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J.B. FINEAN and R.H. MICHELL

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Preface

In the former series of Comprehensive Biochemistry the contributions of membranes to cellular biochemistry were considered in a volume entitled *Cytochemistry* (1964) in which the organelles of the cell were considered individually. Since that time the study of membranes has formed one of the most rapidly expanding fields of biology, and this volume is devoted to a consideration of only one aspect of this progress, namely our current understanding of the relationship between membrane structure and function. Other aspects of membrane biochemistry will be discussed in forthcoming volumes on *Phospholipids* and on *Membrane Transport*. One of the outstanding features of recent research on membrane structure has been a transition from the marked polarisation of views that characterised the 1960s towards a general agreement during the 1970s that all membranes share one basic form of structural organisation. The aims of this volume are to identify general features of membrane structure, to discuss in considerable detail some selected aspects that have been studied intensively in recent years, and to relate some of this molecular information to individual membrane functions.

We anticipate that most of our readers will already have a general knowledge of cell structure and of the roles of individual membranes and organelles in particular cell functions. For those who lack this background information, we would recommend reference to brief monographs such as *Membranes and their Cellular Functions* (J.B. Finean, R. Coleman and R.H. Michell, 2nd ed., 1978, Blackwell, Oxford), *The Biochemistry of Cell Organelles*, (R.A. Reid and R.M. Leech, 1980, Blackie, Glasgow and London) and *Biological Membranes* (R. Harrison and G.G. Lunt, 2nd ed., 1980, Blackie, Glasgow and London).

J.B. Finean
R.H. Michell

Birmingham, August 1980

Contents

Isolation, composition and general structure of membranes

J.B. FINEAN and R.H. MICHELL

Department of Biochemistry, University of Birmingham
P.O. Box 363, Birmingham B15 2TT, U.K.

1. Historical introduction

Awareness of the existence of a discrete plasma membrane at the surface of cells gradually emerged as cell biologists of the late nineteenth century observed a variety of plant cells and single cell organisms and probed their cell boundaries using both physical and chemical techniques [1–3]. From his studies of the permeability of cells to a variety of non-electrolytes, Overton [3] was even able to speculate on the lipid nature of the permeability barrier.

The first significant chemical study of a membrane was not reported until 1925, when Gorter and Grendel [4] extracted lipid from erythrocytes and spread it as a monolayer at an air–water interface in order to compare the area that it might potentially cover with the total surface area of the original erythrocytes. A fortuitous mutual cancellation of experimental errors allowed the correct conclusion that there was sufficient lipid to form a lipid bilayer over all or almost all of the surface of the cell. The probability that the lipid of biological membranes exists predominantly in bilayer form was later reinforced by physical measurements (optical and electrical) made both on biological membranes and on isolated lipid (mainly phospholipid) systems [5]. This has since remained the dominant theme in considerations of membrane structure.

The initial suggestion that protein would probably be closely associated with lipid in plasma membranes (and maybe also in other membranes) was again a speculative one based on surface tension measurements and on the spontaneous association of water-soluble proteins with monolayers of lipid spread at an air–water interface. Although there was no relevant information on the protein components of membranes, Danielli and Davson proposed a general structural scheme [6] for cell membranes which featured a bilayer of lipid coated at its aqueous interfaces with layers of protein. Their first suggestion that protein might penetrate into or through the lipid layer [7] was not based on any direct knowledge of membrane proteins, but was simply a speculative attempt to account for the occurrence of facilitated permeation of solutes through plasma membranes.

Early thoughts on membrane structure were confined to the plasma membrane

Finean/Michell (eds.) Membrane structure

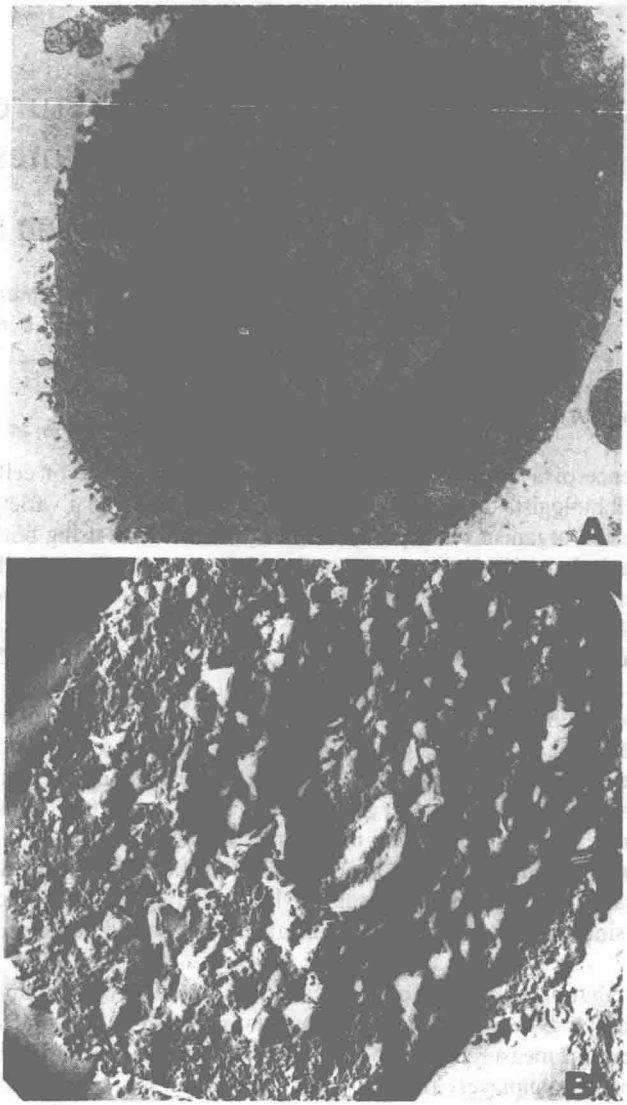

Fig. 1. Electron micrographs of liver cells (hepatocytes) isolated by the procedure of Seglen [37]. (A) Lead citrate-stained section of cells fixed with 1% OsO_4 and 1% tannic acid. ×51000. (B) Freeze-fracture replica of unfixed cell preparation. ×57000.

[8,9]: the more extensive elaboration of membrane-bounded compartments within the cell was not recognised until the 1950s when improvements in the preparation of thin sections of tissues for examination by electron microscopy indicated a similar

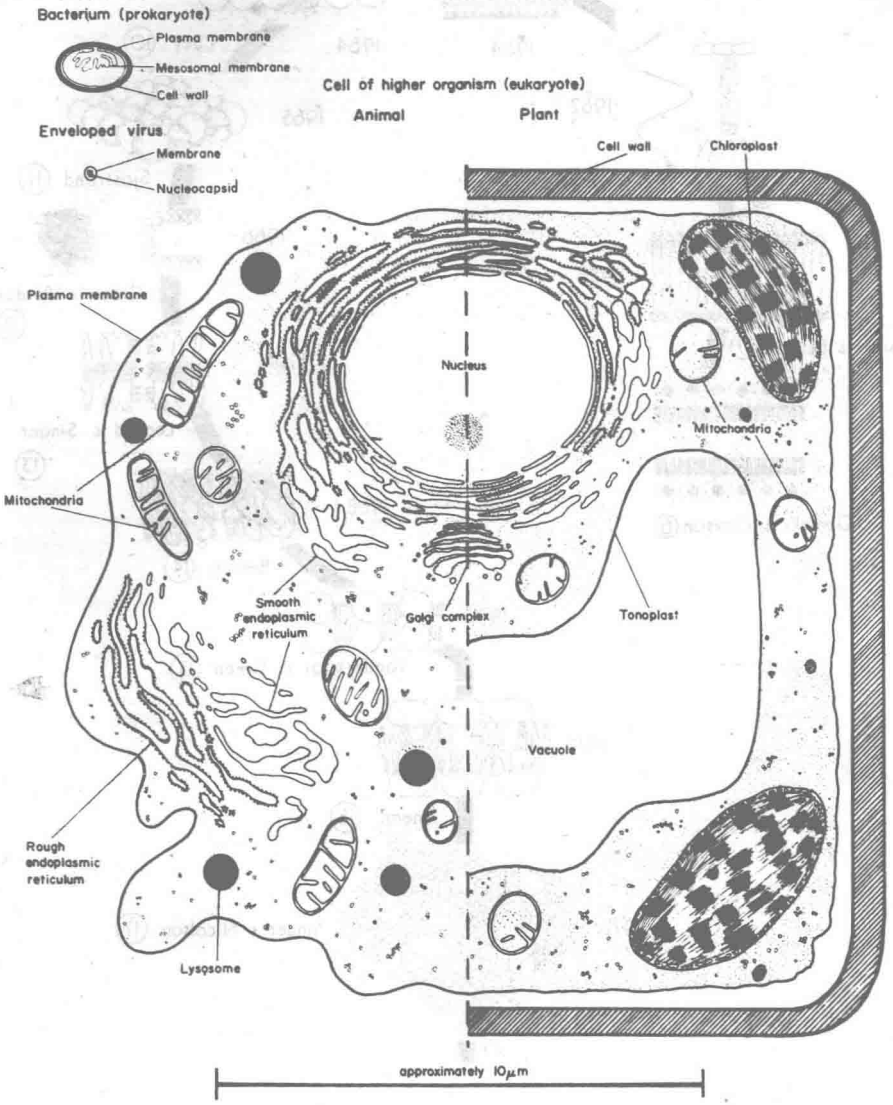

Fig. 2. Diagrammatic illustration of variety of organelles in plant and animal cells as revealed by electron microscopy (from [44]).

general form for both plasma membrane and the membranes of cytoplasmic organelles (Figs. 1 and 2). This emphasised the limitations of the Danielli and Davson membrane model [6] in accounting in structural terms for a much greater range of functions, and hence inspired the proposal of alternative structural arrange-

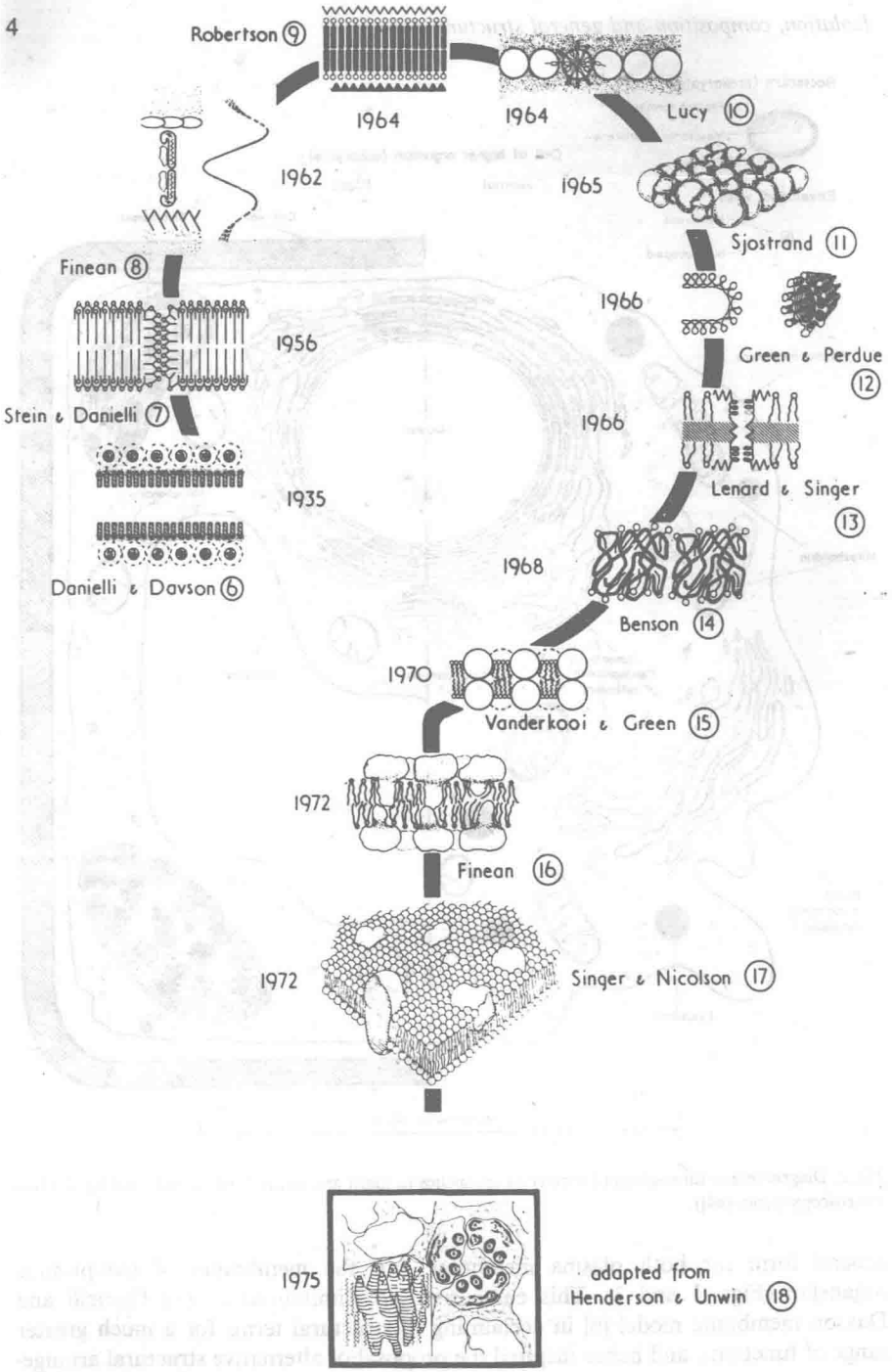

Fig. 3. Diagram illustrating the chronological order in which the most influential models have been proposed.

ments (Fig. 3). In particular, it was realised that: (a) under appropriate conditions some lipids would adopt configurations other than a bilayer; (b) the fine detail of membrane structure as seen at high magnification in some electron micrographs appeared granular; and (c) membranes were dissociated into lipoprotein "particles" by detergent treatment. This encouraged speculation, especially by biochemists, that membranes might consist of laterally aggregated arrays of globular lipoprotein "subunits" (e.g. [10–12,14]).

It has only been as a result of the relatively recent progress in characterisation of membrane proteins that substantial agreement on a general model of membrane structure has been reached. In particular, the identification of membrane proteins in which substantial exposed regions are dominated by non-polar amino acid side chains led to the realisation that such regions would be likely to associate with hydrocarbon regions of the membrane lipid phase; parts of these proteins might therefore be inserted deep into the membrane interior. This, together with an emphasis on disordered or fluid packing of the lipid hydrocarbon chains and on free lateral diffusion of membrane components, was then featured in a new membrane model, the "fluid mosaic" model proposed by Singer and Nicholson [17] in 1972. This has since been generally accepted as a more realistic expression of the general characteristics of membranes than any previous model. It may well be the last of the generalisable membrane models, because experimental work on membranes has now advanced to the stage at which the structural patterns of individual membranes are being defined in some detail [18]. As a result, we now know that individual membranes differ both in the spatial distributions of their molecular components and in the mobilities of these components.

2. *Isolation of membranes*

Some studies of membrane structure can be made using membranes still organised into cells; such studies include microscopical examination of membrane organisation in cells and of membrane-cytoskeletal interactions, X-ray diffraction analysis of cells which possess ordered membrane arrays, some types of measurement of the mobilities of membrane components, and labelling experiments designed to probe the asymmetric orientations of surface membrane components. However, purified membrane preparations are needed for most studies of the chemical composition or spatial organization of membranes.

(a) Criteria for assessing purity

For studies of chemical composition, the chief criterion is that membrane preparations should be pure samples of a single type of membrane [19] but studies of membrane structure also demand that samples of isolated membranes should preserve the spatial interrelationships between different molecules that prevail in the intact, healthy cell. These constraints upon the purity of membrane preparations

used for structural studies are often much more stringent than the requirements to be met by membrane preparations in which the attribute of interest is some organelle-specific function (e.g. an enzyme activity) that can be adequately studied even when the membrane exhibiting it exists only as a component of a membrane mixture. For membranes which contribute substantially to the total membrane complement of cells, achievement of homogeneity requires a purification of only a few-fold, and appropriate techniques may not be unduly complex or difficult to devise. Many membranes, however, constitute only a very small proportion of the total mass of the parent cell, and in such cases very substantial purification (sometimes 50- to 100-fold, or even more) is required to yield a small amount of homogeneous material for analysis.

The monitoring of membrane purification basically consists of following the purification of the required membrane by monitoring some membrane-specific criterion, associated with the simultaneous measurement of a variety of additional criteria specific for all of the possible contaminant structures. Occasionally the morphology of a particular membrane structure remains sufficiently distinctive, even after homogenisation, for electron microscopy and/or phase contrast microscopy to provide a reliable guide to purification (e.g. mitochondria, rough endoplasmic reticulum, intestinal epithelial brush borders, secretory vesicles), but much more often the isolated membrane fragments do not retain a morphology that is sufficiently characteristic for their unequivocal identification (e.g. smooth membrane fragments may come from, among others, smooth endoplasmic reticulum, plasma membrane or Golgi complex). In most cases, therefore, the progress of the required membrane and of contaminants through a fractionation procedure is followed by the assay of a variety of membrane-specific or "marker" criteria; these are usually enzyme activities known to be confined to particular membranes in the cell under study (see, for example, [19] and [20], section 1 of [21], Chapters 1–4 of [22]). A membrane preparation should only be adjudged "pure"; (a) when the purification achieved corresponds to that which would be estimated from consideration of the morphology of the parent cell, and (b) when the concentrations of all known contaminating membranes, as assessed by the activities of their characteristic marker enzymes, have been reduced to levels where it can confidently be calculated that they contribute very little of the mass of the isolated membrane preparation.

In going from an homogenate to an isolated subcellular fraction, such enrichment or depletion in terms of particular membranes is usually expressed in terms of Relative Specific Activities (RSAs) of the chosen marker enzymes, these RSAs being the ratios which compare the specific activities in the final fraction(s) to the specific activities in the initial homogenates [23]. In interpreting RSAs, it is essential to remember that the mass contributed by a particular stucture to an isolated fraction is a function both of the experimentally determined RSA and of the contribution of the particular organelle to the mass of the parent cell. To illustrate this, consider a simplified cell with only two membrane systems, a plasma membrane that contains 1% of the cell protein and mitochondria which contain 20%. From this cell one isolates an 80-fold purified plasma membrane fraction in which the RSA of the

mitochondrial marker enzyme remains 1.0, as in the original homogenate; 20% of the material in this substantially "purified" fraction is contributed by mitochondria. A second fraction from the same cells has a mitochondrial marker RSA of 4.75, but is also enriched 5-fold with respect to the plasma membrane marker: reference to the composition of the original cell shows, however, that 95% of the material in this "contaminated" sample is derived from mitochondria.

(b) The choice of isolation media and of the starting material

In designing a subcellular fractionation scheme with which to isolate a particular membrane, there are a number of technical obstacles to be negotiated. The parent cells must be available in sufficient quantity and adequate purity, a method must be devised for breaking the cells in an appropriate, usually osmotically protective, medium, and physical techniques are required by which the desired membrane can be isolated from the homogenates.

Within cells, membranes normally exist in an aqueous medium rich in small ions and proteins. However, on dilution in an homogenate this high protein concentration is lost. In addition, few cell fractionations are undertaken in predominantly ionic media since such media often cause aggregation of organelles and thus impair the separation. The most common media for subcellular fractionation are physiologically iso-osmotic (approx. 300 mosM) or hyperosmotic solutions of non-permeant neutral solutes such as sucrose or mannitol. A notable exception to this custom is provided by skeletal muscle, where the polymerisation of actinomycin in low ionic strength media means that an ionic medium is sometimes (e.g. [24]), though not always [25], used for the isolation of Ca^{2+}-pump-rich sarcoplasmic reticulum. In addition, mammalian erythrocyte surface membranes, the most widely studied of all membranes, are normally isolated in ionic media (either with or without a divalent cation chelator such as EDTA or EGTA), but most of these diverse media are of lower than physiological ionic strength and osmotic activity [26]. Most of the time, the possible effects on membrane composition and structure of using non-physiological and non-ionic media for membrane isolation are largely ignored, but experience with the red cell suggests that such uncritical attitudes may ultimately have to be abandoned. For example, erythrocyte ghosts made in media of physiological ionic strength and containing small concentrations of divalent cations [27] (or returned rapidly to physiological ionic strength after lysis at lower ionic strength [26]) may be compared with ghosts isolated in almost ion-free media, often in the presence of EDTA or EGTA (e.g. [28]). The former, especially after incubation at 37°C to "reseal" them, tend to be resilient spheres or even somewhat biconcave, they are impermeable to most materials which do not permeate the intact cell, they generate and sustain ion gradients, and they retain the "cytoskeletal" layer of spectrin and actin at their inner surface [26,27,29]. The latter, by contrast, adopt rather irregular shapes, are "floppy" and readily vesiculate, are deficient in spectrin and actin, are permeable even to macromolecules, and may carry "extra" membrane-associated proteins that have become adsorbed at low ionic strengths (e.g.

haemoglobin and maybe also glyceraldehyde-3-phosphate dehydrogenase; see Chapter 5) [26,28,30,31]. Such detailed information on the damaging effects of transferring membranes into environments strikingly different from those prevailing within cells appears to exist only for the erythrocyte plasma membrane, but it might be anticipated that other membranes, especially other plasma membranes, might behave similarly. Renewed attempts to devise effective subcellular fractionation procedures with which to isolate membranes and organelles in media of physiological ionic strength and composition might well yield remarkably interesting, and maybe disquieting, insights.

For details of appropriate isolation conditions for individual membranes, it is usually necessary to consult primary journals: leads into these, and occasionally technical details, can be found in reviews or compilations such as refs. 20–22, 32, 33, and Section 1 of [34]. The starting material for subcellular fractionation of animal cells can be a solid tissue, a population of free-living cells grown in tissue culture, or a suspension of free-living cells from the body: examples of the latter include various types of blood cells and various cell-types which either occur naturally or can be grown in the peritoneal cavity (e.g. macrophages, mast cells, polymorphonuclear leukocytes or free-living neoplastic cells such as Ehrlich ascites). Body fluids normally contain mixed cell populations, so a preliminary to subcellular fractionation is usually the isolation of one cell type in homogeneous form: appropriate techniques include differential and/or density gradient centrifugation, free flow electrophoresis and differential adsorption onto some surface which differentiates between cells as a result either of their intrinsic adhesiveness or their ability to bind to some selective surface-specific ligand (e.g. a lectin or cell-directed immunoglobulin): see, for example, section VB of [35].

Most solid tissues are also heterogeneous, both due to the presence of blood (which can be removed by perfusion) and to the presence of more than one intrinsic cell population. Although this heterogeneity is often ignored, there has been a marked tendency in recent years for individual cell populations to be isolated from tissues before functional studies are undertaken. This has allowed, for example, the properties of hepatocytes [36,37] and of Kupffer cells [38] from mammalian liver to be studied separately. Although potential disadvantages of such techniques include the smaller amounts of starting material that are usually available and the possibility that the tissue dissociating techniques may damage molecular components exposed on the surface of the cells, it is to be hoped that this approach may soon be more widely adopted when isolating membranes for structural studies. There are various techniques for weakening the forces or structures (e.g. collagen fibrils) that hold cells together prior to dissociation of tissues to form cell suspensions, of which the most useful are treatments with either chelators such as EDTA (e.g. [39]) or collagenase (e.g. [36,37]). With some tissues, it is already customary for isolation of a pure cell suspension to precede subcellular fractionation (e.g. fractionation of adipocytes, rather than heterogeneous adipose tissue [40].

When a cell to be fractionated possesses a substantial cell wall (e.g. bacteria, fungi or higher plants) which may both impede its homogenisation and render purification