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Choleretic Effects of Differently Structured Bile Acids in the Guinea Pig (41984)

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Abstract. The effects of 10 differently structured bile acids on bile flow and composition were studied in anesthetized, bile duct-cannulated guinea pigs. At the infusion rates of 2 and 4 μmole/min/kg, all bile acids produced choleresis. The most potent was chenodeoxycholate, which increased bile flow by an average of 31.25 μl/μmole of bile acids excreted in bile. The weakest choleretic was tauroursodeoxycholate (11.02 μ/μmole). When the choleretic activity was plotted against bile acid hydrophobicity (high-performance liquid chromatography retention factor, obtained from the literature), linearity was observed with similarly conjugated bile acids. The order of potency was deoxycholate > chenodeoxycholate > cholate > ursodeoxycholate, both for the glycine and taurine conjugates, and for the unconjugated bile acids as well. Conjugation was also important, and the rank ordering for the choleretic activity (unconjugated bile acids > glycine-conjugates > taurine-conjugates) was the same as that for the hydrophobicity. When the choleretic activity was plotted against bile acid micellar aggregation number (in 0.15 *M* NaCl at 36°C, obtained from the literature), a linear, direct relationship was observed. All bile acids produced similar effects on bile electrolyte concentrations: both bicarbonate and chloride slightly declined during choleresis, whereas bile acid concentrations increased. These studies suggest that, in the guinea pig (1) the differing choleretic activities of differently structured bile acids are not due to their forming micelles in bile of different sizes; (2) either the more hydrophobic bile acids form vesicles, whereas the more hydrophilic form micelles; or bile acids produce choleresis, in part or exclusively, by stimulating an additional secretory mechanism, possibly an inorganic ion pump; or both.

Bile acids exert a number of vital functions within the gastrointestinal system. In the liver, bile acids stabilize the physical state of bile, regulate the biliary excretion of cholesterol and phospholipids (1) and, perhaps more importantly, stimulate bile flow (2). The choleretic properties of bile acids have been documented in a variety of animal species including man (3), and overwhelming evidence has accumulated to indicate that at least a fraction of canalicular bile flow is secondary to the osmotic drive of actively secreted bile acids, or of their counterions (4). However, bile acids are a large class of compounds and both the physicochemical properties and biological effects are closely related to the structural characteristics of their molecules. Thus, differently structured bile acids have different effects on lipid biliary excretion (5–7) and hepatic membrane structure (8, 9) and function (8, 10), and form micellar aggregates in bile of different shape and size (11, 12). Evidence is also available to indicate that the effect of bile acids on bile flow is a function of their molecular structure as well. Trihydroxy and most dihydroxy bile acids are choleretic when administered below their hepatic transport maximum, whereas monohydroxy bile acids are cholestatic (13). Additionally, the choleretic potency of bile acids in a given species seems to be related to their structural characteristics (5–7, 14). However, while the structure–effect relationship on micelle formation and lipid biliary excretion has been investigated extensively, the effect of differently structured bile acids on bile secretion has not. This paper reports the effects of 10 bile acids with different structural characteristics on bile flow and composition in the guinea pig.

Methods. *Animals and chemicals.* Male

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guinea pigs, 450–600 g, were obtained from Perfection Breeders, Douglasville, Pennsylvania, and housed in a temperature-controlled room (22°C) with alternating 12-hr light–dark cycles for at least a week prior to use. They were fed lab chow *ad libitum,* had free access to water, and were fasted for 24 hr before being utilized. Fasting significantly facilitated all surgical procedures within the abdominal cavity.

All bile acids used in these studies, including cholic (C), chenodeoxycholic (CDC), taurocholic (TC), taurodehydrocholic (TDHC), taurodeoxycholic (TDC), taurochenodeoxycholic (TCDC), tauroursodeoxycholic (TUDC), glycocholic (GC), glycodeoxycholic (GDC), and glycochenodeoxycholic (GCDC) were purchased as the respective sodium salts from Calbiochem, La Jolla, California.

Experimental procedure. These studies were carried out in anesthetized, bile duct-cannulated guinea pigs. The surgical procedure and the experimental protocol have been described in detail elsewhere (15). Following surgery, a 60-min equilibration period was allowed after which a constant infusion of a bile acid was started. All bile acids, except CDC, were infused at 2 (30 min) followed by 4 (30 min) μmole/min/kg. CDC was infused at 1 and 2 μmole/min/kg, since severe hemolysis was observed when given at higher rates. At the end of the bile acid infusion, an equilibration period (60 min) was allowed after which, in most experiments, a second bile acid was given (except when CDC was infused) at the same rates and for the same time period. The second bile acid was tested only in those animals in which postcholeretic bile flow was similar to that seen in the precholeretic period, and when no significant hemolysis was present. In all instances, the experimental period was limited to 4–5 hr, during which bile and arterial blood (100 μl) were collected every 10 min. At the end of the experiment, the animal was sacrificed with an overdose of pentobarbital, the liver was quickly removed, and its weight was determined.

All solutions for iv infusion were made in Krebs–Henseleit bicarbonate solution to which 5 mg/ml dextrose, 5 mg/ml bovine serum albumin, and the desired amount of bile acid were added before use. The osmolality of the infusion solution was maintained within the physiological range (280–290 mOsm) using a correction factor of 1.86 mOsm for each meq/liter Na^+ added with bile acids and 27.5 mOsm for 5 mg/ml dextrose. Under these conditions, no hemolysis was observed with most bile acids. However, some hemolysis occurred during infusion of CDC at 2 μmole/min/kg, and TDC, GCDC, GDC, and TCDC at 4 μmole/min/kg. The rate of fluid infusion was carefully monitored throughout the experimental period, using as a reference index the value of the arterial hematocrit and the rate of bile flow. The total rate of fluid infusion ranged from 8 to 15 ml/hr.

Analyses. Bile flow was determined by the difference between the weights of filled and empty collecting vials. Total bile acids in bile were measured by the hydroxysteroid dehydrogenase procedure, as reported previously (16). Sodium and potassium were determined by ion selective electrodes, chloride by coulometric titration, and bicarbonate by the pH rate method (Beckman Astra, Beckman Instruments, Brea, Calif.). Osmolality was measured by freezing-point depression (Osmette, Precision System Inc., Sudbury, Mass.). Statistical differences were established by Student's *t* test.

Results. *Spontaneous bile secretion.* Following cannulation of the common bile duct, the rate of bile flow ranged from 152 to 196 μl/min/kg and, in control experiments, only slightly declined by the end of a 4- to 5-hr collection period (10–15%). Total bile acid concentration in bile during the first 60-min period averaged 2.1 meq/liter (Table II), and their excretion rate 363 nmole/min/kg. Sodium and potassium concentrations in bile and bile osmolality were essentially the same as those in plasma (Table II). However, biliary bicarbonate (53–65 meq/liter) and chloride (63–78 meq/liter) concentrations were approximately 240 and 65% of their respective plasma levels.

Bile acid infusion. All bile acids tested in these studies produced choleresis at the doses employed (1–4 μmole/min/kg). With each bile acid, a linear relationship between bile flow and total bile acid excretion in bile was observed. Figure 1 illustrates such a relationship in four representative experiments in

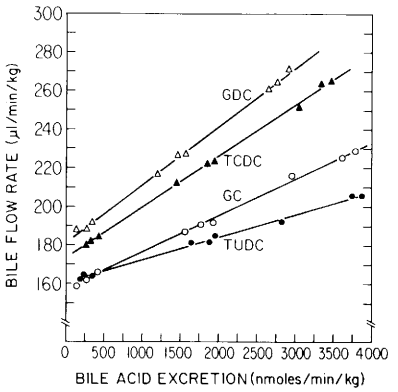

FIG. 1. Relationship between bile flow and total bile acid excretion in bile during administration of four differently structured bile acids at 2 and 4 μmole/min/kg. Lines were obtained from four separate experiments. Note the close linearity for each bile acid infused, and the differences in the slopes of the lines (choleretic activity).

which TUDC, GC, TCDC, and GDC, respectively, were infused at 2 and 4 μmole/min/kg. Except during TDC and GDC infusion, the mean steady-state bile acid excretion in bile accounted for 91.5–99.3% of the infused amount when bile acids were given at 2 μmole/min/kg, and 87.6–97.1% following 4 μmole/min/kg (Table I). During GDC and TDC administration, bile acid excretion accounted, respectively, for 80.6 and 80.4% when infused at 2 μmole/min/kg, and only 72.4 and 74.8% at 4 μmole/min/kg. However, when total bile acid recovery was calculated [the amount excreted during the bile acid infusion (30 min) and postinfusion (20 min) periods], similar values were observed for all bile acids tested (98–105%). This indicates that the diminished excretion observed during GDC and TDC infusion was not due to technical artifacts, e.g., lower infusion rates.

When the choleretic activity of the differently structured bile acids was calculated (slope of the line relating changes in bile flow to those in bile acid excretion), marked differences were observed (Table I). CDC was the most potent, with an average choleretic activity of 31.25 μl/μmole. TUDC was the weakest, and bile flow increased only by 11.02 μl for each micromole of bile acids excreted during its administration. TDHC, a synthetic triketo derivative, was less potent

TABLE I. EFFECT OF DIFFERENTLY STRUCTURED BILE ACIDS ON BILE FLOW AND BILIARY EXCRETION OF TOTAL BILE ACIDS IN THE GUINEA PIG

Bile acid	n	Choleretic activity[a] (μl/μmole)	Total bile acid excretion					
			Infusion rate: 2 μmole/min/kg			Infusion rate: 4 μmole/min/kg		
			μmole/min/kg	%[b]	%[c]	μmole/min/kg	%[b]	%[c]
CDC	3	31.23 ± 2.71	1.94 ± 0.10	97.3 ± 3.5	102.6 ± 3.1	3.63[d]	90.9	101.6
GDC	4	28.28 ± 3.34	1.61 ± 0.11	80.6 ± 5.7	101.8 ± 4.4	2.90 ± 0.14	72.4 ± 3.7	98.6 ± 5.3
TDC	6	27.05 ± 2.67	1.61 ± 0.12	80.4 ± 6.2	99.4 ± 3.7	2.99 ± 0.14	74.8 ± 3.5	98.7 ± 4.7
GCDC	5	24.33 ± 2.64	1.99 ± 0.14	99.3 ± 7.2	103.7 ± 2.8	3.62 ± 0.17	90.6 ± 4.2	103.5 ± 5.2
TCDC	7	23.17 ± 2.31	1.92 ± 0.10	96.1 ± 4.9	100.8 ± 4.4	3.59 ± 0.11	89.7 ± 2.7	97.9 ± 4.6
C	4	20.05 ± 1.88	1.83 ± 0.09	91.5 ± 4.6	97.7 ± 3.3	3.59 ± 0.29	89.9 ± 7.2	98.0 ± 5.0
GC	4	18.94 ± 3.03	1.92 ± 0.06	95.9 ± 3.2	104.5 ± 2.7	3.88 ± 0.29	97.1 ± 7.3	103.4 ± 4.1
TC	7	16.42 ± 2.09	1.87 ± 0.07	93.4 ± 3.4	99.8 ± 4.9	3.70 ± 0.24	92.6 ± 5.9	101.6 ± 6.5
TDHC	6	13.21 ± 1.84	1.85 ± 0.13	92.7 ± 6.7	98.7 ± 5.3	3.50 ± 0.20	87.6 ± 4.9	100.9 ± 5.8
TUDC	8	11.02 ± 2.13	1.97 ± 0.15	98.3 ± 7.5	105.1 ± 6.2	3.58 ± 0.24	89.6 ± 5.8	103.7 ± 4.7

Note. Values are means ± SD (n = number of experiments). The rate of endogenous bile acid excretion observed under spontaneous secretory conditions was not subtracted from the excretion rates seen during unfusion of bile acids.

[a] The choleretic activity is represented by the slope of line relating changes in bile flow to those in bile acid excretion.

[b] Amount recovered in bile at steady-state conditions (third 10-min collection of the 30-min infusion period) when expressed as a percentage of the infusion rate (either 2 or 4 μmole/min/kg).

[c] Total recovery of bile acids (expressed as a percentage of the infused amount) represented by the amount excreted during the 30-min bile acid infusion period plus that in the 20-min postinfusion period.

[d] Only one guinea pig was infused with 4 μmole/min/kg CDC (severe hemolysis was observed when CDC was infused at this high dose).

($P < 0.05$–0.001) than any hydroxy bile acid tested, except TUDC. If the choleretic activity of the differently structured bile acids was plotted against the relative hydrophobicity of their molecules, expressed by the high-performance liquid chromatography retention factor (17), linearity was seen with similarly conjugated bile acids on one hand, and with bile acids of a given number, position, or configuration of hydroxyl groups on the other. As illustrated in Fig. 2, the most hydrophobic TDC was the most potent choleretic among the taurine conjugates. The order of potency was TDC > TCDC > TC > TUDC. Similarly, the order of potency for the glycine conjugates was GDC > GCDC > GC, and the more hydrophobic CDC was choleretically more potent than C. The high-performance liquid chromatography retention factor for TDHC could not be found in the literature, so that this synthetic, presumably more hydrophilic bile acid could not be included in such an analysis. The influence of conjugation on the relationship between bile acid choleretic activity and hydrophobicity is shown in Fig. 3. The unconjugated bile acids are, for a given molecular structure, more hydrophobic than the glycine and the taurine conjugates, and displayed higher choleretic activity. C > GC > TC, CDC > GCDC > TCDC, and GDC > TDC. However, while linearity between the choleretic activity and hydrophobicity was observed with the trihydroxy bile acids, the relationship was not closely linear with the $3\alpha,7\alpha$-dihydroxy derivatives. The choleretic activity of CDC was, in fact, greater than that accounted for solely by its higher hydrophobicity.

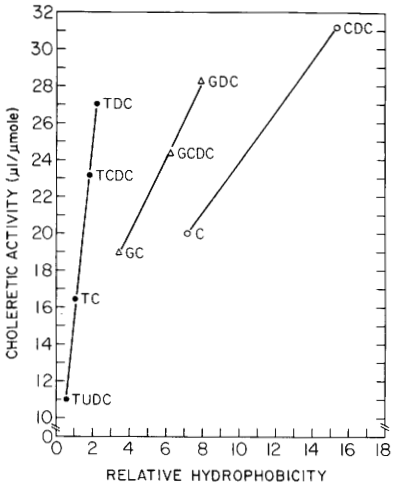

FIG. 2. Relationship between the choleretic activity and relative hydrophobicity of differently structured bile acids. The choleretic activity is represented by the slope of the line relating changes in bile flow to those in bile acid excretion. The hydrophobicity values are the high-performance liquid chromatography retention factors reported in (17). The lines are drawn to show the influence of the number, position, or configuration of hydroxyl groups. The retention factor of TDHC is not available. Values are means (number of experiments are shown in Table II).

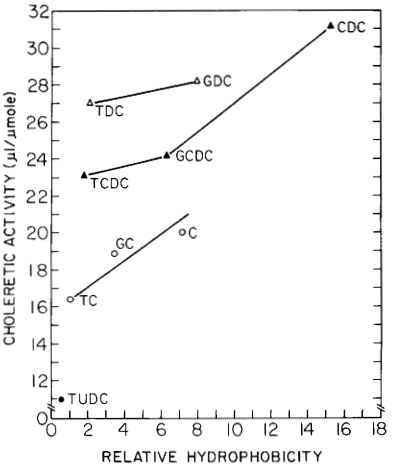

FIG. 3. Relationship between the choleretic activity and relative hydrophobicity of differently structured bile acids. The lines show the influence of conjugation. For more details, see legend to Fig. 2.

When the choleretic activity of the different bile acids was plotted against the micellar aggregation number, determined in 0.15 *M* NaCl at 36°C (11), a linear relationship was observed (Fig. 4). However, the slope of the line was positive, just the opposite of that predicted on a theoretical basis.

Despite the different choleretic activities, all bile acids tested produced similar effects on bile electrolyte concentrations (Table II). The increase in bile acid concentration was invariably associated with a proportional decrease in the concentrations of chloride and bicarbonate, and bile osmolality remained unchanged. Sodium concentrations slightly increased during choleresis produced by most bile acids.

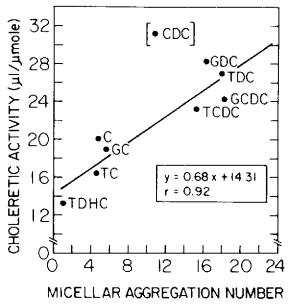

FIG. 4. Relationship between the choleretic activity and the micellar aggregation number of differently structured bile acids. The micellar aggregation numbers are those reported in (11), which were obtained at 36°C in 0.15 *M* NaCl. The number for TDHC is that reported for dehydrocholate at 20°C. The equation shown was obtained with eight bile acids. The aggregation number of TUDC is not available in the literature (under similar conditions), and CDC was not included since, as observed in Fig. 3, the higher choleretic activity of this bile acid cannot be accounted for solely by its greater hydrophobicity. Interestingly, Armstrong and Carey (17) observed the same behavior of CDC when the cholesterol solubilizing capacity of differently structured bile acids was plotted against the hydrophobicity. If CDC is included in the present analysis, the equation is $y = 0.69x + 15.25$ ($r = 0.79$), which is still statistically significant ($P < 0.05$).

Discussion. Previous studies comparing the choleretic effects of differently structured bile acids have demonstrated that, in the rat (7), cat (5), and dog (6, 14), the bile acid with a higher critical micellar concentration always produced a greater increase in bile flow. Such a finding is fully consistent with the classical "osmotic-flow" theory of bile acid-induced choleresis (18) and was, indeed, interpreted accordingly. That is, the bile acid with a higher critical micellar concentration is, for a given concentration in bile, associated with a larger number of osmotically active particles, thereby driving a greater volume of bile. An exception to this view is represented by the studies reported by Klaassen (19), who found that, in the dog, the increase in bile flow produced by equimolar doses of differently structured bile acids was not related to their *in vitro* physicochemical properties. In his experiments, however, bile acids were given as a bolus injection, steady-state conditions were not achieved, and the osmotic activity (µl/µmole of bile acid excreted) was not calculated. Thus, a direct comparison with the results reported in Ref. (5–7, 14) cannot be made.

The present studies have now demonstrated that bile acids with different structural characteristics have differing choleretic activities also in the guinea pig. However, the structure–effect relationship obtained here is exactly the opposite of that found in other animal species. Among 10 bile acids tested, the most hydrophobic (CDC) produced the greatest increase in bile flow and, for a series of bile acids conjugated with the same amino

TABLE II. PLASMA AND BILE ELECTROLYTE CONCENTRATIONS IN THE GUINEA PIG

Fluid	Bile acid infusion	*n*	Na^+ (meq/liter)	K^+ (meq/liter)	Cl^- (meq/liter)	HCO_3^- (meq/liter)	BA[a] (meq/liter)	Osmolality (mOsm)
Plasma		3	141.5 ± 3.6	4.5 ± 0.2	106.7 ± 5.8	24.6 ± 2.7		291.4 ± 3.6
Bile	Controls[b]	25	146.4 ± 3.1	4.6 ± 0.4	70.2 ± 5.1	59.8 ± 4.4	2.1 ± 0.4	300.8 ± 3.1
	TDC[c]	4	+1.4 ± 0.2	NC	−7.5 ± 3.1	−4.7 ± 1.2	12.8 ± 2.5	295.6 ± 3.2
	TC	3	+2.0 ± 0.4	NC	−8.4 ± 3.5	−5.6 ± 1.5	15.3 ± 2.6	302.1 ± 3.8
	TUDC	3	+3.6 ± 0.7	NC	−9.5 ± 3.8	−3.8 ± 1.7	16.1 ± 3.2	307.7 ± 6.8
	C	4	+0.4 ± 0.1	NC	−9.1 ± 2.6	−5.5 ± 1.8	15.2 ± 2.0	304.5 ± 5.7
	TDHC	4	+1.1 ± 0.4	NC	−5.5 ± 3.8	−6.7 ± 3.1	15.8 ± 3.1	300.6 ± 5.2
	GDC	3	−2.0 ± 0.7	NC	−3.7 ± 1.1	−6.2 ± 2.4	10.9 ± 1.9	295.5 ± 4.6
	GCDC	4	−1.8 ± 0.6	NC	−7.4 ± 2.9	−5.8 ± 2.3	13.8 ± 0.7	301.4 ± 5.4

Note. Values are means ± SD (*n* = number of experiments). NC = No measureable change.

[a] Bile acids.

[b] Values were obtained at steady-state conditions prior to administration of bile acids. All the precholeretic values were included in this group.

[c] Maximal change observed during 4 µmole/min/kg bile acid infusion (third 10-min bile collection of the 30-min infusion period), when compared to the precholeretic value.

acid, or having the same number, position, or configuration of hydroxyl groups, the choleretic activity always increased with hydrophobicity. Thus, since the hydrophobicity of bile acids is inversely related to their critical micellar concentration which is, in turn, a function of the micellar aggregation number (11, 12, 17, 20), one apparent conclusion from these studies is that the choleretic activity of the infused bile acids is directly related to their *in vitro* micellar aggregation number (Fig. 4). However, such a relationship cannot be reconciled with the osmotic theory of bile acid choleresis. Hence, it is clear that, in the guinea pig, the differing choleretic activities of various bile acids cannot simply be attributed to their forming micelles of different sizes.

It is possible that most of the bile acids tested in these studies are metabolized in the guinea pig, so that the micellar aggregation number(s) of their metabolites may not be the same as those of the infused species. However, since the relationship between the choleretic activity and the aggregation number was virtually linear, the micellar theory is valid only if we assume that hepatic metabolism inverted the slope of such a relationship, a possibility which seems very unlikely. In such a case, in fact, a more hydrophobic bile acid like GCDC, which is a physiologic species in the guinea pig (21), would have to be transformed into metabolites more hydrophilic than TC, TUDC, or their metabolites; vice versa, a highly hydrophilic compound like TDHC would have to be converted into metabolites more hydrophobic than GDC, or CDC. Alternative explanations seem, therefore, in order.

First of all, the critical micellar concentration and/or the micellar aggregation number of a bile acid in bile may not be the same as those determined *in vitro.* Bile flow in the guinea pig is very high, and bile acid concentration, even during administration of exogenous bile acids at 4 μmole/min/kg, is low when compared to other animal species. Additionally, chloride (Table II), cholesterol, and phospholipid (22) concentrations in bile are low, whereas bicarbonate is high (Table II). Thus, since micelle formation is known to be influenced by the electrolyte and lipid composition of the medium (11, 12), it is not inconceivable that the size of the micelles produced by a given bile acid in the guinea pig bile is quite different from that determined in a standard *in vitro* solution (0.25 *M* NaCl at 36°C). Furthermore, preliminary studies in the dog have demonstrated that administration of TUDC, a more hydrophilic bile acid, results primarily in the formation of vesicles in bile (600–1200 Å), whereas more hydrophobic bile acids, like TC and TCDC, produce mainly mixed micelles (27–35 Å) (23). Were this the case in the guinea pig, and were the number of bile acid molecules aggregated in the vesicles greater than that in the mixed micelles, it could explain the observed relationship between bile acid choleretic activity and hydrophobicity.

Alternatively, choleresis produced by bile acids may not simply be secondary to their osmotic drive, but may, in part or exclusively, result from stimulation of an additional secretory mechanism, perhaps an inorganic ion pump. In the rat, ursodeoxycholate and 7-ketolithocholate are much more potent choleretics than TC, and increase bicarbonate concentrations in bile (24). Such a finding has been interpreted to suggest that bicarbonate transport may be involved in canalicular bile secretion in the rat, and that these two bile acids may also produce choleresis by stimulating such a mechanism. We have looked for such a possibility in the guinea pig, but no compelling evidence was obtained as to implicate stimulation of a specific electrolyte transport by bile acids. In all instances, chloride and bicarbonate concentrations in bile declined during bile acid choleresis. Such a decline appeared to be a compensation for the increase in bile acid concentration. This finding, however, should not be construed to rule out the existence of such a mechanism for bile acid-induced choleresis in the guinea pig. An inorganic ion transport may be stimulated, to varying degrees, by all bile acids and, unlike the rat, the electrolyte composition of the guinea pig bile is quite peculiar so that changes in bicarbonate/chloride concentrations produced by differently structured bile acids are not readily detectable. The finding in other reports (25, 26) and in these studies as well that unconjugated bile acids (more hydrophobic) produce a stronger choleretic effect than their respective glycine and

taurine conjugates is compatible with such a hypothesis. Unconjugated bile acids, in fact, have been shown to undergo conjugation prior to their excretion (25), so that their greater choleretic effect cannot simply be explained in terms of their having higher osmotic activity.

Accordingly, although it remains to be understood why the more hydrophobic the bile acid, the greater is its choleretic activity in the guinea pig, two hypotheses are suggested. Either the more hydrophilic bile acids form vesicles in bile, whereas the more hydrophobic form mixed micelles, or, bile acids stimulate an inorganic ion pump, and the more hydrophobic the bile acid, the greater is its stimulation, or both. Quasielastic light scattering studies and measurements of cholesterol and phospholipids in bile may help clarify this question.

Finally, the observation that the steady-state biliary excretion of bile acids following GDC and TDC infusion was far lower than that observed with all the other bile acids tested merits some comment. Preliminary studies by O'Donovan *et al.* (27) have demonstrated that, in the isolated perfused rat liver, the uptake of differently structured bile acids follows the hydrophilic–hydrophobic ordering and that the presence of albumin in the perfusate diminishes the uptake of more hydrophobic, but not of more hydrophilic bile acids. Thus, it is possible that the lower bile acid excretion observed during GDC and TDC infusion in the present studies reflects a diminished uptake, either because of the high hydrophobicity of these two bile acids and/or their tighter binding to plasma albumin. However, TDC is less hydrophobic than GCDC and, when both bile acids were given at 2 μmole/min/kg, total bile acid excretion accounted for 99.3% during infusion of GCDC, but only 80.4% following TDC administration. Thus, it is likely that, in addition to the hydrophobicity and binding to albumin, other factors as well influence the uptake and/or the biliary excretion of bile acids in the guinea pig.

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