Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 2750–2754, June 1979
Biophysics

Measured work of deformation and repulsion of lecithin bilayers

(phospholipids/membranes/intermolecular forces)

V. A. PARSEGIAN, N. FULLER, AND R. P. RAND

Physical Sciences Laboratory, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland 20205; and Biology Department, Brock University, St. Catharines, Ontario, Canada L2S 3A1

Communicated by Charles P. Bean, March 30, 1979

ABSTRACT **We used three complementary techniques to vary the chemical potential of water in lipid/water mixtures; we measured the work of removing water from the multilayer lattice formed in water by the zwitterionic phospholipid egg lecithin. By x-ray diffraction, we observed the structural consequences of water removal. There are no discrete classes of "bound water" in this system; the work of removal is a continuous function of water content and lattice repeat spacing. From 30 to 3 Å separation between bilayers there exists an exponential "hydration force" repulsion with a 2.6 Å decay length. This interaction translates into a very large force to prevent contact between vesicles and planar membranes. It may be an important feature in controlling vesicle-to-cell fusion. As water is removed, bilayers not only move closer, but thicken as the lipid polar groups on the same bilayer move closer together. It is possible to divide the applied work into that of direct bilayer repulsion and that of bilayer deformation. We thus obtained a first determination of the lateral pressure required to create large increases in bilayer thickness and concomitant decreases in bilayer area. The lateral pressure reaches 25 dynes/cm for a 25% decrease in bilayer area. Systematic measurements of the mechanical properties of bilayers suffering such large deformation will allow critical tests of theories on bilayer stability and phase transition.**

All cell–cell and vesicle–cell interactions requiring contact between membranes probably involve not only molecular interaction between specific membrane constituents, but also nonspecific interactions. The latter include long-range van der Waals attraction, electrostatic repulsion, and the very strong repulsive force that results from having to remove water from the water-soluble groups that cover and stabilize membrane surfaces (1, 2). Durable contacts, as seen in studies of cell adhesion, must reach beyond or through this "wall" of hydration. For example, sparsely distributed glycoproteins, bearing receptors for specific cell contact, would have to reach through the water coating the cell surface. "Hydration" forces probably stop vesicles from close contact with the cell membrane, making spontaneous fusion difficult (3). The triggering events in exocytosis may be a controlled destruction of vesicle–membrane repulsion which must be more than a simple ionic screening or neutralization of coulombic repulsion (4).

We have been exploring all three nonspecific forces acting between phospholipid bilayer membranes. This is done by determining the work needed to remove water from the lamellar lattice formed by phospholipid bilayers in water. In addition to removing water by the osmotic pressure of dextran (1, 2), we now use both a hydrostatic pressure cell and a chamber with controlled vapor pressure to set the chemical potential of water. In using osmotic, hydrostatic, and vapor pressures, we are able to extend our earlier measurements on egg phosphatidylcholine/water multilayers to the full range of water contents.

By x-ray diffraction we see that as water is squeezed from the lattice, the bilayers not only come closer together, but deform to get thicker and to bring closer together molecules on the same surface. We show how to divide the work of water removal into separate components for overcoming bilayer repulsion and effecting bilayer deformation. Most of the work goes into pushing bilayers together with progressive water removal, the proportion of the total work going to deform the bilayer diminishes from 16% to 7% as water is removed.

The direct repulsive pressure between egg lecithin bilayers is first detected at a separation of about 27 Å and grows exponentially, with a decay constant of about 2.6 Å, to reach 1500 atm (1 atmosphere = 1.013×10^5 newtons/m^2 = 1.013×10^6 dyne/cm^2) at 3 Å separation. The direct repulsive force translates into a formidable kinetic barrier preventing lipid vesicles of 100 Å or greater radius approaching each other or approaching a planar membrane. It is much larger than any predicted or measured electrostatic repulsion.

The stress of bilayer deformation, expressed as a lateral pressure or rate of change of molecular free energy with molecular area, is zero at the equilibrium area of about 75 Å per molecule and rises to 25 dynes/cm when the phospholipid area is reduced to 60 Å^2. Because most synthetic phospholipids exhibit ("T_c") transitions between disordered and ordered hydrocarbon chains as their polar groups are laterally compressed, the present method will allow us to test models of those transitions by measuring directly the free energy changes that lead to phase transition. It is now possible also to compare the lateral pressure in bilayer and monolayer films.

Method

The three techniques used to remove the water are (Fig. 1): (*i*) A high molecular weight dextran solution equilibrates with the multilayer and competes with it for water by exerting osmotic pressure ($\lesssim$10 atm). (*ii*) The multilayer is squeezed under pressure P in a cell from which water is allowed to escape via a semipermeable membrane ($6 \leqslant P \leqslant 500$ atm). (*iii*) A saturated salt solution competes for water with the multilayer through a vapor phase whose partial pressure p is less than the vapor pressure P_0 of pure water ($225 \lesssim$ equivalent physical pressure $\lesssim$ 1500 atm). Removal of water under osmotic or hydraulic pressure allows measurement of water activity in a regime where information obtained from vapor pressure is overwhelmed by error because of the proximity to saturation.

The work of transfer of water *from* the multilayer lattice *to* a bulk water phase is set by the chemical potential difference $-\mu_w$, where μ_w is defined in Fig. 1; μ_w is related to P by $\mu_w = -P\overline{V}_w$, in which $\overline{V}_w$ is the molar volume of water.

The use of x-ray diffraction to follow structural changes in this system has been described (1, 2, 5, 6). For each degree of water removal we measure the multilayer repeat distance d and the volume of water v_w per phospholipid molecule. Using the volume of the phospholipid molecule v_l, we can infer the bilayer thickness $d_l = v_l\, d/(v_l + v_w)$ and bilayer separation $d_w = d - d_l$ (Fig. 1).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

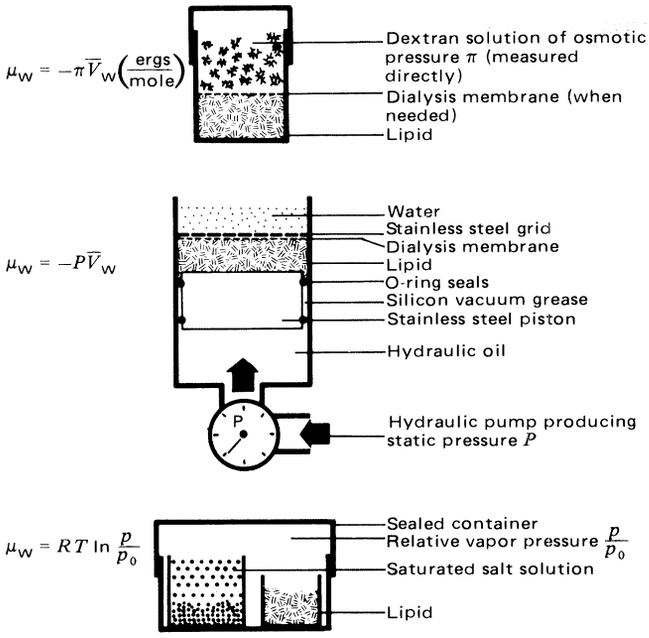

FIG. 1. Scheme of the three methods used to apply (equivalent) pressure P to the phospholipid lamellar lattice. The work, $-\mu_w$, required to transfer a molar volume $\overline{V}_w$ of water from the lamellar phase to bulk water is related to P by $P\overline{V}_w = -\mu_w$.

The average area A available to each phospholipid molecule, a cross-sectional area parallel to the bilayer plane, is determined from the definition that the volume $A \times d$ is equal to the volume $2(v_l + v_w)$ filled by two phospholipid molecules plus the amount of water apportioned to these two phospholipids. This volume of water is determined from the known composition c (dry weight percent) of the lamellar phase. In Å^2 this area is (6)

$$A = \frac{2 \times 10^{24}\, MW_L\, \bar{v}_L}{\phi\, d(\text{Å})\, N_0},$$

in which

$$\phi = \left[1 + \frac{(1-c)\,\bar{v}_w}{c\,\bar{v}_L}\right]^{-1}$$

is the volume fraction of lipid in the lamellar phase and MW_L, the molecular weight of egg lecithin, is 790; N_0 is Avogadro's number; and $\bar{v}_w$ and $\bar{v}_L$ are the partial specific volumes of water and egg lecithin, respectively. We have taken both $\bar{v}_w$ and $\bar{v}_L$ to be equal to 1. [If $\bar{v}_w = 1.00$ and $\bar{v}_L = 0.987$ (7), the areas as presented herein would be underestimated by a maximum of 1.2% or about 1 Å^2.]

For present purposes we have used that definition of the water layer between bilayers that requires fewest additional assumptions—specifically, that all the water forms a separate layer of thickness d_w. Other models (2, 8), where some water is associated with the phospholipid polar groups, can be devised as required, but have little effect on our present interpretation. In accordance with known compressibilities (9), we have assumed no significant weight density changes with removal of water. It is not necessary to make any assumptions about the packing of the hydrocarbon chains or the polar groups of the phospholipids.

Experimental results

All the above parameters for egg lecithin at room temperature have been listed in Table 1. The measured parameters are the applied pressure P, the repeat distance d, and the volume of water v_w per phospholipid molecule. The rest are calculated as described above and in the table legend. Not only does d_w decrease when bilayers are deprived of water, but bilayers also deform to bring polar groups on the same membrane closer together. We observe this deformation as a decrease in area A and an increase in thickness d_l with decreasing water (Table 1). Such a dual response reflects a minimization of the lattice free energy with respect to both d_l and d_w at *each* given amount of water. This minimization comes from a competition between forces between molecules in the same bilayer (changes in d_l) and those between bilayers (changes in d_w).

Table 1. Experimental data for pressure P, repeat spacing d, and volume of water v_w per phospholipid molecule

$\log_{10}P$, dyne/cm^2	v_w, Å^3	d, Å	d_l, Å	d_w, Å	A, Å^2
$P = 0$		62.1	35.1	27	74.8
4.028	1020.39	62.20	35.00	27.20	75.03
4.204	1049.65	62.80	34.90	27.90	75.24
4.681	1027.89	62.40	35.00	27.40	75.03
4.853	992.21	61.80	35.20	26.60	74.60
5.082	1002.52	61.90	35.10	26.80	74.81
5.415	914.67	60.40	35.60	24.80	73.76
5.766	853.45	59.40	36.0	23.40	72.94
5.943	810.23	58.7	36.3	22.40	72.34
6.268	760.54	57.8	36.6	21.2	71.75
(6.502)	693.64	56.7	37.1	19.6	70.78
6.511	714.25	(57.03)	(36.95)	(20.1)	(71.07)
6.671	682.16	(56.45)	(37.15)	(19.30)	(70.69)
(6.738)	670.61	56.2	37.20	19.00	70.59
6.77	632.06	55.70	37.60	18.10	69.84
6.84	602.80	55.30	37.90	17.40	69.29
6.92	565.65	54.80	38.30	16.50	68.56
6.94	647.75	56.00	37.50	18.50	70.03
6.994	610.66	(55.17)	(37.63)	(17.50)	(69.79)
7.02	622.59	55.65	37.75	17.90	69.56
(7.027)	602.56	55.0	37.70	17.30	69.66
7.08	563.70	54.60	38.20	16.40	68.74
7.20	510.99	53.90	38.80	15.10	67.68
7.44	494.07	53.40	38.80	14.60	67.68
7.49	472.29	53.30	39.20	14.10	66.99
7.67	463.41	52.90	39.10	13.80	67.16
7.77	439.89	52.60	39.40	13.20	66.65
7.87	389.64	52.00	40.10	11.90	65.49
7.92	347.56	51.60	40.80	10.80	64.36
8.35	253.53	50.02	41.95	8.10	62.60
8.35	252.17	50.37	42.25	8.12	62.11
8.59	204.88	50.42	43.61	6.81	60.17
8.69	173.92	50.87	44.92	5.95	58.46
8.87	146.53	49.97	44.95	5.02	58.38
9.19	91.99	49.30	46.07	3.23	56.96

Using a molecular volume of 1313 Å^3 per phospholipid molecule, one computes lipid bilayer thickness d_l, bilayer separation d_w, and molecular cross section A. Figures in parentheses are values interpolated from directly measured nonparenthetical values immediately above and below in the same column. Error in pressure as $\log_{10} P$ is about 0.01; in d, ±0.5 Å. To obtain the molar work of transfer of water from the lamellar lattice to a pure water phase, use $-\mu_w = P$ (dynes/cm^2) × 18 cm^3; $-\mu_w$ (cal/mol) = P (dyne/cm^2) × 18 (cm^3)/4.184 × 10^7 (erg/cal) = 4.3 × 10^{-7} P (dyne/cm^2).

Separation of Measured Stress *P* into Inter- and Intrabilayer Forces. We can consider the free energy g per phospholipid molecule as a function of membrane separation d_w and of any variable describing molecular packing. For the latter we use A, the average cross-sectional area per molecule, or d_l, the bilayer thickness, or d_{pp} the average center-to-center distance between polar groups. The increase Δg in molecular free energy with change of volume Δv_w of water is (using our sign convention for pressure P) $\Delta g = -P\,\Delta v_w$. Choosing A and d_w as descriptive variables, we split the change Δv_w in the water

volume into $\Delta v_A = (d_w/2)\Delta A$ and $\Delta v_{d_w} = A(\Delta d_w/2)$, in which, by geometry, $v_w = A(d_w/2)$. Intuitively one can see that the applied pressure P times each of these volume changes gives the change in molecular free energy with deformation and with separation—i.e.,

$$\frac{\partial g}{\partial A} = -P\, d_w/2 \qquad [1]$$

and

$$\frac{\partial g}{\partial (d_w/2)} = -PA \equiv -F_R. \qquad [2]$$

Since $d_l \cdot A = 2v_l$, with v_l the molecular volume of a phospholipid molecule, we have

$$\frac{\partial g}{\partial d_l} = P\,\frac{d_w}{2}\,\frac{A}{d_l} = P\,\frac{v_w}{d_l} \qquad [3]$$

for the rate of change of molecular free energy with bilayer thickness d_l. Assuming hexagonal packing of the phospholipid molecules, we may write $A = (\sqrt{3}/2)\, d_{pp}{}^2$ and the rate of change of molecular free energy with distance between polar groups on one bilayer becomes

$$\frac{\partial g}{\partial d_{pp}} = -P\sqrt{3}\left(\frac{d_w}{2}\right) d_{pp}. \qquad [4]$$

More formally, we could have written the free energy g per phospholipid molecule as a function of any two variables describing the lattice—e.g., (d_w, A), (d, d_w), (d, d_l), (d_l, d_w), etc.—but here have chosen for mathematical convenience the pair of variables (d_w, A). For a given amount of water per phospholipid molecule $v_w = Ad_w/2$, the system takes on a pair of values (d_w, A) that minimize $g(d_w, A)$. That is,

$$\delta_v g = 0 = \frac{\partial g}{\partial d_w}\,\delta\, d_w + \frac{\partial g}{\partial A}\,\delta A = \left(\frac{\partial g}{\partial d_w} - \frac{A}{d_w}\,\frac{\partial g}{\partial A}\right)\delta d_w. \qquad [5]$$

Since the quantity in parentheses must be zero to assure minimization, changes in free energy with phospholipid area $\partial g/\partial A$ must be related to those occurring with bilayer separation $\partial g/\partial d_w$,

$$\frac{\partial g}{\partial A} = \frac{d_w}{A}\,\frac{\partial g}{\partial d_w}. \qquad [6]$$

The work of changing the water volume by Δv_w goes into incremental changes in area ΔA and in separation Δd_w, $2\Delta v_w = A\Delta d_w + d_w \Delta A$. The total work is, again,

$$\begin{aligned}\Delta g &= \frac{\partial g}{\partial A}\,\Delta A + \frac{\partial g}{\partial d_w}\,\Delta d_w \\ &= -(P/2)\,(A\,\Delta d_w + d_w \Delta A).\end{aligned} \qquad [7]$$

From Eqs. **6** and **7** the ratio of the separation work, $(\partial g/\partial d_w)\,\Delta d_w$, to deformation work, $(\partial g/\partial A)\,\Delta A$, is

$$\frac{\dfrac{\partial g}{\partial d_w}\,\Delta d_w}{\dfrac{\partial g}{\partial A}\,\Delta A} = \frac{\Delta d_w/d_w}{\Delta A/A} = \frac{\Delta(\ln d_w)}{\Delta(\ln A)} \equiv X. \qquad [8]$$

The change in molecular free energy with change in spacing d_w is, again (see Eq. **2**),

$$\frac{\partial g}{\partial (d_w/2)} = -P\,\frac{\Delta v_w}{\Delta (d_w/2)}\,\frac{X}{1+X} = -PA \equiv -F_R \qquad [9]$$

and the rate of change of energy with cross-sectional area A (i.e., the lateral pressure on a molecule in a bilayer) is

$$\frac{\partial g}{\partial A} = -P\,\frac{\Delta v}{\Delta A}\,\frac{X}{1+X} = -P\,\frac{d_w}{2}. \qquad [10]$$

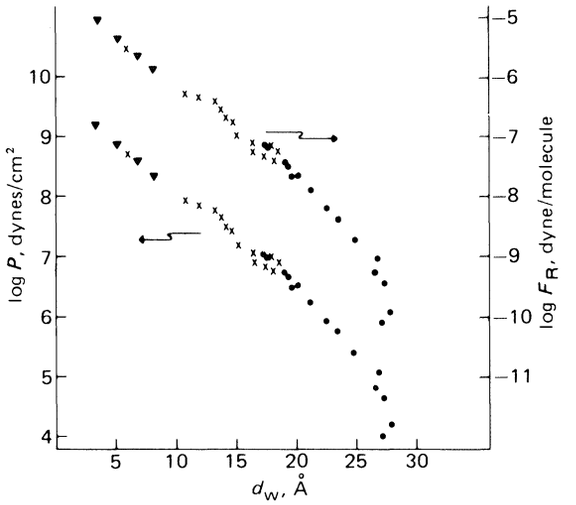

FIG. 2. Applied pressure P and interbilayer force $F_R = PA$ (Eqs. **2** and **9**) per lipid molecule against bilayer separation d_w. Note nearly linear relation between logarithm of stress and separation until appearance of attractive forces near the equilibrium spacing $d_w = 27.5 \pm 0.5$ Å. Points ●, ×, and ▼ are derived, respectively, from osmotic, hydrostatic, and vapor pressure methods for setting the chemical potential of water. Osmotic pressure points are from refs. 1 and 2.

One should note that this derivative, $\partial g/\partial A$, when evaluated below is taken at different values of the separation d_w. Complete separation between interlamellar forces $\partial g/\partial d_w$ and intralamellar forces $\partial g/\partial A$ would require that the change in energy g per molecule be separable as $\partial g/\partial A = f_1(A)$ and $\partial g/\partial d_w = f_2(d_w)$.

Numerical results

The pressure between egg lecithin bilayers, P (Fig. 2), is conveniently described by the form $P(d_w) = P_0\, e^{-d_w/\lambda}$, in which $P_0 = 7.05 \times 10^9$ dynes/cm^2 and $\lambda = 2.56$ Å. The related force $F_R = PA$ on one molecule acting in a direction perpendicular to the bilayer (from Eq. **2** or **9**) is also plotted in Fig. 2. It is described by $F_R(d_w) = F_0\, e^{-d_w/\lambda'}$, $F_0 = 4.15 \times 10^{-5}$ dynes, $\lambda' = 2.62$ Å.

As used here, these exponential relations are purely an empirical representation. No theory is implied, although an order-parameter description (10) does suggest that the force should decay exponentially. We do not insist that P plotted against d_w is purely exponential nor do we neglect the possibility that small kinks in the curves of Fig. 2 might contain useful information. This second point should not be pressed until significantly more data have been collected.

At 27.5 ± 0.5 Å separation, bilayer repulsion is balanced by an attraction that is probably a van der Waals dispersion force (2, 11). We use the language of a Hamaker coefficient H, wherein the attraction between parallel planar bodies of thickness d_l separation d_w has the form (12, 13)

$$(H/(6\pi))(1/d_w{}^3 - 2/(d_w + d_l)^3 + 1/(d_w + 2d_l)^3).$$

Equating this attraction to the pressure $P_0 e^{-d_w/\lambda}$ and extrapolating to $d_w = 27.5 \pm 0.5$ Å, we find the apparent coefficient $H = (6.0 \pm 0.9) \times 10^{-14}$ erg. This procedure has been described in detail (2).

Activity of Boundary Water. It is obvious from Fig. 2 that the work of transfer of water, $-\mu_w = P\,\bar{V}_w$, is a continuous function of water content all the way down to the last two water molecules per phospholipid molecule. (Removal of more water simply destroys the lamellar lattice of egg lecithin.) We take strong exception to the widespread but facile assumption that water near lipid bilayers can be divided into discrete classes of

"bound" and "free." The interested reader can verify for himself that for separations greater than 20 Å, μ_w amounts only to fractions of a calorie per mole. Such small differences in chemical activity, while leading to strong physical forces, are impossible to detect by using most probes of water.

Vesicle Interaction. We have argued elsewhere (1, 4, 14) that the pressure P, or the force PA (Eq. **9**), which occurs between both charged (14) and uncharged (1) bilayers when $d_w < 20$ Å, has an important influence on contact and fusion of lipid vesicles. Assuming its exponential variation (Fig. 2) and applying the Derjaguin approximation (12, 15), we may write the force between two spherical vesicles of radius a as $\pi a\lambda P(d_w)$ and between a vesicle and flat bilayer as $2\pi a\lambda P(d_w)$. The respective energies are $E_{ss} = \pi a\lambda^2 P(d_w)$ and $E_{sp} = 2\pi a\lambda^2 P(d_w)$. Here d_w now refers to the distance of closest approach, and we assume no deformation of the membranes.

These forces and energies translate into significant barriers for even the smallest vesicles. For example, if $a = 300$ Å, roughly the size of a synaptic vesicle, the statistical weightings against mutual approach, $e^{-E_{ss}/kT}$ or $e^{-E_{sp}/kT}$, are less than 10^{-4} for separations <13 Å between spheres and <14.5 Å between sphere and planar bilayer. If a is as small as 99 Å, the apparent limiting size achieved by vigorous sonication of egg phosphatidylcholine (16), then the corresponding distances are 10.2 Å and 11.7 Å, respectively. It is most likely then that membranes must be deformed or otherwise modified before they can make the very close contact apparently necessary for the occurrence of exocytosis or vesicle fusion.

Membrane Deformability and Stability. We plot the force between adjacent phospholipid molecules in the same bilayer as $\partial g/\partial d_{pp}$ (Eq. **4**) in Fig. 3. This force is not easily described by an exponential or inverse-power variation. It does change rapidly—by some 4 orders of magnitude with a 1.2 Å change in average separation.

The ratio of amounts of work going into separation and deformation, given by the parameter $X = \partial(\ln d_w)/\partial(\ln A)$ (Eq. 8), has been determined here by plotting $\ln d_w$ against $\ln A$ and taking the slope of the best fit quadratic. As water is removed, only 7–16% of the incremental work of removal goes into deformation (Fig. 4). The remaining 84–93% of this incremental work goes to pushing bilayers closer together. Consequently, the force F_R perpendicular to the bilayer, because it is plotted on a log scale in Fig. 2, appears to differ little from that assuming no deformation.

The lateral pressure $-\partial g/\partial A$ (Fig. 5) shows an increase from 0 to 25 dynes/cm in changing the area of the phospholipid from 75 Å^2 to 57 Å^2. By integrating $\partial g/\partial A$ numerically, we estimate

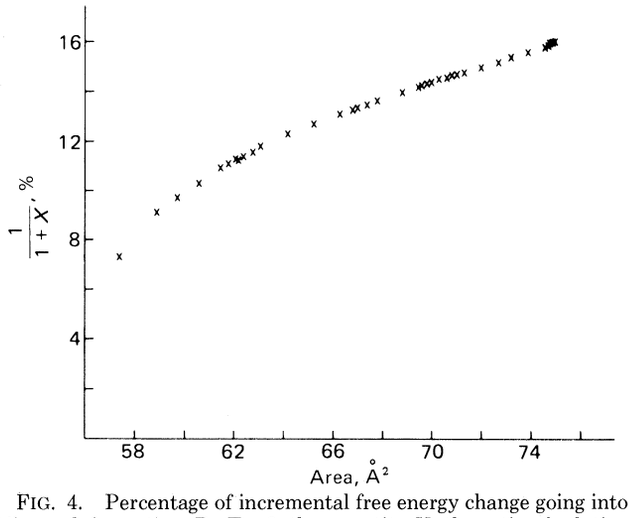

FIG. 4. Percentage of incremental free energy change going into bilayer deformation. By Eq. 8, the quantity X, the ratio of relative rate of change in bilayer separation to relative rate of change in bilayer dimension, is also the ratio of the work of separation to the work of deformation. [$1/(1 + X)$ = % of total energy that deforms the bilayer.] The required derivatives were made from a quadratic fitted to a plot of $\ln A$ against $\ln d_w$. As the cross-sectional area decreases, the bilayer appears progressively stiffer—i.e., relatively more work goes into pushing bilayers together than into deforming them.

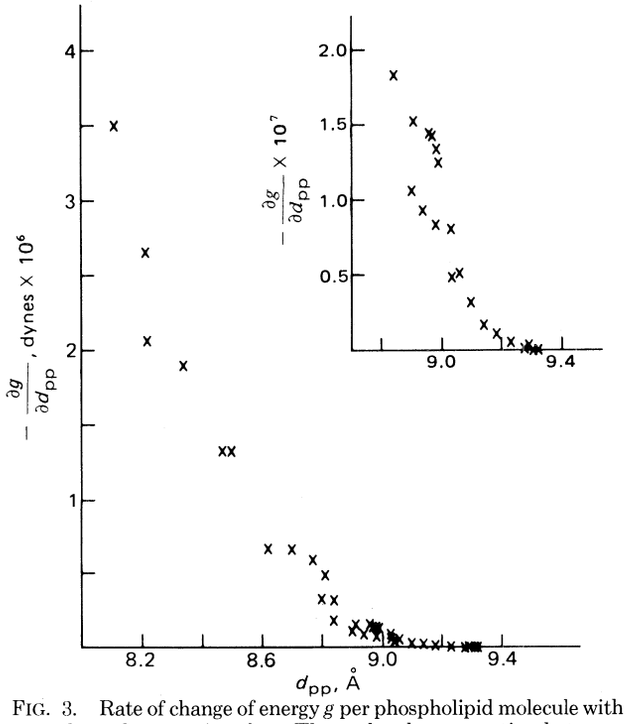

FIG. 3. Rate of change of energy g per phospholipid molecule with average lateral separation d_{pp}. The molecular separation between adjacent molecules on the same bilayer is obtained from the molecular cross section $A = (\sqrt{3}/2)d_{pp}^2$. Values for $\partial g/\partial d_{pp}$ are obtained directly from the data (Table 1) and Eq. **4**.

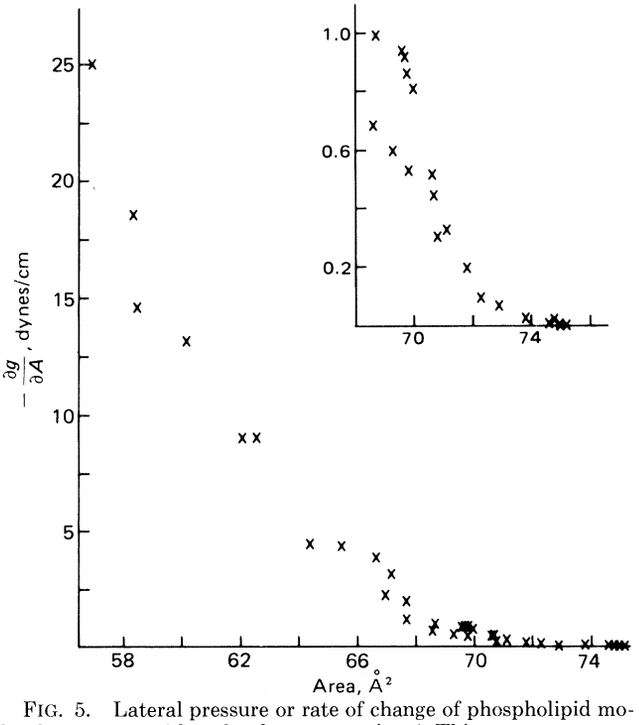

FIG. 5. Lateral pressure or rate of change of phospholipid molecular energy g with molecular cross section A. This pressure appears nonlinear even as it approaches zero at the equilibrium area $A = 75.6$ Å^2. The values of $\partial g/\partial A$ are computed from data in Table 1 by using Eq. **1** or **10**.

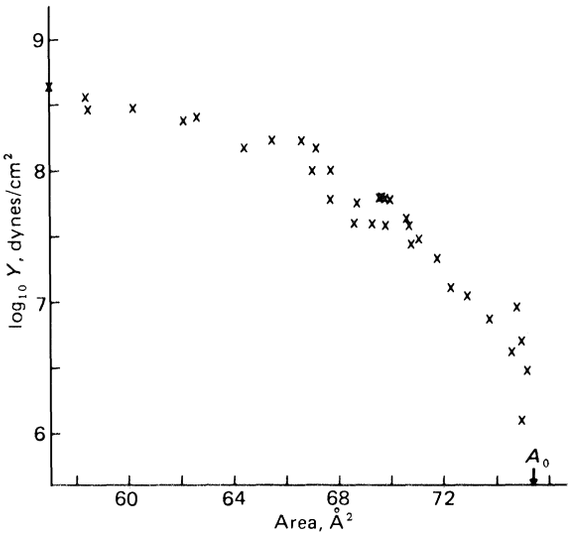

FIG. 6. Apparent modulus of deformability plotted against average molecular crosssection A. The modulus Y is the rate of change of bilayer energy with bilayer thickness, per unit area—i.e., $(2/A)(\partial g/\partial d_l)$ divided by the relative change in thickness from equilibrium $(d_l - d_0)/d_0$. This "modulus" does not approach a constant value as one approaches equilibrium dimensions. The limiting estimate given in the text, 5×10^6 dynes/cm^2, is a linear extrapolation of this plot to the equilibrium area 75.6 Å^2. "Bad" points due to scatter near equilibrium have *not* been omitted.

that the work of deforming the bilayer molecules from A_0 = 75 Å^2 to A = 60 Å^2 is less than 1 kT/molecule. One might expect, in the absence of other interactions, large thermal fluctuations in the area of single phospholipid molecules.

Because the lateral pressure $-\partial g/\partial A$ is not linear in A, the bilayer's mechanical properties cannot be summarized rigorously in terms of conventional moduli. Nevertheless, as a convenient way to compare with related measurements, we compute the rate of change of work required to cause a relative change in thickness of a square centimeter of bilayer, $[(2/A)(\partial g/\partial d_l)]/[(d_l - d_0)/d_0]$, a quantity analogous to Young's modulus (Fig. 6). As $d_l \to d_0$, the equilibrium thickness of a hypothetical isolated bilayer, this modulus is approximately 5×10^6 dynes/cm^2. This value lies between estimates for corresponding moduli found in single bilayer membranes that might contain hydrocarbon solvent: 3.4×10^5 dynes/cm^2 (17) to 10^9 dynes/cm^2 (18–20). The moduli for those membranes were for strain in the opposite direction—i.e., for pulling polar groups apart and making the bilayer thinner. Such artificial bilayers typically break after only 1% deformation. The scatter in our estimates of the modulus is due to the 2% error in determining bilayer dimensions.

The present technique is being applied to a broad class of membrane lipids and provides a practical method for determining membrane interaction and stability. It is becoming clear that the "hydration force" can occur between any two surfaces that are covered by water-soluble groups. We predict, therefore, that this force will be felt between cell membranes as well as between pure phospholipid bilayers. It seems to us that it is these repulsive forces that must be overcome or changed by the biochemical or physical processes governing either the rapid fusion of synaptic vesicles at a nerve terminal or the controlled release of material stored in intracellular vesicles (4).

We can now directly test theories of phase transitions in bilayers that involve changes of hydrocarbon packing and polar headgroup organization. For example, both above and below their hydrocarbon chain phase transitions dilauroyl-, dimyristoyl-, and dipalmitoylphosphatidylcholine as well as egg phosphatidylethanolamine multilayers exhibit forces qualitatively like those seen for egg lecithin (21, 22). The lateral compression of phospholipid bilayers actually induces a transition to the solid-hydrocarbon state above the normal phase-transition temperature. One can measure the force or energy change required to cause phase transitions and compare these with theoretical predictions. It should now be clear not only that studies of membrane interaction and fusion must recognize the strong repulsive forces that exist between membranes, but also that one can now make a systematic study of the physical forces involving lipid aggregates by the marriage of thermodynamics and structure as illustrated here.

We thank Charles Bean for advice on selection of membranes for the hydraulic pressure cell, David Tinker for help in our early attempts at data reduction, and Barry Bunow for several useful conversations on interpretations of results. Meinhart Benkel and Tony Biernacki were indefatigably helpful in building and improving the hydraulic pressure cell. This work was supported in part by Grant A4920 to R.P.R. from the National Research Council of Canada.

1. LeNeveu, D., Rand, R. P. & Parsegian, V. A. (1976) *Nature (London)* **259,** 601–603.
2. LeNeveu, D., Rand, R. P., Parsegian, V. A. & Gingell, D. (1977) *Biophys. J.* **18,** 209–230.
3. Pagano, R. E. & Weinstein, J. N. (1978) *Ann. Rev. Biophys. Bioeng.* **7,** 435–468.
4. Parsegian, V. A. (1977) in *Approaches to the Cell Biology of Neurons*, Society for Neuroscience & Symposia, eds. Cowan, W. M. & Ferrendelli, J. A. (Society for Neuroscience, Bethesda, MD), Vol. 2, pp. 161–171.
5. Luzzati, V. (1968) in *Biological Membranes*, ed. Chapman, D. (Academic, New York), pp. 71–123.
6. Rand, R. P. & Luzzati, V. (1968) *Biophys. J.* **8,** 125–137.
7. Tardieu, A., Luzzati, V. & Reman, F. C. (1973) *J. Mol. Biol.* **75,** 711–733.
8. Tinker, D. O., Pinteric, L., Hsia, J. C. & Rand, R. P. (1976) *Canad. J. Biochem.* **54,** 209–218.
9. Weast, R. C., ed. (1970) *Handbook of Chemistry and Physics* (CRC, Cleveland, OH), 50th Ed.
10. Marcelja, S. & Radic, N. (1976) *Chem. Phys. Lett.* **42,** 129–130.
11. LeNeveu, D., Rand, R. P., Gingell, D. & Parsegian, V. A. (1976) *Science* **191,** 399–400.
12. Verwey, E. J. W. & Overbeek, J. Th. G. (1948) *Theory of the Stability of Lyophobic Colloids* (Elsevier, Amsterdam).
13. Ninham, B. W. & Parsegian, V. A. (1970) *J. Chem. Phys.* **52,** 4578–4587.
14. Cowley, A. C., Fuller, N., Rand, R. P. & Parsegian, V. A. (1978) *Biochemistry* **17,** 3163–3168.
15. Derjaguin, B. V. (1935) *Kolloid-Z.* **69,** 155–164.
16. Huang, C-H. & Mason, J. T. (1978) *Proc. Natl. Acad. Sci USA* **75,** 308–310.
17. White, S. H. (1976) *Biophys. J.* **14,** 155–158.
18. Requena, J., Haydon, D. A. & Hladky, S. B. (1975) *Biophys. J.* 77–81.
19. Wobschall, D. (1972) *J. Colloid Interface Sci.* **40,** 917–923.
20. Alvarez, O. & Latorre, R. (1978) *Biophys. J.* **21,** 1.
21. McAlister, M., Fuller, N., Rand, R. P. & Parsegian, V. A. (1978) *Biophys. J.* **21,** 213a.
22. Parsegian, V. A., Lis, L. J., McAlister, M. J., Fuller, N. L. & Rand, R. P. (1979) *Biophys. J.* **25,** 174a.