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ASSEMBLY OF LIPID-CONTAINING VIRUSES

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Enveloped viruses which form by budding at the cell surface possess a membrane consisting of a lipid bilayer and a small number of virus-coded polypeptides. Since viral polypeptides become integral components of the plasma membrane during assembly, the process of synthesis and incorporation into membranes of these proteins may reflect the pathway of plasma membrane assembly. Electron microscopic studies have suggested that viral envelope proteins are incorporated into discrete, localized regions of the plasma membrane which serve as recognition sites for the viral nucleocapsid. In influenza virus-infected cells, viral polypeptides are associated with cytoplasmic membranes as well as the plasma membrane. The major envelope glycoprotein appears to be synthesized in rough endoplasmic reticulum, and to migrate to smooth membranes after synthesis. Glycosylation is initiated in rough membranes and progresses further in smooth membranes. Unlike the glycoproteins, the major nonglycosylated polypeptide appears to be inserted directly into the plasma membrane. In the presence of 2-deoxyglucose or high concentrations of glucosamine, aberrant viral glycoproteins are detected which appear to be unglycosylated or partially glycosylated; these are associated with membranes and incorporated into virus particles of reduced infectivity. Therefore normal glycosylation is not essential for incorporation of viral glycoproteins into cellular membranes or virus particles, but is required for normal biological activity. The role of the viral neuraminidase in assembly and release has been studied using mutants defective in neuraminidase at restrictive temperature. Under these conditions virus formation occurs, but the progeny form large aggregates at the cell surface. Colloidal iron hydroxide staining indicates that such virus particles contain neuraminic acid, and these residues appear to serve as receptors leading to the extensive aggregation.

INTRODUCTION

Animal viruses with diverse shapes and sizes are assembled by a process of budding at the cell surface. While these agents differ significantly in terms of their structure, biological

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properties, and mechanisms of replication, it has become apparent in recent studies that these viruses all possess limiting membranes which are very similar in their basic structural properties. The details of the composition and arrangement of components in viral membranes are described in several recent reviews (1–5) and will only be summarized briefly here. Some of the general structural features common to lipid-containing viruses which form by budding at the cell surface are as follows: 1) Lipid comprises about 20–30% of the mass of the virus particle and appears to be present as a bilayer structure. Evidence for a lipid bilayer in viral membranes comes from x-ray diffraction (6) as well as electron spin resonance studies (7–9). 2) On the outer surface of the lipid bilayer are surface projections composed of one or more species of glycoproteins. 3) All of the remaining polypeptide components of the virus particle, which are enclosed by the lipid bilayer, are nonglycosylated. 4) The lipids of the virion reflect closely the lipid composition of the cellular plasma membrane, whereas all of the polypeptide components appear to be specified by the viral genome.

Early information on the steps in the assembly process of lipid-containing viruses was obtained mainly by electron microscopic studies of virus-infected cells (10–15). The helical nucleocapsids appear to assemble in the cytoplasm of parainfluenza virus-infected cells, and to align closely beneath areas of the plasma membrane which contain viral envelope proteins. Such altered regions of the membrane give rise to virions by a process of budding or outfolding. Evidence that viral envelope components precede nucleocapsid in arrival at the plasma membrane has been obtained by demonstration of specific hemadsorption or tagging of cell surfaces with ferritin-conjugated antiviral antibody in regions of the membrane which appear morphologically unaltered. Viral components appear to be present in discrete, localized regions of the plasma membrane, as indicated by the absence of identifiable viral spikes or viral antigen in regions of the cell surface just adjacent to budding virus particles. Host cell polypeptides appear to be excluded from those regions of membrane which give rise to virus particles, as indicated by their absence in purified virions. The abrupt transition between virus-specific and host-specific components at the base of budding virus particles is also indicated by staining of neuraminic acid residues on cell surfaces with colloidal iron hydroxide, which stains surfaces of infected cells *except* in regions where influenza and parainfluenza virions are in the process of budding (14). Neuraminic acid is absent from these virions, presumably because they contain a virus-coded neuraminidase as an integral component.

Recently we have undertaken studies of the assembly of influenza virus using cell fractionation procedures (16, 17). These studies have been facilitated by the fact that influenza virus infection inhibits host cell protein synthesis, so that newly synthesized viral polypeptides are readily identified (18, 19). This article will review these studies of the intracellular location and migration of viral polypeptides, and describe more recent observations on the effects of inhibitors of glycosylation on assembly of viral components, and the role of the viral neuraminidase in the assembly and release of virus particles.

MATERIALS AND METHODS

Details of the procedures used have been described previously in the following: growth and purification of the WSN strain of influenza virus (7, 20, 21); isolation and characterization of temperature-sensitive mutants of the WSN strain (22, 23); radioactive labeling and fractionation of infected cells (16, 17); polyacrylamide gel electrophoresis

(24); processing of gels for liquid scintillation counting (17); electron microscopy of infected cells (10), and staining cells with colloidal iron hydroxide (14).

RESULTS

Virion Polypeptides

Figure 1 summarizes the available information on the arrangement of the structural components of the influenza virion and their relationship to polypeptides in a polyacrylamide gel. This model is based on results obtained in many laboratories (7, 21, 25–30), which have been reviewed in detail elsewhere (4, 31–32). The polypeptide of highest molecular weight, designated P, is present in small amounts and it appears to be associated with the viral ribonucleoprotein (33, 34). The largest glycoprotein, designated HA, possesses hemagglutinin activity; under certain conditions it may be cleaved into two polypeptides designated HA_1 and HA_2, although this cleavage is not essential for viral assembly (19, 35–37). The polypeptide subunit of the internal ribonucleoprotein is designated NP, and the other surface glycoprotein, the neuraminidase, is designated NA. The major nonglycosylated polypeptide, designated M, is thought to form a continuous layer on the inner surface of the lipid bilayer.

Polypeptide Synthesis in Influenza Virus-Infected Cells

The extent and kinetics by which host cell polypeptide synthesis is replaced by virus-specific polypeptide synthesis depends on the host cell type and virus strain (18, 19, 38–40). In BHK21-F cells infected with the WSN strain (Fig. 2), there is a high background of cellular polypeptide synthesis at 2 hr post-infection, whereas at 2.5 hr some viral polypeptides can be resolved clearly. At later times the background of host cell synthesis is completely suppressed. The overall pattern of viral polypeptide synthesis does not change markedly between early and late times in the growth cycle. Distinct peaks of polypeptides P and HA, and a major peak of the NP polypeptide, are present at all times. A major nonstructural polypeptide, designated NS, which migrates slightly more rapidly than the M polypeptide, is also a major polypeptide at all time points tested. The M and NS polypeptides are clearly resolved in Fig. 2, and have been shown to be distinct by peptide mapping (19).

The relative amounts of P, HA, NP, and NS synthesized at early and late times after infection appear to be relatively constant. On the other hand, there is a relative increase in the amount of polypeptide M at later times in the growth cycle. In the experiment shown in Fig. 2, the ratio of radioactivity in M:NS is 0.36 at 3.5 hr post-infection, but 0.78 by 10 hr. Since the relative amount of synthesis of M is increasing at times when the overall rate of virus-specific polypeptide synthesis remains constant (19), there is an absolute increase in the rate of synthesis of M rather than a decline in rates of synthesis of the other viral polypeptides.

Cell fractionation studies. Previous studies had indicated that purified plasma membranes contained all of the polypeptides of the virion, and that viral envelope components were the major polypeptides observed in short pulses (19). To determine whether intracellular membranes of infected cells might contain virus-specific polypeptides which could

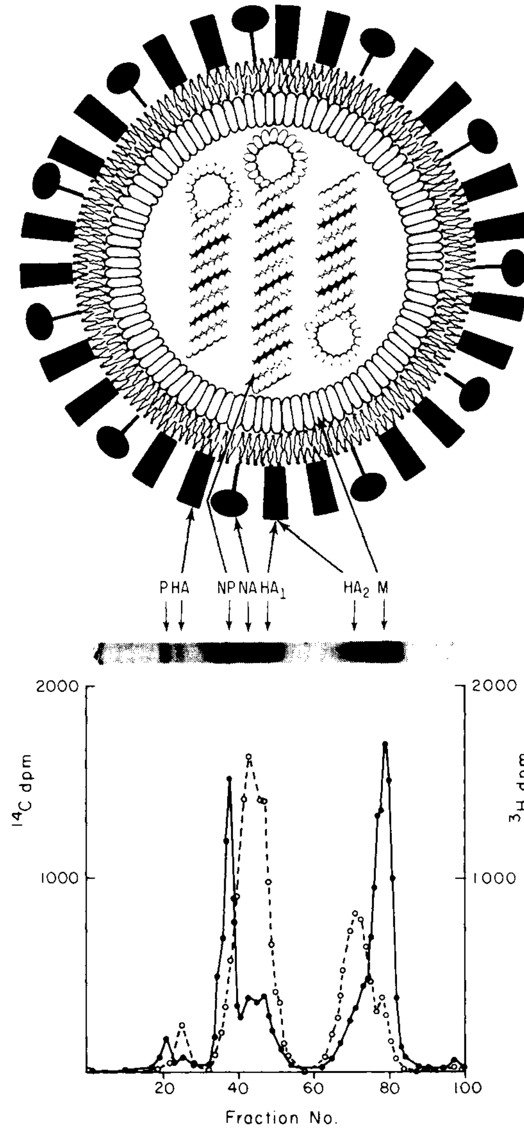


Fig. 1. Schematic diagram summarizing the relationship between structural components of the influenza virion and viral polypeptides in a polyacrylamide gel. From Lenard and Compans (4).

serve as possible precursors of the envelope proteins in the plasma membrane, the procedure of Caliguiri and Tamm (41) was used to isolate cytoplasmic components. This fractionation procedure separates cytoplasmic membranes on the basis of buoyant density in discontinuous sucrose gradients. Figure 3 illustrates the virus-specific polypeptides found in each of the six fractions obtained from influenza virus-infected BHK21-F cells. In fractions 2 and 3, which contain smooth cytoplasmic membranes, the major components are envelope polypeptides HA, NA, and M. Fraction 4, which contains elements of smooth and rough cytoplasmic membranes as well as much of the intracellular viral ribonucleoprotein, shows the NP polypeptide as the major component. Rough microsomal

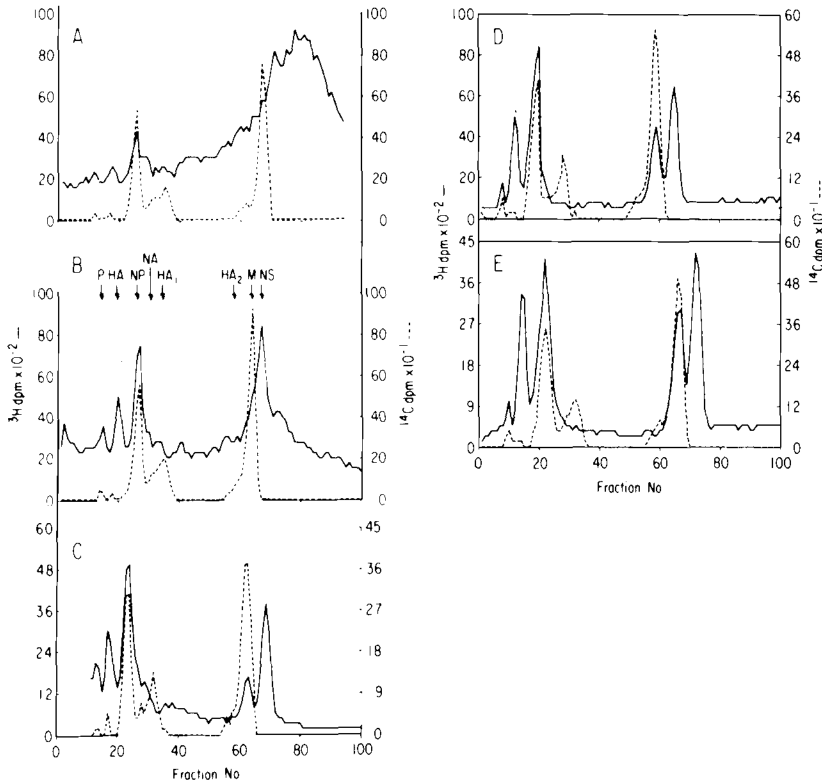


Fig. 2. Polyacrylamide gel electrophoresis of polypeptides synthesized in BHK21-F cells at various times after infection with the WSN strain of influenza virus. A, 2 hr; B, 2.5 hr; C, 3.5 hr; D, 7 hr; E, 10 hr. ^{3}H-leucine–labeled infected cell polypeptides (——); ^{14}C-labeled marker polypeptides from purified virions, (- - -). The designations of polypeptides are indicated in panel B. From Meier-Ewert and Compans (42).

membranes, which band primarily in fractions 5 and 6, contain the HA, NP, and NS polypeptides as major components. After a short pulse label, most of the newly synthesized HA polypeptides were associated with the rough microsomal fraction; when chased with unlabeled amino acids the HA polypeptides appeared to migrate from rough to smooth membranes (16). Subsequently (17) it was observed that the carbohydrates of HA polypeptides associated with smooth and rough cytoplasmic membranes were qualitatively different. Glucosamine was present in the polypeptides in both fractions, whereas fucose was incorporated into polypeptides associated with smooth membranes, but not rough membranes. These results suggested that the synthesis of the HA polypeptide takes place on membrane-bound polyribosomes in the rough endoplasmic reticulum, and that glycosylation is initiated in these membranes. After migration to smooth endoplasmic reticulum, elongation of the carbohydrate side chains continues, and distal residues such as fucose appear to be added in this compartment.

More recent experiments (42) involving much longer chase periods have provided additional information on the process of viral membrane assembly. Figure 4 illustrates patterns of viral polypeptides in BHK21-F cell fractions after a 15 min pulse with ^{3}H-leucine, and after a 15 min pulse followed by a 6 hr chase with unlabeled amino acids.

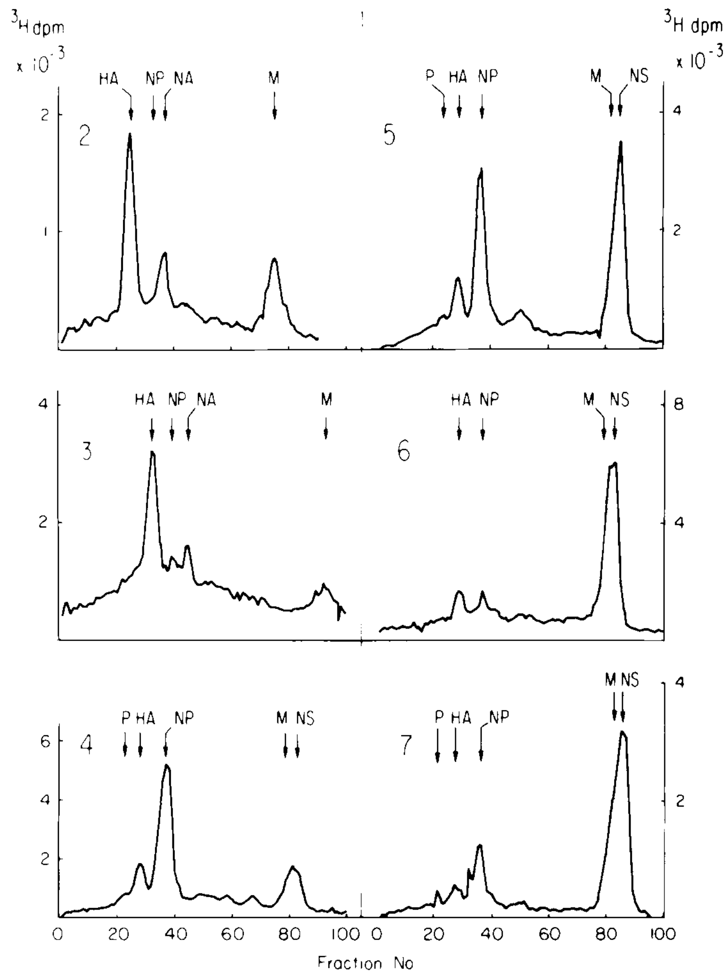


Fig. 3. Polyacrylamide gel electrophoresis of newly synthesized polypeptides associated with cytoplasmic fraction of BHK21-F cells infected with influenza virus. Cells were pulse-labeled with ^{3}H-leucine for 5 min at 4 hr post-infection and "chased" with unlabeled amino acids for 60 min prior to isolation of cytoplasmic fractions. From Compans (16).

A striking difference is the virtual absence of the HA polypeptide in all membrane fractions after the chase period. This observation suggests that these polypeptides have been chased into plasma membranes and virions, providing further support for the view that viral polypeptides in intracellular membranes may serve as precursors of those which are present in plasma membranes.

In contrast, polypeptide M remains associated with intracellular membrane fractions after this prolonged chase period (Fig. 4). This observation, together with the finding of M associated with plasma membranes after short pulse labels (19), suggests that this polypeptide may be inserted into plasma membranes directly after synthesis. The small amount of M associated with intracellular membranes, which is not chased out of these fractions even after prolonged incubation, may not become incorporated into virions.

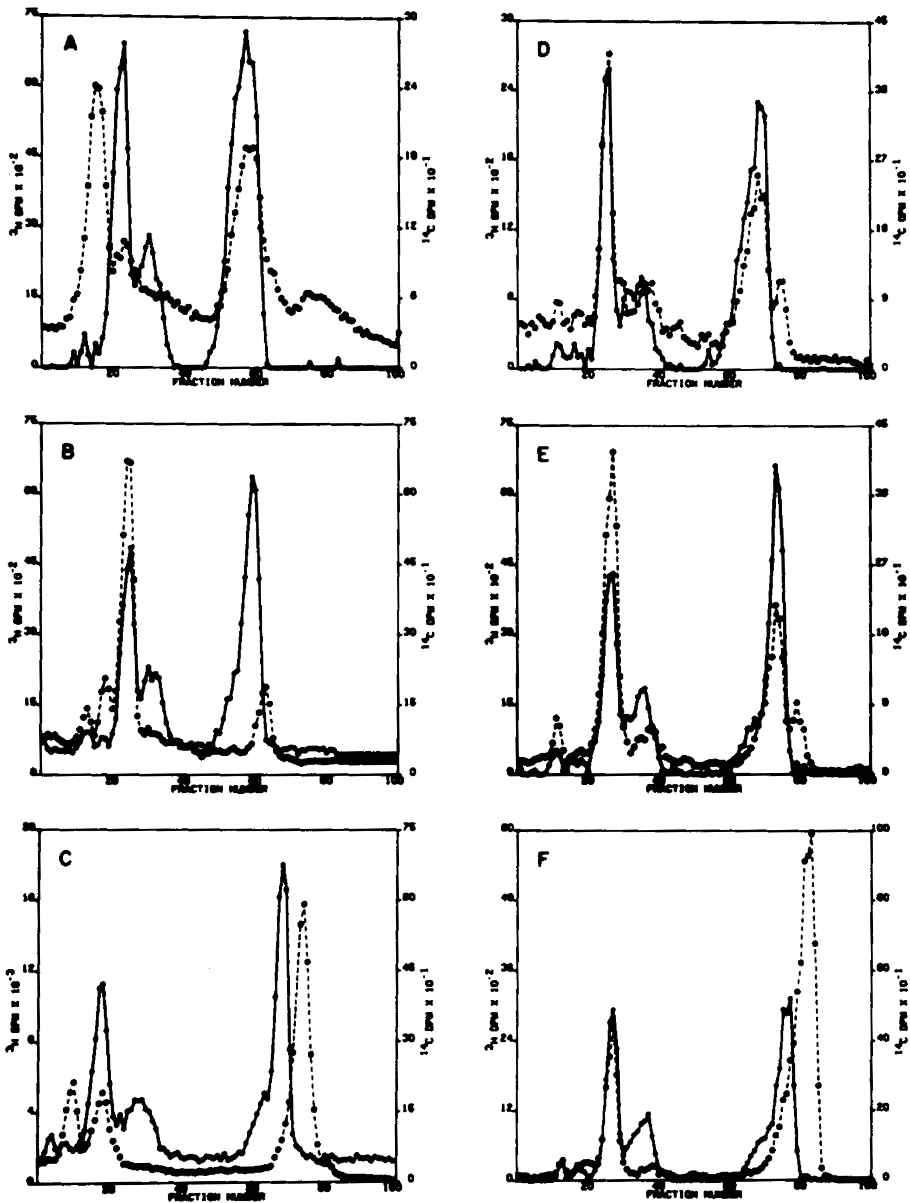


Fig. 4. Polyacrylamide gel electrophoresis of viral polypeptides associated with cytoplasmic fractions of BHK21-F cells. A, B, C, 15 min pulse with ^{3}H-leucine at 4 hr, D, E, F. The 15 min pulse was followed by a 6 hr chase with unlabeled amino acids. A and D, fractions 2 and 3; B and E, fraction 4; C and F, fractions 5 and 6. ^{3}H-labeled polypeptides, (o—o). ^{14}C – labeled marker virion polypeptides, (——). From Meier-Ewert and Compans (42).

Inhibitors of Glycosylation

Observations in several laboratories have demonstrated that the abnormal sugar metabolite 2-deoxyglucose inhibits the replication of influenza virus (43–45). High con-

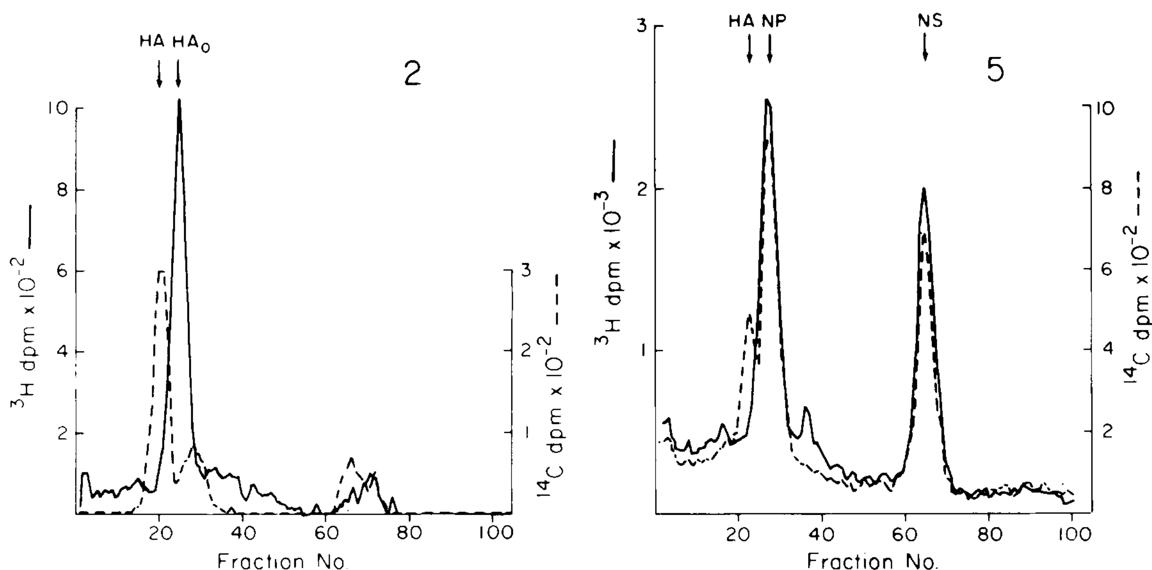
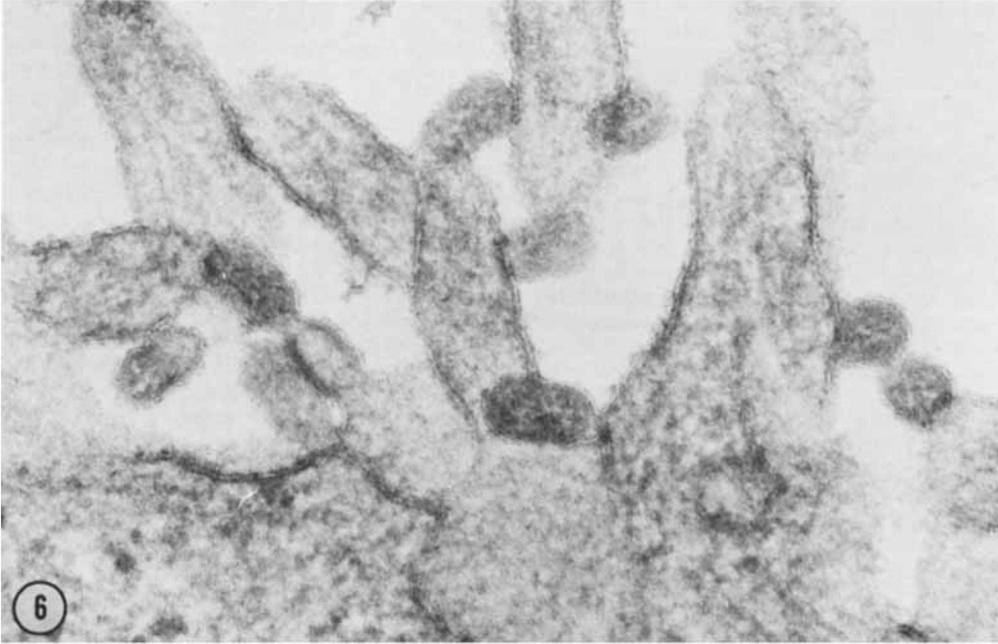


Fig. 5. Coelectrophoresis of ^{3}H-labeled viral polypeptides synthesized in the presence of 40 mM glucosamine (——) and ^{14}C-labeled viral polypeptides (- - -) associated with cytoplasmic fractions. Left panel, smooth membrane fraction 2. Right panel, rough membrane fraction 5. From Compans (48).

centrations of glucosamine have a similar inhibitory effect, and in the presence of these inhibitors the synthesis of the hemagglutinin polypeptide is not detected (44–46). Instead, a different polypeptide component designated HA_0 which migrates more rapidly than the HA polypeptide is produced in inhibitor-treated cells, and it appears that this is an unglycosylated or incompletely glycosylated precursor of the HA polypeptide (46, 47). We have studied the effects of these sugars on the association of polypeptides with intracellular membranes, and on the production of virus particles (48).

Coelectrophoresis experiments indicate that the aberrant polypeptide HA_0, produced in the presence of inhibitors, is present in cytoplasmic membrane fractions (Fig. 5). In the smooth membrane fraction, the more rapidly migrating HA_0 peak can be distinguished readily from the HA polypeptide. In the rough membrane fraction, the absence of HA is also apparent in inhibitor-treated cells and the HA_0 polypeptide can not be identified as clearly because of the large NP polypeptide peak. These results indicate that the aberrant polypeptide HA_0 is incorporated into smooth cytoplasmic membranes, and may also be associated with rough membranes.

At the surfaces of inhibitor-treated cells, virus particles in the process of budding were identified by electron microscopy (Fig. 6). In contrast to control virus particles, which possess a distinct layer of surface projections (Fig. 7), the surfaces of particles budding from inhibitor-treated cells appear to be smooth. The particles produced by cells treated with either deoxyglucose or glucosamine can be purified, and in negatively stained preparations it is possible to resolve projections on their surfaces. Therefore, the smooth surfaces seen in thin-sectioned preparations appear to be due to a lack of staining of the spike layer. Polyacrylamide gel electrophoresis of the virions purified from inhibitor-treated cells indicates that the normal glycoprotein components are absent, and instead polypeptides which appear to be cleavage products of HA_0 are detected (48). The specific



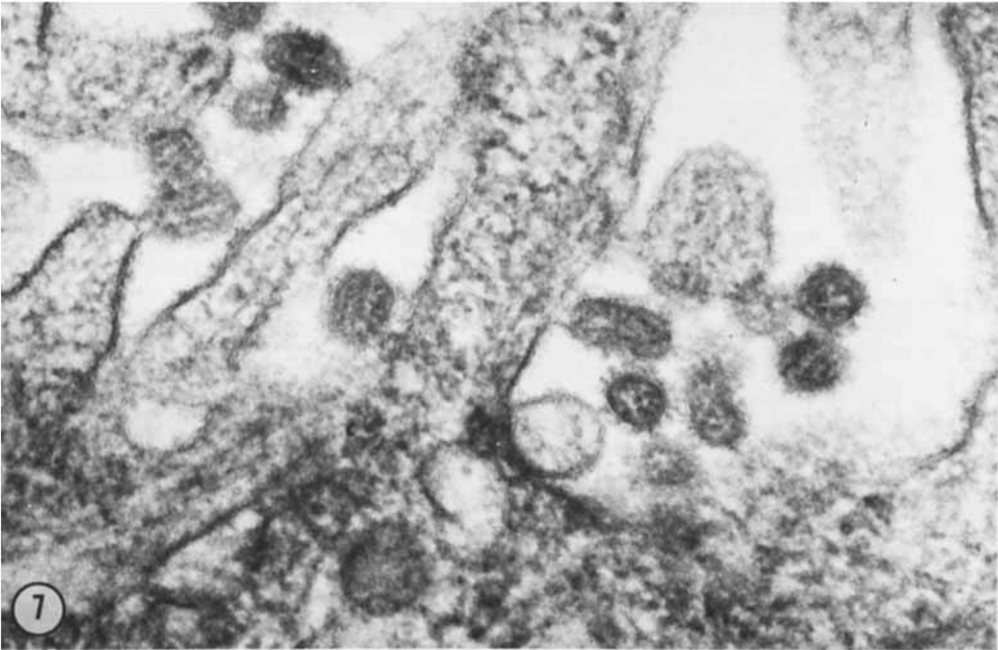


Fig. 6. Smooth-surfaced virus particles budding at the surface of an MDBK cell treated with 2-deoxyglucose. From Compans (48).

Fig. 7. Normal virus particles with surface projections at the surface of an infected cell. From Compans (48).

infectivity and hemagglutinin activity of the particles produced in the presence of inhibitors are markedly lower than control virus preparations.

These observations indicate that the normal glycosylation process is not required for association of viral polypeptides with cytoplasmic membranes or for assembly of mature virus particles. However, the marked reduction in biological activity of the particles containing aberrant glycoproteins indicates that the carbohydrate of the glycoprotein is essential for full biological activity.

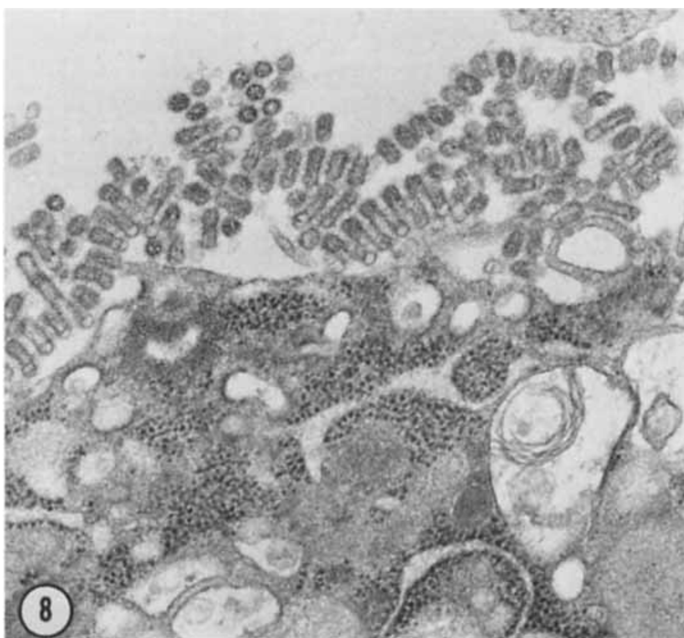
Role of Viral Neuraminidase

Influenza and parainfluenza viruses bind specifically to neuraminic acid-containing receptors on cell surfaces, and it is possible to prevent infection of cells by pre-treatment with neuraminidase (49, 50). The question therefore arises why these viruses contain a virus-coded neuraminidase capable of destroying their own receptor as an integral component of the virus particle. It is clear that the neuraminidase is not essential for the initial stages of the infectious process, since antibody specific for the neuraminidase does not neutralize viral infectivity (51, 52). The viral enzyme has been implicated in the release of progeny virions at the cell surface, since it was found that release was inhibited by specific antibody (52–54). However, it was subsequently found that released virus could be detected in the presence of monovalent F_{ab} antibody fragments, and it was suggested that inhibition of release by bivalent antibody resulted from aggregation of virions and cross-linking of virions to antigens incorporated into the cell surface (55).

We have studied the function of the viral neuraminidase using temperature-sensitive influenza virus mutants which do not produce detectable neuraminidase at nonpermissive temperature (23). In cells infected at nonpermissive temperature, all viral polypeptides are detectable in polyacrylamide gels, but no infectious or hemagglutinating particles are released into the culture medium. Cells infected with two such mutants, ts 3 and ts 11, have been studied by electron microscopy at permissive and non-permissive temperature. These mutants fall in the same complementation group, recombination group IV (23).

At the permissive temperature of 33°, cells infected with the ts mutants were morphologically similar to cells infected with wild-type influenza virus; virus particles were observed in the process of budding, and some completed particles were distributed over the cell surfaces singly or in small groups. In contrast, at the nonpermissive temperature of 39.5° virus particles were also found, but they occurred in the form of large aggregates which covered extensive areas of the cell surface (Figs. 8 and 9). Frequently the aggregated virus particles formed regular hexagonally packed arrays, and it sometimes appeared that the spikes of adjacent particles were in direct contact (Fig. 9). These observations demonstrate that the absence of hemagglutinating activity in the culture medium at nonpermissive temperature is not due to a block in the assembly process, and indicate that virus aggregation occurs at the cell surface.

It is known that influenza and parainfluenza virus particles contain no neuraminic acid, presumably as a result of the viral neuraminidase (14, 56, 57). However, other enveloped viruses which lack neuraminidase contain neuraminic acid bound to viral lipid and proteins (58–60). It therefore seemed likely that the influenza virus particles produced at nonpermissive temperature might contain neuraminic acid because the viral neuraminidase was inactive. If this were the case, these neuraminic acid residues could serve as receptors for the viral hemagglutinin, which would explain the observed aggregation. To examine



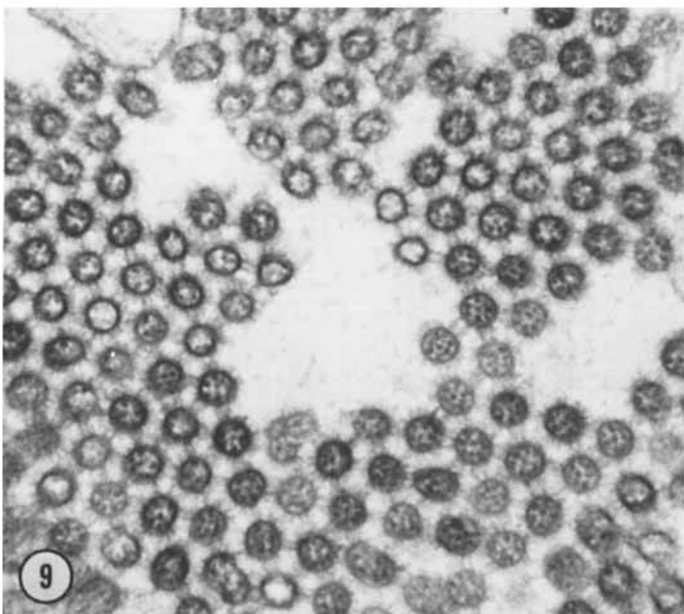


Fig. 8. Region of the surface of an MDBK cell infected with ts 11 and incubated at 39.5°. There is extensive accumulation of virus at the cell surface. From Palese et al. (23).

Fig. 9. Regular array of virus particles at the surface of an MDBK cell infected with ts 3 and incubated at 39.5°. From Palese et al. (23).

this possibility, infected cells were stained with colloidal iron hydroxide which specifically stains neuraminic acid residues on cellular and viral membranes (14).

A region of the surface of a cell infected with wild-type influenza virus and stained with colloidal iron hydroxide is shown in Fig. 10. The iron clearly stains the cell surface, but influenza virions in the process of budding are unstained. In contrast, when cells infected with ts mutants at nonpermissive temperature are examined, the surfaces of virions are densely stained (Fig. 11). Thus the virus particles produced by cells in the absence of a functional neuraminidase appear to contain neuraminic acid.

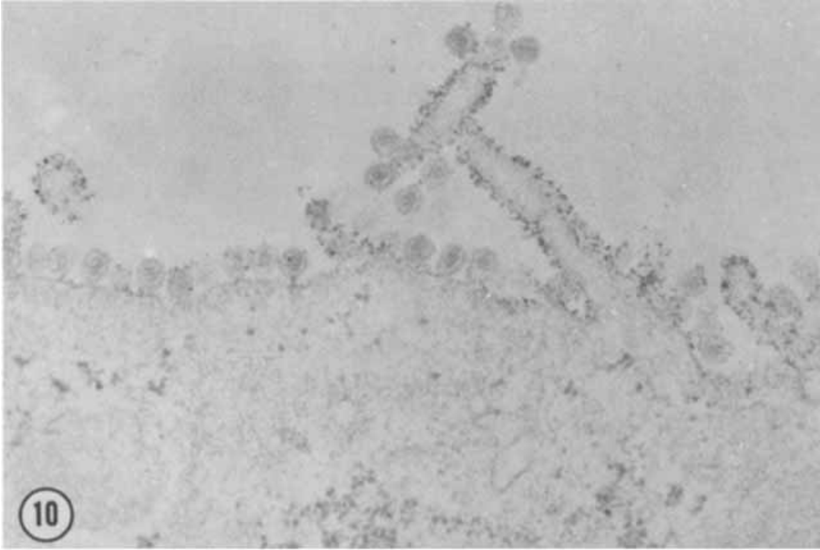
These results indicate that a neuraminidase may be essential for viruses which bind to neuraminic acid moieties if these viruses contain surface components which may function as acceptors for cellular sialyl transferases. In the absence of neuraminidase, neuraminic acid receptors are attached to viral envelope glycoproteins or glycolipids, resulting in extensive aggregation and inhibition of the release of virus particles.

DISCUSSION

The available information on the sequence of events in the assembly of influenza virus is summarized schematically in Fig. 12. Assembly of the ribonucleoprotein appears to occur free in the cytoplasm, since the intracellular ribonucleoprotein structures are not associated with membranes (61). The NP polypeptide appears to be incorporated rapidly into ribonucleoprotein structures, whereas the incorporation of the minor P polypeptides occurs more slowly (34, 62). Envelope glycoproteins appear to be incorporated into the plasma membrane subsequent to their association with intracellular membrane structures, whereas it seems likely that the nonglycosylated M polypeptide is incorporated directly into the plasma membrane (16, 17, 42, 47). Once the envelope polypeptides are present in the plasma membrane in the proper arrangement, the ribonucleoprotein associates with the inner surface of the modified membrane, following which the virus particle is formed by a process of budding.

While the available data suggest that glycosylated and nonglycosylated envelope proteins arrive at the plasma membrane by distinct pathways, it is uncertain whether one component precedes the other in order of association with the membrane. It is possible that M polypeptides only associate with those regions of membrane in which glycoproteins are already present. Alternatively, the proteins could be incorporated independently into different regions of the membrane and associate following lateral diffusion in the plane of the membrane. In either process the precise nature of the specific interaction between internal and external proteins of the membrane remains to be established. The simplest explanation would involve direct protein–protein interaction across the lipid bilayer; however, there is no evidence at present that any viral envelope protein extends across the bilayer. Thus the possibility must also be considered that the association may be mediated by lipid.

The present results indicate that drastic alteration of the carbohydrate moieties on viral glycoproteins have minimal effects on the assembly process, but that the infectivity of particles with altered glycoproteins is markedly reduced. Two different approaches have been used to modify the carbohydrate composition. First, we have employed inhibitors of glycosylation (44, 45) which result in the production of aberrant glycoproteins of higher electrophoretic mobility, which are not labeled by carbohydrate precursors (46). In the presence of such inhibitors, virus particles containing the aberrant



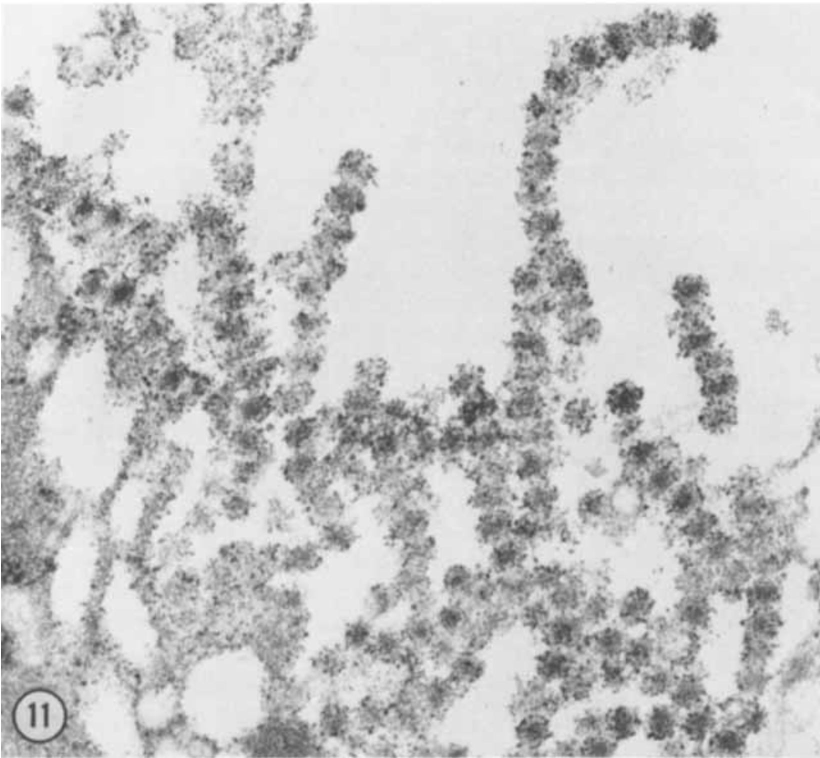


Fig. 10. Colloidal iron hydroxide staining of neuraminic acid residues on the surface of cells infected with wild-type WSN virus. Virus particles are unstained. From Palese et al. (23).

Fig. 11. Staining of neuraminic acid residues at the surface of cell infected with ts 11 and incubated at 39.5°. The staining indicates the presence of neuraminic acid in the virus particles. From Palese et al. (23).

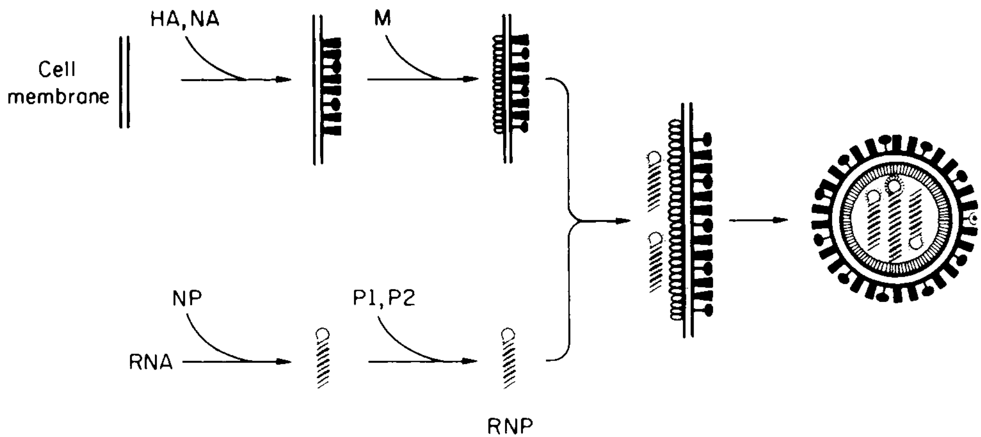


Fig. 12. Diagram of the assembly pathway of influenza virus. There is still some uncertainty about several stages, particularly the distribution of proteins on the external and internal surfaces of the plasma membrane.

glycoproteins are produced, and their infectivity and hemagglutinating activity is markedly lower. Thus the alteration of the carbohydrate does not affect the macromolecular interactions leading to assembly, but may influence the conformation of the glycoprotein so that it does not interact with receptors. The other type of modification involved mutants lacking functional neuraminidase, which appear to produce virions containing neuraminic acid. This marked alteration in surface charge also does not affect the assembly process, but results in virions containing their own receptors that form large aggregates, thus preventing the release of infectious virions. Neuraminic acid has also been attached in vitro to influenza virions by Schulze (63), who demonstrated that this did not inactivate viral infectivity.

The available evidence on the process of assembly of lipid-containing viruses suggests a number of hypotheses concerning the biosynthesis and assembly of plasma membrane components in general:

1. Glycosylated membrane polypeptides are synthesized on membrane-associated polyribosomes.

2. They remain membrane-associated from the time of their synthesis until they arrive at the plasma membrane.

3. The process of glycosylation per se does not determine the final destination of the glycoprotein.

4. Nonglycosylated membrane polypeptides are synthesized on free polysomes and inserted directly into membranes.

These hypotheses are necessarily speculative at present, but may be tested by further studies on viral as well as cellular membrane assembly.

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REFERENCES

1. Choppin, P. W., Compans, R. W., Scheid, A., McSharry, J. J., Lazarowitz, S. G., In "Membrane Research" (C. F. Fox, Ed.) pp. 163–185. Academic Press, New York (1972).
2. Klenk, H. D., in "Biological Membranes" (D. Chapman and D. F. H. Wallach, Eds.), pp. 145–183 Academic Press, London (1973).
3. Blough, H. A., and Tiffany, J. M., Adv. Lipid Res. 11:267 (1973).
4. Lenard, J., and Compans, R. W., Biochem. Biophys. Acta Reviews on Biomembranes 344:51 (1974).
5. Choppin, P. W., and Compans, R. W., in "Comprehensive Virology" (H. Fraenkel-Conrat and R. R. Wagner, Eds.). Plenum Press, New York (in preparation).
6. Harrison, S. C., David, A., Jumblatt, J., and Darnell, J. E., J. Mol. Biol. 60:523 (1971).
7. Landsberger, F. R., Lenard, J., Paxton, J., and Compans, R. W., Proc. Nat. Acad. Sci. U.S. 68:2579 (1971).
8. Landsberger, F. R., Compans, R. W., Paxton, J., and Lenard, J., J. Supramol. Struct. 1:50 (1972).
9. Landsberger, F. R., Compans, R. W., Choppin, P. W., and Lenard, J., Biochemistry 12:4498 (1973).
10. Compans, R. W., Holmes, K. V., Dales, S., and Choppin, P. W., Virology 30:411 (1966).
11. Acheson, N. H., and Tamm, I., Virology 32:128 (1967).
12. Compans, R. W., and Dimmock, N. J., Virology 39:499 (1969).
13. Compans, R. W., Dimmock, N. J., and Meier-Ewert, H., In "The Biology of Large RNA Viruses" (R. D. Barry and B. W. J. Mahy, Eds.). Academic Press, New York (1970).
14. Klenk, H. D., Compans, R. W., and Choppin, P. W., Virology 42:1158 (1970).
15. Compans, R. W., and Choppin, P. W., In "Comparative Virology" (K. Maramorosch and F. Kurstak, Eds.) p. 407. Academic Press, New York (1969).
16. Compans, R. W., Virology 51:56 (1973).
17. Compans, R. W., Virology 55:541 (1973).
18. White, D. O., Taylor, J. M., Haslam, E. A., and Hampson, A. W., In "The Biology of Large RNA Viruses" (R. D. Barry and B. W. J. Mahy, Eds.) p. 602. Academic Press, London (1970).
19. Lazarowitz, S. G., Compans, R. W., and Choppin, P. W., Virology 46:830 (1971).
20. Choppin, P. W., Virology 38:130 (1969).
21. Compans, R. W., Klenk, H.-D., Caliguiri, L. A., and Choppin, P. W., Virology 42:880 (1970).
22. Sugiura, A., Tobita, K., and Kilbourne, E. D., J. Virol. 10:639 (1972).
23. Palese, P., Tobita, K., Ueda, M., and Compans, R. W., Virology, in press. (1974).
24. Caliguiri, L. A., Klenk, H.-D., and Choppin, P. W., Virology 39:460 (1969).
25. Laver, W. G., and Valentine, R. C., Virology 38:105 (1969).
26. Pons, M. W., Schulze, I. T., and Hirst, G. K., Virology 39:250 (1969).
27. Schulze, I. T., Virology 42:890 (1970).
28. Skehel, J. J., and Schild, G. C., Virology 44:396 (1971).
29. Haslam, E. A., Hampson, A. W., Radiskevics, I., and White, D. O., Virology 42:566 (1970).
30. Compans, R. W., Content, J., and Duesberg, P. H., J. Virol. 10:795 (1972).
31. Schulze, I., Adv. Virus Res. 18:1 (1974).
32. Compans, R. W., and Choppin, P. W., in "Comprehensive Virology" (H. Frankel-Conrat and R. R. Wagner, Eds.). Plenum Press, New York (in press) (1974).

33. Bishop, D. H. L., Roy, P., Bean, W. J., Jr., and Simpson, R. W., J. Virol. 10:689 (1972).
34. Caliguiri, L. A., and Compans, R. W., J. Virol 14:191 (1974).
35. Lazarowitz, S. G., Compans, R. W., and Choppin, P. W., Virology 52:199 (1973).
36. Lazarowitz, S. G., Goldberg, A. R., and Choppin, P. W., Virology 56:172 (1973).
37. Rifkin, D. B., Compans, R. W., and Reich, E., J. Biol. Chem., 247:6432 (1972).
38. Skehel, J. J., Virology 49:23 (1972).
39. Klenk, H.-D., Rott, R., and Becht, H., Virology 47:579 (1972).
40. Krug, R. M., and Etkind, P. R., Virology 56:334 (1973).
41. Caliguiri, L. A., and Tamm, I., Virology 42:100 (1970).
42. Meier-Ewert, H., and Compans, R. W., J. Virol., in press (1974).
43. Kilbourne, E. D., Nature 183:271 (1959).
44. Gandhi, S. S., Stanley, P., Taylor, J. M., and White, D. O., Microbios. 5:41 (1972).
45. Kaluza, G., Schottissek, C., and Rott, R., J. Gen. Virol. 14:251 (1972).
46. Klenk, H.-D., Scholtissek, C., and Rott, R., Virology 49:723 (1972).
47. Klenk, H.-D., Wollert, W., Rott, R., and Scholtissek, C., Virology 57:28 (1974).
48. Compans, R. W., (in preparation) (1974).
49. Gottschalk, A., Biochem. Biophys. Acta 23:645 (1957).
50. Rafelson, M. E., In "The Amino Sugars" (E. A. Balazs and R. W. Jeanloz, Eds.) p. 171. Academic Press, New York (1965).
51. Webster, R. G., Laver, W. G., Kilbourne, E. D., J. Gen. Virol. 3:315 (1968).
52. Kilbourne, E. D., Laver, W. G., Schulman, J. L., and Webster, R. G., J. Virol. 2:281 (1968).
53. Seto, J. T., and Rott, R., Virology 30:731 (1966).
54. Compans, R. W., Dimmock, N. J., and Meier-Ewert, H., J. Virol. 4:528 (1969).
55. Becht, H., Hämmerling, U., and Rott, R., Virology 46:337 (1971).
56. Klenk, H.-D., Caliguiri, L. A., and Choppin, P. W., Virology 42:473 (1970).
57. Klenk, H.-D., and Choppin, P. W., Proc. Nat. Acad. Sci. U.S. 66:57 (1970).
58. Klenk, H.-D., and Choppin, P. W., Virology 7:416 (1971).
59. Burge, B. W., and Huang, A. S., J. Virol. 6:176 (1970).
60. McSharry, J. J., and Wagner, R. R., J. Virol. 7:59 (1971).
61. Compans, R. W., and Caliguiri, L. A., J. Virol. 11:441 (1973).
62. Krug, R. M., Virology 50:103 (1972).
63. Schulze, I. T., In "Negative Strand Viruses" (R. D. Barry and B. W. J. Mahy, Eds.). Academic Press, New York (in press) (1974).