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Lipid Polymorphism

PIETER R. CULLIS and COLIN P. TILCOCK

University of British Columbia, Vancouver, British Columbia, Canada

MICHAEL J. HOPE

The Canadian Liposome Co. Ltd., Vancouver, British Columbia, Canada

I. INTRODUCTION

The ability of aqueous dispersions of liquid-crystalline lipids to adopt a variety of structures in addition to the bilayer organization is well established (1,2). It is also becoming generally recognized that these polymorphic capabilities may be directly related to **many functional** capacities of membranes, including membrane fusion (3,4). In this chapter we present a synopsis of the polymorphic capabilities of lipids, discuss the possible theoretical basis for polymorphism, and indicate the implications for the structure and function of **certain** biological membranes.

II. LIPID POLYMORPHISM AND LIPID DIVERSITY: AN OVERVIEW

Biological membranes contain a large variety of different molecular species of lipids. This diversity has naturally led to questions regarding the functional roles of individual lipid components. As yet, this has not resulted in a general framework within which the functions of lipids with differing headgroups and/or fatty acid components can be understood. However, a major theme of this chapter is the proposal that the properties inherent in the polymorphic abilities of lipids **offer** important insights, which may lead to such basic understanding. A particular point is that lipid polymorphism appears to offer more basic insight into lipid function in membranes than can be achieved through rationales of lipid function relying on membrane fluidity arguments.

It is useful to outline the background leading to this statement. Briefly, the recognition that membrane lipids provide a fluid, **liquid-crystalline** bilayer structure in membranes (5) was combined with the realization that acyl-chain composition and headgroup type can strongly influence the gel (frozen) or liquid-crystalline nature of the resulting membrane. It was therefore proposed that the presence of different lipids

is required to **provide** appropriate fluidity characteristic in a given membrane. In addition, local domains of appropriate lipid composition could possibly modulate local fluidity characteristics, possibly influencing protein function. However, for reasons summarized here and elsewhere (1,2,6), this rationale for lipid diversity has proved unsatisfactory. The primary reasons for this include the fact that lipids do not appear to be present in a gel state in the vast majority of biological membranes at physiological temperatures. In addition, there is little evidence to support the contention that local domains of differing fluidity can be readily achieved in **liquid-crystalline** bilayer membranes in response to physiological stimuli such as ionic strength, **pH**, **divalent** cations, or proteins. Finally, the membrane fluidity parameter itself is loosely defined and can lead to confusion. For example, it is commonly assumed that more saturated lipids or the presence of cholesterol makes membranes less "fluid." This is not necessarily the case. Membrane fluidity is rigorously **defined** as the reciprocal of the membrane viscosity, which in turn is inversely proportional to the rotational and lateral diffusion rates (D_R and D_T , respectively) of membrane components (7). Thus, a linear relation between membrane fluidity and D_R and D_T would be expected, which is not observed. Incorporation of cholesterol into phosphatidylcholine (PC) model membranes (at temperatures above the gel to liquid-crystalline transition temperature) has little or no influence on the lateral diffusion rates observed (8,9) and can actually increase the rotational diffusion rates (10). The major influence of cholesterol or decreased unsaturation is to increase the order in the hydrocarbon matrix (11).

Lipid polymorphism appears to offer a more acceptable framework within which to characterize the physical properties of lipids and their functional roles in biological membranes. Reasons for this are detailed at length in subsequent sections of this chapter. Briefly, there are four major points. First, **an** appreciable fraction (30 mol% or more) of lipids in biological membranes either adopt or induce nonbilayer structures in various model systems, and there is strong evidence to suggest that under appropriate conditions, the large majority of membrane lipids can adopt nonbilayer phases. Second, the structural preferences of lipids in pure and mixed lipid systems can be modulated by factors such as ionic strength, **pH**, **divalent** cations, and membrane proteins, indicating the possibility of regulated roles in membrane-mediated phenomena. Third, certain **membrane-mediated** processes, such as fusion, clearly require a local departure from lamellar organization in order to proceed. Finally, detailed consideration of the factors leading to different phase preferences of lipids is leading to an appreciation of parameters such as lipid "**shape**" or "intrinsic membrane curvature." These parameters can strongly influence bilayer membrane properties such as the order in the hydrocarbon region, which may reflect basic conserved properties in membranes.

III. STRUCTURAL PREFERENCES OF LIPIDS

A. Introduction

The subject of lipid polymorphism has been the topic of many reviews (1,2,6) and it is difficult to avoid repetition. However, for the sake of completeness, certain points must be reiterated. **First**, the two major polymorphic phases adopted by pure aqueous dispersions of membrane lipids are the bilayer or lamellar phase and the hexagonal (H_{II}) phase. The

hexagonal (H_{II}) phase consists of hexagonally packed arrays of lipid cylinders where the polar headgroups are oriented toward an aqueous pore of $\sim 20 \text{ \AA}$ diameter. Nonbilayer structures such as the hexagonal H_{II} phase are liquid-crystalline structures; gel-state lipids invariably adopt the bilayer organization. Further, the hexagonal phase per se is not likely to be a major structure available to membrane lipids in vivo, as a permeability barrier could not be maintained. Indeed, the observation of H_{II} structure in vivo appears to be correlated with pathological consequences (12). As indicated below, it is more likely that intermediates between bilayer and H_{II} organization, such as inverted micelles, could provide local, discrete, and transitory departures from bilayer structure compatible with overall membrane integrity.

Techniques commonly employed to monitor lipid phase structure include X-ray procedures, as well as freeze-fracture and nuclear magnetic resonance (NMR) techniques. The advantages and limitations of these techniques have been extensively discussed elsewhere (1,2,13). X-ray analyses provide, in principle, unambiguous information on lipid phase structure. The ^{31}P -NMR technique provides a rapid diagnostic procedure, giving rise to phase assignments entirely consistent with X-ray studies (14). Briefly, bilayer phospholipids give rise to an asymmetrical lineshape with a low-field shoulder, where the dominant motional averaging arises due to rapid axial rotation of the phospholipid about its long axis. In the H_{II} phase, additional motional averaging occurs owing to the ability of the lipids to diffuse laterally around the cylinders characteristic of this phase. This gives rise to line-shapes with reversed asymmetry which are a factor of two narrower (1). Finally, phospholipids in small bilayer systems or in inverted micellar or other nonbilayer alternatives give rise to narrow symmetrical ^{31}P NMR spectra due to the isotropic motional averaging processes available in these structures. Examples of the three types of ^{31}P -NMR response are given in Figure 1.

The application of freeze-fracture techniques to the study of lipid polymorphism has been reviewed elsewhere (15) and provides an ability to visualize local structure in a lipid dispersion. Bilayer systems give rise to flat, relatively featureless fracture planes, whereas hexagonal H_{II} phase systems display corrugated fracture faces (see Fig. 1) arising as the fracture plane cleaves between hexagonally packed lipid cylinders. The greatest utility of the freeze-fracture technique arises from an ability to detect nonbilayer lipid structure giving rise to "lipidic particle" morphology (15) (Fig. 1). NMR and X-ray techniques cannot be employed to detect these systems. This is due to the absence of a regular lattice, which precludes observation by X-ray procedures, and the fact that a variety of structures (e.g., small bilayer systems, micelles) can give rise to the narrow NMR resonances that would be expected to arise from lipidic particles. Limitations of the freeze-fracture procedure include the fact that nonbilayer structures formed only at relatively high temperature ($\geq 30^\circ\text{C}$) are often difficult to capture via freeze-fracture owing to their tendency to revert to a lamellar structure during the freezing process. Also, to avoid formation of ice crystals, cryoprotectants such as glycerol are commonly employed. In some cases, this can influence lipid morphology (16). Finally, nonbilayer structures that have very short lifetimes, including those expected to occur as intermediates in fusion, may well be difficult to observe by freeze-fracture techniques (17).

Thus far we have indicated that isolated species of hydrated, liquid-crystalline membrane lipids adopt the bilayer or H_{II} organization; that

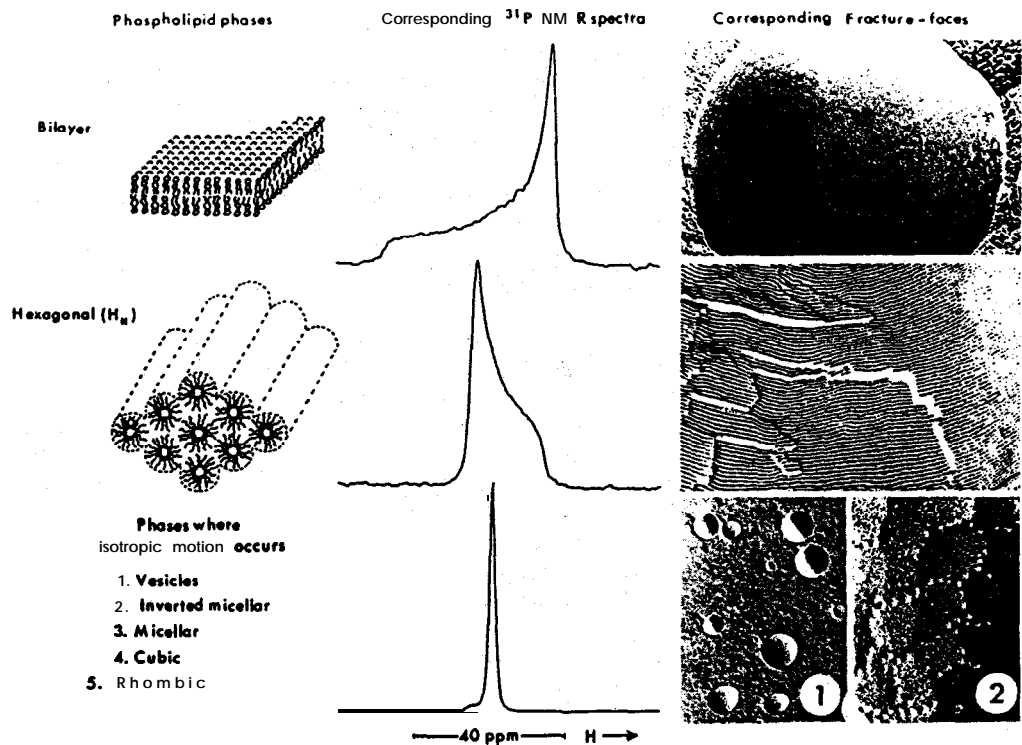


FIGURE 1. ^{31}P NMR and freeze-fracture characteristic of phospholipids in various phases. The bilayer ^{31}P NMR spectrum was obtained from aqueous dispersions of egg yolk phosphatidylcholine, and the hexagonal (H_{II}) phase spectrum from phosphatidylethanolamine (prepared from soybean phosphatidylcholine). The ^{31}P NMR spectrum representing isotropic motion was obtained from a mixture of 70 mol% soya phosphatidylethanolamine and 30% egg yolk phosphatidylcholine after heating to 90°C for 15 min. All preparations were hydrated in 10 mM Tris-acetic acid (pH 7.0) containing 100 mM NaCl, and the ^{31}P NMR spectra were recorded at 30°C in the presence of proton decoupling. The freeze-fracture micrographs represent typical fracture faces obtained from bilayer and H_{II} phase systems as well as structures giving rise to isotropic motional averaging. The bilayer configuration (total erythrocyte lipids) gives rise to a smooth fracture face, whereas the hexagonal (H_{II}) configuration is characterized by ridges displaying a periodicity of 6-15 nm. Common conformations that give rise to isotropic motion are represented in the bottom micrograph: (1) bilayer vesicles (~ 100 nm diameter) of egg phosphatidylcholine prepared by extrusion techniques and (2) large lipid structures containing lipidic particles. The latter system was generated by fusing SUVs composed of egg phosphatidylethanolamine and 20 mol% egg phosphatidylserine which were prepared at pH 7 and then incubated at pH 4 for 15 min to induce fusion.

X-ray, ^{31}P -NMR, and freeze-fracture are useful techniques to visualize these structures; and that intermediate "lipidic particle" arrangements can be detected by freeze-fracture protocols. From the point of view of fusion and other membrane contact phenomena [e.g., tight junctions (18)] the latter structures are of particular interest. Lipidic particles are commonly observed in mixed systems composed of lipids which adopt bilayer structure in isolation and lipids preferring the I-III arrangement. There is now considerable evidence to suggest that these structures are intermediates between bilayer and H_{II} arrangements of lipids (15) and correspond to inverted micelles formed at the nexus of intersecting bilayers (see Fig. 2). It may be noted that the morphology of lipidic particles can vary considerably (15). This can be attributed to the evolution of inverted-micellar contact sites to form inverted tubes [e.g., "line defects" (19)] or to form interlamellar attachment sites (20), or other possibilities as discussed in Section IV.

B. Structural Preferences of Lipids

The polymorphic phase preferences of lipids have been the subject of extensive investigations. A sensible summary of the large amount of data obtained is difficult to achieve in text form. In Table 1 we present a synopsis of these investigations and confine our written remarks to providing an appropriate overview. We address, in turn, the properties of pure lipid systems, mixed lipid systems, and, subsequently, factors modulating these structural preferences.

The phase behavior of the major classes of phospholipids, including phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI), cardiolipin (CL), and sphingomyelin (SM), have all received detailed attention. The results summarized in Table 1 support the following general points. First, effectively all lipids with fully saturated fatty acid constituents only adopt the bilayer organization (either liquid-crystalline or gel state) over the temperature interval 0–100°C, irrespective of hydration, pH, ionic strength, or divalent cation concentration. Second, among the unsaturated lipids, only PE adopts the hexagonal H_{II} phase at "physiological" temperatures, pH values, and salt concentrations. More unsaturated species of PE adopt the hexagonal phase more readily, as indicated by a progressive lowering of the bilayer to H_{II} transition temperature (T_{H}) as the unsaturation is increased. For example, the molecular species 1-palmitoyl-2-oleoyl-PE exhibits a $T_{\text{H}} \approx 75^\circ\text{C}$, whereas for dilinoleoyl-PE T_{H} is less than -10°C . PE's of eukaryotic origin exhibit T_{H} values in the range of 10°C. Among the other phospholipids; unsaturated varieties of PS, PA, and CL can prefer the H_{II} organization at low pH values and/or at Ca^{2+} concentrations of $\geq 2 \text{ mM}$ or higher.

The phase properties of mixed lipid systems are of more direct interest to the properties of biological membranes. Again, the results of Table 1 support a number of general observations. First, all species of phospholipid that adopt the bilayer phase in isolation can stabilize hexagonal-preferring lipids (e.g., unsaturated PE) into an overall bilayer organization in mixed systems. The proportions of bilayer lipid required to achieve this can vary substantially (20–50 mol%). Second, cholesterol has the general ability to induce H_{II} phase structure in mixtures of unsaturated PE with bilayer-stabilizing phospholipids, such as PC and PS. Fatty acids and other fusogenic compounds exhibit a similar ability to induce H_{II} organization. A third

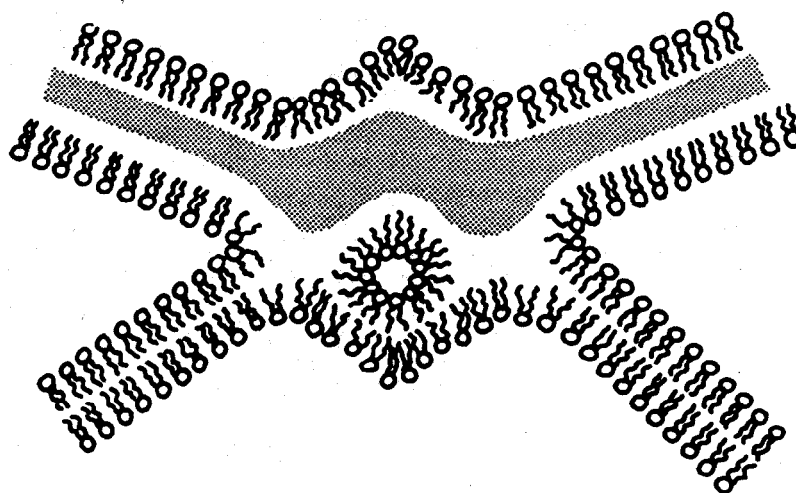
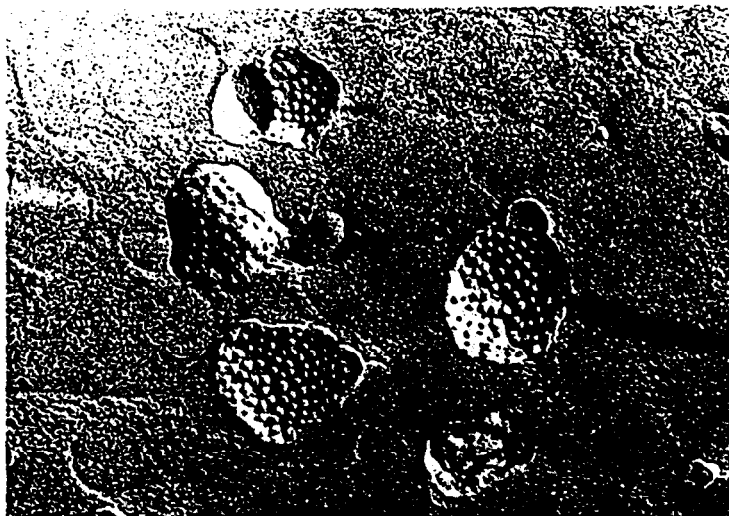


FIGURE 2 Freeze-fracture micrograph of lipidic particles induced by Ca^{2+} in a lipid system consisting of **cardiolipin** and soya phosphatidylethanolamine in the molar ratio of **1:4** (magnification $\sim 80,000$). A model of the lipidic particle as an inverted **micelle** is depicted below the micrograph. The shaded area represents the fracture region.

point concerns the hysteresis effects often observed in the **temperature-** dependent phase behavior of certain mixed **lipid** systems. For example, unsaturated PE-PC systems which adopt the bilayer phase at ambient temperatures can often be converted to systems exhibiting **H_{II}** phase and "isotropic" **motional** averaging components (as detected by ^{31}P NMR) on heating (1,2). Subsequent cooling does not necessarily **result** in conversion back to bilayer phase structure. Indeed, the bilayer phase can often **only** be reset by freezing the sample. Freeze-fracture studies commonly reveal lipidic particles in such systems, and these and other data (1.21) can be interpreted to suggest formation of "honeycomb" structures resulting from

TABLE 1 Phase Behavior of Various Lipids

Species	Phase	Conditions	References
Phosphatidylcholine^a			
Egg	C, H _{II}	5% water, 50°C	46, 48
	L	10% water	46, 48, 49
16:0/16:0	L _α	20% water, 41°C	50, 51
	P _β	36-41°C	51, 52, 53
	L _β	35°C	50, 51
18:1 _c /18:1 _c	L _α	0°C	54
20:4/20:4	La	0-90°C	b
Sphingomyelin			
16:0/16:0	L	10-50°C	58
Egg	L	20°C	59
Bovine brain	L	40% water, 25-50°C	49, 59-61
Phosphonolipids			
16:0/16:0	L	-20-20°C	62
Tetrahymena	L	30°C	63-65
	H _{II}	45°C	63-65
Phosphatidylethanol- amines^c			
Diacyl species			
20:0/20:0	H _{II}	96°C	66
18:0/18:0	H _{II}	110°C	66, 67
16:0/16:0	H _{II}	109-123°C	66, 67
14:0/14:0	H _{II}	85°C	66, 68, 69
16:0/18:1 _c	H _{II}	75°C	70
18:1 _c /16:0	H _{II}	70°C	b
18:1 _c /18:1 _c	H _{II}	60°C	68, 70, 71
18:1 _c /18:1 _c	H _{II}	10°C	68, 72
18:2/18:2	H _{II}	-15°C	68, 72
18:3/18:3	H _{II}	-15°C	70
20:4/20:4	H _{II}	-30°C	70
22:6/22:6	H _{II}	-30°C	70
Rgg	L	pH 79	71
Egg	H _{II}	25-35°C	71
	L	pH 8.5, pressure	73, 74
From egg PC	H _{II}	40-45°C	75
<i>E. coli</i>	H _{II}	55-60°C	71
Human erythrocyte	H _{II}	8°C	71
Porcine erythrocyte	L + H _{II}	20-40°C, 10-90% water	76
Rat liver e.r.	H _{II}	7°C	77
Rabbit s.r.	H _{II}	0°C	78
Soya bean	H _{II}	-10°C	79
Rat mitochondrial	H _{II}	10°C	80
Dialkyl species			
18:1/18:0	H _{II}	80°C	66
16:0/16:0	H _{II}	86°C	66, 67

(continued)

(Table 1, continued)

Species	Phase	Conditions	References
14:0/14:0	H_{II}	93°C , excess water 78°C , salt. NaCl 100°C , excess water 70°C , low water	66,67 66 66 66
Effect of acyl chain linkage			
Vinyl ether	HI1	30°C	80.81
Alkyl ether	HI1	53°C	80
Acyl ester	HI1	68°C	80
Phosphatidylserines			
16:0/16:0	L	60°C , EDTA	82-85
	HI1	Anhydrous	57
	L	70°C + Ca	83
14:0/14:0	L	50°C , EDTA	82,86,87
18:1_t/18:1_t	L	—	88
18:1_c/18:1_c	L	-7°C , EDTA	82.89
Bovine brain	L	EDTA	90
	L	Na, Mg or Ca salt	25,90-93
Egg	L	± Ca	94
Egg	HI1	pH 3 at 40°C	94
Human erythrocyte	L	± Ca	94
Phosphatidylglycerol			
18:0/18:0	L	pH 9.5 , Na salt	95
16:0/16:0	L	pH 9.5 , Na salt	95-97
14:0/14:0	L	100°C	97-100
14:0/14:0	H_{II}	90°C , 1 M CaCl₂	101
12:0/12:0	L	Na, K, NH₄ or Ca salt	95
18:1_c/18:1_c	L	Ca salt	95
Egg	L	30°C	102
<i>E. coli</i>	L	30°C	102
Cardiolipin			
Bovine heart	L	50% water	103-107
	HI1	50% water	103
	HI1	50% water + Ca	103,104
	HI1	50°C + Ca. pH 3 , high salt	107,108
Dilyso	M	20°C , 0.5 M NaCl	108
	L	20°C , 3 M NaCl	108
Monolyso	L	20°C , 3 M NaCl	108
Acyl	HI1	20°C , 3 M NaCl	108
<i>B. subtilis</i>	L	25°C , Na salt	105
	H_{II}	0°C Ca, Mg salt	105
	HI1	25°C Ba salt	105
Phosphatidic acid			
16:0/16:0	L	pH 3.5 12	109
	HI1	104°C , pH 4.6 . 1 M NaCl	110
14:0/14:0	P_β	5°C , pH 13	110
	L	20-55°C	109.112

Species	Phase	Conditions	References
18:1_c/18:1_c	L	pH 4-8	113
	H_{II}	pH 6 + Ca, Mg, Mn	113
	L	pH 8, 30°C	114
	H_{II}	pH 5.5 + Ca. pH 4	114
Egg	L	pH 8-12	115
	H_{II}	pH 6 + Ca	97
Phosphatidylinositol			
Soya	L	25°C ± Ca	116,117
	L	+ Ca	103
Glycosyldiglycerides			
A. laidlawii			
MGluDG	H_{II}	30°C	118-120
DGluDG	L	30°C	118-120
	C	MGDG/DGDG 1.2/1	120
18:0/18:0 MGalDG	L	20°C	121,122
16:0/16:0 MGalDG	HI1	70°C	123
16:1/16:1 MGalDG	H_{II}	38°C	123
18:3/18:3 MGalDG	HI1	20°C	121
Maize galactolipid	H_{II}	-20-100°C, 10% water	124
	c	60-100°C, 10-20% water	124
	L	0-40°C, 20% water	124
Perlargonium leaves			
MGalDG	H_{II}	0-80°C	125
DGalDG	L	0-80°C	125
Sulfoquinovosyl DG	L	20-80°C	125
Spinach MGalDG	HI1	-15°C	126
Wheat chloroplast			
MGalDG	H_{II}	-10-80°C	127
DGalDG	L	-10-80°C	127
Cerebrosides and gangliosides			
Psychosine	Coagel	20% water, 20-70°C	128
	L	20-45% water, 20-70°C	128
	H	50-60% water, 20-70°C	128
	M	70% water, 20-70°C	128
Bovine brain			
Cerebroside	L	20-40% water; 70°C	49,128,129
Palmitoyl-Cer	L	80°C	130
Sulfatide	L	20-40% water, 40°C	128,129
	C	50-60% water, 40°C	128
	M	70% water, 20-80°C	128
Bovine brain			
Ganglioside	H_{II}	18-50% water, 50°C	132
	M	50% water	132
GalCer, GlcCer	L	Mixtures with PC or monoolein	133,134
LacCer, GM₃, GM₁			
GD₁, GT₁			

(continued)

(Table 1, continued)

Species	Phase	Conditions	References
Lysolipids			
18:0 PC	L_β	25°C	135,136
	M	27°C	136
16:0 PC	L	-10°C	137,138
	M	25°C	137
12:0 PC	M	25°C	139
18:3 PE	L	-10°C	140
	HI1	0°C	140
	M	10°C (inverted)	140
18:2 PE	L	-10°C	140
	M	20°C (inverted)	140
18:1 PE	L	0-90°C	140
Lyso-PC	L	+ equimolar cholesterol	141
Lyso-PC	L	+ equimolar fatty acid	142
Effect of polypeptides and proteins			
CL	L	+ Ca and polylysine	143,144
CL/PE	HI1	+ polylysine	143,144
CL	HI1	+ cytochrome c	145
PA	HI1	+ myelin basic protein	146
Axon lipids	HI1	+ cardiotoxin v4	147
PE	HI1	+ gramicidin 1:200 PE	148-150
PC	HI1	+ gramicidin 1:25 PC	148-150
PE	L	+ glycophorin	151
PE	L	+ cytochrome oxidase	152
PE	L	+ chlorophyllase	153
Effect of anesthetics and other lipophilic compounds			
PA	HI1	pH 6 + chlorpromazine	113
CL	HI1	Dibucaine or chlorpromazine	104
Egg-PE	L	2 mM chlorpromazine	154
	L	% MM dibucaine	154
	L	10 mM tetracaine	154
	L	100 mM procaine	154
Egg-PE	L	Triton X-100. 20 mol%	155
	L	Deoxycholate, 5 mol%	155
	L	Octylglucoside, 10 mol%	155
	L	Lyso-PC 5 mol%	155
PE/PS	HI1	+Ca²⁺	
	L	+Ca²⁺ + dibucaine	156
Erythrocyte lipids	HI1	+ palmitoleic acid	36
	HI1	+ retinol	36
	HI1	+ oleic acid	36
	HI1	+ glycerylmonooleate	36
	L	+ glycerylmonostearate	36
PC, PE	HI1	+ glycerylmonooleate	157,158
Egg-PC	HI1	30 mol% diacylglycerol	159

Species	Phase	Conditions	References
Egg-PE	H _{II}	5 mol% diacylglycerol	159
CL/PC (1:1)	L	+ Ca and adriamycin	160
PE/CL (2:1)	H _{II}	+ adriamycin	160
PE/PS (1:1)	H _{II}	+ adriamycin	160
Mixed lipid systems			
PC/PS	L	± Ca, phase separation	25, 46, 86
PC/PA	L	± Ca, phase separation	161, 162.
PC/PG	L	± Ca, phase separation	86
PE/DOPS	L	30 mol% PS, + Mg	163, 164
	L + H _{II}	+ Ca, phase separation, pH 5	164
PE/DLPS	L	± Ca	165
PE/PG	L	30 mol% PG	102
	H _{II}	+ Ca, no phase separation	102
PE/PI	L	15 mol% PI	117
	H _{II}	+ Ca	117
PE/CL	L	30 mol% CL	145
PE/PC	L	20 mol% PC	
PE/PC/Chol	H _{II}	+ equimolar cholesterol	79, 166
PE/DOPS/Chol	L	30°C	165
	H _{II}	+ Ca, no phase separation	165
PE/DLPS/Chol	L	30°C	165
	H _{II}	+ Ca, no phase separation	165
PE/PS/Chol	H _{II}	+ NaCl	167
PC/SM/Chol	L		168
(erythrocyte outer monolayer)			
PC/PE/PS/Chol	H _{II}	+ Ca ²⁺	168
(erythrocyte inner monolayer)			

Unless otherwise stated, the lipids are assumed to be fully hydrated and at neutral pH. Phases are indicated as follows: L = lamellar; H_I = hexagonal HI; H_{II} = hexagonal H_{II}; C = cubic; M = micellar.

^aAll PC's adopt only lamellar gel or liquid-crystal phases except at low hydration. For reviews of gel-liquid crystal behavior see Refs. 55, 56. The mesomorphism of anhydrous and monohydrated PC's has been discussed elsewhere [57].

^bC. P. S. Tilcock (unpublished).

^cGeneral trends are that TH decreases with increasing acyl chain unsaturation, high salt or low hydration. Alkaline pH or high pressures raise TH. For saturated chains, decreasing chain length increases TH. Plasmalogens (vinyl ethers) exhibit lower TH values than the corresponding alkyl or acyl lipids.

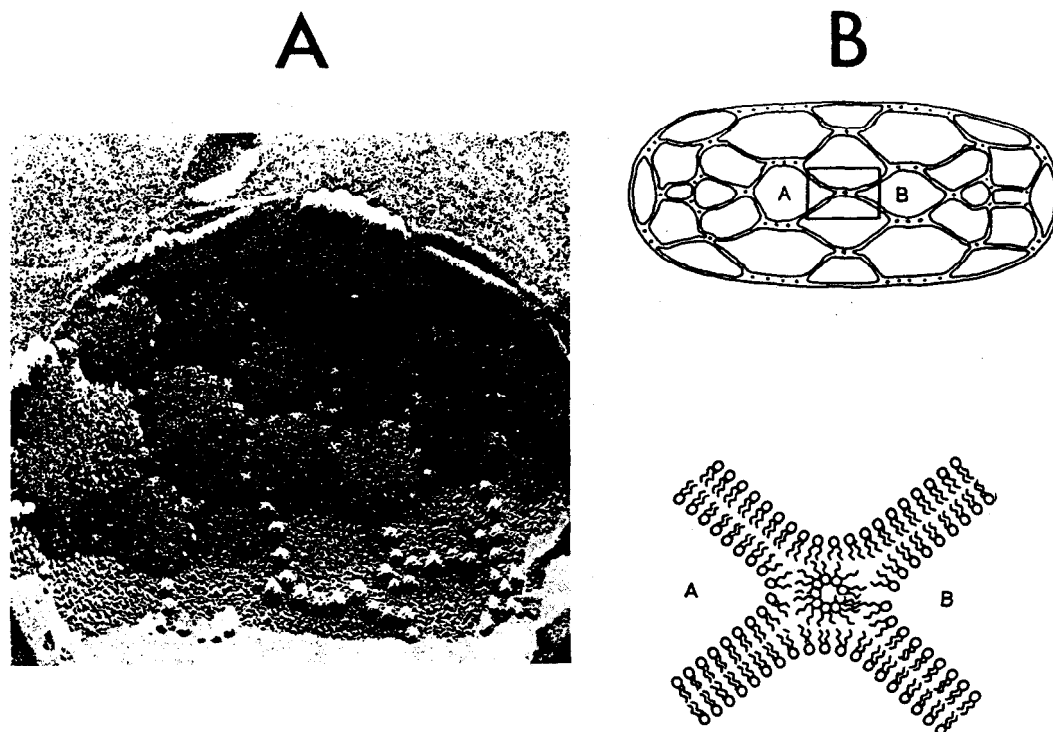


FIGURE 3 (A) Freeze-fracture micrograph of aggregation produced on dialysis of sonicated vesicles (soya PE/egg PS, 5:1) against a $\text{pH} \approx 3.0$ buffer. (B) Honeycomb structure interpretation.

fusion of apposed **bilayers** in multilamellar systems, as shown in Figure 3 (see also Chapter 6). The importance of these observations concerns their possible relation to relatively stable interbilayer connections such as occur in tight junctions (18).

As pointed out previously; in order for nonbilayer lipid structures to play regulated roles in membrane-mediated phenomena, isothermal mechanisms for the generation of such structures *in vivo* must exist. It is, therefore, particularly gratifying that a large variety of biologically relevant variables, such as ionic strength, **pH**, **divalent** cation, and protein, strongly influence the lipid polymorphism. This is apparent in Table 1 and has already been indicated in part by the ability of low **pH** values ($\text{pH} \leq 4.0$) to induce **H_{II}** phase organization in unsaturated PS and PA dispersions as well as the ability of Ca^{2+} to trigger the **H_{II}** phase in CL systems. However, as shown in Table 1, the ability of exogenous factors to modulate the polymorphism of mixed lipid systems is even more pronounced. In (**bilayer**) mixtures of unsaturated PE stabilized by acidic (negatively charged) lipids, for example, the addition of Ca^{2+} can trigger **H_{II}** formation. Alternatively, in similar systems stabilized by unsaturated PS and PA, lower **pH** values again lead to **H_{II}** phase formation. Increased ionic strength can give rise to **H_{II}** structures in previously bilayer PE/PS/cholesterol liposomes. Proteins can have similar bilayer-destabilizing (or bilayer-stabilizing) effects, as indicated by the ability of cytochrome c to induce nonbilayer

lipid organization in CL/PE dispersions. Similar observations have been made for the A₁ basic protein from myelin (30), as well as cardiotoxin and mellitin. Other proteins, such as cytochrome oxidase and glycophorin, can stabilize the bilayer (see Table 1).

C. Mixing Properties of Lipids

As indicated in the previous section, most membrane lipids can adopt H_{II} phase structure under appropriate circumstances. However, in mixed lipid systems, questions concerning the mixing properties of component lipids can be raised, particularly in a multicomponent system where different phase structures (e.g., bilayer, H_{II}, and lipidic particle) are observed to coexist. In such systems it may be expected that lipids preferring the bilayer organization would be predominantly in the bilayer component, whereas H_{II}-preferring lipid would be in the H_{II} component. However, results from this laboratory (22) indicate that ideal lipid mixing is maintained in PC/PE/cholesterol systems for which bilayer, H_{II}, and "isotropic" components are observed by ³¹P NMR. Similar conclusions can be drawn from results obtained for CL/PC systems (23). However, there is some contention in this area, as effects apparently consistent with enrichment of PE in H_{II} phase components existing in PE/PC systems have been observed (24).

In mixed lipid systems where H_{II} phase structure is induced by factors such as pH, ionic strength, or Ca²⁺, two major types of phenomena can occur. The first of these, which has been observed in unsaturated PE/PS systems, concerns the ability of Ca²⁺ to sequester PS into crystalline ("cochleate") domains (25). For example, in DOPS/DOPE systems, the addition of Ca²⁺ can result in lateral phase separation of PS to form cochleate domains, which allows the PE to adopt the H_{II} phase it prefers in isolation (26). However, this behavior is not observed in systems stabilized by more unsaturated varieties of PS or in systems containing extra components such as cholesterol (27). In bilayer DOPS/DOPE/cholesterol (molar ratio 1:1:1) systems, for example, the addition of Ca²⁺ results in complete H_{II}

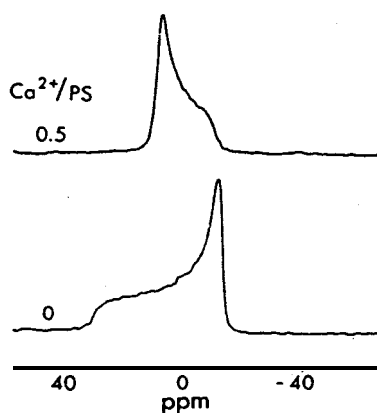


FIGURE 4 81.0 MHz ³¹P NMR spectra at 30°C of a DOPE-DOPC-DOPS-cholesterol (1:1:1:3) mixture in the absence and presence of Ca²⁺. The Ca²⁺ was added to achieve a Ca²⁺/PS molar ratio of 0.5. For further details see Ref. 165.

phase structure where both the PE and the PS (and presumably the cholesterol as well) are contained in the H_{II} organization. The major point of these remarks is to emphasize the conclusion that the large majority of membrane lipids can adopt nonbilayer structure and that stimulation of H_{II} and other nonbilayer phases does not usually result in lateral segregation of component lipids. A dramatic example of this is given in Figure 4, where the ^{31}P NMR characteristics of a DOPC/DOPE/DOPS/cholesterol (molar ratio 1: 1: 1: 3) in the presence and absence of Ca^{2+} are noted. In the absence of Ca^{2+} , a ^{31}P NMR spectrum consistent with lamellar organization is observed, whereas in the presence of Ca^{2+} , complete H_{II} phase formation is observed, as indicated by ^{31}P NMR. This and other (27) evidence show unequivocally that all the DOPC, DOPE, and DOPS adopt the H_{II} organization when Ca^{2+} is present.

IV. THE MOLECULAR BASIS OF LIPID POLYMORPHISM

The ability of lipids to adopt the H_{II} phase and other nonbilayer structures on hydration has naturally stimulated considerable interest in the factors that drive these remarkable structural transitions and the mechanisms involved. The literature in these areas is starting to provide real insight, which, as emphasized in Section II, has general implications for the properties and roles of lipids in membranes.

A. Factors Determining Lipid Phase Structure: The Shape and Curvature Concepts

Progress in this area basically stems from the work of Israelachvili and co-workers (28), who have examined the molecular properties of amphiphiles which form spherical and nonspherical micelles on dispersion in water. A basic packing property that has proved useful to explain these properties is a dimensionless shape parameter defined as $S = V/A_0L_c$. Here A_0 is an "optimum" area per molecule at the lipid-water interface, V is the volume per molecule, and L_c is the length of the fully extended acyl chain. That the S parameter relates to a molecular shape property is easily realized from Figure 5, which defines an additional parameter A_H as the cross-sectional area subtended at the hydrophobic end of the molecule. It is straightforward to show that for $A_H/A_0 < 1$, $S > 1$; for $A_H/A_0 = 1$, $S = 1$; and for $A_H/A_0 > 1$, $S < 1$. Using the language introduced previously (1), lipids that have a preferred shape corresponding to $S < 1$ are referred to as having a "cone" shape, whereas lipids where $S \approx 1$ are cylindrical and lipids where $S > 1$ have an "inverted" cone shape. The relationship between these shape properties and the geometry of the macroscopic lipid aggregate is clear, as lipids in a micellar phase must exhibit an inverted cone shape in order to satisfy geometrical packing constraints, bilayer lipids must be roughly cylindrical, whereas lipids in H_{II} or inverted micellar structures must have a net cone shape.

The shape concept of lipids might appear unduly simplistic. However, it has proven remarkably successful in providing a qualitative but predictive framework for understanding the polymorphic phase preferences of lipid dispersions. Before detailing this success, it is important to realize that lipid shape is an inclusive phenomenological concept which lumps together a large variety of complex molecular forces. For example, A_0 , the optimum cross-sectional area at the lipid-water interface, would be expected to be

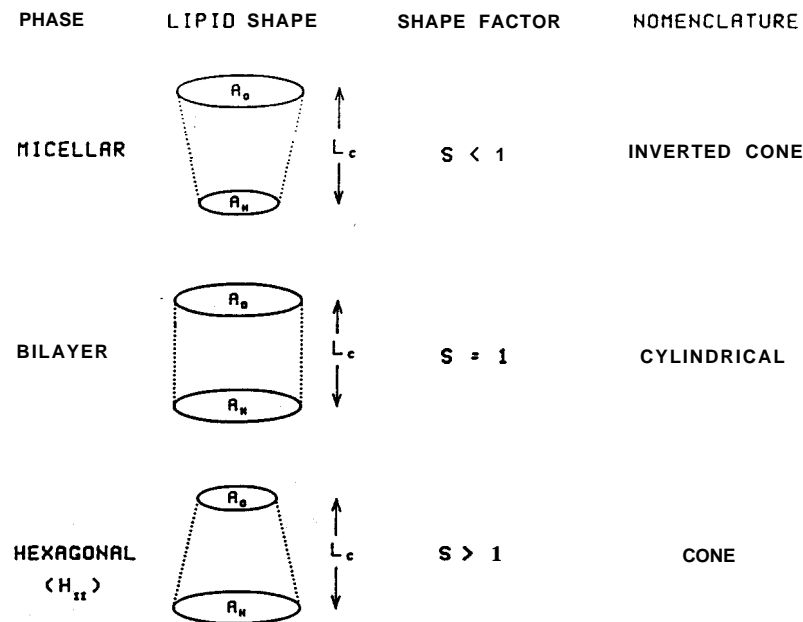


FIGURE 5 Shape features exhibited by membrane lipids. A_o refers to the area subtended by the polar region at the lipid-water interface, whereas A_H refers to the area subtended at the intermonolayer hydrophobic interface. The shape factor $S = V_o/A_o L_c = 1/3[1 + (A_H/A_o)^{1/2} + A_H/A_o]$, where V_o is the volume of the lipid.

sensitive to the size of the lipid headgroup (large headgroup leading to large A_o values), the charge on the headgroup (charged headgroups giving larger effective A_o 's due to inter-headgroup electrostatic repulsion effects), the hydration of the headgroup (lower hydration, smaller A_o), and so on. Alternatively, A_H will be sensitive to factors that modulate the splay at the end of the hydrocarbon chains. Thus, increased acyl chain unsaturation, increased temperature, and increased acyl chain length (for liquid-crystalline lipids) would all be expected to increase the preferred value of A_H , leading to increased cone shape and possible H_{II} phase formation.

The features influencing lipid shape and their predicted influence on the bilayer (L_α) to H_{II} phase transition are summarized in Figure 6. All of these factors modulate lipid polymorphism in the predicted manner. As previously indicated (see Table 1), particularly for PE systems, increased **acyl-chain** unsaturation leads to increased proclivity for H_{II} structure, and increased temperature induces bilayer to H_{II} transitions. The smaller headgroup of PE (as compared to PC) is consistent with **H_{II} organization**. The proclivity of unsaturated PE's for the bilayer phase at $pH \geq 9$ is consistent with deprotonation of the primary amine, resulting in a charged headgroup and thus a larger effective A_o . Similarly, protonation of the PS carboxyl and PA phosphate at lower pH values (≤ 4 , see Table 1) leads to reduced interheadgroup electrostatic repulsion, smaller A_o values, and H_{II} structure. The ability of Ca^{2+} to trigger bilayer H_{II} transitions in CL systems as well as unsaturated PS/PE, PG/PE, PA/PE, and PI/PE systems can be rationalized on a similar basis, as can the ability of high salt concentrations to induce H_{II} organization in PE/PS/cholesterol systems. With

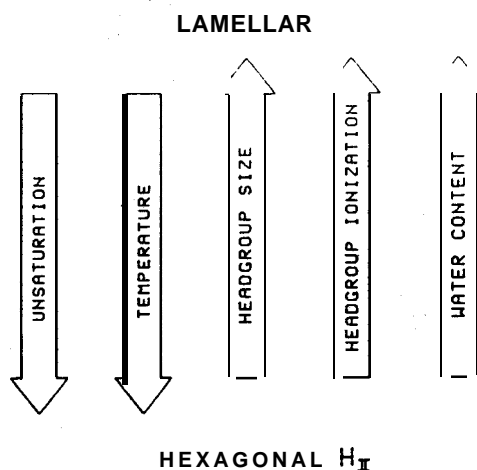


FIGURE 6 Factors influencing the bilayer to hexagonal (H_{II}) phase transition for membrane lipids.

regard to hydration, lower water content has been shown to reduce the TH in a variety of systems, most notably egg PC systems where H_{II} phase structure can be observed under conditions of low water content and high temperature (Table 1) :

In summary, the shape concept offers gratifying correlation between predictions and experiment. With regard to fusion, it is interesting to note that all factors promoting cone-shape character in component lipids (i.e., **promoting H_{II}** organization) also promote fusion (4).

However, the shape parameter is not a well-defined, measurable quantity. For example, as discussed in Section **IV.B**, the lipid shape can vary according to the environment. Theoretical work has therefore been directed toward obtaining a more quantitative measure of forces driving H_{II} phase formation, leading to the "curvature" concept introduced by Gruner and co-workers (29,30). In this approach each monolayer is viewed independently, and the presence of cone-shaped lipid is expressed as a tendency of each monolayer to curl up into cylinders with an equilibrium radius of curvature R_0 . The binding energy for monolayers expressing a radius of curvature R is then **given** (to least significant order in $1/R$) by $E = kc (1/R - 1/R_0)^2/2$ where kc is the elastic compressibility modulus. The tendency of the **mono-**layers to form cylinders is countered by the requirement to fill the **inter-**cylinder spaces which requires acyl-chain stretching. Thus, the actual structure assumed depends on a competition between curvature and hydrocarbon stretching forces.

B. Lipid Shape and Bilayer Packing Properties

The fact that lipid molecules exhibit certain preferred shapes which strongly influence the polymorphic phase adopted (bilayer or H_{II}) leads to interesting possibilities concerning the properties of membranes containing a proportion of lipids that prefer H_{II} organization, but where bilayer structure is

maintained. Clearly, this directly relates to the situation in biological membranes. Assuming a reasonably flat bilayer, the distributed presence of cone-shaped molecules would result in a smaller "optimum" surface area as compared to the optimum hydrophobic area assumed at the hydrophobic (intermonolayer) interface. Least energy considerations would then suggest a lateral compression of the bilayer to counter the large hydration energies associated with increased water penetration into the hydrophobic region. In turn, this compression would be expected to result in larger-order parameters in the hydrocarbon and correspondingly reduced membrane permeability. Such increased hydrocarbon order has been observed (employing ^2H NMR) in bilayer PE/PC systems as compared to PC systems (31), and decreases in K^+ permeability have been observed as PE is titrated into PC bilayers (32). Similar effects may be related to the ability of cholesterol, a cone-shaped molecule, to increase acyl-chain order and reduce membrane permeability (33). The presence of cone-shaped lipids in bilayer membranes therefore appears to lead to increased order in the hydrocarbon. In turn, factors modulating the shape preferences of lipids would be expected to lead to corresponding modulations of hydrocarbon order, assuming bilayer structure is maintained. This could be expressed as changes in membrane permeability or membrane protein function. The appeal of these observations lies in the resulting ability to relate the quantitative shape concepts to the well-defined, measurable hydrocarbon order parameters, which may well represent a conserved, regulated quantity in membranes.

C. Mechanisms and Dynamics of Bilayer Nonbilayer Transitions

The results indicated in the previous sections identify likely forces and stresses that result in an overall preference for bilayer, H_{II} , or other nonbilayer phases. However, they do not address the actual mechanism whereby such reorganizations proceed or the dynamics involved.

An initial observation relating to the mechanism of the bilayer-to- H_{II} transition indicated that the inverted cylinders characteristic of the H_{II} organization form parallel to the planes of closely apposed bilayers (21). Concurrently, the observation was also made that lipidic particles appeared to be intermediate structures between bilayer and H_{II} phases (34), leading to the possibility that lipidic particles (interpreted as inverted micelles) are intermediates in these bilayer-to- H_{II} transitions as well as intermediates in fusion processes. In bilayer-to- H_{II} transitions, a general intermediate role of inverted micelles did not appear warranted, however, as no lipidic particles or narrow isotropic ^{31}P NMR resonances could be observed during such transitions occurring, for example, in unsaturated PE systems. However, Siegel (17,19,20), in an interesting series of papers, provides a thermodynamic analysis of the dynamics of inverted micellar intermediates (IMI) and concludes that the lifetime of IMI is likely to be very short (e.g., $< 10^{-4}$ s) and the steady-state number of IMI will contain only a small fraction of the total lipid. Thus, detection of IMI in bilayer-to- H_{II} transitions would be very difficult by NMR or freeze-fracture techniques. A general model of bilayer-to- H_{II} transitions results, where IMI form between bilayers and provide nucleation points for formation of the cylinders characteristic of the H_{II} phase as the lipid dispersion progressively converts to the H_{II} organization.

Siegel (20) has also provided an elegant analysis of events that may be expected when formation of inverted cylinders from IMI is reduced or inhibited. This may arise in systems incubated just below the bilayer-

to- H_{II} transition temperature, in small unilamellar systems where a limited interface area prevents formation of long interbilayer inverted tubes, as well as in lipid systems that evolve into cubic rather than H_{II} phases. These structures exhibit isotropic NMR resonances and are usually translucent. *In such situations, an evolution of the IMI into interlamellar attachment sites (ILA) is favored.* These interlamellar attachment sites basically represent a completed fusion event.

In summary, the theoretical basis for lipid polymorphism is becoming increasingly well established. The success of the phenomenological shape concept has led to identification of membrane curvature as a quantitative and measurable parameter influencing lipid polymorphism, as well as to increasing insight into the relation between lipid shape properties and hydrocarbon order. Finally, detailed thermodynamic analyses are leading to recognition of the interbilayer inverted micelle as a fundamental intermediate in membrane-membrane interactions, as a precursor to either H_{II} organization, cubic structure, or fusion processes.

V. LIPID POLYMORPHISM AND BIOLOGICAL MEMBRANES

The polymorphic properties of lipids summarized in the previous sections arise from studies performed on model membrane systems. The aim of such studies is, of course, to provide a basic understanding of the physical properties and functional roles of lipids in biological membranes. This has not yet been achieved. However, certain aspects of biological membrane lipid composition and behavior are instructive.

More than 95% of the phospholipid in the erythrocyte is organized in a bilayer organization (35), and similar conclusions may be drawn for most other biological membranes. Chemical fusogens can induce H_{II} structure for erythrocyte "ghost" membranes (3,36). However, no other form of disruption [phospholipase treatment, proteolytic digestion (37)] causes reorganization of membrane bilayer structure, although isolated dispersions of "inner monolayer" lipid exhibit H_{II} organization in the presence of Ca^{2+} (38).

In the case of organelle membranes, the lipid composition and unsaturation result in a much more delicate balance between bilayer structure and other structural alternatives. Limited dehydration of endoplasmic reticulum (microsomal) membranes results in H_{II} organization (41), for example, whereas total lipid extracts of the mitochondrial membrane exhibit a proportion of H_{II} phase structure (40). Similarly, lipid extracts from retinal disk membranes adopt H_{II} organization (39) (indicating a bilayer-stabilizing role for rhodopsin) and lipid extracts from chloroplast membranes exhibit similar behavior (42). It may therefore be suggested that the lipid composition exhibited by these membranes is compatible with the presence of localized nonbilayer lipid structure. This may find expression in fusion processes as well as nonbilayer adhesion points between membranes, such as between the inner and outer mitochondrial membranes (15) and structures related to tight junctions (18).

The observed balance between bilayer and H_{II} phase lipids in membranes has led to the suggestion that maintenance of appropriate proportions of cylindrical and cone-shaped lipids represents conserved quantities in membranes (6). Evidence in support of this hypothesis has been generated employing certain prokaryotes that have rather limited biosynthetic abilities for lipid biosynthesis, allowing appreciable manipulation of lipid composition

and corresponding elucidation of factors regulating lipid composition. *Acholeplasma laidlawii* is perhaps the best example, as inhibition of endogenous fatty acid synthesis has led to the development of strains with an essentially homogeneous fatty acid composition (43). Studies on these systems have led to interesting observations regarding a balance between different molecular species of lipid, which can be interpreted in terms of a requirement for a balance between cone-shaped (**H_{II}** phase) lipids and cylindrical (**bilayer**) species. In particular, it is observed that as the **acyl-chain** length or unsaturation is increased, the ratios of endogenous monoglucosyldiglyceride (**MGluDG**) to diglucosyldiglyceride (**DGluDG**) decrease dramatically (43). As **MGluDG** is a cone-shaped (**H_{II}** phase) lipid and **DGluDG** a cylindrical (bilayer phase) lipid, and as increases in chain length and unsaturation give rise to increased cone-shaped character, the changes in the **MGluDG/DGluDG** ratio are consistent with a need to conserve lipid shape distributions. Similarly, other workers have shown that higher levels of cholesterol (a cone-shaped molecule) result in lower **MGluDG/DGluDG** ratios (44), whereas the inclusion of low levels of inverted cone (anesthetic) molecules in the growth medium has reverse effects (45). These observations have been used to support the contention (6) that conservation of lipid shape properties may be more basic than maintenance of membrane lipid "fluidity" per se. However, it is also possible that the basic conserved quantity is the order in the hydrocarbon, which is modulated (increased) by the presence of cone-shaped lipids (see Section **IV.B**).

VI. CONCLUDING REMARKS

Our phenomenological understanding of the structural properties of lipids found in membranes and factors regulating these polymorphic capabilities is rapidly becoming a mature body of knowledge. This is having two **important** consequences. First, the ability of membrane lipids to adopt transitory or long-lived nonbilayer alternatives has direct application to phenomena, such as fusion, that require local departures from bilayer structure. Second, the observation that biological membranes maintain a balance **between** bilayer and nonbilayer lipids of different shapes may lead to an understanding of conserved quantities, such as curvature or hydrocarbon packing properties, which dictate membrane lipid composition.

NOTE ADDED IN PROOF

Since submission of this chapter, a number of additional findings have been made in this research area. Unfortunately, we have not been able to include all of them. Some recent references are cited at the end of the reference list.

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