Bile salts in submicellar concentrations promote bidirectional cholesterol transfer (exchange) as a function of their hydrophobicity

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Abstract Cholesterol, despite its poor solubility in aqueous solutions, exchanges efficiently between membranes. Movement of cholesterol between different subcellular membranes in the hepatocyte is necessary for assembly of lipoproteins, biliary cholesterol secretion, and bile acid synthesis. Factors which initiate and facilitate transfer of cholesterol between different membranes in the hepatocyte are incompletely understood. It is known that cholesterol secretion into the bile is linked to bile salt secretion. In the present study, we investigated the effects of bile salts of different physicochemical properties at submicellar concentrations (150– 600 μM) on the transfer of [^{14}C]cholesterol from hepatocytes, or crude hepatocellular membranes (donors), to rat high density lipoproteins (acceptor). Bile salts included taurine conjugates of ursodeoxycholic acid (TUDCA), hyodeoxycholic acid (THDCA), cholic acid (TCA), chenodeoxycholic acid (TCDCA), and deoxycholic acid (TDCA). High density lipoprotein (HDL) was separated from hepatocellular membranes and the transfer of [^{14}C]cholesterol from the membranes to HDL was quantitatively determined. In the absence of HDL, [^{14}C]cholesterol remained confined to the membrane fraction. Following addition of HDL, [4-^{14}C]cholesterol in the HDL fraction increased linearly over time. Addition of hydrophilic bile salts (TUDCA and THDCA) increased transfer of [4-^{14}C]cholesterol to HDL only minimally. By contrast, more hydrophobic bile salts stimulated transfer of labeled cholesterol to HDL, and their potency increased in order of increasing hydrophobicity (TCA < TCDCA < TDCA). Both for single bile salts and mixtures of bile salts at a total bile salt concentration of 0.30 mM , the rate of cholesterol transfer exhibited a strong linear correlation with a bile salt monomeric hydrophobicity index (r = 0.95; P < 0.001). We conclude that bile salts at monomeric concentration promote bidirectional transfer (exchange) of cholesterol from hepatocytes and crude hepatic membranes to the acceptor particle (HDL). The cholesterol mobilizing capacity of bile salts appears to be a function of their relative hydrophobicity. We postulate that the ability of bile salts to stimulate cholesterol secretion may result in part from their effect on cholesterol transfer between metabolic compartments within the hepatocyte. —**Vlahcevic, Z. R., E. C. Gurley, D. M. Heuman, and P. B. Hylemon.** Bile salts in submicellar concentrations promote bidirectional cholesterol transfer (exchange) as a function of their hydrophobicity. *J. Lipid Res.* 1990. **31:** 1063–1071.

Supplementary key words bile acids • cholesterol exchange • hepatocytes

Bile salts are polar amphiphiles derived from cholesterol in the liver. Their principal biological function is solubilization of lipids in the gut and in bile. It has long been known that bile salts are required for intestinal absorption (1, 2) and biliary solubilization (3–7) of cholesterol and other lipids. In addition, bile salts play a role in other aspects of hepatic cholesterol metabolism. Bile salts in the enterohepatic circulation down-regulate the rate-limiting enzymes of cholesterol and bile salt biosynthesis pathways, i.e., HMG-CoA reductase and cholesterol 7α-hydroxylase, respectively (8–12). Also, secretion of cholesterol (13–15) and phospholipids (16–18) into the bile is apparently linked to the rate of bile salt secretion by the liver. The mechanism by which bile salts flux through the liver influences biliary secretion of cholesterol and phospholipid is poorly understood.

Armstrong and Carey (19), Salvioli, Lugli, and Pradelli (20), and Rajagopalan and Lindenbaum (21, 22) have shown that potency of bile salts as solubilizers of cholesterol and phospholid (their "detergency") increases with increasing hydrophobicity, as determined by the reversed phase high performance liquid chromatography (HPLC). Recent data suggest that relative hydrophobicity also determines the potency of bile salts as suppressors of HMG-CoA reductase and cholesterol 7α-hydroxylase in the rat (9–11), and may determine their capacity to stimulate bile flow and biliary cholesterol secretion in rats and humans (9, 23–26). These latter findings are somewhat paradoxical because the ability of bile salts to function as deter-

Abbreviations: HPLC, high performance liquid chromatography; CMC, critical micellar concentration; HDL, high density lipoprotein; TCA, taurocholic acid; THDCA, taurohyodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid.

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gents depends on their ability to self-associate in the form of micelles, and formation of micelles occurs only above a critical micellar concentration (CMC) which ranges between 2 and 20 mM for the common dihydroxy and trihydroxy bile salts (3, 27, 28). Bile salts concentrations in this range are found physiologically only within the biliary tract, gallbladder, and small intestine. In the hepatocytes, where most of the aforementioned effects of bile salts take place, bile salt concentration is thought to be well below CMC. Thus, it is difficult to envision a mechanism by which the detergency and the metabolic activities of bile salts within the hepatocyte could be connected directly.

More recent investigations have suggested a possible resolution of this paradox, with the finding that bile salts at concentrations well below their CMC may alter the behavior of cholesterol in model systems. Chijiiwa and Nagai (28, 29) have found that monomeric activity of cholesterol in aqueous solution increases with increasing bile salt concentration. Moreover, the data of Schubert et al. (30–32) indicate that cholesterol/phospholipid bilayers may be disrupted by low concentrations of bile salts. Based on these findings, it is possible that bile salts below their CMC could bind to membranes and initiate, by as yet unknown mechanism, spontaneous release and transfer of membrane cholesterol and phospholipids.

The present investigations were undertaken in order to evaluate the effects of bile salts of different relative hydrophobicity on the movement of cholesterol between hepatic membranes and a cholesterol acceptor. Our data clearly show that bile salts at monomeric concentrations enhance the passive movement of cholesterol into and out of the hepatocyte membranes as a function of their relative hydrophobicity. We hypothesize that many of the regulatory effects of bile salts on aspects of hepatic cholesterol metabolism and transport may result from their ability to stimulate movement of cholesterol or related hydrophobic molecules such as phosphatidylcholine between subcellular membrane compartments.

MATERIALS AND METHODS

Materials

[4-^{14}C]Cholesterol (59.4 mCi/mmol) and [^{3}H]cholesterol (54.8 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Clostridial collagenase was obtained from Worthington. Taurocholic acid, tauroursodeoxycholic acid, taurochenodeoxycholic acid, and taurodeoxycholic acid were obtained from Calbiochem (San Diego, CA). Taurohyodeoxycholic acid was obtained from Steraloids (Wilton, NH). All other reagents were of the highest quality commercially available.

Experimental design

Preparation of hepatocyte cultures from adult rats. Primary monolayer cultures of rat hepatocytes were prepared as previously described (33, 34). Male Sprague-Dawley rats were housed in a light-controlled room in which the dark period was from 0300 to 1500 h. Animals were anesthetized at 0900 h with ether and the liver was perfused in situ with complete culture medium containing 0.036% collagenase. Complete culture medium consisted of a modified Waymouth MB-752 I medium. Isolated cells (3.4×10^6) were plated in 3 ml of culture medium in 60-mm plastic dishes coated with Vitrogen (Collagen Corp., Palo Alto, CA). Hepatocytes were incubated at 37°C in a humidified atmosphere containing 5% CO_2. They were found to be greater than 90% viable as determined by trypan blue dye (0.04%) exclusion.

Preparation of whole cell and membrane samples. Primary hepatocyte monolayer cultures were labeled with [4-^{14}C]cholesterol (6×10^5 dpm/plate) at 4 h after plating and allowed to incubate at 35°C. Radioactive cholesterol was suspended in 95% ethanol and incubated with sterile rat serum for 90 min at 4°C, as previously described (35) before addition to plates. At 24 h after plating, culture medium was changed twice and additions of HDL and/or bile salts were made to whole cell cultures or membrane preparations.

Whole cell culture samples were prepared by changing the media twice at 24 h and making appropriate additions to the plates. The cells were allowed to incubate an additional 24 h and then the media were harvested and frozen at −20°C for further analysis.

In membrane preparations, labeled cells were scraped and broken with a rubber policeman into 3 ml media forming a crude cell suspension. This suspension was transferred to a reaction test tube (17 mm × 100 mm) and HDL (0.3 mg protein) and/or bile acids (0–600 μM) were added. These samples were immediately frozen at −20°C for further analysis on HPLC.

In separate experiments, unlabeled crude hepatocyte membrane preparations were used. These were prepared identical to labeled preparations (above). In these experiments, HDL containing [^{3}H]cholesterol was added to membrane preparations in the presence and absence of bile salts. The decrease of radioactivity from the [^{3}H]HDL peak was determined by HPLC analysis and liquid scintillation spectrometry.

HPLC and GLC analysis of cell culture samples. Frozen samples were quickly thawed, centrifuged for 5 min at 1100 rpm in a clinical centrifuge, and filtered through a 0.45-μm filter. High performance gel filtration chromatography was carried out using a Spherogel-TSK 5000 PW (30 cm × 0.75 cm) column. The column was fitted with a PW precolumn (10 cm × 0.75 cm). The column

was equilibrated with 10 mM $NaPO_4$ buffer, pH 7.0. Two ml of culture media was injected. The flow rate was maintained at 0.85 ml per min and 1.0-min fractions were collected. Radioactivity in each fraction was quantitated by liquid scintillation spectrometry.

Cholesterol mass determinations were made as previously described (33). Five plates of unlabeled hepatocytes were combined and membranes were prepared. Additions of HDL and TCA were made and hepatocyte cell pellet and supernatants were analyzed for cholesterol by gas–liquid chromatography.

Isolation of high density lipoprotein. The HDL fraction was isolated by ultracentrifugation. [^{3}H]HDL was preprared by first labeling serum with [^{3}H]cholesterol (1 μCi per 1 ml of serum) following which HDL was isolated by ultracentrifugation.

Bile salt hydrophobicity index. Hydrophilic-hydrophobic balance of bile salts has been quantified using reverse phase HPLC (mobile phase MeOH–H_2O 70:30, vol/vol). The stationary phase was octadecylsilane C-18 as described previously (23).

HPLC capacity factor (K_i) was calculated from the formula:

$$(K_i) = \frac{V_i - V_o}{V_o},$$

where V_i is the volume of mobile phase required to elute bile salt "i" and V_o is the elution volume of an unretained solute. We define a bile salt monomeric index as:

$$HI_i = \frac{\log (K_i/K_{tc})}{\log (K_{tlc}/K_{tc})}$$

where tc and tlc refer to taurocholate and taurolithocholate, respectively. By this definition: $HI_{tc} = 0$ and $HI_{tlc} = 1$. For a solution containing two or more bile salts:

$$HI = \sum_{i=1}^{n} HI_i \times F_i$$

where F_i is the mole fraction of bile salt "i" in a solution containing n different bile salts. HI_i as defined is linearly related to the free energy of partition of bile salts (either singly or in combination) between the polar mobile phase and nonpolar stationary phase.

RESULTS

The data in **Fig. 1** show the effect of TCA on the transfer of [^{14}C]cholesterol from primary cultured rat hepatocytes to HDL. Hepatocytes were prelabeled with [^{14}C]cholesterol 24 h prior to the initiation of experiments. At the end of 24 h, media were changed and HDL was added with or without taurocholate (TCA). Culture media were harvested after 24 h incubation and fractionated by high performance gel filtration chromatography. There was no detectable transfer of labeled cholesterol from whole cells into the culture medium in the absence of HDL. Addition of bile salts in the absence of HDL also failed to promote transfer of labeled cholesterol. With the addition of HDL the magnitude of transfer was dependent on the concentration of HDL added and was linear with time (up to 24 h). The addition of TCA, in the presence of HDL, resulted in an enhanced, concentration-dependent transfer of labeled cholesterol from the hepatocytes to HDL.

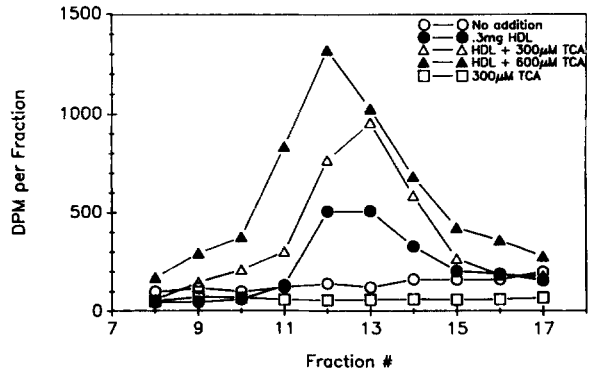

Fig. 1. The effect of addition of TCA on the transfer of cholesterol from the hepatocytes to HDL. TCA (300 or 600 μM) plus HDL (0.3 mg) was added and incubated for 24 h; media were harvested and HDL was separated by high performance gel filtration chromatography.

In order to avoid the potential problem caused by hepatic biotransformation of bile salts (10), we conducted subsequent experiments using crude hepatocyte membrane preparations (hepatocyte lysates) instead of whole cells. In this system, the effects of individual bile salts or mixtures of bile salts on cholesterol transfer could be determined more precisely. The rates of cholesterol transfer in these experiments were determined after 15 min of incubation following which the preparations were frozen at −20°C. High performance gel filtration chromatography was carried out as described in Materials and Methods. **Fig. 2** shows the results of experiments in which crude hepatocyte membranes were incubated in the presence of 300 μM bile salts, with and without addition of HDL. Two relatively hydrophilic bile salts, taurohyodeoxycholate (THDCA) and tauroursodeoxycholate (TUDCA), and three more hydrophobic bile salts, taurocholate (TCA), taurochenodeoxycholate (TCDCA), and taurodeoxycholate (TDCA), were tested in this system. In the presence of HDL alone, slow transfer of labeled cholesterol from membranes to the cholesterol acceptor was observed. THDCA and TUDCA, two hydrophilic bile salts, stimu-

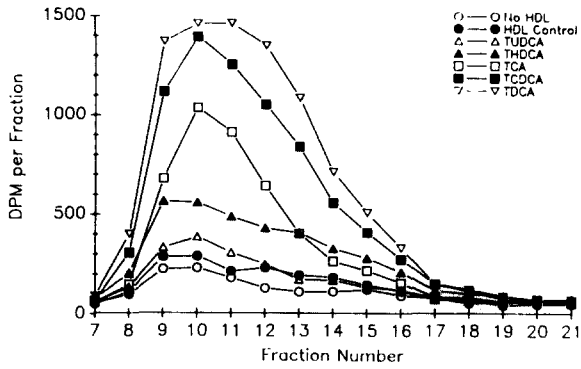

Fig. 2. Effects of different bile salts on rates of cholesterol exchange in a model membrane system. Bile salts (300 μM) and HDL (0.3 mg) were added to hepatocyte membranes, incubated 15 min and the mixtures were immediately frozen at −20°C. After quick thawing, HDL was separated from membranes using HPLC gel-filtration chromatography (TSK PW 5000). Relative rates of [^{14}C]cholesterol transfer to the HDL fraction were determined by separating individual samples on gel filtration chromatography (flow rate 0.85 ml/min). Purified [^{14}C]cholesterol-labeled HDL eluted in fractions 9–16.

lated transfer of [^{14}C]cholesterol only slightly. By contrast, the addition of more hydrophobic bile salts resulted in a pronounced transfer of cholesterol from the membranes to HDL in order of increasing hydrophobicity (TCA < TCDCA < TDCA).

The data in **Fig. 3** demonstrate the effects of varying concentrations of HDL on the transfer of [^{14}C]cholesterol from hepatocyte membranes. Hepatocyte lysates (3.6 mg protein) labeled with [^{14}C]cholesterol were mixed with various concentrations of HDL (0–1.5 mg/plate) in the absence of bile salts. The rate of cholesterol transfer was linear with increasing HDL concentrations up to 1.25 mg/plate.

Fig. 4 demonstrates the time course of [4-^{14}C]cholesterol transfer to the hepatic membranes to HDL acceptor.

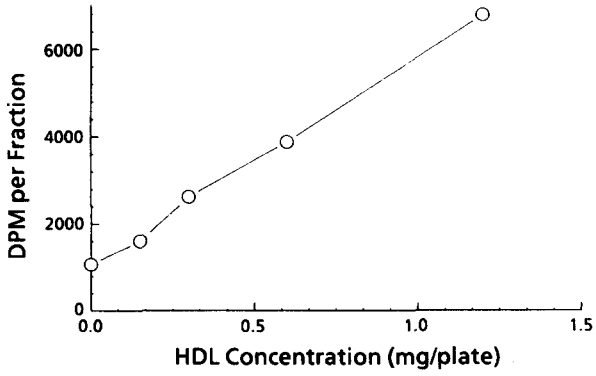

Fig. 3. Effects of HDL concentration on rates of cholesterol transfer in hepatic membranes. The rates of transfer of [^{14}C]cholesterol with varying concentrations of HDL (0 to 1.25 mg). Experiments were carried out under standard conditions as described in Materials and Methods.

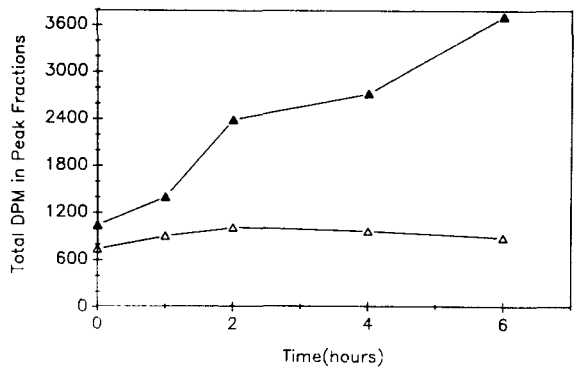

Fig. 4. Time course of [4-^{14}C]cholesterol transfer from hepatic membranes to HDL acceptor. Rates of cholesterol transfer were determined in the presence of 0.3 mg HDL protein alone (△) or HDL 300 μM taurocholate (▲) using conditions described in Materials and Methods.

Rates of cholesterol transfer were determined in the presence of 0.3 mg HDL protein alone or HDL plus 300 μM taurocholate using conditions described in Materials and Methods. The addition of HDL plus 300 μM taurocholate resulted in a threefold increase of [^{14}C]cholesterol bidirectional exchange from hepatic membranes to HDL acceptor. Very little transfer of [^{14}C]cholesterol occurred when HDL was added alone.

Table 1 demonstrates cholesterol content (μg cholesterol per mg protein) in the membranes without addition of HDL (controls) and when HDL alone or HDL plus taurocholate was added. The incubation time was 15 min. There was no change in cholesterol content in the membranes when HDL or HDL plus TCA was added. Cholesterol content in the medium included HDL cholesterol which explains the increase between control and experimental conditions. The important point is that there was no change in cholesterol mass when HDL alone and HDL plus TCA were compared. These data taken together suggest that, under these in vitro experimental conditions, bile salts (TCA) did not induce net transport of cholesterol from the crude cell membrane preparation to the cholesterol acceptor (HDL).

Fig. 5 demonstrates the effects of bile salt hydrophilic-hydrophobic balance on the rates of cholesterol tranfer

TABLE 1. Quantitation of cholesterol in hepatocyte cell membranes and supernatant

Experimental Conditions	Crude Cell Membranes	Supernatant Fluid
	μg cholesterol/mg protein	
Control (no addition)	9.90 ± 0.55[a]	0.63 ± 0.06
HDL alone	9.22 ± 0.26	2.81 ± 0.08
HDL + 300 μM TCA	10.34 ± 0.90	2.70 ± 0.28

[a]Values are means of three samples from one rat.

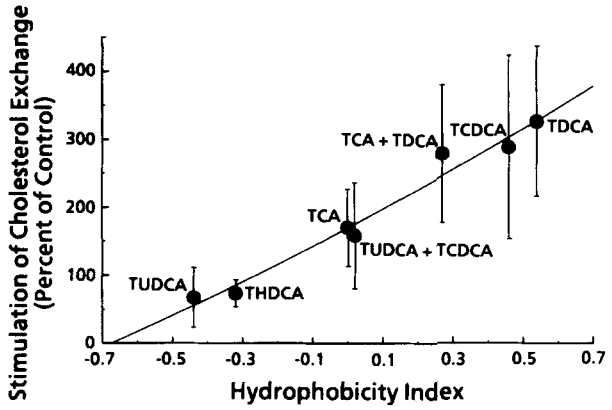

Fig. 5. Effects of bile salt hydrophobicity on [^{14}C]cholesterol transfer between hepatic membranes and HDL. Broken whole cells (3.6 mg protein) labeled with [^{14}C]cholesterol were mixed with HDL (0.6 mg protein) in the presence of individual bile salts (300 μM) or mixture of two bile salts (150 μM of each). The hydrophobicity index of individual bile salts and mixtures of bile salts was determined as previously described (23). HDL was separated from hepatic membranes using HPLC gel filtration with [^{14}C] radioactivity determined in the peak where HDL eluted. The data are expressed as percent above the control.

from hepatic membranes to HDL. Bile salt hydrophobicity index was determined as previously described (23). The hydrophobicity index of individual bile salts and mixtures of bile salts was plotted against percent increase of cholesterol transfer from the hepatic membranes to HDL. A strong positive linear correlation was observed between the bile salt hydrophobicity index and the rate of cholesterol transfer ($r = 0.95$, $P < 0.001$).

Fig. 6 shows that the addition of bile salts affected transfer of cholesterol bidirectionally. In this experiment, HDL was labeled with [^{3}H]cholesterol and unlabeled hepatic membranes served as the cholesterol acceptor. As in the previous experiments, there was a positive linear relationship between the hydrophobicity index of added bile salts and the percent of cholesterol transport from labeled high density lipoprotein to the hepatocyte membranes.

Fig. 7 shows the effects of individual bile salts and their hydrophobicity index on the transfer of labeled cholesterol from the crude hepatocyte membranes in the presence and absence of HDL. The magnitude of cholesterol transfer was linearly related to bile salt hydrophobicity even when HDL was not added. It is apparent that the transfer of [^{14}C]cholesterol from hepatic membranes is much more efficient in the presence of HDL. However, some transfer of labeled cholesterol occurred even in the absence of HDL. The mobilization of cholesterol observed in the absence of HDL is probably due to the release of endogenous membrane vesicles which served as cholesterol acceptors. These vesicles appeared to be similar in size to HDL since they eluted from high performance gel filtration columns in the same fractions.

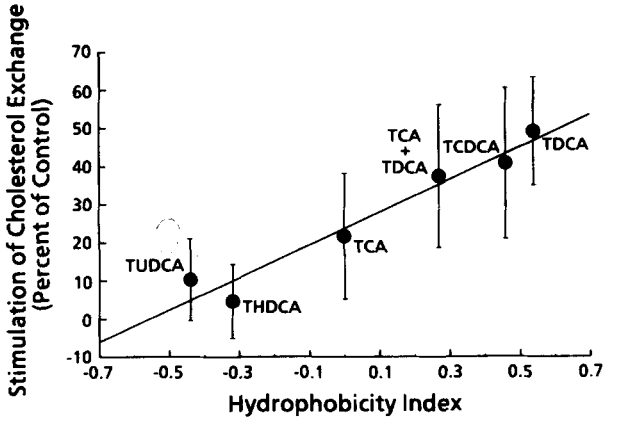

Fig. 6. Effect of bile salt hydrophobicity on [^{3}H]cholesterol transfer from HDL to the hepatocyte membranes. HDL (0.6 mg), labeled with [^{3}H]cholesterol (46,000 dpm) was added to each sample of unlabeled hepatocyte membranes and the transport of cholesterol from labeled HDL to the unlabeled membranes was determined in the presence of individual bile salts (300 μM) or mixture of bile salts. The data are expressed as percent above controls.

DISCUSSION

The present report provides evidence for the role of bile salts at submicellar concentrations in the transfer of cholesterol between cellular membranes. The transfer is bidirectional (exchange) and the data suggest that no changes in net flux of cholesterol took place. Cholesterol transfer was quantitatively determined by the movement of labeled cholesterol from primary hepatocytes in culture or hepatic membranes (donors) to unlabeled HDL (acceptor), with and without addition of bile salts. The data of this study show that, without the addition of HDL, radioactive cholesterol remained confined almost entirely to the hepatic membranes. Addition of HDL to the membranes resulted

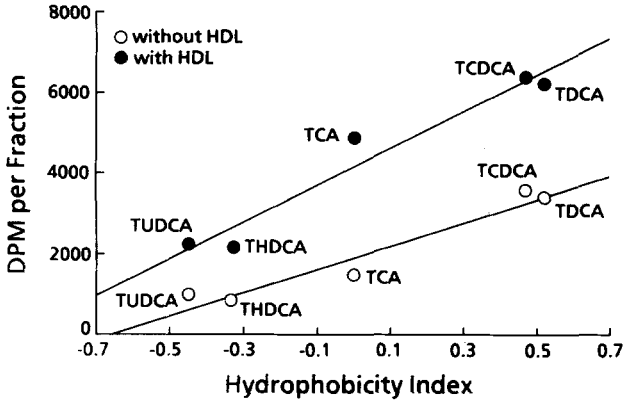

Fig. 7. Effects of bile salt hydrophobicity on cholesterol transfer in hepatocyte cultures with and without addition of HDL. The transfer of [^{14}C]cholesterol from the membranes is plotted against the hydrophobicity index in the presence and absence of HDL. Addition of HDL facilitated the transfer of cholesterol but the linear relationship with the hydrophobicity index is preserved in both experiments.

in a modest transfer of cholesterol. By contrast, cholesterol transfer from the hepatocytes and/or from hepatic membrane fractions to the HDL was markedly augmented by the addition of certain bile salts in concentrations well below their CMC (0.15–0.60 mM). These concentrations of bile salts are thought to be present under physiological circumstances within the hepatocytes (36, 37). Under these in vitro conditions it does not appear that a net transport of cholesterol took place. Thus, our data suggest that bile salts in submicellar concentrations initiate a bidirectional transfer of cholesterol rather than net cholesterol transport. These in vitro data do not necessarily apply to the in vivo situation in which bile salts may conceivably induce net transport of cholesterol between different hepatic organelles.

Our data also demonstrate that the extent of cholesterol transfer between membranes is a function of relative hydrophobicity of bile salts. The addition of hydrophilic bile salts to the hepatic membranes resulted in a minimal stimulation of transfer of labeled cholesterol. By contrast, hydrophobic bile salts affected cholesterol transfer in order of increasing hydrophobicity (TCA < TCDCA < TDCA). These findings are consistent with other in vivo studies that have also shown that many biological activities of bile salts are related to their relative hydrophobicity (9–11). In vivo studies in humans, in which the circulating bile acid pool was acutely altered (25), and acute bile salt perfusion studies in rats (9) have both demonstrated that the amount of cholesterol and phospholipid secretion was roughly proportional to the hydrophobicity of administered bile salts. However Gilmore et al. (38) and Guarantz and Hofmann (39) did not observe predictable relationships between the structure of bile salts and their effect on biliary lipid secretion. The reason for these species-related discrepancies is not known.

In the present studies, both for single bile salts and mixtures of bile salts, the rate of cholesterol transfer exhibited a strong linear correlation with a bile salt monomeric hydrophobicity index ($r = 0.95$, $P < 0.001$). The linear relationship between the hydrophobicity index of bile salts or bile salt mixtures was maintained in the absence of HDL but the cholesterol transfer was much less pronounced. We postulate that in the experiments in which HDL was not added, cholesterol acceptor particles were endogenously produced vesicles mobilized from the hepatocyte membranes by the addition of bile salts. These putative vesicles were eluted on HPLC in the same fractions as HDL, suggesting that both may be of similar size. Interestingly, the studies carried out in the hepatocytes showed that no cholesterol transfer occurred when HDL or HDL plus bile salts were added, suggesting that hepatocytes did not release endogenous vesicles to serve as cholesterol acceptors. Finally, the effect of bile salts on cholesterol transport was bidirectional, i.e., movement from HDL to hepatocyte membranes was also stimulated. The observed quantitative differences in the rates of cholesterol transfer between hepatic membranes and HDL and vice versa were most likely due to different protein, cholesterol, and phospholipid composition of membranes and HDL particles. The important point is that there was a near perfect linear relationship between the hydrophobicity index and cholesterol transfer in both sets of experiments.

Cholesterol, an important constituent of all membranes, is essentially insoluble in water and its aqueous solubility has been reported to be about 10^{-8} M (40). It is well known that, despite its poor aqueous solubility, free cholesterol readily exchanges between the biological membranes, lipoproteins, and liposomes. Two different mechanisms of cholesterol exchange between membranes have been proposed. It has been suggested that cholesterol exchange may take place upon direct contact between membranes as a result of collision. According to this model, collision complexes form between cholesterol-carrying structures which may involve the transitory fusion of the outer leaflets of the membranes bilayers. This hypothesis takes into account the poor solubility of cholesterol in the aqueous environment (41–43). Alternatively, cholesterol transfer may proceed by diffusion of cholesterol through the aqueous medium in a two-step process, i.e., desorption from the donor membranes and adsorption of cholesterol from the aqueous phase and into an acceptor particle (44–47). The latter hypothesis is strongly supported by the findings of Backer and Dawidowicz (47), who reported that cholesterol moves freely between populations of phospholipid vesicles even when the vesicles are physically separated by a dialysis membrane.

Our data could be compatible with either of these hypotheses. Bile salts at low concentrations could stimulate cholesterol exchange by enhancing activation of cholesterol in membranes or by promoting transient fusion of cholesterol-containing particles. Bile salts could reduce the activation energy required for escape of cholesterol from the membrane into the aqueous phase. Such an effect could result from partition of bile salts into the membrane, creating a less favorable hydrophobic environment for membrane cholesterol or, alternatively, it could result from formation of transient bile salt-cholesterol aggregates (possibly dimers) in the aqueous phase. Recently, Nichols (48) has shown that low concentrations of bile salts have marked effects on rates of spontaneous phospholipid transfer between vesicles. He suggested that bile salts in monomeric concentrations partition into the membrane with high affinity and increase the rate of intervesicular transfer of phospholipids. He also postulated that membrane phospholipids in the vicinity of bile salt monomers or dimers become less stable and are more likely to dissociate and translocate. In these experiments hydrophobic bile salts were more effective in translocating phospholipids than hydrophilic bile salts. It is feasible that

monomeric bile salts dislocate cholesterol from the membranes in a manner similar to that observed with phospholipids. Cabral et al. (49) studied the ability of bile salts to traverse membranes using nuclear magnetic spectroscopy. They concluded that the rate constants for transbilayer movement of several unconjugated bile salts was dependent upon the number and location of hydroxy groups, i.e., their relative hydrophobicity. Schubert et al. (32) demonstrated that, at very low bile salt concentration, bile salt-lipid aggregates are formed in the outer vesicle monolayer. The binding strength of hydrophobic bile salt (chenodeoxycholate) to the vesicles was found to be stronger than that of the more hydrophilic bile salt (cholate). Chijiiwa and Nagai (28) reported that cholesterol solubility is enhanced by the presence of taurocholate in the submicellar concentrations. The authors postulate that interaction of cholesterol and bile salt monomers leads to the formation of cholesterol-taurocholate dimers. This explanation may be correct since it is difficult to explain increased cholesterol solubility in the presence of bile salts below their CMC.

Cholesterol transfer within the hepatocytes could be expedited by the presence of sterol carrier proteins (50–56). Sterol carrier proteins are thought to be important in cholesterol biosynthesis (48) and 7α-hydroxylation of cholesterol (51). Similarly, an adrenal sterol carrier protein was identified which presumably shuttles cholesterol to the mitochondrial site, where the cholesterol side chain is oxidized. No attempt was made to eliminate sterol carrier proteins from our hepatic membrane preparation and it is conceivable that some of the exchange stimulated by bile salts resulted from facilitation of the action of these carrier proteins. We are not aware of any studies investigating the interactions of bile salts with hepatic sterol carrier proteins. The possible role of sterol carrier proteins in the transport of cholesterol within the hepatocytes is an important area of investigation which has not been adequately explored.

Hepatocytes have many unique features that are not shared by other cells. They are continuously exposed to the flux of bile salts circulating in the enterohepatic circulation which are known to affect hepatic cholesterol metabolism. It has been well established that cholesterol and phospholipid secretion into the bile is linked to the rate of secretion of bile salts (13–18). In order to maintain this linkage on a continuous basis, a constant supply of cholesterol and phospholipids must be "provided" from the cellular sources. While the biliary lipid secretion of cholesterol and phospholipid is undoubtedly coupled to transcellular fluxes of bile salts, the mechanism by which bile salts trigger the mobilization of cholesterol and phospholipids into the bile is not well understood. Structures known to be capable of transporting cholesterol through aqueous phase are mixed micelles or vesicles. It is not likely that cholesterol is transported in the cells by micelles because the intrahepatic concentration of bile salts is likely to be below CMC. The prevailing view is that cholesterol is transported in the hepatocytes in the form of vesicles. The increased number of vesicles that are observed in the region of biliary canaliculus during bile acid-induced choleresis has been interpreted as accumulation of cellular lipids mobilized from the intracellular sources and destined for secretion into the bile (57–58). It is intriguing that circulating bile salts do not affect the second vectorial transport of cholesterol taking place in the liver, i.e., the translocation of very low density lipoproteins (VLDL) from the smooth endoplasmic reticulum into the plasma.

Bile salts in the enterohepatic circulation are known to have profound effects on several aspects of cholesterol metabolism. They are required for absorption of cholesterol from the intestine (59) and may play a role in the hepatocellular uptake of lipoprotein cholesterol (60, 61). Bile salts down-regulate cholesterol synthesis by suppressing the activity of HMG-CoA reductase. Recent studies suggest that the effect of bile salts on HMG-CoA reductase is probably indirect, i.e., resulting from the bile salt-induced changes in cholesterol absorption (9–11, 62). In addition, biliary bile salt secretion is the principal physiological stimulus for biliary cholesterol secretion (13). The only other quantitatively important pathway of cholesterol elimination, bile acid synthesis, is presumably regulated in a negative feedback manner by the hydrophobic bile salts in the enterohepatic circulation (11, 63). The exact site of action of bile salts on cholesterol metabolism in the hepatocytes in uncertain. Lamri et al. (64) have shown that bile salts are concentrated within the lumen of the tubules of the endoplasmic reticulum and the Golgi apparatus. Thus, the most prominent effect of bile salts on cholesterol transfer might be expected to occur within these two organelles.

There is evidence that the availability of free cholesterol in a pool of cholesterol located in the hepatocytes may be of key importance in the regulation of several aspects of hepatic cholesterol metabolism. Several studies have shown that rapid alterations of the size of this exchangeable pool of cholesterol in the liver can affect the rates of biliary cholesterol secretion. Drugs that inhibit acyl-CoA: cholesterol acyltransferase (ACAT), an enzyme responsible for esterification of cholesterol, or stimulate cholesteryl ester hydrolase have been reported to be capable of altering biliary cholesterol secretion (65, 66). A submicrosomal pool enriched in newly synthesized cholesterol may also be of importance in regulation of bile acid synthesis (67, 68). The ability of bile salts to stimulate passive cholesterol transfer in monomeric concentrations may play a role in regulation of several aspects of hepatic cholesterol metabolism.

This work was supported by the National Institutes of Health Grant (1 PO1 DK38030-03) and the Veterans Administration.

Manuscript received 25 October 1989 and in revised form 2 February 1990.

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