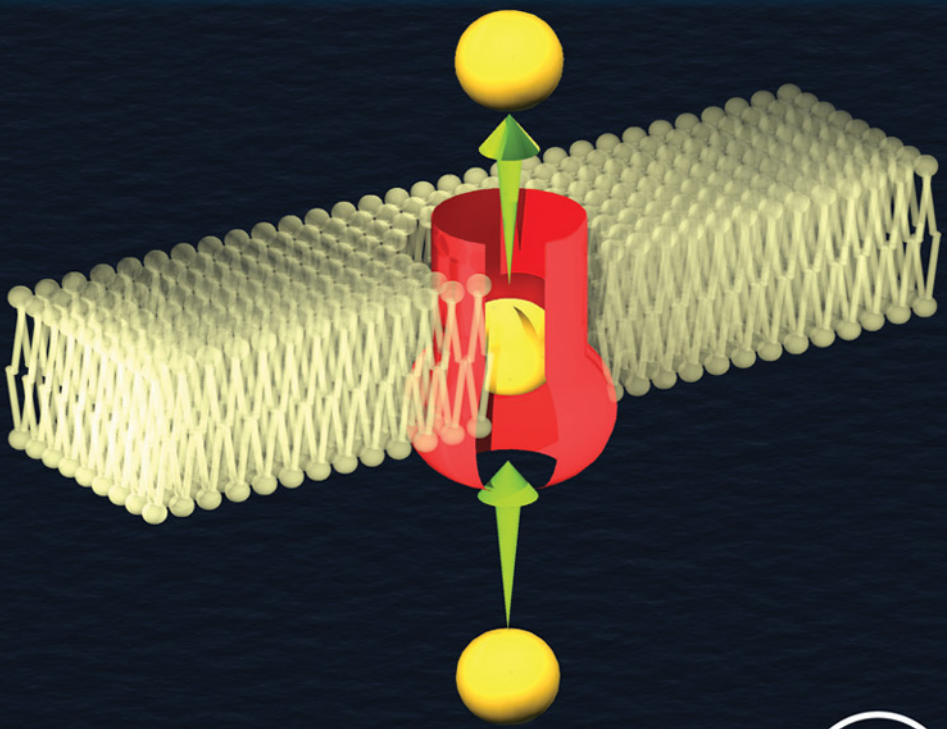
CHANNELS, CARRIERS, AND PUMPS

AN INTRODUCTION TO MEMBRANE TRANSPORT

SECOND EDITION



WILFRED D. STEIN
THOMAS LITMAN

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SECOND EDITION

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AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

First edition 1990

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ISBN: 978-0-12-416579-3

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

Typeset by MPS Limited, Chennai, India
www.adi-mps.com

Printed and bound in the United States of America

The front cover was designed by mmikehortonjerusalem@gmail.com, who also adapted some of the graphical material.

Dedications

To the memory of my friend and colleague William Robert Lieb (1940−2005) for his major contributions over many years to our joint analyses of transport and diffusion

Wilfred D. Stein

In memory of my precious, gentle mentors, Ove Sten-Knudsen (1919−2007) and Bertil Diamant (1930−2005), whose contagious enthusiasm for life and science stay with me each day

Thomas Litman

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Preface to the First Edition

It has been a wonderfully exciting experience, these past three decades, to take part in that great scientific adventure which has led to our present understanding of the channels, carriers, and pumps, the subject of this book. Many of these entities which, 30 years ago, had been mere hypotheses, models, or concepts, have now been isolated and shown to be proteins, and the genes coding for these proteins have been cloned and sequenced. In one or two cases, these transport proteins have been crystallized and their three-dimensional structures determined by crystallographic methods. We know a good deal about transport kinetics and about what regulates transport through the channels, carriers, and pumps. But we do not have, even in one instance, a clear understanding of how these molecules function. We do not know how they distinguish so effectively between their substrates and other similar ions or molecules. We do not know how the transported substrates move through the transporting proteins, nor how these transporting proteins catalyze those movements and, in some cases, link transport to the consumption of metabolic energy.

This book is meant as an introduction to these subjects so that the reader can learn what is already known about them and can prepare to take part in the exploration of the unknown. Readers of this book will thus include those who are about to begin working on membrane transport or have just begun to do so. In addition, those working, for instance, on membrane-bound receptors who want to understand how these receptors function will find this book useful as an introduction to the transport proteins which the receptors help to regulate. The beginning neurobiologist will read it to learn about the function of the channels, carriers, and pumps that are the basis of nervous behavior at the molecular level. The pharmacologist-to-be will find that it provides an approach to understanding cellular and intercellular behavior at the membrane level.

A major aim of the book is to furnish a link between the experimental basis of the subject and theoretical model building. Many examples of experimental work are presented in the illustrations, many taken from original papers, others recalculated and redrawn. Scattered throughout the book are "boxes" in which material of special, often highly current, interest is developed in a detail that might otherwise interfere with the flow of thought in that particular chapter. These boxes are meant, however, to be studied

carefully, and form an integral part of the whole. The selected reference lists at the ends of the chapters are not intended to be comprehensive, but should be sufficient to support the arguments presented and to provide the reader with an entry into subjects that hold a special attraction. My earlier book, *Transport and Diffusion across Cell Membranes*, published by Academic Press in 1986, provides a more comprehensive bibliographic source.

It is a pleasure to acknowledge the help I have received from colleagues who read part or all of earlier drafts of this book. In particular, Hagai Ginsburg, Steven Karlish, and Chana Stein suggested many important improvements. I am much indebted also to an anonymous reader, brought to this task by Academic Press, whose penetrating criticisms, based on a depth of knowledge and understanding, suggested that he or she should really have been the person to write this book! In this and other matters, the staff of Academic Press has been most helpful. Finally, I am very grateful to those who have given permission for the reproduction in this book of their original illustrations, as acknowledged in the figure legends.

Wilfred D. Stein

Preface to the Second Edition

Twenty-five years after the first edition of this book went to press it is, once again, wonderful and exciting to review what has been happening in our field. Whereas previously we had only one or two molecular structures of membrane proteins determined, we now have thousands. As many as 20 structures are known of a single transporter—a calcium pump—in the various states in which it binds its substrate and then finds itself at one and then the other face of the membrane. In the preface to the previous edition we wrote "We do not have, even in one instance, a clear understanding of how these molecules function." Now, we can make convincing models of function for many systems and, with the aid of detailed structural models and molecular dynamics simulations, follow the transport substrates as they cross from one face of the membrane to the other. Many thousands of membrane transporters have had their DNA sequences determined, allowing us to understand the evolution of the transporters and their family interrelationships.

Some things have not changed. Fick's Law is still on the Statute Books. Transport kinetics still provides the basis for posing the problems that structure determinations and molecular dynamics can answer. Thus, we have retained those sections of the first edition which we feel needed little change, but we have described in some detail what we have now learned about structure, the family relationships between the transporters, and evolution. The distinction that the book's title made between channels, carriers, and pumps (for a time questioned) has indeed been strengthened by the new structural information.

Once again, we aim this book at readers who are at the beginning of their work on membrane transport in the hope that its study will help them to make further advances in our field. We envisage that these advances will lie in integrating structure with the process of moving along the transport path itself, to reach a fourth dimension where 3D structure and time are considered together. If there is to be a third edition of this book, surely movies will replace the pictures that lie flat on the present pages.

We have tried to place this book in today's digital world. As far as possible, all first mentions of a membrane protein will show its citation with a ctrl/click. Likewise, all references to the online accessible literature are similarly linked, and protein structures are linked to the Protein Data Bank.

Hard-copy readers are urged to have the online version of the book in easy reach.

The list of those colleagues who have made helpful comments on drafts of sections of this revision includes Suresh Ambudkar, Anthony Carruthers, Flemming Cornelius, Biff Forbush, Lucy Forrest, Hagai Ginsburg, Maria Helena Høyer-Hansen, Ron Kaback, Steve Karlish, Ann Kenworthy, Kazuhiko Kinosita Jr., Hermann Koepsell, Etana Padan, Shimon Schuldiner, Ernest Wright, and Thomas Zeuthen. Of course, the errors that remain are ours. We are most grateful to those publishers who have given permission for the reproduction in this book of their original illustrations, as acknowledged in the figure legends.

Wilfred D. Stein and Thomas Litman

List of Symbols

Symbol	Definition[a]	Page Number[b]
A	Cosubstrate of S	208
A	Membrane area	39
b	Rate of breakdown of carrier–substrate complex	144
C	Electrical capacity	117
d	Distance, or thickness of barrier	44
D	Diffusion coefficient	39
E	Carrier or pump	144
EA	Complex of E with A	207
EAS	Complex of E with A and S	207
ee (superscript)	Equilibrium exchange	151
ES	Complex of E with S	144
f	Rate of formation of carrier–substrate complex	144
F	Faraday constant	46
g	Rate of conformation change of carrier–substrate complex	144
io (subscript)	Parameter in direction inside to outside cell	151
it (superscript)	Infinite trans	151
J	Net flux across membrane	39
k	Rate of conformation change of unloaded carrier	144
K	Affinity	174
K	Partition coefficient	53
K_m	Michaelis parameter	86
L_P	Osmotic permeability coefficient	70
n	Total concentration of carriers	174
oi (subscript)	Parameter in direction outside to inside cell	151
p	Permeability coefficient (GHK equation)	103
P	Alternative substrate	183
P_S	Permeability coefficient of S	51
P	Pressure	45
Q	Charge	117
r	Donnan ratio	335
R	Universal gas constant	45
R (with subscript)	Resistance, transport parameter	174
R	Resistance, reciprocal of diffusion, or permeability coefficient	39
S	Substrate	38
T	Absolute temperature	45
$t_{1/2}$	Half-time	11
U	Chemical potential	45

U°	Standard-state chemical potential	45
v	Unidirectional flux	174
v	Rate of transport or enzyme reaction	86
V	Partial molal volume	45
$\overline{V}$	Partial molal volume of water	70
V_{max}	Maximum velocity	86
Z	Valence	45
zt	(superscript) Zero trans	151
ε	Dielectric constant	82
π	Osmolar concentration	70
ψ	Electrical potential	45
00	(subscript) Zero concentration at both faces	175
1	(subscript) Pertaining to side 1 of membrane	144
12	(subscript) In direction side 1 to side 2	174
2	(subscript) Pertaining to side 2 of membrane	144
21	(subscript) In direction side 2 to side 1	174
I	(subscript) Pertaining to side I	37
I→II	(subscript) In direction side I to side II	38
II	(subscript) Pertaining to side II	37
II→I	(subscript) In direction side II to side I	50

[a]*Symbols used in restricted context are defined locally and are not included in this list.*
[b]*Page on which symbol is first mentioned.*

List of amino acids with their single-letter and triple-letter codes (in **bold type**):

A **Ala**nine	C **Cys**teine	D **Asp**artic acid	E **Glu**tamic acid	F **Phe**nylalanine
G **Gly**cine	H **His**tidine	I Isoleucine (**Ile**)	K **Lys**ine	L **Leu**cine
M **Met**hionine	N Asparagine (**Asn**)	P **Pro**line	Q Glutamine (**Gln**)	R **Arg**inine
S **Ser**ine	T **Thr**eonine	V **Val**ine	W Tryptophan (**Trp**)	Y **Tyr**osine

Chapter 1

Structural Basis of Movement Across Cell Membranes

How do molecules and ions move across cell membranes? How does the cell membrane act as a barrier to such movements? How do special components of the membrane enable specific substrates to overcome this barrier and even allow metabolites to be concentrated within the cell or actively extruded from it? We shall try to answer these questions in this book. But first we need to look at the structure of cell membranes, since this structural information is essential in order to understand how all membrane transport takes place.

1.1 MEMBRANE STRUCTURE: ELECTRON MICROSCOPY OF BIOLOGICAL MEMBRANES

All living cells are enclosed by one or more membranes, which define the cell as a living unit—cutting it off from its environment. Figure 1.1A shows a cross section of the membrane that encloses the human red blood cell. The cell membrane is seen as a double line of material lying diagonally across the photograph. The cytoplasm of the cell is the darker material lying to the right and above this line, while the extracellular environment lies below and to the left of the double line. The thickness of the membrane is some 7.5 nm (75 Å).

Figure 1.1B is an electron micrograph looking down on the surface of the membrane of the human red blood cell. In this case, the membrane was prepared by the "freeze-fracture" method (Figure 1.1C), which allows a researcher to look at the structure within the plane of the membrane. (Cell membranes are rapidly frozen and then fractured with a glass knife. This freeze-fracturing splits the membrane along a plane that appears to lie at the middle of the two lines seen in Figure 1.1A. The frozen and fractured specimen can then be "etched," a process in which the ice layers in a freeze-fractured specimen are sublimed away, exposing also the outermost surface of the membrane.) Look first at the convex surface in the center of Figure 1.1B. This shows the inner surface of half of the membrane, the half that lies on the right of the double line in Figure 1.1A. This is the face that

Channels, Carriers, and Pumps. DOI: http://dx.doi.org/10.1016/B978-0-12-416579-3.00001-0

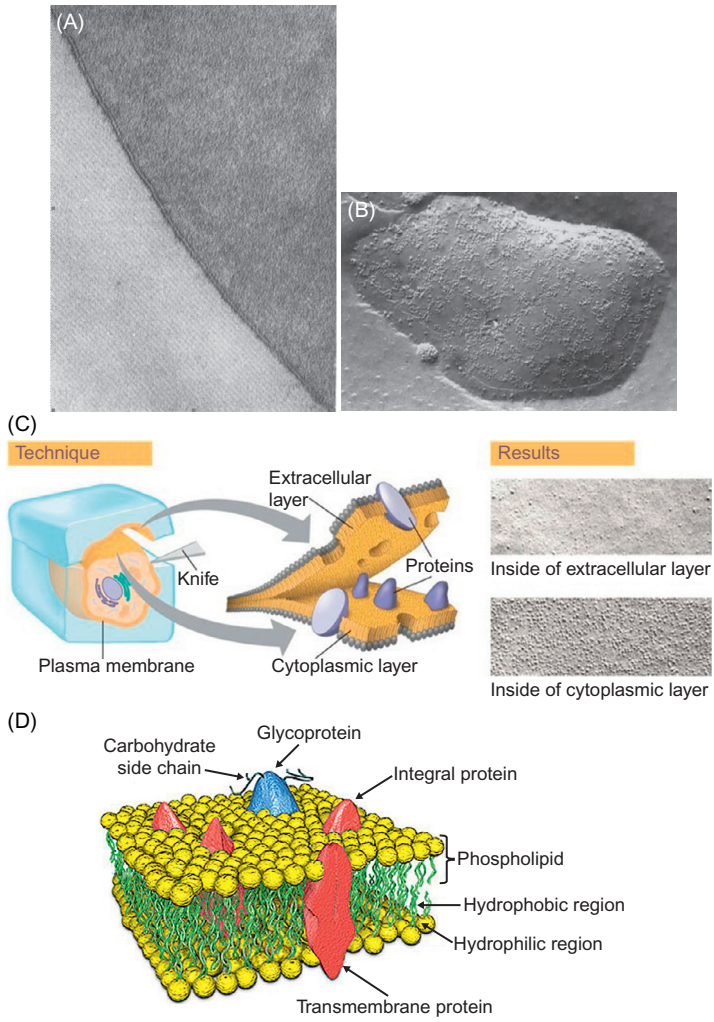

FIGURE 1.1 Structure of the cell membrane. (A) The picture was made using an electron microscope, the cell membrane having been "fixed" prior to microscopy by treatment with a solution of potassium permanganate, a strong oxidizing agent, which reacts with the phospholipid components of the membrane. Magnification: ×280,000. Taken, with kind permission, from Robertson JD, in "Cellular Membranes in Development" (Locke M, ed.), pp. 1–81. Academic Press, New York, 1964. (B) Etched, freeze-fracture image of a ferritin-conjugated red cell membrane. Magnification: ×57,000. Taken, with kind permission, from Branton D, Philos Trans R Soc London B 1971;261:133–138. (C) Diagram to show freeze-fracturing and freeze-etching of a cell membrane. In "Technique," a glass knife is used to split the membrane between its hydrophobic layers. In "Results," the superficial ice is sublimed away to reveal part of the underlying surface. Taken from http://en.wikibooks.org/wiki/Structural_Biochemistry/Lipids/Membrane_Fluidity. (D) Fluid mosaic membrane model. The phospholipid molecules are depicted as the bilayer of yellow heads (the phosphate head-group) with green tails (the lipid region), the protein molecules, as the large irregular shapes embedded in this bilayer. The blue shape is a glycoprotein, with glycosylation sites at its extracellular side. *Taken, with kind permission, from micro.magnet.fsu.edu.*

is adjacent to the cytoplasm of the cell. We can see small globules lying in a smooth matrix material. Around this convex surface is the thin ring of an etched face. This is what one sees from the extracellular medium. We are looking down on the surface that lies to the left of the double line shown in Figure 1.1A. It, too, shows some small globules, somewhat bigger but more sparse than those on the convex surface, embedded in a smooth matrix. (The pale, rather fuzzy surface at the lower left and right edges of the picture is ice, frozen from the extracellular medium. The ice is attached to the extracellular face of the membrane that appears in the picture as emerging from beneath the ice layer.) The small globules shown in Figure 1.1B lie within the center of the double-layered structure in Figure 1.1A and, clearly, also extend out to the extracellular surface. Figure 1.1D is an idealized picture of what the freeze-fracture technique suggests is the structure of the cell's plasma membrane.

What are the materials that compose the cell membrane, this thin but complex structure that separates each cell so effectively from the world around it?

1.2 CHEMICAL COMPOSITION OF BIOLOGICAL MEMBRANES

To determine the chemical composition of cell membranes, we must isolate them. To isolate the plasma membranes (the membranes that bound the cell as opposed to those membranes that bound the intracellular organelles), we first break the cells, bursting them apart by forcing water or bubbles of air into them or else shattering the membranes in a homogenizer. The membranes are then separated from other cell constituents by centrifugation. To prepare membranes from the cellular organelles (mitochondria, chloroplasts, endoplasmic reticulum (ER), lysosomes, and endocytic vesicles), the organelles themselves must first be separated from other cell constituents by differential centrifugation and their membranes then isolated as for the plasma membrane (for more on the cellular organelles, see Alberts et al., 2007). Chemical analysis of the purified membranes shows that they are made up of lipids and proteins, together with smaller amounts of associated carbohydrates. The lipids form the smooth matrix of the image seen in Figure 1.1B; the proteins are the globules seen in that micrograph, with the carbohydrates being attached to some of the lipids or proteins. Different membranes vary greatly in their relative proportions of lipids and proteins, ranging from 20% protein in the case of the myelin layers of membranes that surround nerve cells to 75% protein for the inner membrane of the mitochondrion. In general, those membranes that are the most active metabolically have the greatest proportion of protein. The lipids in any cell membrane are a complex mixture (as we discuss below), and the proteins are very varied. Lipids isolated from cell membranes spontaneously form structured aggregates in water. Certain types of these aggregates, the liposomes, appear in cross

section in the electron microscope rather similar to the double-layered images seen in Figure 1.1A and, in freeze-fracture (Figure 1.1C), as a featureless, complementary pair of plane surfaces. This is the basis for the view that it is the lipid of the cell membrane that forms the matrix, almost uniform in structure, depicted in Figure 1.1B. In contrast, the globular structures seen in Figure 1.1B can be shown by more indirect methods to be proteins. (Indeed, they are visible in Figure 1.1B only because of their attachment to ferritin, an iron-containing molecule and itself a protein, which can be bound specifically to the membrane proteins.) Biological membranes are thus made of a matrix of lipid molecules into which are inserted proteins. Other proteins may be attached to this fundamental structure (Figure 1.1D).

1.2.1 Membrane Lipids

Figure 1.2 shows the chemical structure of a few of the lipids that have been isolated from biological membranes. Many of the most common lipids found in biological membranes are built on the backbone of a glycerol molecule,

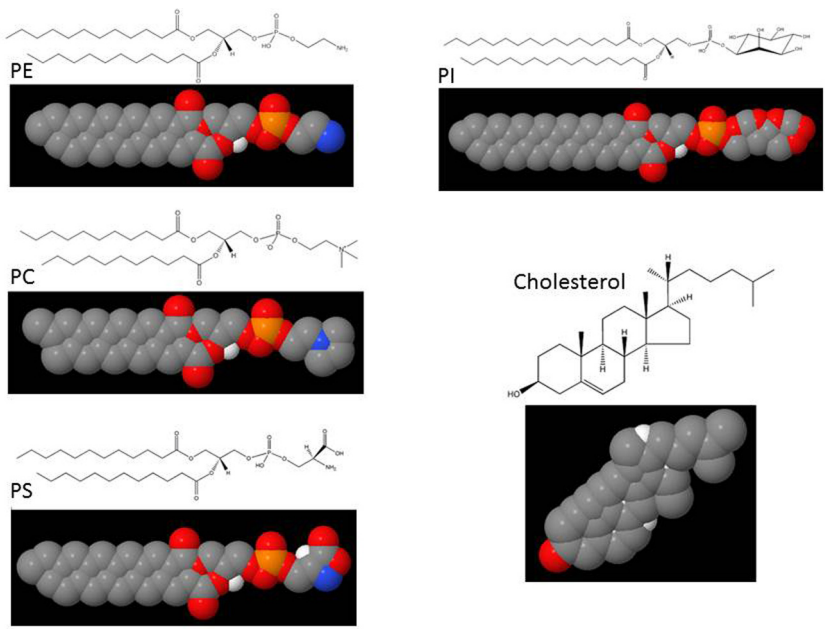

FIGURE 1.2 Structure of some common lipids. The zigzag lines above each subfigure represent the hydrocarbon backbone of the lipids. Below each, these same molecules as 3D structures. PE is phosphatidylethanolamine, PC is phosphatidylcholine, PS is phosphatidylserine, and PI is phosphatidylinositol, with cholesterol also shown. For dynamic visualization of these lipids, just click on (in the on-line version of this book) the links below: PE, PC, PS, PI, and Cholesterol.

esterified at one end by a phosphate residue and, at the other two hydroxyls, by fatty acids.

Lipids of this general class are called phospholipids. The charge on the phosphate can be neutralized by its further esterification by a chain bearing an amino group or choline, or the whole molecule can retain a net negative charge. Half of the fatty acid chains are saturated; the others carry one, two, or more double bonds. Apart from the fatty acid esters listed in Figure 1.2, the sterol cholesterol (seen on the right half of Figure 1.2) is an important constituent of many animal cell membranes; ergosterol takes its place in plant cell membranes. All of these lipids (and the many other types found as more minor constituents in biological membranes) have the essential physical characteristic of a hydrophilic ("water-loving") portion that interacts strongly with water (the phosphate head-group, in many cases, or the hydroxyl-containing portion of the molecule in some other lipids that are not phosphate esters) and a hydrophobic ("water-hating") portion that inserts into an aqueous environment only with difficulty, if at all (the fatty acid chains and all except the hydroxyl residue of the sterol). Such molecules, which have both a polar and a nonpolar portion, are described as being amphiphilic (or, what is the same thing, amphipathic)—penetrating both into the water and into the nonaqueous media.

1.2.2 Membrane Proteins

Many membrane proteins are firmly bound to the membranes and can be removed only by treatment with strong detergents. These are the "intrinsic" or "integral" proteins. The fact that the intrinsic proteins require detergent treatment to release them from cell membranes suggests that, within the lipid matrix, they are bound to the lipid hydrocarbon chains. The detergent, in fact, replaces the hydrocarbon of the lipid. Other proteins are more loosely attached to the cell membrane. They can be removed by treatment with solutions of low ionic strength, often containing EDTA (ethylenediaminetetraacetic acid), to chelate divalent cations. In red cells, for instance, where half the weight of the dried membrane is protein, some one-third of this protein is lightly attached and can be removed by the above treatment. Such loosely bound proteins are known as "extrinsic" or "peripheral" proteins. Many extrinsic proteins are bound to the membrane by being bonded to the intrinsic proteins.

1.2.3 Membrane Carbohydrates

Membrane carbohydrates are attached to the proteins, to the nitrogen of asparagine or the hydroxyl oxygen of hydroxylysine, hyrdoxyproline, serine, or threonine to form the glycoproteins, or to some of the lipid classes, forming the glycosphingolipids. Protein-bound carbohydrate residues are on the extracellular surface of the cell membrane and take part in cell−cell interactions, including those of the immune system.