Binding of Bile Acids to Cholestyramine at Gastric pH Conditions

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Abstract □ The binding of bile salts to cholestyramine was studied under varying conditions of pH and added electrolyte. The taurine-conjugated bile salts were strongly absorbed by the anion-exchange resin at low pH and in the presence of chloride anions. Glycocholic acid binding was very weak at low pH but increased strongly with increasing pH. The presence of chloride ions strongly decreased the amount of glycocholate bound by the anion-exchange resin.

Keyphrases □ Bile acids—binding to cholestyramine at gastric pH □ Cholestyramine—binding of bile acids at gastric pH □ Binding—bile acids to cholestyramine at gastric pH

Recently published evidence shows that the reflux of the duodenal contents into the stomach is frequently the cause of gastric ulcers (1) and gastritis (2). It has been shown that bile salts disrupt the mucosal barrier and render it permeable to hydrogen ions (3, 4). The name "bile reflux gastritis" has been applied to this condition (2).

Cholestyramine resin has been used in recent clinical trials to reduce the intragastric bile salt content (1, 2, 5).

It is not clear, however, from these studies whether the cholestryamine significantly binds bile salts in the stomach or whether the anion-exchange resin acts chiefly to bind bile acids in the intestine, thereby generally reducing the circulating bile acid pool.

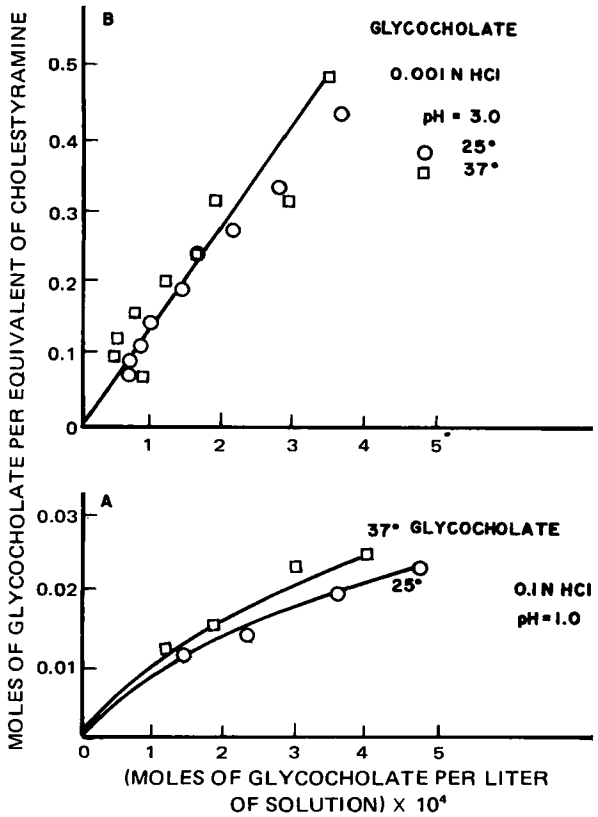

Figure 1—*Isotherms for the binding of glycocholate to cholestyramine at pH 1.0 (A) and 3.0 (B).*

Table I—Binding of Bile Salts by Cholestyramine

Bile Salt	pH	Ionic Strength	Millimoles per Gram of Resin	Grams Bound per Gram of Resin[a]
Glycocholic acid	1.0	0.1	0.08	0.039
	3.0	0.001	2.1	1.00
	3.1	0.1	0.1	0.048
	4.2	0.1	0.26	0.125
Taurocholic acid	1.0	0.1	0.50	0.269
Taurodeoxycholic acid	1.0	0.1	3.63	1.89

[a] The equilibrium concentration of bile salt in the aqueous solution is 4×10^{-4} *M* in each case.

Cholestyramine resin has been shown to be effective in lowering serum cholesterol levels by removing bile salts from the intestine and increasing their fecal elimination (6, 7). The increased fecal loss of bile salts due to binding by the anion-exchange resin leads to increased metabolism of cholesterol to replace the lost bile acids.

Under the relatively high pH conditions prevailing in the small intestine, both *in vitro* (8, 9) and *in vivo* (6, 7) studies showed that bile acids are bound by cholestyramine resin. Since the glycine-conjugated bile acids are weak acids (glycocholic acid, pKa = 3.95; glycodeoxycholic acid, pKa = 4.89) (10), they will be only slightly ionized at the low pH of the stomach and their binding by the anion-exchange resin consequently decreased. The taurine-conjugated bile acids are much stronger acids; even at low gastric pH, a significant fraction of the bile acid will be in the ionized form.

The pH of the stomach will, however, significantly determine the extent of bile acid binding since approximately 75% of the bile acid in adult human bile is glycine conjugated (11).

The objective of this study was to determine quantitatively the binding of bile acids by cholestyramine at acid pH conditions.

EXPERIMENTAL

Glycocholic acid[1], sodium taurodeoxycholate[2], sodium taurocholate, and cholestyramine resin[3] USP were used as received. All other chemicals were reagent grade. The ion-exchange capacity of the resin was determined by argentometric titration of the chloride ions released from the resin in the presence of excess potassium ni-

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Calbiochem, Los Angeles, Calif.
[3] Supplied by Dr. L. Schroeter, Merck Sharp and Dohme, West Point, Pa.

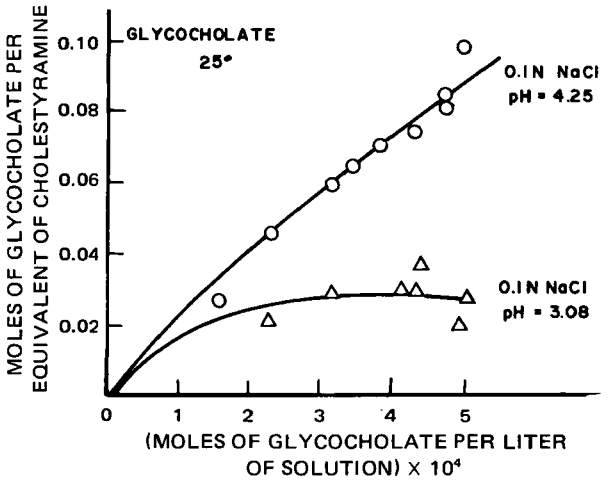

Figure 2—*Isotherms for the binding of glycocholate to cholestyramine in the presence of 0.1* N *NaCl at pH 3.08 and 4.25.*

trate in aqueous solution. The capacity was 3.59 mEq/g. Bile acid analyses were performed spectrophotometrically using the method of Eriksson and Sjovall (12).

Ion-exchange adsorption isotherms were measured by shaking the aqueous bile acid solutions with samples of cholestyramine resin in a constant-temperature bath. Preliminary experiments indicated that overnight shaking was sufficient to attain equilibrium. The solutions were filtered, and the bile acid content of the supernatant liquid was determined. The amount of bile acid absorbed by the ion-exchange resin was calculated as the difference between the amount initially in solution and the amount in solution after equilibration.

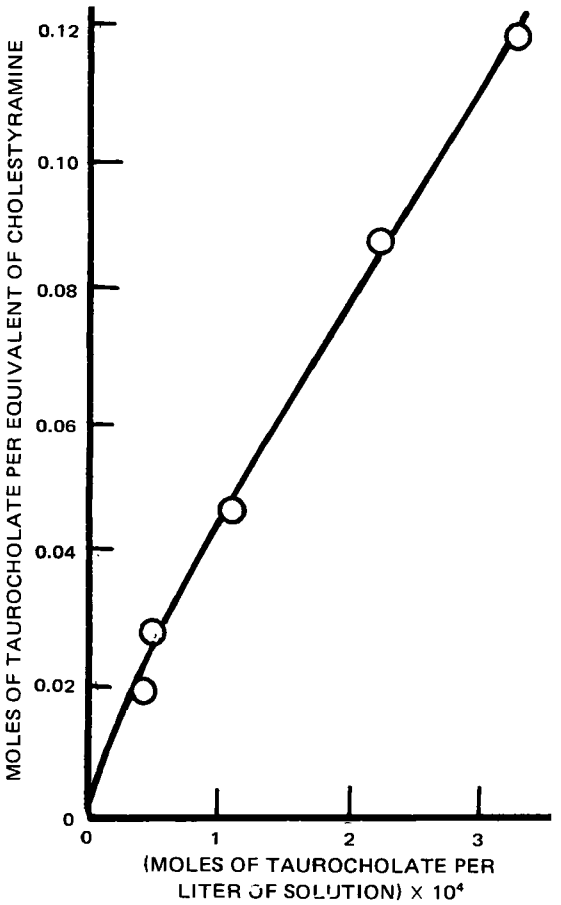

Figure 3—*Isotherms for the binding of taurocholate to cholestyramine at pH 1.0 and 25° in 0.1* N *HCl.*

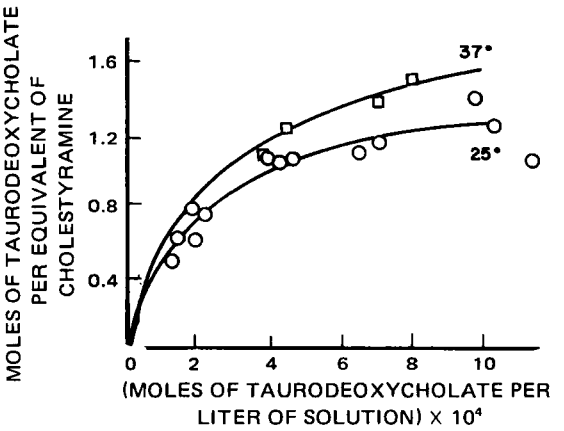

Figure 4—*Isotherms for the binding of taurodeoxycholate to cholestyramine at pH 1.0 in 0.1* N *HCl.*

RESULTS AND DISCUSSION

Ion-exchange isotherms for the distribution of bile salts between cholestyramine resin and aqueous bile salt solution are given in Figs. 1–4. Glycocholate (Fig. 1A) is only slightly absorbed from 0.1 *N* HCl. The binding is weak because the glycocholic acid is only slightly ionized at pH 1.0 and there is competition for the exchange sites by chloride ions. Previous studies of anion-exchange binding of bile salts at higher pH showed that chloride ions compete strongly for the resin anion-exchange sites (9). The isotherms also show that binding is stronger at higher temperature. This result indicates that the enthalpy change for the reaction shown in Scheme I:

$$RCl + A^- \rightleftharpoons RA + Cl^-$$

Scheme I

(where RCl represents cholestyramine resin in the chloride form) is positive, suggesting that the binding of glycocholate ion to ion-exchange resin is caused partly by a hydrophobic interaction between the glycocholate ion and the resin matrix. The interaction between the resin and the glycocholate ion is facilitated by the release of "structured" water surrounding the bile acid anion (13, 14). The temperature effect also may possibly be due in part to a decrease in pKa with increasing temperature.

Much stronger binding of glycocholic acid by the resin is found at pH 3.0 and reduced ionic strength (Fig. 1B). At this pH, more glycocholic acid is in the ionized form and the main interaction with the cholestyramine is probably coulombic. No difference is obtained in the adsorption isotherm between 25 and 37°, suggesting that the hydrophobic contribution is small.

The effect of pH alone on the ion-exchange binding of glycocholic acid is demonstrated better with the data given in Fig. 2. These isotherms at constant ionic strength show that there is a large increase in ion-exchange binding when the pH is increased from 3 to 4. This result suggests that the binding of glycine-conjugated bile acids may be considerably improved by increasing the pH in the gastric lumen.

Taurocholate (Fig. 3) is strongly bound by the resin even at low pH and in competition with 0.1 *M* chloride-ion concentration. Binding of taurodeoxycholate is much stronger and also shows a strong positive temperature dependence. Since taurodeoxycholic acid is a dihydroxy bile acid, it is considerably less polar (more hydrophobic) than the trihydroxy acids and is, therefore, more subject to hydrophobic binding forces.

Further evidence that hydrophobic binding predominates in this case is that the binding capacity for taurodeoxycholic acid, as seen from the ordinate of Fig. 4, exceeds 1.0. Therefore, more than 1 mole of bile acid anion is adsorbed per equivalent of exchange site, suggesting that the hydrophobic resin matrix plays an important role in the binding process.

The effects of pH and added electrolyte on the binding of bile salts by cholestyramine are summarized in Table I. In calculating the amount of bile salt bound per gram of resin, it is assumed that the equilibrium aqueous concentration of bile salt is 4×10^{-4} *M*.

The taurine-conjugated acids are strongly bound even at low pH and in the presence of 0.1 *M* chloride. The glycocholic acid binding is strongly pH dependent, and effective binding is only obtained at low pH in the absence of competing chloride ion. However, previous work (9) showed that much more glycocholate is bound by cholestyramine at higher pH (5.4–6.8), even in the presence of 0.15 *M* sodium chloride or sodium carbonate. Under these conditions, the glycocholate is present almost entirely in the dissociated anionic form.

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Tumor-Inhibitory Agent from *Montezuma speciosissima* (Malvaceae)

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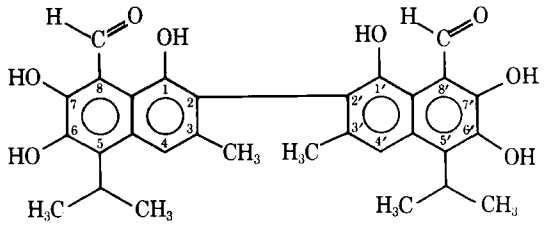
Abstract □ The petroleum ether extract of *Montezuma speciosissima* Sesse and Moc. demonstrated tumor-inhibiting properties in the P-388 lymphocytic leukemia test system (3PS). The constituent responsible for this activity was shown to be a symmetrically substituted 2,2′-dinaphthol, identified as gossypol ($C_{30}H_{30}O_8$).

Keyphrases □ *Montezuma speciosissima*—extract, PMR, UV, IR, and mass spectra, gossypol isolated □ Gossypol—isolated from *Montezuma speciosissima*, PMR, UV, IR, and mass spectra, tumor inhibitory activity □ Tumor inhibition—gossypol, isolated from *Montezuma speciosissima*, PMR, UV, IR, and mass spectra

As a result of the continuing search for plants yielding tumor-inhibiting constituents, it was found that the petroleum ether extract of the woody stems and stem barks of *Montezuma speciosissima* Sesse and Moc. (Malvaceae)[1] showed inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2].

DISCUSSION

The ether-soluble portion of the petroleum ether extract, after mild alkali extraction followed by careful acidification, gave a product which, when subjected to silica gel column chromatography, yielded a brilliant golden-yellow compound. This compound was characterized as 1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl[2,2′-binaphthalene]-8,8′-dicarboxaldehyde, identical to gossypol (I) in all respects. This identity was demonstrated by



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means of its melting point, mixed melting point, elemental analysis, PMR, mass spectrometry, and comparison of UV and IR spectra with authentic sample spectra. Gossypol, the principal yellow pigment of cottonseed, was isolated first by Marchlewski (1) and then synthesized by Edwards (2). The structure of gossypol has been extensively studied by various workers (3).

The petroleum ether extract demonstrated activity of 131% test/control (T/C) at 50 mg/kg, 136% T/C at 25 mg/kg, and 125% T/C at 16 mg/kg. Gossypol demonstrated activity of 150% T/C at 10 mg/kg and 129% T/C at 5 mg/kg in the 3PS test system. Activity in the 3PS system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125% (4).

EXPERIMENTAL[3]

The woody stems and stem barks (10 kg) of *M. speciosissima* were ground and extracted exhaustively in a Lloyd-type extractor with petroleum ether. After removal of the solvent in air, the residue (69 g) was repeatedly extracted with ether and filtered. The

[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resource Laboratory, Plant Genetics and Germ Plasm Institute, Beltsville, Md. A reference specimen was deposited in that herbarium. The plant was collected in Puerto Rico in February 1972.

[2] Of the Division of Cancer Treatment, National Cancer institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected.