Biomembrane phase transitions

In cell membranes, which are among the principal organizational structures of living matter, phase transitions appear to be biologically significant, not just phenomena that happen to occur.

John F. Nagle and Hugh L. Scott

Along with DNA and proteins, cell membranes are among the principal organizational structures of living matter. Over the past decade the study of cell membranes has become a focus of effort ranging from the very biologically oriented to the very physically oriented. One property that is especially appropriate for physical studies is the phase transition that occurs in many cell membranes.

The best documented case is found in the cytoplasmic membrane of *Acholeplasma laidlawii*, which is a primitive organism with a large surface-to-volume ratio. After the cell is grown, at a particular temperature T_g, the membrane is extracted and calorimetric measurements made. These show a specific-heat anomaly about 20 celsius degrees broad centered near or slightly below T_g. When these cells are grown at different growth temperatures, the calorimetric anomaly follows the change in T_g.[1] (For a review, see reference 2.) This suggests that the phase transition is not just some physical phenomenon that happens to occur, but that it has real biological relevance.

There are many other examples of physically induced biological changes related to the membrane phase transition. Organisms that exist in cold environments have membrane components giving rise to reduced phase-transition temperatures. Other properties, such as transport, the response to anesthetics and the immunological response, can be altered by changing the thermodynamic state of the membrane involved.

A molecular sketch of a biomembrane, according to the conventional wisdom, is shown in figure 1. Many important functions of cells are carried out by membrane-bound enzymes, which are proteins; they are indicated by the large objects in the figure. However, the primary structural component of many membranes is the lipid (fat) bilayer, consisting of the more or less regularly spaced molecules shown in figure 1.

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Lipid bilayers

Figure 2 shows an enlarged view of space-filling molecular models of these lipid molecules, as they would appear in the top half of the bilayer in membranes. The lipid molecule is well suited to forming bilayer membranes in water because the hydrocarbon tails, like oil, do not mix well with water, and so are called "hydrophobic." But the charged head groups can reduce their electrostatic energy by associating intimately with water with its high dielectric constant, and so are called "hydrophilic." (Other well known molecules with both properties are soaps or surfactants, which have a single hydrocarbon tail. Apparently the presence of two tails on lipids leads to the formation of bilayers, whereas the single-tailed soaps tend to form small spherical structures in water, called "micelles.")

Experiments have conclusively demonstrated that the phase transition in *Acholeplasma laidlawii* is due exclusively to the lipid component of the membrane.[1,2] Therefore physical techniques that elucidate structure, and theory that deals with extended phases, are appropriate tools to study this phenomenon. Equally important, this means that phase-transition studies performed on "model membranes" made from highly purified and commercially available lipids can be expected to yield useful information. These model membranes are similar in their structural properties to biomembranes; furthermore, they can be much better characterized physically and chemically than living cell membranes, which have a complicated assortment of different lipids. The phase transitions are also much sharper with purified lipids of a single homogeneous type, and this makes quantitative measurement and theoretical interpretation much easier.

The simplest model membrane system is made simply by mixing lipid and water to form a dispersion. Unfortunately, the structures formed in this way are not *single* isolated bilayers but *multi*bilayers, as illustrated by the first sketch in figure 3. However, it appears now that the molecular interactions between adjacent bilayers are small compared to other interactions, so these are appropriate systems for phase-transition studies. These multibilayer systems are similar to lyotropic liquid-crystal systems of the smectic type. Liquid-crystal physicists, such as Peter Pershan of Harvard University, are studying these systems, using birefringence as well as quasielastic and Brillouin-scattering techniques developed for liquid crystals. By these means the elastic properties of lipid bilayers can be determined. These techniques and x-ray diffraction also show that, with low and varying water concentration, lipids have a rich variety of phase behavior in addition to the phase transition in the presence of excess water, which is of primary biological interest.[2]

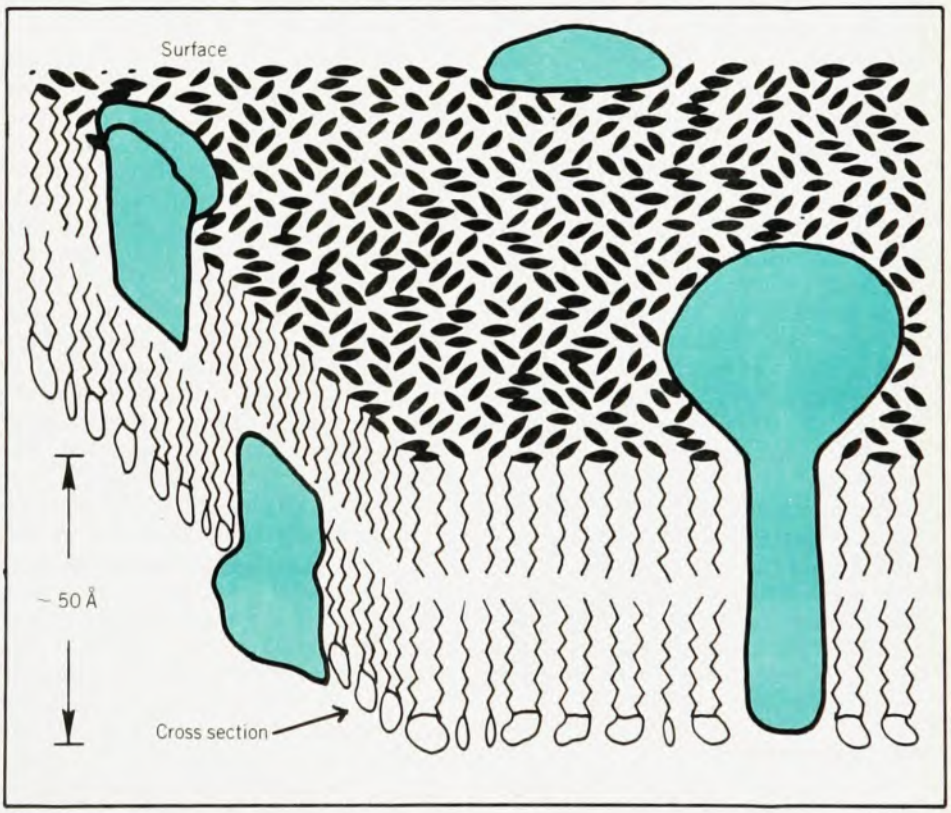
The existence of various phases and the fact that lipids form bilayers in excess water whereas soaps form micelles leads us naturally to ask for a quantitative theory to explain the observed macromolecular structures. Such a theory is likely to be as difficult and remote as a quantitative theory to explain observed

crystal structures in solid-state physics. However, great progress has been made in the study of non-structural phase transitions, such as magnetism, in the solid state by ignoring the question of how the crystal structure arose and accepting it as a given. A similar path can be followed with the phase transition of biological importance in lipids. In particular, the lipids form bilayer structures both above and below this transition, so this structure will be assumed in the further discussion below.

Disordered chains

The basic nature of the transition in multibilayer dispersions is revealed by calorimetry and x-ray diffraction.[2] The transition enthalpy (heat of transition) ΔH is very large, about 9 kcal/mole for phospholipids with chains of 16 carbon atoms. This gives an increase in entropy, $\Delta S = \Delta H/T$, of about 15 R, where R is the gas constant. If the new degrees of freedom were of a simple two-state spin type then, from $\Delta S \approx R \ln 2$, over 20 such degrees of freedom are activated at the transition. This immediately implicates the activation of internal molecular degrees of freedom in the lipid molecules, and the only portions with this many degrees of freedom and the appropriate regularity are the hydrocarbon-chain tails. X-ray studies show that the tails are quite regularly packed below the transition, with a fairly well-defined spacing of 4.8 Å between extended parallel chains. Above the transition, however, the diffraction pattern is diffuse and quite similar to those obtained from long-chain liquid hydrocarbons.

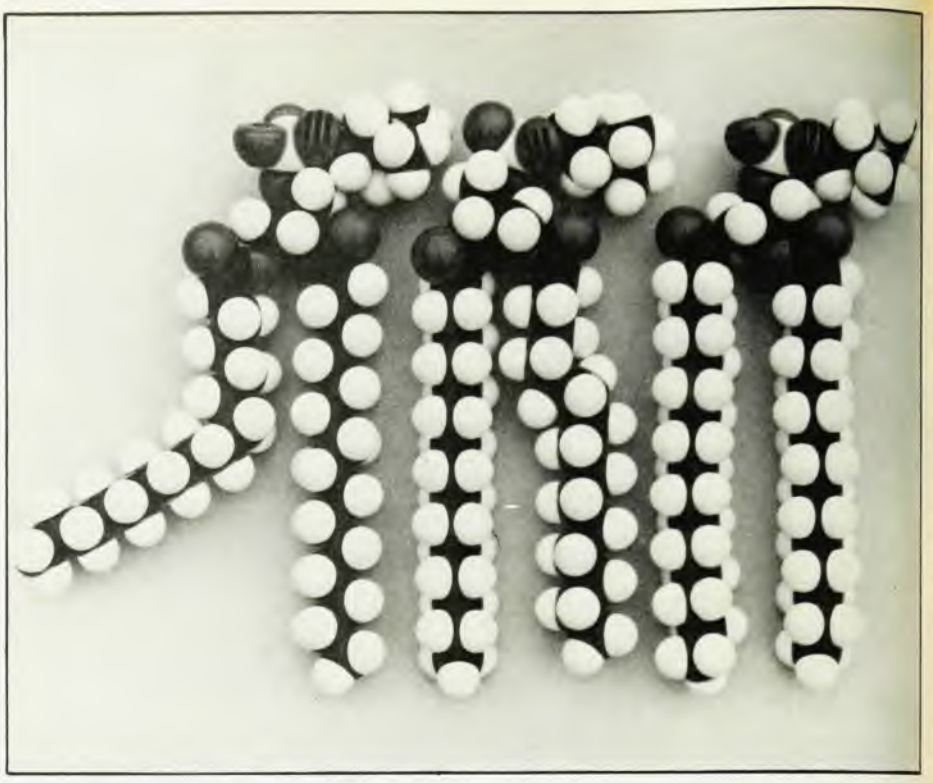
Thermodynamic evidence that the transitions in lipid bilayers mainly involve hydrocarbon-chain disordering comes from recent volume-versus-temperature measurements performed by one of us (Nagle) and Allan Wilkinson. Figure 4 shows a plot of transition temperature versus $1/(n - \delta)$, where n is the hydrocarbon chain length; $\delta = 3$ was chosen to straighten the lines. In the limit $n \to \infty$, at which $1/(n - 3) \to 0$, the transition temperature extrapolates to the transition temperature of polyethylene. The volume change, also shown in this figure, extrapolates as $n \to \infty$ to two thirds of the volume change in polyethylene. However, shorter hydrocarbon chains also have a "premelting transition" a few degrees below the melting transition with a volume change that extrapolates to about one third that of polyethylene. Thus, in the infinite-chain limit the transition in lipid bilayers corresponds to the transition from the premelted state of hydrocarbons to the melted state.



The main features of a biomembrane are the bilayer of lipid (fat) molecules and the proteins, shown in color. At low temperature the hydrocarbon tails of the lipid molecules appear as regular zigzag lines, as the cross section indicates. The tails are joined in pairs by a backbone, shown on the top surface. Omitted from the top but shown on the bottom layer are the head groups that are attached to the backbones of the lipid molecules. Figure 1

In such systems the entropic force that drives the transition is rotation about any one of the carbon–carbon bonds in the hydrocarbon chains. Such rotations are internally impeded, as shown in figure 5. The *rotational isomeric* model, used extensively in polymer research, replaces the continuum of rotational angles by the *trans* angle (lowest in energy) and the two degenerate *gauche* angles, which have energies about 0.5 kcal/mole (RT when T = 250 K). Intermediate angles have much higher energies and are therefore ignored at biological temperatures. The model therefore consists of three states, called "rotamers," for each of the carbon–carbon bonds, one trans rotamer and two gauche rotamers. In the low-temperature phase most of the molecules are in the all-trans state, with the chains parallel to each other. If one tries to change a trans rotamer to a gauche rotamer independently of other rotamers, the free end of that hydrocarbon chain strikes another hydrocarbon chain, as illustrated in figure 2. The molecule on the left in this figure has one jog (one gauche rotation); the middle molecule has one kink (gauche–trans–gauche sequence) and one all-trans chain; the one on the right has two all-trans chains. The excluded-volume interaction therefore requires that the disordering of rotamers be a cooperative effect, which explains the existence of a sharp transition.

In addition to the rotamer energy and the excluded-volume-interaction energy, there is also a strong van der Waals energy, which pulls the hydrocarbon chains towards each other. The transition is accompanied by a volume change of about 4%, which does work against this van der Waals energy. Estimates by one of the authors[3] attribute about half of the measured heat of transition to this increase in van der Waals energy, a little less than half to the formation of gauche rotamers, and much smaller energy changes to the other interactions. Such estimates indicate that the high-temperature phase does not have as many gauche rotamers per carbon–carbon bond as do liquid hydrocarbons. This is reasonable, because the lipid bilayers have an additional constraint: Each hydrocarbon chain is pinned at one end to the water interface. Direct evidence that the chain melting in lipid bilayers is less extensive than in hydrocarbons and polyethylene is that the heat of transition and the volume change per CH_2 group are only about one third as large in the lipids (with 16 CH_2 groups per chain) as for the other systems.



Three molecular models of the same lipid in different configurations. The lipid, dipalmitoyl phosphatidylcholine, consists of two hydrocarbon tails, $(CH_2)_{14}CH_3$, linked to a head group, $(CH_3)_3N^+(CH_2)_2PO_4^-$, by ester linkages and a glycerol backbone, $(OCO)_2(CH_2)_2CH$. The molecule on the left has a single jog (gauche rotation) in one tail, while the other tail is all trans. The middle molecule has a kink (gauche–trans–gauche sequence) in one tail. Figure 2

Resonance studies

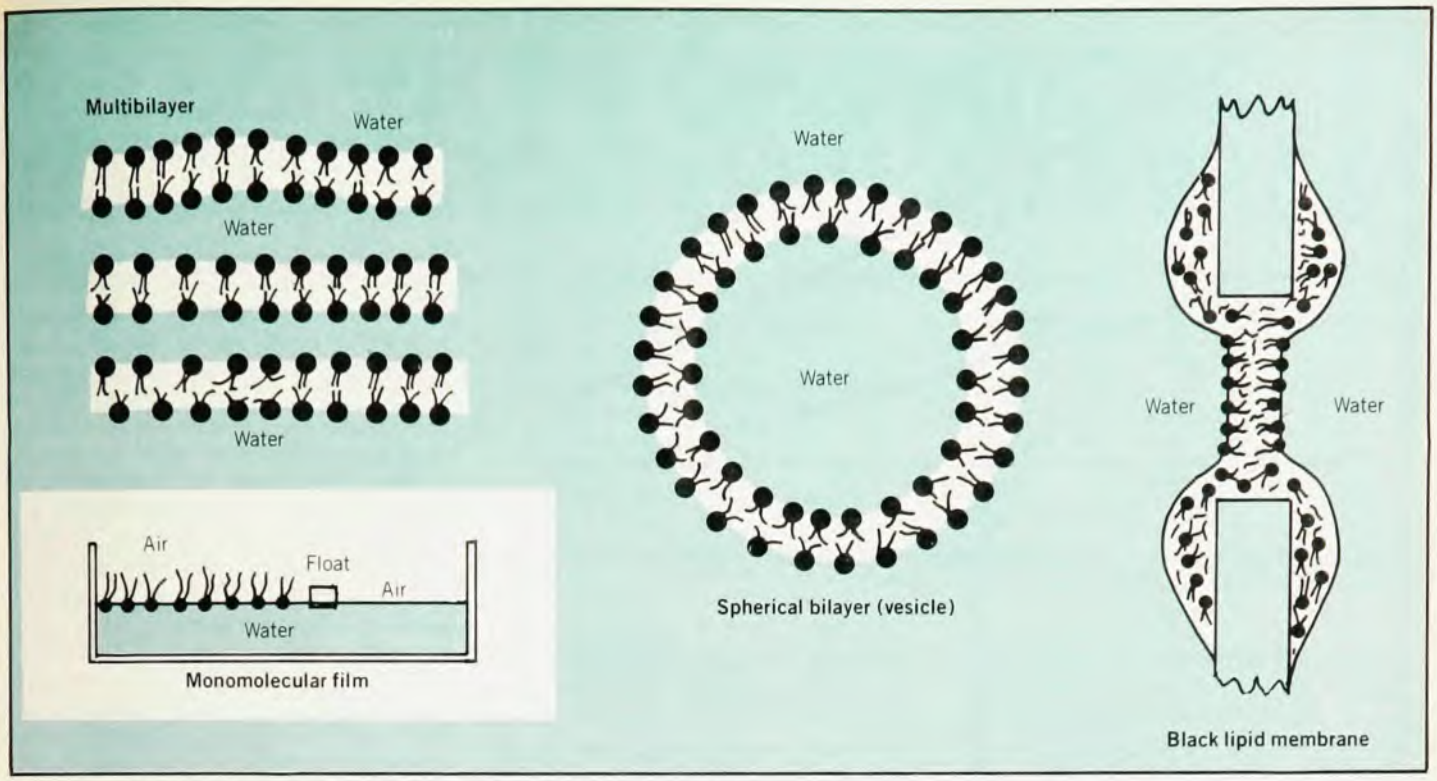
The disordered nature of the hydrocarbon chains in the high-temperature phase is verified by electron spin resonance,[4] nuclear resonance[5] and Raman spectroscopy. The resonance work also measures a directional order parameter of the sort used to define the order in liquid crystals. In the nmr case these order parameters are

$$S_n = \tfrac{1}{2}\langle 3\cos^2\theta_n - 1\rangle$$

where n denotes the position on the hydrocarbon chain from the head group and θ_n is the angular deviation of bond number n from its orientation in an all-trans chain normal to the bilayer surface. However, these order parameters should not be confused with those used in critical phenomena. In critical phenomena the order parameter is identically zero in the high-temperature disordered phase, whereas in bilayers the S_n's are nonzero in the high-temperature phase as well as in the low-temperature phase; they are therefore not the canonical Landau order parameters for the phase transition. The fact that the order parameters S_n are nonzero in both phases simply means that both phases are liquid crystals (of smectic type). Although the description "gel-to-liquid-crystal transition" is often used, the transition is really a polymer type of phase transition within a liquid-crystal phase. Nevertheless, the fundamental question as to the existence of a useful measurable order parameter in the Landau sense remains open. A macroscopic, phenomenological order parameter will be discussed below.

An extraordinary amount of resonance work has been done on lipid bilayers. One interesting discrepancy involves the measurement of the S_n by esr versus nmr. Measurements of S_n by esr, pioneered by Harden McConnell's group at Stanford, require the use of spin probes; these are made by attaching a nitroxide free-radical group with an unpaired electron to the nth position on a hydrocarbon chain. Although the free radical is not excessively large, it does represent a local perturbation, which has been blamed for the difference between the esr and nmr results. Determinations of S_n by nmr use lipids that were synthesized with deuterium substituted in the nth position, and so have negligible perturbation. However, McConnell[4] suggested that the order parameters measured by esr should be different from those measured by nmr because the different hyperfine splittings set different time scales to the S_n averages, 10^{-7}–10^{-9} sec for esr and 10^{-5}–10^{-6} sec for nmr.

The picture of the molecular motions that is proposed by McConnell[4] and Sunney Chan and his co-workers at Cal Tech[5] is that, in addition to rotamer disorder in the high-temperature phase, entire molecules are tilted with respect to the normal to the bilayer and this tilt is measured by esr.[4] The tilting is necessarily cooperative, and in the low-temperature phase it is frozen in with long-range order, so it is seen by x-ray diffraction. However, in the high-temperature phase the bilayer is fluid enough that reorientation of the local tilt is fast on the nmr time scale (and is therefore averaged) but is not fast on the esr time scale. In contrast, rotamer motion is supposed to



Four "model membranes." Parallel sheets of lipid bilayers separated by water form *multibilayers*; the sheets can be rippled. The application of ultrasound converts the multibilayers into *vesicles*, spherical bilayers about 300 Å in radius. Coated carefully on a hole in a plastic sheet and allowed to thin until it appears black, the lipid forms a *black lipid membrane*. The small wiggly lines indicate that some of the hydrocarbon solvent may still be present. A *monomolecular film* may be spread carefully on the surface of a water-filled trough. Such a film, which may be thermodynamically similar to half a bilayer, permits the measurement of the lateral pressure against the float shown. Figure 3

be fast on both time scales. Thus, to compare the results one must first perform an ensemble average of the esr S_n.[4] This possible resolution of the discrepancy is attractive because it supports the contention that good quantitative measurements using different techniques on complicated biological systems do not need to be in direct competition but can be complementary and reveal unsuspected subtleties that further the understanding of the system.

In addition to multibilayer dispersions there are other model systems that are very useful. Ultrasonic agitation breaks up multibilayer dispersions and a large fraction of the material is then in the form of vesicles, each consisting of a single, roughly spherical bilayer enclosing an interior water volume rather like a prototypical cell, as illustrated in the second diagram in figure 3.

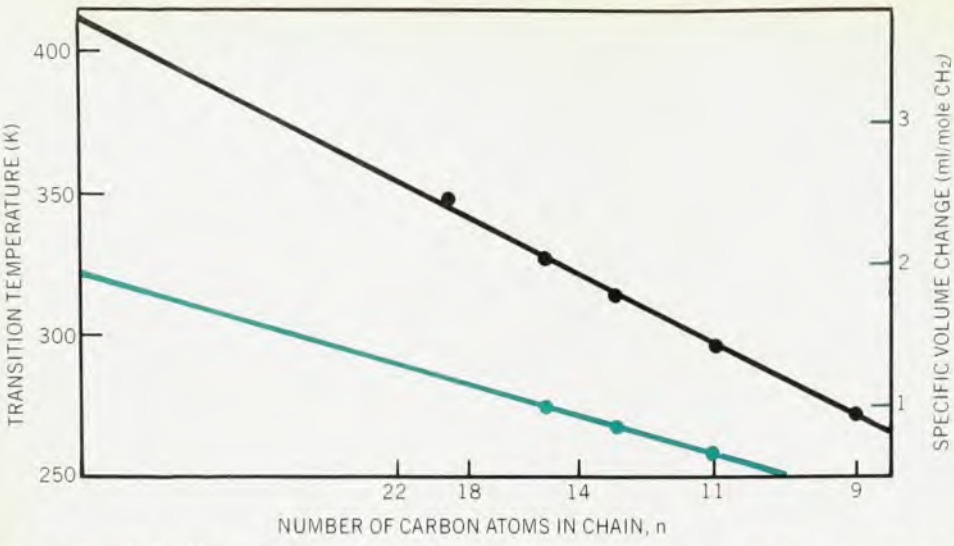
Figure 6, an electron micrograph of a sonicated sample of egg yolk lipid, shows two views of single-walled vesicles as well as small multilamellar structures. Unfortunately, the small diameter of the vesicles (about 300 Å) puts a packing strain on the lipid molecules that is different on the two sides of the bilayer. This is evidenced by a reduction in transition temperature by several degrees and the narrowing of nmr lines due to increased molecular freedom. Although these considerations of the packing strain mean that quantitative phase-transition studies must be interpreted with caution,[6] these sonicated vesicles are very useful in biological studies because of the ease with which substances can be included inside the vesicles during sonication and then removed from the outside solution to form concentration differentials.

Another model system, which allows controlled access to both sides of the membrane, is that of the so-called "black lipid membranes." These are formed by application of a phospholipid–hexane (typically) solution to a small aperture to form the third structure shown in figure 3. It is a single bilayer, which is so thin that it is optically black. Black lipid membranes have been studied extensively and prepared by various techniques.[7] Some problems involving their use as model membranes include their fragile nature and the possibility that they contain some of the hydrocarbon solvent (such as hexane) as well as lipids.

Critical points in monolayers?

It can also be argued that a monomolecular film at an air–water interface, also shown in figure 3, is a model system for membranes.[2,3] One motivation for using monolayers as a model membrane system is that they provide an extra pair of experimentally controllable thermodynamic variables, namely the area per lipid molecule A and the lateral pressure π, which are not experimentally accessible in the other model systems. The intuitive idea that monolayers are model membrane systems is that a bilayer essentially consists of two back-to-back monolayers with only weak interactions between them. Of course, this can only be true if water is prevented from contacting the hydrocarbon region in the single monolayers, as is the case. A slightly less obvious condition for a valid comparison concerns the free water–air interface behind the movable barrier in the figure. This pulls the barrier to the right with a force of 70 dynes/cm due to the surface tension of water, while the free hydrocarbon–air interface on the left pulls it to the left with a force estimated from the surface tension of bulk hydrocarbons to be about 20 dynes/cm. Therefore, to compare monolayers with bilayers, we must apply a lateral surface pressure of about 50 dynes/cm to the monolayer.[3] When this is done the measured transition temperatures for lecithin monolayers and bilayers are in agreement.

Monolayer isotherms, shown in figure 7, definitely show a transition for temperatures below some temperature T_c. Most interpretations of these isotherms are in terms of a two-phase coexistence region, also indicated in the figure. It is not known why the observed isotherms are not flat in the supposed two-phase region, although possibly the speed at which the experiments are done precludes true equilibrium in the transition region into the solid-like phase. In this regard it should be noted that Steve Hui at Roswell Park Memorial Institute, Buffalo, has observed domain structure in the



Transitions in lipid bilayers. The main transition temperatures are shown in black and the volume changes in color; the horizontal axis is linear in $1/(n-3)$. Figure 4

low-temperature phase of homogeneous lipids.[8] The model system in this case is one of single bilayers on an electron-microscope grid, in a special hydrated chamber. This observance of domain structure as well as earlier monolayer work shows that the low-temperature phase becomes rigid and solid-like.

The conventional interpretation of the monolayer experiments[9] suggests a critical point, as shown in figure 7. For surface pressures above π_c or for temperatures above T_c there is no transition. The area change ΔA across the two-phase region in the figure behaves like a typical Landau order parameter, although it is a macroscopic, not a microscopic order parameter. The conjugate field is then the lateral surface pressure π. It is noteworthy that no indication of such a critical point appears when the ordinary three-dimensional pressure P and volume V are studied, either in lipids or polyethylene. This indicates that P and V in bulk lipids are not analogous to π and A. Estimates of the critical surface pressure[9] in 16-carbon phospholipids have placed it near 50 dynes/cm. If the analogy between monolayers and bilayers is taken seriously, this suggests that bilayers at the transition are near a critical point.

One experimental result that supports the contention that the phase transition in multibilayers is near critical is the permeability of the bilayer to sodium ions,[10] which shows an anomalous maximum at T_c. Although this permeability is low compared to its value in real membranes, which have sodium pumps, gated pores or special carrier molecules, it may be a useful probe of bilayer properties. In particular, the rate-limiting step in Na^+ appears to be the transfer of the ions from water through the head-group region and into the hydrocarbon region. This step would be greatly facilitated by critical fluctuations in the lateral packing of the lipids, which would open up small holes where the ions could enter.

Such fluctuations are large near a critical point, at which the lateral compressibility, $-A^{-1}(\partial A/\partial \pi)_T$, is high. As has also been recently suggested by Seb Doniach at Stanford, the anomalous peak in the Na^+ permeability therefore may be evidence for critical fluctuations. However, it must be emphasized that calorimetric and volumetric measurements on multibilayer dispersions show a transition that is only about a degree wide and the precise temperature variation changes from sample to sample. Thus, any critical region must be fairly narrow, at least for these measurements; the conventional presumption is that the phase transition in multibilayers is of first order.

At this point we can speculate on why real biomembranes might like to grow in the thermodynamic region of a phase transition. In order to grow, new material, such as lipids, cholesterol and protein, must be inserted into the membrane because there is no free edge to add on to—as there is in crystal growth. Such insertion is made easier if the lateral compressibility is high, because then local holes could be forced open with less expenditure of energy to accomodate new molecules. If one assumes that there is a growth enzyme already in the membrane, which forces open such holes, then this insertion would not require the vicinity of a critical point but merely a high lateral compressibility, such as occurs in monolayers in the two-phase region.[4] It must be cautioned that many membranes have transition temperatures that are considerably lower than the growth temperature, so a phase transition state is not necessary for these membranes. However, it appears that most membranes do require the disorder or fluidity associated with having the temperature above or within the lipid phase transition.

Lateral movement

In addition to permeability, another dynamical property of lipid bilayers that is biologically relevant is the lateral motion of molecules within the plane of the membrane. The measure of this lateral mobility is the coefficient of lateral diffusion D_L, which is related to the mean-square displacement $\langle r^2 \rangle$ over a time t by the usual relation

$$\langle r^2 \rangle = 4D_L t \qquad (1)$$

Some of the first quantitative measurements of D_L were made by McConnell and his co-workers at Stanford by analyzing the time dependence of the esr spectra that result when multibilayers are prepared with a high concentration of spin label in a localized region.[4] They find $D_L \approx 2 \times 10^{-8}$ cm^2/sec.

Watt Webb and his co-workers at Cornell University have studied the lateral mobility of both lipids and proteins in multibilayers, black lipid membranes and biological membranes, using two novel optical techniques:[11] fluorescence correlation spectroscopy and fluorescence photobleaching recovery. The first technique has been used to measure D_L in black lipid membranes. After the model system is prepared with a small concentration of fluorescent molecules present, a laser is focussed through a microscope onto a small portion of the membrane and the resulting fluorescence intensity is monitored. Over a time τ this intensity fluctuates, due to variations in the concentration of the fluorescent probes in the illuminated region. The usual measure of such fluctuations is the time autocorrelation function

$$g(\tau) = [\langle I(t)\, I(t+\tau)\rangle - \langle I(t)\rangle^2]/\langle I(t)\rangle^2 \qquad (2)$$

where $I(t)$ is the fluorescence intensity at time t. Analysis of the two-dimensional diffusion problem leads to the result

$$g(\tau) = \left[\langle N\rangle \left(1 + \frac{\tau}{\tau_c}\right)\right]^{-1} \qquad (3)$$

where $\langle N\rangle$ is the average number of fluorescent molecules in the illuminated area πr^2 and $\tau_c = r^2/4D_L$ is the characteristic diffusion time for movement out of the illuminated area. From a plot of $g(\tau)$ versus τ, Webb and his co-workers find $D_L \gtrsim 10^{-7}$ cm^2/sec in a variety of black lipid membranes prepared by different techniques and with varying compositions.

The fluorescence photobleaching recovery technique involves monitoring the recovery of fluorescence in a small area of a membrane illuminated by a focussed laser, after the area is irreversibly bleached by a high-intensity laser pulse. The rate of recovery of fluorescence in the area yields the diffusion coefficient through the characteristic time τ_c. From these measurements the Cornell workers find, for membranes of various mammalian cells such as rat myoblasts, $D \approx 2 \times 10^{-10}$ cm^2/sec for the proteins and $D \approx 9 \times 10^{-9}$ cm^2/sec for the lipid components.[11]

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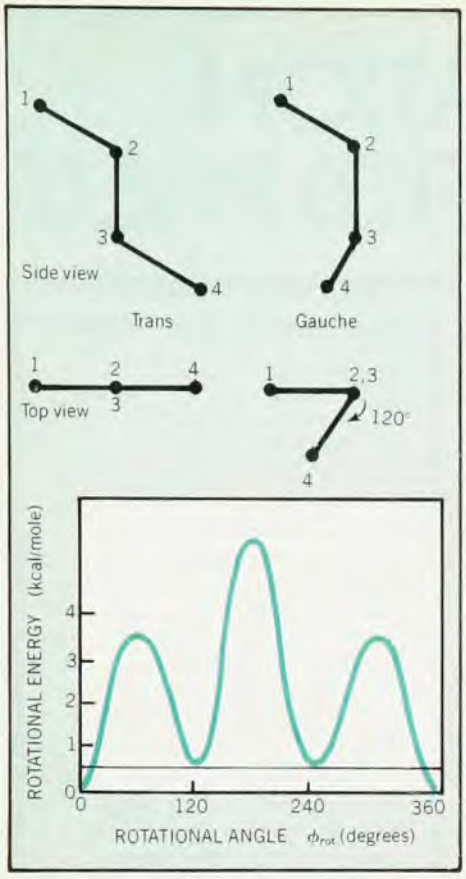

Rotational isomerism affects the shape of a hydrocarbon chain. In the upper diagrams, the left-hand chain is in the ground, trans, state; that on the right has undergone a gauche rotation about the bond connecting carbon atoms 2 and 3. The potential energy as a function of rotation angle shows three minima. Figure 5

With the preceding values of the lateral mobility the time required for a molecule to travel around a cell of size 10 microns can be estimated. Using $D_L \approx 10^{-8}$ cm^2/sec and $\langle r^2 \rangle = (10 \text{ microns})^2$ gives $\tau_c \approx 25$ sec for lipids; using $D_{protein} \approx 2 \times 10^{-10}$ and the same $\langle r^2 \rangle$ gives $\tau_c \approx 20$ min for proteins. This is in good agreement with measured redistribution times for cell-surface antigens after cell fusion.[10] Thus it appears that lateral mobility is, for many cells, biologically operational. Measurements of diffusion constants for various membrane components involved in physiological processes have already shed new light on some of the molecular mechanisms of membrane function, and research continues along these lines in several laboratories. Understanding the immobilization of substantial fractions of various membrane proteins is a current concern.

The previous measurements of D_L were for the high-temperature fluid phase. Below T_c, measurements by Webb and his co-workers show that D_L is smaller by several orders of magnitude for multibilayers. This supports the idea that the low-temperature phase is much more rigid and solid-like. Curiously, however, D_L versus T for black lipid membranes does not show any phase transition. The reason for this difference is imputed to be due to the solvent in the black lipid membranes, which implies that the two model systems are not strictly equivalent, at least in dynamic properties. Paul Fahey and Webb have recently found that D_L does change by several orders of magnitude for large solvent-free single bilayer vesicles formed by a new technique.

The various lipids mentioned in connection with figures 1 and 2 have phase transitions at different temperatures. Roughly, adding two CH_2 groups to each hydrocarbon chain in the membrane raises its T_c by about 15 degrees. Unsaturating the chains to form a single C=C double bond lowers T_c by about 45 degrees. Removing the CH_3 groups from the head regions raises T_c by about 20 degrees. Such perturbations are vitally important biologically. In particular, the way in which cells lower their membrane transition temperatures is to include more short-chain and unsaturated-chain lipids in the membrane composition. However, from a fundamental point of view transition-temperature changes of 10–20 degrees out of 300 K are indeed perturbations and do not indicate drastic qualitative differences.

Mixtures

Some important features of biomembrane phase transitions can not be mimicked by model systems composed of a single kind of lipid but require lipid mixtures. As is typical of all two-component mixtures there are upper and lower transition temperatures, T_{cu} and T_{cl} respectively. For temperatures between the transition points, the lipids A and B in each bilayer are laterally phase-separated into an A-rich (relatively fluid) phase and a B-rich (relatively solid) phase. As the membrane temperature is raised, the A-rich phase grows at the expense of the B-rich phase until there is only a single fluid phase at T_{cu}. As T is lowered to T_{cl} the system may condense into a single solid phase or, depending on the particular lipid mixture, a solution of two solid phases may form.[4]

With more than two lipid components there is, of course, the possibility of more than two coexisting phases. This lateral phase separation leads to another speculation as to why a cell might prefer to grow a membrane that is in a phase-transition region. There are many different membrane-bound proteins that carry out vital biological functions. These proteins may require different lipid environments to function. For example, some proteins may require the presence of lipids with head groups having a net charge, whereas other proteins may require neutral lipids. Clearly, it is possible to accomodate such different requirements in the phase-transition region of a multicomponent mixture of lipids where there are different phases with different lipid characteristics, but this is not possible in a single phase.

Another kind of phase separation that takes place is that the compositions of lipids on the inside layer of the bilayer is often different from that of the outside layer. Differences in composition can be maintained easily because the flip-flop time for a molecule to go from one side of the bilayer to the other is quite long, as McConnell's group showed, using spin labelling.[4] Such a difference, which is called "membrane asymmetry," can be important in preferential binding, in orientation of membrane proteins and in the overall shape of the membrane.

In addition to the preceding speculation concerning the possible effect of lipids on the function of proteins there is esr evidence that the presence of proteins in bilayers affects the lipids that surround proteins lying entirely or almost entirely within the hydrocarbon region of lipid bilayers.[2,4] These studies suggest that each protein is surrounded by layers of "boundary lipid" with the lipids closest to the protein relatively immobile compared to lipids in the high-temperature phase. Such boundary lipids do not contribute to the enthalpy change of the phase transition.

The role played by cholesterol in membranes has been under active investigation for many years, at least in part because of its role in atherosclerosis. The influence of cholesterol upon the lipid phase transition is well known from calorimetric studies. As one adds cholesterol to multibilayers of a single-component lipid, the enthalpy change at the transition decreases, while the transition temperature does not change appreciably, until the transition disappears at 33–50% cholesterol.[2] The disappearance of the transition is generally thought to be due to the rigid nature of the steroid nucleus of the cholesterol molecule, which prevents the cooperative isomerization of the long hydrocarbon chains of the lipids. In any case cholesterol not only hardens the arteries, it also kills the phase transition!

Theoretical approaches

Biological phenomena are often too poorly characterized and involve too many variables to be amenable to the kind of theory developed for physical phenomena. However, the preceding discussion shows that the phase transition in lipid bilayers has been fairly well characterized, so it is not premature to develop theories involving statistical-mechanical calculations and theories of phase transitions; a growing number of theoreticians with backgrounds in physics have turned their attention to this phenomenon.

A proper treatment of transitions requires the calculations of the partition function

$$Z = \sum \exp(-E/kT) \qquad (4)$$

where the sum runs over all configurations

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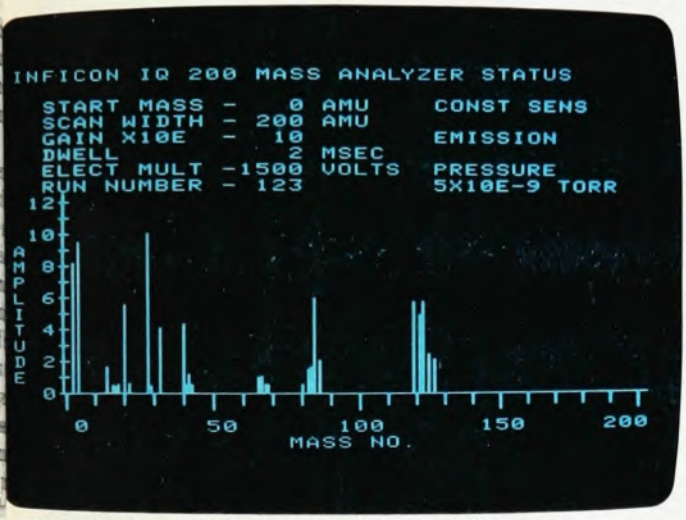

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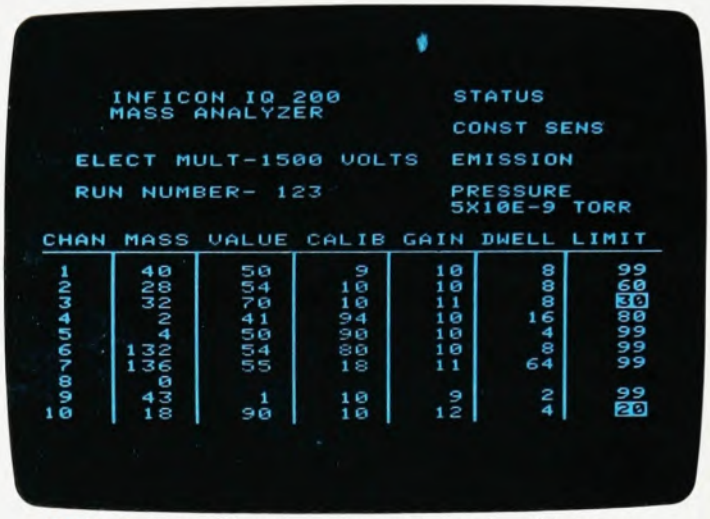

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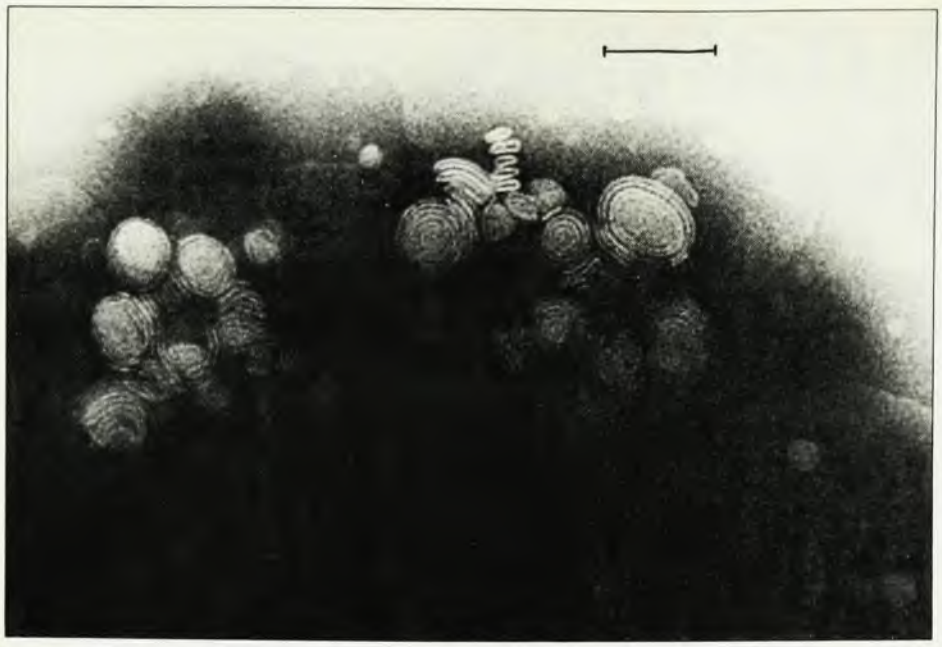
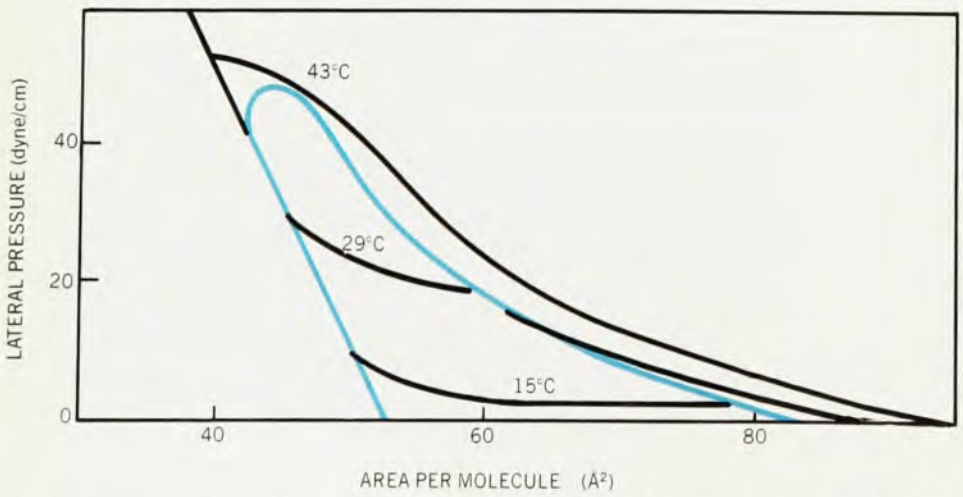


Electron micrograph of sonicated egg-yolk lecithin; the bar indicates a length of 0.1 micron. The bilayers appear bright in contrast to the negative stain of phosphotungstic acid. The larger objects are multilamellar structures. The circular light patches near the edge of the stained drop are interpreted as single-bilayer vesicles, which may be disks or spheres. Also visible are disk-shaped vesicles aggregated in stacks, known as "rouleaux." Figure 6

of the system and E is the energy of a configuration. In a system as complex as a lipid bilayer there are many contributions to E. From the preceding discussion we may write

$$E = E_{\text{xvol}} + E_{\text{rot}} + E_{\text{vdW}} + E_{\text{other}} \quad (5)$$

Here E_{xvol} is the strongly repulsive excluded-volume interaction that prevents atoms from occupying the same volume; E_{rot} is the rotamer energy for the hydrocarbon chains (this equals n_g times about 0.5 kcal/mole, where n_g is the number of gauche rotamers); E_{vdW} is the van der Waals attractive energy between the hydrocarbons (the scale of this energy term has been determined to be 1.84 kcal per mole of CH_2 from sublimation studies on hydrocarbons). Other contributions to the energy, E_{other}, involve the *vibrational and kinetic energies,* which for each degree of freedom will have approximately the same classical value of $\frac{1}{2}kT$ in either phase; *head-group interactions,* including electrostatic interactions and possible weak hydrogen-bond interactions, either direct or mediated by bound water, which by the observed shifts in T_c can be assumed to be small; *interactions between the two layers that form the bilayer,* which appear from the agreement between monolayer and bilayer T_c's to be small, and *interfacial interactions* with the bulk water, which do not change much as long as the basic structure is that of the bilayer. Basic statistical calculations therefore concentrate on E_{xvol}, E_{rot} and E_{vdW}.



The lateral pressure of phospholipid monolayers as a function of their molecular area, taken at three temperatures. The colored line shows the postulated coexistence (two-phase) region. The data are from S. W. Hui and collaborators, reference 9. Figure 7

Even when E_{other} is neglected, the statistical-mechanical problem is formidable because of the excluded-volume interaction as well as the long-range van der Waals interaction, both of which make rigorous calculations intractable even for simple fluid systems. There are basically two alternatives for the theoretician to consider:

- Simplify the model, for example by reducing the dimensionality, so as to obtain a system for which equation 4 may be evaluated exactly.
- Retain the original model and evaluate equation 4 with approximate computational techniques.

These two approaches are complementary and, through study of a wide variety of theoretical models and careful comparison with experiment, a clear picture of the nature of the microscopic interactions that lead to the observed phase properties of bilayers should emerge.

The two authors of this article are involved in theoretical efforts using the two different approaches described above. Nagle simplified the basic model until it could be solved exactly by the Pfaffian-dimer technique in statistical mechanics.[3] In this model the hydrocarbon chains are infinitely long and restricted to a two-dimensional lattice, but the excluded-volume constraint is rigorously satisfied. With no free parameters the calculation gives the correct value for T_c for bilayers and the correct value of the critical point for monolayers. This critical point is of an unusual variety in statistical mechanics, and has been called a "$\frac{3}{2}$-order" critical point.[3]

Scott, on the other hand, has developed an approximation method for the treatment of the hard-core forces in monolayers, which is adapted from the Flory method in polymer statistics.[12] The calculation yields a classical critical point. In addition Scott is performing Monte Carlo simulations of the hydrocarbon region in a monolayer. These calculations emphasize the vital role the hard-core forces play in determining chain conformations, and they shed some additional light on the reason the esr and nmr parameters differ.

Stjepan Marčelja, a physicist at Zagreb University, has developed a mean-field approximation, with which he carefully treats the rotational isomerism.[13] His treatment of the intermolecular interactions is similar to the Maier–Saupe methods for liquid crystals. As is also the case for liquid crystals, the theory produces fairly good quantitative agreement with experiment, but the approximate treatment introduces parameters with values that are either undetermined or at variance with knowledge of intermolecular interactions. Bruce Hudson, Hans Andersen and their co-workers at Stanford have performed calculations that make use of mean-field and scaled-particle theory, and which concentrate on the

behavior of T_c with chain length and on two-component mixtures.[14]

Theoretical model building and statistical-mechanical calculations often focus and sharpen experimental investigation in traditional areas of physics, and the same is true in some of the biomembrane studies discussed here. For example, one of us (Nagle),[3] by analysis of the general energy equation, 5, pointed out that if E_{other} is small the relative volume change $\Delta V/V$ must be in the range 3–4%. In this case the experiments supported the theory. Other cases, yet to be fully supported or rejected by experiment, include the possible subcritical nature of the phase transition in bilayers mentioned earlier, and an hypothesis put forward by one of us (Scott) concerning reasons for the unusual shape of monolayer isotherms.

Prognosis

We have attempted to describe an area of research that is relevant to the biological sciences and also amenable to the types of quantitative experimental and theoretical studies that appeal to physicists. In biomembrane-related research there are of course messy problems that plague experimentalist and theoretician alike but, as we have shown, progress has been made. With continuing contributions from, and interactions between, physicists, biophysicists, chemists, biochemists and biologists, the next decade should see even more rapid advancement.

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