Hepatic transport and secretion of unesterified cholesterol in the rat is traced by the plant sterol, sitostanol

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Abstract The hepatic uptake, transport, and secretion into bile of unesterified cholesterol cannot be directly quantitated because of extensive exchange and equilibration between different pools of unesterified cholesterol. Plant sterols are structurally similar to cholesterol but because of poor intestinal absorption are ordinarily not present in the liver. To quantitate hepatic sterol uptake and transport in the absence of exchange with endogenous sterols, isolated rat livers were perfused with the plant sterol, sitostanol, incorporated in phosphatidylcholine liposomes. Appreciable amounts of sitostanol were taken up by the liver and uptake was independent of the presence of bile salt. In contrast, like unesterified cholesterol, the secretion of sitostanol in bile required bile salt. Sitostanol was detected in bile within 5 min after a perfusion was begun and reached a plateau by about 20 min. The rate of appearance of sitostanol in bile was precisely the same as unesterified cholesterol when both sterols were perfused together. Furthermore, the output of sitostanol in bile was directly proportional to the output of cholesterol. At the peak of biliary sitostanol secretion, the amount of sitostanol relative to unesterified cholesterol was much greater in bile (40–50% of sterols) than in the whole liver (11% of sterols). Selective biliary secretion of sitostanol was associated with much greater concentrations of sitostanol in canalicular membranes than in the interior membranes of the hepatocyte and in newly secreted high density lipoproteins compared to newly secreted very low density lipoproteins. These results indicate that sitostanol parallels the secretion from and distribution of unesterified cholesterol in the liver and suggest that sitostanol can be used as a physiologic analog of unesterified cholesterol to trace the transport of sterols through the liver.—**Robins, S. J., J. M. Fasulo, C. R. Pritzker, and G. M. Patton.** Hepatic transport and secretion of unesterified cholesterol in the rat is traced by the plant sterol, sitostanol. *J. Lipid Res.* 1996. **37:** 15–21.

Supplementary key words bile • bile acids • liver perfusion • high density lipoproteins • cholesterol exchange

A major route for cholesterol elimination from the body is by biliary secretion as unesterified cholesterol (UC). Biliary UC is predominantly preformed, not newly synthesized (1, 2), and must, therefore, derive primarily from the hepatic uptake of lipoprotein-cholesterol. We have previously demonstrated in the live rat (3) that exchange of UC between native plasma lipoproteins and the liver is exceedingly rapid and that the net hepatic uptake of UC from lipoproteins using radiolabeled cholesterol cannot be quantitated without correcting for exchange. We have further demonstrated (4) that UC taken up by the liver from a lipoprotein analog and UC formed in the liver by the hydrolysis of cholesteryl esters rapidly equilibrate with all pre-existing liver UC. Thus, the totality of preformed UC in the liver is in metabolic equilibrium in one single kinetic pool. Because UC that is taken up by the liver is not only extensively mixed within the liver but is also "diluted" by a relatively large pool of endogenous preformed UC, it is not possible to directly quantitate the contribution of lipoprotein-cholesterol to biliary UC secretion.

In an attempt to circumvent both the issue of UC exchange and the equilibration of UC between different intrahepatic pools, we have performed a series of studies with the plant sterol, sitostanol (SIT), that is structurally similar to UC. Relatively little information exists with regard to the hepatic metabolism of SIT, as like all plant sterols, SIT is poorly absorbed by the intestine and is ordinarily present in only trace amounts in plasma and body tissues (reviewed in 5). However, it has been reported that after intravenous administration, humans readily secrete plant sterols in bile (6) and that rats given a prolonged intravenous infusion of plant sterols in liposomes have high hepatic concentrations of these sterols that neither interfere with hepatic cholesterol synthesis nor produce hepatotoxicity (7).

Abbreviations: UC, unesterified cholesterol; SIT, sitostanol; HPLC, high performance liquid chromatography; VLDL, very low density lipoproteins; HDL, high density lipoproteins.

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The present studies were undertaken to assess the feasibility of using SIT as a substitute for radiolabeled UC to measure the hepatic uptake and transport of free sterol in the absence of free sterol exchange and equilibration. Our studies suggest that SIT is secreted in bile by the same mechanisms as UC and may be an appropriate physiologic marker to map the transport of UC through the liver.

MATERIALS AND METHODS

Preparation of liposomes

Multilamellar liposomes were prepared fresh for each liver perfusion by sonicating egg phosphatidylcholine (Lipid Products, S. Nutfield, UK) and [5,6-^{3}H]sitostanol (24α-ethyl-5α-cholestan-3β-ol) (0.1–0.4 Ci/mol) or a combination of SIT and [4-^{14}C]cholesterol (0.05–0.2 Ci/mol) in equimolar amounts. Radiolabeled SIT was obtained from American Radiolabeled Chemicals (St. Louis, MO), [4-^{14}C]UC was obtained from DuPont-New England Nuclear (Boston, MA), and unlabeled sterols were obtained from Sigma Chemical (St. Louis, MO). All sterols were greater than 99% pure by high performance liquid chromatography (HPLC). The molar ratio of phosphatidylcholine:total sterol in liposomes was 2.6:1.0.

Liver perfusions

Perfusions were performed using livers from male Sprague-Dawley rats (Taconic Farms, Germantown, NY) that had been fed Purina Chow ad libitum. Perfusions were performed with the bile duct cannulated and with a recirculating, oxygenated medium of ~100 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), 3% albumin, and 25 mM glucose as previously described (8). When bile salt was administered, sodium taurocholate (A grade, Calbiochem, La Jolla, CA) was infused directly into the portal vein cannula at a constant rate. Liposomes containing sterols were added to perfusions by one of two methods: *1)* as a bolus in the perfusate reservoir, that recirculated for the entire period of perfusion (adding ~35 μmol of sterol) or *2)* as a constant infusion in the portal vein for the first 10 min of perfusion (adding ~30 μmol of sterol).

Samples of perfusate and bile were obtained during the course of perfusions to measure the rate of hepatic uptake of SIT and the biliary secretion of SIT, UC, and other bile lipids. At the conclusion of perfusions, SIT was measured in the liver. SIT was also measured in liver subfractions that were obtained as previously described (9) and in nascent very low density lipoproteins (VLDL) and high density lipoproteins (HDL) that were isolated from the perfusate by centrifugation at d 1.006 g/ml (2.28×10^8 g · min) and d 1.06–1.19 g/ml (5.20×10^8 g · min), respectively.

In vitro sterol transport and exchange

To determine whether SIT would exchange with UC in systems that support UC exchange, two kinds of incubations were performed where, in each case, the reactants could be reliably separated by centrifugation after incubation. First, a triglyceride-rich emulsion that contained [^{14}C]UC was incubated with a reconstituted HDL preparation that contained SIT and [^{3}H]UC (as [2,3-^{3}H]UC from Research Products International, Mt. Prospect, IL). The emulsion and reconstituted HDL were prepared as previously described (10, 11). In a second exchange experiment, liposomes that were prepared with phosphatidylcholine and equimolar amounts of SIT and [^{14}C]UC were incubated with a vesiculated preparation of rat liver plasma membranes that contained [^{3}H]UC. Liposomes were prepared as described above, with a molar ratio of phosphatidylcholine to total sterols of 2.6:1.0. The liver membranes were prepared as indicated previously (9) from a rat that had been injected iv 18 h earlier with ~100 μCi of [^{3}H]UC. Incubations were performed at 37°C in Tris-buffer (pH 7.4) with shaking. Incubations were stopped by adding iced saline to samples. Emulsions were separated from HDL by flotation at 4.2×10^6 g · min at 4°C and liposomes were separated by flotation from the (pelleted) membrane fraction at 3.0×10^6 g · min at 4°C.

Analysis

Lipids were extracted from samples and sterols were separated from other lipids by HPLC, as previously described (12). SIT and UC in the unesterified sterol fractions that were isolated from bile, liver, and perfusate lipoproteins were benzoylated (13), separated by HPLC on an Ultrasphere ODS column (5μ, 2 × 250 mm) with a mobile phase of methanol and at a flow of 0.5 ml/min. Detection was at 230 nm and quantitation by integration of peak areas in conjunction with an internal stigmasterol standard. Triglycerides in perfusate lipoproteins were quantitated by gas chromatography of fatty acid methyl esters (14). The small amount of phospholipids in lipoproteins recovered from perfusates precluded measurement of total phospholipids by phosphorus assay. However, phosphatidylcholines, that we have previously found constitute from 85–90% of the phospholipid content of perfusate lipoproteins (8), could be quantitated by a sensitive HPLC method with integration and an internal standard (15). SIT uptake from perfusate liposomes was determined by the decrease in the concentration of tritium radioactivity in the whole perfusate, measured by liquid scintillation spectrometry.

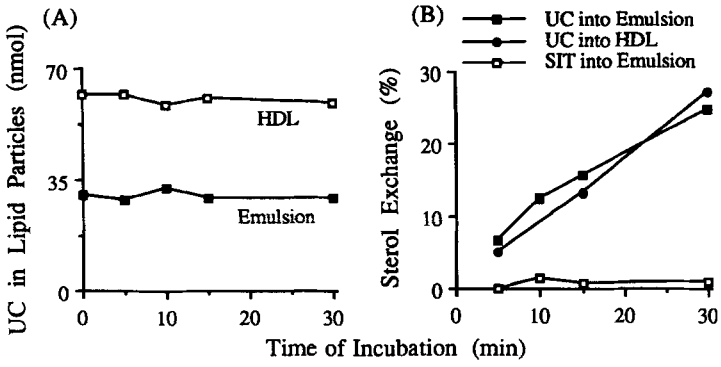

Fig. 1. Exchange and transport of UC and SIT between lipid particles. A triglyceride-rich emulsion made with [^{14}C]UC was incubated with reconstituted HDL made with [^{3}H]UC and SIT. Incubations were performed in duplicate at 37°C in Tris-buffer (pH 7.4) with shaking. After incubation, emulsions were separated from HDL by ultracentrifugation (see Methods). Amounts of sterol in each fraction were determined (A) and the extent of exchange (B) was calculated as a percent by dividing the measured specific activities of UC in emulsions and HDL at each time point by the specific radioactivity of UC in each of these fractions that would be theoretically achieved at equilibrium.

RESULTS AND DISCUSSION

We could detect no SIT in the liver of the chow-fed rat. However, to ensure that the uptake and transport of SIT would not be attributed to the exchange of SIT with endogenous stores of hepatic UC, we measured the rates of transport and exchange of isotopic UC and SIT between lipid particles and between lipid particles and a membrane. For these experiments emulsions were incubated with an HDL analog and liposomes were incubated with rat liver plasma membranes. As shown in **Fig. 1,** during a 60-min period of incubation, there was no net transport of UC between the emulsion and HDL analog. However, UC was readily exchanged and after 30 min of incubation reached ~30% of its value (in both emulsions and HDL) at equilibrium. In contrast to the appreciable exchange of UC, there was negligible transfer of SIT between these particles, that would reflect either net transport of SIT or an exchange of SIT for UC. Similar results were observed with the incubation of liposomes and plasma membranes. In this case UC equilibrated more slowly than between lipid particles and after 60 min of incubation UC had reached 13.1% and 12.3% of its calculated equilibrium values in membranes and liposomes, respectively. After 60 min of incubation, SIT transport from liposomes was again negligible (reaching 0.7% of its value at equilibrium in the plasma membrane).

Studies were next undertaken to compare the biliary secretion of radiolabeled UC and SIT that were perfused together in equimolar amounts. Liposomes with [^{3}H]SIT and [^{14}C]UC were infused directly into the portal vein cannula for 10 min in a non-recirculating system. Perfusions were then continued with fresh perfusate for 5 min to remove residual liposomes and again with fresh perfusate in a recirculating system for a final 20 min (**Fig. 2**). Throughout this period of 35 min, all bile was collected at 2-min intervals. The secretion of radioactivity in bile was normalized for the maximum amount of radioactivity secreted for each sterol. As shown in Fig. 2, the secretion of both radiolabeled SIT and UC in bile increased linearly from 4 to 16 min after the addition of liposomes to the perfusions and, for both sterols, reached a maximum at ~16 min. Slopes of the regression lines of the increase in radioactivity in bile from 4 to 16 min were virtually identical for both sterols, 8.539 ± 0.962 (SD) for SIT and 8.509 ± 0.486 for UC.

After maximum biliary secretion was attained, the secretion of SIT and UC radioactivity was markedly different. While SIT radioactivity in bile rapidly declined after reaching a maximum, UC radioactivity in bile remained at a plateau that was at near-maximum. This difference can most readily be explained by the vast differences in exchange and equilibration of these sterols with the endogenous stores of these sterols in the liver that are appreciable for UC and negligible for SIT.

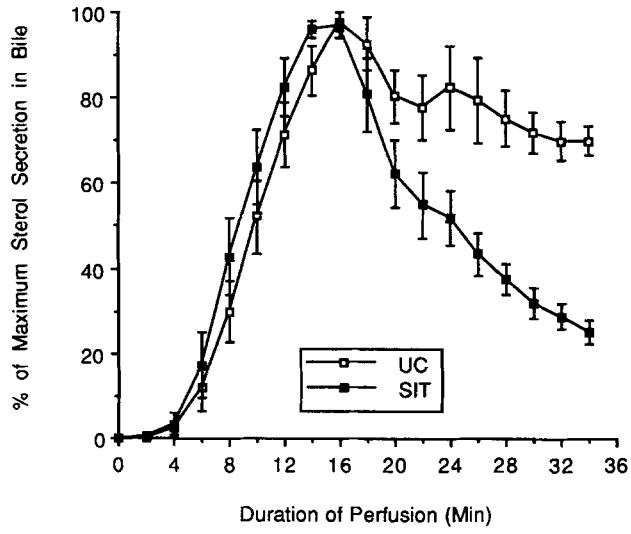

Fig. 2. Biliary secretion of [^{3}H]SIT and [^{14}C]UC by isolated rat livers. Liposomes with an equimolar amount of SIT and UC were added to liver perfusates as a continuous, non-recirculating infusion from 0 to 10 min. The perfusate was changed from 10 to 15 min to wash out residual liposomes. After this wash-out period, the perfusate was again changed and recirculated for an additional 20 min. For the entire 35 min of perfusion, sodium taurocholate was continuously infused at 40 µmol/h. All bile was collected at 2-min intervals after the addition of liposomes. Results are normalized and expressed as a percentage of the maximum secretion of radioactivity for each sterol. Data are shown as mean ± SEM for three perfusions.

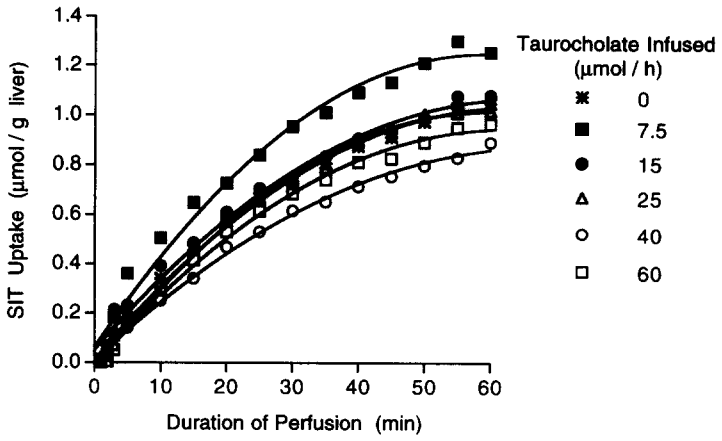

Fig. 3. Hepatic uptake of [^{3}H]SIT by isolated rat livers. Liposomes containing [^{3}H]SIT were added to perfusates at 0 min and recirculated until the end of perfusions. Uptake was calculated from the disappearance of radioactivity from the perfusate at initially 1-min intervals and then at 5-min intervals. Taurocholate was constantly infused at 0, 7.5, 15, 25, 40, or 60 µmol/h, beginning 20 min prior to the addition of liposomes. Data are shown as mean for three perfusions at each infusion rate of taurocholate. Standard error values were similar for all six groups and because of considerable overlap at each time point these are not shown.

In spite of the potential for UC exchange, radiolabeled cholesterol, both as UC and cholesteryl ester, has been repeatedly used to assess the contribution to bile of cholesterol derived from different lipoproteins (16–23). These studies have uniformly found that the contribution of lipoprotein-cholesterol to biliary UC secretion is extremely small, in the rat accounting for 0.1–0.3% of biliary UC secretion per h (16–19, 23). As we have previously indicated (4), this very low number can be predicted by the extent of hydrolysis of cholesteryl esters to UC in the liver and the rapid exchange and equilibration of any radiolabeled UC that derives from lipoproteins with the very large pool of non-radiolabeled UC that is present in the liver. For these reasons, in the present study we could not directly determine the contribution to bile of radiolabeled UC taken up by the liver from liposomes. In contrast, there was no SIT in the liver prior to perfusion and, in the absence of exchange, mixing, or any dilution of SIT, we could directly determine the contribution of this sterol to bile by either isotope or mass measurements.

The hepatic uptake and biliary secretion of SIT in relation to the secretion of other biliary lipids was determined as a function of bile salt secretion. Liposomes with SIT were added to the perfusate reservoir and recirculated for 60 min with taurocholate infused at 0, 7.5, 15, 25, 40, or 60 µmol/h. The hepatic uptake of SIT, measured as the disappearance of [^{3}H]SIT from the perfusate at 1- to 5-min intervals, was best fit to a second-order polynomial at each taurocholate infusion rate (with $R^2 > 0.98$ for each curve) (**Fig. 3**). The disappearance of SIT from the perfusate was not significantly different for different rates of infusion of bile salt (including perfusions without bile salt), except for the infusion of taurocholate at 7.5 µmol/h. For no certain reason, with infusion of taurocholate at 7.5 µmol/h, SIT uptake was greater after 20 min than at other infusion rates of taurocholate.

Bile was collected at 5-min intervals after the start of perfusions with taurocholate (at -20 min) (**Fig. 4**). SIT was added to the perfusates at 0 min. UC secretion was on average greater at the higher rates of taurocholate

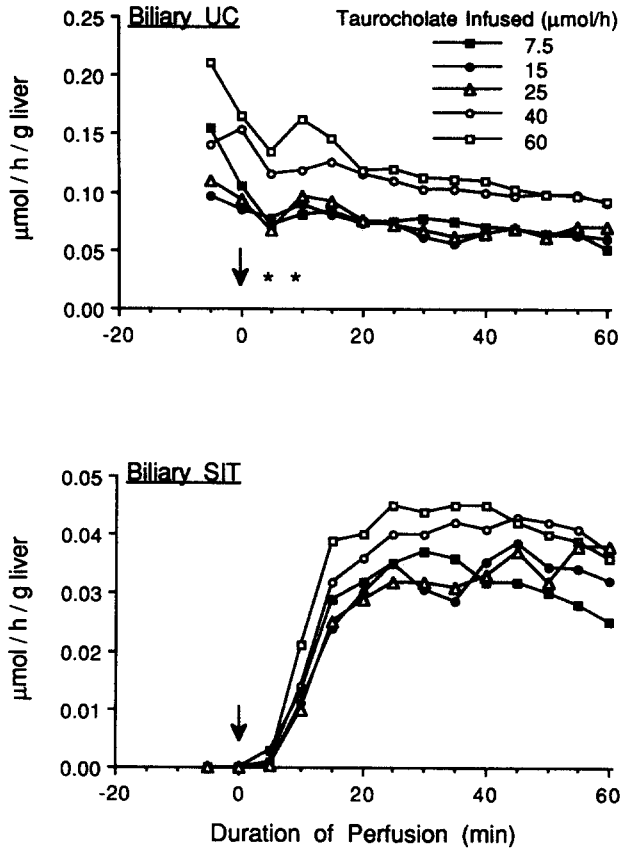

Fig. 4. Biliary secretion of UC and SIT by isolated rat livers. Data are from the same perfusions described in Fig. 3 when taurocholate was infused at 7.5, 15, 25, 40, or 60 µmol/h. Taurocholate infusions were begun at -20 min. Liposomes containing [^{3}H]SIT were added to perfusates at 0 min (arrow) and were recirculated until the end of perfusions. Data are shown as the mean for three perfusions at each bile salt infusion rate. After liposomes were added to perfusions, $P < 0.05$ (asterisks) for UC secretion at 25 vs. 60 µmol/h (at 5 min) and at 7.5 vs. 60 µmol/h (at 10 min).

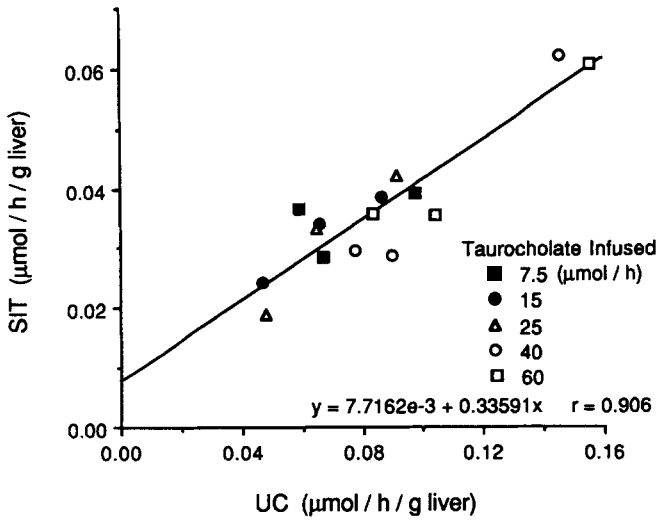

Fig. 5. Relation of UC to SIT secretion in bile from isolated livers perfused with SIT and with different amounts of taurocholate. Data are from the same perfusions described in Figs. 3 and 4 when taurocholate was infused at 7.5, 15, 25, 40, or 60 µmol/h. UC and SIT secretion rates for individual perfusions were calculated from the average of the 20- to 40-min values for each perfusion (comprising five data points), i.e., during the period when SIT secretion had reached a plateau.

infusion (at 40 and 60 µmol/h) than at the lower rates (from 7.5 to 25 µmol/h), but was only significantly different after the start of perfusions with SIT between 60 µmol/h and 25 µmol/h (at 5 min) and 7.5 µmol/h (at 10 min). With the infusion of taurocholate, SIT was detected in bile 5 min after its addition to the perfusate, reached a maximum rate of secretion by about 20 min, and remained stable thereafter until perfusions were concluded (Fig. 4). The secretion of SIT with infusion of taurocholate from 7.5 to 60 µmol/h was highly variable and was not significantly different at these different rates of bile salt infusion. However, when no taurocholate was infused, only trace amounts of SIT were detected in bile (averaging ~0.0002 µmol/h per g liver). Similar to the secretion of SIT, virtually no UC was secreted in bile in the absence of a taurocholate infusion. Maximum biliary transport of SIT, achieved during a period of continuing hepatic uptake of SIT, was about 0.04 µmol/h per g liver.

At the end of perfusions, the amount of SIT in the liver was 0.617 ± 0.146 (SD) µmol/g, almost all of which was present as unesterified SIT (99.44 ± 0.16%). The amount of SIT in the liver was ~13% of the content of hepatic UC (that averaged 4.80 ± 0.39 (SD) µmol/g). In contrast, at the end of perfusions the amount of SIT in bile was about 40–50% of the amount of biliary UC (Fig. 4).

Biliary SIT secretion was strongly and linearly related to biliary UC secretion when taurocholate was infused from 7.5 to 60 µmol/h (**Fig. 5**). In addition, the relation of biliary SIT to bile acids was virtually the same as the relation of biliary UC to bile acids over an approximate 10-fold range, except that there was about half as much SIT as UC in bile (**Fig. 6**). Biliary bile acid secretion closely approximated the amounts of taurocholate infused and the relative concentrations of bile acids in bile generally paralleled secretion rates (ranging from a low of 13.0 ± 2.3 mmol/L when 7.5 µmol/h of taurocholate was perfused to a high of 72.6 ± 7.0 mmol/L when 60 µmol/h of taurocholate was perfused). At the lowest concentration of bile acids, the ratios of both SIT and UC to bile acids were appreciably greater than at all higher concentrations of bile acids (i.e., when taurocholate was infused at 15 µmol/h or greater) (Fig. 6). A greater concentration of UC to bile acids is to be anticipated at low concentrations of biliary bile acids, when micelle formation is less prevalent and there is apt to be proportionately greater solubilization of biliary UC in mixed phospholipid/bile acid vesicles (24). Our data suggest that this also applies to SIT.

To localize SIT within the liver, additional perfusions were performed in the sequence described for the first series of studies that are shown in Fig. 2. Liposomes that contained only [^{3}H]SIT were perfused. As shown in **Table 1,** SIT that was taken up by the liver from liposomes was predominantly concentrated in the canalicular membranes of the hepatocyte, not within the interior membranes of the cell (i.e., mitochondria + Golgi and microsomal fractions). The distribution of SIT in all cell fractions generally paralleled the distribution of UC (with SIT as a

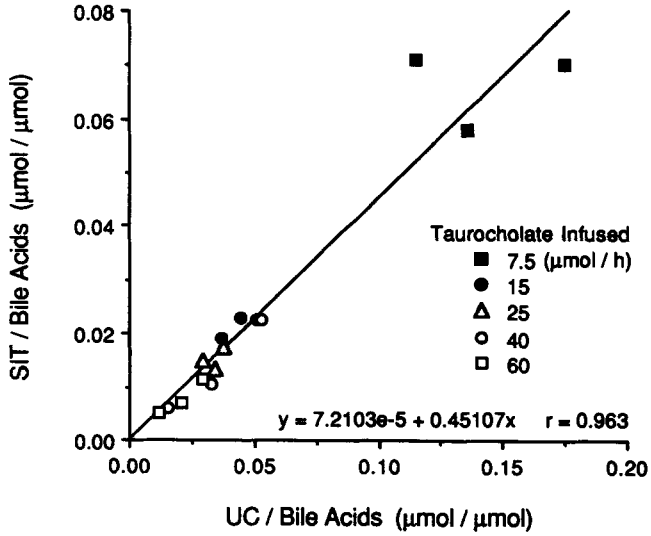

Fig. 6. Relation of the concentrations of UC/bile acids to SIT/bile acids from livers perfused with SIT and with different amounts of taurocholate. Data are from the studies shown in Figs. 3, 4, and 5. Values were calculated from biles that were obtained from 20 to 40 min after SIT was added to perfusions.

TABLE 1. Content of unesterified cholesterol (UC) and sitostanol (SIT) in liver fractions after isolated rat livers were perfused with SIT

Liver Fraction	UC	SIT	Alkaline Phosphatase	UC (C)	SIT (C)
	nmol/mg protein		*U/mg protein*	*nmol/mg protein*	
Whole homogenates	25.0 ± 2.3	0.70 ± 0.21	0.39 ± 0.12	–	–
Mitochondria + Golgi	28.3 ± 7.4	0.62 ± 0.16	0.32 ± 0.10	23.1 ± 8.0	0.46 ± 0.21
Microsomes	52.5 ± 2.2	1.14 ± 0.20	0.40 ± 0.11	45.9 ± 1.9	0.70 ± 0.21
Sinusoidal membranes	82.8 ± 30.6	2.08 ± 1.40	2.95 ± 1.41	35.5 ± 17.5	0.61 ± 0.51
Canalicular membranes	275.3 ± 27.6	8.25 ± 3.65	16.84 ± 4.92	275.3 ± 27.6	8.25 ± 3.65

Livers were perfused in the sequence that is indicated in Fig. 2, adding SIT to perfusates from 0 to 10 min. At the end of perfusions, livers were homogenized and fractions were isolated by differential and gradient centrifugation (see Methods). Alkaline phosphatase activity was used as a marker for canalicular membranes, to correct for contamination of other cell fractions with canalicular membranes (see Results). For this calculation, the assumption was made that only canalicular membranes contain alkaline phosphatase (25) and that canalicular membranes were pure. Corrected values are shown as UC (C) and SIT (C). Data is shown as the mean ± SD for 3 perfusions.

percent of the sterols averaging 2.7% for the whole homogenate and 2.1%, 2.1%, 2.5%, and 2.9% for the mitochondria + Golgi fraction, microsomes, sinusoidal membranes, and canalicular membranes, respectively). Alkaline phosphatase activity, assumed to be exclusively in the canalicular membranes of the hepatocyte (25), was used to correct other cell fractions for canalicular membrane contamination. With this correction, the canalicular membranes of the hepatocyte were even more enriched with SIT and UC than other cell fractions (Table 1).

These same perfusions were used to determine the extent to which SIT was secreted in newly synthesized lipoproteins of hepatic origin. The secretion of SIT in nascent VLDL and HDL was measured during the final 20 min of these perfusions, after the SIT that had been added in liposomes was washed out of the system. As shown in **Table 2,** the content of SIT was appreciably increased in HDL but not in VLDL. Although the origins of HDL have not been delineated with certainty, there is reason to believe that hepatic HDL derives from plasma membranes (8) whereas VLDL most certainly derives from the microsomal-Golgi network of membranes where SIT concentrations were relatively low. It is possible that some of the SIT that was recovered in perfusate HDL was derived from residual liposomes that were not totally eliminated during the 5-min period of washout. However, any major contribution of SIT from liposomes seems unlikely as *1)* liposomes would not be isolated by ultracentrifugation at the density of perfusate HDL, and *2)* the distribution of molecular species of phosphatidylcholines recovered in perfusate HDL was markedly different than the distribution of egg phosphatidylcholines used to make the liposomes (data not shown). Relative to total sterols, the percentage of SIT in HDL was even greater than the percentage of SIT in bile that was obtained during this same period. However, a direct comparison of HDL and bile sterol secretion was not possible, as the kinetics of secretion of HDL have not been determined and may not parallel the secretion of bile SIT (as depicted in Fig. 2).

Our present results with SIT are entirely consistent with two previous studies from our laboratory that pertain to the route by which plasma UC moves through the liver into bile and to the origins of nascent hepatic HDL. In the first study (9), we reported that UC that was exchanged into the liver from plasma lipoproteins is secreted into bile from the canaliculus without first entering the interior of the liver cell. Although the precise mechanism by which the transport of plasma UC into bile takes place has yet to be defined, the principle localization of SIT to the canalicular membrane (Table 1) in the present study suggests that SIT may follow this same

TABLE 2. Lipid composition of perfusate lipoproteins and bile after isolated rat livers were perfused with SIT

	VLDL	HDL	Bile
Triglycerides (nmol)	606 ± 366	7.5 ± 1.6	–
Phosphatidylcholines (nmol)	154 ± 76	16 ± 8	652 ± 31
Cholesteryl esters (nmol)	71 ± 29	10 ± 2	–
UC (nmol)	98 ± 41	20 ± 9	129 ± 58
SIT (nmol)	0.7 ± 0.4	6.4 ± 4.2	25 ± 11
% Unesterified sterols as SIT	0.7 ± 0.5	24 ± 11	10 ± 2

Livers were perfused in the following sequence: liposomes with SIT were added to perfusates from 0 to 10 min, the perfusate was washed free of SIT for 5 min, and then fresh perfusate without SIT was recirculated for a final period of 20 min. Taurocholate was continuously infused at 40 μmol/h and all bile was collected. VLDL and HDL, secreted by the liver during the final 20-min period of perfusion, were isolated from the perfusate by centrifugation (see Methods). Lipoprotein components are shown in nmol per 20-min collection period. Bile sterols are shown in nmol for this same 20-min collection period. Data is the mean ± SD for 3 perfusions.

pathway into bile. In a second earlier study (8), we reported that bile salts do not stimulate the secretion of VLDL but do stimulate the secretion of nascent HDL that is enriched in UC and the specific molecular species of phosphatidylcholine that are present in bile. In the present study, nascent HDL, but not VLDL, was enriched in SIT to an extent that was comparable to bile (Table 2). Thus, results with SIT are consistent with our earlier suggestion that the formation of hepatic HDL and bile lipids are linked and are derived from a similar membrane site and/or by a similar mechanism that is mediated by bile salts.

In summary, in the present studies we have demonstrated that when isolated rat livers are perfused with liposomes that contain SIT: *1)* appreciable amounts of SIT are taken up by the liver; *2)* in the presence of bile salt, SIT is promptly secreted in bile with the same kinetics as UC and in direct proportion to UC; *3)* bile is enriched in SIT compared to the liver; *4)* the distribution of SIT in the liver parallels the distribution of UC and both sterols are preferentially concentrated in the canalicular membranes of the hepatocyte; and *5)* newly secreted HDL, but not VLDL, is greatly enriched in SIT. These results strongly suggest that SIT can be used as a physiologic analog of UC to trace the movement of UC into and out of the liver without being subject to exchange and equilibration that confounds the interpretation of transport when radiolabeled UC is used.

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