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THE MOLECULAR ORGANISATION OF BIMOLECULAR LIPID MEMBRANES

THE EFFECT OF BENZYL ALCOHOL ON THE STRUCTURE

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Summary

The separate effects of benzyl alcohol on the hydrocarbon and polar-head region capacitances and conductances of phosphatidylcholine bimolecular lipid membranes were obtained from measurements of the very low frequency impedance dispersion. It was found that the conductance of the hydrocarbon region (and, to a lesser extent, the polar-head region) increased progressively with increasing concentrations of benzyl alcohol in the external solution. The polar-head capacitance did not show a systematic dependence on the concentration of benzyl alcohol.

At low concentrations of benzyl alcohol (7.5 μM) the capacitance of the hydrocarbon region was not significantly affected by the alcohol. At high concentrations ($\geq$7.5 mM) of benzyl alcohol, however, the capacitance of this region was reduced by $\approx$25%. This is interpreted in terms of an increase in the thickness of this region caused by a straightening of the otherwise kinked, folded (across neighbouring molecules) and perhaps even partially interdigitated hydrocarbon tails of the phosphatidylcholine molecules. This effect of benzyl alcohol is probably closely related also to the apparent increase in the fluidity of the membrane. The effect of benzyl alcohol on the thickness of the hydrocarbon region of the membrane provides a ready insight into its mode of action as a local anaesthetic.

Introduction

Benzyl alcohol, a local anaesthetic, is known from NMR and ESR studies to produce a fluidization of both cell and bimolecular lipid membranes [1,2]. At concentrations up to $\approx$130 mM, benzyl alcohol has a stabilising effect against osmotic haemolysis of human erythrocytes [1,2], and also reduces the critical

potential difference required for electrical breakdown [3]. To extend the utility of probe molecules such as benzyl alcohol in the study of membrane organization and to elucidate its action as a local anaesthetic, it would be desirable to determine the separate effects of benzyl alcohol on the polar-head and hydrocarbon regions of the bimolecular lipid membrane.

Such information can be obtained by measuring the small (2—3%) dispersion in membrane capacitance, C, with frequency in the range 0.1—100 Hz [4]. This, Maxwell-Wagner, dispersion in C and the concomitant dispersion in the conductance, G, arise from interfacial polarisation due to the presence within the membrane of layers having different conduction and dielectric properties. Such substructural layers can therefore be detected and characterised by measurement of the overall C and G of the membrane as a function of frequency. A technique to measure this small dispersion in C and G, in phosphatidylcholine bimolcular lipid membranes at these low frequencies, was recently described [4,5]. In the present communication we present results of experiments in which we have used this technique to determine the separate effects of benzyl alcohol molecules on the electrical parameters of the polar-head and hydrocarbon regions of the bimolecular lipid membrane.

Materials and Methods

(a) Generation of the membranes

The bimolecular lipid membranes formed from a film of egg-phosphatidylcholine in *n*-tetradecane which was painted over a hole (1.2 mm in diameter) in a polycarbonate septum dividing a "Perspex" cell into two compartments. All the results reported here were obtained with membranes which were entirely "black" except for a small region (<1%) occupied by the torus. The cell was filled with a 1 mM KCl solution containing benzyl alcohol in concentrations of 0, 7.5 μM, 7.5 mM or 75 mM. All experiments were performed at 18 ± 2°C.

(b) Measurements of membrane capacitance and conductance

The apparatus which allows us to measure the capacitance and conductance of the bimolecular lipid membrane in the frequency range 0.1—100 Hz with sufficient accuracy to detect the Maxwell-Wagner dispersion has been described in detail previously [4]. It uses a four-terminal measurement to avoid the large errors (relative to the magnitude of the expected total dispersion) which are otherwise introduced by the necessary vectorial subtraction of the complex, frequency-dependent impedance of the solution-electrode system.

(c) Determination of the structural parameters

In the bimolecular lipid membrane each of the two polar-head regions can be represented by a capacitance, C_P, in parallel with a conductance, G_P. These regions sandwich the hydrocrabon region which can also be represented by a capacitance, C_H, and a conductance, G_H.

The overall capacitance and conductance of the bimolecular lipid membrane

is then frequency-dependent and is given by *

$$C_m = \frac{\omega^2 C_H C_P/2(C_P/2 + C_H) + (G_P/2)^2 C_H + G_H^2 C_P/2}{(G_P/2 + G_H)^2 + \omega^2(C_P/2 + C_H)^2}$$

$$G_m = \frac{G_H G_P/2(G_P/2 + G_H) + \omega^2[C_H^2 G_P/2 + (C_P/2)^2 G_H]}{(G_P/2 + G_H)^2 + \omega^2(C_P/2 + C_H)^2} \quad (1)$$

The measured impedance obtained with the four-terminal technique yielded the capacitance and conductance between the equipotential surfaces at which the tips of the potential electrodes were located. These capacitances and conductances refer therefore to those of the bimolecular lipid membrane in series with the two thin slices of solution between the membrane and the potential measuring electrodes. The separate impedance of these slices of solution, although relatively small, was determined from measurements in the absence of the bimolecular lipid membrane and was (vectorially) allowed for in the results presented.

In the unstirred solution layer the presence of the membrane may create regions in which the conductivity is different from that in the bulk solution phase. Corrections made for the electrolyte impedance between the potential electrodes (determined in the absence of a membrane) will not eliminate these additional elements. Such conductance elements would need to be specifically incorporated into the expressions for the total C and G of the membrane system. The electrical equivalent circuit for the complete system is shown in Fig. 1. Assuming that the impedances of the "bulk" electrolyte slices between the bimolecular lipid membrane and the tips of the potential electrodes have already been allowed for, the total capacitance C and conductance G of the membrane system when a surface layer conductance element **, G_ϱ, is present is then given by

$$C = \frac{C_m(G_\varrho/2)^2}{(G_m + G_\varrho/2)^2 + \omega^2 C_m^2}$$

$$G = \frac{G_m G_\varrho/2(G_m + G_\varrho/2) + \omega^2 C_m^2 G_\varrho/2}{(G_m + G_\varrho/2)^2 + \omega^2 C_m^2} \quad (2)$$

From the variation of the total C and G with frequency it is possible to deduce the individual values of C_H, G_H, C_P, G_P and G_ϱ, since each of these parameters affects a different feature of the dispersion curves. This is illustrated in Fig. 2 which also illustrates the sensitivity of the determination of each of the polar-head and hydrocarbon region parameters.

Below 1 Hz, in unmodified phosphatidylcholine bimolecular lipid membranes in 1 mM KCl, the hydrocarbon region conductance is so low that the C and G

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* The polar-head regions are taken to be identical and for calculation purposes they may be lumped together. In a previous publication [4], Eqn. 1 were incorrectly transcribed. However, the numerical results quoted there were correct.

** Such layers adjacent to the membrane will also have an equivalent capacitance (shown dotted in Fig. 1). However, estimates of possible values of this capacitance (e.g. that due to the diffuse ionic double layer [6]) show that the reactive admittances of such elements, certainly at frequencies $\leq$100 Hz, are small compared with the values of G_ϱ estimated from our present results.

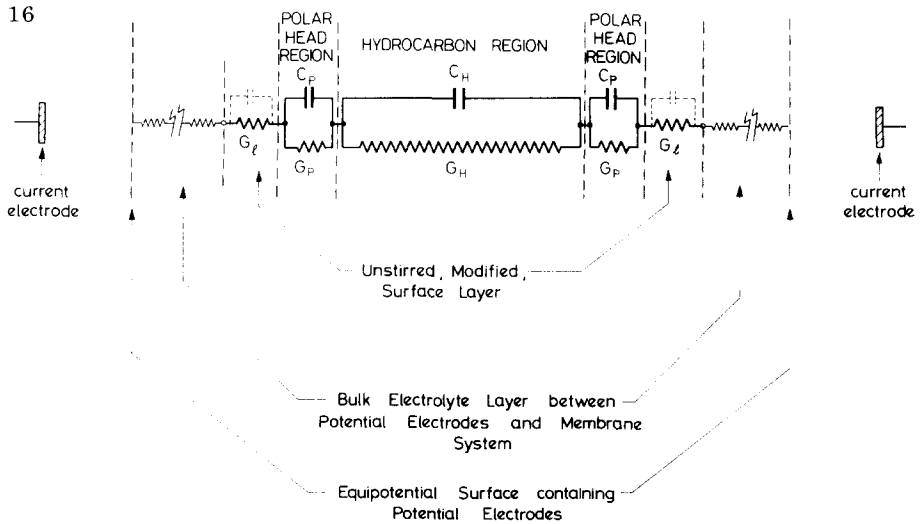

Fig. 1. The equivalent circuit for the bimolecular lipid membrane. Each substructural layer is represented by a parallel combination of an equivalent capacitance and conductance (hydrocarbon, subscript H; polar head, subscript P; modified surface layer, subscript ℓ).

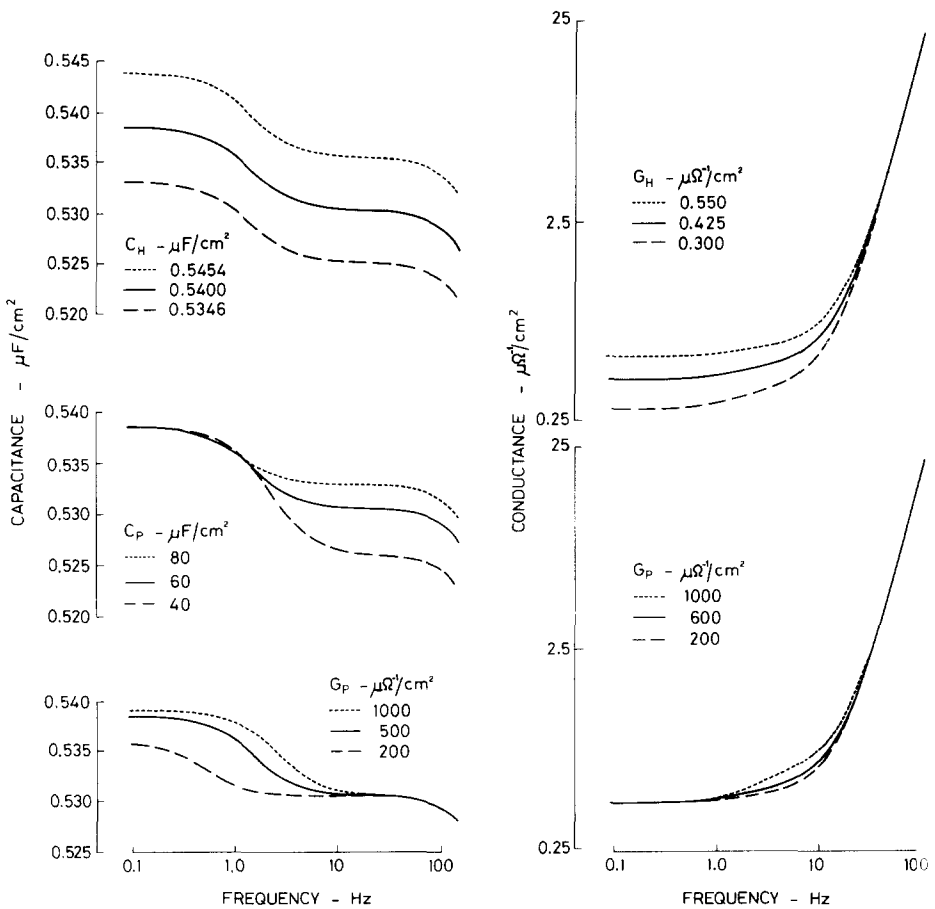

Fig. 2. The effect of varying the parameters C_H, G_H, C_P and G_P on the dispersion characteristics predicted by equations (1) and (2) for the three layer model of the bimoleculat lipid membrane and surface element G_ℓ. The full line in each case is a plot of the equations using a common set of values of the parameters; these are indicated in the legend of the appropriate diagram. In all cases, G_ℓ was 10 000 $\mu\Omega^{-1}/cm^2$. The effect of varying G_ℓ is shown separately in Fig. 3. It is clear that each of the parameters considered affects very different features of the capacitance and conductance dispersion characteristics. By fitting Eqns. 1 and 2 to the experimental data it was possible, for a given membrane, to determine each parameter as follows: G_H and C_H with ±1%, C_P and G_P each within ±10% and G_ℓ within a factor of 2.

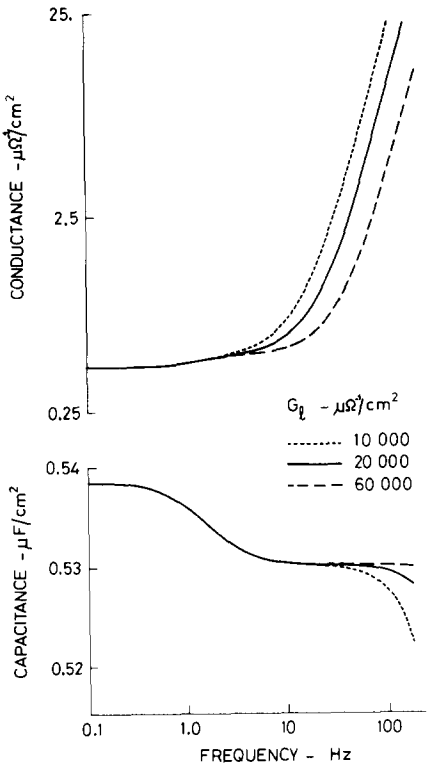

Fig. 3. The effect of the value of the conductance, G_ℓ, of the surface layer in the unstirred solution region on the theoretical dispersion curves. The values of the parameters C_H, G_H, C_P and G_P are the same as for the full line plots shown in Fig. 2. The value of G_ℓ affects the dispersion characteristic in C only at high frequencies.

of the bimolecular lipid membrane are effectively equal to C_H and G_H. The correlation between the experimental data and that predicted by equations (1) and (2) is most sensitive to the values introduced for C_H and G_H; thus a 0.1% change in C_H or a 1% change in G_H produces a decrease of 0.1 in the correlation coefficient * of the corresponding C or G curves (which are typically 0.95 for C and 0.85 for G).

The value of C_P determines the magnitude of the first dispersion in bimolecular lipid membrane capacitance in the frequency range 1—10 Hz. C_P and G_P both affect the frequency range over which this dispersion occurs (see Fig. 8 in ref. 4 and also Fig. 2 of the present results). It was found that for a particular membrane a 10% change in the optimum values for C_P and G_P resulted in a decrease of 25% in the correlation between the experimental data and the theoretical curves.

G_ℓ affects only the high frequency region (≈100 Hz) of the dispersion characteristics. This is illustrated in Fig. 3. When G_ℓ has a value of 10 000 $\mu\Omega^{-1}$ /

* The correlation coefficient used here is given by (see ref. 7). $r = (1 - \Sigma S_{th}^2/S_{ex}^2)^{1/2}$, where S_{th} is the difference between the theoretical and mean experimental values at a given frequency and S_{ex} is the standard deviation of the experimental values at that frequency.

cm^2 (≈ order of magnitude found for bimolecular lipid membranes in 1 mM KCl) it significantly influences the dispersion in C at frequencies ⩾70 Hz. The value of G_Q also has a marked effect on the frequency range where the dispersion in total conductance occurs. When G_Q is greater than 60 000 $\mu\Omega^{-1}/cm^2$ the dispersion curve for the capacitance, up to 100 Hz, is unaffected by the presence of this element. The limited data at these frequencies did not allow us to determine G_Q to better than a factor of 2.

Results

When benzyl alcohol was present in the external solution the "thick" film, from which the bilayer membrane formed, appeared more fluid. This was evident, for example, from the absence of whorls of coloured fringes which are usually seen when benzyl alcohol is not present. With benzyl alcohol present

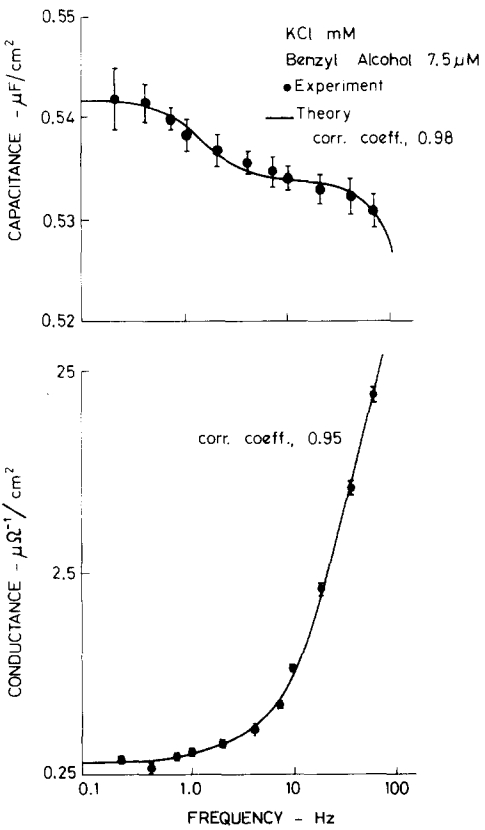

Fig. 4. The capacitance and conductance as a function of frequency of a phosphatidylcholine bimolecular lipid membrane made in 1 mM KCl containing 7.5 μM benzyl alcohol. The points represent the average at each frequency of 5 runs on this membrane. The vertical bars indicate the standard errors (these are very small in results for the conductance). The full lines are the theoretical dispersions predicted by Eqns. 1 and 2 with the following parameters. C_H = 0.543 $\mu F/cm^2$, G_H = 0.28 $\mu\Omega^{-1}/cm^2$, C_P = 60 $\mu F/cm^2$, G_P = 450 $\mu\Omega^{-1}/cm^2$, G_Q = 7000 $\mu\Omega^{-1}/cm^2$. The correlation coefficient between the data points and the theoretical curve was 0.98 for the capacitance and 0.95 for the conductance. The first dispersion in C, over the frequency range 1—10 Hz, is due to the presence of the polar-head region. The additional dispersion ⩾70 Hz is due to the presence of the surface conductance element G_Q.

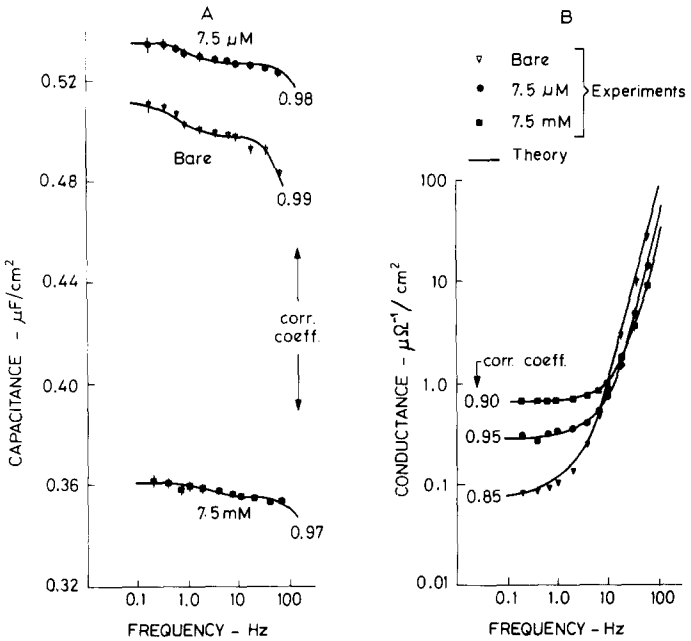

Fig. 5. The dispersion in C and G for bimolecular lipid membranes made in 1 mM KCl containing either 0, 7.5 μM or 7.5 mM benzyl alcohol. At each frequency, the points shown are the average values from 5 runs on the one membrane. The vertical bars indicate the standard errors. The full curves are plots of Eqns. 1 and 2 with the following parameters:

Benzyl alcohol	($\mu F/cm^2$)		($\mu\Omega^{-1}/cm^2$)		
	C_H	C_P	G_H	G_P	G_Q
0 (bare)	0.51	36	0.076	300	2800
7.5 μM	0.543	60	0.28	450	7000
7.5 mM	0.362	35	0.64	540	4000

The correlation coefficients between the data points and the theoretical curves are indicated on the diagram. At 7.5 μM benzyl alcohol had only a relatively small effect on the capacitance, which was within the variation from membrane to membrane for bare bilayers. At 7.5 mM concentration, however, the benzyl alcohol produced a large decrease in the measured capacitance (al all frequencies measured). This (≈25%) decrease in capacitance was due to the drop in C_H, the capacitance of the hydrocarbon region (cf. Fig. 2a). Increasing concentrations of benzyl alcohol progressively increased the low frequency conductance of the membrane; this reflects largely a progressive increase in G_H, the hydrocarbon region conductance (cf. Fig. 2).

the interference fringes were uniform and horizontal which indicated that the film was wedge-shaped and thinning evenly. The breakdown potentials of the bimolecular lipid membranes were invariably below 40 mV (in contrast with a typical value of ≈150 mV in the absence of benzyl alcohol.

Fig. 4 shows the variation with frequency of C and G for a bimolecular lipid membrane made in 1 mM KCl containing 7.5 μM benzyl alcohol.

Our present experimental results indicate the need to include the surface-layer conductance element. However, the measurements were not extended to sufficiently high frequencies, for technical reasons *, to allow us to determine G_Q very accurately.

* At high frequencies the limitations on the maximum sampling rate of the analogue-digital converters forced us to progressively reduce the number of samples of voltage and current taken in each cycle with a subsequent loss of accuracy in the determination of C and G. In a previous study [4], somewhat more severe equipment limitations prevented us from extracting the conductance element G_Q from the impedance of the slices of bulk electrolyte between the potential electrodes and the membrane.

TABLE I

EFFECT OF BENZYL ALCOHOL ON THE SUBSTRUCTURE PARAMETERS OF PHOSPHATIDYLCHOLINE BIMOLECULAR LIPID MEMBRANES

This data was obtained by fitting the experimental dependence of C and G on frequency to Eqns. 1 and 2. The values quoted are mean values obtained from several sets of measurements on different membranes (the number is indicated in brackets in the first column). The errors indicated represent the total scatter in the results.

Concn. of benzyl alcohol		$\mu F/cm^2$		$\mu\Omega^{-1}/cm^2$		
		C_H	C_P	G_H	G_P	G_ℓ
0	(6)	0.51 ± 0.05	36 ± 10	0.07 ± 0.05	450 ± 200	3000 ± 500
7.5 μM	(30)	0.52 ± 0.05	40 ± 15	0.31 ± 0.05	550 ± 200	6000 ± 3000
7.5 mM	(18)	0.39 ± 0.03	35 ± 10	0.59 ± 0.05	500 ± 200	7000 ± 2000
75 mM	(1)	0.25	15	1.05	600	4400

Fig. 5 shows the effect of varying the concentration of benzyl alcohol in the aqueous phase. (Note that the scale for capacitance is much compressed compared with that of Fig. 4). It is clear that at 7.5 mM concentration, benzyl alcohol produced a dramatic decrease in the capacitance of the bilayer at all frequencies <100 Hz. At the lower concentration (7.5 μM) benzyl alcohol did not affect the membrane capacitance in this manner.

Increasing concentrations of benzyl alcohol in the external solution had the effect of progressively increasing the membrane conductance, (See Fig. 5b.)

While the experimental dispersion in C and G of the bilayers could always be fitted to the Eqns. 1 and 2 (with correlation coefficients better than 0.95 for C and 0.85 for G), there were variations between membranes in the individual parameters required for such a fit. A summary of these parameters so determined for solutions containing 0, 7.5 μM, 7.5 mM and 75 mM benzyl alcohol is given in Table I. It is clear that benzyl alcohol at concentrations ⩾7.5 mM dramatically reduced the hydrocarbon region capacitance, C_H. The hydrocarbon region conductance G_H progressively increased with increasing benzyl alcohol concentration.

Similarly the conductance, G_P, of the polar-head region increased with increasing benzyl alcohol concentration, although by not nearly as much as G_H. No systematic dependence of the polar-head capacitance, C_P, or the surface layer conductance, G_ℓ, on benzyl alcohol concentration was apparent. The value of G_ℓ for an unmodified bimolecular lipid membrane in 1 mM KCl corresponds approximately to the conductance of a 100 μm thick slice of the electrolyte.

Discussion

The increase in the conductance of the bimolecular lipid membranes, particularly that of the hydrocarbon region, with increasing concentration of benzyl alcohol in the external solution is not surprising in view of its effect on the fluidity of the membranes.

The unexpected * feature of our present findings was the decrease in the value of the capacitance, C_H, of the hydrocarbon region when the external solution contained $\geqslant$7.5 mM benzyl alcohol. This (>25%) decrease in C_H could, in principle, arise from either: (a) A decrease in dielectric constant, ϵ_H, of the hydrocarbon region (by >25%) and/or (b) An increase in the thickness, δ_H of this region. The dielectric constant of the hydrocarbon region (usually $\approx$2) should not be decreased by the sorption of benzyl alcohol (dielectric constant 13.1 [8]). Considerations of ion partitioning, which would be reflected in the conductance, support this conclusion.

To explain the decrease in the capacitance, C_H, we would then need to postulate that the thickness, δ_H, of the hydrocarbon region increases. The increase in thickness required would depend on the dielectric constant of this region. The reported values of dielectric constants of alkanes (C_nH_{2n+2}) which are unbranched and long ($n > 12$) all exceed 2 [8,10,11]. This value for the ϵ_H of the unmodified bimolecular lipid membrane then gives the lower limit for the thickness, δ_H, of the hydrocarbon region as 3.45 nm. A more likely value of ϵ_H for the bilayer is 2.13 [4,10,11,12]. The corresponding thickness δ_H, is 3.7 nm for the unmodified bimolecular lipid membrane, which agrees with detailed theoretical predictions [9]. On this scheme we would need to postulate that in the unmodified bimolecular lipid membrane, the hydrocarbon chains are partially kinked (for example, double-gauche forms predominate) or the chains are folded across neighbouring phosphatidylcholine molecules, or some interdigitation and/or coiling of the chains occurs (c.f. also ref. 12 in which similar conclusions were reached). In 7.5 mM benzyl alcohol a value of ϵ_H = 2.13 would give $\delta_H \approx$ 4.9 nm. This is some 0.3 nm greater than the fully extended length of two hydrocarbon chains of the predominant phosphatidylcholines present in egg lecithin [11].

We can thus account for the increase in thickness when benzyl alcohol is present at 7.5 mM concentration by a straightening of the otherwise kinked and/or folded fatty acid chains. The small (0.3 nm) increase beyond this may be due to a sparing inclusion of benzyl alcohol molecules between the two halves of the bilayer or retention of *n*-tetradecane.

It is interesting at this juncture to comment briefly on the anaesthetic properties of benzyl alcohol. Thus, for instance, some crucial part of an excitation module in the nerve membrane could simply be buried by the increase ($\approx$1.2 nm) in thickness. When the thickness of the hydrocarbon region of the bilayer is increased in the presence of the anaesthetic the bilayer around the protein modules will need to be distoreted or dimpled to accomodate the hydrophobic portions of the protein units. This would set up a radial compression and axial tension in the conduction module (closing the channel). Alternatively, if the hydrophilic forces between the two ends of the excitation module and the aqueous phases were sufiiciently strong, any imcrease in the width of the hydrocarbon region would tend to stress the module.

If the hydrocarbon region is treated as an incompressible fluid (see also ref. 9), at least up to a thickness equal to twice the fully extended chain length,

* Preliminary experiments in this laboratory also show a decrease in the high frequency (1 KHz) capacitance of the membranes of the marine algae *Valonia ventricosa* in the presence of 10 mM benzyl alcohol.

then the increase in thickness of this region in 7.5 mM benzyl alcohol would need to be interpreted in terms of a decrease in the area per phosphatidylcholine molecule in the bimolecular lipid membrane. It has been suggested that the effect of benzyl alcohol on breakdown [3] and stability [13] in cell membranes may be an indirect effect resulting from alterations in the interactions between the lipids and the proteins of the membrane. On our present findings of the effects of benzyl alcohol on the hydrocarbon chains, such alterations would not be unexpected.

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