FORMATION OF "SOLVENT-FREE" BLACK LIPID BILAYER MEMBRANES FROM GLYCERYL MONOOLEATE DISPERSED IN SQUALENE

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ABSTRACT A simple technique for forming "black" lipid bilayer membranes containing negligible amounts of alkyl solvent is described. The membranes are formed by the method of Mueller et al (*Circulation.* 1962. **26**:1167.) from glyceryl monooleate (GMO) dispersed in squalene. The squalene forms an annulus to satisfy the boundary conditions of the planar bilayer but does not appear to dissolve noticeably in the bilayer itself. The specific geometric capacitance (C_g) of the membranes at 20°C formed by this technique is 0.7771 ± 0.0048 μF/cm^2. Theoretical estimates of C_g for solvent-free bilayers range from 0.75 to 0.81 μF/cm^2. Alkane-free GMO bilayers formed from *n*-octadecane by the solvent freeze-out method of White (*Biochim. Biophys. Acta.* 1974. **356**:8) have values of $C_g = 0.7903 \pm 0.0013$ μF/cm^2 at 20.5°C. The agreement between the various values of C_g strongly suggests that the bilayers are free of squalene. DC potentials applied to the bilayers have no detectable effect on the value of C_g, as expected for solvent-free films. The ability to form bilayers essentially free of the solvent used in the forming solution makes it possible to determine the area per molecule of the surface active lipid in the bilayer. The area per molecule of GMO at 20°C is estimated to be 37.9 ± 0.2 Å^2.

INTRODUCTION

The "black" lipid bilayer membrane, first described by Mueller et al. (1962), has become an important model system for studying biomembrane phenomena such as ion transport. This bilayer system has been often criticized, however, because the alkane or other organic solvent used in the formation of the black lipid film is present in high concentrations in the bilayer per se (Hanai et al., 1965; Andrews et al., 1970; White, 1972). To circumvent this criticism, Montal and Mueller (1972) formed bilayers that were essentially solvent-free, using the technique of Takagi et al. (1965). The septum is pretreated in this method with pentane or hexane solutions of petroleum jelly or silicone grease, which form the annulus required for bilayer stability (White et al., 1976). Such annular constituents have a largely unknown composition and may contain unidentified surface-active compounds that could modify bilayer properties. In addition, this technique requires a rather complex apparatus for bilayer formation. The resulting films are small in diameter and not easily viewed in the microscope. A great advantage of this system is that asymmetric bilayers can be formed.

The purpose of this paper is to report a simple technique for forming apparently solvent-free black lipid bilayer membranes by the method of Mueller et al. (1962). The resulting membranes are stable, large in diameter (~2 mm), and easily observed with a low-power microscope. The idea for the method comes from the work of White (1977), which showed that very long *n*-alkane molecules (greater than 21 carbons or so) should be insoluble in black lipid bilayer membranes formed from glyceryl monooleate. Theoretically, if a black film were formed by using an alkane of sufficient length, the alkane would form a stabilizing annulus but would not dissolve in the bilayer itself. A "solvent-free" black film could thereby be formed. Unfortunately, a normal alkane of sufficient length would be a solid at reasonable working temperature. What was needed was a large, nonsurface-active, long-chain, alkyl molecule, which is liquid at room temperature. Squalene ($C_{30}H_{50}$) is such a molecule. The work of Simon et al. (1977), which demonstrated that squalene is insoluble in dipalmitoyl lecithin dispersions, suggested this solution to the problem.

Thus, a simple method was arrived at for forming "solvent-free" black films: Glyceryl monooleate is dispersed in squalene and the "solution" spread in the usual way across an aperture in a septum separating two aqueous phases. Measurements of the specific geometric capacitance (C_g) of the resulting black lipid film indicate that the bilayer per se contains negligible amounts of squalene. The results of these measurements are reported in this paper. An important spin-off from this work is that the number of monoglycerides per unit area of film (or, alternatively, the area per monoglyceride) can be easily determined. Until now, it has been necessary to use complicated surface chemistry (Cook et al., 1968) or radioactive tracer (Pagano et al., 1972) techniques to determine the area per molecule in black films formed from monoglycerides.

METHODS

Glyceryl-1-monoolein was obtained from Supelco, Inc. (Bellafonte, Penn.) in the crystaline form and was used without further purification. It migrated as a single spot on two different types of thin layer plates using three different developers. Squalene was obtained from Chemical Samples Company (Columbus, Ohio). It was yellow and had a rather strong odor because of various contaminants or breakdown products. These were removed successfully by two passages through absorption alumina. The final product was clear and odorless and was stored under argon at −20°C. The membranes were formed in unbuffered (pH ~6) 0.1 M NaCl solutions made from Heico, Inc. (Delaware Water Gap, Penn.) extreme purity salt and four-times-glass-distilled water. The salt was roasted at 650°C for several hours to destroy any organic contamination. The water was distilled once from a Corning Glass Works, (Corning, N.Y.) AG-3ADA still, followed by three distillations from small stills equipped with Friedrich condensers from Corning Glass Works. In the first of these, the water was distilled from acid $KMnO_4$ and in the second from basic $KMnO_4$. The third was a straight distillation to insure the absence of $KMnO_4$ in the final product. Water prepared in this way had a surface tension of 73 dyn/cm at 20°C measured with a Wilhelmy balance. The electrolyte (100 ml) was equilibrated with the bilayer-forming solution (0.1 ml) at 20°C by vigorous stirring with a clean Teflon stir-bar for 1-2 h.

Black films were formed from a 10 mg/ml dispersion of glyceryl monooleate (GMO) in squalene by the pipette technique of Szabo et al. (1969), as modified by White and Thompson (1973). The GMO was weighed out in a vial and an appropriate volume of sequalene was added. The vial was warmed with body heat from the hands until the GMO melted. The solution was then mixed thoroughly with a small glass rod that had been flamed to ash any organic contaminants. The chamber used for formation was machined from a solid block of polychlorotrifluoroethylene specially molded by Thermech Engineering Corp. (Anaheim, Calif.). It had optically flat windows in either end and was held in a brass thermostat to maintain temperature constant to within ±0.05°C. The chamber was cleaned by thorough scrubbing in a detergent solution, followed by extensive rinsing in water and a 48-h soak in a 20% solution of concentrated HNO_3 in concentrated H_2SO_4. After this step, the chamber was rinsed thoroughly, soaked 24 h. in four-times-distilled water, dehydrated with 200 proof ethanol, and then rinsed and stored in glass-distilled petroleum ether that had been passed through alumina. During assembly, the chamber was handled with disposable polyvinyl surgical gloves to prevent contamination with surface-active lipids from the hands.

The specific geometric capacitance (C_g) of the bilayers was determined from measurements of total bilayer capacitance (C_T) with a high-precision AC bridge (White and Blessum, 1975) and from measurements of membrane area (A_m) by a photographic technique (White, 1970; White and Thompson, 1973). C_g was estimated from the C_T/A_m ratio, as described by White (1973, 1975). The absolute accuracy of the method is estimated to be ±0.7% and the precision to be ±0.3%. The temperature of the electrolyte in the chamber was monitored with an acid-cleaned glass thermistor probe connected to a simple DC bridge constructed in the laboratory. The thermistor-bridge was calibrated against a certified Brooklyn Thermometer Co., Inc. (Farmingdale, N.Y.) type 3522 thermometer. Temperature could be determined with an accuracy ±0.05°C.

RESULTS AND DISCUSSION

The usual colored Fizeau bands were observed in the freshly applied (thick) films but they progressed quite slowly through the various stages preceding the full black state. Sometimes the films remained thick for half an hour or more before making the transition into the black state. Therefore, once the films became gold-colored near the lower edge, a temporary 100-mV potential was used to force the transition. The transition to the full black state, once initiated, took place very rapidly. 2 s or less were required for the "blackness" to propagate over the surface. No systematic efforts were made to establish the average lifetime of the black films, but some films were observed over periods of 3 h or so. Most of the measurements were made over 10–30 min and, at temperatures below 35°C, very few of the membranes ruptured spontaneously during this time. The membranes were reasonably insensitive to mechanical vibrations.

The values of specific geometric capacitance (C_g) of bilayers formed at various temperatures are plotted in Fig. 1. A linear regression curve satisfying the relation $C_g = C_0 + bT$ was calculated from these data by the method of least squares. C_0 was found to equal $0.7824 \pm 0.0048\ \mu F/cm^2$ (SD) and b to equal $-2.68 \pm 1.70 \times 10^{-4}\ \mu F/cm^2/°C$ (SD). The value of C_g for $T = 20°C$ was calculated from this equation to be $0.7771\ \mu F/cm^2$.

A theoretical estimate of C_g for membranes containing no solvent can be made with the equations

$$C_g = \epsilon_0 \epsilon_B/\delta_B, \qquad (1)$$

$$\delta_B = 2V_{mol}/A. \qquad (2)$$

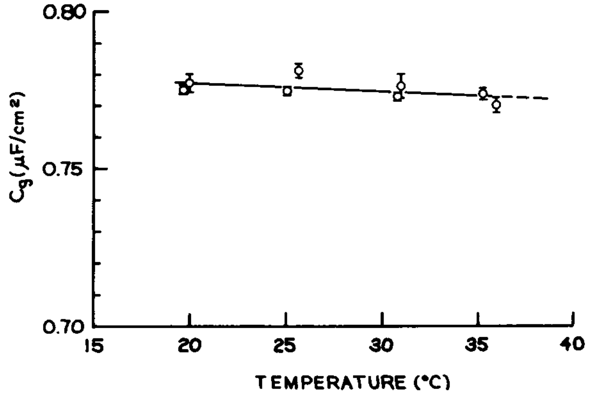

FIGURE 1 Temperature dependence of the specific geometric capacitance (C_g) of squalene-formed GMO black lipid films. The error bars represent the standard error of the means (SEM) of triplicate determinations on three membranes. The straight line through the points is the least squares fit to the equation $C_g = C_0 + bT$, where $C_0 = 0.7824 \pm 0.0048\ \mu F/cm^2$ and $b = -2.68 \pm 1.70 \times 10^{-4}$ F/cm'/°C. The value of C_g calculated from this equation for $T = 20°C$ is 0.7771 $\mu F/cm^2$. This value agrees well with theoretical values for solvent-free bilayers (Table II) and with experimental values determined from other bilayer systems believed to be essentially solvent-free (Table III).

In Eq. 1, $\epsilon_0 = 8.85 \times 10^{-14}$ F/cm, ϵ_B is the dielectric coefficient of the hydrocarbon core comprised solely of GMO acyl chains, and δ_B is the hydrocarbon core thickness. In Eq. 2, V_{mol} is the molecular volume of a single acyl chain and A is the area per acyl chain. Requena and Haydon (1975) have calculated values of ϵ_B and V_{mol} at 20°C and find 2.20 and 4.75×10^{-22} cm^3, respectively. Fettiplace et al. (1971) and Andrews (1970) have measured A for GMO at alkane/0.1 M NaCl interfaces. These values of A are excellent approximations to the values found in alkane-containing black films (Cook et al., 1968; Fettiplace et al., 1975), and I assume similar values will exist in solvent-free bilayers. Using the various values of A reported, theoretical estimates of C_g and δ_B were made. The hypothetical values are shown in Table I. The value of C_g for the squalene-GMO membranes falls within the calculated range of values. Therefore, considering the relative error of A, it is reasonable to assume that the bilayers formed from GMO with the aid of squalene are, for practical purposes, free of squalene.

It could be argued, however, that the area per molecule in the squalene-GMO system changes in such a way that the presence of the squalene in the bilayer is masked. To check this possibility, bilayers were formed by the solvent freeze-out method of White (1974). With this method, bilayers are formed above the melting point of the long-chain alkane solvent used in the forming solution. The temperature is then lowered to below the melting point, which causes the annulus to freeze and the alkane to be frozen out of the bilayer. A large increase in capacitance at the melting point results from the decrease in thickness accompanying the loss of the alkane from the bilayer. If bilayers with values of C_g between 0.75 and 0.80 $\mu F/cm^2$ can be formed by this method, it would be reasonable to assume that there is no fortuitous change in A for squalene-formed bilayers, since the alkyl solvent and circumstances of formation are quite different for the two methods. Measurements of C_g versus temperature for octadecane-GMO membranes are shown in Fig. 2. At 20.5°C, C_g equals 0.7903 ± 0.0013 μF/cm^2, but this value is probably anomalously high. Unlike the earlier measurements on hexadecane-GMO bilayers (Table II) using this technique (White, 1974), these measurements were carried out under equilibrium conditions with respect to alkane distribution. Once the annulus froze at 28°C, the temperature was lowered to 20°C and the total capacitance followed in time until a steady value was reached (about 3 h). The temperature was then increased in small increments and C_g determined after each increase. The membranes were allowed to come to equilibrium at the new temperature (which required about 1 h) before C_g was determined. It is likely, however, that C_g is higher than it should be because GMO is slightly water-soluble and the solubility increases as temperature decreases (White, 1976). The melting point of *n*-octadecane is 28°C and the bilayers must be formed at a temperature higher than this. Thus, as temperature is lowered, the GMO interfacial concentration is likely to decrease due to desorption into the aqueous phase, decreasing the thickness and consequently increasing C_g. It is likely, then, that the bilayer is only in a quasi-equilibrium state with respect to the distribution of GMO in the system. Since the annulus is frozen, it is probably difficult for the GMO to reach true equilibrium in the time available.

The difference in slope of the $C_g(T)$ curves for octadecane-GMO films above and below 28°C deserves comment. White (1976) has demonstrated that these slopes can be used to calculate the enthalpy of solution of the alkane in the bilayer. The change in slope seen in Fig. 2 reflects the difference between the enthalpy of solution of solid and liquid *n*-octadecane. Therefore, the difference in slopes must be related to the latent heat of fusion of the octadecane.

Benz et al. (1975) have measured the specific capacitance of GMO planar bilayers formed by the technique of Takagi et al. (1965), as modified by Montal and Mueller

TABLE I

THEORETICAL VALUES OF THICKNESS AND SPECIFIC GEOMETRIC CAPACITANCE OF SOLVENT-FREE LIPID BILAYER MEMBRANES AT 20°C.

Interface (alkane/0.1 M NaCl)	A	δ_B	C_g
	Å^2/molecule	*Å*	*μF/cm^2*
n-heptane	39.5 ± 1.5	24.1 ± 0.9	0.808 ± 0.029
n-decane	39.5 ± 1.5	24.1 ± 0.9	0.808 ± 0.029
n-tetradecane	36.5 ± 1.5	26.0 ± 1.0	0.749 ± 0.030
n-hexadecane	38	25.0	0.779

δ_B for the various values of A was calculated from Eq. 2 by assuming $V_{\text{mol}} = 4.75 \times 10^{-22}$ cm^3 (Requena and Haydon, 1975). C_g was calculated from Eq. 1 assuming $\epsilon_B = 2.20$ (Requena and Haydon, 1975). The values of A at 20°C for glyceryl monooleate adsorbed at various alkane/0.1 M NaCl interfaces are from Fettiplace et al. (1971) and Andrews (1970). The calculations assume that A in alkane-free bilayers will be identical to values observed at alkane/0.1 M NaCl interfaces.

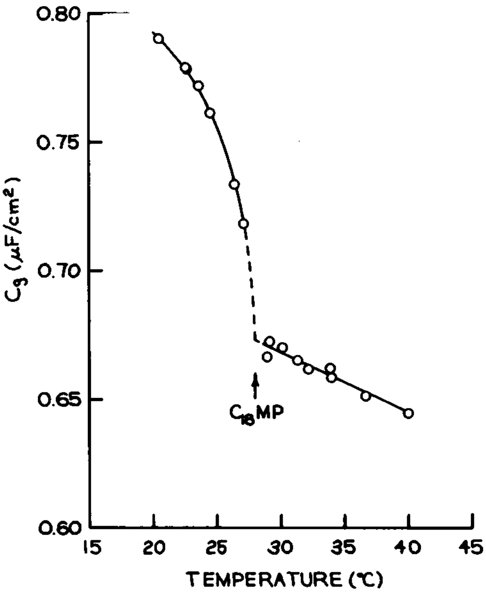

FIGURE 2 Temperature dependence of the specific geometric capacitance (C_g) of black lipid films formed from *n*-octadecane and GMO. Octadecane melts at 28°C. The values of capacitance below this temperature were obtained on several films formed above the melting point and cooled to 20°C. Once a steady value of capacitance was reached, the temperature was increased slightly and held constant until the capacitance was again steady. Each data point above the melting point (arrow) represents the mean value of three membranes. The large increase in slope below 28°C occurs because the octadecane freezes out of the membrane, leaving a solvent-free membrane. The slope is a measure of the enthalpy of solution of *solid* octadecane in the bilayer. At 20.5°, $C_g = 0.7903 \pm 0.0013$ μF/cm^2, essentially the value of capacitance of a solvent-free membrane.

(1972). They obtained a value of 0.745 ± 0.024 μF/cm^2 by assuming the area of the bilayer to be equal to the area of the aperture. This probably results in an overestimate of area, since there must be an annulus surrounding the bilayer that occupies part of the area of the aperture but makes no contribution to the capacitance (White, 1972; White et al. 1976). Thus, this value of C_g is probably somewhat low. The various experimental values of C_g obtained by the various techniques are summarized in Table II.

As a final test of the hypothesis that the squalene-formed bilayers are free of squalene, the voltage dependence of the specific capacitance was examined. Benz et al. (1975) reported that C_g of GMO bilayers formed by the method of Montal and Mueller (1972) is independent of applied voltage. This is expected for solvent-free membranes, since the voltage-dependent capacitance of black films is due to solvent being squeezed out of the bilayer into microlenses (Requena et al., 1975). The specific geometric capacitance of films was therefore measured (23.9°C) in the absence and presence of a 100-mV potential. The values of C_g obtained were 0.7759 ± 0.0019 (SEM) μF/cm^2 and 0.7740 ± 0.0041 (SEM) μF/cm^2, respectively. The bilayers thus appear to be solvent-free by this criterion also. The smallest change in C_g detectable by the experimental method used is about 0.3%. Preliminary measurements of the voltage dependence

(J. E. Hall and S. H. White, unpublished), using the very sensitive method of Alvarez and Latorre (1978), indicate that C_g obeys the equation $C_g(V) = C_g(0)(1 + \alpha V^2)$ with $\alpha \sim 3 \times 10^{-2} V^{-2}$. This is in good agreement with the value of α reported by Alverez and Latorre (1978) for GMO bilayers formed by the technique of Montal and Mueller (1972).

The above data taken together strongly suggest that bilayers formed using squalene as a solvent for the GMO contain negligible (or at least experimentally indetectable) amounts of squalene. If this is true, then we have not only a novel and easy method of forming "solvent-free" bilayers, but also a novel method for estimating the area per molecule of lipid molecules in black lipid membranes and therefore at alkane-water interfaces.

If the squalene-formed GMO bilayers are free of squalene, the area per molecule (A) of the GMO in the bilayer can be calculated by using Eqs. 1 and 2. Rearrangement and substitution yields the following equation of A:

$$A = 2V_{\text{mol}}C_g/\epsilon_0\epsilon_B. \tag{3}$$

However, $V_{\text{mol}} = M/\rho N$ where M is the gram-equivalent molecular weight (237.49) of the acyl chain, ρ is the density, and N is Avogadro's number. Substitution into Eq. 3 yields

$$A = (2M/\epsilon_0 N)C_g/\rho\epsilon_B. \tag{4}$$

C_g, ρ, and ϵ_B are all temperature-dependent. C_g is measured, but the temperature dependence of ϵ_B and ρ must be calculated. At 20°C, $\epsilon_B = 2.20$ and $\rho = 0.8305$ (Requena and Haydon, 1975). The temperature dependence of ϵ_B and ρ can be estimated by the method described by White (1974, 1975), but the estimates require a knowledge of how ρ varies with temperature. This is unknown but I assume that it will be about the same as for the longer n-alkanes. Therefore, I took the temperature coefficient of ρ as the average of the temperature coefficient (h) of the densities of n-dodecane through n-

TABLE II

COMPARISON OF VALUES OF SPECIFIC GEOMETRIC CAPACITANCE OF SOLVENT-FREE GMO BILAYERS FORMED BY VARIOUS METHODS

Method	Reference	T	C_g
		°C	$\mu F/cm^2$
Apposition of monolayers	Benz et al. (1975)	20	0.745 ± 0.024
Solvent "freeze-out" (n-hexadecane, non equilibrium)	White (1974)	10	0.735
Solvent "freeze-out" (n-octadecane, near-equilibrium)	This paper	20	0.7903 ± 0.0013
Solvent exclusion (squalane/GMO)	This paper	20	0.7771 ± 0.0048

TABLE III

PHYSICAL PARAMETERS OF SQUALENE-FORMED BLACK LIPID BILAYER MEMBRANES AT VARIOUS TEMPERATURES

Temperature	A	δ_B	ϵ_B	ρ
°C	A^2/molecule	A		g/cm³
20	37.9 ± 0.2	25.06	2.200	0.8305
25	38.2 ± 0.2	25.02	2.193	0.8270
30	38.4 ± 0.2	24.98	2.186	0.8236
35	38.6 ± 0.2	24.95	2.179	0.8201
40	38.8 ± 0.2	24.92	2.173	0.8167

A is calculated from Eq. 4 and δ_B is calculated from Eq. 1. The calculation of ϵ_B and ρ is described in the text. Four digits are used in the posting of the values of δ_B, ϵ_B, and ρ so that the changes with temperature can be accurately reported. The values of ϵ_B and ρ at 20°C are from Requena and Haydon (1975), who gave the accuracies as 0.7% and 0.1%, respectively. The temperature dependences of ϵ_B and ρ are calculated as described by White (1974, 1975).

octadecane. The resulting equation for the density of the GMO acyl chain is $\rho = g + hT$, where $g = 0.8443$ g/cm³ and $h = 6.910 \pm 0.162 \times 10^{-4}$ g/cm³ per °C. The values of ϵ_B and ρ obtained at several temperatures are given in Table III, along with the values of A, calculated with Eq. 4 and the linear regression data for C_g (Fig. 1). A is plotted as a function of temperature in Fig. 3. The solid line through the points is the least squares-fit linear regression, which has a slope of 0.046 ± 0.002 Å²/°C. If the GMO molecules in the bilayer obey an equation of state of the type $\pi(A - A_0) = kT$, where π is the surface pressure and A_0 is the minimum cross-sectional area of the GMO molecule, then the temperature coefficient A can be estimated to be 0.05 Å²/°C (dashed

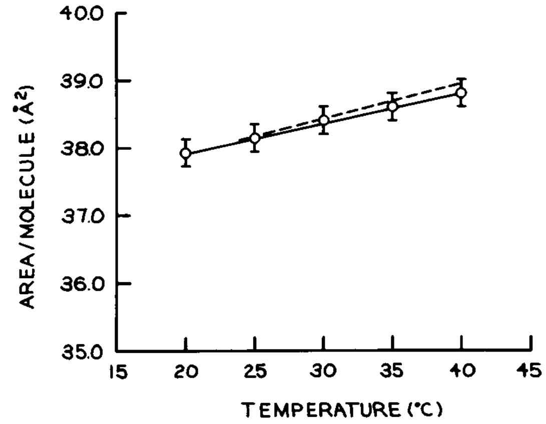

FIGURE 3 Temperature dependence of the area per GMO molecule in squalene-formed lipid bilayer membrane. A was calculated using Eq. 4 and the data for ϵ_B and ρ given in Table IV. The solid curve is the least-squares-fit straight line through the data, which has a slope of 0.046 ± 0.002 Å²/°C. The dashed curve represents the temperature dependence expected if the equation $\pi(A - A^0) = kT$ describes the behavior of the GMO molecules in the bilayers. The changes in A with temperature are those expected if no large changes in GMO interfacial absorption or squalene solubility occur.

TABLE IV
COMPARISON OF VALUES OF AREA PER GMO MOLECULE IN LIPID BILAYERS OBTAINED BY VARIOUS TECHNIQUES

Technique	Comments	Reference	T	A
			°C	A^2/*molecule*
Adsorption isotherm	Heptane/0.1 M NaCl interface	Andrews (1970)	20	39.5 ± 1.5
			25	37.5 ± 1.5
—	Hexadecane/ 0.1 M NaCl interface	—	20	38
Mercury drop	Hexadecane films in 0.1 M NaCl	Pagano et al. (1972)	25	42 ± 4
Specific capacitance	Squalene/GMO films in 0.1 M NaCl	This paper	20	37.9 ± 0.2
			25	38.1 ± 0.2

curve, Fig. 3). Thus, it appears that the changes in A with temperature are simple equation of state-type changes. This means that (*a*) interfacial adsorption of GMO is not strongly temperature-dependent and (*b*) the squalene content of the films is low. The latter conclusion is warranted, since the concentration of squalene in the bilayer should be strongly temperature-dependent (White, 1976) if it is present in significant amounts.

Also shown in Table III are the values of bilayer thickness calculated with Eq. 1. Note that the bilayer thickness does not change significantly over the 20°C temperature range. The change in capacitance is due largely to changes in ϵ_B and the thermal expansion of the bilayer goes almost exclusively into changes in A.

The values of A obtained as outlined above compare favorably with values obtained by other methods. All of the values agree within the experimental errors, as shown in Table IV.

CONCLUSIONS

It appears that "solvent-free" black lipid membranes can be obtained with a GMO-squalene-forming solution and that the area per molecule of the GMO in the bilayer can be simply determined. There is no reason to expect this approach to fail for the other monoglycerides but some caution should be exercised with the longer ones (C_{22} and C_{24}), because the squalene solubility might be significant in these cases. Benz et al. (1975) have shown that hexadecane is significantly more soluble in the C_{22} and C_{24} monoglycerides bilayers than in bilayers formed from the C_{14} through C_{20} monoglycerides. Difficulties may also be encountered if the technique is applied to lecithin bilayers. Fettiplace et al. (1971) found that lecithin-hexadecane films contained little or no hexadecane but that the area of the film expanded steadily in time, becoming much larger than the area of aperture. Such effects are due to fundamental physicochemical differences between the lecithin and monoglyceride black film systems.

Note Added in Proof: The squalene used in these experiments was found to have a small amount (a few percent) of nonsurface-active contaminant upon examination by gas chromatography. The contaminant had a lower boiling point than squalene and was probably a squalene isomer (natural squalene is all-trans) or an isoprene polymer of fewer than 6 units (squalene is a 6 unit isoprene polymer). Experiments performed with contaminant-free squalene prepared by fractional distillation gave results which were statistically identical with those reported here.

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REFERENCES

Alvarez, O., and R. Latorre. 1978. Voltage-dependent capacitance in lipid bilayers made from monolayers. *Biophys. J.* **21**:1–17.

Andrews, D. M., E. D. Manev, and D. A. Haydon. 1970. Composition and energy relations for some thin lipid films, and the chain conformation in monolayers at liquid-liquid interfaces. *Spec. Discuss. Faraday Soc.* **1**:46.

Andrews, D. M. 1970. Ph.D. thesis. University of Cambridge.

Benz, R., O. Frolich, P. Lauger, and M. Montal. 1975. Electrical capacity of black lipid films and of lipid bilayers made from monolayers. *Biochem. Biophys. Acta.* **394**:323.

Cook, G. M. W., W. R. Redwood, A. R. Taylor, and D. A. Haydon. 1968. The molecular composition of black hydrocarbon films in aqueous solutions. *Kolloid-Z. Z. Polym.* **227**:28.

Fettiplace, R., D. M. Andrews, and D. A. Haydon. 1971. The thickness, composition, and structure of some lipid bilayers and natural membranes. *J. Membr. Biol.* **5**:277.

Fettiplace, R., L. G. M. Gordon, S. B. Hladky, J. Requena, H. P. Zingsheim, and D. A. Haydon. 1975. Techniques in the formation and examination of "black" lipid bilayer membranes. *Meth. Membrane Biol.* **4**:1.

Hanai, T., D. A. Haydon, and J. Taylor. 1965. An investigation by electrical methods of lecithin-inhydrocarbon films in aqueous solutions. *Proc. R. Soc. Lond. A Math. Phys. Sci.* **281**:377.

Montal, M., and P. Mueller. 1972. Formation of biomolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. U.S.A.* **69**:3561.

Mueller, P., D. O. Rudin, H. Ti Tien, and W. C. Wescott. 1962. Reconstitution of excitable cell membrane structure in vitro. *Circulation.* **26**:1167.

Pagano, R. E., J. M. Ruysschaert, and I. R. Miller. 1972. The molecular composition of some lipid bilayer membranes in aqueous solution. *J. Membr. Biol.* **10**:11.

Requena, J., and D. A. Haydon. 1975. Van der Waals forces in oil-water systems from the study of thin lipid films. II. *Proc. R. Soc. Lond. A Math. Phys. Sci.* **47**:161.

Requena, J., D. A. Haydon, and S. B. Hladky. 1975. Lenses and the compression of black lipid membranes by an electric field. *Biophys. J.* **15**:77–81.

Simon, S. A., L. J. Lis, R. C. MacDonald, and J. W. Kauffman. 1977. The noneffect of a large linear hydrocarbon, squalene, on the phosphatidylcholine packing structure. *Biophys. J.* **19**:83.

Szabo, G., G. Eisenman, and S. Ciani. 1969. The effects of the macrotetralide antibiotics on the electrical properties of phospholipid bilayer membranes. *J. Membr. Biol.* **1**:346.

Takagi, M., K. Azuma, and U. Kishimoto. 1965. A new method for the formation of bilayer membranes in aqueous solution. *Annu. Rep. Biol. Works Fac. Sci. Osaka Univ.* **13**:107.

WHITE, S. H. 1970. A study of lipid bilayer membrane stability using precise measurements of specific capacitance. *Biophys. J.* **10**:1127.

WHITE, S. H. 1972. Analysis of the torus surrounding planar lipid bilayer membranes. *Biophys. J.* **12**:432.

WHITE, S. H. 1973. Surface charge and double layers of thin lipid films formed from neutral lipids. *Biochim. Biophys. Acta.* **323**:343.

WHITE, S. H. 1974. Temperature-dependent structural changes in planar bilayer membranes: solvent "freeze-out". *Biochim. Biophys. Acta.* **356**:8.

WHITE, S. H. 1975. Phase transitions in planar bilayer membranes. *Biophys. J.* **15**:95.

WHITE, S. H. 1976. The lipid bilayer as a 'solvent' for small hydrophobic molecules. *Nature (Lond.).* **262**: 421.

WHITE, S. H. 1977. A study of the physical chemistry of planar bilayer membranes using high precision measurements of specific capacitance. *Ann. N.Y. Acad. Sci.* **303**:243.

WHITE, S. H., and D. N. BLESSUM. 1975. High precision capacitance bridge for studying lipid bilayer membranes. *Rev. Sci. Instrum.* **46**:1462.

WHITE, S. H., and T. E. THOMPSON. 1973. Capacitance, area, and thickness variations in thin lipid films. *Biochim. Biophys. Acta.* **323**:7.

WHITE, S. H., D. C. PETERSEN, S. SIMON, and M. YAFUSO. 1976. Formation of planar bilayer membranes from lipid monolayers: a critique. *Biophys. J.* **16**:481.