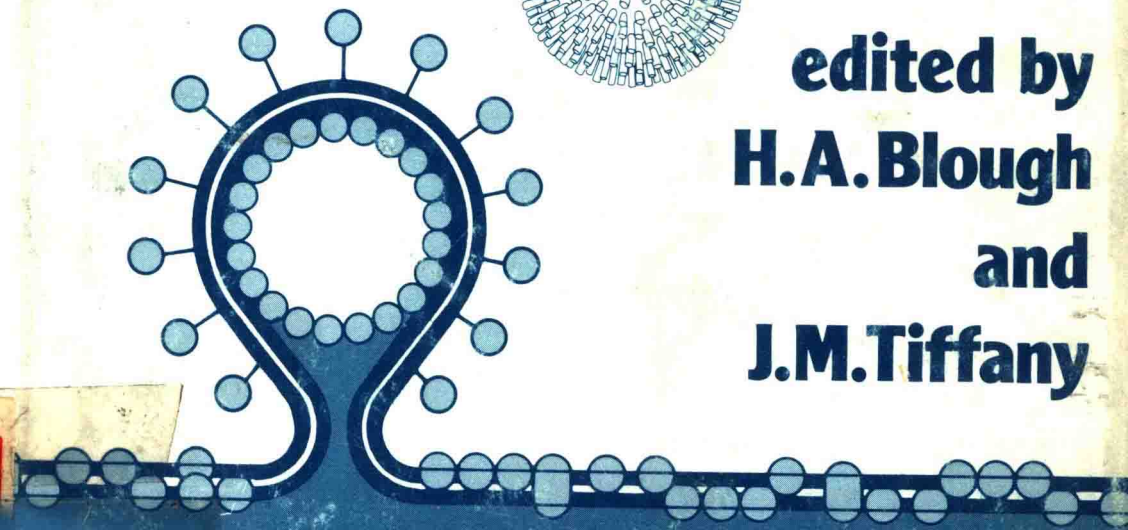
Cell Membranes and Viral Envelopes

Volume 2



edited by
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

11. Composition and Function of Viral Lipids

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1. GENERAL INTRODUCTION

The viral envelope is the region of the virus particle which bears proteins and glycoproteins responsible for the viral functions of release from the host cell and attachment to and penetration of other susceptible cells. The envelope also has the function of protecting the inner genetic component of the virus during transfer from one host cell to another. In most groups of lipid-containing viruses, physical integrity and infectivity are lost or significantly reduced following treatment with detergents or lipid solvents such as ether. Lipid is thus an important structural component of the envelope, and a detailed knowledge of its composition, in relation to that of the region of the cell in which the envelope is assembled, is of importance in elucidating aspects of viral production, structure, stability and function. Other chapters in this book deal in greater detail with membrane lipids and with the biosynthesis of

viral lipids, and with detailed aspects of several virus groups. In this chapter we shall consider: (1) the sites and sequence of assembly of a number of virus classes, and their lipid composition so far as this is known at present and (2) the organization of these lipids in the envelope. Information is limited largely to mammalian viruses and a very small number of others such as the marine bacteriophage PM2. However, similar information is slowly accumulating on a much larger number of lipid-containing plant and invertebrate viruses, some of which we have listed in our earlier review (Blough and Tiffany, 1973); unfortunately too few data are generally available for these virus groups to be included here.

2. LIPID ANALYSES

A. Introduction

(*a*) *Methods of Analysis*

To date, no major difference in lipid classes has been found between viral envelope lipids and other membrane lipids, although certain significant changes occur which can be related to a particular host system or to growth conditions. The major obstacles in analysis of viral lipids are, therefore, the need to establish freedom from contamination of the viral preparation by extraneous lipids (see below) and the available quantities of lipid. This latter factor is particularly important in the case of minor components such as certain glycolipids, which may make up only 0·1–0·2% of total virus dry weight. Methods of lipid extraction do not differ materially from those used for membranes (e.g. Folch *et al.*, 1957; Kritchevsky and Shapiro, 1967; Weinstein *et al.*, 1969, 1970; Blough and Tiffany, 1973), and so are not given here; standard texts may be consulted for the methods of lipid analysis (e.g. Marinetti, 1967; Kates, 1972; Christie, 1973).

(*b*) *Prerequisites for Analysis*

(*i*) *Purity of virus preparations.* Virus particles are generally released from the host cell into the surrounding medium, so that relatively simple techniques of decantation and centrifugal pelleting or banding of virus (to isolate intact particles from incomplete or aberrant forms) can be used for purification. However, some cell systems will normally shed fragments of plasma membrane, both in the infected and the uninfected state (Hoyle, 1950); cells suffering cytopathic effects of viral infection may also release host material as well as virus. When it is required to study the lipid composition of virus particles produced within the cell but not released, the techniques of lysis will enormously increase the amount of membranous material to be separated from the virus particles. While electron microscopy can be used to identify host material

in a virus preparation, the most effective means of monitoring removal of host material from virus is by the isotope dilution assay. In this, material from radioactively labelled uninfected cells is mixed with the crude virus isolate and carried through the purification procedure. Decrease or disappearance of radioactivity thus indicates separation of viral from cellular material (Blough *et al.*, 1967). Obviously, the culture systems used for viral replication must also be free from contamination by microorganisms such as fungus or mycoplasma.

(*ii*) *Uniformity of virus morphology.* When attempting to relate envelope lipid composition to the structure of the virus (e.g. as revealed by electron microscopy), it is important to use a purification method which will give a virus population as uniform as possible with respect to shape and size. Certain strains of influenza virus or paramyxoviruses may be markedly pleomorphic, with considerable variation in size and hence in lipid content per particle (Blough, 1964); rabies virus may contain a variable proportion of short or truncated particles ("T-particles", Hummeler *et al.*, 1967), and Cocal virus (vesicular stomatitis virus) is found with both the characteristic flat-ended bullet shape and a double-ended form (Bergold and Munz, 1967). Some of these apparent variations may in fact be due to the method of preparation for electron microscopy (see below and Chapter 2).

(*c*) *Manner of Assembly*

(*i*) *"Template" and* de novo *assembly.* Electron microscopic examination of the process of formation of virus particles in infected cells shows two major assembly routes. The more common route involves accumulation of the internal component of the virion at or close to a cell membrane, followed by modification of adjacent regions of the membrane by insertion of envelope structural polypeptides. The core material then acquires its envelope by budding through the modified membrane, which then pinches off, and the complete particle is released (if budding takes place at the plasma membrane) or appears in a cytoplasmic vesicle (if budding is at an intracellular membrane). The virus thus uses the structure of an existing cell membrane as a "template" which can be modified by lateral displacement of membrane polypeptides, rather than assembling the envelope *de novo* without direct involvement of a parent membrane. Other viruses (e.g. poxvirus and the marine bacteriophage PM2) are formed by a simultaneous condensation of both lipid and protein around the genetic material of the virus—*de novo* synthesis (Dales, 1963; Cota-Robles *et al.*, 1968). In this case one would not necessarily expect the viral lipid composition to resemble that of any particular cell membrane fraction, whereas many "template" viruses have been shown to have compositions strongly resembling those of their parent uninfected membranes (Blough and Tiffany, 1973).

(*ii*) *Shape-determining factors.* Another basis for classification of lipid-containing viruses is whether the envelope contains an integral membrane protein such as the M polypeptide of influenza virus, which is thought to confer shape and stability, particularly during budding (see Chapter 17). Viruses such as Sindbis and Semliki Forest virus contain, in addition to their core nucleoprotein, only external envelope glycoproteins; it would seem that in these cases the core itself acts as a "former" and determines the regularity of shape of the particle. Other large viruses such as herpesviruses contain a number of envelope structural proteins to which no specific function has yet been assigned (see Chapter 19), and it seems likely that one or more of these could fulfil the same role as the M polypeptide of influenza virus. We have suggested elsewhere (Blough and Tiffany, 1975) that viruses possessing envelopes which can fairly readily be penetrated by negative-contrast electron microscopic stains may all have such an envelope structural protein to give a degree of rigidity to the envelope, independently of a closely packed core, although this may also be present. Rhabdoviruses were suggested to represent an intermediate situation, in which the shape of the coiled nucleocapsid is the major determinant of particle morphology in the intact virion; on the basis of numerical correspondence between nucleocapsid and envelope polypeptides, it is thought that binding exists both between nucleocapsid core and one or more envelope structural proteins (Sokol *et al.*, 1971; Neurath *et al.*, 1972). The particle thus seems to have an envelope capable of maintaining its integrity without the core, but which normally adopts the shape of the core as a "former". More recent results from mild detergent treatment of Semliki Forest virus indicate that the envelope can be isolated intact (Helenius and Söderlund, 1973; see Chapter 12), suggesting that envelope proteins may play a larger role in stability of this virus than was supposed in formulating the "former" theory. It seems possible that the nature of the binding of lipid with the envelope structural protein may influence the composition of viral lipid.

B. Virus Groups Studied

(*a*) *Overall Lipid Composition*

For most groups of viruses so far studied, the lipid content lies in the range 20–35% by weight, but some viruses, notably the pox group, fall considerably below this, e.g. vaccinia virus has only 5% lipid (Zwartouw, 1964). However, these are large complex viruses with a variety of surface and internal components of ill-defined structure, and their lipid and protein may be organized in a different manner from that of the majority of enveloped viruses, with relatively limited lipid regions penetrated by protein units (see Section 3C). For the majority of viruses, the lipid present is roughly the amount required

to form a bimolecular layer at the periphery of the envelope (excluding the surface projections); the very high lipid content of avian leucoviruses (30–35%: Rao *et al.*, 1966; Quigley *et al.*, 1971), which are of comparable size to influenza virus (*ca.* 20%: Blough and Merlie, 1970), may indicate the presence of an additional lipid region as well as a bilayer shell. Such a region has not so far been detected (see below).

All the major classes of lipids present in cell membranes are also found in viruses, although not necessarily in the same proportions as in the uninfected host membranes. Thus in the marine bacteriophage PM2, the two major phospholipid classes are phosphatidylglycerol (65–68%) and phosphatidylethanolamine (28%), the reverse of the proportions of these two classes in the host cell (Braunstein and Franklin, 1971). The distribution of acyl chains in one lipid class frequently differs markedly from that in another class (Tiffany and Blough, 1969a, b; McSharry and Wagner, 1971). In fowlpox virus, the ratio of neutral lipids to phospholipids is unusually high, and the squalene content of the virion is far greater than in the host cell (White *et al.*, 1968). Differences are also noticed in minor components such as sphingoglycolipids, correlated in some cases with the presence of neuraminidase as a viral structural protein (Klenk and Choppin, 1970b; McSharry *et al.*, 1971), although most of these classes resemble those of host membranes. Individual analyses of major groups are discussed below.

(b) Orthomyxoviruses

Probably more attention has been paid to the lipids of influenza virus than of any other virus, leading to a large number of data for numerous strains. Since we intend only to give an outline here, the reader is referred to the original publications or to Blough and Tiffany (1973) for more detailed compositions.

In many earlier studies the virus was inoculated into the allantoic cavity of embryonated hens' eggs and incubated for about 48 h, after which the allantoic fluid, containing virus and fragments of membrane material released from the cells of the chorioallantoic membrane, was harvested. Virus was separated from these "normal cell particles" by centrifugal techniques and by adsorption to and elution from either red blood cells or barium sulphate. Frommhagen *et al.* (1959) found that the egg-grown A_0/PR8/34 strain contained 18·5% lipid, made up of 11·5% phospholipid and 6·5% cholesterol. Phosphatidylcholine formed 28% of the phospholipid and sphingomyelin formed 35%. More extensive analyses of the same strain were reported by Blough *et al.* (1967) and by Blough and Merlie (1970). In these, the virus lipid content was 20–24%, with 10·3% phospholipid and 7·7% cholesterol. Of the phospholipid 33–38% was phosphatidylcholine, 23–24% sphingomyelin, 11·7% phosphatidylethanolamine, 8·8% phosphatidylserine and 7·9% phosphatidic acid. Blough and Merlie (1970) and Blough *et al.* (1969) also analysed the lipids of

incomplete or von Magnus virus produced by passage of high titres of PR8 strain in eggs; relative to the normal virus, this showed less sphingomyelin and phosphatidylcholine and increased amounts of phosphatidylserine and phosphatidylinositol. Almost all neutral lipids were increased, with the exception of cholesterol, which was reduced from 85% to 52% of neutral lipid. The acyl chains present also showed certain significant increases in the proportions of short chains and in the ratio of long unsaturated chains to saturated chains in the phospholipid fraction (Blough *et al.*, 1969). These changes in composition, which would produce a more fluid and expanded envelope lipid region, were considered to be in part responsible for the less regular morphology and other structural characteristics of the incomplete virus. A similar correlation between compositional and morphological changes was also reported for influenza virus grown in the presence of exogenous agents such as vitamin A (Blough, 1964; Blough *et al.*, 1967) and branched-chain fatty acids (Blough and Tiffany, 1969). Lenard *et al.* (1976) compared the phospholipids of normal WSN influenza virus and incomplete virus from up to four undiluted passages in MDBK cells, but found no significant differences. The changes seen in egg-grown incomplete A_0/PR8/34 virus may therefore be related in some way to the properties of the egg culture system.

Comparison has also been made of the acyl chains of three strains of egg-grown influenza virus (A_0, A_2 and one of type B) with chains of "normal cell particles", taken to represent cell surface membranes (Tiffany and Blough, 1969a). The acyl chains of phospholipids of subtypes A_0 and A_2 were much alike, but greater differences were seen in their neutral lipids, where A_2 had considerably more short saturated chains and long polyunsaturated chains than A_0. Neutral lipids of type B also showed considerable differences from both the other viruses in monoenoic/polyenoic and saturated/unsaturated ratios, and the normal cell particles differed from all the viruses in most of these respects. The presence of a much higher $C_{18:1}$ level in neutral lipids of normal cell particles (35% compared to up to 22% in virus) might indicate some contamination by egg yolk lipids in this case, and it is possible that normal cell particles in fact represent a transient and local variation in membrane lipids which promotes pinching off. It was concluded that the differences in composition indicated a selecting force during envelope assembly, and this was thought to be mediated more probably by external envelope proteins than by the core protein, as the fatty acid profiles showed no closer similarities for the subtypes A_0 and A_2 sharing a common group antigen than for the serologically unrelated A and B types.

The fatty acyl chains found in the total phospholipid fraction of the virus are not uniformly represented in each of the individual phospholipid classes; Blough (1971) isolated the four major phospholipids and determined their

individual acyl chain profiles for both A_0 and B/Lee strains of influenza virus grown under similar conditions in embryonated eggs. Wide variation was seen between the content of a particular chain in the phospholipid classes of either virus, although broad resemblance was seen in many cases for a particular chain when comparing the same phospholipid classes in the two viruses. Thus the oleate ($C_{18:1}$) content varied from 9·3% in sphingomyelin to 45·3% in phosphatidylethanolamine of A_0/PR8/34, and from 5·5% to 35·0% in these classes in B/Lee. Some large discrepancies were also seen: phosphatidylethanolamine of B/Lee contained 24·4% of arachidonate ($C_{20:4}$) where A_0/PR8/34 had only 4·1%.

It has been pointed out that the conditions of growth in embryonated eggs involve multiple cycles of infection and release of virus, and suggested that differences seen in the lipids of strains of the same virus may reflect the slightly different strain response of chick cells to these conditions of infection. This would be avoided by a single-cycle infection in cells in tissue culture. The influence of infection on host lipid metabolism is dealt with in greater detail in Chapter 15. Comparison has been made of the lipids of host and virus for influenza virus (MEL/35) grown both in embryonated eggs and in calf kidney cells in tissue culture (Kates *et al.*, 1961). The lipid content of the two viruses was distinctly different (e.g. egg-grown virus and calf kidney virus had respectively 35% and 14% sphingomyelin, 22% and 8% phosphatidylethanolamine, and 22% and 32% of the fatty acyl chains were $C_{16:0}$). However, each virus fairly closely resembled its host in lipid composition, with perhaps the closest resemblance being between calf kidney virus and the host nuclear membrane. A model was proposed to account for this similarity, which involved transport of lipid from the nuclear membrane by viral ribonucleoprotein on exit from the nucleus. This model is now considered to be incorrect, as the apparent correspondence of lipid composition was probably due to inadequacies of the fractionation techniques.

Evidence that newly synthesized as well as preformed lipids are incorporated into viral membranes was obtained by equilibrium labelling of chick embryo fibroblasts with [2-^{14}C]-glycerol, infecting with the A_0/WSN strain of influenza virus and at the same time chasing the [^{14}C]-glycerol and pulsing with [2-^{3}H]-glycerol. Analysis of the lipids of the viral membrane showed that newly synthesized lipids comprised 30–70% of the lipids of the virion with diglyceride and phosphatidylcholine (70% and 50% newly synthesized respectively) predominating (Blough, 1974). These studies were confirmed for NDV by Israel *et al.* (1975) using different techniques.

The glycosphingolipids of four strains of influenza virus (propagated in embryonated eggs) have been investigated by thin-layer and gas–liquid chromatography. The major components were glucosyl- and galactosylceramide. Long chain (C_{19-26}) fatty acids as well as 2-hydroxy fatty acids