Regulation of Bile Salt Transport in Rat Liver

EVIDENCE THAT INCREASED MAXIMUM BILE SALT SECRETORY CAPACITY IS DUE TO INCREASED CHOLIC ACID RECEPTORS

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ABSTRACT Expansion of the bile salt pool size in rats increases maximum excretory capacity for taurocholate. We examined whether increased bile salt transport is due to recruitment of centrolobular transport units or rather to adaptive changes in the hepatocyte. Daily sodium cholate (100 mg/100 g body wt) was administered orally to rats. This treatment was well tolerated for at least 4 d and produced an 8.2-fold expansion of the bile salt pool. This expanded pool consisted predominently (99%) of cholic and deoxycholic acids. Significantly increased bile salt transport was not observed until 16 h after bile acid loading, and maximum elevations of transport capacity to 2.3-fold of control required ~2 d. In contrast, maximum sulfobromophthalein excretion rates increased 2.2-fold as early as 4 h and actually fell to 1.5-fold increase at 4 d. We studied the possibility that this adaptive increase in bile salt secretory transport was due to changes in canalicular surface membrane area, lipid composition, or increased number of putative carriers. Canalicular membrane protein recovery and the specific activities of leucine aminopeptidase, Mg^{++}-ATPase and 5'-nucleotidase activities were unaltered by bile salt pool expansion. The content of free and esterified cholesterol and total phospholipids was unchanged in liver surface membrane fractions compared with control values. In contrast, sodium cholate administration selectively increased specific [^{14}C]cholic acid binding sites twofold in liver surface membrane fractions. Increased numbers of [^{14}C]cholic acid receptors (*a*) was associated with the time-dependent increase in bile salt transport, and (*b*) was selective for the taurine conjugate of cholate and (*c*) was reduced by chenodeoxycholate. Changes in bile acid binding sites 16 h following taurocholate and chenodeoxycholate and the lack of change with glycocholate was associated with comparable changes in bile salt transport. In conclusion, selective bile salts increase bile salt transport in the liver through an adaptive increase in the density of putative bile acid carriers in liver surface membrane.

INTRODUCTION

Bile salts circulate efficiently in an enterohepatic pool whose size is determined, in part, by active transport mechanisms located in ileal epithelial and liver cells. In the liver, bile salts are rapidly cleared from the portal blood by a well characterized sodium-dependent carrier-mediated process (1–3). In contrast, cellular translocation and biliary seretion are poorly understood. Presumably, bile salts are excreted across the canalicular surface membrane by a carrier-mediated process independent of sodium gradients, since it has been shown that secretion reaches a maximum value in the face of a rising concentration in the blood (4), there is competition between different bile salts (5), and ouabain fails to inhibit secretion in isolated hepatocytes (6). Maximum excretory capacity (Tm)[1] for taurocholate, similar to bilirubin (7) and bromosulfophthalein (BSP) (8), has been shown to be the

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[1] *Abbreviations used in this paper*: BSP, bromsulfophthalein; Tm, maximum excretory capacity.

rate-limiting step in its overall transport (9, 10). In bile secretory failure a decrease in the maximum transport capacity may be the only step which is altered (11, 12).

Several lines of evidence have suggested that bile salt carriers are important in the regulation of bile secretion: (*a*) bile salt receptors, whose affinity is independent of the sodium concentration, have been identified and characterized in liver surface membrane fractions (13); and (*b*) maximum taurocholate excretion rates decrease as the number of bile salt receptors decreases (14). In addition to the number of bile salt carriers, membrane lipid composition and fluidity are also major determinants of bile salt excretion (15, 16). These associations have suggested the hypothesis that alterations in either specific bile salt receptors or membrane lipid composition are important determinants of maximum bile salt transport. However, these correlations have been observed only with experimental models of decreased bile salt excretory capacity.

It has been reported that the liver is capable of responding to an increased load of bile salts by increasing bile salt maximum secretory capacity (17). Since bile salt administration produces many intracellular changes in metabolism, the cellular mechanism responsible for increased transport capacity is unclear. The aim of the present study is to determine the cellular mechanism of this effect, namely, whether increased bile salt transport is due to recruitment or to adaptive changes in the liver cell. Specifically, we examined in rats with expanded bile salt pools whether increases in bile salt transport are (*a*) associated with alterations in maximum secretory rates for other organic anions (BSP), (*b*) associated with changes in the surface membrane lipid composition or an increase in the number of bile salt receptors, and (*c*) whether the changes are specific to bile salt structure and/or detergent properties. The results demonstrate that selective bile salts increase hepatic transport through an increase in the number of putative bile salt carriers.

METHODS

Materials. [24-*carboxyl*-^{14}C] Cholic acid (45 mCi/mmol, 99% radiochemically pure) was obtained from New England Nuclear (Boston, MA). The following unlabeled bile acids were obtained from Maybridge Research Chemicals (Cornwall, England): cholic, taurocholic, glycocholic, chenodeoxycholic, taurochenodeoxycholic, taurodeoxycholic, and taurodehydrocholic acids. Ursodeoxycholic acid was kindly supplied as 99+% pure, by Giuliani (Milan, Italy). All bile acids were of the highest purity available and found to be 99% pure by thin-layer chromotography. Triton WR-1339 (oxyethylated tertiary oxtylphenol polymethylene polymer) was obtained from Ruger Chemical Company, Inc. (Irvington, NY). Albumin (bovine), ouabain, AMP, disodium ATP, β-steroid dehydrogenase, 2,6-dichlorophenolindophenol, L-leucyl-β-naphylamide HCl, lactic dehydrogenase, glucose-6-phosphate, and pyruvate kinase were the highest purity available from Sigma Chemical Co. (St. Louis, MO). BSP was obtained from Hynson, Westcott, & Dunning (Baltimore, MD).

Treatment groups. Male Sprague-Dawley rats (Charles River Breeding Laboratories Inc., Wilmington, MA) were used. The animals weighed ~200 g at the start of the experiments. Throughout the studies rats were subjected to darkness between 1800 and 0600, and light between 0600 and 1800. Animals received each evening sodium cholate 100 mg/100 g body wt by oral nasogastric tube under light ether anesthesia. The cholic acid solution was made up in normal saline by carefully adjusting the initial solution to pH 7.4 with 5 N NaOH. The final concentration of cholic acid was 200 mg/ml. Animals had free access to food (Purina Rat Chow, Ralston Purina Co., St. Louis, MO) and tap water, except the evening before killing when food was withheld.

In chronic studies, animals were given cholic acid daily for 4 d and killed the following morning. Randomly selected rats were treated and handled in a similar manner except that 0.9% saline instead of cholic acid was given by nasograstric tube. Daily intake of rat chow and body weight were measured.

In acute experiments (16 h), all bile acids except taurodehydrocholic acid were prepared and administered in a similar manner the evening before study. Taurodehydrocholate and the detergent Triton WR-1339 were administered intraperitoneally to assure complete delivery to the liver.

Bile acid determination. Total bile acid pool size was determined by biliary drainage according to the method of Mok et al. (18). In treated animals, as well as controls, basal hepatic bile acid synthesis was determined from the low point in bile acid secretion and was subtracted from the total amount of bile salt produced over 20 h. Generally, in control animals basal synthesis was reached in the 10–12-h sample, while in treated rats basal synthesis rates were not reached until the 14–16-h collection period. Bile salts were measured using the β-steroid dehydrogenase assay described by Talalay (19). Biliary bile salt species were identified and quantitated by gas-liquid chromatography on samples collected during the first 4 h of biliary drainage. Bile was hydrolyzed in methanol and 4 N NaOH using 5-β-cholanic acid as an internal standard (20). The bile acids were then extracted, methylated with diazomethane, and trimethylsilyl derivatives were prepared (21), gas-liquid chromatography was performed on 6-ft glass columns at 200°C using 1% HiEff-8BP on 100/120 mesh gas chrom Q packing, with helium as the carrier gas at 30 ml/min (20). A Perkin-Elmer model 3920 gas chromatograph (Perkin-Elmer Corp., Instrument Div., Norwalk, CT) was used for all analyses. Bile acid reference standards were those previously used in Dr. Fred Kern's laboratory (22).

Hepatic bile acid and BSP maximal transport capacities. Maximal transport capacity for bile salts and BSP was measured as previously described (14). To determine bile salt Tm, taurocholate was infused through either the subclavian or the femoral vein using a PE 50 catheter. The infusion, using a Harvard pump (Harvard Apparatus Co., Inc., S. Natick, MA) was started immediately after the basal period at a progressive rate from 0.5 to 3.5 μmol/min per 100 g body wt or until cholestasis was noted. Bile was collected in preweighed tubes in 10-min periods for 30 min at each bile salt concentration, through either a PE-10 or PE-50 catheter located just distal to the bifurcation of the common bile duct. Body temperature was maintained at 37±0.5°C with use of

a heating lamp. BSP Tm was determined by an infusion of BSP at a 0.30 μmol/min per 100 g body wt through a femoral vein in control animals, and at 0.50 μmol/min per 100 g body wt in cholate-treated rats. Conjugated BSP was demonstrated in the serum of both animal groups documenting that BSP Tm was achieved.

Analysis of liver surface membrane enzymes and lipids. Liver surface membrane fractions were isolated by the method of Neville (23) through step 12 as described by Pohl et al. (24) and previously characterized in our laboratory (13–16). Surface membrane fraction purification was determined by marker enzymes (13–16). 5'-Nucleotidase (EC 3.1.3.5) was measured by the method of Song and Bodansky (25), glucose-6-phosphatase (EC 3.1.3.9) according to de-Duve et al. (26), succinic dehydrogenase (EC 1.3.99.1) as described by Seubert (27), using 2,6-dichlorophenolindophenol as the donor. The decrease in absorbance was measured at 600 nm, and enzyme activity was calculated using an extinction coefficient of 1610 L cm^{-1} mol^{-1}. Proteins were measured by the method of Lowry et al. (29) using bovine serum albumin as standard, and phosphorous was determined according to Fiske and SubbaRow (30). Leucine aminopeptidase (EC 3.4.11.1) was determined according to Goldberg and Ratenburg (28) with L-leucyl-β-napthylamide HCl as substrate. Magnesium-dependent ATPase was that activity measured in the presence of 2.4 mM ouabain using a coupled enzyme system as described by Schoner et al. (29).

Following chloroform/methanol extraction according to Folch et al. (33), phospholipids were determined by the method of Bartlett (32), assuming 780 average molecular weight for phospholipids. Free and esterified cholesterol was determined after extraction (33) and quantitated by gas-liquid chromatography (34).

Bile acid binding assay. The binding of [^{14}C]cholic acid (45 mCi/mmol, New England Nuclear) was determined as previously described (13). In brief, the binding reaction was started by adding surface membrane fractions to the incubation medium that contained 132 mM monosodium phosphate/disodium phosphate buffer, pH 6.0 and [^{14}C]cholic acid. The samples were incubated in a shaking water bath at 4°C for 20 min and the reaction terminated by rapid vacuum filtration (Hoeffer Scientific Instruments, San Francisco, CA) to separate membrane bound from free ^{14}C-labeled cholic acid. Membrane bound [^{14}C]cholic acid was collected on glass fiber disks (Whatman grade GF/C, Whatman, Inc., Chemical Separation Div., Clifton, NJ) and measured in triplicate.

Free bile acid concentration was estimated by subtracting the total bile acid bound per sample from the total amount of bile acid present in the assay. "Nonspecific" interactions of labeled bile acids with membrane fractions was determined after preincubation of the liver surface membranes at 37°C for 3–5 h to denature the specific bile acid binding sites (13). Specific bile acid binding is assumed to be that fraction remaining after subtracting from the total the value obtained with heat-denatured membranes.

Liver function tests. Serum samples from fasting control and 4-d cholic acid-treated rats were obtained from the portal vein. Serum bilirubin, albumin, and alanine aminotransferase (EC 2.6.1.2; serum glutamic pyruvic transaminase) were determined in the Pediatric Microchemistry Laboratory of the University of Colorado School of Medicine using standard methods (14).

Statistical analysis. Linear regression analyses were performed by the least squares method. Specific cholic acid binding constants were estimated by a nonlinear least squares regression program and variances compared by an F test. Means of groups were compared by Student's *t* test; analysis of variance was used for statistical comparison. *P* values <0.05 were considered significant. Values are expressed as mean±SEM.

RESULTS

General effects of cholic acid loading. Oral administration of sodium cholate for 4 d to male Sprague-Dawley rats does not significantly change growth, liver size, serum tests of liver function, or hepatocellular integrity (Table I). Stools in cholate-fed rats were softer than in controls, although no significant diarrhea was noted. No significant morphological changes were observed in the liver by either light or electron microscopy, and in the small intestine by light microscopy.

Daily cholate administration for 4 d expands the total bile salt pool size 8.2-fold (Table II). As previously found (20), cholic acid (60.9±3.2%) is the major bile salt in rat bile with smaller amounts of deoxycholic (6.0±1.1%) and chenodeoxycholic (7.0±0.7%) acids. Other bile acids found in control samples include β-muricholic, hyodeoxycholic, lithocholic, and ursodeoxycholic acids. These bile acids accounted for

TABLE I
Effect of Cholate Feeding on Growth and Liver Function Tests

	Initial body weight	Weight gain	$\frac{\text{Liver}}{\text{Body}}$ wt × 100	Serum		
				Bilirubin	Albumin	GPT‡
	g	*g/d*		*mg/dl*	*g/dl*	*IU/dl*
Control (6)	206±7	7.3±0.6	3.9±0.2	0.2±0.02	4.3±0.2	42±5
Cholic acid* (5)	197±8	7.5±0.7	3.7±0.2	0.2±0.03	4.3±0.2	47±8
P value	NS	NS	NS	NS	NS	NS

* Cholic acid (100 mg/100 g body wt) was administered orally each day for 4 d, while controls received saline. Values are expressed as mean±SEM. Parentheses represent the number of separate experiments.
‡ Alanine aminotransferase. Wt, weight.

TABLE II
Bile Salt Pool Size and Composition

	Bile salt pool size	Bile acid composition			
		Cholate	Deoxycholate	Chenodeoxycholate	Other
	mg/100 g body wt	*μmol/100 g body wt*			
Control (4)	12.4±3.2	20.5±2.0	2.1±0.5	2.4±0.3	8.5±4.3
Cholic acid (4)	102±21	221.8±28.3	71.6±30.5	0.8±0.8	4.1±4.1
Fold change	8.2	10.8	33.3	0.3	0.5
P value	<0.001	<0.001	<0.001	NS	NS

Cholic acid was administered orally (100 mg/100 g body wt for 4 d). Bile acid pool size was measured by biliary drainage over 24 h on day 5. Bile acid analysis was performed on an aliquot of bile obtained during the initial 4 h of drainage and measured by gas-liquid chromatography (Methods). Values are expressed as mean±SEM.

24.0±3.4% and are combined in Table II under "Other." After cholate administration the bile acid pool was almost entirely converted to cholic and deoxycholic acids, i.e., 99% of the bile acid pool. Chenodeoxycholic and the "other" bile acids were reduced to negligible levels. Indeed, only one of four rats fed cholate had measurable amounts of these bile acids, accounting for the large variation and the lack of a statistically significant decrease in their content.

Hepatic excretory function. In animals with expanded bile acid pools, basal bile flow and bile salt secretion are significantly increased 2- and 3.8-fold, respectively (Table III). In treated rats, basal bile salt secretion rates approached the maximum bile salt excretion capacity of controls. However, in cholate-treated animals maximum taurocholate secretion was increased 2.3-fold, thereby maintaining a significant secretory reserve capacity. Cholate feeding also increased the maximum transport capacity for BSP to 156% of control values.

Rapid recruitment of reserve transport capacity has previously been shown for BSP excretion after bile salt infusions (35, 36). To examine whether similar mechanisms are involved in increasing bile salt Tm, the time course for changes in BSP and bile acid transport were compared (Fig. 1). 4 h after cholic acid administration bile acid Tm is unchanged while BSP Tm is 2.2-fold greater than control values. At 16 h bile salt Tm (135% of control) is now significantly ($P < 0.05$) increased, but does not reach its maximum transport rate until ~2 d and then remains unchanged for at least 4 d during continued cholate administration. In contrast, BSP Tm falls to 156% of control of 16 h and is unchanged for at least 4 d.

Hepatic lipid content. Bile salt infusions are known to change hepatic lipid synthesis and to increase biliary

TABLE III
Effect of Cholate Feeding on Hepatic Excretory Function

	Initial secretion		Tm	
	Bile flow	Bile salt	Taurocholate	BSP
	μl/min/100 g body wt		*μmol/min/100 g body wt*	
Control (9)	7.6±1.8	0.26±0.11	1.0±0.1	0.213±0.003
Cholic acid (5)	15.0±0.8	0.94±0.10	2.3±0.2	0.332±0.022
Percent change	199%	381%	230%	156%
P value	<0.005	<0.005	<0.005	<0.01

Initial biliary secretory function was measured during the first 30 min after cannulation of the bile duct. Taurocholate and BSP Tm were determined as described in Methods. Numbers in parentheses indicate separate determinations. Values are expressed as mean±SEM.

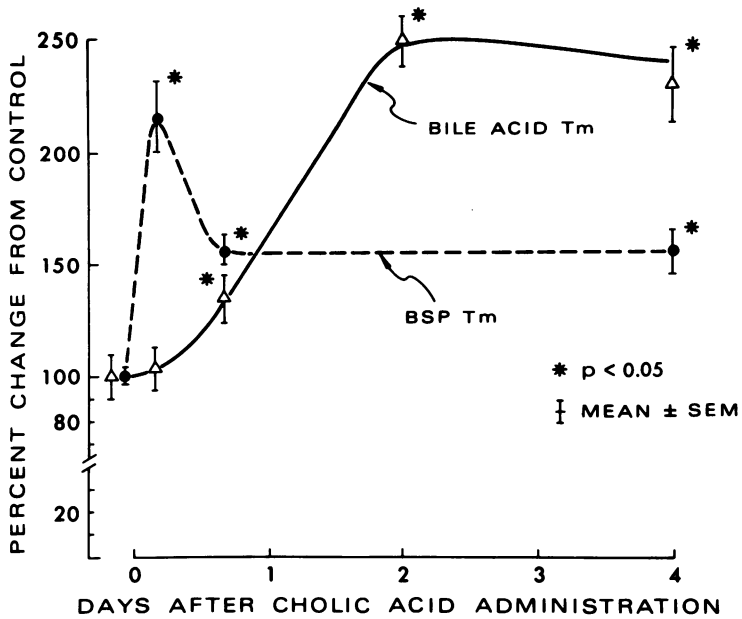

FIGURE 1 Effect of cholic acid feeding on maximum transport capacity for taurocholate and BSP. Sodium cholate (100 mg/100 g body wt, orally) was administered daily, and transport capacity determined (Methods) using PE 10 bile duct cannulas. Each point is the mean±SEM for four to six determinations. The values for control^{-1} taurocholate Tm 1.0±0.1 μmol·min·100 g body wt^{-1} and for BSP Tm = 0.2±0.01 μmol·min·100 g body wt^{-1}. ● - - - ● BSP Tm, △ —— △ bile acid Tm, $^{*}P < 0.05$.

lipid secretion (37–40). To determine whether changes in bile salt transport may be due to alterations in membrane lipid composition, total phospholipids and cholesterol content was determined in liver surface membrane fractions after feeding sodium cholate for 4 d and values compared with control animals. The content of phospholipid (449±21 vs. 416±19 μg/mg protein), free cholesterol (90.8±6.3 vs. 93.4±4.9 μg/mg protein), and esterified cholesterol (4.4±1.3 vs. 3.4±1.6 μg/mg protein) in liver surface membrane fractions was not significantly changed by cholate feeding. Thus, the cholesterol to phospholipid molar ratio, an important determinant of membrane lipid fluidity (41), was not significantly different from controls (0.58±0.05 vs. 0.53±0.06).

Liver surface membrane recovery, enzyme activities, and bile acid binding. Recovery of liver surface membrane fractions after 4 d of cholate feeding was unaltered as judged by protein recovery and marker enzymes (Table IV). Neither specific activities nor relative enrichment of enzymes for the surface membrane including 5′-nucleotidase, Mg^{++}-ATPase, and leucine aminopeptidase were significantly changed. Although glucose-6-phosphatase was unaltered, succinic dehydrogenase, a marker of the mitrochondria, was moderately increased ($P < 0.05$) in surface membrane fractions from sodium cholate-treated rats. In contrast, the maximum number of specific [^{14}C]cholic acid binding sites was increased (23.8±1.4 to 41.3±5.1 nmol/mg protein, $P < 0.01$) by cholate treatment for 4 d (Table IV). The number of nonspecific bile acid binding sites was unchanged compared with control values, indicating that the increase in bile salt receptors was selective.

To examine whether cholic acid treatment increased a heterogeneous number of receptor sites, binding kinetics were analyzed by Scatchard analysis (Fig. 2) (42). Bile acid binding parameters indicate that the dissociation constant (K_d) for [^{14}C]cholic acid was similar in treated (1.20±0.19 mM) and control (1.16±0.12 mM) liver surface membrane fractions, while the maximum binding capacity (N) was significantly ($P < 0.01$) increased twofold (33.8±8.2 vs. 64.2±10.0 nmol/mg protein). These results suggest that cholate feeding increases a single homogeneous set of receptors without evidence for cooperative interactions.

The time course and magnitude of changes in bile acid binding are shown in Fig. 3. These changes are compared to those previously described for taurocholate Tm. Both bile acid binding and transport demonstrate a latent period of at least 4 h, since no significant changes are noted up to that point. Both parameters are significantly increased at 16 h after a single oral feeding of sodium cholate. Specific binding

TABLE IV

Effect of Cholate Administration on Liver Surface Membrane Protein Recovery, Enzyme Activities, and [^{14}C]Cholic Acid Binding

	LSM recovery	5'-Nucleotidase	Mg^{++}-ATPase	Leucine aminopeptidase	Glucose-6-phosphatase	Succinic dehydrogenase	[^{14}C]Cholic acid binding	
							Specific	Nonspecific
	mg protein/ g liver	*μmol Pi/h/mg protein*		*μg β-naphthylamine/ h/ mg protein*	*μm Pi/h/ mg protein*	*μmol dichlorophenol-indeophenol/min/ mg protein*	*nmol/mg protein*	
Control	1.6±0.3	41.4±2.8 (13×)	79.9±7.8 (9×)	1.07±0.07 (5×)	2.8±0.2 (0.5×)	64.5±4.6 (4×)	23.8±1.4	4.3±0.9
Cholic acid	1.4±0.1	41.7±1.6 (13)	86.9±4.8 (11)	0.95±0.2 (5)	2.0±0.3 (0.4)	85.2±7.8 (5)	41.3±5.1	3.6±0.2
Percent change	−14%	+1%	+9%	−11%	−29%	+32%	+74%	−16%
P value	NS	NS	NS	NS	NS	<0.05	<0.01	NS

Liver surface membrane (LSM) fractions were prepared through step 12 of Neville (23) and enzymatic activities determined as described in Methods. [^{14}C]Cholic acid binding was determined (Methods) using an initial concentration of cholic acid of 1.8 mM. Values are mean±SEM. The numbers of separate experiments are shown in parentheses. Cholic acid (100 mg/100 g body wt) was administered orally for 4 d. Values in parentheses ( ) indicate the relative specific activities (surface membrane/homogenate).

and bile salt transport both rise to a maximum between 24–48 h, where despite further cholate administration, the number of [^{14}C]cholic acid receptors and Tm remain unchanged, indicating that a new steady state has been achieved.

Structural specificity for increase in hepatic bile acid binding and transport. To examine whether the structure or detergent properties of bile salts are the important determinants in increasing the number of [^{14}C]cholic acid binding sites, receptors were deter-

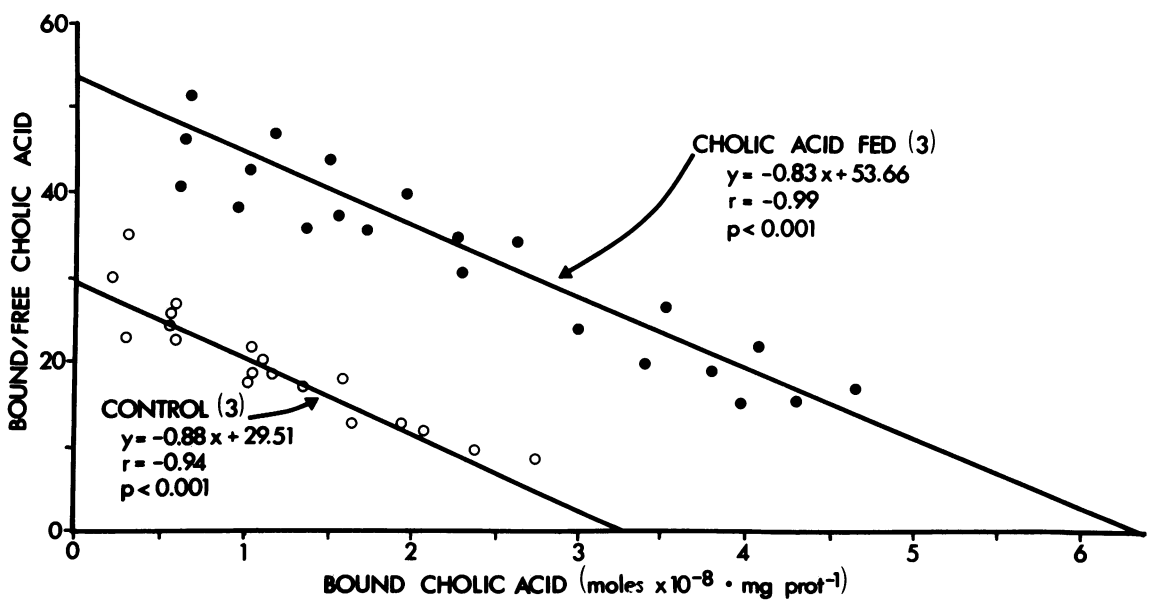

FIGURE 2 Scatchard analysis of specific [^{14}C]cholic acid binding to liver surface membrane fractions from control and cholic acid-fed rats. Plotted data correspond to specific cholic acid binding values determined from binding kinetics between the concentrations of 0.1 and 2.2 mM. The best-fit linear function was determined by least-squares regression. The value of N (maximum binding capacity), estimated by extrapolating the line to the abscissa, is 33.8 and 64.2 nmol/mg membrane protein and of K_d, estimated as 1/slope is 1.1 and 1.2 mM from control and cholic acid-treated rats, respectively. Cholic acid (100 mg/100 g body wt, orally) was administered daily for 4 d and liver surface membrane fractions and [^{14}C]cholic acid binding determined as described in Methods. Numbers in parentheses indicate number of separate experiments in each group.

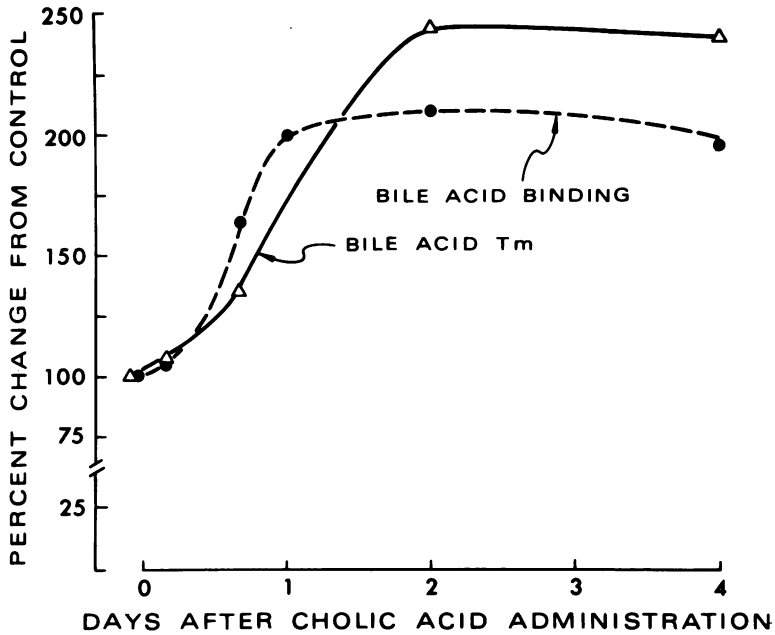

FIGURE 3 Relationship of bile acid Tm to the number of specific [^{14}C]cholic acid binding sites following cholic acid feeding. Taurocholate Tm was determined as described in Methods and previously shown in Fig. 1. [^{14}C]Cholic acid binding was determined in four to six separate determinations in liver surface membrane fractions by ultrafiltration using a cholic acid concentration of 1.8 mM. Control values for taurocholate Tm is 1.0±0.1 μmol/min per 100 g body wt (9 determinations) and for specific cholic acid binding is 26.4±1.1 nmol/mg protein (10 determinations). Each point is the mean of 4–10 determinations. All points from 16 h on were statistically significant at $P < 0.05$.

mined in liver surface membrane fractions 16 h after the oral administration of different bile salts and a nonbile salt detergent (Table V). Both cholate (158%) and taurocholate (199%) increased the maximum number of bile acid receptors compared with control, while glycocholate (113%) failed to significantly increase the number of bile acid receptors. No other tested bile salts or the nonbile salt detergent (Triton WR-1339) significantly increased the maximum number of [^{14}C]cholic acid binding sites. In contrast, chenodeoxycholate significantly reduced to 71% of control the density of bile acid binding sites. This effect was prevented by taurine conjugation of chenodeoxycholate. Furthermore, administration of bile salts did not significantly alter recovery of liver surface membrane fractions since protein recovery and the relative enrichment of marker enzymes was unchanged (Table V).

The apparent specificity for increasing cholic acid binding sites was examined further by determining the effect of administration of different bile salts on bile flow and taurocholate Tm (Table VI). Tauro and glyco conjugates of cholate both significantly increased initial bile flow and bile salt excretion to a similar extent. Oral administration of chenodeoxycholate altered neither bile flow nor bile salt excretion. Although both conjugates of cholate produce similar changes in bile flow, only taurocholate significantly increases taurocholate transport maximum. Similar to its effects on cholic acid binding sites chenodeoxycholate administration proportionately reduces taurocholate Tm. Analysis of the correlation between changes in the number of cholate binding sites and taurocholate Tm after oral administration of different bile salts demonstrates a strong association ($r = 0.993$, $P < 0.01$).

DISCUSSION

Hepatic transport of bile salts is a complex process involving at least the following steps that may be unevenly distributed across a lobular gradient (43): sodium-dependent uptake into hepatocytes (6, 10), translocation across the cell (44), and biliary excretion presumably by a separate carrier-mediated process (45). Under normal conditions, biliary secretory capacity is believed to be the rate-limiting step in hepatic transport (46). In previous studies we have suggested that the number of putative bile acid carriers and/or the lipid composition and structure of the liver surface membrane are important determinants of this maximum bile salt secretory transport rate (14–16).

TABLE V

Effect of Different Bile Salts and Detergents on Specific [^{14}C]Cholate Binding and Liver Surface Membrane Recovery

	Specific [^{14}C]cholate binding	Surface membrane protein recovery	Relative enrichment (Liver plasma membrane homogenate) 5'-Nucleotidase	Succinic dehydrogenase	Glucose-6-phosphatase
	% of control	*mg · g liver^{-1}*			
		(6)	(3)	(3)	(3)
Control	100±9 (23)	1.6±0.3	13±3	4±1	0.5±0.1
Cholate	158±20* (16)	1.4±0.1	13±3	5±1	0.4±0.3*
Taurocholate	199±23* (12)	1.4±0.3	12±2	3±1	0.5±0.2
Glycocholate	113±20 (11)	1.8±0.1	10±1	3±1	0.7±0.1
Chenodeoxycholate	71±15* (7)	1.6±0.1	10±1	2±1	0.5±0.2
Taurochenodeoxycholate	97±14 (9)	2.1±0.5	15±3	5±1	0.5±0.1
Ursodeoxycholate	81±14 (12)	1.6±0.1	11±1	4±1	0.4±0.1
Taurodeoxycholate	86±16 (6)	1.7±0.2	15±1	3±2	0.7±0.6
Taurodehydrocholate	110±25 (6)	1.7±0.3	15±2	4±2	0.4±0.3
Triton WR-1339	101±11 (6)	1.4±0.3	15±1	5±3	0.5±0.2

Bile salts (100 mg/100 g body wt) were administered orally except for taurodehydrocholate and the detergent Triton WR-1339 (22.5 mg/100 g body wt) that were given intraperitoneally 16 h before killing. [^{14}C]Cholate binding and enzyme activities were measured in liver plasma membrane fractions (Methods). Results are expressed as the mean±SD. Numbers in parentheses indicate the individual determinations. * $P < 0.01$.

To examine the hypothesis that bile acid receptors are the in vitro equivalent of the carrier, we sought to examine a model of increased bile acid transport. Adler et al. (17) first demonstrated and Watkins and Klassen (47) confirmed recently that expansion of the bile salt pool with taurocholate is associated with in-

TABLE VI

Effect of Oral Administration of Different Bile Salts on Biliary Secretory Function

Treatment groups	Initial bile flow	Initial bile salt excretion	Taurocholate Tm
	μl · min · g liver^{-1}	*nmol · min · g liver^{-1}*	*nmol · min · g liver^{-1}*
Control (5)	1.76±0.16	46.3±3.3	408.7±7.7
Taurocholate (4)	2.43±0.12*	95.2±10.8*	653.0±109.1‡
Glycocholate (4)	2.45±0.13*	114.0±28.5*	399.5±69.0
Chenodeoxycholate (4)	1.73±0.16	54.0±6.8	313.1±29.4*

Bile salts (100 mg/100 g body wt) were administered orally. 16 h later bile ducts were cannulated with PE 50 tubing and taurocholate Tm measured (Methods). Results are presented as the mean±SEM. Numbers in parentheses indicate separate experiments. * $P < 0.01$, ‡ $P < 0.05$.

creased maximal transport capacity for taurocholate. As has been proposed for several organic anions, two general mechanisms were suggested for this increase in the maximum hepatic excretion of bile acids: (*a*) recruitment of latent sites in hepatocytes from the pericentral zone III, and (*b*) adaptation of the canalicular membrane transport process, including increased canalicular membrane surface area, lipid fluidity, and the numbers of bile salt carriers. The results of our studies strongly suggest that sodium cholate feeding increases bile salt transport in the liver through induction of putative bile acid carriers.

Biliary bile acid analysis demonstrates that cholate administration not only increased the ingested bile acid 11-fold, but was also associated with a dramatic increase (33-fold) in deoxycholate, the major secondary bile salt of cholate. Sodium cholate feeding may also inhibit hepatic synthesis of the primary bile acid chenodeoxycholate and thus the formation of the following secondary bile acids: β-muricholate, hyodeoxycholate, lithocholate, and ursodeoxycholate. Thus, the bile acid pool is converted almost entirely to cholate and deoxycholate (99%) by chronic sodium cholate treatment (Table II). Quantitatively similar changes have been reported in man (48).

As previously shown at 2 d with either oral administration or duodenal infusion of taurocholate (17, 47), expansion of the bile acid pool with oral sodium cholate also increased the maximum transport capacity for taurocholate 2.3-fold (Table III). However, this change in transport capacity was not selective since the Tm of BSP, an organic anion whose transport is separate from bile acids (11), was also increased 1.5-fold. Since the original studies O'Maille et al. (36) on BSP Tm, bile salts have been shown also to increase the biliary excretion of many other organic anions (48–50). Whatever the specific mechanism for increased BSP Tm, the striking difference in its time course for change (<4 h) compared with taurocholate transport (2 d) strongly suggests that increased bile salt transport is an adaptive process rather than a recruitment of latent sites. Furthermore, Watkins and Klassen (47) found that the biliary secretion of phenol-3,6-dibromphthalein and ouabain were not affected by oral taurocholate administration.

It is possible that increased bile acid Tm (as well as BSP Tm) simply represents an increase in the canalicular membrane surface area. Infusions of bile acids at 40 μmol/h have been shown to increase the canalicular diameter in zone III (52). However, these observations do not permit estimation of the canalicular membrane surface. Furthermore, these changes were noted as early as 1 h after infusion of taurocholate. Our data suggest the canalicular membrane surface does not increase since membrane protein recovery was unchanged. However, because this preparation contains a mixed population of membrane surfaces, we examined the effect of cholic acid on three enzymes believed to be primarily localized to the canalicular membrane (Table V): 5′-nucleotidase, leucine aminopeptidase, and Mg^{++}-ATPase. Specific activities and relative specific activities were not changed by cholic acid treatment, suggesting that increased membrane surface area does not account for increased biliary transport.

It is well established that the optimal function of transport processes (53) as well as the activity of many membrane-bound enzymes are influenced by the membrane lipid composition (54). Since bile acid administration is known to inhibit cholesterol synthesis, and β-hydroxy-β-methyl glutarate CoA reductase activity (37), and to increase microsomal synthesis of specific phospholipids (55), it seemed possible that such changes may be reflected in the surface membrane lipid content. In an acute study using large amounts of taurocholate administered intraperitoneally in association with biliary drainage overnight, the phospholipid content of liver plasma membrane fractions was increased twofold (56). Such changes (i.e., decreased cholesterol to phospholipid molar ratios) are usually associated with increased membrane fluidity (41), and could theoretically be responsible for the increased bile acid Tm. However, no change in total phospholipid or cholesterol content was detected in liver surface membrane fractions from rats given cholic acid orally for 4 d. These results suggest that increased transport functions are not directly related to an alteration in membrane lipid fluidity, although it is still possible that specific phospholipids may alter membrane transport.

Previous studies have demonstrated that the number of bile acid receptors may be rate limiting for hepatic transport (14). In this study, cholate administration was associated with an increased number of receptors whose binding kinetics were unchanged. It is unclear whether [^{14}C]cholic acid binding is to the sinusoidal, canalicular, or both membrane domains. Several observations from the present study suggest [^{14}C]cholic acid binding is quantitating canalicular sites: (*a*) Scatchard analysis of binding parameters in the basal state and after induction with cholate feeding demonstrated only a single class of binding sites (Fig. 2); and (*b*) either increases or decreases in the number of cholic acid binding sites was paralleled by similar changes in taurocholate Tm (Fig. 3 and Table VI).

Bile salts indirectly and directly affect many cellular systems, particularly membranes (57, 58). These changes have been shown to have a high degree of structural specificity. Such specificity was observed on the apparent induction of cholic acid binding sites, since only cholate and taurocholate significantly increased the maximum number of receptor sites (Table

V). Since cholate is conjugated with taurine during its initial passage through the liver (20), it is not surprising that the increase with either conjugated or unconjugated cholate is similar, although statistically greater for taurocholate. Unexpectedly, this increase is seen only with the taurine but not the glycine conjugates of cholate (Table V and VI). Although chenodeoxycholate administration does not cause cholestasis, its oral administration significantly decreased the number of binding sites and taurocholate Tm proportionately. The specificity of these changes and the failure of a detergent (Triton WR-1339) to alter either parameter suggests that these receptors are measuring the putative canalicular carrier for bile salts.

In conclusion, cholate administration increases the maximum transport of bile salts probably through an adaptive increase in the maximum number of specific binding sites. This substrate-induced increase is the opposite of that described for most hormone receptors where so-called down regulation is usually seen (59), but is frequently seen with allosteric regulation of enzymes. Whether this increase in the liver surface membrane density of bile acid receptors is mediated through increased synthesis, decreased degradation, or a shifting from a possible intracellular pool to the surface membrane is unknown. Whatever the mechanism, it appears that the number of putative bile acid carriers adapt to the taurocholate pool size.

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