*CHAPTER 5*

# Viral Membranes

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## 1. INTRODUCTION

Many animal viruses, as well as certain plant and bacterial viruses, possess a lipid-containing membrane termed the "viral envelope." For most of these viruses, the envelope is acquired by budding at a cellular membrane, and viral components become an integral part of this membrane during assembly. In this chapter, we review the extensive body of information that has been obtained recently on the fine structure, composition, and assembly processes of the enveloped animal viruses. The replication processes of these viruses have been reviewed in other volumes of this series (e.g., Volumes 3, 4, 10, and 11).

It has become increasingly apparent that viral membranes possess important advantages for studies of membrane structure and assembly; the simplicity of viral membranes in terms of protein composition is probably the most significant advantage for detailed studies of membrane structure. Many lipid-containing viruses are important human and animal pathogens, and their membrane proteins possess



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biologically important functions and immunological properties, which also provide much of the stimulus for research in this area of molecular virology.

Although significant differences in sizes and number of structural proteins occur among lipid-containing viruses, there are several features of membrane structure shared by all these viruses. These include the presence of a lipid bilayer, glycosylated proteins exposed on the external surface of the bilayer, and internal nonglycosylated proteins. The available evidence indicates that the viral genome codes for the protein components of viral membranes, whereas the lipids are derived from the host cell membrane. Carbohydrates, which are covalently linked to viral glycoproteins as well as glycolipids, are also probably specified by cellular enzymes, although some virus-specific modifications of carbohydrates have been identified.

## 2. METHODS FOR ANALYSIS OF VIRAL MEMBRANES

### 2.1. Proteins

Viral membrane proteins resemble proteins of other biological membranes in their solubility properties. While numerous methods have been employed for the study of viral proteins, some general experimental approaches which have been useful are those common to membrane proteins in general. Thus viral membrane proteins can be solubilized by detergents, and polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (Summers *et al.*, 1965) is the most widely used approach for analysis of the polypeptide constituents of enveloped viruses. Other useful general preparative procedures include gel filtration in the presence of guanidine hydrochloride (Lazarowitz *et al.*, 1971; Fleissner, 1971) and isoelectric focussing in the presence of nonionic detergents (Hung *et al.*, 1971). Standard procedures of protein chemistry, including compositional analysis, peptide mapping, and amino acid sequencing, have been applied to certain isolated viral membrane proteins, but much basic information remains to be obtained.

In addition to methods resulting in complete solubilization of virion components, other procedures for the analysis of viral structure rely on the isolation of substructures of the virion. Treatment of enveloped virions with nonionic detergents such as Triton X100 or Nonidet P40, or weak ionic detergents such as deoxycholate, results in solubilization of the viral envelope and release of the internal nucleo-

proteins. Separation of envelope and nucleocapsid proteins can usually then be achieved by centrifugation or phase separation procedures. Affinity chromatography on lectin columns (Hayman *et al.*, 1973) or ion exchange chromatography on phosphocellulose (Strand and August, 1973) has also been useful for isolation of viral glycoproteins.

A variety of approaches have been applied to determine the location of the structural proteins in enveloped viruses, as discussed in detail in sections on individual virus groups. Proteolytic digestion results in selective degradation of viral glycoproteins, which are located on the external surface of the lipid bilayer. The internal proteins remain intact after such treatment and can be isolated as components of glycoprotein-free particles surrounded by an intact lipid bilayer. The external proteins of the virion can also be selectively labeled by surface labeling procedures, including lactoperoxidase-catalyzed iodination, Schiff's base formation with pyridoxal phosphate followed by reduction with borotritide, or reaction with formylmethionylsulfone methylphosphate. Direct electron microscopic examination of subviral components has also frequently enabled their identification as specific structural components of the virion. Using these and other approaches, the location of the major structural polypeptides has been established for many different enveloped viruses. Further analysis of the structure of viral membranes will include identification of nearest-neighbor relationships among viral envelope proteins. Such studies have recently been undertaken using bifunctional cross-linking reagents (Garoff, 1974; Wiley *et al.*, 1977; Dubovi and Wagner, 1977).

## 2.2. Lipid Bilayer

Several biophysical methods have been employed to analyze the organization and physical properties of lipids in viral membranes. X-ray diffraction patterns of virus pellets or concentrated suspensions exhibit a series of concentric circular fringes; the separation and relative intensity of these fringes are related to the variation in electron density as a function of radius from the center of the particle. Fourier analysis of the diffraction data yields a profile of the radial electron density distribution, and the location of the lipid hydrocarbon phase is assumed to correspond to an observed deep minimum in electron density. It has been concluded that only a bilayer arrangement could account for the deep minimum in electron density distribution observed in viral envelopes (Harrison *et al.*, 1971). The proteins on the external surface of the bilayer are also represented in the electron density profiles.

Although it could be concluded that most of the region of the bilayer was occupied by lipid, the possibility of penetration of the bilayer by a small amount of protein probably cannot be excluded.

Electron spin resonance spectroscopy using spin label methods has also provided evidence for a bilayer structure in a variety of enveloped viruses (for review, see Landsberger *et al.*, 1978). This technique employs fatty acid or phospholipid derivatives containing a paramagnetic nitroxide ring, which are incorporated into the lipid bilayer. The resulting ESR spectra have provided evidence concerning the fluidity of lipid fatty acyl chains, as well as other properties of the environment and mobility of lipid molecules. Bilayer structures exhibit a characteristic flexibility gradient, in which the mobility of the fatty acyl chain progressively increases with distance from the polar head group (Hubbell and McConnell, 1971). Spin label ESR spectroscopy has also been used to determine the effects of changes in lipid and protein composition on lipid bilayer fluidity (Landsberger *et al.*, 1973; Landsberger and Compans, 1976) and to study aspects of virus–cell interaction (Maeda *et al.*, 1977).

Nuclear magnetic resonance studies of $^{13}C$-labeled lipids incorporated into enveloped viruses have been employed to analyze the organization of the lipid phase (Stoffel and Bister, 1975). Measurements of spin lattice relaxation times have been carried out with whole virions labeled with $^{13}C$ incorporated into specific positions in lipids, and the results indicate a high degree of rigidity in the envelope structure.

Fluorescence depolarization measurements with probes incorporated into the lipid bilayer have been used to estimate the microviscosity of lipids in viral membranes (Moore *et al.*, 1976; Barenholz *et al.*, 1976). Fluorescent energy transfer from aromatic amino acid residues of viral proteins to a probe incorporated into the lipid bilayer has also been used to estimate the distance separating the donor and acceptor residues (Lenard *et al.*, 1974). Precise distances could not be calculated, however, since it is possible that more than one amino acid residue may be transfering energy to the probe. The precise location of fluorescent probes in viral membranes is not known either, although their solubility properties suggest that they are incorporated into the hydrocarbon phase of the bilayer.

The distribution of lipids between the internal and external monolayers of the lipid bilayer has been investigated by several approaches, including labeling of amino groups with [$^{35}S$]formylmethionylsulfone methyl phosphate (Gahmberg *et al.*, 1972*a*), treatment with phospholipases and phospholipid exchange proteins (Rothman *et al.*,

1976), and measurement of transfer of radioactive cholesterol from virions to lipid vesicles (Lenard and Rothman, 1976). The lipids of the outer monolayer are thought to be involved in these reactions and transfer processes; exchange of lipids between the inner and outer monolayers appears to be a very slow process. These approaches have indicated an asymmetrical distribution of lipids in viral membranes.

Several laboratories have investigated the origin of viral lipids by comparative compositional analyses of lipids of the viral envelope and those of the host cell and its plasma membrane. Either direct chemical determinations or radiolabeling with $^{32}PO_4$ has been employed to determine the distribution of phospholipids. The results of these studies, described in detail in the sections which follow, indicate that viral lipids are derived from the host cell membrane where virus assembly occurs.

## 2.3. Carbohydrates

Carbohydrates are covalently linked to viral glycoproteins as well as glycolipids. Identification of glycoproteins has generally been accomplished by specific labeling with sugar precursors; glucosamine and fucose have been employed most often because they are not converted extensively into other metabolites (Klenk *et al.*, 1970*a*; Strauss *et al.*, 1970). The carbohydrates of viral glycoproteins can also be labeled by exposure to galactose oxidase followed by reduction with tritiated sodium borohydride (Klenk *et al.*, 1978). Further information about the size and structure of the oligosaccharides of viral glycoproteins has been obtained by digestion of the polypeptide backbone with pronase and analysis of the resulting glycopeptide components by gel filtration (Burge and Strauss, 1970). Digestion with specific glycosidases has provided some information of the sequence of sugars in viral oligosaccharides, as described in the sections which follow on rhabdoviruses and togaviruses. Other studies employed methylation of the constituent sugars followed by gas chromatographic analysis as described in the section on influenza virus. Valuable information on the carbohydrate moiety of the viral glycoproteins has been obtained from studies employing inhibitors of glycosylation, such as 2-deoxy-D-glucose, D-glucosamine (Klenk *et al.*, 1972*b*), and tunicamycin (R. Schwarz *et al.*, 1976; Leavitt *et al.*, 1977; Nakamura and Compans, 1978*a*). Tunicamycin has been shown to inhibit the synthesis of lipid-bound precursors of the carbohydrate side chains (Takatsuki *et al.*, 1975; Tkacz and Lampen, 1975), and evidence has been obtained that the other inhibitors act by a similar mechanism (R. Schwarz *et al.*, 1977).

# 3. VIRAL MEMBRANE STRUCTURE AND ASSEMBLY

## 3.1. Arenaviruses

### 3.1.1. Classification, Morphology, Composition

The arenaviruses are a recently identified group of enveloped RNA-containing viruses (Pfau *et al.*, 1974). The members of the group include lymphocytic choriomeningitis virus (LCM), Lassa fever virus, and the Tacaribe complex of viruses (Amapari, Junin, Latino, Machupo, Parana, Pichinde, Tacaribe, and Tamiami viruses). Virions are essentially spherical particles which occur in sizes ranging from 50 to 300 nm in diameter. Distinct surface projections approximately 10 nm in length radiate from the viral envelope. A striking feature of arenavirus morphology is the presence of one or more distinct granules of approximately 20 nm diameter in the interior of virions as observed by thin-section electron microscopy (Dalton *et al.*, 1968; Murphy *et al.*, 1969, 1970). As described below, these granules have been identified biochemically as 80 S ribosomes incorporated into the virion during the assembly process. Although the presence of ribosomes is probably the most distinctive morphological characteristic of the virion, other aspects of virion morphology, including nucleocapsid structure and surface structure, are sufficient to distinguish arenaviruses from the other known groups of animal viruses. Further, ribosomes may not be present in all arenavirus particles (Vezza *et al.*, 1978).

It has been established that arenaviruses contain at least five RNA species, three of which are host cell derived 28 S, 18 S, and 4–6 S RNA species. Virus-specific RNAs of approximately 31 S and 22 S have been observed for several arenaviruses (Carter *et al.*, 1973; Pedersen, 1971, 1973; Anon *et al.*, 1976); in addition, a 15 S RNA species has also been reported for Pichinde (Farber and Rawls, 1975). Convincing evidence has been obtained that the 28 S and 18 S RNAs in arenavirus preparations originate from host cell derived ribosomes (Farber and Rawls, 1975). Whether the ribosomes are essential components of arenaviruses has not been established; however, recent results indicate that some arenaviruses lack significant amounts of the 18 S ribosomal RNA (Vezza *et al.*, 1977, 1978).

The virion proteins of Pichinde virus were the first to be characterized (Ramos *et al.*, 1972), and more recently structural proteins of several other arenaviruses have been identified including those of Junin virus (Martinez-Segovia and DeMitri, 1977), Tacaribe and Tamiami viruses (Gard *et al.*, 1977), and LCM virus (Buchmeier *et al.*, 1977). The available information indicates that all arenaviruses possess

a major carbohydrate-free polypeptide of 66,000–72,000 daltons associated with the nucleocapsid, and one or more envelope glycoprotein species. Pichinde virions contain two glycoproteins with molecular weights of 38,000 and 64,000 (Ramos *et al.*, 1972; Vezza *et al.*, 1977). In contrast to earlier reports indicating that a glycoprotein of Pichinde virus was associated with the viral nucleocapsid (Ramos *et al.*, 1972), recent observations indicate that both glycoproteins of Pichinde virus are located on the surface of the virion, and neither is found as a component of the isolated nucleocapsid (Vezza *et al.*, 1977). In these studies it was observed that both glycoproteins of Pichinde virus are sensitive to digestion by pronase, which produces spikeless particles which contained the major nonglycosylated protein. Further, ribonucleoprotein complexes were isolated from detergent-disrupted virions; these contained the major nucleocapsid protein but were free of either glycoprotein. These results indicate that both glycoproteins of Pichinde virions are present as components of the surface spikes, as found with other enveloped viruses. The two Pichinde glycoproteins are present in approximately equimolar ratios (Vezza *et al.*, 1977). It remains to be determined whether they are present in the same or in different spike structures on the viral surface and whether they contain distinct amino acid sequences.

In contrast to the two glycoproteins of Pichinde virions, only a single glycoprotein size class was detected in Tacaribe or Tamiami virions; these had molecular weights of 44,000 and 42,000, respectively (Gard *et al.*, 1977). These glycoproteins were sensitive to proteolytic digestion with chymotrypsin, which produced spikeless particles containing the major nonglycosylated protein; thus the glycoproteins are located on the viral surface. Junin virions also contained a major glycoprotein of 38,000 daltons, but several additional minor glycoproteins were also reported (Martinez-Segovia and DeMitri, 1977).

Arenaviruses contain lipids as indicated by their inactivation by detergents or lipid solvents (Pfau *et al.*, 1974). Electron microscopic studies indicating the presence of a distinct unit membrane also strongly suggest that these are lipid-containing viruses (Dalton *et al.*, 1968; Murphy *et al.*, 1970). No data are available on viral lipid composition or on carbohydrate components present in arenavirus particles, other than the demonstration that glycosylated polypeptides can be selectively radiolabeled by sugar precursors such as glucosamine.

### 3.1.2. Biosynthesis and Assembly

Little information has been obtained on the biosynthesis of arenavirus proteins in infected cells. In BHK21 cells infected with

Tacaribe virus, synthesis of the major structural proteins could be detected above the background of host cell synthesis (Saleh and Compans, 1978). In addition to the major virion proteins, a virus-induced glycoprotein of ~70,000 daltons was synthesized in large amounts in infected cells; this glycoprotein was not detected in purified virions. It remains to be established whether it is a precursor of the 44,000-dalton virion glycoprotein, a distinct viral gene product, or a virus-induced host cell protein.

The process of morphogenesis of several arenaviruses in Vero cells was studied by Murphy *et al.* (1970). Virus particles form by budding at the plasma membrane in most instances, with occasional budding at intracytoplasmic membranes. Membrane changes characteristic of the viral envelope were observed in regions of the plasma membrane, and a distinct increase in density of the membrane was found in restricted areas large enough to form individual virions. Surface projections were present on the external surface of budding particles, and dense granules (ribosomes) were contained in the interior. In some cells a large proportion of the plasma membrane appears to be involved in the budding process.

No information is available on the molecular interactions involved in viral assembly. The internal nucleoproteins of arenavirions appear as convoluted strands when released from disrupted virions (Gard *et al.*, 1977; Vezza *et al.*, 1977). Most other enveloped viruses which contain strandlike or helical nucleoproteins also contain an internal membrane (M) protein which appears to be involved in assembly of the viral envelope. Since arenaviruses lack such a protein, the nucleoprotein and glycoproteins must determine the assembly process. Further information on the nature of the interactions between these components, as well as the arrangement of the nucleoprotein in the intact virion, is needed to provide insight into the process of virus assembly.

### 3.2. Bunyaviruses

The bunyaviridae include at least 150 distinguishable serological types, 87 of which were classified as the Bunyamwera supergroup of arboviruses (Murphy *et al.*, 1973, Porterfield *et al.*, 1976). They are clearly distinct from the togaviruses both in morphology and in molecular composition. The RNA genome of these viruses consists of three segments, which are contained within circular ribonucleoprotein complexes (Pettersson and von Bonsdorff, 1975; Bouloy *et al.*, 1974; Pettersson and Kääriäinen, 1973). In thin section, virus particles appear

to be roughly spherical with a mean diameter of 100 nm, and a distinct unit membrane suggests a lipid bilayer structure in the envelope (von Bonsdorff and Pettersson, 1975).

Prominent large subunits have been seen by negative staining with uranyl acetate on the external surfaces of Uukuniemi virions (von Bonsdorff and Pettersson, 1975) but not of La Crosse virions (Obijeski *et al.*, 1976). The Uukuniemi morphological units resembled hollow cylinders 10–12 nm in diameter, with a central cavity of about 5 nm. The subunits were closely packed, and clustering as hexagonal arrays with frequent pentons (subunits surrounded by five neighboring units) was observed, suggesting icosahedral symmetry. A $T = 12$ surface lattice was observed in particles where vertex subunits were identified (von Bonsdorff and Pettersson, 1975).

Two glycoproteins with molecular weights of about 65,000 and 75,000 are present in Uukuniemi virions. Both glycoproteins are removed following protease treatment, which removes the surface subunits leaving pleiomorphic smooth surfaced particles (von Bonsdorff and Pettersson, 1975). Thus, like other enveloped viruses, both glycoproteins are located on the external surface of the virion. A small residual peptide was observed after proteolytic digestion, which may represent a protease-resistant segment of the glycoproteins. The virion nucleocapsid consists of RNA and a major nucleocapsid protein (N, 25,000 daltons). Like arenaviruses, the bunyaviruses possess neither an M protein nor an icosahedral nucleocapsid beneath the envelope, and it was therefore suggested (von Bonsdorff and Pettersson, 1975) that interactions between the surface glycoproteins may be important in viral assembly and maintenance of structural stability of the particles. The spikeless particles produced by protease treatment were markedly pleomorphic, which may reflect the role of the glycoproteins in determining the shape of the virion.

Although a similar number of polypeptide components are found in other bunyaviruses, the molecular weights of the components differ from those found for Uukuniemi virus. Three major and one minor protein have been found with four different members of the bunyavirus group (Obijeski *et al.*, 1976). In studies with La Crosse virus, two of the components, with estimated molecular weights of 120,000 and 34,000, were glycosylated, and the two glycoproteins were present in equimolar amounts. Nucleocapsids contained a major protein of 23,000 daltons, and a large protein (180,000 daltons) was present in small amounts in virions. Bromelain treatment removed both glycoproteins and yielded spikeless particles containing the two carbohydrate-free proteins. Lactoperoxidase-catalyzed iodination of intact virions also labeled the

two glycoproteins and not the internal proteins, confirming that they are the only species on the external surface of the envelope. It is not known if the two glycoproteins are components of a single spike or form different spikes on the surface of the virion. Three or four polypeptides including two glycoproteins have also been detected for several other bunyaviruses (McLerran and Arlinghaus, 1973; Rosato *et al.*, 1974; White, 1975; Gentsch *et al.*, 1977).

Virtually nothing is known of the assembly process of bunyaviruses apart from the morphological demonstration of budding. Morphogenesis takes place at Golgi complex and endoplasmic reticulum membranes, within which virions accumulate (Murphy *et al.*, 1973). Precursor particles have not been detected in infected cells by electron microscopy, but the strandlike nucleoprotein structures are probably difficult to resolve in thin section. As discussed above, it has been suggested (von Bonsdorff and Pettersson, 1975) that lateral interactions between the glycoproteins of bunyaviruses may be important in the assembly process.

### 3.3. Coronaviruses

The coronavirus group of RNA viruses is defined by common morphological properties, the most distinctive being the presence of large club-shaped surface projections. The group includes viruses of diverse origins, and insufficient information is now available to determine whether these agents form a unified group in their chemical composition and replication processes. Some biochemical studies have been undertaken with several coronaviruses, including mouse hepatitis, avian infectious bronchitis, transmissible gastroenteritis virus of swine, and a human coronavirus OC43.

The structure of the murine coronavirus A59 has been studied in greatest detail (Sturman, 1977; Sturman and Holmes, 1977). A single nonglycosylated polypeptide of 50,000 daltons is located in the interior of the virion and is probably the structural subunit of the nucleocapsid. On the surface of the virion a number of glycoproteins are detected, but these do not all contain distinct amino acid sequences. A 23,000-dalton glycoprotein designated "E1" and a large glycoprotein designated "E2" appear to be two distinct envelope glycoproteins. E2 is observed in virions as a 180,000- or a 90,000-dalton glycoprotein; however, the two possess common tryptic peptides and the 180,000-dalton component is converted into a 90,000-dalton glycoprotein by trypsin treatment. Thus the 180,000-dalton component appears to be a precursor of the 90,000-

dalton component, or possibly of two cleavage products of similar electrophoretic mobility. Cleavage of the 180,000-dalton glycoprotein had little effect on viral infectivity (Sturman and Holmes, 1977).

The E2 glycoprotein appears to be degraded completely by the proteases bromelain or pronase, which produce particles lacking the surface spikes (Sturman, 1977). In contrast, only about 20% of E1 is removed by protease treatment, and a carbohydrate-free cleavage product of 18,000 daltons is producted (Sturman, 1977; Sturman and Holmes, 1977). It was suggested that the protease-resistant portion of E1 is buried within the lipid bilayer and that E2 forms the spike structure projecting from the surface. This protease-resistant portion of E1 is significantly larger than protease-resistant glycoprotein fragments observed with other lipid-containing viruses. It is therefore conceivable that a large part of this molecule may extend through the bilayer, possibly forming a protein layer on the internal surface of the viral membrane, as suggested by Sturman (1977).

The E1 glycoprotein of murine coronavirus A59 displays unusual properties on SDS gel electrophoresis (Sturman, 1977). Heating to 100°C in the presence of SDS causes the formation of forms of E1 with lower electrophoretic mobility, and sulfhydryl-reducing agents enhance this aggregation.

Studies of polypeptides of other coronaviruses have revealed a larger number of components (Hierholzer *et al.*, 1972; Garwes and Pocock, 1975; Bingham, 1975), but it remains to be established how many of these are virus coded, and whether they contain distinct amino acid sequences. The proteins of the transmissible gastroenteritis virus of swine appear similar to those of murine A59 virions in several respects (Garwes and Pocock, 1975). The largest glycoprotein (200,000 daltons) was removed from virions by treatment with bromelain, and the surface projections were removed concomitantly, indicating that this glycoprotein forms the spike structure. Two low-molecular-weight glycoproteins (30,000 and 28,500) were resistant to protease treatment, as was a nonglycosylated polypeptide of molecular weight 50,000. Six or seven polypeptides including four glycoproteins were identified in preparations of the human coronavirus OC43 (Hierholzer *et al.*, 1972). Two glycoproteins (molecular weights 104,000 and 15,000) were selectively removed by bromelain treatment and appeared to be components of the viral spikes, whereas other glycosylated and nonglycosylated proteins were resistant to protease.

These results indicate that the surface projections of three unrelated coronaviruses apparently contain a high-molecular-weight glyco-

protein. In both murine and porcine coronaviruses, one or two low-molecular-weight glycoproteins appear to be resistant to proteolytic digestion, in part or entirely, and probably penetrate extensively into the viral membrane. Further studies of the interactions between these glycoproteins, and between the envelope proteins and nucleocapsid, are needed to determine the arrangement of coronavirus proteins in the virion and the nature of their association with the viral membrane.

Electron microscopic studies of coronavirus-infected cells indicate that virus assembly occurs by budding at intracytoplasmic membranes, including endoplasmic reticulum and Golgi complex membranes (David-Ferreira and Manaker, 1965; Oshiro, 1973). Virions accumulate in large amount within such vacuoles and are thought to be released by fusion of the vacuolar membrane with the cell surface. At late stages, extensive regions of plasma membranes are observed with adherent virions, but budding at the plasma membrane is not generally observed (Oshiro, 1973).

## 3.4. Herpesviruses

### 3.4.1. Classification, Morphology, Composition

The herpesvirus group contains a large number of viruses, which have been isolated from a variety of animal hosts. Herpes simplex virus (HSV) has been the subject of intensive investigation, as have been certain herpesviruses of other species, notably pseudorabies, a porcine herpesvirus, and equine herpesvirus (EHV). The group is united by common morphological and biochemical properties, but little information is available concerning possible relationships between various herpesviruses. A similar number of structural polypeptides have been identified in HSV types 1 and 2, and EHV (Perdue *et al.*, 1974; Kemp *et al.*, 1974; Cassai *et al.*, 1975). Further, antigenic relationships between certain glycoproteins of HSV type 1 and type 2 have been shown by immune precipitation experiments (Spear, 1976). Detailed reviews of the structure and replication of herpesviruses include those of Roizman and Furlong (1974), O'Callaghan and Randall (1976), and Kaplan (1974).

Herpesviruses are large enveloped DNA-containing viruses with icosahedral nucleocapsids. Virions are usually 150–180 nm in diameter, with their icosahedral capsids measuring 100 nm in diameter. The capsid is a $T = 16$ structure with 162 capsomers. The envelope is a unit

membrane structure when seen in thin sections, and spikelike surface projections about 8–10 nm in length can be resolved on the surface by negative staining (Wildy *et al.*, 1960; Epstein, 1962; O'Callaghan and Randall, 1976). Additional amorphous or fibrous material located between the capsid and envelope has been termed the "tegument" (Roizman and Furlong, 1974).

Herpesvirions are among the most complex of the enveloped viruses. Over 30 structural polypeptides ranging from 18,000 to 275,000 daltons have been identified in purified virions by SDS-polyacrylamide gel electrophoresis (Roizman and Furlong, 1974; O'Callaghan and Randall, 1976). Much information remains to be determined before the precise location of these proteins in the virion can be understood, but many have been localized as components of the capsid or envelope. Isolated nucleocapsids have been obtained by extraction from infected cells or detergent treatment of virions and their polypeptides compared with those of intact virions (Abodeely *et al.*, 1971; Robinson and Watson, 1971; Gibson and Roizman, 1974; Perdue *et al.*, 1974). At least six major proteins and several additional minor proteins are located in the capsid structure, all of which are nonglycosylated proteins.

Herpesvirions contain at least 13 glycoproteins, all of which appear to be components of the viral envelope (Roizman and Furlong, 1974; O'Callaghan and Randall, 1976). By analogy with other enveloped viruses, it is likely that all herpesvirus glycoproteins are exposed on the external surface of the virion, but this has not been definitively established. Some information on the location of virion glycoproteins was obtained by detergent solubilization and selective labeling by tritiated borohydride reduction of Schiff's bases formed between viral proteins and pyridoxal phosphate (Roizman and Furlong, 1974). Four major glycoproteins (VP7, 8, 17, and 18), ranging in size from approximately 60,000 to 130,000 daltons, appeared to be most readily labeled and most easily solubilized, suggesting that they are components present on the external surface of the virion. In addition, six major proteins and four minor proteins were detected as surface components of herpesvirions by lactoperoxidase-catalyzed iodination; most or all of these were glycosylated (Gupta and Rapp, 1977). Interestingly, several glycosylated as well as nonglycosylated envelope proteins of herpesvirions appear to be covalently linked to lipids, as shown by oil red O staining (Abodeely *et al.*, 1971) and specific incorporation of radioactive choline (Perdue *et al.*, 1974). The nature of the linkage of lipid to these proteins and its possible significance in terms of

virus structure and assembly remain to be established. Sulfated structural glycoproteins are also present in herpesvirions (Kaplan and Ben Porat, 1976).

Limited information has been obtained about the lipids of herpesvirions. It is likely that the lipids are arranged in a bilayer corrresponding to the unit membrane in the viral envelope, although the arrangement of lipids has not been investigated. The lipid composition of herpesvirions was found to closely resemble that of the inner nuclear membrane, from which the virus buds, whereas it differed from that of the outer nuclear or cytoplasmic membranes (Ben Porat and Kaplan, 1971). Although most of the viral lipids appeared to be synthesized prior to infection, $^{32}$P-labeled lipids synthesized after infection were preferentially incorporated from the inner nuclear membrane into virions (Ben Porat and Kaplan, 1971). Thus the incorporation of lipids into virions did not seem to occur solely by a random process. The essential role of the envelope lipids for infectivity was indicated by the inactivation of virions by phospholipase C (Spring and Roizman, 1968). Glycolipids of herpesvirions have been analyzed and found to be similar to those observed in infected cells (Brennan *et al.*, 1976).

As described above, both glycoproteins and glycolipids are carbohydrate-containing components of herpesvirions. Glucosamine, galactosamine, galactose, mannose, fucose, and sialic acid have been identified as sugar constituents of the glycoproteins (Roizman and Furlong, 1974). The glycopeptides obtained by pronase digestion of virions or infected cell glycoproteins have been characterized by gel filtration, and up to five species with estimated molecular weights ranging from about 2000 to 10,000 were observed (Honess and Roizman, 1975). Two species with molecular weights of about 4300 and 6800 were the major glycopeptides obtained from glucosamine-labeled virions. The larger glycopeptides contained substantial amounts of sialic acid, which could be released by mild acid hydrolysis.

### 3.4.2. Synthesis and Assembly

Herpesvirus glycoproteins are synthesized with little or no involvement of proteolytic cleavage events (Honess and Roizman, 1973; Spear, 1976). Four immunologically distinct classes of major glycoproteins have been detected; three of these appear to be glycosylated in two discrete stages in infected cells, with partially and fully glycosylated forms that can be resolved by gel electrophoresis (Spear, 1976). Evi-

dence for discrete stages of glycosylation was also obtained by analysis of oligosaccharide units of viral glycoproteins observed after pronase digestion (Honess and Roizman, 1975). In 15-min labeling periods viral glycoproteins contained small glucosamine-containing oligosaccharides of about 2000 daltons, which contained little or no fucose or sialic acid. The completed glycoproteins possess larger oligosaccharides containing fucose as well as sialic acid; only these glycoproteins are observed in virions.

The glycoproteins specified by herpesviruses are found in association with various cellular membranes, including smooth and rough endoplasmic reticulum and plasma membranes (Spear and Roizman, 1970; Spear *et al.*, 1970; Heine *et al.*, 1972). The role of those glycoproteins in virus replication is uncertain, although some may migrate to the inner nuclear membrane where envelopment occurs. In addition, they confer altered immunological properties on the plasma membrane, and may be responsible for herpesvirus-induced cell fusion (Roizman and Furlong, 1974; Ludwig *et al.*, 1974). Virion glycoproteins differ from those present in the inner nuclear membrane of uninfected cells, indicating that host cell proteins are excluded from the envelope of budding virions, although they are not lost from the membrane after infection (Kaplan and Ben Porat, 1970).

As discussed above, the process of herpesvirus envelopment is usually observed at the inner nuclear membrane, particularly at early stages of infection (Darlington and Moss, 1969; Epstein, 1962; Fong *et al.*, 1973; Schwartz and Roizman, 1969). Budding into nuclear or cytoplasmic vacuoles has also been observed. The inner nuclear membrane appears thickened in the region which becomes part of the envelope of the budding virion, presumably because of the presence of viral glycoproteins. By freeze-fracture electron microscopy, normal intramembranous particles were seen to be deleted from specific areas of the inner nuclear envelope in herpesvirus-infected cells, which may represent areas where cellular proteins are displaced by virus-specific proteins (Haines and Baerwald, 1976). Mature enveloped virus particles have been observed in cytoplasmic vacuoles or channels, which are thought to be involved in egress of virions from the nuclear envelope to the cell surface (Morgan *et al.*, 1959; Darlington and Moss, 1969; Schwartz and Roizman, 1969).

Inhibition of glycosylation by 2-deoxyglucose or glucosamine inhibits production of infectious herpesvirus as well as the process of virus-induced cell fusion (Courtney *et al.*, 1973; Gallaher *et al.*, 1973; Ludwig *et al.*, 1974; Knowles and Person, 1976). In the presence of

these inhibitors, changes were observed in the electrophoretic mobility of viral glycoproteins, probably due to production of unglycosylated or partially glycosylated glycoproteins. Inhibition of herpesvirus replication by cytochalasin B may occur by a similar mechanism (Dix and Courtney, 1976). These inhibitors caused a greater inhibition of the production of infectious particles than of physical particles, and the virus particles produced contained aberrant glycoproteins (Courtney *et al.*, 1973; Dix and Courtney, 1976). When radioactive 2-deoxyglucose was added to infected cells, it was found to be incorporated into herpesvirus-specific glycoproteins (Courtney *et al.*, 1973).

## 3.5. Myxoviruses

### 3.5.1. Classification, Morphology, Composition

The orthomyxovirus group includes the influenza viruses of man and animals. Virions are roughly spherical particles ~100 nm in diameter, or filamentous forms of similar width but up to several micrometers in length. By negative staining, prominent surface spikes are seen, and a helical nucleocapsid is contained within the envelope (Horne *et al.*, 1960; Hoyle *et al.*, 1961; Waterson *et al.*, 1961). Thin sections show a distinct unit membrane in the viral envelope, similar to the plasma membrane of the host cell. An additional electron-dense layer on the internal surface (Compans and Dimmock, 1969; Bächi *et al.*, 1969) corresponds to the location of a nonglycosylated internal membrane protein, as described below. Several reviews are available concerning various aspects of influenza virus structure and replication (Schulze, 1973; Laver, 1973; White, 1974; Kilbourne, 1975; Palese, 1977), and a chapter on "Reproduction of Myxoviruses" is contained in Volume 4 of this series (Compans and Choppin, 1975).

The available information indicates that influenza virions consist of about 1–2% RNA, 70–75% protein, 20–24% lipid, and 5–8% carbohydrate (Ada and Perry, 1954; Frommhagen *et al.*, 1959; Schäfer, 1959; Blough *et al.*, 1967). The RNA genome consists of eight distinct single-stranded RNA species, each of which codes for one of the virus-specific polypeptides (Palese, 1977; Pons, 1976; Scholtissek *et al.*, 1976; McGeoch *et al.*, 1976). The virion polypeptides include two glycoproteins, the hemagglutinin and neuraminidase, which form distinct spikes on the external surface (Laver and Valentine, 1969; Webster and Darlington, 1969; Rott *et al.*, 1970). A nucleocapsid subunit, a membrane-associated protein, and three minor high-molecular-weight proteins (P1,

P2, and P3) are internal components of the virion. In Table 1 a list of the envelope proteins of influenza virions along with their accepted designations, estimated molecular weights, and functions is presented.

Classification of influenza viruses is based on serological properties of the viral polypeptides. Three distinct serological types (A, B, and C) have been recognized, which show no immunological cross-reaction between any of their structural components. Both the hemagglutinin and neuraminidase antigens of type A viruses show extensive variation, and distinct serological subtypes have been identified which contain common internal antigens but have surface antigens which appear to be completely unrelated. The appearance of these new subtypes of type A viruses, with distinct surface antigens, is the cause of major pandemics of influenza. The antigenic subtypes of type A influenza viruses are designated according to the serological type of both their hemagglutinin (H) and neuraminidase (N). Four distinct subtypes, $H_0N_1$, $H_1N_1$, $H_2N_2$, and $H_3N_2$, have been identified among human influenza viruses between the first isolation in 1933 and the present time. Type A influenza viruses with hemagglutinin and neuraminidase antigens distinct from those of human influenza viruses have also been isolated from horses, swine, and birds. Only minor variation in immunological properties has been observed with influenza B viruses, and influenza C viruses have not been studied in detail.

### 3.5.2. Fine Structure and Arrangement of Envelope Components

The envelope of influenza virus has probably been studied in more detail than that of any other lipid-containing viruses. The recognized envelope components of influenza A and B viruses include a lipid

**TABLE 1**

**Envelope Proteins of Influenza A Viruses**

| Protein | Designation | Molecular weight of polypeptide | Function |
|---|---|---|---|
| Hemagglutinin | HA | 75,000–80,000 | Binding to receptors |
| | HA1 | 50,000–55,000 | |
| | HA2 | 25,000–30,000 | |
| Neuraminidase | NA | 55,000 | Removal of neuraminic acid |
| Membrane Protein | M | 26,000 | Membrane structure and assembly |

bilayer, external spikes consisting of the two glycoproteins hemagglutinin and neuraminidase, and an internal carbohydrate-free protein, the M protein. The hemagglutinin protein of influenza virus is probably the best-characterized viral envelope protein; there has been a longstanding interest in this protein because of its importance as the major antigen involved in immunity to influenza virus infection. A host-cell-derived carbohydrate antigen has also been detected in association with the viral envelope.

### 3.5.2a. The Hemagglutinin

Influenza virus was the first virus found to agglutinate erythrocytes (Hirst, 1941; McClelland and Hare, 1941); this has proved to be a convenient rapid assay for viruses of many different major groups. In the case of the influenza viruses, hemagglutination is caused by one of the envelope glycoproteins, termed the "hemagglutinin protein." When isolated from SDS-disrupted virus, the hemagglutinin protein was found to be a rodlike subunit ~14 nm in length and 4 nm in diameter (Laver and Valentine, 1969). In the absence of detergents, these glycoproteins were found in characteristic rosettelike aggregates, and it was suggested that one end of each spike may be hydrophobic and may be involved in binding to the viral envelope. End-on views of the hemagglutinin show a triangular shape (Laver, 1973; Griffith, 1975), and the size of the spike suggests that it may be composed of three identical polypeptide chains (see below).

The hemagglutinin spike may be composed of a 75,000-dalton glycoprotein species, designated "HA," or alternatively two cleavage products, designated "HA1" and "HA2" (~50,000 and ~25,000 daltons, respectively). The extent of cleavage of HA into HA1 and HA2 varies with the virus strain and cell type and the presence or absence of serum proteases (Lazarowitz *et al.*, 1971, 1973*a*,*b*; Rifkin *et al.*, 1972; Skehel, 1972; Stanley *et al.*, 1973; Klenk *et al.*, 1975). No cleavage of HA is necessary for virus assembly or receptor binding activity, and virions of some strains containing uncleaved HA are infectious (Lazarowitz *et al.*, 1973*a*; Stanley *et al.*, 1973), but subsequent studies have shown that the infectivity of virions is enhanced by proteolytic cleavage (Klenk *et al.*, 1975; Lazarowitz and Choppin, 1975; Klenk *et al.*, 1977*b*). It was observed that cleavage of HA into polypeptides of sizes similar to HA1 and HA2 could be accomplished by a variety of proteases; however, only cleavage by trypsinlike enzymes resulted in the formation of highly infectious virus (Lazarowitz

and Choppin, 1975; Klenk *et al.*, 1977*b*). Activation of infectivity thus requires cleavage of a specific peptide bond with arginine or lysine in the carboxyl linkage. Dual infection experiments with different influenza virus strains demonstrated that the sensitivity or resistance of the hemagglutinin protein to cleavage is a structural property of each virus glycoprotein (Klenk *et al.*, 1977*b*).

The estimated molecular weight as well as the morphology of the intact hemagglutinin spike suggested that it was a trimer composed of three HA polypeptides, or three HA1-HA2 complexes (Laver, 1973; Griffith, 1975; Schulze, 1975*b*). Further evidence for a trimer structure was obtained by cross-linking experiments with dimethyl suberimidate (Wiley *et al.*, 1977), in which trimers of HA were demonstrated. The available estimates of the number of hemagglutinin polypeptides per virion (Compans *et al.*, 1970; White *et al.*, 1970; Skehel and Schild, 1971; Klenk *et al.*, 1972*a*; Schulze, 1972; Inglis *et al.*, 1976) indicate that 200–350 hemagglutinin spikes are present per particle. Tiffany and Blough (1970) calculated from electron microscopic measurements that a 100-nm-diameter particle possessed 550 spikes and an 80-nm-diameter particle 300 spikes, most of which are hemagglutinin proteins. Since virions occur in a range of sizes, no single value will apply to all virions in a population.

Treatment of influenza virus with the protease bromelain under certain conditions results in degradation of the HA1 polypeptide, producing smooth-surfaced spikeless particles which still contain HA2 (Compans *et al.*, 1970). Therefore, the HA2 segment of the hemagglutinin appears to interact closely with the lipid bilayer, whereas HA1 is located distally in the spike structure. On more extensive proteolytic digestion, HA2 is also cleaved (Schulze, 1970; Klenk *et al.*, 1972*a*) but a small peptide still remains associated with the envelope, which may represent a segment of the glycoprotein which penetrates into the lipid bilayer (Lenard *et al.*, 1976). Treatment of some influenza virus strains with bromelain results in solubilization of a segment of the spike consisting of HA1 as well as a cleavage product of HA2 that is about 5000 daltons smaller than the intact molecule (Skehel and Waterfield, 1975). These solubilized glycoproteins have been crystallized (Brand and Skehel, 1972), and the crystals were shown to exhibit erythrocyte-binding activity (Wiley, 1975). Comparison of the amino acid composition of the intact and bromelain-released glycoproteins indicated a preponderance of hydrophobic amino acids in the segment which is missing after bromelain cleavage (Skehel and Waterfield, 1975), suggesting that this may be a hydrophobic segment which may penetrate into the lipid bilayer. Since the N terminus of HA2 is unmodified by

bromelain treatment, the hydrophobic segment must be located at the C-terminal end of the molecule. Comparison of the N-terminal amino acid sequences of HA1 and HA2 of various human subtypes of type A influenza viruses shows marked conservation of the sequence and a characteristic "palindrome sequence" of amino acids occurs at the N-terminal region of HA2 (Skehel and Waterfield, 1975). This sequence, which is located at the site of the HA polypeptide which is cleaved to form HA1 and HA2, is highly conserved among all influenza A viruses and may be important for recognition by the protease involved in cleavage. Alternatively, since virions with cleaved HA show enhanced infectivity, it is also possible that this region of the molecule undergoes a change in conformation and participates in some aspect of virus–cell interaction. The overall amino acid compositions of HA1 and HA2 subunits of the $A_0$/Bel strain of influenza virus were very similar, except that HA1 was higher in proline and lower in methionine than HA2 (Laver, 1971; Laver and Baker, 1972).

The hemagglutinin glycoprotein contains about 15–20% carbohydrate by weight (Laver, 1971; White, 1974; Schwarz and Klenk, 1974; Nakamura and Compans, 1978*a*). The carbohydrate composition is determined in large part by the host cell, and differences in electrophoretic mobilities are observed in glycoproteins from the same strain of virus grown in different host cells (Compans *et al.*, 1970; Haslam *et al.*, 1970; Schulze, 1970), which are due to differences in carbohydrate content of the glycoproteins (Schwarz *et al.*, 1977; Nakamura and Compans, 1978*b*). An antigenic relationship has also been demonstrated between carbohydrate components obtained from chick allantoic cells and influenza virions grown in these cells (Laver and Webster, 1966).

Until recently, little information was available concerning the carbohydrate portion of the HA glycoprotein. About 80% of the carbohydrate attached to viral proteins was shown to be linked to HA; the glycosyl moieties were shown to be composed of glucosamine, mannose, galactose, and fucose at a molar ratio of 6:4:1:1, respectively, and the total carbohydrate of HA, determined by chemical means, was estimated to be approximately 12,000 daltons (Laver, 1971). Like all myxoviruses containing neuraminidase, influenza A and B viruses lack neuraminic acid. It was also found that HA2 of influenza A virions contains fucose, whereas HA2 of influenza B (GL1760) does not (Choppin *et al.*, 1975). Recently the glycopeptides obtained by pronase digestion of influenza A viruses were characterized (Schwarz *et al.*, 1977; Nakamura and Compans, 1978*b*). Both laboratories showed that HA possesses type I (oligosaccaride side-chain composed of glucosamine,

mannose, galactose, and fucose) and type II (oligosaccharides composed of glucosamine and mannose) glycopeptides. Methylation studies indicated that fucose and galactose are exclusively present in terminal positions. Mannose and glucosamine are in part located in the periphery and in part in more central regions. The occurrence of 2,4-methyl mannose indicates that mannose may be involved in branch formation (Schwarz *et al.*, 1978*a*). Galactose is accessible to surface labeling reactions, such as the action of galactose oxidase followed by reduction with tritiated borohydride (Klenk *et al.*, 1978). The side chains are attached to the polypeptide by *N*-glycosidic linkages between *N*-acetylglucosamine and asparagine; *O*-glycosidic linkages do not exist (Keil *et al.*, 1978). The distribution of type I and type II oligosaccharides on HA1 and HA2 was shown to depend on the virus strain. Schwarz *et al.* (1977) showed that HA1 of an avian influenza virus possessed only type I glycopeptides, whereas both types I and II glycopeptides were shown to be present on HA2. However, Nakamura and Compans (1978*b*) using the WSN strain of influenza found that HA1 possessed both types I and II glycopeptides whereas HA2 contained only type I glycopeptides. Because of such strain differences, it has been suggested that specific amino acid sequences determine whether a type I or type II oligosaccharide is added at a particular site on the glycoprotein (Nakamura and Compans, 1978*b*).

The host cell type may also determine the type of oligosaccharide chains added to a given viral glycoprotein. Thus, although HA2 of WSN strain influenza virions grown in MDBK cells contained only type I glycopeptides, both types I and II glycopeptides were found in HA2 of WSN virions grown in CEF cells (Nakamura and Compans, in preparation).

Schwarz *et al.* (1977) showed that the type I HA glycopeptides of fowl plague and N influenza strains grown in chicken embryo fibroblasts (CEF) had a molecular weight of approximately 2600, whereas type II glycopeptides from the same source had a molecular weight of approximately 2000. The molecular weights of types I and II glycopeptides of the WSN strain of influenza virus grown in CEF cells were also found to be approximately this size (Nakamura and Compans, 1978*b*). HA glycopeptides of virus grown in MDBK cells were slightly larger in size than those of virus grown in CEF cells. Based on the estimated carbohydrate content (12,000 daltons) of the HA glycoprotein and the sizes of the types I and II glycopeptides of influenza virus grown in MDBK cells, it was estimated that HA2 contains a single type I glycopeptide whereas HA1 possesses two type I and one or two type II oligosaccharide side chains for the WSN strain (Nakamura

and Compans, 1978*b*). However, out of five or six side chains present on the fowl plague virus hemagglutinin at least two (both types I and II) appear to be located on HA2 (Schwarz *et al.*, 1977).

Antigenic determinants which react with neutralizing antibody are present on the HA1 portion of the hemagglutinin glycoprotein (Eckert, 1973; Brand and Skehel, 1972; Laver *et al.*, 1974). Immunodiffusion tests with purified hemagglutinin subunits revealed two or three different antigenic determinants (Laver *et al.*, 1974) However, all of the antigenic determinants appeared to be on the same protein molecule, and only one species of hemagglutinin was observed in each virus type.

### 3.5.2b. The Neuraminidase

Enzymatic destruction of receptors on the surface of the erythrocyte by influenza virus provided the first evidence for a virus-associated enzyme (Hirst, 1942). The enzyme was subsequently identified as a neuraminidase (Klenk, 1955; Gottschalk, 1957). The fine structure and function of this enzyme have recently been reviewed (Bucher and Palese, 1975).

The morphology of purified neuraminidase subunits isolated from detergent-disrupted virions is quite distinct from that of the hemagglutinin spikes (Laver and Valentine, 1969; Webster and Darlington, 1969; Rott *et al.*, 1970). The neuraminidase has an oblong head measuring approximately $5 \times 8.5$ nm, from which a fiber $\sim$10 nm long extends. A small knob of $\sim$4 nm is present at the distal end. Like the hemagglutinin subunits, the neuraminidase proteins aggregate into characteristic rosettes in the absence of detergents, with the oblong heads located on the periphery of the rosettes. Therefore, the neuraminidase also appears to possess a hydrophobic end. Protease treatment removes the fiber and releases the oblong head which is still enzymatically active (Seto *et al.*, 1965; Drzeniek *et al.*, 1966*b*) and is seen as a square array of four 4-nm spherical subunits (Wrigley *et al.*, 1973). These observations suggest that the neuraminidase binds to the viral membrane by its hydrophobic end, with the fiber and oblong head projecting outward; however, it has not been possible to clearly resolve the arrangement of neuraminidase subunits on the envelopes of intact virions.

Biochemical evidence has also indicated a tetrameric structure for the intact spikes. The native structure appears to have a molecular weight of $\sim$200,000–250,000 (Drzeniek *et al.*, 1966*b*), and in the presence of SDS and reducing agents it is dissociated into four NA polypeptides of 55,000 daltons (Bucher and Kilbourne, 1972; Lazdins *et*

*al.*, 1972; Kendal and Eckert, 1972). It has been suggested that two NA polypeptides are disulfide linked to form a dimer and that two dimers associate noncovalently to form a tetramer (Lazdins *et al.*, 1972). Several laboratories reported that two different neuraminidase polypeptides of similar molecular weights were present in the X7 recombinant of influenza virus (Webster, 1970; Bucher and Kilbourne, 1972; Lazdins *et al.*, 1972). However, most strains have been shown to possess a single NA polypeptide (Haslam *et al.*, 1970; Laver and Baker, 1972; Skehel and Schild, 1971; Gregoriades, 1972). The possibility that one of the two NA polypeptides in the X7 strain is a proteolytic cleavage product has therefore been considered (Lazdins *et al.*, 1972). Recently it has been shown by analysis of recombinant viruses that a single viral RNA segment codes for the neuraminidase polypeptide (Palese, 1977; Scholtissek *et al.*, 1976), further supporting the conclusion that a single polypeptide is present.

A high proportion of the carbohydrate of the neuraminidase is removed by trypsin treatment (Lazdins *et al.*, 1972; Allen *et al.*, 1977); this removes the fiber and leaves the terminal knob, which is still enzymatically active (Seto *et al.*, 1966).

The NA polypeptide possesses types I and II glycopeptides that appear to be similar to those of the HA polypeptide and both glycoproteins have a similar monosaccharide composition (Allen *et al.*, 1977; Schwarz *et al.*, 1977). Allen *et al.* (1977) and Keil *et al.* (1978) have obtained evidence that the glycopeptides of NA are linked to the polypeptide backbone through an *N*-acetylglucosamine–asparagine linkage.

Recently both the hemagglutinin and neuraminidase of influenza virions were shown to be sulfated glycoproteins (Compans and Pinter, 1975). The sulfate appears to be covalently linked to the oligosaccharide chains of viral glycoproteins (Nakamura and Compans, 1977, 1978*b*). Glycoproteins of enveloped viruses of all other major groups studied also are sulfated, whereas carbohydrate-free polypeptides are not (Pinter and Compans, 1975; Kaplan and Ben Porat, 1976).

The possible function of the neuraminidase in virus replication is discussed below.

### 3.5.2c. The Lipid Bilayer

The envelope of influenza virions is acquired by a process of budding at the plasma membrane (Murphy and Bang, 1952), and there is continuity between the unit membrane of the cell surface and that of

the emerging virus particle (Bächi *et al.*, 1969; Compans and Dimmock, 1969). During this process of morphogenesis, the lipids of the viral envelope are acquired from those of the cellular plasma membrane. It is therefore likely that the arrangement of lipids in the viral envelope reflects that of the plasma membrane.

Evidence for a lipid bilayer structure in influenza virions has been obtained by electron spin resonance (ESR) spectroscopy using spin label methods (Landsberger *et al.*, 1971, 1973). Using stearic acid derivatives with nitroxide-containing functional groups located at different positions on the hydrocarbon chain, a flexibility gradient was observed in the viral envelope, which is characteristic of a lipid bilayer structure (McConnell and McFarland, 1972). When the functional group is located close to the polar head group, it is observed to be in a relatively rigid environment, and fluidity of the environment increases as the nitroxide group is located further down the hydrocarbon chain.

The composition of the virion includes 10–13% phospholipids, 6–8% cholesterol, and at least 1–2% glycolipids (Frommhagen *et al.*, 1959; Blough and Merlie, 1970; Klenk *et al.*, 1972*a*). In early studies it was demonstrated that prelabeled cellular lipids were incorporated into influenza virions and that the lipid composition resembled that of the host cell of origin (Wecker, 1957; Kates *et al.*, 1961). A part of the viral lipid may, however, also be newly synthesized after virus infection (Blough, 1974). Differences in fatty acid composition have been reported for various strains of influenza virus grown in ovo, primarily in the neutral lipid fraction (Tiffany and Blough, 1969*a*,*b*; Blough and Tiffany, 1973). Because of these differences, it has been proposed that viral envelope proteins determine the lipid composition by binding specific lipids; however, extensive and detailed analyses with other virus groups indicate that the viral lipid composition closely reflects that of the host cell plasma membrane and may be influenced by viral proteins only to a very limited extent (see sections on paramyxoviruses and togaviruses). Thus virions obtained from different host cell types contain the same protein components but may show marked differences in lipid composition, which parallel those of the host cell plasma membrane. These differences in lipid composition may result in viral membranes with differences in fluidity of the lipid bilayer as observed by ESR spectroscopy. Thus influenza virions grown in MDBK (bovine kidney) cells were observed to have a more rigid lipid bilayer than virions grown in BHK21 cells (Landsberger *et al.*, 1973). The higher cholesterol content in virions from MDBK cells was thought to be the major determinant of the increased lipid rigidity.

Recently the distribution of lipids between the inner and outer sides of the lipid bilayer of influenza virions has been investigated using phospholipases and phospholipid exchange proteins (Tsai and Lenard, 1975; Rothman *et al.*, 1976). Approximately 30% of the phospholipid was observed to be inaccessible to either phospholipase C digestion or the exchange protein. Nearly all of the phosphatidylinositol, about half of the phosphatidylcholine, 30% of the phosphatidylethanolamine, and 15–25% of sphingomyelin and phosphatidylserine were accessible to either treatment, and it was concluded that these lipids are exposed on the outer monolayer of the viral membrane. The population of lipid molecules which were exchanged in the reaction catalyzed by exchange protein was shown to be the same as that digested by phospholipase C (Rothman *et al.*, 1976). The phospholipid pools accessible to the exchange protein were stable, indicating that exchange between the inner and outer monolayer is extremely slow (a half-time in excess of 30 days for sphingomyelin and 10 days for phosphatidylcholine). The presence of amine-containing phospholipids on the external surface of influenza virions was suggested previously based on staining properties of the viral membrane (Choppin and Compans, 1975). The distribution of lipids in the viral membrane differs from that observed for human erythrocytes in this respect (Bretscher, 1972, 1973). Since less than half of the total phospholipid was observed to be present on the outer monolayer of influenza virions, it was suggested that there is a high glycolipid content on the external side of the bilayer (Rothman *et al.*, 1976). The presence of glycolipid carbohydrates on the external surface of the viral envelope has been demonstrated by binding of specific lectins to protease-treated particles devoid of glycoproteins (Klenk *et al.*, 1972*a*).

An exchange process between virions and lipid vesicles was used to investigate the distribution of cholesterol in the viral envelope (Lenard and Rothman, 1976). Exchange of labeled cholesterol occurred only after treatment with chymotrypsin, which degrades the viral glycoproteins. About half of the cholesterol was rapidly exchangeable, and appeared therefore to be present in the outer monolayer. The half-time for the equilibration of cholesterol between the inner and outer halves of the bilayer was estimated to be at least 13 days.

### 3.5.2d. The M Protein

The M protein was first identified as a distinct structural component by the use of proteolytic enzymes or detergents to selec-

tively remove the surface glycoproteins of influenza virions (Compans *et al.*, 1970; Schulze, 1970; Skehel and Schild, 1971) which enabled the isolation of "spikeless" particles which lacked hemagglutinin and neuraminidase activities. Analysis of their polypeptide composition by SDS-polyacrylamide gel electrophoresis revealed that two major polypeptides were present. The larger of the major polypeptides had been previously identified as the nucleoprotein subunit, but the smaller (~26,000 daltons) was previously unrecognized as a viral structural component. It is now virtually certain that this major protein is associated with the inner surface of the viral envelope, and it has been designated as the M, or membrane, protein. The M protein is the smallest and most plentiful of the virion polypeptides, composing about 40% of the total virion protein by weight. Similar polypeptides have also been identified for other enveloped viruses (see sections on paramyxoviruses and rhabdoviruses).

Electron microscopic studies initially suggested the presence of a protein layer on the inner surface of the viral envelope. Thin sections of influenza virions showed an electron-dense layer on the internal surface of the unit membrane, which is not present on the normal cellular membrane (Apostolov and Flewett, 1969; Bächi *et al.*, 1969; Compans and Dimmock, 1969). It has been calculated that the M protein is present in sufficient quantity to form such a shell beneath the lipid bilayer (Compans *et al.*, 1972; Schulze, 1972). Further evidence for the location of the M protein was obtained by extraction of the lipids of glutaraldehyde or formaldehyde-fixed virions; the resulting particles exhibited a smooth-surfaced membrane surrounding the nucleocapsid, which corresponds to the M protein layer (Schulze, 1972; Reginster and Nermut, 1976). Iodination with chloramine T (Stanley and Haslam, 1971) and fluorescence transfer experiments (Lenard *et al.*, 1974) have also supported the conclusion that the M protein is located beneath the lipid bilayer and external to the viral ribonucleoprotein. The M protein is unaffected when intact virions are exposed to proteolytic enzymes (Compans *et al.*, 1970; Schulze, 1970; Klenk *et al.*, 1972*a*), and it is unreactive with labeling reagents specific for proteins on the external surface of the bilayer (Stanley and Haslam, 1971; Rifkin *et al.*, 1972), indicating that no part of the M protein penetrates through the lipid bilayer to the external surface of the envelope.

The M protein has been purified by gel filtration in the presence of guanidine hydrochloride (Lazarowitz *et al.*, 1971), acid chloroform–methanol extraction (Gregoriades, 1973), and precipitation after solubilization of virions with ammonium deoxycholate (Laver and Downie, 1976). The amino acid composition of the M protein of several

strains of influenza virus has been determined (Laver and Baker, 1972; Gregoriades, 1973); M protein contained ~42% of polar amino acids, similar to the nucleoprotein subunit. Therefore, the overall composition of the M protein is not markedly hydrophobic, although the fact that it is selectively extracted in acid chloroform–methanol indicates hydrophobic properties. Isoelectric focusing of the influenza ($A_0$/WSN) M protein in the presence of NP40 or Triton X100 indicated an isoelectric point of pH 4.6 (Gregoriades, 1973).

The M proteins of various type A influenza viruses are closely related immunologically (Schild, 1972) as well as biochemically, as shown by peptide mapping procedures (Laver and Downie, 1976). A single tryptic peptide was observed to differ when the M proteins of Port Chalmers ($H_3N_2$) and BEL ($H_0N_1$) viruses were compared; all other tryptic peptides were indistinguishable. Influenza B viruses have M proteins which appear to be unrelated immunologically or biochemically to those of type A viruses (Schild, 1972; Laver and Downie, 1976). With type C influenza viruses, a major nonglycosylated polypeptide of 26,000–28,000 daltons is present (Kendal, 1975; Compans *et al.*, 1977), which is thought to correspond to the M protein based on its resistance to protease and absence as a component of purified ribonucleoproteins.

Exposure of intact virions to [$^{14}$C]dansylchloride results in selective labeling of the M protein (Robertson *et al.*, 1978). This observation suggested that the dansyl label became associated with the lipid bilayer, and labeled a portion of the protein which interacted with the bilayer. The dansyl label occurs exclusively on lysine residues; however, several cyanogen bromide peptides are labeled, and further understanding of the basis of the reaction will have to await a better understanding of the molecular structure of the protein.

Several laboratories have recently obtained evidence that the M protein is exposed on the surface of influenza virus infected cells, where it may act as a target for cytotoxic T lymphocytes (Braciale, 1977; Biddison *et al.*, 1977; Ada and Yap, 1977). These observations will undoubtedly stimulate further investigation of the possible functional significance of this membrane association in virus replication and its importance in the immune response to influenza virus.

### 3.5.2e. Sulfated Glycosaminoglycans

The use of $^{35}SO_4$ as a label has revealed a sulfated component in virions migrating near the origin of SDS-polyacrylamide gels, which

was previously unrecognized in studies using amino acid or sugar incorporation or procedures for staining of polypeptides (Compans and Pinter, 1975; Pinter and Compans, 1975). Prelabeling experiments indicate that this component is derived from the host cell (Compans and Pinter, 1975), and it appears likely to correspond to a host cell antigen originally described by Knight (1944, 1946) and subsequently found by others to be a sulfated glycosaminoglycan associated with influenza virions (Haukenes *et al.*, 1965; Howe *et al.*, 1967; Lee *et al.*, 1969). The host antigen has been purified from influenza virions grown in embryonated eggs and shown to be uronic and sialic acid-free sulfated glycosaminoglycan, similar in composition to the keratan sulfate group of acid glycosaminoglycans (Haukenes *et al.*, 1965; Lee *et al.*, 1969). The constituent sugars of this material differ from those of the viral glycoproteins by the presence of glucose and galactosamine and the absence of mannose (Schwarz *et al.*, 1978*a*).

The sulfated glycosaminoglycan is exposed on the surface of the virion, as it can be removed by treatment with proteolytic enzymes. However, it does not appear to be bound firmly to the virion glycoproteins, as a gentle extraction procedure using Triton X100 results in a quantitative separation of the glycoproteins and sulfated glycosaminoglycan (Compans and Pinter, 1975). Therefore, the glycosaminoglycan is either bound loosely to the glycoproteins or attached to the viral lipid directly. If the latter is the case, the binding appears to involve a peptide portion of the molecule, since trypsin treatment quantitatively removes these sulfated components from virions.

### 3.5.2f. Envelope Components of Influenza C Virus

There are marked similarities between the structural components of influenza A and B viruses; however, influenza C virions exhibit some distinct biochemical and morphological properties. These viruses are generally similar to other influenza viruses in particle size and shape, possession of a segmented genome, and morphology of the internal ribonucleoprotein (Ritchey *et al.*, 1976; Cox and Kendal, 1976; Compans *et al.*, 1977). The nucleoprotein subunit and M protein of influenza C virions also are similar to those of other influenza virions in size and relative amounts (Kendal, 1975; Compans *et al.*, 1977). The most distinctive feature of influenza C virions is their lack of detectable neuraminidase activity (Hirst, 1950; Kendal, 1975; Nerome *et al.*, 1976). A receptor-destroying enzyme is present in influenza C virions, but it does not cleave neuraminic acid from various substrates; the

receptors for influenza A and C viruses on erythrocytes appear to be unrelated since both viruses possess receptor-destroying activity for their own receptor but lack activity against the receptor for the heterologous virus. In agreement with the lack of neuraminidase is the finding that influenza C virions contain neuraminic acid (Nerome *et al.*, 1976; Meier-Ewert *et al.*, 1978).

Three glycoprotein species have been detected in influenza C virions: a major glycoprotein of ~88,000 daltons and two minor glycoproteins of ~66,000 and 26,000 daltons (Kendal, 1975; Compans *et al.*, 1977). The two smaller glycoproteins are linked by disulfide bonds (Meier-Ewert *et al.*, 1978), suggesting analogy to HA1 and HA2 of influenza A and B viruses; in the absence of reducing agents only a single glycoprotein peak of ~88,000 daltons is resolved. It is uncertain whether the larger virion glycoprotein seen in the presence of reducing agents is a result of incomplete conversion into the two smaller glycoproteins by proteolytic cleavage or whether it is a distinct viral glycoprotein.

Glycoproteins of influenza C virions are frequently seen in a regular hexagonal arrangement on the surface of the virus (Apostolov *et al.*, 1970; Compans *et al.*, 1977). Such an arrangement is not a regular feature of influenza A viruses, although similar patterns have sometimes been reported.

### 3.5.3. Comparison of the Envelope Structure of Standard and Incomplete Influenza Virus

Noninfectious, hemagglutinating particles of influenza virus are produced upon serial undiluted passage [reviewed in Volume 4 of this series (Compans and Choppin, 1975)]. These particles are termed "incomplete virus" or "von Magnus" virus, since the phenomenon was first described by von Magnus (1954). Frequently incomplete virus is markedly pleomorphic (Rott and Schäfer, 1960; Lenard and Compans, 1975), and altered patterns of surface spikes have been reported (Moore *et al.*, 1962; Almeida and Waterson, 1970). A square array of spikes, observed on some large, pleomorphic particles (Almeida and Waterson, 1970), may represent areas enriched in neuraminidase subunits, which appear as square subunits when viewed end-on (Wrigley *et al.*, 1973).

The polypeptides of standard and incomplete virus of the A/WSN strain grown in MDBK cells were found to differ, in that incomplete virus possessed about twice the relative amount of glycoproteins, while the amount of NP protein was significantly decreased (Lenard and

Compans, 1975). The specific neuraminidase activity was also higher in incomplete virus. A subpopulation of incomplete virus with normal morphology showed the same alterations in protein composition as the large pleomorphic particles. These results indicate that the total amount of glycoproteins per unit area incorporated into the influenza viral membrane may vary with the growth conditions and does not play a primary role in determining viral size and shape. No differences were found in phospholipid content or composition between standard and incomplete virus, nor was any difference in membrane fluidity detected by ESR spectroscopy of spin-labeled virus (Lenard *et al.*, 1976), indicating that these properties of the viral membrane are not determined by the amount of viral glycoprotein present in the envelope. Since lipids of viral envelopes appear to be considerably more rigid than a lipid bilayer of the same composition that is devoid of viral protein (Landsberger and Compans, 1976), these results suggest that the M protein is the major determinant of membrane rigidity (Lenard *et al.*, 1976).

### 3.5.4. Functions of Influenza Virus Glycoproteins

#### 3.5.4a. Adsorption to Receptors

In the case of enveloped viruses, glycoproteins located on the surface of the virion are the components involved in adsorption to cellular receptors, which may or may not be host cell glycoproteins. Hemagglutination by influenza virus has been studied as a model system for adsorption of a virus to receptors, and considerable information has been obtained. Adsorption to the erythrocyte occurs by the hemagglutinin spike binding to sialic acid-containing components on the cellular surface. The receptor molecule has been isolated from chick red blood cells, and it is a major glycoprotein which contains M and N blood group antigens. A detailed summary of the properties of this receptor is given by Schulze (1975*b*). Removal of sialic acid from the receptor molecule by neuraminidase prevents the agglutination of erythrocytes by influenza virus. Hemagglutination can also be inhibited by pretreating the virus with specific antibodies. As has been pointed out above, proteolytic cleavage of HA is not a precondition for hemagglutination, but it is necessary for complete infectivity. Thus it appears that, in addition to its role in adsorption, the hemagglutinin has another function in the infection process, and it has been suggested that it may be involved in penetration (Klenk *et al.*, 1975; Lazarowitz and Choppin,

1975). This notion is supported by the recent observation that the F protein of paramyxoviruses known to be responsible for penetration has a highly hydrophobic amino acid sequence at the N terminus of the F2 fragment (see section on paramyxoviruses) which exhibits almost complete homology with the N-terminal end of HA2 (Skehel and Waterfield, 1975). Thus both glycoproteins might have similar functions.

Antibody to the hemagglutinin protein of influenza virus will neutralize virus infectivity. The specific determinants of viral glycoproteins that are recognized by viral neutralizing antibodies have not been chemically characterized. It has been postulated that antibody molecules may recognize only determinants on the tip of the HA spike of the influenza virion (White, 1974). In reaching these conclusions, it has been assumed that the size of the antibody molecule precludes the possibility that it could make contact with any other part of the spike since the spaces between the spikes are too small for the immunoglobulin to interact with other regions. Electron mciroscopic observations (Lafferty and Oertilis, 1963) indicate that antibody molecules do interact with the tips of surface spikes, and analysis of the tryptic peptides of HA molecules isolated from closely related influenza strains has shown that they rarely differ by more than one or two peptides (Laver and Webster, 1973; Webster and Laver, 1972), suggesting that these strain differences are restricted to small regions or determinants of the HA spike.

The antigenic character exhibited by the glycoproteins of influenza virions is highly variable as evidenced by the many different strains of type A and B influenza. The HA and NA glycoproteins of influenza viruses are antigenically distinct, and they undergo antigenic changes independently of each other (Drzeniek *et al.*, 1966*b*; Seto and Rott, 1966; Webster and Laver, 1967; Kilbourne *et al.*, 1968). The antigenic changes that occur may be gradual, in which case the different virus strains are clearly related to each other with respect to both surface antigens. Antigenic changes of this nature are termed "antigenic drift," which is thought to result from the interplay of viral mutability and immunological selection (Webster and Laver, 1975). The presence of antibody of low avidity may select for single-step mutants, which have an altered amino acid in the key area of the antigenic determinant, giving rise to a new viral strain.

At intervals of 10–15 years sudden and complete changes in the determinants of type A influenza glycoproteins occur; the changes are such that the virus possess glycoproteins that appear completely distinct by peptide mapping and serological tests (Webster and Laver, 1975).

Dramatic changes in the antigenic determinants of the viral glycoproteins are termed "antigenic shifts," and it is these new viruses that cause worldwide influenza pandemics. It has been postulated that antigenic shift may occur because type A strains of human origin may undergo recombination with type A strains of avian or other animal origin. The antigenic determinants of the new HA or NA are sufficiently different that the human host does not possess immunity, and the virus gains a selective advantage. Interestingly, type B influenza viruses do not undergo antigenic shift. The reason for this may lie in the fact that type B influenza viruses have not been isolated from other animal species; thus it is probable that the type B viruses cannot undergo similar recombination events (for review, see Webster and Laver, 1975).

### 3.5.4b. Neuraminidase Activity

Several distinct functions have been proposed for this enzyme. It is postulated that neuraminidase-containing virus lodges in the upper respiratory tract and binds to mucin via the hemagglutinin. The glycosyl residues of mucin are terminated by sialic acid (*N*-acetylneuraminic acid) to which the hemagglutinin binds, and one role of neuraminidase may be to cleave the sialoglycoprotein bond, freeing the bound virion. It is presumed that in this way the virus is released and underlying cell receptors are exposed to which the hemagglutinin can attach (Davenport, 1976).

It is unlikely that neuraminidase is involved in an early event such as penetration because virions remain infectious after inhibition of the activity by specific antibody (Seto and Rott, 1966; Bucher and Palese, 1975). However, the possibility that neuraminidase participates in the release of the budding virion from the infected cell surface has gained support from several types of experiments. By using influenza strains that had different levels of enzyme activity, it was shown that virus strains having low activity were released from cells more slowly than strains with higher enzyme activity (Palese and Schulman, 1974). Further, in the presence of antibody to the viral neuraminidase, which inhibited enzyme activity, virions were formed, but release of virus into culture medium was inhibited (Seto and Rott, 1966; Compans *et al.*, 1969; Webster, 1970). When bacterial neuraminidase was added to the cultures, infectious virus was released from the cells. These studies, however, are complicated by the fact that bivalent antibody can cause cross-linking of virions to viral antigens on cell surfaces, and Becht *et*

*al.* (1971) reported that monovalent Fab fragments inhibited neuraminidase activity without affecting virus release.

More conclusive evidence for the function of the enzyme has been obtained with temperature-sensitive mutants of influenza virus which are defective in neuraminidase activity (Palese *et al.*, 1974). At nonpermissive temperature, no neuraminidase activity is detected; virus particles are produced by cells despite the fact that infectivity titers are markedly reduced. However, the virus particles form large aggregates, and in contrast to wild-type virions these particles contain sialic acid as shown by colloidal iron hydroxide staining. Since influenza virions bind to sialic acid residues, these results indicate that the mutant virus particles aggregate to each other because sialic acid is added to viral carbohydrates, and that the essential function of viral neuraminidase is to remove or prevent the addition of such sialic acid. In support of this conclusion, addition of bacterial neuraminidase to cells infected with these mutants causes a marked enhancement of virus release.

Further evidence which supports this role for the neuraminidase was obtained with a neuraminidase inhibitor, 2-deoxy-2,3-dehydro-*N*-trifluoracetylneuraminic acid (FANA). Influenza virions grown in the presence of FANA contain neuraminic acid on their envelopes and the particles undergo extensive aggregation (Palese and Compans, 1976). A marked reduction in virus yield is observed because of this aggregation, and treatment with a purified neuraminidase results in enhancement of progeny virus yields, apparently by disaggregation of virus. Thus viral neuraminidase is not required for assembly of progeny virions but appears to be essential for the removal of sialic acid from the surface of the virion itself.

### 3.5.5. Biosynthesis of Envelope Components

#### 3.5.5a. Proteins

The synthesis and assembly of influenza virus-specific polypeptides can readily be analyzed in cell cultures because host cell polypeptide synthesis is inhibited after infection. All of the virion polypeptides plus a virus-specific nonstructural polypeptide (designated "NS") can be identified by polyacrylamide gel electrophoresis. The time course of synthesis varies with the specific virus strain and cell type (Lazarowitz *et al.*, 1971; Skehel, 1972; Klenk *et al.*, 1972*b*; Krug and Etkind, 1973; Meier-Ewert and Compans, 1974; Inglis *et al.*, 1976; Lamb and Choppin, 1976).

The intracellular location of viral polypeptide species has been determined using cell fractionation procedures. The HA polypeptide is always membrane associated, and the results of pulse-chase experiments suggest that it is synthesized on membrane-bound polyribosomes in the rough endoplasmic reticulum and that completed chains are transported to the smooth cytoplasmic membrane fraction and from there to the plasma membrane (Lazarowitz *et al.*, 1971; Compans, 1973*a*; Klenk *et al.*, 1974; Hay, 1974; Meier-Ewert and Compans, 1974). This scheme of intracellular migration is also supported by results indicating a progressive addition of sugars to the HA polypeptide (Compans, 1973*b*; Stanley *et al.*, 1973; Klenk *et al.*, 1978). Glucosamine is incorporated into HA polypeptides in the rough as well as the smooth membrane fraction, whereas fucose is present in smooth membranes but absent in the HA polypeptides in the rough membrane fraction (Compans, 1973*b*). Inhibition of protein synthesis by puromycin stops the incorporation of glucosamine almost immediately, whereas fucose is incorporated only after the completed HA polypeptide migrates to the smooth endoplasmic reticulum or Golgi complex membranes in the infected cell (Stanley *et al.*, 1973). Incorporation of sulfate into HA polypeptides also occurs for up to 30 min after inhibition of protein synthesis (Nakamura and Compans, 1977). The initial glycosylation events involve en bloc transfer of the glucosamine–mannose cores of the side chains from a lipid precursor (Schwarz *et al.*, 1977; Klenk *et al.*, 1978). This transfer appears to be extremely rapid, possibly occurring on nascent chains (Klenk, 1974), since it is not possible to detect unglycosylated forms of the HA polypeptide by short pulse labeling (Compans, 1973*a*; Klenk *et al.*, 1974). The site of cleavage of HA into HA1 and HA2 varies with the virus strain. With the WSN strain cleavage occurs at the plasma membrane (Lazarowitz *et al.*, 1971), whereas the fowl plague virus cleavage occurs intracellularly in membranes of the endoplasmic reticulum (Klenk *et al.*, 1974). Cleavage of HA is inhibited if the virus is grown in the presence of protease inhibitors or at reduced temperature (Klenk and Rott, 1973).

The NA polypeptide is present in small amounts in infected cells, and there is less information available on its intracellular location. The NA polypeptide is found along with HA in the smooth membrane fraction (Compans, 1973*a*; Klenk *et al.*, 1974; Hay, 1974), and it appears to be one of the polypeptides to arrive rapidly at the plasma membrane (Lazarowitz *et al.*, 1971; Hay, 1974). These data suggest that the steps in the synthesis and transport of HA and NA to the plasma membrane may be similar. Evidence has been obtained that the hemagglutinin is

incorporated into discrete regions of the plasma membrane, whereas the neuraminidase is more dispersed (Hay, 1974).

The M protein, although the most abundant polypeptide in the virion, is synthesized in limited amounts in infected cells, and it has been suggested that the synthesis of M may be rate limiting for viral assembly (Lazarowitz *et al.*, 1971). In contrast to other viral polypeptides, the rate of synthesis of M increases late in the infectious cycle (Skehel, 1972; Meier-Ewert and Compans, 1974; Lamb and Choppin, 1976). The M protein is found associated with various cytoplasmic membrane fractions as well as the plasma membrane (Lazarowitz *et al.*, 1971; Compans, 1973*a*; Stanley *et al.*, 1973; Klenk *et al.*, 1974; Meier-Ewert and Compans, 1974; Hay, 1974). However, the behavior of M in pulse-chase experiments differs from the behavior of HA, in that no migration of M has been detected between various cellular compartments. M protein is inserted rapidly into the plasma membrane, and it has been suggested that this polypeptide may be synthesized on free polyribosomes and inserted into membranes after synthesis (Compans *et al.*, 1974; Hay, 1974). If this is the case, those molecules found in association with intracellular membranes may be by-products rather than intermediates in assembly. In cells infected at reduced temperature, synthesis of the M protein is selectively inhibited (Klenk and Rott, 1973), and this inhibition results in a block in virus maturation. Similarly, in abortive infection, as it occurs if fowl plague virus is grown in HeLa, L, or BHK cells, synthesis of M protein is suppressed, and virus particles are not formed. However, the lack of M protein has no effect on synthesis and processing of the hemagglutinin. Thus the envelope proteins are synthesized and processed by independent mechanisms (Lohmeyer *et al.*, 1977).

### 3.5.5b. Lipids

The available evidence suggests that the normal cellular processes of lipid biosynthesis are used to generate the lipids incorporated into the viral envelope. Prelabeling of cells with $^{32}PO_4$ before infection was used to demonstrate that preformed host cell lipids were utilized for virus assembly (Wecker, 1957). The lipids are acquired from the plasma membrane during budding and reflect closely the composition of the plasma membrane. The asymmetrical distribution of phospholipids and glycolipids also presumably reflects the distribution on the cell surface. The rate of synthesis of phospholipids was depressed late in the growth

cycle in influenza virus infected cells (Blough and Weinstein, 1973), and it is likely that this may be a secondary effect of inhibition of cellular biosynthesis.

### 3.5.5c. Carbohydrates

Because the genetic information in the viral RNA is accounted for by the known structural and nonstructural viral polypeptides, it is not possible for the virus to direct the synthesis of enzymes involved in carbohydrate biosynthesis. Host cell glycosyltransferases therefore appear to be utilized. Glycosylation of the viral glycoproteins can be detected by addition of radioactive sugars such as glucosamine and fucose to infected cells. It is not known whether carbohydrates present in viral glycolipids are preformed or newly synthesized after infection.

Inhibition of the glycosylation of viral glycoproteins has been reported using the sugars 2-deoxyglucose or glucosamine (Kilbourne, 1959; Gandhi *et al.*, 1972; Kaluza *et al.*, 1972; Klenk *et al.*, 1972*b*) or the antibiotic tunicamycin (R. Schwarz *et al.*, 1977; Nakamura and Compans, 1978*a*). Both the production of the normal HA polypeptide and that of neuraminidase activity are inhibited by these compounds, whereas there is little or no effect on synthesis of carbohydrate-free polypeptides. A new polypeptide designated "HA0"is detected in the presence of inhibitors (Klenk *et al.*, 1972*b*) which appears to be an unglycosylated or partially glycosylated form of the HA polypeptide. Glycosylation of the viral glycoproteins appears to influence their sensitivity to proteolytic cleavage. Thus the aberrant HA0 glycoproteins produced in the presence of glycosylation inhibitors are cleaved into more heterogeneous products resembling HA1 and HA2 (Klenk *et al.*, 1974; Nakamura and Compans, 1978*a*), and in fowl-plague-virus-infected cells treated with tunicamycin the unglycosylated hemagglutinin polypeptide is completely degraded (R. Schwarz *et al.*, 1976). HA0 appears to migrate from rough to smooth cytoplasmic membranes in the same manner as HA (Klenk *et al.*, 1974; Nakamura and Compans, 1978*a*) and in some virus–cell systems the maturation process occurs, although at a reduced level, in the presence of these inhibitors to yield virions containing aberrant glycoproteins (Compans *et al.*, 1974; Nakamura and Compans, 1978*a*). Therefore, full glycosylation is not a requirement for either intracellular migration or assembly of influenza viral glycoproteins into virions. The particles produced in the presence of inhibitors have low specific infectivity, however, indicating that

glycosylation is probably essential for normal biological activity of the glycoproteins.

### 3.5.6. The Assembly Process

Influenza virus was the first virus to be shown to be released from infected cells by a process not involving lysis; virus particles emerged from the cell surface by budding into the extracellular space (Murphy and Bang, 1952). The emerging virions contain a unit membrane like that observed on the cellular plasma membrane, with modifications on the internal and external surfaces (Compans and Dimmock, 1969; Bächi *et al.*, 1969). The external surface contains projections corresponding to the viral glycoproteins, whereas the internal surface shows an electron-dense layer which is thought to represent the internal membrane (M) protein. Viral envelope proteins appear to be incorporated into areas of membrane of normal morphology, which however exhibit specific adsorption of erythrocytes (Compans and Dimmock, 1969). After alignment of the internal viral components, budding occurs by an outfolding of the plasma membrane. Since virions contain little or no host cell proteins, these proteins must be excluded from regions of the cell surface where virus maturation occurs. There is little direct evidence for the nature of the macromolecular interactions involved in assembly that lead to domains on the cell surface from which host cell polypeptides are excluded. With influenza A viruses, there is little morphological evidence for direct interactions between glycoproteins, whereas the M protein appears to form a tightly packed shell with extensive protein–protein interactions. It therefore seems that lateral interactions between M protein molecules, together with transmembrane interactions between glycoproteins and M protein, are likely to be involved in assembly of the envelope.

Recent reports of the presence of actin in enveloped viruses have suggested the possibility that actin microfilaments may be involved in virus maturation (Wang *et al.*, 1976). However, the assembly of influenza virions was not prevented by cytochalasin B (Griffin and Compans, 1978), despite the disruption of cellular microfilaments by the drug. Released virus yields were diminished in the presence of cytochalasin as a result of aggregation of virions at cell surfaces; it was found that this resulted from production of an inactive neuraminidase in the presence of the drug. When purified *V. cholera* neuraminidase was added to cytochalasin-treated cells, a marked enhancement of virus

release was observed. Cytochalasin partially inhibits the glycosylation of influenza virus glycoproteins, which probably results in the formation of inactive neuraminidase.

## 3.6. Paramyxoviruses

### 3.6.1. Classification, Morphology, Composition

The paramyxoviridae family comprises three genera: paramyxovirus (paramyxovirus subgroup), morbillivirus (measles–distemper group), and pneumovirus (respiratory syncytial virus subgroup). Members of the paramyxovirus subgroup include a variety of human and animal viruses, such as Newcastle disease virus (NDV), mumps, and the parainfluenza viruses. The envelope of these viruses has hemagglutinating and neuraminidase activities. The measles–distemper group includes measles virus, canine distemper virus, and rinderpest virus. These viruses contain only hemagglutinin but no neuraminidase. The respiratory syncytial virus (RSV) subgroup includes RSV of man and cattle, and pneumonia virus of mice. These viruses have no neuraminidase activity and RSV has no hemagglutinating activity either. A common feature of almost all paramyxoviruses is their ability to induce cell fusion; RSV causes cell fusion whereas pneumonia virus of mice has not been reported to cause fusion.

Virions are usually spherical, 150 nm or more in diameter, but filamentous forms several micrometers long may be observed. Virions are composed of a helical nucleocapsid and an envelope with surface projections about 10 nm in length. The envelope of paramyxoviruses appears to be particularly flexible, and virus particles exhibit a high degree of pleomorphism.

Virus particles are composed of about 1% RNA, 70% protein, 20% lipid, and 6% carbohydrate (Klenk and Choppin, 1969*a*). The lipids, the carbohydrates, and about 70% of the total viral protein constitute the envelope (Compans and Choppin, 1967; Caliguiri *et al.*, 1969). The carbohydrate is present in the form of glycoprotein (Klenk *et al.*, 1970*a*), glycolipid (Klenk and Choppin, 1970*b*), and mucopolysaccharide (Pinter and Compans, 1975).

Detailed studies have been carried out on the envelopes of NDV and two parainfluenza viruses: Sendai virus and simian virus 5 (SV5). Less information is available on morbillivirus and pneumovirus envelopes, but there is increasing evidence that they follow the same general rules for structure, function, and biosynthesis. Reviews on

paramyxovirus replication have been published in Volume 4 of this series (Choppin and Compans, 1975), and elsewhere (Kingsbury, 1973).

### 3.6.2. Fine Structure and Arrangement of Envelope Components

The paramyxovirus envelope is composed of a lipid bilayer, an inner carbohydrate-free protein (M protein), and external spikes consisting of glycoproteins. Table 2 presents a list of the envelope proteins, their estimated molecular weights, and functions.

The envelope can be disrupted and its components can be solubilized by detergents (Rott and Schäfer, 1960). The glycoprotein spikes and the M protein have different solubilities in detergent–salt solutions and can thus be separated from each other (Scheid *et al.*, 1972). The spikes are removed from the virus particles by extensive protease treatment (Chen *et al.*, 1971). It is not clear if the spikes completely penetrate the lipid layer and thus are able to directly interact with the M protein. Glucosamine, mannose, galactose, and fucose have been identified as carbohydrate constituents of the spike glycoproteins (Klenk *et al.*, 1970*a*). By means of their carbohydrate complement the spikes are able to bind to phytoagglutinins (Becht *et al.*, 1972).

Paramyxoviruses possess two different glycoprotein species, as was shown first with SV5 (Klenk *et al.*, 1970*a*), Sendai virus (Content and Duesberg, 1970), and NDV (Mountcastle *et al.*, 1971), and has been subsequently further substantiated with several other members of this group. The available evidence indicates that each glycoprotein species constitutes a distinct type of spike (Scheid *et al.*, 1972; Seto *et al.*, 1973; Shimizu *et al.*, 1974). By means of gradient centrifugation (Scheid *et al.*, 1972), affinity chromatography to agarose-bound fetuin (Scheid and Choppin, 1973), or isoelectric focussing (Shimizu *et al.*, 1974), both types have been separated from each other and characterized in detail. From a comparison of the structural and functional data of all paramyxovirus glycoproteins analyzed to date (Table 2) the following picture emerges: One spike type consists of a glycoprotein which in the case of NDV, SV5, Sendai virus, and mumps virus has hemagglutinin and neuraminidase activities and has, therefore, been designated HN. With measles virus, this glycoprotein has only hemagglutinin activity, and with RSV it has none of these activities. The other type of spike is composed of glycoprotein F and with all viruses has cell-fusing and hemolytic activity. The data from which this general conclusion has been derived will now be discussed in more detail.

## TABLE 2

### Envelope Proteins of Paramyxoviruses[a]

| | Paramyxovirus | | Morbillivirus | | Pneumovirus[c] | |
|---|---|---|---|---|---|---|
| Designation[b] | Molecular weight | Biological activity | Molecular weight | Biological activity | Molecular weight | Biological activity |
| HN | 65,000–74,000 | Hemagglutinin, neuraminidase | 80,000 | Hemagglutinin | 48,000 | Hemagglutinin[e] |
| F F1,2 | 54,000–68,000 | Hemolysis and cell fusion | 62,000 | Hemolysis and cell fusion | n.a.[g] | Cell fusion[f] |
| F F1 | 48,000–56,000 | | 40,000[d] | | 42,000 | |
| F F2 | 10,000–16,000 | | 15,000 | | n.a. | |
| M | 40,000 | — | 37,000 | | 27,000 | — |

[a] For references, see text.
[b] Nomenclature of Scheid and Choppin (1977).
[c] Localization and function of pneumovirus proteins have not been firmly established yet.
[d] This polypeptide is not glycosylated with measles virus.
[e] Hemagglutination is observed only with pneumonia virus of mice.
[f] Cell fusion is not observed with penumonia virus of mice.
[g] n.a., Not analyzed.

### 3.6.2a. The Hemagglutinin–Neuraminidase Spike

In contrast to influenza virus which has hemagglutinin and neuraminidase on distinct glycoprotein molecules, in paramyxoviruses both of these activities are associated with one glycoprotein species, the HN glycoprotein (Scheid *et al.*, 1972; Scheid and Choppin, 1973; Seto *et al.*, 1973; Tozawa *et al.*, 1973; Shimizu *et al.*, 1974). HN of Sendai virus, SV5, and NDV has a molecular weight in the range of 65,000–74,000 as determined by polyacrylamide gel electrophoresis in the presence of SDS and reducing agents. Under nonreducing conditions a dimer is observed, suggesting that the hemagglutinin–neuraminidase spike is composed of two disulfide-linked HN glycopolypeptides (Scheid *et al.*, 1978). Chymotrypsin treatment of this spike yields a water-soluble protein with neuraminidase activity (Drzeniek and Rott, 1963) which contains the larger polypeptide fragment (HN-c1) as a disulfide-linked dimer (Scheid *et al.*, 1978). The other cleavage product(s) are lipophilic and contain that part of HN which anchors the spike in the virus membrane. Failure to detect an N-terminal amino acid on HN or HN-c1 suggests that the hydrophobic tail of HN contains the C terminus (Scheid *et al.*, 1978).

In some strains of NDV, an 82,000-dalton precursor to the HN glycoprotein designated "HNO" is incorporated into virions (Nagai *et al.*, 1976*b*; Nagai and Klenk, 1977). Such particles have reduced hemagglutinin and neuraminidase activities as will be described in detail below.

### 3.6.2b. The Fusion Spike

The fusion spike is composed of glycoprotein F, which consists of two disulfide-linked fragments, F1 (MW 48,000–56,000) and F2 (MW 10,000–16,000). Under nonreducing conditions the two fragments are not separated from each other and migrate on polyacrylamide gels as a complex (MW 54,000–68,000) which has been designated "F1,2" (Table 2). The fragments are derived by proteolytic cleavage from a common precursor F0 (MW ~65,000) which under certain conditions is incorporated into NDV and Sendai virus particles (see below). Since only the cleavage product but not the uncleaved precursor is biologically active, it could be clearly demonstrated that glycoprotein F is responsible for hemolysis and cell fusion (Homma and Ohuchi, 1973; Scheid and Choppin, 1974*a*; Shimizu *et al.*, 1974; Nagai *et al.*, 1976*b*). Similar conclusions have been drawn from a study using monospecific

antibodies (Seto *et al.*, 1974). Of the two cleavage fragments only the larger one (F1) was first identified and was then designated "F" (Scheid and Choppin, 1974). Subsequently evidence has been obtained that a smaller cleavage fragment F2 is also present on the spike (Shimizu *et al.*, 1974; Homma *et al.*, 1975; Nagai *et al.*, 1976*a*), and recently this fragment has been isolated and characterized in some detail (Scheid and Choppin, 1977). With Sendai virus, SV5, and NDV, F1 as well as F2 is glycosylated, but F2 contains relatively more carbohydrates than F1. No free N terminus was detected on F0 or F2 of Sendai virus, whereas N-terminal phenylalanine was found on F1. This suggests that the order of the F0 polypeptide is X-NH-F2-Phe-F1-COOH. Analysis of the N-terminal sequence of the F1 polypeptide revealed a high degree of hydrophobicity and of homology among all three viruses (Scheid *et al.*, 1978; Gething *et al.*, 1978).

### 3.6.2c. The Lipid Bilayer

Paramyxoviruses derive their lipids from the plasma membrane of the host, where maturation occurs. This concept is based to a large extent on a series of comparative lipid analysis of SV5 and isolated plasma membranes of different host cells (Klenk and Choppin, 1969*b*, 1970*a*,*b*). These studies demonstrated that all lipid species observed in the plasma membrane are also found in the viral envelope. The only exception are gangliosides, which because of the action of the viral neuraminidase are not incorporated into the virion. The envelope lipids are present in proportions typical for plasma membranes; i.e., there is a high cholesterol content with a molar ratio of cholesterol to phospholipid close to 1, and there are relatively high amounts of sphingomyelin and of glycolipids. The close similarity between the viral envelope and the plasma membrane is further emphasized when the patterns of the individual glycolipid species are analyzed which show distinct variations depending on the host cell. For instance, galactosyl-galactosyl-glucosylceramide is found in rhesus monkey kidney cells and in virions grown in them, but not in bovine kidney (MDBK) cells or virus from MDBK cells (Klenk and Choppin, 1970*b*). Lipids of Sendai virus and of several strains of NDV grown *in ovo* have also been analyzed, and differences mainly in the fatty acid composition have been interpreted to demonstrate that the viral proteins have the ability to select preferentially certain lipid classes (Blough and Lawson, 1968; Tiffany and Blough, 1969*b*). However, the studies on SV5 described above clearly show that it is primarily the plasma membrane of the host cell that determines the lipid composition of the paramyxovirus envelope.

Electron spin resonance studies have provided evidence that the lipids of SV5 are found in bilayer structures with fluid lipid phases similar to those observed for other biological membranes (Landsberger *et al.*, 1973). All viral membranes were shown to be substantially more rigid than the corresponding host cell plasma membranes. Proteolytic removal of the glycoprotein spikes did not appreciably alter the fluidity of the lipid bilayer of the envelope. Thus it was concluded that the rigidity of viral membranes may be determined by the M protein and not by the viral glycoproteins. However, another factor in determining envelope rigidity was the lipid composition, since differences in the rigidity of different host cell membranes were reflected in the viral lipid bilayer.

### 3.6.2d. The M Protein

Like influenza and rhabdoviruses, paramyxoviruses possess two major nonglycosylated proteins. One of those is the nucleocapsid protein; the other one (MW ~40,000, Table 2) is associated with the viral membrane and has therefore been designated the M protein. The first evidence for a protein associated with the inner side of the lipid bilayer came from electron microscopic studies (see section on influenza viruses). This location was further substantiated by the observation that the M protein is resistant to protease treatment, which removes the spikes from the surface of intact virus particles (Chen *et al.*, 1971). The M protein is insoluble in water and nonionic detergents but can be solubilized by nonionic detergents in the presence of high salt. On the basis of these properties purification procedures have been developed, and chemical analysis revealed that the M protein of SV5 contains a high proportion of hydrophobic amino acids (Scheid *et al.*, 1972; McSharry *et al.*, 1975). The M protein has high affinity for membranes, as indicated by cell fractionation studies which revealed that it is present in infected cells only in membrane-containing fractions (Nagai *et al.*, 1976*a*; Lamb and Choppin, 1977).

### 3.6.2e. The Envelopes of Mumps, Measles, and Respiratory Syncytial Viruses

Mumps, measles, and respiratory syncytial viruses are by far not as well analyzed as Sendai virus, NDV, and SV5. The data on protein composition available to date (Table 2) suggest, however, that their envelopes are organized by the same general principles.

Mumps virus contains two glycoproteins. The larger one has been found to possess hemagglutinin and neuraminidase activities. Evidence

has also been obtained for the presence of an M protein as judged by its solubility in Triton X100 and high-salt solution (Jensik and Silver, 1976).

The experimental evidence obtained in a series of studies on measles virus indicates that this virus also has two glycoproteins. One of these glycoproteins has a molecular weight of ~80,000 as indicated by polyacrylamide gel electrophoresis in the presence of mercaptoethanol (Bussell *et al.*, 1974; Mountcastle and Choppin, 1977), whereas under nonreducing conditions it migrates as a dimer (Hardwick and Bussell, 1976). It resembles therefore the HN glycoprotein of Sendai virus in these properties. The other glycoprotein exhibits structural features typical for F. It is composed of two disulfide-linked fragments F1 and F2, and a precursor F0 has also been identified. In contrast to Sendai virus, SV5, and NDV, F1 appears to be unglycosylated with measles virus (Hardwick and Bussell, 1976; Mountcastle and Choppin, 1977; Scheid *et al.*, 1978). These data are compatible with immunological studies in which two distinct antigens have been identified, one with hemagglutinating and the other one with hemolytic activity (Norrby and Gollmar, 1975).

The polypeptides of RSV have also been analyzed by polyacrylamide gel electrophoresis, but so far an association with biological activities has not been established. Two glycoproteins have been found, and there is suggestive evidence for an M protein (Wunner and Pringle, 1976).

### 3.6.3. Biogenesis and Assembly of the Envelope

The envelopes of paramyxoviruses are assembled on preformed cellular membranes, and the final step in assembly is a budding process at the plasma membrane. The available evidence, which is derived mainly from electron microscopic and cell fractionation studies and will be discussed in detail below, suggests that envelope maturation is a multistep process involving sequential incorporation of viral proteins into cellular membranes.

#### 3.6.3a. Biosynthesis of Glycoproteins

Fractionation studies on cells infected with NDV (Nagai *et al.*, 1976*a*) and Sendai virus (Lamb and Choppin, 1977) have shown that the polypeptide chains of the glycoproteins are synthesized on the rough

endoplasmic reticulum. The available evidence indicates that they migrate via the smooth endoplasmic reticulum and the Golgi apparatus to the plasma membrane.

In the course of migration, the polypeptides of the glycoproteins undergo posttranslational modifications. These involve, as can be assumed from analogy to influenza virus (see Section 3.5.5a), sequential glycosylation on the rough endoplasmic reticulum and on smooth membranes. Inhibitors of glycoprotein synthesis such as 2-deoxy-D-glucose (Scholtissek *et al.*, 1974; Hodes *et al.*, 1975) and tunicamycin (Takatsuki and Tamura, 1971) block paramyxovirus replication. There is evidence that the inhibitors interfere with glycosylation (H. D. Klenk, R. T. Schwarz, M. F. G. Schmidt, and K. Bortfeldt, manuscript in preparation) as has been demonstrated in the case of other enveloped viruses such as orthomyxoviruses or togaviruses (see Sections 3.5.5c and 3.10.3).

The other type of posttranslational modification which has been studied extensively with paramyxoviruses is proteolytic cleavage. Cleavage is involved in the biosynthesis of both envelope glycoproteins. As described above, the F1 and F2 subunits of glycoprotein F are derived from a common precursor F0. This precursor has been identified with Sendai virus (Homma and Ohuchi, 1973; Scheid and Choppin, 1974*a*), SV5 (Peluso *et al.*, 1977), measles virus (Scheid *et al.*, 1978), and a whole series of different strains of NDV (Samson and Fox, 1973; Hightower *et al.*, 1975; Nagai *et al.*, 1976*a*,*b*). Cleavage of F0 takes place on smooth internal membranes and on the plasma membrane (Nagai *et al.*, 1976*b*). With two strains of NDV, a precursor HNO has been observed which is converted, again by proteolytic cleavage, into glycoprotein HN (Nagai *et al.*, 1976*a*). It is not clear whether synthesis of HN0 is a general phenomenon occurring with all paramyxoviruses. However, suggestive evidence has been obtained by *in vitro* translation studies that HNO might be synthesized by another NDV strain in which the precursor cannot be detected if analyzed under *in vivo* conditions (Clinkscales *et al.*, 1977).

Cleavage is not required for virus assembly, but it is necessary for the expression of the biological activities of each glycoprotein, i.e., hemolysis and cell fusion in the case of F (Homma and Ohuchi, 1973; Scheid and Choppin, 1974*a*; Nagai *et al.*, 1976*a*) and hemagglutinating and neuraminidase activity in the case of HN (Nagai *et al.*, 1976*a*; Nagai and Klenk, 1977). As will be shown later, these activities reflect the role of the glycoproteins in the penetration process. It is therefore not surprising that virus particles with uncleaved precursors have reduced infectivity.

Whether cleavage occurs depends on the virus strain and on the host cell. Sendai virus (Homma and Ohuchi, 1973; Scheid and Choppin, 1974*a*) and certain strains of NDV (Nagai *et al.*, 1976*a*) in appropriate host cells produce virus particles with one or both glycoproteins present in the inactive precursor form, whereas if grown in other host systems the virions are fully infectious and contain both glycoproteins in the cleaved form. This observation indicates that host enzymes, which are present in a given cell type and absent in another one, are responsible for cleavage. On the other hand, in a given host cell the glycoproteins of one NDV strain may be cleaved, whereas those of another one are not cleaved, and evidence has been presented that these variations in cleavage are based on structural differences of the glycoproteins (Nagai *et al.*, 1976*a*).

Virus particles with uncleaved glycoproteins, which are obtained with Sendai virus and certain NDV strains, can be activated by *in vitro* treatment with proteases and provide suitable substrates to determine the specificity of these enzymes. It was found that the F glycoprotein of these viruses is cleaved and activated exclusively by trypsin (Scheid and Choppin, 1976; Nagai and Klenk, 1977). With Sendai virus, mutants with an altered protease sensitivity have been isolated that are not susceptible to trypsin but are activated by other enzymes such as chymotrypsin and elastase (Scheid and Choppin, 1976). In contrast to glycoprotein F0, which requires a specific protease, glycoprotein HNO was cleaved and activated by a series of enzymes of different specificities (Nagai and Klenk, 1977). Thus there is a striking difference in the susceptibility of the two glycoproteins to proteolytic enzymes, and it is reasonable to assume that HNO has a much higher chance to encounter an appropriate protease in a given host cell and that it is, therefore, more readily cleaved than F0. This might explain why, so far, HNO could be detected only with two NDV strains, whereas F0 has been observed with almost all paramyxoviruses analyzed.

### 3.6.3b. Biosynthesis of the M Protein

The association of the M protein with membranes of infected cells and its incorporation into mature envelopes have been investigated in cell fractionation studies (Nagai *et al.*, 1976*b*; Lamb and Choppin, 1977). The M protein was found to be incorporated into plasma membranes and into virions immediately after its synthesis. It appears that insertion of the M protein into the plasma membrane is one of the final steps in envelope assembly.

Evidence has been obtained with NDV that M is inserted into membrane regions which already contain glycoproteins (Nagai *et al.*, 1976*b*). The mechanism by which M recognizes such areas is not known. Direct interaction across the lipid bilayer between the envelope glycoproteins and the M protein has not been demonstrated with paramyxoviruses, but reconstitution studies with isolated components suggest that the M protein mediates the binding of the spikes to the nucleocapsid (Yoshida *et al.*, 1976).

The data described so far support the concept that incorporation of M into the envelope precursor results in the formation of mature envelope with morphologically distinct spikes appearing on the outer and nucleocapsid strands being attached to the inner side of the envelope. It is reasonable to assume that on its incorporation into the membrane the M protein serves as a binding site for the nucleocapsid (Shimizu and Ishida, 1975; McSharry *et al.*, 1971, 1975).

Little is known about the events that lead to the appearance of the spikes, but it has to be assumed that extensive rearrangement of the glycoproteins occurs. Recently evidence has been obtained that the rearrangement might involve patch formation. It has been found that the NDV glycoproteins that are incorporated into smooth membranes of infected cells have a high degree of mobility in the plane of the membrane and show random distribution, whereas in the mature envelope they have a low mobility and are aggregated in patches (Nagai *et al.*, 1975). Thus it appears that insertion of the M protein into the envelope and patch formation of the glycoproteins are closely linked events. However, it is not yet known whether patch formation is a precondition for attachment of M, or whether insertion of M promotes the aggregation of the glycoproteins.

### 3.6.3c. Budding

Our knowledge of the budding process is derived mainly from electron microscope and cell fractionation studies. As described in the preceding section, cell fractionation studies suggest that the immediate precursor to the viral envelope is a discrete patch of plasma membrane containing virus-specific proteins. The concept of patch formation is supported by electron microscopic studies which show that (1) a layer of surface projections covers the virus particle but is absent on the adjacent cell surface (Compans *et al.*, 1966), (2) ferritin-labeled antibody binds to the entire surface of the budding virions but not to adjacent cell membrane (Choppin *et al.*, 1971), and (3) neuraminic acid

residues are present on the nonaltered cell surface but are absent on the budding virus particle (Klenk *et al.*, 1970*b*). Host cell polypeptides appear to be excluded from regions of membrane which give rise to virus particles as indicated by their absence in the mature virion.

An important feature of the assembly of the virus is that during the budding process the membrane of the viral envelope is continuous with and morphologically similar to the plasma membrane of the host cell. This suggests that the lipids in the unaltered plasma membrane are easily exchangeable by lateral diffusion with those in the budding virus particles. It is therefore not surprising that the lipids in the envelopes very closely resemble those of the host cell membrane.

There is thus good evidence that the viral envelope is derived from a patch of plasma membrane modified by the incorporation of virus-specific proteins. However, little is known about the mechanism which promotes protrusion of this patch to induce bud formation. Since the M protein and the nucleocapsid are the last virus-specific components to arrive in these patches, it is perhaps not unreasonable to assume that either one or both of these structures might be directly responsible for budding. In this respect, it is of interest to note that under certain conditions virus particles lacking nucleocapsid can be isolated (Rott *et al.*, 1963). This observation suggests that the M protein plays a more important role in the budding process than the nucleocapsid.

### 3.6.4. Biological Function of Envelope Glycoproteins

#### 3.6.4a. Role in Initiation of Infection

The glycoproteins of paramyxoviruses play essential roles in the initiation of infection. HN appears to be responsible for adsorption and F is involved in penetration. This conclusion can be drawn from numerous studies on the function of these glycoproteins. Paramyxoviruses are particularly suitable for such studies for the following two reasons: (1) There are specific activities associated with each glycoprotein that can be easily measured and thus can serve as model reactions. (2) Virus particles with biologically inactive precursors are available that can be activated by *in vitro* treatment with specific proteases, e.g., strain Ulster of NDV HNO can be activated by chymotrypsin and F0 by trypsin. Thus the activity of each individual glycoprotein can be analyzed independently.

*i. The HN Glycoprotein.* The ability of paramyxoviruses to interact and agglutinate a wide spectrum of erythrocytes has been

known for many years. That it is justified to use hemagglutination as a model reaction for adsorption is demonstrated by the observation described above that NDV particles with the biologically inactive precursor HN0 have a reduced infectivity (Nagai *et al.*, 1976*b*; Nagai and Klenk, 1977). This concept is also supported by the observation that a mutant of Sendai virus forms virions that lack the HN glycoprotein as well as infectivity (Portner *et al.*, 1975). Both of these findings demonstrate that HN is essential for infection.

The glycoprotein nature of the myxovirus receptor on erythrocytes and the essential role of neuraminic acid for its function have been known for many years (Klenk *et al.*, 1955). Since then much information has been accumulated on the receptor (for reviews, see Bächi *et al.*, 1977). There appears to be little specificity concerning the structure to which neuraminic acid is linked, because a whole series of different neuraminic acid containing glycoproteins have been shown to bind. Moreover, gangliosides are also active (Huang *et al.*, 1973; Haywood, 1975).

There is evidence that the same active center on the HN glycoprotein is responsible for hemagglutinating and neuraminidase activities. This is suggested by binding studies of a neuraminic acid analogue (Scheid and Choppin, 1974*b*) and by activation studies with proteases (Nagai and Klenk, 1977). The neuraminidase of paramyxoviruses has a broader substrate specificity than the enzyme of influenza viruses (Drzeniek, 1967; Huang and Orlich, 1972). As has been pointed out above, lipids and carbohydrates in the virus particle are determined largely by the host. The neuraminidase appears to be responsible for the only distinct virus-specific modification, i.e., the absence of neuraminic acid in glycoproteins and glycolipids (Klenk and Choppin, 1970*b*; Klenk *et al.*, 1970*a*,*b*). Whereas there is general agreement that hemagglutination reflects the role of the HN glycoprotein in adsorption, less is known about the biological significance of the enzymatic activity. It has been suggested that the neuraminidase may be important for virus release, but the evidence for this concept has been obtained mostly from studies with influenza virus (see Section 3.5.4b).

*ii. The F glycoprotein.* There is now wide agreement that the common principle underlying cell fusion and hemolysis is insertion of the viral envelope into the cell membrane by fusion. Experimental evidence for insertion and subsequent dispersion of envelope antigens in the plasma membrane has been obtained from electron microscopic studies (Hoyle, 1962; Howe and Morgan, 1969; Apostolov and Almeida, 1972; Bächi *et al.*, 1977). Hemolysis appears to require insertion of a highly permeable envelope in order to allow leakage of ions, swelling of

the cells, and finally rupture and egress of hemoglobin (Shimizu *et al.*, 1976; Homma *et al.*, 1976). Cell fusion occurs if the envelope incorporated into the plasma membrane fuses with another cell. Hemolysis as well as cell fusion requires the presence of glycoprotein F in its cleaved form as outlined above. Since this glycoprotein is also necessary for infectivity, it is a fair assumption that envelope–membrane fusion is also the mechanism of paramyxovirus penetration.

Cell fusion and hemolysis have found wide application as model systems to study envelope–membrane fusion (for a review, see Hosaka and Shimizu, 1977), and recent studies suggest that the virus alters the structure of the lipid bilayer of the target cell membrane and that there is an exchange of lipid between the viral envelope and the cell membrane. Evidence for lipid exchange has been obtained by Hoyle (1962), who reported that radioactively labeled phospholipid was transferred from influenza virus to membrane vesicles prepared from virus-infected cells, and Drzeniek *et al.* (1966), who showed that lipid antigens were transferred from paramyxovirions into erythrocytes on exposure of cells to viruses. Recently lipid exchange mediated by NDV between cells in culture could be demonstrated by means of a fluorescent lipid probe (Huang, 1977).

Further insight into the molecular mechanism involved in the fusion process came from studies employing spin-labeled probes (Maeda *et al.*, 1975, 1977; Lyles and Landsberger, 1977). These studies suggest that first the envelope bilayer of the Sendai virion and the membrane of the erythrocyte are brought in close contact by the action of the HN glycoprotein. Subsequently the lipids of the cell membrane are destabilized and lipid intermixing between both bilayers occurs. For destabilization and lipid exchange glycoprotein F must be present in the cleaved form; virions devoid of F or virions containing the uncleaved form have very low activity. Similar results have been obtained by nuclear magnetic resonance studies of chick embryo cells exposed to NDV which also showed that the virus alters the bilayer structure of the target cell membrane and that this perturbation depends on cleavage of F (Klenk *et al.*, 1977*a*). The mechanism of the perturbation induced by glycoprotein F appears to be a biophysical rather than a biochemical event since the chemical composition of the membrane lipids is not changed in the fusion process (Elsbach *et al.*, 1969; Diringer and Rott, 1976). As has been pointed out above, the F protein has a highly hydrophobic region at the N terminus of the F1 segment, and it is tempting to speculate that this region might be responsible for the interaction with the target cell membrane.

### 3.6.4b. Envelope Glycoproteins as Determinants of Host Range and Pathogenicity

It has been pointed out above that proteolytic cleavage of the viral glycoproteins is determined by the structure of these molecules as well as by the disposition of the appropriate enzymes by the host. Depending on the host cell and the virus strains, Sendai virus (Scheid and Choppin, 1976) and NDV (Nagai *et al.*, 1976*b*) form either infectious particles with cleaved glycoproteins or particles with reduced infectivity and uncleaved glycoproteins. Thus there are differences in host range, and these differences proved to be of high importance for the pathogenicity of NDV. Five virulent and five avirulent strains have been analyzed that were grown in five different host systems. For avirulent strains, only a few host systems were permissive, i.e., they produced infectious virus and allowed replication under multiple cycle conditions; most host cells were nonpermissive for these strains, i.e., they produced inactive virus and did not allow multiple cycle replication. In contrast, all host systems were permissive for virulent strains. It is a reasonable assumption that the wide host range of the virulent strains should facilitate spread of infection in the organism and thus promote outbreak of disease.

## 3.7. Poxviruses

### 3.7.1. Classification, Morphology, Composition

The poxviridae family comprises six different genera and several other members not yet allocated to a specific genus, such as molluscum contagiosum virus. Natural hosts are a wide range of vertebrates and invertebrates. Most of the information on structure and replication has been obtained from studies on vaccinia virus (genus orthopoxvirus). The replication of poxviruses has been reviewed in Volume 3 of this series (Moss, 1974).

Poxviruses are large, brick-shaped or ovoid particles 300–450 nm long and 170–260 nm wide. The chemical composition of vaccinia virus has been shown to be about 92% protein, 3% DNA, 5% lipid (1.2% cholesterol, 2.1% phospholipid, 1.7% neutral lipid), and 0.2.% non-DNA carbohydrates (Hoagland *et al.*, 1940; Smadel *et al.*, 1940; Zwartouw, 1964). Biochemical studies and electron microscopy have demonstrated that the external envelope of vaccinia virus meets all the

criteria for biological membranes. Chemically it has been demonstrated to contain protein and lipid. Morphologically it possesses a trilaminar or unit membrane structure of the same width as that of cell membranes and exhibits tubular elements at the outer surface (Peters, 1956; Dales and Siminovitch, 1961; Nagington and Horne, 1962; Westwood *et al.*, 1964; Dales and Mosbach, 1968). A second membrane may be present in the core structure, but it has not been so well studied as the surface membrane (Morgan *et al.*, 1962; Easterbrook, 1966; Mitchiner, 1969).

### 3.7.2. Components of the Envelope

Because of the complexity of the virus structure, our knowledge on composition and architecture of the envelope is far from complete.

#### 3.7.2a. Proteins

In vaccinia virions 20–30 polypeptides ranging in molecular weight between about 8000 and 200,000 have been identified by polyacrylamide gel electrophoresis (Holowczak and Joklik, 1967; Moss and Salzman, 1968; Katz and Moss, 1970; Moss and Rosenblum, 1972; Sarov and Joklik, 1972). Out of 28 carbohydrate-free polypeptides observed in whole virus in one of these studies, 17 have been found in cores that were prepared by treating virions with Nonidet P40 and 2-mercaptoethanol followed by iodoacetamide, sonication, and banding in sucrose density gradients. Five carbohydrate-free polypeptides appeared to be envelope proteins, since they were found to be located at or near the surface of the virus. This conclusion was based on their ability to react in situ with surface labeling agents and on the ability of chymotrypsin and Nonidet P40 to release them from virions (Sarov and Joklik, 1972). One of these five polypeptides (MW 58,000) appears to constitute the tubules at the surface of the virus (Stern and Dales, 1976).

In contrast to the apparently complex pattern of carbohydrate-free envelope proteins, the glycoprotein composition of vaccinia virus is relatively simple. Only one (Holowczak, 1970; Garon and Moss, 1971; Moss *et al.*, 1973) or perhaps two glycoproteins (Sarov and Joklik, 1972) could be identified in the virion. The vaccinia virus glycoprotein differs remarkably in its carbohydrate composition from the glycoproteins of other enveloped viruses, since glucosamine has been found to be

the only constituent sugar. Glycopeptides obtained after pronase digestion of virions grown in avian and mammalian cell lines were indistinguishable and a similar glycopeptide was not detected in uninfected cells (Garon and Moss, 1971). The precise location of the glycoprotein within the viral envelope or even within the virion is unknown. Holowczak (1970) and Garon and Moss (1971) noted that a major portion of the glucosamine-labeled glycoprotein was removed from virions by treatment with Nonidet P40 and a reducing agent. This procedure was originally devised by Easterbrook (1966) to remove the viral envelopes. The glycoprotein was also removed by tryptic digestion under conditions in which the lipids were not removed, viral DNA was not released, and no loss of plaque-forming units occurred (Moss *et al.*, 1973). These results, which would suggest that the glycoprotein is located at the surface of the viral envelope, are difficult to reconcile with the observations of Sarov and Joklik (1972), who reported that the glycoproteins could not be removed by Nonidet P40 treatment in the absence of reducing agents or by chymotrypsin and that the glycoproteins are not accessible to surface labeling agents.

### 3.7.2b. Lipids

The vaccinia virus envelope in its trilaminar structure resembles cellular membranes and the envelopes of viruses that are assembled on such membranes. Unlike the budding viruses, however, vaccinia does not derive its lipid constituents by emerging from preexisting cellular membranes but rather most probably by a process of autonomous self-assembly within cytoplasmic "virus factories" (Dales and Mosbach, 1968). As a consequence of this mode of membrane biogenesis, the lipid composition of vaccinia virus is distinct from that of the host cell plasma membrane, notably with respect to a decreased amount of phosphatidylethanolamine and the presence of much greater quantities of an as yet unidentified phospholipid (Stern and Dales, 1974). Both preformed and nascent phospholipids entered the virion during and after particle maturation. *In vitro* transfer of phospholipids from liposomes to virus was demonstrated in the presence of either beef heart or HeLa cell phospholipid exchange protein, implying that such a protein may catalyze the *in vivo* transfer of phospholipid from endoplasmic reticulum to assembling vaccinia envelopes (Stern and Dales, 1974).

Evidence has been obtained that virus infection affects the lipid metabolism of the cell and that the resulting alterations in lipid composition are reflected in the host. Thus, after infection with vaccinia

virus, there is a shift in HeLa and L cells from complex to less complex glycolipids. Purified virus contains the same relative amounts of glycolipid as do the host cells whose composition has been altered by the infection. Evidence has been obtained that these changes may be the consequence of virus-mediated suppression of host protein synthesis, thereby affecting also the activity of enzymes involved in production of glycolipids (Anderson and Dales, 1978). Similarly, fowlpox virus has been found to contain significant amounts of squalene, which is a metabolic precursor of cholesterol and is not observed in the uninfected host cell (White *et al.*, 1968).

### 3.7.3. Biogenesis of the Envelope

The envelopes of vaccinia and other poxviruses are assembled *de novo* in cytoplasmic foci termed viroplasmic "matrices" or "factories." Such envelopes appear first within the factories and are not continuous with preexisting cellular membranes. The envelopes develop progressively from arc-shaped trilamellar structures, possessing an external coat of a closely packed layer of spicules. Ultimately they are formed into closed spheres enclosing immature forms of vaccinia. With time, progressive differentiation into a core and lateral bodies takes place inside the envelopes, while the external surface undergoes poorly understood morphological alterations, such as transformation in which spicules disappear and surface tubules appear (Dales and Mosbach, 1968). Rifampicin blocks the formation of virus particles at an early stage and membranes lacking the spicule layer accumulate within the cytoplasm. When rifampicin is removed, virtually all membranes become coated with spicules within 10 min (Moss *et al.*, 1969).

### 3.7.4. Virus-Induced Modifications of Cellular Membranes

Despite the apparent lack of involvement of the host plasma membrane in poxvirus assembly, there are indications that this structure is modified during virus replication. Alterations occurring early after infection are the appearance of virus-specific antigens at the cell surface (Miyamoto and Kato, 1968; Ueda *et al.*, 1969). Other modifications are hemagglutination and cell fusion. Interestingly, there is evidence that in some of these phenomena virus-specific proteins might be involved that are not structural components of the virions.

### 3.7.4a. Hemagglutination

Vaccinia virus produces a hemagglutinin which is found only in infected cells, not in virions. It is a late antigen and is located at the cell surface (Ichihashi and Dales, 1971). The hemagglutinin might be identical with the major plasma membrane glycoprotein synthesized after infection, which is thought to be virus specific (Weintraub and Dales, 1974). Such nonstructural glycoproteins have been observed in relatively large amounts in membrane fractions of infected cells (Moss *et al.*, 1973) and have been found to differ from the glycoprotein present in the virus particle in electrophoretic mobility, size of the oligosaccharide, and carbohydrate composition (Garon and Moss, 1971).

### 3.7.4b. Cell Fusion

Vaccinia virus induces cell fusion, which requires both DNA replication and protein synthesis, occurs only after mature particles have been assembled, and is not elicited from the outside by inoculum virus (Dales *et al.*, 1976). Cell fusion can be suppressed by antiserum prepared against isolated surface tubules of the virion. Such treatment also neutralizes virus infectivity (Stern and Dales, 1976). These observations support the concept derived from electron microscopic studies that the virus penetrates into the host cell by fusion of the envelope with the plasma membrane (Chang and Metz, 1976; Dales *et al.*, 1976). Moreover, these findings suggest that the fusing agent is located at the virus surface. It remains to be seen, however, whether this agent is the surface tubule itself or another component of the envelope.

## 3.8. Rhabdoviruses

### 3.8.1. Classification, Morphology, Composition

The rhabdoviridae family comprises the genus vesiculovirus, with the prototype vesicular stomatitis virus (VSV), and the genus lyssavirus, with the prototype rabies virus. Natural hosts of these viruses are vertebrates and arthropods. In addition, there are members of this family that multiply in plants.

Virions are usually bullet shaped, 130–300 nm long and 70 nm wide. They contain a long tubular nucleocapsid with helical symmetry

that is surrounded by an envelope. They are composed of about 3–4% RNA, 65% protein, 20–25% lipid, and 3–13% carbohydrate (McSharry and Wagner, 1971*a*; Schneider and Diringer, 1976). The carbohydrate is present in the form of glycoprotein, glycolipid (Klenk and Choppin, 1971), and mucopolysaccharide (Pinter and Compans, 1975).

We will confine ourselves here to VSV, which is one of the best-studied enveloped viruses, and to rabies virus. Various aspects of rhabdovirus structure and replication have been reviewed previously in Volume 4 of this series (Wagner, 1975) and elsewhere (Emerson, 1976; Schneider and Diringer, 1976).

### 3.8.2. Fine Structure and Arrangement of Envelope Components

The rhabdovirus envelope is composed of a lipid bilayer, one or two inner carbohydrate-free M proteins, and external spikes consisting of a single glycoprotein designated G. Table 3 presents a list of the envelope proteins of VSV and rabies virus.

The envelope proteins of VSV have been separated from the nucleocapsid by treatment with deoxycholate (Kang and Prevec, 1969; Cartwright *et al.*, 1970), digitonin (Wagner *et al.*, 1969), hydrochloric acid at pH 1.5 (Mudd, 1973), or Triton X100 in the presence of 0.3 M NaCl (Emerson and Wagner, 1972). In low-ionic-strength solution, Triton X100 (Kelley *et al.*, 1972) and other nonionic detergents, such as Nonidet P40 (Arstila, 1973; Cartwright *et al.*, 1970) and Triton N101 (Dietzschold *et al.*, 1974), solubilize selectively the G protein of VSV. Similarly, the glycoproteins of rabies virus have been solubilized by the

**TABLE 3**

**Envelope Proteins of Rhabdoviruses**

| Vesicular stomatitis virus Indiana serotype | | Rabies virus | | | |
|---|---|---|---|---|---|
| | | HEP flury strain | | PM strain | |
| Designation[a] | Molecular weight[a] | Designation[a] | Molecular weight[b] | Designation[a] | Molecular weight[c] |
| G | 69,000 | G | 80,000 | G1 | 78,000 |
| | | | | G2 | 65,000 |
| M | 29,000 | M2 | 25,000 | M2 | 22,000 |

[a] Wagner *et al.* (1972*b*).
[b] Sokol *et al.* (1971).
[c] Neurath *et al.* (1972).

action of Triton X100 and purified from contaminating proteins by isoelectric focusing (Schneider and Diringer, 1976).

Although the rhabdovirus envelope is composed predominantly of virus-specific proteins, there is evidence that host cell proteins are incorporated at levels too low to be detected by biochemical means. Thus the L-cell histocompatibility antigen has been found in VSV purified from these cells (Hecht and Summers, 1972).

### 3.8.2a. The Spikes

Rhabdoviruses are covered with closely spaced glycoprotein spikes that are approximately 10 nm in length as shown by electron microscopic studies. The spikes can be removed by proteases, such as trypsin (Cartwright *et al.*, 1970; Schloemer and Wagner, 1974), and in intact virions only the spike glycoprotein can be labeled by surface labeling agents (Eger *et al.*, 1975). The available evidence indicates that the spikes are composed of a single glycoprotein species termed the "G protein." With VSV it has a molecular weight of approximately 69,000 (Table 3). With rabies virus, occasionally two glycoprotein peaks, G1 and G2 (Table 3), can be observed on polyacrylamide gels, which, however, appear to be modifications of the same species. The proportions of G1 and G2 varied depending on the virus strain, the virus preparation, and storage (Sokol *et al.*, 1971; Neurath *et al.*, 1972). This suggests that the lower-molecular-weight component G2 is a degradation product of the high-molecular-weight component G1.

The glycoproteins of rhabdoviruses are amphipathic like those of other enveloped viruses. After protease treatment of VSV a fragment of the G protein was demonstrated to be associated with the intact virion (Mudd, 1974). More recently Schloemer and Wagner (1975*b*) have isolated a small nonglycosylated portion of the protein from the envelope of protease-treated VSV virions. The fragment was found to have a molecular weight of 5200, approximately equivalent to 50 amino acids. Amino acid analysis showed that there was a preponderence of hydrophobic amino acids. The hydrophobic fragment of the G protein is long enough to penetrate the lipid bilayer. Conclusive evidence for such penetration has not been obtained, but cross-linking experiments with glutaraldehyde suggest that interactions may occur between G proteins and the internal M proteins (Brown *et al.*, 1974).

The carbohydrates linked to the G protein of VSV have been studied in detail. The glycoprotein is 9–10% carbohydrate by weight and has been found to contain mannose, galactose, *N*-acetylgluco-

samine, and neuraminic acid as the major sugar components with lesser amounts of *N*-acetylgalactosamine and fucose (McSharry and Wagner, 1971*b*). The presence of *N*-acetylgalactosamine would imply *O*-glycosidic linkages of carbohydrate chains to the polypeptide. However, such linkages have not been observed in the G protein, and in recent analyses *N*-acetylgalactosamine could indeed not be detected (Hunt and Summers, 1976*b*).

The attachment of the oligosaccharide to the polypeptide is most probably via a $\beta$-*N*-glycosidic linkage between asparagine and *N*-acetylglucosamine. This conclusion is based on the susceptibility of the oligosaccharide to endo-$\beta$-*N*-acetylglucosaminidase and to strong alkali and its resistance to mild alkaline hydrolysis, which would cleave *O*-glycosidic bonds involving serine and threonine (Moyer *et al.*, 1976).

The carbohydrate side chains of the VSV glycoprotein have been characterized in quite some detail. Analysis of the glycopeptides obtained by pronase digestion followed by gel chromatography revealed that there is only one type of side chain (Moyer *et al.*, 1976). From the size of the pronase glycopeptides (MW 3000–3400), the molecular weight of the glycoprotein, and the amount of carbohydrate present in the glycoprotein, it has been calculated that the G protein contains only two carbohydrate side chains (Etchison and Holland, 1974*a*,*b*; McSharry and Wagner, 1971*b*).

Analysis of the tryptic glycopeptides of the G protein of VSV showed that the oligosaccharides are attached at two specific sites (Robertson *et al.*, 1976). These sites are apparently strongly conserved, since tryptic glycopeptides were indistinguishable in wild-type VSV and in a mutant which had been chosen by repeated selection for thermolability and resistance to neutralizing (anti-G) antibody, and therefore presumably possessed multiple alterations in its G protein (Robertson and Summers, 1977).

The structure of the oligosaccharide side chain resembles the structures of influenza virus and togavirus glycoproteins in possessing a core structure of mannose and *N*-acetylglucosamine followed by branches containing additional residues of *N*-acetylglucosamine, galactose, and terminal fucose and neuraminic acid (Etchison *et al.*, 1977). The oligosaccharide is sulfated, but the attachment site of the sulfate is not clear (Pinter and Compans, 1975). The presence of neuraminic acid on the envelope of VSV has been demonstrated by treating the virus with colloidal iron hydroxide, which stains neuraminic acid residues (Klenk *et al.*, 1970*b*). The oligosaccharide side chains lack neuraminic acid when VSV is grown in mosquito cells, because these cells lack neuraminosyltransferase (Schloemer and Wagner, 1975*b*). Previous studies had also

shown that the size and composition of the carbohydrate moieties are variable, depending on the cell type in which the virions were grown (Burge and Huang, 1970; Etchison and Holland, 1974*a*). Likewise, the sequence of the carbohydrate within the glycosyl side chains may exhibit cell dependence (Moyer and Summers, 1974).

### 3.8.2b. The Lipid Bilayer

The lipids of VSV are composed of phospholipids and neutral lipids with high ratios of cholesterol to phospholipid and of sphingolipids to glycerophospholipids. The lipid composition of the virions reflects, but does not exactly mimic, the lipid composition of the host cell plasma membrane (McSharry and Wagner, 1971*a*). In virions grown in BHK 21-F cells, relatively large amounts of hematoside are observed, which is also the predominant glycolipid of the host cell (Klenk and Choppin, 1971). A similar lipid pattern has been found in rabies virus (Diringer *et al.*, 1973).

The lipids are present in the envelope as a bilayer. This has been demonstrated with VSV by electron spin resonance studies using spin labels (Landsberger and Compans, 1976) and by $^{13}C$ nuclear magnetic resonance (Stoffel and Bister, 1975; Stoffel *et al.*, 1976). Neuraminidase treatment of intact and spikeless particles of VSV grown in BHK cells converted gangliosides into neuraminic acid-free glycolipids, demonstrating that these were present exclusively on the outer surface of the viral bilayer (Stoffel *et al.*, 1975). Treatment of VSV grown in BHK cells with a nonpenetrating reagent showed that only 36% of the phosphatidylethanolamine was available for reaction and was therefore present on the outer surface (Fong *et al.*, 1976).

The envelope lipids of VSV are derived from the host and reflect compositional differences of the host. Such differences are paralleled by physical changes of the envelope (Landsberger and Compans, 1976). Envelope rigidity, however, is also dependent on the envelope proteins. Landsberger and Compans (1976) have observed that when the glycoproteins of VSV are removed by proteases the envelope becomes more fluid, indicating that the viral glycoproteins may contribute to the rigidity of the envelope. However, Landsberger and Compans postulated also that the major effect on bilayer fluidity is exerted by the M protein, since the fluidity of the lipid bilayer is altered only slightly when the G protein is removed by proteases, whereas vesicles prepared from extracted viral lipids were much more fluid than lipids in virions. Moore *et al.* (1976) and Barenholz *et al.* (1976) have shown that the

envelope of VSV has a higher microviscosity than the plasma membranes from which the virus budded. The increased viscosity was attributed in part to the insertion of the hydrophobic regions of the glycoproteins into the envelope bilayer. Stoffel and Bister (1975) using NMR spectra of $^{13}C$-labeled lipids also demonstrated that the envelope lipids of VSV are highly rigid, due either to lipid–lipid or to lipid–protein interactions. In general, it may be concluded therefore that the glycoprotein does interact with the lipid bilayer and may affect the rigidity of the membrane to some extent, but that the M protein may be of equal or greater importance in determining membrane rigidity.

### 3.8.2c. The M Protein

Only one type of M protein is found in VSV. Rabies virus has been reported to possess two different types of this protein (M1 and M2), but there is no evidence that M1 is really an envelope protein. The M protein is located on the inner side of the lipid bilayer as indicated by its resistance to protease treatment of the intact virus particle (Cartwright *et al.*, 1970; McSharry *et al.*, 1971; Schloemer and Wagner, 1974) and its inaccessibility to surface labeling agents (Eger *et al.*, 1975). The amino acid composition of the M protein of VSV has been analyzed, and no unusual distributions have been found (Moore *et al.*, 1974).

The M protein of VSV is antigenic (Cartwright *et al.*, 1970*b*) and monospecific antibodies against purified M protein clearly demonstrated that it is an antigen distinct from the G protein and the nucleocapsid protein (Dietzschold *et al.*, 1974). The anti-M serum does not neutralize the infectivity of virions but reacts specifically with the M protein in either Ouchterlony or complement fixation assays.

## 3.8.3. Biogenesis and Assembly of the Envelope

Maturation of the rhabdovirus envelope is a multistep process involving sequential incorporation of viral proteins into preformed cellular membranes. In general, the envelope appears to be assembled by mechanisms similar to those that have been observed with ortho- and paramyxoviruses (see Sections 3.5.6 and 3.6.3).

### 3.8.3a. Biosynthesis of the Glycoprotein

The G protein of VSV is synthesized on membrane-bound ribosomes (Grubman *et al.*, 1974; Morrison and Lodish, 1975; Wirth

*et al.*, 1977), and glycoproteins are always found associated with intracellular or plasma membranes but are never free in the cytoplasm (Wagner *et al.*, 1972*a*; Hunt and Summers, 1976). Pulse-chase experiments have shown that during and shortly after biosynthesis the glycoprotein is associated with the rough endoplasmic reticulum. At later times it is associated with a smooth cytoplasmic membrane fraction and finally, after about 20 min, it appears at the plasma membrane (David, 1973; Lafay, 1974; Hunt and Summers, 1976*a*; Knipe *et al.*, 1977*a*). With *ts* mutants of complementation group V of VSV the G protein is arrested at the nonpermissive temperature at the rough endoplasmic reticulum. Budding of virions occurs only when the block in migration to smooth membranes is released by a shift to the permissive temperature. Thus there is a correlation between the migration of the G protein and virus assembly (Lafay, 1974; Knipe *et al.*, 1977*b*).

Further insight into the relationship between polypeptide synthesis, insertion into membranes, and glycosylation of the G protein is obtained from studies analyzing the translation of this protein in a cell-free system. The protein which is synthesized in the presence of endoplasmic reticulum membranes appears to be about 2000 daltons larger than that prepared in the absence of membranes. This extra material probably represents carbohydrate as shown by its susceptibility to digestion by endoglycosidases, and by the fact that protein synthesized in the presence of membranes binds concanavalin A, while that prepared in the absence of membranes does not (Katz *et al.*, 1977; Toneguzzo and Ghosh, 1977, 1978). Further, the glycoprotein produced in the presence of membranes is inaccessible to proteolytic digestion, except for about 5% of the total protein, which has been found by peptide analysis to comprise the C terminus of the polypeptide chain (Katz *et al.*, 1977; Toneguzzo and Ghosh, 1978). Membranes must be added before peptide chain synthesis is about 15% complete in order for glycosylation and proper insertion to occur (Rothman and Lodish, 1977; Toneguzzo and Ghosh, 1978). This shows that initiation of polypeptide synthesis can precede membrane binding. These observations are in agreement with the "signal hypothesis" according to which polypeptide synthesis is initiated by the synthesis of a short amino acid sequence at the N terminus that is responsible for attachment of the protein to and transport through the membrane (Blobel and Dobberstein, 1975).

The available evidence suggests that in the *in vitro* system the carbohydrates are attached to the polypeptide as two distinct entities which correspond to the cores of the two oligosaccharide side chains of the molecule (see above) (Rothman and Lodish, 1977). The inability to detect intermediates containing a smaller number of sugar residues sug-

gests that each core is added in a single step presumably by transfer from lipid intermediates. Pulse-chase experiments in infected cells cannot distinguish between the time course of incorporation of amino acids and core sugars to the completed glycoprotein, indicating that the cores are added in the course of, or promptly on completion of, the polypeptide chain. Fucose, galactose, and neuraminic acid are added later during processing. The oligosaccharide is completed before the protein arrives at the cell surface (Knipe *et al.*, 1977*c*). Similar conclusions on the mechanisms involved in glycosylation have been drawn previously from studies with influenza virus (Compans, 1973*b*; Klenk *et al.*, 1974, 1978).

Glycosylation of the G protein of VSV can be inhibited by tunicamycin, also supporting the concept that lipid intermediates are involved in this reaction. Virus particles are not produced under these conditions, but the unglycosylated G protein is synthesized and appears to be stable in the cell (Leavitt *et al.*, 1977).

### 3.8.3b. Biosynthesis of the M Protein

The M protein is synthesized independently from the G protein and by different mechanisms. It is synthesized on soluble ribosomes, and is found largely in the cytoplasm without being strictly bound to cellular membranes. The M protein is rapidly incorporated into the plasma membrane, where it appears in pulse chase experiments already after 2 min (Knipe *et al.*, 1977*a*). The M protein is also more rapidly and efficiently incorporated into virions than the G protein (Lafay, 1974).

### 3.8.3c. Envelope Assembly

The available evidence presented above suggests that, on arrival of the G protein at the site of assembly, the M protein and subsequently the nucleocapsid associate with this area. Assembly occurs usually by budding from cellular membranes. The budding site of VSV is in most instances the plasma membrane, but occasionally maturation at intracellular membranes has been observed. The New Jersey strain of this virus was observed to bud primarily from plasma membranes of L or Vero cells, and almost entirely at intracytoplasmic membranes of pig kidney cells (Zee *et al.*, 1970). This report indicated that the site of maturation of a specific virus type may vary depending on the host cell.

Several sites of virus assembly have also been observed with rabies

virus. In cell cultures, virus budding regularly occurs at the plasma membrane and within the cytoplasm from preexisting membranes of the endoplasmic reticulum (Hummeler *et al.*, 1967; Matsumoto and Kawai, 1969). Virus assembly at *de novo*-synthesized membranes in or near the matrix as described by Hummeler *et al.* (1967) seems to be a rarer event which possibly is virus strain dependent (Schneider and Dir-inger, 1976). Budding from the Golgi complex has been observed in cultivated mammalian neurons (Matsumoto *et al.*, 1974).

### 3.8.4. Biological Function of the Envelope Glycoprotein

It is reasonable to assume that rhabdovirus glycoproteins are involved in the initiation of infection, like the glycoproteins of other enveloped viruses such as myxoviruses. Thus spikeless VSV particles which have been produced by treatment with proteolytic enzymes were shown to be non infectious. Infectivity was restored, however, when these particles were reconstituted with purified G protein isolated from the same virus strain or from a different serotype. Antibody directed against the homologous G protein used for reconstitution effectively neutralized the virus, but antibody directed against the serotype of the spikeless particle was ineffective in neutralization if glycoproteins from a different serotype were used for reconstitution (Bishop *et al.*, 1975).

The G protein may be involved in the adsorption process. This is suggested by the observation that VSV (Arstila *et al.*, 1969; Arstila, 1972) as well as rabies virus (Halonen *et al.*, 1968) are able to agglutinate goose erythrocytes and that, at least in the case of VSV, the G protein has been identified as the hemagglutinin (Arstila, 1973).

Little is known about how rhabdoviruses penetrate the plasma membrane. Evidence has been presented that VSV may penetrate by a fusion process (Heine and Schneitman, 1971), and, in appropriate cells, this virus may induce cell fusion (Takehara, 1975). However, it is not clear whether the G protein plays a direct role in this process.

Antibody against the G protein will neutralize the infectivity of VSV, and vaccination of mice with purified G protein effectively protects them against challenge with infective virions (Dietzschold *et al.*, 1974). The antibody to G protein of VSV is type specific and forms the basis for division of this virus into serotypes (Kang and Prevec, 1969). The G protein of rabies virus is also responsible for induction of virus neutralizing antibodies (Schneider *et al.*, 1973; Wiktor *et al.*, 1973) and for conferring immunity to animals against a lethal challenge infection (Atanasiu *et al.*, 1974).

## 3.9. Retroviruses: C-Type Particles

The retrovirus group includes viruses found in various avian and mammalian species, with the murine and avian C-type viruses being the best-studied members. The viruses have a distinctive appearance and are unique among RNA viruses in replicating through a DNA intermediate. Although most of the viruses are oncogenic, certain nononcogenic viruses such as visna and progressive pneumonia viruses of sheep also possess the morphological and biochemical characteristics of the group. A number of reviews are available which provide more details of the structure and replication process of these viruses (Bolognesi, 1974; Bauer, 1974; Bader, 1974; Shapiro and August, 1976; Vogt, 1977; Eisenman and Vogt, 1978).

### 3.9.1. Envelope Components

#### 3.9.1a. Surface Proteins and Glycoproteins

One or two glycoproteins have been recognized as virus-specific components on the external surface of the viral envelope. Avian RNA tumor viruses, including Rous sarcoma virus (RSV) and avian myeloblastosis virus (AMV), possess two glycoproteins with apparent molecular weights of 85,000 and 37,000, designated "gp85" and "gp37," respectively (Hung *et al.*, 1971; Rifkin and Compans, 1971; Bolognesi *et al.*, 1972). Murine leukemia viruses (MuLVs), including Rauscher, Moloney, and Friend leukemia viruses, possess a single major glycoprotein of molecular weight ~70,000, designated "gp70" (Moroni, 1972; August *et al.*, 1974). In many preparations, two closely adjacent bands designated "gp69" and "gp71" have been observed (Strand and August, 1973), whereas in other systems only a single component has been identified. However, the two MuLV glycoprotein bands cannot be distinguished by peptide mapping (Elder *et al.*, 1977), and it is therefore likely that a single functional type of glycoprotein is present, with possible minor differences in size or charge due to heterogeneity upon secondary modifications. Minor glycoproteins of molecular weight 45,000–52,000 have also been identified in murine leukemia virus preparations (Moroni, 1972; Moenning *et al.*, 1974; Pinter and Compans, 1975; Marquardt *et al.*, 1977), and their possible origin is discussed below.

Both glycoproteins of avian RNA tumor viruses, as well as gp70 of murine leukemia viruses, are located on the external surface of the viral

envelope. This has been demonstrated by proteolytic digestion experiments, in which the glycoproteins were selectively removed, leaving smooth-surfaced particles which still contained the internal carbohydrate-free viral proteins (Rifkin and Compans, 1971; Witter *et al.*, 1973). In addition, surface labeling procedures resulted in selective labeling of the glycoproteins (Robinson *et al.*, 1971; Witte *et al.*, 1973; McLellan and August, 1976). The glycoproteins can be isolated in the form of rosettelike aggregates after detergent treatment, which morphologically resemble aggregates of the projections on the viral envelope (Bolognesi *et al.*, 1972). Further, the fact that these isolated glycoproteins possess the type-specific antigens of the virus which react with neutralizing antibody (Duesberg *et al.*, 1970; Tozawa *et al.*, 1970; Bolognesi *et al.*, 1972) indicates their location on the surface of the virion.

Evidence has been obtained that the two glycoproteins of avian RNA tumor viruses, gp85 and gp37, are distinct gene products (Mosser *et al.*, 1977) linked by disulfide bonds (Leamnson and Halpern, 1976). The resulting complex has an electrophoretic mobility slightly less than that of gp85, and it appears to consist of one molecule each of gp85 and gp37. As discussed below, these two glycoproteins also appear to be synthesized as a single large precursor molecule (England *et al.*, 1977). Recently, an additional carbohydrate-free polypeptide has been resolved in murine leukemia virus preparations, which also appears to be located on the external surface of the viral envelope and is designated p15(E) (Ikeda *et al.*, 1975; Ihle *et al.*, 1976; Famulari *et al.*, 1976; Van Zaane *et al.*, 1976). This protein tends to aggregate in the absence of detergents and is found in the void volume using gel filtration in guanidine hydrochloride. It can be resolved from an internal virion polypeptide of similar electrophoretic mobility by high pH discontinuous SDS gel electrophoresis (Famulari *et al.*, 1976). Recent studies with Moloney murine leukemia virus (Leamnson *et al.*, 1977) indicate that gp70 and p15(E) are also linked by disulfide bonds. While it is uncertain whether this is the case in all murine leukemia viruses, it is likely that p15(E) is associated with gp70, and it may play a role in binding of the spike structure to the viral envelope. The association of gp70 and p15(E) is also suggested by recent observations that they are synthesized in the form of a common precursor polypeptide (Famulari *et al.*, 1976; Witte and Weismann, 1976; Van Zaane *et al.*, 1976).

The exact size of the spike structure of RNA tumor viruses and the number of polypeptide chains which compose the spikes have not been determined. Bolognesi *et al.* (1972) have suggested that gp37 of AMV forms the spike and gp85 is located as peripheral knob in the spike

structure. Sedimentation of RSV glycoproteins in sucrose gradients in the presence of Tween 20 resolved peaks at 9 S and 12 S, and it was suggested that these consisted of two or three disulfide-linked complexes of gp85 plus gp37 (Leamnson and Halpern, 1976). However, it is uncertain whether either of these corresponds to the native spike structure. The major glycoproteins of Friend leukemia virus, after release from virions by osmotic shock, sedimented as a single peak at 4.05 S, corresponding to a molecular weight of 58,000 (Moennig *et al.*, 1974). Thus, under these conditions, MuLV glycoproteins appear to be released from virions as individual glycoprotein molecules.

It is apparent that the glycoproteins of RNA tumor viruses can be dissociated easily from the viral envelope by a variety of conditions, which are not generally feasible for isolation of glycoproteins of other virus groups. This is particularly true for murine leukemia viruses, in which release of glycoproteins has been demonstrated using osmotic shock, freeze-thawing, ultrasonication, and the use of chaotropic agents (Moenning *et al.*, 1974; Strand and August, 1976; Marquardt *et al.*, 1977). The glycoprotein (gp70) preparations obtained appeared free of detectable levels of p15(E), suggesting that no covalent linkage existed between these envelope proteins. These observations indicate that the nature of the interactions which bind RNA tumor virus glycoproteins to the viral membrane may differ from those observed with other enveloped viruses. There may be a weak interaction between gp70 and p15(E), so that gp70 is readily dissociated from the envelope whereas the interaction of p15(E) with the bilayer may resemble that observed with hydrophobic segments of other viral glycoproteins.

As discussed above, a minor glycoprotein of 45,000–52,000 daltons has been observed in many murine leukemia virus preparations. Several possibilities have been suggested for the origin of this component. Marquardt *et al.* (1977) have isolated both gp70 and gp45 from Rauscher leukemia virus and compared their carbohydrate content. Their data suggest that gp70 contains about 32% of carbohydrate by weight and that gp45 contains 6–7%; both glycoproteins appeared to have polypeptide chains of about 45,000 daltons. On the other hand, it has recently been observed that brief trypsin treatment results in conversion of gp70 to a glycoprotein of 45,000–52,000 daltons (Krantz *et al.*, 1977; Kemp *et al.*, 1978), suggesting that the minor glycoprotein is a proteolytic cleavage product of gp70. It has also been suggested that the minor glycoprotein may represent host-cell-coded H-2 antigen (Bubbers and Lilly, 1977). Whether a single mechanism will account for the results obtained in various virus–cell systems remains to be established. In the case of avian C-type viruses, tryptic peptide analysis

has demonstrated that gp37 is not a cleavage product of gp85 (Mosser *et al.*, 1977), whereas similar experiments with murine viruses show that gp45 and gp70 have common peptide species (Elder *et al.*, 1977). Thus it appears that there is no analogy between the minor glycoproteins of murine and avian viruses.

The glycoproteins of avian C-type viruses define the viral host range as well as the classification into subgroups based on interference and neutralization properties (Vogt and Ishizaki, 1966; Ishizaki and Vogt, 1966; Tozawa *et al.*, 1970). The major glycoprotein of murine C-type viruses also is essential for binding to cellular receptors (DeLarco and Todaro, 1976). Proteolytic digestion of the surface glycoproteins inactivates the infectivity of both avian and murine C-type viruses (Rifkin and Compans, 1971; Witter *et al.*, 1973). A hemagglutination assay for mouse leukemia viruses requires the presence of intact glycoproteins on the viral surface (Witter *et al.*, 1973).

Comparisons of tryptic peptides of gp70 molecules isolated from a large number of murine C-type viruses reveal extensive strain differences (Kennel, 1976; Elder *et al.*, 1977). Further, gp70 molecules isolated from different tissues of the same mouse strains also showed differences, suggesting that modifications of a prototype gene may occur during differentiation. On the other hand, murine C-type viruses with similar host range also showed similarities in gp70 primary structure.

### 3.9.1b. Lipid Bilayer

The lipids of RNA tumor viruses are arranged in a bilayer structure, as shown by electron spin resonance spectra of spin-labeled fatty acid derivatives incorporated into Rauscher leukemia virus (Landsberger *et al.*, 1972). Lipids were shown to compose about 20% of the mass of Rous sarcoma virions (Quigley *et al.*, 1971). The lipid composition of the virus was shown to be similar to that of the cellular plasma membrane, and enveloped viruses of several major groups were all found to have very similar lipids when grown in the same host cell (Rao *et al.*, 1966; Quigley *et al.*, 1971). However, the content of phosphatidylcholine in the virion was lower, and that of sphingomyelin and phosphatidylethanolamine was higher, than in the plasma membrane, and the ratio of cholesterol to phospholipid was also significantly higher in the virion than in the plasma membrane (Quigley *et al.*, 1972). It was suggested that these compositional differences might result from preferential budding of virus particles at sites which differ in composition from the average composition of the overall isolated membrane.

### 3.9.1c. Internal Membrane Proteins

At least four or five carbohydrate-free polypeptides are present as internal components of C-type viruses, and it is uncertain whether one or more of these is closely associated with the viral envelope. The core structure appears to be an icosahedral capsid (Nermut *et al.*, 1972) which contains the viral RNA as well as two major proteins (Bolognesi *et al.*, 1973). One or more additional internal proteins (10,000 and 19,000 daltons in the case of avian and 12,000 daltons in murine viruses) are not found in isolated core structures, and it has been suggested that these polypeptides are associated with the envelope or located between the core and the viral membrane (Bolognesi *et al.*, 1973; Stromberg *et al.*, 1974). Both the p10 polypeptide of AMV and p12 of MuLV are selectively labeled with dansyl chloride, which suggests that they are exposed on or near the surface of the virion (Bolognesi *et al.*, 1973). Further information is needed to determine the exact location of the various internal polypeptide components of C-type viruses.

### 3.9.1d. Carbohydrate Components

The oligosaccharides of glycoproteins of various RSV strains have been characterized by gel filtration after extensive pronase digestion (Lai and Duesberg, 1972). A single broad peak, with an estimated molecular weight of 3900–5100, was observed. The glycopeptides in viruses obtained from transformed cells were consistently larger than those obtained from nontransformed cells; such differences were observed in viruses of various RSV subgroups. The structure of the oligosaccharide groups has been partially characterized, and they have been shown to contain glucosamine, mannose, galactose, and sialic acid (Krantz *et al.*, 1976).

Host cell derived mucopolysaccharide components also appear to be present in avian and murine RNA tumor viruses (Lai and Duesberg, 1972; Pinter and Compans, 1975). The polysaccharide components as well as the major and minor glycoproteins of murine C-type viruses are sulfated; the ratio of sulfate to glucosamine appears to be 3 times higher in the minor glycoprotein (gp52) than in gp70 (Pinter and Compans, 1975). The possible significance of this glycoprotein sulfation, as well as the presence of host-cell-derived mucopolysaccharides in virions, remains to be investigated.

Evidence has been presented which indicates that the carbohydrate

components are not required for several biological activities of RNA tumor virus glycoproteins, including their antigenic determinants, interference properties, or ability to adsorb neutralizing antibodies, as shown by retention of these properties after removal of about 70% of the carbohydrate with a mixture of glycosidases (Bolognesi *et al.*, 1975; Schäfer *et al.*, 1977). However, indirect hemagglutination activity by the glycoprotein was inactivated by the glycosidase treatment.

### 3.9.2. Synthesis and Assembly of Envelope Components

#### 3.9.2a. Identification of Viral Proteins in Infected Cells

Unlike many cytocidal viruses, productive infection by RNA tumor viruses occurs without marked effects on cellular biosynthesis, and virus-specific proteins compose a minor fraction of the proteins being synthesized in infected cells. Immunoprecipitation and gel electrophoresis have therefore been employed to detect newly synthesized radiolabeled viral proteins. In initial studies, it was demonstrated that the internal virion proteins are synthesized as a single precursor molecule which is subsequently cleaved to yield the virion structural proteins (Vogt and Eisenmann, 1973; Vogt *et al.*, 1975; Naso *et al.*, 1975; Van Zaane *et al.*, 1975). More recently, it has been demonstrated that the envelope proteins are also synthesized in the form of a precursor molecule. In the murine leukemia viruses, the major external proteins gp70 and p15(E) are found as a precursor of about 85,000 daltons (Famulari *et al.*, 1976; Naso *et al.*, 1976; Witte *et al.*, 1977). Antiserum to either gp70 or p15(E) could precipitate the precursor protein, and it was converted to the two virion proteins in pulse chase experiments. In cells infected with avian C-type particles, a 90,000-dalton precursor has been identified using antiserum to the virion glycoprotein gp85 (England *et al.*, 1977). In pulse-chase experiments, this precursor appears to be cleaved to yield both gp85 and gp37. It has also been reported that a 70,000-dalton precursor, thought to represent incompletely glycosylated gp85, could be identified in infected cells (Halpern *et al.*, 1974; Moelling and Hayami, 1977) but this species was not observed in other studies (England *et al.*, 1977).

Some information on the intracellular location of MuLV glycoproteins and their precursors has been obtained by Witte *et al.* (1977). The precursor glycoprotein was predominant in a high-density cytoplasmic membrane fraction, whereas fractions of lower density contained increasing amounts of gp70 and a component believed to be p15(E).

None of the precursor molecules appeared accessible to reagents which label the external surface of the cell, indicating that cleavage to yield gp70 occurred prior to or simultaneously with the appearance of the glycoproteins on the cell surface. The precursor glycoproteins also appear to lack an appreciable amount of sialic acid, which is added prior to arrival of glycoprotein on the cell surface. Studies of the kinetics of appearance of newly synthesized viral proteins into mature virions (Fleissner *et al.*, 1975; Witte and Weissman, 1976) have indicated that core proteins are incorporated rapidly into virions, whereas there is a significant delay before envelope glycoproteins are incorporated. This may reflect intracellular events involved in secondary modification and transport of the glycoproteins to the cell surface. Further, the results indicate that a large intracellular pool of glycoproteins is available for continued incorporation into virions over a long time period.

### 3.9.2b. Assembly and Maturation of Virions

Formation of progeny virions occurs by budding at the cell membrane. In contrast to most other virus groups, preformed nucleocapsid or core structures are not generally observed in the cytoplasm; rather, formation of the nucleocapsid occurs by a process of progressive enlargement as the budding particle emerges. Crescent-shaped core structures are observed during the process of budding. No information is available on the precise molecular interactions which occur during budding. A structural alteration in C-type viruses also occurs after their release from the infected cell, which involves condensation of the core structure and has been termed the "maturation process." Comparison of the RNA and polypeptide profiles of rapidly harvested virions with those obtained after longer intervals indicates differences in both the RNA and proteins (Canaani *et al.*, 1973; Cheung *et al.*, 1972). The mechanism and significance of this phenomenon is not understood; however, newly formed virions possess higher infectivity (Smith, 1974), indicating that the "maturation" process may not be beneficial for the virions.

### 3.9.2c. Assembly of Spikeless Particles

Both morphological and biochemical evidence has been obtained which demonstrates that spikeless particles lacking viral glycoproteins

can be produced by cells infected with avian C-type virus mutants deficient in the gene coding for envelope protein (Scheele and Hanafusa, 1971; DeGiuli *et al.*, 1975; Halpern *et al.*, 1976). These observations indicate that glycoproteins are not required for assembly and release of progeny virus particles. It is, however, uncertain whether some portion of the spike is present in these particles. The particles thus obtained are noninfectious (Weiss, 1969; Hanafusa *et al.*, 1976) and appear similar to particles from which glycoproteins have been removed by proteolytic digestion. Formation of particles lacking glycoproteins has not been generally observed with other groups of enveloped viruses.

### 3.9.2d. Effects of Inhibitors

The effects of several inhibitors of glycosylation on assembly and release of RNA tumor viruses have been described. Three inhibitors, glucosamine, 2-deoxy-D-glucose, and tunicamycin, have been employed; based on results with other viruses it is likely that only the last is completely effective in preventing glycosylation of virion glycoproteins. Glucosamine was observed to inhibit the release of infectious Rous sarcoma virus as well as physical particles as measured by [$^3$H]uridine incorporation (Hunter *et al.*, 1974). The effects of 2-deoxyglucose on Kirsten murine leukemia sarcoma virus also included inhibition of the formation of infectious progeny virions; however, release of physical particles did not appear to be impaired (Prochownik *et al.*, 1975). The protein components of such particles appeared to be markedly different from those of normal virions. Tunicamycin did not prevent the release of physical particles of Rous sarcoma virus and the resulting particles appeared to completely lack glycoproteins (Schwarz *et al.*, 1976), thus resembling the noninfectious spikeless particles produced by defective deletion mutants of RSV. These results support earlier observations indicating that glycoproteins are not required for the assembly process of C-type virus particles.

### 3.9.2e. Aberrant Particles Produced by Virus Mutants

A temperature-sensitive mutant of RSV designated LA334 possesses a defect in replication which leads to the production of aberrant virus particles (Hunter and Vogt, 1976; Hunter *et al.*, 1976; Friis *et al.*, 1976; Rohrschneider *et al.*, 1976). The particles bud atypically and

are more heterogeneous in size and density than wild-type virions. Evidence has been obtained that the defect in this mutant leads to aberrant cleavage of the precursor to the internal proteins of the virion (Hunter *et al.*, 1976; Rohrschneider *et al.*, 1976). However, immunoferritin staining of viral glycoproteins was also markedly reduced on the external surface of budding particles, suggesting that the alteration in internal proteins may affect the incorporation of glycoproteins into the viral membrane.

A number of physiologically distinct temperature-sensitive mutants of MuLV have been examined by electron microscopy (Yeger *et al.*, 1976), and the results indicate that the maturation process can be blocked at several discrete stages. These include mutants blocked at the stage of formation of a slight outfolding of the plasma membrane, with an underlying crescent of core material, and other mutants blocked at a point near the termination of the budding process in which an almost completely formed core can be seen. Other mutants produce complete virus particles of aberrant morphology. The precise definition of the biochemical lesions in these mutants should provide new insight into the events in C-type virus assembly.

## 3.10. Togaviruses

### 3.10.1. Classification, Morphology, Composition

The togaviridae family comprises four genera: alphavirus (previously known as arboviruses of group A), flavivirus (previously known as arboviruses of group B), rubivirus, and pestivirus. All alphaviruses and probably all flaviviruses multiply in arthropods as well as vertebrates.

Virions are spherical, with a diameter of 40–70 nm. They contain an icosahedral nucleocapsid that is surrounded by an envelope with spikes about 7 nm in length. Alphaviruses are composed of about 6% RNA, 60% protein, 6% carbohydrate, and 30% lipid (Pfefferkorn and Hunter, 1963*a*; Strauss *et al.*, 1970; Laine *et al.*, 1973).

Of the many members of the togavirus family, Semliki forest virus (SFV) and Sindbis virus have been studied in most detail as far as structure and replication are concerned. We will therefore concentrate here on these two viruses which are both alphaviruses. In addition, some data will be reported on flaviviruses.

### 3.10.2. Fine Structure and Arrangement of Envelope Components

The envelope of alphaviruses consists of a lipid layer studded with spikes that are composed of glycoproteins. There is a high degree of organization as shown by surface analysis of negatively stained Sindbis virus. Evidence has been obtained that the glycoproteins are organized as 240 trimers clustering in a $T = 4$ icosahedral surface lattice (von Bonsdorff and Harrison, 1975). Alphaviruses do not possess a carbohydrate-free membrane (M) protein at the inner side of the lipid bilayer as do other enveloped viruses, such as rhabdoviruses and myxoviruses. It is reasonable to assume that, with the alphaviruses, the function of the M protein is exerted by the core protein. Flavivirus, however, appears to contain a nonglycosylated envelope protein (MW ~7000–9000) that might be comparable to the M protein (Table 4).

The togavirus envelope can be disintegrated by detergents, and the action of Triton X100, sodium dodecylsulfate, and sodium deoxycholate on SFV has been studied extensively. The solubilization process begins with binding of detergents to the virus, then it proceeds with increasing detergent concentration to lysis of the membrane, solubilization into lipid–protein–detergent complexes, and finally complete delipidization of the proteins (Helenius and Söderlund, 1973; Becker *et al.*, 1975; Helenius *et al.*, 1976). Thus the envelope proteins of SFV and Sindbis virus have been purified by chromatography on hydroxylapatite in the presence of SDS (Garoff *et al.*, 1974) or on

**TABLE 4**

**Envelope Proteins of Togaviruses**

| Alphaviruses | | Flaviviruses | |
|---|---|---|---|
| Designation[a] | Molecular weight[b] | Designation[c] | Molecular weight[d] |
| E1 | ~50,000 | | |
| E2 | ~50,000 | E | 53,000–58,000 |
| E3[e] | 10,000 | | |
| | — | M | 7000 |

[a] Baltimore *et al.* (1976).
[b] Schlesinger *et al.* (1972), Garoff *et al.* (1974), Ivanic (1974), Pedersen *et al.* (1974).
[c] Trent (1977).
[d] Shapiro *et al.* (1971), Stollar (1969), Trent and Qureshi (1971), Westaway (1975), Westaway and Reedman (1969).
[e] Occurs only in SFV.

DEAE-cellulose in the presence of Triton X100 (Burke and Keegstra, 1976). Other isolation procedures employed preparative isoelectric focusing in the presence of Triton X100 (Dalrymple *et al.*, 1976) and sucrose gradient centrifugation in the presence of sodium deoxycholate (Helenius *et al.*, 1976).

### 3.10.2a. The Spikes

The spikes of togaviruses are composed of glycoproteins. Whereas flaviviruses contain only one glycoprotein, three species (E1, E2, E3) have been observed in alphaviruses (Table 4). E1 and E2, which both have molecular weights around 50,000, appear to be regularly present in the virion and have been observed with Eastern equine encephalitis, Semliki forest, Sindbis, Venezuelan equine encephalitis, and Western equine encephalitis viruses (Schlesinger *et al.*, 1972; Garoff *et al.*, 1974; Ivanic, 1974; Pedersen *et al.*, 1974). The smallest glycoprotein, E3 (MW ~10,000), which is highly glycosylated, has been observed only in SFV, not in other alphaviruses. Using cross-linking agents and immunoprecipitation with specific antisera, evidence has been obtained that the spike structure of SFV is a trimer containing one each of E1, E2, and E3 (Garoff, 1974).

That the glycoproteins form the spikes at the virus surface can be demonstrated by surface labeling techniques (Gahmberg *et al.*, 1972*a*; Sefton *et al.*, 1973), by treatment with proteolytic enzymes, which results in the formation of spikeless particles from which the glycoproteins have been removed (Calberg-Bacq and Osterrieth, 1966; Compans, 1971), and by the agglutinability of the virus with concanavalin A (Oram *et al.*, 1971; Birdwell and Strauss, 1973).

The envelope glycoproteins are amphipathic molecules. Whereas amino acid analysis of the whole glycoprotein does not show a significantly higher proportion of hydrophobic amino acids (Garoff *et al.*, 1974; Burke and Keegstra, 1976), evidence has been obtained for small hydrophobic regions. After proteolytic treatment of virions, these regions remain as fragments of the glycoproteins in the bilayer (Gahmberg *et al.*, 1972*b*). Both E1 and E2 contain such peptides, which are rich in hydrophobic amino acids (Utermann and Simons, 1974). Through these hydrophobic segments the isolated membrane glycoproteins are able to aggregate into star-shaped oligomers or rosettes (Mussgay and Rott, 1964; Simons *et al.*, 1973; Helenius and von Bonsdorff, 1976) and to bind detergents such as Triton X100 and

deoxycholate (Helenius and Simons, 1972, 1975; Utermann and Simons, 1974; Becker *et al.*, 1975).

Through the hydrophobic segments, the spikes are anchored in the lipid bilayer. The fact that the SFV glycoproteins can be cross-linked to the nucleocapsid by dimethyl suberimidate suggests that at least one of the glycoproteins spans the bilayer (Garoff and Simons, 1974). Treatment of Sindbis virus with formaldehyde results in cross-linking of envelope proteins to the nucleocapsid, suggesting that with this virus the glycoproteins span the lipid layer, too (Brown *et al.*, 1974).

The carbohydrate moieties of the SFV and Sindbis virus glycoproteins have been isolated after pronase digestion and their sugar composition has been determined (Burge and Huang, 1970; Burge and Strauss, 1970; Strauss *et al.*, 1970; Sefton and Keegstra, 1974; Keegstra *et al.*, 1975; Burke and Keegstra, 1976; Mattila *et al.*, 1976). Side chains of type A (nomenclature of Johnson and Clamp, 1971), containing glucosamine, mannose, galactose, fucose and neuraminic acid, and side chains of type B, containing only glucosamine and mannose, have been found. E1 and E2 of Sindbis virus contain one A-type and one B-type side chain while SFV contains one or two A-type side chains in E1 and E3, and one A- and two or three B-type chains in E2.

Sequential degradation of the oligosaccharide side chains with exo- and endoglycosidases has been carried out, and the structures for the A chains of SFV which appear to be heterogenous have been deduced (Pesonen and Renkonen, 1976; Renkonen *et al.*, 1976). The largest A chains are present on E3 and appear to be to be the most exposed ones on the intact virus, because they react most readily with neuraminidase and galactose oxidase (Luukkonen *et al.*, 1977*a*). With Sindbis virus, the A-type oligosaccharide of E2 appears to be the most exposed one, because it is the only side chain which can be removed by glycosidase treatment of intact virions (McCarthy and Harrison, 1977).

Virions which lack neuraminic acid in their envelope proteins, either after enzymatic removal or when grown in mosquito cells, retain their infectivity and hemagglutinating activity (Kennedy, 1974; Stollar *et al.*, 1976). Even the subterminal glucosamine and galactose residues may be lacking without decreased biological activity, suggesting that the glucosamine–mannose core of the A-type oligosaccharide is sufficient for these activities (Schlesinger *et al.*, 1976).

The available evidence indicates that the primary polypeptide structure of the envelope proteins determines the number and type of the oligosaccharide chains, which are added, however, by cellular enzymes (Burge and Huang, 1970; Grimes and Bruge, 1971; Sefton,

1976). Thus there are host-dependent variations in the oligosaccharides (Keegstra *et al.*, 1975; Schlesinger *et al.*, 1976; Stollar *et al.*, 1976).

### 3.10.2b. The Lipid Bilayer

Thirty-seven percent of the alphavirus envelope consists of lipids (Laine *et al.*, 1973). The main components are phospholipid and cholesterol. The lipids are derived from the host cell (Pfefferkorn and Hunter, 1963*b*), and the composition resembles that of the plasma membrane of the host (Renkonen *et al.*, 1971, 1972*a*,*b*). This is true also for the fatty acid composition within the different phospholipid classes (Laine *et al.*, 1972) and for the glycolipids (Renkonen *et al.*, 1971; Hirshberg and Robbins, 1974). The host-dependent lipid variation is particularly striking when virions grown in mammalian and mosquito cells are compared. There was nearly 50% change in phospholipid head group composition, and an even larger overall change if the nature of the linkage between the hydrocarbon chain and the glycerol moieties was also taken into account (Luukkonen *et al.*, 1976). Further, cholesterol is present in 5–6 times higher amounts in SFV from BHK than from mosquito cells, although the ratio of phospholipid to protein is similar in particles from both sources (Luukkonen *et al.*, 1977*b*). The stability of the virion may depend to some extent on the lipid composition (Sly *et al.*, 1976).

The lipids in the alphavirus envelope form a bilayer as demonstrated by X-ray diffraction studies on Sindbis virus (Harrison *et al.*, 1971). Electron spin resonance studies also indicated a bilayer structure (Sefton and Gaffney, 1974). The bilayer appears to be asymmetrical. Treatment of SFV from mosquito cells with a nonpenetrating reagent suggested that the outer layer possessed relatively more ceramide phosphoethanolamine and less phosphatidylethanolamine than the inner layer (Luukkonen *et al.*, 1976). Furthermore, neuraminidase treatment of intact and spikeless particles of Sindbis virus completely removed neuraminic acid from gangliosides, demonstrating that these were present exclusively on the outer surface of the viral bilayer (Stoffel and Sorgo, 1976).

Less than 10% of the bilayer is occupied by the penetrating segment of the envelope glycoproteins (Harrison *et al.*, 1971). Nevertheless, the envelope proteins appear to stabilize the organization of the lipids in the bilayer, since the microviscosity is higher in the presence of the envelope proteins or hydrophobic fragments than in liposomes

made of viral lipids or in cell membranes (Sefton and Gaffney, 1974; Hughes and Pedersen, 1975; Moore *et al.*, 1976).

### 3.10.3. Biogenesis and Assembly of the Envelope

The structural proteins of alphavirus are translated from 26 S mRNA (Cancedda *et al.*, 1974*b*; Clegg and Kennedy, 1974*b*, 1975*a*,*b*,*c*; Simmons and Strauss, 1974*b*; Wengler *et al.*, 1974; Glanville *et al.*, 1976*a*). The 26 S RNA polysomes are membrane bound (Kennedy, 1972; Wirth *et al.*, 1977), as is the synthesis of structural proteins (Friedman, 1968*a*). The structural proteins appear to be translated as a polyprotein with a molecular weight of 130,000–140,000 (Schlesinger and Schlesinger, 1973; Keränen and Kääriäinen, 1975; Lachmi *et al.*, 1975; Kaluza, 1976; Kaluza *et al.*, 1976). Evidence has been obtained with SFV by sequential labeling that the core protein is translated first followed by the envelope proteins in the order E3, E2, and E1 (Clegg, 1975; Clegg and Kennedy, 1975*b*,*c*; Lachmi and Kääriäinen, 1976).

The capsid protein is cleaved from the nascent polyprotein *in vivo* (Burke, 1975; Strauss and Strauss, 1976) and *in vitro* (Cancedda *et al.*, 1974*a*,*b*; Clegg and Kennedy, 1974*b*; Wengler *et al.*, 1974; Glanville and Ulmanen, 1976), probably immediately after the ribosome has finished the translation of the sequences coding for capsid protein (Clegg, 1975; Söderlund, 1976). The cleavage enzyme might be virus specific, as suggested by mixed infection experiments with Sindbis *ts* mutants (Scupman *et al.*, 1977). The cleavage between the envelope proteins takes place at a time when most of the polyprotein has been translated (Söderlund, 1976). The products of this cleavage are the envelope protein E1 and a common precursor to E2 and E3 (MW 62,000–68,000) designated "p62," "NSP68," "NVP68," or "PE2" (Schlesinger and Schlesinger, 1973; Simons *et al.*, 1973*b*; Garoff *et al.*, 1974; Lachmi *et al.*, 1975).

The envelope proteins are transferred from the endoplasmic reticulum to the plasma membrane and finally into released virus (Richardson and Vance, 1976). In the plasma membrane the envelope proteins of Sindbis virus can be demonstrated as early as 2 hr after infection (Birdwell and Strauss, 1974). Studies with *ts* mutants of Sindbis virus suggest that both E1 and PE2 are incorporated into the plasma membrane, where subsequently cleavage of PE2 takes place. In wild-type virus, cleavage of PE2 can be prevented by antiserum against E1, suggesting that both glycoproteins are closely associated with each

other at the outside of the plasma membrane (Bracha and Schlesinger, 1976*b*; Smith and Brown, 1977).

Valuable information on glycosylation mechanisms has been obtained from studies in which this reaction has been blocked. In the presence of specific inhibitors such as 2-deoxy-D-glucose (Kaluza *et al.*, 1973; Scholtissek *et al.*, 1975; Schmidt *et al.*, 1976), D-glucosamine (Duda and Schlesinger, 1975), 2-fluorohexoses (Schmidt *et al.*, 1976), and tunicamycin (Schwarz *et al.*, 1976; Leavitt *et al.*, 1977; Ogura *et al.*, 1977) and in glucose-free medium (Kaluza, 1975), the envelope glycoproteins and their immediate precursors are synthesized in an unglycosylated form. Studies on Sindbis virus have shown that, after release of the block, carbohydrate is not attached to these unglycosylated polypeptides. Furthermore, under normal conditions unglycosylated Sindbis virus glycoproteins are not found in the infected cell. From these and other observations it has been concluded that glycosylation is initiated on the nascent polypeptide chain before release from the polysomes (Sefton, 1977). However, glycosylation appears to take place after cleavage of E1 and PE2, since the common precursor of both proteins (MW ~100,000) (Strauss *et al.*, 1969; Schlesinger and Schlesinger, 1973) has been reported to be carbohydrate free (Sefton and Burge, 1973). This first step of glycosylation probably involves the transfer of the cores of the oligosaccharides consisting of glucosamine and mannose (MW ~1800) (Sefton, 1977) from a lipid intermediate to the polypeptide as suggested by glycosylation studies in a cell-free system (Krag and Robbins, 1977). Similar results have also been obtained by *in vitro* glycosylation of SFV proteins (Schwarz *et al.*, 1978*b*).

The addition of the distal sugars such as glucosamine, galactose, and neuraminic acid takes place later after about 20 min (Sefton, 1977). At this stage part of the the mannose residues are probably removed from the "core" before addition of the distal sugars (Sefton and Burge, 1973).

The unglycosylated form of the precursor p62 is metabolically stable. A possible explanation for this observation is that cleavage requires proper glycosylation (Kaluza, 1975, 1976). Antibodies specified against the envelope glycoproteins react readily with the glycosylated precursor p62, but only poorly with its unglycosylated form. Thus glycosylation appears to be necessary for the proper antigenic configuration of the glycoprotein (Kaluza, 1976).

Little is known about the biosynthesis of the envelope proteins of flaviviruses. Tentative evidence for posttranslational processing has been presented in the case of the glycoprotein of Kunjin virus, but the nature of this processing is unclear (Westaway and Shaw, 1977).

### 3.10.4. Morphogenesis of the Envelope

In mammalian cells alphaviruses mature usually by budding from the plasma membrane (Acheson and Tamm, 1967; Erlandson *et al.*, 1967; Bykovsky *et al.*, 1969; Waite *et al.*, 1972). Prior to budding the nucleocapsid aligns under the plasma membrane. It has been proposed that because of transmembrane interaction between the glycoproteins and the core protein the spikes are arrested in patches, whereas host proteins are excluded from these areas by lateral movement, and that this mechanism initiates budding (Garoff and Simons, 1974). The interaction between spikes and nucleocapsid cannot be highly specific, since phenotypic mixing occurs with alphaviruses (Burge and Pfefferkorn, 1966; Lagwinska *et al.*, 1975).

In mosquito cells alphaviruses mature at intracellular membranes by budding into vacuoles, which release the virus particles into the medium by fusing with the cell membrane (Whitfield *et al.*, 1971; Gliedman *et al.*, 1975).

Flavivirus are usually found in association with internal membranes of the infected cells. However, there is conclusive evidence neither that this is the site of assembly nor that assembly takes place by budding (for references, see Schlesinger, 1977).

### 3.10.5. Biological Activities of the Envelope

Alphaviruses and flaviviruses have hemagglutinating capacity (Clarke and Casals, 1958). Titrations are usually carried out on goose erythrocytes. It is reasonable to assume that the spikes are responsible for this activity. This has been demonstrated with Sindbis virus and SFV, where hemagglutinating activity was found to be associated specifically with glycoprotein E1 (Dalrymple *et al.*, 1976; Helenius *et al.*, 1976). Also in Venezuelan equine encephalitis virus the hemagglutinin seems to be only one of the envelope proteins (Pederson and Eddy, 1974).

Flaviviruses, particularly primary isolates of dengue virus, induce significant fusion and syncytium formation in cultures of *Aedes albopictus* cells (Paul *et al.*, 1969). This effect occurs several days after infection and therefore represents fusion from within. Thus fusion appears to depend on membrane alterations occurring late in infection and might be associated with incorporation of viral glycoproteins into the cell membrane. Similar observations have been made in another togavirus which appears to be neither an alpha- nor a flavivirus (Igarashi *et al.*, 1976).

## 4. CONCLUSIONS

### 4.1. Viral Membrane Structure

Recent models of membrane structure include the fluid lipid bilayer–globular protein mosaic in which membrane proteins are free to migrate in the plane of the membrane, as well as models in which extensive lateral interaction between protein subunits fixes the location of these proteins in the membrane. The general features of the fluid mosaic model have been supported by morphological, biochemical, and biophysical observations on various membrane systems. Knowledge concerning the composition and arrangement of membrane components of lipid-containing viruses has emerged in parallel with similar studies on cellular membranes. Aspects of both a fluid mosaic model and protein subunit model apply to some features of viral envelope structures. Viral envelopes possess a lipid membrane with glycoproteins and glycolipids exposed on the external surface and carbohydrate-free proteins in the interior. Cell surface membranes are similar in asymmetry with respect to localization of all carbohydrate constituents on the external surfaces. Viral glycoproteins appear to be amphipathic globular proteins, as have been proposed in the fluid mosaic model for cellular membrane glycoproteins. Recently evidence has been obtained for asymmetrical distribution of various phospholipid classes between the internal and external sides of the bilayer in both cellular and viral membranes. Amine-containing phospholipids are preferentially located on the internal surface and phosphatidylcholine and sphingomyelin on the external surface. The origin of this asymmetry is an interesting problem for future investigation.

A variety of biophysical methods, including X-ray diffraction, electron spin resonance, nuclear magnetic resonance, differential calorimetry, and fluorescence polarization spectroscopy have been applied to analyze the structure of the lipid phase of viral and cellular membranes. The results have indicated that a lipid bilayer structure is universally present as the fundamental matrix of all biological membranes. The fluidity of viral lipids, as indicated by spin label probes, appears to be significantly less than that of corresponding host cell membranes or vesicles prepared from extracted viral lipids. These observations, along with data indicating that viral membrane fluidity is affected only slightly by variation in glycoprotein content or by proteolytic digestion of the surface glycoproteins, have suggested that internal membrane proteins (M proteins) are primary determinants of viral membrane rigidity in myxoviruses, paramyxoviruses, and rhabdoviruses. The M

proteins of these viruses are major proteins that appear to form a continuous layer of protein subunits on the internal surface of the lipid bilayer. Interactions between M proteins and viral nucleocapsids (and probably viral glycoproteins as well) are undoubtedly important features in the specificity of viral membrane assembly.

An important distinction is made in the fluid mosaic model between "peripheral" and "integral" membrane proteins, which differ with respect to their modes of interaction with the lipid bilayer. Integral proteins are those considered to be at least partially embedded in the lipid phase and to be associated with membranes by strong hydrophobic interactions. Generally they are not dissociated from membranes unless drastic conditions such as detergent treatment are used. Peripheral proteins appear to be associated with the surface of the membrane, possibly by electrostatic interactions, and are dissociated by conditions such as changes in ionic strength or use of chelating agents. Viral glycoproteins generally appear to be integral membrane proteins with a strong hydrophobic association with the bilayer. Evidence for partial penetration of the bilayer by a hydrophobic segment of the glycoprotein has been obtained for certain viruses, in which residual hydrophobic peptides remain associated with the lipid phase after extensive proteolytic digestion. Individual virus groups may differ in this respect, however. The major glycoprotein of C-type retroviruses appears more loosely associated with the bilayer, being released by procedures such as osmotic shock or freezing and thawing. The precise nature of the interaction of internal viral membrane (M) proteins with the bilayer has not been investigated. These proteins may resemble cellular proteins which associate with the cytoplasmic surfaces of the membranes, such as the protein termed "spectrin" on the inner surface of the erythrocyte which has properties of a peripheral membrane protein. Spectrin forms fibrous aggregates, and similar structures are seen with isolated viral M proteins. Such proteins may be involved in transmembrane interactions with surface glycoproteins, and phenomena such as antibody- or lectin-mediated patching or capping of surface glycoproteins are therefore influenced by such internal membrane proteins.

It is evident from the above comments that viral envelopes and cellular membranes are similar with respect to important features of the arrangement and properties of molecular components. Certain aspects of viral membrane structure may differ from the fluid mosaic model of cellular plasma membrane organization, however. The ability of certain cell surface proteins to undergo lateral diffusion in the plane of the membrane is a well-established and important finding in support of the fluid mosaic model. Whether viral membrane proteins are able to

undergo such lateral diffusion is uncertain in the case of surface glycoproteins, and unlikely in the case of M proteins because of their location in a tightly packed layer, probably with extensive lateral interaction. Whether virion glycoproteins may be restricted in lateral mobility because of transmembrane interactions with internal proteins has not been firmly established for most virus groups.

It is likely that extensive lateral protein–protein interactions may also occur in specialized regions of cellular membranes, such as junctional complexes. Such interactions could occur at three levels: extracellularly, on the internal side of the bilayer, or in the hydrocarbon phase. No evidence has been obtained for the latter type of interaction between viral membrane proteins, and the available data from proteolytic digestion of glycoproteins and freeze fracture electron microscopy suggest that it is unlikely that enough protein is present in the hydrocarbon phase for such interaction. Lateral interactions between envelope proteins may occur predominantly on the external surface of the virion for viruses in which glycoproteins comprise a large percentage of the total structural protein (e.g., togaviruses, bunyaviruses), whereas with other groups such as the myxo- and paramyxoviruses such interactions may occur at the level of the M protein on the internal surface of the bilayer.

## 4.2. Biogenesis and Assembly of the Envelope

The envelopes of many viruses are assembled on preformed cellular membranes. In most instances assembly takes place by budding from the plasma membrane, although occasionally other cellular membranes serve as budding sites. Envelope maturation is a multistep process involving sequential incorporation of viral proteins into cellular membranes.

Viral glycoproteins are always bound to cellular membranes; they do not appear free in the cytoplasm. Translation takes place on the rough endoplasmic reticulum, and there is some evidence suggesting that a "signal sequence" at the N terminus of the nascent polypeptide chain directs attachment of the polyribosome to specific structures in the endoplasmic reticulum, which in turn facilitate transmembrane passage of the polypeptide into the lumen of the canaliculi. Subsequently the glycoproteins migrate via smooth endoplasmic reticulum and Golgi complex to the plasma membrane, where budding takes place. In the course of migration the polypeptides undergo posttranslational modifications, such as glycosylation and proteolytic cleavage.

The biosynthesis of the carbohydrate side chains of the glycoproteins occurs in a stepwise manner, with different saccharide residues added in distinct cellular compartments. Glycosylation is initiated at the rough endoplasmic reticulum on the nascent polypeptide chain as has been observed first with influenza virus and subsequently with toga and rhabdoviruses. The available evidence indicates that, at this stage, oligosaccharides containing glucosamine and mannose are transferred *en bloc* from a polyisoprenol derivative to the polypeptide. Fucose, galactose, and neuraminic acid appear to be attached later after migration of the glycoprotein to the smooth endoplasmic reticulum and the Golgi apparatus. The process of glycosylation does not appear to play an important role in determining the intracellular migration of viral glycoproteins. Although this has been suggested as a possible function for carbohydrate components of glycoproteins, the available data using inhibitors of glycosylation suggest that, at least with myxo- and oncornaviruses, it is possible to inhibit the glycosylation process without preventing the migration of the glycoproteins to the cell surface. The glycosylation site and the type of the carbohydrate side chains appear to be determined by the primary structure of the polypeptide. On the other hand, the side chain may exhibit host-dependent variations. Host-specific carbohydrate sequences may in turn be modified by virus-specific enzymes, such as the myxovirus neuraminidase. Thus the carbohydrate moiety of viral glycoproteins is the result of a complex interaction of virus- and host-specific factors.

Formation of precursors that are subsequently cleaved by proteolysis appears to be a fairly common event in the biogenesis of the envelope glycoproteins. Cleavage is involved in the processing of the influenza hemagglutinin, of both paramyxovirus glycoproteins, of the envelope glycoproteins of togaviruses, and of the envelope proteins of oncornaviruses. It is particularly interesting that cleavage was always found to be paralleled by induction of biological activity, if it was possible to obtain the precursor relatively free of its cleavage products. This has been observed with both glycoproteins of paramyxoviruses and with the hemagglutinin of influenza virus. With myxoviruses it could be demonstrated that strain-specific differences exist in the susceptibility of the glycoproteins to proteolytic enzymes. Furthermore, circumstantial evidence has been obtained that these enzymes are host-specific proteases and that a given protease may be present in some host cells but not in others. Thus proteolytic cleavage depends on the structure of the viral glycoprotein as determined by the viral genome as well as on the disposition of the appropriate enzyme by the host. It has also been

shown that activation by proteolytic cleavage may be an important factor in determining host range and pathogenicity of a virus.

One of the final steps in envelope assembly appears to be the insertion of a carbohydrate-free protein such as the M protein of myxo- and rhabdoviruses or the core protein of alphaviruses. The M protein was found to be incorporated immediately after its synthesis into regions of the plasma membrane which already contain viral glycoproteins. The mechanism by which M recognizes such areas might be interaction with the glycoproteins that penetrate the membrane. However, so far only with some viruses reasonable evidence has been obtained that the glycoproteins span the membrane. Also, the interaction between the proteins cannot be very specific, since phenotypic mixing occurs between the envelope proteins of viruses belonging to a large variety of groups. An important feature of the assembly of the virus is that during the budding process the membrane of the viral envelope is continuous with and morphologically similar to the plasma membrane of the host cell. This suggests that the lipids in the unaltered plasma membrane area easily exchangeable by lateral diffusion with those in the budding virus particle. It is therefore not surprising that the lipids in the envelopes closely resemble those of the host cell membrane. There is thus good evidence that the viral envelope is derived from a patch of plasma membrane modified by the incorporation of virus-specific proteins. Little is known about the mechanism by which host proteins are excluded from this area. The budding process itself is not fully understood, either. However, the observation that budding does not occur under conditions where all envelope components are synthesized except the M protein suggests that this protein plays an important role.

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## 5. REFERENCES


Abodeely, R. A., Palmer, E., Lawson, L. A., and Randall, C. C., 1971, The proteins of enveloped and de-enveloped equine abortion (herpes) virus and the separated envelope, *Virology* **44**:146.

Acheson, N. H., and Tamm, I., 1976, Replication of Semliki Forest virus: An electron microscopic study, *Virology* **32**:123.

Ada, G. L., and Perry, B. T., 1954, The nucleic acid content of influenza virus, *Aust. J. Exp. Biol. Med. Sci.* **32**:453.

Ada, G. L., and Yap, K. L., 1977, Matrix protein expressed at the surface of cells infected with influenza viruses, *Immunochemistry* **14**:643.

Allen, A. K., Skehel, J. J., and Yuferov, V., 1977, The amino acid and carbohydrate composition of the neuraminidase of B/Lee/40 influenza virus, *J. Gen. Virol.* **37**:625.

Almeida, J. D., and Waterson, A. P., 1970, Two morphological aspects of influenza virus, in: *The Biology of Large RNA Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 27–51, Academic Press, New York.

Anderson, R., and Dales, S., 1978, Biogenesis of poxviruses: Glycolipid metabolism in vaccinia-infected cells. *Virology* **84**:108.

Anon, M. C., Grau, O., Martinez Segovia, Z. M., and Franze-Ferandez, M. T., 1976, RNA composition of Junin virus, *J. Virol.* **18**:833.

Apostolov, K., and Almeida, J. D., 1972, Interaction of Sendai (HVJ) virus with human erythrocytes: A morphological study of haemolysis and cell fusion, *J. Gen. Virol.* **15**:227.

Apostolov, K., and Flewett, T. H., 1969, Further observations on the structure of influenza viruses A and C, *J. Gen. Virol.* **4**:365.

Apostolov, K., Flewett, T. H., and Kendall, A. P., 1970, Morphology of influenza A, B, C, and infectious bronchitis virus (IBV) virions and their replication, in: *The Biology of Large RNA Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 3–26, Academic Press, New York.

Arstila, P., 1972, Two hemagglutinating components of vesicular stomatitis virus, *Acta Pathol. Microbiol. Scand.* **80**:33.

Arstila, P., 1973, Small-sized hemagglutinin of vesicular stomatitis virus released spontaneously with Nonidet P40, *Acta Pathol. Microbiol. Scand.* **81**:27.

Arstila, P., Halonen, P. E., and Salmi, G., 1969, Hemagglutinin of vesicular stomatitis virus, *Arch. Ges. Virusforsch.* **27**:198.

Atanasiu, P., Tsiang, H., Perrin, P., Favre, S., and Sisman, J., 1974, Extraction d'un antigène soluble (glycoprotéine) par le Triton X100, *Ann. Microbiol.* **125B**:539.

August, J. T., Bolognesi, D. T., Fleissner, E., Gilden, R. V., and Nowinski, R., 1974, A proposed nomenclature for the virion proteins of oncogenic RNA viruses, *Virology* **60**:595.

Bächi, T., Gerhard, W., Lindenmann, J., and Mühlethaler, K., 1969, Morphogenesis of influenza A virus in Ehrlich ascites tumor cells as revealed by thin-sectioning and freeze-etching, *J. Virol.* **4**:769.

Bächi, T., Deas, J. E., and Howe, C., 1977, Virus–erythrocyte interactions, in: *Virus Infection and the Cell Surface*, Vol. 2 of *Cell Surface Reviews* (G. Poste and G. L. Nicolson, eds.), pp. 83–128, North-Holland, Amsterdam.

Bader, J. P., 1974, Reproduction of RNA tumor viruses, *Comp. Virol.* **4**:253.

Baltimore, D., Burke, D. C., Horzinek, M. C., Huang, A. S., Kääriäinen, L., Pfeffer-

korn, E. R., Schlesinger, M. J., Schlesinger, S., Schlesinger, R. W., and Scholtissek, C., 1976, Proposed nomenclature for alphavirus polypeptides, *J. Gen. Virol.* **30**:273.

Barenholz, Y., Moore, N. F., and Wagner, R. R., 1976, Enveloped viruses as model membrane systems: Microviscosity of vesicular stomatitis virus and host cell membranes, *Biochemistry* **15**:3563.

Bauer, H., 1974, Virion and tumor cell antigens of C-type RNA tumor viruses, *Adv. Cancer Res.* **20**:273.

Becht, H., Hämmerling, U., and Rott, R., 1977, Undisturbed release of influenza virus in the presence of univalent antineuraminidase antibodies, *Virology* **46**:337.

Becht, H., Rott, R., and Klenk, H.-D., 1972, Effect of concanavalin A on cells infected with enveloped viruses, *J. Gen. Virol.* **14**:1.

Becker, R., Helenius, A., and Simons, K., 1975, Solubilization of the Semliki Forest virus membrane with sodium dodecyl sulfate, *Biochemistry* **14**:1835.

Ben Porat, T., and Kaplan, A. S., 1971, Phospholipid metabolism of herpesvirus-infected and uninfected rabbit kidney cells, *Virology* **45**:252.

Ben Porat, T., and Kaplan, A. S., 1972, Studies on the biogenesis of herpesvirus envelope, *Nature (London)* **235**:165.

Biddison, W. E., Doherty, P. C., and Webster, R. G., 1977, *J. Exp. Med.* **146**:690.

Bingham, R. W., 1975, The polypeptide composition of avian infectious bronchitis virus, *Arch. Virol.* **49**:207.

Birdwell, C. R., and Strauss, J. H., 1973, Agglutination of Sindbis virus and of cells infected with Sindbis virus by plant lectins, *J. Virol.* **11**:502.

Birdwell, C. R., and Strauss, J. H., 1974, Replication of Sindbis virus. IV. Electron microscope study of the insertion of viral glycoproteins into the surface of infected chick cells, *J. Virol.* **14**:366.

Bishop, D. H. L., Repik, P., Obijeski, J. F., Moore, N. F., and Wagner, R. R., 1975, Restitution of infectivity to spikeless vesicular stomatitis virus by solubilized viral components, *J. Virol.* **16**:75.

Bittman, R., Majuk, Z., Honig, D. S., Compans, R. W., and Lenard, J., 1976, Permeability properties of the membrane of vesicular stomatitis virions, *Biochim. Biophys. Acta.* **433**:63.

Blobel, G., and Dobberstein, B., 1975, Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma, *J. Cell Biol.* **67**:835.

Blough, H. A., 1974, Newly synthesized lipids incorporated into influenza virus membranes, *Nature (London)* **251**:333.

Blough, H. A., and Lawson, D. E. M., 1968, The lipids of paramyxoviruses: A comparative study of Sendai and Newcastle disease virus, *Virology* **36**:286.

Blough, H. A., and Merlie, J., 1970, The lipids of incomplete influenza virus, *Virology* **40**:685.

Blough, H. A., and Tiffany, J. M., 1973, Lipids in viruses, *Adv. Lipid Res.* **11**:267.

Blough, H. A., and Weinstein, D. B., 1973, Effect of influenza virus infection on lipid metabolism and chick embryo fibroblasts, in: *Biology of the Fibroblast* (E. Kulonen and J. Pikkarainen, eds.), pp. 303–308, Academic Press, London.

Blough, H. A., Weinstein, D. B., Lawson, D. E. M., and Kodicek, E., 1967, *Virology* **33**:459.

Bolognesi, D. P., 1974, Structural components of RNA tumor viruses, *Adv. Virus Res.* **19**:315.

Bolognesi, D. P., Bauer, H., Gelderblom, H., and Huper, G., 1972, Polypeptides of avian RNA tumor viruses. IV. Components of the viral envelope, *Virology* **47**:551.

Bolognesi, D. P., Luftig, R., and Shapper, J. H., 1973, Localization of RNA tumor virus polypeptides. I. Isolation of further virus substructures, *Virology* **56**:549.

Bolognesi, D. P., Collins, J. J., Leis, J. P., Moennig, V., Schäfer, W., and Atkinson, P. H., 1975, Role of carbohydrate in determining the immunochemical properties of the major glycoproteins (gp71) of Friend murine leukemia viruses, *J. Virol.* **16**:1453.

Bouloy, M., Krams-Czden, S., Horodmiceanu, F., and Hannoun, C., 1974, Three-segment RNA genome of Lumbo virus (bunyavirus), *Intervirology* **2**:173.

Bracha, M., and Schlesinger, M. J., 1976, Defects in $RNA^+$ temperature-sensitive mutants of Sindbis virus and evidence for a complex of PE2-E1 viral glycoproteins, *Virology* **74**:441.

Braciale, T. J., 1977, Immunologic recognition of influenza virus-infected cells. II. Expression of influenza A matrix protein on the infected cell surface and its role in recognition by cross-reactive cytotoxic T cells, *J. Exp. Med.* **146**:673.

Brand, C. M., and Skehel, J. J., 1972, Crystalline antigen from the influenza virus envelope, *Nature* (*London*) *New Biol.* **238**:145.

Brennen, J. P., Steiner, S. M., Courtney, P. J., and Skelly, J., 1976, Metabolism of galactose in herpes simplex virus-infected cells, *Virology* **69**:216.

Bretscher, M., 1972, Asymmetrical lipid bilayer structure for biological membranes, *Nature* (*London*) *New Biol.* **236**:11.

Bretscher, M., 1973, Membrane structure: Some general principles, *Science* **181**:622.

Brown, F., Smale, C. J., and Horzinek, M. C., 1974, Lipid protein organization in vesicular stomatitis and Sindbis viruses, *J. Gen. Virol.* **22**:455.

Bubbers, J. E., and Lilly, F., 1977, Selective incorporation of H-2 antigenic determinants into Friend virus particles, *Nature* (*London*) **266**:459.

Bucher, D. J., and Kilbourne, E. D., 1972, $A_2(N_2)$ neuraminidase of the X-7 influenza virus recombinant: Determination of molecular size and subunit composition of the active unit, *J. Virol.* **10**:60.

Bucher, D., and Palese, P., 1975, The biologically active proteins of influenza virus: Neuraminidase, in: *The Influenza Virus and Influenza* (E. D. Kilbourne, ed.), p. 83, Academic Press, New York.

Buchmeier, M. J., Gee, S. R., and Rawls, W. E., 1977, Antigens of Pichinde virus. I. Relationship of soluble antigens derived from infected BHK-21 cells to the structural components of the virion, *J. Virol.* **22**:175.

Burge, B. W., and Huang, A. S., 1970, Comparison of membrane protein glycopeptides of Sindbis virus and vesicular stomatitis virus, *J. Virol.* **6**:176.

Burge, B. W., and Pfefferkorn, E. R., 1966, Phenotypic mixing between group A arboviruses, *Nature* (*London*) **210**:1397.

Burge, B. W., and Strauss, J. H., 1970, Glycopeptides of the membrane glycoprotein of Sindbis virus, *J. Mol. Biol.* **47**:449.

Burke, D. C., 1975, Processing of alphavirus proteins in infected cells, *Med. Biol.* **53**:352.

Burke, D. J., and Keegstra, K., 1976, Purification and composition of the proteins from Sindbis virus grown in chick and BHK cells, *J. Virol.* **20**:676.

Bussell, R. H., Waters, D. J., Seals, M. K., and Robinson, W. S., 1974, Measles, canine distemper and respiratory syncytial virions and nucleocapsids: A comparative study of their structure polypeptide and nucleic acid composition, *Med. Microbiol. Immunol.* **160**:105.

Bykovsky, A. F., Yershov, F. I., and Zhdanov, V. M., 1969, Morphogenesis of Venezuelan equine encephalomyelitis virus, *J. Virol.* **4**:496.

Calberg-Bacq, C. M., and Osterrieth, P. M., 1966, Morphological modifications of Semliki Forest virus after treatment with pronase, *Acta Virol. Prague* **10**:266.

Caliguiri, L. A., Klenk, H.-D., and Choppin, P. W., 1969, The proteins of the parainfluenza virus SV5. I. Separation of virion polypeptides by polyacrylamide gel electrophoresis, *Virology* **39**:460.

Canaani, E., Helm, K. V. D., and Duesberg, P., 1973, Evidence for 30–40 S RNA as precursor of the 60–70 S RNA of Rous sarcoma virus, *Proc. Natl. Acad. Sci. USA* **70**:401.

Cancedda, R., Swanson, R., and Schlesinger, M. J., 1974*a*, Effects of different RNAs and components of the cell-free system on in vitro synthesis of Sindbis viral proteins, *J. Virol.* **14**:652.

Cancedda, R., Swanson, R., and Schlesinger, M. J., 1974*b*, Viral proteins formed in a cell-free rabbit reticulocyte system programmed with RNA from a temperature-sensitive mutant of Sindbis virus, *J. Virol.* **14**:664.

Carter, M. F., Biswal, N., and Rawls, W. E., 1973, Characterization of the nucleic acid of Pichinde virus, *J. Virol.* **11**:61.

Cartwright, B., Talbot, P., and Brown, F., 1970, The proteins of biologically active subunits of vesicular stomatitis virus, *J. Gen. Virol.* **7**:267.

Cassai, E. N., Sarmiento, M., and Spear, P. G., 1975, Comparison of the virion proteins specified by herpes simplex virus types 1 and 2, *J. Virol.* **16**:1327.

Chang, A., and Metz, D. H., 1976, Further investigations on the mode of entry of vaccinia virus into cells, *J. Gen. Virol.* **32**:275.

Chen, C., Compans, R. W., and Choppin, P. W., 1971, Parainfluenza virus surface projections: Glycoproteins with hemagglutinin and neuraminidase activities, *J. Gen. Virol.* **11**:53.

Cheung, K. S., Smith, R. E., Stone, M. P., and Joklik, W. K., 1972, Comparison of immature (rapid harvest) and mature Rous sarcoma virus particles, *Virology* **50**:851.

Choppin, P. W., and Compans, R. W., 1975, Reproduction of paramyxoviruses, in: *Comprehensive Virology*, Vol. 4 (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 95–178, Plenum Press, New York.

Choppin, P. W., Klenk, H.-D., Compans, R. W., and Caliguiri, L. A., 1971, The parainfluenza virus SV5 and its relationship to the cell membrane, in: *Perspectives in Virology*, Vol. VII (M. Pollard, ed.), pp. 127–156, Academic Press, New York.

Choppin, P. W., Lazarowitz, S. G., and Goldberg, A. R., 1975, Studies on proteolytic cleavage and glycosylation of the hemagglutinin of influenza A and B viruses, in: *Negative Strand Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 105–119, Academic Press, New York.

Clarke, D.-H., and Casals, J., 1958, Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses, *Am. J. Trop. Med. Hyg.* **7**:561.

Clegg, J. C. S., 1975, Sequential translation of capsid and membrane protein genes in arbovirus-infected cells, *Nature* (*London*) **254**:454.

Clegg, J. C. S., and Kennedy, S. I. T., 1974, In vitro synthesis of structural proteins of Semliki Forest virus directed by isolated 26 S RNA from infected cells, *FEBS Lett.* **42**:327.

Clegg, J. C. S., and Kennedy, S. I. T., 1975*a*, Translation of Semliki Forest virus intracellular 26 S RNA: Characterization of the products synthesized in vitro, *Eur. Mol. Biol.* **53**:175.

Clegg, J. C. S., and Kennedy, S. I. T., 1975*b*, Initiation of synthesis of the structural proteins of Semliki Forest virus, *J. Mol. Biol.* **97**:401.

Clegg, J. C. S., and Kennedy, S. I. T., 1975*c*, Translation of the genes for the structural proteins of alphaviruses, *Med. Biol.* **53**:383.

Clinkscales, C. W., Bratt, M. A., and Morrison, T. G., 1977, Synthesis of Newcastle disease virus polypeptides in a wheat germ cell-free system, *J. Virol.* **22**:97.

Compans, R. W., 1971, Location of the glycoprotein in the membrane of Sindbis virus, *Nature (London) New Biol.* **229**:114.

Compans, R. W., 1973*a*, Influenza virus proteins. II. Association with components of the cytoplasm, *Virology* **51**:56.

Compans, R. W., 1973*b*, Distinct carbohydrate components of influenza virus glycoproteins in smooth and rough cytoplasmic membranes, *Virology* **55**:541.

Compans, R. W., and Choppin, P. W., 1967, Isolation and properties of the helical nucleocapsid of the parainfluenza virus SV5, *Proc. Natl. Acad. Sci. USA* **57**:541.

Compans, R. W., and Choppin, P. W., 1975, Reproduction of myxoviruses, in: *Comprehensive Virology*, Vol. 4 (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 179–252, Plenum Press, New York.

Compans, R. W., and Dimmock, N. J., 1969, An electron microscopic study of single-cycle infection of chick embryo fibroblasts by influenza virus, *Virology* **39**:499.

Compans, R. W., and Pinter, A., 1975, Incorporation of sulfate into influenza virus glycoproteins, *Virology* **66**:151.

Compans, R. W., Holmes, K. V., Dales, S., and Choppin, P. W., 1966, An electron microscopic study of moderate and virulent virus–cell interactions of the parainfluenza virus SV5, *Virology* **30**:411.

Compans, R. W., Dimmock, N. J., and Meier-Ewert, H., 1969, Effect of antibody to neuraminidase on the maturation and hemagglutinating activity of an influenza virus $A_2$, *J. Virol.* **4**:528.

Compans, R. W., Klenk, H.-D., Caliguiri, L. A., and Choppin, P. W., 1970, Influenza virus proteins. I. Analysis of polypeptides of the virion and identification of spike glycoproteins, *Virology* **42**:880.

Compans, R. W., Landsberger, F. R., Lenard, J., and Choppin, P. W., 1972, Structure of the membrane of influenza virus, in: *International Virology 2* (J. L. Melnick, ed.), pp. 130–132, S. Karger, Basel.

Compans, R. W., Meier-Ewert, H., and Palese, P., 1974, Assembly of lipid-containing viruses, *J. Supramol. Struct.* **2**:496.

Compans, R. W., Bishop, D. H. L., and Meier-Ewert, H., 1977, Structural components of influenza C virions, *J. Virol.* **21**:658.

Content, J., and Duesberg, P., 1970, Electrophoretic distribution of the proteins and glycoproteins of influenza virus and Sendai virus, *J. Virol.* **6**:707.

Courtney, R. J., Steiner, S. M., and Benyesh-Melnick, M., 1973, Effects of 2-deoxy-D-glucose on herpes simplex virus replication, *Virology* **52**:447.

Cox, N. J., and Kendal, A. P., 1976, Presence of a segmented single-stranded RNA genome in influenza C virus, *Virology* **74**:239.

Dales, S., and Mosbach, E. H., 1968, Vaccinia as a model for membrane biogenesis, *Virology* **35**:564.

Dales, S., and Siminovitch, I., 1961, The development of vaccinia virus in strain L cells as examined by electron microscopy, *J. Biophys. Biochem. Cytol.* **10**:475.

Dales, S., Stern, W., Weintraub, S. B., and Huima, T., 1976, Genetically controlled surface modifications by poxvirus influencing cell–cell and cell–virus interactions, in: *Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones, and Small Molecules* (R. F. Beers, Jr., and E. G. Bassett, eds.), pp. 253–270, Raven Press, New York.

Dalrymple, J. M., Schlesinger, S., and Russell, P. K., 1976, Antigenic characterization of two Sindbis envelope glycoproteins separated by isoelectric focusing, *Virology* **69**:93.

Dalton, A. J., Rowe, W. P., Smith, G. H., Wilsnack, R. W., and Pugh, W. E., 1968, Morphological and cytochemical studies on lymphocytic choriomeningitis virus, *J. Virol.* **2**:1465.

Darlington, R. W., and Moss, L. H., 1969, The envelope of herpes virus, *Progr. Med. Virol.* **11**:16.

Davenport, F. M., 1976, Influenza virus, in: *Viral Infections of Humans* (A. S. Evans, ed.), pp. 273–296, Plenum Press, New York.

David, A. E., 1973, Assembly of the vesicular stomatitis virus envelope: Incorporation of viral polypeptides into the host cell plasma membrane, *J. Mol. Biol.* **76**:135.

David-Ferreira, J. F., and Manaker, R. A., 1965, An electron microscope study of the development of a mouse hepatitis virus in tissue culture cells, *J. Cell Biol.* **24**:57.

DeGiuli, C., Kawai, S., Dales, S., and Hanafusa, H., 1975, Absence of surface projections on some noninfectious forms of RSV, *Virology* **66**:253.

DeLarco, J., and Todaro, G. J., 1976, Membrane receptors for murine leukemia viruses: Characterization using the purified viral envelope glycoprotein, gp71, *Cell* **8**:365.

Dietzschold, B., Schneider, L. G., and Cox, J. H., 1974, Serological characterization of the three major proteins of vesicular stomatitis virus, *J. Virol.* **19**:1.

Diringer, H., and Rott, R., 1976, Metabolism of preexisting lipids in baby hamster kidney cells during fusion from within, induced by Newcastle disease virus, *Eur. J. Biochem.* **65**:155.

Diringer, H., Kulas, H.-P., Schneider, L. G., and Schlumberger, H. D. 1973, The lipid composition of rabies virus, *Z. Naturforsch.* **28c**:90.

Dix, R. D., and Courtney, R. J., 1976, Effects of cytochalasin B on herpes simplex virus type 1 replication, *Virology* **70**:127.

Drzeniek, R., 1973, Viral and bacterial neuraminidase, *Curr. Top. Microbiol. Immunol.* **59**:35.

Drzeniek, R., and Rott, R., 1963, Abspaltung einer neuraminidase-haltigen Komponente aus Newcastle disease virus (NDV), *Z. Naturforsch.* **18b**:1127.

Drzeniek, R., Saber, M. S., and Rott, R., 1966*a*, Veränderung der Erythrozytenoberfläche durch Newcastle disease virus. II. Auftreten eines Forssman und eines "Mononucleose"-Antigens an Newcastle disease virus-behändelten Erythrozyten, *Z. Naturforsch.* **21b**:254.

Drzeniek, R., Seto, J. T., and Rott, R., 1966*b*, Characterization of neuraminidase from myxoviruses, *Biochim. Biophys. Acta* **128**:547.

Dubovi, J. E., and Wagner, R. R., 1977, Spatial relationships of the proteins of vesicular stomatitis virus: Induction of reversible oligomers by cleavable protein cross-linkers and oxidation, *J. Virol.* **22**:900.

Duda, E., and Schlesinger, M. J., 1975, Alterations in Sindbis viral envelope proteins by treating BHK cells with glucosamine, *J. Virol.* **15**:416.

Duesberg, P. H., Martin, G. S., and Vogt, P. K., 1970, Glycoprotein components of avian and murine RNA tumor viruses, *Virology* **41**:631.

Easterbrook, K. B., 1966, Controlled degradation of vaccinia virus in vitro: An electron microscopic study, *J. Ultrastruct. Res.* **14**:484.

Eckert, E. A., 1973, Properties of an antigenic glycoprotein isolated from influenza virus hemagglutinin, *J. Virol.* **11**:183.

Eger, R., Compans, R. W., and Rifkin, D. B., 1975, The organization of the proteins of vesicular stomatitis virions: Labeling with pyridoxal phosphate, *Virology* **66**: 610.

Eisenman, R. M., and Vogt, V. M., 1978, The biosynthesis of oncovirus proteins, *Biochim. Biophys. Acta* **473**:187.

Elder, J. H., Jensen, F. C., Bryant, M. L., and Lerner, R. A., 1977, Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: Virion associated and differentiation antigens encoded by a multi-gene family, *Nature* (*London*) **267**:23.

Elsbach, P., Holmes, K. V., and Choppin, P. W., 1969, Metabolism of lecithin and virus-induced cell fusion, *Proc. Soc. Exp. Biol. Med.* **130**:903.

Emerson, S. U., 1976, Vesicular stomatitis virus: Structure and function of virion components, *Curr. Top. Microbiol. Immunol.* **73**:1.

Emerson, S. U., and Wagner, R. R. 1972, Dissociation and reconstitution of the transcriptase and template activities of vesicular stomatitis B and T virions, *J. Virol.* **10**:297.

England, J. M., Bolognesi, D. P., Dietzschold, B., and Halpern, M. S., 1977, Evidence that a precursor glycoprotein is cleaved to yield the major glycoprotein of avian tumor virus, *J. Virol.* **21**:810.

Epstein, M. A., 1962, Observations on the fine structure of mature herpes simplex virus and on the composition of its nucleoid, *J. Exp. Med.* **115**:1.

Erlandson, R. A., Babcock, V. I., Southam, C. M., Bailey, R. B., and Shipkey, F. H., 1976, Semliki Forest virus in HEp2 cell cultures, *J. Virol.* **1**:996.

Etchison, J. R., and Holland, J. J., 1974*a*, Carbohydrate composition of the membrane glycoprotein of vesicular stomatitis virus, *Virology* **60**:217.

Etchison, J. R., and Holland, J. J., 1974*b*, Carbohydrate composition of the membrane glycoprotein of vesicular stomatitis virus grown in four mammalian cell lines, *Proc. Natl. Acad. Sci. USA* **71**:4011.

Etchison, J. R., Robertson, J. S., and Summers, D. F., 1977, Partial structural analysis of the oligosaccharide moieties of the vesicular stomatitis virus glycoprotein by sequential chemical and enzymatic degradation, *Virology* **78**:375.

Famulari, N. G., Buchhagen, D. L., Klenk, H.-D., and Fleissner, E., 1976, Presence of murine leukemia virus envelope proteins gp70 and p15 (E) in a common polyprotein of infected cells, *J. Virol.* **20**:501.

Farber, F. E., and Rawls, W. E., 1975, Isolation of ribosome-like structures from Pichinde virus, *J. Gen. Virol.* **26**:21.

Fleissner, E., 1971, Chromatographic separation and antigenic analysis of proteins of the oncornaviruses, *J. Virol.* **8**:778.

Fleissner, E., Ikeda, H., Tund, J.-S., Vitetta, E. S., Tress, E., Hardy, W., Jr., Stockert, E., Boyse, E. A., Pincus, T., and O'Donnell, P., 1975, Characterization of murine leukemia virus-specific proteins, *Cold Spring Harbor Symp. Quant. Biol.* **34**:1057.

Fong, B. S., Hunt, R. C., and Brown, J. C., 1976, Asymmetric distribution of phosphatidylethanolamine in the membrane of vesicular stomatitis virus, *J. Virol.* **20**:658.

Fong, C. K. Y., Tenser, R. B., Hsiung, G. D., and Gross, P. A., 1973. Ultrastructural studies of the envelopment and release of guinea pig herpes-like virus in cultured cells, *Virology* **52**:468.

Friedman, R. M., 1968, Protein synthesis directed by an arbovirus, *J. Virol.* **2**:26.

Friis, R. R., Ogura, H., Gelderblom, H., and Halpern, M. S., 1976, The defective maturation of viral progeny with a temperature sensitive mutant of avian sarcoma virus, *Virology* **73**:259.

Frommhagen, L. H., Knight, C. A., and Freeman, N. K., 1959, The ribonucleic acid, lipid and polysaccharide constituents of influenza virus preparations, *Virology* **8**:176.

Gahmberg, C. G., Simons, K., Renkonen, O., and Kääriäinen, L., 1972*a*, Exposure of proteins and lipids in the Semliki Forest virus membrane, *Virology* **50**:259.

Gahmberg, C. G., Uterman, G., and Simons, K., 1972*b*, The membrane proteins of Semliki Forest virus have a hydrophobic part attached to the viral membrane, *FEBS Lett.* **28**:179.

Gallaher, W. R., Levitan, D. B., and Blough, H. A., 1973, Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and herpes simplex viruses, *Virology* **55**:193.

Gandhi, S. S., Stanley, P., Taylor, J. M., and White, D. O., 1972, Inhibition of influenza viral glycoprotein synthesis by sugars, *Microbios* **5**:41.

Gard, G. P., Vezza, A. C., Bishop, D. H. L., and Compans, R. W., 1977, Structural proteins of Tacaribe and Tamiami virions, *Virology* **83**:84.

Garoff, H., 1974, Cross-linking of the spike glycoproteins in Semliki Forest virus with dimethylsuberimidate, *Virology* **62**:385.

Garoff, H., and Simons, K., 1974, Location of the spike glycoproteins in the Semliki Forest virus membrane, *Proc. Natl. Acad. Sci. USA* **71**:3988.

Garoff, H., Simons, K., and Renkonen, O., 1974, Isolation and characterization of the membrane proteins of Semliki Forest virus, *Virology* **61**:493.

Garon, C. F., and Moss, B., 1971, Glycoprotein synthesis in cells infected with vaccinia virus. II. A glycoprotein component of the virion, *Virology* **46**:233.

Garwes, D. J., and Pocock, D. H., 1975, The polypeptide structure of transmissible gastroenteritis virus, *J. Gen. Virol.* **29**:25.

Gentsch, J., Bishop, D. H. L., and Obijeski, J. F., 1977, The virus particle nucleic acids and proteins of four bunyaviruses, *J. Gen. Virol.* **34**:257.

Gething, M. J., White, J. M., and Waterfield, M. D., 1978, The purification and structural characterization of the fusion factor of Sendai virus, *Top. Infect. Dis.* **3**:213.

Gibson, B., and Roizman, B., 1974, Proteins specified by herpes simplex virus. X. Staining and radiolabeling properties of B-capsid and virion proteins in polyacrylamide gels, *J. Virol.* **13**:155.

Glanville, N., and Ulmanen, I., 1976, Biological activity of in vitro synthesized protein: Binding of Semliki Forest virus capsid protein to the large ribosomal subunit, *Biochem. Biophys. Res. Commun.* **71**:393.

Glanville, N., Morser, J., Uomala, P., and Kääriäinen, L., 1976, Simultaneous translation of structural and nonstructural proteins from Semliki Forest virus RNA in two eukaryotic systems in vitro, *Eur. J. Biochem.* **64**:167.

Gliedman, J. B., Smith, J. F., and Brown, D. T., 1975, Morphogenesis of Sindbis virus in cultured *Aedes albopictus* cells, *J. Virol.* **16**:913.

Gottschalk, A., 1957, Neuraminidase: The specific enzyme of influenza virus and *Vibrio cholerae*, *Biochim. Biophys. Acta* **23**:645.

Gregoriades, A., 1972, Isolation of neuraminidase from the WSN strain of influenza virus, *Virology* **49**:333.

Gregoriades, A., 1973, The membrane protein of influenza virus: Extraction from virus and infected cell with acidic chloroform–methanol, *Virology* **54**:369.

Griffin, J., and Compans, R., 1978, Effects of cytochalasin B on the maturation of enveloped viruses, Abst. ASM Meet., p. 257.

Griffith, I. P., 1975, The fine structure of influenza virus, in: *Negative Strand Viruses* (R. D. Barry and B. W. J. Mahy, eds.), p. 121, Academic Press, New York.

Grimes, W. J., and Burge, B. W., 1971, Modification of Sindbis virus glycoprotein by host-specified glycosyl transferase, *J. Virol.* **7**:309.

Grubman, M. J., Ehrenfeld, E., and Summers, D. F., 1974, In vitro synthesis of proteins by membrane-bound polyribosomes from vesicular stomatitis virus-infected HeLa cells, *J. Virol.* **19**:560.

Gupta, P., and Rapp, F., 1977, Identification of virion polypeptides in hamster cells transformed by herpes simplex virus type 1, *Proc. Natl. Acad. Sci. USA* **74**:372.

Haines, H., and Baerwald, R. J., 1976, Nuclear membrane changes in herpes simplex virus-infected BHK-21 cells as seen by freeze-fracture, *J. Virol.* **17**:1038.

Halonen, P. E., Murphy, F. A., Fields, B. N., and Reese, D. R., 1968, Hemagglutination of rabies and some other bullet-shaped viruses, *Proc. Soc. Exp. Biol.* **127**:1037.

Halpern, M. S., Bolognesi, D. P., and Friis, R. R., 1976, Viral glycoprotein synthesis studied in an established line of Japanese quail embryo cells infected with Bryan high titer strain of Rous sarcoma virus, *J. Virol.* **18**:504.

Hanafusa, T., Hanafusa, H., Metroka, C. E., Haguard, W. S., Rettenmier, C. W., Sawyer, R. C., Dougherty, R. M., and DiStefano, H. S., 1976, Pheasantvirus: A new class of ribodeoxy virus, *Proc. Natl. Acad. Sci. USA* **73**:1333.

Hardwick, J. M., and Bussell, R. H., 1976, Glycoproteins of measles virus under reduced and nonreduced conditions, *Abstr. Am. Soc. Microbiol.*, p. 232.

Harrison, S. C., David, A., Jumblatt, J., and Darnell, J. E., 1971, Lipid and protein organization in Sindbis virus, *J. Mol. Biol.* **60**:523.

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

Haukenes, G., Harboe, A., and Mortensson-Egnund, K., 1965, A uronic and sialic acid free chick allantoic mucopolysaccharide sulphate which combines with influenza virus HI-antibody to host material, *Acta Pathol. Microbiol. Scand.***64**:534.

Hay, A. J., 1974, Studies on the formation of the influenza virus envelope, *Virology* **60**:398.

Hayman, M. J., Skehel, J. J., and Crumpton, M. J., 1973, Purification of virus glycoproteins by affinity chromatography using *Lens culinaris* phytohemagglutinin, *FEBS Lett.* **29**:185.

Haywood, A., 1975, Model membranes and Sendai virus: Surface–surface interaction,

in: *Negative Strand Viruses* (B. W. J. Mahy and R. D. Barry, eds.), pp. 923–928, Academic Press, London.

Hecht, T. T., and Summers, D. F., 1972, Effect of vesicular stomatitis virus infection on the histocompatibility antigen of L cells, *J. Virol.* **10**:578.

Heine, J. W., and Schnaitman, C. A., 1971, Entry of vesicular stomatitis virus into L cells, *J. Virol.* **8**:786.

Heine, J. W., Spear, P. G., and Roizman, B., 1972, The proteins specified by herpes simplex virus. VI. Viral proteins in the plasma membrane, *J. Virol.* **9**:431.

Helenius, A., and Simons, K., 1972, The binding of detergents to lipophilic and hydrophilic proteins, *J. Biol. Chem.* **247**:3656.

Helenius, A., and Simons, K., 1975, Solubilization of membranes by detergents, *Biochim. Biophys. Acta* **415**:29.

Helenius, A., and Soderlund, H., 1973, Stepwise dissociation of the Semliki Forest virus membrane with Triton X-100, *Biochim. Biophys. Acta* **307**:287.

Helenius, A., and von Bonsdorff, C.-H., 1976, Semliki Forest virus membrane proteins: Preparation and characterization of spike complexes soluble in detergent-free medium, *Biochim. Biophys. Acta* **436**:895.

Helenius, A., Fries, E., Garoff, H., and Simons, K., 1976, Solubilization of the Semliki Forest virus membrane with sodium deoxycholate, *Biochim. Biophys. Acta* **436**:319.

Hierholzer, J. C., Palmer, E. L., Whitfield, S. G., Kaye, H. S., and Dowdle, W. R., 1972, Protein composition of coronavirus OC 43, *Virology* **48**:516.

Hightower, L. E., Morrison, T. G., and Bratt, M. A., 1975, Relationships among the polypeptides of Newcastle disease virus, *J. Virol.* **16**:1599.

Hirschberg, C. G., and Robbins, P. W., 1974, The glycolipids and phospholipids of Sindbis virus and their relation to the lipids of the host cell plasma membrane, *Virology* **61**:602.

Hirst, G. K., 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, *Science* **94**:22.

Hirst, G. K., 1942, Adsorption of influenza hemagglutinins and virus by red blood cells, *J. Exp. Med.* **76**:195.

Hirst, G. K., 1950, The relationship of the receptors of a new strain of virus to those of the mumps-NDV-influenza group, *J. Exp. Med.* **91**:177.

Hirst, G. K., and Pons, M. W., 1973, Mechanism of influenza virus recombination. II. Virus aggregation and its effect on plaque formation by so-called noninfectious virus, *Virology* **56**:620.

Hoagland, C. L., Smadel, J. E., and Rivers, T. M., 1940, Constituents of elementary bodies of vaccinia. I. Certain basic analysis and observations on lipid components of the virus, *J. Exp. Med.* **71**:737.

Hodes, D. S., Schnitzer, T. J., Kalica, A. R., Camargo, E., and Chanock, R. M., 1975, Inhibition of respiratory syncytial, parainfluenza 3, and measles viruses by 2-deoxy-D-glucose, *Virology* **63**:201.

Holowczak, J. A., 1970, Glycopeptides of vaccinia virus. I. Preliminary characterization and hexosamine content, *Virology* **42**:87.

Holowczak, J. A., and Joklik, W. K., 1967, Studies on the proteins of vaccinia virus. I. Structural proteins of virions and cores, *Virology* **33**:726.

Homma, M., and Ohuchi, M., 1973, Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural difference of Sendai virus grown in eggs and tissue culture cells, *J. Virol.* **12**:1457.

Homma, M., Tozawa, H., Shimizu, K., and Ishida, N., 1975, A proposal for designation of Sendai virus proteins, *Jpn. J. Microbiol.* **19**:467.

Homma, M., Shimizu, K., Shimizu, Y. K., and Ishida, N., 1976, On the study of Sendai virus hemolysis. I. Complete Sendai virus lacking in hemolytic activity, *Virology* **71**:41.

Honess, R. W., and Roizman, B., 1973, Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpesvirus polypeptides in the infected cell, *J. Virol.* **12**:1347.

Honess, R. W., and Roizman, B., 1975, Proteins specified by herpes simplex virus, *J. Virol.* **16**:1308.

Horne, R. W., Waterson, A. P., Wildy, P., and Farnham, A. E., 1960, The structure and composition of the myxoviruses. I. Electron microscope studies of the structure of the myxovirus particles by negative staining techniques, *Virology* **11**:79.

Hosaka, Y., and Shimizu, K., 1977, Cell fusion by Sendai virus, in: *Virus Infection and the Cell Surface*, Vol. 2 of *Cell Surface Reviews* (G. Poste and G. L. Nicolson, eds.), pp. 129–156, North-Holland, Amsterdam.

Howe, C., and Morgan, C., 1969, Interactions between Sendai virus and human erythrocytes, *J. Virol.* **3**:70.

Howe, C., Lee, L. T., Harboe, A., and Haukenes, G., 1967, Immunochemical study of influenza virus and associated host tissue components, *J. Immunol.* **98**:543.

Hoyle, L., 1962, The entry of myxoviruses into the cell, *Cold Spring Harbor Symp. Quant. Biol.* **27**:113.

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

Huang, R. T. C., 1977, Transfer of glycolipid between membranes of tissue culture cells, using dansylcerebroside as a model, *Z. Naturforsch.* **32c**:379.

Huang, R. T. C., and Orlich, M., 1972, Substrate specificities of the neuraminidase of Newcastle disease virus and fowl plague virus, *Z. Physiol. Chem.* **353**:318.

Huang, R. T. C., Rott, R., and Klenk, H.-D., 1973, On the receptor for influenza viruses. I. Artificial receptor for influenza virus, *Z. Naturforsch.* **28c**:342.

Hubbell, W. L., and McConnell, H. M., 1971, Molecular motion in spin-labeled phospholipids and membranes, *J. Am. Chem. Soc.* **93**:314.

Hughes, F., and Pedersen, C. E., 1975, Paramagnetic spin label interactions with the envelope of a group A arbovirus: Lipid organization, *Biochim. Biophys. Acta* **394**:102.

Hummeler, K., Koprowski, H., and Wiktor, T. J., 1967, Structure and development of rabies virus in tissue culture, *J. Virol.* **1**:152.

Hung, P. P., Robinson, H. L., and Robinson, W. S., 1971, Isolation and characterization of proteins from Rous sarcoma virus, *Virology* **43**:251.

Hunt, L. A., and Summers, D. F., 1976*a*, Association of vesicular stomatitis virus proteins with HeLa cell membranes and released virus, *J. Virol.* **20**:637.

Hunt, L. A., and Summers, D. F., 1976*b*, Glycosylation of vesicular stomatitis virus glycoprotein in virus-infected HeLa cells, *J. Virol.* **20**:646.

Hunter, E., Friis, R. R., and Vogt, P. K., 1974, Inhibition of avian sarcoma virus replication by glucosamine, *Virology* **58**:449.

Hunter, E., Hayman, M. J., Rongey, R. W., and Vogt, P. K., 1976, An avian sarcoma virus mutant which is temperature-sensitive for virion assembly, *Virology* **69**:35.

Ichihashi, Y., and Dales, Y., 1971, Biogenesis of poxviruses: Interrelationship between hemagglutinin production and polykaryocytosis, *Virology* **46**:533.

Igarashi, A., Harrap, A. A., Casals, J., and Stollar, V., 1976, Morphological, biochemical and serological studies on a viral agent (CFA) which replicates and causes fusion of Aedes albopictus (Singh) cells, *Virology* **74**:174.

Ihle, J. M., Hanna, M. G., Jr., Schäfer, W., Hunsmann, G., Bolognesi, D. P., and Huper, G., 1975, Polypeptides of mammalian oncornaviruses. III. Localization of p15 and reactivity with natural antibody, *Virology* **63**:60.

Ihle, J. N., Lee, J. C., Collins, J. J., Fischinger, P. J., Pazmino, N. H., Moenning, V., Schäfer, W., Hanna, M. G., and Bolognesi, D. P., 1976, Characterization of the immune response to the major glycoprotein (gp71) Friend leukemia virus. II. Response in C57BL/6 mice, *Virology* **75**:88.

Ikeda, H., Hardy, W. J., Tress, E., and Fleissner, E., 1975, Chromatographic separation and antigenic analysis of proteins of the oncornaviruses V. Identification of a new murine viral protein, *J. Virol.* **16**:53.

Inglis, S. C., Carroll, A. R., Lamb, R. A., and Mahy, B. W. J., 1976, Polypeptides specified by the influenza virus genome, *Virology* **74**:489.

Ishizaki, R., and Vogt, P. K., 1966, Immunological relationships among envelope antigens of avian tumor viruses, *Virology* **30**:375.

Ivanic, S., 1974, Identification of two envelope proteins of Semliki Forest virus before and after treatment with Triton X-100, *Arch. Ges. Virusforsch.* **44**:164.

Jensik, S. C., and Silver, S., 1976, Polypeptides of mumps virus, *J. Virol.* **17**:363

Johnson, I., and Clamp, J. R., 1971, The oligosaccharides of human type L immunoglobulin M (macroglobulin), *Biochem. J.* **123**:739.

Joseph, B. S., and Oldstone, M. B. A., 1974, Antibody-induced redistribution of measles virus antigens on the cell surface, *J. Immunol.* **113**:1205.

Kaluza, G., 1975, Effect of impaired glycosylation on the biosynthesis of Semliki Forest virus glycoproteins, *J. Virol.* **16**:602.

Kaluza, G., 1976, Early synthesis of Semliki Forest virus-specific proteins in infected chicken cells, *J. Virol.* **19**:1.

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

Kaluza, G., Schmidt, M. F. G., and Scholtissek, C., 1973, Effect of 2-deoxy-D-glucose on the multiplication of Semliki Forest virus and the reversal of the block by mannose, *Virology* **54**:179.

Kaluza, G., Kraus, A. A., and Rott, R., 1976, Inhibition of cellular protein synthesis by simultaneous pretreatment of host cells with fowl plague virus and actinomycin D: A method for studying early protein synthesis of several RNA viruses, *J. Virol.* **17**:1.

Kang, C. Y., and Prevec, L., 1969, Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens, *J. Virol.* **3**:404.

Kaplan, A. S., 1974, *The Herpes Viruses*, Academic Press, New York.

Kaplan, A. S., and Ben-Porat, T., 1970, Synthesis of proteins in cells infected with herpesvirus. VI. Characterization of proteins of the viral membrane, *Proc. Natl. Acad. Sci. USA* **66**:799.

Kaplan, A. S., and Ben-Porat, T., 1976, Synthesis of proteins in cells infected with herpes virus. XI. Sulfated, structural glycoproteins, *Virology* **70**:561.

Kates, M., Allison, A. C., Tyrrell, D. A., and James, A. T., 1961, Lipids of influenza virus and their relation to those of the host cell, *Biochim. Biophys. Acta* **52**:455.

Katz, E., and Moss, B., 1970, Formation of a vaccinia virus structural polypeptide from a higher molecular weight precursor: Inhibition by rifampicin, *Proc. Natl. Acad. Sci. USA* **66**:677.

Katz, F. V., Rothman, J. E., Leingappa, V. R., Blobel, G., and Lodish, H. R., 1977, Membrane assembly in vitro: Synthesis, glycosylation, and asymmetric insertion of a transmembrane protein, *Proc. Natl. Acad. Sci. USA* **74**:3278.

Keegstra, K., Sefton, B., and Burke, D., 1975, Sindbis virus glycoproteins: Effect of the host cell on the oligosaccharide, *J. Virol.* **16**:613.

Keil, W., Klenk, H.-D., and Schwarz, R. T., 1978, Charakterisierung des Bindungstyps zwischen dem Kohlenhydrat-und Proteinanteil von Influenzavirus-glykoproteinen, *Z. Physiol. Chem.* **359**:283.

Kelley, J. M., Wagner, R. R., and Emerson, S. U., 1972, The glycoprotein of vesicular stomatitis virus is the antigen that gives rise to and reacts with neutralizing antibody, *J. Virol.* **10**:1231.

Kemp, M. C., Perdue, M. L., Rogers, H. W., O'Callaghan, D. J., and Randall, C. C. 1974, Structural polypeptides of the hamster strain of equine herpes virus type 1: Products associated with purification, *Virology* **61**:361.

Kemp, M. C., Wise, K. S., Edlund, L. E., Acton, R. T., and Compans, R. W., 1978, Origin of minor glycoproteins of murine leukemia viruses. *J. Virol.* **28**:84.

Kendal, A. P., 1975, A comparison of "influenza C" with prototype myxoviruses: Receptor-destroying activity, neuraminidase and structural polypeptides, *Virology* **65**:87.

Kendal, A. P., and Eckert, E. A., 1972, The preparation and properties of $^{14}$C-carboxyamidomethylated subunits from $A_2$/1957 influenza neuraminidase, *Biochim. Biophys. Acta* **258**:484.

Kennedy, S. I. T., 1972, Isolation and identification of the virus-specified RNA species found on membrane-bound polyribosomes of chick embryo cells infected with Semliki Forest virus, *Biochem. Biophys. Res. Commun.* **38**:1254.

Kennedy, S. I. T., 1974, The effect of enzymes on structural and biological properties of Semliki Forest virus, *J. Gen. Virol.* **23**:129.

Kennel, S. J., 1976, Purification of a glycoprotein from mouse ascites fluid by immunoaffinity chromatography which is related to the major glycoprotein of murine leukemia viruses, *J. Biol. Chem.* **251**:6197.

Keranen, S., and Kääriäinen, L., 1975, Proteins synthesized by Semliki Forest virus and its 16 temperature-sensitive mutants, *J. Virol.* **16**:388.

Kilbourne, E. D., 1959, Inhibition of influenza virus multiplication with a glucose antimetabolite (2-deoxy-D-glucose), *Nature* (*London*) **183**:271.

Kilbourne, E. D., 1975, *The Influenza Viruses and Influenza*, Academic Press, New York.

Kilbourne, E. D., Laver, W. G., Shulmann, J. L., and Webster, R. G., 1968, Antiviral activity of antiserum specific for an influenza virus neuraminidase, *J. Virol.* **2**:28.

Kingsbury, D. W., 1973, Paramyxovirus replication, *Curr. Top. Microbiol. Immunol.* **59**:1.

Klenk, E., Faillard, H., and Lempfried, H., 1955, Über die enzymatische Wirkung von Influenzavirus, *Z. Physiol. Chem.* **301**:235.

Klenk, H.-D., 1974, Viral envelopes and their relationship to cellular membranes, *Curr. Top. Microbiol. Immunol.* **68**:29.

Klenk, H.-D., and Choppin, P. W., 1969*a*, Chemical composition of the parainfluenza virus SV5, *Virology* **37**:155.

Klenk, H.-D., and Choppin, P. W., 1969*b*, Lipids of plasma membranes of monkey and hamster kidney cells and of parainfluenza virions grown in these cells, *Virology* **38**:255.

Klenk, H.-D., and Choppin, P. W., 1970*a*, Plasma membrane lipids and parainfluenza virus assembly, *Virology* **40**:939.

Klenk, H.-D., and Choppin, P. W., 1970*b*, Glycosphingolipids of plasma membranes of cultured cells and an enveloped virus (SV5) grown in these cells, *Proc. Natl. Acad. Sci. USA* **66**:57.

Klenk, H.-D., and Choppin, P. W., 1971, Glycolipid content of vesicular stomatitis virus grown in baby hamster kidney cells, *J. Virol.* **7**:416.

Klenk, H.-D., and Rott, R., 1973, Formation of influenza virus proteins, *J. Virol.* **11**:823.

Klenk, H.-D., Caliguiri, L. A., and Choppin, P. W., 1970*a*, The proteins of the parainfluenza virus SV5. II. The carbohydrate content and glycoproteins of the virion, *Virology* **42**:473.

Klenk, H.-D., Compans, R. W., and Choppin, P. W., 1970*b*, An electron microscopic study of the presence or absence of neuraminic acid in enveloped viruses, *Virology* **42**:1158.

Klenk, H.-D., Rott, R., and Becht, H., 1972*a*, On the structure of the influenza virus envelope, *Virology* **47**:579.

Klenk, H.-D., Scholtissek, C., and Rott, R., 1972*b*, Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose, *Virology* **49**:723.

Klenk, H.-D., Wöllert, W., Rott, R., and Scholtissek, C., 1974, Association of influenza virus proteins with cytoplasmic fractions, *Virology* **57**:28.

Klenk, H.-D., Rott, R., Orlich, M., and Blödorn, J., 1975, Activation of influenza A viruses by trypsin treatment, *Virology* **68**:426.

Klenk, H.-D., Nagai, Y., Rott, R., and Nicolau, C., 1977*a*, The structure and function of paramyxovirus glycoproteins, *Med. Microbiol. Immunol.* **164**:35.

Klenk, H., Rott, R., and Orlich, M. 1977*b*, Further studies on the activation of influenza virus by proteolytic cleavage of the hemagglutinin, *J. Gen. Virol.* **36**:151.

Klenk, H.-D., Schwarz, R. T., Schmidt, M. F. G., and Wollert, W., 1978, The structure and biosynthesis of the carbohydrate moiety of the influenza virus hemagglutinin, *Top. Infect. Dis.* **3**:83.

Knight, C. A., 1944, A sedimentable component of allantoic fluid and its relationship to influenza viruses, *J. Expl. Med.* **30**:83.

Knight, C. A., 1946, Precipitin reactions of highly purified influenza viruses and related materials, *J. Expl. Med.* **83**:281.

Knipe, D. M., Baltimore, D., and Lodish, H. F., 1977*a*, Separate pathways of maturation of the major structural proteins of vesicular stomatitis virus, *J. Virol.* **21**:1128.

Knipe, D. M., Baltimore, D., and Lodish, H. F., 1977*b*, Maturation of viral proteins in cells infected with temperature-sensitive mutants of vesicular stomatitis virus, *J. Virol.* **21**:1149.

Knipe, D. M., Lodish, H. F., and Baltimore, D., 1977*c*, Localization of two cellular forms of the vesicular stomatitis viral glycoprotein, *J. Virol.* **21**:1121.

Knowles, R. W., and Person, S., 1976, Effects of 2-deoxyglucose, glucosamine, and mannose on cell fusion and the glycoproteins of herpes simplex virus, *J. Virol.* **18**:644.

Krag, S. S., and Robbins, P. W., 1977, Sindbis envelope proteins as endogenous acceptors in reactions of guanosine diphosphate-($^{14}$C)mannose with preparations of infected chicken embryo fibroblasts, *J. Biol. Chem.* **252**:2621.

Krantz, M. J., Lee, Y. C., and Hung, P. P., 1976, Characterization and comparison of the major glycoprotein from three strains of Rous sarcoma virus, *Arch. Bioch. Biophys.* **174**:66.

Krantz, M. J., Strand, M., and August, J. T., 1977, Biochemical and immunological characterization of the major envelope glycoprotein gp69/71 and degradation fragments from Rauscher leukemia virus, *J. Virol.* **22**:804.

Krug, R. M., and Etkind, P. R., 1973, Cytoplasmic and nuclear virus-specific proteins in influenza virus-infected MDCK cells, *Virology* **56**:334.

Lachmi, B., and Kääriäinen, L., 1976, Sequential translation of nonstructural proteins in cells infected with a Semliki Forest virus mutant, *Proc. Natl. Acad. Sci. USA* **73**:1936.

Lachmi, B., Glanville, N., Keranen, S., and Kääriäinen, L., 1975, Tryptic peptide analysis of nonstructural and structural precursor proteins from Semliki Forest virus mutant-infected cells, *J. Virol.* **16**:1615.

Lafay, F., 1974, Envelope proteins of vesicular stomatitis virus: Effect of temperature-sensitive mutations in complementation groups III and IV, *J. Virol.* **14**:1120.

Lafferty, K. J., and Oertilis, S., 1963, The interaction between virus and antibody III. Examination of virus–antibody complexes with the electron microscope, *Virology* **21**:91.

Lagwinska, E., Stewart, C. C., Adless, C., and Schlesinger, S., 1975, Replication of lactic dehydrogenase virus and Sindbis virus in mouse peritoneal macrophages: Induction of interferon and phenotypic mixing, *Virology* **65**:204.

Lai, M. M., and Duesberg, P. H., 1972, Differences between the envelope glycoproteins and glycopeptides of avian tumor viruses released from transformed and from nontransformed cells, *Virology* **50**:359.

Laine, R., Kettunen, M.-L., Gahmberg, C. G., Kääriäinen, L., and Renkonen, O., 1972, Fatty chains of different lipid classes of Semliki Forest virus and host cell membranes, *J. Virol.* **10**:433.

Laine, R., Soderlund, H., and Renkonen, O., 1973, Chemical composition of Semliki Forest virus, *Intervirology* **1**:110.

Lamb, R. A., and Choppin, P. W., 1976, Synthesis of influenza virus proteins in infected cells: Translation of viral polypeptides, including three P polypeptides, from RNA produced by primary transcription, *Virology* **74**:504.

Lamb, R. A., and Choppin, P. W., 1977, The synthesis of Sendai virus polypeptides in infected cells. II. Intracellular distribution of polypeptides, *Virology* **81**:371.

Landsberger, F. R., and Compans, R. W., 1976, Effect of membrane proteins on the lipid bilayer structure: A spin label ESR study of vesicular stomatitis virus, *Biochemistry* **15**:2356.

Landsberger, F. R., Lenard, J., Paxton, J., and Compans, R. W., 1971, Spin label ESR study of the lipid-containing membrane of influenza virus, *Proc. Natl. Acad. Sci. USA* **68**:2579.

Landsberger, F. R., Compans, R. W., Choppin, P. W., and Lenard, J., 1973, Organization of the lipid phase in viral membranes: Effects of independent variation of the lipid and the protein composition, *Biochemistry* **12**:4498.

Landsberger, F. R., Lyles, D. S., and Choppin, P. W., 1978, Enveloped viruses: Their

structure and interaction with cells, in: *Negative Strand Viruses and the Host Cell* (R. D. Barry and B. W. J. Mahy, eds.), Academic Press, New York.

Laver, W. G., 1971, Separation of two polypeptide chains from the hemagglutinin subunit of influenza virus, *Virology* **45**:275.

Laver, W. G., 1973, The polypeptides of influenza virus, *Adv. Virus Res.* **18**:57.

Laver, W. G., and Baker, N., 1972, Amino acid composition of polypeptides from influenza virus particles, *J. Gen. Virol.* **17**:61.

Laver, W. G., and Downie, J. C., 1976, Influenza virus recombination. 1. Matrix protein markers and segregation during mixed infection, *Virology* **70**:105.

Laver, W. G., and Valentine, R. C., 1969, Morphology of the isolated hemagglutinin and neuraminidase subunits of influenza virus, *Virology* **38**:105.

Laver, W. G., and Webster, R. G., 1966, The structure of influenza viruses. IV. Chemical studies of the host antigen, *Virology* **30**:104.

Laver, W. G., and Webster, R. G., 1972, Studies on the origin of pandemic influenza. II. Peptide maps of the light and heavy polypeptide chains from the hemagglutinin subunits of $A_2$ influenza viruses isolated before and after the appearance of Hong Kong influenza, *Virology* **48**:445.

Laver, W. G., Downie, J. C., and Webster, R. G., 1974, Studies on antigenic variation of influenza virus: Evidence for multiple antigenic determinants on the hemagglutinin subunits of A/Hong Kong/68 ($H_3N_2$) virus and the A/England/72 strains, *Virology* **59**:230.

Lazarowitz, S. G., and Choppin, P. W., 1975, Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the hemagglutinin polypeptide, *Virology* **68**:440.

Lazarowitz, S. G., Compans, R. W., and Choppin, P. W., 1971, Influenza virus structural and nonstructural proteins in infected cells and their plasma membranes, *Virology* **46**:830.

Lazarowitz, S. G., Compans, R. W., and Choppin, P. W., 1973*a*, Proteolytic cleavage of the hemagglutinin polypeptide of influenza virus: Function of the uncleaved polypeptide HA, *Virology* **52**:199.

Lazarowitz, S. G., Goldberg, A. R., and Choppin, P. W., 1973*b*, Proteolytic cleavage by plasmin of the HA polypeptide of influenza virus: Host cell activation of serum plasminogen, *Virology* **56**:172.

Lazdins, I., Haslam, E. A., and White, D. O., 1972, The polypeptides of influenza virus. VI. Composition of the neuraminidase, *Virology* **49**:758.

Leamnson, R. N., and Halpern, M. S., 1976, Subunit structure of the glycoprotein complex of avian tumor virus, *J. Virol.* **18**:956.

Leamnson, R. N., Shander, M. H. M., and Halpern, M. S., 1977, A structural protein complex in Moloney leukemia virus, *Virology* **76**:437.

Leavitt, R., Schlesinger, R., and Kornfeld, S., 1977, Tunicamycin inhibits glycosylation and multiplication of Sindbis and vesicular stomatitis viruses, *J. Virol.* **21**:375.

Lee, L. T., Howe, C., Meyer, K., and Choi, H. U., 1969, Quantitative precipitin analysis of influenza virus host antigen and of sulfated polysaccharides of chicken embryonic allantoic fluid, *J. Immunol.* **102**:1144.

Lenard, J., and Compans, R. W., 1974, The membrane structure of lipid-containing viruses, *Biochim. Biophys. Acta* **344**:51.

Lenard, J., and Compans, R. W., 1975, Polypeptide composition of incomplete influenza virus, *Virology* **65**:418.

Lenard, J., and Rothman, J. E. 1976, Transbilayer distribution and movement of cholesterol and phospholipid in the membrane of influenza virus, *Biochemistry* **73**:391.

Lenard, J., Landsberger, F. R., Wong, C. Y., Choppin, P. W., and Compans, R. W., 1974, Organization of lipid and protein in viral membranes: Spin label and fluorescent probe studies, in: *Negative Strand Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 823–833, Academic Press, New York.

Lenard, J., Tsai, D. K., Compans, R. W., and Landsberger, F. R., 1976, Observations on the membrane organization of standard and incomplete influenza grown in MDBK cells, *Virology* **71**:389.

Lohmeyer, J., Talens, L. T., and Klenk, H.-D., 1977, Biosynthese der Membrankomponenten des Influenzavirus im abortiven Zyklus, *Z. Physiol. Chem.* **358**:271.

Ludwig, H., Becht, H., and Rott, R., 1974, Inhibition of herpes virus-induced cell fusion by concanavalin A, antisera, and 2-deoxy-D-glucose, *J. Virol.* **14**:307.

Luukkonen, A., Kääriäinen, L., and Renkonen, O., 1976, Phospholipids of Semliki Forest virus grown in cultured mosquito cells, *Biochim. Biophys. Acta* **450**:109.

Luukkonen, A., Gahmberg, C. G., and Renkonen, O., 1977*a*, Surface labeling of Semliki Forest virus glycoproteins using galactose-oxidase, *Virology* **76**:55.

Luukkonen, A., von Bonsdorff, C.-H., and Renkonen, O., 1977*b*, Characterization of Semliki Forest virus grown in mosquito cells: Comparison with the virus from hamster cells, *Virology* **78**:331.

Lyles, D. S., and Landsberger, F. R., 1977, Sendai virus-induced hemolysis: Reduction in heterogeneity of erythrocyte lipid bilayer fluidity, *Proc. Natl. Acad. Sci USA* **74**:1918.

Maeda, T., Asano, A., Ohki, K., Okada, Y., and Ohnishi, S.-I., 1975, A spin-label study on fusion of red blood cells induced by hemagglutinating virus of Japan, *Biochemistry* **14**:3736.

Maeda, T., Asano, A., Okada, Y., and Ohnishi, S.-I., 1977, Transmembrane phospholipid motions induced by F glycoprotein in hemagglutinating virus of Japan, *J. Virol.* **21**:232.

Majuk, Z., Bittman, R., Landsberger, F. R., and Compans, R. W., 1977, Effects of filipin on the structure and biological activities of enveloped viruses, *J. Virol.* **24**:883.

Marquardt, H., Gilden, R. V., and Oroszlan, S., 1977, Envelope glycoproteins of Rauscher murine leukemia virus: Isolation and chemical characterization, *Biochemistry* **16**:710.

Martinez-Segovia, Z. M., and DeMitri, M. I., 1977, Junin virus structural proteins, *J. Virol.* **21**:579.

Matsumoto, S., and Kawai, A., 1969, Comparative studies on development of rabies virus in different host cells, *Virology* **39**:449.

Matsumoto, S., Schneider, L. G., Kawai, A., and Yonezawa, T., 1974, Further studies on the replication of rabies and rabies-like viruses in organized cultures of mammalian neural tissues, *J. Virol.* **19**:981.

Mattila, K., Luukkonen, A., and Renkonen, O., 1976, Protein-bound oligosaccharides of Semliki Forest virus, *Biochim. Biophys. Acta* **419**:435.

McCarthy, M., and Harrison, S. C., 1977, Glycosidase susceptibility: A probe for the distribution of glycoprotein oligosaccharides in Sindbis virus, *J. Virol.* **23**:61.

McClelland, L., and Hare, R., 1941, The adsorption of influenza virus by red cells and

a new in vitro method of measuring antibodies for influenza virus, *Can. Pub. Health J.* **32**:530.

McConnell, H. M., and McFarland, B. G., 1972, The flexibility gradient in biological membranes, *Ann. N.Y. Acad. Sci.* **195**:207.

McGeoch, D., Fellner, P., and Newton, C., 1976, Influenza virus genome consists of eight distinct RNA species, *Proc. Natl. Acad. Sci. USA* **73**:3045.

McLellan, W. L., and August, J. T., 1976, Analysis of the envelope of Rauscher murine oncornavirus: In vitro labeling of glycopeptides, *J. Virol.* **20**:627.

McLerran, C. J., and Arlinghaus, R. B., 1973, Structural components of a virus of the California encephalitis complex: La Crosse virus, *Virology* **53**:247.

McSharry, J. J., and Wagner, R. R., 1971*a*, Lipid composition of purified vesicular stomatitis viruses, *J. Virol.* **7**:59.

McSharry, J. J., and Wagner, R. R., 1971*b*, Carbohydrate composition of vesicular stomatitis virus, *J. Virol.* **7**:412.

McSharry, J. J., Compans, R. W., and Choppin, P. W., 1971, Proteins of vesicular stomatitis virus and phenotypically mixed vesicular stomatitis–simian virus 5 virions, *J. Virol.* **8**:722.

McSharry, J. J., Compans, R. W., Lackland, H., and Choppin, P. W., 1975, Isolation and characterization of the non-glycosylated membrane protein and a nucleocapsid complex from the paramyxovirus SV5, *Virology* **67**:365.

Meier-Ewert, H., and Compans, R. W., 1974, Time course of synthesis and assembly of influenza virus proteins, *J. Virol.* **14**:1083.

Meier-Ewert, H., Compans, R. W., Bishop, D. H. L., and Herrler, G., 1978, in: *Negative Strand Viruses and the Host Cell* (R. D. Barry and B. W. J. Mahy, eds.), pp. 127–133, Academic Press, New York.

Miller, G. L., Lauffer, M. A., and Stanley, W. M., 1944, Electrophoretic studies on PR8 influenza virus, *J. Exp. Med.* **80**:549.

Mitchiner, M. B., 1969, The envelope of vaccinia and of viruses: An electron-cytochemical investigation, *J. Gen. Virol.* **5**:211.

Miyamoto, H., and Kato, S., 1968, Immune hemadsorption by cells infected with poxvirus, *Biken J.* **11**:343.

Moelling, K., and Hayami, M., 1977, Analysis of precursors to the envelope glycoproteins of avian RNA tumor viruses in chicken and quail cells, *J. Virol.* **22**:598.

Moennig, V., Frank, H., Hunsmann, G., Schneider, Il, and Schäfer, W., 1974, Properties of mouse leukemia viruses. VII. The major viral glycoprotein of Friend leukemia virus. Isolation and physicochemical properties, *Virology* **61**:100.

Moore, D. H., Davies, M. C., Levine, S., and Englert, M. E., 1962, Correlation of structure with infectivity of influenza virus, *Virology* **17**:470.

Moore, N. F., Kelley, J. M., and Wagner, R. R., 1974, Envelope proteins of vesicular stomatitis virions: Accessibility to iodination, *Virology* **61**:292.

Moore, N. F., Barenholz, Y., and Wagner, R. R., 1976, Microviscosity of togavirus membranes studied by fluorescence depolarization: Influence of envelope proteins and the host cell, *J. Virol.* **19**:126.

Morgan, C., Rose, H. M., Holden, M., and James, E. P., 1959, Electron microscopic observations on the development of herpes simplex virus, *J. Exp. Med.* **110**:643.

Morgan, C., Rifkin, R. A., and Rose, H. M., 1962, The use of ferritin-conjugated antibodies in electron microscopic studies of influenza and vaccinia viruses, *Cold Spring Harbor Symp. Quant. Biol.* **27**:57.

Moroni, C., 1972, Structural proteins of Rauscher leukemia virus and Harvey sarcoma virus, *Virology* **47**:1.

Morrison, T. G., and Lodish, H. F., 1975, Site of synthesis of membrane and non-membrane proteins of vesicular stomatitis virus, *J. Biol. Chem.* **250**:6955.

Moss, B., 1974, Reproduction of poxviruses, in: *Comprehensive Virology*, Vol. 3 (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 405–474, Plenum Press, New York.

Moss, B., and Rosenblum, E. N., 1972, Hydroxylapatite chromatography of protein–sodium dodecyl sulfate complexes: A new method for the separation of polypeptide subunits, *J. Biol. Chem.* **247**:5194.

Moss, B., and Salzman, N. P., 1968, Sequential protein synthesis following vaccinia virus infection, *J. Virol.* **2**:1016.

Moss, B., Rosenblum, E. N., Katz, E., and Grimley, P. M., 1969, Rifampicin: A specific inhibitor of vaccinia virus assembly, *Nature (London)* **224**:1280.

Moss, B., Rosenblum, E. N., and Garon, C. F., 1973, Glycoprotein synthesis in cells infected with vaccinia virus. III. Purification and biosynthesis of the virion glycoprotein, *Virology* **55**:143.

Mosser, A. C., Montelaro, R. C., Rueckert, R. R., 1977, Proteins of Rous-associated virus type 61: Polypeptide stoichiometry and evidence that glycoprotein gp37 is not a cleavage product of gp85, *J. Virol.* **23**:1079.

Mountcastle, W. E., and Choppin, P. W., 1977, A comparison of the polypeptides of four measles virus strains, *Virology* **78**:463.

Mountcastle, W. E., Compans, R. W., and Choppin, P. W., 1971, Proteins and glycoproteins of paramyxoviruses: A comparison of Simian virus 5, Newcastle disease virus and Sendai virus, *J. Virol.* **7**:47.

Moyer, S. A., and Summers, D. F., 1974, Vesicular stomatitis virus envelope glycoprotein alterations induced by host cell transformation, *Cell* **2**:63.

Moyer, S. A., Tsang, J. M., Atkinson, P. H., and Summers, D. F., 1976, Oligosaccharide moieties of the glycoprotein of vesicular stomatitis virus, *J. Virol.* **18**:167.

Mudd, J. A., 1973, Effects of pH on the structure of vesicular stomatitis virus, *Virology* **55**:546.

Mudd, J. A., 1974, Glycoprotein fragment associated with vesicular stomatitis virus after proteolytic digestion, *Virology* **62**:573.

Murphy, F. A., Webb, P. A., Johnson, K. M., and Whitfield, S. G., 1969, Morphological comparison of Machupo with lymphocytic choriomeningitis virus: Basis for a new taxonomic group, *J. Virol.* **4**:535.

Murphy, F. A., Webb, P. A., Johnson, K. M., Whitfield, S. G., and Chappell, W. A., 1970, Arenoviruses in vero cells: Ultrastructural studies, *J. Virol.* **6**:507.

Murphy, F. A., Whitfield, S. G., Webb, P. A., and Johnson, K. M., 1973, Ultrastructural studies of arenaviruses, in: *Lymphocytic Choriomeningitis Virus and Other Arenaviruses* (F. Lehmann-Grube, ed.), pp. 273–285, Springer, Berlin.

Murphy, J. S., and Bang, F. B., 1952, Observations with the electron microscope on cells of the chick chorio-allantoic membrane infected with influenza virus, *J. Exp. Med.* **95**:259.

Mussgay, M., and Rott, R., 1964, Studies on the structure of a hemagglutinin component of a group A arbovirus (Sindbis), *Virology* **23**:573.

Nagai, Y., and Klenk, H.-D., 1977, Activation of precursors to both glycoproteins of NDV by proteolytic cleavage, *Virology* **77**:125.

Nagai, Y., Yoshida, T., Yoshii, S., Maeno, K., and Matsumoto, T., 1975, Modification

of normal cell surface by smooth membrane preparations from BHK-21 cells infected with Newcastle disease virus, *Med. Microbiol. Immunol.* **161**:175.

Nagai, Y., Klenk, H.-D., and Rott, R., 1976*a*, Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus, *Virology* **72**:494.

Nagai, Y., Ogura, H., and Klenk, H.-D., 1976*b*, Studies on the assembly of the envelope of Newcastle disease virus, *Virology* **69**:523.

Nagington, J., and Horne, R. W., 1962, Morphological studies of orf and vaccinia viruses, *Virology* **16**:248.

Nakamura, K., and Compans, R. W., 1977, The cellular site of sulfation of influenza virus glycoproteins, *Virology* **79**:381.

Nakamura, K., and Compans, R. W., 1978*a*, Effects of glucosamine, 2-deoxy-D-glucose and tunicamycin on glycosylation, sulfation and assembly of influenza virus glycoproteins, *Virology* **84**:303.

Nakamura, K., and Compans, R. W., 1978*b*, Glycopeptide components of influenza viral glycoproteins, *Virology* **86**:432.

Naso, R. B., Arcement, L. J., and Arlinghaus, R. B., 1975, Biosynthesis of Rauscher leukemia viral proteins, *Cell* **4**:31.

Naso, R. B., Arcement, L. J., Karshin, W. L., Jamjoom, G. A., and Arlinghaus, R. B., 1976, A fucose-deficient glycoprotein precursor to Rauscher leukemia virus gp69, 71, *Proc. Natl. Acad. Sci. USA* **73**:2326.

Nermut, M. V., Frank, H., and Schäfer, W., 1972, Properties of mouse leukemia virus. III. Electron microscopic appearance as revealed after conventional preparation techniques as well as freeze-drying and freeze-etching, *Virology* **49**:345.

Nerome, K., Ishida, M., and Nakayama, M., 1976, Absence of neuraminidase from influenza C virus, *Arch. Virol.* **50**:241.

Neurath, A. R., Vernon, S. K., Dobkin, M. B., and Rubin, B. A., 1972, Characterization of subviral components resulting from treatment of rabies virus with tri-(*n*-butyl) phosphate, *J. Gen. Virol.* **14**:33.

Norrby, E., and Gollmar, Y., 1975, Identification of measles virus-specific hemolysis-inhibiting antibodies separate from hemagglutination-inhibiting antibodies, *Infect. Immun.* **11**:231.

Obijeski, J. F., Bishop, D. H. L., Murphy, F. A., and Palmer, E. L., 1976, The structural proteins of La Crosse virus, *J. Virol.* **19**:985.

O'Callaghan, D. J., and Randall, C. C., 1976, Molecular anatomy of herpesviruses: Recent studies, *Progr. Med. Viol.* **22**:152.

Ogura, H., Schmidt, M. F. G., and Schwarz, R. T., 1977, Effect of tunicamycin on the morphogenesis of Semliki Forest virus and Rous sarcoma virus, *Arch. Virol.* **55**:155.

Oram, J. D., Ellwood, D. C., Appleyard, G., and Stanley, J. C., 1971, Agglutination of an arbovirus by concanavalin A, *Nature (London) New Biol.* **233**:50.

Oshiro, L. S., 1973, Coronaviruses, in: *Ultrastructure of Animal Viruses and Bacteriophages* (A. J. Dalton and F. Haguenau, eds.), pp. 331–335, Academic Press, New York.

Palese, P., 1977, The genes of influenza virus, *Cell* **10**:1.

Palese, P., and Compans, R. W., 1976, Inhibition of influenza virus replication in tissue culture by 2-deoxy-2,3-dehydro-*N*-trifluoroacetylneuraminic acid (FANA): Mechanism of action, *J. Gen. Virol.* **33**:159.

Palese, P., and Schulman, J. L., 1974, Isolation and characterization of influenza

virus recombinants with high and low neuraminidase activity: Use of 2-(3'-methoxyphenyl) *N*-acetylneuraminic acid to identify clone populations, *Virology* **57**:227.

Palese, P., Tobita, K., Ueda, M., and Compans, R. W., 1974, Characterization of temperature sensitive influenza virus mutants defective in neuraminidase, *Virology* **61**:397.

Paul, S. D., Singh, K. R. P., and Bhat, U. K. M., 1969, A study of the cytopathic effect of arboviruses on cultures from *Aedes albopictus* cell line, *Indian J. Med. Res.* **57**:339.

Pedersen, I. R., 1971, Lymphocytic choriomeningitis virus RNAs, *Nature* (*London*) *New Biol.* **234**:112.

Pedersen, I. R., 1973, Different classes of ribonucleic acid isolated from lymphocytic choriomeningitis virus, *J. Virol.* **11**:416.

Pedersen, C. E., and Eddy, G. A., 1974, Separation, isolation, and immunological studies of the structural proteins of Venezuelan equine encephalomyelitis virus, *J. Virol.* **14**:740.

Pedersen, C. E., Marker, S. C., and Eddy, G. A. 1974, Comparative electrophoretic studies on the structural proteins of selected group A arboviruses, *Virology* **60**:312.

Peluso, R. W., Lamb, R. A., and Choppin, P. W., 1977, Polypeptide synthesis in SV5-infected cells, *J. Virol.* **23**:177.

Perdue, M. L., Kemp, M. C., Randall, C. C., and O'Callaghan, D. J., 1974, Studies of the molecular anatomy of the L-M cell strain of equine herpes virus type 1: Proteins of the nucleocapsid and intact virion, *Virology* **59**:201.

Pesonen, M., and Renkonen, O., 1976, Serum glycoprotein-type sequence of monosaccharides in membrane glycoproteins of Semliki Forest virus, *Biochim. Biophys. Acta* **455**:510.

Peters, D., 1956, Morphology of resting vaccinia virus, *Nature* (*London*) **178**:1453.

Pettersson, R., and Kääriäinen, L., 1973, The ribonucleic acids of Uukuniemi virus, a non-cubical tick-borne arbovirus, *Virology* **56**:608.

Pettersson, R., and von Bonsdorff, C.-H., 1975, Ribonucleoproteins of Uukuniemi virus are circular, *J. Virol.* **15**:386.

Pfau, C. J., Bergold, G. H., Casals, J., Johnson, K. M., Murphy, F. A., Pedersen, I. R., Rawls, W. P., Webb, P. A., and Weissenbacher, M. C., 1974, Arenaviruses, *Intervirology* **4**:207.

Pfefferkorn, E. R., and Hunter, H. S., 1963*a*, Purification and partial chemical analysis of Sindbis virus, *Virology* **20**:443.

Pfefferkorn, E. R., and Hunter, H. S., 1963*b*, The source of the ribonucleic acid and phospholipid of Sindbis virus, *Virology* **20**:446.

Pinter, A., and Compans, R. W., 1975, Sulfated components of enveloped viruses, *J. Virol.* **16**:859.

Pons, M. W., 1976, A re-examination of influenza single- and double-stranded RNAs by gel electrophoresis, *Virology* **69**:789.

Porterfield, J. S., Casals, J., Shumakov, M. P., Gaidamovich, S. Y., Hannoun, C., Holmes, I. H., Horzinek, M. C., Mussgay, M., Oker-Blom, N, and Russell, P. K., 1976, Bunyaviruses and bunyaviridae, *Intervirol* **6**:13.

Portner, A., Scroggs, R. A., Marx, P. A., and Kingsbury, D. W., 1975, A temperature-sensitive mutant of Sendai virus with an altered hemagglutinin–neuraminidase polypeptide: Consequence for virus assembly and cytopathology, *Virology* **67**: 179.

Prochownik, E. V., Panem, S., and Kirsten, W. H., 1975, Biological and physical modifications of a murine oncornavirus by 2-deoxy-glucose, *J. Virol.* **15**:1323.

Quigley, J. P., Rifkin, D. B., and Reich, E., 1971, Phospholipid composition of Rous sarcoma virus, host cell membranes and other enveloped RNA viruses, *Virology* **46**:106.

Quigley, J. P., Rifkin, D. B., and Compans, R. W., 1972, Isolation and characterization of ribonucleoprotein substructures from Rous sarcoma virus, *Virology* **50**:65.

Ramos, B. A., Courtney, R. J., and Rawls, W. E., 1972, Structural proteins of Pichinde virus, *J. Virol.* **10**:661.

Rao, P. R., Bonar, R. A., and Beard, J. W., 1966, Lipids of the BAI strain A avian tumor virus and of the myeloblast host cell, *Exp. Mol. Pathol.* **5**:374.

Reginster, M., and Nermut, M. V., 1976, Preparation and characterization of influenza virus cores, *J. Gen. Virol.* **31**:211.

Renkonen, O., Kääriäinen, L., Simons, K., and Gahmberg, C. G., 1971, The lipid class composition of Semliki Forest virus and of plasma membranes of the host cells, *Virology* **36**:318.

Renkonen, O., Gahmberg, C. G., Simons, K., and Kääriäinen, L., 1972*a*, The lipids of the plasma membrane and endoplasmic reticulum from cultured baby hamster kidney cells (BHK-21), *Biochim. Biophys. Acta* **255**:66.

Renkonen, O., Kääriäinen, L., Gahmberg, C. G., and Simons, K., 1972*b*, Lipids of Semliki Forest virus and host cell membranes, in: *Current Trends in the Biochemistry of Lipids* (J. Ganguly and R. M. S. Smellie, eds.), pp. 407–422, Academic Press, New York.

Renkonen, O., Pesonen, M., and Mattila, K., 1976, Oligosaccharides of the membrane glycoproteins of Semliki Forest virus, in: *Structure of Biological Membranes* (L. Abrahamson and I. Pascher, eds.), pp. 409–423, Plenum Press, New York.

Richardson, C. D., and Vance, D. E., 1976, Biochemical evidence that Semliki Forest virus obtains its envelope from the plasma membrane of the host cell, *J. Biol. Chem.* **251**:5544.

Rifkin, D. B., and Compans, R. W., 1971, Identification of the spike proteins of Rous sarcoma virus, *Virology* **46**:485.

Rifkin, D. B., Compans, R. W., and Reich, E., 1972, A specific labeling procedure for proteins on the outer surface of membranes, *J. Biol. Chem.* **247**:6432.

Ritchey, M. B., Palese, P., and Kilbourne, E. D., 1976, RNAs of influenza A, B, and C viruses, *J. Virol.* **18**:738.

Robertson, B. H., Bhown, A. S., Compans, R. W., and Bennett, J. C., 1978, in: *Negative Strand Viruses and the Host Cell* (R. D. Barry and B. W. J. Mahy, eds.), pp. 213–219, Academic Press, New York.

Robertson, J. S., and Summers, D. F., 1977, Glycosylation of the glycoprotein of a thermolabile mutant of vesicular stomatitis virus, *J. Supramol. Struct. Suppl.* **1**:5.

Robertson, J. S., Etchison, J. R., and Summers, D. F., 1976, Glycosylation sites of vesicular stomatitis virus glycoprotein, *J. Virol.* **19**:871.

Robinson, D. J., and Watson, D. H., 1971, Structural proteins of herpes simplex virus, *J. Gen. Virol.* **10**:163.

Robinson, W. S., and Robinson, H. L., 1971, Envelope proteins of the avian tumor viruses, in: *Membrane Research* (C. Fred Fox, ed.), pp. 187–203, Academic Press, New York.

Rohrschneider, J. M., Diggelmann, H., Ogura, H., Friis, R. R., and Bauer, H., 1976, Defective cleavage of a precursor polypeptide in a temperature sensitive mutant of avian sarcoma virus, *Virology* **75**:177.

Roizman, B., and Furlong, D., 1974, The replication of herpesviruses, in: *Comprehensive Virology*, Vol. 3 (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 229–403, Plenum Press, New York.

Roizman, B., and Spear, P. G., 1971, The role of herpesvirus glycoproteins in the modification of membranes of infected cells, in: *Nucleic Acid–Protein Interactions: Nucleic Acid Synthesis in Viral Infection* (D. W. Ribbons, J. F. Woessner, and J. Schultz, eds.), pp. 435–455, North-Holland, New York.

Rosato, R. R., Dalrymple, J. M., Brandt, W. E., Cardiff, R. D., and Russell, P. K., 1974, Biophysical separation of major arbovirus serogroups, *Acta Virol.* **18**:25.

Rothman, J. E., and Lodish, H. F., 1977, Synchronised transmembrane insertion and glycosylation of a nascent membrane protein, *Nature (London)* **269**:775.

Rothman, J. E., Tsai, D. K., Dawidowicz, E. A., and Lenard, J., 1976, Transbilayer phospholipid asymmetry and its maintenance in the membrane of influenza virus, *Biochemistry* **15**:2361.

Rott, R., and Schäfer, W., 1960, Research on the hemagglutinizing non-infectious particles of influenza virus. 1. The production of "incomplete forms" of the virus of the classical fowl plague (Von Magnus phenomenon), *Z. Naturforsch.* **15b**:691.

Rott, R., Frank, H., and Schäfer, W., 1961, Isolierung und Eigenschaften der hemagglutinierenden Komponente des Virus der Newcastle disease, *Z. Naturforsch.* **16b**:625.

Rott, R., Reda, I. M., and Schäfer, W., 1963, Charakterisierung der verschiedenen, nach Infekten mit Newcastle disease Virus auftretenden, nichtinfektiosen, hemagglutinierenden Teilchen, *Z. Naturforsch.* **18b**:188.

Rott, R., Drzeniek, R., Saber, S., and Reichert, E., 1966, Blood group substances, Forssman and mononucleosis antigen in lipid-containing RNA viruses, *Arch. Ges. Virusforsch.* **19**:273.

Rott, R., Drzeniek, R., and Frank, H., 1970, On the structure of influenza viruses, in: *The Biology of Large RNA Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 74–85, Academic Press, London.

Rutter, G., and Mannweiler, K., 1976, Antibody-induced redistribution of virus antigens on the surface of influenza virus-infected cells, *J. Gen. Virol.* **33**:321.

Saleh, F., and Compans, R. W., 1978, Polypeptide synthesis in Tacaribe virus-infected cells, *Abstr. Annu. Meet. Am. Soc. Microbiol.*, p. 249.

Samson, A. C. R., and Fox, C. F., 1973, Precursor protein for Newcastle disease virus, *J. Virol.* **12**:579.

Sarov, I., and Joklik, W. K., 1972, Studies on the nature and location of the capsid polypeptide of vaccinia virions, *Virology* **50**:579.

Schäfer, W., 1959, The comparative chemistry of infective virus particles and of other virus-specific products: Animal viruses, in: *The Viruses*, Vol. 1 (F. M. Burnet and W. M. Stanley, eds.), pp. 475–504, Academic Press, New York.

Schäfer, W., Fischinger, P. J., Collins, J. J., and Bolognesi, D. P., 1977, Role of carbohydrate in biological functions of Friend murine leukemia virus gp71, *J. Virol.* **21**:35.

Scheele, C. M., and Hanafusa, H., 1971, Proteins of helper-dependent RSV, *Virology* **45**:401.

Scheid, A., and Choppin, P. W., 1973, Isolation and purification of the envelope proteins of Newcastle disease virus, *J. Virol.* **11**:263.

Scheid, A., and Choppin, P. W., 1974*a*, Identification and biological activities of paramyxovirus glycoproteins: Activation of cell fusion, hemolysis and infectivity by proteolytic cleavage of an inactive precursor of Sendai virus, *Virology* **57**:475.

Scheid, A., and Choppin, P. W., 1974*b*, The hemagglutinating and neuraminidase (HN) protein of a paramyxovirus: Interaction with neuraminic acid in affinity chromatography, *Virology* **62**:125.

Scheid, A., and Choppin, P. W., 1976, Protease activation mutants of Sendai virus: Activation of biological properties by specific proteases, *Virology* **69**:277.

Scheid, A., and Choppin, P. W., 1977, Two disulfide-linked polypeptide chains constitute the active F protein of paramyxoviruses, *Virology* **80**:54.

Scheid, A., Caliguiri, L. A., Compans, R. W., and Choppin, P. W., 1972, Isolation of paramyxovirus glycoproteins: Association of both hemagglutinating and neuraminidase activities with the larger SV5 glycoproteins, *Virology* **50**:640.

Scheid, A., Graves, M. C., Silver, S. M., and Choppin, P. W., 1978, Studies on the structure and function of paramyxovirus proteins, in: *Negative Strand Viruses and the Host Cell* (B. W. J. Mahy and R. D. Barry, eds.), pp. 181–193, Academic Press, New York.

Schlesinger, M. J., and Schlesinger, S., 1973, Large-molecular-weight precursors of Sindbis virus proteins, *J. Virol.* **11**:1013.

Schlesinger, M. J., Schlesinger, S., and Burge, B. W., 1972, Identification of a second glycoprotein in Sindbis virus, *Virology* **47**:539.

Schlesinger, R. W., 1977, Dengue virus, in: *Virology Monographs* (S. Gard and C. Hallauer, eds.), Springer-Verlag, New York.

Schlesinger, S., Schlesinger, M. J., and Burge, B. W., 1972, Defective virus particles from Sindbis virus, *Virology* **48**:615.

Schlesinger, S., Gottlieb, C., Feil, P., Gelb, N., and Kornfeld, S., 1976, Growth of enveloped RNA viruses in a line of chinese hamster ovary cells with deficient *N*-acetyl-glucosaminyltransferase activity, *J. Virol.* **17**:239.

Schloemer, R. H., and Wagner, R. R., 1974, Sialoglycoprotein of vesicular stomatitis virus: Role of the neuraminic acid in infection, *J. Virol.* **14**:270.

Schloemer, R. H., and Wagner, R. R., 1975*a*, Mosquito cells infected with vesicular stomatitis virus yield unsialylated virions of low infectivity, *J. Virol.* **15**:1029.

Schloemer, R. H., and Wagner, R. R., 1975*b*, Association of vesicular stomatitis virus glycoprotein with membranes: Isolation and characterization of a lipophilic fragment of the glycoprotein, *J. Virol.* **16**:237.

Schmidt, M. F. G., Schwarz, R. T., and Scholtissek, C., 1976*a*, Interference of nucleoside diphosphate derivatives of 2-deoxy-D-glucose with the glycosylation of virus-specific glycoproteins in vivo, *Eur. J. Biochem.* **70**:55.

Schmidt, M. F. G., Schwarz, R. T., and Ludwig, H., 1976*b*, Fluorosugars inhibit biological properties of different enveloped viruses, *J. Virol.* **18**:819.

Schneider, L. G., and Diringer, H., 1976, Structure and molecular biology of rabies virus, *Curr. Top. Microbiol. Immunol.* **75**:153.

Schneider, L. G., Dietzschold, B., Dierks, R. E., Matthaeus, W., Enzmann, P.-H., and Strohmaier, K., 1973, The rabies group-specific ribonucleoprotein (RNP) antigen and a test system for grouping and typing of rhabdoviruses, *J. Virol.* **11**:748.

Scholtissek, C., Rott, R., Hau, G., and Kaluza, G., 1974, Inhibition of the multiplication of vesicular stomatitis and Newcastle disease virus by 2-deoxy-D-glucose, *J. Virol.* **13**:1186.

Scholtissek, C., Kaluza, G., Schmidt, M. F. G., and Rott, R., 1975, Influence of sugar derivatives on glycoprotein synthesis of enveloped viruses, in: *Negative Strand Viruses*, Vol. 2 (B. W. J. Mahy and R. D. Barry, eds.), pp. 669–683, Academic Press, New York.

Scholtissek, C., Harms, E., Rohde, W., Orlich, M., and Rott, R., 1976, Correlation between RNA fragments of fowl plague virus and their corresponding gene functions, *Virology* **74**:332.

Schulze, I. T., 1970, The structure of influenza virus. I. The polypeptides of the virion, *Virology* **42**:890.

Schulze, I. T., 1972, The structure of influenza virus. II. A model based on the morphology and composition of subviral particles, *Virology* **47**:181.

Schulze, I. T., 1973, Structure of the influenza virion, *Adv. Virus Res.* **18**:1.

Schulze, I. T., 1975*a*, Effects of sialylation on the biological activities of the influenza virion, in: *Negative Strand Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 161–175, Academic Press, New York.

Schulze, I. T., 1975*b*, The biologically active proteins of influenza virus: The hemagglutinin, in: *The Influenza Viruses and Influenza* (E. D. Kilbourne, ed.), pp. 53–82, Academic Press, New York.

Schwartz, J., and Roizman, B., 1969, Concerning the egress of herpes simplex virus from infected cells: Electron microscopic observations, *Virology* **38**:42.

Schwarz, H., Hunsmann, G., Moenning, V., and Schäfer, W., 1976, Properties of mouse leukemia virus. VI. Immunoelectron microscopic studies on viral antigens on the cell surface, *Virology* **69**:169.

Schwarz, R. T., and Klenk, H.-D., 1974, Inhibition of glycosylation of influenza virus hemagglutinin, *J. Virol.* **14**:1023.

Schwarz, R. T., Rohrschneider, J. M., and Schmidt, M. F. G., 1976, Suppression of glycoprotein formation of Semliki Forest, influenza, and avian sarcoma virus by tunicamycin, *J. Virol.* **19**:782.

Schwarz, R. T., Schmidt, M. F. G., Anwer, U., and Klenk, H. D., 1977, Carbohydrates of influenza virus. I. Glycopeptides derived from viral glycoproteins after labeling with radioactive sugars, *J. Virol.* **23**:217.

Schwarz, R. T., Fournet, B., Montreuil, J., Rott, R., and Klenk, H.-D., 1978*a*, The carbohydrates of influenza virus. II. Gas chromatographic analysis of glycopeptides derived from viral glycoproteins and mucopolysaccharides, *Arch. Virol.* **56**:251.

Schwarz, R. T., Schmidt, M. F. G., and Lehle, L., 1978*b*, In vitro glycosylation of Semliki Forest- and influenza virus glycoproteins and its suppression by nucleotide 2-deoxy-sugar, *Eur. J. Biochem.* **85**:163.

Scupman, R. K., Jones, K. J., Sagik, B. P., and Bose, H. R., 1977, Virus-directed post-translational cleavage in Sindbis virus-infected cells, *J. Virol.* **22**:568.

Sefton, B. M., 1976, Virus-dependent glycosylation, *J. Virol.* **17**:85.

Sefton, B. M., 1977, Immediate glycosylation of Sindbis virus membrane proteins, *Cell* **10**:659.

Sefton, B. M., and Burge, B. W., 1973, Biosynthesis of the Sindbis virus carbohydrates, *J. Virol.* **12**:1366.

Sefton, B. M., and Gaffney, B. J., 1974, Effect of the viral proteins on the fluidity of the membrane lipids in Sindbis virus, *J. Mol. Biol.* **90**:343.

Sefton, B. M., and Keegstra, K., 1974, Glycoproteins of Sindbis virus: Preliminary characterization of the oligosaccharides, *J. Virol.* **14**:522.

Sefton, B. M., Wickus, G. G., and Burge, B. W., 1973, Enzymatic iodination of Sindbis virus proteins, *J. Virol.* **11**:730.

Seto, J. T., and Rott, R., 1966, Functional significance of sialidase during influenza virus multiplication, *Virology* **30**:731.

Seto, J. T., Drzeniek, R., and Rott, R., 1966, Isolation of low molecular weight sialidase (neuraminidase) from influenza virus, *Biochim. Biophys. Acta* **123**:402.

Seto, J. T., Becht, H., and Rott, R., 1973, Isolation and purification of surface antigens from disrupted paramyxoviruses, *Med. Microbiol. Immunol.* **159**:1.

Seto, J. T., Becht, H., and Rott, R., 1974, Effect of specific antibodies on biological function of the envelope components of Newcastle disease virus, *Virology* **61**:354.

Shapiro, S. Z., and August, J. T., 1976, Proteolytic cleavage events in oncornavirus protein synthesis, *Biochim. Biophys. Acta Cancer Rev.*

Shapiro, D., Brandt, W. E., Cardiff, R. D., and Russell, P. K., 1971, The proteins of Japanese encephalitis virus, *Virology* **44**:108.

Shimizu, K., and Ishida, N., 1975, The smallest protein of Sendai virus: Its candidate function of binding nucleocapsid to envelope, *Virology* **67**:427.

Shimizu, K., Shimizu, Y. K., Kohama, T., and Ishida, N., 1974, Isolation and characterization of two distinct types of HVJ (Sendai virus) spikes, *Virology* **62**:90.

Simmons, D. T., and Strauss, J. H., 1974, Translation of Sindbis virus 26 S RNA and 49 S RNA in lysates of rabbit reticulocytes, *J. Mol. Biol.* **86**:397.

Simons, K., Keranen, S., and Kääriäinen, L., 1973, Identification of a precursor of one of the Semliki Forest virus membrane proteins, *FEBS Lett.* **29**:87.

Skehel, J. J., 1972, Polypeptide synthesis in influenza virus-infected cells, *Virology* **49**:23.

Skehel, J. J., and Schild, G. C., 1971, The polypeptide composition of influenza A viruses, *Virology* **44**:396.

Skehel, J. J., and Waterfield, M. D., 1975, Studies on the primary structure of the influenza virus hemagglutinin, *Proc. Natl. Acad. Sci. USA* **72**:93.

Sly, W. S., Lagwinska, E., and Schlesinger, S., 1976, Enveloped virus acquires membrane defect when passaged in fibroblasts from I-cell disease patients, *Proc. Natl. Acad. Sci. USA* **73**:2443.

Smadel, J. E., Lavin, G. I., and Dubos, R. J., 1940, Some constituents of elementary bodies of vaccinia virus, *J. Exp. Med.* **71**:373.

Smith, J. F., and Brown, D. T., 1977, Envelopment of Sindbis virus: Synthesis and organization of protein in cells infected with wild type and maturation defective mutants, *J. Virol.* **22**:662.

Smith, R. E., 1974, High specific infectivity avian RNA tumor viruses, *Virology* **60**:543.

Soderlund, H., 1976, The post-translational processing of Semliki Forest virus structural polypeptide in puromycin treated cells, *FEBS Lett.* **63**:56.

Sokol, F., Stancek, D., and Koprowski, H., 1971, Structural proteins of rabies virus, *J. Virol.* **7**:241.

Spear, P. G., 1976, Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells, *J. Virol.* **17**:991.

Spear, P. G., and Roizman, B., 1970, Protein specified by herpes simplex virus. IV. Site of glycosylation and accumulation of viral membrane proteins, *Proc. Natl. Acad. Sci. USA* **66**:730.

Spear, P. G., Keller, J. M., and Roizman, B., 1970, Proteins specified by herpes simplex virus, *J. Virol.* **5**:123.

Spring, S. B., and Roizman, B., 1968, Herpes simplex virus products in productive and abortive infection. III. Differentiation of infectious virus derived from nucleus and cytoplasm with respect to stability and size, *J. Virol.* **2**:979.

Stanley, P., and Haslam, E. A., 1971, The polypeptides of influenza virus. V. Localization of polypeptides in the virion by iodination techniques, *Virology* **46**:764.

Stanley, P., Gandhi, S. S., and White, D. O., 1973, The polypeptides of influenza virus. VII. Synthesis of the hemagglutinin, *Virology* **53**:92.

Stern, W., and Dales, S., 1974, Biogenesis of vaccinia: Concerning the origin of the envelope phospholipids, *Virology* **62**:293.

Stern, W., and Dales, S., 1976, Biogenesis of vaccinia: Isolation and characterization of a surface component that elicits antibody suppressing infectivity and cell–cell fusion, *Virology* **75**:232.

Stoffel, W., and Bister, K., 1975, $^{13}C$ Nuclear magnetic resonance studies on the lipid organization of enveloped virions (vesicular stomatitis virus), *Biochemistry* **14**:2841.

Stoffel, W., and Sorgo, W., 1976, Asymmetry of the lipid-bilayer of Sindbis virus, *Chem. Phys. Lipids* **17**:324.

Stoffel, W., Anderson, R., and Stahl, J., 1975, Studies on the asymmetric arrangement of membrane-lipid-enveloped virions as a model system, *Z. Physiol. Chem.* **356**:1123.

Stoffel, W., Bister, K., Schneider, C., and Tunggal, B., 1976, $^{13}C$ NMR studies of the membrane structure of enveloped virions (vesicular stomatitis virus), *Z. Physiol. Chem.* **357**:905.

Stollar, V., 1969, Studies on the nature of dengue viruses. IV. The structural proteins of type 2 dengue virus, *Virology* **39**:426.

Stollar, V., Stollar, B. D., Koo, R., Harrap, K. A., and Schlesinger, R. W., 1976, Sialic acid contents of Sindbis virus from vertebrate and mosquito cells: Equivalence of biological and immunological viral properties, *Virology* **69**:104.

Strand, M., and August, J. T., 1973, Structural proteins of oncogenic ribonucleic acid viruses: Interspec II, A new interspecies antigen, *J. Biol. Chem.* **248**:5627.

Strand, M., and August, J. T., 1976, Structural proteins of ribonucleic acid tumor viruses, *J. Biol. Chem.* **251**:559.

Strauss, J. H., and Strauss, E. G., 1976, Togaviruses, in: *The Molecular Biology of Animal Viruses* (D. P. Nayak, ed.), pp. 111–166, Marcel Dekker, New York.

Strauss, J. H., Burge, B. W., and Darnell, J. E., 1969, Sindbis virus infection of chick and hamster cells: Synthesis of virus-specific proteins, *Virology* **37**:367.

Strauss, J. H., Jr., Burge, B. W., and Darnell, J. E., Jr., 1970, Carbohydrate content of the membrane protein of Sindbis virus, *J. Mol. Biol.* **47**:437.

Stromberg, K., Hurley, N. E., Davis, N. L., Rueckert, R. R., and Fleissner, E., 1974, Structural studies of avian myeloblastosis virus: Comparison of polypeptides in virion and core components by dodecyl sulfate polyacrylamide gel electrophoresis, *J. Virol.* **13**:513.

Sturman, L. S., 1977, Characterization of a coronavirus. I. Structural proteins: Effects of preparative conditions on the migration of proteins in polyacrylamide gels, *Virology* **77**:637.

Sturman, L. S., and Holmes, K. V., 1977, Characterization of a coronavirus. II. Glycoproteins of the viral envelope: Tryptic peptide analysis, *Virology* **77**:650.

Summers, D. F., Maizel, J. V., Jr., and Darnell, J. E., 1965, Evidence for virus-specific noncapsid proteins in poliovirus-infected HeLa cells, *Proc. Natl. Acad. Sci. USA* **54**:509.

Takatsuki, A., and Tamura, G., 1971, Tunicamycin, a new antibiotic. III. Reversal of

antiviral activity of tunicamycin by aminosugars and their derivatives, *J. Antibiot.* **24**:224.

Takatsuki, A., Khono, K., and Tamura, G., 1975, Inhibition of biosynthesis of polyisoprenol sugars in chick embryo microsomes by tunicamycin, *Agr. Biol. Chem.* **39**:2089.

Takehara, M., 1975, Polykaryocytosis induced by vesicular stomatitis virus infection in BHK-21 cells, *Arch. Virol.* **49**:297.

Tiffany, J. M., and Blough, H. A., 1969*a*, Myxovirus envelope proteins: A directing influence on the fatty acids of membrane lipids, *Science* **163**:573.

Tiffany, J. M., and Blough, H. A., 1969*b*, Fatty acid composition of three strains of Newcastle disease virus, *Virology* **37**:492.

Tiffany, J., and Blough, H., 1970, Models of structure of the envelope of influenza virus, *Proc. Natl. Acad. Sci. USA* **65**:1105.

Tkacz, D. S., and Lampen, J. O., 1975, Tunicamycin inhibition of biosynthesis of polyisoprenol sugars in chick embryo microsomes by tunicamycin, *Biochem. Biophys. Res. Commun.* **65**:248.

Toneguzzo, F., and Ghosh, H. P., 1977, Synthesis and glycosylation in vitro of glycoprotein of vesicular stomatitis virus, *Proc. Natl. Acad. Sci. USA* **74**:1516.

Toneguzzo, F., and Ghosh, H. P., 1978, In vitro synthesis and insertion into membranes of vesicular stomatitis virus membrane glycoprotein, *Proc. Natl. Acad. Sci. USA* **75**:715.

Tozawa, H., Bauer, H., Graf, T., and Gelderblom, H., 1970, Strain specific antigen of the avian leukosis sarcoma virus group, *Virology* **40**:530.

Tozawa, W., Watanabe, M., and Ishida, N., 1973, Structural components of Sendai virus: Serological and physico-chemical characterization of hemagglutinin subunit associated with neuraminidase activity, *Virology* **55**:242.

Trent, D. W., 1977, Antigenic characterization of flavivirus structural proteins separated by isoelectric focusing, *J. Virol.* **22**:608.

Trent, D. W., and Qureshi, A. A., 1971, Structural and nonstructural proteins of Saint Louis encephalitis virus, *J. Virol.* **7**:379.

Tsai, K. H., and Lenard, J., 1975, Asymmetry of influenza virus membrane bilayer demonstrated with phospholipase C, *Nature* (*London*) **225**:554.

Ueda, V., Ito, M., and Tagaya, I., 1969, A specific surface antigen induced by poxvirus, *Virology* **38**:180.

Uterman, G., and Simons, K., 1974, Studies on the amphipathic nature of the membrane proteins in Semliki Forest virus, *J. Mol. Biol.* **85**:569.

Van Zaane, D., Gielkens, A. L. J., Dekker-Michielsen, M. J. A., and Bloemers, H. P. J., 1975, Virus-specific precursor polypeptides in cells infected with Rauscher leukemia virus, *Virology* **67**:544.

Van Zaane, D., Dekker-Michielsen, M. J. A., and Bloemers, H. P. J., 1976, Virus-specific precursor polypeptides in cells infected with Rauscher leukemia virus: Synthesis, identification, and processing, *Virology* **75**:113.

Vezza, A. C., Gard, G. P., Compans, R. W., and Bishop, D. H. L., 1977, Structural components of the arenavirus Pichinde, *J. Virol.* **23**:776.

Vezza, A. C., Clewley, J. P., Gard, G. P., Abraham, N. Z., Compans, R. W., and Bishop, D. H. L., 1978, The virion RNA species of the arenaviruses Pichinde, Tacaribe and Tamiami, *J. Virol.* **26**:485.

Vogt, P. K., 1977, Genetics of RNA tumor viruses. in: *Comprehensive Virology*, Vol. 9 (H. Fraenkel-Conrat and R. R. Wagner, eds.), pp. 341–455, Plenum Press, New York.

Vogt, P. K., and Ishizaki, R., 1966, Patterns of viral interference in the avian leukosis and sarcoma complex, *Virology* **30**:368.

Vogt, V. M., and Eisenman, R., 1973, Identification of a large polypeptide precursor of avian oncornavirus proteins, *Proc. Natl. Acad. Sci. USA* **70**:1734.

Vogt, V. M., Eisenman, R., and Diggelman, H., 1975, Generation of avian myeloblastosis virus structural proteins by proteolytic cleavage of a precursor polypeptide, *J. Mol. Biol.* **96**:471.

von Bonsdorff, C. H., and Harrison, S. C., 1975, Sindbis virus glycoproteins form a regular icosahedral surface lattice, *J. Virol.* **16**:141.

von Bonsdorff, C. H., and Pettersson, R., 1975, Surface structure of Uukuniemi virus, *J. Virol.* **16**:1296.

von Magnus, P., 1954, Incomplete forms of influenza virus, *Adv. Virus Res.* **2**:59.

Wagner, R. R., 1975, Reproduction of rhabdoviruses, in: *Comprehensive Virology*, Vol. 4 (H. Fraenkel-Conrat and R. R. Wagner, eds.), Plenum, New York.

Wagner, R. R., Schnaitman, T. C., Snyder, R. M., and Schnaitman, C. A., 1969, Protein composition of the structural components of vesicular stomatitis virus, *J. Virol.* **3**:611.

Wagner, R. R., Kiley, M. P., Snyder, R. M., and Schnaitman, C. A., 1972*a*, Cytoplasmic compartmentalization of the protein and ribonucleic acid species of vesicular stomatitis virus, *J. Virol.* **9**:672.

Wagner, R. R., Prevec, L., Brown, F., Summers, D. F., Sokol, F., and McLead, R., 1972*b*, Classification of rhabdovirus proteins: A proposal, *J. Virol.* **10**:1228.

Waite, M. R. F., Brown, D. T., and Pfefferkorn, E. R., 1972, Inhibition of Sindbis virus release by media of low ionic strength: An electron microscope study, *J. Virol.* **10**:537.

Wang, E., Wolf, B. A., Lamb, R. A., Choppin, P. W. and Goldberg, A. R., 1976, The presence of actin in enveloped viruses, in: *Cell Motility* (R. Goldman, T. Pollard, and J. Rosenbaum, eds.), p. 589, Cold Spring Harbor Press, New York.

Waterson, A. P., Rott, R., and Schäfer, W., 1961, The structure of fowl plague virus and virus N, *Z. Naturforsch.* **16b**:154.

Webster, R. G., 1970, Estimation of the molecular weights of the polypeptide chains from the isolated hemagglutinin and neuraminidase subunits of influenza viruses, *Virology* **40**:643.

Webster, R. G., and Darlington, R. W., 1969, Disruption of myxoviruses with Tween 20 and isolation of biologically active hemagglutinin and neuraminidase subunits, *J. Virol.* **4**:182.

Webster, R. G., and Laver, W. G., 1967, Preparation and properties of antibody directed specifically against the neuraminidase of infleunza virus, *J. Immunol.* **99**:49.

Webster, R. G., and Laver, W. G., 1972, Studies on the origin of pandemic influenza I. Antigenic analysis of $A_2$ influenza viruses isolated before and after the appearance of Hong Kong influenza using antisera to the isolated hemagglutinin subunits, *Virology* **48**:433.

Webster, R. G., and Laver, W. G., 1975, Antigenic variation of influenza viruses, in: *The Infleunza Viruses and Influenza* (E. D. Kilbourne, ed.), pp. 270–314, Academic Press, New York.

Wecker, E., 1957, Die Verteilung von $^{32}P$ in Virus der klassischen Geflügelpest bei verschiedenen Markierungsverfahren, *Z. Naturforsch.* **12b**:208.

Wengler, G., Beato, M., and Hackemack, B.-A., 1974, Translation of 26 S virus-specific RNA from Semliki Forest virus-infected cells in vitro, *Virology* **61**:120.

Weintraub, S., and Dales, S., 1974, Biogenesis of poxviruses: Genetically controlled modifications of structural and functional components of the plasma membrane, *Virology* **60**:96.

Weiss, R. A., 1969, The host range of Bryan strain RSV synthesized in the absence of helper virus, *J. Gen. Virol.* **5**:511.

Westaway, E. G., 1975, The proteins of Murray Valley encephalitis virus, *J. Gen. Virol.* **27**:283.

Westaway, E. G., and Reedman, B. M., 1969, Proteins of group B arbovirus Kunjin, *J. Virol.* **4**:688.

Westaway, E. G., and Shaw, M., 1977, Proteins and glycoproteins specified by the flavivirus Kunjin, *Virology* **80**:309.

Westwood, J. C. N., Harris, W. J., Zwartouw, H. T., Titmuss, D. H. J., and Appleyard, G., 1964, Studies on the structure of vaccinia virus, *J. Gen. Microbiol.* **34**:67.

White, A. B., 1975, Structural polypeptides of California encephalitis virus, BFS-283, *Arch. Virol.* **49**:281.

White, D. O., 1974. Influenza viral proteins: Identification and synthesis, *Curr. Top. Microbiol. Immunol.* **63**:1.

White, D. O., Taylor, J. M., Haslam, E. A., and Hampson, A. W., 1970, The polypeptides of influenza virus and their biosynthesis, in: *The Biology of Large RNA Viruses* (R. D. Barry and B. W. J. Mahy, eds.), pp. 602–618, Academic Press, London.

White, H. B., Powell, S. S., Gafford, L. G., and Randall, C. C., 1968, The occurrence of squalene in lipid of fowlpox virus, *J. Virol. Chem.* **243**:4517.

Whitfield, S. G., Murphy, F. A., and Sudia, W. D., 1971, Eastern equine encephalomyelitis virus: An electron microscopic study of *Aedes triseriatus* (Say) salivary gland infection, *Virology* **43**:110.

Wiktor, T. J., Gyorgy, E., Schlumberger, H. D., Sokol. F., and Koprowski, H., 1973, Antigenic properties of rabies virus components, *J. Immunol.* **110**:269.

Wildy, P., Russell, W. C., and Horne, R. W., 1960, The morphology of herpes virus, *Virology* **12**:204.

Wiley, D. C., 1975, Crystalline hemagglutinin from influenza virus, *International Virology 3* (abstr.), p. 265.

Wiley, D. C., Skehel, J. J., and Waterfield, M., 1977, Evidence from studies with cross-linking reagent that the haemagglutinin of influenza virus is a trimer, *Virology* **79**:446.

Wirth, D. F., Katz, F., Small, B., and Lodish, H., 1977, How a single-stranded Sindbis virus mRNA directs the synthesis of one soluble protein and two integral membrane glycoproteins, *Cell* **10**:253.

Witte, O. N., and Weissman, I. L., 1976. Oncornavirus budding: Kinetics of formation and utilization of viral membrane glycoprotein, *Virology* **69**:464.

Witte, O. N., Weissman, I. L., and Kaplan, H. S., 1973, Structural characteristics of some murine RNA tumor viruses studied by lactoperoxidase iodination, *Proc. Natl. Acad. Sci. USA* **70**:36.

Witte, O. N., Tsukamoto-Adey, A., and Weissman, I. L., 1977, Cellular maturation of oncornavirus glycoproteins: Topological arrangement of precursor and product forms in cellular membranes, *Virology* **76**:539.

Witter, R., Frank, H., Moennig, V., Hunsmann, G., Lange, J., and Schäfer, W., 1973, Properties of mouse leukemia viruses. IV. Hemagglutination assay and characterization of hemagglutinating surface components, *Virology* **54**:330.

Wrigley, N. G., Skehel, J. J., Charlwood, P. A., and Brand, C. M., 1973, The size and shape of influenza virus neuraminidase, *Virology* **51**:525.

Wunner, W. H., and Pringle, C. R., 1976, Respiratory syncytial virus proteins, *Virology* **73**:228.

Yagi, M. J., and Compans, R. W., 1977, Structural components of mouse mammary tumor virus. I. Polypeptides of the virion, *Virology* **76**:751.

Yeger, H., and Kalnins, 1976, Electron microscopy of mammalian type-C RNA viruses: Use of conditional lethal mutants in studies of virion maturation and assembly, *Virology* **74**:459.

Yoshida, T., Nagai, Y., Yoshii, S., Maeno, K., and Matsumoto, T., 1976, Membrane (M) protein of HVJ (Sendai virus): Its role in virus assembly, *Virology* **71**:143.

Zee, Y. C., Hackett, A. J., and Talens, L., 1970, Vesicular stomatitis virus maturation sites in six different host cells, *J. Gen. Virol.* **7**:95.

Zwartouw, H. T., 1964, The chemical composition of vaccinia virus, *J. Gen. Microbiol.* **34**:115.