Hoekstra, D., de Boer, T., Klappe, K., & Wilschut, J. (1984) *Biochemistry 23*, 5675–5681.
Homma, M., Shimizu, K., Shimizu, Y.-K., & Ishida, N. (1976) *Virology 71*, 41–47.
Hsu, M.-C., Scheid, A., & Choppin, P. W. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 5862–5866.
Israel, S., Ginsberg, D., Laster, Y., Zakai, N., Milner, Y., & Loyter, A. (1983) *Biochim. Biophys. Acta 732*, 337–346.
Koppel, D. E., & Sheetz, M. P. (1981) *Nature (London) 293*, 159–161.
Koppel, D. E., Axelrod, D., Schlessinger, J., Elson, E. L., & Webb, W. W. (1976) *Biophys. J. 16*, 1315–1329.
Loyter, A., & Volsky, D. J. (1982) *Cell Surf. Rev. 8*, 215–266.
Lyles, D. S., & Landsberger, F. R. (1979) *Biochemistry 18*, 5088–5095.
Maeda, T., Eldridge, C., Toyama, S., Ohnishi, S.-I., Elson, E. L., & Webb, W. W. (1979) *Exp. Cell Res. 123*, 333–343.
Maeda, T., Kuroda, K., Toyama, S., & Ohnishi, S.-I. (1981) *Biochemistry 20*, 5340–5345.
Markwell, M. K. A., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978) *Anal. Biochem. 87*, 206–210.
Micklem, K. J., Nyaruwe, A., & Pasternak, C. A. (1985) *Mol. Cell. Biochem. 66*, 163–173.
Peretz, H., Toister, Z., Laster, Y., & Loyter, A. (1974) *J. Cell Biol. 63*, 1–11.
Poste, G., & Pasternak, C. A. (1978) *Cell Surf. Rev. 5*, 306–367.
Rimon, G., Meyerstein, N., & Henis, Y. I. (1984) *Biochim. Biophys. Acta 775*, 283–290.
Ryan, J. L., Albert, R. D., Dickler, H. B., & Henkart, P. A. (1975) *J. Exp. Med. 142*, 814–826.
Schlessinger, J., Axelrod, D., Koppel, D. E., Webb, W. W., & Elson, E. L. (1977) *Science (Washington, D.C.) 195*, 307–309.
Shimizu, Y. K., Shimizu, K., Ishida, N., & Homma, M. (1976) *Virology 71*, 48–60.
Steck, T. L., & Kant, J. A. (1974) *Methods Enzymol. 31*, 172–175.
Struck, D. K., & Pagano, R. E. (1980) *J. Biol. Chem. 255*, 5404–5410.
Struck, D. K., Hoekstra, D., & Pagano, R. E. (1981) *Biochemistry 20*, 4093–4099.
Uchida, T., Yamaizumi, M., & Okada, Y. (1977) *Nature (London) 266*, 839–840.
Vainstein, A., Hershkowitz, M., Israel, S., Rabin, S., & Loyter, A. (1984) *Biochim. Biophys. Acta 773*, 181–188.
van Meer, G., Davoust, J., & Simons, K. (1985) *Biochemistry 24*, 3593–3602.
Wey, C.-L., Cone, R. A., & Edidin, M. A. (1981) *Biophys. J. 33*, 225–232.
White, J., Kielian, M., & Helenius, A. (1983) *Q. Rev. Biophys. 16*, 151–195.
Wolf, D. E., Edididn, M., & Dragsten, P. R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2043–2045.

Low Concentrations of Bile Salts Increase the Rate of Spontaneous Phospholipid Transfer between Vesicles†

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ABSTRACT: The rate of 1-palmitoyl-2-[12-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]phosphatidylcholine (P-C_{12}-NBD-PC) transfer between dioleoylphosphatidylcholine vesicles was measured by a technique based on resonance energy transfer between P-C_{12}-NBD-PC and *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine [Nichols, J. W., & Pagano, R. E. (1982) *Biochemistry 21*, 1720–1726]. Addition of bile salts at concentrations below their critical micelle concentrations increased the rate of spontaneous P-C_{12}-NBD-PC transfer without disrupting the vesicles. The effectiveness in increasing the transfer rate was dependent on the structure of the bile salt. In general, conjugated bile salts were more effective than unconjugated, and mono- and dihydroxy bile salts were more effective than trihydroxy. The kinetics of intervesicular P-C_{12}-NBD-PC transfer in the presence of cholate were found to be consistent with a mass action kinetic model based on the premise that bile salts bind to the vesicles, alter the dissociation and/or association rate constants for phospholipid monomer–vesicle interaction, and increase the rate of phospholipid transfer via the diffusion of soluble monomers through the aqueous phase. Temperature dependence studies indicated that cholate binding to vesicles is an entropy-driven process and that cholate binding lowers the free energy of activation for phospholipid monomer–vesicle dissociation by producing compensatory decreases in both the enthalpy and entropy of activation.

Phospholipids spontaneously transfer between membranes by the diffusion of soluble monomers through the aqueous bulk phase (Duckwitz-Peterlein et al., 1977; Roseman & Thompson, 1980; McLean & Phillips, 1981, 1984; Nichols & Pagano, 1981, 1982; Massey et al., 1982a,b; Decuyper et al., 1983; Ferrell et al., 1985). This paper demonstrates that concentrations of bile salts below their critical micelle concentrations bind to membranes and increase the spontaneous rate of intervesicular phospholipid transfer. The simplest explanation for this effect is that bile salts increase the rate of spontaneous transfer by partitioning into the membranes and altering the dissociation and/or association rate constants for phospholipid monomer–vesicle interaction. This paper presents a model based on this premise using the principles of mass action kinetics and demonstrates that this model can predict the initial

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rate kinetics of bile salt stimulated intervesicular phospholipid transfer. According to this model, bile salts increase the rate of phospholipid transfer between vesicles by increasing the rate constant for phospholipid dissociation from vesicles. Measurements of the temperature dependence of the bile salt stimulated transfer indicate that bile salts lower the free energy of activation for this process.

Control and bile salt stimulated initial rates of intervesicular transfer for the fluorescently labeled phospholipid 1-palmitoyl-2-[12-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]phosphatidylcholine (P-C_{12}-NBD-PC)[1] were measured by a technique based on resonance energy transfer between P-C_{12}-NBD-PC and *N*-(lissamine-rhodamine B sulfonyl)-dioleoylphosphatidylethanolamine (*N*-Rh-PE) (Nichols & Pagano, 1982). A preliminary report of this work has appeared elsewhere (Nichols, 1985a).

Experimental Procedures

Materials and Routine Procedures. DOPC and P-C_{12}-NBD-PC were purchased from Avanti Biochemical Corp. *N*-Rh-PE was synthesized and purified as previously described (Struck et al., 1981). [^{3}H]DOPC was a gift of Richard Pagano (Pagano et al., 1981). [^{14}C]Cholate was purchased from New England Nuclear. [^{3}H]DOPC and [^{14}C]cholate were tested for purity by silica gel thin-layer chromatography and liquid scintillation counting. Greater than 98% of the ^{14}C and 95% of the ^{3}H counts migrated with cholate and DOPC, respectively. Bile salts were purchased from Sigma Chemical Co. Bile sallts were tested for purity by silica gel thin-layer chromatography in choroform/methanol/acetic acid/water, 65:25:2:4. Densitometric analysis of the plates following sulfuric acid charring indicated that all of the bile salts used in these studies were greater than 97% pure. Phospholipids were stored at −20 °C, periodically monitored for purity by thin-layer chromatography, and repurified when necessary. Phospholipid concentrations were determined by a lipid phosphorus assay (Ames & Dubin, 1960). Bile salt concentrations were determined by a fluorescence assay (Beher et al., 1981).

Vesicle Preparation. Lipids were mixed in desired proportions and their storage solvents removed by evaporation under nitrogen followed by a minimum of 4 h of vacuum desiccation. Vesicles were prepared by ethanol injection (Kremer et al., 1977) as follows. Dried phospholipids were dissolved in ethanol (10–40 μmol/mL), injected into NaCl–HEPES at room temperature, and dialyzed against 4 L of NaCl–HEPES overnight at 4 °C. The resulting vesicles (final concentration 0.5–2.0 mM) were used on the day following injection.

Fluorescence Measurements. Fluorescence was recorded from a Perkin-Elmer MPF-44E fluorescence spectrophotometer. Peak absorbance of samples was kept to <0.1 to reduce inner filter effects. Solutions in the cuvette were stirred by a magnetic stirrer, and the temperature was controlled by a circulating water bath. Concentrations of P-C_{12}-NBD-PC and *N*-Rh-PE in vesicles were determined by disrupting the vesicles with Triton X-100 (1% final concentration) and measuring NBD and rhodamine fluorescence. Triton X-100 disruption eliminates energy transfer, allowing P-C_{12}-NBD-PC and *N*-Rh-PE concentrations to be determined by comparison to a standard curve made from stock solutions of known concentration.

Table I: Percent Increase in the Initial Rate of Intervesicular P-C_{12}-NBD-PC Transfer in the Presence of Bile Salts[a]

bile salt	% increase in rate	bile salt	% increase in rate
lithocholate	31	chenodeoxycholate	297
cholate	106	deoxycholate	309
glycocholate	134	glycolithocholate	341
taurocholate	197	taurochenodeoxycholate	464
glycodeoxycholate	296	taurodeoxycholate	483

[a] Donor vesicles: P-C_{12}-NBD-PC/*N*-Rh-PE/DOPC, mole ratio 1:1:98, final concentration 38 μM. Acceptor vesicles: DOPC, final concentration 38 μM. Bile salts: final concentration 100 μM; temperature, 25 °C.

Initial Rate Measurements of P-C_{12}-NBD-PC Intervesicular Transfer. Resonance energy transfer between P-C_{12}-NBD-PC and *N*-Rh-PE was used to measure the rate of transfer of P-C_{12}-NBD-PC from donor to acceptor vesicles as described previously (Nichols & Pagano, 1982). Briefly, the method is as follows. Donor vesicles containing P-C_{12}-NBD-PC/*N*-Rh-PE/DOPC (mole ratio 1:1:98, total phospholipid 500 μM) were added to NaCl–HEPES + bile salts in the fluorescence cuvette. Resonance energy transfer between P-C_{12}-NBD-PC and *N*-Rh-PE quenches the NBD fluorescence (excitation 475 nm, emission 530 nm). Unlabeled acceptor vesicles (DOPC, 500 μM) were added to the cuvette, and an increase in NBD fluorescence was recorded as P-C_{12}-NBD-PC moved into the nonquenching environment of the acceptor vesicles. *N*-Rh-PE moves between the vesicles at a much slower rate than P-C_{12}-NBD-PC (Nichols & Pagano, 1983) and can be considered as nonexchangeable on the time scale of these experiments. Initial rates (relative fluorescence units per minute) were determined from the recordings and converted to the amount transferred per minute from a standard curve prepared from P-C_{12}-NBD-PC contained in DOPC vesicles at nonquenching concentrations (0.1 mol %). The fluorescence yield of the standard vesicles was determined for each temperature.

Measurement of the Cholate–Vesicle Equilibrium Constant. The cholate–vesicle equilibrium constant was measured by dialysis; 100 μM [^{14}C]cholate and varying amounts of [^{3}H]-DOPC vesicles in 1.0 mL of NaCl–HEPES were placed on the cis side of a multichambered Plexiglas dialysis apparatus and allowed to equilibrate (approximately 12 h) across a Spectrapor 6 dialysis membrane (50 000 molecular weight cutoff) with NaCl–HEPES on the trans side. Concentrations of [^{3}H]DOPC and [^{14}C]cholate in both chambers were determined by liquid scintillation counting. The cholate concentration in the trans chamber is the free monomer concentration. The concentration of vesicle-bound cholate was obtained by subtracting free monomer minus the total cholate concentration on the cis side. The slope of a plot of the ratio of vesicle-bound to free cholate vs. vesicle concentration is equal to the equilibrium constant (K_b^I; see eq A15 in the Appendix). For the calculation of thermodynamic parameters, K_b^I was converted to the mole fraction partition coefficient by multiplying by the molar concentration of water (55 mM).

Results and Discussion

The data presented in Table I indicate that low concentrations of bile salts in solution with phospholipid vesicles increase the rate of intervesicular phospholipid transfer. Under the conditions used for these rate measurements (0.15 M NaCl, pH 7.4, 25 °C), all of the bile salt molecules tested in

[1] Abbreviations: P-C_{12}-NBD-PC, 1-palmitoyl-2-[12-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]phosphatidylcholine; *N*-Rh-PE, *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; NaCl–HEPES, 0.9% NaCl in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4; HDL, high-density lipoprotein.

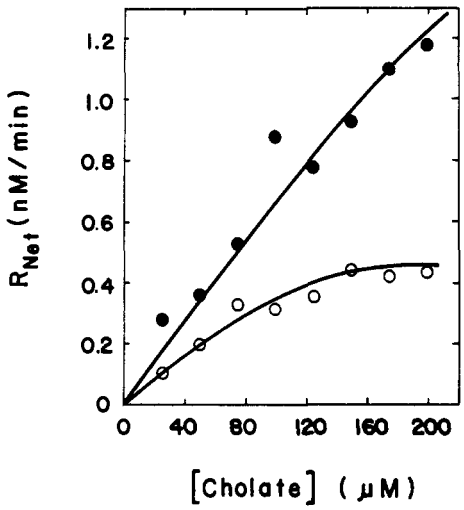

FIGURE 1: Cholate concentration dependence of intervesicular P-C_{12}-NBD-PC transfer. R_{net} is the cholate-dependent increase of the initial rate over the control. Donor vesicles, P-C_{12}-NBD-PC/*N*-Rh-PE/DOPC, mole ratio 1:1:98; acceptor vesicles, DOPC. (○) Donor vesicle concentration 50 μM, acceptor vesicle concentration 20 μM; (●) donor vesicle concentration 50 μM, acceptor vesicle concentration 200 μM.

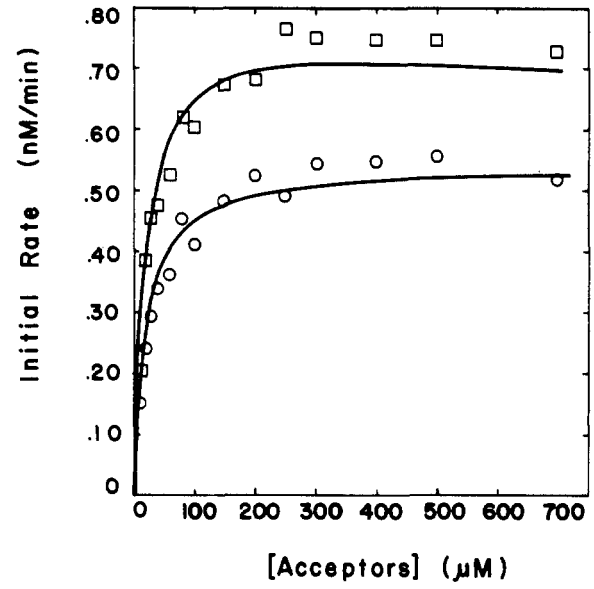

FIGURE 2: Acceptor concentration dependence of intervesicular P-C_{12}-NBD-PC transfer. Donor vesicles, P-C_{12}-NBD-PC/*N*-Rh-PE/DOPC, mole ratio 1:1:98, final concentration held constant at 20 μM; acceptor vesicles, DOPC. (○) Initial rate without cholate; (□) initial rate in the presence of 50 μM cholate.

Table I exist predominantly as unprotonated salts with critical micelle concentrations greater than 1 mM (Carey & Small, 1969, 1972). At these low concentrations of bile salts, the integrity of the phospholipid vesicles remained intact, as evidenced by only a slight change in the extent of resonance energy transfer between P-C_{12}-NBD-PC and *N*-Rh-PE. If the vesicles had been disrupted by the bile salts, the two probes would be physically separated and the extent of energy transfer would be significantly reduced. We therefore propose that bile salts increase the rate of intervesicular transfer of phospholipids between vesicles by binding to the vesicle and increasing the rate of spontaneous monomer diffusion through the aqueous phase. A model based on this mechanism is presented in the Appendix and has been tested for its ability to predict the initial rate kinetics of intervesicular phospholipid transfer stimulated by the presence of bile salts.

The model assumes for simplicity that at low mole fractions of bile salts bound to the vesicles the phospholipid association and dissociation rate constants are altered by a constant factor when in the vinicity of a bile salt molecule. This assumption was true for cholate concentrations below 50 μM even at the lowest concentrations of vesicles used in these experiments (Figure 1). At concentrations of bile salt below the critical micelle concentration, the amount of bile salt bound to the donor vesicles is a linear function of the total amount of bile salt added to solution (eq A18). Thus, the increase in the initial rate due to addition of bile salts (R_{net}) would be expected to be a linear function of the total bile salt concentration given a constant fractional change in rate constants in the vicinity of a bile salt molecule. The effect of bile salts begins to saturate at higher concentrations, probably due to bile salt molecules interacting with each other as opposed to adjacent phospholipids.

A representative experiment demonstrating the ability of the model to predict the effect of acceptor vesicle concentration on the initial transfer rate is presented in Figure 2. Although the model (eq A21) has three unknown parameters, two of the unknowns (k^I_{D-} and K^I_b) were determined independently, leaving a single parameter (f^I_{D-}) to fit to the data. The cholate–vesicle equilibrium constant (K^I_b) was measured to be 627 M^{-1} at 25 °C (see Experimental Procedures; data not shown). The off-rate constant from the vesicles was calculated from the spontaneous rate of P-C_{12}-NBD-PC transfer in the absence of bile salt [$k^I_{D-} = (3.6 \pm 0.8) \times 10^{-5}$ s^{-1}; average ± standard deviation of three independent experiments]. The initial rate of P-C_{12}-NBD-PC transfer in the presence of 50 μM cholate was predicted from the best fit of the model while holding k^I_{D-} and K^I_b constant at the above-mentioned values ($f^I_{D-} = 13.0 \pm 1.8$; average ± standard deviation of three independent experiments). The theoretical curve accurately predicted the dependence of the initial rate of P-C_{12}-NBD-PC transfer on acceptor vesicle concentration.

The model also predicts that at very high acceptor vesicle concentrations the initial rate will return to that of the control. This was not observed in these experiments because the affinity of cholate for the vesicles (K^I_b) was so low that addition of the acceptor vesicle concentration required to see this effect was not experimentally possible. The rationale for this prediction is that at high acceptor vesicle concentrations most of the bile salt will be bound to the acceptors, leaving an insignificant amount bound to the donors. At high acceptor concentrations, the rate-limiting step for monomer diffusion is the dissociation rate from the donors.

Since previous studies (Nichols, 1985b) demonstrated that the activation energy for phospholipid monomer dissociation from vesicles is dominated by the activation enthalpy, with only a small contribution due to the entropy of activation, we were interested in determining the effect of cholate on the enthalpy and entropy of activation. The temperature dependence of the dissocation rate constant was measured in the presence and absence of cholate and is presented as Arrhenius plots in Figure 3.

Since the apparent rate constant in the presence of cholate depends both on the affinity constant (K^I_b) and also on its effect on the dissociation constant (f^I_{D-})

$$k^I_{D-}\left[1 + \frac{(f^I_{D-} - 1)\alpha[b]_T}{[A]_I + [B]_{II} + (K^I_b)^{-1}}\right]$$

the temperature dependence of the affinity constant was measured independently (data not shown). A van't Hoff plot of the partition coefficient vs. the inverse of temperature indicates that the binding of cholate to DOPC vesicles is only

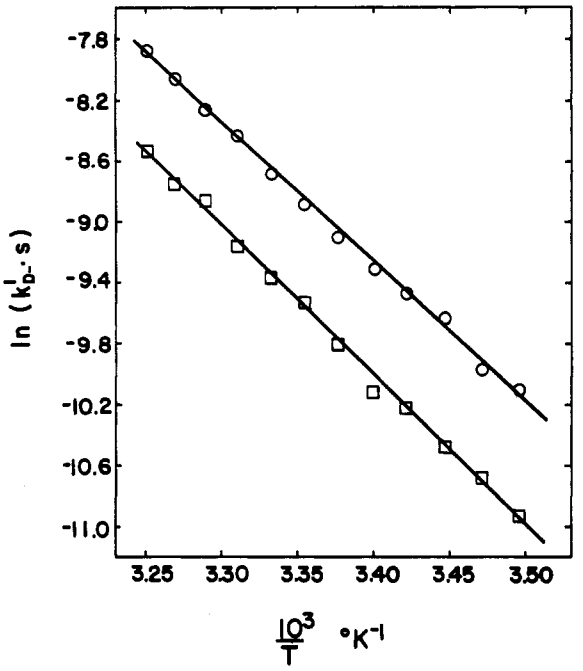

FIGURE 3: Arrhenius plots of dissociation rate constants. Effect of cholate on P-C_{12}-NBD-PC dissociation from DOPC vesicles. Donor vesicles, P-C_{12}-NBD-PC/*N*-Rh-PE/DOPC, mole ratio 1:1:98, final concentration 50 μM; acceptor vesicles, DOPC, final concentration 50 μM. (□) Control; (○) +100 μM cholate. Linear regression analysis was used to calculate the slopes and *y* intercepts ± standard deviations (Snedecor & Cochran, 1967). Control: slope = −9830 ± 170 K; *y* intercept = 23.4 ± 0.6. With cholate: slope = −9150 ± 160 K; *y* intercept = 21.8 ± 0.5. Predicted ln ($k_{D-}^{I}s$) at 25 °C for control equals −9.58 ± 0.05 and with cholate equals −8.86 ± 0.04.

slightly dependent on temperature and thus is an entropy-driven reaction ($\Delta G°$ = −6.2 ± 0.4 kcal/mol; $\Delta H°$ = +0.7 ± 0.3 kcal/mol; $T\Delta S°$ = 6.9 ± 0.3 kcal/mol; K_b^I = 627 M^{-1} at 25 °C). Therefore, the temperature dependence of the apparent phospholipid monomer–vesicle dissociation rate constant in the presence of cholate was interpreted to result solely from changes in the dissociation rate constant and not from changes in the cholate–vesicle affinity constant. Thus, the effect of cholate on the activation energies for dissociation can be determined (Table II). An Arrhenius plot of the experiment (Figure 3) yielded two straight lines (with and without cholate) indicating that the enthalpy of activation for both was independent of temperature in this range. A theoretical description of amphiphile monomer–micelle dissociation developed by Aniansson et al. (1976) was used to calculate the enthalpy, entropy, and free energy of activation from the Arrhenius plots (Table II). These results indicated that cholate binding to phospholipid vesicles lowered the free energy of activation for P-C_{12}-NBD-PC dissociation. Under the conditions used in these experiments (total cholate concentration equals 100 μM and donor and acceptor vesicle concentrations equal 50 μM DOPC), the ratio of cholate bound to DOPC in the donor vesicles can be calculated from eq A18 to be 0.06. At 25 °C, this mole ratio of cholate to DOPC lowered the free energy of activation for monomer–vesicle dissociation by 0.4 kcal/mol (equivalent to a ≈2-fold increase in the rate of transfer). The net reduction in the free energy of activation resulted from larger but compensatory decreases in the enthalpy of activation ($\Delta H^{\ddagger\circ}$ = −1.3 kcal/mol) and the entropy of activation ($T\Delta S^{\ddagger\circ}$ = −0.9 kcal/mol).

The three hydroxyl groups on the cholate molecule are α oriented, and the aliphatic chain terminating in the carboxyl group is flexible such that all the polar groups lie on the same plane of the steroid nucleus (Carey & Small, 1972). Considering this structure, it is unlikely that the individual cholate molecules intercalate into the phospholipid acyl chain region of the bilayer as does cholesterol. Two possible alternatives are that individual cholate molecules lie on the surface of the vesicle with the apolar surface of the steroid nucleus penetrating only a few carbons deep into the acyl chain region and the polar plane remaining exposed to the water in the headgroup region or that hydrogen bonding of the hydroxyl groups on the cholate molecules results in the formation of dimers that are able to intercalate into the phospholipid bilayer, exposing only their apolar surface to the hydrophobic acyl chain region (Mazer et al., 1980). In either case, according to the kinetic data presented above, phospholipids in the vicinity of a cholate monomer or dimer are less stable and more likely to dissociate from the bilayer.

Table II: Thermodynamic Parameters for P-C_{12}-NBD-PC Monomer Dissociation from DOPC Vesicles ± Cholate[a]

parameter	control	+cholate	change[b]
k_{D-} ($\times 10^4$ s^{-1})	0.69 ± 0.03	1.42 ± 0.06	2.1
$\Delta G^{\ddagger\circ}$ (kcal/mol)	20.7 ± 0.03	20.3 ± 0.02	−0.4
$\Delta H^{\ddagger\circ}$ (kcal/mol)	19.5 ± 0.3	18.2 ± 0.3	−1.3
$T\Delta S^{\ddagger\circ}$ (kcal/mol)	−1.2 ± 0.3	−2.1 ± 0.3	−0.9

[a] Activation energies for monomer–vesicle dissociation were calculated by using the following theoretical description of amphiphile monomer–micelle dissociation developed by Aniansson et al. (1976): $k_{D-}^{I} = [D_m/(l_b)^2] \exp(-\Delta G^{\ddagger\circ}/RT)$. At constant pressure, $\Delta G^{\ddagger\circ} = \Delta H^{\ddagger\circ} - T\Delta S^{\ddagger\circ}$, and thus, $\ln k_{D-}^{I} = \Delta H^{\ddagger\circ}/RT + \Delta S^{\ddagger\circ}/R + \ln [D_m/(l_b)^2]$. *R* and *T* have the usual meanings, D_m is the diffusion constant for the exiting monomer (assumed to be 5×10^{-6} cm^2/s), and l_b is the width of the barrier that is *RT* energy units below its maximum (assumed to be 0.7 Å). $\Delta H^{\ddagger\circ}$ and $\Delta S^{\ddagger\circ}$ were obtained respectively from the slope and *y* intercept of the Arrhenius plot in Figure 3. The ± values are the standard deviations propagated from the calculated standard deviations of the slopes, *y* intercepts, and predicted ln ($k_{D-}^{I}s$) from Figure 3. [b] For k_{D-} the change is the quotient (+cholate/control); for activation energies, the change is the difference of the two.

The structure of the bile salts determines their effectiveness in increasing the rate of intervesicular phospholipid transfer (Table I). For a given amount of bile salt added to the vesicle solution, the degree to which the initial rate is increased depends both on the extent of binding to the vesicles (determined by K_b^I) and on the extent to which it increases the dissociation rate constant for phospholipids in its vicinity (f_{D-}^{I}). In general, conjugated bile salts are more effective than unconjugated, and mono- and dihydroxy bile salts are more effective than trihydroxy.

This mechanism for increasing the rate of intervesicular phospholipid transfer should apply to any number of different molecules that bind to membranes and nonspecifically reduce the stability of phospholipids in the bilayer. For example, in the same assay presented above, the addition of 5 μM lysolecithin increases the rate of P-C_{12}-NBD-PC 2-fold without disrupting the vesicles (data not shown). Furthermore, the nonspecific lipid transfer protein isolated from beef liver (Crain & Zilversmit, 1980) has been proposed to function by a similar mechanism (Nichols & Pagano, 1983).

Concentrations of bile salt in the same range as used in this sudy are found postprandial in the hepatic portal vein of humans (Ahlberg et al., 1977), and 30–40% of the bile salt in the plasma is bound to HDL particles (Kramer et al., 1979). Low concentrations of bile salt in hepatic portal blood may function to increase the rate of movement of lipids between HDL and other membranous compartments. The kinetic model for bile salt stimulated monomer diffusion of phospholipids between vesicles will be useful in pursuing the physiological significance of low levels of plasma bile salt. In addition, this model should be generally useful for studying

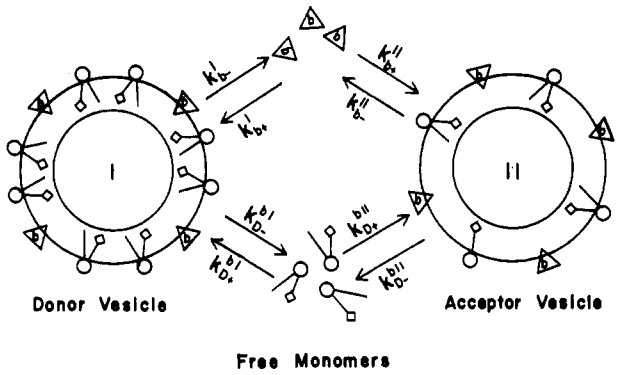

FIGURE 4: Schematic diagram of bile salt stimulated phospholipid diffusion between vesicles and definition of rate constants.

other molecules thought to stimulate the diffusion of lipids between membranes.

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APPENDIX

Model for Bile Salt Stimulated Phospholipid Diffusion between Vesicles. A model describing the diffusion of phospholipid monomers between phospholipid vesicles has been presented previously (Nichols & Pagno, 1981, 1982). The effect of bile salts on this process is modeled by assuming that bile salts partition rapidly between the vesicle and water phases. Bile salts are assumed to alter the phospholipid monomer–vesicle dissociation and/or association rate constants of a given vesicle population as a linear function of their mole fraction in the vesicle population.

Rate equations for the spontaneous diffusion-mediated model (Nakagawa, 1974; Thilo, 1977; Nichols & Pagano, 1981, 1982) can be written by assuming that the rate at which a phospholipid monomer escapes from the surface of a vesicle is proportional to its concentration on that surface and that the association rate is proportional to the product of the free monomer concentration and the surface area of the membrane (Figure 4). The following rate equations describing the transfer of the phospholipid probe molecule D between two populations of vesicles composed of phospholipid A and B can be written by using these equations:

$$d[D]_I/dt = k^I_{D+}[D]_m(S_D[D]_I + S_A[A]_I) - k^I_{D-}[D]_I \quad (A1)$$

$$d[D]_{II}/dt = k^{II}_{D+}[D]_m(S_D[D]_{II} + S_B[B]_{II}) - k^{II}_{D-}[D]_{II} \quad (A2)$$

k^I_{D+} and k^{II}_{D+} are the on-rate constants of the D phospholipid into vesicle populations I and II, respectively. k^I_{D-} and k^{II}_{D-} are the corresponding off-rate constants. $[D]_m$ is the free monomer concentration in bulk solution of the D phospholipid, and $[D]_I$, $[D]_{II}$, $[A]_I$, and $[B]_{II}$ are the concentrations in bulk solution of the D, A, and B phospholipids residing in the outer leaflet of the subscripted vesicle populations. S_A, S_B, and S_D are the surface area per mole of the subscripted lipid. The free monomer concentration is assumed to reach steady-state equilibrium rapidly in relation to the net transfer process, and therefore

$$d[D]_I/dt + d[D]_{II}/dt = 0$$

and

$$[D]_m = \frac{k^I_{D-}[D]_I + k^{II}_{D-}[D]_{II}}{k^I_{D+}(S_D[D]_I + S_A[A]_I) + k^{II}_{D+}(S_D[D]_{II} + S_B[B]_{II})} \quad (A3)$$

If $[D]_I$ and $[D]_{II}$ are a small fraction of $[A]_I$ and $[B]_{II}$, respectively, then

$$S_D[D]_I + S_A[A]_I \approx S_A[A]_I \quad (A4)$$

and

$$S_D[D]_{II} + S_B[B]_{II} \approx S_B[B]_{II} \quad (A5)$$

Substituting eq A3–A5 into eq A2, the initial rate of transfer (R), where $[D]_{II} = 0$, is predicted by

$$R_{spont} = \frac{d[D]_{II}}{dt} = \frac{k^{II}_{D+}k^I_{D-}[D]_I S_B[B]_{II}}{k^I_{D+}S_A[A]_I + k^{II}_{D+}S_B[B]_{II}} \quad (A6)$$

The effect on the rate of transfer of the binding of small amounts of bile salt to the vesicles can be modeled in an analogous manner:

$$R_{bile} = \frac{k^{bI}_{D+}k^{bI}_{D-}[D]_I S_B[B]_{II}}{k^{bI}_{D+}S_A[A]_I + k^{bII}_{D+}S_B[B]_{II}} \quad (A7)$$

where R_{bile} is the initial rate of phospholipid transfer in the presence of bile salt and k^{bI}_{D-}, k^{bI}_{D+}, and k^{bII}_{D+} are the off- and on-rate constants for the two vesicle populations in the presence of bile salt. Assume that at low mole fractions of bile salt in the vesicles there are two off-rate constants: that for phospholipids not in the vicinity of a bound bile salt molecule (k^I_{D-}) and that for phospholipids in the vicinity of a bound bile salt molecule ($f^I_{D-}k^I_{D-}$), where the spontaneous rate constant (k^I_{D-}) is either increased or decreased by a constant factor (f^I_{D-}). The relative contribution of each rate constant to the apparent rate constant (k^{bI}_{D-}) is dependent on the mole fraction of phospholipid in the vicinity of a bound bile salt molecule ($\alpha[b]_I/[A]_I$), where $[b]_I$ and $[A]_I$ are the concentrations per bulk solution of bile salt and phospholipid in vesicle population I and α is the number of phospholipids in the vicinity of one bile salt molecule. Thus

$$k^{bI}_{D-} = k^I_{D-}\{1 - \alpha[b]_I/[A]_I + f^I_{D-}\alpha[b]_I/[A]_I\} \quad (A8)$$

Similarly

$$k^{bI}_{D+} = k^I_{D+}\{1 - \alpha[b]_I/[A]_I + f^I_{D+}\alpha[b]_I/[A]_I\} \quad (A9)$$

$$k^{bII}_{D+} = k^{II}_{D+}\{1 - \alpha[b]_{II}/[B]_{II} + f^{II}_{D+}\alpha[b]_{II}[B]_{II}\} \quad (A10)$$

Substituting eq A8–A10 into eq A7 gives

$$R_{bile} = \{k^{II}_{D+}\{1 + (f^{II}_{D+} - 1)\alpha[b]_{II}/[B]_{II}\}k^I_{D-}\{1 + (f^I_{D-} - 1)\alpha[b]_I/[A]_I\}[D]_I S_B[B]_{II}\}/\{k^I_{D+}\{1 + (f^I_{D+} - 1)\alpha[b]_I/[A]_I\}S_A[A]_I + k^{II}_{D+}\{1 + (f^{II}_{D+} - 1)\alpha[b]_{II}/[B]_{II}\}S_B[B]_{II}\} \quad (A11)$$

If the bile salt is assumed to equilibrate rapidly between the two populations of vesicles, the amount of bile salt in each can be calculated from its affinity for and the concentration of each vesicle type as demonstrated below. The bile salt is distributed between vesicle populations I and II and the aqueous phase:

$$[b]_T = [b]_I + [b]_{II} + [b]_{aq} \quad (A12)$$

$[b]_T$ is the total concentration of bile salt in solution, and $[b]_{aq}$ is the concentration of unbound bile salt in solution.

Rate equations for bile salt binding to vesicles can be written in a manner analogous to phospholipid–vesicle binding:

$$d[b]_I/dt = k^I_{b+}[b]_{aq}[A]_I - k^I_{b-}[b]_I \quad (A13)$$

$$d[b]_{II}/dt = k^{II}_{b+}[b]_{aq}[B]_{II} - k^{II}_{b-}[b]_{II} \quad (A14)$$

At equilibrium

$$d[b]_I/dt = d[b]_{II}/dt = 0$$

and since by definition

$$k^{I}_{b+}/k^{I}_{b-} = K^{I}_{b} \quad \text{and} \quad k^{II}_{b+}/k^{II}_{b-} = K^{II}_{b}$$

then

$$[b]_{aq} = [b]_{I}/K^{I}_{b}[A]_{I} \tag{A15}$$

and

$$[b]_{aq} = [b]_{II}/K^{II}_{b}[B]_{II} \tag{A16}$$

and

$$[b]_{I}/[b]_{II} = K^{I}_{b}[A]_{I}/K^{II}_{b}[B]_{II} \tag{A17}$$

Substituting eq A15–A17 into eq A12 results in

$$[b]_{I} = \frac{[b]_{T}K^{I}_{b}[A]_{I}}{K^{I}_{b}[A]_{I} + K^{II}_{b}[B]_{II} + 1} \tag{A18}$$

$$[b]_{II} = \frac{[b]_{T}K^{II}_{b}[B]_{II}}{K^{I}_{b}[A]_{I} + K^{II}_{b}[B]_{II} + 1} \tag{A19}$$

Substituting eq A18 and A19 into eq A11 gives the initial rate equation for bile salt stimulated phospholipid transfer between vesicles:

$$R_{bile} = \left[k^{II}_{D+}k^{I}_{D-}[D]_{I}S_{B}[B]_{II}\left\{1 + \frac{(f^{II}_{D+} - 1)\alpha[b]_{T}K^{II}_{b}}{K^{I}_{b}[A]_{I} + K^{II}_{b}[B]_{II} + 1}\right\}\left\{1 + \frac{(f^{I}_{D-} - 1)\alpha[b]_{T}K^{I}_{b}}{K^{I}_{b}[A]_{I} + K^{II}_{b}[B]_{II} + 1}\right\}\right] \Big/ \left[k^{I}_{D+}S_{A}[A]_{I}\left\{1 + \frac{(f^{I}_{D+} - 1)\alpha[b]_{T}K^{I}_{b}}{K^{I}_{b}[A]_{I} + K^{II}_{b}[B]_{II} + 1}\right\} + k^{II}_{D+}S_{B}[B]_{II}\left\{1 + \frac{(f^{II}_{D+} - 1)\alpha[b]_{T}K^{II}_{b}}{K^{I}_{b}[A]_{I} + K^{II}_{b}[B]_{II} + 1}\right\}\right] \tag{A20}$$

For the special case where vesicle populations I and II are composed of the same lipids, $k^{I}_{D-} = k^{II}_{D-}$, $k^{I}_{D+} = k^{II}_{D+}$, $f^{I}_{D-} = f^{II}_{D-}$, $f^{I}_{D+} = f^{II}_{D+}$, $S_A = S_B$, and $K^{I}_{b} = K^{II}_{b}$, eq A20 simplifies to

$$R_{bile} = \frac{k^{I}_{D-}[D]_{I}[B]_{II}\{1 + (f^{I}_{D-} - 1)\alpha[b]_{T}/[[A]_{I} + [B]_{II} + (K^{I}_{b})^{-1}]\}}{[A]_{I} + [B]_{II}} \tag{A21}$$

and eq A6 simplifies to

$$R_{spont} = \frac{k^{I}_{D-}[D]_{I}[B]_{II}}{[A]_{I} + [B]_{II}} \tag{A22}$$

Thus, R_{net}, the bile salt stimulated increase of the initial rate over the spontaneous rate, equals

$$R_{net} = R_{bile} - R_{spont} = \frac{(f^{I}_{D-} - 1)k^{I}_{D-}\alpha[D]_{I}[B]_{II}[b]_{T}}{([A]_{I} + [B]_{II})[[A]_{I} + [B]_{II} + (K^{I}_{b})^{-1}]} \tag{A23}$$

Registry No. DOPC, 10015-85-7; lithocholate, 434-13-9; cholate, 81-25-4; glycocholate, 475-31-0; taurocholate, 81-24-3; glycodeoxycholate, 360-65-6; chenodeoxycholate, 474-25-9; deoxycholate, 83-44-3; glycolithocholate, 474-74-8; taurochenodeoxycholate, 516-35-8; taurodeoxycholate, 516-50-7.

REFERENCES

Ahlberg, J., Angelin, B., Björkhem, I., & Einarsson, K. (1977) *Gastroenterology 73*, 1377–1382.

Ames, B. N., & Dubin, D. T. (1960) *J. Biol. Chem. 235*, 769–775.

Aniansson, E. A. G., Wall, S. N., Almgren, M., Hoffman, H., Kielman, I., Ulbricht, W., Zana, R., Lang, J., & Tondre, C. (1976) *J. Phys. Chem. 80*, 905–922.

Beher, W. T., Stradnieks, S., Lin, G. J., & Sanfield, J. (1981) *Steroids 38*, 281–295.

Carey, M. C., & Small, D. M. (1969) *J. Colloid Interface Sci. 31*, 382–396.

Carey, M. C., & Small, D. M. (1972) *Arch. Intern. Med. 130*, 506–527.

Crain, R. C., & Zilversmit, D. B. (1980) *Biochemistry 19*, 1433–1439.

DeCuyper, M., Joniau, M., & Dangreau, H. (1983) *Biochemistry 22*, 415–420.

Duckwitz-Peterlein, G., Eilenberger, G., & Overath, P. (1977) *Biochim. Biophys. Acta 469*, 311–325.

Ferrell, J. E., Jr., Lee, K.-J., & Huestis, W. H. (1985) *Biochemistry 24*, 2857–2864.

Kramer, W., Buscher, H., Gerok, W., & Kurz, G. (1979) *Eur. J. Biochem. 102*, 1–9.

Kremer, J. M. H., v. d. Esker, M. W. J., Pathmamanoharan, C., & Wiersema, P. H. (1977) *Biochemistry 16*, 3932–3935.

Massey, J. B., Gotto, A. M., Jr., & Pownall, H. J. (1982a) *Biochemistry 21*, 3630–3636.

Massey, J. B., Gotto, A. M., Jr., & Pownall, H. J. (1982b) *J. Biol. Chem. 257*, 5444–5448.

Mazer, N. A., Benedek, G. B., & Carey, M. C. (1980) *Biochemistry 19*, 601–615.

McLean, L. R., & Phillips, M. C. (1981) *Biochemistry 20*, 2893–2900.

McLean, L. R., & Phillips, M. C. (1984) *Biochemistry 23*, 4624–4630.

Nakagawa, T. (1974) *Colloid Polym. Sci. 252*, 56–64.

Nichols, J. W. (1985a) *Biophys. J. 47*, 246a.

Nichols, J. W. (1985b) *Biochemistry 24*, 6390–6398.

Nichols, J. W., & Pagano, R. E. (1981) *Biochemistry 20*, 2783–2789.

Nichols, J. W., & Pagano, R. E. (1982) *Biochemistry 21*, 1720–1726.

Nichols, J. W., & Pagano, R. E. (1983) *J. Biol. Chem. 258*, 5368–5371.

Pagano, R. E., Martin, O. C., Shroit, A. J., & Struck, D. K. (1981) *Biochemistry 20*, 4920–4927.

Roseman, M. A., & Thompson, T. E. (1980) *Biochemistry 19*, 439–444.

Snedecor, G. W., & Cochran, W. G. (1967) *Statistical Methods*, 6th ed., The Iowa State University Press, Ames, IA.

Struck, D. K., Hoekstra, D., & Pagano, R. E. (1981) *Biochemistry 20*, 4093–4099.

Thilo, L. (1977) *Biochim. Biophys. Acta 469*, 326–334.