CELL SURFACE SCIENCE IN MEDICINE AND PATHOLOGY

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The principal object of natural philosophy is not the material elements and their composition but the totality of the form, independently of which they have no existence.

Aristotle. *De partibus animalium* Book 1, *circa* 384–322 BC

Introduction: How to read *Cell Surface Science in Medicine and Pathology*

This book is an integrated work that uses the basic sciences of biochemistry, pharmacology and immunology to explain how research effort is progressing in important areas of clinical research. The clinician or pathologist may find interest in reading first about endotoxaemia, rheumatoid arthritis, diabetic vascular disease, hypertension, cancer or the leukaemias, and then they can delve into the background that is given in the introductory chapters. Conversely, pharmacologists could start with Chapter 3 because they will then learn how understanding of receptors applies to the cells of the immune system. Biochemists should start with Chapters 1 and 5 because this is not exactly traditional biochemistry. Immunologists will be pleased to find that they have an interest in almost every chapter, but they should note that immunology in this text is seen in the context of microbiology and the host body defences.

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Newcastle NE3 3DE

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1
The cell membrane: structure and constituents

STRUCTURE OF THE LIPID BILAYER

In the 1930s J.F. Danielli proposed that a cell membrane is made up of two layers of phospholipids so forming a sandwich that has a hydrophobic centre. In the lipid bilayer the cholesterol : phospholipid ratio is 1 : 1. The accompanying proteins are attached to the polar head groupings of the phospholipids on the inner and the outer sides. The result is a membrane 7 nm thick, which has a high electrical resistance, and which is impermeable to ions and other polar molecules. Thus the lipid membrane is a 'phase' or zone of stability that separates aqueous zones outside or within the cell where chemical reactions occur.

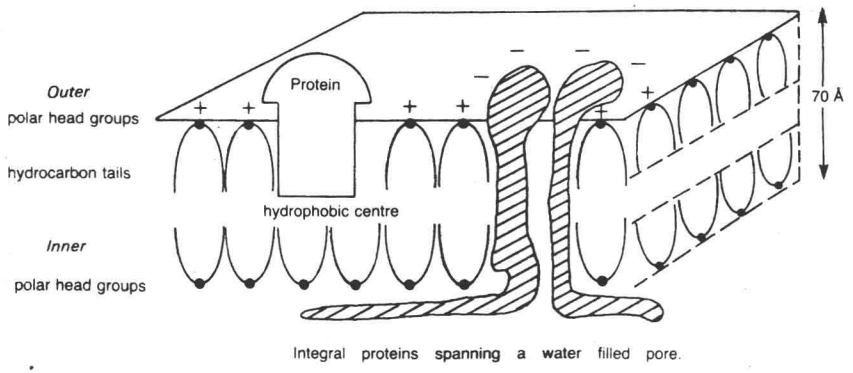


Figure 1 A cell-membrane lipid bilayer with protein inserts

Clearly one has to understand the phospholipids[2], otherwise known as 'glycerophosphatides', and their constituents. Each consists of a glycerol molecule to which are attached fatty acids at positions 1 and 2, and at position 3 there is a phosphate group to which a nitrogenous base is attached (Figure 2). If this is choline, then the phospholipid is phosphatidycholine (PC) otherwise known as lecithin. PC forms the 'surfactant' that lines the surface of the alveoli of the lungs and lowers the surface tension so that they do not collapse.

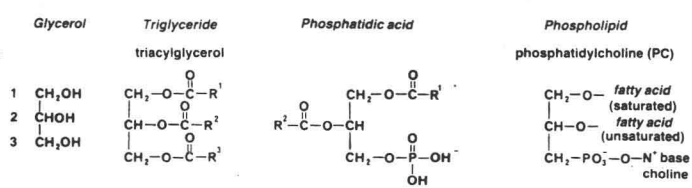


Figure 2 Phospholipid structure

The fatty acid at position 1 of the phospholipid is usually saturated, whilst that at position 2 is arachidonate which is unsaturated (C20 : n4 or C20 : ω4). The acyl chains account for most of the molecular motion. For example, there are rotations about single bonds and there is a swinging motion. The phospholipid molecules can 'translate' sidewards in the plane of the bilayer, and occasionally they can 'flip-flop' from one layer to another (Figure 29).

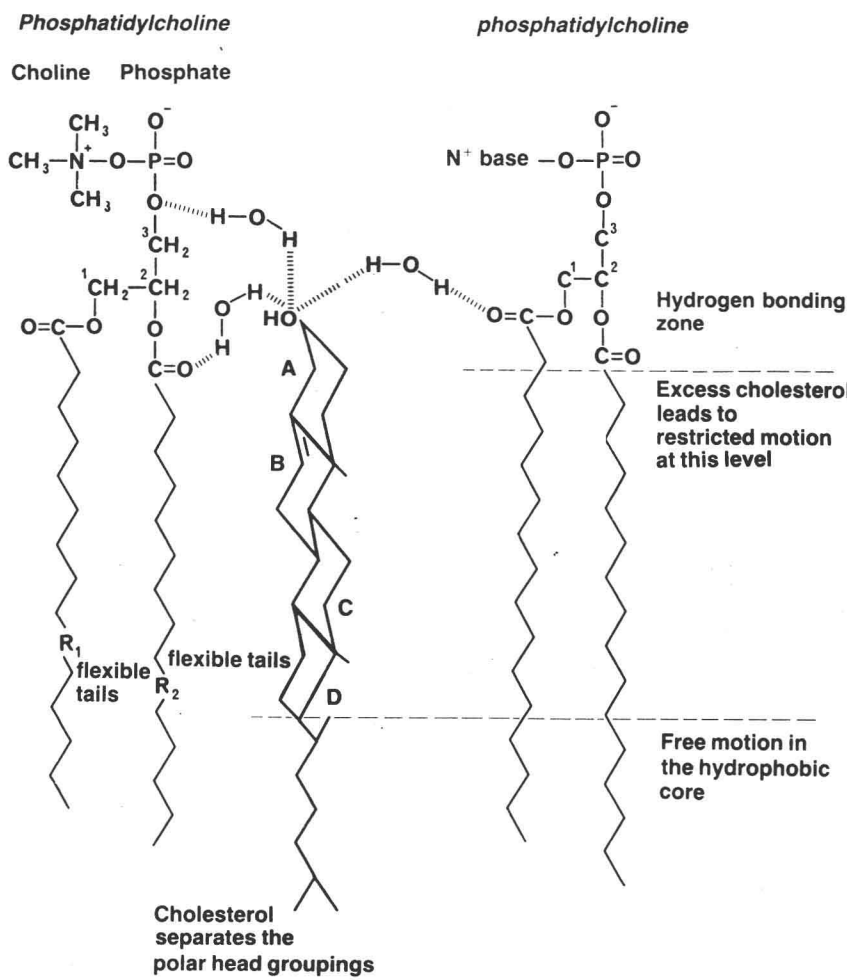


Figure 3 Lipid monolayer insertion of cholesterol

In 1925 Leathes first noted that cholesterol has a 'condensing effect' on phospholipid monolayers at an air–water interface. Due to an increase of local van der Waal's forces it increases the efficiency of packing of the fatty acyl chains near the surface, but inside it actually allows a greater freedom of rotation of the fatty acid tails. Thus there is a 100–200 fold gradient of restriction of movement, expressed as 'microviscosity', from the highly ordered polar head groups and glycerol backbones that are scarcely free to move to the fluid centre of the bilayer where the fatty acid tails wave around. These motions can be demonstrated by means of ^{13}C nuclear magnetic resonance (NMR), by electron spin resonance (esr) fatty acid probes and by fluorescent probes.

The probes also reveal that at physiological temperatures the membrane lipids segregate into 'domains'. Where the fatty acyl chains are tightly packed the lipid is in gel form, and where they are free to move the lipid is liquid. Proteins can be inserted into either form of domain.

Cholesterol also renders the phospholipid bilayer less permeable to water, glucose and other hydrophilic molecules. The impediment to diffusion might depend on the 'condensing effect' but more likely it depends on the presence of a 'hydrogen belt' within the membrane (cf. Anaesthesia, page 48, and Figure 46). In fact the presence of cholesterol increases the hydration of the polar head groups so that water becomes bound and accounts for 20% by weight of the membrane. Unsaturated phospholipids are more hydrated than those that are saturated. Phosphatidylethanolamine binds less water than phosphatidylcholine, whilst phosphatidylserine binds none.

Intercellular junctions (Figure 4)

Only recently has the nature of intercellular junctions been clarified[3]. Between cells there is a transition between the lipid bilayer and cylindrical micelles. In them the lipids are packed tightly into cylinders with the hydrocarbon chains facing outwards. They are thus lipids of a cylindrical hexagonal II phase[4].

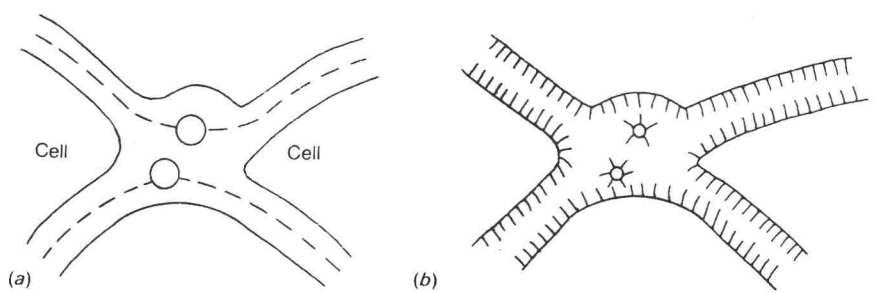


Figure 4 Structure of the intercellular junctions

CELL MEMBRANE LIPIDS

Figure 5 indicates the structure of the various phospholipids, and Figure 7 depicts the glycosphingolipids which contain the base sphingosine instead of glycerol.

Phospholipids (glycerophosphatides)

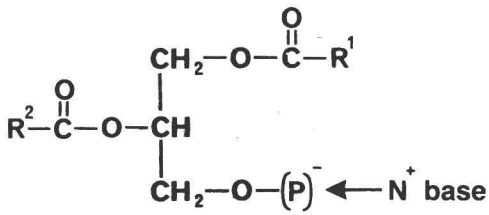
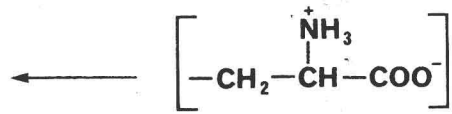


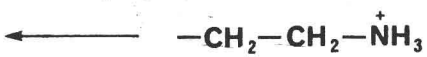
Figure 5 The phospholipids (glycerophosphatides)

Individuality depends on the bases which are as follows:

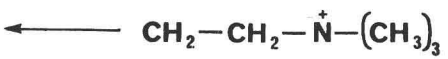
(1) Phosphatidylserine (PS)



(2) Phosphatidylethanolamine (PE)

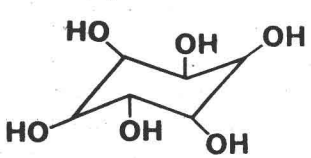


(3) Phosphatidylcholine (PC)



(4) Phosphatidic acid, i.e. phosphatidylglycerol. In this case there is no nitrogen base at position 3.

(5) Phosphatidylinositol (PI), called 'myoinositol', based on inositol.



(6) Cardiolipin. This is a 1,3-bis-phosphatidylglycerol that occurs in mitochondrial membranes (phosphatidic acid-glycerol-phosphatidic acid).

The complexity of the phosphoinositides is determined by the amount of phosphorylation, as indicated in Figure 6. The phosphate groups turn over rapidly, and the particular physiological role will become apparent on page 91. Phosphatidylinositides are synthesized by the transfer of a phosphatidyl group from CDP-diglycerides to free inositol (Figure 10).

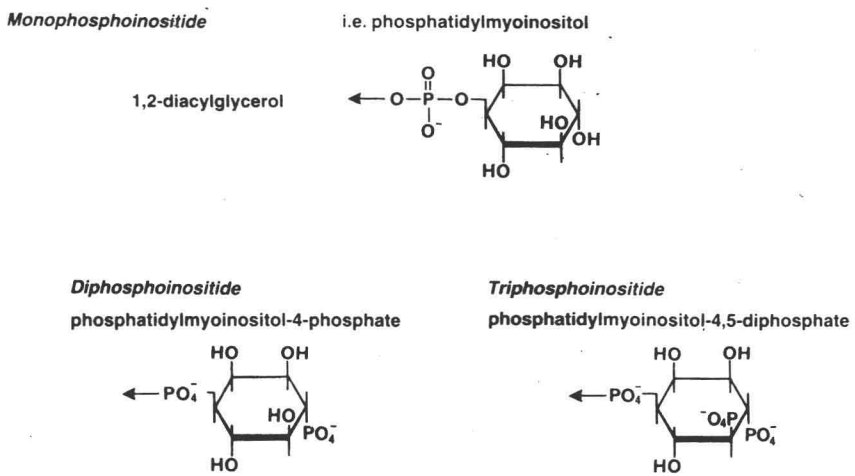
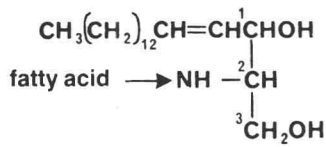


Figure 6 The phosphoinositides

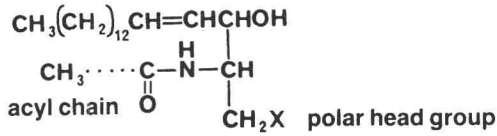
Sphingolipids (Glycosphingolipids)

The second major class of cell membrane lipids is derived from sphingosine. Figure 7 shows that its structure is also based on the glycerol molecule. In *N*-acylsphingosine 1-penadecene forms the side-chain with an unsaturated bond attached at position 1, there is a fatty acid attached by an amide bond at position 2, and there is a polar group *X* attached at position 3. *X* may be phosphorylcholine (in sphingomyelin), a simple sugar (glucocerebroside or galactocerebroside) or its derivative as in sulphatides, or finally a complex polysaccharide that can be neutral or acidic (as in gangliosides) (page 132).

***N*-Acylsphingosine**

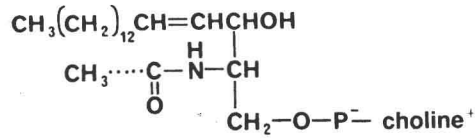


Ceramide



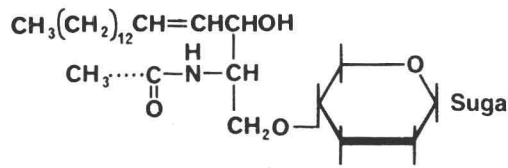
Sphingomyelins

e.g. ceramide P-choline and ceramide P-ethanolamine



Cerebrosides

e.g. lactosylceramide (see Figure 102)



Gangliosides

***N*-acylsphingosine**

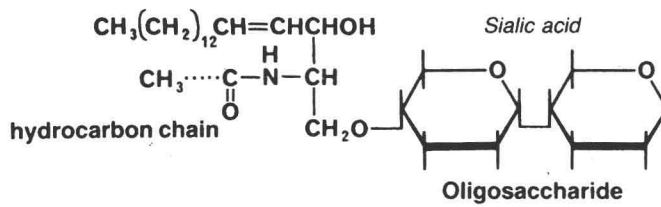


Figure 7 Structure of the glycosphingolipids

The various phospholipids are not evenly distributed between the inner and outer layers of the cell membrane but asymmetrically, as is shown for the erythrocyte in figure 8 (see also Table 12, page 97). Since these

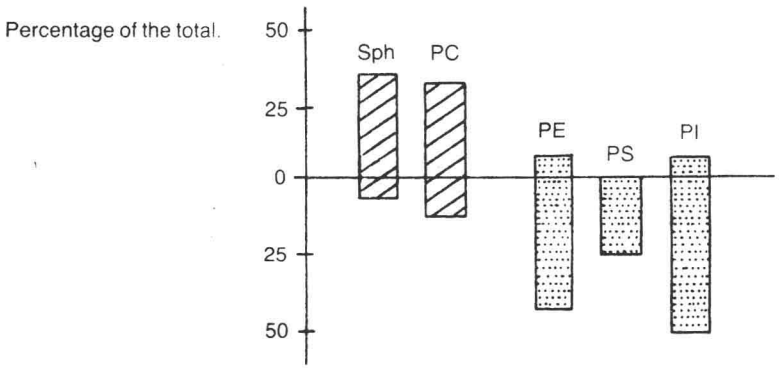


Figure 8 Asymmetric distribution of phospholipids in the erythrocyte. Outer layer: phosphatidylcholine (PC), sphingomyelin (Sph). Inner layer: phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) Increase of Sph : PC increases membrane rigidity, whilst increase of PC : Sph increases membrane fluidity

phospholipids contain arachidonic acid and inositol that are liberated for important intracellular reactions by the action of membrane phospholipases, the actions of these particular enzymes are indicated in Figure 9.

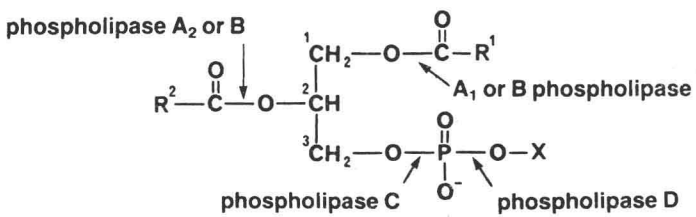


Figure 9 The phospholipases

It will also be useful at this stage to have a reminder of the biosynthetic scheme of the phospholipid molecules (Figure 10).

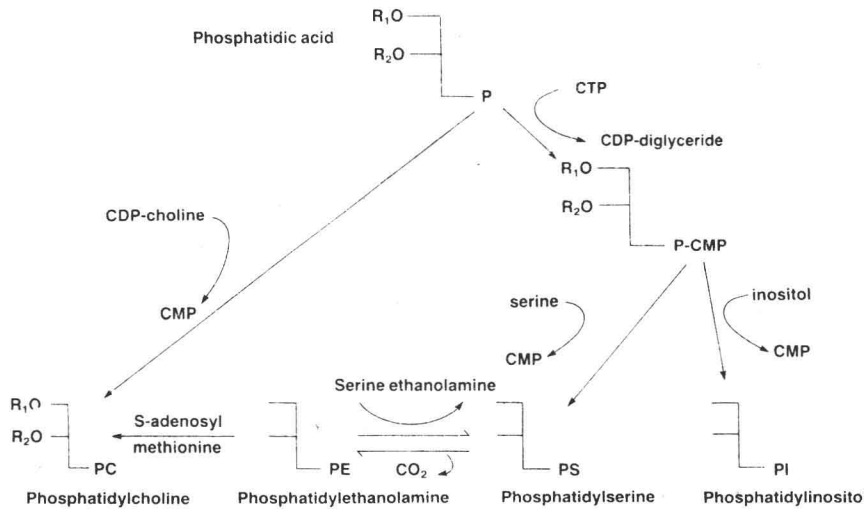


Figure 10 Biosynthesis of the phospholipids (Cf. Figure 75, page 96)

Cell membrane characteristics

The cell membrane consists of a bilayer of phospholipids with two types of protein attached. 'Extrinsic' or peripheral proteins are bound to the membrane surfaces by polar interactions, directly perpendicular to the plane of the bilayer, whilst 'intrinsic' or integral proteins span the whole thickness of the membrane and are anchored beneath inside the cell. The latter proteins are bound to lipids by hydrophobic interactions parallel to the plane of the lipid bilayer. Hence solubilization of integral proteins requires strong membrane disrupting agents. There is commonly a 1 : 1 ratio of protein to lipid in membranes, but the value can vary greatly, e.g. from 0.25 in myelin to 3.0 in bacterial membranes. Table 1 gives some illustrative data.

Table 1 Chemical composition of membranes (as % total dry weight)

Compound	*Erythrocyte*	*Myelin*	*Mitochondrion*	*Bacterial*
Protein	60	22	76	75
Total lipid	40	78	24	25
Total phospholipid	24	33	22	25
Cholesterol	9	17	0.2	–
Glycolipid	0.1	22	0.05	–

One example, which will later come in for much attention, is the platelet whose membranes contain 25% protein, 70% phospholipid and 5% glycolipid. The bilayer is coated on its outer surface by PAS-staining glycoproteins, and it is the sialic acid of this outer layer that gives the normal negative charge to the cell surface. The membrane phospholipids of the platelet are phosphatidylcholine (37%), phosphatidylethanolamine

(27%), sphingomyelin (17%), phosphatidylserine (10%) and phosphatidylinositol (5%).

On account of molecular motion the lipids can be considered to be in a fluid state. This has considerable relevance when one thinks of the processes of phagocytosis and secretion, of membrane biogenesis, cell fusion and intercellular junction formation. X-ray diffraction studies and other techniques have shown that lipid bilayers undergo 'phase transitions' with changes of temperature. At a temperature below the 'transition' the fatty acid side-chains of the phospholipids are in a relatively rigid crystalline state. At a temperature above the 'transition' these side-chains acquire more fluid random motion. Just as energy is required to melt ice, so energy has to be absorbed by a membrane to 'melt' the ordered array of the fatty acid chains. Above 15–20 °C the packing of the fatty acid side-chains is loosened, and there is greater freedom of movement of the hydrocarbon tails. The temperature at which phase transition occurs depends on the length of the fatty acid side-chains and on their degree of saturation. Long and saturated hydrocarbon chains will result in a higher transition temperature. Saturated chains can be packed so tightly that the viscosity of the membrane is high, whilst the unsaturated fatty acids that have numerous mobile double bonds spread themselves out, so that there is mobility of the chains and a fluid membrane (Figure 11)[1]. The fatty acid composition of neutral lipids

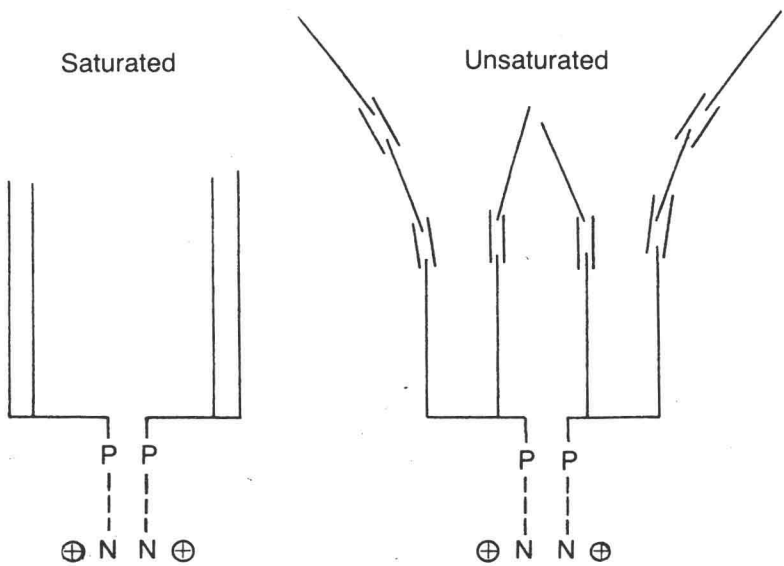


Figure 11 Mobility of saturated and unsaturated fatty acids

really shows little or no variation between different organs or in different species. However, the fatty acid composition of the phospholipids varies considerably between individual organs.

Figure 12 shows that the principal fatty acids are C_{16} (palmitic) and $C_{18:1}$ (oleic) in the neutral fats; and C_{16} (palmitic), C_{18} (stearic), $C_{18:1}$ (oleic) and $C_{18:2}$ (linoleic) in the phospholipids. Arachidonic acid ($C_{20:4}$) is an important constituent of the phospholipids for it is used as substrate for prostaglandin synthesis.

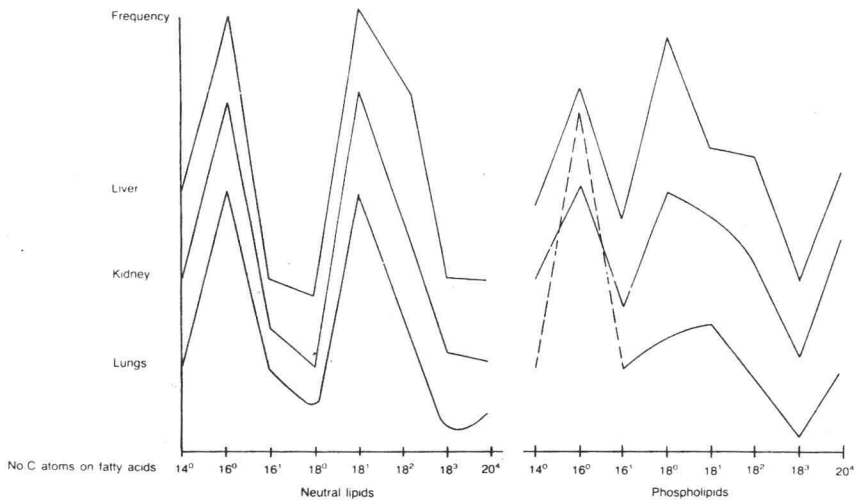


Figure 12 Fatty acid chain length and unsaturation in rat organs (Adapted from reference 5, with permission.)

The ratio of unsaturated : saturated fatty acids in a membrane correlates with the activity of membrane-bound enzymes. It is measured as the 'double bond index'/saturated fatty acid ratio[6]. Thus the Hill coefficient for inhibition of Mg-ATPase by means of fluoride shows a close correlation with this ratio. Studies have shown that the double bond index/saturated fatty acid ratio is raised in the liver of diabetic subjects[7] but is low in alcoholic cirrhosis on account of deficiency of linoleate and linolenate[7].

The subject of lipids and membrane fluidity is so important that vast works have been written[8–10]. It can, for example, explain the action of many drugs on cells. At the 'transition temperature' there is a phase change of the glycerophospholipids and sphingolipids from the gel state, in which there is crystalline order of the molecules, to a liquid/crystalline

state. At that temperature there will be a marked change in the bilayer viscosity. Changes of microviscosity of synthetic liposomes made of different lecithins, as determined by DPH depolarization studies, is plotted against the reciprocal of the absolute temperature in Figure 13.

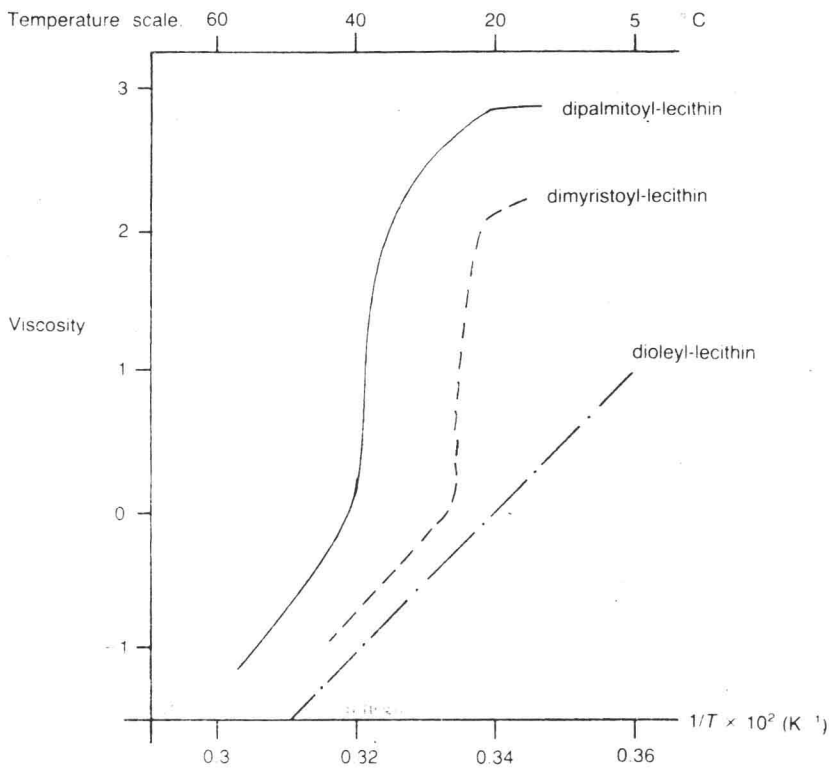


Figure 13 Apparent microviscosity and reciprocal of the absolute temperature. Note the abrupt change at the transition temperature. Dimyristoyl-lecithin is akin to erythrocyte membrane

Note the 10-fold change in microviscosity at the gel to liquid phase transition.

The temperature at which the transition occurs depends in part on the hydration of the lipid. In the anhydrous state the temperature is higher, whereas it is lowered by water. The transition also increases as the chain length of the fatty acids increases, but is lowered when there is unsaturation and branching of the hydrocarbon chains. In fact for naturally occurring membrane lipids the transition can vary from 50 °C down to 0 °C. *Cis*-unsaturated double bonds prevent close hexagonal packing of the phospholipids, as do branched methyl or hydroxyl groupings.