Basic Biology Course

5 Cell Membranes

BASIC BIOLOGY COURSE
UNIT 3
REGULATION WITHIN CELLS

BOOK 5

Cell Membranes

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Foreword

This book is part of a Basic Biology Course for undergraduates written by the Nuffield Inter University Biology Teaching Project team at Sussex.

The main aim of the book is to provide you with an insight into the structure and function of cell membranes, sufficient for you to examine critically some of the models proposed for their structure and to enable you to appreciate the vitally important, yet complex role which they play in regulating life processes.

Book 5 is in fact one of five books (Books 5 to 9 inclusive) comprising a unit called 'Regulation within cells' (See the outline of course structure at front of book.) It also provides a necessary background for Books 10 and 11 dealing with aspects of 'Communication between cells', such as conduction of impulses by neurons and hormonal action.

Sussex, 1974

Michael A. Tribe
Michael R. Eraut
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Contents

5.0. Introduction

5.0.1. Discussion

The best starting point for the investigation of any system is an analysis of its input and output. So it is logical to begin a study of the cell with an account of cell membranes. The plasma membrane determines what goes into the cell and what comes out of it; and the cytomembranes determine the input and output of each of the cell's subsystems, such as the nucleus and the mitochondrion. Taken together these cell membranes normally constitute 50 per cent of the dry mass, which remains when a cell is broken up and centrifuged and its 'soluble' cytoplasm poured off; in some instances the membranous fraction may account for 70–80 per cent of the dry mass.

Present-day research into the structure and function of biological membranes is extremely active and uses sophisticated biophysical and biochemical techniques.

The importance of cell membranes, particularly the plasma membrane, in determining selective permeability and controlling the transport of substances into and out of cells is now well established. Recent research, however, is revealing other fascinating roles for the plasma membrane. For example, it is concerned in cell recognition, now an important aspect of cancer research. Whereas normal cells exhibit auto-immune responses and can distinguish 'self' from 'non-self', it appears that cancerous cells do not recognize 'self', and it is possible that differences in the plasma membrane may be responsible. Another role is the provision of receptor sites for hormones or man-made drugs.

Lastly, since the plasma membrane is that part of the cell which comes into contact with other cells or surfaces, it is intimately involved in activities such as cell movement, cell adhesion, cell fusion, phagocytosis, pinocytosis, and secretory processes of various kinds.

5.0.2. Overview

Preceding books in this course were concerned with:

(1) The variety and fine structure of different cells in living organisms as revealed by light and electron microscopy (Books 1, 2 and 3).
(2) The dependence of organisms on their environment; the degree of tolerance shown by organisms with respect to changeable environmental conditions; and the pattern of inter-relationships between organisms, culminating in the concept of ecosystems (Book 4).

In this unit (Unit 3), consisting of Books 5 to 9 inclusive, we aim to:

(1) examine evidence for the function of organelles;
(2) examine the inter-relationships which exist between the various inclusions of the cell and point out the advantages of cellular organization;
(3) indicate those factors which tend to limit the size of cells; and
(4) examine some of the mechanisms by which a cell is able to control its growth and metabolism.

5.0.3. Preknowledge requirements

Basic knowledge of electron microscopy and cell structure as presented in Books 1 and 2 (Unit 1) in this series.

A basic knowledge of the following chemical substances and terms: gases, liquids, solids; solute, solvent; fat solvents (e.g. chloroform, benzene, etc); diffusion, osmosis; acids, bases and electrolytes; polar and non-polar groups. An idea of the approximate molecular size and chemical composition of sugars, starches, lipids and proteins (stereochemical details or knowledge of secondary and tertiary structures of proteins is not assumed).

5.0.4. Objectives

At the end of this book students should be able to:

(1) Define and give examples of the following terms:
diffusion, facilitated diffusion, osmosis, active transport.

(2) Present models explaining the structure and some of the functions of cell membranes as deduced from experimental evidence.

(3) Given experimental data, deduce whether transport is active or passive.

(4) Indicate the effect of lipid solubility and molecular size of non-electrolytes on their rate of penetration into cells.

(5) Recognize the specific problems of transporting strong electrolytes (particularly Na^+, K^+ and Cl^- ions) across cell membranes and briefly describe the involvement of Na^+ ions in glucose and amino acid transport in the mammalian intestine.

(6) Define and explain the terms phagocytosis, pinocytosis, antigen, antibody, opsonin, with special reference to blood cells, capillary blood vessel cells, fibroblasts and amoebae.

5.0.5. Instructions on working through programmed sections

In the programmed sections, questions and answers are arranged sequentially down the page. You are provided with a masking card and probably a student response booklet. Sections 5.1 and 5.2 of this book are programmed. Cover each page in turn, and move the masking card down to reveal two thin lines.

This marks the end of the first question on that page. Record your answer to the question under the appropriate section heading in the response booklet provided. Then *check* your answer with the answer given. If your answer is correct, move the masking card down the page to the next double line and so on. If any of your answers are incorrect retrace your steps and try to find out why you answered incorrectly. If you are still unable to understand the point of a given question, make a note of it and consult your tutor.
The single thick line

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is a demarcation between one frame and the next.
A double bold line indicates a convenient stopping point in the programme, since it is unlikely that you will have time to read through the whole book in one session.

5.1. The structure and function of cell membranes

5.1.1. Structure

1 Those of you who read Book 2 (*Electron microscopy and cell structure*) in this series, will recall that fixatives such as osmium tetroxide and potassium permanganate clearly reveal the position of cell membranes by reacting with *the lipids and proteins that form the basis of membrane structure.*

Another line of enquiry which was begun by Overton at the beginning of the century, looked at the capacity of various substances to penetrate the plasma membrane.

The following table shows the type of results obtained.

Table 1

Penetration				
Very rapid	**Rapid**	**Slow**	**Very slow**	**Virtually none**
Gases: CO_2	Water	Glucose	Strong electrolytes (e.g. inorganic salts)	Proteins
O_2		Amino acids		
N_2		Glycerol	Acids	Polysaccharides
Alcohol			Bases	Phospholipids
Ether		Fatty acids		
Chloroform			Sucrose	
Benzene			Maltose	
Carbon tetrachloride			Lactose	

You will notice from the table that organic solvents penetrate the membrane at a faster rate than water, and that strong electrolytes penetrate very slowly. What does this suggest about the nature of the plasma membrane?

That it is predominantly non-polar, i.e. lipid, in character

2 What reasons can you give to account for the finding that water, which is more polar than glycerol, penetrates the membrane more rapidly than glycerol?

(i) The small size of the water molecule might account for its relatively rapid penetration because small molecules diffuse faster than large ones. This would also explain why monosaccharides like glucose penetrate faster than disaccharides such as sucrose.

(ii) The presence of small polar 'pores' in the membrane could account for the rapid penetration of water and other small polar molecules.

---

3 Proteins, polysaccharides and phospholipids are known to exist inside cells but cannot penetrate the cell membrane. So how do they get inside?

They have to be made inside the cell itself from smaller components which can be imported. (Though this is generally true there are a few exceptional mechanisms for importing these compounds, which we will discuss later.)

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4 This characteristic of the plasma membrane is called selective permeability (see Glossary) and gives the cell some considerable control over its input and output. In what ways might such control be beneficial to the cell?

(i) It prevents sudden changes in the concentration of all but the most rapidly penetrating molecules.
(ii) It keeps many chemicals which are essential to the life of the cell within the boundaries of the cell. (*Note.* If the plasma membrane is torn with a fine needle the contents ooze out.)
(iii) It allows the rapid penetration of gases such as CO_2, O_2, and N_2 which, as we noted in Book 4 (*Ecological principles*), are important factors in the interaction between many organisms and their environment.

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5 The predominantly non-polar character of membranes separates them quite clearly from their environment, which is aquatic on both sides, and therefore polar. What advantages can you suggest for having internal cytomembranes as well as a plasma membrane?

By creating internal compartmentation they permit a greater degree of organization. This is one of the factors which enables the cell to perform its functions in a manner more similar to that of a production line than that of a cottage industry.

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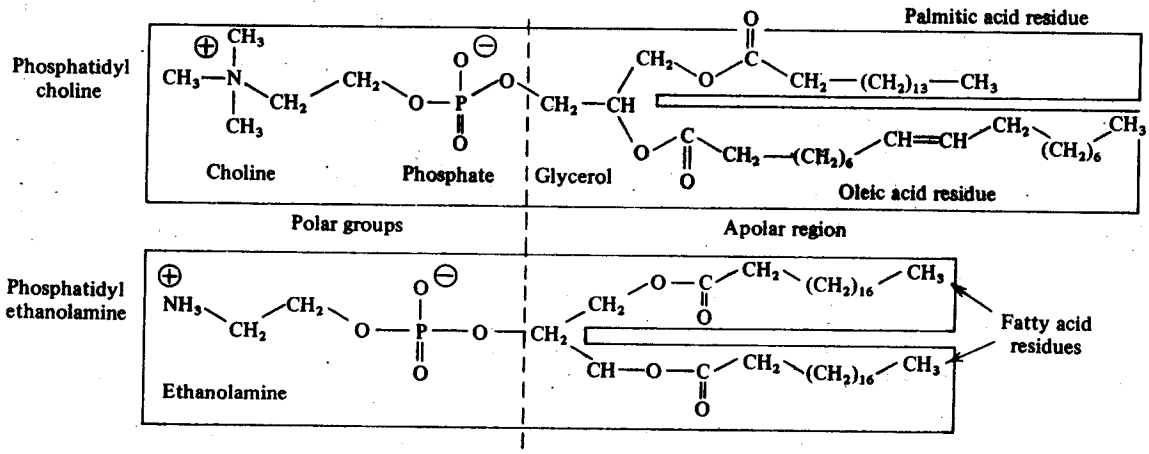
6 Let us now examine membranes more closely, beginning with the lipid component.

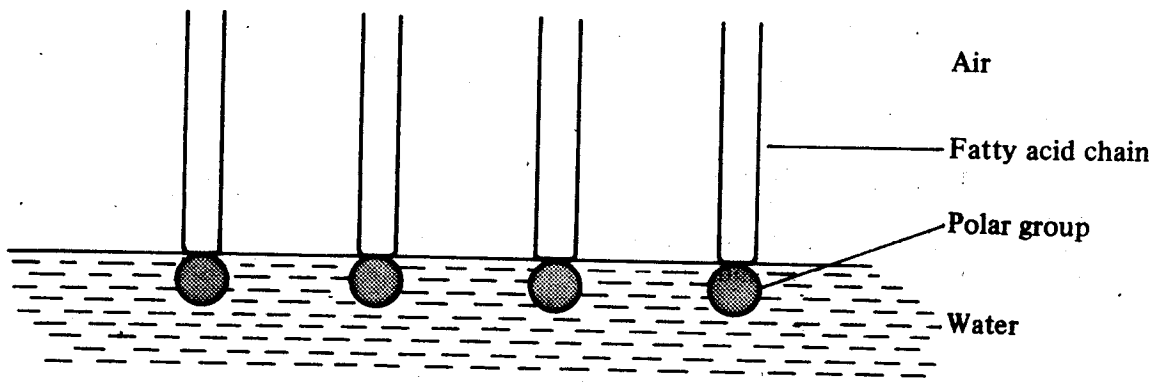
Overpage you will see two different phospholipid molecules commonly

found in biological membranes; namely, a phosphatidyl choline (or lecithin) and a phosphatidyl ethanolamine. Each molecule resembles a two-pronged peg, in which the choline and ethanolamine residues respectively are soluble in water and are called *polar* groups because they carry a positive electric charge. The long chain fatty acid residues (or side chains) are insoluble in water and are often referred to as the *apolar* region of the molecule. Notice too, that the two side chains are often derived from different fatty acid origins. The phosphatidyl choline illustrated, for example, has one side chain derived from palmitie acid (a saturated fatty acid because it contains no double bonds between carbon atoms) and the other side from oleic acid (an unsaturated acid since –C=C– is present).



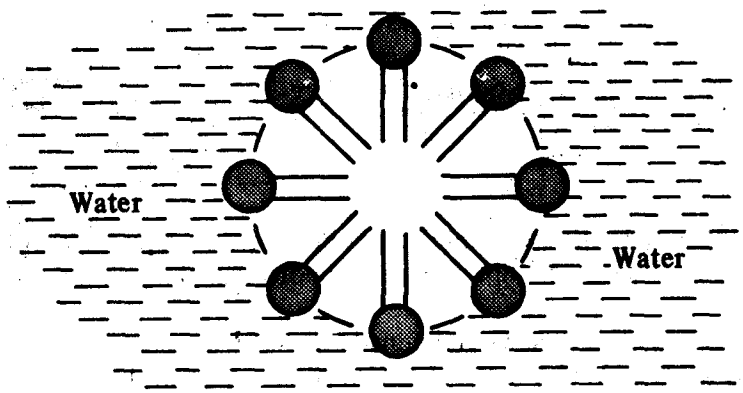
If a thin layer of these molecules is spread over a water surface, how would the molecules orientate themselves with respect to this surface?

With their polar groups (residues) in the water and the insoluble fatty acid residues uppermost.



Orientated molecules at an air/water interface form a monolayer or

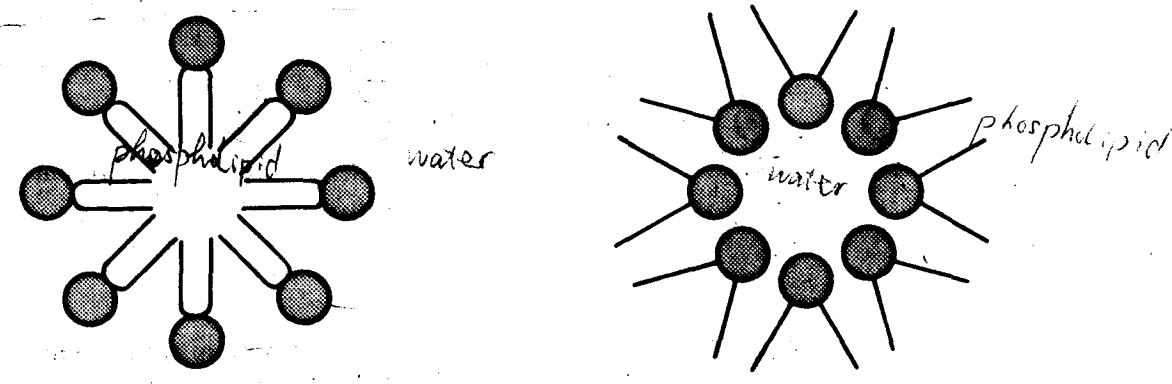
unimolecular layer. When the phospholipid concentration is sufficiently high to more than saturate the interface, micelles are formed, e.g.



7 What would happen (*a*) if a little oil is shaken in an excess of water?
(*b*) if a little water is shaken in an excess of oil?

(*a*) Little drops of oil would be scattered throughout the water.
(*b*) Little drops of water would be scattered throughout the oil.

8 Draw diagrams to illustrate the orientation of phospholipid micelles when
(*a*) a little phospholipid is present in an excess of water
(*b*) a little water is present in an excess of phospholipid.
(*Hint.* Use the answers to frames 6 and 7)



(i) (ii)

9 In 1925, Gorter & Grendel tried to estimate the thickness of the red blood cell membrane. They took a known number of cells (with defined surface area) and extracted the lipids from the red cell plasma membrane. The extracted lipid was then spread over a water surface. The area of the coherent unimolecular film (which was approx. 2 nm thick) proved to be twice as large as the total surface area of the red blood cells from which it was derived.

What explanation can you give for this result?

The result suggests that the lipid component of the plasma membrane consisted essentially of a lipid bilayer (bimolecular film) about 4 nm thick.

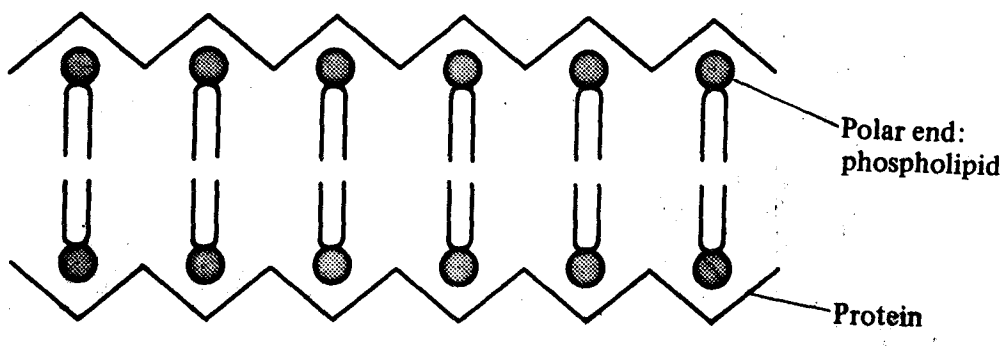
Note. Although Gorter & Grendel made the correct deductions from their experimental data, they actually made an error in their calculations of cell surface area, but this error was cancelled out because (unknown to them) they also failed to extract all the lipids in the red blood cell membranes because the extraction techniques at that time were inadequate. A remarkable coincidence in Science!

10 The other important components of the plasma membrane are proteins. Evidence for their presence is inferred from electron microscope studies together with biochemical analysis.

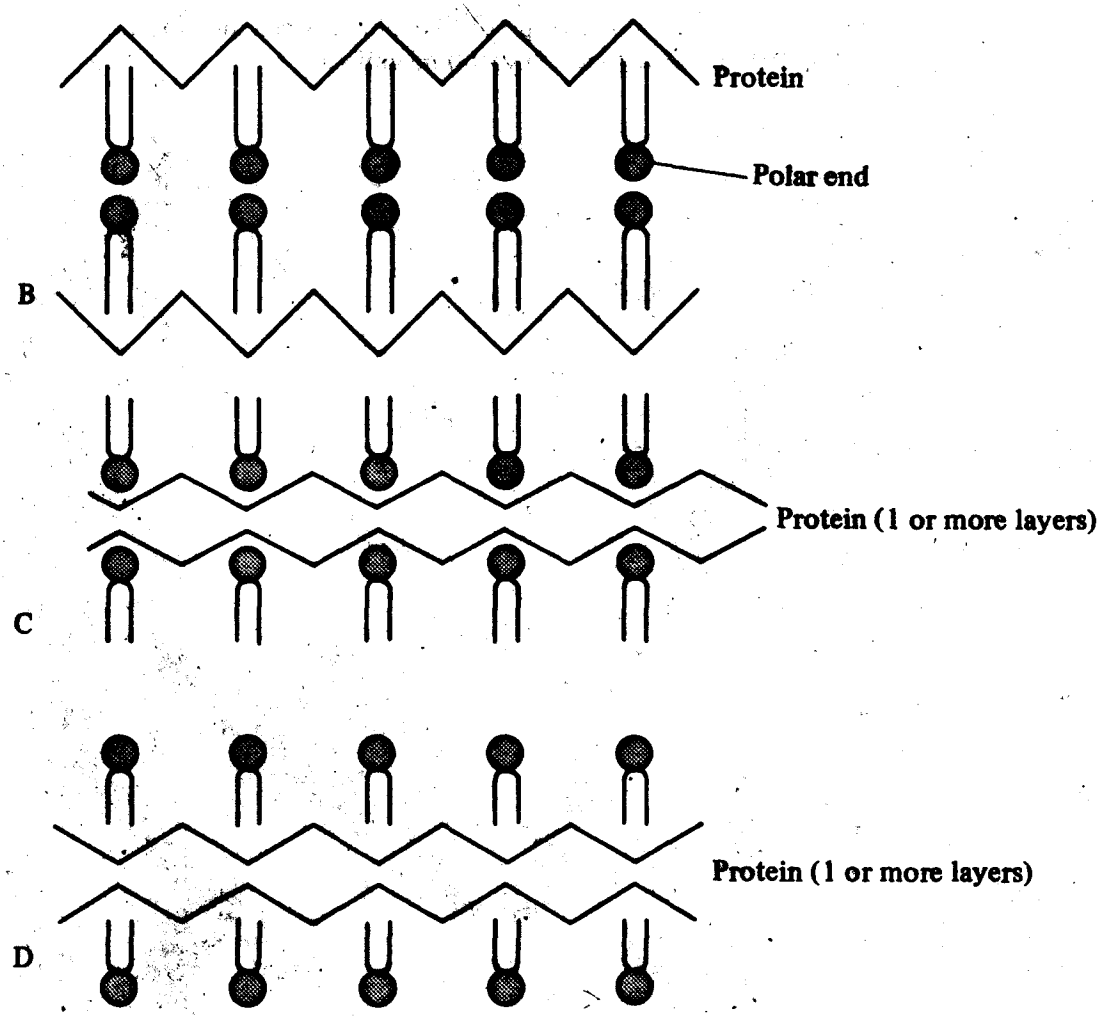
Our evidence so far indicates, therefore, that a lipid bilayer is present together with protein. The plasma membrane is in other words a lipoprotein complex. The question we need to ask is how the protein is arranged in relation to the lipid.

One such model we could propose is as follows:

A



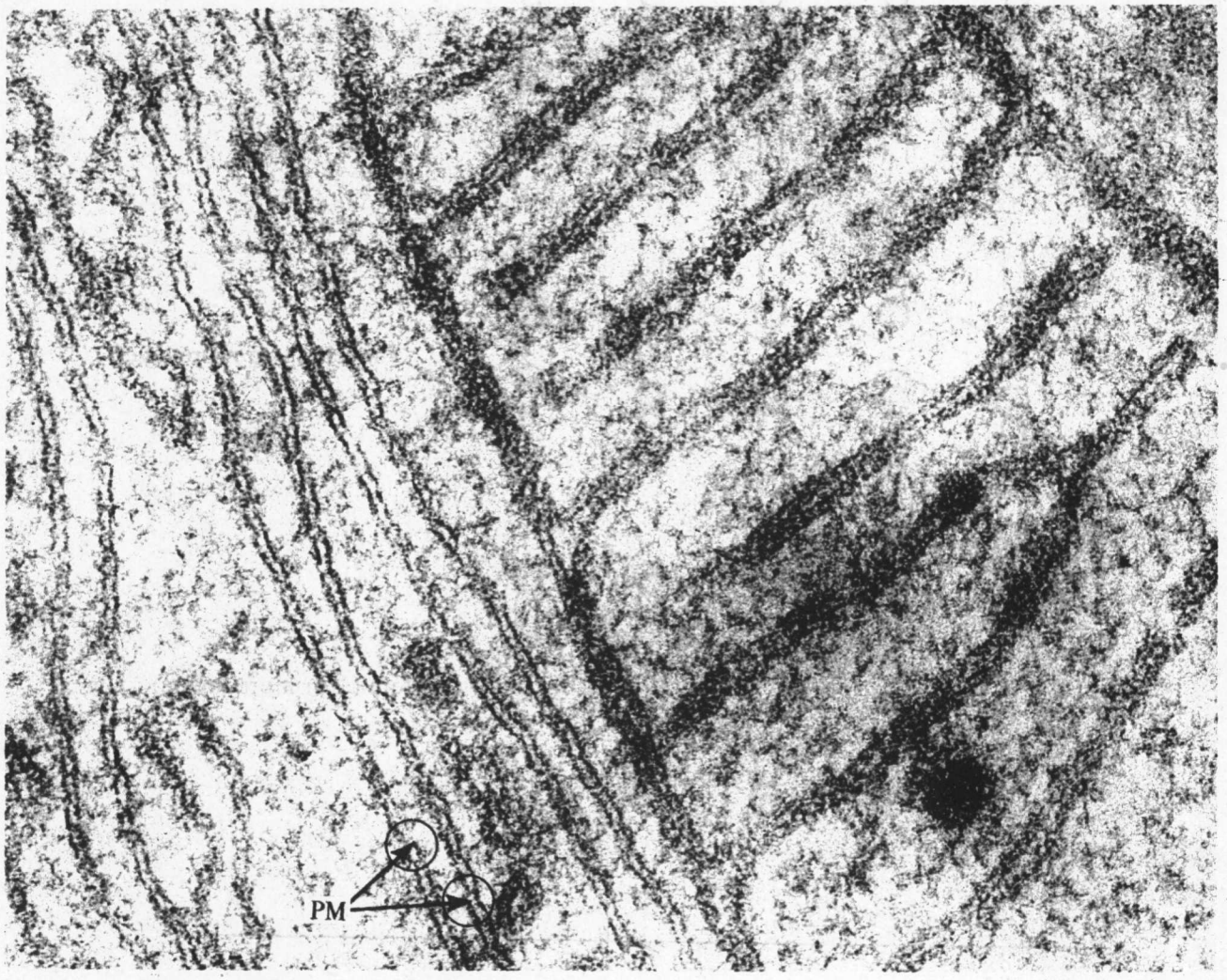
Here the polar lipid heads are linked to the outer protein coat by ionic and/or hydrogen bonds. What other arrangements of the protein in relation to the phospholipid are possible?



11 We mentioned earlier that the environment both within and outside the cell is essentially aqueous. Which of the above models does this piece of evidence rule out?

C. It is unlikely that the non-polar groups of the phospholipids would project into an aqueous medium.

12 Below is an electron micrograph of two adjacent cell membranes (marked PM and arrowed) × 300 000 magnification.

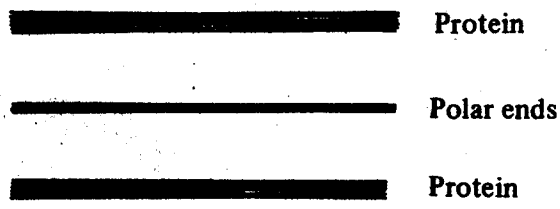


Each membrane (ringed PM) is approximately 7.5 nm thick and would not, therefore, be visible under the light microscope.

The fixative and staining used here reveals the protein as dark bands. Most electron microscopic evidence from work with isolated lipid bilayers suggests that the polar ends of the phospholipid molecules become darkly stained in preference to the non-polar fatty acid residues. On the basis of this evidence refer to the models A, B, C, D proposed earlier (frame 10).

Which model best interprets the electron microscopic evidence?

Model A, in which the non-polar portions of the phospholipid molecules are directed inwards and the lipid bilayer is stabilized by the adsorption of monolayers of hydrophilic protein on the outside. If model B or D were correct we should expect to see three dark bands:



If model C were correct (see previous objection) then only one dark band would be seen.

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13 There is one other feature of importance in animal cells.
Look at the area between the two adjacent cell membranes, it is approximately 15 nm wide. How would you interpret this clearer area? (Seen better on page 12, B.)

(i) It may represent a fluid-, solid- or gel-filled space.
(ii) The plasma membranes may be covered with a thick outer surface coat, which does not react with OsO_4 or $KMnO_4$.
(iii) It may be an artefact produced by fixation of the specimen.

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14 What microscopic evidence could distinguish (i) from (ii)?

The amount of variation in the observed intercellular distance. In fact this is fairly constant at 15–20 nm; and it is now known that the outer layer of protein is coated with a mucopolysaccharide (large complex sugar) forming a mucoprotein or glycoprotein.

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15 In 1959, Robertson proposed a 'unit membrane' hypothesis to account for the basic structure of all biological membranes,* and the model that he proposed resembled A in frame 10.

Much of the physico-chemical evidence for the hypothesis was based on data originally collected by Danielli & Davson in 1935, whilst Robertson's own contribution to the theory was based on electron microscopic evidence as shown in A and B below. In obtaining these excellent micrographs Robertson used potassium permanganate as fixative.

*Although Robertson proposed that all biological membranes have a uniform type of structure, with a characteristic trilaminar appearance under the EM, he was *not* saying that all biological membranes are the same.