Watabe, T., and Akamatsu, K. (1972), *Biochim. Biophys. Acta 279*, 297.
Watabe, T., Kiyonaga, K., Akamatsu, K., and Hara, S. (1971), *Biochem. Biophys. Res. Commun. 43*, 1252.
Zimmerman, D. C. (1966), *Biochem. Biophys. Res. Commun. 23*, 398.

Organization of the Lipid Phase in Viral Membranes. Effects of Independent Variation of the Lipid and the Protein Composition†

Frank R. Landsberger,* Richard W. Compans, Purnell W. Choppin, and John Lenard‡

ABSTRACT: Spin-label electron spin resonance (esr) methods have been used to investigate the effects of independent variation of the lipid and protein composition on the organization of the lipid in viral membranes. Influenza and parainfluenza SV5 virions were grown in BHK21-F and MDBK cells and labeled with stearic acid derivative spin labels. Since the lipid composition of the virus reflects that of the plasma membrane of the host cell but the proteins are virus specified, two different viruses grown in the same cell line contain membranes with similar lipids and different proteins. The esr spectral splittings of such virions were found to be indistinguishable. Growing the same virus in different cells permitted a comparison of membranes with similar proteins but different lipids. The esr spectra of these virions showed significant differences. These results indicate that the rigidity of the viral membrane depends largely on the lipid composition, and is not affected by the differences in the protein composition of the two viruses. Evidence is presented that the lipids of parainfluenza virions are arranged in a bilayer structure.

Enveloped viruses, such as influenza and parainfluenza viruses, possess lipid-containing membranes which are acquired during the process of assembly by budding at the cell surface. The viral lipid composition reflects the composition of the plasma membrane of the host cell, while the proteins are virus specific (Klenk and Choppin, 1969, 1970a,b; Choppin *et al.*, 1971, 1972). The influenza virion contains seven polypeptides (Compans *et al.*, 1970; Schulze, 1970). Four of these are glycoproteins and form projections or spikes on the outer surface of the virion. A major nonglycosylated protein appears to be associated with the inner surface of the viral lipid membrane. The SV5 virion, often used as a model for the parainfluenza group, contains five major proteins, two of which are the glycoproteins forming the surface spikes, and one is a carbohydrate free protein thought to be associated with the viral membrane (Caliguiri *et al.*, 1969; Klenk *et al.*, 1970; Chen *et al.*, 1971; Mountcastle *et al.*, 1971). As discussed previously (Choppin *et al.*, 1972), viral membranes provide useful systems for the study of lipid–protein interactions for several reasons. (1) They are easily obtained in a high degree of purity. (2) They are composed of a limited number of protein species, and there is considerable information about the arrangement of the polypeptides associated with the viral lipid layer. (3) It is possible to alter the lipid composition of the membrane, without altering the proteins, by growing the same strain of viruses in cell types with differing plasma membrane lipids. (4) Since the proteins associated with viral membranes are coded for entirely by the viral genome, one can obtain viruses with a similar lipid composition but a completely different set of membrane-associated proteins by growing two different viruses in the same host cell.

Recently we have studied the organization of the lipid phase in influenza and Rauscher murine leukemia viruses by electron spin resonance (esr) using spin-label methods (Landsberger *et al.*, 1971a, 1972). The results indicate that in each virus the lipids are organized in a bilayer structure. Complete removal of the spikes from influenza virus particles by protease treatment (Compans *et al.*, 1970) had no detectable effect on the organization of the viral lipid (Landsberger *et al.*, 1971a), suggesting that the spikes do not penetrate through the lipid bilayer.

The present communication presents evidence for a lipid bilayer in parainfluenza virus and describes the results of esr studies on the lipid-containing membrane of influenza and parainfluenza viruses grown in different host cells. The effects of independently varying the lipid and the protein composition on the organization of the lipid phase have been determined.

Materials and Methods

Virus and Cells. The WSN strain of influenza virus A_0 and the W3 strain of the parainfluenza virus SV5 were used. Viruses were grown in the MDBK line of bovine kidney cells or the BHK21-F line of baby hamster kidney cells, as described previously (Choppin, 1964, 1969; Compans *et al.*, 1970; Holmes and Choppin, 1966).

† From the Department of Chemistry, Indiana University, Bloomington, Indiana 47401 (F. R. L.), The Rockefeller University, New York, New York 10021 (R. W. C. and P. W. C.), and the Division of Endocrinology, Sloan-Kettering Institute for Cancer Research, New York, New York 10021 (J. L.). *Received June 25, 1973.* This work was supported by National Science Foundation Grants GB34297 and GB-36789; U. S. Public Health Service Grants AI05600, AI10884, RR7031, and CA08748; and a grant from the American Cancer Society IN-46L.

‡ Work performed during tenure of an Established Investigatorship from the American Heart Association. Present address: Department of Physiology, College of Medicine and Dentistry of New Jersey–Rutgers Medical School, Piscataway, N. J. 08854.

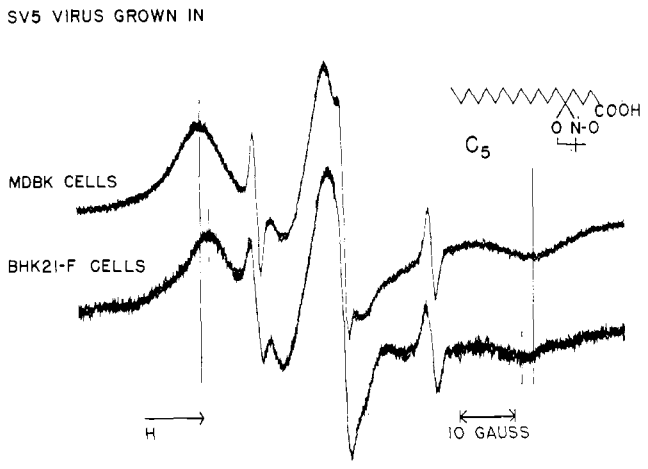


FIGURE 1: Comparison of the esr spectra of C_5 spin-labeled SV5 virions grown in MDBK and BHK21-F cells to determine the effect of a change in the lipid composition of the viral membrane. To demonstrate the greater splitting of the spectrum of virions grown in MDBK cells, a line is drawn through the high and low magnetic field peaks of the spectrum obtained from these virions and extended through the spectrum of the virions grown in BHK21-F cells. The short line is drawn through the corresponding peaks in the BHK21-F grown virions.

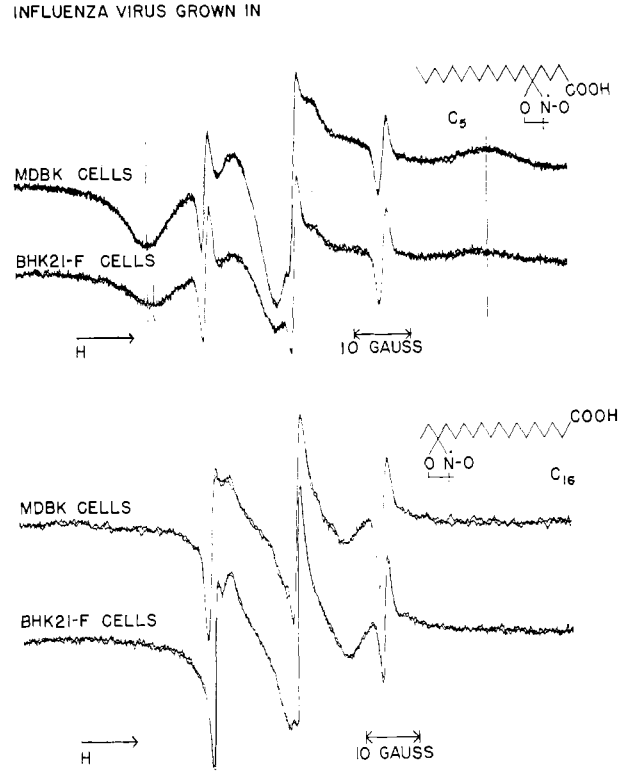
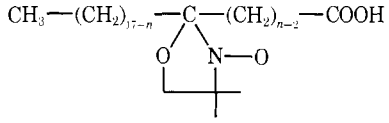


FIGURE 2: Comparison of the esr spectra of C_5 and C_{16} spin-labeled influenza virions grown in MDBK and BHK21-F cells to determine the effect of a change in the lipid composition of the viral membrane.

Virus Purification. Virions were purified by precipitation with polyethylene glycol followed by equilibrium centrifugation in a 5–40% potassium tartrate gradient (Landsberger *et al.*, 1971a). Samples were dialyzed overnight against phosphate-buffered saline (PBS) (Dulbecco and Vogt, 1954) prior to spin labeling.

Spin Labeling. Viruses were labeled with nitroxide derivatives of stearic acid having the general structure



where the nitroxide ring is located on the *n*th carbon (C_n) counting from the carboxyl group. Three spin labels were used: C_5, C_{12}, and C_{16}. Labeling with C_5 and C_{16} was accomplished by incubating the purified virus with a spin-label–bovine serum albumin complex as described previously (Landsberger *et al.*, 1971a). For labeling with C_{12} a different procedure was used. A small amount of C_{12} label dissolved in chloroform was gently evaporated to dryness on the bottom of a small erlenmeyer flask. Virus samples (0.5–2 mg in 1–2 ml) were applied to the film and stirred for 4 hr at room temperature. After labeling by either procedure, unincorporated label was removed by repurification of the virus in a potassium tartrate gradient. Prior to esr measurements, samples were dialyzed against PBS, concentrated by pelleting, and resuspended in PBS.

Chemicals. Polyethylene glycol was obtained from Amend Drug and Chemical Co., Irvington, N. J. Bovine serum albumin (less than 0.01% fatty acid) was purchased from Sigma Chemical Co., St. Louis, Mo., and the spin labels from Syva Corp., Palo Alto, Calif. Both C_5 and C_{16} were used without additional purification. The C_{12} label was further purified by preparative thin-layer chromatography.

Esr Spectroscopy. The spectra were recorded on Varian Associates E-4 and E-12 esr spectrometers. A Varian aqueous sample cell was used, except when a sample could not be properly resuspended in PBS. The wet pellet was then applied directly to a flat quartz Scanlon tissue cell with a quartz cover plate (J. F. Scanlon Co., Whittier, Calif.).

Results

The esr spectra of spin-labeled influenza and SV5 virions grown in MDBK and BHK21-F cells shown in Figures 1, 2, and 3 are characterized by the superposition of two types of spectra, a "broad-line" spectrum and a "liquid-line" spectrum. The latter consists of three sharp equally spaced lines and is due to rapid isotropic tumbling of the label. The observed splittings between these three lines is consistent with spin label in the buffering medium. A portion of the "liquid-line" spectrum is due to spin label incompletely removed from the suspending medium. The remainder is a result of spin label diffusing out of the lipid membrane. In practice this last contribution is only significant for the C_{12} label. The "liquid-line" spectrum cannot be readily interpreted in terms of membrane structure. The "broad-line" spectrum is due to spin label intercalated into the lipid phase of the viral particles (Keith *et al.*, 1968; Hubbell and McConnell, 1969a,b; Landsberger *et al.*, 1971a,b, 1972). In general, the magnitude of the splitting between the high and low magnetic field peaks increases as the motion of the spin label is restricted by its environment (Hubbell and McConnell, 1971; Jost *et al.*, 1971).

It has previously been found that SV5 virions grown in MDBK and BHK21-F cells are characterized by a marked difference in lipid composition, reflecting the differences in lipid composition of the host cell plasma membrane (Klenk and Choppin, 1969, 1970a,b; Choppin *et al.*, 1971). These differences include a higher cholesterol content of MDBK grown virus, *i.e.*, a molar ratio of cholesterol to phospholipid of 0.84 and 0.64 in virions from MDBK and BHK21-F cells, respec-

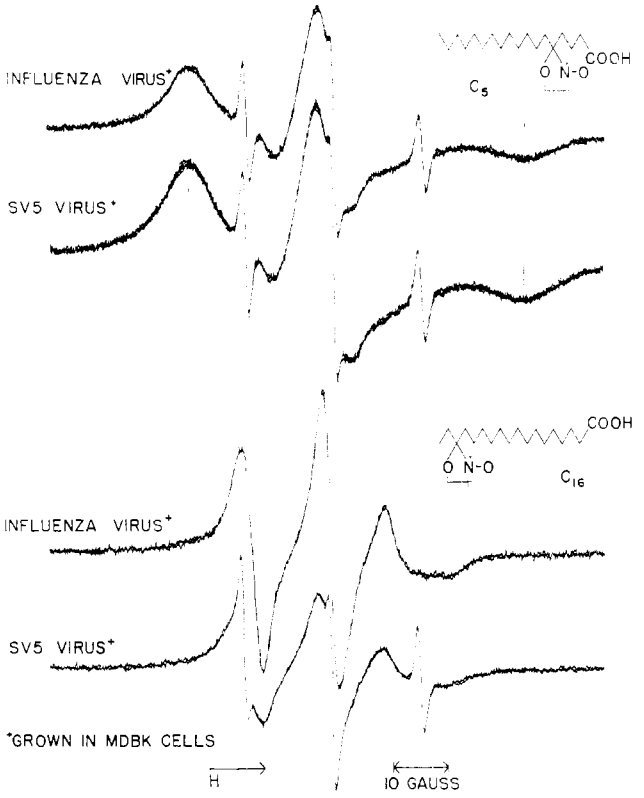


FIGURE 3: Comparison of the esr spectra of C_5- and C_{16}-labeled influenza and SV5 viruses grown in MDBK cells to determine the effect of a change in the complete set of membrane associated proteins. The identity of the spectral splitting of the two types of virions is shown by the single line drawn through the high and low magnetic field peaks of both spectra.

tively (Choppin *et al.*, 1971). To investigate the rigidity of the lipid membrane of the SV5 virions grown in cells with differing lipid composition, the esr spectra of SV5 virus grown in MDBK and BHK21-F cells are compared in Figure 1. The splitting between the high and low magnetic field peaks for C_5-labeled SV5 virions from MDBK cells is greater, and hence the environment near the polar head group is more rigid in these virions than those grown in BHK21-F cells.

Analogously, to investigate the effect of lipid composition on the structure of the lipid bilayer of influenza virus, the esr spectra of influenza virus grown in MDBK and BHK21-F cells are compared in Figure 2. The results for the C_5-labeled influenza virions are similar to those for C_5-labeled SV5 particles, *i.e.*, the region in the vicinity of the lipid polar head groups is more rigid when the lipid of influenza virus is derived from MDBK rather than BHK21-F plasma membranes. In Figure 2, it can be seen that the spectral splitting from C_{16}-labeled influenza particles is considerably smaller than C_5-labeled virions indicating the greater fluidity in the interior nonpolar region of the viral membrane. In contrast with the C_5 results, the spectral splittings of C_{16}-labeled influenza virus grown in MDBK and BHK21-F cells appears to be identical implying that the environments probed by the C_{16} label are not detectably different in terms of fluidity, although small differences might be obscured due to the interference between the high magnetic field peaks of the "liquid-line" and "broad-line" spectra. Spectra obtained from C_{12}-labeled influenza virions (not shown) demonstrate that the environment of this probe is intermediate in rigidity between that of C_5 near the polar head group and that of the deeper hydrocarbon region of C_{16}. It is difficult to obtain satisfactory spectra of C_{12}-labeled virions because of the intensity of the associated "liquid-line" spectrum due in part to spin label diffusing out of the virus. The strong interference between the "liquid-line" and the "broad-line" spectra prevents a reliable comparison between BHK21-F and MDBK grown C_{12}-labeled influenza virus.

The present results indicate that by changing the host cell from BHK21-F to MDBK the rigidity of the lipid membrane is increased in the vicinity of the polar head groups, whereas there is no detectable difference in the deep hydrocarbon region.

SV5 and influenza virions possess no common polypeptide components (Caliguiri *et al.*, 1969; Compans *et al.*, 1970; Compans and Choppin, 1971; Choppin *et al.*, 1972). To determine whether the completely different protein compositions of the SV5 and influenza viral membranes are reflected in the rigidity of the lipid phase of these membranes, the esr spectra of SV5 and influenza virions grown in MDBK cells were compared (Figure 3). The spectral splittings of C_5-labeled SV5 and influenza virions are identical. Similarly, no difference is detected in the esr splittings of C_{16}-labeled virions. Spectra of SV5 and influenza virions labeled with C_{12} indicated that the rigidity of the probe's environment is less than that of the C_5 label near the polar head groups and greater than that of the C_{16} label in the deeper hydrocarbon region. Thus the lipid organization of both viruses is characterized by a flexibility gradient, and from Figure 3, it can be seen that the gradients are indistinguishable. Therefore, although SV5 and influenza virions contain completely different membrane polypeptides, there is no detectable difference in the rigidity of the lipid layers of such virions grown in the same cell, and thus containing similar lipids.

The effect of variation of the viral proteins was also investigated by comparing influenza and SV5 virions grown in a second cell type, BHK21-F. The spectral splittings and hence the rigidity of the local environment of the C_5 spin label are identical when both influenza and SV5 are grown in BHK21-F cells, confirming the corresponding experiment with MDBK grown virions.

Discussion

Several physical methods have shown that the lipids of biological membranes, including erythrocytes and *Mycoplasma laidlawii* membranes, are organized in a bilayer structure (Steim *et al.*, 1969; Knutton *et al.*, 1970; Engelman, 1971; Wilkins *et al.*, 1971). Spin-label esr studies of these membranes and synthetic lipid bilayers have indicated that the environment of the spin label becomes increasingly more rigid as the nitroxide moiety is moved closer to the polar carboxyl head group of the spin label (Hubbell and McConnell, 1969b, 1971; Rottem *et al.*, 1970; Jost *et al.*, 1971; Landsberger *et al.*, 1971a,b; Simpkins *et al.*, 1971a,b). The observation of a rigidity gradient in lipid membranes has been recently confirmed by nonprobe techniques such as ^{13}C and ^{1}H nmr (Metcalfe *et al.*, 1971; Chan *et al.*, 1972; Horwitz *et al.*, 1972; Lee *et al.*, 1972; Levine *et al.*, 1972). The pronounced similarity in the flexibility gradient between influenza and Rauscher murine leukemia viruses and erythrocytes led to the conclusion that the lipids in both of these viruses were organized in a lipid bilayer structure (Landsberger *et al.*, 1971a, 1972). Therefore, since a similar flexibility gradient has been found in SV5 virions, we propose that the lipid phase of SV5 is also organized in a bilayer structure. By X-ray diffraction, it has been shown that the lipids in Sindbis virus (Harrison *et al.*, 1971b) and in the bacteriophage PM2 (Harrison *et al.*, 1971a)

are also organized in a bilayer. It would thus appear that the lipid bilayer structure is a ubiquitous feature of enveloped viruses. The present results indicating that the rigidity of the viral membrane depends on its lipid composition also suggests the existence of a lipid bilayer. Furthermore, since the esr spectra of the spin labels used do reflect changes in the viral lipid composition, these results provide additional evidence that the spin labels are in fact incorporated into the lipid phase of the viral membrane.

The differences in the rigidity of the viral lipid bilayers of influenza and SV5 viruses when grown in MDBK or BHK21-F cells may be attributable to at least four categories of differences in the lipid composition of the viral envelope: (1) cholesterol content, (2) phospholipid pattern, (3) fatty acid pattern of the phospholipids, and (4) types of glycolipids (Klenk and Choppin, 1969, 1970a,b; Choppin *et al.*, 1971). We cannot identify with certainty which of these variations accounts for differences in the lipid bilayer between MDBK and BHK21-F grown virions. Klenk and Choppin (1969, 1970a) have found that the molar ratio of cholesterol to phospholipid of SV5 virus grown in MDBK cells is 0.84, and in BHK21-F cells is 0.64. Since these data suggest that the cholesterol content difference is one of the more striking differences in the lipid composition of MDBK and BHK21-F grown virus (Klenk and Choppin, 1969, 1970a,b), it may be one of the more significant factors in the observed differences in membrane rigidity. The experimental results (Figures 1 and 2) indicate that the lipid bilayer of SV5 or influenza virions is more rigid when derived from MDBK than from BHK21-F cell plasma membranes. This is consistent with model membrane studies which have shown that for lipids in the liquid crystalline state the rigidity of a lipid bilayer increases with cholesterol content (DeGier *et al.*, 1969; Waggoner *et al.*, 1969; Phillips *et al.*, 1970; Butler *et al.*, 1970; Long *et al.*, 1970; Oldfield and Chapman, 1971; Darke *et al.*, 1972; Hsia *et al.*, 1972; McConnell and McFarland, 1972; Keough *et al.*, 1973; Schreier-Muccillo *et al.*, 1973). It is also possible that to some degree the greater rigidity of the SV5 lipid envelope derived from MDBK cells rather than BHK21-F cells is in part due to the higher phosphatidylethanolamine content of the MDBK grown SV5 virions (Klenk and Choppin, 1969, 1970a; Ladbrooke and Chapman, 1969). Furthermore, it cannot be ruled out that some of the observed changes in lipid organization may be due to differences in the saturated fatty acids (Klenk and Choppin, 1969, 1970a; Tourtellotte *et al.*, 1970; Rottem *et al.*, 1970; Henry and Keith, 1971; Raison *et al.*, 1971; Eletr and Keith, 1972). The data indicate that if the cholesterol content is largely responsible for the difference in BHK21-F and MDBK grown virions the condensing effect of cholesterol at these lipid compositions on the lipid bilayer of these virions is greater in the vicinity of the polar head groups than in the deep hydrocarbon region. This is consistent with studies on lecithin–cholesterol membranes which indicate that cholesterol affects the deep hydrocarbon region of the membrane to a far lesser extent than the region near the glycerol backbone (Darke *et al.*, 1972; McConnell and McFarland, 1972; Keough *et al.*, 1973).

The present data show that while the rigidity of the lipid bilayer of enveloped viruses is dependent on the lipid composition it is not altered by a complete change in the proteins associated with the viral envelope. The independence of the rigidity of the lipid bilayer on the detailed nature of the membrane proteins extend the results of previous experiments (Landsberger *et al.*, 1971a), which showed that complete removal of the glycoprotein spikes from the surface of influenza virus does not alter the rigidity of the lipid bilayer. Thus within the resolution of spin-label esr experiments, the organization of the bilayer is not altered by either removal of some membrane proteins or a change in the complete set (differing in number, size, and amino acid sequence) of membrane proteins. However, there is a nonglycosylated protein on the inner surface of the bilayer which may contribute to the rigidity of the lipid phase. For example, Rottem *et al.* (1970) and Tourtellote *et al.* (1970) found that the lipid phase of the *Mycoplasma laidlawii* membrane was more rigid than dispersed mycoplasmal membrane lipids. Similarly, Hong and Hubbell (1972) showed that the addition of rhodopsin to phosphotidyl choline bilayers increased the rigidity of the bilayer.

The lipid-containing membrane of enveloped viruses is acquired during the budding process at the plasma membrane of the host cell. To the extent that the resulting viral membranes reflect the structural features of biological membranes, the above results may be cautiously generalized to the structural organization features of cellular membranes: (1) the major portion of membrane lipid is organized as a bilayer, and the structural rigidity of the lipid bilayer does depend on the lipid composition; and (2) whereas the absolute rigidity of the bilayer may depend on the presence of protein, the rigidity does not depend on *differences* in the membrane associated proteins.

Acknowledgments

The authors gratefully acknowledge Dr. John Paxton of Varian Associates, Springfield, N. J., for his collaboration in obtaining the initial spin-label esr spectra of influenza virus particles. Miss Lynne Martin is thanked for her excellent technical assistance, and Miss Nancy Eklund for the preparation of the figures.

References

Butler, K. W., Smith, I. C. P., and Schneider, H. (1970), *Biochim. Biophys. Acta 219*, 514.

Caliguiri, L. A., Klenk, H. D., and Choppin, P. W. (1969), *Virology 39*, 460.

Chan, S. I., Seiter, C. H. A., and Feigenson, G. W. (1972), *Biochem. Biophys. Res. Commun. 46*, 1488.

Chen, C., Compans, R. W., and Choppin, P. W. (1971), *J. Gen. Virol. 11*, 53.

Choppin, P. W. (1964), *Virology 23*, 224.

Choppin, P. W. (1969), *Virology 39*, 130.

Choppin, P. W., Compans, R. W., Scheid, A., McSharry, J. J., and Lazarowitz, S. G. (1972), *in* Membrane Research, Fox, F., Ed., New York, N. Y., Academic Press.

Choppin, P. W., Klenk, H. D., Compans, R. W., and Caliguiri, L. A. (1971), *Perspect. Virol. 7*, 127.

Compans, R. W., and Choppin, P. W. (1971), *in* Comparative Virology, Maramorosch, K., and Kurstak, E., Ed., New York, N. Y., Academic Press.

Compans, R. W., Klenk, H. D., Caliguiri, L. A., and Choppin, P. W. (1970), *Virology 42*, 880.

Darke, A., Finer, E. G., Flook, A. G., and Phillips, M. C. (1972), *J. Mol. Biol. 63*, 265.

DeGier, J., Mandersloot, J.G., and Van Deenen, L. L. M. (1969), *Biochim. Biophys. Acta 173*, 143.

Dulbecco, R., and Vogt, M. (1954), *J. Exp. Med. 99*, 167.

Eletr, S., and Keith, A. D. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 1353.

Engelman, D. M. (1971), *J. Mol. Biol. 58*, 153.

Harrison, S. C., Caspar, D. L. D., Camerini-Otero, R. D., and Franklin, R. M. (1971a), *Nature (London), New Biol. 229*, 197.

Harrison, S. C., David, A., Jumblatt, J., and Darnell, J. E. (1971b), *J. Mol. Biol. 60*, 523.

Henry, S. A., and Keith, A. D. (1971), *J. Bacteriol. 106*, 174.

Holmes, K. V., and Choppin, P. W. (1966), *J. Exp. Med. 124*, 501.

Hong, K., and Hubbell, W. L. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2617.

Horwitz, A. F., Horsley, W. J., and Klein, M. P. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 590.

Hsia, J. C., Long, R. A., Hruska, F. E., and Gesser, H. D. (1972), *Biochim. Biophys. Acta 290*, 22.

Hubbell, W. L., and McConnell, H. M. (1969a), *Proc. Nat. Acad. Sci. U. S. 63*, 16.

Hubbell, W. L., and McConnell, H. M. (1969b), *Proc. Nat. Acad. Sci. U. S. 64*, 20.

Hubbell, W. L., and McConnell, H. M. (1971), *J. Amer. Chem. Soc. 93*, 314.

Jost, P., Libertini, L. J., Hebert, V. C., and Griffith, O. H. (1971), *J. Mol. Biol. 59*, 77.

Keith, A. D., Waggoner, A. S., and Griffith, O. H. (1968), *Proc. Nat. Acad. Sci. U. S. 61*, 819.

Keough, K. M., Oldfield, E., and Chapman, D. (1973), *Chem. Phys. Lipids 10*, 37.

Klenk, H. D., Caliguiri, L. A., and Choppin, P. W. (1970), *Virology 42*, 473.

Klenk, H. D., and Choppin, P. W. (1969), *Virology 38*, 255.

Klenk, H. D., and Choppin, P. W. (1970a), *Virology 40*, 939.

Klenk, H. D., and Choppin, P. W. (1970b), *Proc. Nat. Acad. Sci. U. S. 66*, 57.

Knutton, S., Finean, J. B., Coleman, R., and Limbrick, A. R. (1970), *J. Cell Sci. 7*, 357.

Ladbrooke, B. D., and Chapman, D. (1969), *Chem. Phys. Lipids 3*, 304.

Landsberger, F. R., Compans, R. W., Paxton, J., and Lenard, J. (1972), *J. Supramol. Struct. 1*, 50.

Landsberger, F. R., Lenard, J., Paxton, J., and Compans, R. W. (1971a), *Proc. Nat. Acad. Sci. U. S. 68*, 2579.

Landsberger, F. R., Paxton, J., and Lenard, J. (1971b), *Biochim. Biophys. Acta 266*, 1.

Lee, A. G., Birdsall, N. J. M., Levine, Y. K., and Metcalfe, J. C. (1972), *Biochim. Biophys. Acta 255*, 43.

Levine, Y. K., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1972), *Biochemistry 11*, 1416.

Long, R. A., Hruska, F., Gesser, H. D., Hsia, J. C., and Williams, R. (1970), *Biochem. Biophys. Res. Commun. 41*, 321.

McConnell, H. M., and McFarland, B. G. (1972), *Ann. N. Y. Acad. Sci. 195*, 207.

Metcalfe, J. C., Birdsall, N. J. M., Feeney, J., Lee, A. G., Levine, Y. K., and Partington, P. (1971), *Nature (London) 233*, 199.

Mountcastle, W. E., Compans, R. W., and Choppin, P. W. (1971), *J. Virol. 7*, 47.

Oldfield, E., and Chapman, D. (1971), *Biochem. Biophys. Res. Commun. 43*, 610.

Phillips, M. C., Kamat, V. B., and Chapman, D. (1970), *Chem. Phys. Lipids 4*, 409.

Raison, J. K., Lyons, J. M., Mehlhorn, R. J., and Keith, A. D. (1971), *J. Biol. Chem. 246*, 4036.

Rottem, S., Hubbell, W. L., Hayflick, L., and McConnell, H. M. (1970), *Biochim. Biophys. Acta 219*, 104.

Schreier-Muccillo, S., Marsh, D., Dugas, H., Schneider, H., and Smith, I. C. P. (1973), *Chem. Phys. Lipids 10*, 11.

Schulze, I. T. (1970), *Virology 42*, 890.

Simpkins, H., Panko, E., and Tay, S. (1971a), *J. Membrane Biol. 5*, 334.

Simpkins, H., Tay, S., and Panko, E. (1971b), *Biochemistry 10*, 3579

Steim, J. M., Tourtellotte, M. E., Reinert, J. C., McElhaney, R. N., and Rader, R. L. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 104.

Tourtellote, M. E., Branton, D., and Keith, A. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 909.

Waggoner, A. S., Kingzett, T. J., Rottschaeffer, S., and Griffith, O. H. (1969), *Chem. Phys. Lipids 3*, 245.

Wilkins, M. H. F., Blaurock, A. E., and Engelman, D. M. (1971), *Nature (London), New Biol. 230*, 72.