Drug–Cholestyramine Interactions I: Physicochemical Factors Affecting *In Vitro* Binding of Sodium Fusidate to Cholestyramine

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Abstract □ The *in vitro* binding of the steroidal antibiotic, sodium fusidate, to cholestyramine was investigated. Equilibrium data were interpreted by means of the Langmuir adsorption isotherm, and rate of binding data were interpreted by use of a second-order kinetic model. The results indicated that the anionic exchange resin had a marked affinity for the drug. It was shown that the extent of the fusidate–resin interaction at equilibrium was affected insignificantly by temperature. However, the second-order rate constant governing the binding process was found to increase with increasing temperature, the apparent energy of activation associated with the rate process being 4.47 kcal./mole. The addition of physiologic concentrations of the inorganic electrolytes, sodium chloride and sodium bicarbonate, produced a significant decrease in both the extent and rate of the interaction. The available evidence suggests that the drug–resin interaction involves both primary electrostatic and secondary nonelectrostatic forces. In addition, it was demonstrated that physiologic bile salt and fatty acid anions effectively compete with fusidate anions for the available binding positions on the resin. These *in vitro* findings strongly suggest that the resin may interfere with the normal GI absorption pattern of sodium fusidate.

Keyphrases □ Cholestyramine–sodium fusidate *in vitro* binding—effects of temperature, organic and inorganic electrolytes □ Sodium fusidate–cholestyramine *in vitro* binding—effects of temperature, organic and inorganic electrolytes □ Drug–anionic exchange resin interactions—sodium fusidate and cholestyramine binding

Cholestyramine is a pharmacologically important, quaternary ammonium, anionic exchange resin which has been shown clinically to lower elevated plasma cholesterol and lipid levels (1–5). Its mechanism of action is thought to involve primarily its ability to bind bile salt anions present in the luminal fluids of the small intestine, thus interrupting the normal enterohepatic circulation of these physiologic surfactants (6, 7).

In previous articles (8–10) the authors reported on the results of *in vitro* investigations designed to quantify the binding tendencies of cholestyramine for physiologic bile salt and fatty acid anions and to ascertain the influence of various physicochemical factors thereon. In view of the fact that other drugs may be orally administered during chronic cholestyramine therapy, the possibility exists that drug–cholestyramine interactions could also occur. Such interactions could significantly decrease the GI absorption of these drugs.

The ability of cholestyramine to interact with drug molecules has received limited attention in the literature (11–16). The results of the *in vitro* portions of these investigations permit no conclusions to be drawn as to the possible mechanism(s) or the magnitude of the forces involved in the binding process. In addition, no investigation reported to date has considered the dynamics of the binding process and the potential influence of physiologic inorganic and organic electrolytes on the binding tendencies of the resin for the test drugs.

The objectives of the present investigation were to establish and to quantify the *in vitro* binding tendencies of cholestyramine for a representative drug which could potentially interact with the resin in a direct fashion. In addition, studies were conducted to ascertain the influence of temperature and added inorganic and organic physiologic electrolytes on both the extent and rate of interaction of the test drug with the resin. These *in vitro* studies were designed so as to provide information necessary for an understanding of the binding process *in vivo*, which must take place in the presence of other electrolytic species contained in normal GI fluids.

Sodium fusidate, an antibiotic employed in the treatment of staphylococcus infections, was chosen as the test drug. Chemically, sodium fusidate possesses a cyclopentanoperhydrophenanthrene or steroid nucleus and, in this respect, is structurally similar to the physiologic bile salt anions. This structural similarity was an important consideration in the selection of this particular drug, since it was anticipated that some of our previously reported findings on the bile salt anion–cholestyramine interaction (8–10) could be utilized in the interpretation of, and in comparison with, those obtained in the present drug–resin studies.

EXPERIMENTAL

Materials—The sodium fusidate[1] was protected from light at all times and stored in a vacuum desiccator until used. The sodium salts of taurocholic acid[2], glycodeoxycholic acid[3], and lauric acid[4] were dried *in vacuo* for at least 48 hr. prior to use. The cholestyramine[5] was pharmaceutical grade, the particle size of which was 100% < 100 mesh and 80% < 200 mesh. Reagent grade sodium chloride, concentrated sulfuric acid, glacial acetic acid, hydrochloric acid, acetic anhydride, and chloroform were used as received.

Procedure for Equilibrium Adsorption Studies—A series of aqueous solutions of the antibiotic was prepared over the concentration range of 0.5–6.0 m*M*. Twenty-five-milligram samples of cholestyramine were accurately weighed and placed into 50-ml. glass-stoppered conical flasks, together with a 25.0-ml. portion of the adsorbate solution. At each concentration, a control flask was prepared containing a similar quantity of the solution under investigation but no cholestyramine. These latter control solutions, which were assayed concomitantly with the solutions exposed to cholestyramine, were used to prepare the required Beer's law plots. All flasks were closed securely, protected from light, and mechanically shaken[6] at 25 or 37° until equilibrium was established; this normally occurred within 24–48 hr. The equilibrated samples were subjected to Millipore filtration (0.45-

[1] Generously supplied by E. R. Squibb and Sons, New Brunswick, N. J., Batch B14.
[2] Obtained from Calbiochem, Los Angeles, CA 90063. Grade A.
[3] Obtained from Mann Research Laboratories, New York, NY 10006. Enzymatic grade (reported to be >99% pure by TLC).
[4] Obtained from Eastman Organic Chemicals, Rochester, N. Y.
[5] Generously supplied by Merck & Co., Inc., Rahway, N. J.
[6] Precision Shaker, Precision Scientific Co., Chicago, IL 60647

μ pore size), the filtrates were suitably diluted, and the equilibrium fusidate concentration was determined (see *Assay Procedure*).

The experimental protocol for those studies designed to determine the binding tendencies of cholestyramine for fusidate anions in the presence of added physiologic inorganic and organic electrolytes was essentially the same, with the exception that the desired concentrations of these species were added initially to the binding system.

Procedure for Equilibrium Desorption Studies—To determine the desorption characteristics of the bound adsorbate molecules, 25.0-ml. quantities of each fusidate-containing solution were prepared and shaken with 25.0 mg. of cholestyramine in 125-ml. glass-stoppered conical flasks. After attainment of equilibrium, the samples were diluted with 50.0-ml. portions of distilled water and agitated until equilibrium was again established. After filtration and appropriate dilution, the filtrates were assayed for free fusidate concentration.

In both the equilibrium adsorption and desorption studies, the amount of antibiotic bound to cholestyramine was calculated from the difference between the initial concentration of adsorbate introduced into the system and the concentration present free in solution at equilibrium. All equilibrium binding experiments were performed at least in duplicate, with the amounts of adsorbate bound to the resin falling within the limits of experimental error (a 5% range).

Procedure for Rate of Binding Studies—All binding rate studies were performed using a 250-ml., three-necked, round-bottom flask maintained at either 25, 37, or 47 ± 0.1°. Constant agitation of the reaction medium was accomplished by the use of a constant-torque overhead stirrer[7] (60 ± 1 r.p.m.) equipped with a Teflon-coated, three-blade propeller (blade diameter 5.0 cm.) immersed 3.8 cm. into the reaction medium. In all experiments the concentration of fusidate initially present was maintained at 1.0 m*M*, and an equivalent amount of cholestyramine, based on an equivalent weight for the resin of 230, was employed. Each of the binding rate experiments was performed at least in duplicate, with the rate constants obtained therefrom falling within the limits of experimental error (a 5% range).

In the case of pure aqueous systems, 57.5 mg. of cholestyramine, dry weight, was allowed contact with 240 ml. of deionized distilled water at 37° for 0.5 hr. prior to the introduction of a 10-ml. aliquot of a concentrated stock solution of sodium fusidate. For those studies involving the influence of added secondary physiologic inorganic and organic electrolytes on the rate of binding of fusidate molecules to cholestyramine, identical procedures were followed except that the resin was placed into only 230 ml. of water. After 30 min., 10.0-ml. quantities of concentrated solutions of secondary substances and sodium fusidate were simultaneously added to the reaction flask.

Samples were withdrawn from the reaction flask at appropriate time intervals using a 3-ml. pipet fitted with a glass wool prefilter; the samples were suitably diluted and spectrophotometrically assayed for the concentration of fusidate remaining free in solution.

For each experiment the concentration of primary adsorbate (fusidate) remaining free or unreacted after the reaction had reached completion was determined. Two hundred and fifty milliliter volumes of solutions containing the same concentration of fusidate anion, or fusidate and added secondary substances, and the same amount of cholestyramine as that employed in the rate studies were placed into appropriately sealed bottles and shaken at 37° until equilibrium was established. Equilibration was normally attained with a 24–72-hr. period. These equilibrium data were utilized in the kinetic interpretation of the results obtained in the fusidate–cholestyramine interaction rate experiments.

Assay Procedure—The concentration of sodium fusidate in the aqueous samples was determined by a spectrophotometric method involving use of the Liebermann–Burchard (L–B) reagent[8] (17). An aliquot of the aqueous sample was acidified with 1 *N* HCl, and the free acid was extracted with a 15.0-ml. quantity of chloroform. A 5.0-ml. portion of the chloroform phase was transferred to a test tube, and the chloroform solution was evaporated to dryness in a water bath maintained at 70°. The tubes were then cooled to room temperature, the residue was dissolved in 5.0 ml. of L–B reagent, and the solution was allowed to remain at 25° for exactly 20 min. Subsequently, the tubes were placed into an ice bath until the absorbance of the green-colored sample was read on a colorimeter[9] at 585 nm. using as the blank a water sample carried through the entire assay procedure. Fusidic acid in the L–B reagent was found to obey the Beer–Lambert relationship over the concentration range of 0.03–0.30 m*M*.

The presence of taurocholate, glycodeoxycholate, or laurate anions in the assay samples was found not to interfere with the determination of sodium fusidate. All fusidate-containing solutions were protected from direct exposure to light at all times.

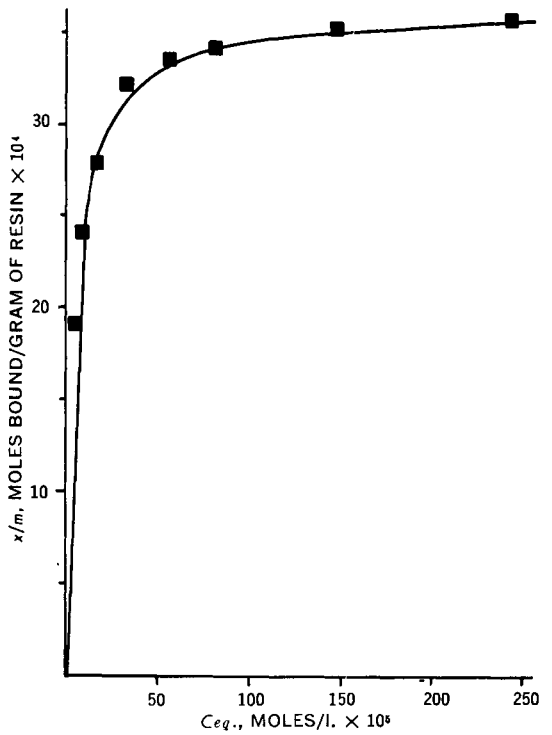

Figure 1—*Adsorption isotherm for the binding of fusidate anion to cholestyramine at 25°.*

RESULTS AND DISCUSSION

All *in vitro* fusidate–resin binding experiments were conducted in unbuffered aqueous solutions. Under these conditions, the pH of the binding systems under investigation, both before and after equilibration with cholestyramine, remained essentially constant and independent of the initial fusidate concentration. The pH was also independent of the type and concentration of various secondary species (*e.g.*, inorganic and organic physiologic electrolytes) initially added to the fusidate–cholestyramine binding system. Based on the pH range of 6.40–7.15 observed following equilibration of cholestyramine with fusidate alone or in the presence of secondary species, and the reported pKa value for sodium fusidate of 5.35 (18), it was determined that the antibiotic was present almost entirely in the ionized form (from 91.8 to 98.5%).

Equilibrium Adsorption Studies—The data for the equilibrium adsorption of sodium fusidate onto cholestyramine at 25°, plotted in accordance with the nonlinear form of the Langmuir adsorption equation (19) given here, is shown in Fig. 1:

$$x/m = \frac{k_1 k_2 C_{eq.}}{1 + k_1 C_{eq.}} \qquad \text{(Eq. 1)}$$

where $C_{eq.}$ is the concentration of unbound fusidate present in solution at equilibrium; x/m is the amount of fusidate bound per

[7] Cole-Parmer Co., Chicago, Ill.

[8] The L-B reagent was composed of 25 ml. of concentrated sulfuric acid, 75 ml. of glacial acetic acid, and a sufficient quantity of acetic anhydride to make 250 ml. Five grams of anhydrous sodium sulfate was added to keep the reagent anhydrous. After preparation the reagent was protected from light, stored under refrigeration, and used within 1 week.

[9] Bausch & Lomb Spectronic 20.

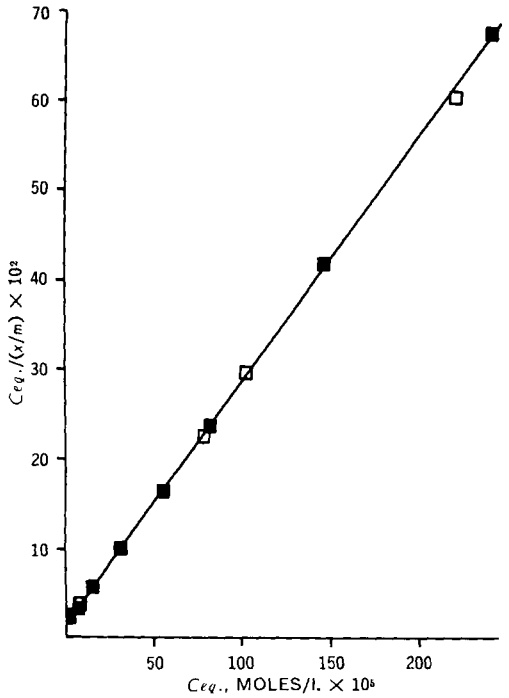

Figure 2—*Langmuir adsorption isotherm for the binding of fusidate anion to cholestyramine. Key:* ■, *25°; and* □, *37°.*

unit weight of resin; k_1 is the affinity constant, which is related to the strength of the forces involved in the interaction; and k_2 is a measure of the maximum amount of fusidate that can be monomolecularly adsorbed per unit weight of cholestyramine. The general shape of the representative plot (Fig. 1) indicates that the amount of fusidate bound per gram of resin at 25° remains constant at high $C_{eq.}$ values and that only a monolayer of fusidate anions was adsorbed onto the resin.

Equation 1 may be rearranged to yield a linear form of the Langmuir adsorption isotherm:

$$C_{eq.}/(x/m) = \frac{1}{k_1 k_2} + \frac{C_{eq.}}{k_2} \qquad \text{(Eq. 2)}$$

From the form of this equation, it is readily apparent that a plot of $C_{eq.}/(x/m)$ *versus* $C_{eq.}$, on rectilinear coordinates, should yield a straight line from which one can obtain the interaction constants k_1 and k_2. The data for the adsorption of fusidate onto cholestyramine at 25 and 37°, plotted according to Eq. 2, are presented in Fig. 2. The excellent linearity obtained is indicative of the adherence of the binding process to the Langmuir adsorption isotherm. There is essentially no difference between the binding tendencies of resin for the fusidate anions at 25 and 37°. In fact, least-squares analysis of the 37° data yielded Langmuir affinity (k_1) and capacity (k_2) constants of 2.61×10^4 l./mole and 0.863 mole of fusidate bound per mole-equivalent of resin, respectively. These values almost coincide with the corresponding values of 2.35×10^4 l./mole and 0.837 mole/mole-equivalent calculated for the binding process at 25°. This negligible temperature dependence of the fusidate–cholestyramine interaction is consistent with results previously obtained with fatty acid anion–cholestyramine and bile salt anion–cholestyramine interactions (8, 9) and with ion-exchange processes in general (20). The magnitude of the capacity constants suggests that the interaction approximates a 1:1 stoichiometry. The fact that the actual capacity constants determined from the data are less than the theoretical value of unity can be attributed to the presence of inaccessible binding positions on the interior surfaces of the resin bead.

Table I—Langmuir Adsorption Constants for the Binding of Sodium Fusidate and Selected Glycine-Conjugated Bile Salt Anions to Cholestyramine at 25°

Adsorbate Species	Langmuir Affinity Constants (k_1), Liters/Mole $\times 10^{-4}$	Langmuir Capacity Constants (k_2)[a], Moles Bound/Mole Equivalent of Resin
Fusidate	2.35	0.837
Glycocholate[b]	0.891	0.863
Glycodeoxycholate[b]	4.25	0.941

[a] Based on a monomer equivalent weight for cholestyramine of 230.
[b] Data obtained from *Reference 9.*

Table II—Effect of Varying Concentrations of Sodium Chloride and Sodium Bicarbonate on the Binding of Sodium Fusidate and Selected Glycine-Conjugated Bile Salts to Cholestyramine at 25°

Adsorbate Species[a]	Added Inorganic Electrolyte	x/m (Moles of Adsorbate Bound per Gram of —Cholestyramine $\times 10^4$—, Concentration of Added Inorganic Electrolyte, mM 0.0	50.0	100.0	150.0
Fusidate	NaCl	28.3	20.6	18.0	16.7
Glycocholate[b]	NaCl	26.8	14.1	10.7	9.18
Glycodeoxycholate[b]	NaCl	26.5	24.9	22.8	22.3
Fusidate	$NaHCO_3$	28.3	22.3	19.4	18.1
Glycocholate[b]	$NaHCO_3$	26.8	16.8	12.1	10.5
Glycodeoxycholate[b]	$NaHCO_3$	26.5	27.9	26.5	25.7

[a] The initial concentration of adsorbate was held constant at 3.0 mM.
[b] Data obtained from *Reference 8.*

Since endogenous bile salt anions are structurally similar to the fusidate anion, they can potentially function *in vivo* as competitors in the resin–fusidate interaction. Hence, it was of interest to compare the inherent binding tendencies of the resin for fusidate anions with that for representative dihydroxy and trihydroxy conjugated bile salt anions. The glycodeoxycholate and glycocholate anions were chosen for this comparison because they represent two of the major bile salts present in human bile. An examination of the data presented in Table I indicates that only very slight differences exist among the capacity constants for the fusidate and bile salt anions. However, a comparison of the affinity (k_1) constants for the adsorbates reveals that the fusidate anion binds strongly to cholestyramine and that the strength of interaction is on the same order of magnitude as that observed for the two conjugated bile salts. The actual interaction strength was found to decrease in the order: glycodeoxycholate > fusidate > glycocholate.

The desorption characteristics of bound fusidate were studied at 2 and 4 mM initial concentrations of antibiotic. Assay of equilibrated fusidate–resin systems before and after dilution with water yielded x/m values (*i.e.*, the moles of fusidate anion bound per gram of resin) of 19.4×10^{-4} and 34.7×10^{-4} before and 19.2×10^{-4} and 33.7×10^{-4} after dilution for the 2 and 4 mM initial concentrations of the antibiotic, respectively. These results indicate that the original equilibrium conditions that existed between free and bound fusidate were not significantly affected by moderate dilution of the binding system.

Effect of Physiologic Inorganic Electrolyte—Under *in vivo* conditions, the binding of the fusidate anion to cholestyramine must of necessity occur in the presence of various physiologic inorganic electrolytes. Consequently, studies were initiated to determine the influence of varying physiologic concentrations of sodium chloride and sodium bicarbonate on the extent of binding of fusidate to

Table III—Apparent Second-Order Rate Constants for the Binding of Sodium Fusidate to Cholestyramine at 37° Alone and in the Presence of Varying Concentrations of Sodium Chloride

Adsorbate[a] Species	Concentration of Sodium Chloride, mM	Apparent Second-Order Rate Constants (k'), l. · mole^{-1} min.$^{-1}$ $\times 10^{-2}$
Fusidate	—	7.03
Fusidate	100.0	5.64
Fusidate	150.0	4.58

[a] The concentration of free adsorbate remaining unreacted after the reaction reached completion with respect to the available binding positions on cholestyramine were: fusidate alone, 0.295 mM; fusidate at 100 mM NaCl, 0.445 mM; and fusidate at 150 mM NaCl, 0.472 mM.

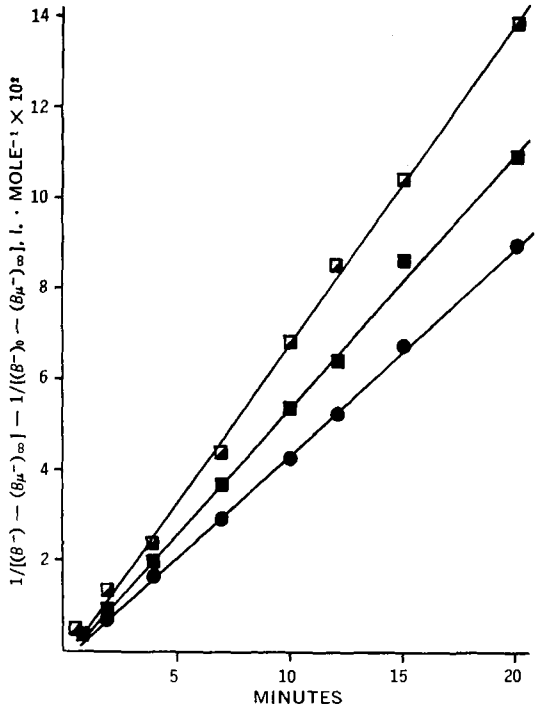

Figure 3—*Rate of binding of fusidate anion to cholestyramine at 37° in the absence and presence of varying initial concentrations of NaCl. Agitation intensity = 60 r.p.m. Key: ◪, 0.0 mM NaCl; ■, 100.0 mM NaCl; and ●, 150.0 mM NaCl.*

cholestyramine. The results of these experiments, expressed as the amount of adsorbate bound per unit weight of adsorbent (x/m), are presented in Table II. For comparison purposes, results for a representative trihydroxy-(glycocholate) and a dihydroxy-(glycodeoxycholate) bile salt are also included in this table (8). Both electrolytes have the ability to reduce significantly the binding capacity of the resin for the fusidate anion, and the degree of binding decreases with increasing concentrations of added inorganic electrolyte. At an electrolyte concentration of 150 m*M*, the binding of fusidate is reduced 41 and 36% by sodium chloride and sodium bicarbonate, respectively. In the presence of any one concentration of either inorganic electrolyte (NaCl or $NaHCO_3$), a comparison of the (x/m) values for fusidate with those obtained for the two bile salt anions reveals that the inhibitory effect of added electrolyte on the binding of these three adsorbate species decreases in the order: glycocholate>fusidate>glycodeoxycholate.

The inhibitory effect of inorganic electrolytes on the binding process would primarily occur at the site of ion–ion interaction, where the added inorganic anion could compete for available binding positions on the resin or effectively reduce the charge density on the two ionic reactants and by so doing weaken the attractive, electrostatic forces between the adsorbate and adsorbent species. Hence, adsorbate species whose significant binding to cholestyramine is strongly dependent on the existence of electrostatic interactions (*e.g.*, the glycocholate anion) would be more drastically affected by the presence of inorganic anions than those

Table IV—Effect of Temperature[a] on the Rate of Binding of Sodium Fusidate with Cholestyramine at an Agitation Intensity of 60 r.p.m.

Temperature	Apparent Second-Order Rate Constant (k'), l.·mole^{-1} min.$^{-1}$ × 10^{-2}
25°	4.92
37°	7.03
47°	8.15

[a] The concentration of free fusidate remaining unreacted after the reaction reached completion with respect to available binding positions on cholestyramine was essentially temperature independent.

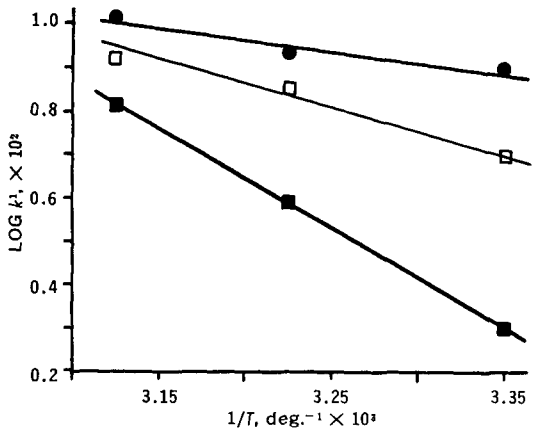

Figure 4—*Arrhenius relationship for adsorbate–cholestyramine interactions. Key: ●, glycodeoxycholate; □, fusidate; and ■, glycocholate. (Data for the two bile salt anions obtained from* Reference 10.)

adsorbate species whose significant interaction with the resin occurs *via* nonelectrostatic forces (*e.g.*, the glycodeoxycholate anion) (8, 9). Based on these results, it may be proposed that although the fusidate–cholestyramine interaction involves an electrostatic component, it is significantly stabilized by fairly strong nonelectrostatic forces. This nonelectrostatic contribution to the overall interaction appears to be intermediate between that for the glycocholate and glycodeoxycholate anions.

Rate of Binding Studies—The influence of cholestyramine on drug absorption should be maximal under those conditions where a significant fraction of the dose of the drug has strongly interacted with the resin in the GI fluids. If it is assumed that sodium fusidate is absorbed rapidly from solution by a passive diffusion process, the resin must not only preferentially and extensively interact with the antibiotic anions, but it must do so at a rapid rate (*i.e.*, the rate constant for the *adsorption* process must be significantly greater than that associated with the process of drug *absorption*). Conse-

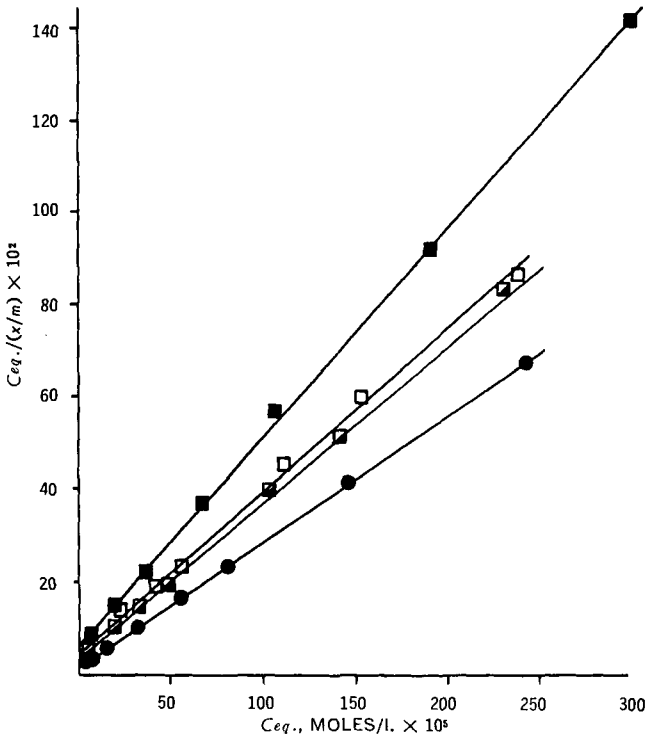

Figure 5—*Langmuir adsorption isotherms for the binding of fusidate anion to cholestyramine at 25° alone and in the presence of 2.0 mM initial concentrations of organic anions. Key: alone, ●; and in the presence of: taurocholate, ◪; glycodeoxycholate, □; and laurate, ■.*

Table V—Apparent Second-Order Rate Constants and Langmuir Adsorption Constants for the Binding of Sodium Fusidate to Cholestyramine, Alone and in the Presence of Added Physiologic, Organic Electrolytes

Added Inhibitor Substance		Apparent Second-Order Rate Constants at 37°	Langmuir[b] Affinity Constants at 25°	Langmuir[b] Capacity Constants at 25° (k_2), Moles of Fusidate Bound/Mole Equivalent of Resin
Species[a]	Initial Concentration, mM	(k'), l.·mole^{-1} min.$^{-1}$ × 10^{-2}	(k_1), l./mole fusidate × 10^{-4}	
—	—	7.03[a]	2.35[b]	0.837[b]
Taurocholate	2.0	4.97[a]	0.890	0.670
Glycodeoxycholate	2.0	2.82[a]	0.731	0.657
Laurate	2.0	2.46[a]	0.727	0.513

[a] The concentration of free fusidate remaining unreacted after the reaction reached completion with respect to the available binding positions on cholestyramine were: alone, 0.295 mM; 2.0 mM taurocholate, 0.378 mM; 2.0 mM glycodeoxycholate, 0.530 mM; and 2.0 mM laurate, 0.620 mM. [b] The Langmuir affinity and adsorption constants for the binding process at 37° were essentially the same as those determined at 25°, being 2.61 × 10^4 and 0.862, respectively.

quently, *in vitro* experiments were conducted to determine the rate of interaction of sodium fusidate with the resin and the influence of several physicochemical factors thereon.

Effect of Inorganic Electrolyte—The rate of binding of sodium fusidate to cholestyramine was conducted in pure aqueous medium and as a function of added inorganic electrolyte (NaCl) concentration. The rate data from these 37° studies were subjected to kinetic analysis with the aid of the following integrated second-order rate expression (10):

$$\frac{1}{(B^-) - (B_\mu^-)_\infty} - \frac{1}{(B^-)_0 - (B_\mu^-)_\infty} = k't \qquad \text{(Eq. 3)}$$

where $(B^-)_0$ and (B^-) represent the total concentration (mM) of fusidate anion present free in solution at time *zero* and anytime, t, respectively; $(B_\mu^-)_\infty$ is the concentration of fusidate remaining unreacted after the reaction has reached completion with respect to the *available* binding positions on the resin; and k' is the apparent second-order rate constant governing the interaction. The rate data, plotted according to Eq. 3, are shown in Fig. 3. The apparent second-order rate constants, obtained from the least-squares slopes of these linear plots, are listed in Table III. The magnitude of the second-order rate constant observed in the absence of added inorganic electrolyte (*i.e.*, 7.03 × 10^2 l.·mole^{-1} min.$^{-1}$) indicates that sodium fusidate rapidly interacts with the resin and that it does so at a rate comparable to conjugated bile salt anions. For example, the rate constant for the glycodeoxycholate–resin interaction under identical experimental conditions was previously shown to be 8.61 × 10^2 and that for the glycocholate anion to be 3.91 × 10^2 l.·mole^{-1} min.$^{-1}$ (10). In addition, the rate of interaction of the fusidate anion is depressed significantly in the presence of physiologic concentrations of sodium chloride. This inhibitory effect appears to increase with increasing electrolyte concentration. The meachanism(s) by which this inhibitory effect is produced is most probably the same as that mentioned previously in connection with the effect of inorganic electrolytes on the extent of interaction of fusidate with cholestyramine.

Effect of Temperature—It was previously demonstrated that over the temperature range of 25–37° the affinity and capacity constants observed for the fusidate–cholestyramine binding process remained constant. However, these two constants are equilibrium parameters and do not necessarily provide any information concerning the influence of temperature on the *rate* of adsorption. From Table IV it may be observed that as the temperature was increased from 25 to 47°, the rate of binding of the fusidate anion to cholestyramine increased.

To ascertain whether the kinetic temperature data for the fusidate interaction process obeyed the Arrhenius relationship, a plot was made of the logarithm of the apparent second-order rate constant *versus* the reciprocal of the temperature (°K) (Fig. 4). The data followed this relationship over the temperature range investigated. For comparison purposes, the curves for glycodeoxycholate and glycocholate (10) are also included in this figure. From the least-squares slopes of these plots, the apparent energies of activation (E_a) for the three adsorbates were calculated to be 10.6, 4.47, and 2.39 kcal./mole for the glycocholate, fusidate, and glycodeoxycholate systems, respectively. This order suggests that an intermediate *energy barrier* exists to the binding of fusidate to the resin. This finding is consistent with the previous observation that the fusidate anion is capable of an intermediate level of interaction strength with cholestyramine (*i.e.*, the observed Langmuir affinity constants paralleled the calculated energies of activation for the respective adsorbates).

Influence of Organic Electrolytes on the Binding of Fusidate to Cholestyramine—In addition to *inorganic* electrolytes, the fluids of the GI tract also contain various *organic* anions. Consequently, it was important to establish and quantify, *via in vitro* equilibrium and rate of binding experiments, whether these physiologic, organic electrolytes could interfere with the binding of fusidate to cholestyramine.

The equilibrium adsorption data, analyzed by the Langmuir-type adsorption equation (Eq. 2), for the fusidate–cholestyramine adsorption process alone and in the presence of 2 mM initial concentrations of the organic anions (taurocholate, glycodeoxycholate, and laurate) are shown in Fig. 5, with the Langmuir constants appearing in Table V. It is apparent that all of the inhibitor species are capable of effectively competing with fusidate anions for the available binding positions on the resin. A closer examination of the equilibrium data reveals that the three chemically diverse, inhibitor species are equally effective in producing a reduction in the affinity of interaction between the fusidate anion and the resin. For example, the affinity constant for fusidate was reduced by 62.1, 68.9, and 69.1% in the presence of 2 mM initial concentrations of taurocholate, glycodeoxycholate, and laurate, respectively. Even though the differences are rather small, the percent reduction values do parallel the inherent affinity constants for the inhibitors alone (*i.e.*, 1.99 × 10^4, 4.25 × 10^4, and 5.64 × 10^4 l./mole for the taurocholate, glycodeoxycholate, and laurate systems, respectively).

As may also be observed from Table V, not only are the equilibrium affinity and capacity constants for the fusidate–resin interactions reduced markedly in the presence of the organic inhibitors, but also the apparent second-order rate constant governing the rate of uptake of the fusidate anion by the exchange resin is diminished. Moreover, there appears to be significant differences between the three inhibitors as to their ability to interfere with the fusidate–cholestyramine interaction rate. The taurocholate anion is the least effective, producing only a 29.3% reduction, while the other two anions decrease the rate by 60–65%.

The present investigation demonstrated that the antibiotic, sodium fusidate, binds strongly and rapidly to cholestyramine. In addition, both the extent and rate of the interaction are drastically reduced by the presence of physiologic concentrations of inorganic (*e.g.*, sodium chloride and sodium bicarbonate) and organic (*e.g.*, conjugated bile salt and fatty acid anions) electrolytes. However, these electrolytic species, which are normal constituents of GI fluids, do not completely prevent the antibiotic–resin interaction from taking place.

In view of this *in vitro* evidence, it is quite conceivable that the GI absorption pattern of sodium fusidate may be affected when cholestyramine is concomitantly present in the GI tract.

REFERENCES

(1) S. S. Bergen, T. B. Van Itallie, D. M. Tennent, and W. N. Sebrell, *Circulation*, **20,** 981(1959).

(2) D. M. Tennent, H. Siegel, M. E. Zanetti, G. W. Kuron, W. H. Ott, and J. F. Wolf, *J. Lipid Res.*, **1**, 469(1960).
(3) T. B. Van Itallie and S. A. Hashim, *Med. Clin. N. Amer.*, **47**, 629(1963).
(4) D. Berkowitz, *Amer. J. Cardiol.*, **12**, 834(1963).
(5) S. A. Hashim and T. B. Van Itallie, *J. Amer. Chem. Soc.*, **192**, 289(1965).
(6) M. E. Zanetti and D. M. Tennent, *Proc. Soc. Exp. Biol. Med.*, **112**, 991(1963).
(7) J. B. Carey, Jr., *J. Clin. Invest.*, **40**, 1028(1961).
(8) W. H. Johns and T. R. Bates, *J. Pharm. Sci.*, **58**, 179(1969).
(9) *Ibid.*, **59**, 329(1970).
(10) *Ibid.*, **59**, 788(1970).
(11) D. G. Gallo, K. R. Bailey, and A. L. Sheffner, *Proc. Soc. Exp. Biol. Med.*, **120**, 60(1965).
(12) K. D. G. Edwards, *Med. J. Aust.*, **2**, 925(1965).
(13) K. D. G. Edwards and M. McCredie, *ibid.*, **4**, 534(1967).
(14) F. Bergman, P. A. Heedman, and W. Vander Linden, *Acta Endocrinol.*, **53**, 256(1966).
(15) R. C. Northcutt, J. N. Stiel, J. W. Hollifield, and E. G. Stant, Jr., *J. Amer. Med. Ass.*, **208**, 1857(1969).
(16) R. Saral and V. L. Spratt, *Arch. Int. Pharmacodyn. Ther.*, **167**, 10(1967).
(17) "Encyclopedia of Industrial Chemical Analysis," vol. 5, S. Snell and J. Hilton, Eds., Interscience, New York, N. Y., 1967, p. 517.
(18) W. Godtfredsen, K. Roholt, and L. Tybring, *Lancet*, **1962**, 928.
(19) I. Langmuir, *J. Amer. Chem. Soc.*, **38**, 2221(1916).
(20) F. Helfferich, "Ion Exchange," McGraw-Hill, New York, N. Y., 1962, p. 166.

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Drug–Cholestyramine Interactions II: Influence of Cholestyramine on GI Absorption of Sodium Fusidate

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Abstract □ The results of a previously reported *in vitro* study provided evidence that the hypocholesterolemic agent, cholestyramine, was capable of strongly and rapidly interacting with the antibiotic, sodium fusidate. Based on these findings, the influence of this pharmacologically important anionic exchange resin on the *in vivo* absorption pattern of sodium fusidate was studied in the rat. Serum antibiotic levels were determined microbiologically as a function of time following oral administration of the antibiotic alone and in the presence of the resin. Concurrent administration of the resin yielded statistically significant reductions in serum drug levels at all experimental time intervals. Peak serum levels of fusidate were found to decrease by 33–77% of control values as the resin-to-drug dose ratio administered was varied from 0.14:1 to 0.72:1. At dose ratios of greater than 1:1 (resin–drug) but far less than the ratio of the average therapeutic, single dose of each drug (*i.e.*, 10:1, cholestyramine–fusidate), there were no detectable serum antibiotic levels. This latter observation indicated that the presence of the insoluble resin in the GI tract totally prevented drug absorption. The time interval between the oral administration of the resin and antibiotic was found also to influence peak serum antibiotic levels.

Keyphrases □ Cholestyramine effect—GI absorption of sodium fusidate, rats □ Sodium fusidate, GI absorption—effect of cholestyramine, rats □ Drug–anionic exchange resin interactions—effect of cholestyramine on GI absorption of sodium fusidate, rats □ Absorption, GI, sodium fusidate—effect of cholestyramine, rats

The water-insoluble, anionic exchange resin, cholestyramine, lowers serum cholesterol levels by binding bile salt anions in the small intestine (1–7). The reduction in bile salt concentration decreases the intestinal absorption of exogenous cholesterol and lipids and increases the hepatic metabolism of endogenous (serum) cholesterol into additional bile salts, which are subsequently bound by the resin. The resin is, therefore, an important therapeutic agent for the treatment of biliary cirrhosis and those conditions normally associated with high blood cholesterol and lipid levels (*e.g.*, atherosclerosis and thrombotic vascular disease).

Since anionic drugs may be concurrently administered during chronic cholestyramine therapy, the possibility exists that a drug–cholestyramine interaction could occur within the GI tract. Such an interaction is of considerable importance clinically because it might result in a decrease in the rate and/or extent of drug absorption and, hence, the onset and/or intensity of drug activity. However, only a limited number of studies have been reported in the literature pertaining to this potential drug–resin therapeutic incompatibility (8–13). These investigations are informative, but their experimental protocols preclude any rigorous interpretation of the *in vivo* absorption data. In addition, no attempts were made by these investigators to determine the influence of relative times of administration of the test drug(s) and cholestyramine on the absorption characteristics of the drug.

It was previously demonstrated *in vitro* (14) that cholestyramine possesses a marked affinity for the steroidal antibiotic, sodium fusidate. This investigation