FORMATION OF PLANAR BILAYER MEMBRANES
FROM LIPID MONOLAYERS
A CRITIQUE

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ABSTRACT The formation of planar bilayer membranes from lipid monolayers as
described by Montal and Mueller (Proc. Natl. Acad. Sci. 1972. 69:3561) is analyzed. Bilayers absolutely free of alkane solvents or other nonpolar hydrocarbons can be
formed on polytetrafluoroethylene (PTFE) (e.g. Teflon) septa only if certain boundary
conditions are satisfied. Measurements have been made of the contact angles between
monolayer-coated water and PTFE in the presence and absence of alkane solvents. The
measurements suggest that the boundary conditions for formation of stable bi-
layers can be satisfied only when a nonpolar solvent is present. We conclude that the
bilayer must be surrounded by a torus of alkane solvent, petroleum jelly, or silicone
grease depending upon the details of technique used to form the bilayer. The non-
polar solvent used in the formation of the bilayer may or may not be present in the
bilayer depending upon the water solubility and size of the solvent molecule relative
to the size of the alkyl chain of the lipid. Detailed sketches describing the formation of
bilayers from monolayers are presented.

INTRODUCTION
An important advance in biomembrane methodology was established when Mueller
et al. (1962) reported a method for forming planar lipid bilayer membranes separating
two aqueous phases. A dispersion of phospholipid or other surface-active lipid in a
nonpolar solvent such as decane is spread beneath an aqueous phase across an aperture
several millimeters in diameter drilled through a partition (septum) of some nonpolar
material such as polyethylene (PE), polytetrafluoroethylene (PTFE), or polychlorotrifluoroethylene (PCTFE). The bilayer forms spontaneously in the manner shown in
Fig. 1 and results in a system consisting of the bilayer in quasi equilibrium with an
annulus of bulk solution (also called the torus or Plateau-Gibbs border) which forms a
transition zone between the bilayer and septum. This system has been studied exten-
sively (see monographs by Jain, 1972 and Tien, 1974) as a model for the lipid bilayer of
cell membranes but its usefulness has been questioned because the thin lipid film con-
Summary of the process of formation of planar lipid bilayers from surface-active lipids dispersed in alkane solvents using the technique of Mueller et al. (1962). The lipid solution is applied beneath water across an aperture in a nonpolar material such as polychlorotrifluoroethylene (PCTFE). Note that the lipid solution "wets" the septum and forms an annulus or Plateau-Gibbs border around the boundary of the bilayer. The initially thick lipid film thins spontaneously because of the Plateau-Gibbs border suction. The cross-sectioned isometric sketch was made utilizing the analysis of the annulus given by White (1972).

Figure 1 Summary of the process of formation of planar lipid bilayers from surface-active lipids dispersed in alkane solvents using the technique of Mueller et al. (1962). The lipid solution is applied beneath water across an aperture in a nonpolar material such as polychlorotrifluoroethylene (PCTFE). Note that the lipid solution "wets" the septum and forms an annulus or Plateau-Gibbs border around the boundary of the bilayer. The initially thick lipid film thins spontaneously because of the Plateau-Gibbs border suction. The cross-sectioned isometric sketch was made utilizing the analysis of the annulus given by White (1972).

contains solvent molecules distributed within the bilayer per se and as microlenses floating in the bilayer (Henn and Thompson, 1968; Andrews and Haydon, 1968; Pagano et al., 1972; White and Thompson, 1973). To what extent the presence of the solvent limits the usefulness of the planar bilayer has not been established but it is clearly important to be able to form solvent-free planar bilayers.

One technique for forming bilayers containing little or no solvent is that reported by White (1974) in which the solvent is "frozen out" of the bilayer by lowering the temperature of bilayers formed by the technique of Mueller et al. (1962) to below the melting point of the solvent.

A method for forming bilayers from lipid monolayers which is supposed to eliminate solvent has been developed by Montal and Mueller (1972). The technique as described by Montal (1974) is illustrated in Fig. 2. Two PTFE troughs containing electrolyte are separated by a thin (6.5–25 μM) septum through which a hole 0.1–0.5 mm in diameter is punched. A monolayer is spread from pentane or hexane on each electrolyte solution. After the solvent has evaporated, the water level in one and then the other trough is raised above the top of the aperture bringing the alkyl chains of the monolayers into apposition. The Montal-Mueller (1972) technique is a particularly significant achievement because asymmetric bilayers can be formed (Montal, 1973) and
FIGURE 2 The process of forming a "solvent-free" bilayer from lipid monolayers as proposed by Montal and Mueller (1972). The septum (PTFE) is shown to have about the same thickness as the bilayer but this is an artistic convenience. In reality, the septum is about $10^3$ times thicker than the bilayer. The angle $\theta$ is the contact angle of the monolayer-coated aqueous phase on the PTFE septum. $\theta$ is shown here as being greater than 90° as it must be if the formation is to take place as shown. In fact, $\theta \ll 90°$ so that this scheme for formation will not work.

FIGURE 3 One portrayal of the junction between the bilayer and the septum assuming no solvent is present. This scheme is impossible if the bilayer film has a nonzero surface tension ($\gamma_f$) which must be balanced by an equal and opposite tension ($\gamma_{AW}$) in the monolayers on the surface of the septum. The surface monolayer is normal to the bilayer and cannot therefore exert a force parallel to the bilayer (see force diagram).

FIGURE 4 An alternate portrayal of the junction between the bilayer and septum in which there is assumed to be an "air pocket" forming a transition zone (annulus). The angle $\alpha$ is estimated to be about 89° while $\beta$ is estimated to be 155° for glycerol monooleate. This combination of angles is unlikely to result in a stable membrane. The force diagram illustrates the balance of forces ($\gamma_f = 2\gamma_{AW} \cos \alpha$) necessary for a stable membrane.

proteins can be incorporated by first introducing them into the monolayers (Montal and Korenbrot, 1973). Because of these advantages it is important to understand how and under what conditions a bilayer may be formed from the monolayers. Work in our laboratory and the laboratories of other leads us to conclude that the bilayer is not being formed as shown in Fig. 2. The purpose of this paper is to set forth the necessary conditions for the formation of bilayers from lipid monolayers.

QUESTIONS AND OBSERVATIONS

It must be understood at the outset that the septum is at least three orders of magnitude thicker than the bilayer. The septum in Fig. 2 is shown to have about the same thickness as the bilayer but this is strictly for artistic convenience. The thinnest septum recommended by Montal (1974) is $6.5 \times 10^{-6}$ m as compared with $2.5 \times 10^{-9}$ m for the bilayer. Assuming the aperture has a right cylindrical geometry and the bilayer is located roughly in the midplane of the septum, Fig. 3 more accurately portrays the bilayer-septum junction. This portrayal further assumes that there is no torus or other transition zone between bilayer and septum, as must be true if absolutely no solvent is
present. Is such a configuration reasonable or even possible? The answer is no if the film tension $\gamma_f$ is not zero, as the following arguments will show. Benz et al. (1975) have measured $\gamma_f$ for glycerol monooleate bilayers formed by the Montal-Mueller technique and find a value of 1.2 dyn/cm. Each monolayer on the surface of the aperture must, at equilibrium, exert an equal and opposite tension, otherwise the bilayer will collapse. For the arrangement shown in Fig. 3, the tension of the monolayers on the aperture surface is normal to that of the bilayer and can exert no tension parallel to the plane of the bilayer (see force diagram, Fig. 3). Therefore Fig. 3 must be an incorrect representation.

An alternate portrayal of the boundary of a solvent-free bilayer is shown in Fig. 4 in which there is a transition zone (called a torus, annulus, or Plateau-Gibbs border) of air. The surface monolayer is shown as making an angle $\alpha$ with the bilayer and an angle $\beta$ with the septum surface. The angle $\alpha$ is determined by the equation $\gamma_f = 2\gamma_{AW} \cos \alpha$ where $\gamma_{AW}$ is the interfacial tension at an air-water interface with an adsorbed monolayer (see force diagram, Fig. 4). The equilibrium spreading pressure $\pi$ of glycerol monolaurin at 25°C is known to be about 35 dyn/cm and probably the value for glycerol monooleate is not much different (Phillips and Hauser, 1974). $\gamma_{AW}$ can be determined using the equation $\gamma_{AW} = \gamma_o - \pi$, where $\gamma_o = 72$ dyn/cm is the interfacial tension of a clean air-water interface. $\gamma_{AW}$ is found to be about 37 dyn/cm and $\alpha$ to be about 89°. The portrayal to this point seems reasonable provided $\beta \leq 90^\circ$. Is this true? To answer this question the contact angle $\theta = 180^\circ - \beta$ (see Fig. 2) of water coated with a glycerol monooleate (GMO) monolayer on a clean PTFE surface was measured. A droplet of water ($\sim 10^{-2} \mu$L) was placed on a horizontal PTFE surface and illuminated from behind. A small glass rod ($\sim 1$ mm in diameter) was dipped into a dispersion of GMO in hexane, removed, the solvent allowed to evaporate, and then touched to the droplet surface. The contact angle immediately decreased from greater than 90° to about 20-30°. A photograph of the monolayer-coated water droplet in air on the PTFE surface is shown in Fig. 5. This result is significant, for it means that water coated with a glycerol monooleate monolayer wets PTFE ($\theta < 90^\circ$). In Figs. 2 and 4 it has been assumed that the PTFE is not wetted ($\theta > 90^\circ$) so that the monolayer acyl chains come naturally into apposition as the bilayer is formed. Taking $\theta = 25^\circ$, $\beta = 155^\circ$ so the condition $\beta \leq 90^\circ$ cannot be satisfied. Therefore, it is unlikely that glycerol monooleate bilayers can be formed as shown in Fig. 4.

Montal and Mueller (1972) were in fact able to form bilayers in some way approximating the scheme of Fig. 2. How is this possible? The answer is to be found in the work of Benz et al. (1975) who observed: "When monoglyceride was added as an n-hexane solution to both water surfaces and a long time (several minutes) was allowed for the solvent to evaporate, no membrane could be obtained on a carefully cleaned plastic septum. With shorter waiting times, or where a small amount of n-hexane was

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1Thermodynamically, $\beta$ may be greater than 90° leading to a convex torus. It seems unlikely to us that such a geometry could be established during formation.
added to the solvent-free monolayers, bilayers could be formed." Montal (1974) notes: "To improve the stability of the membranes the septum may be preconditioned by cleaning with pentane, hexane, or a 2% solution of petroleum jelly in pentane." Thus, a necessary condition for bilayer formation appears to be the presence of an alkane solvent or other nonpolar hydrocarbon on the surface of the septum. The solvent probably acts to form a transition zone (i.e. torus or annulus) between the bilayer and septum. This transition region permits the necessary boundary conditions of the bilayer to be satisfied (White, 1972).

A hydrocarbon solvent can aid the formation of the bilayer through the creation of an annulus provided the angles $\alpha$ and $\beta$ are each less than 90° and $\alpha + \beta \leq 90°$ (White, 1972). Haydon and Taylor (1968) have shown that $\alpha \sim 2°$ for an annulus containing an alkane solvent. An approximate value of $\beta$ can be determined from photographs of droplets of water resting on a PTFE surface immersed in a hydrocarbon solvent in which glycerol monooleate has been dispersed. Fig. 6 shows such a photograph for $n$-decane in which $\theta = 150°$ giving $\beta = 180 - 150° = 30°$. Therefore, $\alpha + \beta = 32° < 90°$. Note that now, in the presence of the hydrocarbon solvent, the water droplet with absorbed glycerol monooleate monolayer does not "wet" the PTFE
FIGURE 7  A sequence of sketches showing what happens if one attempts to form bilayers from monolayers in the absence of an alkane or other nonpolar solvent. The raising of the left-hand water level "peels off" the monolayer on the right aqueous phase shown here protruding through the aperture (third and fourth frames). The septum is shown for artistic convenience as being thin. In reality, the septum is much thicker (see Fig. 3).

surface. This permits the alkyl chains of the monolayers to have a favorable arrangement for coming together to form a bilayer.

FORMATION OF BILAYERS FROM MONOLAYERS

With the observations and conclusions given above, we can now speculate as to how and under what conditions bilayers are formed from monolayers. Before doing so, however, one additional observation is important. If one observes the aperture through a microscope from the front as the rear monolayer-coated aqueous phase is raised above the aperture (the water level in the front chamber being below the aperture), the water can be seen to break through to the front surface and form a meniscus over the aperture. This occurs by capillary action as expected from the fact that the monolayer-coated aqueous phase wets the PTFE. If the rear aqueous phase is then lowered, a droplet of water remains in the aperture. This droplet is coated on both surfaces by a monolayer and, under proper lighting conditions, a "soap" film is seen to form complete with the colored Fizeau bands. These observations mean that the bilayer, if it forms, forms on the front surface of the septum and not in the midplane of the aperture.

Consider now the attempted formation of a bilayer in the absence of a nonpolar solvent. The steps in the formation are shown in Fig. 7. Note that the monolayer-coated aqueous phase wets the PTFE surface. The third frame of the figure shows the situation with the rear water level raised and the front water level in the process of being raised. The geometry is such that as the front monolayer is raised it becomes continuous with the monolayer on the aqueous layer penetrating through the aperture. As it is raised further, the monolayer covering the aperture's aqueous phase is simply "peeled off" leaving the aqueous phases on either side of the septum in direct contact. This scheme explains why Benz et al. (1975) were unable to form bilayers when no solvent was present.

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Next consider what happens when some nonpolar solvent remains associated with the monolayer (Fig. 8). The solvent wets the surface ($\theta = 15^\circ$ for heptane on PTFE [see Bargeman, 1972]) and the contact angle between the monolayer-coated aqueous phase and PTFE septum is greater than $90^\circ$. The monolayers now have the proper geometry for coming together to form a bilayer as shown in the third and fourth frames of Fig. 8. The advancing solvent phases meet and form an annulus of solvent as the bilayer is formed. The bilayer with annulus probably shifts back somewhat into the aperture after the bilayer is formed. The presence of the annulus permitted Benz et al. (1975) to “bulge” the bilayer by differences in the front and back water levels. That bulging did occur was indicated by increases in capacitance.

CONCLUSIONS

It appears to us that bilayers formed from monolayers are qualitatively equivalent to bilayers formed from dispersions of lipids in alkane solvents. A necessary condition for formation is the presence of a nonpolar solvent to form an annulus to satisfy the boundary conditions of the film. This can be fulfilled by either the spreading solvent or the petroleum jelly and silicone grease used under some circumstances in the preparation of the septum. This raises the question of whether or not the bilayer contains solvent. The specific capacitance of bilayers formed from hexane-glycerol monooleate increases with time after formation and asymptotically approaches a limiting value. This was interpreted to mean that solvent was present initially in the bilayer causing it to be thicker and consequently have a lower capacitance.\(^2\) Hexane has a limited solubility in water (see Bell, 1973) and in time probably diffuses into the aqueous phase

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\(^2\)The bilayers were stable for only a few minutes after the limiting value of capacitance was reached. We interpret this to mean that some solvent remained in the annulus but was lost in a few minutes to the aqueous phase thereby creating an instability.
and ultimately escapes into the atmosphere. The loss of solvent causes the bilayer to become thinner and the capacitance to increase. The limiting value of capacitance probably represents that of a solvent-free bilayer. It appears that petroleum jelly can act as an annulus also (Benz et al., 1975). In this case, however, if it is in the bilayer it probably appears as microlenses and not as part of the bilayer per se because the petroleum jelly is composed of long-chain hydrocarbons. White (1975) and Benz et al. (1975) have shown that as the chain length of the solvent approaches that of the alkyl chains of the lipid, the solvent tends to be excluded from the bilayer structure per se. Even though a solvent may be too large to fit into the bilayer structure, it nevertheless forms a satisfactory annulus and can exist as microlenses floating in the bilayer.

We thus believe that bilayers formed from monolayers are not necessarily completely free of solvent. We do not, however, wish to imply that the technique of Montal and Mueller (1972) is not important or should be discarded. It is a convenient way of forming asymmetric bilayers and offers new possibilities for the incorporation of proteins into bilayers. The findings presented here do suggest, however, that care be exercised in interpreting experiments using the technique. Solvent may be present in the membranes and the model for formation shown in Fig. 2 is probably not correct.

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This research was supported by grants from the National Science Foundation (GB-40054) and the National Institute of Neurological Disease and Stroke (NS-10837) and an ORN contract number N0014-67-A-0251-0022. Dr. Petersen was supported by a Small College Faculty Award awarded by the NSF as a supplement to GB-40054.

Received for publication 15 September 1975.

REFERENCES


WHITE ET. AL. *Formation of Planar Bilayer Membranes from Lipid Monolayers* 489