Shipolini, R. A., Callewaert, G. L ., Cottrell, R. C., Doonan, S., Vernon, C. A., & Banks, B. E. C. (1971) *Eur. J. Biochem. 20*, 459–468.
Siminovitch, D. J., & Jeffrey, K. R. (1981) *Biochim. Biophys. Acta 645*, 270–278.
Smith, I. C. P., Jennings, H. J., & Deslauriers, R. (1975) *Acc. Chem. Res. 8*, 306–313.
Takahashi, T., Sugahara, T., & Ohsaka, A. (1981) *Methods Enzymol. 71*, 710–725.
Tsai, M.-D. (1982) *Methods Enzymol. 87*, 235–279.
Tsai, M.-D. (1983) in *^{31}P NMR: Principles and Applications* (Gorenstein, D., Ed.) Academic Press, New York (in press).
Tsai, M.-D., & Bruzik, K. (1983) *Biol. Magn. Reson. 5*, 129–181.
Tsai, M.-D., Bruzik, K., & Gupte, S. M. (1982) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 41*, 860.
Van Deenen, L. L. M., & de Haas, G. H. (1963a) *Biochim. Biophys. Acta 70*, 469–471.
Van Deenen, L. L. M., & de Haas, G. H. (1963b) *Biochim. Biophys. Acta 70*, 538–553.
Vasilenko, I., DeKruijff, B., & Verkleij, A. J. (1982) *Biochim. Biophys. Acta 685*, 144–152.
Verheij, H. M., Volwek, J. J., Jansen, E. H. J. M., Puyk, W. C., Dijkstra, B. W., Drenth, J., & de Haas, G. H. (1980) *Biochemistry 19*, 743–750.
Villafranca, J. J., & Raushel, F. M. (1980) *Annu. Rev. Biophys. Bioeng. 9*, 363–392.
Volwerk, J. J., & de Haas, G. H. (1982) in *Lipid–Protein Interactions* (Jost, P. C., & Griffith, O. H., Eds.) Vol. 1, pp 69–149, Wiley, New York.
Webb, M. R. (1982) *Methods Enzymol. 87*, 301–316.
Wells, M. A. (1974) *Biochemistry 13*, 2258–2264.

Deuterium Nuclear Magnetic Resonance Studies of Bile Salt/Phosphatidylcholine Mixed Micelles†

Ruth E. Stark,* Joanne L. Manstein, William Curatolo, and Barry Sears

ABSTRACT: Mixed micelles of deoxycholate (DOC) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) have been prepared in which the POPC was specifically deuterated in the 2-, 6-, 10-, or 16-position of the palmitoyl chain or in the *N*-methyl position of the choline head group. The deuterium nuclear magnetic resonance (^2H NMR) spectrum of each of these specifically deuterated mixed micelles consists of a singlet whose line width depends upon the position of deuteration. Spin–spin relaxation times indicate a gradient of mobility along the POPC palmitoyl chain in the mixed micelle, with a large increase in mobility on going from the 10- to the 16-position. Spin–lattice relaxation times (T_1's) demonstrate a similar gradient of mobility. Both trends in NMR relaxation behavior are consistent with a bilayer arrangement for the solubilized POPC. ^2H T_1 times for DOC/POPC micelles are significantly shorter than those measured in other bilayer systems, indicating unusually tight phospholipid acyl chain packing in the mixed micelle.

Bile salts play a crucial role in the digestion of fats and in the pathogenesis of cholesterol gallstones. Their physiological activity derives from the ability to form micelles, small molecular aggregates which can solubilize fatty acids, monoglycerides, phospholipids, and cholesterol—all hydrophobic species which would otherwise form insoluble dispersions in water. Normal gallbladder bile contains mixed bile salt/phosphatidylcholine/cholesterol (BS/PC/CH)[1] micelles, where small portions of the PC/CH bilayer are thought to be present within each BS aggregate [reviewed by Carey & Small (1972)]. If too much cholesterol is present, the solubilizing capacity of the micelles is exceeded; crystals of cholesterol may precipitate and subsequently grow into gallstones (Redinger & Small, 1972).

During the last 15 years, significant progress has been made toward a molecular understanding of the physiological functions of bile. Phase diagrams have been determined for mixtures of bile salts with phosphatidylcholine and cholesterol (Small, 1970), and a detailed structural picture has begun to emerge with the aid of various physical and spectroscopic techniques (Zimmerer & Lindenbaum, 1979; Mazer et al., 1980; Müller, 1981; Claffey & Holzbach, 1981).

Nuclear magnetic resonance (NMR) has been a widely used tool for the study of conformation and dynamics of model membranes (Jacobs & Oldfield, 1981; Chan et al., 1981; Browning, 1981) as well as micellar lipid assemblies (Ribeiro & Dennis, 1976; Burns & Roberts, 1980). For BS/PC mixtures, an early ^1H NMR study revealed that small additions of sodium cholate can produce high-resolution spectra for the lipids in egg phosphatidylcholine, though just a few molecular groupings are identifiable (Small et al., 1969). ^{13}C spectra and relaxation times (T_1's) are potentially more informative regarding segmental motion of the lipid acyl chains (London & Avitabile, 1977), but natural-abundance studies have often

† From the Department of Chemistry, Amherst College, Amherst, Massachusetts 01002 (R.E.S. and J.L.M.), and the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (W.C. and B.S.). *Received October 18, 1982.* This work was supported by grants from the National Institutes of Health (BRSG S074407110 and GM/NS-28149), the National Science Foundation (TFI-8021037), and the Research Corporation (C-1403). NMR experiments performed at the National Magnet Laboratory were supported by the National Institutes of Health (RR00995) and the National Science Foundation (C-670). This work was presented, in part, at the Biophysical Society Annual Meeting, Boston, MA, Feb 1982.

* Correspondence should be addressed to this author at the Department of Chemistry, Massachusetts Institute of Technology.

[1] Abbreviations: BS, bile salt; CH, cholesterol; NMR, nuclear magnetic resonance; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; $\Delta\nu$, line width; τ_{eff}, effective correlation time; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DOC, deoxycholate; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/83/0422-2486$01.50/0

been hampered by incomplete resolution of NMR signals from individual sites (Sears, 1975; Godici & Landsberger, 1974). We have chosen instead to probe acyl chain dynamics with ^2H NMR, using BS/PC micelles in which the PC has been selectively deuterated at key molecular sites.

In this study, spin–lattice relaxation times (T_1's) have been measured for 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) solubilized in sodium deoxycholate (DOC) micelles. The molecular mobility of the polar head group and along the saturated 1-chain has been evaluated and compared with previous investigations of lipids in bilayer and micellar forms. In addition, spin–spin relaxation times (T_2's) have been derived from spectral line widths ($\Delta\nu$'s); these data are considered in terms of possible contributions of slow motions to the relaxation behavior and by comparison with trends observed for sonicated bilayer vesicles. Finally, the deuterium NMR relaxation data are considered in light of structural models for the BS/PC mixed micelle.

Experimental Procedures

Materials

Deoxycholate (DOC) was purchased from Sigma Chemical Co. (St. Louis, MO) and was treated with charcoal before use. Triton X-100 was purchased from J. T. Baker Co. Dideuteriopalmitate labeled in the 2-, 6-, and 10-positions was synthesized by the method of Tulloch (1977). Palmitate-*16,16,16-d_3* was purchased from Serdary Labs (London, Ontario) and was checked for purity by thin-layer chromatography and mass spectroscopy. Oleic acid was purchased from Nu Chek Prep (Elysian, MN).

Methods

Dipalmitoylphosphatidylcholine (DPPC) was synthesized by acylation of glycerylphosphocholine with the acylimidazole of the appropriate deuterated palmitic acid (Boss et al., 1975). 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) was synthesized by phospholipase A_2 digestion of DPPC and reacylation (Gupta et al., 1977). POPC-*N-Me$_3$-d_9* was synthesized as follows: Dipalmitoylphosphatidic acid was prepared by digestion of DPPC by phospholipase D (Dawson & Hemington, 1967). Choline-*tri(N-methyl-d_3)* was synthesized by reaction of C^2H_3I and ethanolamine and was purified by ion-exchange chromatography (Brulet & McConnell, 1976). Choline-*tri(N-methyl-d_3)* and dipalmitoylphosphatidic acid were then reacted to give DPPC-*N-Me$_3$-d_9*, as previously described (Sears et al., 1976). POPC-*N-Me$_3$-d_9* was prepared from DPPC-*N-Me$_3$-d_9* as described above. All lipids were purified by silicic acid chromatography and exhibited only one spot on thin-layer chromatography in $CHCl_3/CH_3OH/H_2O$ (65:25:4).

Mixed micelle samples for NMR were prepared as follows: The appropriate POPC was mixed with DOC in organic solvent. Samples were dried under N_2, desiccated overnight under vacuum, and hydrated with 10 mM Tris-HCl (pH 9) in deuterium-depleted H_2O (Sigma Chemical, St. Louis, MO). Triton/POPC mixtures were prepared by adding a solution of the detergent in Tris buffer (see above) to dry phospholipid; vortex mixing gave a solution which remained optically clear on standing.

Phospholipid vesicles were prepared as follows: Deuterated PC (80 μmol) was dried under N_2, desiccated under vacuum overnight, lyophilized twice from 0.3 mL of deuterium-depleted H_2O, and finally hydrated with 2 mL of deuterium-depleted H_2O. POPC-*10,10-d_2* was sonicated for 5 min at 3 °C by using a Heat Systems-Ultrasonics W-370 probe sonicator. Titanium fragments were removed by centrifugation at 10000*g* for 20 min at 5 °C. DPPC-*10,10,10′,10′-d_4* vesicles were prepared by sonication at 45 °C for 7 min, followed by centrifugation for 10 min at 38 °C, a temperature above the phase transition temperature of sonicated DPPC vesicles. ^2H NMR studies of DPPC-*10,10,10′,10′-d_4* vesicles were performed immediately after preparation.

NMR Spectroscopy. ^2H NMR measurements at 15.29 MHz were performed with a JEOL FX-100Q spectrometer. A capillary which contained a saturated aqueous solution of LiCl was inserted in each 10-mm sample tube to provide a field-frequency lock signal; all spectra were obtained without sample spinning. The temperature of the solutions was controlled to within 0.5 K by heating and/or passing nitrogen-cooled air through the sample compartment; sample temperatures were monitored before and after each experiment via ^1H chemical-shift differences for a methanol sample immersed in aqueous LiCl. ^2H NMR measurements at 41.4 MHz were carried out with a Bruker HX-270 spectrometer, equipped with a Nicolet 1080 data system.

Spin–lattice relaxation times (T_1's) were measured by the inversion–recovery method (Vold et al., 1968), using a waiting time of at least five T_1's between applications of a 180°–τ–90° pulse sequence. A typical experimental run required 1.2 h of signal averaging and included 20 values of the increment τ ranging from 0.2 to 2.5 T_1, in addition to several "∞ values" corresponding to $\tau = 5\ T_1$'s. T_1 values were determined from the least-squares slope of a plot of $[M(\infty) - M(\tau)]/[2M(\infty)]$ vs. τ, where $M(\tau)$ is the amplitude of the deuterium peak (spin magnetization) at time interval τ. Spin–spin relaxation times (T_2's) were estimated from the relation $T_2 = 1/(\pi\Delta\nu)$, where $\Delta\nu$ represents a ^2H spectral line width corrected for contributions from magnetic field inhomogeneities.

Results

Line Widths and Spin–Spin Relaxation Times. All experiments were performed on mixtures with a DOC:POPC ratio of 2:1, in which mixed micelles (and a population of simple BS aggregates) are likely to be present (Mazer et al., 1980). Attention was focused on the NMR characteristics of POPC, since this lipid is a constituent of normal human bile (Ahlberg, et al., 1981).

The electric quadrupole moment (eQ) of the ^2H nucleus can interact with electric field gradients (eq) produced by its electronic environment, leading to distinctive NMR spectra and spin relaxation behavior. Each POPC resonance is a single Lorentzian line, as expected when molecular reorientation occurs rapidly compared with the magnitude of the quadrupole coupling constant (e^2qQ/h). In DOC/POPC mixed micelles, the ^2H NMR line widths ($\Delta\nu$'s) are smallest for choline and terminal methyl groups (see Table I) but as much as 40-fold larger for intermediate positions along the saturated acyl chain. A typical profile of $\Delta\nu$ vs. chain position is shown in Figure 1, exhibiting a moderate decrease from positions 2 to 10 and a dramatic falloff at the 16-methyl group. All $\Delta\nu$'s decrease with temperature (see Table I), but little variation in the shape of the profile is observed between 4 and 51 °C. These trends are in qualitative agreement with results reported previously for ^2H $\Delta\nu$'s of fatty acid probes intercalated in single-bilayer vesicles of egg PC (Stockton et al., 1976), as well as for ^2H quadrupole splittings in multibilayer dispersions (Seelig & Seelig, 1974). Sonicated vesicles composed of specifically deuterated POPC's show similar trends (W. Curatolo and B. Sears, unpublished results).

For ^2H resonances which are appreciably broader than the instrumentally limited line width (2-, 6-, and 10-positions), a value of the spin–spin relaxation time (T_2) may be found

Table I: ^{2}H NMR Line Widths for Bile Salt/Phosphatidylcholine Mixtures[a]

temp (°C)	line width, $\Delta\nu$ (Hz)[b] for POPC site				
	$N^+(CD_3)_3$[c]	2	6	10	16[c]
4		199 (10)	158 (8)	155 (8)	<10 (5)
10	9 (3)	187 (9)	153 (8)	122 (6)	4 (1)
17	6 (2)	151 (8)	143 (7)	65 (3)	5 (2)
24		107 (5)	89 (4)	55 (3)	
28	4 (1)	101 (5)	85 (4)	61 (3)	5 (2)
32	7 (2)	100 (5)	75 (4)	40 (2)	3 (1)
37		75 (4)	61 (3)	40 (2)	
41		93 (5)	78 (4)	51 (3)	5 (2)
45	5 (2)	83 (4)	79 (4)	44 (2)	8 (3)
51	3 (1)	65 (3)	68 (3)	31 (2)	6 (2)

[a] Solutions were 25 mM in POPC and 50 mM in POPC, prepared as described under Experimental Procedures. [b] Full line width at half-height, corrected for contributions from magnetic field inhomogeneities. All measurements were carried out at 15.29 MHz. Error limits, estimated from repeated measurements, are indicated in parentheses. [c] Line widths for these resonances are determined principally by inhomogeneity of the external magnetic field.

Table II: ^{2}H NMR Spin Relaxation Times for Bile Salt/Phosphatidylcholine Mixtures[a]

temp (°C)	spin-lattice relaxation time, T_1 (ms),[b] for POPC site					spin-spin relaxation time, T_2 (ms),[b] for POPC site		
	$N^+(CD_3)_3$	2	6	10	16	2	6	10
4	27.8 (2.1)	4.06 (0.30)	5.16 (0.39)	6.31 (0.47)	91.7 (6.9)	1.60 (0.08)	2.01 (0.09)	2.05 (0.10)
10	32.9 (2.5)	4.58 (0.34)	5.48 (0.41)	6.76 (0.51)	132 (10)	1.70 (0.08)	2.08 (0.10)	2.61 (0.12)
17	45.9 (3.4)	5.33 (0.40)	6.63 (0.50)	7.37 (0.55)	159 (12)	2.11 (0.11)	2.23 (0.11)	4.90 (0.22)
24	56.2 (4.2)	5.82 (0.44)	7.47 (0.56)	9.04 (0.68)	202 (15)	2.97 (0.13)	3.58 (0.16)	5.79 (0.30)
28	68.2 (5.1)	6.04 (0.45)	6.94 (0.52)	8.53 (0.64)	233 (17)	3.15 (0.15)	3.74 (0.16)	5.22 (0.25)
32	76.9 (5.8)	6.42 (0.48)	7.30 (0.55)	10.3 (0.8)	210 (16)	3.18 (0.15)	4.24 (0.21)	7.96 (0.38)
37	80.8 (6.1)	6.42 (0.48)	8.20 (0.62)	11.2 (0.8)	204 (15)	4.24 (0.21)	5.22 (0.25)	7.96 (0.38)
41	84.9 (6.4)		7.85 (0.59)	10.9 (0.8)	244 (18)	3.42 (0.17)	4.08 (0.20)	6.24 (0.35)
45	100 (8)	7.14 (0.54)	9.13 (0.68)	11.6 (0.9)	321 (24)	3.84 (0.18)	4.03 (0.19)	7.23 (0.31)
51	123 (9)	7.41 (0.56)	9.37 (0.70)	14.3 (1.1)	348 (26)	4.90 (0.22)	4.68 (0.20)	10.27 (0.62)
	5.6[c] (0.6)	2.2[c] (0.3)	2.2[c] (0.4)	3.0[c] (0.5)	4.4[c] (0.9)			

[a] Solutions were 25 mM in POPC and 50 mM in POPC, prepared as described under Experimental Procedures. [b] All measurements were carried out at 15.29 MHz. Error limits, as estimated from repeated measurements, are indicated in parentheses. [c] Activation energy (in kilocalories).

from the relation $T_2 = 1/(\pi\Delta\nu)$. These values appear in Table II, where they are compared with spin–lattice relaxation times (T_1's) (vide infra) obtained under identical experimental conditions.

Spin–Lattice Relaxation Times. Deuterium spin relaxation is caused by interactions between the nuclear quadrupole moment and surrounding electric field gradients which fluctuate during molecular reorientation. The characteristic rates T_1 and T_2 depend on the efficiency with which these fluctuations occur at the ^{2}H NMR frequency, which depends in turn on the reorientation rate of a given C–D segment in the lipid molecule. For this reason, ^{2}H relaxation provides a sensitive probe of local mobility at each chain site, with short T_1's indicative of slow motions. T_1 data for DOC/POPC mixtures at several temperatures are summarized in Table II.

As with line widths or T_2's, it is useful to examine profiles along the acyl chain; the variation in relaxation parameters at three representative temperatures appears in Figure 2. We find for DOC/POPC micelles a near-plateau in the relaxation profile between positions 2 and 10 and a dramatic increase in T_1 at the terminal methyl group, in agreement with patterns observed previously in multilayer and fatty acid systems (Brown et al., 1979; Davis et al., 1978). Our results are also in reasonable accord with ^{13}C relaxation trends reported for micellar short-chain phospholipids (Burns & Roberts, 1980), but they contrast sharply with the smooth variation of ^{13}C T_1's found for free hydrocarbon chains (Levine et al., 1973) and for monomeric PC's (Burns & Roberts, 1980; Brown et al., 1979).

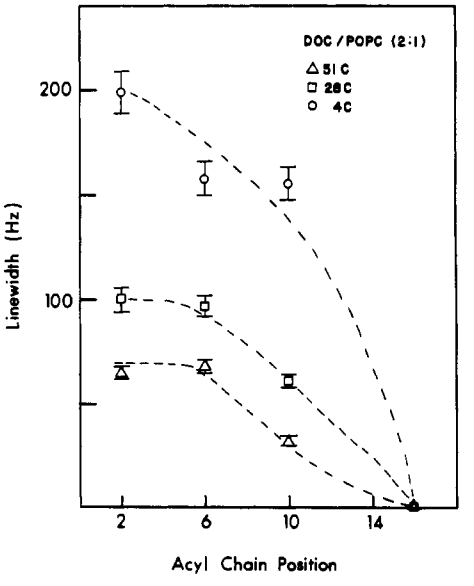

FIGURE 1: ^{2}H line widths at 15.29 MHz as a function of acyl chain position for DOC/POPC (2:1) mixtures at three temperatures. The narrow resonance from POPC-*16,16,16-d*$_3$ has a line width which is determined principally by inhomogeneity of the applied magnetic field. Results for the full range of temperatures are summarized in Table I.

Both T_1's and T_2's increase with temperature in DOC/POPC mixtures between 4 and 51 °C, implying that rapid molecular reorientation is responsible for the relaxation process.

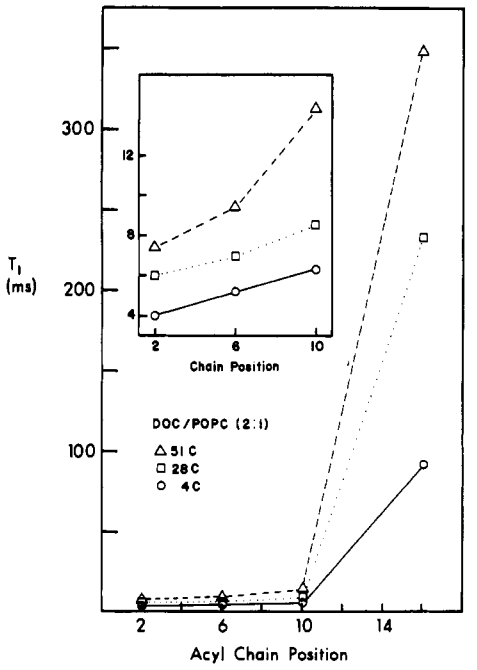

FIGURE 2: ^2H T_1's at 15.29 MHz as a function of acyl chain position for DOC/POPC (2:1) mixtures at three temperatures. Differences among the 2-, 6-, and 10-positions are small but reproducible. Complete results appear in Table II.

Application of standard formulas for mobile liquids (Abragam, 1961) yields effective correlation times (τ_{eff}) in the range 7×10^{-12} to 6×10^{-10} s so that the extreme narrowing condition ($\omega_0^2\tau_{eff}^2 \ll 1$, $\omega_0 = 9.61 \times 10^7$ rad/s) would seem to be satisfied. However, we find $T_1 > T_2$ at all lipid sites in this mixed micellar system, with discrepancies of up to a factor of 3. Similar behavior has been noted previously in sonicated vesicle preparations and attributed to restrictions on the internal molecular motions, i.e., partial ordering of lipid acyl chains within the bilayer (Stockton et al., 1976).

The variation of ^2H T_1's with temperature is summarized in Table II, where the relaxation parameters have been fit to an Arrhenius equation of the form $\ln T_1 = \ln T_1^0 - E_a/(RT)$. Activation energies obtained in this fashion range between 2.2 and 5.6 kcal/mol; the values are generally smaller than corresponding E_a's obtained previously for bilayer preparations of dipalmitoylphosphatidylcholine (DPPC) (Brown et al., 1979) and potassium palmitate (Davis et al., 1978).

Finally, ^2H NMR T_1 relaxation times have been determined for deuterated POPC in sonicated vesicles and in mixed micelles with the nonionic detergent Triton X-100. These results are presented in Table III, where they are compared with data for DOC/POPC micelles at the same temperature. T_1's for deuterated POPC in DOC/POPC mixed micelles are significantly shorter than those observed for either POPC vesicles or Triton-solubilized POPC.[2]

Discussion

Our deuterium relaxation profiles (Figures 1 and 2) are consistent with a bilayer arrangement for POPC in the DOC/POPC mixed micelle, though alternative models are not rigorously excluded. Quantitative differences with NMR data reported for other bilayer systems also reveal several distinguishing properties of bile salt solubilized POPC.

Table III: ^2H T_1 Relaxation Times for Selectively Deuterated POPC in Various Aggregates

temp (°C)	sample[a]	T_1 (ms)[b] 15.3 MHz	41.4 MHz
24	2:1 DOC:POPC-*10,10-d*$_2$ micelles	9.04 (0.68)	16.5 (1.2)
24	POPC-*10,10-d*$_2$ vesicles		22.4 (1.7)
24	2:1 DOC:POPC-*6,6-d*$_2$ micelles	7.47 (0.56)	15.2 (1.1)
24	8:1 Triton:POPC-*6,6-d*$_2$ micelles	10.7 (0.8)	
31	2:1 DOC:POPC-*10,10-d*$_2$ micelles	10.3 (0.8)	
31	POPC-*10,10-d*$_2$ vesicles	17.4 (1.3)	

[a] DOC/POPC micellar solutions were 50 mM in DOC and 25 mM in POPC. Triton/POPC mixed micelles were 80 mM in Triton X-100 and 10 mM in POPC. [b] Error limits, as estimated from repeated measurements, are indicated in parentheses.

As noted above, ^2H line widths in DOC/POPC mixtures exhibit variations which are typical of saturated acyl chains in bilayer systems; yet deuterons at the 2-, 6-, and 10-positions give rise to appreciably narrower peaks than those observed previously in phospholipid vesicles (Stockton et al., 1976; W. Curatolo and B. Sears, unpublished results). This sensitivity of line width to aggregate structure has also been noted previously for ^1H and ^{13}C resonances in egg PC/detergent mixed micelle systems (Sears, 1975; Ribeiro & Dennis, 1976; Lichtenberg et al., 1979). Spin–spin relaxation is dominated by relatively slow molecular processes, which could include off-axis (perpendicular) motion of the chains, lateral diffusion of individual PC's on the bilayer surface, collective bilayer fluctuations, and overall aggregate tumbling (Chan et al., 1981). Our narrowed peaks (longer T_2's) suggest that some of these motions are considerably less hampered when portions of the POPC bilayer are incorporated in a DOC micelle. It is expected that the last process in particular is rapid enough to account for the small line widths, since micellar aggregates formed in 2:1 BS:PC mixtures are only 40–50 Å in diameter (Mazer et al., 1980).

By contrast, spin–lattice relaxation is usually attributed to more rapid reorientation effected by segmental motions. The nearly constant POPC T_1's observed in the mid region of the saturated acyl chain (Figure 2) are typical of aggregated systems; this phenomenon is understandable if segmental motions (trans–gauche isomerizations) are cooperative (Brown et al., 1979). Our T_1's rise more dramatically at the chain terminus than in other bilayer systems (Davis et al., 1978), indicating a larger mobility gradient when the phospholipid is solubilized in DOC. Moreover, the ^2H spin–lattice relaxation rates in DOC/POPC micelles are 40–70% faster than values obtained for POPC in vesicles or Triton micelles (Table III). These results imply that segmental motions are unusually sluggish for PC's solubilized in bile salt micelles and suggest a mixed-aggregate structure which involves tight molecular packing.

If all of the motional processes which contribute to T_1 and T_2 were sufficiently rapid, then the white spectrum/extreme narrowing approximation (Abragam, 1961) would predict equal values for the two spin relaxation times. A different picture emerges when both fast (local) and slow (overall) motions are important; this interplay has been treated theoretically for a number of ordered molecular systems, including

[2] Our value of T_1 for sonicated POPC-*10,10-d*$_2$ vesicles (22.4 ms at 24 °C, 41.4 MHz) is significantly shorter than that which we observe for DPPC-*10,10,10′,10′-d*$_4$ vesicles (36.6 ms) at a similar reduced temperature with respect to the acyl chain order–disorder transition. This restriction of palmitoyl chains by adjacent oleoyl chains is currently under investigation.

bilayer vesicles (Stockton et al., 1976;Chan et al., 1981), multilayer dispersions (Brown et al., 1979; Brown, 1982), and thermotropic liquid crystals (Freed, 1977; Poupko et al., 1980). Either a single effective tumbling time or a sum of long and short correlation times may be assumed; in the latter case, the two terms may produce opposing effects on the T_1 value as sample temperature is varied so that anomalously low activation energies will be observed. Additional theoretical predictions include the inequality of T_1 and T_2, a dependence of relaxation rates on the applied magnetic field (even when the apparent tumbling time satisfies the extreme narrowing condition), and the dependence of relaxation on both motional and ordering parameters. As shown in Tables II and III, NMR relaxation data for DOC/POPC mixtures confirm these expectations. By conducting relaxation experiments at two field strengths, it should be possible to determine both correlation times as well as the order parameter at a given acyl chain site—even though no resolved quadrupole splittings appear in the deuterium NMR spectra. Measurements of this type are currently in progress in our laboratories.

Small et al. (1966) have proposed a molecular model of the bile salt/phosphatidylcholine micelle in which a PC bilayer disk is solubilized by a layer of BS molecules around its perimeter. Because PC molecules on the periphery are in contact with the hydrophobic face of the BS, their acyl chains are sequestered from the aqueous environment. As a refinement to this model, Mazer et al. (1980) have suggested that hydrogen-bonded bile salt dimers are also found in the interior of the BS/PC disk. Although spectroscopic evidence for this "mixed-disk" proposal has been somewhat conflicting (Zimmerer & Lindenbaum, 1979; Castellino & Violand, 1979; Mazer et al., 1980; Müller, 1981; Claffey & Holzbach, 1981), the model gains support from the observation that BS/PC micelles can accommodate only small quantities of cholesterol (Small et al., 1966). Regardless of the structural model, most bile salt molecules should be situated at the disk perimeter in our 2:1 BS:PC mixtures. Thus, PC molecular packing is tightened by constraints at the perimeter, even in the absence of substantial BS incorporation within the PC bilayer. ^2H T_1 studies for both components in mixtures of varying composition are currently in progress in efforts to learn more about the micellar structure.

Acknowledgments

We thank Dr. M. F. Roberts and D. M. Small for helpful discussions during the course of this investigation. We also acknowledge the assistance of Richard W. Storrs and Peter D. Leff in performing some of the NMR relaxation experiments.

Registry No. DOC·Na, 302-95-4; POPC, 6753-55-5.

References

Abragam, A. (1961) *Principles of Nuclear Magnetism*, Chapter 8, Oxford University Press, London.
Ahlberg, J., Curstedt, T., Einarsson, K., & Sjovall, J. (1981) *J. Lipid Res. 22*, 404.
Boss, W., Kelley, C., & Landsberger, F. (1975) *Anal. Biochem. 64*, 289.
Brown, M. F. (1982) *J. Chem. Phys. 77*, 1576.
Brown, M. F., Seelig, J., & Häberlen, U. (1979) *J. Chem. Phys. 70*, 5045.
Browning, J. L. (1981) in *Liposomes: From Physical Structure to Therapeutic Applications* (Knight, C. G., Ed.) pp 189–242, Elsevier/North-Holland Press, Amsterdam.
Brulet, P., & McConnell, M. M. (1976) *J. Am. Chem. Soc. 98*, 1314.
Burns, R. A., Jr., & Roberts, M. F. (1980) *Biochemistry 19*, 3100.
Carey, M. C., & Small, D. M. (1972) *Arch. Intern. Med. 130*, 506.
Castellino, F. J., & Violand, B. N. (1979) *Arch. Biochem. Biophys. 193*, 543.
Chan, S. I., Bocian, D. F., & Petersen, N. O. (1981) in *Membrane Spectroscopy* (Grell, E., Ed.) pp 1–50, Springer-Verlag, New York.
Claffey, W. J., & Holzbach, R. T. (1981) *Biochemistry 20*, 415.
Davis, J. H., Jeffrey, K. R., & Bloom, M. (1978) *J. Magn. Reson. 29*, 191.
Dawson, R. M., & Hemington, N. (1967) *Biochem. J. 102*, 76.
Freed, J. H. (1977) *J. Chem. Phys. 66*, 4183.
Godici, P. E., & Landsberger, F. R. (1974) *Biochemistry 13*, 362.
Gupta, C. M., Radhakrishnan, R., & Khorana, H. G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4315.
Jacobs, R. E., & Oldfield, E. (1981) *Prog. Nucl. Magn. Reson. Spectrosc. 14*, 113.
Levine, Y. K., Partington, P., & Roberts, G. C. K. (1973) *Mol. Phys. 25*, 497.
Lichtenberg, D., Zilberman, Y., Greenzaid, P., & Zamir, S. (1979) *Biochemistry 18*, 3518.
London, R. E., & Avitabile, J. (1977) *J. Am. Chem. Soc. 99*, 7765.
Mazer, N. A., Benedek, G. B., & Carey, M. C. (1980) *Biochemistry 19*, 601.
Müller, K. (1981) *Biochemistry 20*, 404.
Poupko, R., Vold, R. L., & Vold, R. R. (1980) *J. Phys. Chem. 84*, 3444.
Redinger, R. N., & Small, D. M. (1972) *Arch. Intern. Med. 130*, 618.
Ribeiro, A. A., & Dennis, E. A. (1976) *J. Colloid Interface Sci. 55*, 94.
Sears, B. (1975) *J. Membr. Biol. 20*, 59.
Sears, B., Hutton, W. C., & Thompson, T. E. (1976) *Biochemistry 15*, 1635.
Seelig, A., & Seelig, J. (1974) *Biochemistry 13*, 4839.
Small, D. M. (1970) *Adv. Intern. Med. 16*, 243.
Small, D. M., Bourges, M., & Dervichian, D. G. (1966) *Nature (London) 211*, 816.
Small, D. M., Penkett, S. A., & Chapman, D. (1969) *Biochim. Biophys. Acta 176*, 178.
Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hazan, F., & Smith, I. C. P. (1976) *Biochemistry 15*, 954.
Tulloch, A. P. (1977) *Lipids 12*, 92.
Vold, R. L., Waugh, J. S., Klein, M. P., & Phelps, D. E. (1968) *J. Chem. Phys. 48*, 3831.
Zimmerer, R. O., & Lindenbaum, S. (1979) *J. Pharm. Sci. 68*, 581.