the olefin absorption region. Very low concentrations of nitrogen oxide (less than 0.01 ppm) were present in the dilution air. Despite these very small amounts and ratios of olefin to nitrogen oxide greater than 1000:1, at least part of the background conversions may arise from reactions induced by nitrogen oxide.

Although conversions of aldehyde and olfefin mixtures (Table 2) are slower in sunlight than in laboratory irradiations, the amounts consumed are significant. Comparisons of the rates of reactions in this new series of reactions with those under comparable conditions, but involving nitrogen oxides, are difficult. Laboratory irradiation sources do not reproduce accurately the ratio of total solar ultraviolet to the fraction of ultraviolet less than 3400 Å. Ultraviolet measurements in Los Angeles in October 1965 indicated that at that time of the year about 20 percent of the ultraviolet light was below 3400 Å (*12*). An initial comparison of laboratory results (Table 1) and sunlight irradiations (Table 2) of ethylene or 1-butene with aldehyde or nitric oxide indicates that the difference in rates of reaction is about an order of magnitude; that is, the rate of reaction of a hydrocarbon in the presence of optimum amounts of nitrogen oxides may be about ten times faster than for the same hydrocarbon in the presence of an aldehyde.

The relation of this class of reaction systems to the desirability of reducing atmospheric nitrogen oxides is of practical significance. Even the limited results now available suggest that the rates of reaction and product yields in atmospheric reactions may not approach zero as nitrogen oxides are reduced even to very low concentrations.

Aldehydes are emitted to the atmosphere directly by combustion processes. Furthermore, aldehyde yields in photooxidations of hydrocarbon in the presence of nitrogen oxide in the laboratory remain high even at low nitrogen oxide levels (*3*). The present results could have a direct bearing on efforts to determine the improvment in purity of the atmosphere attainable by control of nitrogen oxides from combustion sources.

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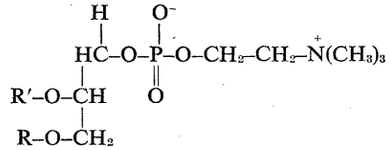
Forces between Lecithin Bimolecular Leaflets Are Due to a Disordered Surface Layer

Abstract. *The long-range repulsion observed between bileaflets of lecithin cannot be explained either with the usual view that the polar groups are arrayed coplanar with the bileaflet surface or by the assumption that charges protrude straight into the aqueous environment. Statistical-thermodynamic analysis of experimental data suggests rather that structure of the leaflet surface is better described as a diffuse charge layer. Forces between leaflets are caused largely by entropy changes in the surface with leaflet separation.*

Recent x-ray diffraction studies on lamellar liquid-crystals of pure cell-membrane lecithin phospholipids in water (*1*) offer an unusual opportunity for quantitative study of their bimolecular leaflet properties (Fig. 1). These data have been interpreted as showing (*2*) that there is a long-range potential field around the electrically neutral leaflet that has a strength comparable to forces within the leaflet. This field, acting to repel the leaflets, causes an increase in the thickness of the lipid aggregates as the water fraction decreases. The x-ray data can be accounted for by applying a statistical-thermodynamic theory previously developed (*3*) to explain phase transitions between liquid-crystalline states of fatty acid salts and water. This analysis indicates that the lecithin molecules tend to aggregate in bilayers with maximum disorder, and this disorder itself acts as a thermodynamic force when perturbed.

The lecithin molecules,



each have two hydrocarbon chains (16 to 22 carbons long) esterified to the glycerol backbone which merge to form a nonpolar region (Fig. 1a). The zwitterionic polar group is part of the boundary region between hydrocarbon and water. In the one-phase liquid-crystal system (*1*), planar lipid lamellae alternate with layers of water. For each lipid concentration set in the experiment, the system assumes a measurable repeat distance or long spacing d to minimize its free energy. Measured densities and molecular weights of components allow one to calculate a volume fraction ϕ = (volume hydrocarbon)/(total volume) from the weight concentration. Mean thickness of the hydrocarbon of the bilayer, then, is $d_{hc} = \phi d$ and of the water layer, plus any polar parts of the lipid molecule, is $d_{aq} = (1 - \phi)\ d$. The interfacial area S allotted per molecule can also be calculated from the spacing d, since $S \cdot d_{hc} = 2\ V_{hc}$, where V_{hc} is the volume of hydrocarbon per molecule (*3*, *4*).

The generally assumed picture (*5*) of the bileaflet surface has been that the zwitterionic polar groups are coplanar with the hydrocarbon-water interface. A cardinal feature of this model is that all stabilizing interactions are confined to the individual lipid leaflets; there is only a weak force field outside the leaflets (*5*). The fact that lamellar thickness changes with concentration is strong evidence against this. If all stabilizing interactions were confined to the same leaflet its size would not be changed by the approach of other structures. Instead, when water is removed from the one-phase system (that is, ϕ is increased) interaction between leaflets causes them to thicken (d_{hc} increases). Water layer thickness d_{aq}

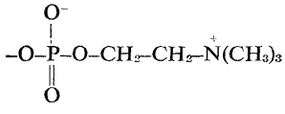
Table 1. Calculations on x-ray spacing data. Weight fraction lecithin c and long spacing d are used to find volume fraction hydrocarbon moiety ϕ, hydrocarbon and aqueous region thickness d_{hc} and d_{aq}. The other column headings: ξ designates the fraction of tetramethylamine groups within 5 Å of phosphate if allowed to diffuse freely; $[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}(x)$ is a function relating to ϕ and d, which should give the same value of all measured pairs of variables; ϕ = (volume hydrocarbon and glycerol chains/total volume). "Hydrocarbon" refers to all the lecithin molecule except for the phosphoryl choline moiety. The hydrocarbon density employed is 0.95 g/cm³, and 1.07 g/cm³ for total lecithin density. Measured molecular weight of the egg lecithin used was 775. The system was free of small ion salts. Temperature was 22°C [data of Small and Bourges (*1*) and of Small, Bourges, and Dervichian (*6*)].

c	ϕ	d(Å)	d_{hc}(Å)	d_{aq}(Å)	ξ(%)	$[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}(x)$*
0.56	0.468	64.1	30.0	34.1	79	2.529
.62	.520	60.5	31.5	29.0	80	2.591
.7	.591	56.4	33.3	23.1	83	2.639
.8	.680	52.5	35.7	16.8	87	2.641
.85	.725	51.	37.	14.	90	2.613

* Average value, 2.602; average r.m.s. deviation, 1.6 percent.

does not decrease as fast as it would if d_{hc} remained constant. This can be due only to a repulsion between leaflets (*2*). The plot of d_{aq} versus ϕ in Fig. 1b shows that this repulsion occurs at distances of the order of 30 Å between surfaces with no net charge.

In an unsuccessful effort to identify a source of repulsion between leaflets, I considered a model in which the



groups were part of the aqueous phase and stood out at right angles to the lamellar surface. This was to bring the positive charges on opposing leaflets as close together as possible. The set of parallel charge pairs on the same leaflet was fixed on a hexagonal net to minimize their mutual repulsion. I then calculated the interaction force between such an array and a reference charge pair on an opposing leaflet; the pair was in the minimum energy position above the space between three molecules in the hexagonal array. Since the observed repulsion must occur at constant concentration, a displacement of the reference charge pair toward the array (reducing d_{aq}) must be accompanied by a separation of the pairs in the net (increase in $S, d_{aq}/d_{hc}$ is constant). Under these conditions it turns out that there is actually a weak attraction between the opposing leaflets. The charge groups tend to "interdigitate." Since even this fixed-charge model failed completely, a different approach was used —one that does not try to define a given structure for the leaflet.

Imagine a situation where the $-CH_2-N^+-(CH_3)_3$ part of the choline molecule is again in the aqueous region but acts as an independent counterion whose distance from the phosphate group at the lipid-water boundary can vary although it is limited by the highly flexible $-CH_2-CH_2-$ linkage between them (about 5 Å maximum separation). The electrostatic potential due to diffusion of free ions from the negatively charged surface and the distribution of counterions in the aqueous region are calculated approximately by use of a planar Poisson-Boltzmann equation. Electrostatic potential will be a function of surface charge density (phosphate charges) and water layer thickness, both of which can be found from the repeat distance d and volume fraction ϕ. It is important that, even if ions are allowed to diffuse freely, the majority of them would stay within the 5-Å limit (column ξ, Table 1). To a first approximation, then, one can treat the $-CH_2-N^+-(CH_3)_3$ group as a free counter-ion in formulating an electrostatic free energy for the system.

Electrostatic free energy, G^e, is found by calculating the electrostatic energy of the potential field at any state of partial charge and reversibly charging the system from a discharged state in the usual manner for ionic solutions (*3*). The G^e will be a function of surface charge density and lamellar separation; it has been shown (*3*) that

$$G^e = G^e(d, \phi) = kT\left(\frac{x}{\tan x} - 1 + \ln\frac{2x}{\sin 2x}\right)$$

per molecule, where

$$x \tan x = \frac{\pi e^2}{2\varepsilon\, kT\, V_{hc}}\,\phi(1-\phi)d^2$$

(e is the unit of electronic charge; V_{hc}, the volume of the hydrocarbon and glycerol chains of one lecithin molecule; ε, the dielectric constant of water; k, Boltzmann's constant; and T, temperature).

A second important contribution to the free energy will come from energy of the boundary surface between lipid and water regions. Since the interfacial area per molecule, S, (55 to 70 Å² in Fig. 1b) is greater than the area of the glycerol group, much of this boundary surface will necessarily be occupied by hydrocarbon material. In this case, as in previous work on ionic lipid aggregates (*3*), the surface free energy, G^s, is considered proportional to the interfacial area plus constant terms G^s_0. Then $G^s = \gamma S + G^s_0$, where γ is a constant surface coefficient.

The interior hydrocarbon region and the aqueous region in the discharged state give additional terms to the free energy. However, these are bulk regions and their free energy contribution will be taken as an increment G_0 (bulk) which does not vary strongly with changes in system geometry.

The total free energy

$$G = G(d, \phi) = G^e(d, \phi) + G^s(d, \phi) + G_0$$

is a function of two independent variables. At each given concentration ϕ, the system will assume a spacing d such that G is at a minimum. Here

$$\left(\frac{\partial G}{\partial d}\right)_\phi = 0 \text{ or } \left(\frac{\partial G^e}{\partial d}\right)_\phi = -\left(\frac{\partial G^s}{\partial d}\right)_\phi$$

a relation between ϕ and d that one should observe in the experimental data. By introducing the functions given for G^e and G^s, this relation can be rewritten (*3*) as

$$[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}(x) = \gamma[\pi e^2 V_{hc}/2\epsilon\,(kT)^3]^{\frac{1}{2}}$$

The parameter used above in G^e is $x = x(d, \phi)$; $f_{st}(x)$ is a well-defined monotonic increasing function of x.

No quantity in $\gamma[\pi e^2 V_{hc}/2\epsilon\,(kT)^3]^{\frac{1}{2}}$ should change with d or ϕ, if one assumes that the surface coefficient and dielectric constant are independent of concentration. In other words

$$[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}[x(d, \phi)]$$

must have the same value for all observed pairs of (d, ϕ). The present model can be critically tested by calculating

$$[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}(x)$$

for each ϕ and corresponding d (which vary by 45 percent and 23 percent, respectively) and finding its root-

mean-square (r.m.s.) percentage deviation. Macroscopic values of dielectric constant and density are used for water (see Table 1). The theory accounts for the experimental observation with a deviation of only 1.6 percent, which is comparable to experimental variation. (An error of 1 percent in ϕ will vary

$$[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}(x)$$

by more than 1 percent.) Typically, experimental error in d is $\pm$ 0.5 Å and in c, $\pm$ 2 percent.

By verifying the constancy of

$$[\phi/(1-\phi)]^{\frac{1}{3}} f_{st}$$

one has also obtained a characteristic value of $\gamma[\pi e^2 V_{hc}/2\epsilon(kT)^3]^{\frac{1}{3}}$ and hence of the surface coefficient $\gamma = 9.85 \pm 0.16$ erg/cm^2 for the system, independent of concentration. Two significant properties of the hydrocarbon chain component are its volume per molecule V_{hc} = (mol. wt. hydrocarbon + glycerol)/(Avogadro's No. $\times$ density) and the energy of unit area interface with water γ. This puts one in a position to calculate these quantities and predict their role in aggregate formation.

Already some correspondence with molecular structure is suggested. Previous estimates of γ were approximately 18.5 erg/cm^2 for fatty acid salts with saturated hydrocarbon chains (12 to 18 carbons long) and 15 erg/cm^2 for the singly unsaturated C_{18} chain (*3*). The estimate of 9.85 erg/cm^2 in this sample of egg lecithin may be related to the fact that 16 percent of the side chains are the doubly unsaturated 18-carbon chain of linoleic acid (*6*). However, this connection between unsaturation and the value of γ is still tentative, especially in view of the approximation necessary in finding the electrostatic free energy.

In the aqueous region the electrostatic free energy includes the energy of interactions between charges as well as an entropy term dependent on their arrangement. The electrostatic energy part, E^e, of the free energy $G^e = E^e - TS^e$ is again a simple function of d and ϕ. The present theory can be used to show that a relatively small part of $(\partial G^e/\partial d)_\phi$ is due to the $(\partial E^e/\partial d)_\phi$ contribution. The dominant term of $(\partial G^e/\partial d)_\phi$ is from $-T(\partial S^e/\partial d)_\phi$; at $\phi = 0.468$, for example, 81 percent of the variation in electrostatic free energy $(\partial G^e/\partial d)_\phi$ is due to $-T(\partial S^e/\partial d)_\phi$. This suggests that there are changes in the probability of arrangements of the polar groups with the approach of another bileaflet. These changes are thermodynamically more important than variation in the actual energy of interaction.

It is almost axiomatic in studying biological materials to associate a particular structure or conformation with the functioning state of the molecule in question. While this is a reliable working assumption in most cases, it may be of limited validity when applied to components that must be flexible and resilient in order to act in their biologi-

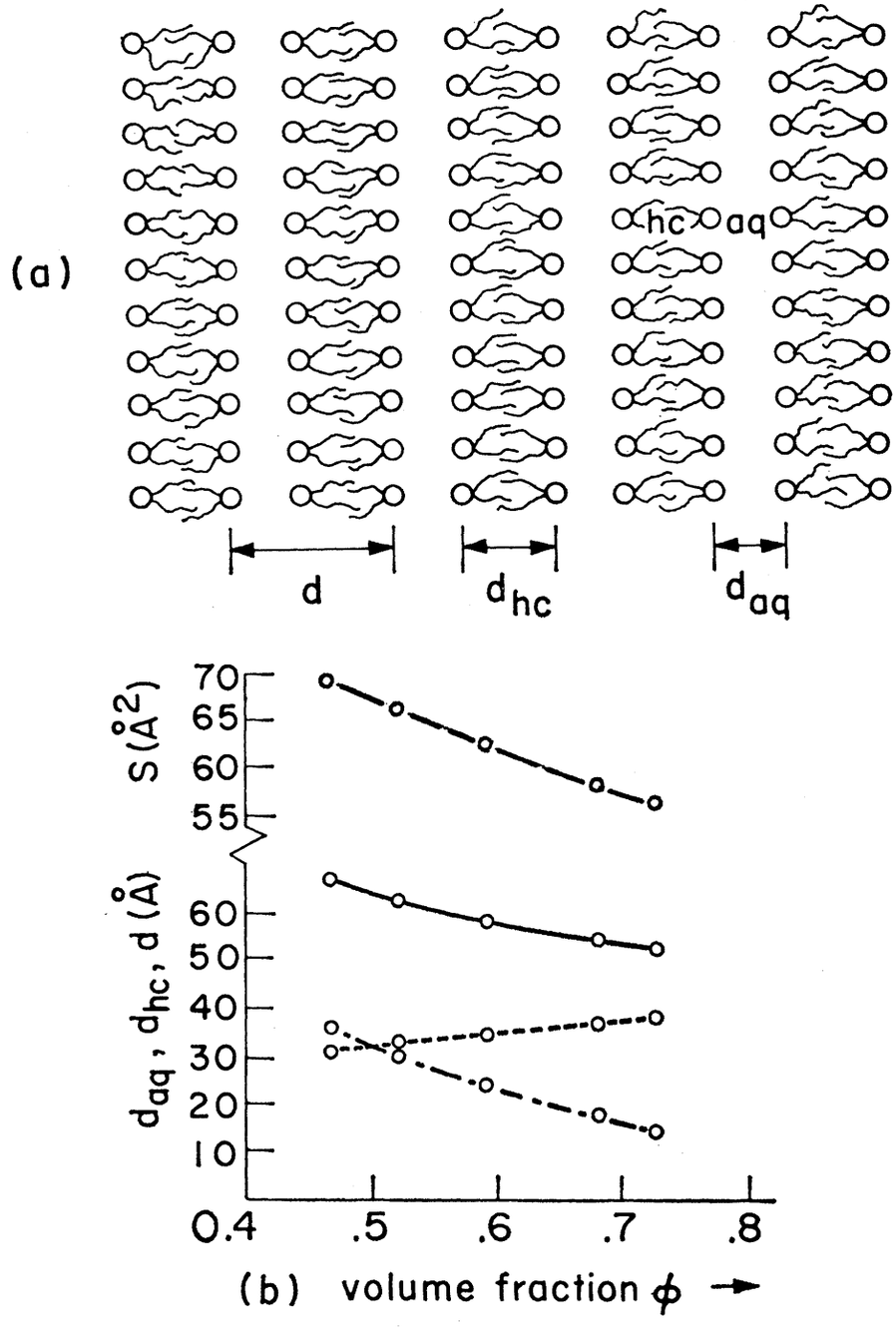


Fig. 1. (a) Planar bimolecular leaflets alternating with aqueous regions in one-dimensional liquid-crystal lattice. Abbreviations: *hc* designates the hydrocarbon interior of the leaflet; *aq*, the pure water or water-plus-choline region. Circles denote the interface between hydrocarbon and water where the glycerol backbone is located. (b) Measured repeat spacing d, leaflet thickness d_{hc}, interleaflet separation d_{aq}, and interfacial area per molecule S plotted as function of lipid volume fraction ϕ. The leaflet thickness of d_{hc} is taken to include the glycerol and hydrocarbon chains; the phosphoryl choline groups are considered as part of the aqueous region. Density of hydrocarbon taken to be 0.95 g/cm^3. Putting the entire lecithin molecule in d_{hc} would shift d_{aq} and d_{hc} down and up respectively but would not change the slope significantly.

cal environment. Resemblance of the lecithin bimolecular leaflet to the arrangement of the lipid fraction in our current picture of the cell membrane may be only superficial (*7*); however, both the forces that stabilize assemblies of this component and the leaflet's influence on its aqueous and ionic environment are probably involved in the organization and behavior of the cell membrane.

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Sex Differences in Taste Preference for Glucose and Saccharin Solutions

Abstract. *Taste preferences of mature male and female rats for caloric and noncaloric sweet solutions have been found to differ. Although females do not drink more water than males, they consume significantly greater quantities of a slightly sweet 3 percent glucose and a very sweet 0.25 percent saccharin solution. When given a choice, males switch their initial preference for a saccharin solution to a preference for a glucose solution after several days, while females maintain a preference for the saccharin solution. Females also prefer significantly higher concentrations of saccharin than males do.*

With the exception of sexual and maternal behavior, there are relatively few categories of response tendencies in which reliable differences between male and female rodents have been reported. The presence of ovarian-dependent activity cycles in the female has been well described (*1*). Less clear are the reports of sex differences in emotionality based upon open-field (*2*) and avoidance tests (*3*).

With a growing interest in the genetics of behavior (*4*) and in the possibility that steroid hormones administered at critical periods may influence the central nervous system to develop in a feminine or masculine direction (*5*), behavior tests that demonstrate sex differences are likely to be useful. While studying the factors that influence taste preferences in animals, we observed that, in general, male and female rats respond differently to solutions of glucose or saccharin.

Mature rats of the Holtzman albino strain (68 males and 55 females) were housed in individual cages and had no experience with sweet solutions prior to testing. Different rats were used in each of three experiments; they were adapted to the test cages for approximately 1 week. Because mature male and female rats differ in weight at the same age, we used groups of animals that varied in age from 80 to 325 days and varied in average weight from 211 to 420 g. In all experiments, Purina Lab Chow and two different solutions in bottles were available to the animals at all times. Each day the 24-hour fluid consumption was measured, bottles were washed, and fresh solutions were provided. Spilled fluid was captured in plastic cylinders mounted under each drinking tube. The solutions were mixed with distilled water; concentrations are expressed in terms of grams in a total of 100 ml of fluid. The saccharin used was sodium saccharin (sodium-*o*-benzoic sulfimide).

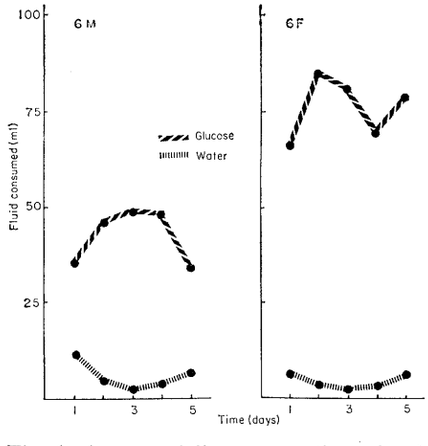

Fig. 1. Average daily consumption of a 3 percent glucose solution and tap water by male (M) and female (F) rats. The average body weight for males at the start of the experiment was 301 g; it was 250 g for females.

In the first experiment six males and six females were given a choice between tap water and a 3 percent glucose (0.165 mole) solution that is just perceptibly sweet to humans. Fluid consumption was measured daily for 5 consecutive days. Both sexes preferred the glucose solution, but the females consumed significantly more of this fluid (Fig. 1). In view of the fact that females consume less water in general (average daily consumption of water is 30 ml for females and 35 ml for males) when this is the only fluid available, the results suggest that the female, in comparison to the male, exhibits an exaggerated response to the sweet solution.

In a second experiment (repeated three times), groups of female and male animals were provided with a choice between a 3 percent glucose solution and a 0.25 percent saccharin (0.01 mole) solution that is almost sickeningly sweet for humans. This saccharin solution is approximately at the point of maximum preference judged by the fact that rats tend to consume less of solutions with either much higher or lower concentrations (*6*). Animals of both sexes consumed more of the saccharin solution at first, but by the 3rd or 4th day the males started to switch to a preference for glucose (Fig. 2). In contrast, the females consumed greater amounts of the saccharin solution and did not switch to a preference for glucose. Regardless of the age and weight of these mature animals, average daily consumption of the saccharin solution reached an asymptote at approximately 60 ml for the females and 35 ml for the males. Although males prefer a 0.25 percent solution of saccharin to tap water for a prolonged period of time, they gradually switch their preference when provided with a less sweet glucose alternative. The females, which show a greater positive response to mildly sweet glucose so-