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# One- and two-dimensional NMR relaxation studies of dynamics and structure in bile salt-phosphatidylcholine mixed micelles

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**A series of one- and two-dimensional $^1$H-NMR relaxation measurements has been conducted on simple and mixed micellar aggregates of taurocholate, diphenylvaleroylphosphatidylcholine (diPVPC) and egg yolk phosphatidylcholine (egg PC). The results are interpreted to provide structural and dynamic comparisons between micelles and vesicles, between phospholipids of varying chain length, and between different lipid components within the same micellar aggregate. Both chemical shift changes and two-dimensional nuclear Overhauser effect cross peaks suggest direct interaction of taurocholate and PC chemical sites, although the latter observations may also be accounted for by PC-PC interactions. These experiments demonstrate the promise of NMR relaxation techniques for investigations of molecular organization in model substrate for lipolytic enzymes.**

## Introduction

Biliary lipids have been the subject of numerous physical studies in recent years [1–8] because of their role as physiological detergents. Bile salts themselves form small micellar aggregates [9], and in assemblies with other amphiphiles they are essential to bile formation [10] and fat digestion [11]. Both mixed micelles [1–6] and unilamellar vesicles [7,8] may form in taurocholate-egg phosphatidylcholine mixtures (Figs. 1 and 7), for instance, although the detailed molecular arrangement of such aggregates is still in question [2–4]. Bilayer conformation, packing and dynamics have all been probed in bile salt-PC solutions, and the data have been compared with those of model membrane systems [12]. Such comparisons are particularly important in light of the dependence of lipolytic enzyme activity on the aggregation state of the phospholipid substrate [13].

Using a combination of $^1$H- and $^2$H-NMR techniques [5,6], it has been possible to develop a qualitative picture of acyl chain motion and micellar structure in bile salt-PC mixtures. Unusually sluggish segmental motions and tight packing were inferred from $^2$H relaxation measurements in micelle-solubilized PCs that were selectively deuterated at various acyl chain sites. Complementary $^1$H linewidth studies revealed that polar portions of the phospholipids retain substantial motional freedom during the growth of mixed micellar ag-

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Abbreviations: NMR, nuclear magnetic resonance; $R_1$, spin-lattice relaxation rate; $R_1$(ns), nonselective relaxation rate; $R_1$(s), selective relaxation rate; PC, phosphatidylcholine; diPVPC, diphenylvaleroylphosphatidylcholine.

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gregates, whereas bile salt motions become more restricted.

Used extensively to elucidate the structure and conformation of macromolecules in solution, nuclear Overhauser effect spectroscopy is a valuable technique which potentially provides both structural and dynamical information for bile salt-PC mixtures [14–16]. It is well known that the dipolar cross relaxation responsible for the nuclear Overhauser effect depends on both spatial proximity and relative motions of the interacting sets of nuclei [16]. Recently, cross relaxation rates have been obtained in a two-dimensional nuclear Overhauser effect (NOESY) [17,18] study of sonicated PC vesicles [19]. In the present work, similar experiments reveal contrasting patterns for PCs organized as vesicles [19,20] or as mixed micelles, and for long- and short-chain PCs solubilized in bile salt aggregates. With the aid of $^1H$ spin-lattice relaxation experiments and NMR data obtained previously [5,6], the two-dimensional nuclear Overhauser enhancement spectroscopy results have been given a structural interpretation.

## Experimental procedures

### *Materials*

Sodium taurocholate was purchased from Calbiochem-Behring (San Diego, CA) and purified as described previously [5]. Egg yolk PC was obtained as a Grade I product from Lipid Products (Redhill, Surrey, U.K.) in a $CHCl_3/CH_3OH$ (2 : 1, v/v) solution. Diphenylvaleroylphosphatidylcholine (diPVPC) was synthesized by acylation of glycerolphosphocholine with the acylimidazole of ω-phenylvaleric acid (Aldrich Chemical, Milwaukee, WI) [21,22]. This compound was provided by Mr. Larry Coury. Each phospholipid gave one spot on thin-layer chromatography in $CHCl_3/CH_3OH/CH_3COOH$ (70 : 30 : 1).

Micellar solutions of 20 mM taurocholate and diPVPC were prepared by dissolving the dry compounds in 1 mM EDTA in 99.96 atom% $^2H_2O$ (Aldrich Chemical, Milwaukee, WI). (Critical micelle concentrations of the lipids are 3 mM [9] and 0.5 mM [22].) Mixed taurocholate/PC solutions for NMR were prepared by coprecipitation [23]: lipid solutions in $CH_3OH/CHCl_3$ (2 : 1) were mixed in a 1 : 1 molar ratio, dried under a stream of $N_2$ and at reduced pressure, and then hydrated with 99.96 atom% $^2H_2O$ to a final total lipid concentration of 12.5 mg/ml. Solutions were incubated for 24 h and vortex-mixed prior to the NMR measurements.

### *NMR spectroscopy*

$^1H$-NMR experiments were conducted on several spectrometers: (a) a homebuilt 500 MHz instrument at the Francis Bitter National Magnet Laboratory (M.I.T., Cambridge, MA); (b) a Varian XL-200 instrument at the University of Massachusetts (Amherst, MA); (c) a JEOL GX-400 instrument at Hunter College (C.U.N.Y., New York, NY), and (d) a JEOL FX-100Q instrument at Amherst College (Amherst, MA). Measurements were made at 25–40°C on 300–500 μl samples (sampling conditions are noted in the figures and tables).

Spin-lattice relaxation rates ($R_1 = 1/T_1$) were obtained with the inversion-recovery (IRFT) pulse sequence [24]. The return of the nuclei to equilibrium was monitored either after nonselective inversion of all spin populations ($R_1$(ns)) or after selective inversion of a single resonance ($R_1$(s)).

Two-dimensional nuclear Overhauser spectroscopy was performed in the 'pure absorption' mode [25]. The sequence $[90^\circ_\Phi\text{-}t_1\text{-}90^\circ_\Phi\text{-}\tau_M\text{-}90^\circ_\Phi\text{-acquire }(t_2)]_n$ [18] was employed with 512 $t_1$ values, mixing times $\tau_M$ of 150–600 ms, and $n = 32$. The phase-cycling scheme used in the acquisition of pure absorption mode spectra removes J(1) and J(2) terms arising from scalar coupling, but J(0) contributions are not so removed. Because the latter terms diminish at longer mixing times (such as those employed here) [26], no further measures were used to remove scalar coupling effects. Two-dimensional Fourier transformation provided spectra as a function of frequencies $f_1$ and $f_2$; resulting data are represented conveniently in contour plots. A typical experiment required 16 h of spectrometer time.

## Results

### *Two-dimensional nuclear Overhauser spectroscopy*

One-dimensional $^1H$-NMR spectra of simple micelles of taurocholate and diphenylvaleroylphosphatidylcholine (diPVPC) are shown in Figs. 1 and 2. The excellent resolution obtained at high

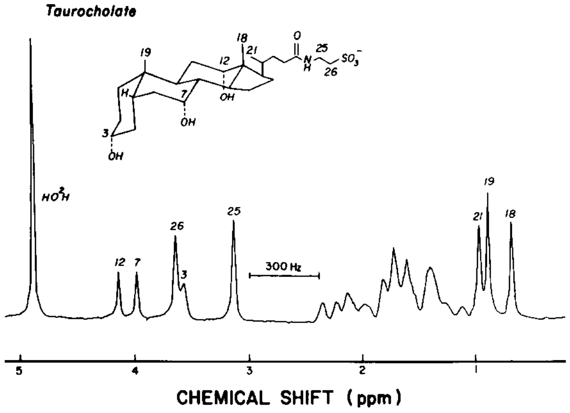

Fig. 1. 500 MHz $^1$H-NMR spectrum of a 20 mM micellar solution of sodium taurocholate. Resonance assignments are made by reference to published spectra [27], using the numbering scheme shown above.

magnetic field strength permits identification of key hydrophobic and hydrophilic sites on each detergent. The two-dimensional spectrum of the simple taurocholate micelles (Fig. 3) indicates dipolar cross relaxation between several protons on the steroid rings, as well as weak interactions of these upfield steroid protons with nearby methyl [18,19,21] and with carbinol [7,12] sites. The adjacent sites 25 and 26 on the taurocholate sidechain are also linked by nuclear Overhauser effects. By contrast, micelles of the short-chain phospholipid diPVPC (and of the related compound ω-phenyldecanoate) exhibit no cross peaks in two-dimensional nuclear Overhauser enhancement (NOESY) spectra obtained with mixing times up to 600 ms (spectra not shown).

The one-dimensional spectrum of a 50 : 50 mixture of taurocholate and diPVPC is found in Fig. 4, and a number of spectral features indicate changes in the magnetic environments of several protons, relative to those found in the pure components. Proton 26 moves upfield in the mixed micelle, thus reversing the relative chemical shift positions of taurocholate protons 3 and 26. The glycerol $CH_2O$ protons shift downfield in the mixed micelle; the phosholipid aromatic protons shift significantly upfield in the mixed micelle, relative to the pure phospholipid. Unlike the spectra observed for the pure components, the two-dimensional nuclear Overhauser enhancement spectra of the mixed micelles (Fig. 5) indicate that extensive dipolar cross-relaxation is occurring. Changes in environment (structural or dynamic) upon formation of mixed particles provide a plausible expanation for both chemical shift changes and increases in dipolar connectivities.

As seen in Fig. 5, cross peaks between steroid, methyl and carbinol sites are enhanced in intensity and in number relative to the pure-component micelles. In addition, cross relaxation occurs between the different methyl groups and also between more distant methyl [18,21] and carbinol [7,12] sites (not shown). However, the nuclear Overhauser effects between side chain sites 25 and

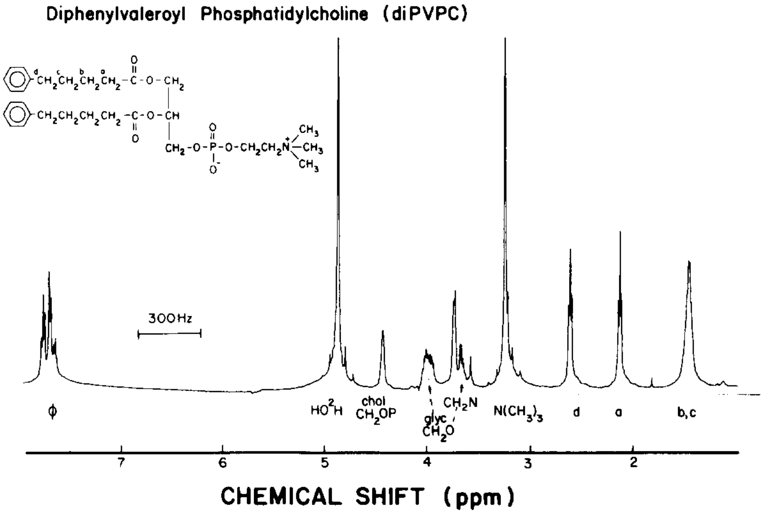

Fig. 2. 500 MHz $^1$H-NMR spectrum of a 20 mM micellar solution of diphenylvaleroylphosphatidylcholine. The assignment scheme is indicated above and is based on prior studies of similar compounds [28,29].

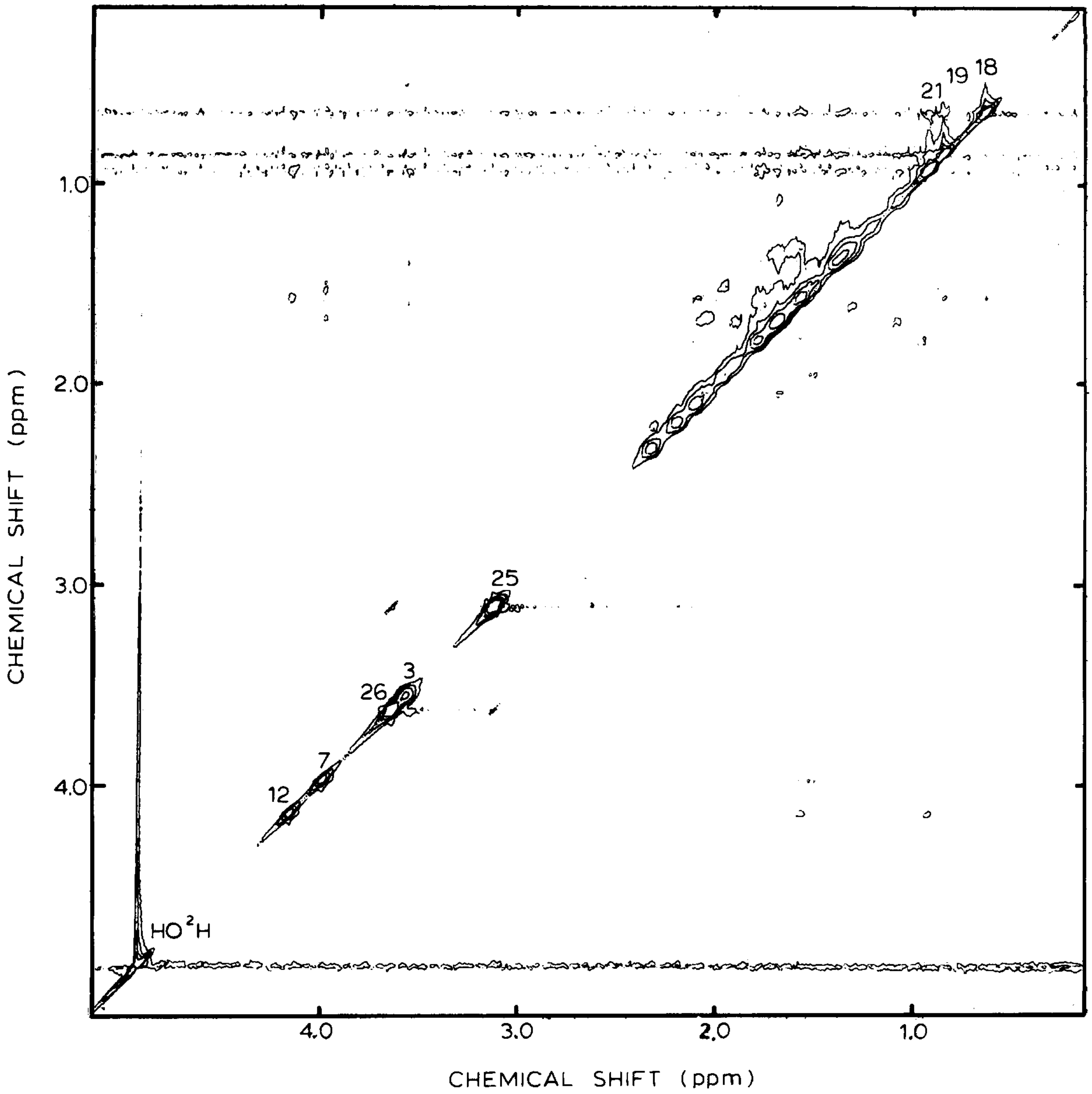

Fig. 3. Two-dimensional nuclear Overhauser spectroscopy of 20 mM sodium taurocholate micelles. A mixing time of 600 ms was used; the lowest contour of this display has an intensity 0.1% of that of the largest peak in the spectrum.

26 are absent in the taurocholate-diPVPC mixed micelles (negative results not shown). Within the short-chain phospholipid, no spin communication is observed for phenyl, acyl chain, or choline headgroup moieties (negative results not shown), but prominent cross peaks link the protons of the glycerol backbone (Fig. 6). Unambiguous identification of taurocholate-diPVPC cross peaks is difficlt due to spectral overlap, but several steroid and phospholipid acyl chain groupings may be linked (Fig. 5, dashed lines).

Finally, two-dimensional nuclear Overhauser enhancement studies have been conducted for a previously characterized mixture [6] of taurocholate with egg phosphatidylcholine (egg PC) (Fig. 7). (Spectral linewidths in pure egg PC samples

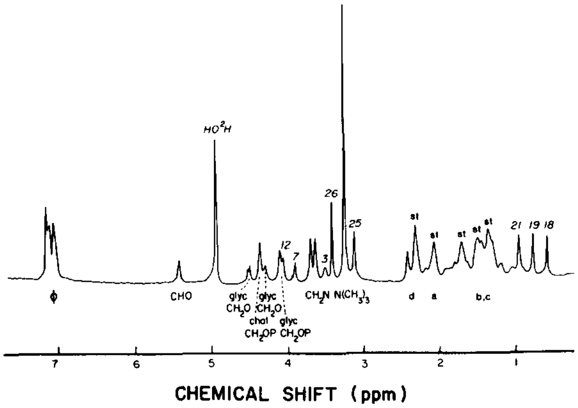

Fig. 4. 500 MHz $^1$H-NMR spectrum of sodium taurocholate-diphenylvaleroylphosphatidylcholine mixed micelles, prepared as described in the Materials section.

are large enough as to preclude evaluation of possible chemical shift changes in the mixed-micelle system relative to the pure components.) As in the mixed lipid aggregate described above, taurocholate cross peaks are greater in number in the taurocholate-egg PC micelle than in simple micellar solutions. In the egg PC mixed micelle the cross peak between adjacent side chain sites 25 and 26 is retained (see Fig. 8) but, in contrast, the adjacent methylenes of the PC headgroup ($CH_2N$ and chol $CH_2OP$) are not linked in the two-dimensional nuclear Overhauser enhancement spectrum (a weak crosspeak is observed for a mixing time of 600 ms). The choline methyls ($N(CH_3)_3$) cross relax with several spin groupings that could belong to taurocholate or PC. Cross peaks are also observed among glycerol backbone sites and between acyl chain moieties. For example, the CH=CH and CHO protons included in the broad resonance at 5.3 ppm communicate with glycerol $CH_2OP$ methylenes, as well as (weakly) with the choline methyls and with a variety of upfield methylene and methyl groupings that absorb between 1 and 2 ppm. Possible spin communication between the taurocholate and egg PC components of the aggregate is treated further in the Discussion section.

*Selective and nonselective relaxation rates*

The fact that taurocholate-egg PC micelles possess pairs of adjacent methylene groups (taurocholate 25 and 26, PC $CH_2N$ and chol $CH_2OP$) with distinctly different two-dimensional nuclear Overhauser enhancement cross peak behavior suggests that these pairs of methylenes also have differing mobilities in the aggregates. In order to test this hypothesis and to establish more generally how the two-dimensional nuclear Overhauser enhancement results depend on site-specific motional effects, a series of spin-lattice relaxation experiments was performed. The taurocholate-egg PC micellar mixture, for which the dynamic state has been characterized previously by other NMR methods [5,6], was chosen to illustrate the relaxation phenomena for a variety of proton types.

Table I summarizes $^1$H spin-lattice relaxation rates obtained under several experimental conditions for key resonances in the 50:50 taurocholate-egg PC mixture. A distinct spread of values is observed among the various spin groupings, and in each case $R_1(\mathrm{ns})$ depends clearly on both temperature and magnetic field strength. In addition, the observed differences between $R_1(\mathrm{ns})$ and $R_1(\mathrm{s})$ indicate significant contributions from the cross relaxation term $\Sigma\sigma_{ij}$ for most nuclei [16,30].

Assuming a dipolar mechanism and isotropic motion, the relaxation rates are given by [16,30]

$$R_i(\mathrm{ns}) = \sum_j \rho_{ij} + \sum_j \sigma_{ij} \tag{1}$$

where

$$\rho_{ij} = R_i(\mathrm{s}) = \frac{\gamma^4 \hbar^2}{10\, r_{ij}^6} \left\{ \tau_c + \frac{3\tau_c}{1+\omega^2\tau_c^2} + \frac{6\tau_c}{1+4\omega^2\tau_c^2} \right\} \tag{2}$$

and

$$\sigma_{ij} = \frac{\gamma^4 \hbar^2}{10\, r_{ij}^6} \left\{ \frac{6\tau_c}{1+4\omega^2\tau_c^2} - \tau_c \right\} \tag{3}$$

$\gamma$ is the proton gyromagnetic ratio, $\tau_c$ is the correlation time modulating the internuclear dipolar interaction, $r_{ij}$ is the internuclear separation, and $\omega$ is the proton Larmor frequency.

The observation of frequency-dependent $R_1$ values as well as negative cross-relaxation terms (Table I) suggests that molecular motions are slow i.e., $\tau_c^2 \gtrsim 5/4\omega^2$. The largest nonselective $R_1$ values are observed for $CH_2N$ and $N(CH_3)_3$ of PC and for proton 18 taurocholate; larger values of $R_1(\mathrm{ns})$ indicate (relatively) enhanced mobility of

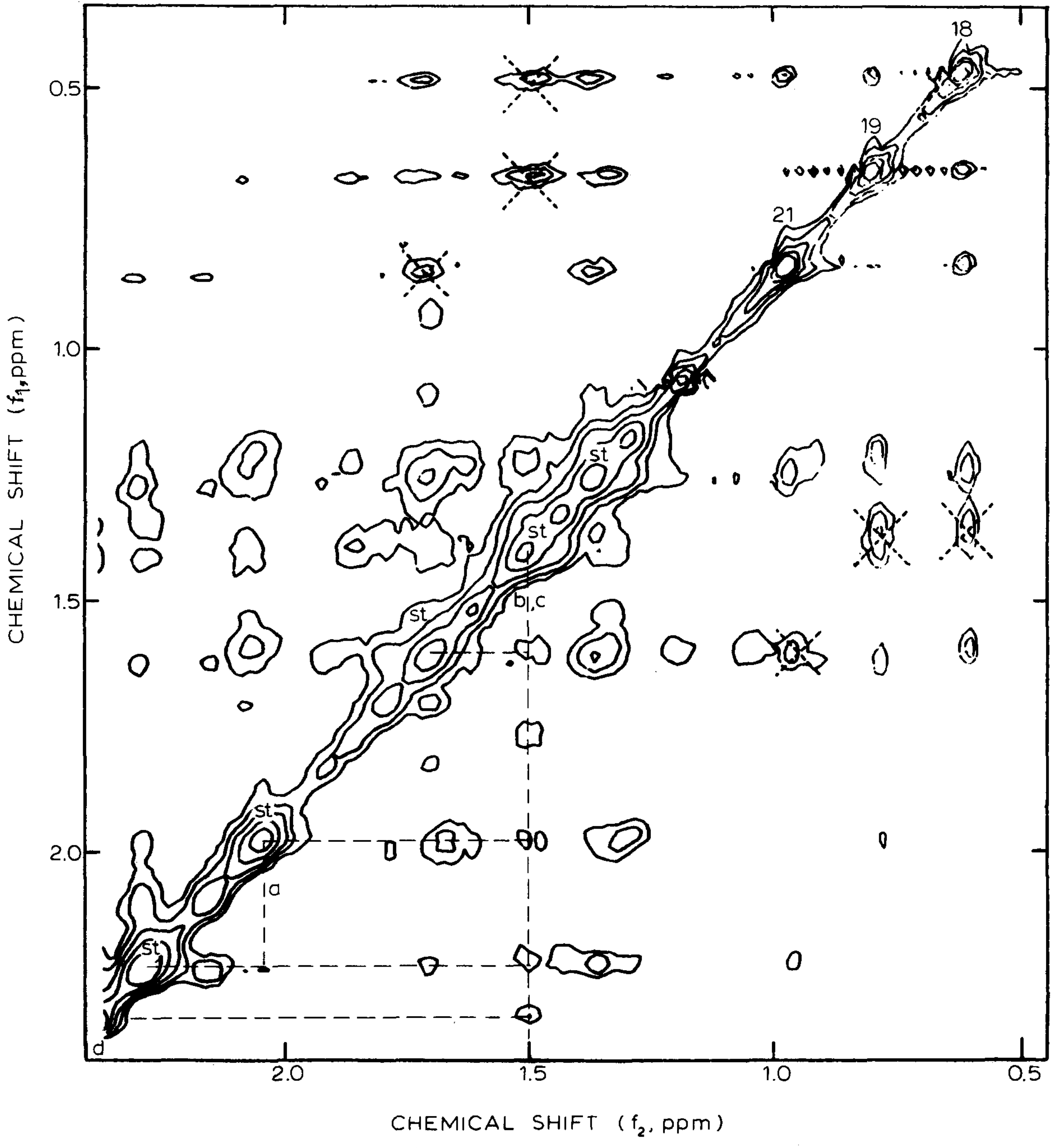

Fig. 5. Two-dimensional nuclear Overhauser enhancement spectrum of a 50:50 taurocholate-diPVPC mixture. Experimental conditions matched those described in Fig. 3. The displayed region includes taurocholate methyl groups [18,19,21] and steroid ring sites (st) and also diPVPC acyl chain protons (a–d). The dashed crosses indicate cross peaks observed for taurocholate micelles alone. The dashed lines connect possible cross peaks between bile salt and phospholipid molecules.

these polar groups, consistent with the results of prior $^1$H linewidth studies [6]. We have attempted to analyze the relaxation data using a number of theoretical models for internal and/or anisotropic motions. It is possible to reproduce the frequency dependence and general behavior of $R_1$(ns) and $R_1$(s), but only limited quantitative agreement with the experimental values is obtained. This result is

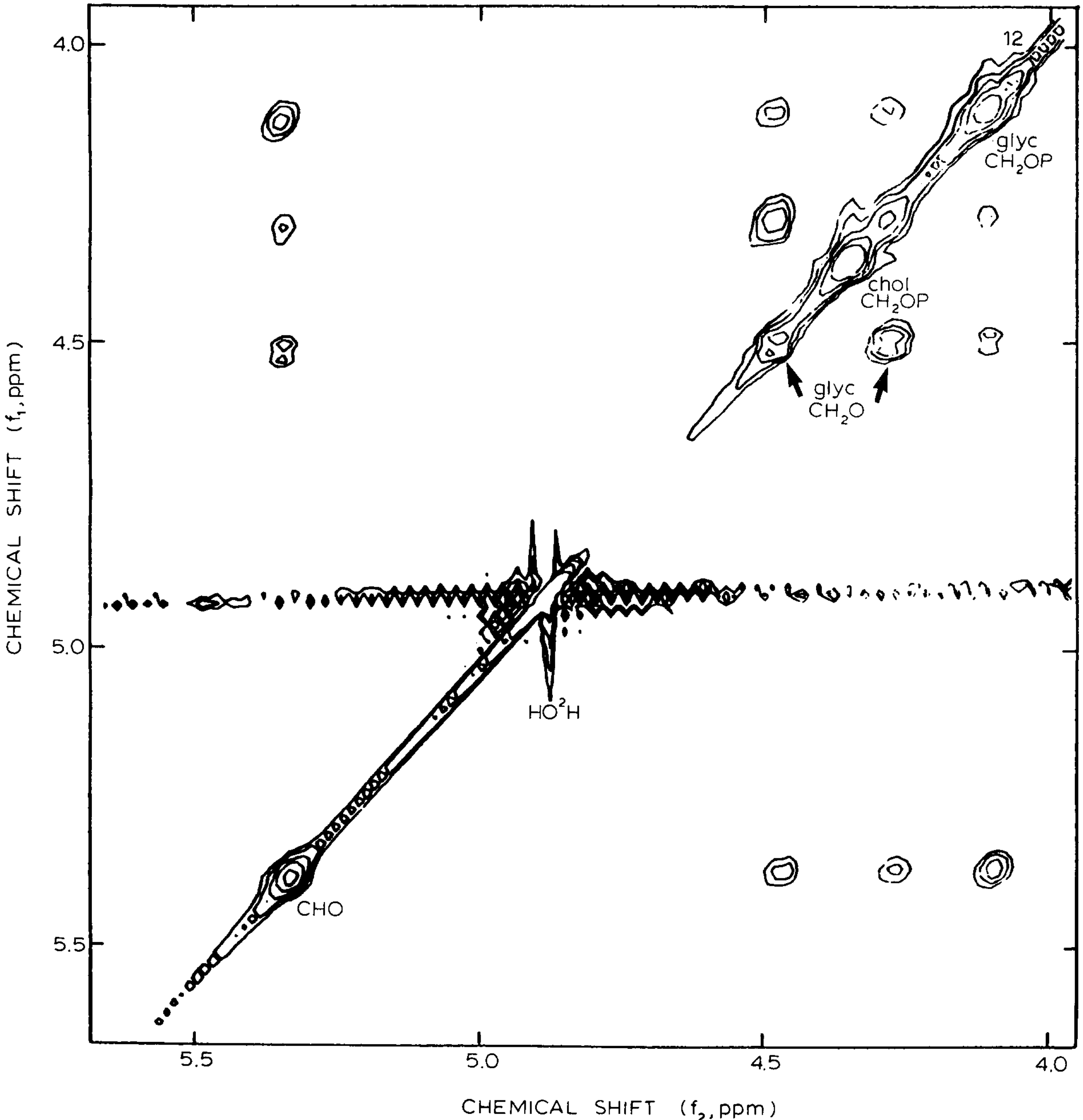

Fig. 6. Two-dimensional nuclear Overhauser enhancement spectrum of a 50:50 taurocholate-diPVPC mixture. The experimental conditions are as described in Fig. 5. This region includes glycerol backbone protons of the phospholipid (as well as residual protons in the $^2H_2O$ solvent).

to be expected, given the small number of data points relative to the number of parameters that describe complex molecular motions. The generalized conclusion of these modeling studies is that several dynamic modes are important, with correlation times on the order of $\omega^2\tau_c^2 \gtrsim 1$. If slow isotropic or simple anisotropic motions are assumed, the models predict increasing relaxation rates with increasing temperature. With more complex motional formulations, it is possible to account qualitatively for the opposite temperature dependence observed experimentally. It is also instructive to contrast the cross relaxation rate ($\sigma$) observed for $CH_2N$ protons of the egg PC ($\Sigma\sigma_{ij} \approx$

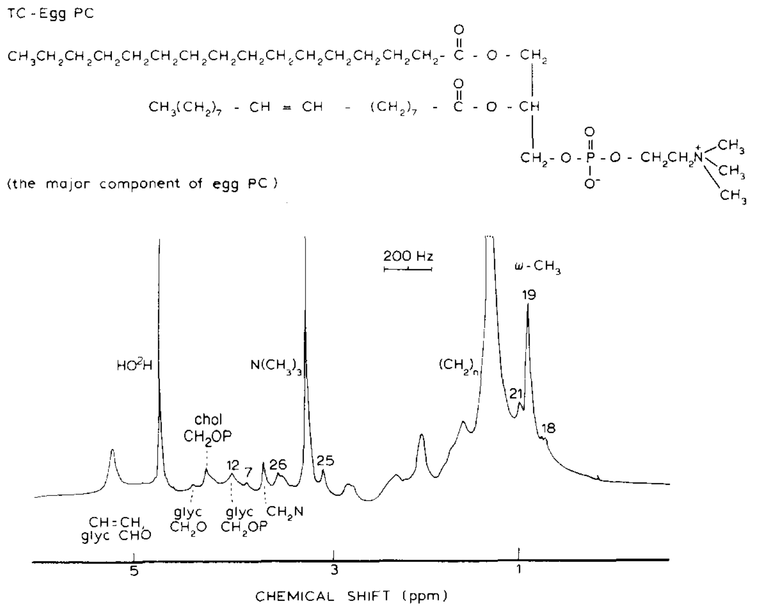

Fig. 7. 500 MHz $^1$H-NMR spectrum of sodium taurocholate-egg phosphatidylcholine (TC-egg PC) mixed micelles. Solutions are 9.5 mM in each component, prepared as described in the Materials section. Resonance assignments are made with reference to published spectra [27,28].

0) with that for methylene groups 25 and 26 of the taurocholate ($\Sigma\sigma_{ij} < 0$). The $CH_2N$ spins are those for which spin communication is notably absent in the two-dimensional nuclear Overhauser enhancement spectra. Conversely (since $\sigma_{ij}$ becomes negative when $\tau_c^2$ exceeds $5/4\ \omega^2$), those bonded methylenes which are more restricted motionally do exhibit two-dimensional nuclear Overhauser enhancement cross peaks.

## Discussion

These studies of bile salt and phospholipid mixtures represent the first application of the $^1$H-$^1$H two-dimensional nuclear Overhauser enhancement (NOESY) experiment to micellar aggregates; related work has been conducted recently on polypeptides in a micellar matrix [31] and on phospholipids organized in vesicular structures (Ref. 19 and Gabriel, N.E. and Roberts, M.F., unpublished results). From a practical point of view, it is clear that this approach provides a simple and more efficient alternative to the conventional one-dimensional nuclear Overhauser effect experiment. In what follows, the observed cross relaxation patterns are interpreted in tems of both structure and dynamics, by comparing different lipid systems and with the aid of independent one-dimensional NMR relaxation data.

The dependence of nuclear relaxation parameters on tumbling times is described in Eqns. 1–3, and it is the $\sigma$-to-$\rho$ ratio that determines the magnitudes of one- and two-dimensional nuclear Overhauser effects [16,30]. For simple taurocholate detergent micelles, cross relaxation occurs primarily between sites of the rigid steroid backbone, whereas more extensive, and different, spin communication networks exist when, according to current models [1,2], the bile salt is constrained at the perimeter of diPVPC or egg PC mixed micelles. If the appearance of two-dimensional nuclear Overhauser enhancement cross peaks (negative nuclear Overhauser effects) is associated with slow molecular tumbling [16], then relative immobilization at sites 25 and 26 of the taurocholate sidechain may be deduced in both back-to-back bile salt micelles [9] and in bile salt-egg PC mixtures [2,6]. No two-dimensional nuclear Overhauser enhancement cross peaks link the resonances of these nuclear spins when the solute is the short-chain phosphatidylcholine diPVPC, suggesting that in this latter system the taurocholate side chain extends more freely into the aqueous surroundings.

Taurocholate solubilization also alters PC structure in a manner that depends on acyl chain length. Whereas proton groupings in diPVPC and other short-chain phospholipid micelles are apparently too floppy to exhibit two-dimensional

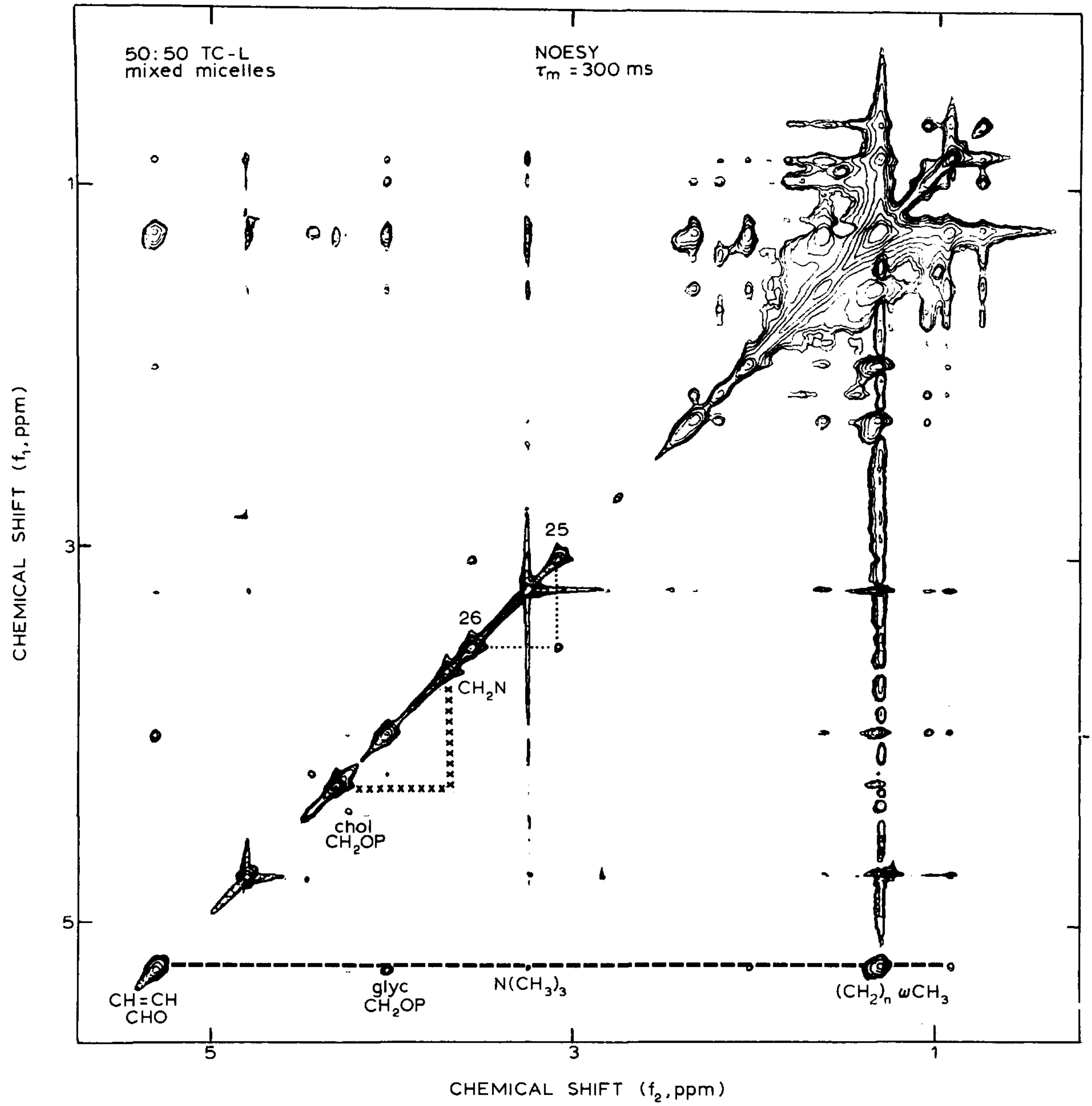

Fig. 8. Two-dimensional nuclear Overhauser enhancement (NOESY) spectrum for 50 : 50 taurocholate-egg PC mixture (TC-L), shown with a mixing time of 300 ms in order to accentuate differences in cross peak intensities at different molecular sites. The lowest contour has an intensity 0.2% of that of the largest spectral peak.

nuclear Overhauser enhancement cross peaks (N.E. Gabriel and M.F. Roberts, unpublished results), glycerol backbone sites become rigid enough to form a cross relaxation network when the diPVPC is present in a bile salt detergent matrix. For egg PC in the same taurocholate environment, spin communication is observed between numerous PC sites (and possibly also between taurocholate and PC molecules). A greater degree of motional restriction is inferred for this phospholipid, yet for $CH_2N$ (and perhaps $N(CH_3)_3$), correlation times as short as approx. 0.5 ns are estimated from the equality of selective and nonselective relaxation rates.

TABLE I

PROTON RELAXATION RATES FOR MODEL BILIARY MIXTURES

| Molecular site | $R_1$(ns) [a] | | | $R_1$(s) [a] | $\sum_{i \neq j} \sigma_{ij}$ [b] | Temp. |
|---|---|---|---|---|---|---|
| | 100 MHz | 200 MHz | 400 MHz | 400 MHz | 400 MHz | |
| CH=CH/CHO | 4.1 | 2.7 | 1.6 | | | 25°C |
| $N(CH_3)_3$ | 3.5 | 2.8 | 2.4 | 2.7 | −0.3 | |
| $(CH_2)_n$ | 4.1 | 2.8 | 2.0 | | | |
| 25 | 3.0 | 2.1 | 1.4 | 1.8 | −0.4 | |
| 26 | 3.6 | | 1.2 | 2.0 | −0.8 | |
| $CH_2N$ | 5.1 | 3.3 | 2.5 | 2.6 | −0.1 | |
| $CH_3$/19 | 3.6 | 2.0 | 1.4 | 1.8 | −0.4 | |
| 18 | | 3.1 | 2.3 | | | |
| CH=CH/CHO | | 2.7 | 1.3 | | | 30°C |
| $N(CH_3)_3$ | 3.0 | 2.6 | 2.0 | 2.0 | 0.0 | |
| $(CH_2)_n$ | 3.9 | 2.8 | 1.7 | 2.0 | −0.3 | |
| 25 | | 2.1 | 1.2 | 1.5 | −0.3 | |
| 26 | | 2.4 | 1.1 | 1.7 | −0.6 | |
| $CH_2N$ | | 3.2 | 2.2 | 2.4 | −0.2 | |
| $CH_3$/19 | 3.1 | 2.1 | 1.1 | 1.4 | −0.3 | |
| 18 | | 3.1 | 1.9 | | | |
| CH=CH/CHO | | 2.4 | 1.2 | | | 35°C |
| $N(CH_3)_3$ | 2.5 | 2.3 | 1.8 | | | |
| $(CH_2)_n$ | 3.6 | 2.5 | 1.6 | 1.9 | −0.3 | |
| 25 | | 1.8 | 1.2 | 1.5 | −0.3 | |
| 26 | | 2.0 | 1.0 | 1.6 | −0.6 | |
| $CH_2N$ | | 2.5 | 2.1 | 2.1 | 0.0 | |
| $CH_3$/19 | 2.8 | 1.7 | 0.9 | 1.3 | −0.4 | |
| 18 | | 3.0 | 1.8 | | | |
| CH=CH/CHO | | 2.4 | 1.2 | | | 40°C |
| $N(CH_3)_3$ | 2.5 | 2.2 | 1.7 | 1.8 | −0.1 | |
| $(CH_2)_n$ | 3.5 | 2.5 | 1.5 | 1.8 | −0.3 | |
| 25 | | 1.7 | 1.2 | 1.4 | −0.2 | |
| 26 | | 2.1 | 1.1 | 1.5 | −0.4 | |
| $CH_2N$ | | | 1.8 | 1.9 | −0.1 | |
| $CH_3$/19 | 2.7 | 1.9 | 1.0 | | | |
| 18 | | 2.9 | 1.8 | | | |

[a] $R_1$(ns) and $R_1$(s) indicate nonselective and selective spin relaxation rates, respectively.

[b] Defined as the difference $R_1(ns) - R_1(s)$.

Because enzymatic lipolysis rates are influenced by both the presence and identity of substrate aggregates [13], it is particularly informative to compare the structural and dynamic characteristics of phospholipid micelles and vesicles. For instance, the cross peak between protons at 5.3 and 0.9 ppm is unique to the micelle-solubilized egg PC system (compare with Ref. 19); it may be attributed to efficient spin diffusion [32] involving the CH=CH and $\omega CH_3$ acyl chain groups if aggregate packing results in restricted segmental motions. $^1H$ and $^2H$ $R_1$ values are enhanced in this system as compared with relaxation arising from fast motions in vesicular aggregates [33,34]; these results are in accord with more restricted motion in the micellar systems and support a (restricted) motional origin of this cross peak. An alternative explanation of this two-dimensional nuclear Overhauser enhancement cross peak invokes a hydrophobic interaction between the PC acyl chain and $CH_3$ 19 of the taurocholate molecule. As noted above and displayed in Fig. 5, the taurocholate-di-

PVPC mixed micelle may exhibit an analogous intermolecular nuclear Overhauser effect between chain $CH_2$'s and steroid backbone sites.

In contrast to the above, prominent cross peaks expected between nearby $CH_2N$ and $CH_2OP$ methylenes of the choline headgroup are absent at room temperature for sonicated egg PC vesicles [19], mixed-chain PC vesicles (N.E. Gabriel and M.F. Roberts, unpublished results) and both types of taurocholate-PC micelles (this work). Given that the floppy $CH_2N$ segment [6] constitutes a break in the spin communication network it is probable that $N(CH_3)_3$ cross peaks at 1.3 ppm represent interactions with taurocholate rather than with the PC acyl chains. Intermolecular cross relaxation effects have also been proposed between PC moieties in sonicated vesicle systems [19], though they remain unconfirmed in mixed chain length PC vesicle assemblies (N.E. Gabriel and M.F. Roberts, unpublished results).

The picture which emerges from this work is that significant dynamic alterations to the phospholipid components accompany changes of the aggregate form from vesicle to micelle to mixed micelle. Interaction of bile salt and phospholipid is also chain-length dependent: the taurocholate side chain is located in a motionally unrestricted site in mixtures with a short-chain PC, whereas this same taurocholate side chain is motionally restricted in both the taurocholate-egg PC system and in the pure taurocholate micelle. Direct interaction of taurocholate and PC chemical sites is suggested by both chemical shift changes and two-dimensional nuclear Overhauser enhancement cross peaks, though the latter features may also arise from PC-PC interactions. Either structural explanation requires some restriction of the PC chain motions.

Taken together, these NMR relaxation studies of simple and mixed lipid aggregates demonstrate the usefulness of two-dimensional nuclear Overhauser enhancement (NOESY) techniques to address structural and dynamic questions for micelles and vesicles. One-dimensional $R_1$ values help to delineate the motional contributions to two-dimensional nuclear Overhauser enhancement cross peak intensities, and comparisons among various model substrates offer new insight into those aspects of molecular organization that influence enzymatic attack rates.

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