Biochemical Pharmacology, Vol. 38, No. 15, pp. 2543–2549, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

EFFECT OF INHIBITION OF PROTEIN SYNTHESIS ON CHOLESTASIS INDUCED BY TAUROLITHOCHOLATE, LITHOCHOLATE, AND A MANGANESE–BILIRUBIN COMBINATION IN THE RAT

LENA DAHLSTRÖM-KING and GABRIEL L. PLAA*
Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Québec, Canada, H3C 3J7

(*Received* 13 *July* 1988; *accepted* 19 *December* 1988)

Abstract—Taurolithocholate, lithocholate, and a manganese–bilirubin combination produced a rapid reduction in bile flow after i.v. injection in the rat. The effect was diminished or blocked completely by pretreating the animals with cycloheximide or ethionine, known inhibitors of protein synthesis. The injection sequence and time period between administration of the inhibitor of protein synthesis and the cholestatic agent influenced the degree to which they modulated the cholestatic effect. The results indicate that uninterrupted protein synthesis is required for the expression of maximal reduction of bile flow by taurolithocholate, lithocholate, and a manganese–bilirubin combination.

The term intrahepatic cholestasis has generally been employed to describe the diminution or cessation of bile flow occurring in the absence of gross anatomic obstruction. The lesion has been observed as a low incidence, toxic effect of certain drugs [1], as a genetic disorder [2], in pregnancy [3], and in newborns [4].

Several chemicals produce reduced bile flow in laboratory animals and are employed as models to elucidate mechanisms involved in cholestasis. The three cholestatic agents used in this study (taurolithocholate, lithocholate, and a manganese–bilirubin combination) show common features. The onset of modulation of bile flow caused by the bile salts in the rat is almost instantaneous following intravenous injection. The reduction in bile flow caused by the manganese–bilirubin combination also occurs promptly after bilirubin administration, if the injection sequence of manganese followed by bilirubin 15 min later is respected [5, 6]. The distortion of the bile canalicular membrane observed by electron microscopy is similar with all three models [7, 8].

Yousef *et al.* [9] showed that pretreatment of rats with cycloheximide, an inhibitor of protein synthesis, completely abolishes the effects of lithocholate on bile flow. The present study was conducted to investigate if inhibition of protein synthesis would modify the cholestatic effect of the two bile salts and a manganese–bilirubin combination to the same extent and if it was an additional common feature of the three chemical models of cholestasis.

METHODS

Chemicals. Albumin, crystalline bilirubin from bovine gallstone, cycloheximide (Cx), *dl*-ethionine, sodium taurolithocholate (TLCh) and urethane were purchased from the Sigma Chemical Co. (St Louis, MO). Sodium lithocholate (LCh) was obtained from the Calbiochem–Behring Corp. (La Jolla, CA) and monohydrated manganese sulfate ($MnSO_4 \cdot H_2O$) from the Fisher Scientific Co. (Fair Lawn, NJ).

Animal treatments. Male Sprague–Dawley rats (Charles River Canada, St-Constant, Québec) weighing 230–290 g were used in all experiments. The rats were maintained on standard laboratory pelleted diet (Charles River Rat Chow No. 5012) and water *ad lib* in animal quarters with a constant temperature of 22° and alternating 12-hr light and dark cycles. After an acclimation period of 4 days, the rats were pretreated as follows: (a) cycloheximide was dissolved in saline and injected intraperitoneally in doses of 0.89 to 7.11 μmol/kg in several different time sequences prior to the intravenous administration of TLCh, LCh, or the manganese–bilirubin combination (Table 1); (b) *dl*-ethionine (suspended in saline with a few drops of concentrated HCl for dissolution) was administered intraperitoneally either 18 hr or 42 and 18 hr prior to intravenous injection of TLCh, 30 μmol/kg; (c) rats serving as controls were injected intraperitoneally with the vehicle (saline or acidified saline) in the same manner as the experimental animals. Each experimental unit consisted of six animals.

Experimental protocol. The rats were anesthetized with urethane (1 g/kg, i.p.); a femoral vein and the common bile duct were cannulated with polyethylene tubing, PE-50 and PE-10, respectively. Body temperature was monitored with a rectal thermoprobe (YSI thermoregulator) and maintained at 37° with a thermostatically controlled infrared lamp. Bile was collected over 15- to 30-min periods, measured volumetrically, and bile flow calculated from the volume collected. Sodium TLCh was dissolved in a vehicle containing 10% albumin and 0.45% NaCl; sodium LCh was suspended in the same vehicle. Manganese

* Correspondence: Dr Gabriel L. Plaa, Département de pharmacologie, Faculté de médecine, Université de Montréal, C.P. 6128, Succursale A, Montréal, Québec, Canada H3C 3J7.

Table 1. Pretreatment sequences: Intraperitoneal administration of cycloheximide prior to intravenous injection of taurolithocholate, lithocholate, and a manganese–bilirubin combination

Pretreatment time				
66 hr	42 hr	18 hr	3 hr	Intravenous challenge
●	●	●	●	Taurolithocholate (40 μmol/kg)
●	●	●		
●	●	●		
	●	●		
		●		
●	●	●		Taurolithocholate (30 μmol/kg)
		●		Lithocholate (63 and 75 μmol/kg)
		●		Manganese (89 μmol/kg)–Bilirubin (26 μmol/kg)
	●	●		Manganese (89 μmol/kg)–Bilirubin (34 μmol/kg)

sulfate was dissolved in 0.9% NaCl, and bilirubin was suspended in a solution containing 0.52 g NaCl and 0.52 g Na_2CO_3 per 100 ml. For the manganese–bilirubin combination, manganese was injected first, followed 15 min later by bilirubin.

Statistical methods. Data were submitted to analysis of variance. Differences between treatment means were subsequently tested using the Student–Newman–Keuls procedure [10]. A probability of less than 0.05 was chosen as the criterion of significance.

RESULTS

The three cholestatic models. TLCh, LCh, and the manganese–bilirubin combination produced a dose-dependent decrease in bile flow (Fig. 1). With TLCh, maximal diminution of bile flow occurred in the 15-min collection period immediately following i.v. administration, whereas with LCh maximal reduction was observed in bile collected 15–45 min after injection. The manganese–bilirubin combination produced its maximal effect in bile collected 30–60 min after the bilirubin injection. The dose-dependency for LCh and manganese–bilirubin manifested itself in the intensity of the maximal decrease in bile flow. TLCh, on the other hand, exhibited no dose-dependent difference in maximal intensity of effect with 30 and 40 μmol/kg, but the higher dose increased the duration of the severely reduced bile flow.

Protein inhibition on taurolithocholate-induced cholestasis. TLCh (40 μmol/kg) produced a very intense cholestasis (~75% reduction in bile flow) for the entire collection time. Cycloheximide given 3 hr prior to TLCh did not alter the cholestatic profile (Table 2A). When cycloheximide was administered 18 hr before TLCh, the decrease in bile flow was less marked and of shorter duration (Table 2B). The effect was statistically significant in bile collected during the first 15 min after TLCh administration with 7.11 μmol Cx/kg and in bile collected 15–75 min for all doses of cycloheximide. Administration of cycloheximide i.p. 18 hr prior to an i.v. challenge of the vehicle for TLCh and LCh had no detectable effect on bile flow (data not shown).

To determine the sequence of administration of cycloheximide that would result in maximal attenuation of the cholestatic response, the effect of cycloheximide given for 3 consecutive days (Table 2C), two injections 42 and 18 hr (Table 2D) or 66 and 42 hr prior to TLCh (Table 2E), and a single injection 42 hr before TLCh (Table 2F) were compared. The control animals received saline for 3 days instead of cycloheximide; the administration of saline for 3 hr, 18 hr, or 3 days did not affect the TLCh response. The initial dramatic reduction in bile flow in bile collected 0–15 min after injection was altered by all treatment sequences with the exception of cycloheximide (3.55 μmol/kg) administered as a single injection 42 hr prior to TLCh (Table 2F). The percent decrease in bile flow from base line in the cycloheximide-pretreated animals was ~50–60% compared to ≥75% for the saline-treated rats. In bile collected 15–75 min after injection, all injection sequences essentially abolished the reduction in bile flow provoked by TLCh. Multiple doses of cycloheximide did not affect bile flow over 3 hr in vehicle (no TLCh) challenged rats: control group, 6.27 to 6.60 μl/min/100 g; cycloheximide group 6.53 to 6.92 μl/min/100 g.).

Table 3 depicts the influence of cycloheximide on the reduced bile flow by TLCh (30 μmol/kg). The same pattern as with the higher dose was discernible. Cycloheximide administered 42 and 18 hr prior to TLCh was more effective in modulating the cholestatic effect than a single injection 18 hr prior to TLCh. Comparison of individual pairs with 2 days of pretreatment indicated statistical differences

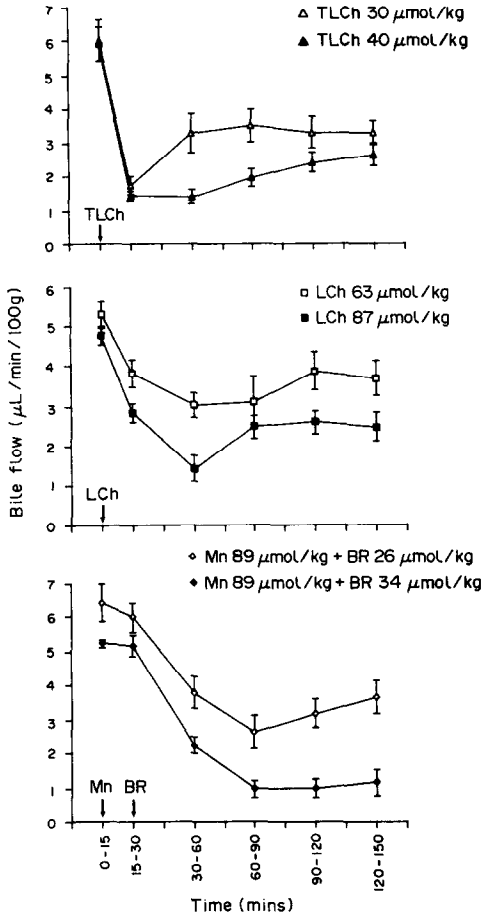

Fig. 1. Bile flow following intravenous administration of taurolithocholate (TLCh), lithocholate (LCh) and a manganese–bilirubin (Mn–BR) combination. Values are means ± SE, N = 6.

between cycloheximide- and saline-treated animals at all collection periods (Table 3B). Cycloheximide (1.78 and 3.55 μmol/kg) in a single injection altered bile flow significantly only 45–75 min after the TLCh challenge (Table 3A).

Limited experiments were performed with ethionine as an inhibitor of protein synthesis (Table 4). Two consecutive injections of ethionine (1.22 mmol/kg) were required to diminish TLCh-induced cholestasis. This inhibitor of protein synthesis did not reduce the cholestatic response to TLCh as effectively as cycloheximide.

Cycloheximide and lithocholate-induced reduction in bile flow. The lower dose of LCh (63 μmol/kg) resulted in ~45% reduction in bile flow in saline-treated animals; a single dose of cycloheximide administered 18 hr earlier blocked this reduction (Table 5A). This effect was statistically significant for 0.89, 1.78, and 7.11 μmol Cx/kg when individual pairs were compared. LCh (75 μmol/kg) resulted in a 40–70% suppression of bile flow; pretreatment with cycloheximide abolished the effect completely (Table 5B). Larger doses of LCh (to effect >80% reduction in bile flow) could not be used, because these doses are lethal in rats. Multiple treatments of cycloheximide were not investigated, since the single administration abolished LCh-induced reduction in bile flow.

Cycloheximide pretreatment followed by manganese–bilirubin combinations. Small doses of manganese are not cholestatic in rats [6], and manganese (89 μmol/kg) alone had no effect on the bile flow in these experiments. Manganese followed by bilirubin (26 μmol/kg) produced a 40–60% reduction in bile flow. Cycloheximide (0.89 to 3.55 μmol/kg) modified the effect of this treatment sequence, since bile flow in cycloheximide-treated rats was reduced by only 10–20% (Table 6A). In the manganese–bilirubin model, the severity of the reduction in bile flow is related directly to the dose of bilirubin [6]. The intense cholestatic response produced by a larger dose of bilirubin (~80% reduction in bile flow) was inhibited less efficiently (Table 6B). Cycloheximide pretreatment resulted in less severely reduced bile flow than that observed in saline-treated animals; however, the flow was still depressed in relation to the initial bile flow. Multiple injections of cycloheximide over 2 days were no more effective than a single dose at 18 hr in modifying the effect of the manganese–bilirubin combination (Table 6B and 6C).

DISCUSSION

From Fig. 1, one observes that the three experimental models used in this study produced dose-dependent reduction in bile flow; their temporal patterns, however, differed. TLCh yielded a pronounced and more rapid onset in the reduction of bile flow than LCh. The manganese–bilirubin combination resembled LCh.

The effect of TLCh was diminished by cycloheximide pretreatment. The injection sequence and time period between cycloheximide administration and the i.v. injection of the bile salt had a profound impact on the final response. The initial drop in bile flow (collection period 0–15 min) was most difficult to modulate; the major effect of a single injection of cycloheximide was on bile flow observed 15–75 min after TLCh. Thus, a single treatment with the inhibitor of protein synthesis appears to affect duration rather than the initial response. Multiple administration of cycloheximide over 2 or 3 days, however, resulted in a marked effect on both duration and the initial response. Cycloheximide, depending on dose and frequency of administration, can decrease the duration of TLCh-induced bile flow reduction and its initial intensity. Inhibition of protein synthesis appears to be a reasonable explanation for the protective effect of cycloheximide because of the following: (a) cycloheximide alone had no effect on bile flow, yet it modified the cholestatic effect of TLCh; (b) the doses of cycloheximide employed are in the range known to suppress protein synthesis in the rat [11]; (c) ethionine, another inhibitor of protein synthesis, also reduced the cholestatic effect of TLCh.

Maximal suppression of *de novo* protein synthesis in liver by cycloheximide occurs 2–6 hr after adminis-

Table 2. Temporal aspects of cycloheximide pretreatment on reduced bile flow induced by taurolithocholate (40 μmol/kg)

Cycloheximide (μmol/kg)	Bile flow/Time period (μl/min/100 g) −15–0 min	0–15 min	15–45 min	45–75 min
A. Cycloheximide i.p. 3 hr prior to an i.v. injection of TLCh at 0 min				
Vehicle	5.24 ± 0.42	1.27 ± 0.23	1.29 ± 0.42	1.88 ± 0.52
1.78	6.19 ± 0.47	0.86 ± 0.06	1.05 ± 0.21	1.94 ± 0.33
7.11	5.30 ± 0.47	1.12 ± 0.24	1.68 ± 0.76	1.91 ± 0.62
B. Cycloheximide i.p. 18 hr prior to an i.v. injection of TLCh at 0 min				
Vehicle	5.94 ± 0.48	1.44 ± 0.13	1.40 ± 0.19	1.97 ± 0.26
1.78	6.44 ± 0.44	1.97 ± 0.23	3.06 ± 0.28*	3.53 ± 0.29*
3.55	5.46 ± 0.32	1.47 ± 0.20	2.51 ± 0.44*	3.42 ± 0.41*
7.11	6.35 ± 0.35	2.60 ± 0.36*†	4.06 ± 0.48*†	4.60 ± 0.55*
C. Cycloheximide i.p. repetitively prior to an i.v. injection of TLCh at 0 min injection sequence (66, 42, and 18 hr)				
Vehicle	4.47 ± 0.21	0.92 ± 0.12	1.29 ± 0.36	1.44 ± 0.32
0.89	5.34 ± 0.29	2.20 ± 0.12*	4.80 ± 0.31*	5.11 ± 0.37*
1.78	5.83 ± 0.26	3.24 ± 0.54*‡	5.56 ± 0.54*	5.31 ± 0.49*
3.55	6.01 ± 0.48	3.02 ± 0.48*‡	5.67 ± 0.55*	5.60 ± 0.54*
D. Cycloheximide i.p. repetitively prior to an i.v. injection of TLCh at 0 min injection sequence (42 and 18 hr)				
3.55	5.65 ± 0.48	2.62 ± 0.30*§	5.26 ± 0.41*§	5.11 ± 0.37*§
E. Cycloheximide i.p. repetitively prior to an i.v. injection of TLCh at 0 min Injection sequence (66 and 42 hr)				
3.55	6.63 ± 0.48*§	2.56 ± 0.35*§	6.40 ± 0.42*§	6.27 ± 0.44‡,§
F. Cycloheximide i.p. 42 hr prior to an i.v. injection of TLCh at 0 min				
3.55	5.10 ± 0.45	1.56 ± 0.33	4.31 ± 0.59*§	4.18 ± 0.56*§

Values are means ± SE of six animals.
* Significantly different ($P < 0.05$)from respective vehicle group.
† Significantly different ($P < 0.05$) from cycloheximide, 3.55 μmol/kg.
‡ Significantly different ($P < 0.05$) from cycloheximide, 3.55 μmol/kg, administered 42 hr prior to TLCh.
§ Significantly different ($P < 0.05$) from the vehicle group in panel C.

Table 3. Temporal aspects of cycloheximide pretreatment on reduced bile flow induced by taurolithocholate (30 μmol/kg)

Cycloheximide (μmol/kg)	Bile flow/Time period (μl/min/100 g) −15–0 min	0–15 min	15–45 min	45–75 min
A. Cycloheximide i.p. 18 hr prior to an i.v. injection of TLCh at 0 min				
Vehicle	6.04 ± 0.60	1.75 ± 0.26	3.30 ± 0.59	3.51 ± 0.50
1.78	6.08 ± 0.42	3.70 ± 0.48	5.70 ± 0.43	5.64 ± 0.41*
3.55	6.41 ± 0.60	3.08 ± 0.39	4.90 ± 0.69	5.51 ± 0.37*
7.11	5.95 ± 0.70	2.74 ± 0.68	4.15 ± 0.76	4.55 ± 0.68
B. Cycloheximide i.p. 42 and 18 hr prior to an i.v. injection of TLCh at 0 min				
Vehicle	4.70 ± 0.54	1.31 ± 0.12	2.18 ± 0.25	2.54 ± 0.32
0.89	5.48 ± 0.16	3.51 ± 0.22*	6.11 ± 0.54*	6.08 ± 0.45*
1.78	5.46 ± 0.14	2.95 ± 0.32*	5.02 ± 0.46*	5.00 ± 0.50*
3.55	5.12 ± 0.31	2.71 ± 0.56*	4.54 ± 0.54*	4.05 ± 0.58*†

Values are means ± SE of six animals.
* Significantly different ($P < 0.05$) from respective vehicle group.
† Significantly different ($P < 0.05$) from cycloheximide, 0.89 μmol/kg.

tration in the rat [11]. The lack of protective effect 3 hr after cycloheximide and the appearance of an effect at 18 hr can be explained by the presence of a protein pool. The effect of cycloheximide administered at various time intervals before TLCh suggests that it may be the consequence of a disruption of synthesis of one or several proteins with a turnover of about 2 days.

Table 4. Effect of ethionine pretreatment on taurolithocholate-induced reduction in bile flow

Ethionine (mmol/kg)	Bile flow/Time period (μl/min/100 g) −15–0 min	0–15 min	15–45 min	45–75 min
A. Ethionine i.p. 18 hr prior to an i.v. injection TLCh (30μmol/kg) at 0 min				
Vehicle	6.62 ± 0.19	2.54 ± 0.23	3.87 ± 0.40	4.37 ± 0.42
0.31	6.46 ± 0.69	2.28 ± 0.22	3.35 ± 0.36	3.36 ± 0.33
1.22	7.51 ± 0.38	3.07 ± 0.32	3.90 ± 0.55	4.40 ± 0.50
B. Ethionine i.p. 42 and 18 hr prior to an i.v. injection of TLCh (30 μmol/kg) at 0 min				
Vehicle	6.47 ± 0.31	2.08 ± 0.13	2.83 ± 0.42	3.13 ± 0.41
0.31	5.55 ± 0.41	2.22 ± 0.20	2.85 ± 0.26	2.70 ± 0.22
1.22	6.80 ± 0.70	4.11 ± 0.55*†	4.86 ± 0.53*†	4.82 ± 0.38*†

Values are means ± SE of six animals.
* Significantly different ($P < 0.05$) from vehicle.
† Significantly different ($P < 0.05$) from ethionine, 0.31 mmol/kg.

Table 5. Bile flow following cycloheximide pretreatment i.p. 18 hr prior to a lithocholate challenge

Cycloheximide (μmol/kg)	Bile flow/Time period (μl/min/100 g) 0–15 min	15–30 min	30–60 min	60–90 min
A. Lithocholate (63 μmol/kg)				
Vehicle	5.81 ± 0.34	3.83 ± 0.37	3.02 ± 0.32	3.09 ± 0.64
0.89	6.19 ± 0.31	5.54 ± 0.27*	5.68 ± 0.31*†	5.61 ± 0.34*
1.78	6.81 ± 0.42	6.80 ± 0.45*†	7.36 ± 0.43*†	6.88 ± 0.32*†
3.55	5.82 ± 0.27	4.86 ± 0.58	3.64 ± 0.93	4.03 ± 0.64
7.11	6.94 ± 0.39	5.78 ± 0.40*	5.93 ± 0.83*†	6.56 ± 0.78*†
B. Lithocholate (75 μmol/kg)				
Vehicle	4.78 ± 0.22	2.84 ± 0.23	1.42 ± 0.32	2.48 ± 0.29
0.89	5.28 ± 0.32	5.18 ± 0.35*	5.49 ± 0.42*	5.58 ± 0.42*
1.78	5.10 ± 0.39	5.13 ± 0.36*	4.37 ± 0.56*	4.28 ± 0.68*
3.55	6.29 ± 0.36‡	5.38 ± 0.41*	4.34 ± 1.31*	5.15 ± 0.78*

Values are means ± SE of six animals.
* Significantly different ($P < 0.05$) from respective vehicle group.
† Significantly different ($P < 0.05$) from cycloheximide, 3.55 μmol/kg.
‡ Significantly different ($P < 0.05$) from all other values listed horizontally.

Cycloheximide administered 18 hr before LCh essentially abolished the effect on bile flow exerted by the bile salt. This observation confirms the finding by Yousef *et al.* [9] that cycloheximide inhibits LCh action. The less severe attenuation of bile flow may explain why a single injection of cycloheximide was sufficient to block the effect of LCh, whereas administration of cycloheximide for 2 days was required for TLCh.

The impact of cycloheximide on the reduction of bile flow after the manganese–bilirubin combination was more dependent on the severity of the manganese–bilirubin response than on the temporal pattern of cycloheximide administration. The 40–60% reduction following the smaller dose of bilirubin (26 μmol/kg) was virtually blocked by a small dose of cycloheximide (0.89 μmol/kg) 18 hr earlier, whereas the 80% reduction in bile flow following a larger dose of bilirubin (34 μmol/kg) was merely attenuated. Multiple doses of cycloheximide were not more effective than a single dose. Thus, cycloheximide protection in the manganese–bilirubin combination was quantitatively different than that observed with TLCh.

These results are highly indicative that unaltered protein synthesis is necessary for expression of maximal cholestasis in the three models. Involvement of proteins in the handling of bile salts has been demonstrated throughout the enterohepatic cycle [12–17]. In particular, cytosolic proteins acting as carriers, and reducing the concentration of free bile salts in the cell [18] and binding sites on the canalicular membrane for carrier-mediated expulsion from the hepatocyte into the bile [15, 19], have been invoked. A bilirubin-binding protein, distinct from a bile salt-binding protein, has been identified in liver plasma membrane [20]. However, even if binding of bile salts and other cholephilic anions to proteins has been demonstrated, their functional role in the generation and regulation of bile flow is not clear [18]. Given the interdependence of proteins and compounds involved in the generation of bile flow, it is not surprising that altered protein synthesis or degradation has an impact on agents modifying bile flow. Gonzalez *et al.* [21] proposed that cycloheximide diminishes taurocholate-induced choleresis because of a reduced number of binding sites. On the other hand, Yousef *et al.* [9] speculated that

Table 6. Effect of cycloheximide pretreatment on manganese–bilirubin induced reduction in bile flow

Cycloheximide (μmol/kg)	Bile flow/Time period (μl/min/100 g) −15–0 min	0–15 min	15–45 min	45–75 min	75–105 min
A. Cycloheximide i.p. 18 hr prior to an i.v. injection of manganese (89 μmol/kg) at 0 min, followed by bilirubin (26 μmol/kg) at 15 min					
Vehicle	6.44 ± 0.54	6.01 ± 0.43	3.81 ± 0.46	2.63 ± 0.49	3.18 ± 0.41
0.89	6.70 ± 0.26	6.60 ± 0.23	5.89 ± 0.40*	5.56 ± 0.46*	5.10 ± 0.49*
1.78	7.33 ± 0.37	7.09 ± 0.44	6.02 ± 0.47*	5.42 ± 0.40*	5.58 ± 0.35*
3.55	7.48 ± 0.45	7.26 ± 0.34	6.33 ± 0.47*	5.93 ± 0.45*	6.09 ± 0.63*
B. Cycloheximide i.p. 18 hr prior to an i.v. injection of manganese (89 μmol/kg) at 0 min, followed by bilirubin (34 μmol/kg) at 15 min					
Vehicle	5.25 ± 0.11	5.19 ± 0.31	2.25 ± 0.18	0.96 ± 0.25	0.99 ± 0.27
0.89	5.74 ± 0.26	5.44 ± 0.25	3.05 ± 0.47	2.25 ± 0.83	2.30 ± 0.78
1.78	6.35 ± 0.40	5.92 ± 0.43	3.94 ± 0.39*	3.82 ± 0.52*	3.22 ± 0.67*
3.55	5.93 ± 0.22	5.52 ± 0.09	3.37 ± 0.19	2.90 ± 0.51	3.23 ± 0.36*
C. Cycloheximide i.p. 42 and 18 hr prior to an i.v. injection of manganese (89 μmol/kg) at 0 min, followed by bilirubin (34 μmol/kg) at 15 min					
Vehicle	7.12 ± 0.55	6.93 ± 0.59	2.84 ± 0.46	1.33 ± 0.48	1.35 ± 0.54
0.89	7.36 ± 0.29	6.43 ± 0.17	3.28 ± 0.56	2.58 ± 0.77	2.51 ± 0.69†
1.78	6.95 ± 0.39	6.52 ± 0.40	3.98 ± 0.58†	3.75 ± 0.77*†	3.99 ± 0.71*†
3.55	6.57 ± 0.49	5.58 ± 0.50	1.88 ± 0.28	0.70 ± 0.12	0.69 ± 0.18

Values are means ± SE of six animals.
* Significantly different ($P < 0.05$) from respective vehicle group.
† Significantly different ($P < 0.05$) from cycloheximide, 3.55 μmol/kg.

cycloheximide interferes with the cytosolic binding of LCh, either by inhibition of binding proteins or by competition with LCh.

An alternative hypothesis relates to the role of bile salts as active regulators of bile flow, perhaps by regulating solute pumps [22, 23]. This could explain the somewhat contradictory observation that cycloheximide modulates the cholestatic effect of TLCh/LCh as well as the choleretic properties of taurocholate. It is possible that cycloheximide inhibits a protein acting as a solute pump or modulator of a solute pump secreting ions into the bile; certain bile salts such as LCh and TLCh may elicit a negative feed-back, while others, such as taurocholate, would increase its solute-pumping activity. Cholestatic models such as TLCh, LCh and the combination manganese–bilirubin could be useful tools in the search to unravel the complexity of bile formation.

Acknowledgements—Supported by the Medical Research Council of Canada and the Fonds pour la formation de chercheurs et l'aide à la recherche, Québec, Canada. The expert technical assistance of Ms Johanne Couture and Ms Thérèse Vaillancourt is gratefully acknowledged.

REFERENCES

1. Plaa GL and Priestly BG, Intrahepatic cholestasis induced by drugs and chemicals. *Pharmacol Rev* **28**: 207–273, 1976.
2. Reichen J and Simon FR, Cholestasis. In: *The Liver: Biology and Pathobiology* (Eds. Arias IM, Popper H, Schachter D and Shafritz DA), pp. 785–800. Raven Press, New York, 1982.
3. Reyes H, The enigma of intrahepatic cholestasis of pregnancy: lessons from Chile. *Hepatology* **2**: 87–96, 1982.
4. Balistreri WF, Neonatal cholestasis. *J Pediat* **106**: 171–184, 1985.
5. de Lamirande E and Plaa GL, Role of manganese, bilirubin and sulfobromophthalein in manganese–bilirubin cholestasis in rats. *Proc Soc Exp Biol Med* **158**: 283–287, 1978.
6. de Lamirande E and Plaa GL, Dose and time relationships in manganese–bilirubin induced cholestasis. *Toxicol Appl Pharmacol* **49**: 257–263, 1979.
7. Kakis G and Yousef IM, Pathogenesis of lithocholate- and taurolithocholate-induced intrahepatic cholestasis in rats. *Gastroenterology* **75**: 595–607, 1978.
8. de Lamirande E, Tuchweber B and Plaa GL, Morphological aspects of manganese–bilirubin induced cholestasis. *Liver* **2**: 22–37, 1982.
9. Yousef IM, Tuchweber B and Weber A, Prevention of lithocholate-induced cholestasis by cycloheximide, an inhibitor of protein synthesis. *Life Sci* **33**: 103–110, 1983.
10. Winer BJ, *Statistical Principles in Experimental Design*. McGraw-Hill, New York, 1971.
11. Rothblum LI, Devlin TM and Ch'ih JJ, Regulation of mammalian protein synthesis *in vivo*. *Biochem J* **156**: 151–157, 1976.
12. Gallagher K, Mauskopf J, Walker JT and Lack L, Ionic requirements for the active bile salt transport system. *J Lipid Res* **17**: 572–577, 1976.
13. Roda A, Cappelleri G, Aldini R, Roda E and Barbara L, Quantitative aspects of the interaction of bile acids with human serum albumin. *J. Lipid Res* **23**: 490–495, 1982.
14. Delahunty T and Feldkamp T, A study of endogenous *N*-cholylglycine distribution among serum proteins using radioimmunoassay. *Steroids* **36**: 439–449, 1980.
15. Accatino L and Simon FR, Identification and characterisation of a bile acid receptor in isolated liver surface membranes. *J. Clin Invest* **57**: 496–508, 1976.
16. Anwer MS, Kroker R, Hegner D and Petter A, Cholic

acid binding to isolated rat liver plasma membranes. *Hoppe–Seyler's Z Physiol Chem* **358**: 543–553, 1977.
17. von Dippe P and Levy D, Characterization of the bile acid transport system in normal and transformed hepatocytes. *J Biol Chem* **258**: 8896–8901, 1983.
18. Strange RC, Hepatic bile flow. *Physiol Rev* **64**: 1055–1102, 1984.
19. Meier PJ, St. Meier-Abt A, Barrett C and Boyer JL, Mechanisms of taurocholate transport in canalicular and basolateral rat liver plasma membrane vesicles. *J Biol Chem* **259**: 10614–10622, 1984.
20. Stremmel W and Berk PD, Hepatocellular uptake of sulfobromophthalein and bilirubin is selectively inhibited by an antibody to the liver plasma membrane sulfobromophthalein/bilirubin binding protein. *J Clin Invest* **78**: 822–826, 1986.
21. Gonzalez MC, Sutherland E and Simon FR, Regulation of hepatic transport of bile salts. Effect of protein synthesis inhibition on excretion of bile salts and their binding to liver surface membrane fractions. *J Clin Invest* **63**: 684–694, 1979.
22. Erlinger S, Does Na^+–K^+ ATPase have any role in bile secretion? *Am J Physiol* **273**: G243–G247, 1982.
23. Klaassen CD and Watkins JB, Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacol Rev* **36**: 1–67, 1984.