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PLANAR BILAYER MEMBRANES FROM PURE LIPIDS

R.C. WALDBILLIG and G. SZABO

Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550 (U.S.A.)

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Summary

Planar lipid bilayer membranes are formed from mixtures of pure lipids in the absence of non-biological solvents. The solventless bilayers are characterized by a large specific capacitance (586—957 nF/cm^2) comparable to that of cell membranes but considerably greater than that of conventional lipid/decane bilayers. Hydrocarbon solvents, such as *n*-alkanes or squalene, thicken the bilayer.

Membrane dielectric thickness is used as an indicator of bilayer lipid composition. For membranes made from pure monoglyceride/triglyceride mixtures the thickness of the solventless lipid bilayer is independent of both the chain length (11—22 carbons) and mol fraction (0.1—0.9) of triglyceride in the bulk mixture. In contrast, the thickness of the bilayer (2.0—3.3 nm) depends strongly upon the length (16—24 carbons) of the monoglyceride component. Molecular volume considerations lead to the conclusion that the bulk lipid mixture disproportionates to yield bilayer membranes composed of nearly pure monoglyceride. The dielectric thickness of the monoglyceride bilayer is consistent with the notion that the lipid fatty acyl chains are fluid.

Introduction

The molecular organization of cell membranes is a matter of considerable interest in biology and medicine. Fundamental insights regarding the structure and function of cell membranes have emerged from detailed studies of simplified systems such as squid axons and bimolecular lipid membranes [1—6]. A major difficulty in using the planar lipid membrane as an electrophysiological model system, however, has been the fact the membranes are contaminated with anesthetic-like solvent compounds such as *n*-alkanes [7].

In principle, a planar bilayer can be formed over a submerged aperture when-

ever a specific set of interfacial boundary conditions pertaining to the organic, aqueous and solid phases are satisfied. In practice, these conditions have been met by adding a secondary non-polar compound, such as *n*-alkane, to the surface-active lipid [6,8,9]. Unfortunately, it has been found that hydrocarbon solvents penetrate into the thin lipid membrane and significantly alter its structure. For example, *n*-alkanes of decreasing chain length increasingly swell planar bilayers [10].

A considerable effort has been made to rid the bilayer membrane of these non-biological compounds. It is known that large hydrocarbon molecules such as hexadecane and squalene have only a limited solubility in the membrane phase and this fact has been exploited in efforts to form so-called solvent-free bilayers [4,9]. Other maneuvers aimed at minimizing the solvent content of the bilayer have included freezing of the membrane supporting torus [10], or otherwise decreasing the solvent activity in this organic bulk phase [11]. Also an empirical method has been developed to form solventless bilayers from lipid films spread at the air-water interface [12]. However, these bilayers are not likely to be strictly solvent free. This is so because materials such as petroleum jelly or silicone oil, introduced in a pretreatment procedure necessary for bilayer stability [8,13], may enter the membrane. All of the earlier efforts to rid the bilayer of non-lipid materials have been marred by a lack of independent criteria that could convincingly demonstrate the absence of solvent compounds in the membrane phase.

In view of this circumstance we have reconsidered the long-standing assumption that hydrocarbons are required to satisfy the boundary conditions for the formation of stable membranes. We have been encouraged in this direction by a previous report [14] that bilayers could be formed from solventless alkylamines. Thus, we depart here from convention by forming planar bilayer membranes from a variety of pure lipid mixtures. Furthermore, we show by independent criteria, that bilayers of nearly unitary (i.e. pure monoglyceride) composition can be formed from binary mixtures of mono- and triglycerides.

Methods

Planar black lipid films were formed using a modification of the conventional Mueller-Rudin technique [6]. The modification involves the total exclusion of non-lipid materials (i.e. solvents) from the organic phase. The high viscosity of the organic phase made it necessary to paint the membrane with the aid of a spatula-like tool (Teflon, polypropylene, etc.). With the exception of these differences the technique followed conventional practice [15]. Care was taken to assure that the bilayers were both planar and circular [13].

The torus material was made solely from high purity (greater than 99%) monoglycerides and triglycerides (Nu-Check, Supelco, Sigma). Binary monoglyceride/triglyceride lipid mixtures were prepared by weighing small volumes (less than 10 μl) of the melted lipids into glass vials (Supelco, 0.1 ml). The two lipids were thoroughly mixed with a miniature stirring rod fashioned from a freshly flamed glass micropipette. Particular care was taken to avoid contact with organic solvents (e.g. *n*-alkanes) and to maintain the purity of the glycerides.

The 1 M NaCl was prepared with attention to surface chemical details. The reagent grade salt was roasted at greater than 625°C to ash trace organics. Water was purified by a high quality ion-exchange-carbon system (Millipore, Inc., Milli-Q). This water was found to be free of surfactants ($\gamma = 72.5 \pm 0.3$ dyne/cm, 22°C) and was experimentally indistinguishable from water prepared using a quartz distillation apparatus.

The total geometrical capacitance of the bilayer was determined using a precision (1%) bridge adapted from the design of White [16,17]. The bridge was driven by a 1 kHz ($\pm 1.0 \cdot 10^{-3}$ Hz) sine wave of ±5 mV peak amplitude. The bridge output was passed through a 1 kHz bandpass filter (Rockland 852) and displayed as a Lissajous pattern on an oscilloscope. Calibration tests showed the parallel R/C bridge system to maintain 1% accuracy for series resistance loads of up to 3 kΩ. The resistance of the electrolyte in the absence of a membrane was less than 250 Ω.

The performance of the bridge system was further verified by direct admittance measurements of test circuits as well as bilayer membranes. In this case a ±25 mV (peak) pink input voltage noise signal was applied. The resulting output current noise as well as the input signal were amplified, filtered to prevent aliasing, and digitized. The magnitude and phase of the transfer function were computed from the ratio of the Fourier-transformed output and input signals. A non-linear least-squares Marquardt-Levenberg algorithm was used to fit an equivalent circuit to the magnitude and phase of the transfer function. Transfer function analysis and a.c. bridge methods always agreed to better than 1.5% for calibration circuits.

The total geometrical capacitance was divided by the membrane area to determine the specific geometrical capacitance (C_g) of the bilayer. The membrane area was measured by viewing the brightly illuminated bilayer with a low-power microscope fitted with a graticule eyepiece. The magnification of the microscope was calibrated with reference to a precision optical ruling (Bausch and Lomb Co.). The microscope-chamber optical system was then calibrated by viewing a 200 mesh electron microscope grid immersed near the plane of the chamber aperture. This calibration procedure established the accuracy of the area measurement to be better than 1%.

Results

The transparently simple and heretofore overlooked notion that solventless bilayers can be formed from solventless lipids was tested. A variety of pure lipid mixtures were found to form bilayers of large surface area (greater than 1.25 mm^2). Observations of numerous black films indicate that these bilayers are generally more stable than conventional lipid/alkane bilayers. They also appear to be free of the pinpoint solvent lenses which characterize conventional bilayers.

We have used dielectric analysis techniques [9] to compare the properties of bilayers made from lipid mixtures to those made using conventional dilute lipid/alkane solutions. Black films were formed from a binary mixture containing only glyceryl monopalmitolein and glyceryl triolein. We find that bilayers made from this solventless lipid mixture have specific capacitance (C_g)

values near 956 nF/cm^2. This specific capacitance may be compared with a reported [13] value of 445 nF/cm^2 for bilayers made from dilute monopalmitolein/decane solutions. Axonal membranes have C_g values near 920 nF/cm^2 [2]. The profound difference in the specific capacitance of the pure lipid and solvent-containing bilayers suggests that the solvent-free bilayers have a hydrophobic thickness (assuming $\epsilon = 2.2$) of only 2.0 nm whereas the decane-containing bilayers have a thickness near 4.4 nm.

In view of the novel character of our techniques and the high specific capacitance of the bilayers made from the monopalmitolein/triolein mixture we have taken care to control for possible limitations associated with the use of bridge techniques. Experiments were carried out to evaluate the dielectric properties of the bilayer by direct admittance measurements. Transfer function analysis was used to evaluate the equivalent circuit of the bilayer and surrounding electrolyte. The phase and magnitude of the system admittance could be fit to a circuit having a parallel RC component (the bilayer) in series with a resistive component (the electrolyte, typically less than 250 Ω). When normalized for membrane area the bilayer equivalent RC gave $C_g = 939$ nF/cm^2 and $R_m^* = 67$ kΩ · cm^{-2}. Thus the results obtained by the transfer function analysis are in excellent agreement with the results obtained using the bridge system. The combined results support the conclusion that lipid bilayers made from monopalmitolein/triolein mixtures have capacitative properties associated with an ultrathin membrane structure.

It is known [18—21] that the dielectric thickness of the planar bilayer depends upon the length (i.e. volume) of the membrane lipid fatty acyl chain. We have used this relationship to determine the lipid composition of bilayers made without solvents. In one series of studies membranes were made from mixtures in which the chain length of the monoglyceride component of the torus phase was held constant while the chain length of the triglyceride component was varied. It can be seen from the results illustrated in Fig. 1 that the bilayer specific capacitance and therefore thickness is independent of the length of the triglyceride chain. The fact that the internal volume of the membrane is independent of the hydrophobic volume of the triglyceride component in the torus mixture is well illustrated by the results obtained using monoolein with different triesters. In this case C_g remains nearly constant at 858 ± 8 nF/cm^2 for bilayers made from binary mixtures of monoolein with triesters that have acyl chains ranging from 11 to 22 carbons. Thus by using hydrophobic volume as a criterion for evaluating the chemical composition of the membrane it can be concluded that the thin bilayer contains little or no triglyceride.

We have also used lipid chain length to assay the monoglyceride content of the solventless lipid bilayers. In this second series of studies bilayers were formed from binary lipid mixtures in which the chain length of the triglyceride

* The specific resistance of 67 kΩ · cm^{-2}, deduced here on the basis of admittance measurements, is essentially the same as the value determined by direct current measurements. Evidently the conductance of monopalmitolein/triolein bilayers ($1.5 \cdot 10^{-5}$ siemens · cm^{-2}) is much greater than that of conventional solvent containing bilayers (less than 10^{-8} siemens · cm^{-2}) and it may approach leakage conductance of biological membranes [39]. The large conductance appears to result from the extreme thinness of the bilayer since the intrinsic conductance is greatly reduced by increasing the pure lipid bilayer thickness ($G \approx 10^{-8}$ siemens · cm^{-2} for monoerucin/triolein).

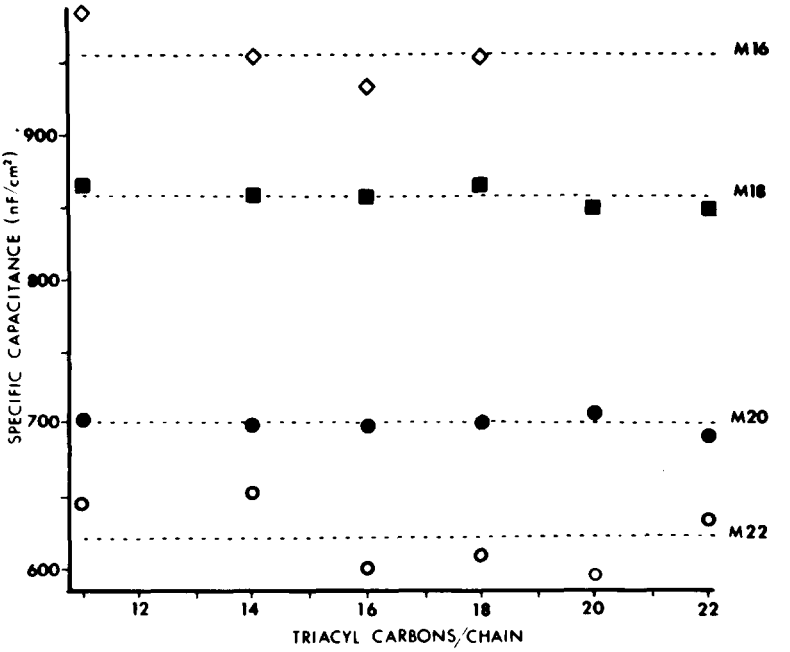

Fig. 1. Specific capacitance for bilayers formed from solventless binary lipid mixtures of glyceryl mono- and triesters having various fatty acyl chain lengths. The membrane capacitance is independent of the number of carbons in the triacyl lipid chain but is inversely related to the number of carbons in the monoacyl (M) lipid chain. The interrupted lines are the mean values for bilayers from triglycerides mixed with monopalmitolein (◇); monoolein (■); monoeicosenoin (●) and monoerucin (○).

was held constant and the length of the monoglyceride component was varied. These results, also shown in Fig. 1, indicate that C_g is inversely related to the length of the monoglyceride component of the bulk mixture. The fact that the bilayer thickness increases with increasing monoacyl chain length is well illustrated by the monoeicosenoin (20 carbon) mean capacitance of 701 nF/cm^2 and the monoerucin (22 carbon) mean capacitance of 622 nF/cm^2. Interestingly mononervonin (24 carbon)/triolein mixtures yield bilayers only at temperatures greater than 28°C where C_g is near 586 nF/cm^2. Since the bilayer thickness is independent of the length of the triglyceride chain yet depends upon the length of the monoglyceride chain we must conclude that the binary torus mixture disproportionates to give rise to bimolecular lipid membranes of unitary (monoglyceride) composition.

In addition to lipid chain length we have used another criterion to further evaluate the distribution of the two lipids between the bulk torus and the thin bilayer phases. We have recently shown for conventional bilayers [11] that the *n*-alkane content of the membrane is strongly influenced by the *n*-alkane concentration in the torus material. Here we use the concentration dependence of C_g as an additional index of the relative miscibility of the torus lipids in the membrane phase. Bilayers were formed from solventless binary lipid mixtures containing various concentrations of the triglyceride component. It can be seen from the results in Table I that the bilayer specific capacitance is independent

TABLE I

A SUMMARY OF THE MEAN SPECIFIC CAPACITANCE VALUES FOR BIMOLECULAR LIPID MEMBRANES FORMED FROM VARIOUS MIXTURES

M, monacyl carbons; T, triacyl carbons/chain; X_T, triglyceride mol fraction in torus; C_g, specific capacitance; X_S, squalene mol fraction in torus.

M	T	X_T	C_g	S.D.	M	T	X_T	C_g	S.D.
16	11	0.73	984	33	20	11	0.81	703	26
16	14	0.74	954	28	20	14	0.82	700	23
16	16	0.81	934	12	20	16	0.80	700	18
16	18	0.83	954	12	20	18	0.80	702	15
16	18	0.53	974	36	20	18	0.47	679	9
16	18	0.12	980	10	20	18	0.17	666	13
18	11	0.73	866	24	22	11	0.88	645	9
18	14	0.87	858	17	22	14	0.82	653	7
18	16	0.75	857	17	22	16	0.79	601	26
18	18	0.74	866	5	22	18	0.35	610	10
18	18	0.11	879	12	22	18	0.56	620	14
18	18	0.38	859	14	22	18	0.91	613	14
18	18	0.61	846	37	24	18	0.84	586	24
18	18	0.90	861	20					
18	20	0.78	850	24					
18	22	0.65	848	13					

M	X_S	C_g	S.D.
14	0.80	977	13
16	0.76	873	28
18	0.96	794	13
20	0.77	648	11
22	0.91	558	32
24	0.83	520	27

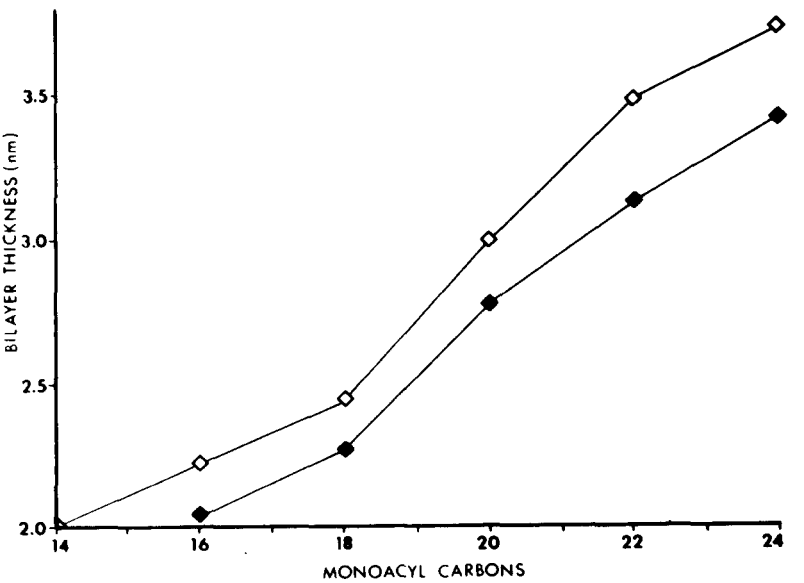

Fig. 2. Bilayer dielectric thickness as a function of the number of carbons in the monoglyceride fatty acyl chain. Membranes made from monoglyceride/squalene dispersions (◇) are thicker than bilayers made from pure lipid mixtures (◆). Monoacyl dielectric coefficients taken from Ref. 19.

of the mol fraction of triglyceride in the torus mixture. Here again C_g depends solely upon the chain lenght of the monoglyceride component. The absence of a concentration dependence of C_g reinforces the conclusion that the bilayers are composed of virtually pure monoglyceride.

It has recently been reported that so-called 'solventless' bilayers can be formed from monoglyceride/squalene mixtures [9]. Since squalene is a linear hydrocarbon solvent ($C_{30}H_{50}$) we have compared membranes made from lipid/squalene dispersions to those made from lipid/lipid mixtures. Dispersions containing purified squalene and monoglycerides of differing chain length were used to form bilayers. The results presented in Table I reveal an expected increase in bilayer thickness with increasing monoglyceride chain length. However, when the squalene results are compared to those obtained from solventless lipid/lipid mixtures (Fig. 2) it is evident that bilayers which are exposed to a squalene-containing torus are always thicker than those made from the pure lipid system. If the lipid area/molecule is the same in both cases then prevailing concepts of bilayer structure suggest that a measurable volume of squalene is retained in bilayers made from monoglyceride/squalene dispersions.

Discussion

The most direct way of forming pure lipid bilayers is to begin with materials which are free of non-lipid components. We have successfully substituted triglycerides for the customary solvents of the bimolecular lipid membrane system. The exclusion of anesthetic-like alkane solvents from both the bilayer and its supporting torus yields a chemically defined model membrane system which is free of biologically obtrusive components. Moreover the apparent disproportionation of the bulk phase lipids during the thinning of the bilayer provides a new avenue for understanding the physicochemical processes which control intramembrane mixing of hydrophobic molecules.

There is no clear precedent for forming bilayers of the Mueller-Rudin type from pure lipids. It would appear that earlier workers were dissuaded from using a secondary lipid as a bilayer 'solvent' because of their concern for the interfacial factors which govern the formation and stability of bilayers [9]. While interfacial factors [22] are critical to our understanding of membrane organization it is becoming increasingly apparent that lipid bilayers also behave as solutions. For example, we have recently demonstrated that, contrary to simple surface chemical expectations, the residual solvent content of lipid/alkane bilayers is strongly influenced by the concentration of alkane in the membrane torus [11]. Others have subsequently confirmed and extended the relationship of the torus activity to the composition of the thin bilayer [23] and related studies [24] have raised questions regarding the applicability of Regular Solution theory [25] to the intrabilayer environment.

The results of this study make it clear that triglycerides can satisfy the boundary requirements for bilayer stability. The use of triglycerides as a torus material is advantageous in that the triesters are expected to be compatible with biological systems. In addition to biological considerations our selection of triglyceride as a bilayer support fluid stems from the fact that these natural oils have many of the physicochemical characteristics of very long chain length

aliphatic hydrocarbons [26]. Previous studies [10,13] have established that the residual solvent content of the bilayer is inversely related to the molar volume of the *n*-alkane ($n > 10$) solvent. Indeed this relationship is the basis for the recent re-introduction [9,27] of the large linear alkene, squalene, as a solvent. For comparison it is interesting to note that the molar volume of squalene is about 480 cm^3/mol while that of the triglyceride compound trierucin is approximately 1120 cm^3/mol.

The results presented here indicate that the monoglyceride component of the binary lipid mixture dominates the interfacial system. In this regard it is noteworthy that triglycerides are very weak surfactants when compared to monoglycerides. For example, it is known [28] that tripalmitin lowers the tension of the paraffin oil/water interface by only 2 dynes/cm whereas monostearin decreases the tension by 46 dynes/cm. Moreover the near 30 dyne/cm tension of a triglyceride/water interface has been shown to decrease to less than 5 dynes/cm in the presence of monoolein [29]. These changes are similar to those which have been reported for alkane-water interfaces in the presence of adsorbed monoglycerides [30]. The weak surface activity of triglycerides is also consistent with their inability to form uniform monolayers at the air-water interface [31] while this is not the case with monoglycerides [32,33]. We have observed that monolayers spread from monoglyceride/triglyceride binary mixtures have the characteristics of the monoester component (unpublished results). This apparent affinity of monoglycerides for water is in agreement with the reported phase behavior of hydrated monoglycerides and triglycerides [34,35].

The results indicate that bilayers made from binary lipid mixtures have about one-half the thickness of black membranes made from conventional dilute monoglyceride/decane solutions [36]. The available evidence, summarized in Fig. 3, suggests that bilayers exposed to decane have a maximum thickness whereas bilayers exposed to triglycerides are of minimum thickness. That is to say that decane appears to swell the membrane to a dielectric thickness which approaches but does not exceed the dimensions of two opposed, extended and untilted lipid fatty acyl chains. It has been speculated by others that removing the solvent from the bilayer leads to a collapse of the lipid chains into a coiled or folded configuration [13]. If the lipid area/molecule remains constant at these two limits of thickness then the results presented here cannot easily be explained by a simple extended chain molecular model. Our results are consistent with a liquified lipid chain model of the intrabilayer environment. However, we have no means of excluding alternative models in which the lipid chains have an exaggerated tilt and/or are completely interdigitated.

Others have recently used measurements of the bilayer specific geometrical capacitance to estimate the lipid area/molecule in the bilayer [9]. This procedure requires the assumption of a lipid chain molecular volume and dielectric coefficient derived from the incremental properties of bulk liquid alkanes and alkenes [19]. If the C_g values of pure monoglyceride bilayers reported here are treated in this manner, then the monoglyceride area/molecule is estimated to be near 0.40 nm^2 (see Table II). This calculated area is very near the value of 0.39 nm^2 reported from oil-water interfacial studies of monoolein [30] and the value of 0.43 nm^2 obtained by direct sampling of the monoolein/alkane bilayer

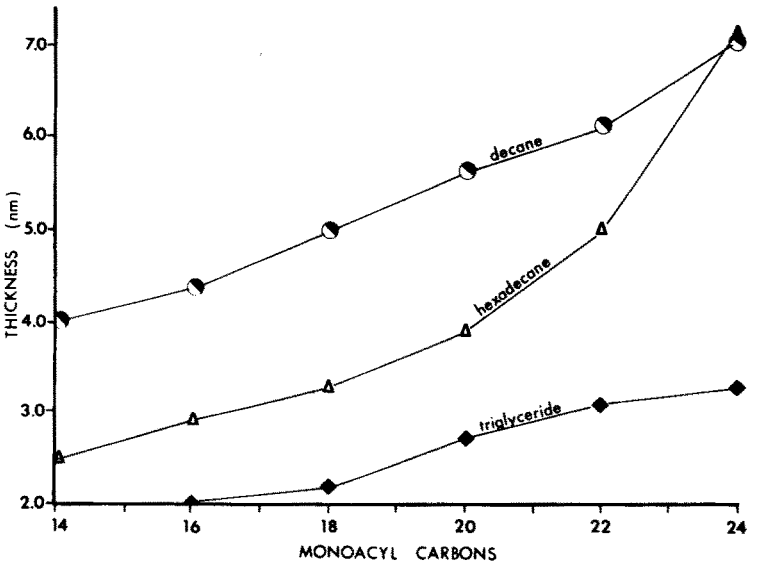

Fig. 3. A comparison of the dielectric thickness of planar bilayers made with and without alkane solvents. Bilayers containing the maximum amount of decane are nearly twice as thick as pure lipid membranes. Decane and hexadecane bilayer thicknesses were calculated from the capacitance data of Ref. 13 using the dielectric coefficients of Ref. 19.

[7]. This close agreement is also consistent with the fact that the lipid area/molecule remains constant above the critical micelle concentration [37] and is practically unaltered by different solvents [7,30].

It is noteworthy that the present results suggest that the lipid area/molecule may change somewhat with the length of the monoglyceride fatty acyl chain. Earlier workers [13] neglected to consider the effects of variations in the lipid area/molecule with chain length in their studies of the thickness of bilayers made from different monoglycerides. Since the thickness of the membrane depends upon both the lipid area/headgroup as well as the volume of the low dielectric segment of the lipid chain, caution must be exercised when comparing bilayers of different lipids.

As noted previously there is a recent report [9] that so-called solventless

TABLE II

THE ESTIMATED AREA/HEADGROUP OF VARIOUS MONOGLYCERIDES IN PURE LIPID MEMBRANES

Volume and dielectric coefficients of monoacyl dielectric segments taken from Ref. 19.

Monoacyl carbons	Dielectric coefficient	Bilayer dielectric thickness (nm)	Dielectric volume/chain (nm^3)	Area/ headgroup (nm^2)
16	2.202	2.04	0.421	0.413
18	2.200	2.27	0.475	0.418
20	2.198	2.78	0.529	0.381
22	2.196	3.13	0.583	0.373
24	2.195	3.32	0.637	0.384

bilayers can be formed from monoglyceride dispersed in squalene. It is known from calorimetric studies that squalene is virtually immiscible in phospholipid vesicles [38] but there is no such evidence regarding monoglyceride systems. While the results presented here confirm and extend the earlier data obtained for bilayers from monoolein/squalene dispersions [9] the present results do not support the earlier conclusion that squalene is virtually immiscible in monoglyceride bilayers. For example if the dielectric volume of the fatty acyl chain of monoolein is taken to be 0.475 nm^3/molecule [19] then the lipid/squalene bilayer has about 7% excess volume occupied by squalene molecules, assuming a constant lipid area/molecule and dielectric coefficient. Alternatively it is possible, although unlikely, that bilayers supported by the squalene dispersion have a membrane lipid area/molecule which is about 0.04 nm^2 less than the same monoglyceride supported by the pure lipid mixture. Other alkane solvents are known to thicken bilayers by occupying space within a membrane instead of altering the lipid area/molecule [7,30]. Moreover the notion that bilayers supported by different torus fluids are analogous to monolayers at different points on a pressure vs. area curve is not supported by the fact that very substantial changes in the molecular structure of the triester fluid (11—22 carbons/chain) have no measurable effect upon the thickness (i.e. area/molecule) of the thin monoglyceride bilayer. Thus our squalene results are most simply explained by supposing that a small but measurable quantity of residual squalene remains inside of the monoglyceride bilayer.

While the evidence presented here indicates that triglycerides are virtually immiscible in monoglyceride bilayers it would be thermodynamically unrealistic to suppose that there is absolutely no triglyceride in the planar bilayer. However, the results do suggest limits for the maximum amount of triester which might go undetected. It is clear that monester chain volume increments corresponding to two $-CH_2-$ moieties are readily detected by the capacitance method. Since alkanes have volume increments near 0.027 nm^3/CH_2 [19] it follows that at a constant monoglyceride area/molecule volume changes of the order of 0.05 nm^3/lipid should be readily detectable. If the hydrophobic molecular volume of monoolein is taken to be 0.475 nm^3 [19] then it is evident that a 10% change in lipid volume would be easily detected. Thus if the hydrophobic molecular volume of a triester such as trierucin is approximately 1.8 nm^3/molecule (i.e. three 22 carbon chains) then greater than 3% triester excess volume in the membrane phase would be detected without difficulty.

A more realistic estimate of the maximum triglyceride volume which might escape detection can be made by considering the variability of the C_g data for a particular monoglyceride such as monoolein. Since the standard deviation of the monoolein data of Fig. 2 is 8 nF/cm^2 it can be estimated that the bilayer thickness uncertainty is 0.021 nm which, at an area/monoolein of 0.418 nm^2, corresponds to a volume/monoolein uncertainty of 0.009 nm^3/chain. Again if it is assumed that the hydrophobic molecular volume of trierucin is near 1.8 nm^3 then it can be reasoned that triglyceride volumes of less than 0.5% might be unresolved. While it must be recognized that the several assumptions of these estimates are untested, it is entirely reasonable to expect that trace quantities of triglyceride lipid in the monoglyceride lipid bilayer would not be deleterious in the modeling of biological processes.

Preliminary experiments indicate that it is also possible to form stable lipid bilayers from biological phospholipids mixed with di- or triglycerides. Further studies are required to establish whether or not significant amounts of the boundary glycerides are present in these membranes. It is anticipated, however, that the phospholipid/glyceride system will further provide useful synthetic analogs of cell membranes.

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References

1 Hille, B. (1978) Biophys. J. 22, 283—294
2 Takashima, S. (1978) Biophys. J. 22, 115—119
3 Racker, E., Knowles, A.F. and Eytan, E. (1975) Ann. N.Y. Acad. Sci. 264, 17—31
4 Haydon, D.A. (1975) Ann. N.Y. Acad. Sci. 264, 2—15
5 Waldbillig, R.C., Robertson, J.D. and McIntosh, T.J. (1976) Biochim. Biophys. Acta 448, 1—14
6 Mueller, P., Rudin, D.O., Tien, H.T. and Wescott, W.C. (1962) Nature 194, 979
7 Pagano, R.E., Ruysschaert, J.M. and Miller, I.R. (1972) J. Membrane Biol. 10, 11—30
8 White, S.H., Petersen, D.C., Simon, S. and Yafuso, M. (1976) Biophys. J. 16, 481—488
9 White, S.H. (1978) Biophys. J. 23, 337—347
10 White, S.H. (1977) Ann. N.Y. Acad. Sci. 303, 243—265
11 Waldbillig, R.C. and Szabo, G. (1978) Nature 272, 839—840
12 Montal, M. and Mueller, P. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3561—3566
13 Benz, R., Frohlich, O., Lauger, P. and Montal, M. (1975) Biochim. Biophys. Acta 394, 323—334
14 Szabo, G. (1969) Ph.D. Dissertation, University of Chicago, p. 75
15 Szabo, G., Eisenman, G. and Ciani, S.J. (1969) J. Membrane Biol. 1, 346—382
16 White, S.H. (1969) Ph.D. Dissertation, University of Washington
17 Waldbillig, R.C. (1973) Ph.D. Dissertation, University of Rochester
18 Requena, J., Billett, D.F. and Haydon, D.A. (1975) Proc. R. Soc. Lond. A347, 141—159
19 Requena, J. and Haydon, D.A. (1975) Proc. R. Soc. Lond. A347, 161—177
20 McIntosh, T.J., Waldbillig, R.C. and Robertson, J.D. (1976) Biochim. Biophys. Acta 448, 15—33
21 McIntosh, T.J., Waldbillig, R.C. and Robertson, J.D. (1977) Biochim. Biophys. Acta 466, 209—230
22 Kermer, J.M.H., Agterof, W.G.M. and Wiersema, P.H. (1977) J. Colloid Interface Sci. 62, 396—405
23 White, S. (1979) Biophys. J. 25 (2,II), 10a
24 Simon, S.A., Stone, W.L. and Bennett, P.B. (1979) Biochim. Biophys. Acta 550, 38—47
25 Hildebrand, J.H., Prausnitz, J.M. and Scott, R.L. (1970) Regular and Related Solutions, Van Nostrand Reinhold Co., New York
26 Lutton, E.S. (1972) J. Am. Oil Chem. Soc. 49, 1—9
27 Mueller, P., Rudin, D.O., TiTien, H. and Wescott, W.C. (1963) J. Phys. Chem. 67, 534—535
28 Hartman, L. (1964) J. Am. Oil Chem. Soc. 41, 519—520
29 Gros, A.T. and Feuge, R.O. (1951) J. Am. Oil Chem. Soc. 28, 1—4
30 Fettiplace, R., Andrews, D.M. and Haydon, D.A. (1971) J. Membrane Biol. 5, 277—296
31 Baumeister, W., RIngeli, U.P., Hahn, M., Kopp, F. and Seredynski, J. (1976) Biophys. J. 16, 791—810
32 Dreher, K.D., Schulman, J.H. and Hofmann, A.F. (1967) J. Colloid Interface Sci. 25, 71—83
33 Merker, D.R. and Daubert, B.F. (1964) J. Phys. Chem. 68, 2064—2066
34 Lutton, E.S. (1965) J. Am. Oil Chem. Soc. 42, 1068—1070
35 Brokaw, G.Y. and Lyman, W.C. (1958) J. Am. Oil Chem. Soc. 35, 49—52
36 Requena, J., Brooks, D.E. and Haydon, D.A. (1977) J. Colloid Interface Sci. 58, 26—35
37 Adamson, A.W. (1976) Physical Chemistry of Surfaces, John Wiley and Sons, New York
38 Simon, S.A., Lis, L.J., McDonald, R.C. and Kauffman, J.W. (1977) Biophys. J. 19, 83—90
39 Adrian, R.H. (1964) J. Physiol. 175, 134—159