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SMR.961 - 2

**WORKSHOP ON:
PROTEINS, MEMBRANES and their INTERACTIONS**

22 JULY - 2 AUGUST 1996

"Lipid polymorphism and Membrane Structure and Function"

PART I

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22 July- 2 August 1996

Lipid Polymorphism and Membrane Structure and Function

- Cell membranes
- Biological lipid chemical structures
- Lipid types
- Lipid biosynthesis and compositions
- Amphiphilic properties of biological lipids
- Phase identification and structure: diffraction
- Lamellar phases
- Non-lamellar phases
- Role of non-lamellar structures in cell membranes
- Effects of hydrostatic pressure
- Phase transitions: epitaxy
- Effects of solutes

Lipid Polymorphism and Membranes: Bibliography

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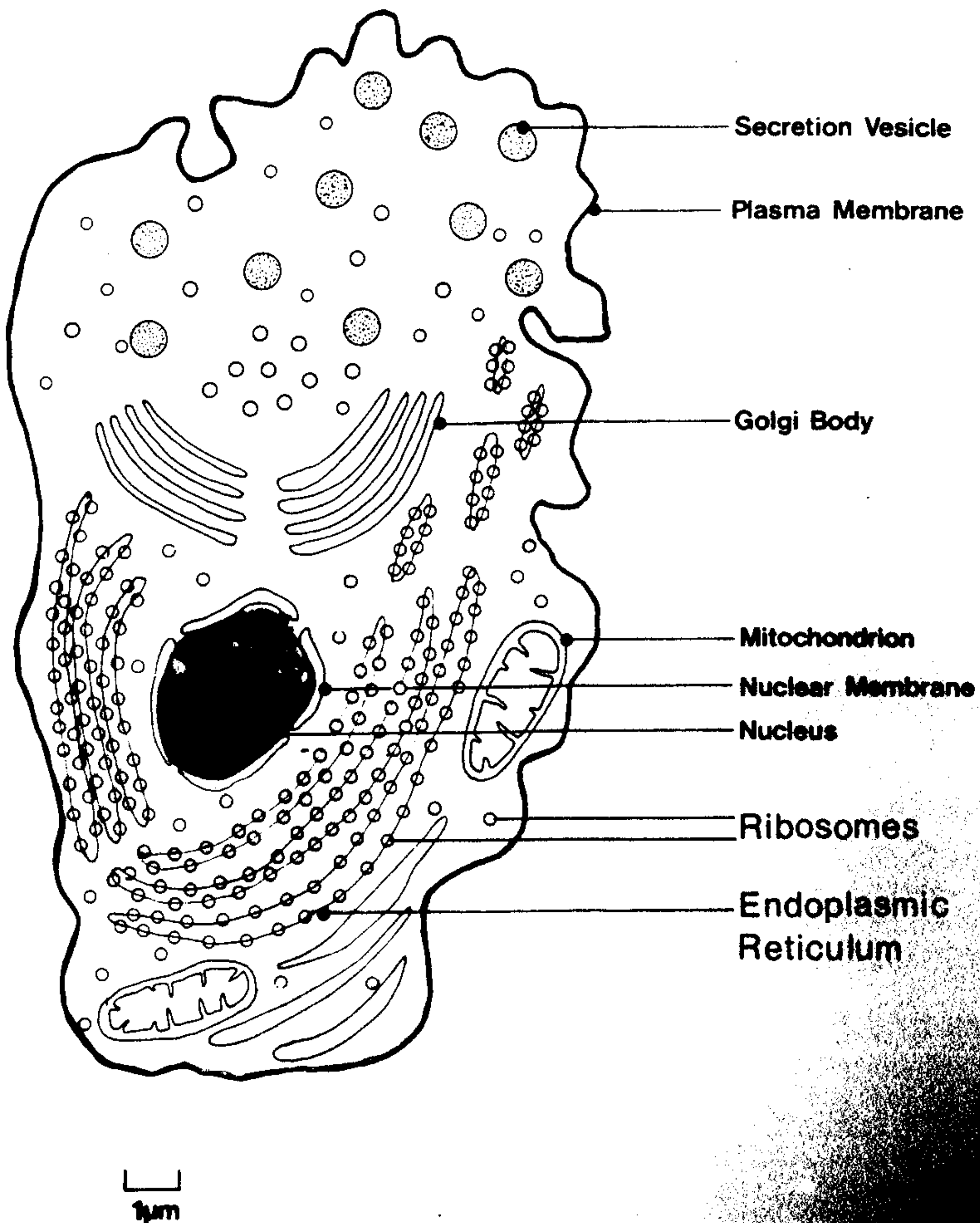
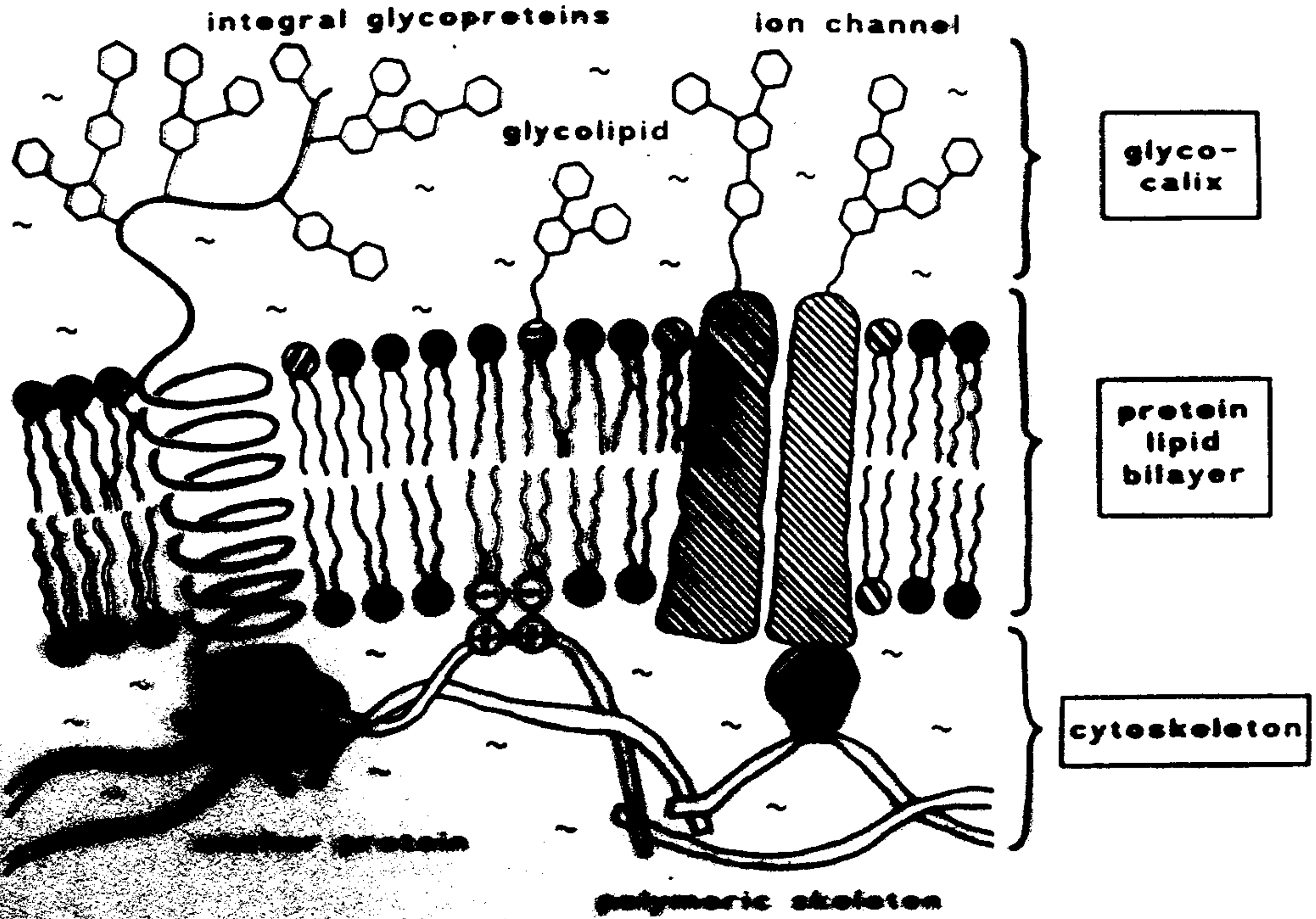
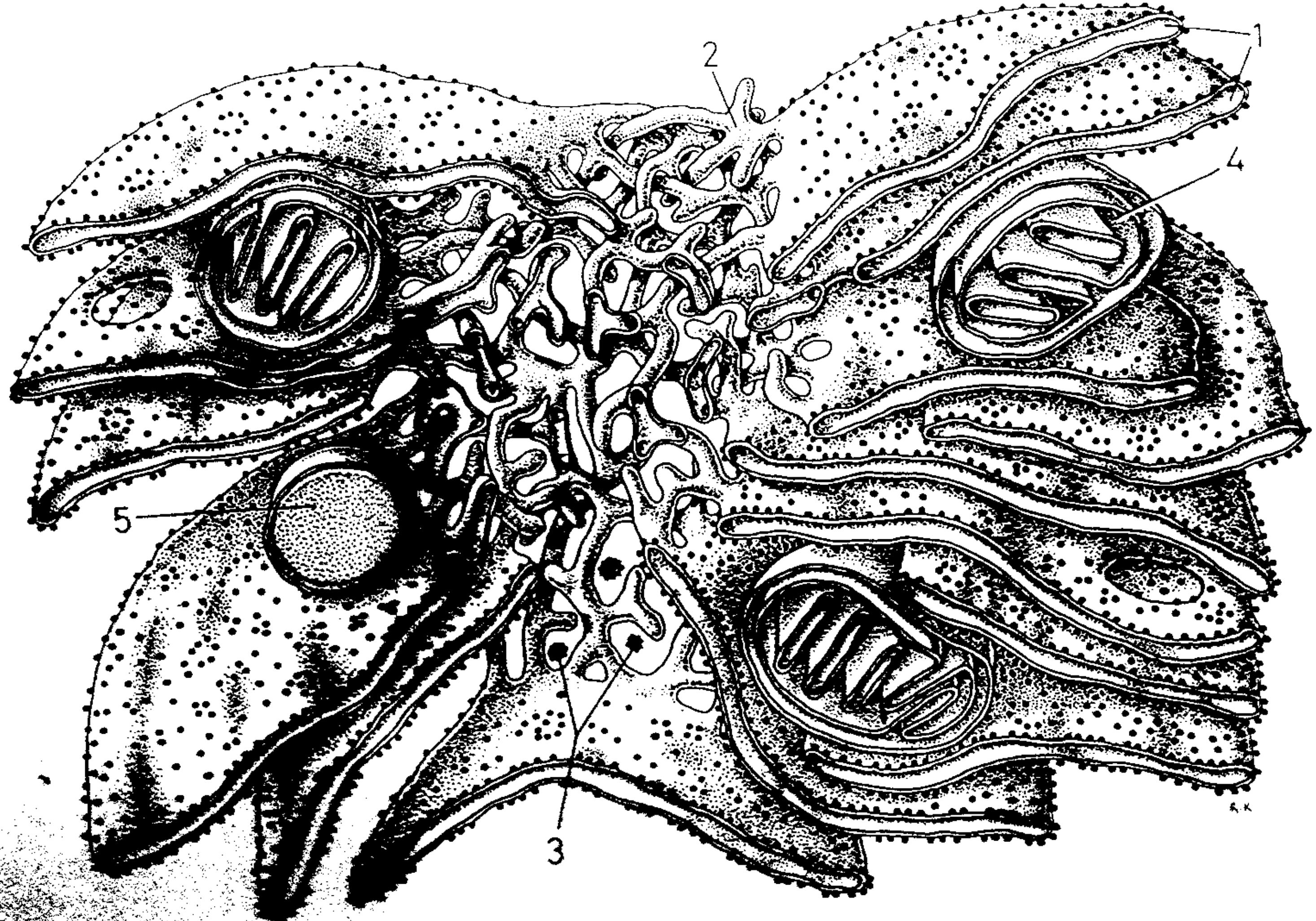

Secretion Vesicle
Plasma Membrane
Golgi Body
Mitochondrion
Nuclear Membrane
Nucleus
Ribosomes
Endoplasmic Reticulum
1µm

Functions of biomembranes

- Permeability barrier to ions and other polar solutes
- Intracellular organisation and compartmentalisation
- Cell surface receptors (recognition and signalling)
- Cell adhesion
- Ion transport
- Intracellular calcium storage (sarcoplasmic reticulum)
- Nerve conduction and insulation
- Oxidative phosphorylation (mitochondrion)
- Protein synthesis (rough endoplasmic reticulum)
- Lipid synthesis (smooth endoplasmic reticulum)
- Packaging of secretory molecules
- Vision (photoreceptor membranes)
- Photosynthesis (plant thylakoid membranes)


integral glycoproteins
ion channel
glycolipid
glyco-
calix
protein
lipid
bilayer
cytoskeleton
polymeric skeleton


1
2
3
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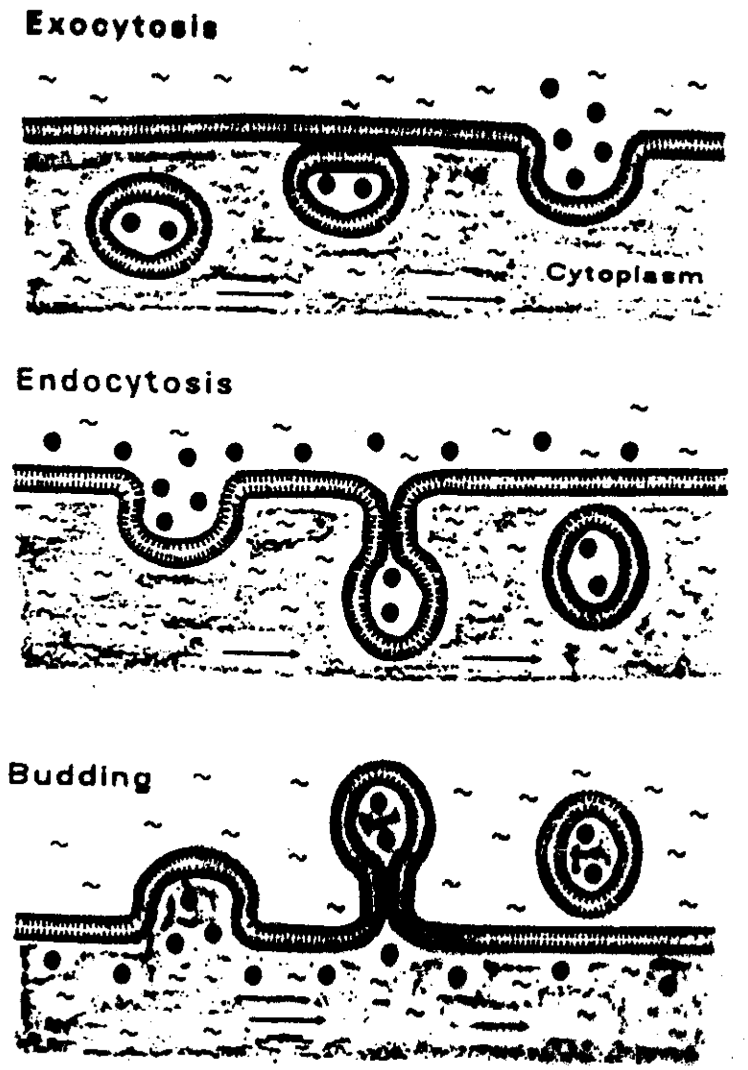
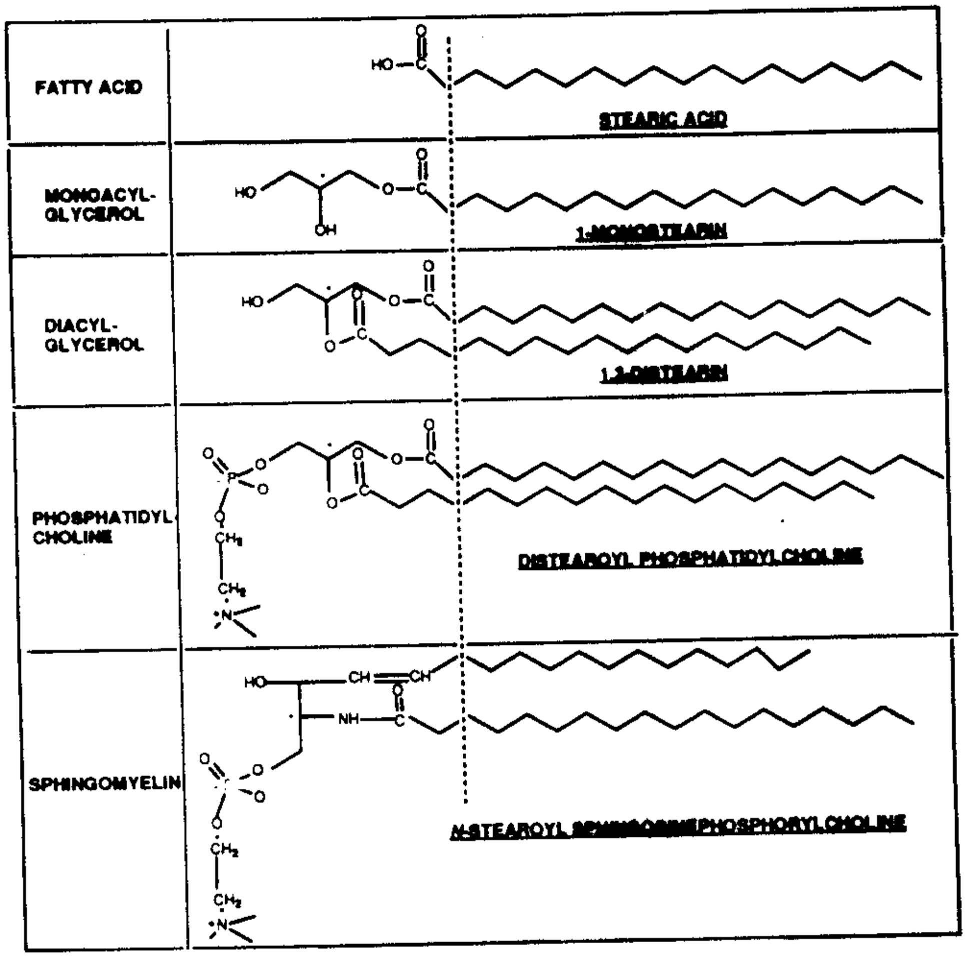
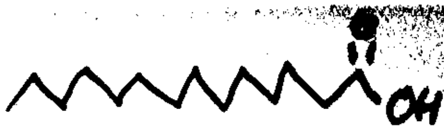

Exocytosis
Cytoplasm
Endocytosis
Budding


FATTY ACID
STEARIC ACID
MONOACYL-GLYCEROL
1-MONOSTEARIN
DIACYL-GLYCEROL
1,2-DISTEARIN
PHOSPHATIDYL-CHOLINE
DISTEAROYL PHOSPHATIDYLCHOLINE
SPHINGOMYELIN

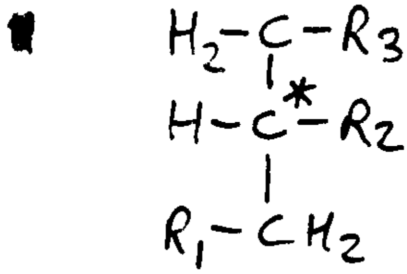
Fatty Acids

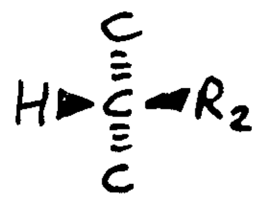


- Simplest amphiphile.
- Too weakly polar to form LC phases in water when fully protonated.
- Increasing pH causes dissociation: COOH → COO⁻
- Such soap forms micelles or LC phases.
- pK for a free COOH is 4·6, but is raised to pH ~ 8 for long chain fatty acids (>C_{12})
- Free fatty acids usually found only in small amounts in cell membranes
- They may be produced by action of lipases on phospholipids or fats (triacylglycerols).
- Fatty acids are soluble in phospholipid bilayers to very high amounts (> 80 mol %).
- They strongly modify the lipid phase behaviour and structure.
- In certain diseases, fatty acids [illegible] destroying the membrane and [illegible]

Mono acyl Glycerol

- Widespread in food and pharmaceuticals.
- Very minor component of membranes.
- Sufficiently hydrophilic to form a wide range of lyotropic phases in water.
- Lipids based on glycerol are usually chiral:



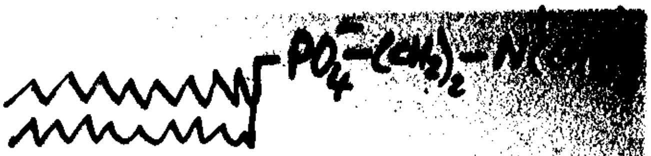


The chiral centre is denoted by an asterisk: *

Diacyl glycerol

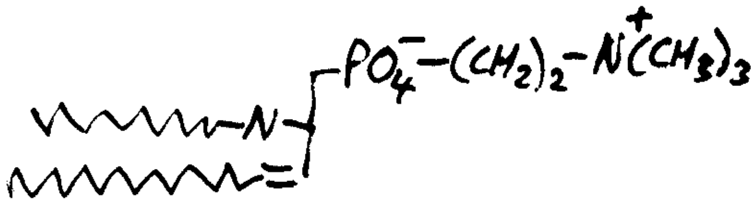
- Important intermediate in phospholipid biosynthesis.
- Like fatty acids, it is too weakly polar to form any lyotropic phases in water.
- Soluble to > 80 mol% in lipid bilayers
- Key molecule in cell signalling

Phosphatidylcholine (PC)

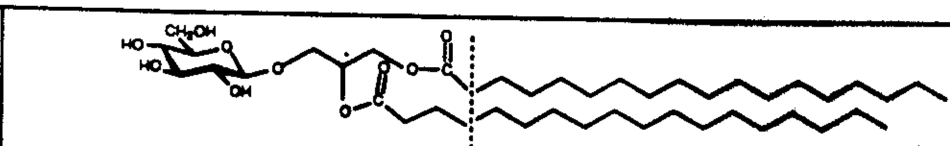
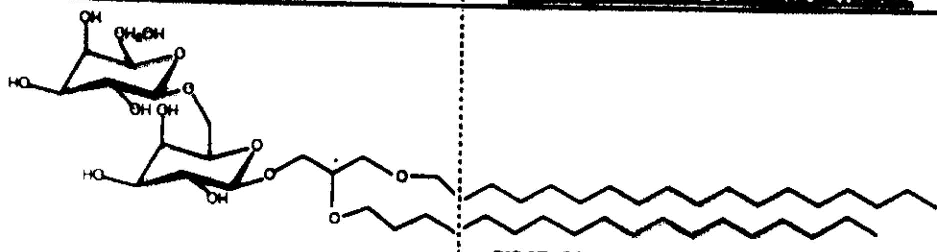
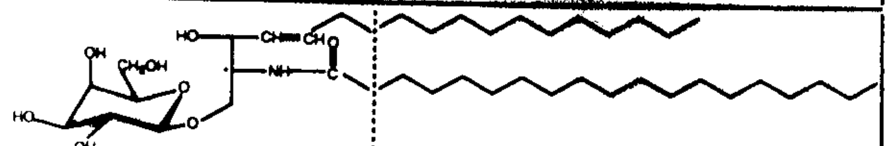
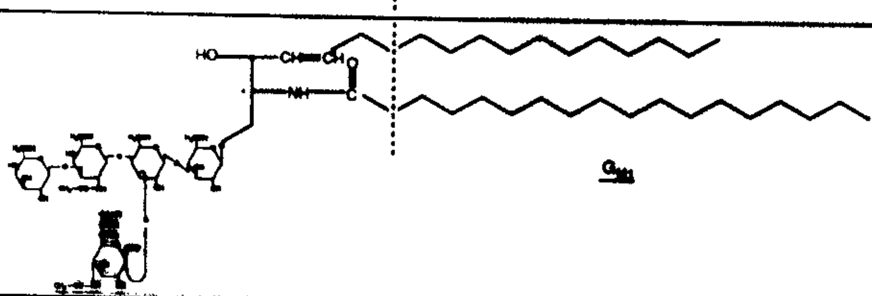


- Abundant in animal cell membranes
- Most studied model system.
- Forms lamellar phases under almost all conditions of T, p, H_2O, pH etc.
- Zwitterionic headgroup (but pH insensitive).

Sphingomyelin



- Same headgroup as PC.
- Derived from sphingosine.
- Similar phase behaviour to PC.
- Abundant in plasma membrane.

MONOGLUCOSYL-DIACYLGLYCEROL	 DISTEAROYL GLUCOPYRANOSYL GLYCEROL
DIGALACTOSYL-DIALKYLGLYCEROL	 DIOCTADECYL GALACTOPYRANOSYL GLYCEROL
GALACTO-CEREBROSIDE	 N-STEAROYL GALACTOSYLSPHINGOSINE
GANGLIOSIDE	 G_{M1}

Monoglucosyl diacylglycerol (MGlcDG)

- Formed by linking glucose to diacylglycerol.
- Common in membranes of microorganisms (E. coli).
- Nonionic: uncharged.
- Possibility for extensive hydrogen-bonding.

Monogalactosyl DG (MGalDG)

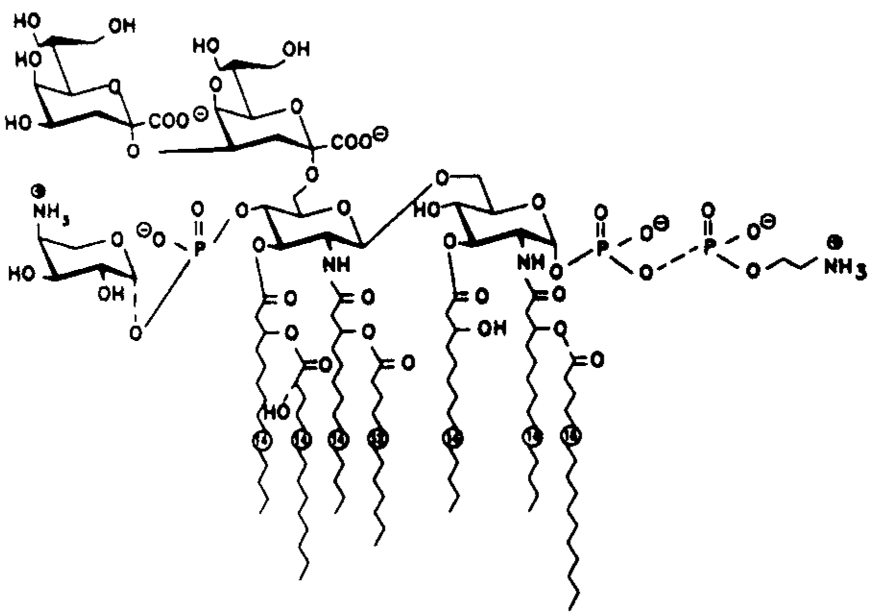
- Major lipid in plant cells.
- Galactose differs from glucose in the configuration of the OH group on carbon C4 of the sugar ring:

  Gal: axial   Glc: equatorial

- Galactose-based lipids are by far the most abundant lipids on Earth.

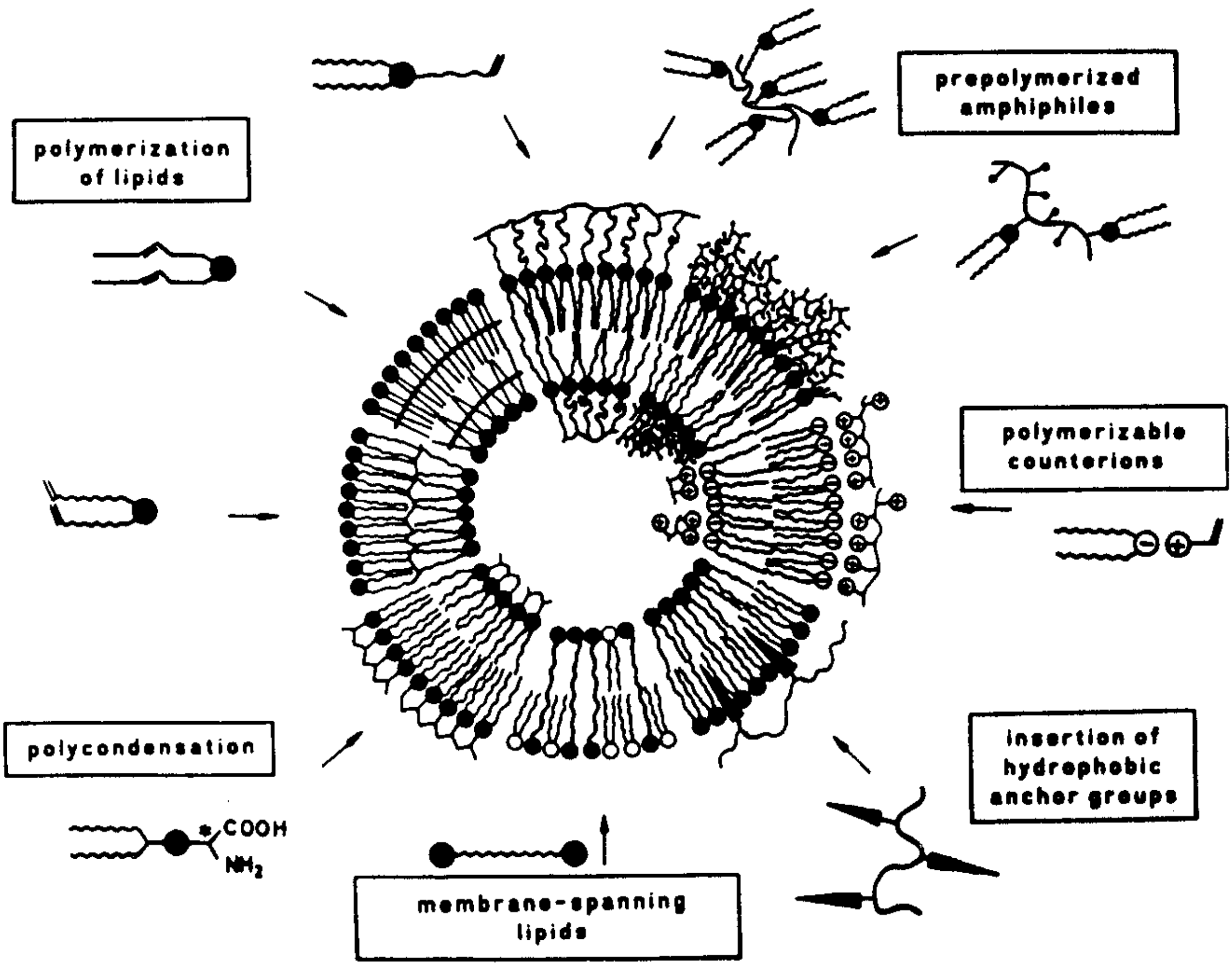
- Galactose lipids also important in animal cell membranes, as cerebrosides or gangliosides.

Lipopolysaccharide from *S. minnesota*



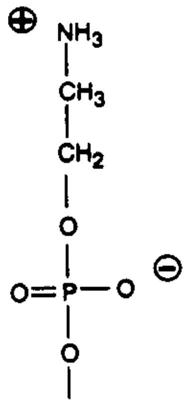
From: K. Brandenburg et al. (1992) J. Struct. Biol. 108, 93.

Polymerizable and membrane-spanning lipids



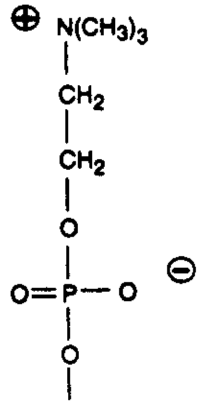
From: H. Ringsdorf et al., Angew. Chem. Int. Ed. Engl., 1988, 27, 113-158

Polar headgroups of phospholipids



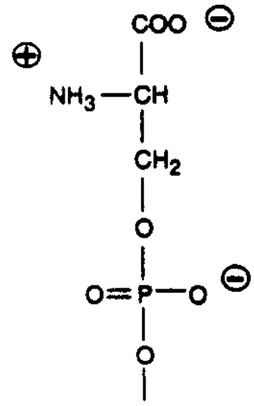
Phosphatidylethanolamine

(PE)



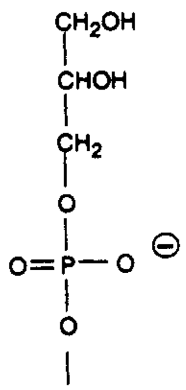
Phosphatidylcholine

(PC)



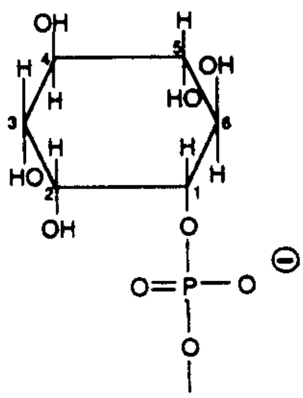
Phosphatidylserine

(PS)



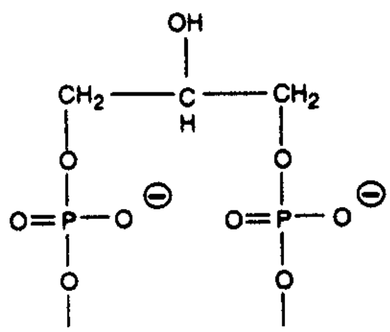
Phosphatidylglycerol

(PG)



Phosphatidylinositol

(PI)



Cardiolipin (diphosphatidylglycerol)

(CL)

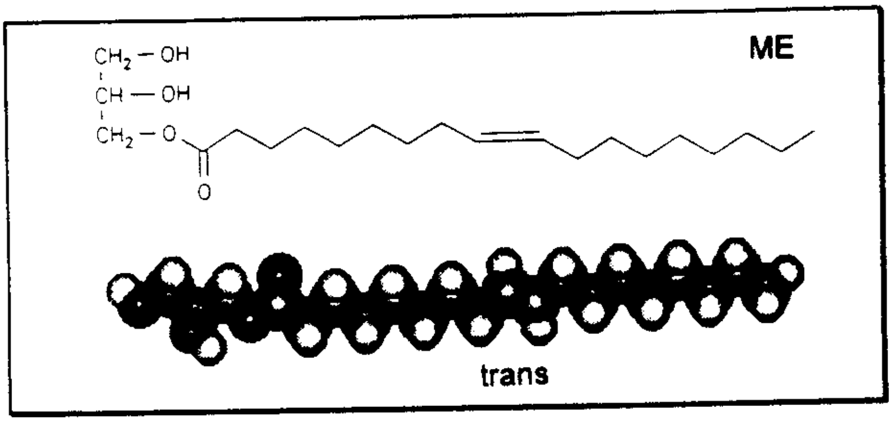
From: D. Fennel Evans and H. Wennerström, The Colloidal Domain, 1990, VCH, Weinheim.

Hydrocarbon chains of lipids

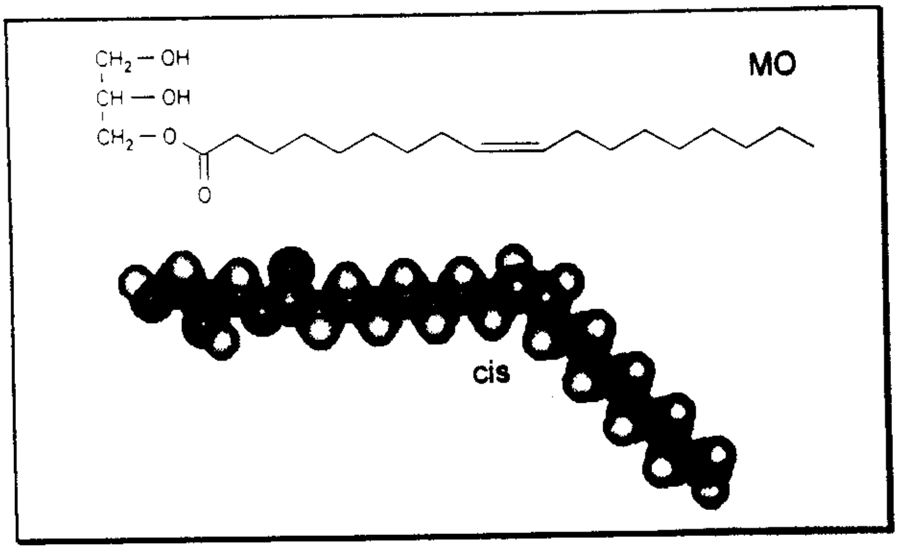
- **Number:** 1-7 (but usually 2)
- **Linkage:** ester (-COO-) or ether (-CO-)
- **Length:** mainly C12-C22 (but can be up to C90)
- **Asymmetry in length:** usually small
- **Unsaturation:** up to 4 double bonds, usually *cis-*
- **Branching:** methyl, cyclopropane, isoprenoyl, etc

Hydrocarbon chain unsaturation

trans-unsaturation (monoelaidin)



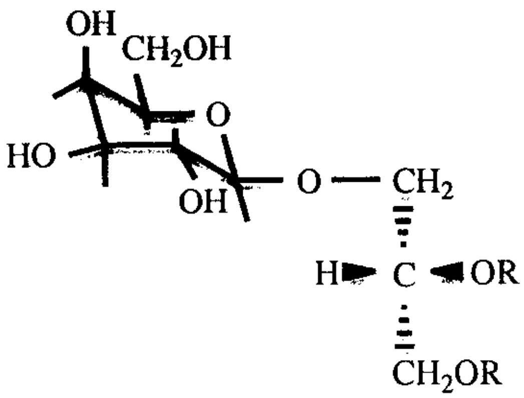
cis-unsaturation (monoolein)



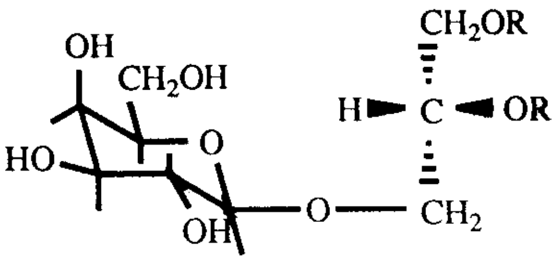
From: C. Czeslik et al., Biophys. J., 1995, 68, 1423-1429

THE *sn*-3 (A) AND *sn*-1 (B) STEREOISOMERS OF DIALKYL -Oβ-D-GALACTOPYRANOSYL GLYCEROLS.

A

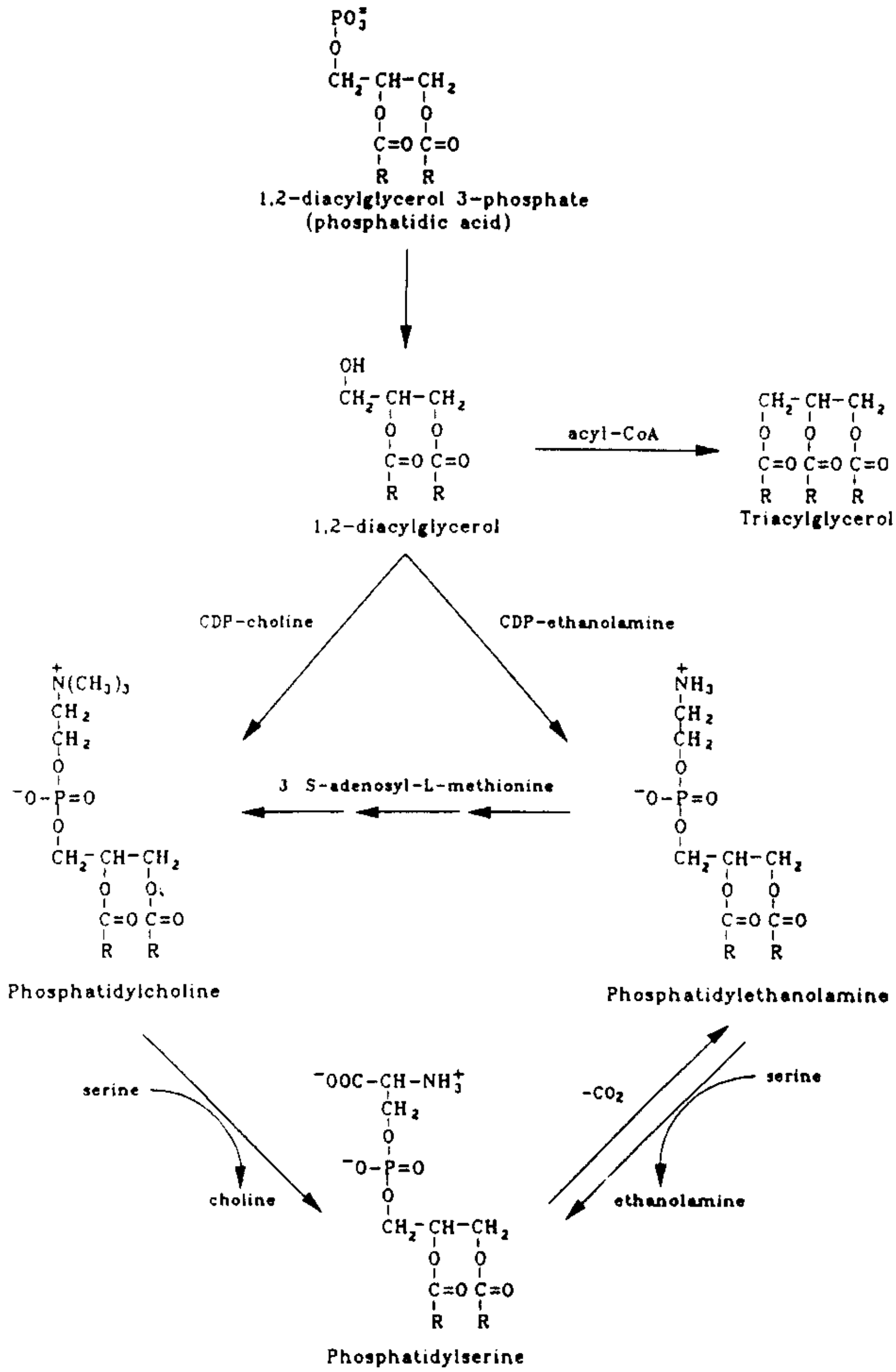


B



n	R
1	$(CH_2)_{11}CH_3$
2	$(CH_2)_{12}CH_3$

Phospholipid biosynthesis



From: K. J. Longmuir, in Phospholipids Handbook (G. Cevc, Ed.), 1993, Marcel Dekker, New York.

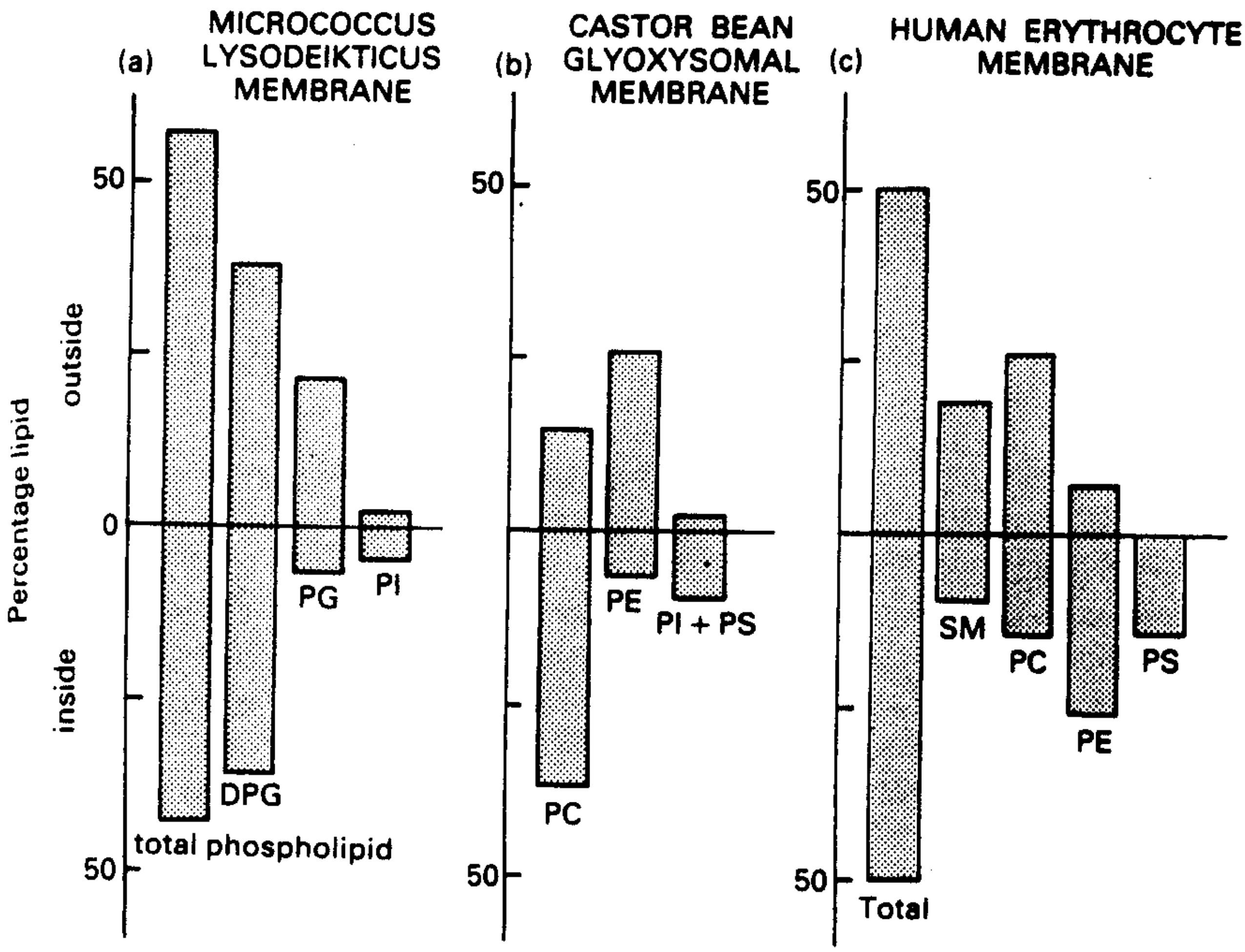
Lipid compositions of membranes

	Membrane (% total lipid)				
	Chloroplast (spinach)	*Protoplast (B. megaterium)*	*Mitochondrion (rat)*	*Erythrocyte (rat)*	*Myelin (rat)*
Lipid: protein	1 : 1	1 : 3	1 : 3	1 : 3	3 : 1
Phospholipid	12	48	90	61	41
PC	tr	0	40	34	12
PE + PI + PS	tr	19	41	11	26
PG	12	26	—	—	—
DPG	—	3	7	—	—
SPH	—	—	2	16	3
Glycolipid	80	52	–	11	42
MGDG	41	—			
DGDG	23	—			
SQDG	16	—			
Sterol. sterol ester	tr	0	tr	28	17
Acylglycerols	—	—	10		—
Pigments	8	—	—		—

PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PS = phosphatidyl serine; PG = phosphatidyl glycerol; SPH = sphingomyelin, MGDG = monogalactosyldiacylglycerol; DGDG = digalactosyldiacylglycerol, SQDG = sulpholipid, DPG – diphosphatidylglycerol.

From: M.I. Gurr and J.L. Harwood, Lipid Biochemistry, 1991, Chapman and Hall, London.

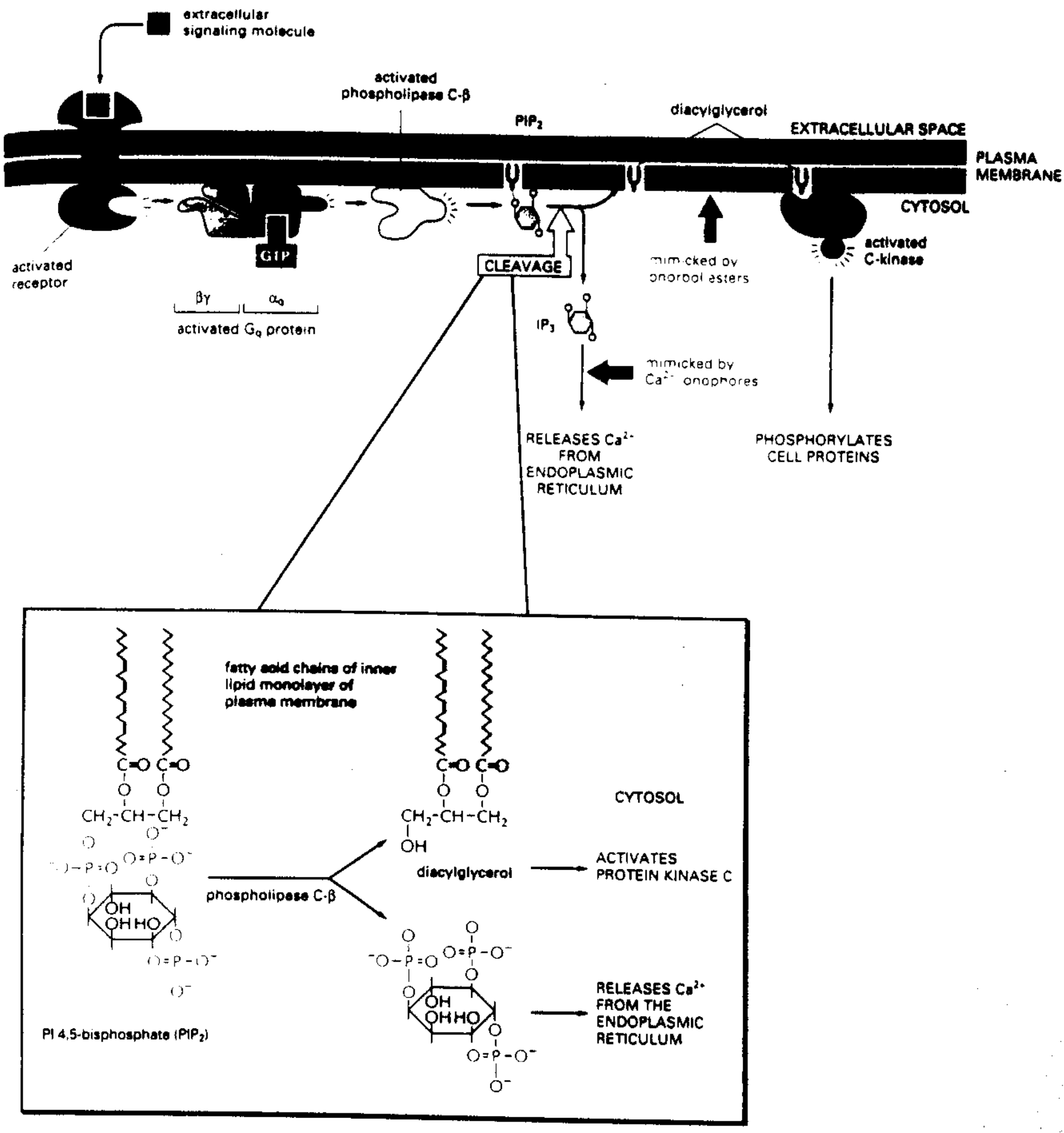
Asymmetry of Membrane Lipids



DPG: Diphosphatidylglycerol
PG: Phosphatidylglycerol
PI: Phosphatidylinositol
PC: Phosphatidylcholine
PE: Phosphatidylethanolamine
PS: Phosphatidylserine
SM: Sphingomyelin

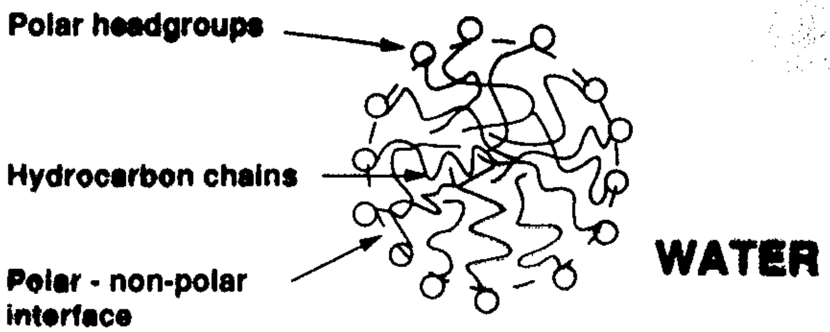
From: M.I. Gurr and J.L. Harwood, Lipid Biochemistry, 1991, Chapman and Hall, London.

Inositol phospholipids in cell signalling

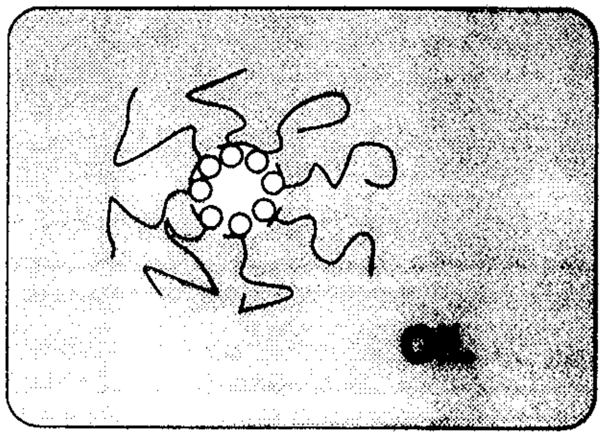


From: B. Alberts et al., 1994, *Molecular Biology of the Cell*, 3rd Edn, Garland, Publishing Inc, New York.

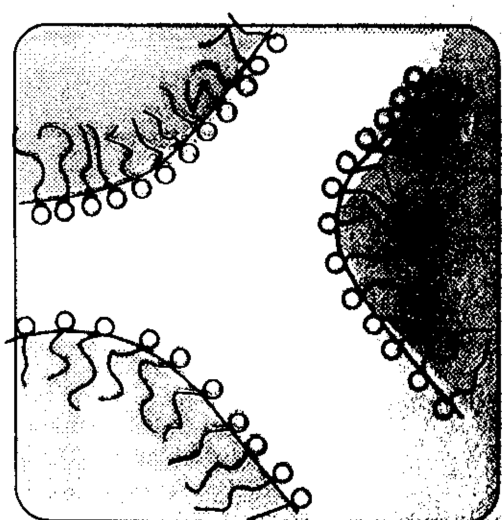
MICELLE



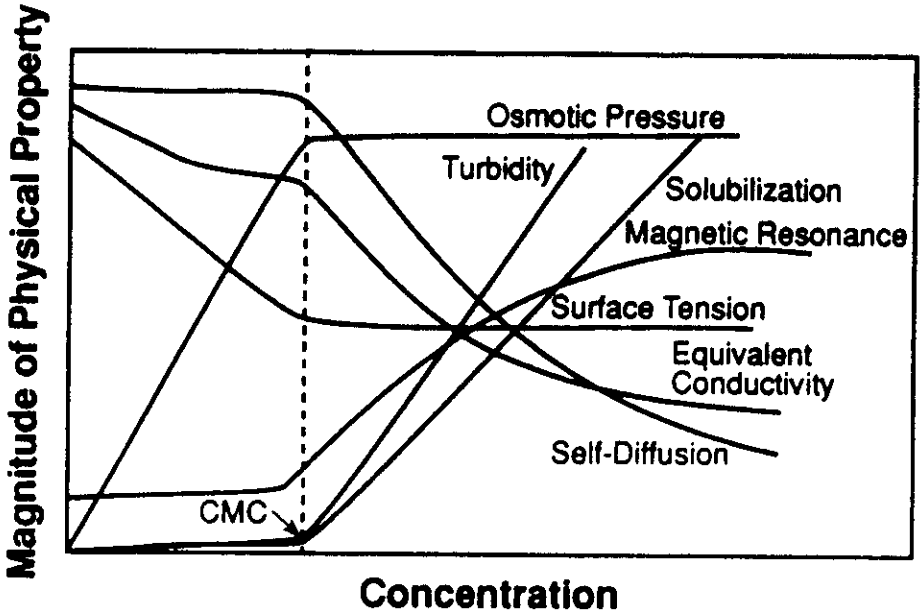
REVERSE MICELLE



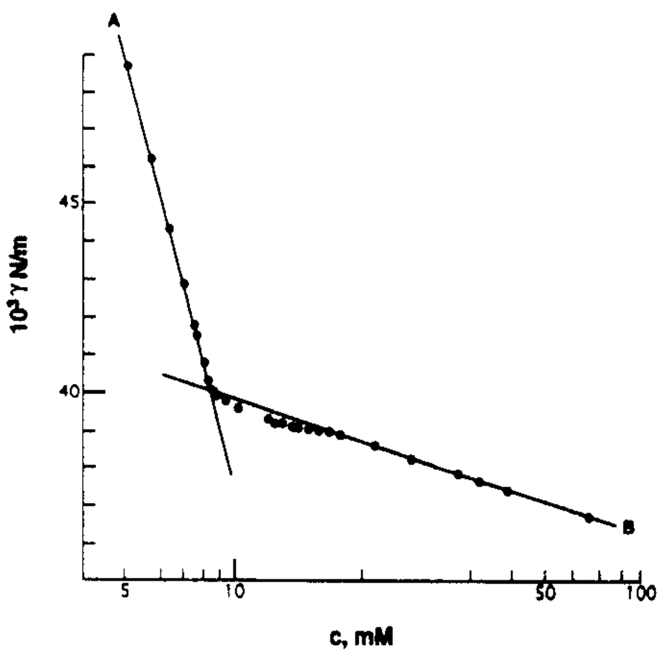
BICONTINUOUS MICROEMULSION



Experimental determination of the critical micelle concentration (cmc)

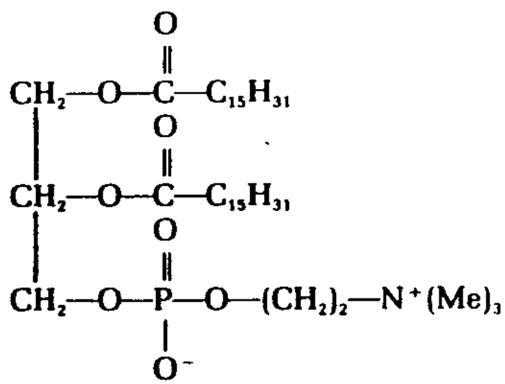


Surface tension versus concentration for SDS

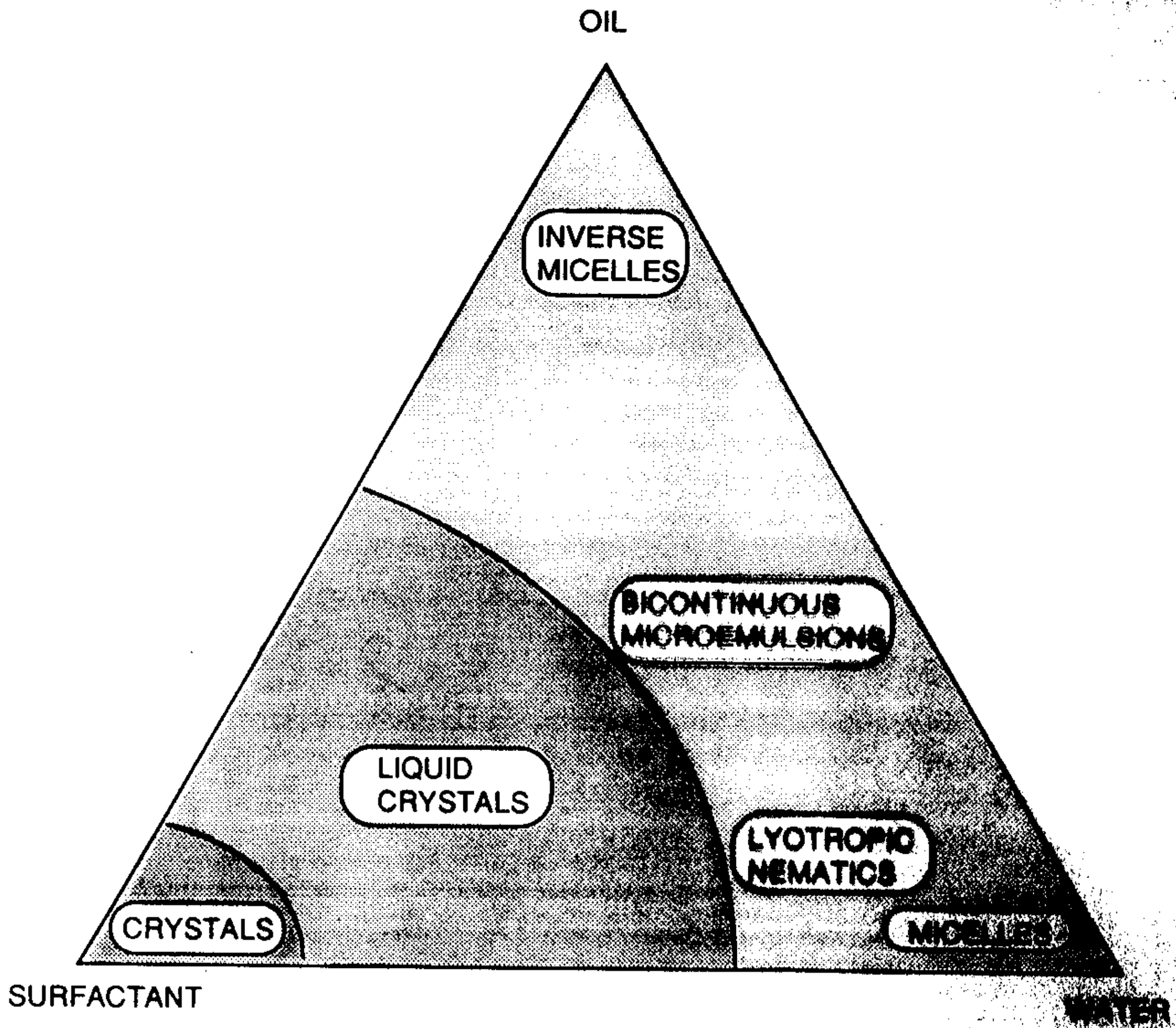


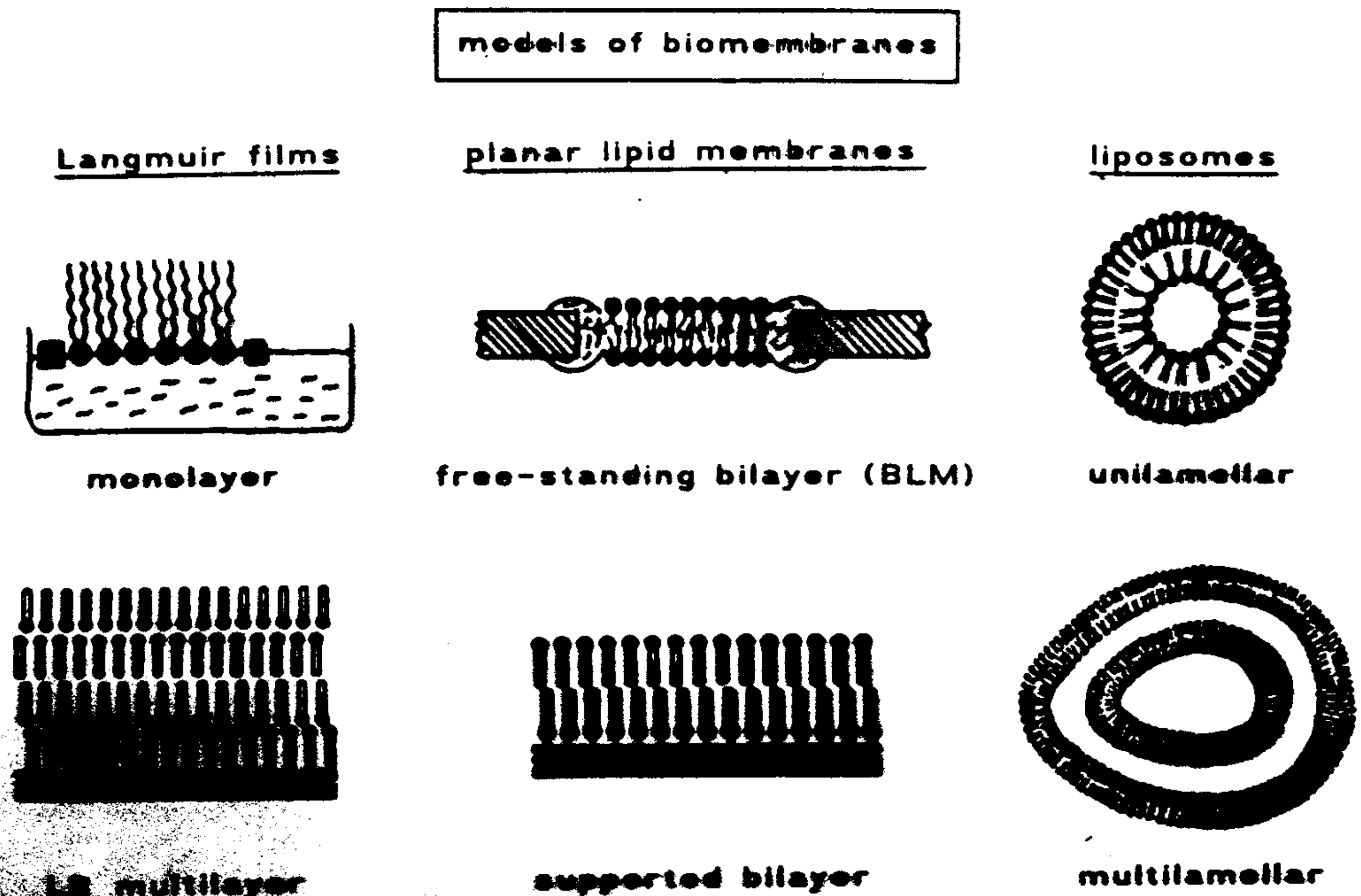
From: D. Fennel Evans and H. Wennerström, 1994, *The Colloidal Domain*, VCH, Weinheim.

cmc values for surfactants and lipids

Structure	Amphiphile	Critical Micelle Concentration (*M*)	Temperature (°C)
$C_{12}H_{25}OSO_3Na$	Sodium dodecyl sulfate	8.1×10^{-3}	25
$C_{12}H_{25}CO_2Na$	Sodium dodecanoate	2.44×10^{-3}	25
$C_{12}H_{25}N(CH_3)_3Br$	Dodecyltrimethylammonium bromide	1.5×10^{-2}	25
$C_{12}H_{25}NH_3Br$	Dodecylammonium bromide	1.2×10^{-2}	30
$C_{12}H_{25}N(CH_3)_2(CH_2)_3SO_3$	Dodecyldimethyl propane sultaine	3.57×10^{-3}	30
	Dipalmitoyl phosphatidylcholine	5×10^{-10}	41
$C_{12}H_{25}(OCH_2CH_2)_6OH$	Hexaethylene glycol dodecylether	8.7×10^{-5}	25
$C_{10}H_{21}N(CH_3)_2O$	Decyldimethylamine oxide	1.9×10^{-2}	25

From: D. Fennell Evans and H. Wennerström, 1994, *The Colloidal Domain*, VCH, Weinheim.


OIL
INVERSE MICELLES
BICONTINUOUS MICROEMULSIONS
LIQUID CRYSTALS
LYOTROPIC NEMATICS
CRYSTALS
MICELLES
SURFACTANT
WATER


models of biomembranes
Langmuir films
planar lipid membranes
liposomes
monolayer
free-standing bilayer (BLM)
unilamellar
LB multilayer
supported bilayer
multilamellar

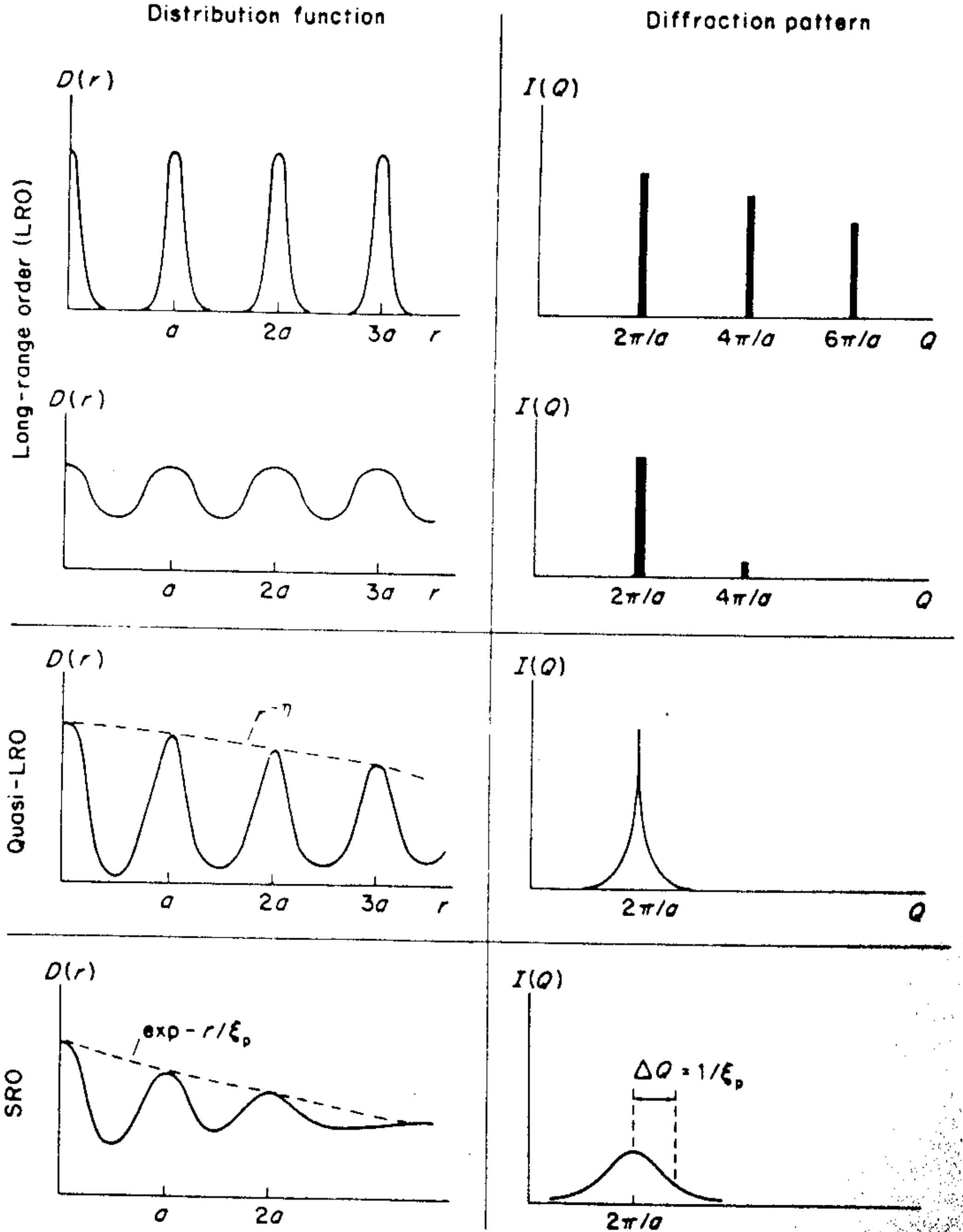
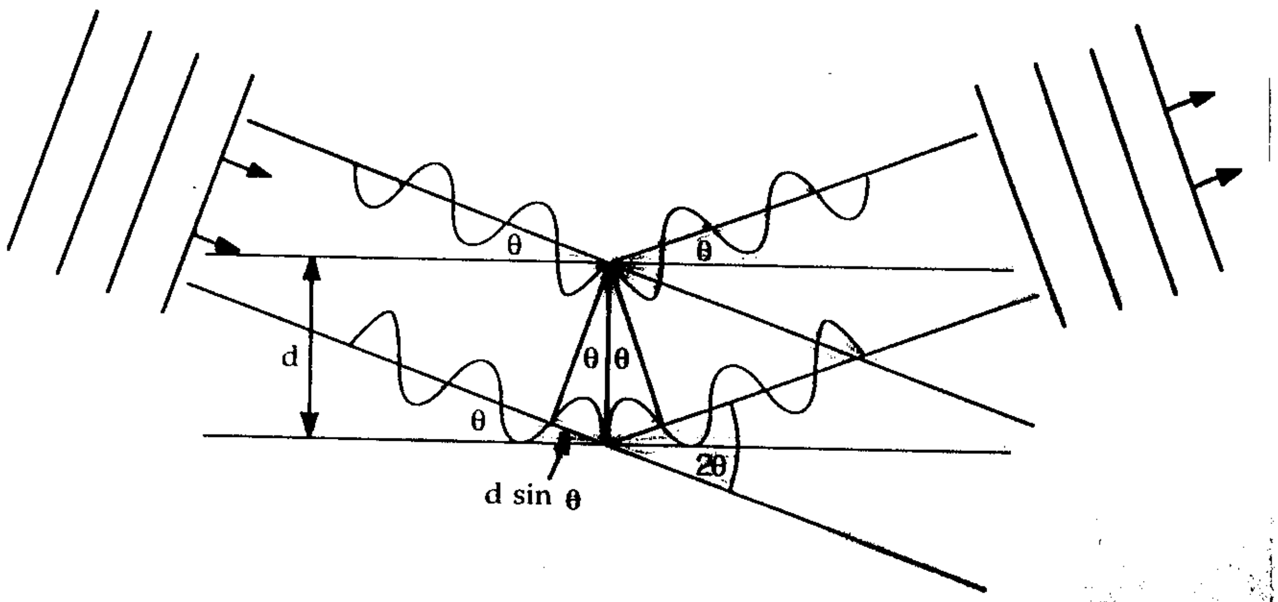
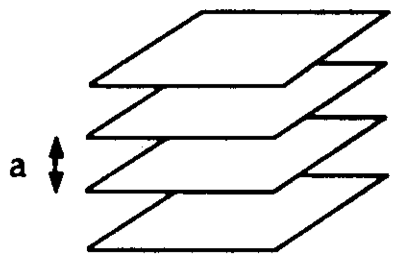
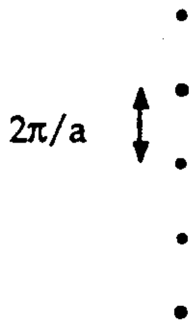
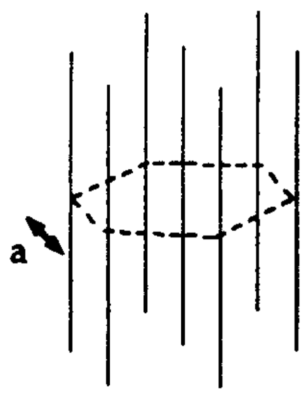
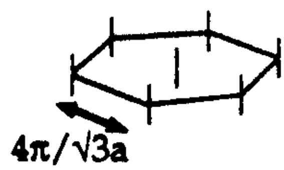
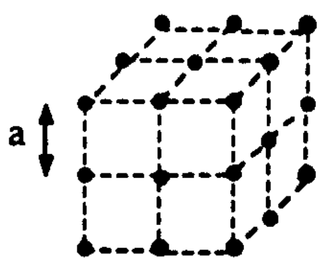
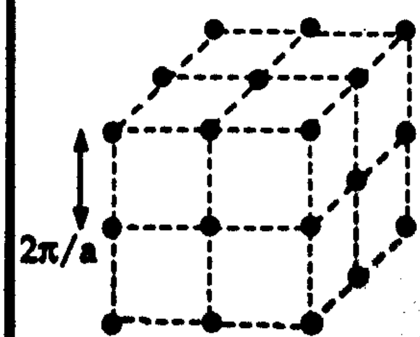


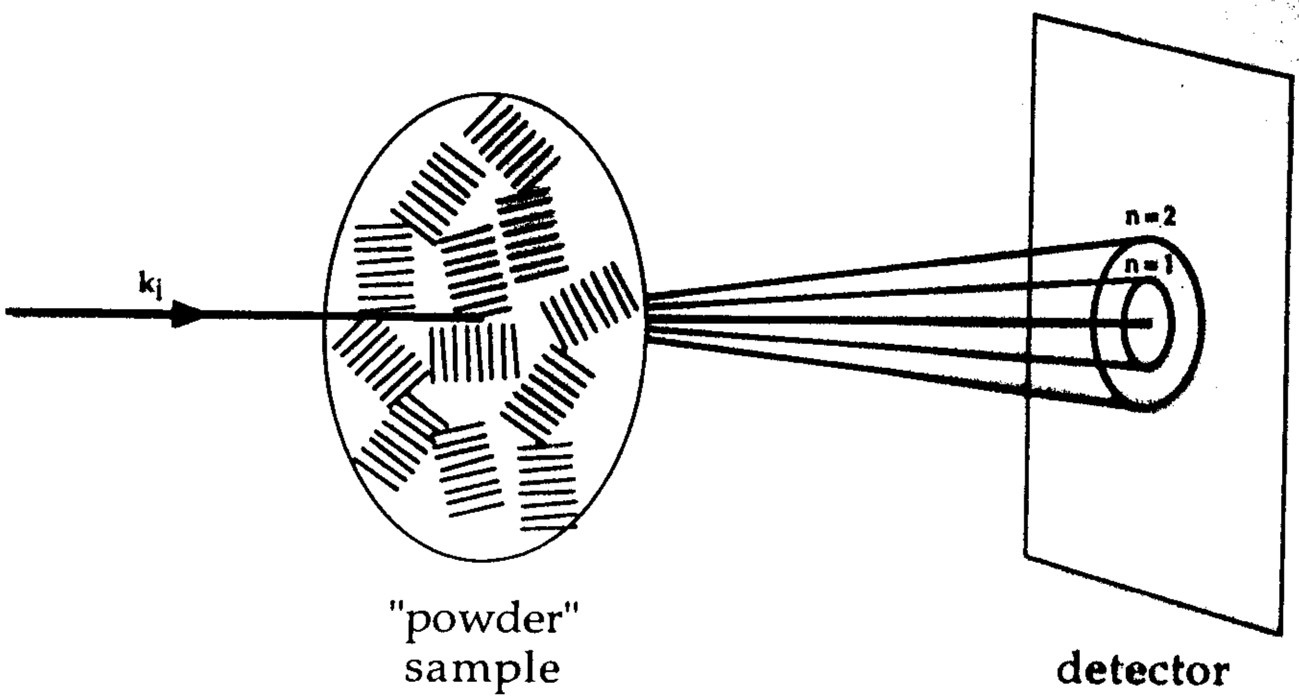
Figure 1.3 Molecular distribution functions and corresponding diffraction patterns for different kinds of positional order.

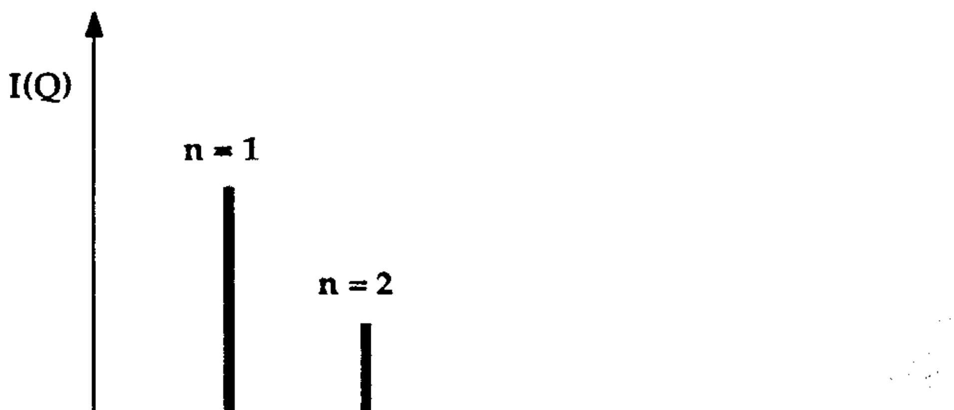
BRAGG'S LAW

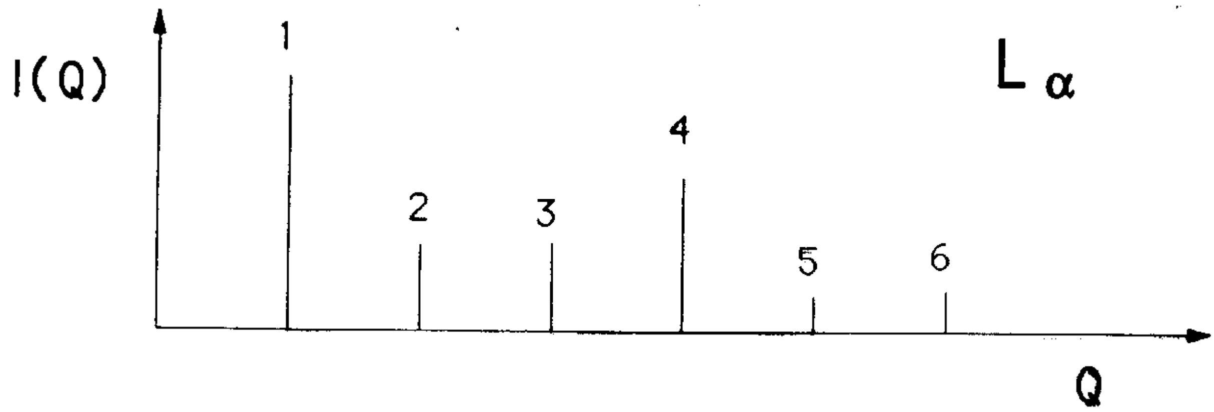


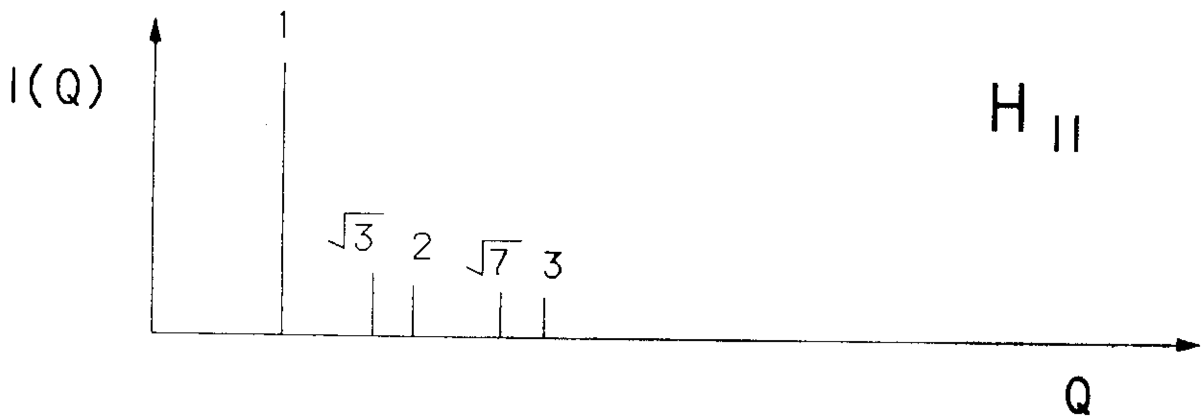
$$2d\sin\Theta = n\lambda$$

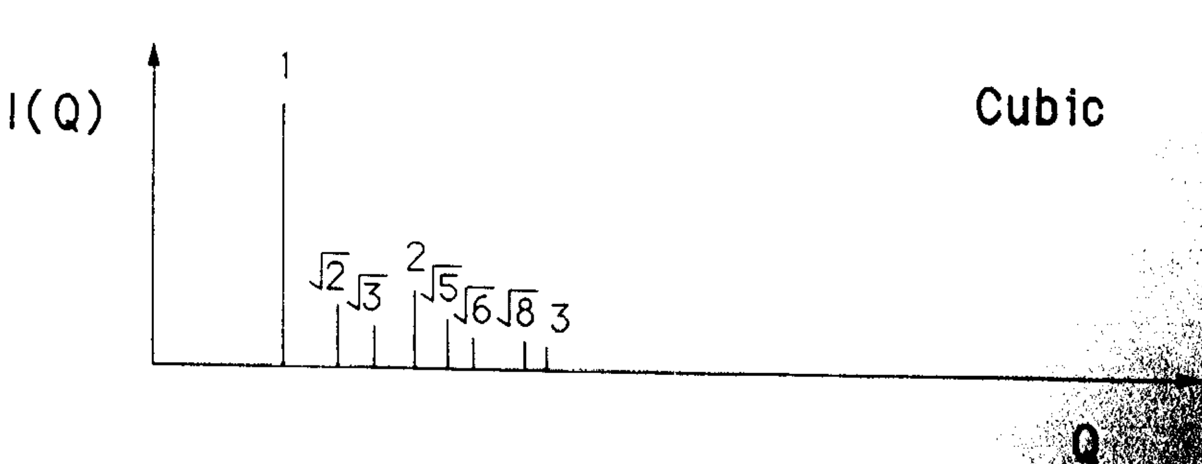
	REAL SPACE $\rho(\underline{r})$	RECIPROCAL SPACE $F(\underline{Q})$
1-D		
2-D		
3-D		


k_i
n = 2
n = 1
"powder"
sample
detector


I(Q)
n = 1
n = 2
Q = 4π sinθ / λ


I(Q)
1
2
3
4
5
6
L α
Q


I(Q)
1
√3
2
√7
3
H II
Q

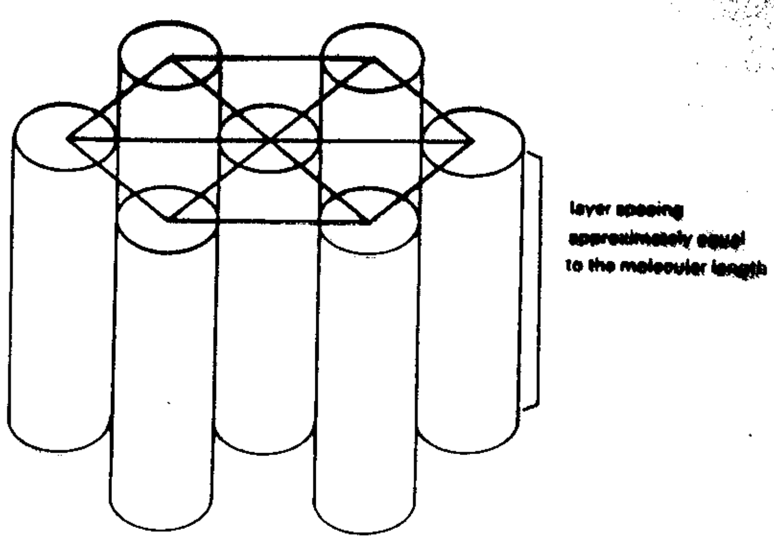

I(Q)
1
√2
√3
2
√5
√6
√8
3
Cubic
Q

Spacings formulae

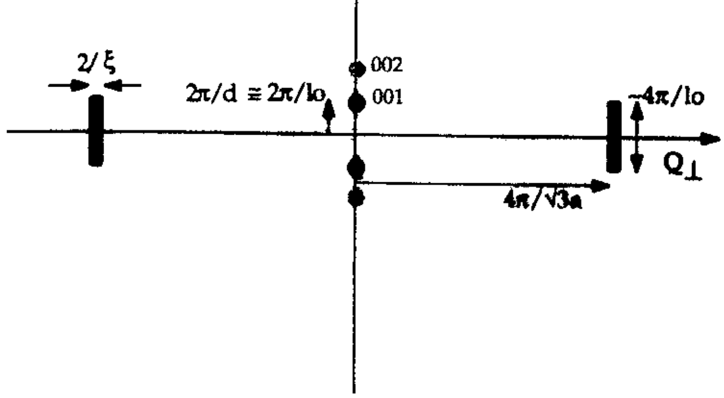
Phase	Reciprocal spacings	Ratios
Lamellar	$s_l = 1/d$	1,2,3,4...
Hexagonal	$s_{hk} = 2(h^2+k^2-hk)^{1/2} / \sqrt{3}a$	$1, \sqrt{3}, 2, \sqrt{7}, 3, \sqrt{12}, \sqrt{13}$..
Cubic	$s_{hkl} = (h^2+k^2+l^2)^{1/2} / a$	$1, \sqrt{2}, \sqrt{3}, 2, \sqrt{5}, \sqrt{6}, \sqrt{8}, 3$..

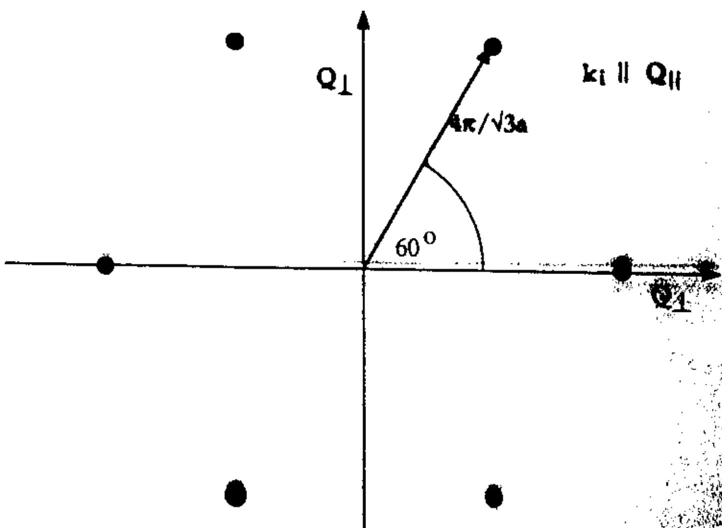
Cubic spacegroups: allowed reflections

Pm3n (Q^{223})	Pn3m (Q^{224})	Fd3m (Q^{227})	Im3m (Q^{229})	Ia3d (Q^{230})	RATIO
					1
110	110		110		$\sqrt{2}$
	111	111			$\sqrt{3}$
200	200		200		2
210					$\sqrt{5}$
211	211		211	211	$\sqrt{6}$
220	220	220	220	220	$\sqrt{8}$
	221				3
310	310		310		$\sqrt{10}$
	311	311			$\sqrt{11}$
222	222	222	222		$\sqrt{12}$
320					$\sqrt{13}$
321	321		321	321	$\sqrt{14}$
400	400	400	400	400	4


layer spacing
approximately equal
to the molecular length

S_B


Q||
k_i ⊥ Q||
2/ξ
2π/d ≅ 2π/lo
002
001
4π/lo
Q⊥
4π/√3a


Q⊥
k_i || Q||
4π/√3a
60°
Q⊥