INTERACTIONS BETWEEN NEUTRAL PHOSPHOLIPID BILAYER MEMBRANES

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ABSTRACT We have obtained force vs. separation relations between bilayers in 10 different phospholipid preparations: dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, or dioleoylphosphatidylcholine (PC); egg phosphatidylethanolamine; cholesterol-containing bilayers of dipalmitoyl PC and of egg PC. The chemical potential of water in the multilamellar lattice is determined at all water contents and changes continuously with bilayer separation; no discrete classes of water are observed. The interbilayer van der Waals force is estimated from the balance of forces at the bilayer separation where the multilayer lattice is in equilibrium with pure water. Although quantitative differences are evident for different phospholipids, all force curves but one show a clear, exponentially decaying "hydration repulsion" whose decay distance is 2–3 Å. Estimates of forces between bilayer vesicles show great sensitivity to the identity of the phospholipid polar group and to the packing of the hydrocarbon acyl chains. One major implication of this is the likelihood of local structural changes and lipid segregation in the area of closest approach of interacting vesicles of mixed phospholipids.

INTRODUCTION

Quantitative estimates of forces between membranes and of membrane deformability are essential to a satisfactory understanding of cell membrane contact or vesicular adsorption, aggregation, and fusion (1, 2). Long-range electrostatic and electrodynamic forces, felt during the initial mutual approach of membranes, are relatively well understood theoretically and experimentally (3–10). It is known, for example, that electrodynamic forces are strong enough to explain multilayer stacking of phospholipid bilayers (7, 9, 10). Under limited conditions, these interactions are observed to occur between erythrocytes and substrates (11–13). Differences in electrodynamic, or van der Waals, interaction can confer some specificity in attraction between like bodies (7, 9, 14, 15).

Short-range forces occurring near contact between specific chemical groups have traditionally come within the purview of biochemistry and the quest to identify particular molecular receptors, antigens, bridging agents, etc. that create strong and specific cell contact (see e.g., reference 16). What should be implicit in any picture of stable molecular contact at a membrane surface is that the contacting groups prefer each other to the water that would otherwise separate them.

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We have been measuring the forces between phospholipid bilayers in order to model intact membranes and to provide some insight into the contact and fusion of phospholipid vesicles. Our earlier results (17–20) provided experimental support for the theoretical estimation of van der Waals forces of attraction between phospholipid bilayers (18, 19). We have also measured and characterized the electrostatic repulsive force between phospholipid bilayers of various charge densities. Beyond 30-Å separation these forces are in good agreement with the predictions of nonlinear double-layer theory (21).

It has become apparent, though, that phospholipid bilayer membranes experience a strongly repulsive "hydration force" consequent to the work of removing water from the polar groups that cover the bilayer and allow the lipid body to remain stable in water. Below 20–30-Å separation, these forces easily dominate any electrostatic repulsion (21) and, as they grow exponentially with a growth constant of ~2.5 Å(20), pose a significant energetic barrier to bilayer contact (17, 19, 22).

As suggested from data reported here and from that on other, nonlipid, systems (22–24), a repulsive force due to work of dehydration is likely to be characteristic of all interacting hydrophilic surfaces. It can be strong enough to prevent contact between membranes (e.g., synaptic vesicles poised next to the presynaptic membrane [22]), and require special mechanisms or a statistically infre-

quent event to disrupt the hydrophilic surface to allow membrane fusion (25, 26).

To determine differences in these hydration forces that result from polar group identity and packing density, we have measured interbilayer forces between 10 kinds of neutral bilayers. Our earlier studies (20) described three methods for measuring the work of removing of water from a phospholipid multilayer; this work was combined with x-ray diffraction by the water-deprived multilayer to learn the structural consequences of water removal.

In all cases but one (distearoylphosphatidylcholine) the hydration force for pure neutral phospholipids is exponential, with decay lengths roughly the size of a water molecule. Phosphatidylethanolamine (PE) shows a stronger net attraction than a corresponding phosphatidylcholine. Spreading of polar groups on the bilayer surface, by addition of cholesterol, sometimes increases bilayer separation and, by inference, increases bilayer repulsion. Inasmuch as these hydration forces are negligibly dependent on membrane charge and on the ionic strength of the medium (21, 27, 28), we can expect similar differences in vesicular attraction with changes in polar group identity and packing.

From the data presented below, we now predict energy minima between vesicles near contact. Between PE vesicles, attraction can be great enough to cause aggregation and adhesion. In vesicles of mixed composition, it can also promote separation to put more attractive components near the region of contact (29).

We have recently shown that the stress technique used to determine the interbilayer forces referred to above, can also determine intrabilayer forces (20); measurements analogous to those made on monolayers at the air-water or oil-water interface. In the succeeding paper, we describe the deformation characteristics of these bilayer systems.

MATERIALS

Egg PE purchased from Nutfield Lipid Products (Surrey, England) and cholesterol from Calbiochem-Behring Corp. (San Diego, Calif.) were used without further purification. Three sources of dipalmitoylphoshatidylcholine (DPPC) were used: some was purchased from Serdary Research Laboratories (London, Ontario) and used without further purification; some of this latter was chromatographed through Al_2O_3; other DPPC was synthesized in this laboratory using the method of Robles and Van den Berg (30). DPPC from each of the above sources produced the same equilibrium spacing in excess water as determined by x-ray diffraction. Dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC) and dioleoylphosphatidylcholine (DOPC) were obtained from Sigma Chemical Company (St. Louis, Mo.). All lipids were checked periodically for purity using thin-layer chromatography and showed <1% contamination. All lipids were stored under nitrogen at -18°C until used. Mixtures of DPPC or egg yolk lecithin with cholesterol were prepared by dissolving the DPPC and cholesterol in chloroform, combining the solutions and removing the solvent by rotary evaporation and final drying under vacuum. We expect there to be negligible residual solvent. Dextran (T-2,000; 2,000,000 mol wt) was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Water, double-distilled in glass, was used for all lipid and dextran samples.

METHODS

Measurement of Structural Parameters

We used x-ray diffraction both to characterize the structures within the lipid-water mixtures and to measure the structural parameters of the lamellar phases formed by the lipid in water (20). The x-ray camera was of the Guinier type operating *in vacuo*. The $CuK_{\alpha 1}$ line ($\lambda = 1.540$ Å was isolated using a bent quartz crystal monochrometer. Diffraction patterns were recorded photographically. The samples were sealed between mica windows 1 mm apart. Samples requiring heating were controlled to ±0.2°C with thermoelectric elements. All samples contained powdered teflon, mixed directly with the sample, which acted as an internal standard to measure the repeat spacings of the lamellar phases (±0.1Å), which are made up of alternating layers of water and bimolecular lipid leaflets.

In a model wherein the lipid and water form separate layers, i.e., the water does not penetrate into the lipid layer plane, the thickness of the bilayer d_l and the distance between bilayers d_w can be calculated according to their volume concentrations. That is,

$$d_l = \phi d \qquad \text{and} \qquad d_w = d - d_l, \tag{1}$$

where ϕ is the volume fraction of the lipid in the sample and d the lattice repeat spacing.

$$\phi = [1 + (1 - c)\bar{v}_w/c\bar{v}_L]^{-1}, \tag{2}$$

where c is the weight fraction of lipid in sample, and $\bar{v}_w$ and $\bar{v}_L$ are the partial specific volumes of water and phospholipid, respectively. $\bar{v}_L$ was taken as 1.00 for lipid systems with melted hydrocarbon chains and 0.95 for those with frozen hydrocarbon chains (31).

The average cross-sectional area available to one phospholipid molecule is

$$\begin{aligned} A &= 2(V_w + V_L)/d \\ &= 2V_w/d_w \\ &= \frac{2 \times 10^{24}\, MW_L\bar{v}_L}{d(\text{Å})N_0}\ (\text{in Å}^2) \end{aligned} \tag{3}$$

where V_w is the volume of water per lipid molecule, V_L the volume of each lipid molecule, MW_L the molecular weight of the phospholipid, and N_0 Avogadro's number. When cholesterol/phospholipid mixtures (29, 32) are considered, these quantities refer to water and to total lipid normalized per phospholipid molecule.

We emphasize that estimates of cross-sectional area are independent of the assumptions used to divide the repeat spacing d into water layer and lipid layer. The repeat spacing d was measured, and parameters d_l, d_w, and A derived for the full range of lipid-water ratios. Lipid and water were mixed by weight in small weighing bottles. After equilibration for ~48 h, the samples were transferred to the x-ray sample holder at the temperature at which the diffraction was to be performed.

Measurement of Bilayer Repulsion and Deformation

We have described elsewhere in detail a technique for determining the pressure between bilayers and the lateral pressure within bilayers (20). It is briefly summarized here. The chemical potential, μ_w, of the water within the multilayer is enforced by one of three methods. In the first, the lipid is equilibrated with dextran solution of known concentration and osmotic pressure (measured directly). Since the dextran molecule is too large to enter into the lattice structure, the lipid must compete with dextran for water. The strength of this competition is measured as the osmotic pressure exerted by the dextran solution. In the second method the lipid is allowed to imbibe water across a dialysis membrane against a directly measured mechanical pressure exerted on it by a hydraulically

driven piston. In the third, the lipid is equilibrated with known vapor pressures generated by saturated salt solutions (33). The sample is weighed both dry and at equilibrium with the vapor and thus the final concentration of the lipid determined.

Each of these three methods imposes on the lipid-water mixture a preset chemical potential. At equilibrium, that potential is the work of transfer of water between the multilayer system and the water phase. In combination, the three methods cover the range of pressures from 0 to 10^{10} dynes/cm^2 (i.e., up to 10^4 atm).

X-ray diffraction of the equilibrated samples gives the structural parameters of the resultant lamellar phase as described above. Experiments using the dextran and the hydraulic pressure methods did not allow direct measurement of the concentration of lipid in the lamellar phase. In these cases we refer to our measurements of repeat spacing d vs. lipid concentration measurements where lipid and water thicknesses are known. Since d is monotonic with concentration, we match the d achieved under stress with the same repeat spacing d in the gravimetric mixture. (It is obvious that the same structure results when the amount of water in the lipid is restricted, either gravimetrically or by the pressure techniques [17, 19].) Thus we can determine the relation between external pressure P and the structural parameters d_w, A, d_l, and V_w.

Removal of water from a lamellar phase that has been fully swelled in excess water is found to have two major structural consequences. (*a*) The lipid molecules come closer together in a direction normal to the plane of the membrane to decrease d_w; (*b*) the molecules also pack more closely within each bilayer to decrease A and to increase bilayer thickness d_l. The method for separating energies or forces required to effect each of these changes has been described in detail (20).

We have divided the total work of water removal $P=dg/dV_w$ into an interlamellar force on a single molecule $\partial g/\partial d_w = F_R(d_w;A)$ and a lateral pressure $\partial g/\partial A = F_{LP}(A;d_w)$. In practice (20), we have treated the data as though these functions were separable, such that $F_R(d_w;A) = F_R(d_w)$, and $F_{LP}(A;d_w) = F_{LP}(A)$. While we believe this assumption to be useful in practice, it should not be considered proven. For the present we recognize that the 25% changes in A accompanying 10^3 or 10^4 factor changes in P assure that our estimate of an exponential decay distance in F_R would not be affected appreciably had a constant area A been assumed. A similar argument cannot be made so easily for the lateral pressure function F_{LP} in the event that one cannot treat it as a function of only one variable. In later applications we expect to introduce specific models for $\partial g/\partial A_{(d_w\to\infty)}$ and $\partial g/\partial d_{w(A,\text{ constant})}$ to test these models using total measured energies dg/dV_w and the observed concomitant changes in A and d_w.

Bilayer Repulsion

The change in molecular free energy with bilayer separation, $\partial g/\partial(d_w/2) = PA = F_R$, is the net repulsive force on one molecule acting in a direction perpendicular to the bilayer. We describe it as the difference $F_R = F_H - F_A$ between a repulsive hydration force F_H of the form $P = P_o^{-d_w/\lambda}$ or $F_H = F_o\exp(-d_w/\lambda')$ (20) and a van der Waals attractive force F_A taken to be of the form (15–16).

$$F_A(d_w; d_l) = \frac{HA}{6\pi}\left[\frac{1}{d_w^3} - \frac{2}{d^3} + \frac{1}{(d + d_w)^3}\right]. \qquad (4)$$

F_R is zero at the equilibrium separation d_w^o. It is at this point that the effective London-Hamaker coefficient H can be evaluated (19, 20) by comparing F_A with F_H extrapolated to d_w^o. Energies (as opposed to forces) of repulsion are obtained by integrating F_R over d_w from d_w^o.

We apply the Derjaguin approximation (34, 35) to estimate the energy of interaction E_{ss} between two spherical vesicles of outer radius a, bilayer thickness d_l and separation d_w. Specifically,

$$E_{ss} = E_R - E_A,$$

$$E_R = a\lambda^2\pi P(d_w),$$

$$E_A = V(z,a,a) - 2V(z,a,a - d_l) + V(R,a - d_l, a - d_l), \qquad (5)$$

where

$$V(R,a_1,a_2) = (H/3)\{\}$$

$$\{\}a_1a_2\,[1/(R^2 + (a_1 + a_2)^2) + 1/(R^2 + (a_1 - a_2)^2)]$$

$$+ \tfrac{1}{2}\ln\,[(R^2 - (a_1 + a_2)^2)/(R^2 - (a_1 - a_2)^2)] \qquad (6)$$

and $z = d_w + 2a$.

This approximation assumes that the spherical vesicles can be considered big enough to exhibit the same kind of average macroscopic behavior demonstrated by planar membranes. Local fluctuations of lipid packing might affect the use of this approximation.

RESULTS

Structure of Lamellar Phases

We first determine the lamellar repeat spacings d as a function of lipid concentration for the lipid preparations (symbols × or Δ) in Fig. 1. In all cases d increases with the amount of water until a limiting spacing is reached. Any additional water has no apparent effect on the lamellar lattice repeat. (We do not go to the very high water contents, >95% water, where budding phospholipid vesicles are reported to appear [36, 37]).

Upon progressive dehydration, wide angle x-ray diffraction of several preparations reveals a sudden change in the packing of hydrocarbon chains. DLPC at 25°C and DMPC at 25°C (rather than the 27°C case plotted in Fig. 1) show a transition from the disorder characteristic of the liquid-crystal to the order of the gel state. This transition, at 25°C rather than the −1.8°C (DLPC) (38) or 23°–23.9°C (DMPC) (38, 39) seen for these lipids in excess water is consistent with the rise in melting temperature upon dehydration expected from phase diagrams (40). DSPC, already in a gel state at 25°C, shows a change in chain tilt with lipid concentration. At very high lipid concentrations, one often sees evidence for nonlamellar phases and a number of different crystalline structures. We have not analyzed this region in detail. It has been characterized by Tardieu et al. (31) for many phosphatidylcholines; our results are consistent with theirs.

From the repeat spacings and lipid concentration we derive bilayer separation d_w, bilayer thickness d_l and the cross-sectional area normalized per phospholipid head group A by methods described above. All these parameters reach limiting values at excess water. A and d_w increase monotonically while d_l decreases with increasing amounts of water. The extent of these changes varies widely among the different lipids. Agreement is good with observations of Janiak et al. (41) on DMPC and DPPC and of Inoko and Mitsui (42) on DPPC (except that, in concurrence with Inoko and Mitsui (42), we find greater swelling of DPPC in excess water than assumed in reference [41]).

In Fig. 2 we plot d_w vs. A for several phospholipids. It is along these lines that lipid free energies are accessible.

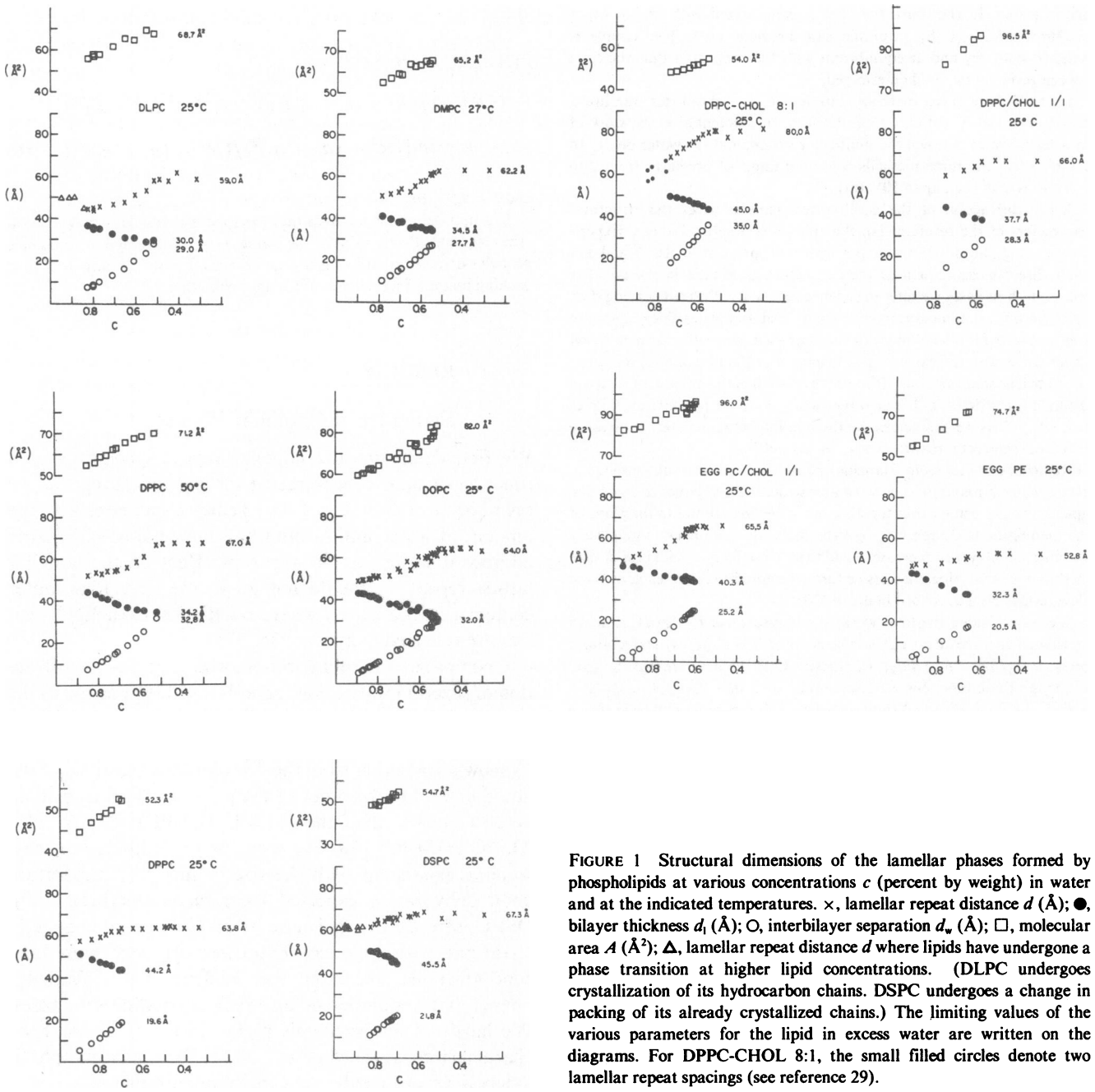

FIGURE 1 Structural dimensions of the lamellar phases formed by phospholipids at various concentrations c (percent by weight) in water and at the indicated temperatures. ×, lamellar repeat distance d (Å); ●, bilayer thickness d_l (Å); ○, interbilayer separation d_w (Å); □, molecular area A (Å^2); △, lamellar repeat distance d where lipids have undergone a phase transition at higher lipid concentrations. (DLPC undergoes crystallization of its hydrocarbon chains. DSPC undergoes a change in packing of its already crystallized chains.) The limiting values of the various parameters for the lipid in excess water are written on the diagrams. For DPPC-CHOL 8:1, the small filled circles denote two lamellar repeat spacings (see reference 29).

Interaction between Bilayers

Fig. 3 and Table I summarize the results on the interaction between like phospholipid bilayers either as the distance between bilayers varies or as the number of water molecules per lipid varies.

F_R (Fig. 3) is the repulsive force, in dynes, acting on one phospholipid molecule, or one phospholipid molecule and its portion of a cholesterol molecule. Most of the relationship between log F_R and d_w can be described by the best-fit exponential whose parameters are given in Table I and which are plotted as lines in Fig. 3. The corresponding best-fit exponential relationships between the experimentally measured pressures P and bilayer separation are also given in Table I. For comparison of the lipids, these latter relationships are all plotted in Fig. 4 over the range of d_w covered by the data points.

A second part of the relationship between interbilayer force and bilayer separation is the deviation from the exponential near the equilibrium spacing in water; the latter is indicated by a vertical arrow on the d_w axis in Fig. 3. The deviation results because near equilibrium in water the van der Waals attractive force becomes comparable to the repulsive force (19) to which it must be equal at the equilibrium separation in pure water. We have extrapolated the exponential repulsive line to the equilibrium

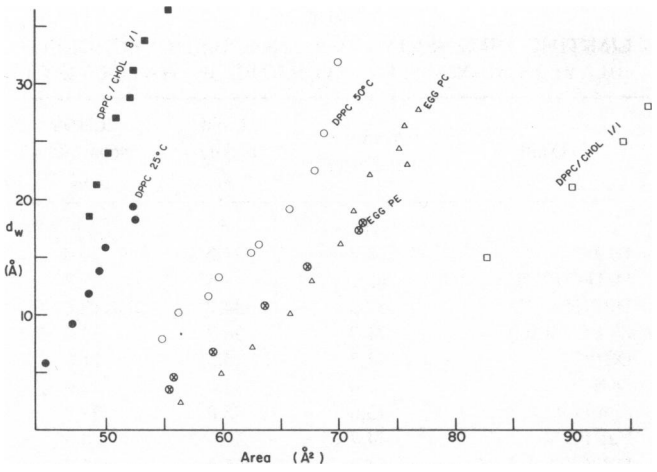

FIGURE 2 Bilayer separations (d_w) vs. areas (A).

spacing in water to estimate a Hamaker coefficient H for each lipid system as in Eq. 4. These coefficients, too, are listed in Table I.

DISCUSSION

Taking the entire set of force vs. distance data (Figs. 3, 4, and Tables), we observe one clear consistency. Over four decades the repulsive force for solid and liquid hydrocarbon chain bilayers, for natural and synthetic zwitterionic lipids, for two kinds of polar group, and for mixtures of phosphatidylcholines with different amounts of cholesterol, varies as an exponential hydration force with a decay distance close to the diameter of a water molecule. We agree with Radic and Marcelja (43) that the decay of the repulsive force is due mainly to the behavior of water near a hydrophilic boundary surface rather than to the molecular structure of that surface. If it is the properties of water that determine the form of the repulsive force, we can expect exponentially decaying forces, similar to those reported here, between virtually all surfaces stabilized by permanently and closely bound water-soluble groups.

Force measurements using electrically charged phosphatidylglycerol and phosphatidylinositol confirmed the correctness of standard double-layer theory but showed that, for separations <20–30 Å(depending on the degree of charge on the lipid), "hydration" forces such as seen here overwhelmed electrostatic forces (21). It is these stronger forces that will dominate the interaction of membranes near contact.

Even with the qualitative similarity among the forces reported here, there are distinct differences. A number of

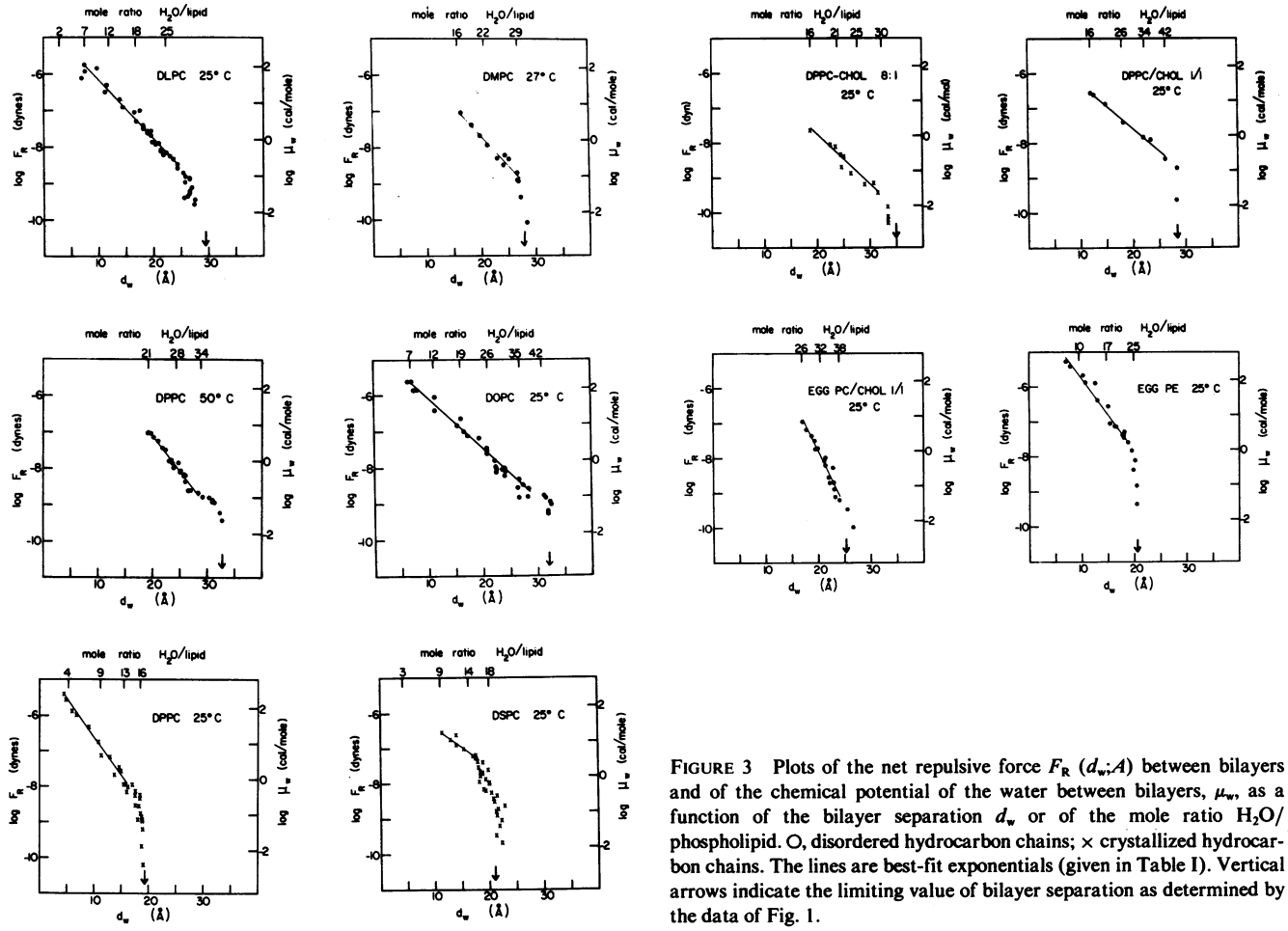

FIGURE 3 Plots of the net repulsive force F_R (d_w;A) between bilayers and of the chemical potential of the water between bilayers, μ_w, as a function of the bilayer separation d_w or of the mole ratio H_2O/phospholipid. O, disordered hydrocarbon chains; × crystallized hydrocarbon chains. The lines are best-fit exponentials (given in Table I). Vertical arrows indicate the limiting value of bilayer separation as determined by the data of Fig. 1.

TABLE I
INTERACTION PARAMETERS AND RELATIONSHIPS

Lipid	$P_0e^{-d_w/\lambda}$ (P)	$F_0e^{-d_w/\lambda'}$ (F_R)	H*
	dynes/cm²	*dynes*	*ergs* × 10^{14}
DLPC	$10^{9.72}e^{-d_w/2.6}$	$10^{-4.57}e^{-d_w/2.7}$	4.9
DMPC (27°C)	$10^{9.94}e^{-d_w/2.6}$	$10^{-4.3}e^{-d_w/2.6}$	7.9
DPPC	$10^{9.83}e^{-d_w/2.0}$	$10^{-4.54}e^{-d_w/2.1}$	6.1
DPPC (50°C)	$10^{10.99}e^{-d_w/2.2}$	$10^{-3.15}e^{-d_w/2.2}$	3.1
DSPC‡	$10^{9.13}e^{-d_w/3.6}$	$10^{-5.27}e^{-d_w/3.8}$	
DOPC	$10^{9.6}e^{-d_w/2.9}$	$10^{-4.64}e^{-d_w/3.0}$	5.6
Egg PC	$10^{9.76}e^{-d_w/2.6}$	$10^{-4.50}e^{-d_w/2.7}$	8.2
Egg PE	$10^{10.57}e^{-d_w/2.1}$	$10^{-3.71}e^{-d_w/2.1}$	32.5
DPPC/chol,8:1	$10^{9.47}e^{-d_w/3.0}$	$10^{-4.90}e^{-d_w/3.0}$	2.2
DPPC/chol,1:1	$10^{9.19}e^{-d_w/3.2}$	$10^{-4.98}e^{-d_w/3.3}$	11.0
EggPC/chol,1:1	$10^{12.60}e^{-d_w/1.4}$	$10^{-1.48}e^{-d_w/1.4}$	1.2

*An ambiguity of 1 Å in d_w^0 creates a ±30% range in H. Changes in the convention defining d_w Eq. 1 will affect estimates of H (cf. reference 19).
‡Because of changes in the DSPC acyl chain packing, smooth exponential decay is not observed nor is a reliable Hamaker coefficient obtained.

comparisons can be made, particularly regarding bilayer thickness and bilayer separation. To detect systematic changes, we compare the values of these parameters taken from Fig. 1 when the lipid is in equilibrium in excess water and the net force between bilayers is zero (Table II).

Effect of Hydrocarbon Chain Conformation

A comparison of all the phosphatidylcholines without cholesterol, and particularly of DPPC at 25°C with DPPC at 50°C shows that chain melting, associated with a decrease in bilayer thickness of ~10 Å is accompanied by an increase in bilayer separation of ~10 Å.

Effect of Hydrocarbon Chain Length

Comparison of DLPC, DMPC, and DPPC, all with melted chains, or of DPPC and DSPC with frozen chains

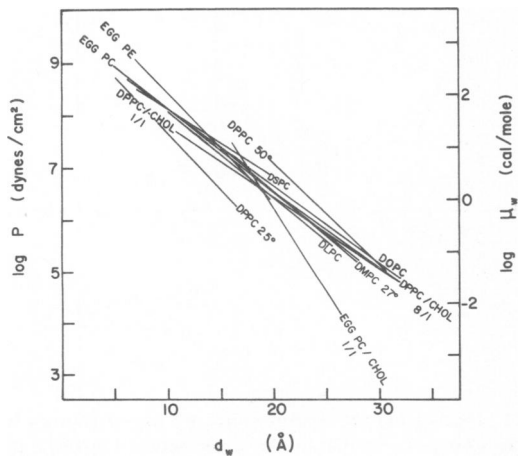

FIGURE 4 Plots of the best-fit exponentials for the relationships between the pressure between bilayers and bilayer separation. μ_w is the chemical potential of the water between bilayers relative to bulk water. The exponential parameters are given in Table I.

TABLE II
LIMITING DIMENSIONS FOR PHOSPHATIDYLCHOLINE BILAYERS MAXIMALLY HYDRATED IN WATER (25°C)

Lipid	Area per head group	Lipid thickness d_l	Bilayer separation d_w
	Å²	*Å*	*Å*
DLPC	68.7	30.0	29.0
DMPC (27°C)	65.2	34.5	27.7
DPPC*	52.3	44.2	19.6
DPPC (50°C)	71.2	34.2	32.8
DSPC	54.7	45.5	21.8
DOPC	82.0	32.0	32.0
Egg PC‡	75.6	35.0	27.5
Egg PE	74.7	32.3	20.5
DPPC/chol, 8:1§	54.0	45.0	35.0
DPPC/chol, 1:1§	96.5	37.7	28.3
Egg PC/chol, 1:1§	96.0	40.3	25.2

*From M. J. McAlister (1978) M. Sci. Thesis, Brock University, St. Catharines, Ontario.
‡From reference 20.
§From reference 29.

shows that not only does d_l increase with increasing chain length as expected but that the bilayer separation increases systematically as well. The effect is much smaller than that of changing hydrocarbon chain conformation.

Effect of Cholesterol

Compare DPPC (50°C) with DPPC:chol 1:1, each with disordered chains, or egg PC with egg PC:chol 1:1 each with heterogeneous disordered chains. It appears that cholesterol at this high level results in only a slight decrease in bilayer separation (in addition to a "condensing effect" resulting in increased bilayer thickness [32]).

However, a comparison of DPPC at 25°C with DPPC:chol 8:1, where the hydrocarbon chains are still ordered, shows that cholesterol causes little increase in bilayer thickness but a very large increase in bilayer separation. This increase is consonant with the large repeat spacings previously observed by Ladbrooke et al. (44).

The preparations described here do not show the phase separations expected from the multilayer (45) and monolayer studies of Tajima and Gershfeld (46), although in a separate study (29) we have established that in this region, i.e., of slightly lower cholesterol content than reported here, there coexist two lamellar phases. The effects of cholesterol on DPPC bilayer structures are complex; the indications here are that the effects on bilayer separation can be large at low concentrations.

Effect of Polar Group

Comparison of egg PC with egg PE shows that with the removal of the methyl groups from the PC polar group, both the bilayer thickness and the bilayer separation

decrease. Among the pure PC, it is worth noting that the group of bilayers (DLPC, egg PC, DOPC at 25°C, DMPC at 27°C, and DPPC at 50°C) showing greater limiting areas A_0 per molecule also take on larger limiting separations d_w^0 than do those with smaller areas A_0 (DPPC, DSPC at 25°C). Spreading of polar groups from A_0 = 70 Å^2 to A_0 = 100 Å^2 by adding cholesterol causes a drop in limiting separation. We would have expected that, at least among the PCs, the different forces would be monotonically related to the density of polar groups. They are not.

It must be kept in mind that we have used a model for the lamellar lattice assuming complete division of the lipid and water components. In fact, there is likely to be water mixed with the phospholipid polar groups; bilayer separation d_w is likely to be less than the numbers quoted. For example, imagine that five water molecules were associated with each bilayer phospholipid region rather than with the intervening fluid. Estimate of d_w would be less by ~4.5 Å for bilayers with molecular area A = 70 Å. It may be that hydrocarbon chain freezing entails a decrease in the number of associated waters even to an extent that would account for the sudden decrease in d_w upon freezing. Estimates of decay distances λ are not affected by such adjustments in d_w nor are cross-sectional areas A in any way affected by assignment of the water to bilayer or intervening fluid. Hamaker coefficients H, Eq. 4, will appear smaller if a smaller d_w is used to describe a given attractive force (19).

Chemical Potential of Water

The quantity μ_w in Fig. 3 is the difference in chemical potential between bulk water and the water in the interbilayer space. Although μ_w is very small near equilibrium separations, and insignificant as a chemical potential of solvation, it translates into large repulsive interactions between bilayers of any significant area (see E_{ss} below). Nuclear magnetic resonance probes (45–47), differential scanning calorimetry (48), measurements of diffusivity (49–50), and studies with solubility probes (51–53) of interlamellar water suggest that much of this water appears indistinguishable from bulk water. This is so because such probes are not sensitive to perturbations of less than a few calories per mole even though the consequences of such perturbations on physical forces are enormous.

Indeed we have verified (18, 19) that small sugars, sucrose and glucose, can easily dissolve in most of the water between egg lecithin bilayers as had been demonstrated earlier for several solutes with DMPC by Katz and Diamond (53–55). The forces of diffusion are easily great enough to allow such small solutes to displace water whose chemical activity is so little different from that of bulk water. When nearly all of the water is stripped from the bilayer, the chemical potential of the remaining water extrapolates to that of the hydration energies of ions and cell surface polar groups (33).

In view of the continuously changing chemical potentials μ_w now observed (Fig. 3), we strongly suggest that modeling used to interpret probes of water near bilayers should no longer assume discrete classes of bound and free water. Rather, it seems much more likely that the water molecules observed by nuclear magnetic resonance, for example, are showing the effects of averaging over a continuum of states. It would probably be more accurate to fit resonant data with a perturbation decaying exponentially from the bilayer surfaces.

Vesicle-Vesicle Interaction

To illustrate the consequences of these forces in systems of interacting vesicles, we have calculated the work needed to bring together unilamellar phospholipid vesicles of 300-Å Diam. Fig. 5 shows the sphere-sphere interaction energy E_{ss}/kT (over the range of -1 to $+2$ kT units) vs. the distance between the vesicles for several of the lipid systems studied here.

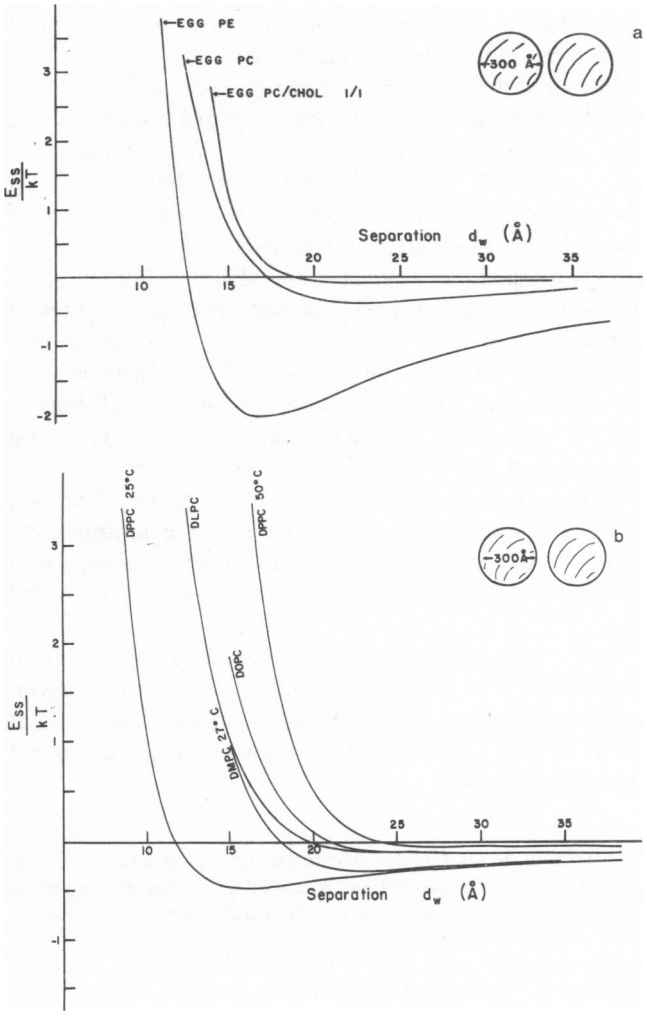

FIGURE 5 Vesicle-vesicle interactions inferred from the forces given in Fig. 3 introduced into Eqs. 5 and 6. Data for egg PC are from reference 20.

Curvature creates an equilibrium separation several Ångstroms smaller than occurs between parallel planar bilayers. Among the several lipid species, vesicles of egg PE or of frozen DPPC are expected to approach the most closely. These both arrive at equilibrium separations of ~17 Å.

Differences in depth and location of the energy minimum for vesicles of different lipids suggest to us that the lipid molecules in vesicles of mixed composition will redistribute within the vesicle surface as the vesicles are brought together. For example, PE might become concentrated near, or cholesterol be driven from (29) regions of closest approach.

We have not included the possibility of spherical bilayer deformation induced by contact. Such deformations would, in any case, act to increase the area of bilayer contact between more attractive species and would thereby act to amplify the differences pointed out here between different lipids.

We have argued elsewhere (17, 20, 22) that hydration forces pose a formidable barrier to aggregation and fusion of the bilayer membranes. Diminution or elimination of these forces diminishes vesicle stability.

Phosphatidylserine (PS) in the absence of Ca^{++} shows electrostatic and hydration repulsion (56) essentially like that reported for phosphatidylglycerol (21). Addition of Ca^{++} causes PS bilayers to collapse together (25, 56), an obvious indication that PS bilayer repulsion is lost with added Ca^{++}, and that water is displaced from the polar groups by the more favorably bound Ca^{++}. For the same reason PS vesicles fuse (57) or aggregate (58) only with the addition of Ca^{++}. These same Ca^{++}/PS vesicles will fuse also when the PS is mixed with PE, but their fusion is prevented by addition of the more repellent PC (cf. Figs. 3 and 4) (59). The difference between the aggregation properties of PS/PE and PS/PC vesicles is not due to a difference in their ability to bind Ca^{++} ions (60). The ease of PE vesicle aggregation compared with the improbability of PC vesicle aggregation (61) also correlates well with hydration and van der Waals forces shown in Figs. 3 and 4.

Recognition of interlamellar forces should prove useful in promoting or analyzing fusion of vesicles in artificial suspensions and in cellular systems. A fuller discussion of forces in the context of biological phenomena is given in a current review (62).

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