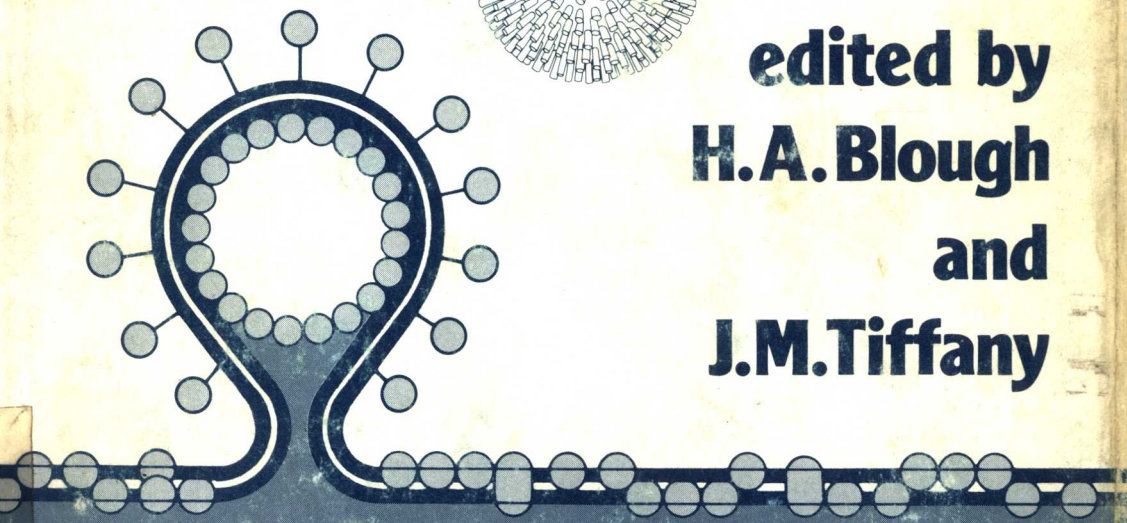
Cell Membranes and Viral Envelopes

Volume 1



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H.A.Blough
and
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Preface

Over the past twenty or twenty-five years, a very large body of information has been accumulated on the composition, assembly, structure and function of both cell membranes and viruses, and the manner of synthesis and assembly of their component parts. More recently, improvements in instrumentation, and techniques of *in vitro* assembly of membrane components, have led to increased knowledge of the dynamic aspects of membrane function. Books about the composition and properties of viruses and membranes tend to deal mainly with one or the other topic, rather than attempting to cover both, or at least the middle ground between them. Yet it is impossible to consider the attachment of a virus particle to a cell, its engulfment or penetration through the cell membrane, and all the subsequent steps of the replicative cycle culminating in assembly and release of virus progeny, without some knowledge of the nature of the membranes involved in these steps. This is particularly true of the enveloped viruses, in which the infectious nucleic acid is surrounded and protected by a lipoprotein shell with functional groups on its outer surface. The envelope is, in the majority of cases, assembled at a cell membrane, and derives some, if not all, of its lipid from the membrane. Knowledge of the structure of the parent membrane, of the manner of insertion and post-translational modification of newly synthesized materials, and of the associative properties of typical membrane constituents, is obviously then of great importance in studying viral envelope assembly. Conversely, because viral envelopes contain representatives of the same types of molecules as are found in membranes (lipids of all classes, glycoproteins, etc.), but have a much smaller range of functions and number of components, and can also be obtained in high purity, the envelope can be used as a simplified model in membrane studies.

In view of the reciprocal interaction of structure and function, it seems to us of the greatest importance to have as clear an idea as possible of the chemical nature of the constituents of cell membranes and envelopes, in addition to functional information—we might term this the *what* and *where*, as well as the *how* or *why* (or even the *when*) of these systems. A vast amount of this information is available in the literature, and reviews of various aspects are published with ever-increasing frequency. The degree of detail in these is often far greater than is needed by any save those who are working

directly in the field of the review (and who, in any case, are likely to be familiar with the primary literature). Thus we feel there is still a need for a work drawing together review chapters on composition, biogenesis and assembly, and ways of determining these, for both membranes and virus envelopes. It is unfortunately inevitable, because of the delay between writing and publication of any book, that new information will have accrued or that new techniques will have been introduced. This book has, for a number of reasons, taken a long time to compile; some of the contributors have fully updated their material, while others have added only brief mention of new developments. We hope, however, that the groundwork laid here will still be found of substantial value despite refinements of detail.

The book is divided into two volumes, largely for convenience in handling. The first volume deals mainly with membranes and the second with viral envelopes, although they are in no way intended to stand separately as a membrane book and a virus book. In Volume 1, the opening chapters deal with some of the more effective methods of investigation of the structure of membranes (and also the application of these methods to viruses), and discusses the results obtained so far in this very active field of enquiry. X-ray diffraction and spectral probe methods, despite their difficulties of interpretation, can be expected to continue to advance our knowledge of both static and dynamic structures, while electron microscopy remains probably the most powerful single technique for the determination of structure. The later chapters in this volume deal with the main structural components of the normal cell membrane; these are divided for convenience into proteins, lipids and carbohydrates, although one must expect some overlap between these categories. Synthesis, composition and assembly, and associative or structure-forming properties are considered. Of the last two chapters, one deals with the problems of reconstructing a functional membrane system (in this case of viral origin) from its components; this approach goes a step further in relation to function than the many experiments in recent years on the formation of bimolecular lipid layers incorporating a single protein. The remaining chapter in this volume considers membrane fusion, which lies at the heart of almost all membrane-related phenomena, including viral penetration and release, cell division, pinocytosis, cellular secretion, and many other cellular functions.

The first half of the second volume deals with both compositional and biosynthetic aspects of lipids, proteins and carbohydrates in viral envelopes. The next four chapters deal in much greater detail with examples of some of the most intensively studied groups of enveloped viruses, as a means of showing the application of the methods of analysis described in earlier chapters to specific problems of structure. The final chapter reviews the information available about the interaction of viruses with cell membranes.

Despite the length of this work, a number of interesting topics have had to be omitted, and some readers may feel slighted if it is their favourite which has been dropped. Chapters 2–4 in Volume 1 obviously do not contain all the major techniques of structure determination, but rather some of those which have so far made substantial contributions to our knowledge of structure. We have almost entirely omitted material relating to the replication process within the cell, and properties of viral or cellular nucleic acids. The membranes referred to are in almost all cases the cell plasma membranes, and not those of specialized organelles. While as editors we regret this, we must accept that complete coverage would make the book far more expensive and would require a multi-volume format. The chapters included here contain a sufficient number of references to recent and more specialized reviews for the interested reader to follow up.

Among the many people who have helped in the preparation of this work, we should particularly like to thank Mrs Drita Taraila, Miss Lu Foley and Mrs Dolores Scholz for their typing and review of manuscripts; Ms Charlotte Urbano for her careful proof-reading of all the manuscripts, galley-proofs and page-proofs, including the many revisions introduced into these; Ms Jane Broughan for undertaking the immense task of compiling the index. Herbert A. Blough is especially indebted to Dr Harold G. Scheie, Founding Director of the Scheie Eye Institute and Emeritus Professor of Ophthalmology, University of Pennsylvania, for his encouragement during the preparation of the text and his keen appreciation of the need for interdisciplinary studies of the kind we have endeavoured to provide here.

December 1979

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Contents of Volume 1

Contents of Volume 2

1. Introduction

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The interaction of membranous viruses with the plasma membrane of susceptible cells has opened Pandora's box to the widest range of phenomena that reflect the structure, function and control principles of biological membranes. When one considers each of the component events in the cyclical process which starts with the invasion of the cell by a single virus and which terminates with the extrusion from the cell of large numbers of copies of the same virus, it is obvious that both the viral membrane and the plasma membrane must be endowed with a multiplicity of capabilities to make possible this cyclical process. Any acceptable theory of membrane structure and function must provide a rationalization for all these capabilities. It may be useful merely to catalogue the capabilities that the viral cycle requires, be it in the host membrane or in the viral membrane, or in both. The host membrane must contain a receptor protein that will react selectively with the complementary molecule(s) in the envelope of the virus and this interaction must initiate a sequence of structural and functional changes which allow the virus to enter the host membrane. Thus, the host membrane must contain a complex biochemical system which, when triggered by interaction with a viral protein, will in turn trigger the operation of a control mechanism that sets in motion the changes in the membrane prerequisite for entry of the virus into the membrane. Intrinsic to viral invasion of the cell and viral release from the cell is the phenomenon of the fusion of viral and host membranes and of the "defusion" of such fused membranes. Thus, both the viral and host membranes must have the capability for undergoing a cycle of fusion and defusion and both must respond to the same ions (e.g. Ca^{2+}) that physiologically induce this cycle. The "uncoating" of viral nucleic acid as it enters the cell and the encapsulation and envelopment of viral nucleic acid as it leaves the cell are two manœuvres that undoubtedly call for specialized molecular devices, both in the host and viral membranes. The introduction

into the cell membrane of proteins whose synthesis is virally induced and which are earmarked to be intrinsic to the viral envelope again is a process that is crucial to the tactic of virus formation. This cannot be a random process and to imagine anything other than a very precise and controlled sequence of events would be a serious error of judgement. The same considerations would apply with equal force to the introduction into the cell membrane of the complement of lipids which will be associated with the viral proteins. In the phase when the cell is committed to the synthesis of viral proteins, nucleic acid and lipids, there has to be a continuous flow of precursors from the plasma into the cell and this flow requires a galaxy of transport systems in the plasma membrane, both active and passive. We must also presume that the viral membrane itself has a modicum of transport capabilities essential for the life cycle of the virus. Finally, the structural characteristics of the viral membrane—the highly ordered and regularly spaced arrays of projections from the membrane, the wrapping of the membranous envelope around the nucleic acid in the interior space, the interdigitation of protein and lipid in precise proportions in the viral membrane—all reflect precision assembly and the minimization of random interrelationships of the component elements. One could extend even further the list of capabilities and properties that the viral and plasma membranes must possess to implement the viral invasion cycle, but what has been enumerated is sufficient to make the point that only a very sophisticated model of membrane structure and function could be equal to the task of rationalizing this multiplicity of capabilities and properties possessed by both the plasma and viral membranes.

As our understanding of the nature of energy coupling in transducing membranes has grown, more and more structural and functional features of transducing membranes have had to be invoked. There is thus a close parallelism between developments in the field of viral membranes and developments in the field of transducing membranes with respect to the more stringent requirements which an acceptable model of membrane structure and function must meet. The era of primitive models which deal with one limited aspect of membrane structure is over and the era of strict accountability for *all* relevant aspects of membrane structure and function has begun.

Is a viral or a mitochondrial membrane *sui generis*, or do all membranes share common constructional principles adaptable to the full range of functions subserved by different membranes? At present, no one could answer that question with finality, but at least it can be said that biochemistry has taught us that the most fundamental principles—e.g. the principles of energy coupling and heredity—and the fundamental molecules of the cell—e.g. the vitamins, the coenzymes, ATP, the building blocks of proteins and

nucleic acid—are universal (Green and Goldberger, 1967). By that token, the history of biochemistry is on the side of the universality of the principles of membrane construction and our further remarks will be premised on that universality. At the level of fundamental constructional principles, all biological membranes, regardless of their function, may be assumed to be alike.

It would be useful to sketch a few of the concepts which will have to be woven into an acceptable model of membrane structure and function. The two basic components of membranes are proteins and lipids (predominantly phospholipids). We may think of protein and bilayer phospholipid as separate though closely associated domains, but not all the lipid is in the bilayer lipid domain. A significant moiety, 20–30% in the case of the mitochondrion, is encapsulated within membrane-spanning lipoproteins which are intrinsic to the protein domain. The lipid in these lipoproteins appears to play a very central role in membrane function, particularly in the transport of ions and polar molecules (Tyson *et al.*, 1976). Membrane-derived glycoproteins are usually lipoproteins and several glycoproteins have already been implicated in a variety of transport phenomena (Sandri *et al.*, 1976).

Until very recently, the idea of the complexes and proteins of the protein domain spanning the membrane (Green *et al.*, 1972) was strongly resisted, but by now, it is generally accepted that the protein domain, like the bilayer phospholipid domain, spans the membrane. In the protein domain, there can be sets of proteins which collectively span the membrane or individual proteins such as lipoproteins which can span the membrane. The protein molecules which are in the interior of the membrane or which span the membrane must have an amino acid composition (low polarity on their external surface) compatible with such an assignment (Vanderkooi and Capaldi, 1972). A large number of intrinsic protein molecules with such low polarity have in fact been isolated from membranes.

In addition to the intrinsic proteins, there are the extrinsic membrane proteins which are electrostatically linked, to either the lipid or protein domain, and readily extracted from the membrane in high salt solutions.

The preoccupation of workers in the membrane field with bilayer phospholipid has delayed recognition of the important functional role of lipid encapsulated within lipoproteins or within crevices in the proteins of the protein domain. Blough and Tiffany, in their incisive review of viral envelopes, have emphasized this functional aspect of viral lipids (Blough and Tiffany, 1975). The discovery of the so-called boundary lipid (lipid with immobilized fatty chains) has pointed up that a significant moiety of the total lipid is not in the bilayer modality (Jost *et al.*, 1973) and that such immobilized lipid may serve as a continuous phase with solvent properties in the interior of proteins. Lipoproteins with hollow interiors filled with such encapsulated lipid can thus provide internal tracks for ionophore-dependent

movement of ions and polar molecules across the membrane or for movement of ions between fixed protein components in the membrane (Green, 1974).

We are just at the beginning of defining the characteristics of the protein domain. In membranes with highly ordered arrays of projecting elements (the mitochondrion, sarcoplasmic reticulum, viral membranes) there is electron microscopic evidence that the protein domain is a continuum of repeating units—each unit being associated with a projecting element. In the mitochondrion, the continuum nature of the protein domain now rests on very solid evidence, both biochemical and electron microscopic (Green, 1976). The continuity of the protein domain rationalizes the well known cooperative behaviour of membranes such as the plasma membrane and the response of an entire membrane to interactions at one receptor site.

The two main functions of the plasma membrane (transport and immunochemical) need not be carried out by a single protein continuum. There could be separate continua—one for transport and the other for immunochemical processes (Green, 1976). In the mitochondrion, there are indeed separate continua for the electron transfer chain and for the systems which implement coupled synthesis and hydrolysis of ATP, as well as transport, both active and passive (Green, 1976).

Finally, the notion of a control mechanism subject to regulation by Mg^{2+} and Ca^{2+} has to be added to the now well established capabilities of membrane systems. Membranes with such control mechanisms can exist in two alternative states with different functional capabilities (Southard and Green, 1974; Hunter *et al.*, 1976). It is the protein domain that fluctuates between these two states and that is subject to the control mechanism. The phenomenon of fusion and defusion may well depend upon such two-state transitions—Ca^{2+}, for example, favouring fusion, and Mg^{2+} favouring defusion. In the mitochondrion, the transition is triggered by the collapse of the projecting headpiece-stalk into the membrane (Green, 1976). Is a comparable cycle possible for the projecting elements of the viral membrane?

These are a few examples of the new concepts which will have to be woven into the warp and woof of future models of membrane structure and function. One would do well to take *cum grana saltis* many of the models currently in vogue.

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2. Electron Microscopic Methods of Investigating Membrane and Virus Structure

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1. INTRODUCTION

Since Danielli and Davson (1935) proposed a model of a unit membrane containing a bilayer of lipids, some other models of a more or less static membrane structure have emerged (Robertson, 1966; Sjöstrand, 1970; Bretscher, 1971; Bretscher and Raff, 1975). However, it has become apparent that biological membranes represent dynamic systems (Capaldi, 1974) displaying many specialized functions. Another interesting model proposed by Singer and Nicolson (1972) as the fluid mosaic model describes movement of structural as well as functional proteins in the plane of the membrane. Such a dynamic system of membrane components necessitates instantaneous fixation of membranes prior to a direct study by electron microscopy.

The aim of this chapter is to discuss the significance and applications of biological transmission electron microscopy particularly by the use of low-temperature preparation and labelling techniques, and scanning electron microscopy. A survey is also given on image analysis by conventional optical diffraction or by electron diffraction on hydrated membranous material. Possible image analysis by a computer for the future study of membranes is also included (Goldfarb and Frank, 1978).

2. GENERAL CONSIDERATIONS ON BIOLOGICAL ELECTRON MICROSCOPY

A. Resolution

Although modern transmission electron microscopes have a resolving power of approximately 0·3 nm (3 Å), the resolution in biological material is usually limited to approximately 2–3 nm (Beer *et al.*, 1975). Occasionally a resolution of 0·7 nm has been achieved on a regular array of biological molecules (Unwin and Henderson, 1975). This discrepancy between instrumental and practical resolution is due to some problems in handling of the specimen and imaging. The scanning electron microscope, on the other hand, usually allows a resolution of 15 nm (Hearle *et al.*, 1972; Reimer, 1978). Since the specimens are usually examined in a vacuum they have to be chemically or physically fixed prior to dehydration and analysis. Furthermore, the contrast in the specimen has to be increased, because membranes are composed of elements

FIG. 1. (a) A schematic drawing of a positively stained particle, with heavy atoms bound to its surface, placed on a support film. The projection of an osmium-stained cholesterol particle is shown in the image plane. (b) A schematic drawing of a negatively stained particle surrounded by an unevenly distributed, heavy metal derivative. The projection resulting from electron scattering through a cholesterol particle, surrounded by neutralized phosphotungstic acid, is shown in the image plane. (c) *Above*: Isolated membrane fragments of human erythrocytes negatively stained by 1% ammonium molybdate. *Below*: Purified membrane proteins of *Acholeplasma* (see Johansson and Hjertén, 1974).