The Biophysical Characterisation of the Cell Surface

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Preface

"கற்றது நகை மண்ணள
கல்லாதது உலகளவு"

ஔவையார்

What we have learnt is a fistful of earth
What we are yet to learn is as vast as the Earth*

St Avvaiyyar
(Tamil Poetess 11th Century, India)

A major part of the research activity in the field of membrane biology has been concerned with the cell surface and has involved the characterisation of the components of the membrane surface, their organisation and topographical distribution. The cell surface owes this privileged position to its ubiquitous participation, mediated by the macromolecular components, in diverse biological events such as cell division, growth, differentiation, morphogenesis, neoplasia, cell recognition, antigenicity, and in the communication of environmental information to the cell. Therefore little need be said in justification of a book which aspires to review the field of cell surface biology, notwithstanding the limitations imposed on it by the state of my knowledge and interpretation of the events. This branch of membrane biology is so vast that I have approached it with a sense of humility which has been impressed on me by the quoted verse written by the 11th Century Tamil poetess St Avvaiyyar,* and which also serves to emphasise the finite state of our current knowledge of the complexities of biological phenomena.

The growth of science and the advance of scientific thought has, as the history of science would show, been generally non-uniform, with bursts

* Translation by courtesy of Dr M. S. Lakshmi.

of scientific activity interspersed with relatively more quiet periods. The peaks of activity have nearly always accompanied the invention and development of new technology. Bernal's* description of science as "ordered technique" aptly describes this association. Therefore science as a whole or any of its branches can be treated in two different ways, namely as unfolded by technical innovation and advance or as a compilation of observation and discussion. In this book I have taken the former course, and have discussed the theoretical aspects of some biophysical methods and have examined their application in the characterisation of the cell surface. I have then attempted a collation and integration of the different kinds of data relating to the cell surface in its normal state and as affected by some disease processes. I hope I have succeeded in giving the book a sense of cohesion rather than let it appear as a mixture of methods and results. In the main the book is about the cell surface as visualised by a number of bioelectric and electrokinetic techniques.

The scope of this book is wide simply by virtue of the subject being treated. Although the book would appear somewhat specialised in the sense that it deals only with the cell surface, I expect that it would prove its relevance in several fields of study such as cell differentiation, embryology, cancer research, cell biology, immunology and virology. It has been intended for use at the research level but I feel confident that it would prove useful also at the undergraduate level. If indeed it did, I would consider the time taken to write it well spent.

I am grateful to my many friends and colleagues who read through parts or whole of the manuscript and offered valuable criticism. I would like especially to acknowledge the help I received from the late Professor J. A. V. Butler, FRS; Professor David Kessel; Dr M. S. Lakshmi; Professor J. S. Mitchell, FRS; Professor K. R. Rees and Dr P. A. Riley. I am thankful to the large number of fellow scientists and publishers who most graciously allowed me to reproduce their published data and figures. Most of the work in my laboratory while at the University College Hospital Medical School and at the Chester Beatty Research Institute was done in collaboration with Dr Lakshmi without whose help perhaps there would have been little research of my own, and without whose constant encouragement I could not have written this book. I received financial support for my research from The Beit Memorial Fellowship, The Damon Runyon Memorial Fund, The Lord Dowding Fund for Humane Research, The Medical Research Council, The Peel Medical Research Trust, The Tenovus and The Williams Fellowship and

*Bernal, J. D. (1959) "Science in History". Penguin Books, England, p. 3.

the Central Research Fund of London University, to whom I am deeply indebted. Finally, I would like to thank Academic Press for the most cordial treatment accorded to me and to my book. It has been a considerable pleasure to work with them.

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Abbreviations

ADP	Adenosine diphosphate
ALS	Antilymphocyte serum
APF	Aggregation promoting factor
CCD	Counter current distribution
Con A	Concanavalin A (lectin from *Canavalia ensiformis*)
c.p.m.	counts per minute
CPDS	6,6′-Dithiodinicotinic acid, carboxypyridine disulphide
DAB	Dimethylaminoazobenzene
DFP	Diisopropylfluorophosphate
DMSO	Dimethylsulphoxide
EB virus	Epstein-Barr virus
EDTA	Ethylene diaminetetraacetic acid
EF	Encephalitogenic factor
EI	Ethyleneimine
EKZ	Electrokinetic zone
EM	Electron microscope
EO	Ethylene oxide
EPM	Electrophoretic mobility
e.s.u.	Electrostatic units
FDNB	Fluorodinitrobenzene
5-HT	5-Hydroxytryptamine
IEF	Isoelectric focusing
IEZ	Isoelectric zone
LPS	Bacterial lipopolysaccharide
LVD	Low viscosity dextran
MEM	Macrophage electrophoretic mobility
MSF	Macrophage slowing factor
MW	Molecular weight
NANA	*N*-Acetylneuraminic acid (sialic acid)
NANase	Neuraminidase (RDE, receptor destroying enzyme)
4-OHA	4-Hydroxyanisole
PAGE	Polyacrylamide gel electrophoresis

PAS	Periodic acid-Schiff reagent
PEG	Polyethylene glycol
PFU	Plaque forming unit
pH	Hydrogen ion concentration
pI	Isoelectric point
pIE	Isoelectrophoretic point
pII	Isoionic point
pK	Ionisation constant
PO	Propylene oxide
PPD	Protein derivative of tubercle bacillus (used as antigen in macrophage electrophoretic mobility test)
PPHE	Post-pH equilibrium
PTSC	Paratoluenyl sulphonyl chloride
PVS	Polyvinyl sulphate
Py	Polyoma virus
Py3T3	Polyoma virus-transformed 3T3 mouse fibroblasts
RDE	Receptor destroying enzyme (neuraminidase)
RNA	Ribonucleic acid
RNAase	Ribonuclease
RSPD	Receptor saturation pI differential value
RSV	Rous sarcoma virus
SDS	Sodium dodecyl sulphate
SL	Stationary level (phase) in electrophoretic cell
SV-40	Simian virus-40
SV-CHK	Simian virus-40-transformed Chinese hamster kidney cells
SV-TRK	Simian virus-40-transformed rabbit kidney cells
SV-3T3	Simian virus-40-transformed 3T3 mouse fibroblasts
TU	Tiselius unit for EPM ($=10^{-5}$ cm $sec^{-1}V^{-1}$cm)
WGA	Wheat germ agglutinin

Symbols

A	Hamaker constant, area
Å	Ångström (1 Å = 10^{-8} cm)
d	Thickness of electrical double layer
D	Dielectric constant of water (78·54 at 25°C)
D	Diffusion constant
e	Electronic charge $4{\cdot}8 \times 10^{-10}$ e.s.u.
η	Viscosity of solvent
E	Potential gradient in V cm^{-1}
E_m	Membrane potential
f	pH compensation factor for calculating EPM from isoelectric data
F	The Faraday 96 500 coulombs mol^{-1}
H	Distance between particles
i	Current in amperes
I	Ionic strength
k	Boltzmann constant ($1{\cdot}3803 \times 10^{-23}$ $J°K^{-1}$)
K	Specific conductance
K	Debye–Hückel function; partition coefficient
M	Molarity of solution, gram mole
N	Normality of solution
N	Avogadro's number ($6{\cdot}023 \times 10^{23}$ mol^{-1})
P	Potential of the surface of particle
ψ	Potential at the interface
Q	Net surface charge
r	Radius of curvature of particle
R	Molar gas constant (8·3144 J mol^{-1} $°K^{-1}$)
R	Resistance in ohms
S	Svedberg unit
σ	Electrical charge density
t	Time
T	Absolute temperature (absolute zero = −273·15°C)
v	Electrophoretic mobility
V	Velocity; volume
V	Volt

X Field strength
ζ Zeta potential
z Valency of ion

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4. Cell Electrophoresis

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1. Membrane Structure and Organisation

INTRODUCTION

Cellular membranes perform several functions essential to the life of the cell, and account for 80% of the dry weight of a cell (O'Brien, 1967). Membranes may be subdivided into three groups, namely the plasma membrane, the cytoplasmic and the organelle membranes. The plasma membrane forms the interface between the cell and its environment and maintains the structural integrity of the cell as a stable but dynamic unit and acts as a complex control system for the passage of water, electrolyte ions and other materials required for the metabolic activity of the cell. The plasma membrane also serves as a link in the communication of environmental information to the cell and controls cell division, growth and metabolism. In addition, it plays a significant role in differentiation and morphogenesis, and in cell recognition and antigenicity. Most of these functions are mediated by the macromolecular components of the membrane. Characterisation of cell membrane components and elucidation of their topographical distribution and organisation have therefore formed a major part of research in membrane biology. This area of research is so vast and the growth of the literature so rapid that it would be too ambitious to attempt to survey the whole field. This book is therefore restricted to the discussion of biophysical data, especially bioelectric and electrokinetic, relating to the cell membrane. The purpose of this chapter is to provide a brief description of the salient features of the structure and organisation of the membrane, in order to put the discussions in subsequent chapters in proper perspective. An exhaustive and complete discussion of this subject may be found in the recent reviews by Nicolson (1974a,b, 1975, 1976a,b).

MEMBRANE STRUCTURE

LIPID BILAYER STRUCTURE

Overton (1895) first suggested that membranes were composed of lipids. This was based on the readiness with which lipid-soluble substances penetrated the plasma membrane of the cell. In 1927 Gorter and Grendel extracted lipids from erythrocyte membranes. When these lipids were spread as a monolayer at an air–water interface, they covered an area twice as much as the erythrocyte surface area. This observation led to the postulation of the lipid bilayer. But the surface tension of the cell membrane is much lower than if the membrane had consisted of the lipid bilayer alone. Thus from considerations of surface tension, permeability characteristics and electrical conductivity measurements, Danielli and Davson (1935) deduced that the lipid bilayer is coated on both sides by proteins. The structure of unimolecular films of phospholipids and cholesterol at air–water interface indicated that these lipids were orientated in such a way that their polar groups projected into the aqueous phase. Therefore the lipid bilayer was visualised as a bimolecular leaflet with its non-polar fatty acyl chains orientated inwards perpendicular to the membrane surface. The polar groups of phospholipids were postulated to occur at the external surface, coated in addition by proteins and polysaccharides. This was the early concept of membrane structure generally accepted as the "sandwich" or "unit" model (Robertson, 1959; Davson and Danielli, 1952).

MICELLAR STRUCTURE

Alternative proposals for membrane structure include the globular or hexagonal micelle structure. Electron microscopy has revealed globular or hexagonal micelles in some membrane systems (Sjorstrand, 1963a,b,c; Lucy and Glauert, 1964). Sjorstrand described globular components of approximately 50 Å diameter in membranes from mouse kidney cells, and proposed that membranes may be composed of these globular units with protein molecules between them. This possibility was supported by the earlier finding of Fernandez-Moran (1957) and by subsequent work of Gent *et al.* (1964), Robertson (1963) and Blasie *et al.* (1965). Lucy and Glauert (1964) suggested, on the basis of their work on artificial lipid mixtures, that penta- or hexagonal micelles of lecithin and cholesterol occurred in plasma membranes. Pores existed between the lipid micelles. Proteins, of course, were postulated to occur as a layer on the surface.

FLUID MOSAIC MODELS

The lipid bilayer concept, although an attractive one and accounts for several properties of the cell membrane, does nonetheless present a static picture of membrane structure. It appears from recent work that many components of the cell surface are liable to and capable of rapid redistribution (Fig. 1). Surface immunoglobulins and antigens show aggregation and patching (Taylor, 1971; De Petris and Raff, 1972, 1973; Davis, 1972; Edidin and Weiss, 1972; Kourilsky *et al.*, 1972) leading to the endocytosis of some of the immunoglobulin complexed with the surface (De Petris and Raff, 1974). In heterokaryons produced by cell fusion, an intermixing of the surface antigens occurs in 30–40 minutes at 37°C (Frye and Edidin, 1970; Edidin and Weiss, 1972). Cell surface receptors of lectins are also known to move laterally and aggregate (Comoglio and Filigamo, 1973; Inbar *et al.*, 1973b; Inbar and Sachs, 1973; Nicolson, 1972a, 1973, 1974b; Bretton *et al.*, 1972; Rosenblith *et al.*, 1972; Garrido *et al.*, 1974).

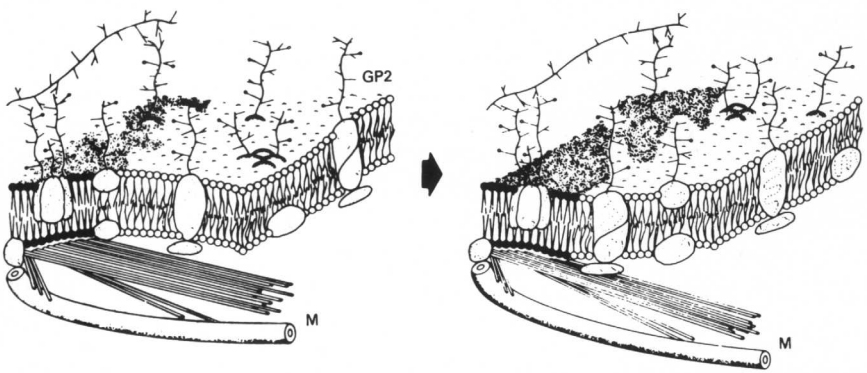

Fig. 1. Fluid mosaic model for the cell membrane showing lateral diffusion of glycoprotein complex GP2. M is membrane-associated cytoskeletal elements (from Nicolson, 1976).

The lipid bilayer concept has since been refined and restated by Singer and Nicolson and described as the fluid mosaic model (Singer, 1972; Singer and Nicolson, 1972; see also Vanderkooi, 1973; Vanderkooi and Green, 1970). This model takes into consideration the motional properties of lipid molecules arranged in a bimolecular leaflet. These dynamic properties include (a) rapid internal motion within each lipid molecule, (b) a lateral diffusion of molecules in the plane of the bilayer, (c) a rotational motion along the axis of the molecule and (d) a "flip-flop" motion of the moleculė from one side of the membrane to the other.