicated times the incubation was terminated by scraping the cells with 0.1% SDS. Lipids were then extracted from cellular debris and separated by thin layer chromatography as described in Materials and Methods.

cellular lipids were extracted and separated using thin-layer chromatography to allow quantitation of the amount of cholesterol converted into ester. In the absence of enzyme, 0.002 pmol of cellular ester appeared after 2 h, and its level increased only 3.5-fold after 12 h (Table III). However, in the presence of CEase, the level of cholesterol ester increased 39-fold in the same time period. Thus, after 12 h, the CEase pathway is the origin of about 90% of the cholesterol which is subsequently processed intracellularly to ester.

## DISCUSSION

The principal intracellular fate of cholesterol in the enterocyte is esterification and subsequent packaging into chylomicrons. This study demonstrates that the majority of externally derived cholesterol entering the enterocyte for the esterification pathway is assisted by pancreatic CEase. In a bile salt and sterol concentration dependent fashion, pancreatic CEase stimulated not only the rate but also the extent of uptake (Figure 1). This enhanced cholesterol uptake is accompanied by the appearance of a significantly higher level (39-fold) of cholesteryl ester in the cell, indicating that the sterol is moving into a physiologically important pool. In contrast, uptake in the absence of exogenous enzyme leads to little subsequent metabolism. These findings suggest a specific, novel, and quantitatively important role for pancreatic CEase in regulating free cholesterol transport into the enterocyte.

The movement of cholesterol in the absence of protein mediation has been under active investigation (Steck et al., 1988; Thurnhofer & Hauser, 1990; McClean & Phillips, 1981). For example, the uptake of cholesterol by brush border membranes has been shown to be a second-order process, indicating the uptake of sterol most likely occurs by collision between brush border membrane and donor vesicle (Thurnhofer & Hauser, 1990). Similarly, the appearance of sterol in Caco-2 cells, in the presence or absence of CEase, is also a second-order process.

Absorption of free cholesterol has previously been viewed as the diffusion of an insoluble lipid from one aqueous environment, the small intestinal lumen, to another aqueous environment, the cellular compartment. An important barrier to this transport process is the unstirred water layer, a series of water lamellas surrounding the cell membrane which become progressively less stirred as the solute approaches the membrane. In a series of elegant studies (Thomson & Dietschy, 1977; Thomson, 1980), the transport velocity ($J$) across this layer was related to its thickness ($d$) and surface area ($S_w$), the diffusion coefficient ($D$) and concentration ($C$) of the lipid, the Michaelis constant ($K_m$) of transport, and finally the true maximal transport rate ($J_m$):

$$J = \frac{S_w D}{2d}\left[C + K_m + \frac{J_m d}{S_w D} - \left[\left(C + K_m + \frac{J_m d}{S_w D}\right)^2 - 4C\frac{J_m d}{S_w D}\right]^{1/2}\right]$$

Using this equation and the same values for the various constants used by Thomson and Dietschy (1977), the physical–chemical basis for the results found here can be better understood. For example, if simple basal cholesterol transport in the absence of CEase is characterized by the values $S_w$ = 1 cm$^2$ (100 mg of intestine)$^{-1}$, $D = 30 \times 10^{-5}$ cm$^2$ min$^{-1}$, $J_m$ = 100 nmol (100 mg of intestine)$^{-1}$ min$^{-1}$, $d = 1 \times 10^{-2}$ cm, $C$ = 1 mM, and $K_m$ = 5 mM, the transport velocity can be calculated to be 11.1 nmol/(100 mg of intestine)$^{-1}$ min$^{-1}$. The CEase-stimulated pathway is governed by many of the same constants since the unstirred water layer and the diffusion constant of cholesterol remain the same; however, the presence of CEase bound to the membrane can change either the $K_m$ or the maximal transport rate. For example, if the CEase-stimulated pathway has a 25-fold tighter Michaelis constant (0.2 mM), then the velocity of transport becomes 27.8 nmol (100 mg of intestine)$^{-1}$ min$^{-1}$ or almost 3 times greater than that found in the absence of enzyme. Alternatively, if the Michaelis constant remains the same, but the maximal transport rate increases 25-fold to 2500 nmol (100 mg of intestine)$^{-1}$ min$^{-1}$, then the transport rate becomes 28.5 nmol (100 mg of intestine)$^{-1}$ min$^{-1}$ or once again about 3-fold greater than that found in the absence of enzyme. By altering either or both of these parameters, CEase allows the cell to take up sterol more efficiently even though the intestinal milieu and especially the unstirred water layer remain unchanged.

While these quantitative studies have provided a powerful framework for the description of cholesterol flux, they do not take into account the cellular fate of sterol. Indeed, as pointed out above, the most striking effect of CEase on sterol uptake is on subsequent ester formation. After 12 h, in the absence of enzyme, there is low-level esterification, representing less than 1–2% of the initial sterol in the incubation medium, a result that is similar to that reported by Field et al. (1987) using the Caco-2 cell line and an assay system similar to that used here. On the other hand, in the presence of CEase, after 12 h, 15–20% of the applied cholesterol is converted to ester. The mechanism for this enhanced esterification remains unknown, but there are currently two known enzyme systems which could account for this observation. For example, it has been proposed that the formation of esters within the intestinal cell is catalyzed solely by ACAT (Field, 1984). The higher level of esterification seen here could then be due to a functionally higher intracellular concentration of cholesterol, produced by the transport properties of CEase, thereby enhancing the activity of ACAT. On the other hand, it has been found using immunocytometry that CEase actually crosses the brush border membrane (Gallo et al., 1980). This finding, plus previous kinetics studies (Kyger et al., 1990) showing that the ability of CEase to function as a cholesterol ester synthase is enhanced by low taurocholate concentrations, a condition which might pertain within the cell, indicate that ester formation may be due to this pancreatic enzyme as well. In the first model, CEase behaves as a "shuttle" providing more substrate for ACAT, while in the second, the esterase plays a much more complete role in cholesterol absorption. Experiments in progress will help distinguish between these two different roles for pancreatic CEase.

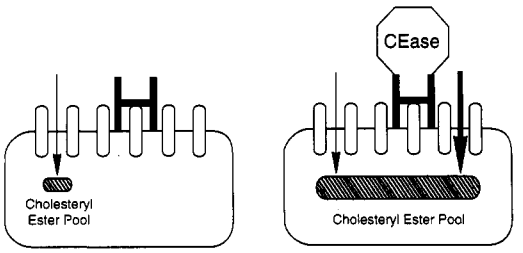


FIGURE 4: Model for the CEase-stimulated uptake of unesterified cholesterol. (Left) The enterocyte can convert small amounts of free cholesterol into cholesterol ester, as signified by the small arrow and small hatched box. (Right) CEase binds to heparin (H) on the surface of the enterocyte. While bound, the enzyme hydrolyzes cholesteryl ester to free cholesterol and fatty acid and also catalyzes the uptake of cholesterol derived from either the diet or the liver. By this process, cholesterol is efficiently converted to cholesteryl ester as signified by the bold arrow and large hatched area.

The different amounts of cholesterol transported and esterified under these two conditions may be due to different pathways of absorption and processing. Such segregation of various cholesterol pools within the cell has been reported previously. For example, Field et al. (1987) noted in uptake studies performed in the absence of CEase that the intracellular preformed pool of cholesterol was not affected by the new, cell-associated cholesterol. Similarly, Stange et al. (1983) found in rat intestinal crypt and villus cells that there were at least three distinct cholesterol pools. Because the principal intracellular fate of cholesterol in the enterocyte is esterification prior to secretion, all the data are consistent with the hypothesis that, in the absence of the CEase-mediated pathway, new cell-associated cholesterol enters a functional pool very slowly, if at all.

Accordingly, the present findings and previous evidence that pancreatic CEase is anchored to the intestinal membrane through heparin indicate that this enzyme may play a pivotal role in the modulation of intestinal cholesterol absorption. As shown in Figure 4, cholesterol can become associated with the intestinal cell by one of two pathways. Basal uptake occurs without the assistance of CEase, is associated with low levels of esterification, and may direct cholesterol to outer monolayer locations. On the other hand, the CEase-mediated pathway not only is much more efficient as a cholesterol transport system but also leads to high levels of ester formation within the cell. While it is not clear why there are two pathways for cholesterol uptake, an intriguing possibility is that the protein-mediated pathway plays a role in chylomicron formation and hence constitutes absorption while the other is used for maintenance of the enterocyte membrane. Current studies will distinguish between these two possibilities.

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