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The Membranes of Animal Cells

Third Edition

A.P.M.Lockwood and A·G.Lee

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General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

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Readers' comments will be welcomed by the Institute.

1984

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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 Third Edition

Over the five years since the Second Edition was produced there has been increased development in knowledge of the biochemical structure and functioning of membrane systems. To do justice to the increasing importance of these aspects this new edition endeavours to provide a balanced introduction not only to the microscopical structure and physiology of membranes but also to their biochemistry.

Southampton, 1984 A.P.M.L.
A.G.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?

Before the advent of the electron microscope, the only membrane recognized with any degree of certainty was the *plasma membrane* that surrounds all cells and which defines the boundaries of the cell. For prokaryotic organisms (bacteria and blue-green algae) this is indeed often the only membrane present. However, visualization of many eukaryotic cells (cells of animals, plants and fungi) in the electron microscope showed the presence of a variety of membranous structures within the cell. Some of these membranes surround organelles such as the *nucleus* and *mitochondria*, whereas others form interconnecting networks within the cell, such as the *endoplasmic reticulum* and the Golgi apparatus. The most commonly found structures are listed in Table 2–1 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.

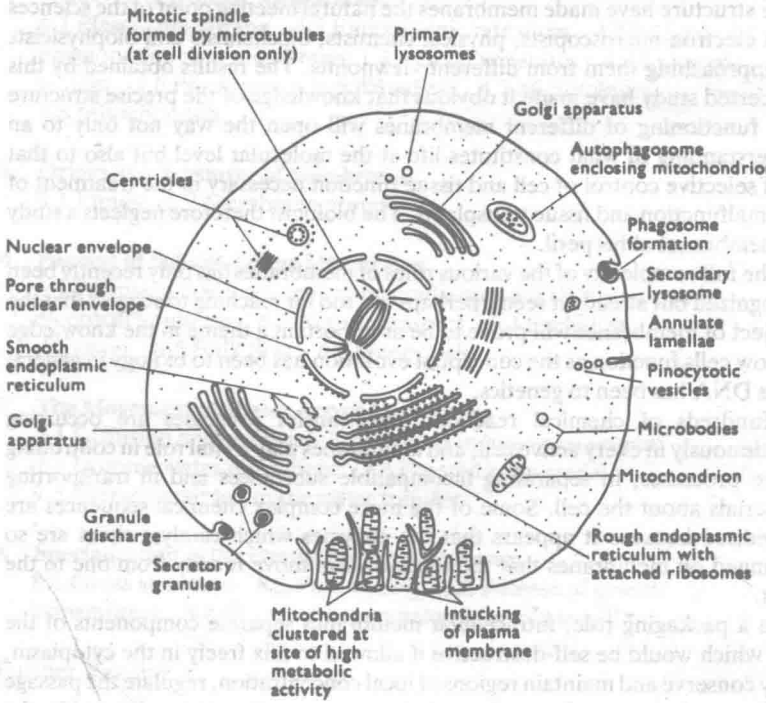


Fig. 2–1 Diagrammatic representation of the major membrane-bounded structures found in cells. N.B. Few, if any, cells will contain all these organelles simultaneously.

Table 2–1 Cell organelles and their primary functions.

Plasma membrane	Diffusion barrier, active transport of ions, nutrients etc., electrically excitable membrane
Rough endoplasmic reticulum	Covered with ribosomes, engaged in synthesis of proteins
Smooth endoplasmic reticulum	Free of ribosomes, involved in detoxification, synthesis of phospholipids and steroids
Golgi apparatus	'Packaging' of enzymes to be used elsewhere
Lysosomes	Vesicles containing enzymes used in autodigestion and breakdown of materials engulfed during phagocytosis and absorptive endocytosis
Phagosomes	Vesicles containing particulate material from outside the cell; formed by sealing off portions of the plasma membrane
Pinocytotic vesicles	Small vesicles containing soluble material formed by sealing off portions of the plasma membrane
Microsomes	Name given to fragments of membrane, principally those of endoplasmic reticulum and Golgi apparatus, produced as a result of homogenization of cells and separation by centrifigation
Mitochondria	Membrane-surrounded organelles responsible for energy production (ATP) in the cell
Peroxisome	Also called microbodies – contain enzymes involved in oxidation of amino acids etc.
Nuclear envelope	Surrounds the nucleus; consists of an inner and an outer membrane, fused in places to form pores through which RNA can pass between the nucleus and the cytoplasm
Microtubules and Microfibrils	Filamentous structures, making up the cytoskeleton of the cell, involved in controlling the shape and changes of shape, of cells. In contact in various ways with cell membranes

The number and relative amounts of these different organelles varies between cells, and reflect the function of the cell. The mammalian red blood cell (erythrocyte) contains only the plasma membrane. In most mammalian cells, however, the endoplasmic reticulum, consisting of a system of flattened tubes or sacs, is very extensive and fills most of the cytoplasm. In macrophages, which are designed to locate, sequester and kill invading pathogenic micro-organisms, phagosomes are very common. Mitochondria contain the enzymes involved in the oxidation of food stuffs by oxygen and produce the 'carrier' of energy in the cell, adenosine triphosphate or ATP for short. Mitochondria are found in greatest number in cells which are energetically very active, such as muscle cells. In plants ATP can in addition be produced in organelles called *chloroplasts* during the process of photosynthesis.

It is clear then that membranes are a very important structural feature of cells. Correspondingly, they are likely to have major functional roles in the cell. The most obvious function of the plasma membrane is to define the boundaries of the cell: it has to keep the inside of the cell inside and the outside,

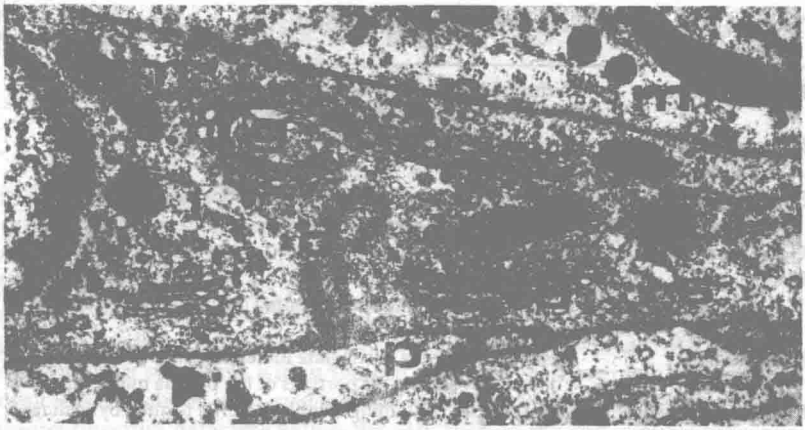


Fig. 2–2 Portion of an avian parathyroid gland parenchymal cell showing the principal organelles: **c**, centrioles; **g**, Golgi apparatus; **m**, mitochondrion; **n**, nuclear membrane; **p**, plasma membrane; **r**, rough endoplasmic reticulum; **s**, smooth endoplasmic reticulum. (× 1700.) (Reproduced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

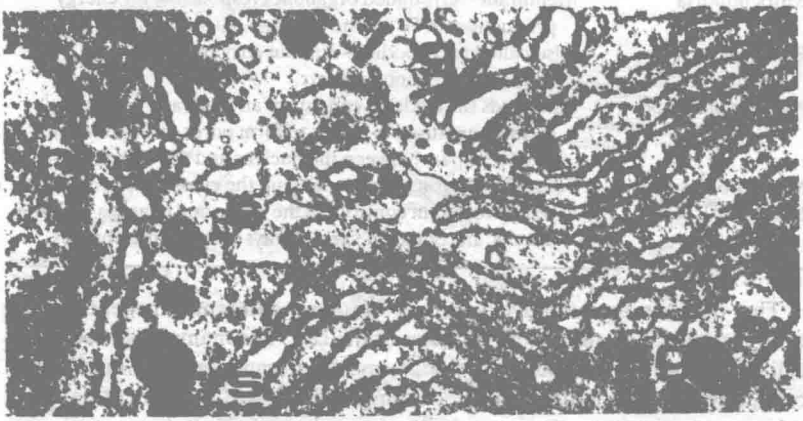


Fig. 2–3 Portion of a rat anterior pituitary cell: **g**, Golgi apparatus; **n**, nuclear membrane; **p**, plasma membrane; **r**, rough endoplasmic reticulum; **s**, smooth endoplasmic reticulum; **sg**, secretory granule. (× 28 200.) (Reproduced by courtesy of Mr R.P. Gould.)

outside. In this capacity it has more than merely a passive role to play; the membrane has to be flexible to allow for the distortions that occur with bodily movements, it needs selectively to allow nutrients to enter the cell and waste products to be eliminated and (particularly for electrically excitable cells such as nerves and muscles) it must provide a high degree of electrical insulation and have the capacity to regulate the active and passive movement of inorganic

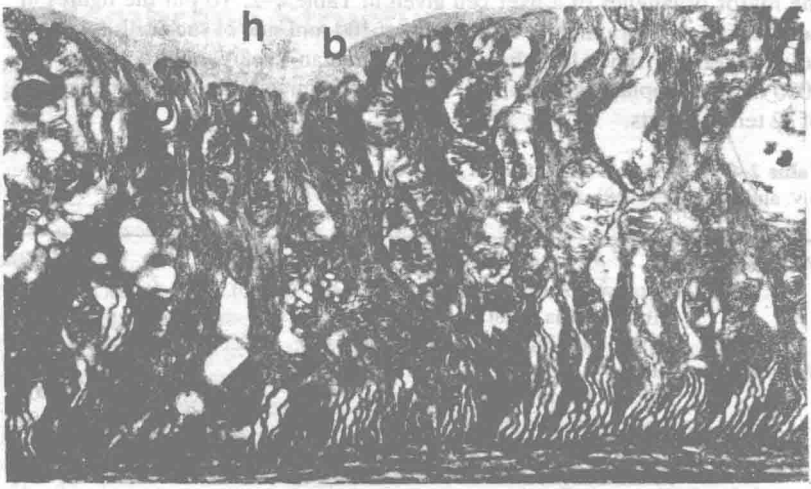


Fig. 2–4 Part of an ion-transporting epithelial cell of the gill of *Gammarus duebeni* (Amphipoda) showing the typical intuckings of the apical plasma membrane in a transporting cell: **o**, plasma membrane intuckings; **m**, mitochondrion; **c**, cuticle at external surface of the gill; **b**, basement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

ions. Finally, as the outermost part of the cell, the plasma membrane is responsible for control of the passage of information between the cell and its environment. Before any message can be passed from the environment, it must first be received and recognized by the membrane. This is made possible by the presence in the membrane of specific receptors for external stimuli. For example, it is important that the body distinguishes between its own cells and foreign cells, such as those of bacteria and viruses. Such a distinction is made by specific 'markers' in the plasma membrane together with receptors that can distinguish between correct and incorrect markers. This ability of cells to recognize other cells which do not originate in the same body constitutes a problem for transplant surgery which has to be overcome by immobilizing the recipient's immune response system.

Metabolic processes are too numerous and too complex to be managable in free solution in the cytoplasm of cells and perhaps the major role of intracellular membranes is in compartmentalization, in separating specific regions of submicroscopic order where particular complex sequences of biochemical reactions can occur. The membranes themselves, by providing a surface on which the enzymes responsible for a particular reaction sequence are associated, contribute directly to the orderly passage of reactants. An important example is in the mitochondrion where sequences of reactions carried out by enzymes in the mitochondrial membrane ensure the efficient production of ATP from nutrients. The relatively enormous area which can be available for this purpose is illustrated by the estimate of the size and number of

the major organelles of a liver cell given in Table 2–2. 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 2–2 Organelles of a single rat liver cell. (From WIENER, J., LOUD, A.V., KIMBERG, D.V. and SPIRO, D. (1968). *Journal of Cell Biology*, **37**, 47–61.)

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		

In the next chapter we will look at some of the molecules that are used to construct cell membranes, and see how the properties of membranes can be deduced from the properties of the constituent molecules. This is currently an active area of research but many large gaps still exist in our understanding. It is the sheer complexity of the functions necessarily performed by a typical membrane which makes them so difficult to study, and, at a time when microelectronics is revolutionizing our lives by putting ever more functions on to ever smaller silicon chips, it is salutary to remember the wonders of miniaturization which have been achieved by the membranes of cells.

3 Membrane Structure and Composition

3.1 General features of biological membranes

The processes of nature are essentially conservative: the same basic molecules and structures are found in all cells. This applies to biological membranes as much as to any other feature of cell architecture, and all cell membranes show a number of common features.

(1) Membranes are generally very thin, being between 6 and 10nm across. This implies that they can be only a few molecules thick.

(2) Although the chemical compositions vary, membranes consist mainly of lipid and protein, with some carbohydrate. The carbohydrate is not free but covalently attached to the molecules of lipid and protein.

(3) The lipid component of the membrane provides the basic matrix of the membrane, and serves as a permeability barrier.

(4) The protein component of the membrane is responsible for the specific functions of the membrane.

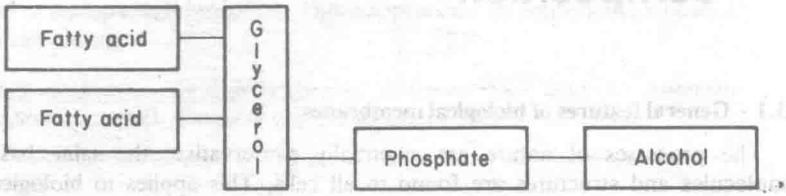
(5) The lipid and protein components of the membrane are held together by non-covalent interactions. The protein can be pictured as being in a two-dimensional solution of lipid.

3.2 The lipids

The lipids are a rather unusual class of biological molecules. Most components of cells are water soluble, since the cell is largely made up of water. The lipids however are insoluble in water but highly soluble in organic solvents such as methanol or chloroform. Some lipids, like the triglycerides (fats), are used in biological systems as energy stores, and anyone who has had the dubious pleasure of washing the greasy plates after Sunday lunch will be well aware of the fact that fat is not readily miscible with water. This immiscibility of lipid and water is put to good use in the design of biological membranes, as we will see. In the membrane, the lipids are not triglycerides, but most commonly phospholipids, glycolipids and cholesterol.

Despite their apparent chemical diversity membrane lipids all have one common feature upon which rests the structure of the membrane. This feature is that they are *amphipathic*, which is to say that one end of the molecule is charged or polar and mixes well with water whereas the other end of the molecule is non-polar and mixes poorly with water. Remembering this common feature, we can look at some structures. The most abundant of all the membrane lipids are the phospholipids. Phospholipids are based either on glycerol or on sphingosine with the former class, the glycerophospholipids, being the more important. The structure of a glycerophospholipid consists of

three parts: a glycerol backbone, a phosphorylated alcohol making the head-group, and two fatty acid chains:



By varying the fatty acid and alcohol groups, a wide variety of chemically distinct structures can be generated.

The fatty acids found in the lipids of animal cells are unbranched, with an even number of carbon atoms, generally between 14 and 24. Many contain double bonds in the *cis* configuration. Fatty acids containing double bonds are referred to as 'unsaturated' whereas those with no double bonds are 'saturated'. Some fatty acids are listed in Table 3–1. Most phospholipids contain one saturated and one unsaturated fatty acid chain.

Table 3–1 Fatty acids commonly found in phospholipids.

Name	*Structure*	*Notation*
Palmitic acid	$CH_3(CH_2)_{14}COOH$	16:0
Stearic acid	$CH_3(CH_2)_{16}COOH$	18:0
Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	18:1
Linoleic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOH$	18:2
Arachidonic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	20:4

Unsaturated fatty acids have *cis* double bonds. The short-hand notation gives the number of carbon atoms in the chain followed by the number of double bonds.

The phosphate group of the phospholipid is joined to a variety of alcohols, the most common of which are choline, ethanolamine and serine giving, respectively, the phosphatidylcholines (or lecithins), phosphatidylethanolamines and phosphatidylserines (Fig. 3–1). All the phospholipids carry a phosphate group which will have a negative charge at physiological pH. In addition, choline and ethanolamine contain amino groups which will be positively charged at pH 7, so that phosphatidylcholines and phosphatidylethanolamines will be zwitterions with no net charge. Serine, however, has one negative and one positive charge at pH 7, thus phosphatidylserines will carry a single net negative charge.

In the sphingolipids, the backbone of the molecule is not glycerol, but rather an amino alcohol, however, as shown in Fig. 3–1 the overall structures of the sphingolipids and glycerophospholipids are very similar. In sphingomyelin the head group is the same as that in phosphatidycholine. A sugar group such as galactose or glucose can also be attached to give the cerebrosides. More

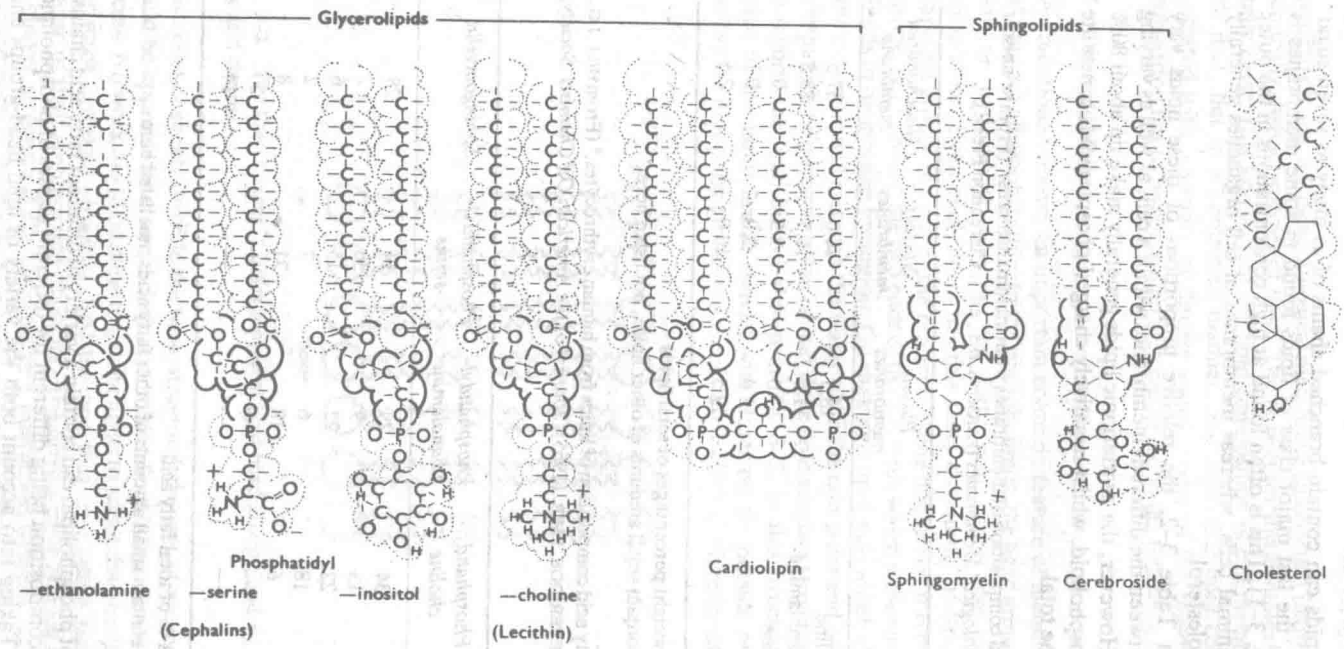


Fig. 3–1 The chemical formulae of the major lipids of membranes drawn to illustrate their approximate spatial appearance. (From FINEAN, J.B. (1961). In *Chemical Ultrastructure in Living Tissue*, CHARLES, C. THOMAS (ed.). Illinois.)

complex glycolipids can contain branched chains of as many as seven sugar groups. Finally, the last major class of lipids found in some membranes is cholesterol (Fig. 3–1). This is often found at high concentrations in the outer membrane of animal cells, whereas membranes of cell organelles generally contain little cholesterol.

As shown in Table 3–2, the relative proportions of these lipids vary considerably between the different membranes within a cell, as well as varying between cells. However, the zwitterionic lipids generally make up about 80% of the total phospholipid, whilst negatively charged phospholipids constitute about 20% of the total.

Table 3–2 Lipid composition of membranes from mammalian cells.[a] (From ROBINSON, G.B. (1975). In *Biological Membranes* (PARSONS, D.S., ed.). Clarendon Press.)

Lipid class[b]	*Plasma membranes*	*Nuclear membranes*	*Mitochondrial membrane*
Phosphatidylcholine	18.5	44.0	37.5
Phosphatidylethanolamine	11.5	16.5	28.5
Phosphatidylserine	7.0	3.5	0
Sphingomyelin	12.0	3.0	0
Cholesterol	19.5	10.0	2.5

a. Expressed as weight percentages of total lipids
b. Membranes contain small amounts of other lipids, not listed here

Table 3–3 Fatty acid composition of lipids from human erythrocytes.[a] (From HILL, J.G., KUKSIS, A. and BEVERIDGE, J.M.R. (1965). *Journal of the American Oil Chemists' Society*, **42**, 137.)

Fatty acid[b]	*Phosphatidyl-choline*	*Phosphatidyl-ethanolamine*	*Phosphatidyl-serine*	*Sphingomyelin*
16:0	34	29	14	28
18:0	13	9	36	7
18:1	22	22	15	6
18:2	18	6	7	2
20:4	6	18	21	8
24:0	—	—	—	20
24:1	—	—	—	14

a. Expressed as % of total fatty acid
b. Membranes contain small amounts of other fatty acids, not listed here

Each class of phospholipid can contain a variety of different fatty acid chains, the fatty acid composition being different for different classes of phospholipid (Table 3–3). Taking into account both the variety of lipid head-groups and

fatty acid chains, a typical biological membrane can contain many hundreds of chemically distinct species of lipid. The reason for this complexity is still not totally clear. It is known that the lipid composition of cell membranes varies with diet, but in a controlled manner, and abnormal lipid composition is associated with a variety of disease states. Some of the reasons why lipid composition has to be controlled are considered in the following section.

3.3 The lipid bilayer

As mentioned above, an important common feature of membrane lipids is that they are all amphipathic. In the phospholipids, the head group is relatively polar or hydrophilic whereas the fatty acid chains are non-polar or hydrophobic. Even cholesterol has this same dichotomy of structure with a polar–OH group, the rest of the molecule being non-polar. It is the difference in properties between the two ends of the molecule which is the basis of the role of lipids in membrane formation. On shaking a lipid with water, the polar end of the molecule will mix with the water while the non-polar end will not. These incompatible requirements can be met if the lipid adopts a structure made up of two layers, the lipid bilayer shown in Fig. 3–2. In this structure, the polar groups of the lipids are in contact with water on the outside of the bilayer whereas the non-polar groups are sequestered within the interior of the bilayer.

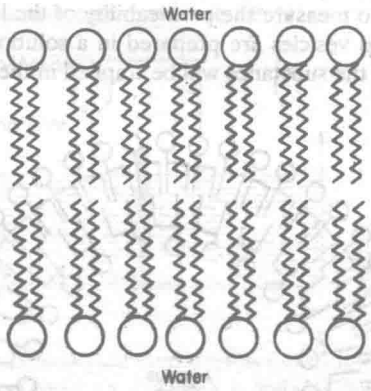


Fig. 3–2 The lipid bilayer. Circles represent lipid head groups, and zig-zag lines the fatty acid chains.

These bilayer sheets provide the basic structural element of the membrane. It should be appreciated that the formation of the lipid bilayer is a self-assembly process. No work has to be done to get the lipids to adopt this arrangement: it is their most favoured structure, and the one that they adopt spontaneously because of their amphipathic character. The driving force making lipids adopt the bilayer structure is the unfavourable interaction between water and the non-polar parts of the lipids. The origin of the effect lies in the unusually strong

interaction between water molecules. The water molecule is polar, with small positive charges on the H atoms and a small negative charge on the O atom. In liquid water, the water molecules tend to arrange themselves so that the oxygen of one water molecule is close to the hydrogens of adjacent water molecules, to form hydrogen bonds. Now imagine a fatty acid chain introduced into the water. Such a chain will effectively form a cavity in the water and transiently disrupt the hydrogen bonding network for the water molecules in its vicinity. The water molecules will then re-organize themselves around the fatty acid chains in such a way that they can again form hydrogen bonds between themselves, and, in doing so, the water molecules around the fatty acid chain will become much more ordered than ordinary water. This ordering is unfavourable (cf. Second Law of Thermodynamics). Conversely, removing a fatty acid chain from water is highly favoured, and this is achieved by the formation of a lipid bilayer. The exclusion of non-polar groups from water is referred to as the hydrophobic effect.

Because of their biological importance, the properties of lipid bilayers have been studied extensively. Lipid bilayers can be made simply by vigorously shaking phospholipids in water. The bilayers tend to close on themselves to give sealed spherical vesicles (Fig. 3–3), so that there are no ends with fatty acid chains exposed to water. The diameter of the vesicles can be anything between 50nm and about 1μm, depending on the exact details of preparation. These vesicles can be used to measure the permeability of the lipid bilayer to small molecules. If the lipid vesicles are prepared in a solution of a substance of interest then some of the substance will be trapped in the aqueous interior of

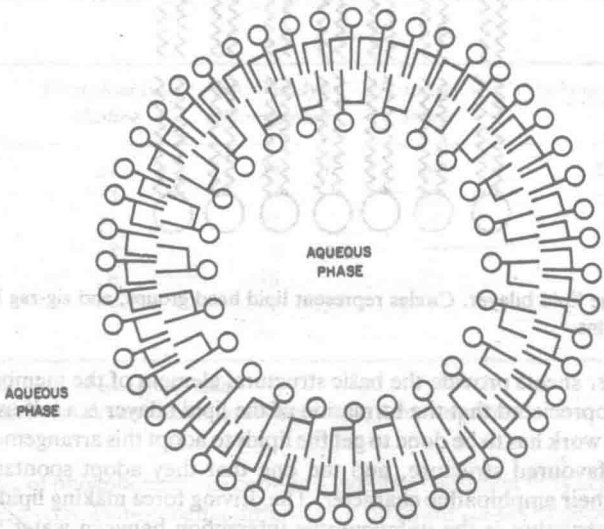


Fig. 3–3 Sealed lipid vesicles.