The Institute of Biology's
Studies in Biology no. 27

The Membranes of Animal Cells

Second Edition

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General Preface to the Series

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1978

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Preface to the First Edition

Unfortunately for the student, the literature on membranes is already voluminous and not without its conflicts of opinion and duplication of terminology. The primary function of this booklet, therefore, is to provide an elementary account for the beginner shorn of at least some of the 'ifs' and 'buts'. Over-simplification will be obvious to the specialists but the author will have achieved his aim if the student is stimulated to extend his reading to more detailed accounts of the topic.

I am much indebted to Dr F. S. Billett for reading the draft of this booklet and for pointing out various errors.

Southampton, 1971

A. P. M. L.

Preface to the Second Edition

There is a calculation in Lull's *Organic Evolution* to the effect that if all the progeny of a single *Paramecium* were to survive and divide in their turn then, after 9000 generations (fifteen years) the mass of organisms 'would exceed the confines of the known universe and the rate of growth would be extending the circumference into space with the velocity of light'. The literature expansion on the topic of membranes in the seven years since this booklet was first published suggests that an almost equally daunting explosive growth is in progress. The extent of this research interest is to be welcomed since it reflects the recognition of the importance of membranes in almost every facet of cell function though it does have the unfortunate corollary that still greater selection and over-simplification is necessary in a basic summary text such as this.

Southampton, 1978

A. P. M. L.

Contents

1 Introduction

Biological theories, like sartorial fashions, not infrequently turn full circle. A hundred years ago the distinguished microscopist C. G. Ehrenberg claimed to be able to detect a complex series of internal organs within the cells of protozoa but his ideas were derided. The last thirty years have shown, however, that, though his views were over-extravagant, he was at least correct in supposing that cells have a highly organized internal structure composed of membrane-bounded vesicles.

Interest in these intracellular membranes has become increasingly intense with the realization that they do not just play a passive role in segregating different regions of the cell but that their functions embrace every facet of cell activity.

The multiple nature of their metabolic involvement and the complexity of their structure have made membranes the natural meeting point of the sciences with electron microscopists, physical chemists, biochemists and biophysicists all approaching them from different viewpoints. The results obtained by this concerted study have made it obvious that knowledge of the precise structure and functioning of different membranes will open the way not only to an understanding of what constitutes life at the molecular level but also to that vital selective control of cell and tissue function necessary in the treatment of cell malfunction and tissue transplants. The biologist therefore neglects a study of membranes at his peril.

The full complexity of the various roles of membranes has only recently been recognized but already it seems perhaps not too far reaching to suggest that the subject of membranes will prove to be as important a theme in the knowledge of how cells function as the concept of evolution has been to biology in general or as DNA has been to genetics.

Hundreds of chemical reactions and transfer processes are occurring continuously in every active cell, and membranes play a vital role in controlling these processes, in separating incompatible substances and in transporting materials about the cell. Some of the more complex chemical sequences are expedited because it appears that the enzymes which catalyse them are so arranged on membranes that the reactants can move readily from one to the next.

In a packaging role, intracellular membranes separate components of the cell which would be self-destructive if allowed to mix freely in the cytoplasm, they conserve and maintain regions of local concentration, regulate the passage of inorganic ions and complexes between compartments and provide the principal means for the trammelling, ordering and regulation of the metabolic processes which constitute life.

2 What and Where are the Membranes?

The advent of the electron microscope has greatly extended knowledge of intracellular membranes. Forty years ago the only membrane systems recognized with any degree of certainty were the *plasma membrane* separating the cytoplasm from the medium at the cell boundary, the *nuclear membrane* separating the nucleoplasm from the cytoplasm and an enigmatic system of vesicles, the *Golgi apparatus*, about whose reality controversy was rife. Various other cellular inclusions such as mitochondria, vacuoles and mitotic figures were known but that they were membranous structures remained to be established. Since then the number of intracellular organelles known to be associated with membranes has grown considerably. The most commonly represented

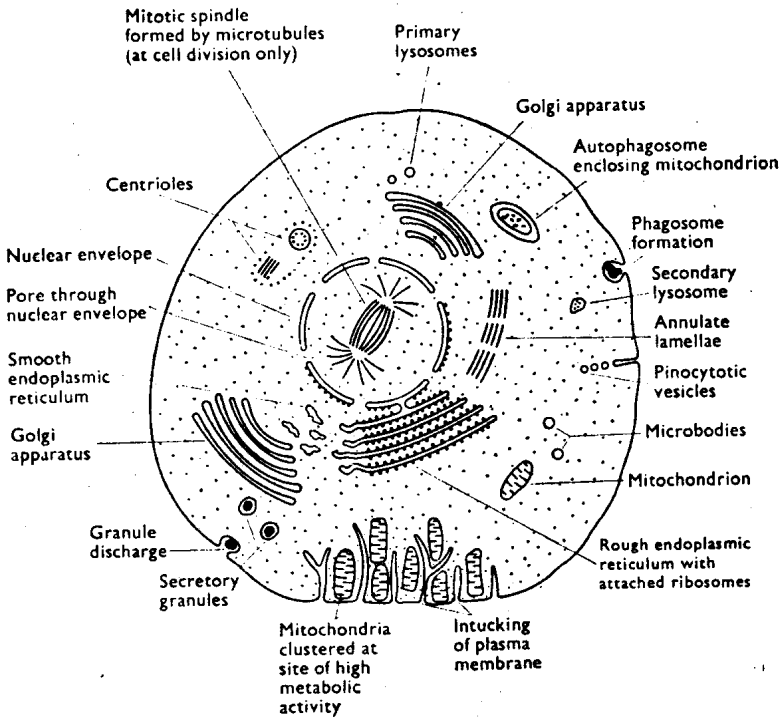


Fig. 2–1 Diagrammatic representation of the major membrane-bounded structures found in cells. N.B. Few, if any, cells will contain all these organelles simultaneously.

structures are listed in Table 1 and illustrated diagrammatically in Fig. 2–1. Their appearance in the electron microscope can be seen by reference to Figs 2–2, 2–3 and 2–4.

Not every type of cell has a full complement of the organelles outlined in Table 1 and the degree to which they are represented in different tissues

Table 1 Cell organelles and their primary functions

Plasma membrane	Diffusion barrier, active transport of sodium outwards, electrically excitable membrane. Principal enzymes include Na–K–Mg ATPase, glucose-6-phosphatase
Rough endoplasmic reticulum	Synthesis of protein
Smooth endoplasmic reticulum	Steroid metabolism, transport of materials from rough endoplasmic reticulum to Golgi apparatus
Golgi apparatus	Storage vesicles; 'packaging' of enzymes to be used elsewhere
Lysosomes	Vesicles containing enzymes used in autodigestion and breakdown of materials brought in by phagosomes and pinocytosis
Phagosomes	Vesicles, containing particulate material from outside the cell; formed by inversion of portions of the plasma membrane
Pinocytotic vesicles	Microscopic vesicles, formed by plasma membrane inversion, containing materials adsorbed onto the membrane
Multivesicular vesicles	Bodies containing smooth membranes. These belong to the lysosome family of organelles
Microtubules	Fine cylindrical structures which subserve a variety of roles in the cell
Microsomes	The name given to small fragments of cytomembrane, principally those of the endoplasmic reticulum and Golgi apparatus, produced as an artefact of homogenization and separated by centrifugation
Mitochondria	Membranous organelles responsible for the production of most of the energy donor substance, ATP, formed by cells
Microbodies	Vesicles containing the enzyme catalase and often involved in uric acid metabolism
Nuclear envelope	A two-layered membranous diffusion barrier between the cytoplasm and the nucleoplasm
Centrioles	A pair of self-replicating cylindrical bodies containing 11 parallel fibrils. They function in organizing the poles of the mitotic figure during cell division
Basal bodies	Structurally similar to centrioles, associated with the base of flagellae
Annulate lamellae	Present primarily in oocytes, function unclear
Microfibrils	Filaments, usually contractile, which are involved in aspects of cell or organelle movement, e.g. constriction of the cleavage furrow of dividing cells

also varies widely. Thus the endoplasmic reticulum is particularly extensive in cells, e.g. those of the pancreas, which manufacture and secrete protein; lysosomes are well developed in macrophages, which ingest particulate matter; pinocytotic vesicles are common in liver; annulate lamellae are characteristic of egg cells; Golgi vesicles are

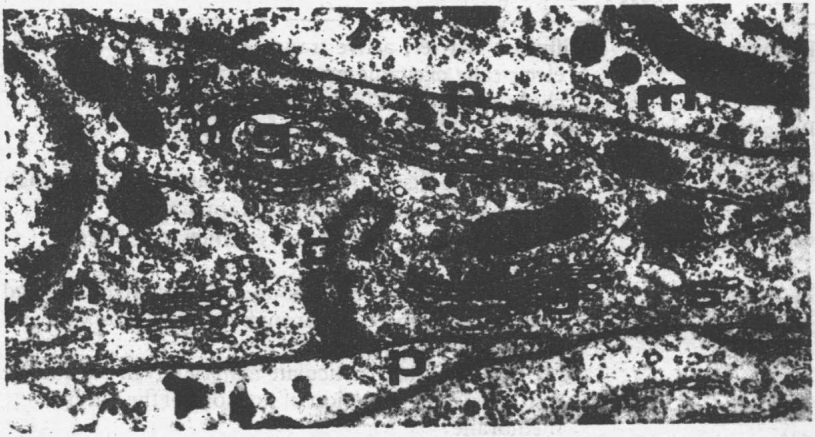


Fig. 2–2 Portion of an avian parathyroid gland parenchymal cell showing the principal organelles. c, centrioles; g, Golgi apparatus; m, mitochondrion; n, nuclear membrane; p, plasma membrane; r, rough endoplasmic reticulum; s, smooth endoplasmic reticulum. (× 1700.) (Reproduced by courtesy of Mr R. P. Gould.)

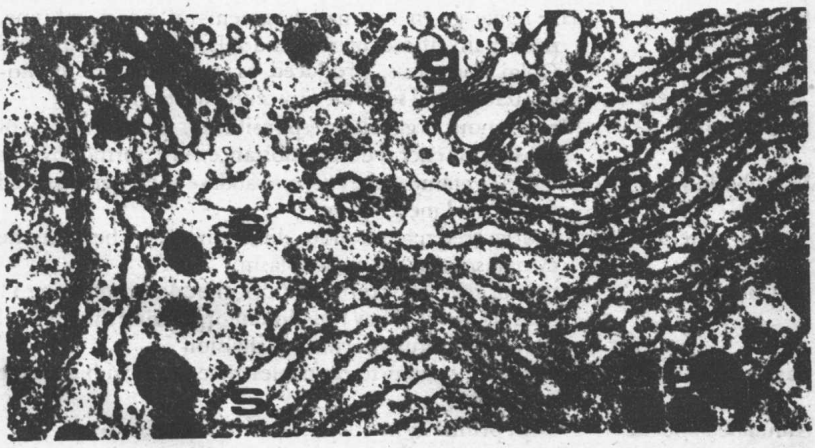


Fig. 2–3 Portion of a rat anterior pituitary cell. g, Golgi apparatus; n, nuclear membrane; p, plasma membrane; r, rough endoplasmic reticulum; s, smooth endoplasmic reticulum; sg, secretory granule. (× 28 200.) (Reproduced by courtesy of Mr R. P. Gould.)

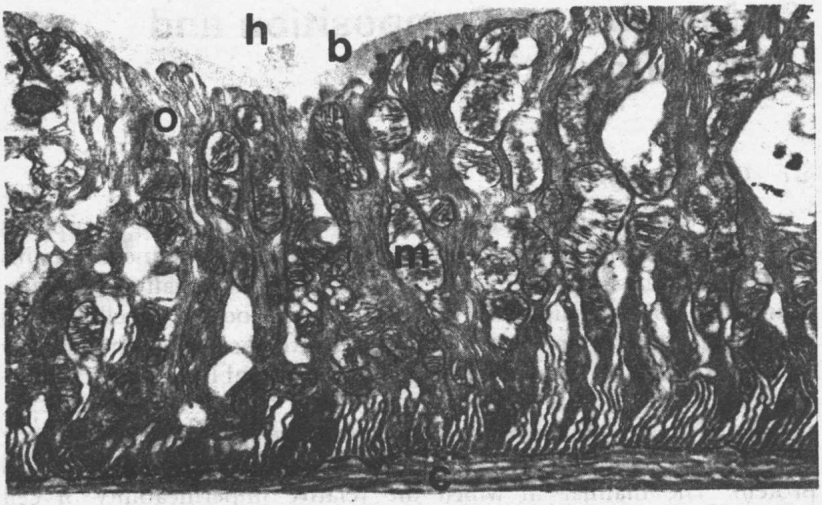


Fig. 2–4 Part of an ion-transporting epithelial cell of the gill of *Gammarus duebeni* (Amphipoda) showing the typical intuckings of the apical plasma membrane in a transporting cell. o, plasma membrane intuckings; m, mitochondrion; c, cuticle at external surface of the gill; b, basement membrane; h, haemolymph (blood). (× 6000).

enlarged in storage tissues and mitochondria concentrate in regions of high energy expenditure in the cell.

One of the primary functions of membranes is to provide a surface for the location of enzymes. The relatively enormous area which can be available for this purpose is clearly illustrated by the estimate of the size and number of the major organelles of a liver cell given in Table 2. To put the figures in perspective one should note that a cube of 100 mm side of such cells (giving a volume some 3 times less than that of a human liver) would contain over 9000 m^2 of endoplasmic reticulum. This is approximately equivalent to the area of 32 tennis courts.

Table 2 Organelles of a single rat liver cell. (From WEINER, J., LOUD, A. V., KIMBERG, D. V. and SPIRO, D. J. (1968). *Cell Biol.*, 37.)

Volume μm^3		
	Total cytoplasm of cell	5 100
	Mitochondria (total)	995
	Lysosomes (total)	10
Membrane area μm^2		
	Smooth endoplasmic reticulum	17 000
	Rough endoplasmic reticulum	30 400
	Mitochondrial outer membrane	7 470
	Mitochondrial inner membrane	39 600
Total number of mitochondria 1160		

3 Membrane Composition and Configuration

3.1 Configuration

Anyone who has had the dubious pleasure of washing the greasy plates after Sunday lunch will be well aware of the two facts that meat contains fat and that fat is not readily miscible with water, especially cold water. This immiscibility of lipid and water is put to good use in cellular and intracellular membranes, most of which owe their low permeability to water-soluble materials to the high proportion of lipids in their composition.

Isolated plasma membranes of erythrocytes (red blood cells) and many other cells contain about 40% lipid, 0–10% carbohydrate and 50–60% protein. The manner in which the relative impermeability of cell membranes derives from their lipid content may be understood by consideration of what happens at the molecular level when lipid and water interact. Fatty acids [e.g. compounds with the general structure $(CH_3(CH_2)_nCOOH)$] have a polar end (—COOH) which can interact with water molecules and a non-polar, strongly hydrophobic, group (CH_3—) at the other end of the hydrocarbon chain (see p. 7). In consequence fatty acid molecules placed at an air-water interface tend to orientate themselves in a layer one molecule thick with the polar groups in contact with the water and the non-polar hydrocarbon chains sticking up into the air. If just sufficient fatty-acid molecules are present to cover the water surface a close-packed array of molecules is formed (Fig. 3–1a) which has a high measure of impermeability to hydrophilic substances.*

When more molecules are present than are required to form a monolayer a bimolecular leaflet of lipid may be produced (Fig. 3–1b).

Early measurements of the amount of lipid present in the cell membrane of red blood cells showed that there was just sufficient present to cover the surface area of the cell with such a bimolecular leaflet and it was therefore suggested that the cell membrane consists of a lipid bilayer which is coated on each side by proteins (Fig. 3–2). This hypothesis, due to H. Davson and J. F. Danielli, at first proved most attractive since it accounted for a number of properties of cell membranes including their dimensions and appearance in electron micrographs, the presence of lipid and protein and the fact that lipid soluble substances penetrate membranes preferentially. However, studies over the last few years have

* This property has, incidentally, been utilized in reservoirs in the warmer parts of the world to slow the loss of water by evaporation. For example, experiments in which a monolayer of cetyl alcohol was spread on Lake Umberumberka in Australia reduced evaporative water loss by up to 50%.

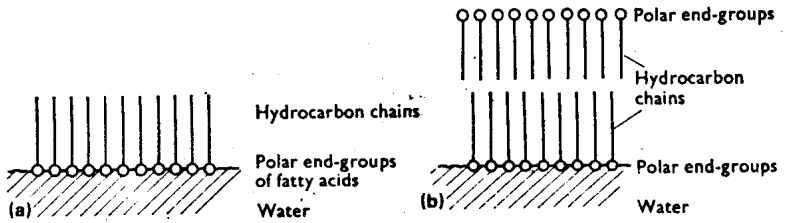


Fig. 3–1 Interaction between lipid molecules and water. (a) Close-packed molecules orientated at an air–water interface with the polar (hydrophil) groups in contact with the water and the non-polar (hydrofuge) hydrocarbon chains sticking up into the air. (b) A 'doublet' of lipid molecules is formed when too much lipid is present to be contained by a monolayer.

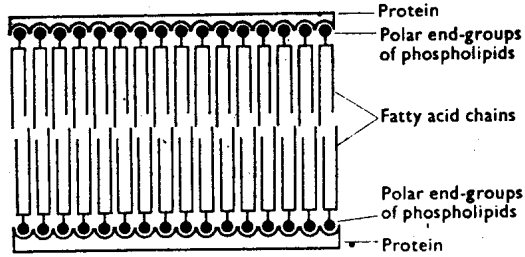


Fig. 3–2 Stylized drawing of the molecular organization of a membrane according to the 'unit membrane' theory. A lipid bilayer is coated on either side by protein layers which interact with the polar head-groups of phospholipid.

suggested that, though the concept of the 'unit membrane' based on a ground structure of a bimolecular leaflet of lipid can still be accepted in broad terms, the original Davson–Danielli concept as regards the location of the proteins must be revised. It now appears that the proteins are actually incorporated in the body of the membrane and may even, in some cases, be so located through the membrane that they project on either side (Fig. 3–3). Evidence for this conclusion is based on observations which suggest that:

(1) membrane proteins are essentially globular rather than having the expanded structure to be expected if they were superficially located;

(2) some protein molecules can be tracer labelled, or experimentally attacked by enzymes from both sides of the membrane whilst others (those thought to be restricted to a single surface of the membrane) can only be labelled from one side;

(3) globular structures of similar dimensions to membrane proteins can be seen in the interstices within the lipid bilayers when membranes are frozen and then fractured in the horizontal plane.

The finding that some proteins are quite firmly bound to lipids and that certain enzymes, including glucose-6-phosphatase and Na–K–Mg

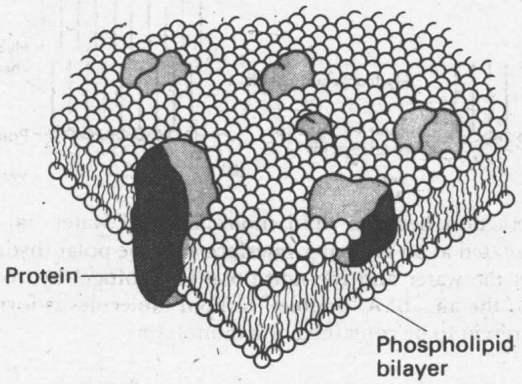


Fig. 3–3 The current lipid-globular protein mosaic model of the plasma membrane. Lipid molecules are shown as hollow circles with the hydrocarbon tails projecting into the body of the membrane; proteins are the larger masses incorporated into the membrane or projecting right through. The upper face, as shown, will be that in contact with the cytoplasm. The carbohydrate elements of the glycoproteins which project from the external surface are not visible in this diagram. (After SINGER, S. J. and NICHOLSON, G. L. (1972) *Science*, 175, 720–31.)

ATPase will not function in the absence of lipid also suggests that the proteins are constituent components of the membrane.

Membranes should not be regarded as rigid, static structures. The overall viscosity, as pointed out by G. L. Nicholson, is comparable with that of light machine oil so that the general consistency is fluid. Considerable mobility of the molecules in the plane of the membrane is therefore possible though it appears that there is essentially an 'upstairs downstairs' separation of the two lipid bilayers in so far as 'flip flop' interchange of a phospholipid molecule from one layer to the other is a relatively rare event by comparison with alteration of position within a layer. The ability of molecules to move laterally and change their associations with one another makes it possible for membranes to become mosaics of different structural and functional regions in both space and time. Both such associations of molecules form specialized *domains* within membranes and the variety of molecules composing membranes determine the configuration and function.

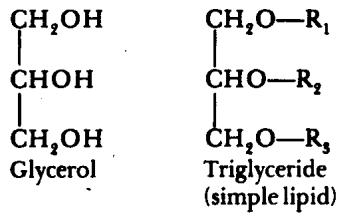
3.2 Chemical composition

Lipids and proteins together account for almost the entire composition of membranes; but each of these two general types of compound occur in a wide variety of forms.

3.2.1 Lipids

Two general classes of lipid occur in organisms: (1) simple lipids, (2) compound lipids.

Simple lipids are composed of glycerol and fatty acids and have the generalized formula shown below.



where R_1, R_2 and R_3 are fatty acids.

Simple lipids are important in fat deposits but, though present in muscle plasma membranes, they do not normally contribute appreciably to cellular membranes.

The fatty acids combined in both the simple and compound lipids have a variety of chain lengths. Three of the most commonly occurring forms are palmitic, stearic and oleic acids.

Palmitic acid	$CH_3(CH_2)_{14}COOH$
Stearic acid	$CH_3(CH_2)_{16}COOH$
Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$

Fatty acids which, like oleic acid, contain double bonds are termed 'unsaturated'. Those with no double bonds, as in palmitic and stearic, are 'saturated'.

Compound lipids are more complex in composition than the simple lipids and, in addition to fatty acids, may also include glycerol, or similar compounds and nitrogenous bases. Steroids are commonly classed with this group though they do not contain fatty acids. The three most important groups of compound lipids are (1) glycerophosphatides, (2) sphingolipids and (3) steroids.

(1) Glycerophosphatides are a group of compound lipids based on simple lipids in which one of the fatty acids is substituted. Several types occur:

(a) phosphatidyl choline (lecithin) composed of glycerol, two fatty acids, phosphate and choline;
(b) phosphatidyl ethanolamine composed of glycerol, two fatty acids, phosphate and ethanolamine;
(c) phosphatidyl serine composed of glycerol, two fatty acids, phosphate and serine;
(d) phosphatidyl inositol composed of glycerol, two fatty acids, phosphate and inositol.

These last three compounds are sometimes referred to as *cephalins* though this term is more usually restricted to phosphatidyl ethanolamine and phosphatidyl serine alone.

The virtual absence of lipid-bound amino groups on the outward facing surface of the membrane suggests that phosphatidyl choline (lecithin) is the dominant lipid of the outer bilayer whilst the amino-acid lipids (phosphatidyl serine and phosphatidyl ethanolamine) are the principal phospholipids of the inner bilayer. Glycolipids are thought to be restricted to the outer layer.

The amino-acid phospholipids are usually present in a somewhat lower concentration than the sum of lecithin and the glycolipids which is compatible with the suggested distribution since the inner bilayer would be expected to have a lower lipid component than the outer so that the layer may incorporate the associated proteins (cf. Fig. 3–3).

Membranes often contain small proportions of materials which, though otherwise comparable to the four listed above, give aldehyde reactions. These are known respectively as phosphatidal choline, phosphatidal ethanolamine, phosphatidal serine and phosphatidal inositol.

A more complex glycerophosphatide, cardiolipin, isolated from heart membranes, contains two glycerols, four fatty acids and two phosphate groups.

(2) Sphingolipids differ from the glycerophosphatides in lacking glycerol: instead they contain the nitrogenous base sphingosine. The two major compounds of the group are (a) *sphingomyelin* (sphingosine, one fatty acid, phosphate and choline) and (b) *cerebroside* (sphingosine, one fatty acid and galactose).

(3) Cholesterol. This steroid is a major component of plasma membranes but only occurs to a minor extent in intracellular membranes. Most of the cholesterol in plasma membranes is associated with the outer bilayer.

The approximate steric structure of these compound lipids is illustrated in Fig. 3–4.

Minor components of membranes include sialic acid, phosphatidic acid, glycolipids and inorganic ions, particularly calcium, but, by comparison with the lipids mentioned above and proteins, they occur in only trivial amounts.

Although compound lipids contribute to the membranes of every cell they are by no means constant in amount in different tissues or membrane systems or indeed even in one type of plasma membrane under different environmental circumstances. Such variation reflects membrane specialization and the need to maintain a specific viscosity in order to function normally.

The degree of mobility of lipid molecules is dependent upon whether their *phase transition temperature* (effectively the melting point) is above or

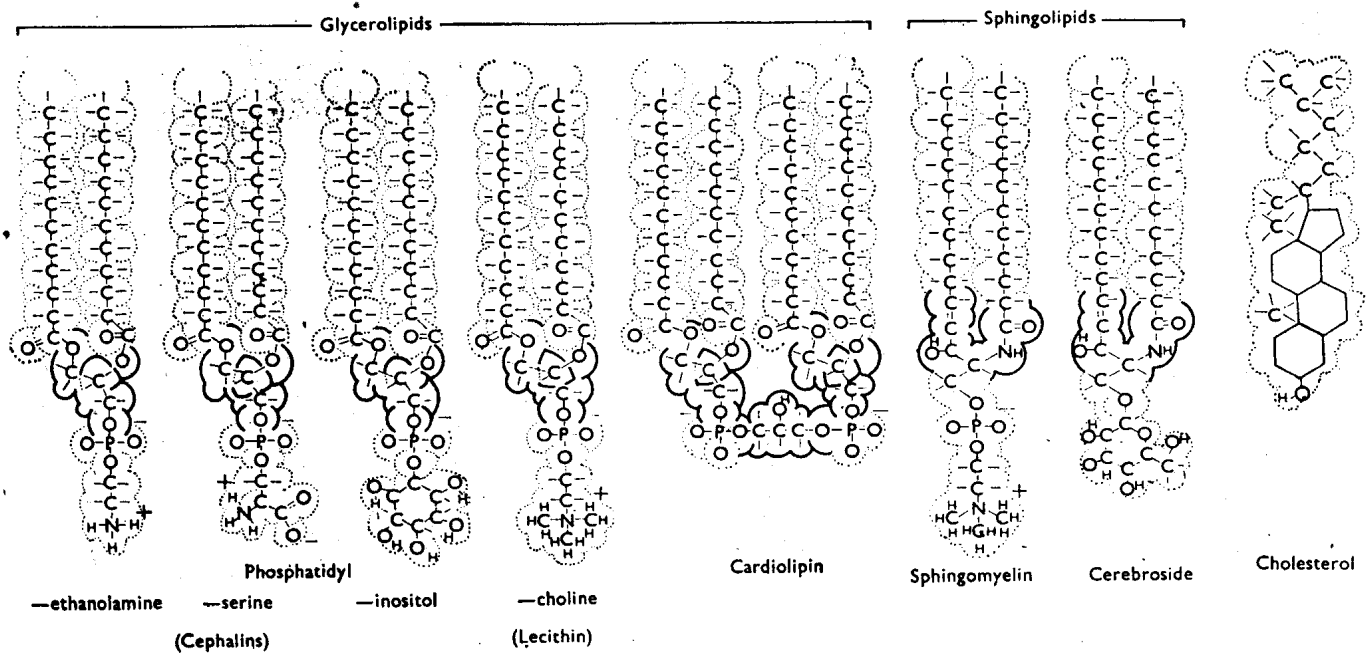


Fig. 3–4 The chemical formulae of the major lipids of membranes drawn to illustrate their approximate spatial appearance. (From FINEAN, J. B. (1961). In *Chemical Ultrastructure in Living Tissue*. Charles C. Thomas, Illinois.)

below the ambient temperature. The phase transition temperature is determined by the chain length and degree of saturation of the molecule. The shorter the chain length and the greater the degree of unsaturation, the lower is the melting point. In addition the phase transition can be influenced by the presence of cholesterol. Cholesterol serves to blur the distinction between the solid ('frozen') and liquid ('melted') phases since it has the effect of decreasing molecular mobility in the liquid phase and increasing it in the solid phase. The appropriate degree of mobility is important to normal functioning of membranes and there is evidence from both dietary and temperature studies of the dynamic regulation of this property. Thus, although unusual dietary lipid intake can result in variation of the lipid composition of membranes there is a tendency for a balance to be maintained between the saturated and unsaturated components such that a more or less constant overall viscosity is maintained. Similarly, response to temperature change can involve adjustments within membranes. When the protozoan *Tetrahymena*, cultured at 28°C, are suddenly exposed to 10°C there is some degree of gelling (i.e. freezing) of the plasma membrane. Within 24 h, however, changes in the membrane components lower the overall freezing point restoring the fluid nature of the membrane.

Despite such control of average viscosity, segregation of particular lipid types can create zones within a membrane of a greater or lesser mobility than the mean. There are also considerable variations in the composition of different membranes confirming the proposition that there must be a variety of ways in which membranes can be assembled from component molecules and that differences in composition can be related to membrane function. Myelin (from nerve sheaths), chloroplast membranes, mitochondrial membranes and red cell plasma membranes illustrate these differences with respect to their content of glycerophosphatides and sphingolipids. The glycerophosphatide content of mitochondrial membranes is high (over 75% of the total lipid); it is smaller in myelin and red cell ghosts (about 33% of the lipid) and still less in chloroplast membranes (12%). Sphingolipids are high in myelin (25%), less important in red cell ghosts (15%), and still lower in mitochondria.

Not only does the total content of phospholipid vary but the fatty acids they incorporate also differ from membrane to membrane. The lipids of myelin are rich in saturated long chain fatty acids admixed with those of medium chain length; medium chain length unsaturated fatty acids are most frequent in red cell ghosts and mitochondrial membranes are also rich in unsaturated fatty acids. That such differences in composition reflect the functional role of a membrane, or of specialized regions within a membrane, is indicated by experimental attempts to reconstitute the oxidative phosphorylation system after disruption of mitochondrial membranes. Success is dependent upon the relative proportions of phosphatidyl ethanolamine and lecithin present.