EFFECT OF BENZYL ALCOHOL ON LIPID BILAYERS

A COMPARISON OF BILAYER SYSTEMS

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ABSTRACT The effect of the small anesthetic molecule, benzyl alcohol, on the structure of various bilayer systems has been studied by optical, electrical, and x-ray diffraction techniques. We find that the modifications in bilayer thickness caused by benzyl alcohol differ dramatically for planar (or black lipid) bilayers containing solvent, planar bilayers containing little or no solvent, and vesicular bilayers. Benzyl alcohol increases the thickness of planar bilayers containing *n*-alkane solvents, yet decreases the thickness of "solvent-free" planar bilayers. The effect of benzyl alcohol on vesicular bilayers below the phase transition temperature also depends on whether solvent is present in the bilayers. Without solvent, gel-state bilayers are reduced in thickness by benzyl alcohol, whereas in the presence of solvent, the thickness is unchanged. Above the phase transition temperature, benzyl alcohol has no measurable effect on vesicular bilayer thickness, whether solvent is present or not. These results indicate that different model membrane systems respond quite differently to a particular anesthetic.

INTRODUCTION

Model membrane systems enjoy wide popularity for many studies which cannot be conveniently carried out in vivo (1, 2). Vesicle systems are often used to reconstitute isolated enzyme systems; black lipid films are used to study electrical behavior of model transport processes conveniently. These systems differ from one another and from real biological membranes, but we do not as yet have a clear quantitative understanding of the differences. We do know that the interior of a membrane bilayer is unlike an isotropic liquid, even though for many purposes it can be treated approximately as a thin film of hydrocarbon (3–5).

Most of the physiologically important activities of biological membranes are probably promoted by enzymes and other molecules residing in the bilayer. Thus, knowledge of the physical chemistry of membrane structure is as important to the understanding of membrane processes as knowledge of the structure and properties of water is to understanding solution chemistry.

In this paper, we will examine the response to the local anesthetic, benzyl alcohol, of three membrane systems, all in current use as models for biological membranes. Benzyl alcohol was chosen because Ashcroft et al. (6) have shown that this molecule decreases membrane capacitance—and thus increases bilayer thickness—by a factor of two in lecithin bilayers

formed with tetradecane as a solvent. We are interested in testing the generality of this result with other model membranes.

A number of different model membrane systems are in current use. We will consider two major categories: *"Vesicular bilayers"* are formed by dispersing suitable lipids in aqueous solution (7). This procedure results in the formation of multilamellar vesicles which can be used in x-ray diffraction or permeability studies. Single *"planar (black lipid) bilayers"* can be formed so that they contain organic solvent (5, 8) or are almost solvent-free (9–11). This immediately raises the question of what effect the solvent has on the properties of the membranes. Unlike vesicular bilayers, both solvent-containing and solvent-free planar bilayers are in equilibrium with a torus (10). It is likely that the manner in which solvent partitions from the torus into the bilayer region of the membrane will be a factor of considerable importance.

MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine (DPL) was obtained from Sigma Chemical Co. (St. Louis, Mo.); egg phosphatidylcholine from Lipid Products, North Surry, England; and bacterial phosphatidylethanolamine (BPE) from Supelco, Inc. (Bellefonte, Pa.). These lipids were checked for purity by thin-layer chromatography (one spot) and for neutrality by microelectrophoresis (12) (mobility of neutral lipids was 0 in 0.01 M KCl). Water was distilled in an all quartz still. Salts were roasted at 300°C for 24 h to remove organic impurities.

Decane was from Eastman Kodak Co., Rochester, N.Y., and squalene from Chemical Samples, Columbus, Ohio, and was passed through alumina and found to have only very slight impurity by gas chromatography. Benzyl alcohol was purchased from Fisher Scientific Co. (Pittsburgh, Pa.).

"Solvent-free" Montal-Mueller planar films (9) (M-M type black films) were formed by spreading 10 μl of a 12.5-mg/ml solution of BPE in *n*-pentane over a 1.0-M KCl solution of 2 cm^2 surface area. The monolayers were then brought together to form a bilayer (9, 10). The membrane capacitance was obtained by measuring the current response to a voltage ramp. The area of the hole in the Teflon partitions was either 7.3×10^{-4} or 5.0×10^{-3} cm^2 and the area of the film was taken as equal to that of the hole. The capacitance changes we report were all from the same membrane and are accurate to $\pm 5\%$. Typical membrane capacitance for our M-M BPE bilayers is 0.63 $\mu f/cm^2$. For these experiments, various aliquots of benzyl alcohol were added to the aqueous phases, a not entirely satisfactory procedure because of the long time required for equilibration, but sufficient for qualitative purposes.

Black films of the Mueller-Rudin (8) type were formed by spreading a solution of BPE in decane (12.5 mg/ml) on a 1.7-mm hole in a Teflon partition separating two compartments filled with 1 M KCl and benzyl alcohol. The capacitance of such membranes was typically 0.39 $\mu f/cm^2$. Because we found that benzyl alcohol required a long time to equilibrate with the aqueous phases, solutions preequilibrated for several days at various concentrations of benzyl alcohol were used. Planar bilayers using BPE dissolved in squalene (6.0 mg/ml) were prepared in the same manner and equilibrated with different aliquots of the same solutions of 1 M KCl used for BPE-decane experiments.

Capacitance was measured by applying a triangle wave of amplitude 10 mV and frequency of ~1 kHz to the membrane. The resulting current was essentially a square wave whose rms amplitude was taken to be proportional to the capacitance. Calibration was done by substituting a known capacitance for the membrane and adjusting the frequency of the triangle wave so that the rms voltage was equal in magnitude to the known capacitance.

Membrane diameter was measured using a calibrated reticle and a dissecting microscope at $\times 100$ or $\times 200$. The area was calculated by using two diameters at right angles to each other and applying the formula for the area of an ellipse.

Liposomes used in turbidity measurements were formed by the method outlined by Hill (13). The lipid concentration of the dispersion was 0.5 mg/ml. Samples were cycled through the transition temperature until consecutive runs gave identical curves.

Optical density of vesicle suspensions vs. temperature was measured using a Beckman-spectrophotometer at a wavelength of 450 nm (Beckman Instruments, Inc., Fullerton, Calif.). The temperature was regulated by flowing water through a jacketed curvette and measured using a calibrated thermister. The dispersion was stirred by a small magnet inside the curvette, which was sealed to prevent loss of material through evaporation.

For x-ray diffraction experiments, lipids were evaporated from a chloroform solution, hydrated in excess (>>50%) water or 75 mM benzyl alcohol, concentrated by brief centrifugation with a bench centrifuge, and sealed in thin-walled x-ray capillary tubes. Diffraction patterns were recorded using a pin-hole collimator with nickel filter and a flat plate film cassette loaded with three or more sheets of Ilford Industrial (Ilford, England) G x-ray film. A Jarrell-Ash stationary anode generator was the source for copper Kα x-radiation. Exposure times were between 2 and 5 h. The diffraction data were processed by standard methods. Densitometer traces were recorded on a Joyce-Loebl microdensitometer model MK IIIC, the background curve was subtracted, and integrated intensities $I(h)$, where h is the order number, were measured. The standard Lorentz-polarization factor for unoriented specimens was applied, and structure amplitudes $|T(h)| = [h^2 I(h)]^{1/2}$ were obtained. Electron density profiles, $\rho(x)$, were calculated by use of the formula $\rho(x) = \Sigma_h |T(h)| \phi(h) \cos/(2\pi x h)/d$ where $\phi(h)$ is the phase for each lamellar order h, and must be either + or − for these centrosymmetric bilayers. The correct phase combination for pure DPL bilayers in water has previously been obtained (14, 15). The phase choice for DPL in 75 mM benzyl alcohol was made by selecting the one phase combination which gave a profile consistent with a bilayer structure.

RESULTS

Optical Density vs. Temperature

The transition temperature of lipid dispersions was determined from the displacement in absorbance (at 450 nm) vs. temperature plots (13). Fig. 1 shows such a curve (upper scan) for

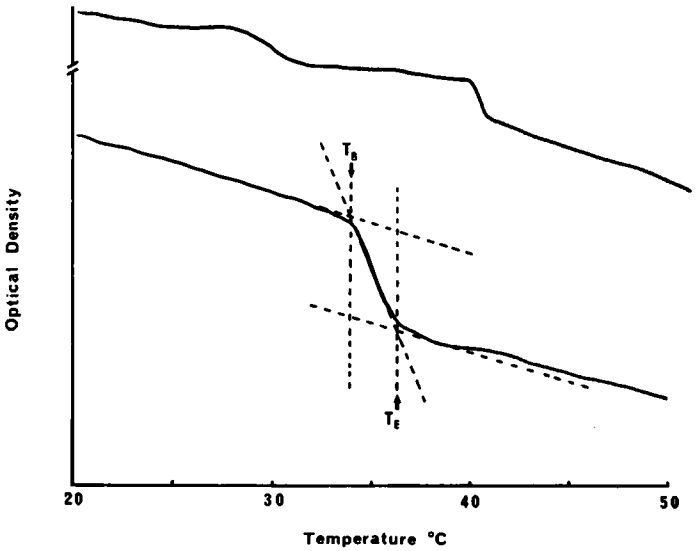

FIGURE 1 The effect of increasing the temperature at a rate of 2°C/min on the optical density of dispersions of DPL vesicles in 0.1 M NaCl buffered with 10^{-3} M sodium phosphate to pH 7.0 (upper curve). The lower curve is similar to the upper except that 42×10^{-3} M benzyl alcohol was equilibrated with the dispersion before heating. The symbols T_B and T_E indicate the beginning and end of melting, respectively.

DPL dispersed in 0.1 M NaCl, 10^{-3}M phosphate buffer (pH 7.0). There are two distinct breaks in the curve. The first, at 31°C, reflects the pretransition, whereas the second, at 41°C, is the main endothermic transition. The lower scan in Fig. 1 shows that the addition of 42 mM benzyl alcohol to DPL abolishes the pretransition break and lowers and broadens the main transition. The onset and end of the transition temperatures are taken as the intersections of the extrapolated portions of the appropriate straight line segments as shown on the figure.

Fig. 2 shows the combined results of the data from experiments at a variety of benzyl alcohol concentrations up to 60 mM in the aqueous phase. Fig. 2 A shows the dependence of the beginning of the transition (T_B) and of the end of the transition (T_E) as a function of the aqueous phase concentration of benzyl alcohol. The curves in Figs. 2 A and B consist of two straight lines with a break to a smaller slope at 20 mM benzyl alcohol. In Fig. 2 B, the temperature at the end of the transition, T_E, is plotted vs. percent saturation of benzyl alcohol in the aqueous phase; the slope in the low concentration region (0–20 mM) is -1°C/5.3 mM and that in the high concentration region (20–60 mM) is -1°C/16 mM. A break at 20 mM benzyl alcohol was also reported by Metcalfe et al. (16) and by Colley and Metcalfe (17) for the partition coefficient of benzyl alcohol into erythrocyte membranes and lecithin bilayers, respectively.

We also investigated the effects on the DPL phase transition of tetradecane, a molecule differing greatly from benzyl alcohol in both size and symmetry, and one commonly used as a solvent for planar bilayers (1) (Fig. 3, middle trace). Tetradecane in DPL at molar ratio of 2:1 abolishes the pretransition, diminishes the magnitude, and slightly raises the temperature of the main transition, and causes the appearance of a new event at 48°C. Similar results have also been determined by differential scanning calorimetry.[1]

The addition of benzyl alcohol to DPL-tetradecane dispersions eliminates the high temperature phase and lowers the main transition temperature to 36°C, a temperature only slightly higher than that of DPL treated with benzyl alcohol alone (Fig. 3).

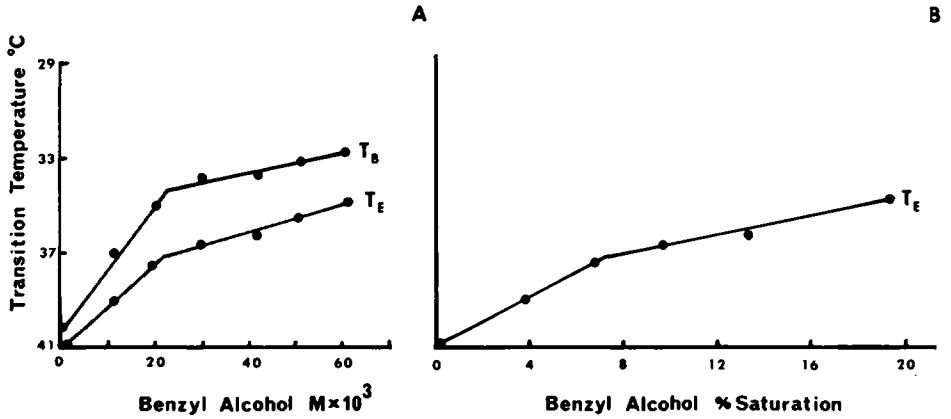

FIGURE 2 Composite results of data obtained from optical density vs. temperature heating scans of DPL vesicles in 0.1 M NaCl buffered with 10^{-3} M sodium phosphate to pH 7.0 with various benzyl alcohol concentrations. (A) The lines labeled T_B and T_E represent the beginning and end of the main endothermic transition obtained as shown in Fig. 1; (B) Graph of T_E vs. percent saturation of benzyl alcohol in the aqueous phase. The slope of the line at lower benzyl alcohol concentrations was used to calculate the DPL-water partition coefficient.

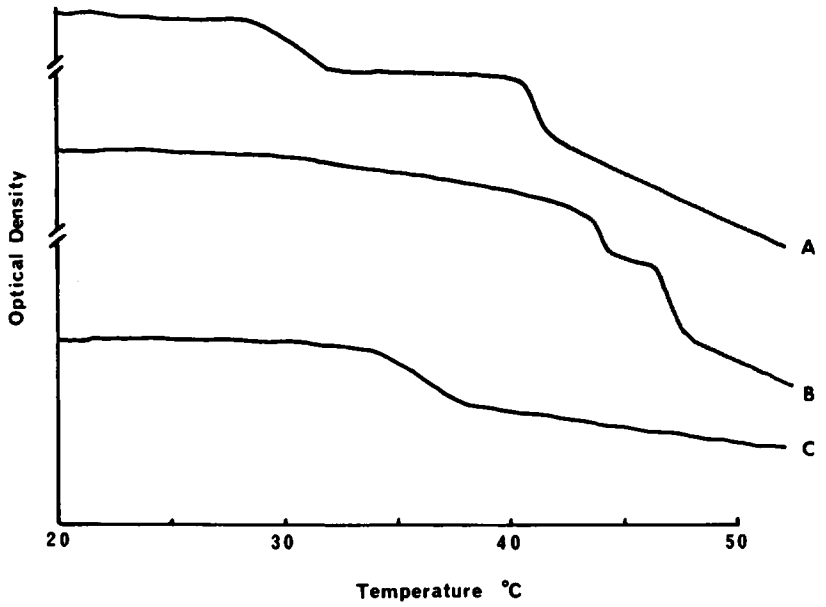

FIGURE 3 The effect of increasing the temperature at a rate of 2°C/min on the optical density of dispersions of DPL in 0.1 M NaCl buffered to pH 7.0 with 10^{-3} M sodium phosphate. Curve (A) is the control as described in Fig. 1. Curve (B) is the heating scan of dispersions of DPL: *n*-tetradecane at a mole ratio of 1:5.1. This high mole ratio of alkane to lipid was used to try and simulate the large excess solvent found in Mueller-Rudin planar bilayers. Curve (C) is the same dispersion as (B) equilibrated with 75 mM benzyl alcohol. This was accomplished by heating dispersion (B) for 1 hr at 60°C in nitrogen atmosphere. All curves were cycled at least twice. Upon completion of the main endothermic transition, and before cooling, the dispersion was kept above its transition temperature for ~10 min to minimize kinetic effects. With this procedure, the shapes of the heating and cooling curves were similar.

X-Ray Diffraction

The parameters obtained from x-ray diffraction experiments on multilamellar vesicular bilayers are given in Table I. Benzyl alcohol had no detectable effect on bilayers when added above the phase transition temperature. However, below the phase transition temperature it produced a marked effect on hydrocarbon chain packing, as indicated both by electron density profiles (Fig. 4) and wide-angle diffraction from the hydrocarbon chains (see below).

The repeat period for gel-state dipalmitoyllecithin at $T=20$°C is 14 Å larger in water than in 75 mM benzyl alcohol. Electron density profiles (Fig. 4) clearly show that this difference in repeat period is the result of a difference in bilayer thickness and not to a change in fluid layer thickness. The lowest density region of these profiles is located at the geometric center of the bilayer (at 0 Å) and corresponds to the low density terminal methyl groups of the hydrocarbon chain. The medium density plateau on either side of this central trough correspond to the rest of the hydrocarbon chain region of the bilayer. The width of the terminal methyl trough is resolution-limited and becomes sharper in higher resolution profiles (14, 15). The high density peaks, centered at ~ ± 21 Å for the DPL in water profile and at ±15 Å for the DPL in 75 mM benzyl alcohol, correspond to the DPL head groups. The low density regions on the outside of both bilayers correspond to the aqueous layers between the multilayers. Note that the width of the aqueous layers is about the same in both profiles in Fig. 4. However, the separation

TABLE I
X-RAY DIFFRACTION DATA

Fully hydrated lipid	Additions	Long spacing d_l	Short spacing d_s	Angle of acyl chains with respect to plane	T
		Å	Å		°C
DPL	—	64 ± 2	4.2 ± 0.05	30	20
	75 mM benzyl alcohol	50 ± 2	4.2 ± 0.05	0	20
	—	67 ± 2	4.5 ± 0.1	—	45
	75 mM benzyl alcohol	67 ± 2	4.5 ± 0.1	—	45
	1:2 *n*-tetradecane	72 ± 2	4.2 ± 0.05	0	20
	1:2 *n*-tetradecane + 75×10^{-3} M benzyl alcohol	72 ± 2	4.2 ± 0.05	0	20
Egg lecithin	—	64 ± 2	4.5 ± 0.1	—	20
	75 mM benzyl alcohol	64 ± 2	4.5 ± 0.1	—	20
	1:2 *n*-tetradecane	64 ± 2	4.6 ± 0.1	—	20
	75 mM benzyl alcohol 1:2 *n*-tetradecane	64 ± 2	4.6 ± 0.1	—	20

between lipid head groups is 10–14 Å greater for the DPL bilayer in water than in benzyl alcohol solution.

Wide-angle diffraction indicates that benzyl alcohol changes the hydrocarbon chain organization of the lecithin molecules below the phase transition. For DPL in water, the wide-angle pattern contains a sharp reflection at $(4.2\ \text{Å})^{-1}$ with a more diffuse reflection centered at $\sim(4.0\ \text{Å})^{-1}$. Tardieu et al. (14) showed that this type of pattern is caused by the lipid hydrocarbon chain having a tilt of ~30° with respect to the normal to the plane of the bilayer. However, the wide-angle pattern for DPL in 75 mM benzyl alcohol consists of a single sharp reflection at $(4.2\ \text{Å})^{-1}$, indicating that in this case the hydrocarbon chains are oriented approximately perpendicular to the plane of the bilayer (14).

In contrast to the decrease of the long spacing in DPL bilayers produced by benzyl alcohol, *n*-tetradecane increases the repeat period from 64 to 72 Å. The wide-angle patterns indicate that the hydrocarbon chain tilt is eliminated by the addition of tetradecane. The addition of benzyl alcohol to the DPL *n*-tetradecane mixture at 20°C has no detectable effect on the x-ray diffraction patterns. Under these conditions, benzyl alcohol still apparently enters the membrane since it reduces the transition temperature in the presence of *n*-tetradecane (Fig. 3).

At a temperature of 45°C, which is above the phase transition temperature of DPL, the wide-angle pattern becomes a broad reflection at 4.5 Å and the long spacing increases to 67 Å.

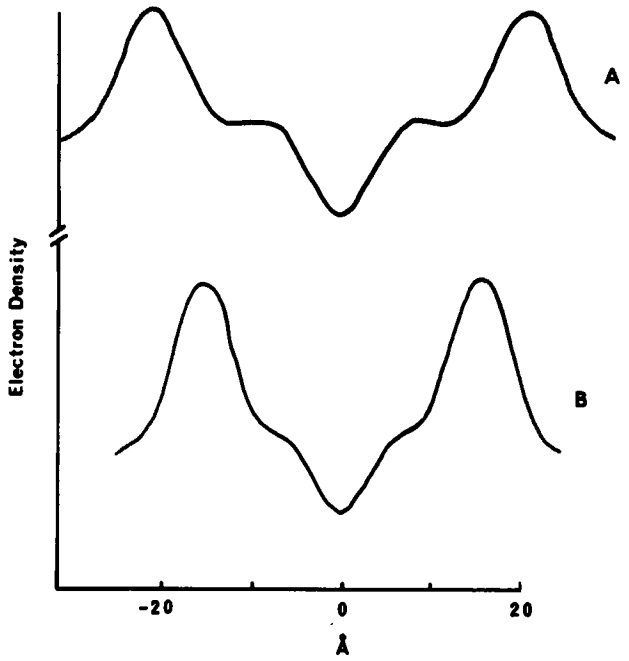

FIGURE 4 Electron density profiles at ~13 Å resolution of DPL in water (A) and in 75 mM benzyl alcohol (B). In both cases, data was collected at 20°C, below the lipid phase transition.

The presence of benzyl alcohol has no effect on these parameters. Similarly, for egg lecithin in the liquid crystalline state at T = 20°C there was no difference in the x-ray diffraction parameters in the presence and absence of benzyl alcohol or *n*-tetradecane (Table I).

Capacitance Measurements

Fig. 5 shows that benzyl alcohol increases the capacitance of planar BPE bilayers which do not contain solvent, whether they are formed using squalene, a solvent shown not to be present in the bilayer by several methods (11, 18), or by joining two monolayers to form a bilayer. For BPE bilayers formed from monolayers, the capacitance of the bilayer in the absence of benzyl alcohol was 0.63 ± 0.03 $\mu f/cm^2$, which corresponds to a membrane thickness of 29.5 Å. The capacitance increased linearly, with 7% increase at 75 mM benzyl alcohol. These results are in essential agreement with those obtained by Reyes and Latorre (19), which are shown for comparison. For squalene-BPE bilayers, the original bilayer capacitance was 0.69 ± 0.03 $\mu f/cm^2$ corresponding to a thickness of 26.9 Å. In this case, the capacitance again increased linearly with concentration so that at 5 mM benzyl alcohol, the capacitance increased 16.5%. These data fit a straight line with a regression coefficient of 0.99. In contrast to these results with solvent-free bilayers, Ashcroft et al. (6) found that the addition of benzyl alcohol to egg lecithin bilayers containing *n*-tetradecane decreases the capacitance. To demonstrate that when solvent is present this decrease is not unique to a particular lipid-solvent combination, we repeated these experiments using BPE-decane bilayers. Quantitatively similar results were obtained at comparable concentrations (Fig. 5).

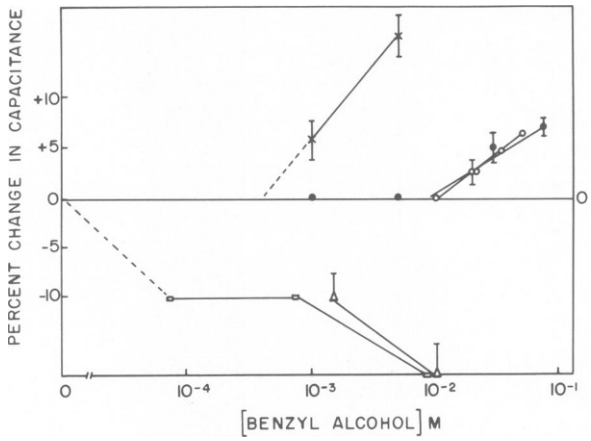

FIGURE 5 The percent changes in capacitance of BPE bilayers made by: raising two BPE monolayers [(●) this work, (o) reference 19], BPE-squalene (X), egg phosphatidylcholine-tetradecane-(□) (reference 6) and BPE-decane (△) as a function of the aqueous benzyl alcohol concentration. The base-line values of the capacitance are given in the text. Experiments were performed at room temperature 20 ± 2°C.

DISCUSSION

In this paper we have shown several differences between various bilayer systems. It is clear that not all bilayer systems are equivalent in the manner in which they respond to even a relatively simple molecule like benzyl alcohol. We found the effects of benzyl alcohol differ dramatically in the planar bilayers containing solvent, planar bilayers containing little or no solvent, and vesicular bilayers. The interaction of benzyl alcohol with different types of model systems provides a useful example of how apparently trivial alterations in a bilayer system can have great consequences.

From the "freezing point depression" produced by benzyl alcohol in DPL, one may calculate the bilayer-buffer partition coefficient. Following the analysis of Hill (13), we write

$$\frac{\delta \Delta T}{\delta r} = \frac{RT^2}{\Delta Hm} KS, \tag{1}$$

where T is the temperature, ΔT is the freezing point depression, S is the concentration of benzyl alcohol at saturation, r is the fraction of saturation, ΔHm is the latent heat of the transition, and K is the partition coefficient in mole fraction units. Using the low concentration range data of Fig. 2, along with thermodynamic values for the transition temperature and transition enthalpy of DPL (20), we obtain a value for the membrane-water partition coefficient of 440 in mole fraction units. Assuming a volume of 550 cm^3/mol for the hydrocarbon region of DPL (see below), this is equivalent to 14.4 in molar units. The partition coefficient obtained by this method compares favorably with that directly measured for the dimyristoyllecithin-water system at 40°C, which is 13.9 in molar units (21).

From the partition coefficient and the aqueous concentration, we can calculate the volume fraction of benzyl alcohol in vesicle bilayers. First, we calculate the volume of the hydrocarbon region of the phospholipid from the empirical formula (22),

$$V = [27.4 + 26.9\, N_c]\text{Å}^3, \tag{2}$$

where N_c is the number of carbon atoms in the acyl chain. For DPL, the calculation yields a hydrocarbon volume of 550 cm^3/mol. If we now assume the partial molar volume of benzyl alcohol to be identical to its molar volume (104 cm^3/mol), then at an aqueous phase concentration of 20 mM, the alcohol occupies 3% of the hydrocarbon volume of the DPL bilayer.

The fact that the slopes of the graphs in Fig. 2 decrease for aqueous benzyl alcohol concentrations (greater than) $\sim 20 \times 10^{-3}$ M is likely to be an "activity effect" of the alcohol in the membrane (23). It is well known that alcohol may associate in nonpolar liquids at these concentrations. It is unlikely that there is a significant structural reorganization of the bilayer because 75 mM benzyl alcohol does not significantly alter the DPL properties measured by x-ray diffraction above the transition temperature (Table I).

Table I shows the results of the x-ray data for DPL with benzyl alcohol below the lipid's transition temperature. At 75 mM benzyl alcohol, the long spacing (which is directly proportional to the membrane thickness) decreases by 14 Å. This reduction occurred despite a reduction in chain tilt which would have tended to increase the thickness of the bilayer. The reduction in thickness can be explained by considering that benzyl alcohol is anchored to the interface by its OH group (17) and extends to a depth of only three to four methylene groups into the acyl region of the bilayer. Consequently the chains must bend around the benzyl alcohol because creation of large holes in the acyl region is energetically very unfavorable. The perturbation of the chains induces the formation of additional rotational isomers or "kinks" which reduce the thickness of the bilayer by ~ 1.3 Å per kink (24). Because the interaction between chains is highly cooperative, this effect is expected to be maximal for bilayers below their transition. For vesicular bilayers above their phase transition temperature, the effects of benzyl alcohol on bilayer thickness are below experimental error (± 2 Å long spacing). We note that Turner and Oldfield (25), using high-field deuterium nuclear magnetic resonance spectroscopy, calculate a thickness reduction of <2 Å for liquid-crystalline state bilayers of dimyristoyllecithin in the presence of large amounts of benzyl alcohol (3:1 benzyl alcohol: lipid mole ratio).

In contrast to the decrease in the thickness produced by benzyl alcohol in DPL, *n*-tetradecane increases the thickness of the bilayer below the transition temperature. It does this primarily by removing the chain tilt as shown in Table I. A similar result has been noted by Tardieu et al. (14) for decane in DPL bilayers. We have shown from electron density profiles (26), that *n*-tetradecane is located in each monolayer of the bilayer and not between the monolayers. Consequently, *n*-tetradecane must be located parallel to the acyl chains with one of its terminal methyl groups near the interface.[1] For bilayers above their transition temperature, such as egg lecithin at 20°C and DPL at 45°C, the incorporation of *n*-tetradecane does not change either the long or short spacing (Table I). This is because the *n*-tetradecane tends to align parallel to the lipid acyl chains (26). Thus, both above and below the lipid phase transition, *n*-tetradecane is located parallel to the lipid acyl chains. Only below the transition is there a significant change in repeat period, because of removal of hydrocarbon chain tilt.

For bilayers containing *n*-tetradecane either above or below the phase transition temperature, the addition of benzyl alcohol does not change the x-ray diffraction parameters.

[1]McIntosh, T. J., S. A. Simon, and R. C. MacDonald. Submitted for publication.

However, for DPL bilayers below the transition, we know that benzyl alcohol is present, as it reduces the effects on the transition produced by *n*-tetradecane (Fig. 3).

It is of particular interest that benzyl alcohol does not reduce the width of gel-state DPL when tetradecane is present. A possible explanation for this is that tetradecane could fill the potential void in the center of the bilayer created by the benzyl alcohol molecule anchored at the interface. Thus, the DPL chains do not have to bend to fill this void, and the bilayer does not get thinner. Regardless of the possible molecular interpretations, the optical densities (Fig. 3) and x-ray diffraction results show that the presence of solvent can affect the properties of vesicular bilayers.

The effects of benzyl alcohol on planar bilayers, as shown in Fig. 5, reveal that there are also significant differences between solvent-free and solvent-containing planar bilayers. Benzyl alcohol decreases the capacitance of planar bilayers containing decane or tetradecane, but increases the capacitance of solvent free bilayers. The specific capacitance, *Cg*, of a bilayer is given by

$$Cg = 8.85\frac{\epsilon}{\tau}, \tag{3}$$

where Cg = specific capacitance in $\mu f/cm^2$, ϵ = dielectric constant; and τ = thickness in angstroms.

Because the dielectric constant of benzyl alcohol (13.1 at 20°C) is much larger than the dielectric constant of hydrocarbon (2.1), one might expect that the change in dielectric constant would greatly effect *Cg*. However, this dielectric constant effect appears to be relatively small. We note that if the specific capacitance changes were primarily due to increases in ϵ then *Cg* should increase for both solvent-containing and solvent-free bilayers. Clearly this is not the case (Fig. 5). There are two possible reasons why the benzyl alcohol-induced change in dielectric constant is not large. First, the effective dielectric constant of benzyl alcohol in the hydrocarbon region of a bilayer is probably much lower than 13.1. We note that the dielectric constant of toluene is 2.38 (at 21°C) and that it is the presence of the OH group that raises the bulk dielectric constant of benzyl alcohol relative to toluene. However, since the OH group is confined to the interface, it is not likely to contribute appreciably to the membrane's dielectric constant. Secondly, under these experimental conditions relatively small amounts of benzyl alcohol partition into the bilayer hydrocarbon region. For example, at a benzyl alcohol concentration of 7.5×10^{-4} M, benzyl alcohol would occupy only 0.1% of the volume of the bilayer. Thus, since the observed changes in *Cg* (Fig. 5) can not be due primarily to changes in dielectric constant, they must be due, in large part, to changes in bilayer thickness.

Benzyl alcohol could increase the thickness of solvent containing bilayers by decreasing the chemical potential of the solvent (and lipid). The solvent would then increase its solubility in the film by partitioning in the region behind the benzene ring of benzyl alcohol along the acyl chains of the phospholipid molecule. This should have the effect of straightening out the chains of the lipid molecule, thus increasing its thickness. It is interesting to note that the large changes that are observed in thickness for planar bilayers with solvents are not observed in multilamellar dispersions by x-ray diffraction techniques. Again, there are differences between vesicular bilayers (made even with excess tetradecane) and planar bilayers in equilibrium with a torus in the manner in which they respond to benzyl alcohol.

For bilayers made from monolayers, our results essentially agree with those obtained by Reyes and Latorre (19) (Fig. 5). From Eq. 3, assuming no change in ϵ, we calculate that the membrane thickness decreased from 29.5 in water to 27.6 Å in 75 mM benzyl alcohol. The benzyl alcohol-free bilayer thickness agrees well with the hydrocarbon thickness obtained from x-ray diffraction of a 50-wt percent dispersion of BPE in 0.1 M NaCl which is between 28 and 30 Å.[2] This change of thickness of 2 Å could not be detected from low resolution x-ray patterns from fully hydrated multilayered vesicles.

While the data of Fig. 5 indicate that BPE-squalene membranes are more sensitive to benzyl alcohol than membranes made from monolayers, this is probably a consequence of the way in which the experiments were carried out. The monolayer-formed film experiments were done by adding small aliquots of benzyl alcohol, whereas the BPE-squalene experiments were done using solutions pre-equilibrated with benzyl alcohol. Thus, the monolayer-formed bilayer experiments are probably only semiquantitative. They nevertheless show clearly that benzyl alcohol increases rather than decreases the capacitance.

In conclusion, we have shown that there are quantitative differences between various bilayer systems in the manner in which they respond to the small molecule benzyl alcohol. This is especially evident for planar bilayers made by the technique of Mueller et al. (8) using small nonpolar molecules in the membrane forming solution. The incorporation of a third component in the system acts to change the chemical potential of the alkane (also lipid) in a manner than increases the alkane solubility in the bilayer. The resulting increase in membrane thickness, which does not occur in "solventless" planar bilayers and multilamellar vesicles (or probably in biological membranes), suggests the use of caution in developing theories of anesthesia using these systems.

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