Quantification of the Binding Tendencies of Cholestyramine III: Rates of Adsorption of Conjugated Bile Salt Anions onto Cholestyramine as a Function of Added Inorganic Electrolyte Concentration, Temperature, and Agitation Intensity

WILLIAM H. JOHNS* and THEODORE R. BATES

Abstract ☐ The *in vitro* rate of binding of several conjugated bile salt anions to cholestyramine was studied at 37°, alone and in the presence of varying concentrations of sodium chloride. The kinetic studies were conducted under relatively mild agitation conditions to simulate reasonably the *in vivo* situation existing in the small intestinal lumen. A second-order kinetic model was found to represent the interaction data most suitably. The rate constants for the adsorption process were found to decrease in the presence of increasing concentrations of inorganic electrolyte, the reductive effect being more pronounced with the trihydroxy conjugates than with the dihydroxy derivative. Langmuir affinity constants were found to parallel the apparent second-order rate constants for systems devoid of added inorganic electrolyte. From studies concerning the influence of temperature on the rate of interaction, calculated apparent energies of activation indicated the existence of a substantially lower *energy barrier* to the binding of the dihydroxy glycine-conjugated derivative, glycodeoxycholate, than for its trihydroxy counterpart, the glycocholate anion. Variation in the intensity of agitation at which the reaction was conducted had a more pronounced effect on the rate of uptake of the glycodeoxycholate anion by the resin than on the glycocholate anion. A log–log relationship was found to exist between the second-order interaction rate constant and the speed of agitation. The results obtained from the temperature and agitation studies suggested that the binding of bile salt anions to cholestyramine apparently occurs by means of a diffusion-controlled process.

Keyphrases ☐ Cholestyramine binding—conjugated bile salt anions ☐ Electrolyte-concentration effect—cholestyramine binding ☐ Agitation, temperature effects—anion adsorption, cholestyramine ☐ Rate constants—anion adsorption, cholestyramine ☐ Colorimetric analysis—spectrophotometer

It has been shown that oral administration of the anionic-exchange resin, cholestyramine, results in the lowering of intestinal bile salt concentrations (1) and the interruption of normal enterohepatic circulation of bile salts (2). Cholestyramine also decreases the absorption of dietary cholesterol (3), fatty acids (4), and other materials, both exogenous and naturally occurring in the gastrointestinal tract, which rely on the surface-active properties of bile salts for their efficient intestinal absorption (5, 6). It has been proposed previously that the forces involved in the bile salt anion–cholestyramine interaction are primarily electrostatic in nature and are reinforced by secondary nonelectrostatic forces (7, 8).

The high doses required for efficacy in both clinical (9) and animal studies (10) suggest that cholestyramine is relatively inefficient in its ability to reduce intestinal bile salt levels, even though the capacity expected of such an ion-exchange resin (11) and approached by *in vitro* equilibrium experimentation with cholestyramine (7) would indicate otherwise.

In vivo, the physiologically active glycine- and taurine-conjugated bile salts present in bile are secreted into the lumen of the duodenal segment of the small intestine *via* the common bile duct, where they exist and function as endogenous surfactants in essentially the ionized form (12). Conservation of the secreted bile salts is effectively accomplished by their active reabsorption from the ileal segment of the small intestine (13). Therefore, the resin must interact with bile salt anions during their residence in the duodenal and jejunal regions of the small intestine, prior to their reabsorption from the ileum. If the binding process is inherently slow, or if the presence of physiologic electrolytes or other substances normally present in the intestinal tract tends to retard the rate of adsorption onto the resin, the resin may pass into the large intestine having only been partially utilized, thereby lowering the apparent efficiency of this pharmacologically important adsorbent.

In view of the fact that there would exist two dynamic, competitive reactions in the fluids of the small intestine, that is, the bile salt anion–cholestyramine interaction and the active reabsorption of bile salts from the ileum, it was of interest to investigate the *in vitro* kinetics of the former reaction alone and under the influence of the physiologic electrolyte, sodium chloride. Sodium chloride was previously shown to have an inhibitory effect on the interaction of bile salt anions with cholestyramine, the decrease in apparent capacity being greater for trihydroxy bile salts (*e.g.*, glycocholate and taurocholate) than for dihydroxy derivatives (*e.g.*, glycodeoxycholate) (7). The authors proposed that the electrolyte functioned principally to reduce the charge density on the charged adsorbate and adsorbent species, thereby weakening the electrostatic component of the interaction.

The literature is almost devoid of investigations concerned with the rates of binding of biliary constituents to adsorbents of the ion-exchange type, with the exception of the study of Sawchuk and Nairn (14) on the interaction of the bile pigment, bilirubin, with a series of anionic-exchange resins, including Dowex 1-X2. This work was primarily concerned with the rate of binding as it related to the degree of crosslinkage in the polyvinylstyrene skeleton of the resin polymer and the dependency of the adsorption rate on the particle size of the resin material.

The objectives of the present investigation were to establish and compare the *in vitro* rates of binding of several physiologic, conjugated bile salts to cholesty-

ramine at 37°. Studies of the adsorption-rate process in the presence of varying concentrations of added inorganic electrolyte at 37° were performed in order to appreciate more fully the role these endogenous ions may play in the *in vivo* rates of interaction. The influence of temperature and rate of agitation on the kinetics of the bile salt anion–cholestyramine interaction were also explored to obtain an appreciation for the magnitude of the energy of activation associated with the rate process and to determine whether the adsorption process was film diffusion- or particle diffusion-controlled. Particle diffusion-controlled reactions are rate limited by diffusion of exchangeable ions within the ion-exchange particle itself, whereas film diffusion-controlled exchange is a result of limiting diffusion rates in the diffuse film surrounding the ion-exchange particle.

EXPERIMENTAL

Materials and Methods—The sodium salts of taurocholic,[1] glycocholic,[1] and glycodeoxycholic[1] acids were dried *in vacuo* for at least 48 hr. prior to use. Reagent grade furfuraldehyde,[2] sulfuric and glacial acetic acids, and sodium chloride were used as received. The cholestyramine[3] employed in this investigation was pharmaceutical grade (7).

All binding-rate studies were performed using a 250-ml., three-necked, round-bottom flask maintained at 37 ± 0.1°. Constant agitation of the reaction medium was accomplished by the use of a Cole-Parmer[4] constant torque overhead stirrer (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 adsorbate initially present was maintained at 1.0 millimolar (m*M*)[5] 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 (*i.e.*, a 5% range.)

In the case of the aqueous systems, 57.5 mg. of cholestyramine, dry weight, was allowed contact with 240 ml. of deionized, distilled water at 37° for a period of 0.5 hr. prior to the introduction of a 10-ml. aliquot of a bile salt stock solution. For the studies involving added inorganic electrolyte, identical procedures were followed except that the resin was placed in only 230 ml. of water. Ten-milliliter quantities of concentrated sodium chloride and bile salt stock solutions were simultaneously added to the reaction flask after a half hour.

Samples were withdrawn from the reaction flask at appropriate time intervals using a 1-ml. pipet fitted with a glass wool prefilter; the samples were suitably diluted, if necessary, and spectrophotometrically assayed for the concentration of bile salt remaining free in solution (see *Assay Procedure*).

Since previous work (7) had shown that sodium chloride effectively reduced the capacity of cholestyramine for certain bile salt anions, the concentration of bile salt anion remaining free or unreacted after the reaction had reached completion was determined. Two hundred and fifty-milliliter volumes of a solution containing the same concentration of bile salt, or bile salt and sodium chloride, 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 established within a 24–48-hr. period. These equilibrium data were utilized in the kinetic analysis of the results obtained in this investigation.

The experimental protocol for those studies designed to establish the role of temperature and agitation intensity on the rate of the bile salt anion–cholestyramine interaction was essentially the same as that described previously. The temperature studies were performed in an aqueous medium at 25, 37, and 47°. The agitation intensity was maintained constant at 60 r.p.m.

The influence of agitation intensity on the reaction rate was studied in pure aqueous medium at a constant temperature of 37° and speeds of agitation of 40, 60, 70, and 100 r.p.m. In these latter studies, the resin was exposed to the medium, which was agitated at 60 r.p.m., for 30 min. After this period, the intensity of agitation was adjusted to the desired level and the bile salt stock solution was added to the system.

Assay Procedure—The concentrations of sodium glycocholate and taurocholate remaining free in solution at the time of sampling were assayed using essentially the same method as reported by Irvin *et al.* (15). The 1-ml. sample, or a similar volume of a suitable dilution thereof, was heated for 0.5 hr. at 65° in the presence of 6.0 ml. of 16 *N* sulfuric acid and 1.0 ml. of a 3% furfuraldehyde solution. Stabilization of the blue color, which developed on heating, was accomplished by the addition of 5.0 ml. of glacial acetic acid. The absorbance (maximum at 660 mμ), which obeyed the Beer's law relationship, was read using a Bausch and Lomb Spectronic-20, equipped with appropriate photomultiplier tube and filter. A solution containing no bile salt and treated in an identical manner served as the blank for these colorimetric determinations. The concentrations of glycodeoxycholate were determined spectrophotometrically as previously described (7).

RESULTS AND DISCUSSION

Theory—The formation of a 1:1 irreversible complex between an anion and an anionic-exchange resin may be expressed by the following equation, the molecularity of which is based on well-established evidence for reactions of ion-exchange resins in general (16, 17):

$$(B^-) + (C^+) \xrightarrow{k} (B\text{-}C) \qquad \text{(Eq. 1)}$$

where (B^-) represents the total concentration (m*M*) of anion present free in solution at any time *t;* (C^+) the total *concentration* (meq.) of ionized binding sites on the anionic-exchange resin unoccupied by anions at any time *t;* (B-C) the concentration (meq.) of anion-resin complex formed at any time *t;* and *k* the second-order rate constant for the reaction. A similar reaction scheme, which assumes that the binding process is virtually irreversible, was suggested by Helfferich (18) as being valid in describing ion-exchange reactions, and has been employed by Sawchuk and Nairn (14) to interpret kinetically the binding between bilirubin and an anionic-exchange resin.

In the bile salt–cholestyramine system under discussion in this paper, Eq. 1 must be modified so as to take account of the binding sites on the anionic-exchange resin that have been previously shown (7) to be unavailable for binding bile salt anions. The concentration of available binding sites on cholestyramine (C^+_a) is therefore equal to the total concentration of sites, (C^+), minus those that are unavailable, (C^+_u). Unless this correction is made, the reaction would appear to be incomplete.

Since a 1:1 stoichiometric relationship exists between the cholestyramine and the bile salt anions, (C^+_a) at any time *t* must equal the concentration of unreacted or free bile salt available for binding to these sites, (B^-_a). Any measurable concentration of bile salt remaining unreacted after the reaction has reached completion is, therefore, a measure of the concentration of bile salt unavailable for binding, (B^-_u), and would equal the concentration of unavailable unoccupied sites, (C^+_u).

The magnitude of (C^+_a) will depend on the accessibility of binding positions on the interior surfaces of the resin bead which, in turn, is a function of the chemical structure of the bile salt anion and thus its ability to assume an optimal orientation for interaction with these sites.

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[1] Obtained from Calbiochem, Los Angeles, Calif., Grade A.

[2] Obtained from Distillation Products, Inc., Eastman Kodak, Rochester, N. Y.

[3] Supplied by Merck and Co., Inc., Rahway, N. J.

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

[5] The pH values of 1.0 m*M* aqueous solutions of the sodium salts of taurocholic, glycocholic, and glycodeoxycholic acids, before and after contact with cholestyramine, remained constant at 5.30, 6.80, and 6.60, respectively. These values were insignificantly influenced by the presence or concentration of the inorganic electrolyte used in some of the studies. Based on the pH values obtained and the highest pKa values previously reported for these acids (7), it was determined that the bile acids existed almost completely in the ionized form (*i.e.*, 98.9, 99.6, and 99.8% ionized, respectively).

Table I—Apparent Second-Order Rate Constants Governing the Binding of Conjugated Bile Salts to Cholestyramine at Various Sodium Chloride Concentrations[a]

Bile Salt Anion[b]	Concn. of NaCl, m*M*	Apparent Second-Order Rate Constant, k' (l. mole^{-1} min.$^{-1}$ × 10^{-2})	Langmuir[c] Affinity Constants at 25°, k_1 (l. mole^{-1} × 10^{-4})
Glycocholate	0.00	3.91	0.891
Glycocholate	50.0	1.48	—
Taurocholate	0.00	5.41	1.99
Taurocholate	25.0	3.32	—
Taurocholate	50.0	2.53	—
Taurocholate	75.0	1.98	—
Glycodeoxycholate	0.00	8.61	4.25
Glycodeoxycholate	50.0	8.17	—

[a] Temperature, 37°; agitation intensity, 60 r.p.m. [b] The concentrations of free bile salt remaining unreacted after the reaction had reached completion with respect to the available binding positions on cholestyramine were: glycocholate, at 0 and 50 m*M* concentrations of NaCl, 0.25 and 0.38 m*M*; taurocholate, at 0, 25, 50, and 75 m*M* concentrations of NaCl, 0.21, 0.26, 0.30, and 0.33 m*M*, respectively; and glycodeoxycholate, at 0 and 50 m*M* concentrations of NaCl, 0.01 and 0.05 m*M*. [c] These equilibrium Langmuir constants were obtained from *Reference 7*. The equilibrium binding of bile salt anions to cholestyramine appears to be essentially temperature independent.

The addition of inorganic electrolyte to the bile salt–cholestyramine system has been shown previously to reduce the capacity of the resin to bind bile salt anions (7). The extent of this reduction, which may either result from a competitive reaction between bile salt and electrolyte anions for available, unoccupied positions on the resin or from the ability of the electrolyte to reduce the charge density on the resin, has been found to be not only a function of electrolyte concentration but also of the structure of the bile salt anion. In any event, (C^+_a) will be further reduced below that for the binary bile salt anion–cholestyramine system.

Since the reaction under investigation is bimolecular, and essentially irreversible for the bile salts employed (7), the rate expression for the disappearance of bile salt anion from solution, based only on available binding positions on the resin, may be expressed by the following equation:

$$\frac{-d(B^-_a)}{dt} = k'(B^-_a)(C^+_a) \qquad \text{(Eq. 2)}$$

The experimental design of the binding-rate studies was such that the initial total concentrations of the reactants were equal [*i.e.*, $(B^-)_0 = (C^+)_0$]. A similar equality exists between $(B^-_a)_0$ and $(C^+_a)_0$. Therefore, at any time t the concentration of free bile salt available for binding, (B^-_a), would be equal to the concentration of unreacted available adsorption sites on the resin, (C^+_a). The term (B^-_a) is equivalent to (B^-) minus (B^-_u), or (B^-) minus $(B^-_u)_\infty$, since the concentration of free bile salt unavailable for binding is a constant and therefore independent of time. Substitution of these conditions into Eq. 2, and subsequently integrating the resulting rate expression, yield the apparent irreversible second-order kinetic relationship utilized in the analysis of the bile salt uptake data:

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

where k' is the apparent second-order rate constant governing the reaction of bile salt anions with *available* binding positions on cholestyramine. The concentration of bile salt remaining unreacted in solution after the reaction has reached completion $[(B^-_u)_\infty]$, under the various experimental conditions, are listed as footnotes to Tables I–III.

Attempts to fit all of the binding-rate data to the well-established integrated rate expression (19) for the reversible reaction, $(B^-) + (C^+) \rightleftarrows$ (B-C), yielded, in a majority of cases, curvilinear rather than the linear plots predicted by this reversible rate expression (19). This finding further supports the use of the apparent irreversible reaction scheme given by Eq. 1.

Effect of Inorganic Electrolyte—The validity of the assumptions made concerning the interactions studied is evidenced by the excellent linearity of the data interpreted by utilization of Eq. 3 (Figs. 1–3). The apparent second-order constants, k', were calculated from the slope of a regression line through the data points subse-

Table II—Effect of Temperature on the Rate of Interaction of Glycine-Conjugated, Bile Salt Anions with Cholestyramine at an Agitation Intensity of 60 r.p.m.

Bile Salt Anion[a]	Temperature	Apparent Second-Order Rate Constant, k' (l. mole^{-1} min.$^{-1}$ × 10^{-2})
Glycocholate	25	2.00
	37	3.91
	47	6.60
Glycodeoxycholate	25	7.89
	37	8.61
	47	10.4

[a] The concentrations of free bile salt remaining unreacted after the reaction had reached completion with respect to the available binding positions on cholestyramine were essentially temperature independent (Table I, Footnote[b]).

Table III—Effect of Agitation Intensity on the Rate of Interaction of Glycine-Conjugated, Bile Salt Anions with Cholestyramine at 37°

Bile Salt Anion[a]	Agitation Intensity, r.p.m.	Apparent Second-Order Rate Constant, k' (l. mole^{-1} min.$^{-1}$ × 10^{-2})
Glycocholate	40	2.99
	60	3.91
	70	4.78
	100	5.91
Glycodeoxycholate	40	5.33
	60	8.61
	70	10.7
	100	14.3

[a] The concentrations of free bile salt remaining unreacted after the reaction had reached completion with respect to the available binding positions on cholestyramine were independent of agitation intensity (Table I, Footnote[b]).

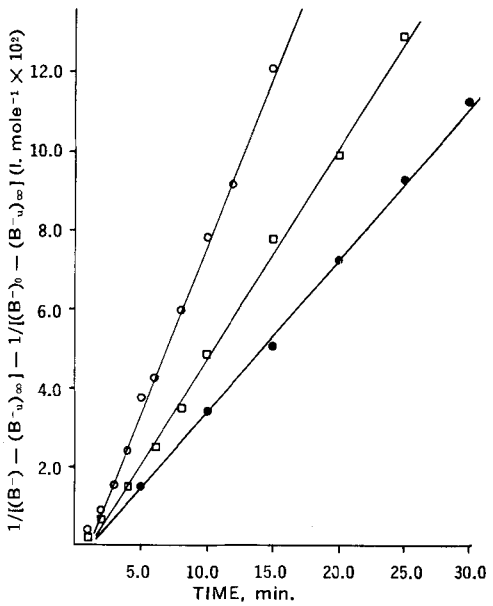

Figure 1—*Rate of binding of conjugated bile salt anions to cholestyramine at 37°. Agitation intensity, 60 r.p.m. Key: glycodeoxycholate* (○), *taurocholate* (□), *and glycocholate* (●).

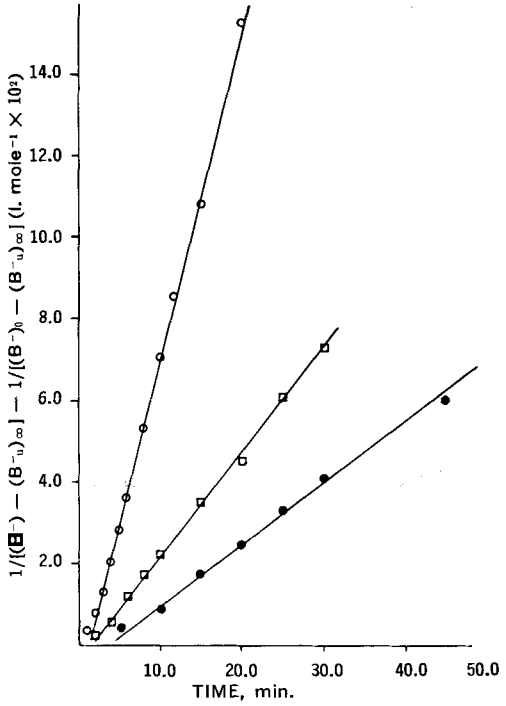

Figure 2—*Rate of binding of conjugated bile salts to cholestyramine in the presence of a 50.0 mM concentration of sodium chloride at 37°. Agitation intensity, 60 r.p.m. Key: glycodeoxycholate* (○), *taurocholate* (□), *and glycocholate* (●).

quent to the initial nonlinear phase. For all of the systems under examination, the apparent lag times (*i.e.*, the extrapolated, least-squares intercepts on the time axis of the linear segments of the plots shown in Figs. 1–3) ranged from 1 to 3 min. As is apparent from an examination of the plots shown in Fig. 1 and the rate-constant data summarized in Table I, the rate constant governing the bile salt–cholestyramine binding process, from aqueous pure medium, is significantly greater for the dihydroxy derivative, glycodeoxycholate, than for the trihydroxy salts investigated. A comparison of the rate-constant data for the two trihydroxy bile salts studied indicates that the taurine-conjugated derivative, taurocholate, is adsorbed onto the resin at a faster rate than the

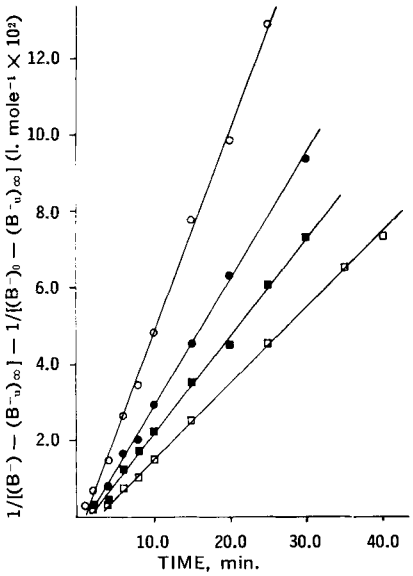

Figure 3—*Rate of binding of taurocholic anion to cholestyramine in the presence of varying concentrations of sodium chloride at 37°. Agitation intensity, 60 r.p.m. Key: 0.00 mM NaCl* (○), *25.0 mM NaCl* (●), *50.0 mM NaCl* (■), *and 75.0 mM NaCl* (□).

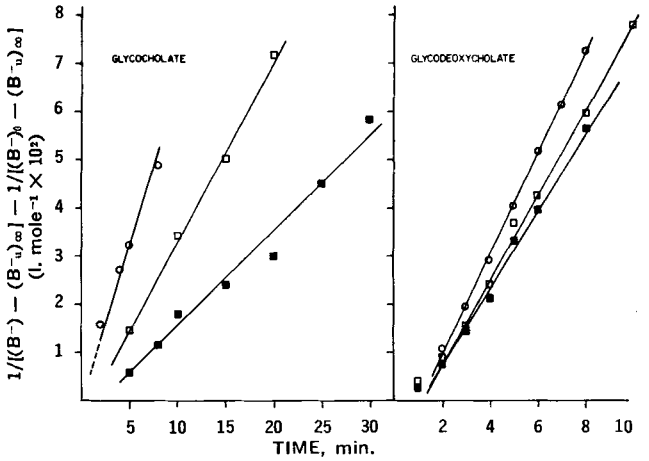

Figure 4—*Effect of temperature on the rate of binding of glycine-conjugated, bile salt anions with cholestyramine at an agitation intensity of 60 r.p.m. Key: 25°* (■), *37°* (□), *and 47°* (○).

glycine-conjugated derivative, glycocholate. Also shown in Table I are the affinity or association constants obtained from a Langmuir interpretation of equilibrium binding data for the bile salts alone (7). The rank order relationship existing between this parameter and the k' values for the binary bile salt–cholestyramine systems suggests that the affinity, which signifies the strength of interaction between adsorbate and adsorbent species, functions as a driving force for the interaction, the rate of binding increasing with increasing interaction strength. This conclusion is in agreement with the statements of Helfferich (20, 21) concerning the role of selectivity in the rate of uptake of simple ions by ion-exchange resins.

The observed rates of interaction in the ternary, bile salt–electrolyte–cholestyramine systems are significantly reduced in comparison to those in the absence of added electrolyte, as is evidenced by a comparison of the appropriate curves presented in Figs. 1 and 2 and the rate constants listed in Table I. The diminution in the rate of adsorption is decidedly more pronounced in the taurocholate- or glycocholate-containing systems than in the glycodeoxycholate-containing system. For example, at an added inorganic electrolyte concentration of 50 mM, there are approximately 53 and 62% decreases in the rates of interaction, respectively, for the former two trihydroxy anions; whereas there is only a 5% reduction in the rate of binding for the latter dihydroxy bile salt anion. These results tend to add supportive evidence to the proposed contributory role of nonelectrostatic interactions in the adsorption process (8),

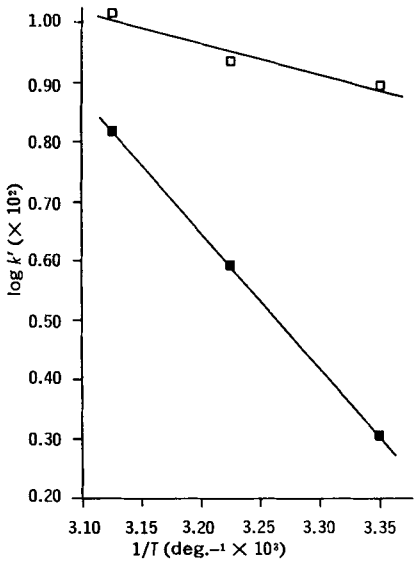

Figure 5—*Arrhenius relationship for glycine-conjugated, bile salt anion–cholestyramine interaction. Key: glycodeoxycholate* (□) *and glycocholate* (■).

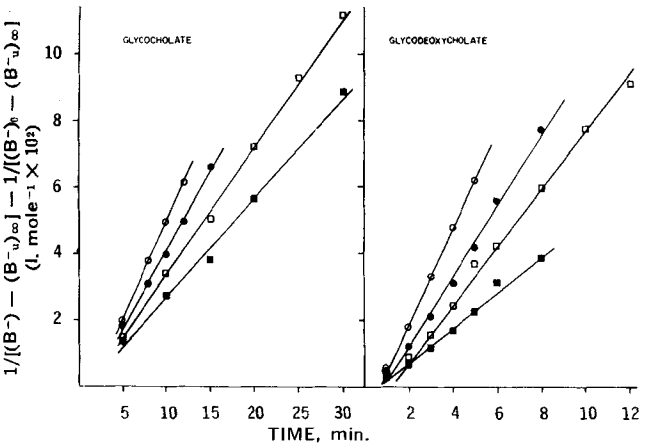

Figure 6—*Effect of agitation intensity on the rate of binding of glycine-conjugated, bile salt anions with cholestyramine at 37°. Key: 100 (○), 70 (●), 60 (□), and 40 (■) r.p.m.*

since electrolytes would be expected to have less of an effect on binding processes which are more nonelectrostatic in nature (*i.e.*, the dihydroxy bile salt–cholestyramine interaction) than on those interactions which are primarily due to an electrostatic mechanism (*i.e.*, trihydroxy bile salt–cholestyramine interactions).

The second-order rate constants appear to decrease with increasing electrolyte concentration, as is clearly exemplified by Fig. 3 and the rate constants listed in Table I for the taurocholate-containing systems. This primary salt effect is most probably due to a reduction in the charge density associated with the two charged reactants produced by the *screening* effect of the added inorganic electrolyte ions. The overall result is a weakening in the electrostatic forces between the two reactants and hence a corresponding decrease in the rate of interaction. A competition between bile salt and added chloride anions for the charged binding positions on cholestyramine may also be operable in the observed reduction in the uptake rates of taurocholate and glycocholate by the resin.

Effect of Temperature—Presented in Fig. 4 and listed in Table II are the results of the studies concerned with the temperature dependency of the initial rate of interaction between bile salt anions and cholestyramine, at a constant agitation intensity of 60 r.p.m. It is readily apparent from an examination of the rate-constant data that both glycocholate and glycodeoxycholate show an increase in their rate of binding with increasing temperature. The 10° temperature coefficient for the reaction was estimated to be approximately 2.1 for the former anion and 1.1 for the latter bile salt anion. These temperature coefficient values are of the order of magnitude similar to that reported for other heterogeneous, diffusion-controlled reactions (23, 24).

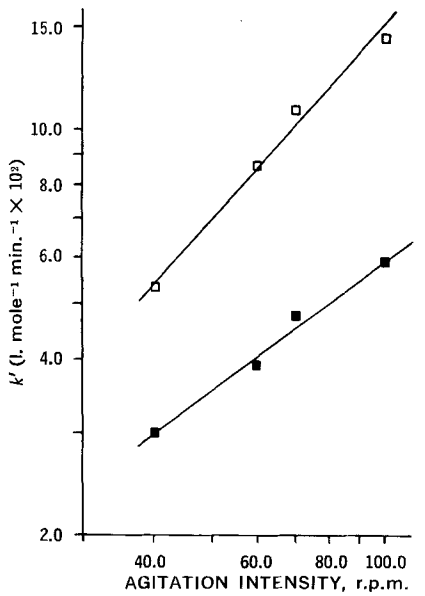

Figure 7—*Log apparent second-order rate constant* versus *log speed of agitation (r.p.m.) for the interaction of glycine-conjugated, bile salt anions with cholestyramine at 37°. Key: glycodeoxycholate (□) and glycocholate (■).*

Apparent energies of activation (E_a) associated with the rates of interaction of glycocholate and glycodeoxycholate with cholestyramine were calculated by means of the Arrhenius equation,

$$\log k' = \log A - \frac{E_a}{2.303R}\frac{1}{T} \qquad \text{(Eq. 4)}$$

in which k' represents the apparent second-order rate constant, A is the frequency factor, E_a is the energy of activation, R is the gas constant (1.987 cal./deg.-mole), and T is the absolute temperature. The temperature data plotted according to Eq. 4 are shown in Fig. 5. The energy of activation for each adsorbate, as determined from the least-squares slope of the linear plots, was found to be +2.39 kcal./mole for the glycodeoxycholate anion and +10.6 kcal./mole for the glycocholate anion. These findings are consistent with those previously obtained in that the dihydroxy bile salt anion, glycodeoxycholate, was suggested to be capable of a higher degree of nonelectrostatic interaction with cholestyramine than was the trihydroxy derivative, glycocholate (8).

Effect of Agitation Intensity—In order to ascertain whether the speed of agitation, to which the reaction medium was subjected, influenced the rate of the bile salt anion-cholestyramine interaction, studies were performed at a constant temperature of 37° and agitation intensities of 40, 60, 70, and 100 r.p.m. The results of these studies are depicted in Fig. 6 and the second-order rate constants, calculated from the slopes of these linear plots, are listed in Table III. The data obtained indicate that the rates of binding of both glycodeoxycholate and glycocholate anions to cholestyramine increase with increasing rate of agitation of the reaction medium, the former anion being more influenced by this parameter than the latter.

A log–log plot of the apparent second-order rate constants *versus* speed of agitation (r.p.m.) is presented in Fig. 7. The excellent linearity of these curves suggests that agitation intensity affects the rate of interaction in accordance with the following empirical relationship:

$$\log k' = b \log S + \log a \qquad \text{(Eq. 5)}$$

where S represents the speed of agitation (r.p.m.), k' is the apparent second-order rate constant governing the interaction, and b and a are constants. This mathematical relationship has been commonly employed to aid in distinguishing the process controlling a heterogeneous reaction rate (25). Generally, if the reaction in question is diffusion controlled, then the value of the constant b should closely approach or be equal to unity, which is in agreement with the Nernst-Brunner film theory (25). The applicability of this equation to bile salt anion–cholestyramine interactions becomes evident if one considers the adsorption process, which in essence involves the removal of adsorbate molecules from solution to essentially an undissolved state, as being the reverse of a dissolution process. The magnitude of the constant b for the two adsorbates was obtained from linear regression slope values of the plots illustrated in Fig. 7. The values of b, so determined, were 0.760 and 1.09 for the glycocholate and glycodeoxycholate anions, respectively, which suggest that the adsorption process is primarily diffusion controlled. It appears, therefore, that the rate of binding of bile salt anions to cholestyramine is dependent on the rate of diffusion of the adsorbate through a stagnant film surrounding the individual resin particle beads which is characteristic of film diffusion-controlled exchange reactions. As a result, as the speed of agitation of the reaction medium is increased, the thickness of this film is reduced and hence there is a potentiation in the rate of interaction. The lower b value, as well as the lower second-order rate constant at any speed of agitation, noted for the glycocholate–cholestyramine interaction as compared to that for the glycodeoxycholate anion can be readily explained based on the fact that trihydroxy bile salt anions are hydrated to a greater extent than dihydroxy derivatives (26). The higher apparent molecular weight of the glycocholate anion results in a lower diffusion coefficient for this anion, thus producing both a decrease in its rate of diffusion through the stag-

nant film as well as a decrease in its rate of interaction with this pharmacologically important anionic exchange resin.

SUMMARY

The present investigation has established, apparently for the first time, that the bile salt anion–cholestyramine reaction occurs by means of apparent second-order kinetics and that the reaction rates are dependent on the chemical structure of the bile salt anion participating in the interaction. Under conditions of mild agitation, it was shown that the reaction rates decrease in the following order: glycodeoxycholate > taurocholate > glycocholate, which parallels the affinity with which they bind to the resin. It is also of physiological interest that the addition of inorganic electrolyte to the system markedly depresses the rate of binding of the taurocholate and glycocholate anions to cholestyramine, which helps to explain why the resin is not as efficient as it should be *in vivo* based on the recommended dosage levels prescribed.

Both an increase in the temperature and the agitation intensity employed in the kinetic studies potentiated the rate of interaction between the glycocholate or glycodeoxycholate anion and cholestyramine. These data suggest that the binding process is most probably film diffusion controlled (27).

Additional kinetic studies are being conducted in this laboratory in order to ascertain the influence of such parameters as resin particle size and the influence of other endogenous substances on this pharmacologically important resin–bile salt interaction.

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* Predoctoral Fellow, U. S. Public Health Service.

Toxogonin: Blood Levels and Side Effects after Intramuscular Administration in Man

FREDERICK R. SIDELL and WILLIAM A. GROFF

Abstract □ Toxogonin, given intramuscularly to 10 healthy young men in doses of 2.5–10 mg./kg., produced dose-related oxime whole blood levels of 6.3–26.5 mcg./ml. and had a plasma half-time of 82.8 min. Associated side effects included tachycardia, hypertension, and a dose-independent symptom complex consisting of peroral warmth, paresthesia and hypalgesia, and a menthol taste. Some published data on the relative effectiveness of toxogonin and pralidoxime (chloride and methanesulfonate) suggest further inquiry into toxogonin's therapeutic potential may be needed.

Keyphrases □ Toxogonin, intramuscular injection—oxime blood levels □ Plasma half-life—toxogonin □ Dose relation, toxogonin—plasma blood levels □ Urinary excretion—toxogonin □ Side effects—toxogonin

Until about a decade ago the only therapy for poisoning by cholinesterase inhibitors was atropine or another anticholinergic drug plus supportive therapy. In the 1950's the oximes were developed and their usefulness as adjuncts to atropine was established (1, 2).

On the basis of therapeutic potency, relatively low toxicity in man, and other factors, the oxime generally adopted for use is pralidoxime chloride (2-PAMCl;