SUPPORTED PHOSPHOLIPID BILAYERS

LUKAS K. TAMM AND HARDEN M. MCCONNELL
Stauffer Laboratory for Physical Chemistry, Stanford University, Stanford, California 94305

ABSTRACT Phospholipid bilayers have been formed on glass, quartz, and silicon surfaces by a sequential transfer of two monolayers at a pressure of ~40 dyn/cm from the air-water interface to the solid substrates. Lateral diffusion measurements of L-α-dipalmitoylphosphatidylcholine (DPPC) bilayers supported on oxidized silicon wafers reveal two sharp phase transitions at temperatures similar to those found in multilayer systems with several different techniques. The diffusion measurements obtained using fluorescence recovery after pattern photobleaching provide evidence for the existence of an intermediate (probably $P_{\beta'}$ or ripple) phase in single bilayers. While in the intermediate and high temperature (liquid-crystalline L_α) phase, the diffusion coefficients do not vary very much with temperature, a strong temperature dependence is observed in the low temperature (gel $L_{\beta'}$) phase. This is attributed to defect-mediated diffusion. Lipids in silicon supported bilayers made from L-α-dioleoylphosphatidylcholine (DOPC) or L-α-dimyristoylphosphatidylcholine (DMPC) diffuse rapidly above their respective chain-melting transition temperatures. Arrhenius plots show straight lines with activation energies of 40.9 and 43.7 kJ/mol, respectively. Supported DPPC bilayers on oxidized silicon form long tubular liposomes when heated through their chain-melting-phase transition, as viewed with epifluorescence microscopy. It is suggested that this is a consequence of the expansion of the lipid on the fixed solid support. Conversely, DOPC bilayers form large void areas on this substrate upon cooling. Large circular membrane defects (holes) are observed under rapid coating conditions. The formation of these defects is modulated by including small amounts of lyso-L-palmitoyl phosphatidylcholine in the DMPC-supported bilayers. A simple model describes the dependence of hole size and hole number on the concentration of lysolecithin.

INTRODUCTION

Membrane-membrane interactions have attracted the interest of cell biologists, biochemists, and biophysicists for a long time. In recent years, a number of different approaches have been used to mimic effector-cell/responder-cell interactions by replacing the effector or responder cell with liposomes or reconstituted membranes. Especially in the field of immunology, many typical cellular responses can be triggered by model membranes (Kinsky and Nicoletti, 1977; Littman et al., 1979; Hafeman et al., 1980; Herrmann and Mescher, 1981; Cartwright et al., 1982; Balakrishnan et al., 1982; Albert et al., 1983). To investigate the contact region of the two interacting membranes at a microscopic level, a planar membrane model system was developed in this laboratory some years ago (von Tscharner and McConnell, 1981*b*: Hafeman et al., 1981). Briefly, a lipid monolayer is transferred from the air-water interface onto a previously alkylated (i.e., hydrophobic) solid substrate. Especially when combined with the technique of fluorescence excitation in an evanescent radiation field, substrate-supported monolayers become very attractive for membrane-cell interaction studies (Weis et al., 1982).

One limitation of this system is the difficulty of reconstituting transmembrane proteins into monolayers. (This problem can be circumvented to some extent by trypsinizing the protein before incorporation; Nakanishi et al., 1983.) Bearing this in mind, we have developed a substrate-supported bilayer system. Here we report lateral-diffusion measurements in bilayers of different phospholipids (DPPC, DMPC, and DOPC)[1] supported on oxidized silicon. These results provide a first physical characterization of lipid bilayers on solid supports, and show that the diffusivity of lipid molecules in these membranes is virtually identical to that in the conventional multilayer systems. These measurements should answer some prevailing questions regarding the relationships of certain physical properties of supported lipid monolayers, supported lipid bilayers, isolated lipid bilayers, and multibilayers.

MATERIALS AND METHODS

DPPC, DMPC, and lyso-L-palmitoylphosphatidylcholine were purchased from Sigma Chemical Co. (St. Louis, MO) or Calbiochem-Behring Corp. (La Jolla, CA); DOPC purchased from Sigma Chemical Co. or Avanti Polar Lipids, Inc. (Birmingham, AL); NBD-PE and NBD-DPPE purchased from Avanti. Lipids were used without further purification.

Silicon wafers (p-type, 111) were obtained from Aurel (Santa Clara, CA). To prevent fluorescence quenching by the semiconducting material, ~800-nm thick surface oxide layers were grown thermally. After stan-

[1] *Abbreviations used in this paper:* DPPC is L-α-dipalmitoylphosphatidylcholine; DMPC is L-α-dimyristoylphosphatidylcholine; DOPC is L-α-dioleoylphosphatidylcholine; DSPC is L-α-distearoylphosphatidylcholine; NBD-PE is *N*-(7-nitro-2,1,2-benzoxodiazol-4-yl) egg phosphatidylethanolamine, and NBD-DPPE is *N*-(7-nitro-2,1,2-benzoxodiazol-4-yl) dipalmigoylphosphatidylethanolamine.

dard cleaning procedures, oxidation was performed in a furnace at 1,000°C in the presence of high O_2 (10 min), H_2/high O_2 (225 min) and high O_2 (10 min).

The thick oxide wafers were cut to the desired shape, boiled in diluted Linbro 7× detergent (1:5) for 10 min, sonicated for 30 min, and rinsed with distilled water for 3 h. An 8-min treatment with $H_2O/H_2O_2/NH_4OH$ (5:1:1) was followed by a 10-min treatment with $H_2O/H_2O_2/HCl$ (5:1:1) and immersion in diluted HF (1:50) for 1 min. After each step the wafers were rinsed extensively with distilled water. After the last rinse they were dried in an oven at 150°C for 1 h. Immediately before coating with lipids the wafers were cleaned in a plasma cleaner (Harrick Scientific Corp., Ossining, NY) for 10 min using an argon plasma. Glass and quartz slides were boiled in diluted Linbro 7× (1:5) for 10 min, sonicated for 30 min in a bath sonifier, and rinsed with distilled water for 3 h. They were then dried in an oven for 1 h at 150°C and plasma cleaned for 10 min immediately before use.

Monolayers were spread at the air-water interface in a Langmuir trough similar to that described previously (von Tscharner and McConnell, 1981*a*). Milli-Q purified water was used as subphase (Millipore/Continental Water Systems, Bedford, MA). Lipids were spread as a 1.25 mM 9:1 hexane/ethanol solution. Fluorescent-probe concentrations of 0.5% mol were usually used for diffusion measurements, whereas 2% mol was employed when taking epifluorescence photographs. The surface pressure was measured with a Wilhelmy plate made from a Millipore filter (0.22 μm, GS Triton free) (Millipore/Continental Water Systems) 2.5 cm wide and attached to a torsion balance. After spreading, 15–20 min were usually allowed for solvent evaporation. The lipid monolayers were then slowly compressed to the desired surface pressure.

The monolayers were left at this pressure for at least 3 h for solid membranes (30 min for fluid membranes) for equilibration. During this time period the lateral pressure usually dropped slightly (e.g., for DPPC, a pressure drop from 42 to 35 dyn/cm was typical), and was then readjusted to its original value. A clean hydrophilic piece of a silicon wafer (or glass coverslip or quartz slide) was then immersed vertically through the monolayer into the trough. No significant change in surface pressure was observed at this step. The wafer was then pulled out at a speed just slow enough to permit water to drain from the surface. A substantial drop in surface pressure was then observed and compensated for by reducing the area of the trough. The loss of surface area was approximately equal to twice the area of the substrate. The substrate was then mounted onto the tip of a Pasteur pipette under suction and pushed through the air-water interface horizontally. The surface pressure decreased again and recompression yielded an area decrease corresponding to roughly 1.5 times the area of the substrate. A glass coverslip was attached to the substrate with double-stick tape under water.

The lateral diffusion of the lipid was measured by pattern photobleaching (Smith and McConnell, 1978; Smith et al., 1980) with a photomicroscope (Zeiss III, Carl Zeiss Inc., Thornwood, NY) using a 40× water-immersion objective. The sample was placed in a small temperature-controlled water bath on the microscope stage and a thermocouple was attached in sliding contact with the coverslip close to the region of inspection. Epifluorescence photographs were taken with the built-in microscope camera using 1,000 ASA recording film (Eastman Kodak Co., Rochester, NY). The sample was illuminated with the bleach beam (~10 W/mm²) of the optical system used for photobleaching. Exposure times of only a fraction of a second were typically used.

RESULTS

Pressure-area isotherms of three different phospholipid monolayers at the air-water interface are shown in Fig. 1. The lipids used are DPPC, DMPC (saturated fatty acyl chains) and DOPC (containing a *cis*-double bond in both fatty acyl chains). Their calorimetric chain–melting-phase transitions ($P_{\beta'}$ to L_α) are 41°C, 24°C, and −22°C, respectively. Pressure-area curves are taken at 21°C (DPPC and DOPC) and 22°C (DMPC).

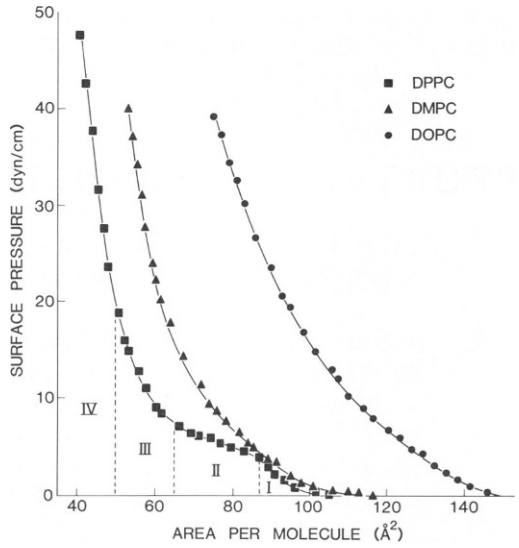

FIGURE 1 Pressure-area isotherms for phosphotidylcholines spread on deionized distilled water are illustrated. ■, DPPC at 21°C; ▲, DMPC at 22°C; ●, DOPC at 21°C.

The DPPC isotherm at 21°C shows the characteristic features described by several different laboratories (e.g., Albrecht et al., 1978, and references therein). Briefly, four different regions can be readily distinguished. Region I represents a homogeneous fluid monolayer with a decreasing compressibility as the area per molecule is decreased. At the high molecular area end of this region, heterogeneous lipid distribution has been observed that is thought to be due to the coexistence of a fluid and a gaseous lipid phase (Lösche et al., 1983; Tamm, L., R. Weis, and H. McConnell, unpublished results). Region II has been shown to be a coexistence region of solid and fluid lipid (Peters and Beck, 1983) where solid lipid domains form periodic patterns (McConnell et al., 1984). In region III, solid lipid domains are interconnected, but still coexist with fluid lipid. Long-range lateral diffusion is therefore slow in this region. Finally, in region IV all lipid is in a solid state. In this region, lateral compressibility is low and the aliphatic chains are thought to be densely packed. DMPC and DOPC exhibit isotherms at 22 and 21°C, respectively, characteristic for one single phase at all measured pressures. This is a homogeneous fluid phase, similar to that in region I of the DPPC curve.

Compressed monolayers of DPPC, DMPC, and DOPC have been transferred from the air-water interface onto hydrophilic substrates in two sequential steps. Glass, quartz, and oxidized single-crystal silicon wafers have been used as substrates. The first monolayer is taken up by the hydrophilic surface of the substrate when it is slowly pulled through the interface at a 90° angle. A reduction of the total trough area by ~2× (±10–20%) the substrate area readjusts the surface pressure to its previous value. This is

expected if both faces of the substrate are coated with a continuous monolayer of approximately the same molecular density that it had on the air-water interface. When 0.5 mol % of NBD-PE was incorporated into a DPPC monolayer and transferred onto a silicon wafer, inspection of the (dry) monolayer-coated substrate under the epifluorescence microscope reveals a surface that is uniformly fluorescent. All three phospholipid monolayers show no lateral diffusion under these conditions. In a second step the monolayer-coated substrate is apposed horizontally to the recompressed monolayer at the air-water interface for a few seconds to allow for equilibration and then pushed through the air-water interface. A coverslip is placed over the bilayer surface using double-stick tape. On recompression the total trough area is decreased by ~1.5 times the area of the substrate. (A factor of >1 is presumably due to some lipid deposited on the backside of the substrate.) Visualization under the epifluorescence microscope again shows uniformly fluorescent surfaces with all three phospholipids. The fluorescence intensity increases by about a factor of two after the second monolayer has been transferred onto a thick oxide silicon wafer.

Lateral diffusion measurements have been carried out at various temperatures. Fig. 2 shows the diffusion coefficients obtained from DPPC bilayers on silicon wafers as a function of temperature. The figure includes data from

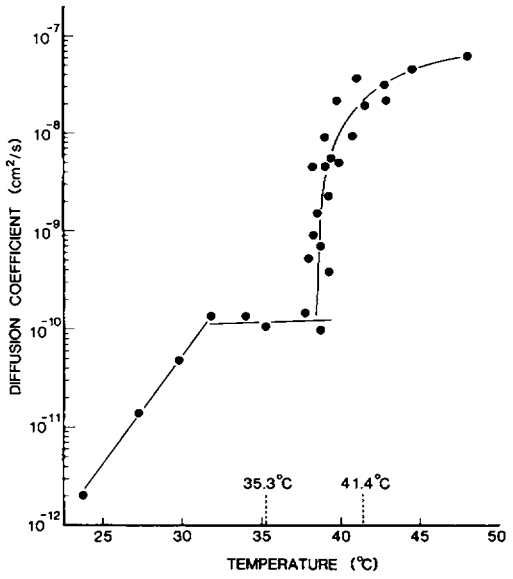

FIGURE 2 Lateral diffusion coefficients of the fluorescent lipid probe NBD-PE (0.5 mol %) in single DPPC bilayers supported on oxidized silicon are shown. Thermal phase transitions occur at ~32 and 40°C. Markings at 35.3 and 41.4°C indicate the midpoints of the pretransition and the chain-melting-phase transition, respectively, obtained by differential scanning calorimetry on multilayered DPPC liposomes (Mabrey and Sturtevant, 1976). The bilayers were coated at 42–45 dyn/cm. Each data point is an average of at least four individual bleach experiments, and data from nine different samples are included in this plot. Data are taken on heating and cooling. These data provide evidence for the existence of an intermediate (probably $P_{\beta'}$) phase in single bilayers.

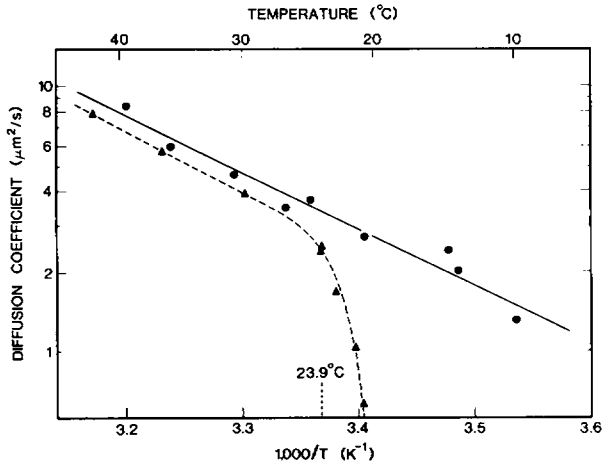

FIGURE 3 Arrhenius plot of the lateral diffusion coefficients of the fluorescent lipid probe NBD-PE (0.5 mol %) in single DOPC (●) and DMPC (▲) bilayers supported on oxidized silicon is shown. The bilayers were coated at 36.5 dyn/cm. Data points are averages of 4–6 individual bleach experiments. Activation energies are 40.9 kJ/mol (DOPC) and 43.7 kJ/mol (DMPC, >28°C). Data are taken on heating.

nine different samples that were all coated at 42–45 dyn/cm. Each datapoint is an average diffusion coefficient computed from at least four individual fluorescence recovery curves fitted to a single exponential. In most cases the fits to a single exponential were very good, and full (100%) recovery was observed. In a few cases the recovery curves were more complex; these were not included in the present analysis.[2] Three different regions can clearly be distinguished in Fig. 2: (*a*) a low temperature region between 24 and 32°C with diffusion coefficients steeply increasing from $\sim 2 \times 10^{-12}$ to 10^{-10} cm²/s; (*b*) a plateau region between 32 and 39°C where D is close to 10^{-10} cm²/s; and (*c*) a region with sharply increasing diffusion coefficients (39–42°C) leveling off between 42 and 48°C. The diffusion coefficients approach a value of $5–6 \times 10^{-8}$ cm²/s at 46°C. This value is typical of diffusion coefficients of lipids in fluid bilayers. The three regions are separated very clearly by two sharp transitions that occur at ~32 and 40°C. These values are very close to the thermal phase transitions observed in multibilayer systems of DPPC under a variety of techniques.

Diffusion data obtained from DMPC and DOPC bilayers supported on silicon wafers are presented in Fig. 3 in the form of Arrhenius plots. At any temperature above their chain-melting phase-transition temperature, diffusion is fast ($1–8 \times 10^{-8}$ cm²/s) and recovers 100% according to a single exponential. Straight lines are

[2]It is possible that some of the samples were slightly heterogeneous with respect to lipid diffusion, i.e., in some small areas the bottom leaflet of the bilayer may interact with the substrate more strongly and thus lead to double-exponential recovery curves. This behavior is only occasionally observed with DPPC at low temperatures and in the transition region. All samples exhibit uniform monophasic diffusion in the liquid-crystalline phase.

obtained in the Arrhenius plots yielding activation energies of 43.7 and 40.9 kJ/mol for the two lipids, respectively. These diffusion coefficients and activation energies are in good agreement with earlier lipid diffusion measurements on multibilayers (for a review, see Vaz et al., 1982).

Under certain conditions, inhomogeneous lipid structures or bilayer defects can be observed on solid supports. In the first two cases that we describe here, the distortions are generated by temperature variations, and in the third case, membrane defects are introduced by rapid coating and by the addition of lysolecithin.

(*a*) When DPPC bilayers are coated at 42–45 dyn/cm and room temperature and then heated to 43°C (where the chain–melting-phase transition is complete), we observe the formation of very long tubular liposomes (Fig. 4 *a*). These lipid tubes grow from the supported bilayer into the surrounding aqueous medium. The planar bilayer still remains continuous and shows long-range lateral diffusion.

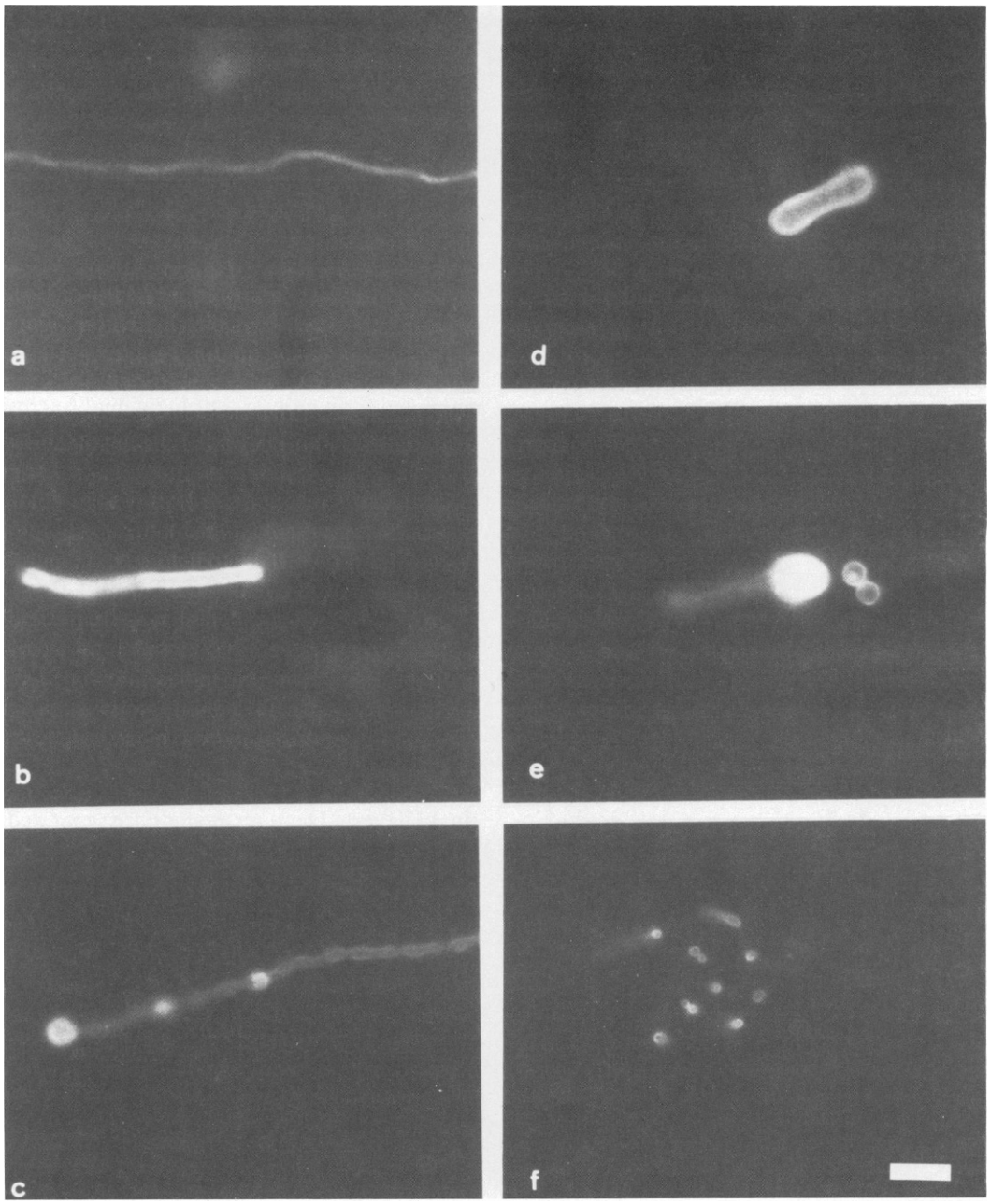

FIGURE 4 Epifluorescence microscope photographs of DPPC liposomes (containing 2 mol % NBD-PE) attached to silicon-supported DPPC bilayers at temperatures ≥43°C are shown. The bilayers were prepared at room temperature and showed uniform epifluorescence at temperatures <41°C. Each section as marked shows the following: (*a*) thin highly flexible tube, (*b*) more rigid, wider tube, (*c*) helix formed from two thin tubes, (*d*) tube contracting to a large spherical liposome, (*e*) and (*f*) spherical liposomes. Bar is 10 μm. These various structures result from the thermally induced expansion of an otherwise uniform supported bilayer.

The tubes can be over 200 μm long with a diameter of ~1 μm. Often they are attached to one end to the planar bilayer with their other end moving freely in solution. Lipid diffusion along these tubes is fast. It is likely that the wall of these tubes consists of a single bilayer. These long tubes are stable for several minutes, but then often contract rapidly into tubes that are much shorter, typically ~3 × 40 μm (Fig. 4 *b*). Their fluorescence is very bright, suggesting that they are multilamellar. Occasionally, helical structures are seen (Fig. 4 *c*; see Lin et al., 1982). Tubes such as those seen in Fig. 4 *b* contract slowly (Fig. 4 *d*) until they assume a spherical shape (Fig. 4, *e* and *f*). Many of these spherical liposomes remain attached to the supported bilayer, but some of them float freely in solution. The mobile liposomes interfere with diffusion measurements at high temperatures. The release of lipid from the supported bilayer into a different (vesicle) phase is partially reversible upon cooling, as shown by the addition of fluorescent vesicles to a nonfluorescent supported bilayer.

(*b*) DOPC bilayers formed at 35 dyn/cm and room temperature on silicon wafers are usually uniformly fluorescent. When this membrane is cooled to 8°C, large black regions (20–40 μm) with irregular boundaries are observed in the epifluorescence microscope (Fig. 5). When the bilayer is reheated to the original temperature, these regions remain. To a first approximation, lateral lipid diffusion is reversible in these membranes.

(*c*) Defects are also introduced into supported lipid bilayer membranes when the first monolayer is coated rapidly. (Some water remains associated with the slide.) This is demonstrated in Fig. 6, *a–d* for DMPC bilayers containing 2 mol % NBD-DPPE and supported on quartz slides. Under these conditions a gradient of membrane defects with increasing size and number is established from about the center of the slide to the edge that has been coated last with the first monolayer (Fig. 5, *a–d*). It is clear that the dark areas in Fig. 6 actually represent holes in the membrane, since the employed fluorescent phospholipid probe NBD-DPPE has been shown to partition into both solid and fluid lipid phases (McConnell et al., 1984). Hole sizes range from ~2–8 μm. When 1 mol % lyso-L-palmitoyl-phosphatidylcholine is included in the monolayer at the air-water interface, rapidly coated bilayers still show a similar gradient of holes, but the hole size is decreased, whereas the hole number is increased compared with the bilayers containing no lysolecithin (range ~0.5 to 4 μm) (Fig. 6, *e–h*). Smaller lysolecithin concentrations gave intermediate results (data not shown).

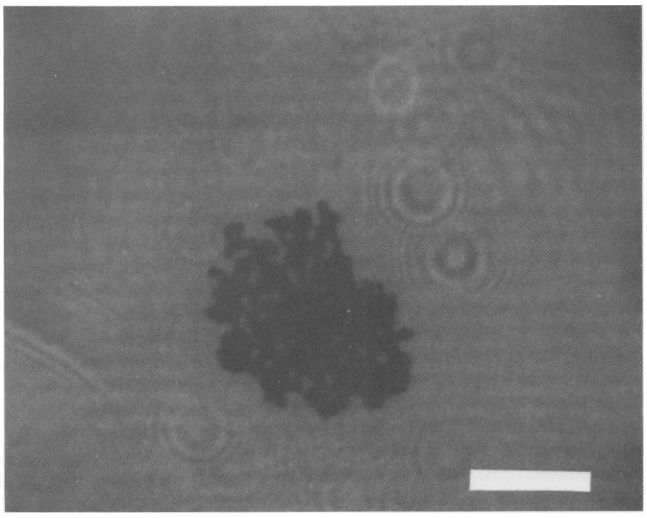

FIGURE 5 Epifluorescence microscope photograph of a large membrane defect induced by cooling an originally uniform DOPC bilayer (containing 2 mol % NBD-PE) from 24°C to 8°C is shown. The bilayer is supported on an oxidized silicon wafer. Bar is 20 μm. This defect results from the thermally induced contraction of an otherwise uniform supported lipid bilayer.

DISCUSSION

Our results show that it is possible to support planar lipid bilayers on various hydrophilic solid substances. The nearly stoichiometric transfer of two monolayers and the fact that many physical properties are the same for supported lipid films as for multibilayer systems suggests very strongly that we have indeed formed substrate-supported lipid bilayers.

Long-range lateral diffusion has been observed for all three phospholipids that we have employed in this study. The measured diffusion coefficients agree very well with those measured on multibilayers. It is interesting that at least to the resolution of our experiment the lipids diffuse at the same rate in both leaflets of the bilayer (see below). Diffusion measurements on DPPC bilayers clearly show two thermal transitions, one at ~32°C and one at 40°C. These temperatures are very close to the transition temperatures that have been measured for DPPC by differential scanning calorimetry (Mabrey and Sturtevant, 1976) and other techniques. This provides convincing evidence for the existence of an intermediate phase in single bilayers. It is very likely that this is the $P_{\beta'}$ phase, well known from studies of multibilayer systems. In this phase the diffusion coefficients of DPPC bilayers show little temperature dependence. Recent NMR evidence (Wittebort et al., 1982) as well as theoretical considerations (Falkowitz et al., 1982; Marder et al., 1984) suggest that some of the lipid is in a quasi-fluid state in the $P_{\beta'}$ phase. This might account for the lack of significant activation energy for diffusion.

There is a strong temperature dependence of the diffusion coefficients of DPPC bilayers below the pretransition. When the data are analyzed using an Arrhenius plot, we find an apparent activation energy of 400 kJ/mol. This energy is much too high for a unimolecular diffusion process. It is possible that the mechanism of lateral diffusion in this phase involves submicroscopic defect structures (Sackmann et al., 1980). Smith and McConnell (1978) and Schneider et al. (1983) measured lateral diffusion in DMPC multilayers between 10 and 23°C. In both cases a

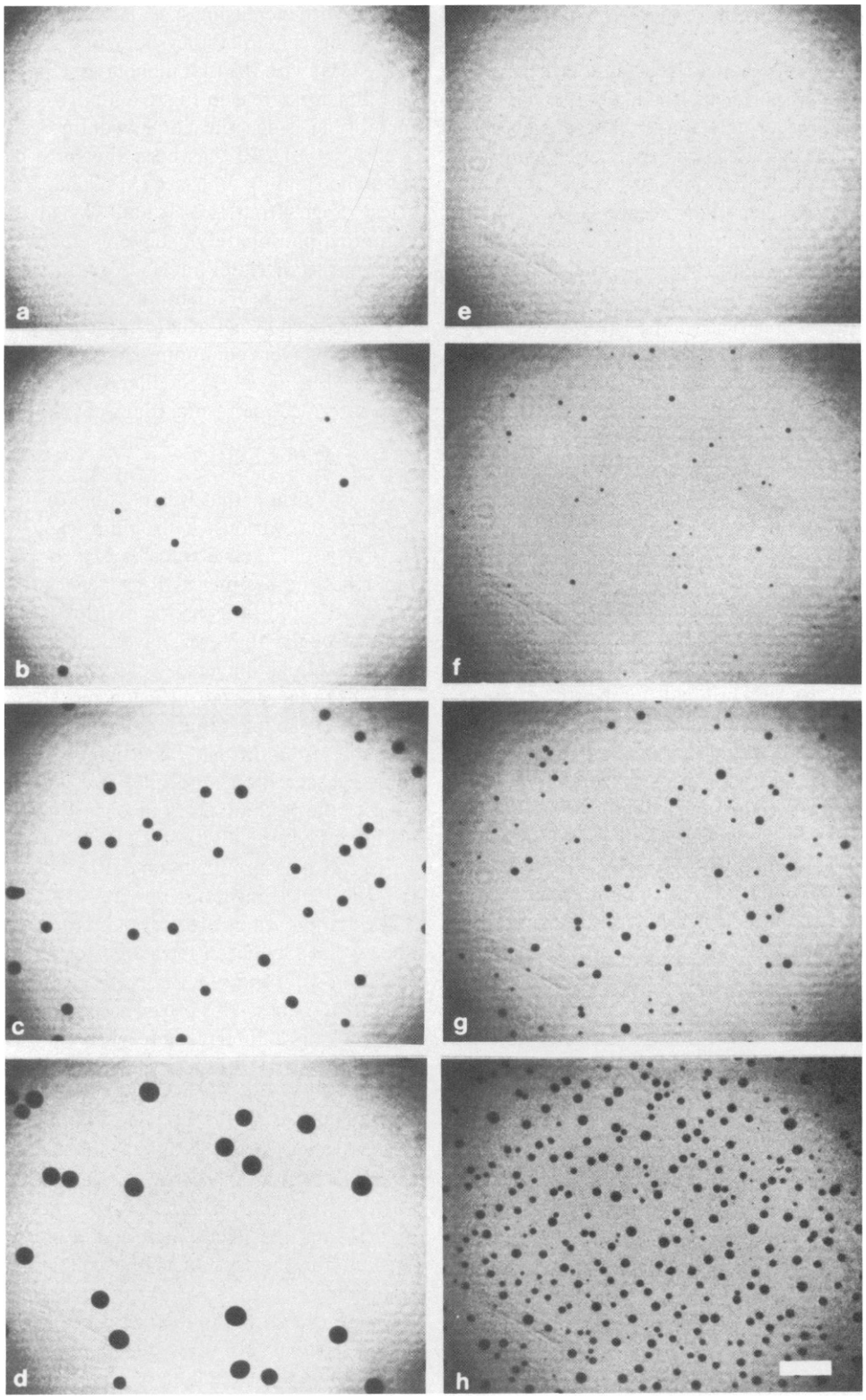

FIGURE 6 Epifluorescence microscope photographs of membrane defects formed by rapid coating of the first monolayer are shown. DMPC bilayers containing 2 mol % NBD-DPPE and supported on quartz slides. The sample shown in *e–h* in addition includes 1 mol % lyso-L-palmitoyl-phosphatidylcholine. A gradient of defects is formed from about the center of the substrate to the edge that has been coated last (*a–d* and *e–h*, respectively) (see text). NBD-DPPE partitions into solid and fluid membranes, and the dark areas therefore represent regions devoid of any lipid, i.e., holes, in the membrane. Bar is 20 μm.

change in the diffusion coefficients at the pretransition (14.2°C for DMPC) was not clearly resolved. The reason for this could be that in the earlier studies few data were collected in the $L_{\beta'}$ phase. In addition, multilayered systems offer the possibility of defects between layers that may well affect long-range diffusion (Schneider et al., 1983; Asher and Pershan, 1979). These probably are also the reasons for the lower activation energies reported for the multilayered DMPC preparations (151 kJ/mol; Smith and McConnell, 1978). Rotational diffusion measurements on DSPC and DPPC multibilayers in the $L_{\beta'}$, phase yielded activation energies of ~140 kJ/mol (Smith et al., 1981). Since long distance displacements of the fluorescent probe are required for translational diffusion measurements by photobleaching techniques, we would expect higher apparent activation energies for lateral diffusion than for rotational diffusion, if defect-mediated mechanisms are assumed.

The transfer of phospholipid monolayers onto hydrophilic substrates by a Langmuir-Blodgett technique has been attempted before (Levine et al., 1968), but has not found wide application in biophysical research compared with the numerous studies on fatty acid and soap films (for an excellent review see Kühn et al., 1972). Korenbrot and co-workers (Korenbrot and Pramik, 1977; Korenbrot and Jones, 1979) have used Langmuir-Blodgett phospholipid films containing rhodopsin for structural and spectroscopic investigations. Langmuir-Blodgett films of phospholipids have also been used for device fabrication (Procarione and Kauffman, 1974; Taylor and Mahboubian-Jones, 1982).

One might ask what forces hold a phospholipid bilayer on a hydrophilic substrate and still allow rapid lateral diffusion of the constituent lipids. It is well known that silicious materials exhibit free silanols at the surface that become deprotonated at neutral pH. All solid surfaces that we have used here are therefore negatively charged. Although phosphatidylcholines bear no net charge, a phosphatidylcholine monolayer (or bilayer) establishes an interfacial potential that can attract the charges on the surface of the solid substrate. From the diffusion data we conclude that there must be a water-filled space between the bilayer and the solid support. The equilibrium distance is probably established by an interplay of electrostatic attraction and the chemical potentials for hydration of the two surfaces. Long-range van der Waals forces also may play a significant role in the substrate-bilayer interactions (see Parsegian, 1973). Similar considerations were taken into account to explain interbilayer spacings of uncharged lipid liposomes (Rand, 1981).

The absolute lateral pressure (equals the gradient of the sum of all interfacial molar free energies in the system) of a free or supported bilayer at equilibrium is not known. Certainly, deducing lateral bilayer pressures from the lateral monolayer pressures is not straightforward. Simpler, however, is a comparison of the average molecular areas that can be measured in monolayers and bilayers individually. When preparing supported phospholipid bilayers, we have used lateral monolayer pressures at areas per molecule that closely correspond to the known average areas occupied by a solid or fluid phospholipid molecule in a bilayer at equilibrium pressure, i.e., 40.8 $Å^2$ (solid, untilted chains) and ~58 $Å^2$ (fluid, saturated chains), respectively (see Luzzati, 1968; Schindler and Seelig, 1975). Bilayers formed under these conditions appear to be stable and close to equilibrium. But evidently, a given bilayer supported by a substrate of fixed geometry is stable only in a certain temperature-pressure regime. This is clear from Figs. 4, 5, and 6. It is well known that DPPC bilayers expand laterally at the chain–melting-phase transition by ~20% in area (Träuble and Sackmann, 1972). At the same time the bilayer thickness decreases by ~10–12 Å (Seelig and Seelig, 1980) and the total bilayer volume increases by only ~1.5%. This lateral expansion creates an excess lateral pressure in the membrane that is sufficient to squeeze lipids out into liposomes of different shapes (Fig. 4). This is an analogous situation to the collapse of monolayers at the air-water interface at high lateral pressures. Therefore, in this particular system the transition is not strictly first order, but coupled to the formation of a new phase (i.e., liposomes). This may account for the asymmetric shape of the transition curve in Fig. 2. Note, however, that this process is not fully reversible and that some slow kinetics may play a role here.

Similar considerations can be applied in discussing the defects seen in Figs. 5 and 6. In these two cases the bilayer on the solid support probably is under tension (i.e., the actual lateral bilayer pressure is smaller than its two-dimensional equilibrium pressure). If the tension becomes high enough (lateral pressure low enough), the bilayer will finally rupture. DOPC bilayers in the liquid-crystalline phase have highly expanded chains (see Fig. 1) and, most certainly, a relatively large thermal expansion coefficient. Chain condensation at low temperatures (but still above the chain–melting-phase transition of this lipid) can easily lead to defects such as those seen in Fig. 5.

Tension was applied to a DMPC bilayer on quartz by a rapid first-coating step. Circular holes of increasing number and diameter are formed along an increasing (putative) tension gradient (Fig. 6). Once formed, the holes appear to be fixed on the solid support, suggesting either some interaction of the hole perimeter with the substrate or the accumulation of only minute amounts of impurities from solution on the exposed surface. The formation of these holes is modulated by including small amounts of lysolecithin. Lysolecithin is a surface-active compound, and we assume that it accumulates at the perimeter of the membrane holes. It is expected that the number of holes increases and the diameter decreases as the amount of included lysolecithin is increased (see Appendix). This is indeed what we observe (Fig. 6). Note that the first

monolayer, although obviously deposited under tension, does not show the holes when inspected with the epifluorescence microscope. It is only when the second monolayer (and water) is deposited that the underlying tension pattern is revealed. The mechanism of the hole formation is not known. The view that membrane tension can lead to circular defects is also supported by the observation of very similar circular defects — often arranged in periodic patterns — in monolayers at the air-water interface that have been subjected to local tension by some brief surface aspiration (Weis, R., L. Tamm, and H. McConnell, unpublished results).

In conclusion, we have shown that there is a reliable method for forming large, planar phospholipid-bilayer membranes on solid supports. These bilayers are physically very similar to lipid monolayers and bilayers in other model systems. Excluding some extreme temperature and pressure conditions, the supported bilayers are uniform and continuous, at least to the resolution of the light microscope. Techniques of higher resolution are required for the detection of still smaller defects. However, all our data presented here are consistent with a single continuous bilayer on the solid support. The reconstitution of membrane proteins into these bilayers will be interesting in its own right, but should also provide a novel approach for studying the interaction with cells.

APPENDIX

Assume a bilayer is under tension and wants to reduce its area by an amount A. Enough lysolecithin is added to make this possible by the formation of N holes each of area a and radius r. It is assumed that lysolecithin accumulates at the circumference of the holes at a fixed density ℓ per unit length of the hole perimeter. The total number of lysolecithin molecules L located in these rings is

$$L = \ell 2\pi r N. \tag{A1}$$

The equilibrium constant for lysolecithin partitioning into rings is

$$K = L/cF, \tag{A2}$$

where c is the total lysolecithin concentration and F is the area of the bilayer. Substituting Eq. A2 into Eq. A1 yields

$$c = 2\pi r \ell N/KF. \tag{A3}$$

The total area unoccupied by bilayer is

$$A = Na = N\pi r^2. \tag{A4}$$

By combining Eqs. A3 and A4 and by introducing the dimensionless fractional area $\overline{A} = A/F$, we obtain

$$r = 2\ell\overline{A}/Kc \tag{A5}$$

and

$$N = (1/4\pi\ell^2\overline{A})FK^2c^2. \tag{A6}$$

Hence, for constant A, r decreases as $1/c$ and N increases quadratically with increasing lysolecithin concentration c. Note that c is not necessarily the actual input concentration, since some partitioning into the aqueous environment may occur. This effect, however, is thought to be negligible for the small lysolecithin concentrations used here ($\leq$1 mol %).

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REFERENCES

Albert, F., B. Boyer, L. D. Leserman, and A.-M. Schmitt-Verhulst. 1983. Immunopurification and insertion into liposomes of native and mutant H-2K^b: Quantification by solid phase radioimmunoassay. *Mol. Immunol.* 20:655–667.

Albrecht, O., H. Gruler, and E. Sackmann. 1978. Polymorphism of phospholipid monolayers. *Le Journal de Physique.* 39:301–313.

Asher, S. A., and P. S. Pershan. 1979. Alignment and defect structures in oriented phosphatidylcholine multilayers. *Biophys. J.* 27:393–422.

Balakrishnan, K., F. J. Hsu, A. D. Cooper, and H. M. McConnell. 1982. Lipid hapten containing membrane targets can trigger specific immunoglobulin E-dependent degranulation of rat basophil leukemia cells. *J. Biol. Chem.* 257:6427–6433.

Cartwright, G. S., L. M. Smith, E. W. Heinzelmann, M. J. Ruebush, J. W. Parce, and H. M. McConnell. 1982. H-2K^k and vesicular stomatitis virus G proteins are not extensively associated in reconstituted membranes recognized by T cells. *Proc. Natl. Acad. Sci. USA.* 79:1506–1510.

Falkowitz, M. S., M. Seul, H. L. Frisch, and H. M. McConnell. 1982. Theory of periodic structures in lipid bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 79:3918–3921.

Hafeman, D. G., J. T. Lewis, and H. M. McConnell. 1980. Triggering of the macrophage and neutrophil respiratory burst by antibody bound to a spin-label phospholipid hapten in model lipid bilayer membranes. *Biochemistry.* 19:5387–5394.

Hafeman, D. G., V. von Tscharner, and H. M. McConnell. 1981. Specific antibody-dependent interactions between macrophages and lipid haptens in planar lipid monolayers. *Proc. Natl. Acad. Sci. USA.* 78:4552–4556.

Herrmann, S. H., and M. F. Mescher. 1981. Secondary cytolytic T lymphocyte stimulation by purified H-2K^k in liposomes. *Proc. Natl. Acad. Sci. USA.* 78:2488–2492.

Kinsky, S. C., and R. A. Nicoletti. 1977. Immunological properties of model membranes. *Annu. Rev. Biochem.* 46:49–67.

Korenbrot, J. I., and O. Jones. 1979. Linear dichroism of rhodopsin in air-water interface films. *J. Membr. Biol.* 46:239–254.

Korenbrot, J. I., and M.-J. Pramik. 1977. Formation, structure, and spectrophotometry of air-water interface films containing rhodopsin, *J. Membr. Biol.* 37:235–262.

Kühn, H., D. Möbius, and H. Bücher. 1972. Spectroscopy of monolayer assemblies. *In* Physical Methods of Chemistry. Vol. I, Part IIIB. A. Weissberger, and B. W. Rossiter, editors. John Wiley & Sons, Inc., New York. 577–702.

Levine, Y. K., A. I. Bailey, and M. H. F. Wilkins. 1968. Multilayers of phospholipid bimolecular leaflets. *Nature. (Lond.).* 220:577–578.

Lin, K.-C., R. M. Weis, and H. M. McConnell. 1982. Induction of helical liposomes by Ca^{2+}-mediated intermembrane binding. *Nature. (Lond.).* 296:164–165.

Littman, D. R., S. E. Cullen, and B. D. Schwartz. 1979. Insertion of Ia and H-2 alloantigens into model membranes. *Proc. Natl. Acad. Sci. USA.* 76:902–906.

Lösche, M., E. Sackmann, and H. Möhwald. 1983. A fluorescence microscopic study concerning the phase diagram of phospholipids. *Ber. Bunsen-Ges. Phys. Chem.* 87:848–852.

Luzzati, V. 1968. X-ray diffraction studies of lipid-water systems. *In* Biological Membranes. D. Chapman, editor. Academic Press, Inc., New York. 71–123.

Mabrey, S., and J. M. Sturtevant. 1976. Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. *Proc. Natl. Acad. Sci. USA.* 73:3862–3866.

Marder, M., H. Frisch, J. Langer, and H. M. McConnell. 1984. Theory of the intermediate ripple phase of phospholipid bilayers. *Proc. Natl. Acad. Sci. USA.* In press.

McConnell, H. M., L. K. Tamm, and R. M. Weis. 1984. Periodic structures in lipid monolayer phase transitions. *Proc. Natl. Acad. Sci. USA.* In press.

Nakanishi, M., A. A. Brian, and H. M. McConnell. 1983. Binding of cytotoxic T-lymphocytes to supported lipid monolayers containing trypsinized H2-K^k. *Mol. Immunol.* 20:1227–1231.

Parsegian, V. A. 1973. Long-range physical forces in the biological milieu. *Annu. Rev. Biophys. Bioeng.* 2:221–255.

Peters, R., and K. Beck. 1983. Translational diffusion in phospholipid monolayers measured by fluorescence microphotolysis. *Proc. Natl. Acad. Sci. USA.* 80:7183–7187.

Procarione, W. L., and J. W. Kauffman. 1974. The electrical properties of phospholipid bilayer Langmuir films. *Chem. Phys. Lipids.* 12:251–260.

Sackmann, E., D. Ruppel, and C. Gebhard. 1980. Defect structure and texture of isolated bilayers of phospholipids and phospholipid mixtures. *In* Liquid Crystals of One- and Two-Dimensional Order. W. Helfrich and G. Heppke, editors. Springer-Verlag New York, Inc., New York. 309–326.

Schindler, H., and J. Seelig. 1975. Deuterium order parameters in relation to thermodynamic properties of a phospholipid bilayer. A statistical mechanical interpretation. *Biochemistry.* 14:2283–2287.

Schneider, M. B., W. K. Chan, and W. W. Webb. 1983. Fast diffusion along defects and corrugations in phospholipid $P_{\beta'}$ liquid crystals. *Biophys. J.* 43:157–165.

Seelig, J., and A. Seelig. 1980. Lipid conformation in model membranes and biological membranes. *Q. Rev. Biophys.* 13:19–61.

Smith, B. A., and H. M. McConnell. 1978. Determination of molecular motion in membranes using periodic pattern photobleaching. *Proc. Natl. Acad. Sci. USA.* 75:2759–2763.

Smith, L. M., J. L. R. Rubenstein, J. W. Parce, and H. M. McConnell. 1980. Lateral diffusion of M-13 coat protein in mixtures of phosphatidylcholine and cholesterol. *Biochemistry.* 19:5907–5911.

Smith, L. M., R. M. Weis, and H. M. McConnell. 1981. Measurement of rotational motion in membranes using fluorescence recovery after photobleaching. *Biophys. J.* 36:73–91.

Taylor, D. M., and M. G. B. Mahboubian-Jones. 1982. The electrical properties of synthetic phospholipid Langmuir-Blodgett films. *Thin Solid Films.* 87:167–179.

Träuble, H., and E. Sackmann. 1972. Studies of the crystalline-liquid crystalline phase transition of lipid model membranes. III. Structure of a steroid-lecithin system below and above the lipid-phase transition. *J. Am. Chem. Soc.* 94:4499–4510.

Vaz, W. L. C., Z. I. Derzko, and K. A. Jacobson. 1982. Photobleaching measurements of the lateral diffusion of lipids and proteins in artificial phospholipid bilayer membranes. *In* Membrane Reconstitution. G. Poste and G. Nicolson, editors. North Holland Publishing Company, Amsterdam. 83–135.

von Tscharner, V., and H. M. McConnell. 1981*a*. An alternative view of phospholipid phase behavior at the air-water interface. *Biophys. J.* 36:409–419.

von Tscharner, V., and H. M. McConnell. 1981*b*. Physical properties of lipid monolayers on alkylated planar glass surfaces. *Biophys. J.* 36:421–427.

Weis, R. M., K. Balakrishnan, B. A. Smith, and H. M. McConnell. 1982. Stimulation of fluorescence in a small contact region between rat basophil leukemia cells and planar lipid membrane targets by coherent evanescent radiation. *J. Biol. Chem.* 257:6440–6445.

Wittebort, R. J., A. Blume, T.-H. Huang, S. K. Das Gupta, and R. G. Griffin. 1982. Carbon-13 nuclear magnetic resonance investigations of phase transitions and phase equilibria in pure and mixed phospholipid bilayers. *Biochemistry.* 21:3487–3502.