Effect of Cholesterol on Viscoelastic Properties of Dipalmitoylphosphatidylcholine Multibilayers As Measured by a Laser-Induced Ultrasonic Probe†

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Received December 9, 1985; Revised Manuscript Received February 27, 1986

ABSTRACT: Using a novel laser-induced ultrasonic probe, we have examined the bulk viscoelastic properties of fully hydrated dipalmitoylphosphatidylcholine (DPPC) aligned multibilayers in terms of the anisotropic in-plane elastic stiffness ($C_{11}$) and viscosity ($\eta_1$). Our measurements of $C_{11}$ are in accord with those reported on Brillouin light scattering on a similar system. Our measurements on viscosity are the first of their kind and are, on the average, a factor of 10 lower than microviscosities estimated by spectroscopic techniques. We report the first comprehensive study of the effects of cholesterol on the bulk mechanical properties of DPPC multibilayers. At temperatures above the phase transition temperature of DPPC ($T_c$), an increase in both $C_{11}$ and $\eta_1$ is noticed when cholesterol is incorporated in the multibilayers. However, at temperatures below $T_c$, no measurable changes are detected in either $C_{11}$ or $\eta_1$. These results, reflecting changes in the bulk viscoelastic properties of the multibilayers, differ from the changes reported by local fluidity parameters in that the latter indicate a decrease in the bilayer fluidity in the presence of cholesterol above $T_c$ and an increase below $T_c$ ("dual effect" of cholesterol). Our data suggest that the "dual effect" of cholesterol is noticeable only on a molecular scale. Increasing cholesterol concentrations higher than 20 mol % cease to further affect $C_{11}$ or $\eta_1$ of the DPPC multibilayers. This agrees with various results reported in the literature, by techniques measuring the local effects of cholesterol, and supports the changes in molecular organization postulated to occur when cholesterol concentration reaches 20 mol % in the lipid bilayers.

†This work was supported by NSF Grant DMR84-16343 and NIH Grant 5R01 GM32205.

C

Cholesterol is an important and abundant constituent of most eukaryotic membranes. In cell membranes, cholesterol constitutes up to 50 mol % of the lipid. The physiological significance of cholesterol and its effect on membrane fluidity has been the subject of many recent articles and reviews (Shinizky et al., 1983a,b). The interactions of cholesterol with phospholipid molecules (the other major lipid component of cell membranes) have been actively examined through model systems (bilayers, vesicles, and micelles) for the past decade (Jain, 1975; Phillips, 1972; Demel & De Kruijff, 1976). Various techniques including electron spin resonance (ESR) spectroscopy (Rocquet, 1981; Shimshick & McConnell, 1973), NMR spectroscopy (Cullis, 1976; Tilcock et al., 1982), electron microscopy (Copeland, & McConnell, 1980; Lentz et al., 1980), IR spectroscopy (Cortijo & Chapman, 1981), fluorescence spectroscopy (Vanderkooi, 1974; Shinizky & Barenholz, 1978), and differential scanning calorimetry (DSC) (Papahadjopoulos et al., 1973; Mabrey et al., 1978; Estep et al., 1978) have been used to study the effect that cholesterol has on the mechanical properties and fluidity of the cholesterol/phospholipid model system. All these techniques have different definitions of the membrane fluidity. The term "membrane fluidity" has been used in the literature to describe two different types of motion: (1) the vibrational or rotational movement of a group on a molecule, often measured by NMR and ESR; (2) the diffusivity or translational motion of a molecule. Although both classes of properties depend on the "free space" available in a two-dimensional lattice (Blank, 1962; Blank & Britten, 1965), when different samples are compared, the trends can be easily masked by a large variety of competing processes (Schreier et al., 1978). Furthermore, all these techniques measure changes in the molecular environment of the bilayer rather than bulk changes in the bilayer plane.

More recently, viscosity and lateral compressibility measurements have been performed on multibilayers according to the theories of wave propagation through lipid multibilayers.
Materials and Samples

Dipalmitoylphosphatidylcholine (Sigma) was used without further purification. Cholesterol (Calbiochem) was dissolved in chloroform and filtered through a pore size of 0.1 μm to remove dust particles. The CHCl₃ was blown off and the dried stock stored desiccated in a freezer. HEPES [N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid] (Sigma) was made to 50 mM, pH 7.5, and also filtered to remove dust particles. The CHCl₃ was blown off and the dried sample was allowed to rest while the HEPES buffer diffused into the bilayers. This hydration process was monitored by using the LIPS experiment (both the frequency and attenuation of the launched acoustic wave are sensitive to percent hydration (T. A. Guion et al., unpublished results]). Hydration was assumed to be complete when no change in the frequency and attenuation of the signal was detected in a 0.5-h time period. This usually required 2–3 h.

Cholesterol content in the samples was assayed by a FeCl₃ colorimetric assay (Zlattis et al., 1953).

Methods

Briefly, the typical experiment proceeds as follows (see Figure 1). The aligned lipid multibilayers are exposed to two crossed, picosecond excitation pulses having the same wavelength and polarization. Constructive and destructive interference in the crossing volume produces a sinusoidally varying pattern of intensity peaks and nulls, which launches an acoustic standing wave. The acoustic wavelength and orientation match the interference-pattern geometry (Fayer, 1982, 1984). The periodic density (and thus the refractive index) variations associated with the acoustic wave propagation act as a diffractive grating for a variably delayed probe pulse incident at the Bragg angle. The changes in diffracted probe intensity as a function of delay time following the grating excitation are used to calculate the velocity and attenuation of the induced ultrasonic wave (Eyring & Fayer, 1984).
The LIPS technique involves crossing two excitation laser pulses to optically generate longitudinal (compressional) acoustic waves with a well-defined wave vector and monitoring the resulting density grating with a third probe pulse. This means that both the wavelength and the propagation direction of the acoustic wave are known. By varying the acoustic wave propagation direction with respect to the bilayer normal, the anisotropic properties of the multilayer are examined. By varying the acoustic wavelength, a range of physical properties can be probed (Nelson et al., 1982).

There are two mechanisms of acoustic wave generation. (1) One is thermal absorption. The sample experiences a weak absorption due to the forbidden $v = 0 \rightarrow v = 3$ C–H stretch transition where the two IR pulses constructively interfere. A rapid relaxation translates this absorption to heat. Thus, on a 100-ps time scale, a sinusoidal temperature variation is set up in the sample. The expansion of the heated areas produces two effects: First, the expansion launches counterpropagation acoustic waves having the grating wave vector. Second, the expansion itself becomes a nonpropagating density grating, less dense in the heated areas and more dense in the unheated areas. This density grating decays by thermal diffusion (microsecond time scale).

Previous estimates (Eyring & Fayer, 1985) show temperature increase in the intensity peaks to be $\sim 10^3$ K per shot. Thus, the generation of the acoustic waves is an extremely mild perturbation of the system.

The same crossed IR excitation pulses generate a second set of counterpropagating acoustic waves because of coupling of the electromagnetic field of the laser pulses with the sample through the photoelastic constant. This process, also known as electrostriction, generates longitudinal acoustic waves having the wave vector of the IR interference grating (Nelson, 1982). These acoustic waves differ from the thermally generated acoustic waves only in phase and amplitude.

Thus, density variation produced by the standing longitudinal acoustic waves generated by thermal absorption and SBS add to the nonpropagating thermal density grating. Since in our experiments the system is monitored over 20 ns, the thermal density grating appears to be static. The acoustic waves, on the other hand, propagate and decay on a nanosecond time scale.

These density variations are monitored by a third pulse that is brought into the sample at the Bragg angle to the density grating. The intensity of the Bragg diffracted probe pulse is related to the magnitude of the density variations by (Nelson et al., 1982)

$$I(t) = |A[1 - \cos \omega t \exp(-\alpha Vt)] - B \sin \omega t \exp(-\alpha Vt)|^2$$

(2)

where $A$ and $B$ are constants indicating relative amplitudes of the thermal absorption and SBS mechanisms, respectively, and $\omega$ is the circular frequency of the traveling acoustic waves having velocity $V$ (cm/s) and attenuation $\alpha$ [meper (Nm)/cm]. The damped cosine term corresponds to the thermally generated acoustic wave, and the damped sine term corresponds to the SBS generated wave. Two aspects of this function need to be illuminated. First, since $1 - \cos \omega t = 2 \cos (\omega/2)t$, the thermal absorption term appears to have half the frequency of the SBS term. Second, at long time $t \gg (\alpha V)^{-1}$, $I(t) \propto A^2$ i.e., the probe detects only from the static thermal density grating. Figure 3 shows typical data that illustrate the various aspects of eq 2. Figure 3A is for a sample of DPPC with 0 mol % cholesterol at 73 °C, while Figure 3B is for DPPC with 30 mol % cholesterol at the same temperature. In Figure 3B the acoustic damping is considerably faster than in Figure 3A, as evidenced by the more rapid decrease in the size of the oscillations. Notice that between the large peaks at the beginning of the trace are small peaks. The smaller peaks are

![Diagram](image_url)
The velocity measured in the experiment. The elastic constant bilayer plane at constant layer spacing elastic constants, denoted by $C_{11}$ is a measure of the area compressibility of the lipid in the parameters are essentially independent, and reproducible fits are obtained. Since the wavelength of the acoustic waves is given by $q_2 = \frac{2\pi f}{V}$ and $V = \frac{A}{f}$.

Determination of Elastic Stiffness and Viscosity. The two physical constants obtained by the LIPS experiment are acoustic velocity $V$ and attenuation $\alpha$. The velocity is related to the elastic stiffness constants $C_0$ of the system. It is implicit that the derivatives are strongly frequency-dependent processes with characteristic relaxation times $\tau_\gamma$. The latter are more or less related to the shear viscosity. However, a comparison can be made by using eq 8 and noting that in most liquids $\eta_3 \approx \eta_1$ in the high-frequency range (Candau & Letcher, 1978). For example, in the nematic phase of the liquid crystal MBBA $[N-(p$-methoxybenzylidene)-p-butyryl]-aniline, the shear and volume viscosities have been independently measured (Candau & Letcher, 1978). It is found that $\eta_2 = 0.41$ P and $\eta_3 = 0.43$ P. Therefore, for comparison purposes it is reasonable to take the shear viscosity to be $\eta_2 = \eta_3$. In the work described here the wave vector is in the plane of the bilayer, so that

$$V_1^2 = 0$$

and

$$V_2^2 = \rho^{-1}C_{11}$$

where $\rho$ is the macroscopic density. $V_2$ will be designated $V_\omega$, the velocity measured in the experiment. The elastic constant $C_{11}$ is a measure of the area compressibility of the lipid in the bilayer plane at constant layer spacing $d$:

where $A$ is the area per molecule and $P$ is the lateral pressure that tends to change $A$. It is implicit that the derivatives are taken at constant entropy and water concentration.

Acoustic damping has several sources and is written in general as $\alpha = \alpha_s + \alpha_l + \alpha_v$, where $\alpha_s$ is the classical viscoelastic damping, $\alpha_l$ is damping due to a coupling with lipid internal degrees of freedom, and $\alpha_v$ is damping due to coupling of the acoustic field with critical fluctuations seen at phase transition. $\alpha_l$ and $\alpha_v$ are strongly frequency-dependent processes with characteristic relaxation times $\tau_\gamma$. Their contribution to damping is found under the summation in eq 6. $\alpha_s$ is the limiting viscosity at high frequency. Gamble and Schimmel (1978) fit their ultrasonic absorption data on DPPC vesicles near the phase transition temperature with a single relaxation time of 10 ns (16 MHz) and saw no relaxation processes above 150 MHz. Since our study involved 600-MHz waves, eq 6 reduces to

$$\frac{\alpha}{f^2} = B^* + \sum_i A_i \tau_i (1 - \omega^2 \tau_i^2)^{-1}$$

where $B^*$ describes the limiting viscosity at high frequency. Thus we expect to see no critical damping in the region of the phase transition or frequency-dependent damping due to rotational isomerization of the lipid chains.

The damping constant, $B^*$, is related to the elements of the viscosity tensor. Just as with the acoustic velocity, the symmetry of the multibilayer array requires that the attenuation be anisotropic; i.e., its magnitude depends on the acoustic wave vector (Martin et al., 1972). For the case of longitudinal waves propagating in the plane of the bilayer, the expression for $\alpha/f^2$ simplifies to (Candau & Letcher, 1978)

$$\frac{\alpha}{f^2} = \frac{2\pi^2}{\rho V^3} (\eta_2 + \eta_3) = \frac{2\pi^2}{\rho V^3} \eta_1$$

where $\eta_2$ and $\eta_3$ are two of the five viscosities required to describe a compressible smectic system, in the notation of Martin et al. (1972), and $\eta_1$ is equivalent to their sum (Forster et al., 1971). $\eta_1$ is the in-plane shear viscosity, where $\eta_3$ has contributions from the volume viscosity. The membrane viscosities obtained from ultrasonic attenuation measurements are not directly comparable to the microviscosities deduced from rotational and translation diffusion studies (Shimshick & McConnell, 1973; Shnitzky & Barenholz, 1978) since the latter are more or less related to the shear viscosity. However, a comparison can be made by using eq 8 and noting that in most liquids $\eta_3 \approx \eta_1$ in the high-frequency range (Candau & Letcher, 1978). For example, in the nematic phase of the liquid crystal MBBA $[N-(p$-methoxybenzylidene)-p-butyryl]-aniline, the shear and volume viscosities have been independently measured (Candau & Letcher, 1978). It is found that $\eta_2 = 0.41$ P and $\eta_3 = 0.43$ P. Therefore, for comparison purposes it is reasonable to take the shear viscosity to be $\eta_2 = \eta_3$.

Densities in eq 8 and 5 were obtained from Gersfeld (1978). The error introduced into $C_{11}$ and $\eta_3$ by uncertainty in the density is always less than half the experimental error. The experimental error in $\omega$ values was generally very small ($<2\%$) and insensitive to the signal to noise ratio (S/N) in the LIPS scans. However, the $\alpha V$ values had a larger experimental error ($<15\%$) and were found to be sensitive to the quality of
Cholesterol effects on DPPC multibilayers

**RESULTS**

**Cholesterol Effects on Viscosity** ($\eta_{11}$). The effect of cholesterol content on the viscosity (eq 8) of the DPPC bilayers at various temperatures is shown in Figure 4. In the liquid-crystalline phase, the viscosity increases with increasing cholesterol, up to $\sim 22$ mol % cholesterol, after which increasing cholesterol content has no further effect on the viscosity. The initial increase in viscosity agrees with the established behavior of the changes detected in “microviscosities” of bilayers as measured by other techniques (Jain, 1975) under similar conditions. Cholesterol incorporation into a lipid bilayer in the gel phase is reported to decrease the “microviscosity” (Jain, 1975). We detect no measurable change in the viscosity in the gel phase (Figure 4). This result will be discussed later in this report.

Another notable trend in Figure 4 is that the changes in $\eta_{11}$ due to cholesterol incorporation in the multibilayers are at a maximum in the vicinity of $T_c$, decreasing as the temperature is increased above $T_c$, showing no change by $80^\circ$C, and decreasing when the temperature is decreased below $T_c$, showing no change by $35^\circ$C. This kind of behavior agrees with the concept that the lipid bilayer is most susceptible to cholesterol perturbations in the vicinity of the phase transition temperature since cholesterol is thought to disrupt the cooperativity between the hydrocarbon chains (Presti et al., 1982).

**Cholesterol Effect on Elastic Stiffness** ($C_{11}$). Figure 5 shows the changes in the elasticity of DPPC bilayers as cholesterol content is increased at various temperatures.

In the liquid-crystalline phase, incorporation of cholesterol in the bilayer up to $10$ mol % has no measurable effect on the $C_{11}$. An increase in $C_{11}$ is noticed as cholesterol concentrations are increased from $15$ to $\sim 20$ mol % cholesterol. Further increasing the cholesterol concentration has little or no further effect on the measured $C_{11}$. In the vicinity of the phase transition temperature ($40$ and $43$ °C) $C_{11}$ values with increasing cholesterol concentrations is noticed until $\sim 20$ mol % cholesterol. Below the phase transition temperature, the $C_{11}$ parameter responds in an analogous manner to the viscosity, showing no measurable changes in the presence of cholesterol.

**Cholesterol Effects on Phospholipid Phase Transition.** The presence of cholesterol has been reported to broaden the first-order phase transition of lipid bilayers (Jain, 1975). This phenomenon is seen in both the viscosity (Figure 6) and the elastic stiffness (Figure 7) measurements. The phase transition break at $41.5$ °C for DPPC multibilayers in the absence of cholesterol is particularly apparent in Figure 6, where a nonlinear behavior of viscosity vs. temperature is noticed. As cholesterol is introduced into the multibilayers, the break becomes less obvious, and by $20$ mol % cholesterol, its presence is questionable in view of the error in our measurements (Figure 6). In bilayers, where the cholesterol content is higher than $20$ mol %, the $\eta_{11}$ parameter appears to increase linearly with decreasing temperature.

**DISCUSSION**

There exist in the literature only a few papers where measurements of vesicle/bilayer viscoelastic properties are reported. Mitaku et al. (1978) reported the bulk modulus $K$ of DPPC multilamellar vesicles at various temperatures with
temperatures where the lipid bilayer exists in the liquid-crystalline phase. However, a difference is observed in the gel phase of DPPC/DPPG (di-palmitoylphosphatidylglycerol) bilayers. The range of $C_{11}$ change from 30 to 70 °C for the sample with varying cholesterol concentrations is as follows: 0 mol %, (30-20) × 10^2 g cm^-3 s^-2; 5 mol %, 30-20; 10 mol %, 30-20; 15 mol %, 30-20; 20 mol %, 31-21; 25 mol %, 31-21; 30 mol %, 30-21; 35 mol %, 30-21. This information enables the estimation of $C_{11}$ at any point on the plot.

<table>
<thead>
<tr>
<th>Table 1: Elastic Stiffness Measurements by Different Techniques</th>
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<tr>
<td>elastic stiffness (x10^2 dyn/cm)</td>
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<td>---------------------------------</td>
</tr>
<tr>
<td>$K^*$</td>
</tr>
<tr>
<td>$C_{11}$</td>
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<tr>
<td>$C_{12}$</td>
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<td>$C_{22}$</td>
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Sakanishi et al., 1979. ^LePesant et al., 1978. This work. *Forty-nine mole percent cholesterol in bilayers. /Thirty-three mole percent cholesterol in bilayers. /Thirty-five mole percent cholesterol in bilayers.

The difference between the $K$ values reported by Sakanishi et al. (1979) and the $C_{11}$ values reported here and by LePesant et al. (1978) can be explained by the difference in the definition of $K$, which measures the elastic response to acoustic waves traveling at all orientations to the bilayer, and $C_{11}$, which measures the elastic response to waves traveling only in the bilayer plane. Acoustic waves having a vector component normal to the bilayer may not experience the stiffening effect of the phase transition to the same extent as acoustic waves with in-plane wave vectors. Thus $K$ would be expected to be lower than $C_{11}$ for the same system. The difference between our $C_{11}$ values and those of LePesant et al. could be caused by the sensitivity of both techniques to the optical quality of the sample. The poor optical quality of the gel phase of DPPC multibilayers greatly increases the scattered light, resulting in a decreased signal to noise ratio. This problem is more severe for the Brillouin scattering than for the LIPS technique.

The term microviscosity has been used to describe the fluidity of biomembranes. The literature documents a wide range of values for this parameter. For example, fluorescent spectroscopy, with perylene as a probe, obtained a value of
1 P for DPPC dispersions at 45 °C (Cogan et al., 1973), while with pyrene as a probe, it obtained a value of 0.6 P for dimyristoylphosphatidylcholine (DMPC) dispersions at 30 °C (Vanderkooi, 1974). 13C NMR data on DMPC sonicated dispersions at 52 °C estimate a microviscosity near the center of the bilayer of 2.4 P (Lee, 1975). It is important to remember that all these measurements mainly reflect the orientational constraints of the probe in the bilayer and they do not directly reflect the anisotropic bulk viscosity in the bilayer plane. The experiments presented here are the first to directly measure the bulk viscosity in the plane of the lipid bilayer. These measurements of the bulk η11 on DPPC multibilayers are a factor of 10–15 smaller than the previously reported microviscosities. (Typical values from our experiments are 0.1–0.15 P.) These differences emphasize that the microviscosity and the bulk viscosities of lipids are two very different properties and that one should not project from one to the other without extreme caution.

The generally accepted view of phospholipid/cholesterol interaction is that cholesterol has a "fluidizing" effect on the phospholipid bilayer below Tc and a "gelling" effect above Tc, such that at ~20 mol % cholesterol the cooperativity of the lipid-chain melting at the phase transition temperature has been removed and a state of intermediate fluidity is maintained that is relatively insensitive to temperature changes (Jain, 1975). The phenomenon has been called the "dual effect" of cholesterol and has been observed within 5 °C above and below the Tc of the phospholipid.

Our η11 and C11 measurements support the "gelling" effect seen with the addition of cholesterol to DPPC multibilayers above Tc. However, in the gel state, addition of cholesterol showed no decrease in the measured C11 or η11. Furthermore, though cholesterol did abolish the cooperativity of the hydrocarbon-chain melting at ~20 mol % (as detected by the disappearance of the sharp break in viscosity around Tc, Figures 6 and 7), the resulting state was still temperature sensitive, showing a decrease in C11 and η11 with increasing temperature. This strongly suggests that the "dual effect" of cholesterol as measured by local fluctuations is not a macroscopic phenomenon.

The various special effects measured at 20 mol % cholesterol by different physical techniques (Jain, 1975) have recently been incorporated into a molecular model proposed by Presti et al. (1982). This model proposes a molecular separation below 20 mol % cholesterol, where cholesterol–phospholipid complexes (1:2) coexist with free phospholipid domains. This separation is hypothesized to cease at 20 mol % cholesterol, at which concentration the free phospholipid domains are hypothesized to disappear. It is important to note that while this is a microscopic model, we detect changes in bulk properties of the multibilayers, i.e., the viscosity η11 and elastic stiffness C11, in the vicinity of 20 mol % cholesterol (Figures 4 and 5). This suggests that the model describes changes in the macroscopic as well as microscopic properties of these systems.

A recent article by Knoll et al. (1985) reports small-angle neutron scattering (SANS) and freeze–fracture electron microscopy work on DMPC–cholesterol mixed vesicles. Their work does not indicate phase separation below 20 mol % cholesterol at temperatures above the Tc of the lipid. It is therefore in variance with the conclusions based on previous spectroscopic techniques. The authors argue that the molecular motions measured by these techniques depend on the local packing density of the lipid molecules and that nonideal behavior of the molar areas (or volumes) of a mixture may lead to breaks in the probe partitioning [e.g., the ESR probe 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO)] or molecular mobilities. It is suggested that these could be erroneously interpreted in terms of phase boundaries. The LIPS technique used in this work measures bulk properties and is not sensitive to such local inhomogeneities, and yet we see a change in the trends of our data above Tc, at ~20 mol % cholesterol (Figures 4 and 5). Although the observed trends cannot be proven to be indicative of phase separations, they lend additional credibility to the results obtained with spectroscopic techniques, demonstrating that the anomalies reported by these techniques above Tc, at 20 mol % cholesterol, are not due to inhomogeneous probe distributions but reflect actual properties of the bilayers under investigation.

Hui and He (1983), using X-ray and electron diffraction, have monitored the increase in the width of the wide-angle diffraction signal as the cholesterol concentration is increased in DMPC. By associating the diffraction peak with the coherence length of lipid packing, they conclude that addition of cholesterol decreases the coherence length of lipid packing up to ~20 mol % cholesterol, where a limit in cooperation between DMPC molecules is reached. Hui and He argue that their results are compatible with the Presti model and thus may be interpreted as a quantification of the model. What is remarkable is the high degree of correlation between the coherence length of the DMPC molecules measured by Hui and He and our measurement of the in-plane viscosity of DPPC. This correlation suggests a molecular interpretation for the behavior of the in-plane bulk viscosity.

The LIPS experiment described here allows the examination of the anisotropic bulk properties of lipid multibilayers. We have reported quantitative values of the elastic stiffness and viscosity of these model membranes as a function of cholesterol concentration and temperature. These are important parameters in model membrane studies, which previously have been unobtainable or extrapolated from molecular models. This work emphasizes the difference between the anisotropic bulk and microscopic properties of lipid multibilayers. The values obtained for the viscosity of fully hydrated DPPC multibilayers was an order of magnitude smaller than reported microviscosities. Furthermore, the trends in C11 and η11 measurements did not always follow those reported in local "fluidity" measurements or order parameters. It is not within the scope of this work to propose a model that accounts for the difference between the microscopic observables and the bulk properties of DPPC–cholesterol multibilayers. However, the results presented here provide a different perspective from which to examine the viscoelastic properties of these model systems.

Acknowledgments
We express our thanks to Professor W. Huestis for the generous use of her laboratory and for many stimulating discussions that helped guide this work. We also thank Professor Auld for his assistance in the understanding of acoustic wave propagation, Dr. Greg Eyring for his help in the early stage of this research, and Dr. Frank Patterson for his technical assistance with the Nd:YAG laser.

Registry No. DPPC, 2644-64-6; cholesterol, 57-88-5.

References
Effect of Short-Chain Primary Alcohols on Fluidity and Activity of Sarcoplasmic Reticulum Membranes

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Received December 19, 1985; Revised Manuscript Received March 20, 1986

ABSTRACT: Intramolecular excimer formation with the fluorescent probe 1,3-di(1-pyrenyl)propane, differential scanning calorimetry, and X-ray diffraction were used to assess the effect of ethanol, 1-butanol, and 1-hexanol on the bilayer organization in model membranes, sarcoplasmic reticulum (SR) lipids and native SR membranes. These alcohols have fluidizing effects on membranes and lower the main transition temperature induced in the lipid bilayer. Depending on the chain length, the alcohols interact with the SR membranes in different domains, perturbing differently the Ca2+-pump activity. Nevertheless, the energetic efficiency of the pump (Ca2+/ATP) is not significantly affected by ethanol or 1-butanol at high concentrations. The different effects of alcohols on the bilayer organization in model membranes, sarcoplasmic reticulum (SR) lipids and native SR membranes. These alcohols have fluidizing effects on membranes and lower the main transition temperature (DMPC), but only 1-hexanol alters the cooperativity of the phase transition with 1-butanol at high concentrations. The different effects of alcohols on the bilayer organization in model membranes, sarcoplasmic reticulum (SR) lipids and native SR membranes. These alcohols have fluidizing effects on membranes and lower the main transition temperature induced in the lipid bilayer. Depending on the chain length, the alcohols interact with the SR membranes in different domains, perturbing differently the Ca2+-pump activity.

Short-chain primary alcohols affect the functional and structural states of several biological membranes (Chin &

Goldstein, 1977; Stokes & Harris, 1982; Waring et al., 1981; Swartz et al., 1974; Retig et al., 1977; Kondo & Kassai, 1973; Garda & Brenner, 1984).

Traditional pharmacology groups alcohols with other chemically unrelated compounds, i.e., anesthetic agents (Seeman, 1972), sharing in common the ability to perturb membrane organization (Seeman, 1972; Paterson et al., 1972;