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In Vitro Binding of Drugs to Colestipol Hydrochloride

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Abstract □ The *in vitro* binding of drugs by colestipol hydrochloride depended not only upon ionic strength, pH, and type of competing ion but also upon whether association could occur with other molecules. Where feasible, the initial input ratio of drug to binding agent was equivalent to that if both were administered orally in therapeutically effective amounts. The water-soluble drugs chlorpropamide-^{35}S, niacin-6-^{14}C, ascorbic acid, aspirin, salicylic acid, phenobarbital-^{14}C, sulfadiazine, penicillin G, and lincomycin hydrochloride were less than 30% bound to colestipol hydrochloride in tromethamine–sodium chloride buffer (pH 7.5, μ = 0.15), while warfarin and tetracycline hydrochloride were bound 59 and 30%, respectively. The binding of a drug determined under these conditions is an estimate of the upper limit of the percentage of the dose of orally ingested drug that would remain bound *in vivo*. The binding of tetracycline hydrochloride, sulfadiazine, benzyl penicillin, and lincomycin hydrochloride to colestipol hydrochloride was reversible, and only a small fraction of warfarin was irreversibly bound to colestipol hydrochloride. As a positive control, binding of the drugs (or bile salts) to cholestyramine was also studied. Cholestyramine bound most of the drugs investigated to a greater extent than did colestipol hydrochloride. The binding of water-soluble drugs to colestipol hydrochloride decreased in the combined presence of monoolein, oleic acid, and taurocholate. The colestipol hydrochloride binding of compounds of low aqueous solubility, vitamins A_1, D_2, and K_1, and cholesterol-^{14}C increased as the concentration of taurocholate decreased. Monoolein increased, while oleic acid decreased, the binding of taurocholate to colestipol hydrochloride. Oleic acid increased slightly the binding of taurocholate to cholestyramine; monoolein had no effect. The data are consistent with the hypotheses that: *(a)* taurocholate associates with and thus transfers compounds of low aqueous solubility to polymer binding sites, *(b)* the fraction of vitamin A_1 and cholesterol-^{14}C associated with the polymer binding sites and the fraction in solution depends upon the concentration of taurocholate, and *(c)* the binding of taurocholate depends upon the composition of micelle formed.

Keyphrases □ Colestipol hydrochloride—*in vitro* binding to various drugs, effects of ionic strength, pH, and competing ion, compared to cholestyramine □ Binding—colestipol hydrochloride and various drugs *in vitro*, effects of ionic strength, pH, and competing ion, compared to cholestyramine □ Drug binding—colestipol hydrochloride and various drugs *in vitro*, effects of ionic strength, pH, and competing ion, compared to cholestyramine □ Cholesterol-reducing agents—*in vitro* binding of various drugs to colestipol hydrochloride

Colestipol hydrochloride is a copolymer that indirectly lowers serum cholesterol in experimental animals and humans and is believed to do so through binding bile acids and their conjugates in the GI tract (1). Cholestyramine (a bile salt-sequestering agent) interferes with the absorption of thyroxine in humans (2) and other drugs in rats and dogs (3). It was conceivable that concurrently ingested drugs would be similarly bound to colestipol hydrochloride and thus be poorly absorbed.

To identify potential drug–polymer interaction problems prior to *in vivo* binding studies, the comparative *in vitro* binding to colestipol hydrochloride and cholestyramine was determined for different drugs. Initial drug–polymer ratios typical of clinical usage were employed under experimental conditions (pH and ionic strength) approximating those of the distal portion of the ileum, one of the later absorbing sites in the GI tract.

EXPERIMENTAL

Materials—Sodium taurocholate-7-^{3}H had a specific activity of 46.3 mCi/mmole[1], and sodium taurocholate-24-^{14}C had a specific activity of 6.6 μCi/mmole[2]. Unlabeled sodium taurocholate[3] was used as carrier. These compounds were homogeneous by TLC. Tetracycline-7-^{3}H hydrochloride (1200 mCi/mmole[4]), niacin-6-^{14}C (26.2 mCi/mmole[4]), D-biotin-carbonyl-^{14}C (57.5 mCi/mmole[4]), chlorpropamide-^{35}S (1.8 mCi/mmole[4]), potassium benzyl penicillin-^{14}C (20–40 mCi/mmole[4]), phenobarbital-2-^{14}C (2.97 mCi/mmole), and cholesterol-26-^{14}C (46 mCi/mmole[5]) were of 95–99% radiochemical purity as determined by paper or thin-layer chromatography.

The binding of these compounds to colestipol hydrochloride[6] and to cholestyramine[7] was studied.

[1] Supplied by Nuclear Research Chemicals Co., Orlando, Fla.
[2] Prepared by Dr. R. C. Thomas, The Upjohn Co., Kalamazoo, Mich.
[3] Grade A, Calbiochem Co., Los Angeles, Calif.
[4] Amersham/Searle Co., Chicago, Ill.
[5] New England Nuclear, Boston, Mass.
[6] Colestid, supplied by The Upjohn Co., Kalamazoo, Mich.
[7] Cuemid, Merck, Sharp and Dohme, West Point, Pa.

The following buffers (all concentrations are final concentrations) were used: sodium phosphate containing 0.15 *M* phosphate with μ = 0.4 and pH 7.5; sodium phosphate containing 0.23 *M* phosphate with μ = 0.4 and pH 6.5; sodium acetate containing 0.15 *M* sodium acetate adjusted to pH 6.0 with acetic acid; imidazole hydrochloride containing 0.15 *N* HCl adjusted to the following pH's with imidazole, pH 5.95, 6.58, 6.98, 7.47, and 7.96; tromethamine–sodium chloride containing 0.05 *N* HCl and 0.1 *N* NaCl adjusted to pH 7.5 with tromethamine (Buffer I, μ = 0.15). The buffer employed in a particular experiment is indicated in the tables.

Apparatus—The UV spectra of free unlabeled drugs were measured with a spectrophotometer[8]. The radioactivity of free labeled drugs was measured with scintillation counters[9].

Methods—Fifteen milliliters of the appropriate buffer, 100 mg of colestipol hydrochloride (or cholestyramine), and an amount of bile acid conjugate (or drug whose binding was to be tested) were added to a 50-ml glass-stoppered centrifuge tube (round bottom).

The amount of sequestrant and drug added corresponded to 1/150 of the daily human dose (unlabeled drug was added to labeled drug to make up the required amounts). The samples were vigorously mixed at 275 oscillations/min during equilibration. Periodically, the shaker[10] was stopped and the tubes were shaken manually so that grains of resin adhering to the sides of the tubes were washed down. In most experiments, the samples were equilibrated for 1 hr at 23.7°. Other times are indicated in the text.

After equilibration, the samples were centrifuged at about 1000×*g* for 5 min. With radioactive samples, 0.5 ml of supernate was mixed with 15 ml of scintillator[11] [toluene–dioxane–methanol (350:350:210 by volume) containing 73 g of 2,5-diphenyloxazole and 0.08 g of 1,4-bis-2-(5-phenyloxazolyl)benzene/liter] and counted. For the zero time control, 0.5 ml of solution that had not been exposed to polymer was counted. For the unlabeled drugs, *e.g.*, vitamin A, the amount of drug in the supernate was measured by the absorbance of the solution at the wavelength of maximum absorptivity. The percent drug bound (B) at various equilibration times was calculated according to the formula:

$$B = 100(1 - C/C_0) \qquad \text{(Eq. 1)}$$

where C is the number of radioactive counts or absorbance (concentration) of free drug in the supernate after equilibration, and C_0 is the radioactive count or absorbance (concentration) in the absence of polymer.

RESULTS AND DISCUSSION

The binding of various drugs was studied in different buffers, at different pH values, and, in some cases, at different concentrations of drug. As model compounds, aspirin and taurocholate were studied more intensively.

Binding of Taurocholate at Different Equilibration Times—The binding of taurocholate to colestipol hydrochloride was sufficiently rapid that the binding process was about 94% complete in 60 min (Buffer I, μ = 0.15 and pH 7.5). Equilibration was established in about 3 hr (Fig. 1), and the time required was independent of buffer ionic strength in the range studied (0.01–0.25). With cholestyramine, taurocholate required 1–2 hr to establish equilibrium (Fig. 2). To approximate the degree of equilibration that might be encountered *in vivo*, the equilibration time in most experiments was limited to 1 hr.

Ionic Strength—The binding of taurocholate was found to decrease with increasing ionic strength (2) (Figs. 1 and 2) and was probably due to competition of the other ions for the same sites or to shielding. Such relationships with ionic strength suggest that at least some binding occurs between oppositely charged particles (4). Since taurocholate is negatively charged at pH 7.5, colestipol hydrochloride and cholestyramine must be positively charged according to this hypothesis. In the case of cholestyramine, this expectation is confirmed since it is a copolymer of styrene cross-linked

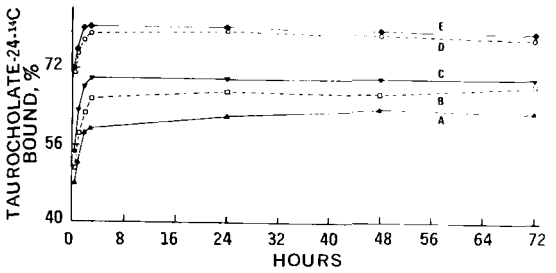

Figure 1—*Effect of ionic strength at pH 7.5 on the binding of taurocholate to colestipol hydrochloride. Key: A, μ = 0.25, tromethamine, 0.05 N HCl, 0.2 N NaCl; B, μ = 0.15, phosphate; C, μ = 0.15, tromethamine, 0.05 N HCl, 0.1 N NaCl; D, μ = 0.05, tromethamine, 0.05 N HCl; and E, water.*

with 2% divinylbenzene[12] and is a strongly basic anion-exchange resin with quaternary ammonium functional groups (5). The ion-exchange group in colestipol hydrochloride can be considered to be weakly basic secondary and tertiary amines.

An ionic strength of 0.15 approximates the lower limit of ionic strength of GI secretions. Gastric juice, gastric mucus, pancreatic juice, hepatic duct bile, and jejunal secretions are nearly isotonic (6) with blood plasma. Almost all of the osmotic pressure is contributed by monovalent ions—*viz.*, Na^+, H^+, Cl^-, and HCO_3^-. Thus, the lower limit of the ionic strength of GI secretions is approximately equal to that of physiological saline. The contribution of polyvalent ions such as proteins to ionic strength could be substantial (about 0.1) but would depend upon the kind of protein, pH, and ion concentration. Ingested substances would further alter the ionic strength to many other values. An ionic strength of 0.15 was employed as a standard condition.

Binding of Taurocholate and Aspirin at Different pH's—To study the effect of pH on the binding of a drug and bile acid, the binding of aspirin and taurocholate was studied in imidazole hydrochloride buffers (ionic strength of 0.15). The pH values were varied from 5.95 to 7.96. The effect of pH on taurocholate was much greater than on aspirin. As the pH was increased over this range, aspirin binding (60-min values) to colestipol hydrochloride decreased from 28.3 to 20.9%. For taurocholate, the binding (60-min values) in this system decreased from 20.4 to 7% as the pH was increased over the same range.

As shown in Fig. 3, the data suggested that a weak basic ionizing group in colestipol hydrochloride, with a pKa in the region of pH 7.2–7.3, was involved in the binding of taurocholate and aspirin. At pH 7.5, the binding contribution by this group was relatively minimal. Since some residual binding was still present around pH 8, some groups could be strongly basic amines or quaternary nitrogens.

In fasting humans, pH's between 2.5 and 7.5 in the duodenum, between 6.5 and 7.5 in the jejunum, and between 3.5 and 8 in the ileum have been recorded (7). For fed humans, the pH values ranged from 5.3 to 8 in these regions but tended to increase from 6

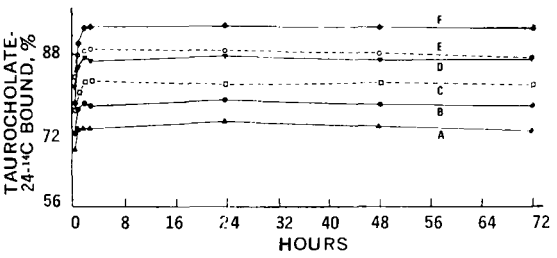

Figure 2—*Effect of ionic strength at pH 7.5 on the binding of taurocholate to cholestyramine. Key: A, μ = 0.25, tromethamine, 0.05 N HCl, 0.2 N NaCl; B, μ = 0.15, tromethamine, 0.05 N HCl, 0.1 N NaCl; C, μ = 0.10, tromethamine, 0.05 N HCl, 0.05 N NaCl; D, μ = 0.05, tromethamine, 0.05 N HCl; E, μ = 0.15, phosphate; and F, water.*

[8] Hitachi model 124, supplied by Coleman Instruments Co., Maywood, Ill.

[9] Models 3375 and 314EX2A, Packard Instrument Co., Downers Grove, Ill.

[10] Utility shaker, Clay Adams, New York, N.Y.

[11] Diotol, supplied by Burdick and Jackson, Muskegon, Mich.

[12] Dowex 1-X2.

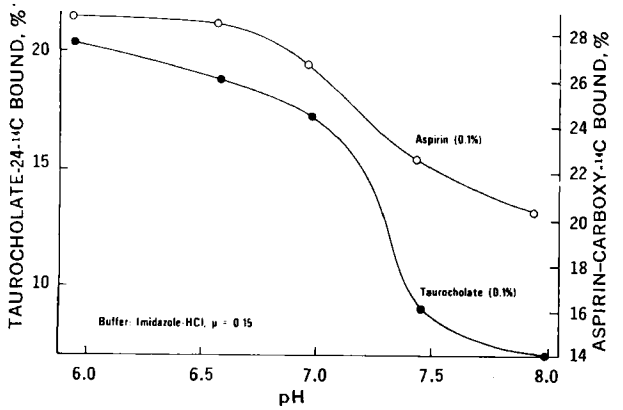

Figure 3—*Binding to colestipol hydrochloride as a function of pH.*

to 7 or 8 as the intestinal contents proceeded from the duodenum to the lower ileum (6). Binding of *ionized* acidic compounds is expected to be greater at the lower pH's and would be dependent on the relative positions of the compound and colestipol hydrochloride in the GI tract.

Weak acids, which must be unionized to be efficiently absorbed, should tend to be bound less to colestipol hydrochloride when the pH in the tract is below the pKa of the weak acid. It is expected that drugs bound at the lower pH's (when the drug–polymer combination is at positions in the tract near the stomach) would tend to be released as lower portions of the small intestine and higher pH's are reached. If a drug is more strongly bound at the higher pH's, then the determination at the higher pH's would be an estimate of the maximum binding.

A pH of 7.5 was chosen to estimate the amount of drug binding that might be expected at the distal portion of the ileum, where bile acid conjugate absorption takes place. Since absorption of the drug may have taken place at prior points in the GI tract, this binding value represents the "last chance" binding for drug not previously absorbed and is an estimate of the upper limit of the *dose* of the drug bound.

Aspirin Binding as a Function of Aspirin Concentration—The percent binding of taurocholate to colestipol hydrochloride is highly concentration dependent. It was of interest to determine if the binding of aspirin (a model compound) to colestipol hydrochloride would also be concentration dependent in the same range, 1.85–46.3 m*M*. In this concentration range, the binding of aspirin

Table I—Effect of Sodium Taurocholate, Monoolein, and Oleic Acid on Drug Binding to Colestipol Hydrochloride

Drug	Input Concentration, mg/ml	Percent Drug Bound after 1 hr Equilibration: Buffer Alone[a]	Percent Drug Bound after 1 hr Equilibration: Buffer + Sodium Taurocholate, Monoolein, and Oleic Acid
Chlorpropamide-^{35}S	0.16	6.4	−5.9
Niacin-6-^{14}C	0.13	5.5	2.3
Biotin-^{14}C	0.05	0.5	−1.7
Aspirin (UV 266 nm)	1.67	10.8[b]	—[c]
Salicylic acid (UV 229 nm)	1.67	28.8	—[c]
Ascorbic acid (UV 264 nm)	0.553	4.5	—[c]
Benzyl penicillin-^{14}C	0.27	20.0	10.6
Tetracycline-7-^{3}H hydrochloride	0.27	48.3	30.7

[a] Buffer: tromethamine, 0.05 *N* HCl, and 0.1 *N* NaCl with a final pH of 7.5. [b] This value was corrected for the 10.5% (relative to the initial molar concentration of aspirin) salicylic acid formed. [c] Experiment not run.

Table II—Effect of Sodium Taurocholate, Monoolein, and Oleic Acid on Drug Binding to Cholestyramine

Drug	Concentration, mg/ml	Percent Drug Bound after 1 hr of Equilibration: Buffer Alone[a]	Percent Drug Bound after 1 hr of Equilibration: Buffer + Sodium Taurocholate, Monoolein, and Oleic Acid
Chlorpropamide-^{35}S	0.16	53.5	31.8
Niacin-6-^{14}C	0.13	9.4	−0.8
Biotin-^{14}C	0.05	11.8	−1.1
Aspirin (UV 266 nm)	1.67	17.0[b]	—[c]
Salicylic acid (UV 229 nm)	1.67	60.2	—[c]
Ascorbic acid (UV 264 nm)	0.553	6.4	—[c]
Benzyl penicillin-^{14}C	0.27	43.8	17.6
Tetracycline-7-^{3}H hydrochloride	0.27	55.8	42.8

[a] Buffer: tromethamine, 0.05 *N* HCl, and 0.1 *N* NaCl with a final pH of 7.5. [b] This value was corrected for the 3.5% (relative to the initial molar concentration of aspirin) salicylic acid formed. [c] Experiment not run.

(together with any small amount of salicylic acid formed) to colestipol hydrochloride (average 8.9%, range 8.1–9.4% bound, n = 11) was not greatly concentration dependent, if at all.

This finding may indicate that aspirin (unlike taurocholate or glycocholate) does not undergo transformation to a new molecular species (such as micelles or dimers) when the concentration is increased and/or that the new molecular species have the same selectivity coefficient.

Since the binding of drugs to the copolymers is expected to be concentration dependent, the binding was studied where the relative amounts of drug to copolymer approximate the ratio of the daily therapeutic dose of drug and copolymer (except for compounds with assay end-points of low absorptivity and where higher concentrations were required).

Choice of Suitable Buffer for Drug Comparisons—Ion-exchange resins bind some ions more than others and, on the basis of mass action, another ion can displace a given ion from a binding site. Therefore, drug binding could vary with the ionic environment. The initial objective was to find a buffer that would satisfactorily control pH and not greatly inhibit binding.

Chlorpropamide-^{35}S, niacin-6-^{14}C, biotin-^{14}C, ascorbic acid, aspirin, phenobarbital-^{14}C, and taurocholate-24-^{14}C were bound to colestipol hydrochloride to an extent less than or equal to 6.2% in sodium phosphate buffer at pH 6.5 or 7.5 (ionic strength equal to 0.4). Binding of the drugs tended to be less at the lower pH. The binding of taurocholate greatly increased (from an average of 0.9 to 21.9%) when the ionic strength of the phosphate buffer was reduced from 0.4 to 0.004 (pH 6.5).

At the same ionic strength and pH (μ = 0.15 and pH 7.5), sodium phosphate buffer reduced taurocholate binding to colestipol hydrochloride more than Buffer I (Fig. 1). When cholestyramine was used as a binding agent, the reverse was true. On an equimolar basis, however, the presence of phosphate greatly reduced taurocholate binding to both colestipol hydrochloride and cholestyramine below that found when other anions (chloride or acetate) were substituted. Since the binding of most compounds in phosphate buffers appeared to be relatively low, another buffering system was sought as a standard system for drug comparisons.

The binding of drugs to colestipol hydrochloride and cholestyramine was studied in Buffer I at pH 7.5 and μ = 0.15 (Tables I and II). The binding of the drugs to colestipol hydrochloride varied from zero for lincomycin hydrochloride to as high as 59% for warfarin. Except for warfarin and tetracycline hydrochloride, less than 30% of the initial inputs of the drugs was bound to colestipol hydrochloride. In this system, the drugs studied were more greatly bound by cholestyramine than by colestipol hydrochloride (Tables I and II).

The relatively strong binding of tetracycline to the polymers is

Table III—Effect of Monoolein, Oleic Acid, and Taurocholate on the Binding of Phenobarbital to Colestipol Hydrochloride

Compounds Present[a]					Taurocholate Binding		Phenobarbital Binding	
Colestipol	Phenobarbital	Taurocholate	Monoolein	Oleic Acid	Free, dpm	% Bound	Free, dpm	% Bound
+	+	−	−	−	—	—	6315	14.3
+	+	+	−	−	20,436	55.8	6250	15.2
+	+	+	+	−	18,235	60.6	6305	14.5
+	+	+	+	+	29,724	35.7	6353	13.8
+	+	−	−	+	—	—	6277	13.8
+	−	+	−	−	20,378	55.9	—	—
+	−	+	+	−	18,098	60.9	—	—
+	−	+	+	+	29,497	36.2	—	—
+	−	+	−	+	29,922	35.3	—	—
+	+	+	−	+	29,505	36.2	6357	13.7
−	+	+	−	−	46,258	—	7370	—

[a] Plus = compounds added; minus = compounds not added. Final concentrations of the compounds added were: 0.5% sodium taurocholate-^{3}H, 0.00067% phenobarbital-^{14}C, 0.025% monoolein, and 0.140% oleic acid. The solutions were buffered by tromethamine, 0.067 *N* HCl, and 0.067 *M* NaCl at pH 7.5. There was 100 mg of colestipol hydrochloride in each 15 ml of solution.

Table IV—Effect of Monoolein, Oleic Acid, and Taurocholate on the Binding of Phenobarbital to Cholestyramine

Compounds Present[a]					Taurocholate Binding		Phenobarbital Binding	
Cholestyramine	Phenobarbital	Taurocholate	Monoolein	Oleic Acid	Free, dpm	% Bound	Free, dpm	% Bound
+	+	−	−	−	—	—	4257	42.2
+	+	+	−	−	12,715	72.5	5203	29.4
+	+	+	+	−	11,835	74.4	5134	30.3
+	+	+	+	+	12,671	72.6	5650	23.3
+	+	−	−	+	—	—	4570	38.0
+	−	+	−	−	12,270	73.4	—	—
+	−	+	+	−	12,350	73.3	—	—
+	−	+	+	+	11,759	74.6	—	—
+	−	+	−	+	11,164	75.9	—	—
+	+	+	−	+	12,771	72.3	5581	24.2
−	+	+	−	−	46,258	—	7370	—

[a] Plus = compounds added; minus = compounds not added. Final concentrations of the compounds were: 0.5% sodium taurocholate-^{3}H, 0.00067% phenobarbital-^{14}C, 0.025% monoolein, and 0.140% oleic acid. The solutions were buffered by 0.067 μ tromethamine, 0.067 *N* HCl, and 0.067 *M* NaCl at pH 7.5. There was 100 mg of cholestyramine for each 15 ml of solution.

interesting since tetracycline would only be partially negatively charged at pH 7.5 with pKa's at 7.82 and 9.57 (8). Whether the binding involves the nitrogen hydrogens of the polymers and the two 1,3-diketones of tetracycline to form rings through ionic, hydrogen bonding, and van der Waals' forces is not known. Another possibility is that the ionized tetracycline molecules form relatively insoluble salts with the polymers and the binding reaction continues as additional tetracycline associates until a final equilibrium is established.

The final pH's after equilibration of the drugs and the polymers was within 0.1–0.2 of a pH unit from pH 7.5. Thus, the Buffer I system was considered satisfactory and was employed in further studies.

Table V—Effect of Tromethamine, Monoolein, and Oleic Acid on Taurocholate-^{3}H Binding to Colestipol Hydrochloride[a]

T	Sodium Chloride	Monoolein	Oleic Acid	Final pH	Supernatant Radioactivity, dpm	Percent Bound
−	2+	+	+	8.95	19,614	17.7
+	+	+	+	7.90	15,219	36.2
+	+	−	−	8.00	17,655	26.0
+	+	−	+	7.90	22,624	5.1
+	+	8+	−	7.90	16,379	31.3
+	+	−	−	8.10	23,844[b]	—

[a] The 15 ml of solution consisted of 0.05 *M* $NaHCO_3$ plus the following compounds. T: plus is 0.05 *N* HCl + tromethamine to pH 7.5; sodium chloride: plus is 0.05 *N*, 2+ is 0.1 *N*; monoolein: plus is 3.8 mg (0.025% final concentration), 8+ is 26.4 mg (0.176% final concentration); oleic acid: 21 mg (0.140% final concentration); sodium taurocholate: 40 g (4.96 m*M* or 0.267% final concentration). Minus is no addition of the compound was made. [b] No copolymer present.

Reproducibility of Binding Experiments—To obtain an estimate of the precision of two different levels of drug binding, advantage was taken of the fact that taurocholate binding to colestipol hydrochloride is dependent on taurocholate concentration. For 15% binding, the standard deviation was 1.0% (absolute); for 55% binding, the standard deviation was 2.7%.

Effect of Taurocholate, Monoolein, and Oleic Acid on Drug Binding: Binding of Aqueous Soluble Compounds—Since taurocholate, monoolein, and oleic acid together form micelles (9), it was of interest to determine if the binding of some drugs would be increased by the presence of these substances. As shown in Tables I and II, chlorpropamide-^{35}S, niacin-6-^{14}C, biotin-^{14}C, benzyl penicillin-^{14}C, and tetracycline-7-^{3}H hydrochloride were each bound less by cholestyramine or colestipol hydrochloride when sodium

Table VI—Effect of Tromethamine, Monoolein, and Oleic Acid on Taurocholate-^{3}H Binding to Cholestyramine[a]

T	Sodium Chloride	Monoolein	Oleic Acid	Final pH	Supernatant Radioactivity, dpm	Percent Bound
−	2+	+	+	8.88	4199	82.7
+	+	+	+	8.08	4237	82.2
+	+	−	−	8.09	5955	75.0
+	+	−	+	8.95	4099	82.8
+	+	8+	−	8.10	6026	74.7
+	+	−	−	8.10	23,844[b]	—

[a] The 15 ml of solution consisted of 0.05 *M* $NaHCO_3$ plus the following compounds. T: plus is 0.05 *N* HCl + tromethamine to pH 7.5; sodium chloride: plus is 0.05 *N*, 2+ is 0.1 *N*; monoolein: plus is 3.8 mg, 8+ is 26.4 mg; oleic acid: 21 mg (0.140% final concentration); sodium taurocholate: 40 mg (4.96 m*M* or 0.267% final concentration). Minus is no addition of the compound was made. [b] No resin present.

Table VII—Colestipol Hydrochloride Precipitation or Binding of Fat-Soluble Vitamins Dissolved in Micelles

Vitamin	Initial Concentration, μg/ml	Equilibration Time, hr	Percent Taurocholate-^{3}H[a] Bound	Percent Vitamin Bound or Precipitate[b]
A_1[c]	8.8	1.0	60.7	96
		2.0	63.8	96
D_2[c]	9.1	1.0	60.5	104
		2.0	64.2	99
K_1[d]	1300	1.0	62.7	58
		2.0	66.0	85

[a] ^{3}H radioactivity. [b] Absorbance of the free vitamin was measured. [c] Solubilizing agent: 1% sodium taurocholate-^{3}H in Buffer I (μ = 0.15 and pH 7.5). [d] Solubilizing agent: 1% sodium taurocholate-^{3}H and 2.0 mg/ml monoolein in Buffer I (μ = 0.15 and pH 7.5).

taurocholate, monoolein, and oleic acid were present in combination. Taurocholate, monoolein, and oleic acid, alone or in combination, were tested as factors in altering phenobarbital binding to cholestyramine or to colestipol hydrochloride. Both taurocholate ($p < 0.05$) and oleic acid ($p < 0.01$) significantly reduced (Table IV) binding of phenobarbital to cholestyramine. Only oleic acid was significant ($p < 0.01$) in reducing the binding of phenobarbital to colestipol hydrochloride (Table III).

The decreased binding could be the result of decreased availability of binding sites (for anions) on the polymers, *i.e.*, a reduction in the number of binding sites and/or polarity of the medium. These results plus those shown in Tables I and II indicated that the *in vivo* binding of these water-soluble drugs may be expected to decrease when ingested together with fatty foods.

The separate and combined effects of monoolein and oleic acid on the binding of taurocholate were investigated. Taurocholate binding to colestipol hydrochloride increased with the composition of the media as follows (Tables III and V): buffer + oleic acid < buffer + monoolein + oleic acid < buffer < buffer + monoolein. Oleic acid decreased ($p < 0.01$), while monoolein increased ($p < 0.05$), the binding of taurocholate to colestipol hydrochloride. The data are compatible with: *(a)* greater binding of taurocholate as micelles to colestipol hydrochloride, with taurocholate micelles having less affinity than taurocholate–monoolein micelles; and *(b)* oleic acid competing for the binding sites of colestipol hydrochloride. The results suggest that the *in vivo* binding of taurocholate may be increased by monoglycerides derived from food and decreased by fatty acids.

The binding of taurocholate to cholestyramine was less affected by the presence of monoolein or oleic acid (Table IV) with taurocholate at a concentration of 0.5%. The range of binding values was only 3.6% (median 73.4%). At the lower concentration of 0.267%, the range of values was 8.1% (median 82.2%) (Table VI). The greatest binding was observed when oleic acid was present ($p < 0.01$) with or without monoolein.

Such observations are compatible with oleic acid increasing slightly the binding of taurocholate as micelles of taurocholate–oleic acid or taurocholate–oleic acid–monoolein. Monoolein alone did not affect ($p > 0.05$) the binding of taurocholate to cholestyramine. Thus, under these conditions, micelles do not play as great a relative role in the binding of taurocholate to cholestyramine and/or their affinity for cholestyramine is not greatly different from single taurocholate molecules.

Table VIII—Cholestyramine Precipitation or Binding of Fat-Soluble Vitamins Dissolved in Micelles

Vitamin	Initial Concentration, μg/ml	Equilibration Time, hr	Percent Taurocholate-^{3}H[a] Bound	Percent Vitamin Bound or Precipitated[b]
A_1[c]	8.8	1.0	73.4	96
		2.0	74.2	94
D_2[c]	9.1	1.0	74.0	106
		2.0	75.4	104
K_1[d]	1300	1.0	75.1	81
		2.0	76.0	82

[a] ^{3}H radioactivity. [b] Absorbance of the free vitamin was measured. [c] Solubilizing agent: 1% sodium taurocholate-^{3}H in Buffer I (μ = 0.15 and pH 7.5). [d] Solubilizing agent: 1% sodium taurocholate-^{3}H and 2.0 mg/ml monoolein in Buffer I (μ = 0.15 and pH 7.5).

Table IX—Effect of Different Concentrations of Taurocholate on Colestipol Hydrochloride Precipitation or Binding of Vitamin A_1

Supernatant Taurocholate Concentration[a]		Colestipol Hydrochloride, mg	Vitamin A_1 in Supernate: Absorbance minus Blank	Percent Bound
Input, %	After Equilibration, %			
1.0	0.35–0.38	100.0	0.019	98.7
1.0	1.0	0.0	1.46 (9.4 μg/ml)	
2.5	1.75	100.0	1.29	16.2
2.5	2.5	0.0	1.54 (9.9 μg/ml)	

[a] Buffer I (μ = 0.15 and pH 7.5).

Binding of Compounds with Low Aqueous Solubility—The binding of a substance is difficult to study in water when that substance is not very water soluble, *e.g.*, fat-soluble vitamins, cholesterol, fats, and nonpolar drugs.

Bile salts have been implicated in the GI absorption of lipid-soluble vitamin D (10, 11), fats (12, 13), and certain drugs of low aqueous solubility (14). Bile salts have been postulated to increase the aqueous solubility of a compound by forming micelles which entrap or envelop the otherwise "insoluble" compound. Conceivably, such micelles could be bound by an ion-exchange resin.

Vitamins A_1, D_2, and K_1—The possible binding of the lipid-soluble vitamins to colestipol hydrochloride and cholestyramine in the presence of bile salts was studied. Sodium taurocholate (1%, about 18 m*M*) was added to solubilize vitamins A_1 and D_2. The further addition of monoolein to taurocholate was required to improve the solubility of vitamin K_1. After equilibration with colestipol hydrochloride and cholestyramine, the vitamins were almost entirely absent from the supernates in both copolymer experiments (Tables VII and VIII). Such results could occur if the vitamins were being tightly bound by the copolymers and/or if the water solubility of the vitamin–taurocholate micelles were being lowered by the loss of taurocholate in the supernates to the copolymers.

To check whether the amount of taurocholate was a factor, the removal of vitamin A_1 from the supernate was measured in the presence of 1 and 2.5% (initial concentration) sodium taurocholate plus 100 mg of colestipol hydrochloride (Table IX). If the final taurocholate concentration were not a factor limiting the solubility of the vitamin–taurocholate micelles, nearly quantitative binding would be expected for both concentrations of taurocholate. With the higher initial concentration of 2.5% taurocholate, the removal

Table X—Extent of Vitamin A_1 Precipitation when the Concentration of Sodium Taurocholate is Lowered

Final Sodium Taurocholate Concentration, %	Vitamin A_1 Absorbance of the Solution		Percent Vitamin A_1 Precipitated Relative to Initial Absorbance of 2.5% Solution (1.464)
	After Dilution[a]	After Shaking (1 hr)	
1.75	1.46 (9.4 μg/ml)	1.45	0.7
1.00	1.46	1.45	1.0
0.40	1.35	1.17	20.1

[a] All solutions had equal inputs of vitamin A_1; Buffer I (μ = 0.15 and pH 7.5).

Table XI—Effect of Different Concentrations of Taurocholate on Precipitation or Binding of Cholesterol-^{14}C to Colestipol Hydrochloride

Input Supernatant Sodium Taurocholate Concentration[a], %	Colestipol Hydrochloride, mg	^{14}C dpm minus Background[b]	Percent Binding or Precipitation
0.4	0.0	580	8.2
1.00	0.0	637	−0.8
1.75	0.0	615 } Mean = 632	2.7 } Mean = 0%
2.50	0.0	643	−1.7 (by definition)
1.00	100.0	20	96.8
2.50	100.0	454	28.2

[a] Buffer I (μ = 0.15 and pH 7.5). [b] Input cholesterol concentration: 4 ng/ml.

of vitamin A_1 from the supernate was reduced to only 16.2%; therefore, taurocholate concentration was a factor in the removal of vitamin A_1 from the supernate.

Since the taurocholate concentration in the supernate decreased from 2.5% to about 1.75% after equilibration, an adequate amount of taurocholate was present for retaining the vitamin in a micellar solution. Thus, the 16.2% removal of vitamin A_1 from the supernate with the higher taurocholate concentration likely represents nonionic adsorption to the taurocholate–colestipol hydrochloride surface or binding of taurocholate–vitamin A micelles to colestipol hydrochloride.

About 30% taurocholate was bound when the initial concentration was 2.5%. If the initial ratio of vitamin A_1 to taurocholate were maintained after equilibration with the copolymer, then the binding of vitamin A_1 would also have been 30%. Since the binding was less than this value, the act of binding taurocholate to the copolymer decreased the micellar association of vitamin A_1 with the taurocholate in the copolymer.

Since the presence of taurocholate helped to solubilize vitamin A, the extent that vitamin A_1 precipitated external to the copolymer due to the removal of taurocholate was estimated by the following experiment. Vitamin A_1 was dissolved in 7.5% sodium taurocholate and diluted into 0.4, 1.0, and 1.75% sodium taurocholate (final concentrations) with Buffer I (μ = 0.15 and pH 7.5). The 0.4 and 1.75% values corresponded to the equilibrated supernatant sodium taurocholate concentrations that would be obtained when 1.0 and 2.5% sodium taurocholate solutions are equilibrated with 100 mg of colestipol hydrochloride.

The initial input of vitamin A_1 was chosen to approximate the concentrations employed in the "binding" experiment. If the binding phenomenon previously observed had been due to precipitation of vitamin external to the copolymer, then almost quantitative vitamin A_1 precipitation should occur for the 0.4% sodium taurocholate concentration, and 16% vitamin A_1 precipitation should occur for the 1.75% sodium taurocholate concentration.

The results (Table X) indicated that less vitamin A_1 precipitated in the absence of colestipol hydrochloride than would be expected if the binding observed in the presence of the copolymer were due only to precipitation external to the copolymer. The vitamin A_1 remaining in the supernate in the absence of colestipol hydrochloride is an indication of the solubility of vitamin A_1 in the presence of the different concentrations of sodium taurocholate. The final concentrations of vitamin A_1 left in the supernate after equilibration with colestipol hydrochloride (Table XI) do not exceed the solubility limit of vitamin A_1 at any of the final concentrations of taurocholate in the supernate. Thus, the values for the "percent bound" represent the amount bound to the copolymer only and no precipitation of the vitamin likely occurred external to the copolymer under the experimental conditions.

In the GI tract, the presence of ingested lipids should help solubilize vitamin A and hence reduce any precipitation of the fat-soluble vitamin external to colestipol hydrochloride. The binding of the vitamin to the copolymer depends on the concentration of bile salts present and the competition for copolymer–bile salt sites by other lipids.

Cholestyramine bound vitamin D_3-^{3}H to a considerable extent *in vivo* and *in vitro* (10). *In vitro*, 100% of vitamin D_3-^{3}H was bound by cholestyramine (100 mg) in the presence of taurocholate (20 m*M*). However, further additions of linoleic acid and monoolein decreased the amount of the vitamin bound. At levels of cholestyramine sufficient to cause steatorrhea, absorption of vitamin D_3-^{3}H in rats was decreased from a control value of 65 ± 4% to 28 ± 14.7% ($p < 0.001$). In dicumarol-treated dogs, low doses of cholestyramine (201 mg/kg/day) did not affect absorption of vitamin K_1, but doses of 1 g/kg and greater decreased and delayed absorption of the vitamin (15).

When 2% cholestyramine (equivalent to 12–15 g/day as percentage of food intake in humans) was added to a diet marginal in vitamin K activity, prothrombin times of chicks slightly lengthened after 2 weeks but not after 4 weeks (16). Vitamin K stores were lower in chicks fed cholestyramine. Body weight gains of weanling rats were reduced when 2% cholestyramine was added to their high-fat diet containing only growth-limiting amounts of vitamin A. When larger dietary amounts of vitamin A were given, no effect on weight gains was observed but liver storage of the vitamin was reduced. Thus, vitamin deficiency was avoided when the vitamin intake was more than minimal (16).

Although the *in vitro* and *in vivo* binding of lipid-soluble compounds by bile acid sequestrants can occur, clinical signs of deficiency are uncommon at therapeutic hypocholesterolemic doses (17, 18). No abnormalities in prothrombin times or other evidence of fat-soluble vitamin deficiencies occurred in healthy male subjects on normal diets and 12–15 g of colestipol hydrochloride/day[13]. After a year of colestipol hydrochloride administration to dogs at a maximum dose equivalent to approximately 10 times the human therapeutic dose of 15 g/day, no laboratory or clinical signs of fat-soluble vitamin deficiencies occurred (19). The utilization of fat-soluble vitamins was not evidently impaired in weanling rats fed laboratory food[14] containing up to 2000 mg of colestipol hydrochloride/kg daily for 18 months[15].

Cholesterol-^{14}C—The binding or precipitation of cholesterol-^{14}C was studied in the presence of taurocholate (1 and 2.5% initial

Table XII—Recovery of Water-Soluble Drug Bound to Colestipol Hydrochloride after Four Equilibrations with Fresh Buffer

Drug	Input Concentration, mg/ml	Assay	Percent Bound[a] in 1 hr	Percent Recovery after Four Washes[a]
Tetracycline hydrochloride	1.42	UV (361 nm)	30	98
Sulfadiazine	0.0203	UV (255 nm)	23	100
Benzyl penicillin	0.50	^{14}C	23	102
Lincomycin hydrochloride	0.568	^{14}C	0	100
Warfarin	0.070	UV (305 nm)	59	85

[a] Buffer and wash: tromethamine + 0.05 *N* HCl + 0.1 *N* NaCl (pH 7.5).

[13] Dr. K. Gundersen cited in Ref. 19.
[14] Purina.
[15] Dr. H. D. Webster cited in Ref. 19.

concentration) and colestipol hydrochloride (Table XI). Results were similar to those found for the lipid-soluble vitamins. Quantitative precipitation or binding of cholesterol-^{14}C was observed in the presence of an initial taurocholate concentration of 1%, but only 28% was precipitated or bound on or in colestipol hydrochloride in the presence of an initial concentration of 2.5% sodium taurocholate. Only 8% of the cholesterol-^{14}C precipitated in 0.4% sodium taurocholate in the absence of colestipol hydrochloride (the supernatant concentration of sodium taurocholate was 0.4% after 15 ml of initially 1% sodium taurocholate was equilibrated with 100 mg of colestipol hydrochloride).

No cholesterol-^{14}C precipitated in the absence of colestipol hydrochloride when the concentration of sodium taurocholate was reduced to 1.75% (the supernatant concentration of sodium taurocholate after 15 ml of initially 2.5% sodium taurocholate was equilibrated with 100 mg of colestipol hydrochloride). Thus, cholesterol-^{14}C likely did not precipitate and the binding values found represent cholesterol-^{14}C bound to colestipol hydrochloride. The binding is markedly dependent on sodium taurocholate concentration. The binding of cholesterol to colestipol hydrochloride in the presence of bile salt provides another mechanism by which cholesterol may be removed from enterohepatic circulation.

Recovery of Aqueous-Soluble Drugs Bound to Colestipol Hydrochloride—In the GI tract, drugs bound to colestipol hydrochloride would be repeatedly bathed by fluids. Some unbound drug would be continuously removed by GI absorption. This process was simulated by determining the recovery of colestipol-bound drug after repeated equilibration with fresh buffer. Drugs were equilibrated with colestipol hydrochloride in Buffer I for 1 hr, and the amount of unbound drug was determined. The clear portion of the solution was passed through 45-μm filters[16] and the filtrate was saved.

Fifteen milliliters of Buffer I and the filter just used were added to the centrifuge tube containing the colestipol hydrochloride and any drug that was bound. The contents of the centrifuge tube were reequilibrated by shaking for 15 min and then the material was again filtered with a fresh filter. In this manner the colestipol hydrochloride and bound drug were washed four times. The results of such experiments are shown in Table XII. Some of these drugs were among the strongest bound of the aqueous-soluble drugs tested. All were recovered quantitatively in the filtrate except for warfarin, and even warfarin was 85% recovered. Thus, the binding of these drugs to colestipol is completely or largely reversible and colestipol hydrochloride may, to some extent, serve as a depot source of drug.

In the GI tract, where the concentration of free drug is reduced by absorption and the GI contents compete for binding sites, the final percentage of the *dose* of ingested drug remaining on the copolymer at the ileum and beyond is expected to be less than the percentage of drug bound to the copolymer in the *in vitro* experiments (single equilibration). Indeed, Phillips *et al.* (20) found that the area under the serum concentration–time curve of aspirin-^{14}C, sulfadiazine-^{35}S, or phenobarbital-2-^{14}C in rats was not affected by concurrent ingestion of therapeutic doses of colestipol hydrochloride. Three times the therapeutic dose of colestipol hydrochloride was required before the bioavailability of aspirin-^{14}C was decreased by only 15% and those of sulfadiazine-^{35}S and phenobarbital-2-^{14}C were affected little or not at all.

[16] HA Millipore.

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