Theoretical Aspects of Structure and Assembly of Viral Envelopes

HERBERT A. BLOUGH and JOHN M. TIFFANY[1,2]

With 2 Figures

Table of Contents

I. Introduction 1

II. Methods of Investigation of the Viral Envelope 4
- A. Information from Intact Virus Particles 4
- B. Information from Disrupted Virions 6
- C. Information from Reconstituted Viral Membrane Systems 8

III. Biosynthesis of Envelope Components 8
- A. Lipids 8
- B. Carbohydrates 10
- C. Proteins 11

IV. The Assembly Process — Theoretical Considerations 12
- A. Production of Materials 12
- B. Transport to the Assembly Site 13
- C. Insertion into the Parent Membrane 13
- D. Organisation of the Envelope Prior to Budding 15
- E. Bud Growth and Aberrant Forms of the Envelope 16
- F. Release 17
- G. Forces Operating during Assembly and in Maintenance of Structure 18
- H. The Role of the M Protein in Envelope Structure 20

References 23

I. Introduction

Viruses which can be seen in the electron microscope to have a coherent envelope structure are all found to contain lipid, and this has in the past

1 Division of Biochemical Virology and Membrane Research, Scheie Eye Institute, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104, USA. JMT present address: Nuffield Laboratory of Ophthalmology, Oxford University, Oxford, England.

2 Supported by grants from the National Cancer Institute (CA 15370); Damon Runyon Memorial Fund for Cancer Research (DRG-1254); National Eye Institute (EY 01067-02) and a contract from the U.S. Army Medical Research and Development Command (DADA 17-72C-2147).

W. Arber et al. (eds.), *Current Topics in Microbiology and Immunology*
© Springer-Verlag, Berlin · Heidelberg 1975

been used as a means of classifying viruses, secondary to the type of nucleic acid they contain (COOPER, 1961). Another common feature which these viruses have more recently been found to possess is carbohydrate (other than that in their nucleic acid), and in all cases so far studied their distribution has been found to be asymmetric—the glycoproteins being found only in the exterior of the viral coat. The envelope may therefore be defined as a protective lipoprotein container for the genetic material, bearing, embedded in its surface, glycoproteins and glycolipids responsible for cell surface recognition of the virus and for the preliminary stages of infection (attachment and penetration). The virus is generally sensitive to agents such as detergents or lipid solvents, which disrupt the lipid region of the envelope. Virus assembly generally occurs at a specific cell membrane, where virally-specified materials are incorporated into the membrane to begin forming the envelope, followed by formation of a bud which eventually surrounds the viral core material.

The general field of virus assembly and structure is largely covered by a number of recent and more extensive reviews (LAVER, 1973; LENARD and COMPANS, 1974; SCHULZE, 1973) and we have recently published a review of many aspects of lipid involvement in viruses (BLOUGH and TIFFANY, 1973). It would be largely superfluous to attempt to cover the same ground in the present review; rather, we propose to consider methods of investigating structure and assembly, and to point out limitations which must be placed on their interpretation. We shall also consider the process of assembly, in particular those aspects yet to be elucidated by experiment, with the hope of suggesting aspects amenable to practical testing and perhaps of drawing attention to larger structural resemblances between different virus classes. We include some models derived from existing data on several viruses, together with estimates of the magnitude of forces likely to operate within the envelope to maintain a given structure.

The role of lipid in virus envelopes must be carefully considered, as well as the reliability of methods used to probe its functions, since it is intimately involved in the process of insertion of viral surface proteins into the template membrane, and bud formation. In recent years lipid has been variously considered to be passively incorporated, although with a structural function (e.g. KINGSBURY, 1972), selected from an available pool of preformed host lipids, or as a fortuitous space-filler. Since lipids are synthesized by the host cell, the effect of virus infection on host cell lipid metabolism is of obvious importance. We refer to a considerable amount of recent work from this laboratory which tends to disprove earlier ideas about the origins of viral lipids. Mention is also made in Section III of some aspects of biosynthesis of viral polypeptides and carbohydrates.

Many of the points raised or structures suggested have no experimental basis as yet, and indeed many workers in the field of virology are dubious of the value of model-building. However, considerations of the overall geometry of the envelope and its constituents have proved to be useful in determining the number of surface projections of the virion (TIFFANY and BLOUGH, 1970b),

Table 1. Enveloped viruses of vertebrates and some representative members[a]

Classes	Representative members
1. *RNA viruses*	
Orthomyxoviruses	Influenza (human, equine, avian, porcine) viruses
Paramyxoviruses	Newcastle disease, parainfluenza, mumps, measles [b] viruses
Alphaviruses (Arbovirus group A)	Sindbis, Semliki Forest, equine encephalitis, rubella [b] viruses
Flaviviruses (Arbovirus group B)	Yellow fever, Dengue, Japanese B encephalitis viruses
Rhabdoviruses	Vesicular stomatitis, rabies viruses
Oncornaviruses	Rous sarcoma, mouse leukemia (e.g. Rauscher, Moloney, Friend), mouse mammary tumor, visna viruses
Coronaviruses	Avian infectious bronchitis, mouse hepatitis, human respiratory viruses
Arenaviruses	Lymphocytic choriomeningitis, Lassa, Parana viruses
2. *DNA viruses*	
Herpesviruses	Herpes simplex, varicella, infectious bovine rhinotracheitis, Epstein-Barr, Marek's disease viruses
Iridoviruses	Amphibian polyhedral cytoplasmic (frog virus, FV3) virus
Poxviruses	Vaccinia, orf, smallpox, fowlpox, Yaba tumor viruses

[a] Modified from WILDY (1971).
[b] Tentative classification.

the number of different polypeptides in the virion and the volume they will occupy, and indeed in predicting the existence of an envelope structural polypeptide for influenza virus (TIFFANY and BLOUGH, 1970a). Model building has served a valuable function in determining the structure of many macromolecules, and of viruses possessing cubic symmetry, and if properly applied can offer considerable help in elucidating the structure of the virus envelope.

Table 1 is taken in part from BLOUGH and TIFFANY (1973) and indicates most of the major groups of lipid-containing mammalian viruses, as well as some others which have been extensively studied and which cast light on the general principles of virus structure. As with our review of viral lipids (BLOUGH and TIFFANY, 1973), attention centers chiefly on the myxoviruses, and many illustrative examples are drawn from the literature on this group. This reflects the intense study over a considerable number of years applied to every aspect of infection by these viruses, and the relative paucity of data for many of the other virus groups. In addition, we shall omit consideration of chemical or physical properties of the internal nucleoprotein of the virus, unless the conditions of viral assembly are such that it imposes a particular form on the envelope.

II. Methods of Investigation of the Viral Envelope

A. Information from Intact Virus Particles

The essential prerequisite for study of viral structure is purity of the virus preparation. The techniques used may depend on the system used for virus growth—i.e. whether the virus is harvested from tissue culture supernatant fluids, from allantoic fluid following growth in embryonated eggs, by disruption of host cells to release viral aggregates, etc. Unless host material can be rigorously excluded, the chemical composition of the virus cannot be accurately determined. Information derived from physical probes (e.g. electron spin resonance spectra of spin-labelled lipids incorporated into whole virus) may also be suspect if insufficiently-purified virus is used, since the structure of lipid regions of the virus envelope is possibly different from that in the host cell membrane fragments which are also present.

Primary information on assembly, release and structure of the intact virus particle is derived from electron microscopy. There are, however, many limitations to this technique and its derivatives, which should be borne in mind in interpreting the images obtained. The preparation of positively-stained thin sections involves fixation, dehydration, and embedding of the virus, and the use of solvents in these stages may remove lipid; this can be avoided by the use of water-miscible embedding media, but at the price of more frequent polymerization problems. The image also depends on the staining procedure used (COMPANS and DIMMOCK, 1969; NERMUT et al., 1972), and generally definition of ultrastructure in poor. The negative contrast technique has unique capability in resolving surface structure, but problems of interpretation may arise when superimposition of images from the upper and lower surfaces of the virus particle is seen (VERNON et al., 1972). Isolated subunits of the virus have been visualized by this method (LAVER and VALENTINE, 1969). Approximate molecular weights of subunits can be obtained from their dimensions, if a value is assumed for their partial specific volume. However this technique has not been extensively used in the determination of infrastructure (as opposed to surface morphology) because the envelope may disrupt or break up into small "rosettes" under the action of surface forces during drying, or following treatment with agents such as ether (HOYLE et al., 1961); there is no way of telling whether this disruption accompanies phase transitions of lipid components in the envelope. Other drawbacks of the negative-staining method include shrinkage and distortion of the virus (NERMUT and FRANK, 1971) and interaction between stain and specimen. Freeze-dried particles (with or without freeze-etching) require metal shadowing, which will add at least 15 Å to the apparent size of any structure such as surface projections and will increase the apparent smoothness of etched or fractured surfaces. It has the advantage that no fixation or solvent dehydration is required, and except for the thickness of the metal shadow, probably approaches closer to the original dimensions of the particle than any other method. This and related techniques are dealt with in more detail by ZINGS-

HEIM (1972). More complex techniques, such as identification of surface chemical groups by antibody or lectin binding, may also be combined with electron microscopy to show the site of binding (LAFFERTY, 1963; AOKI et al., 1970) and to indicate that the particles are still intact.

X-ray diffraction to determine the disposition of envelope lipids and proteins has been used successfully only on Sindbis and PM2 viruses (HARRISON et al., 1971a, b). Interpretation of the results for more complicated viruses will be extremely difficult. Even for these comparatively simple examples (which contain a very small number of envelope polypeptides), it is hard to distinguish between icosahedral and truly spherical structures. Fourier synthesis of diffraction data leads to a radial electron density distribution for the particle, from which the dimensions and thickness of the lipid region and surrounding protein-rich areas can be deduced. The question of whether protein protrudes through the lipid bilayer region in these viruses is not yet satisfactorily resolved, but X-ray diffraction techniques do not seem to give an unequivocal answer.

A variety of spectroscopic techniques can be used to investigate the state of mobility of the lipid regions of the intact particle. These involve adding a lipid-soluble substance to the virus, and comparing its characteristic spectrum with that of the same probe molecule in structurally-defined surroundings; e.g., one may use nitroxy-stearic acid for electron spin resonance studies (LANDSBERGER et al., 1971, 1973; KORNBERG and MCCONNELL, 1971), or perylene for fluorescence polarization studies (RUDY and GITLER, 1972), and compare each of these with their behaviour either in solution or in a known (or presumed) lipid bilayer structure. The results for influenza virus have been interpreted as showing that the viral lipid is in the form of a fluid bilayer when the reference probe is incorporated into a red blood cell membrane (LANDSBERGER et al., 1971). JOST et al. (1973) have concluded, from ESR studies by this method on reconstituted lipoprotein membranes, that a monomolecular layer of immobilized lipid surrounds membrane proteins, while the remainder of the lipid behaves as a fluid bilayer. This indicates a significant drawback of the spin probe method when applied to intact virus: the probe must be added and allowed to be incorporated into the envelope solely by diffusion, whereas reconstitution of membranes as carried out by JOST et al. (1973) can be performed in the presence of the label. If there is specific interaction between envelope structural proteins and at least a small proportion of the lipid, as indicated by the results of TIFFANY and BLOUGH (1969a, b) for influenza and Newcastle disease viruses, the probe molecule will not diffuse as readily into this tightly-bound region as into loosely-bound or non-specific lipid regions. Thus the ESR spectra will not indicate any great degree of interaction between protein and lipid, and will tend to imply that all the viral lipid is in a fluid state. LESSLAUER et al. (1972) have pointed out that the bulky nitroxy spin label group may in fact alter the molecular architecture of the membrane, and prevent ready substitution of labelled for unlabelled molecules. The "melting" behaviour of the lipids, and hence their apparent

fluidity, may be affected by the presence of these bulky groups, as pointed out by HUBBELL et al. (1970) in relation to the original work of HUBBELL and MCCONNELL (1968). Fluorescent electron transfer techniques (WU and STRYER, 1972) show a great deal of promise in measuring the distance of lipid polar head groups from membrane structural proteins; however these techniques have not yet been applied to viral systems. Unfortunately, many of these probe techniques are limited by the inability of the extrinsic probe to detect subtle changes in membrane structural polypeptides.

B. Information from Disrupted Virions

As with intact virus, electron microscopy is frequently the first line of approach, to determine what effect disrupting agents have had on the particle, and what shapes are adopted by released subunits. Freeze-fracture methods appear to reveal some of the polypeptides located in or around apolar regions of the envelope (BÄCHI et al., 1969; NERMUT and FRANK, 1971; BROWN et al., 1972; BÄCHI and HOWE, 1973), since the plane of fracture follows these regions (DEAMER and BRANTON, 1967); no details of organisation of the lipid can be seen, since although BRANTON (1969) has shown recognisable differences to exist between the cleavage planes of lamellar and hexagonal phospholipid phases, an insufficient extent of lipid is revealed within a virus envelope by cleavage.

The isolated substructures obtained depend largely on the methods of disruption and the agents used. For purposes of revealing polypeptides, the most widely-used are detergents and lipid solvents such as Tween 20, sodium dodecyl sulphate (SDS), Nonidet P40, sodium deoxycholate and diethyl ether. Generally these techniques involve extraction of lipid and stabilization of remaining exposed hydrophobic regions of protein; an exception is proteolysis using enzymes such as bromelain where the aim is selective destruction as a means of identification of the sites of constituent proteins (COMPANS et al., 1970). Once disrupted, the released polypeptides are generally resolved by polyacrylamide gel electrophoresis; this technique has largely superseded earlier methods such as cellulose acetate strip electrophoresis (LAVER, 1964) which in some cases failed to resolve viral polypeptides adequately. It is of great use in finding the number of polypeptides (COMPANS et al., 1970; HASLAM et al., 1970; SKEHEL and SCHILD, 1971; LAZAROWITZ et al., 1971) and in determining the degree of contamination by host cell polypeptides (SPEAR and ROIZMAN, 1972; HOLLAND and KIEHN, 1970). Detergent disruption may be followed by alkylation and reduction before separating the products on polyacrylamide gels, and by appropriate pulse-chase studies the times of synthesis of virus-specific materials in the infected cell can be established. Molecular weights are frequently quoted, using appropriate protein standards, but it must be borne in mind that the proportion of carbohydrates in glycoproteins may materially affect the results (SEGREST et al., 1971). Alternative

methods of separation of isolated polypeptides include affinity columns (e.g. phytohemagglutinin linked to Sepharose (HAYMAN et al., 1973).

Extensions of the technique of BRETSCHER (1971) to label accessible ε-amino groups and amino-containing phospholipids (GAHMBERG et al., 1972a), or lactoperoxidase with ^{125}I to label surface-accessible tyrosine residues (PHILLIPS and MORRISON, 1971) have been applied to viruses. Polyacrylamide gel electrophoresis of solubilized polypeptides then distinguishes between external (envelope) and internal polypeptides, or even between those on the envelope surface and those in its interior (STANLEY and HASLAM, 1971; KATZ and MARGALITH, 1973). If done in conjunction with radioisotope labelling of monosaccharides, these methods show all the envelope glycoproteins to be external (CARTWRIGHT et al., 1970; COMPANS et al., 1970; KLENK et al., 1972). Enzymatic localization of envelope glycoproteins has also been done using chymotrypsin (SCHULZE, 1970), bromelain (COMPANS et al., 1970) and caseinase (REGINSTER and CALBERG-BACQ, 1968).

Additional techniques include fluorescent labelling, e.g., dansylation of both polypeptides and hexosamines (BOLOGNESI et al., 1973), or the use of lectins to locate glycolipids (OKADA and KIM, 1972; KLENK et al., 1972) as well as specific glycosidases (BIKEL and KNIGHT, 1972). The use of pure phospholipases to localize structures within the viral envelope has not yet been applied to the same extent as for red blood cell ghosts (VERKLEIJ et al., 1973; ZWAAL et al., 1971), although preliminary studies of the action of purified phospholipases on the envelope of Semliki Forest virus show evidence of asymmetry of distribution of lipids between interior and exterior of the envelope (BLOUGH and RENKONEN, unpublished data).

Few amino acid compositions of virus envelope structural polypeptides have so far been determined; this work is limited to a large extent by the availability of material. Techniques are now being developed to permit production of larger quantities of viral components (STANLEY et al., 1973; GREGORIADES, 1973). Earlier studies on the tryptic digests of viral polypeptides are vitiated by the fact that many of these "maps" were done on impure preparations. However "fingerprints" of purified nucleocapsid and envelope polypeptides have been obtained for Semliki Forest virus (SIMONS et al., 1973).

The way in which lipid and protein are embedded in the envelope has not been resolved satisfactorily by electron microscopy. Attempts to digest the viral envelope suffer from the drawback that one may at the same time be damaging structural portions of the envelope. This has of course in many cases been controlled by limiting proteolysis times and/or concentrations (e.g. COMPANS et al., 1970). The structure of the envelope following proteolytic digestion may however be quite different from that of the native envelope, due to rearrangement of the components as belancing forces are disturbed, although spin resonance studies on intact but spikeless influenza virus particles fail to reveal such changes (LANDSBERGER et al., 1973). It has been shown that tryptic digestion of red blood cell ghosts causes an aggregation of the 70 Å intramembranous particles (PINTO DA SILVA and BRANTON, 1970).

C. Information from Reconstituted Viral Membrane Systems

Direct studies on the interaction of viral lipids and proteins or glycoproteins have been limited thus far to recombinations of the components to form rather ill-defined vesicles, which however do indicate specificity of lipid-protein binding and exhibit similar envelope surface properties to the intact virus (HOSAKA and SHIMIZU, 1972a, b). Exactly how these moieties may combine to form a viral envelope is still not entirely clear; we shall discuss some of the remaining conceptual difficulties in Section IV. A major drawback to the interpretation of structure from such reconstitutions is that, while undoubtedly complex vesicular structures are produced, they commonly exhibit the same surface features on both inner and outer faces, whereas these features are shown on only one face of the intact virus envelope. It scarcely seems possible, considering the data available on asymmetry of distribution of both glycoproteins and lipids, that the same lateral cohesive forces can operate in a reconstituted vesicular membrane showing the same type of polarity on both sides. It is impossible, in this type of experiment, to control the distribution of phospholipids so that the outer layer of a lipid bilayer shall predictably contain a specified excess of phosphatidylcholine or deficit of phosphatidylethanolamine over the inner layer (THOMPSON and SEARS, 1974; ISRAELACHVILI, 1973; MICHAELSON et al., 1973). Obviously, probe and other studies (ESR, NMR, etc.) will have to be done to monitor and evaluate these reconstitution studies; promising techniques using asymmetric phospholipid vesicles are also being developed which may surmount some of the technical problems involved (THOMPSON and SEARS, 1974).

Specific binding between isolated lipids and proteins has not been examined with the same thoroughness for viruses as for mitochondria (GREEN and PERDUE, 1966), chloroplasts (JI and BENSON, 1968) or high density lipoproteins (SCANU and TARDIEU, 1971); however, GREGORIADES (1973) has isolated a membrane structural polypeptide from influenza virus and determined its amino acid composition (which includes about 10% of amino acids with hydrophobic sidechains), and this will lend itself to detailed lipid binding studies. Similar preparations and analyses have also been done by LAVER and BAKER (1972) and on the paramyxovirus SV5, which has as many as 64% hydrophobic amino acids (MCSHARRY et al., 1972).

III. Biosynthesis of Envelope Components

A. Lipids

Since we have already discussed the biosynthesis of viral envelope lipids in some detail in our previous review (BLOUGH and TIFFANY, 1973), we shall only present a few selected points here. Lipid must be considered a major structural component of enveloped viruses, since in most cases it makes up 20–35% of the weight of the particle; there are some obvious exceptions to

this, such as vaccinia virus, which contains only 5% lipid. Until recently, lipids have been thought to be entirely preformed, i.e. derived from lipids already present in the host cell at the time of infection (KATES et al., 1961; PFEFFERKORN and HUNTER, 1963). In cells infected with influenza or Sindbis virus, the specific activity of ^{32}P-labelled phospholipids following maximal growth of the virus was the same for both the virus and the host cell membranes. However, ^{32}P-orthophosphate is a poor label for measurement of turnover in eukaryotic cells, since it becomes compartmentalized in slowly turning over organic pools, making measured turnover rates unreliable (WEINSTEIN and BLOUGH, in press).

A more reliable method of measuring rapid turnover is by pulse-chase studies using 2-^{14}C- and 2-^{3}H-glycerol (BLOUGH and WEINSTEIN, 1973; BLOUGH et al., 1973; GALLAHER et al., 1973b; GALLAHER and BLOUGH, in press); these studies show that the half-life of major phospholipids in the cell is 2–3 hours, i.e. considerably less than the single-cycle growth time of mammalian viruses. Also, in contrast to earlier studies, when chick embryo fibroblasts were labelled with 2-^{14}C-glycerol, infected with influenza virus (strain A_0/WSN) and then pulsed with 2-^{3}H-glycerol, 30–70% of the viral lipid was found to contain ^{3}H-glycerol, suggesting that newly-formed lipids as well as preformed lipids are incorporated into the virion (BLOUGH, 1974). The presence of unique sequences of hydrophobic amino acids of membrane or envelope structural polypeptides would provide a possible method for preferential selection of lipids bearing certain acyl chains. Alternatively, strong polarity such as that shown by the envelope structural polypeptide of PM2 virus, which has an isoelectric point of pH 12.3 and hence is normally strongly positively charged (SCHAEFER et al., 1974), could select phospholipids on the basis of charge. Thus the so-called "hot spots" where envelope biogenesis is occurring (BEN-PORAT and KAPLAN, 1972) may be sites where newly-synthesized lipid is incorporated into membranes to produce virus of high specific activity. It appears that assembly of the envelope requires a coordinated synthesis and breakdown of lipids (BLOUGH et al., 1973). If turnover is decreased, or if there is lack of coordination between synthesis and turnover of phospholipids or neutral lipids, an abortive or an incomplete infection may occur. In support of this hypothesis, it was found that turnover rates for phospholipids were greatly increased in HeLa cells persistently infected with mumps virus (BLOUGH, 1973); in this case neutral lipids were unaffected. In addition, alternative biosynthetic pathways appear to be operative in cells in the carrier state (BLOUGH, unpublished observations). The function of lipids is not clear at this point, but it was suggested by BLOUGH and TIFFANY (1973), and elsewhere in this review, that lipids are important for the transport of certain hydrophobic membrane-type M proteins to the site of assembly of the virus envelope. The function of glycolipids is at present also unknown, although it has been suggested by BLOUGH and LAWSON (1968) that they play an important role in cell fusion.

B. Carbohydrates

The synthesis of oligosaccharides is controlled by a non-template mechanism consisting of host cell glycosyltransferases. These enzymes are located in membranes of the Golgi apparatus and the endoplasmic reticulum and are responsible for initiation, elongation and termination of oligosaccharides attached to nascent polypeptide chains and ceramides (HAGOPIAN et al., 1968; CACAM and EYLAR, 1970; BRADY and FISHMAN, 1973; GRIMES and BURGE, 1971).

These glycosyltransferases appear to be altered in some way following virus infection, in configuration and/or specificity, since different sequences of monosaccharides are added to viral polypeptides and glycolipids in comparison to those found in normal uninfected plasma membranes or endoplasmic reticulum (FROGER and LOUISOT, 1972a, b; DEFRENE and LOUISOT, 1973). Increased activities of mannosyl and *N*-acetyl-glucosaminyltransferases have been observed in cells infected with Sindbis virus or myxoviruses in comparison to uninfected controls. The K_m of the enzymes was unchanged, although minor changes were observed in pH optima, suggesting that two slightly different host cell glycosyltransferases were involved following infection with Sindbis virus (GRIMES and BURGE, 1971). Using exogenous acceptors and measuring CMP-sialyl- and fucosyltransferase activities, no differences were noted in specific activity or acceptor specificity. GRIMES and BURGE (1971) concluded from these results that the carbohydrate portion of viral glycopeptides is therefore host-specified. This is undoubtedly true for many of the smaller viruses, but may not be so for larger viruses such as herpes simplex virus, which has a genomic molecular weight of about 100 million and codes for 47 polypeptides (HEINE and ROIZMAN, 1973). One or more of these may in fact be a glycosyltransferase. RAY and BLOUGH (1974) have recently shown that the glucosyltransferase activity of herpes simplex virus (HSV-1)-infected cells is markedly enhanced during infection, while the mannosyltransferase activity is unchanged.

By inhibiting glycosylation of viral polypeptides and glycolipids by using sugar analogues such as 2-deoxy-D-glucose, the sequence of incorporated monosaccharides may be altered and the oligosaccharide chain terminated (KALUZA et al., 1972; GALLAHER et al., 1973a; RAY and BLOUGH, 1974). Such sugar analogues offer a convenient means of determining structure and function in component parts of the virion (COURTNEY et al., 1973).

The major function of the sugar moiety seems to be to ensure the transport of glycoproteins from one membrane (i.e. the site of synthesis) to the final site of assembly or function—the plasma membrane for myxoviruses, or the nuclear envelope for herpesviruses. Support for this hypothesis has recently been provided at the membrane level by MELCHERS (1973) for monomeric IgG_1 immunoglobulin, where the addition of intermediate sugars such as galactose is necessary for transport from the rough to the smooth endoplasmic reticulum. Completed oligosaccharide chains are also necessary for full ex-

pression of a viral function such as attachment (BIKEL and KNIGHT, 1972). Sugar molecules may confer the necessary tertiary or quaternary structure on envelope structural polypeptides as well as permit function of non-structural polypeptides such as the glycoprotein responsible for cell fusion by herpesvirus (LEVITAN and BLOUGH, in the press).

It is not clear from any published work on the carbohydrate composition of the virion or of infected or transformed membranes (MORA et al., 1971) exactly how the sequence of sugars is altered from that of normal cells. Detailed information on all aspects of glycosylation in the normal and infected cell will be necessary if the role of glycoproteins and glycolipids in viral infection is to be fully understood. An additional factor not mentioned above is post-synthetic modification of carbohydrate by either endogenous or exogenous glycosidases, to produce glycoproteins and glycolipids with monosaccharide sequences different from those originally synthesized. The only evidence of this at present is in the absence of sialic acid from the envelope hemagglutinin and glycolipids of myxoviruses (KLENK and CHOPPIN, 1970; KLENK et al., 1970).

C. Proteins

Viral structural proteins are synthesized on cytoplasmic ribosomes and must then migrate to the parts of the cell where virus assembly takes place. Appropriate pulse-chase studies have shown that the movement of viral polypeptides from smooth endoplasmic reticulum to plasma membrane is exceptionally rapid, and that with both RNA and DNA viruses (myxoviruses, rhabdoviruses and poxviruses) glycoprotein synthesis is detected as early as one hour after infection (MUDD and SUMMERS, 1970; PRINTZ and WAGNER, 1971).

The messenger RNA of poxviruses is reported to consist of two classes—late messenger RNA with a half-life of 13 minutes and early messenger RNA with a half-life of 120 minutes (SEBRING and SALZMAN, 1967), although others find no difference in the stability of early and late messenger RNAs (ODA and JOKLIK, 1967).

The number of polypeptides incorporated into the virion can be extremely small, as in the case of the alphaviruses; these may have only two envelope proteins, in addition to the nucleocapsid protein (SCHLESINGER et al., 1970; SIMONS et al., 1973). Large viruses such as herpesvirus, on the other hand, code for as many as 47 polypeptides, of which about 25 may be present in the virion (SPEAR and ROIZMAN, 1972). The number of glycosylated polypeptides is also variable, as is the complexity of the carbohydrate moiety. Some simpler viruses contain a large variety of monosaccharides, whereas with the complex poxvirus the two glycoproteins (SAROV and JOKLIK, 1972) contain only the simple sugar *N*-acetyl-glucosamine (GARON and MOSS, 1971).

Several examples are known of post-translational cleavage of viral polypeptides: proteolytic enzymes are capable of producing the paramyxoviruses SV5

and Sendai with enhanced infectivity, fusion and hemolytic activity (HOMMA and TAMAGAWA, 1973; SCHEID and CHOPPIN, 1974); the hemagglutinin of influenza virus may be modified by plasmin so that its dimeric structure (LAVER, 1971) is cleaved into two large and two small fragments without affecting the biological activity of the virus (LAZAROWITZ et al., 1973). Additional changes which might influence the charge density of polypeptides include phosphorylation, which has been described for rhabdoviruses (SOKOL and CLARK, 1973) and murine leukemia viruses (STRAND and AUGUST, 1971), among others.

IV. The Assembly Process — Theoretical Considerations

We shall consider in this section some of the still unresolved problems involved in assembly of enveloped viruses. Since information is not complete for any group of viruses, we must deal with the subject in rather general terms; some reference to specific virus examples is included, but we shall cover certain aspects of particular groups in more detail in Section V. There is essentially no difference in principle between acquisition of the envelope and release by budding from the plasma membrane, and assembly either by budding into cytoplasmic vesicles which then void following fusion with the plasma membrane, or by budding from the nucleus through the inner nuclear membrane (herpesviruses), so we shall refer only to "the membrane" as the site of assembly. Poxviruses generally assemble by a *de novo* aggregation within the cytoplasm, but an envelope may also be acquired by budding through the plasma membrane. It has been suggested that only the fully enveloped particles are infectious. This group is also more resistant to disruption by organic solvents than other enveloped viruses, perhaps indicating a different membrane structure. The remarks here on envelope assembly may therefore not be applicable to the poxviruses.

A. Production of Materials

Summarising what has been mentioned above under biosynthesis, virally-coded polypeptides may be produced on polyribosomes either in the required size or as part of a larger precursor which must be cleaved within the cell before incorporation into a functional envelope can take place (KLENK and ROTT, 1973; KATZ and MOSS, 1970). Further modification may take place after release, but without obvious structural requirement (LAZAROWITZ et al., 1973). Post-translational modification also includes glycosylation to form glycoproteins and glycolipids (and may involve elaboration and then shortening by endogenous glycosidases) as a sequential procedure determined by host cell enzymes.

Lipid metabolism of the cell is considerably altered following viral infection, to the extent that a large proportion of viral lipids are newly-

synthesized, and are not simply drawn from pre-existing pools of normal cell membrane lipids. Otherwise the same classes of lipid are generally found in the envelope as in the parent membrane, although the proportions of polar classes and the distribution of acyl chains within each class may vary (BLOUGH and TIFFANY, 1973).

B. Transport to the Assembly Site

Once synthesized, viral components must be transported to the actual site of assembly. This may present no difficulty in the case of lipids and some polypeptides, but structural proteins having sizable non-polar regions used in hydrophobic bonding require some form of protection in transit to preserve their unique tertiary structure. This could take the form of specific binding of lipids to form a soluble lipoprotein complex, or of a detergent-like action of lipid in forming a "hemi-micelle" surrounding the non-polar regions of the protein. In either of these cases a means would also be offered for introduction of specifically-tailored lipids into the assembly region. In this sense the viral polypeptide could act like the lipid carrier protein of uninfected cells (WIRTZ and ZILVERSMIT, 1968).

C. Insertion into the Parent Membrane

The sequence of insertion of components into the membrane, and conversion of an area of membrane into a budding patch, may be determined by the type of virus to be formed. Viruses formed by budding can be divided into (a) those having a loose envelope of rather variable shape which seems not to be tightly attached to the underlying nucleocapsid core (e.g. influenza, herpesviruses), and (b) those where the envelope is tightly packed onto the core (e.g. arboviruses, and rhabdoviruses except for the flattened end region of the particle). In group (a) myxoviruses are known to have an envelope structural protein or M protein forming a coherent but somewhat flexible shell within the envelope. This shell has insufficient cohesion in the absence of viral lipid to be insolated as such without preliminary cross-linking with glutaraldehyde (SCHULZE, 1970). The presence of a similar protein or group of proteins has not been demonstrated for herpesviruses, but at least ten structural polypeptides have been identified which are neither glycosylated (envelope outer surface) or core, and hence might fulfil this function (HEINE and ROIZMAN, 1973). In (b), the nucleocapsid core is complete and tightly packed before budding begins (e.g. Semliki Forest virus, GRIMLEY and FRIEDMAN, 1970). The same is true of the rhabdoviruses except that ordering of the nucleocapsid into a tight cylindrical spiral takes place during rather than before budding (HOWATSON, 1970). Rhabdoviruses are thought to contain envelope M protein (KANG and PREVEC, 1970), and specific binding may take place between envelope proteins and nucleocapsid protein, on the basis of numerical correspondence between these polypeptide species (SOKOL et al., 1971; NEU-

RATH et al., 1972). This seems to be indicated also by phenotypic mixing experiments involving double infection with vesicular stomatitis virus (VSV) and paramyxovirus SV5; particles contained surface glycoproteins from either virus, but VSV nucleoprotein was associated only with VSV membrane structural protein (McSHARRY et al., 1971). However, the rhabdovirus envelope appears to have integrity quite distinct from that conferred by nucleocapsid binding, in the flattened base region where a ballooning or re-entrant form may be shown. Thus the important factor in rhabdovirus envelope assembly seems to be the time sequence of coiling of nucleocapsid, and this could conceivably be under the influence of a very small number of molecules of "morphopoietic factor". The critical requirement for both (a) and (b) is for a structural component capable of forming a base on which the envelope lipids and surface polypeptides can be assembled. It seems likely also that the characteristic curvature of the envelope both in budding and in the mature particle will be determined by this structural factor, perhaps also aided in part by lateral repulsion between external projections of the virion.

It is not known exactly how proteins such as the glycosylated surface proteins of the virus are inserted through the lipid region of the host membrane, nor how, once inserted, they are anchored in position. We have suggested a possible method, based on a structural model for influenza virus (BLOUGH, 1969; TIFFANY and BLOUGH, 1970a), involving local phase changes of the membrane lipid region from a bilayer to a micellar form, which would permit passage of the external proteins and leave them anchored to M protein bases with the lipid remaining in micellar form (Fig. 1A). If the lipid of the envelope is, in fact, in bilayer form, as is suggested from spin resonance studies of influenza virus and X-ray diffraction analysis of Sindbis virus, rearrangement of lipid from micellar to bilayer form could follow the insertion. Calculations by OHKI and AONO (1970) indicate that bilayer-hexagonal lipid phase rearrangements may take place within the range of net charge of 0–2e per phospholipid molecule, with the bilayer being the lowest energy form. Higher net charge densities at the polar end of envelope surface proteins may aid in the preliminary conversion, and the passage into the membrane of the apolar inner end of the surface protein might then reduce local net charge sufficiently to induce reversion to a bilayer configuration of lipid. In the proposed influenza model, subsequent surface proteins were inserted alongside the first with lateral interactions between M protein bases holding the units together until a patch of cell membrane was entirely infiltrated by viral material (Fig. 1B). Prior insertion of such units might facilitate addition of further units, but this is not strictly necessary to the model. Conceivably a mechanism of this type could operate in the normal insertion of glycosylated cell membrane proteins which then show lateral mobility (SINGER and NICOLSON, 1972), as an alternative to the reverse-pinocytosis method proposed by HIRANO et al. (1972). A similar process of fusion between virus-specific cytoplasmic vesicles and the plasma membrane has been suggested for myxovirus assembly (CHOPPIN et al., 1972; Fig. 1C). If lateral mobility exists, then the point of insertion

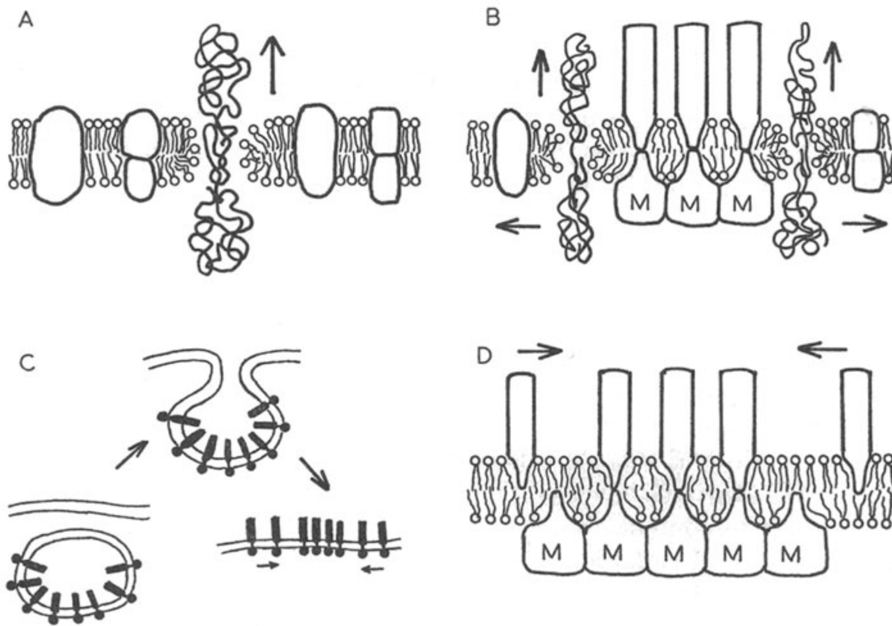

Fig. 1 A–D. Models of assembly of virus-specific areas of membrane. Note that a complete penetration of the membrane by spike and M protein is not an essential part of any of these models. The models are not meant to indicate one-to-one correspondence between spikes and M protein units. (A) Insertion of spike protein (shown here already associated with M protein) through the membrane. A local phase change of membrane lipid aids insertion. The membrane structure shown is the fluid mosaic model of SINGER and NICOLSON (1972). (B) Presence of an inserted spike makes further insertion favorable, with a similar lipid phase change in each case. In the completed region the lipid is shown to have reverted to a bilayer configuration. Membrane proteins are gradually displaced laterally since a coherent "raft" of viral M protein is formed. (C) Method of insertion by fusion of virus-specific vesicles with the membrane as suggested by CHOPPIN et al. (1972). (D) Model showing lateral mobility of spikes and M protein. Lateral cohesion of M proteins prevents redispersal

of viral glycoproteins may not, in fact, be at the subsequent site of budding (Fig. 1 D). If more random entry of surface proteins is postulated, budding itself must be preceded by the presence at the inner membrane surface of the major envelope structural factor—a firm nucleocapsid "former" or a raft or island of structural M protein.

D. Organisation of the Envelope Prior to Budding

If insertion of envelope external proteins is essentially a random process, lateral diffusion of surface units, floating in a sea of membrane lipid, would take place until the area over the "former" contained a full complement of surface proteins (not the full amount required for the envelope, but enough to initiate budding). A major problem at this point, however, is the mechanism

of recognition across the lipid region between surface proteins and "former". It has been generally assumed, in envelope models postulating a lipid bilayer (CHOPPIN et al., 1972; KLENK, 1973), that surface proteins of the envelope have a hydrophobic end which is inserted into the outer leaflet of the bilayer in the same manner as in the fluid mosaic membrane model (SINGER and NICHOLSON, 1972); this is to some extent supported by evidence on the manner of aggregation of isolated envelope subunits (LAVER and VALENTINE, 1969) and from the extensive non-polar amino acid composition of Semliki Forest virus glycoprotein (GAHMBERG et al., 1972b). No evidence exists at the moment for a corresponding insertion of "former" protein from the inner side of the bilayer (LENARD et al., 1974), but it is hard to see how laterally-mobile surface proteins could be constrained to aggregate in a patch overlying the "former" unless some direct interaction through the bilayer anchors them into position (Fig. 2A). This anchoring is an essential part of our model for the influenza virus envelope (TIFFANY and BLOUGH, 1970a), although we are prepared to concede that the lipid region may be substantially in the form of a bilayer. In addition, the fluidity indicated to exist in the lipid region of the influenza virus envelope (LANDSBERGER et al., 1971, 1973) from spin-resonance studies would tend to make interaction between M protein and surface proteins across an intact inner bilayer leaflet even more difficult. The relative numbers of spike and envelope polypeptides calculated for rabies (NEURATH et al., 1972; SOKOL et al., 1971) and vesicular stomatitis viruses (CARTWRIGHT et al., 1972), suggest that surface protein units may be anchored to the "former" in the rhabdovirus envelope. Unless such an anchoring takes place, it is hard to explain recognition between external and internal proteins of the envelope, sufficient to maintain cohesion of the "raft" without loss by lateral diffusion, and to displace host membrane proteins from the area, since none are found in mature virus (HOLLAND and KIEHN, 1970). Fig. 2B shows a possible method, which involves specific interaction between inner bilayer leaflet lipids and the M protein; lipid fluidity would be limited largely to the outer leaflet in this model.

E. Bud Growth and Aberrant Forms of the Envelope

At the time of initiation of budding, it is unlikely that all the envelope materials required have already entered the membrane. Subsequent enlargement of the bud must then take place by lateral diffusion and/or direct insertion into a "growing ring" surrounding the line of attachment of the bud to the membrane. It is in this stage of assembly that anomalies of envelope structure become apparent for myxoviruses grown in the presence of detergents (BLOUGH, 1963a) or exogenous lipids (BLOUGH, 1963b, 1964) or high titre passage "incomplete" or von Magnus virus (BLOUGH et al., 1969; BLOUGH and MERLIE, 1970). These forms of virus are markedly pleomorphic, and strains which are normally spherical frequently show filamentous particles. Exogenous branched chain fatty acids have been shown to be incorporated

into phospholipids of influenza virus, thus changing the composition of viral lipids (BLOUGH and TIFFANY, 1969), and the lipid metabolism of the host cell is considerably altered during production of von Magnus virus (BLOUGH and WEINSTEIN, 1973). Several mechanisms can be postulated for this change in shape:

a) The presence of exogenous lipids or shifts in availability of cellular lipids causes "expansion" of the lipid region and weakening of forces within the envelope, thus making it more flexible.

b) Selective binding of certain lipids to M protein produces two types corresponding roughly to "penton" and "hexon" of regular viruses, in which relatively few "pentons" are available, leaving predominantly "hexon", which tends to form tubular filaments of indeterminate length.

c) There may actually be two closely-related M proteins of very similar molecular weight and physical properties, which have a hexon-like or penton-like function. The relative availability of these may depend on host type and growth conditions.

d) Modification of normal viral lipid composition may be less important than the time sequence of changes in infected cell metabolism leading to restrictions in the proportions of envelope materials available at the budding site (possibly through influencing lipid-aided transport of proteins). Thus although enough "penton" material may be available to initiate budding, the local supply may fall off so that less is subsequently available during the rounding-up and pinching off of the virus bud, and the resultant local excess of "hexon" produces filaments.

Normally filamentous strains of influenza (e.g. influenza C) or SV5 may be produced in a similar manner, but here the nucleocapsid may act to a greater extent as a "former" since it appears to be uniformly helically wound in a continuous strand against the inner wall of the envelope, unlike the loosely-bundled nucleocapsid of influenza A and B.

F. Release

Bud termination in loosely-enveloped viruses may also be determined by local availability of "penton" material, resulting in a sudden necking down of the envelope bud. Little is known of the mechanism of pinching off and resealing of membrane and envelope at this time, but the process presumably resembles that involved in pinching off membrane fragments (such as the "normal cell particles" released from the allantois of embryonated hen's eggs) or in intracytoplasmic vesicle formation; it has in fact been suggested that virus release is only a modification of this normal exfoliation phenomenon (KINGSBURY, 1972). After pinching off, release of the particle from the cell (assuming it buds at the cell membrane) may be influenced by accessibility of receptor groups for the virus on the surrounding cell surface. Influenza virus is pinched off but remains bound at the cell surface if antibody directed against viral neuraminidase is present (SETO and CHANG, 1969; DOWDLE et al.,

1974). Pretreatment of the cell during the eclipse phase with the antibody tends also to produce filamentous rather than spherical particles. Conceivably an effect similar to that in Sec. IV E (b) or (c) might operate, in which neuraminidase was preferentially bound to the "penton" rather than the "hexon" component; the presence of antibody to neuraminidase would than sequester the enzyme and cause "hexon" and hence filamentous structures to predominate.

G. Forces Operating during Assembly and in Maintenance of Structure

Until recently, relatively little attention has been given to the types of interaction involved in assembly and maintenance of the integrity of the viral envelope. It is common practice to use the terms hydrophobic and hydrophilic loosely to justify a particular arrangement of molecules within a particle, but this may not be sufficient to determine whether the postulated structure will be stable, or whether a logical series of assembly steps can take place to produce this structure.

The information at present available on the types of forces actively contributing to envelope stability comes partly from experiments with various disruptive agents, and partly by an extension of the available information on cell membrane structural factors to the case of the viral envelope. Thus by a judicious choice of agent (detergents, lipid solvents, proteases or lipases) or changes in pH or ionic strength (WAITE et al., 1972), virus particles may be partly dismantled, and the ease with which this is done gives some indication of the milieu of subunit molecules prior to release. Another approach involves mixing isolated viral components (e.g. lipids and proteins) in an attempt to reconstruct the particle under controlled conditions, but this is subject to experimental difficulties as mentioned above.

One should also perhaps be wary of attempts to predict forces or structural arrangements in the viral envelope from data on the organisation of the parent membrane at which the virus was assembled. Although the trilamellar staining pattern of the parent membrane seems to continue into the virus envelope during budding, host proteins are not present in the budding region (HOLLAND and KIEHN, 1970; AOKI et al., 1970), and a quite different pattern of interaction between protein and lipid may exist in the virus envelope from that in the unperturbed membrane without change in the stained electron-optical appearance. Since there is no synthesis or turnover of envelope components in the mature virus and its necessary functions are few, it seems likely that the fluidity and fluctuating composition which are desirable in a functional membrane will be unnecessary or possibly structurally undesirable in the virus. In addition, the procedures used in preparation of specimens for electron microscopy may themselves introduce an apparent similarity of structure.

Nevertheless, the same general principles must apply within virus envelopes as in other structures involving protein and lipid molecules, since similar polar, ionic and non-polar chemical groups are present in both cases. The

possible magnitude of these interacting forces can be assessed, although of course many variations can occur in an actual viral system through minor changes in composition, etc.

The effects likely to be of importance are electrostatic or ionic forces, permenent and induced dipole interactions, interpeptide or sidechain hydrogen bonds in proteins, and dispersion or London-van der Waals forces. The magnitudes of some of these have been estimated by SALEM (1962a, b): electrostatic interaction energies (attractive or repulsive) are in the region of 5 kcal/mol for a pair of univalent charged groups 5 Å apart, depending in part on the effective (microscopic) dielectric constant of the medium between them; induced dipole energies (e.g. between an ionic or polar group and a dipole induced in a $—CH_2$ group) are typically less than 0.1 kcal/mol and can probably be ignored; dispersion forces are attractive but fall off very rapidly ($\propto 1/D^6$) with increasing separation D of the interacting groups, and energies between two groups are small (ca. 0.1 kcal/mol), but if summed over all the groups of closely-apposed parallel hydrocarbon chains the resultant may be as high as 8 kcal/mol for a pair of stearoyl chains 5 Å apart in a monomolecular film. Similar magnitudes are also possible between strong permanent dipole groups. Van der Waals forces are not limited to hydrocarbons, but can also operate between saturated or largely apolar regions of proteins, and between proteins and lipids. These forces are of particular importance in extensive and closely-packed water-insoluble regions such as the hydrocarbon tail region of a phospholipid bilayer, where the net ionic interaction is either near zero or repulsive. Because of their extreme sensitivity to separation of the interacting groups, Salem has termed these summed dispersion forces "distance-sensitive" (SALEM, 1962a, b). These forces may hence be of comparable magnitude to ionic forces; however, the hydrogen-bonding nature of water itself may disturb these interactions (KAUZMANN, 1959) and entropic factors due to limitation of rotation of the hydrogen-bonded groups may influence their effectiveness. Hydrogen bonding of water could be of considerable importance in retention or loss of tertiary structure when a structural protein is extracted from the virus and purified for use in reconstitution experiments. It should be noted that the additivity of ionic forces operates in the opposite direction to that of van der Waals forces even when arrays of positive and negative charges are present; the net interaction energy of such on ionic array is always at least slightly repulsive, whereas van der Waals forces are always attractive.

The contribution of each of the types of force mentioned above to envelope structure can be at least partially estimated from the response to disruptive agents. Ionic forces, especially those depending on net charge neutralization with divalent cations, will be disrupted if the ionic strength of the medium is sharply raised. Hydrogen bonding will be weakened in the presence of high concentrations of a hydrogen-bonding solute such as urea, and disulfide bonds in the same way with sulfhydryl reagents. Lipid solvents and non-ionic detergents, by their ability to generate dispersion forces with non-polar regions

of the envelope, will weaken "hydrophobic" structural relationships. Anionic or cationic detergents (such as sodium dodecyl sulfate or sodium deoxycholate) have a similar effect, but are frequently more dependent on other components of the system which may influence their micellar behaviour. These effects may be reversible on withdrawal of the disruptive agent from an isolated fraction of the envelope.

H. The Role of the M Protein in Envelope Structure

The detection and isolation of a virus envelope internal structural protein (the M protein) is one of the most important advances of recent years in the study of enveloped viruses. Such a protein had been predicted for influenza virus (BLOUGH, 1969; TIFFANY and BLOUGH, 1970b) on the basis of the then known dimensions and amounts of protein in the virion. Thus it was known that the envelope "unit membrane" appeared thicker on the inner side, depending on the electron microscopic staining procedure used (COMPANS and DIMMOCK, 1969), and after prolonged protease digestion (KENDAL et al., 1969). Further evidence soon followed (COMPANS et al., 1970) of a protein identifiable on polyacrylamide gels as an envelope component but not one of the external glycoproteins. Since then, envelope structural proteins have also been identified for rhabdoviruses, and classified (WAGNER et al., 1972), andsimilar proteins will in due course be discovered for other virus groups. Because of its site in the envelope, the M protein must inevitably be considered in relation to the viral lipid, and a number of points remain unresolved with regard to the packing and interrelationship of the two.

TIFFANY and BLOUGH (1970a) gave calculations indicating that a considerable excess of lipid appeared to be present in the influenza virion over that necessary to form a bilayer (80–100%), and the actual volume available in the bilayer would be further reduced by penetration of surface proteins in the same manner as in SINGER and NICOLSON's model (1972) for the cell membrane, as is indicated for many groups of viruses (SCHULZE, 1973; GAHMBERG et al., 1972b; VERNON et al., 1972). The problem is even more acute in the case of leukoviruses, which have as much as 35% lipid, although here the possibility of lipid in the particle core remains unresolved. It is of course hazardous to perform calculations with a virus as notoriously variable in size and shape as influenza, as can be seen on recalculating the particle size necessary to accommodate the lipid content of the A_0/PR_8 strain (average diameter 1000 Å); thus there is 80% more bilayer area in a particle of 1270 Å overall diameter than in a 1000 Å particle (mean bilayer diameter increasing from 760 Å to 1030 Å). It seems clear that the effect of various methods of preparation for electron microscopy on the dimensions of the virion requires closer study (NERMUT and FRANK, 1971). In addition, as mentioned in BLOUGH and TIFFANY (1973), the calculations depend closely on the value taken for the molecular weight of the whole virion; LANDSBERGER et al. (1971) calculate that there is just the amount of lipid required for a bilayer by using a particle

MW of 250×10^6, whereas a more reliable figure is 360×10^6, based both on the protein data of REIMER et al. (1966) and the MW of viral RNA (SKEHEL, 1971). Interpretation of the size of substructures of the envelope from electron micrographs is subject to variation also. SCHULZE (1972) quotes 60 Å both for the M protein shell thickness and the bilayer thickness, whereas X-ray data on lipid bilayers show a thickness of 40 Å (WILKINS et al., 1971) and an alternative interpretation of the staining pattern of the M protein region (30–40 Å thick) is given by NERMUT (1972).

The original model of TIFFANY and BLOUGH (1970a) attempted to overcome this excess of lipid by postulating that the M protein formed specific associations with lipids in the same way as the chloroplast lamellar protein studied by JI and BENSON (1960). This was related to the variations in lipid composition seen in different strains of the same virus type (TIFFANY and BLOUGH, 1969a, b; MCSHARRY and WAGNER, 1971; DAVID, 1971; BLOUGH, 1971; BLOUGH et al., 1967). From the degree of variation both in polar groups and in acyl chains, it would seem that the binding of at least a small proportion of the lipid to M protein is indicated, without specifying whether it is a polar or a hydrophobic interaction. Fig. 2B shows a type of polar interaction which could take place with a non-penetrating M protein, to provide a partially-immobilized lipid region in the neighbourhood of the M protein "raft", capable of serving as a recognition site for floating and laterally-mobile spike proteins. We are still inclined to favour the penetration of the inner bilayer leaflet by M protein (Fig. 2A) as offering assembly and structural advantages, and possibly also as explaining the discrepancies of staining patterns noted between SCHULZE (1972) and NERMUT (1972).

It is of interest to see whether the influenza M protein data of GREGORIADES (1973) may be used in the calculation method of TREMAINE and GOLDSACK (1968), to determine the ability of this protein to form a shell by non-polar interaction alone in the same way as capsid proteins of small regular viruses (BANCROFT et al., 1967). This calculation depends on the assumption that all polar amino acids are located on the inner and outer faces of a spherical shell formed of the protein units. We assume also that the units are cylindrical and that their partial specific volume is 0.72 cc/g. The volume of the protein unit is then 30900 Å^3 from its MW of 25900 (SCHULZE, 1970), and its polar area from the TREMAINE and GOLDSACK calculation (1968) is 1411 Å^2. This corresponds to a mean cylindrical diameter of 30 Å and a shell thickness of 44 Å, in good agreement with earlier data. Using REIMER et al. (1966) value of 4.2×10^{-16} g protein per particle and assuming 50% of this is M protein (SCHULZE, 1970), we calculate that there are 4890 M protein units. This is higher than the values of 3300–3700 units quoted by SCHULZE (1972) who however has used a value of 42% for the proportion of M protein, apparently taken from COMPANS et al. (1970). The area of the hydrophobic sides of the units ($2\pi rh$) is 4100 Å^2 or 75% of the total area. CHOTHIA (1974) suggests a hydrophobic interaction energy of 24 cal/mole/Å^2 for such surfaces, giving $4100 \times 24 = 99$ kcal/mole per M protein unit. This seems to provide a sub-

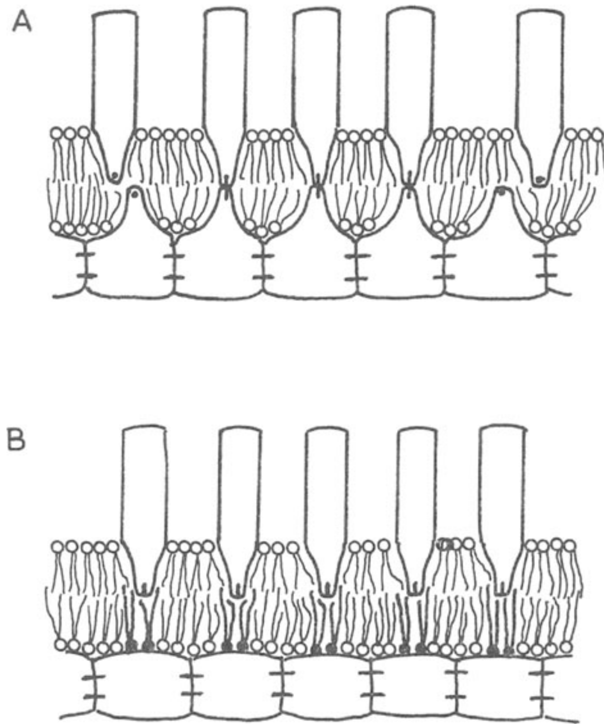

Fig. 2A and B. Penetrating and non-penetrating M protein models. Each M protein unit shown here may consist of about 6 individual molecules. (A) Assembly by lateral diffusion of "floating" spikes into the region where a coherent "raft" of M protein occurs. Spikes remain anchored and the patch remains free of host membrane proteins. Black bars represent hydrophobic interactions. (B) Non-penetrating M protein. The "raft" is coherent, but specific lipid binding in the inner lipid leaflet is necessary to provide a site for recognition by spike proteins. (Note that only a small proportion of viral lipid would be strain-specific in the case, involving only moderate differences between host membrane and viral lipid compositions

stantial stabilizing force within the envelope, and by further interaction between protein and lipid, a robust envelope structure which does not require an internal nucleocapsid former to maintain its integrity (BLOUGH, 1963a).

Rhabdoviruses were considered above to occupy an intermediate position between those viruses whose envelope form is determined by their M protein, and those which depended on a dense core to act as a former during formation of the envelope. From the numerical correspondence of the envelope structural proteins of rabies virus (SOKOL et al., 1971; NEURATH et al., 1972) and of vesicular stomatitis virus (CARTWRIGHT et al., 1972), it appears that there is specific binding between the nucleocapsid protein and an envelope protein, and this has been made the basis for interesting structural models (VERNON et al., 1972; CARTWRIGHT et al., 1972). Both of these models show the surface spike glycoproteins penetrating the lipid region, but the nature of the anchoring is not clear. We have calculated from analysis of rabies virus lipids (BLOUGH,

AASLESTAD and TIFFANY, unpublished data) that there is slightly less lipid per particle than is required to form a bilayer, and this may indicate specific binding between spike proteins and envelope protein rather than "floating" spikes. It has been noted that rabies virus is structurally more resistant to ether and detergent treatment than some other enveloped viruses (CRICK and BROWN, 1970), suggesting that lipid plays less part in maintaining the integrity of the envelope. The model of VERNON et al. (1972) accounts for the large hexagons which are apparently a structural feature of some strains of rabies virus examined by negative-contrast electron microscopy, as artifacts produced by superimposition of images from the upper and lower surfaces of the particle and the strong parallel banding of the helical nucleocapsid core. KUWERT et al. (1972) calculated that there were about 580 such hexagons with a centre-to-centre spacing of 100 Å; this appears to be based on mismeasurement of their micrographs, which in fact show a 200 Å spacing and hence about 140 hexagons per particle. The connection between this number and that of the spikes, which has been variously estimated as 790 (BLOUGH and TIFFANY, 1973) and 1072–1453 (VERNON et al., 1972), is not yet clear. The number of surface projections on vesicular stomatitis virus is estimated to be about 500 (CARTWRIGHT et al., 1972).

The exact architecture of the rhabdovirus envelope cannot be worked out with the data currently available, but it would seem that the M protein plays a two-fold role which is not seen (or only partially seen) in the myxoviruses: it acts as a base for the spike glycoproteins on the outer side of the envelope, and it also contributes to the stability of the inner helix of ribonucleoprotein within the particle.

It will be seen in Figs. 1 and 2 that we have indicated an apparent one-to-one correspondence between surface spikes and M protein units in the envelope. The numbers of M protein molecules calculated above and by SCHULZE (1972) are considerably greater than the number of spikes calculated from their surface spacing and the viral diameter, corresponding roughly to 6 molecules per spike unit. We do not consider it entirely improbable that the M protein will form sub-aggregates in this way, and each molecule need contribute only a very small "tail" of hydrophobic character to the penetration of the lipid layer. Such a tail would almost certainly be overlooked in the isolated molecule.

References

AOKI, T., BOYSE, E. A., OLD, D. T., HARVEN, E. DE, HÄMMERLING, U., WOOD, H. A.: G (Gross) and H-2 cell-surface antigens: Location on Gross leukemia cells by electron microscopy with visually labelled antibody. Proc. nat. Acad. Sci. (Wash.) **65**, 569–576 (1970)

BÄCHI, T., GERHARD, W., LINDENMANN, J., MÜHLETHALER, K.: Morphogenesis of influenza virus in Ehrlich ascites tumor cells as revealed by thin-sectioning and freeze-etching. J. Virol. **4**, 769–776 (1969)

BÄCHI, T., HOWE, C.: Morphogenesis and ultrastructure of respiratory syncytial virus. J. Virol. **12**, 1173–1180 (1973)

BANCROFT, J. B., HILLS, G. J., MARKHAM, R.: A study of the self-assembly process in a small spherical virus. Formation of organized structures from protein subunits *in vitro*. Virology **31**, 354–379 (1967)
BEN-PORAT, T., KAPLAN, A. S.: Studies on the biogenesis of herpesvirus envelope. Nature (Lond.) **235**, 165–166 (1972)
BIKEL, I., KNIGHT, C. A.: Differential action of *Aspergillus* glycosidases on the hemagglutinating and neuraminidase activities of influenza and Newcastle disease viruses. Virology **49**, 326–332 (1972)
BLOUGH, H. A.: The role of the surface state in the morphogenesis of influenza virus filaments. Virology **19**, 112–114 (1963a)
BLOUGH, H. A.: The effect of vitamin A alcohol on the morphology of myxoviruses. I. The production and comparison of artificially produced filamentous virus. Virology **19**, 349–358 (1963b)
BLOUGH, H. A.: Role of the surface state in the development of myxoviruses. In: Ciba Symposium, Cellular Biology of Myxovirus infections, G. W. WOLSTENHOLME and J. KNIGHT (eds.), p. 120–143. London: J. & A. Churchill 1964
BLOUGH, H. A.: Studies on the structure of influenza virus envelope. Bull. Wld. Hlth. Org. **41**, 487–488 (1969)
BLOUGH, H. A.: Fatty acid composition of individual phospholipids of influenza virus. J. gen. Virol. **12**, 317–320 (1971)
BLOUGH, H. A.: The effect of chronic viral infections on the synthesis and turnover of lipids in cultured animal cells. Ninth Intl. Cong. Biochem. P407 (1973)
BLOUGH, H. A.: New lipids incorporated into the membrane of influenza virus. Nature (Lond.) **251**, 333–335 (1974)
BLOUGH, H. A., GALLAHER, W. R., WEINSTEIN, D. B.: Viral lipids: Host cell biosynthetic parameters. In: Membrane mediated information, P. W. KENT (ed.), **1**, p. 183–199. Lancaster: Medical & Technical Publishing Co. 1973
BLOUGH, H. A., LAWSON, D. E. M.: The lipids of paramyxoviruses: a comparative study of Sendai and Newcastle disease viruses. Virology **36**, 286–292 (1968)
BLOUGH, H. A., MERLIE, J. P.: The lipids of incomplete influenza virus. Virology **40**, 685–692 (1970)
BLOUGH, H. A., MERLIE, J. P., TIFFANY, J. M.: The fatty acid composition of incomplete influenza virus. Biochem. biophys. Res. Commun. **34**, 831–834 (1969)
BLOUGH, H. A., TIFFANY, J. M.: Incorporation of branched-chain fatty acids into myxoviruses. Proc. nat. Acad. Sci. (Wash.) **62**, 242–247 (1969)
BLOUGH, H. A., TIFFANY, J. M.: Lipids in viruses. Advanc. Lipid Res. **11**, 267–339 (1973)
BLOUGH, H. A., WEINSTEIN, D. B.: Effect of influenza virus infection on lipid metabolism of chick embryo fibroblasts. In: Biology of the fibroblast, E. KULONEN and J. PIKKARAINEN (eds.), p. 303–308. London: Academic Press 1973
BLOUGH, H. A., WEINSTEIN, D. B., LAWSON, D. E. M., KODICEK, E.: The effect of vitamin A on myxoviruses. II. Alterations in the lipids of influenza virus. Virology **33**, 459–466 (1967)
BOLOGNESI, D. M., LUFTIG, R., SHAPER, J. H.: Localization of RNA Tumor virus polypeptides. I. Isolation of further virus substructures. Virology **56**, 549–564 (1973)
BRADY, R. O., FISHMAN, P. H.: Alterations in membrane glycolipids in tumorigenic virus-transformed cell lines. In: Membrane mediated information, P. W. KENT (ed.), **1**, p. 18–63. Lancaster: Medical & Technical Publishing Co. 1973
BRANTON, D.: Membrane structure. Ann. Rev. Plant Physiol. **20**, 209–238 (1969)
BRETSCHER, M. S.: Human erythrocyte membranes: specific labelling of surface proteins. J. molec. Biol. **58**, 775–781 (1971)
BROWN, D. T., WAITE, M. R. F., PFEFFERKORN, E. R.: Morphology and morphogenesis of Sindbis virus as seen with freeze-etching techniques. J. Virol. **10**, 524–536 (1972)
CACAM, J. F., EYLAR, E. H.: Glycoprotein biosynthesis: Purification and characterization of a glycoprotein: galactosyl transferase from Ehrlich ascites tumor cell membranes. Arch. Biochem. Biophys. **137**, 315–323 (1970)
CARTWRIGHT, B., SMALE, C. J., BROWN, F., HULL, R.: Model for vesicular stomatitis virus. J. Virol. **10**, 256–260 (1972)
CARTWRIGHT, B., TALBOT, P., BROWN, F.: The proteins of biologically active subunits of vesicular stomatitis virus. J. gen. Virol. **7**, 267–272 (1970)

CHOPPIN, P. W., COMPANS, R. W., SCHEID, A., MCSHARRY, J. J., LAZAROWITZ, S. G.: Structure and assembly of viral membranes. In: Membrane research, C. F. Fox (ed.), p. 163–185. London-New York: Academic Press 1972

CHOTHIA, C.: Hydrophobic bonding and accessible surface area in proteins. Nature (Lond.) **248**, 338–339 (1974)

COMPANS, R. W., DIMMOCK, N. J.: An electron microscope study of single-cycle infection of chick embryo fibroblasts by influenza virus. Virology **39**, 499–515 (1969)

COMPANS, R. W., KLENK, H.-D., CALIGUIRI, L. A., CHOPPIN, P. W.: Influenza virus proteins. I. Analysis of polypeptides of the virion and identification of spike glycoproteins. Virology **42**, 880–889 (1970)

COOPER, P. D.: A chemical basis for the classification of animal viruses. Nature (Lond.) **190**, 302–305 (1961)

COURTNEY, R. J., STEINER, S. M., BENYESH-MELNICK, M.: Effect of 2-deoxy-D-glucose on Herpes simplex virus replication. Virology **52**, 447–455 (1973)

CRICK, J., BROWN, F.: In: The biology of large RNA viruses, R. D. BARRY and B. W. J. MAHY (eds.), p. 133. New York: Academic Press 1970

DAVID, A. E.: Lipid composition of Sindbis virus. Virology **46**, 711–720 (1971)

DEAMER, D. W., BRANTON, D.: Fracture planes in an ice-bilayer model membrane system. Science **158**, 655–657 (1967)

DEFRENE, A., LOUISOT, P.: Glycoprotein biosynthesis in animals infected with a myxovirus. II. Study of physicochemical parameters of liver *N*-acetyl-glucosaminyl transferase. Int. J. Biochem. **4**, 249–258 (1973)

DOWDLE, W. R., DOWNIE, J. C., LAVER, W. G.: Inhibition of virus release by antibodies to surface antigens of influenza viruses. J. Virol. **13**, 269–275 (1974)

FROGER, C., LOUISOT, P.: Comportement des mannosyltransférases microsomiques dans les cellules porteuses d'une infection à Arbovirus. C. R. Acad. Sci. (Paris) **274**, 737–740 (1972a)

FROGER, C., LOUISOT, P.: Glycoprotein synthesis in arbovirus-infected cells. II. Study of microsomic mannosyl transferase activity. Int. J. Biochem. **3**, 613–622 (1972b)

GAHMBERG, C. G., SIMONS, K., RENKONEN, O., KÄÄRIÄINEN, L.: Exposure of proteins and lipids in the Semliki Forest virus membrane. Virology **50**, 259–262 (1972a)

GAHMBERG, C. G., UTERMANN, G., SIMONS, K.: The membrane proteins of Semliki Forest virus have a hydrophobic part attached to the viral membrane. FEBS Letters **28**, 179–182 (1972b)

GALLAHER, W. R., BLOUGH, H. A.: Syntheses and turnover of lipids in monolayer cultures of BHK-21 cells. Arch. Biochem. Biophys. (in press)

GALLAHER, W. R., LEVITAN, D. B., BLOUGH, H. A.: Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and Herpes simplex viruses. Virology **55**, 193–201 (1973a)

GALLAHER, W. R., WEINSTEIN, D. B., BLOUGH, H. A.: Rapid turnover of principal phospholipids in BHK-21 cells. Biochem. biophys. Res. Commun. **52**, 1252–1256 (1973b)

GARON, C. F., MOSS, B.: Glycoprotein synthesis in cells infected with vaccinia virus. II. A glycoprotein component of the virion. Virology **46**, 233–246 (1971)

GREEN, D. E., PERDUE, J. F.: Membranes as expressions of repeating units. Proc. nat. Acad. Sci. (Wash.) **55**, 1295–1302 (1966)

GREGORIADES, A.: The membrane protein of influenza virus: extraction from virus and infected cell with acidic chloroform-methanol. Virology **54**, 369–383 (1973)

GRIMES, W. J., BURGE, B. W.: Modification of Sindbis virus glycoprotein by host-specified glycosyl transferases. J. Virol. **7**, 309–313 (1971)

GRIMLEY, P. M., FRIEDMAN, R. M.: Development of Semliki Forest virus in mouse brain: an electron microscopic study. Exp. molec. Path. **12**, 1–13 (1970)

HAGOPIAN, A., BOSMANN, H. B., EYLAR, E. H.: Glycoprotein biosynthesis: the localization of polypeptidyl: N-acetylgalactosaminyl, collagen:glucosyl, and glycoprotein:galactosyl transferases in HeLa cell membrane fractions. Arch. Biochem. Biophys. **128**, 387–396 (1968)

HARRISON, S. C., CASPAR, D. L. D., CAMERINI-OTERO, R. D., FRANKLIN, R. M.: Lipid and protein arrangement in bacteriophage PM2. Nature (Lond.) New Biol. **229**, 197–201 (1971a)

Harrison, S. C., David, A., Jumblatt, J., Darnell, J. E.: Lipid and protein organization in Sindbis virus. J. molec. Biol. **60**, 523–528 (1971 b)

Haslam, E. A., Hampson, A. W., Radiskevics, I., White, D. O.: The polypeptides of influenza virus. III. Identification of the hemagglutinin, neuraminidase and nucleocapsid proteins. Virology **42**, 566–575 (1970)

Hayman, M. J., Skehel, J. J., Crumpton, M. J.: Purification of virus glycoproteins by affinity chromatography using *Lens culinaris* phytohemagglutinin. FEBS Letters **29**, 185–188 (1973)

Heine, J. W., Roizman, B.: Proteins specified by Herpes simplex virus. IV. Contiguity of host and viral proteins in the plasma membrane of infected cells. J. Virol. **11**, 810–813 (1973)

Hirano, H., Parkhouse, B., Nicolson, G. L., Lennox, E. S., Singer, S. J.: Distribution of saccharide residues on membrane fragments from a myeloma cell homogenate: its implications for membrane biogenesis. Proc. nat. Acad. Sci. (Wash.) **69**, 2945–2949 (1972)

Holland, J. J., Kiehn, E. D.: Influenza virus effects on cell membrane proteins. Science **167**, 202–205 (1970)

Homma, M., Tamagawa, S.: Restoration of the fusion activity of L cell-borne Sendai virus by trypsin. J. gen. Virol. **19**, 423–426 (1973)

Hosaka, Y., Shimizu, Y. K.: Artificial assembly of envelope particles of HVJ (Sendai virus). I. Assembly of hemolytic and fusion factors from envelopes solubilized by Nonidet P40. Virology **49**, 627–639 (1972a)

Hosaka, Y., Shimizu, Y. K.: Artificial assembly of envelope particles of HVJ (Sendai virus). II. Lipid components for formation of the active hemolysin. Virology **49**, 640–646 (1972b)

Howatson, A. F.: Vesicular stomatitis and related viruses. Advanc. Virus Res. **16**, 195–256 (1970)

Hoyle, L., Horne, R. W., Waterson, A. P.: The structure and composition of the myxoviruses. II. Components released from the influenza virus particle by ether. Virology **13**, 448–459 (1961)

Hubbell, W. L., McConnell, H. M.: Spin label studies of the excitable membranes of nerve and muscle. Proc. nat. Acad. Sci. (Wash.) **61**, 12–16 (1968)

Hubbell, W. L., Metcalfe, J. C., Metcalfe, S. M., McConnell, H. M.: The interaction of small molecules with spin-labelled erythrocyte membrane. Biochim. biophys. Acta (Amst.) **219**, 415–427 (1970)

Israelachvili, J. N.: Theoretical considerations on the asymmetrical distribution of charged phospholipid molecules on the inner and outer layers of curved bilayer membranes. Biochim. biophys. Acta (Amst.) **323**, 659–663 (1973)

Ji, T. H., Benson, A. A.: Association of lipids and proteins in chloroplast lamellar membrane. Biochim. biophys. Acta (Amst.) **150**, 686–693 (1968)

Jost, P. C., Griffith, O. H., Capaldi, R. A., Vanderkooi, G.: Evidence for boundary lipid in membranes. Proc. nat. Acad. Sci. (Wash.) **70**, 480–484 (1973)

Kaluza, G., Scholtissek, C., Rott, R.: Inhibition of the multiplication of enveloped RNA-viruses by glucosamine and 2-deoxy-D-glucose. J. gen. Virol. **14**, 251–259 (1972)

Kang, C. Y., Prevec, L.: Proteins of vesicular stomatitis virus. II. Immunological comparisons of viral antigens. J. Virol. **6**, 20–27 (1970)

Kates, M., Allison, A. C., Tyrrell, D. A. J., James, A. T.: Lipids of influenza virus and their relation to those of host cells. Biochim. biophys. Acta (Amst.) **52**, 455–466 (1961)

Katz, E., Margalith, E.: Location of vaccinia virus structural polypeptides on the surface of the virus particle. J. gen. Virol. **18**, 381–384 (1973)

Katz, E., Moss, B.: Formation of a vaccinia virus structural polypeptide from a higher molecular weight precursor: inhibition by rifampicin. Proc. nat. Acad. Sci. (Wash.) **66**, 677–684 (1970)

Kauzmann, W.: Some factors in the interpretation of protein denaturation. Advanc. Protein Chem. **14**, 1–63 (1959)

Kendal, A. P., Apostolov, K., Belyavin, G.: The effect of protease treatment on the morphology of influenza A, B and C viruses. J. gen. Virol. **5**, 141–143 (1969)

Kingsbury, D. W.: Paramyxovirus replication. Curr. Topics Microbiol. and Immunol. **59**, 1–34 (1972)

KLENK, H.-D.: Glycoproteins and glycolipids in viral envelopes. In: Membrane mediated information (P. W. KENT, ed.), vol. 1, p. 200–211. Lancaster: Medical and Technical Publishing Co. 1973

KLENK, H.-D., COMPANS, R. W., CHOPPIN, P. W.: An electron microscopic study of the presence or absence of neuraminic acid in enveloped viruses. Virology **42**, 1158–1162 (1970)

KLENK, H.-D., CHOPPIN, P. W.: Glycosphingolipids of plasma membranes of cultured cells and an enveloped virus (SV5) grown in these cells. Proc. nat. Acad. Sci. (Wash.) **66**, 57–64 (1970)

KLENK, H.-D., ROTT, R.: Formation of influenza virus proteins. J. Virol. **11**, 823–831 (1973)

KLENK, H.-D., ROTT, R., BECHT, H.: On the structure of the influenza virus envelope. Virology **47**, 579–591 (1972)

KORNBERG, R. D., MCCONNELL, H. M.: Lateral diffusion of phospholipids in a vesicle membrane. Proc. nat. Acad. Sci. (Wash.) **68**, 2564–2568 (1971)

KUWERT, E., BÖHME, U., LICKFELD, K. G., BÖHME, W.: Zur Oberflächenstruktur des Tollwutvirion (TWV). Zbl. Bakt. I. Abt. Orig. A **219**, 39–45 (1972)

LAFFERTY, K. J.: The interaction between virus and antibody. II. Mechanism of the reaction. Virology **21**, 76–90 (1963)

LANDSBERGER, F. R., COMPANS, R. W., CHOPPIN, P. W., LENARD, J.: Organisation of the lipid phase in viral membranes. Effects of independent variation of the lipid and the protein composition. Biochemistry (Wash.) **12**, 4498–4502 (1973)

LANDSBERGER, F. R., LENARD, J., PAXTON, J., COMPANS, R. W.: Spin-label electron spin resonance study of the lipid-containing membrane of influenza virus. Proc. nat. Acad. Sci. (Wash.) **68**, 2579–2583 (1971)

LAVER, W. G.: Structural studies on the protein subunits from three strains of influenza virus. J. molec. Biol. **9**, 109–124 (1964)

LAVER, W. G.: Separation of two polypeptide chains from the hemagglutinin subunit of influenza virus. Virology **45**, 275–288 (1971)

LAVER, W. G.: The polypeptides of influenza viruses. Advanc. Virus Res. **18**, 57–103 (1973)

LAVER, W. G., BAKER, N.: Amino acid composition of polypeptides from influenza virus particles. J. gen. Virol. **17**, 61–67 (1972)

LAVER, W. G., VALENTINE, R. C.: Morphology of the isolated hemagglutinin and neuraminidase subunits of influenza virus. Virology **38**, 105–119 (1969)

LAZAROWITZ, S. G., COMPANS, R. W., CHOPPIN, P. W.: Influenza virus structural and non-structural proteins in infected cells and their plasma membranes. Virology **46**, 830–843 (1971)

LAZAROWITZ, S. G., GOLDBERG, A. R., CHOPPIN, P. W.: Proteolytic cleavage by plasmin of the HA polypeptide of influenza virus: host cell activation of serum plasminogen. Virology **56**, 172–180 (1973)

LENARD, J., COMPANS, R. W.: The membrane structure of lipid-containing viruses. Biochim. biophys. Acta (Amst.) **344**, 51–94 (1974)

LENARD, J., WONG, C. Y., COMPANS, R. W.: Association of internal membrane protein with the lipid bilayer in influenza virus. Biochim. biophys. Acta (Amst.) **332**, 341–349 (1974)

LESSLAUER, W., CAIN, J. E., BLASIE, J. K.: X-ray diffraction studies of lecithin bimolecular lipid leaflets with incorporated fluorescent probes. Proc. nat. Acad. Sci. (Wash.) **69**, 1499–1503 (1972)

LEVITAN, D. B., BLOUGH, H. A.: Preliminary biochemical characterization of the herpesvirus fusion factor(s). Virology (in press)

MCSHARRY, J. J., COMPANS, R. W., CHOPPIN, P. W.: Proteins of vesicular stomatitis virus and of phenotypically mixed vesicular stomatitis virus and simian virus 5 virions. J. Virol. **8**, 722–729 (1971)

MCSHARRY, J. J., COMPANS, R. W., LACKLAND, H., CHOPPIN, P. W.: Isolation of viral membrane proteins, p. 215. Abstr. 72nd Ann. Mtg. Amer. Soc. Microb. Phila., 1972

MCSHARRY, J. J., WAGNER, R. R.: Lipid composition of purified vesicular stomatitis viruses. J. Virol. **7**, 59–70 (1971)

MELCHERS, F.: Biosynthesis, intracellular transport, and secretion of immunoglobulins. Effect of 2-deoxy-D-glucose in tumor plasma cells producing and secreting immunoglobulin G_1. Biochemistry (Wash.) **12**, 1471–1476 (1973)

MICHAELSON, D. M., HORWITZ, A. F., KLEIN, M.: Transbilayer asymmetry and surface homogeneity of mixed phospholipids in cosonicated vesicles. Biochemistry (Wash.) **12**, 2637–2645 (1973)

MORA, P. T., CUMAR, F. A., BRADY, R. O.: A common biochemical change in SV40 and polyoma virus transformed mouse cells coupled to control of cell growth in culture. Virology **46**, 60–72 (1971)

MUDD, J. A., SUMMERS, D. F.: Protein synthesis in vesicular stomatitis virus infected HeLa cells. Virology **42**, 328–340 (1970)

NERMUT, M. V.: Further investigation on the fine structure of influenza virus. J. gen. Virol. **17**, 317–331 (1972)

NERMUT, M. V., FRANK, H.: Fine structure of influenza A_2 (Singapore) as revealed by negative staining, freeze-drying and freeze-etching. J. gen. Virol. **10**, 37–51 (1971)

NERMUT, M. V., FRANK, H., SCHÄFER, W.: Properties of mouse leukemia viruses. III. Electron microscopic appearance as revealed after conventional preparation techniques as well as freeze-drying and freeze-etching. Virology **49**, 345–358 (1972)

NEURATH, A. R., VERNON, S. K., DOBKIN, M. B., RUBIN, B. A.: Characteristics of subviral components resulting from treatment of rabies virus with tri(n-butyl) phosphate. J. gen. Virol. **14**, 33–48 (1972)

ODA, K., JOKLIK, W. K.: Hybridization and sedimentation studies on "early" and "late" vaccinia messenger RNA. J. molec. Biol. **27**, 395–419 (1967)

OHKI, S., AONO, O.: Phospholipid bilayer-micelle transformation. J. Colloid and Interface Sci. **32**, 270–281 (1970)

OKADA, Y., KIM, J.: Interaction of concanavalin A with enveloped viruses and host cells. Virology **50**, 507–515 (1972)

PFEFFERKORN, E. R., HUNTER, H. S.: The source of the ribonucleic acid and phospholipid of Sindbis virus. Virology **20**, 446–456 (1963)

PHILLIPS, D. R., MORRISON, M.: Exposed protein on the intact human erythrocyte. Biochemistry (Wash.) **10**, 1766–1771 (1971)

PINTO DA SILVA, P., BRANTON, D.: Membrane splitting in freeze-etching. J. Cell Biol. **45**, 598–605 (1970)

PRINTZ, P., WAGNER, R. R.: Temperature-sensitive mutants of vesicular stomatitis virus: synthesis of virus-specific proteins. J. Virol. **7**, 651–662 (1971)

RAY, E. K., BLOUGH, H. A.: Biosynthesis and transport of herpesvirus glycoproteins and glycolipids., p. 202 Abstr. 74th Ann. Mtg. Amer. Soc. Microbiol. Chicago, 1974

REGINSTER, M., CALBERG-BACQ, C.-M.: Further observations on the effects of caseinase C on the envelope of influenza and Newcastle disease viruses. J. Ultrastruct. Res. **23**, 144–152 (1968)

REIMER, C. B., BAKER, R. S., NEWLIN, T. E., HAVENS, M. L.: Influenza virus purification with the zonal ultracentrifuge. Science **152**, 1379–1381 (1966)

RUDY, B., GITLER, C.: Microviscosity of the cell membrane. Biochim. biophys. Acta (Amst.) **288**, 231–236 (1972)

SALEM, L.: Attractive forces between macromolecular chains of biological importance. Nature (Lond.) **193**, 476–477 (1962a)

SALEM, L.: The role of long-range forces in the cohesion of lipoproteins. Canad. J. Biochem. **40**, 1287–1298 (1962b)

SAROV, I., JOKLIK, W. K.: Studies on the nature and location of the capsid polypeptides of vaccinia virions. Virology **50**, 579–592 (1972)

SCANU, A. M., TARDIEU, A.: Temperature transitions of lipid mixtures containing cholesterol esters. Relevance to the structural problem of serum high density lipoprotein. Biochim. biophys. Acta (Amst.) **231**, 170–174 (1971)

SCHAEFER, R., HINNEN, R., FRANKLIN, R. M.: Further observations on the structure of the lipid-containing bacteriophage PM2. Nature (Lond.) **248**, 681–682 (1974)

SCHEID, A., CHOPPIN, P. W.: Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**, 475–490 (1974)

SCHLESINGER, M. J., SCHLESINGER, S., BURGE, B. W.: Identification of a second glycoprotein in Sindbis virus. Virology **47**, 539–541 (1970)

SCHULZE, I. T.: The structure of influenza virus. I. The polypeptides of the virion. Virology **42**, 890–904 (1970)

SCHULZE, I. T.: The structure of influenza virus. II. A model based on the morphology and composition of subviral proteins. Virology **47**, 181–196 (1972)

SCHULZE, I. T.: Structure of the influenza virion. Advanc. Virus Res. **18**, 1–56 (1973)

SEBRING, E. D., SALZMAN, N. P.: Metabolic properties of early and late vaccinia virus messenger ribonucleic acid. J. Virol. **1**, 550–558 (1967)

SEGREST, J. P., JACKSON, R. L., ANDREWS, E. P., MARCHESI, V. T.: Human erythrocyte membrane glycoprotein: a re-evaluation of the molecular weight as determined by SDS polyacrylamide gel electrophoresis. Biochem. biophys. Res. Commun. **44**, 390–395 (1971)

SETO, J. T., CHANG, F. S.: Functional significance of sialidase during influenza virus multiplication: an electron microscope study. J. Virol. **4**, 58–66 (1969)

SIMONS, K., KERÄNEN, S., KÄÄRIÄINEN, L.: Identification of a precursor for one of the Semliki Forest virus membrane proteins. FEBS Letters **29**, 87–91 (1973)

SINGER, S. J., NICOLSON, G. L.: The fluid mosaic model of the structure of cell membranes. Science **175**, 720–731 (1972)

SKEHEL, J. J.: Estimations of the molecular weight of the influenza virus genome. J. gen. Virol. **11**, 103–109 (1971)

SKEHEL, J. J., SCHILD, G. C.: The polypeptide composition of influenza A viruses. Virology **44**, 396–408 (1971)

SOKOL, F., CLARK, H. F.: Phosphoproteins, structural components of rhabdoviruses. Virology **52**, 246–263 (1973)

SOKOL, F., STANCEK, D., KOPROWSKI, H.: Structural proteins of rabies virus. J. Virol. **7**, 241–249 (1971)

SPEAR, P. G., ROIZMAN, B.: Proteins specified by Herpes simplex virus. V. Purification and structural proteins of the virus. J. Virol. **9**, 143–159 (1972)

STANLEY, P., CROOK, N. E., STREADER, L. G., DAVIDSON, B. E.: The polypeptides of influenza virus. VIII. Large-scale purification of the hemagglutinin. Virology **56**, 640–645 (1973)

STANLEY, P., HASLAM, E. A.: The polypeptides of influenza virus. V. Localization of polypeptides in the virion by iodination techniques. Virology **46**, 764–773 (1971)

STRAND, M., AUGUST, J. T.: Protein kinase and phosphate acceptor proteins in Rauscher murine leukamia virus. Nature (Lond.) New Biol. **233**, 137–140 (1971)

THOMPSON, T. E., SEARS, B.: Non-ideal mixing of phosphatidylcholine and cholesterol in single-walled bilayer vesicles. (Abstract.) Fed. Proc. **33**, 1551 (1974)

TIFFANY, J. M., BLOUGH, H. A.: Myxovirus envelope proteins: a directing influence on the fatty acids of membrane lipids. Science **163**, 573–574 (1969a)

TIFFANY, J. M., BLOUGH, H. A.: Fatty acid composition of three strains of Newcastle disease virus. Virology **37**, 492–494 (1969b)

TIFFANY, J. M., BLOUGH, H. A.: Models of structure of the envelope of influenza virus. Proc. nat. Acad. Sci. (Wash.) **65**, 1015–1112 (1970a)

TIFFANY, J. M., BLOUGH, H. A.: Estimation of the number of surface projections of myxo- and paramyxoviruses. Virology **41**, 392–394 (1970b)

TREMAINE, J. H., GOLDSACK, D. E.: The structure of regular viruses in relation to their subunit amino acid composition. Virology **35**, 227–237 (1968)

VERKLEIJ, A. J., ZWAAL, R. F. A., ROELOFSEN, B., COMFURIUS, P., KASTELIJH, D., VAN DEENEN, L. L. M.: The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy. Biochim. biophys. Acta (Amst.) **323**, 178–193 (1973)

VERNON, S. K., NEURATH, A. R., RUBIN, B. A.: Electron microscopic studies on the structure of rabies virus. J. Ultrastruct. Res. **41**, 29–42 (1972)

WAGNER, R. R., PREVEC, L., BROWN, F., SUMMERS, D. F., SOKOL, F., MACLEOD, R.: Classification of rhabdovirus proteins: a proposal. J. Virol. **10**, 1228–1230 (1972)

WAITE, M. R., BROWN, D. T., PFEFFERKORN, E. R.: Inhibition of Sindbis virus release by media of low ionic strength: an electron microscope study. J. Virol. **10**, 537–544 (1972)

WEINSTEIN, D. B., BLOUGH, H. A.: Kinetics of incorporation of ^{32}P into several cell lines and the inability to turn it over. Biochim. biophys. Acta (Amst.) (in press)

WILDY, P.: Classification and nomenclature of viruses. Monogr. Virol. **5**, 1–81 (1971)

WILKINS, M. H. F., BLAUROCK, A. E., ENGELMAN, D. M.: Bilayer structure in membranes. Nature (Lond.) New Biol. **230**, 72–76 (1971)

WIRTZ, K. W., ZILVERSMIT, D. B.: Exchange of phospholipids between liver mitochondria and microsomes *in vitro*. J. biol. Chem. **243**, 3596–3602 (1968)

WU, C.-W., STRYER, L.: Proximity relationships in rhodopsin. Proc. nat. Acad. Sci. (Wash.) **69**, 1104–1108 (1972)

ZINGSHEIM, H. P.: Membrane structure and electron microscopy. The significance of physical problems and techniques (freeze-etching). Biochim. biophys. Acta (Amst.) **265**, 339–366 (1972)

ZWAAL, R. F. A., ROELOFSEN, B., COMFURIUS, P., VAN DEENEN, L. L. M.: Complete purification and some properties of phospholipase C from *Bacillus cereus*. Biochim. biophys. Acta (Amst.) **233**, 474–479 (1971)