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Research Article

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Cyclical Oxidation–Reduction of the C_3 Position on Bile Acids Catalyzed by 3α-Hydroxysteroid Dehydrogenase

II. Studies in the Prograde and Retrograde Single-Pass, Perfused Rat Liver and Inhibition by Indomethacin

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Abstract

[3β-^{3}H, 24-^{14}C]Lithocholic, chenodeoxycholic, and cholic acids were administered in tracer bolus doses either prograde or retrograde in the isolated perfused rat liver. Little ^{3}H loss from cholic acid was observed, whereas with the other bile acids, 20–40% of the administered ^{3}H was lost in a single pass from perfusate to bile. Most of the ^{3}H loss occurred rapidly (5 min) and was recovered as [^{3}H]water in perfusate. Excretion of bile acids was delayed with retrograde administration, and ^{3}H loss was more extensive. In both prograde and retrograde studies, indomethacin markedly inhibited the excretion of the bolus of bile acid into bile. Indomethacin inhibited the extraction of glycocholate (50 μM) during steady state perfusion without affecting transport maximum for excretion. At lower glycocholate concentration (5 μM), indomethacin inhibited both extraction and excretion. A greater effect was seen on excretion in the latter case, which suggests that displacement of bile acid from the cytosolic protein lead to redistribution in the hepatocyte as well as reflux into the sinusoid. These data suggest that binding of bile acids to cytosolic 3α-hydroxysteroid dehydrogenases occurs extensively during hepatic transit and is important in mediating the translocation of bile acids from the sinusoidal to canalicular pole of the cell.

Introduction

The process by which bile acids are translocated intracellularly from sinusoidal to canalicular pole of hepatocytes is poorly understood. Bile acid binding by cytosolic proteins has been proposed as having an important role in intracellular transport of bile acids. We have characterized previously the binding properties of the major cytosolic bile acid binding proteins in rat liver (1–4). These proteins include GSH S-transferases (1–3), Y′ bile acid binders (1), and Z protein (4). Recently, we have reported that Y′ bile acid binders are 3α-hydroxysteroid dehydrogenases (3α-HSD),[1] which can catalyze oxidation or reduction of the 3 position (C_3) of bile acids depending on cofactor concentrations and pH (5). Under physiological redox and pH conditions, this cytosolic enzyme in pure form or in hepatocytes catalyzes the net conversion of 3-oxo bile acid to 3α-hydroxy bile acid and not vice versa (5). This enzyme also exclusively catalyzes the back-and-forth equilibrium cycling of bile acids (3α-OH ⇌ 3-oxo) without net conversion to 3-oxo bile acids in hepatocytes. Thus, by using [3β-^{3}H, 24-^{14}C]bile acids, loss of ^{3}H can be used to specifically probe the interaction of bile acids with cytosolic 3α-HSD in hepatocytes. Furthermore, indomethacin, a competitive inhibitor of 3α-HSD, can be used to assess the effects of inhibition of the interaction of bile acids with 3α-HSD. In the accompanying manuscript, we found that indomethacin significantly shifted the equilibrium distribution of bile acids from cells to media without affecting initial uptake rates. In those experiments, isolated hepatocytes have lost their polarity and the bile acids have the opportunity to cycle in and out of the cells. The significance of bile acid binding to 3α-HSD as reflected in ^{3}H loss, in the vectorial transport and extent of equilibrium cycling during a single transit from sinusoid to bile could not be assessed in this model. Therefore, in the present paper we have extended this work to a more physiologic model, the isolated perfused rat liver. With this model, we have assessed the extent of ^{3}H loss during one transit from perfusate to bile in the periportal or pericentral zones and have examined the effect of indomethacin on ^{3}H loss, rate of excretion, and extraction of bile acid.

Methods

Preparation of [3β-^{3}H, 24-^{14}C]bile acids. [3β-^{3}H]Lithocholic, chenodeoxycholic, and cholic acids were synthesized from respective 3-oxo forms by [^{3}H]sodium borohydride ([^{3}H]$NaBH_4$) (500 mCi/mmol, Amersham Corp., Arlington Heights, IL) treatment (6). Each [3β-^{3}H]bile acid was mixed with [24-^{14}C]bile acids: [^{14}C]lithocholic acid (55 mCi/mmol, Amersham Corp.), [^{14}C]chenodeoxycholic acid (60 mCi/mmol, Amersham Corp.), and [^{14}C]cholic acid (52 mCi/mmol, New England Nuclear, Boston, MA), to yield ^{3}H/^{14}C ratios between 5 and 10.

Isolated liver perfusions. Male Sprague–Dawley rats (Hilltop Lab Animals, Inc., Scottdale, PA) that weighed 250–300 g were used. The animals had free access to water and food (Purina rodent chow; Ralston-Purina Co., St. Louis, MO) until the time of the experiments.

Rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and the common bile duct was cannulated with a PE-10 tube (Clay Adams, Div. of Becton-Dickinson & Co., Parsippany, NJ) after abdominal incision. Prograde liver perfusion was started 10 min after bile duct cannulation.

Livers were perfused in situ, single-pass, with Krebs–Ringer bicarbonate buffer gassed to equilibrium with a ratio of 95% O_2 to 5% CO_2 at pH 7.4 and 37°C according to the method of Sies (7), as previously reported (8). In retrograde perfusion, the direction of perfusion was switched from prograde to retrograde 10 min after the start of prograde perfusion. Perfusion rates were 4 ml/min per g liver in either direction.

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1. *Abbreviations used in this paper:* 3α-HSD, 3α-hydroxysteroid dehydrogenases; TLC, thin-layer chromatography; T_{max}, transport maximum.

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In each case, [^{3}H, ^{14}C]bile acid (15 nmol in 2–6 μl of ethanol) was injected in the inlet line 40 min after the beginning of bile duct cannulation. In some experiments, infusion of indomethacin (50 μM) was started 10 min before the injection of [^{3}H, ^{14}C]bile acids and continued until the end of the experiments. Bile samples were collected every 5 min up to 60 min after bile acid injection into preweighed polyethylene tubes. In some antegrade experiments, bile samples were collected every 2 min. Aliquots of bile samples and pooled perfusates collected during the 1st 5 min after injection were counted for ^{3}H and ^{14}C. ^{3}H/^{14}C ratios of the samples were calculated after the correction of the crossover of ^{14}C counts into the ^{3}H channel.

During all perfusion studies, O_2 uptake (2 μmol/min per g liver) and the hydrostatic perfusion pressure (~ 5 cm H_2O) remained constant. Bile flow immediately decreased after bile duct cannulation (~ 50–25 μl/h per g body wt) presumably because of the decrease of biliary bile acid excretion. After injection of bile acids, bile flow continued to decline gradually; ~ 20% decrease of bile flow was observed during 60 min. Infusion of indomethacin increased bile flow ~ 40% in prograde perfusion only. There was no detectable cell damage during all perfusions, as monitored by glutathione S-transferase activity in perfusates, which were checked every 10 min by the method of Habig (9).

Exogenous bile acid excretion. Bile acid concentrations in bile samples were determined by the method of Paumgartner (10) using bacterial 3α-HSD. Initial bile acid outputs after cannulation were 95±21 nmol/min per g liver. The output rapidly decreased and remained between 1.5 and 2.5 nmol/min per g during the experimental intervals before and up to 60 min after tracer administration in the presence or absence of indomethacin.

Thin-layer chromatography (TLC) analysis of radioactive bile acids. Aliquots of pooled bile samples collected in the 60-min interval after the injection of bile acid were analyzed by TLC. Bile samples were subjected to deconjugation by cholylglycine hydrolase at 37°C for 16 h, as previously reported (6). After incubation, the mixture was adjusted to pH 8 with sodium phosphate buffer and applied onto prewashed Sep Pak C_{18} cartridges. Bile acids were eluted from the cartridges with methanol after washing with water (6). Evaporated samples were redissolved in methanol and applied to silica gel TLC plates (K6F; Whatman Chemical Separation Inc., Clifton, NJ) and developed with the solvent system of isooctane/ethylacetate/acetic acid (10:10:2 vol/vol). Lanes corresponding to mono-, di-, and trihydroxy bile acids were scraped and ^{3}H and ^{14}C counts measured.

Steady state perfusion with [^{14}C]glycocholic acid. Prograde perfusion of the rat liver was performed as described above. Continuous perfusion of glycocholic acid (50 or 5 μM with [^{14}C]glycocholic acid (52.5 mCi/mmol, New England Nuclear) was begun 20 min after bile duct cannulation. Continuous infusion of indomethacin (50 μM) was added between 20 and 40 min after starting the glycocholic acid infusion. Radioactivity in the bile and perfusate was counted every 5 min.

Results

^{3}H loss from all three double-labeled bile acids was observed during a single transit through the isolated rat liver perfused in a prograde or retrograde fashion. However, ^{3}H loss from double-labeled cholic acid was much less extensive than from lithocholic and chenodeoxycholic acids (Figs. 1–3). The rate of decrease of ^{3}H/^{14}C ratios of double-labeled bile acids appearing in the bile was similar in prograde and retrograde studies. A marked difference in the rate of biliary excretion of radiolabeled bile acids was observed in comparing prograde and retrograde studies. The peak biliary excretion of ^{14}C representing bile acids occurred within the 1st 5 min for three bile acids perfused prograde, whereas biliary excretion of ^{14}C was always delayed in retrograde perfusion. Detailed analysis of the excretion patterns after substraction of dead space in the first few

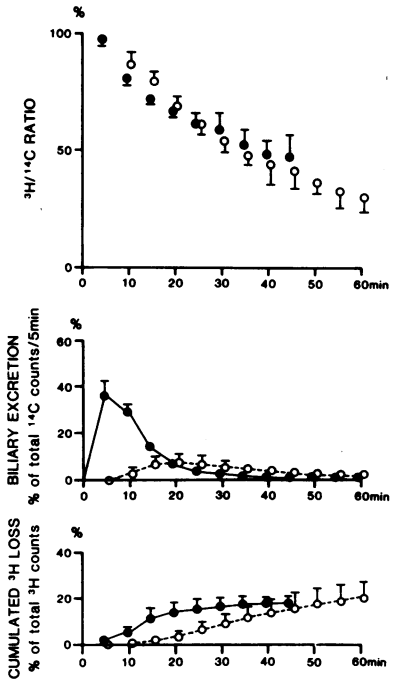

Figure 1. Comparison of biliary excretion of bile acid and ^{3}H loss after administration of a tracer bolus of [3β-^{3}H, 24-^{14}C]lithocholic acid in the prograde and retrograde perfused rat liver. The top figure shows the ^{3}H/^{14}C ratio of bile acids excreted in bile over time. The middle figure shows the excretion rate in sequential 5 min collections of bile as a percentage of the total bolus of [^{14}C]lithocholic acid administered. The bottom figure shows the cummulative ^{3}H that was lost from the bile acids excreted in bile after hepatic transit. (•) represents results from prograde and (○), from retrograde studies.

minutes indicated that peak excretion occurred almost immediately. The recovery of ^{14}C radioactivity in bile during 60 min perfusion was complete in prograde studies; 101±3% for lithocholic acid, 104±4% for chenodeoxycholic acid (means±SD, n = 3), and 95% for cholic acid (mean of two experiments). However, the recovery of administered ^{14}C in bile was not complete during 60 min retrograde perfusion: 49±15% for lithocholic acid, 94±5% for chenodeoxycholic acid (means±SD), and 66% for cholic acid. Cummulated ^{3}H loss from bile acids appearing in bile during 60 min was higher in

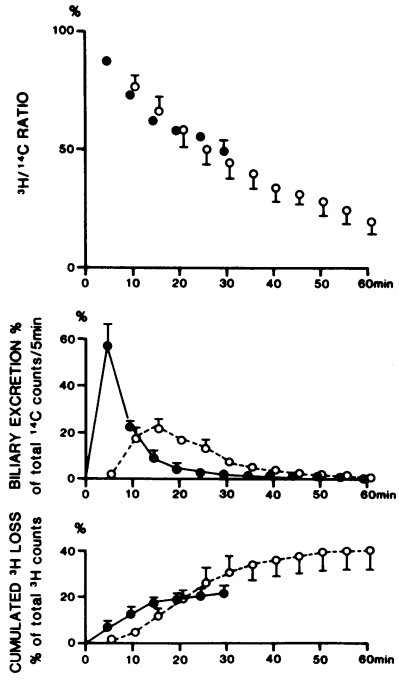

Figure 2. Comparison of biliary excretion of bile acid and ^{3}H loss after administration of a tracer bolus of [3β-^{3}H, 24-^{14}C]chenodeoxycholate acid in the prograde (•) and retrograde (○) perfused rat liver. See legend to Fig. 1 for description.

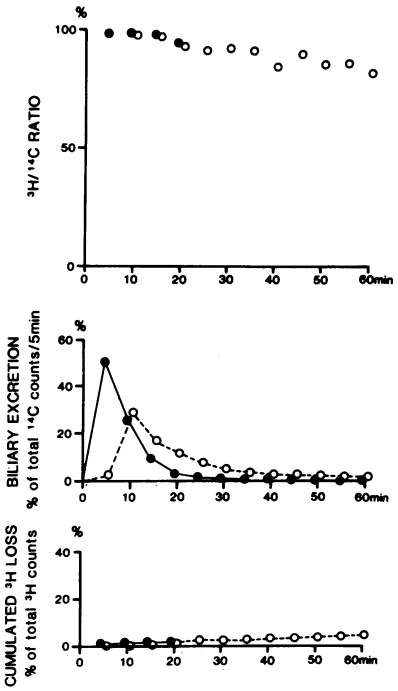

Figure 3. Comparison of the biliary excretion of bile acid and ^3H loss after administration of a tracer bolus of [3β-^3H, 24-^{14}C]cholic acid in the prograde (●) and retrograde (○) perfused rat liver. See legend to Fig. 1 for description.

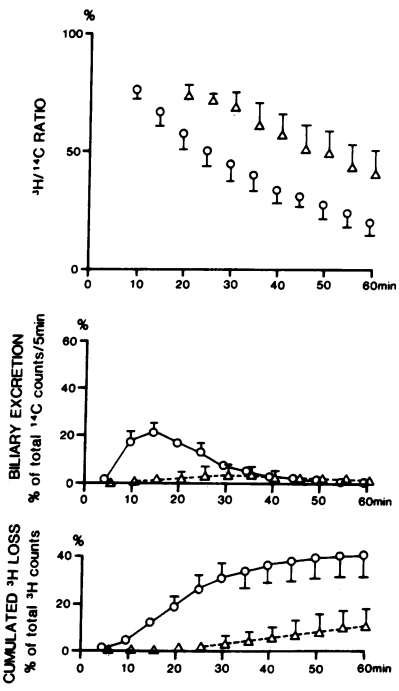

Figure 5. Effect of indomethacin on the biliary excretion and ^3H loss after administration of a tracer bolus of [3β-^3H, 24-^{14}C]chenodeoxycholic acid in the retrograde perfused rat liver. See legend to Fig. 1 for description. (○) no indomethacin and (△) 50 μM indomethacin by continuous perfusion.

retrograde (40.7±9.0%) than prograde (21.4±3.5%) perfusion ($P < 0.02$) with double-labeled chenodeoxycholic acid (Fig. 2). This difference was not observed with lithocholic acid probably because of the marked delay in its excretion when administered retrograde.

Indomethacin inhibited ^3H loss from double-labeled chenodeoxycholic acid excreted into bile both in prograde (Fig. 4) and retrograde (Fig. 5) studies. Indomethacin also delayed the excretion of labeled chenodeoxycholic acid into bile (Figs. 4 and 5). The recoveries of ^{14}C in bile after administering double-labeled chenodeoxycholic acid during 60 min perfusion were 53±24% (mean±SD) in prograde and 17.6±6.7 in retrograde studies in the presence of 50 μM indomethacin ($P < 0.02$ for each vs. control). Cummulated ^3H loss was also lowered by indomethacin both in prograde and retrograde perfusions (Figs. 4 and 5).

Most of the ^3H lost from 3β position of bile acids was recovered in perfusates. During the 1st 5 min after the injection of double-labeled lithocholic and chenodeoxycholic acids, considerable amounts of ^3H counts were recovered in perfusates both in prograde and retrograde perfusion (Table I). In comparing this initial ^3H loss into perfusates, significantly greater release from [3β-^3H]lithocholic acid was found in retrograde as compared with prograde studies. A similar trend was seen in studies with radiolabeled chenodeoxycholic acid, but did not reach statistical significance. In the presence of indomethacin, less ^3H was released from radiolabeled chenodeoxycholic acid into the perfusates (Table I). Because of the small sample size, this difference reached significance only in the

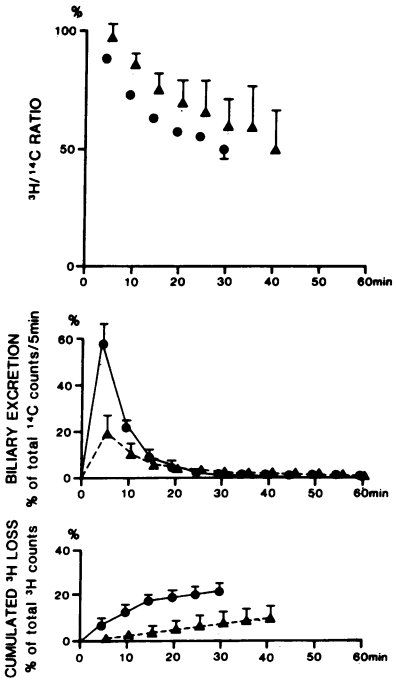

Figure 4. Effect of indomethacin on the biliary excretion of bile acid and ^3H loss after administration of a tracer bolus of [3β-^3H, 24-^{14}C]chenodeoxycholic acid in the prograde perfused rat liver. See legend to Fig. 1 for description. (●) no indomethacin and (▲) 50 μM indomethacin by continuous perfusion.

Table I. Percentage of Administered ^3H Counts Recovered in Effluent Perfusates during the 1st 5 min of Perfusion

	Lithocholic acid	Chenodeoxycholic acid	Chenodeoxycholic acid plus indomethacin	Cholic acid
Prograde	11.0±1.0	17.3±1.9	12.9±5.3	1.1
Retrograde	14.7±1.4*	23.6±7.9	12.9±2.7‡	1.2

Values are means±SD of the percentages of total ^3H counts from three to four or mean of two experiments. No ^{14}C counts were detected in these samples. These results should be compared with the cumulated ^3H loss from bile acids excreted in bile in 60 min. These comparisons were by unpaired *t* test. The other comparisons did not reach significance.

* $P < 0.02$ (retrograde vs. prograde lithocholic acid).

‡ $P < 0.02$ retrograde chenodeoxycholic acid plus indomethacin vs. retrograde chenodeoxycholic acid control.

retrograde case. After the 1st 5 minutes, the ^3H concentration in perfusate samples was too low to allow for the accurate comparison of sinusoidal release with and without indomethacin.

Extraction of all three bile acids administered as tracer boluses either prograde or retrograde was complete in the presence or absence of indomethacin. Release of ^{14}C into perfusates was negligible (< 2% of total activity) during 60 min perfusions (not shown). Neither extraction of the tracer bolus of bile acid nor reflux of bile acid into the perfusate was affected by indomethacin.

Table II shows the extent of hydroxylation of double-labeled lithocholic and chenodeoxycholic acids appearing in bile after a single transit through the isolated perfused rat liver. Significantly greater hydroxylation of lithocholic and chenodeoxycholic acids occurred in retrograde than prograde perfusions. Indomethacin had no significant effect on the hydroxylation of chenodeoxycholic acid in prograde or retrograde perfusions (Table II).

Table III shows $^3H/^{14}C$ ratios of hydroxylated forms of bile acids in pooled 60 min bile collections. Greater ^3H loss was observed in retrograde than prograde perfusions. In the experiments with double-labeled lithocholic acid, little ^3H loss of the parent lithocholic acid was observed. Esterification of the 3α-OH position of lithocholic acid did not occur extensively (11±3% of lithocholic acid in prograde and 27±5% of lithocholic acid in retrograde perfusions). Thus, the lack of ^3H loss could not be accounted for by esterification of the 3α-OH group of lithocholic acid, which would prevent equilibrium cycling by 3α-HSD.

Fig. 6 shows the effects of indomethacin on the continuous prograde infusion of glycocholic acid in isolated rat liver. With 50 μM glycocholic acid, indomethacin increased bile flow and decreased the concentration of glycocholic acid in bile. However, the biliary excretion rate of glycocholic acid did not change during concomitant infusion with indomethacin. The net steady state extraction of glycocholic acid was reversibly decreased by indomethacin. The inhibition of extraction was not accompanied by a fall in excretion. With 5 μM glycocholic acid, indomethacin inhibited both extraction and excretion (Fig. 6). Excretion appeared to diminish more than extraction. There was no evidence for nonspecific toxicity by indomethacin in these experiments as monitored by O_2 consumption, perfusion pressure, or cytosolic enzyme release.

Discussion

In the preceding paper, we have documented the validity of monitoring ^3H loss from the 3β position of bile acids as a probe of their interaction with a cytosolic enzyme, 3α-HSD. This enzyme under physiologic conditions catalyzed bidirectional oxidoreduction of the C3 position on bile acids, without net conversion to 3-oxo bile acids. Our previous studies, which utilized isolated hepatocytes, demonstrated the potential for use of the double-labeled bile acid probes. However, isolated cells in suspensions lose their polarity, and bile acids can cycle back and forth across the plasma membrane. Due to these limitations, we turned to a more physiologic model, the isolated perfused liver. Using this model, the extent of interaction of bile acids with cytosolic 3α-HSD, as assessed by ^3H loss, could be determined during a single transit from perfusate to bile. Furthermore, the perfused liver model provides the opportunity to assess the effect of direction of flow or zonation on ^3H loss. Others have shown that bile acid excretion during prograde perfusion is much more rapid than in retrograde perfusion, whereas bile acid metabolism (hydroxylation) is much more extensive in the retrograde perfusion (11). These observations suggest that zone III hepatocytes retain and more extensively biotransform bile acids as compared with zone I hepatocytes.

Our findings were in general agreement with zonal differences reported by others with respect to the rate of excretion (11, 12) and extent of hydroxylation (11). Double-labeled cholic, chenodeoxycholic, or lithocholic acids given as tracer boluses were virtually completely extracted in prograde and retrograde studies. In prograde studies, nearly complete recovery of the bile acids occurred in bile in 1 h. In the retrograde studies excretion of bile acid was delayed, so that the recovery was incomplete after 1 h bile collection. Minimal ^3H loss from cholic acid was observed during one transit through the liver into bile, which could be anticipated based on the lower affinity of 3α-HSD for cholic acid (5). In contrast, in the case of lithocholic or chenodeoxycholic acids, extensive ^3H loss was observed. Approximately 20% of the ^3H was lost with either bile acids given prograde; the bulk of this ^3H loss from biliary bile acids occurred in the 1st 15 min after prograde administration and was reflected in the recovery of most of the lost ^3H in perfusate samples within the 1st 5 min. With retrograde administration, ^3H loss from bile acids excreted in bile was

Table II. Hydroxylation of Bile Acids in Pooled Bile for 60 min Perfusion

	Lithocholic acid		Chenodeoxycholic acid		Chenodeoxycholic acid plus indomethacin	
	Prograde	Retrograde	Prograde	Retrograde	Prograde	Retrograde
Monohydroxy	39±3	11±1*	—	—	—	—
Dihydroxy	18±1	8±1*	52±19	13±7‡	24±3	11±3*
Trihydroxy	43±3	81±1*	48±19	87±7‡	76±3	89±3*

Values are mean percentages±SD of three experiments on three hydroxylated forms of bile acids in the bile. All comparisons of prograde vs. retrograde were significantly different (*$P < 0.01$, ‡$P < 0.05$, unpaired t test). There was no significant difference in percentage of individual hydroxy forms when comparing perfusion without and with indomethacin in the retrograde and prograde studies with chenodeoxycholic acid.

Table III. Percentage of the Initial $^3H/^{14}C$ Ratio of Hydroxylated Forms of Bile Acids in Pooled 60 min Biles

	Lithocholic acid		Chenodeoxycholic acid	
	Prograde	Retrograde	Prograde	Retrograde
Monohydroxy	89.1±3.5	83.6±9.6	—	—
Dihydroxy	72.4±4.8	37.2±1.0*	83.1±1.8	58.5±6.3*
Trihydroxy	64.8±2.4	54.2±3.0*	66.9±3.9	57.3±8.8

Values are mean percentages±SD of three experiments.
* $P < 0.01$ by unpaired t test, prograde vs. retrograde.

more extensive for chenodeoxycholic (~ 40%) than for lithocholic acid (~ 20%). With the former, the bulk of ^3H loss from biliary bile acids occurred in the 1st 25–30 min, but in the case of the latter, ^3H loss was continuing to increase over 60 min. Despite the delay in bile acid excretion and greater ^3H loss in retrograde studies, two thirds to three fourths of total ^3H lost from biliary bile acids in one hour was recovered in the perfusate in the 1st 5 min, during which time no labeled bile acid had been excreted in bile. Thus, it would appear that ^3H loss occurs very rapidly in zone III and, therefore, delayed excretion from zone III into bile is not the major cause of greater ^3H loss from chenodeoxycholic acid. Possible differences in the content of 3α-HSD or in nucleotide cofactor redox state may contribute to greater ^3H loss in retrograde studies.

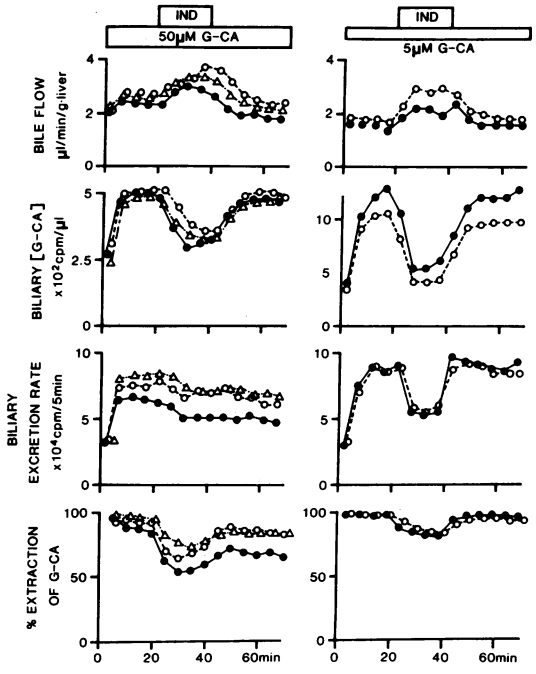

Figure 6. Effect of indomethacin on the steady state extraction and excretion of [^{14}C]glycocholic acid. Studies on the left represent three experiments with 50 μM glycocholic acid (G-CA) and on the right, two experiments with 5 μM G-CA. As indicated by the horizontal bar, a step infusion of indomethacin (50 μM) was carried out for 20 min. From top to bottom are depicted bile flow, biliary concentration, and excretion rate of [^{14}C]glycocholic acid and the percent extraction from the perfusate ([inflow concentration − outflow concentration]/inflow concentration × 100).

Indomethacin has been reported to be a potent inhibitor of 3α-HSD (13, 14). We previously demonstrated its ability to inhibit 3β-^3H loss from bile acids catalyzed by the pure enzyme and in hepatocyte suspensions. Our studies in cells revealed the very interesting finding that indomethacin caused a shift in the equilibrium distribution of bile acids (displacement from the cells into the media) without affecting initial rates of uptake. In the present studies, the perfused liver model provided a means to further assess the significance of inhibition of 3α-HSD by indomethacin in the overall translocation of bile acids from perfusate to bile.

During perfusion with indomethacin there was both a marked inhibition of ^3H loss (in bile acid excreted in bile in 1 h) as well as in ^3H appearance in effluent perfusate in the 1st 5 min. In parallel with this inhibition of 3α-HSD, there was an inhibition of bile acid excretion into bile in both prograde and retrograde studies. In the prograde studies, peak excretion into bile occurred almost immediately in the absence or presence of indomethacin. The peak height and subsequent rate of excretion were significantly decreased in the presence of indomethacin. In the retrograde studies, peak excretion not only was inhibited but was significantly delayed.

In contrast to our findings in isolated hepatocytes, indomethacin did not displace bile acids from the liver into the perfusate. Rather, it caused increased retention of bile acids in the liver. The different experimental conditions of the two models may explain the apparent discrepancy. In the cell studies, bile acid concentrations accumulating in 2×10^6 cells incubated in 1 ml buffer (5 μM bile acid) could lead to saturation of intracellular sites for bile acids. Therefore, when indomethacin displaced bile acids from 3α-HSD, bile acids were released into the medium possibly because the cells were loaded with bile acids. In the perfused liver studies, only tracer amounts of bile acids were administered to livers largely depleted of endogenous bile acids. Therefore, indomethacin did not cause the net release of the tracer bile acid, but delayed its excretion. Although inhibition of excretion could be due to a direct effect of indomethacin on the excretory step, the data with glycocholate infusions suggests that this is not the case. Therefore, such a delay in excretion implies that displacement of bile acids from 3α-HSD leads to possible redistribution within the cells to a compartment(s) that contributes to the delay in transit through the hepatocyte. This suggests that cytosolic compartmentation of bile acids is involved in the excretion and that redistribution out of the cytosol to undefined sites in the hepatocytes delays excretion. These findings are consistent with the importance of binding to 3α-HSD in retaining bile acids in the cytosolic compartment from which these molecules undergo transport into bile.

Having demonstrated that indomethacin delayed the excretion of a tracer bolus of chenodeoxycholic acid, we then examined the steady state, single-pass extraction and excretion of glycocholate. The 50-μM concentration of administered bile acid probably exceeded the transport maximum (T_{max}) of excretion. Indomethacin caused an immediate and reversible fall in the net steady state extraction of the bile acid. Since the decrease in net extraction was unaccompanied by a decrease in excretion, the decreased extraction must still have been sufficient to supply bile acid at or above T_{max} for excretion. This assumption was verified by demonstrating that at much lower perfusate bile acid concentration, indomethacin inhibited both extraction and excretion. These findings complement the

tracer bolus injection studies by indicating that indomethacin does not directly affect the excretory capacity for bile acids.

Comparison of constant infusions of 5 and 50 μM glycocholate in the perfused liver demonstrates how displacement of bile acids from 3α-HSD in cytosol can explain the effects of indomethacin to displace bile acids in the cell studies and to delay excretion of tracer boluses of bile acid in the perfusion studies. Depending on the bile acid load (Fig. 6), indomethacin has a greater effect on extraction or excretion. At the high load, the concentration of glycocholate in the liver exceeds the excretory maximum, and displacement from 3α-HSD by indomethacin leads to regurgitation into the sinusoid and a consequent fall in net extraction. There also may be subcellular redistribution within the liver, but the results indicate that sufficient bile acid is still available to the canalicular transport to maintain the same maximum excretion rate. Thus, the fall in extraction and possible redistribution caused by indomethacin were insufficient to limit the saturating hepatic concentration of bile acid available for excretion. At infusion rate of 5 μM glycocholate, well below T_{max}, when indomethacin displaces bile acid from 3α-HSD, the displaced bile acid can either regurgitate (fall in net extraction) or undergo subcellular redistribution in the liver. If only regurgitation occurred, the fractional fall in extraction would determine and equal that in excretion. However, the fall in excretion greatly exceeded the fall in extraction. Therefore, the displaced bile acid must also have redistributed in the liver. Extending this interpretation to the tracer bolus perfusion experiments, displacement of the very small bile acid load by indomethacin leads to subcellular redistribution and delayed excretion with no detectable fall in extraction. Thus, displacement of bile acid from 3α-HSD by indomethacin will result under the same perfusion conditions in inhibition of extraction and/or excretion of the bile acid depending on the load. In the isolated hepatocyte studies, the cells become loaded with bile acid so that displacement from the cells would be expected (analogous to decreased net extraction). Thus, the perfused liver studies with different loads of glycocholate provide evidence that explains apparent differences between the indomethacin-induced displacement from isolated cells and delayed tracer bolus excretion from the perfused liver.

Since we have shown in isolated cells that indomethacin does not affect initial uptake rates of bile acids, the fall in extraction of glycocholate is consistent with displacement from intracellular cytosolic sites and efflux (reverse transport). Thus, extraction represents a net result of bidirectional movement across the plasma membrane that may be determined by the free concentration of bile acid outside and inside the cell. Indomethacin may increase reverse transport by displacing bile acids from cytosolic protein, especially 3α-HSD, into the intracellular pool of free bile acid. It is also possible that displacement from GSH S-transferases contributes to the effect, but is less likely due to its much lower affinity for indomethacin as compared with 3α-HSD (Takikawa, H., A. Stolz, and N. Kaplowitz, unpublished observations).

In summary, our work using ^3H loss from double-labeled bile acids and inhibition by indomethacin indicates the probable importance of the interaction of bile acids with a specific cytosolic component, 3α-HSD, in the intracellular transit of bile acids from sinusoid to canaliculus.

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