Advances in

VIRUS RESEARCH

VOLUME 18

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PREFACE

In the Preface to Volume 1 of *Advances in Virus Research,* written two decades ago, the editors expressed the intent of bringing together essays on viruses of man, of the higher animals, of insects, of plants, and of bacteria, essays focused on the virus rather than the disease. Volume 1 and succeeding volumes have followed this general pattern; Volume 18 is typical. The first two essays deal with influenza virus. The third deals with the mode of action of an antibiotic and pox virus. The fourth review compares the properties of the hereditary carbon dioxide sensitivity factor of *Drosophila* sigma virus with the morphologically similar vesicular stomatitis virus of mammals. The fifth essay discusses the initial effects in both bacterial and animal cells of virus infection. There follows a discussion of five large RNA viruses of animals: reoviruses, rhabdoviruses, paramyxoviruses, myxoviruses, and oncornaviruses. The final two essays deal primarily with the properties of plant rhabdoviruses and the viruses causing yellowing diseases in beets.

The Editors of *Advances in Virus Research* believe that responsible scientists have the right to treat their subjects from their own points of view even when some of the editors strongly hold different interpretations. The Editors frequently correspond with an author about a controversial issue and in the end publish the author's final version. One such issue is encountered in the present volume in the essay entitled "Relationship of Sigma Virus to Vesicular Stomatitis Virus." Dr. Karl Maramorosch has expressed the following view:

"For some twenty years, sigma, the 'CO_2 sensitivity factor' of *Drosophila,* has been termed a 'cytoplasmically inherited virus.' The evidence that sigma is a rhabdovirus has been derived from observations in 1965 and 1968 of particles in thin sections of *Drosophila.* Attempts to purify the presumptive virus, to link observed particles with infectivity, or to observe such particles in cultured cells in which a high-infectivity titer was obtained, have consistently failed. It should, therefore, be recognized that the view that sigma is a virus is still hypothetical."

Max A. Lauffer
Frederik B. Bang
Karl Maramorosch
Kenneth M. Smith

CONTENTS

Initial Effects of Viral Infection in Bacterial and Animal Host Cells

ALEXANDER KOHN AND PINHAS FUCHS

Nucleocapsids of Large RNA Viruses As Functionally Active Units in Transcription

A. G. BUKRINSKAYA

Plant Rhabdoviruses

R. I. B. FRANCKI

The Yellowing Virus Diseases of Beet

JAMES E. DUFFUS

STRUCTURE OF THE INFLUENZA VIRION

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I. Introduction

This review brings together information about the influenza virus which is presently available from biochemical and biophysical studies and from electron microscopy. An attempt has been made to design a model of the particle based on this information. Although much is known about these viruses, the available information is incomplete in

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[2] The author wishes to thank Ms. S. Lazarowitz and Drs. P. Choppin, D. Compans, M. V. Nermut, M. Pons, and R. Webster for making information from their work available to me prior to its publication. Except for references citing those investigations, this review is based on information published by the end of 1972.

many respects. For example, detailed information about a specific portion of the virion or about a specific biochemical process may be available, but from one virus strain only. Attempts to formulate a detailed model are also frustrated by the limitations of the experimental procedures themselves. Procedures employed to prepare a sample for viewing in the electron microscope may alter the structure of the virus. Disrupting the virion and separating its components for biochemical and biophysical analyses provides further opportunities for artifacts. However, these procedures also provide complementary information about the structure of the virion. Since we must rely on observations without full knowledge of what has produced them, seeing alone is not believing. Facts about the structure can be derived from the evidence at hand only when a number of seemingly independent observations converge on and mutually support a conclusion. The structure proposed here is, therefore, intended to be a working model, based on our present state of knowledge. It is put forth at this time as much to provoke questions as to answer them.

A. Antigenic Properties of the Influenza Viruses

The influenza viruses, originally designated myxoviruses and now referred to as orthomyxoviruses (Melnick, 1971) are of human, avian, equine, and porcine origin. These viruses are very similar in chemical composition, in appearance as viewed by electron microscopy, and in biological activity, independent of whether they are of mammalian or of avian origin.

Influenza viruses are designated as antigenic type A, B, or C, according to the kind of complement-fixing antigen (ribonucleoprotein) which is present within the virion. To date, type A antigen has been found in viruses isolated from birds, lower mammals, and man, whereas types B and C antigens have been found in viruses of human origin only. Human-porcine and human-avian recombinant type A strains can be obtained with fair ease under laboratory conditions; these recombinants probably arise in nature as well and may be involved in the emergence of new pandemic strains (see review by Webster, 1973).

Two components of the viral envelope, the hemagglutinin and the neuraminidase, constitute the major antigenic determinants on the surface of the virion. The hemagglutinin is responsible for the attachment of the virus to host cells and to erythrocytes, and it induces the production of neutralizing antibody. The viral neuraminidase removes terminal sialic acid residues from glycoproteins on the surface of cells and causes elution of the virion. It also induces the formation of specific antibodies.

Human type A viruses have in the past been classified as subtypes A_0, A_1, or A_2, depending on the antigenic properties of the hemagglutinin. At present, the antigenic properties of both the neuraminidase and the hemagglutinin are used in the identification of newly isolated strains (World Health Organization Report, 1971).

B. Comparison of Orthomyxoviruses and Paramyxoviruses

The group of viruses which most closely resembles the influenza viruses in chemical and physical properties is the paramyxoviruses. No antigenic relationships exist between the two groups. The paramyxoviruses are enveloped viruses which also have neuraminidase and hemagglutinin molecules on their surfaces. Some paramyxoviruses have hemolytic and cell fusion capacities whereas the orthomyxoviruses do not. Virions of both groups contain an RNA-dependent RNA polymerase. Although their genetic material is in both cases single-stranded RNA, the genome of the orthomyxoviruses is segmented, whereas that of the paramyxoviruses is in one piece. The paramyxoviruses undergo little antigenic change in nature and do not show high levels of recombination. They produce defective particles with less facility than do orthomyxoviruses. Finally, the synthesis of the influenza viruses is inhibited by actinomycin D, whereas that of paramyxoviruses is not. Facts about the paramyxoviruses will be used in appropriate sections to augment the available information or to make comparisons with the influenza viruses.

C. Nomenclature

All influenza A strains discussed here are of human origin except the Rostock strain of fowl plague virus, designated A_0/FPV/Rostock. Commonly known strains are designated by the antigenic subtype and the name assigned to them at the time of isolation. For recombinant A strains, the revised system of nomenclature for influenza viruses (World Health Organization Report, 1971) is used so that the antigenic subtype (A_0, A_1, A_2, or A_3) of both the hemagglutinin and the neuraminidase can be indicated.

The virion polypeptide designations used here are those adopted in 1971 at the Workshop on Influenza Virus Polypeptides and Antigens (Kilbourne *et al.*, 1972; Laver, this volume).

II. Physical Properties of Influenza Virus Particles

A. Morphology of the Virion

Most influenza virus particles are roughly spherical; some filamentous or rod-shaped particles are, however, observed in preparations of viruses

of all three types. Both the spherical and the rod-shaped particles are covered with evenly spaced radial projections which are called "spikes." Influenza A and B strains have nearly identical surface morphology, whereas influenza C differs somewhat, displaying areas, sparsely covered with spikes, which reveal an underlying lattice of hexagonal and pentagonal units (Archetti *et al.*, 1967; Waterson *et al.*, 1963; Flewett and Apostolov, 1967). The influenza viruses are variable in size and shape by comparison to small icosahedral structures such as the picornaviruses. However, much of the pleomorphism observed by negative staining is induced during storage or during preparation of virus samples for electron microscopy (Choppin *et al.*, 1961; Nermut and Frank, 1971;

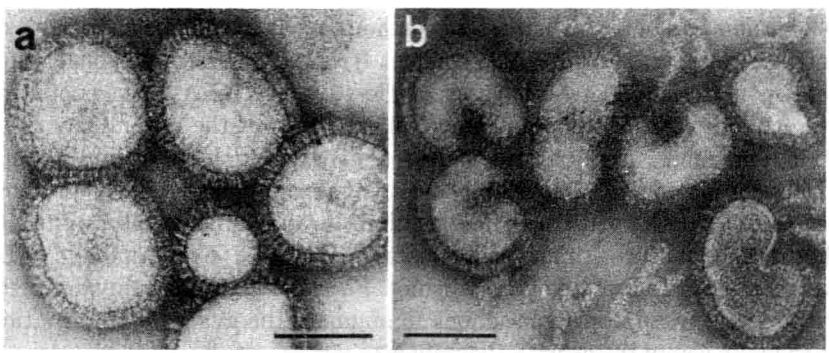

FIG. 1. Effects of storage on the morphology of purified influenza virus. (a) Newly prepared A_0/WSN virus, negatively stained with potassium phosphotungstate at pH 7.0. Modified from Schulze (1972). (b) Virus after approximately 1 week of storage at 5° in 0.1 *M* NaCl containing 0.05 *M* Tris·HCl, pH 7.5 and 10^{-3} *M* EDTA. Particles are invaginated and bean-shaped. (I. T. Schulze, unpublished electron micrograph.) a and b: Marker = 1000 Å.

Schulze, 1972). Although newly prepared virus is sufficiently stable to retain a quasi-spherical shape when subjected to negative staining, freeze-drying or freeze-etching procedures produce more uniform images. Changes in the viral envelope that occur during storage, or can be induced by mild protease treatment, permit even greater distortion of the particles during electron microscopy (Fig. 1).

Newly isolated strains of virus, i.e., strains which have been grown for only a few passages in embryonated eggs or in cell cultures, appear to be more pleomorphic and to contain more filamentous forms than do virus strains which have been grown for many generations under laboratory conditions (see Laver, this volume). Since a period of adaptation is usually required before a high proportion of infectious virus is

obtained from a new host, this pleomorphism may also result from distortion of poorly formed and therefore unstable particles.

A number of factors appear to be involved in determining whether a virion will be spherical or filamentous. It has been suggested that in nature human strains of virus may be predominantly filamentous; Burnet and Lind (1957) observed that, concomitant with adaptation of a newly isolated human strain of virus to growth in the chorioallantoic

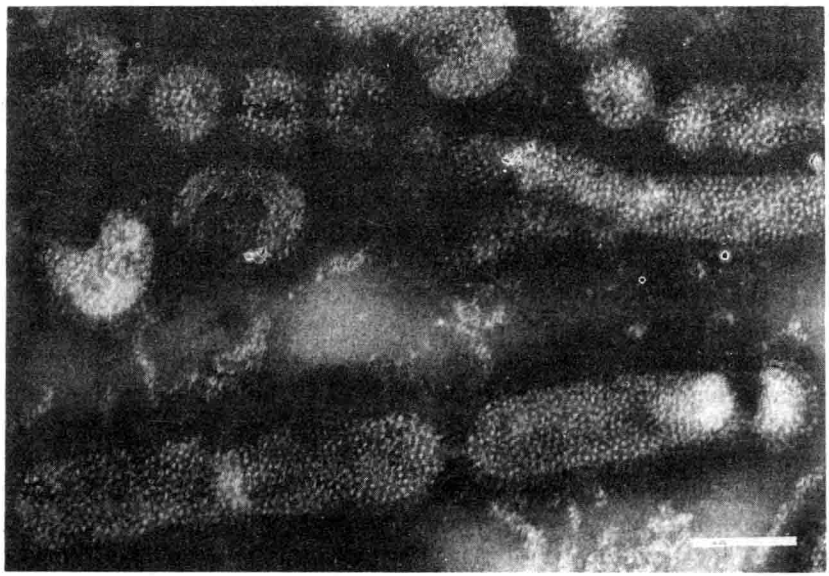

FIG. 2. A preparation of purified virus showing filamentous, ellipsoid and spherical particles. A_0/Mel virus grown in the allantoic sac of chick embryos was purified by centrifugation to equilibrium in a sucrose gradient as described by Pons and Hirst (1968a); the preparation was negatively stained with potassium phosphotungstate. (I. T. Schulze, unpublished electron micrograph.) × 140,000; marker = 1000 Å.

membrane of the chick embryo, the number of filaments was reduced. Such changes could result from mutation, coupled with a growth advantage for spherical particles. There is good evidence that the filamentous characteristic of some strains of virus is genetically determined (Murphy and Bang, 1952; see review by Kilbourne, 1963). However, surface-active agents have been reported to induce the formation of filaments and large pleomorphic particles from a strain of virus usually found to be predominantly spherical (Blough, 1963). Thus, the presence of filaments is not determined solely by the genetic character of the virus. Figure 2 shows filamentous and spherical particles recovered from a sucrose

density gradient. Evidently, the two types of particles either have the same density or one type of particle was formed from the other within the sample. Preparations of virus which contain a predominance of filamentous particles can have high infectivity to particle ratios (Ada *et al.*, 1958). Therefore, it seems likely that, at least in some cases, filaments represent either multiple incomplete genomes capable of complementation or multiple nucleocapsids enclosed in one elongated envelope. Such particles could arise by incomplete separation during budding or by fusion.

Early evidence suggesting that influenza virus might have icosahedral symmetry was reported by Hoyle *et al.* (1953) who found that some A_0/DSP virus particles appeared to have hexagonal outlines after shadowing. In addition, Almeida and Waterson (1967) have pointed out similarities between the packing of the spikes on the surface of the influenza virion and the packing of capsid proteins in naked icosahedral virions. By using freeze-drying and freeze-etching techniques, Nermut and Frank (1971) have obtained somewhat more convincing evidence that influenza virus can resemble an icosahedron (Fig. 3). Shadows cast by individual particles resemble those cast by an icosahedron and particles pack together in an hexagonal array. In addition, isolated virions with hexagonal outlines could occasionally be seen following negative staining. As pointed out by Nermut and Frank, particles which are enclosed in a lipoprotein envelope would not be expected to exhibit acute angles and flat surfaces like those observed with naked icosahedral virions. They have described the influenza virus particle as a "plastic icosahedron."

B. Heterogeneity of the Virus Population

Deductions about the composition and structure of influenza virus are based on information derived from populations of virions rather than from single particles; it is important therefore to know the ratio of infectious particles to total particles present in virus preparations. Under optimal conditions about one out of ten spherical particles in an influenza virus preparation produces progeny (Donald and Isaacs, 1954). However, influenza virus is notorious in its ability to form biologically active but genetically deficient particles (von Magnus, 1954). Thus, after even one passage at a high multiplicity of infectious virions per cell, virus yields show a 10- to 100-fold increase in defective particles. By using consecutive passages at high multiplicities, populations can be obtained which contain one infectious virion per 10^5 defective particles. Fortunately, the quality of a virus preparation can be readily deter-

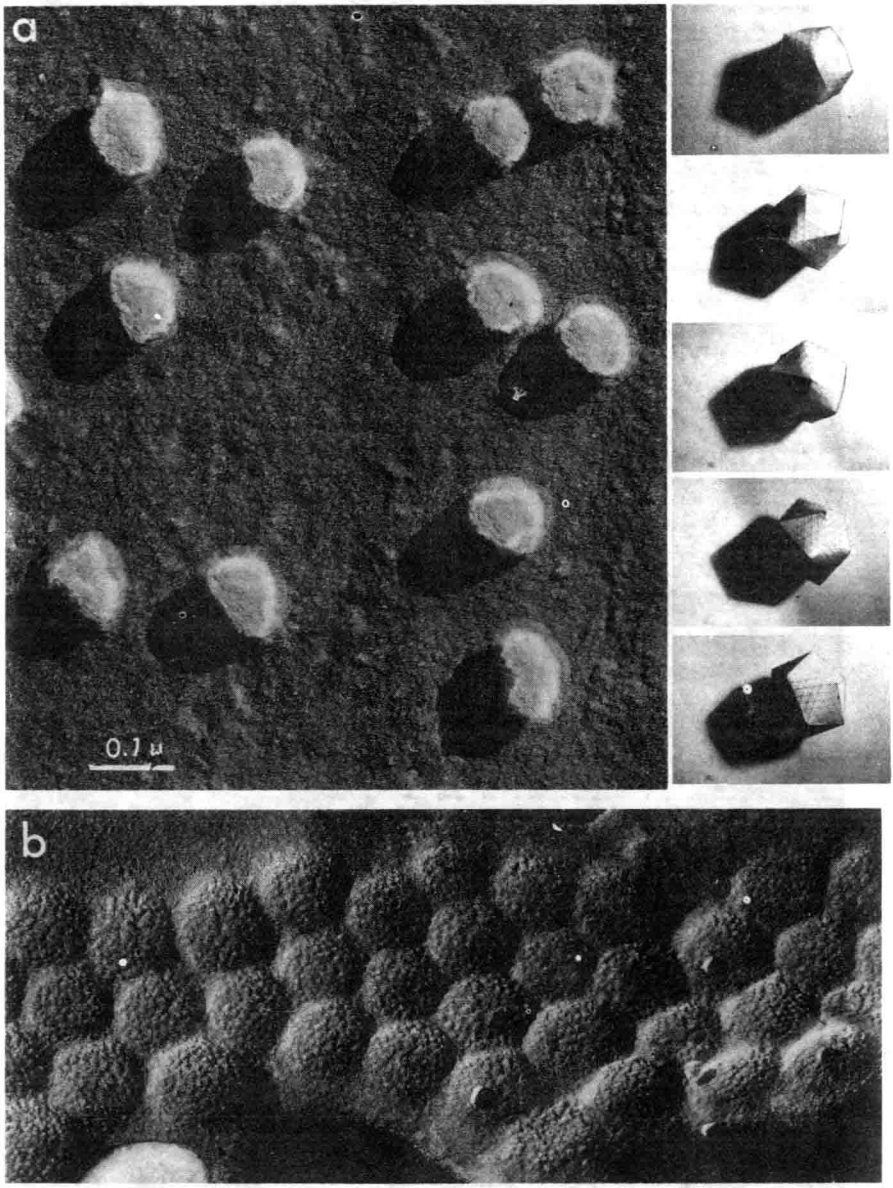

FIG. 3. Morphology of purified A_2/Singapore virus. Virus was grown in the allantoic cavity of chick embryos and was purified by cycles of adsorption to and elution from red blood cells. (a) Particles were prepared for electron microscopy by freeze-drying and shadowing. (b) Surface of closely packed particles as revealed by freeze-etching and shadowing. Marker = 1000 Å. From Nermut and Frank (1971).

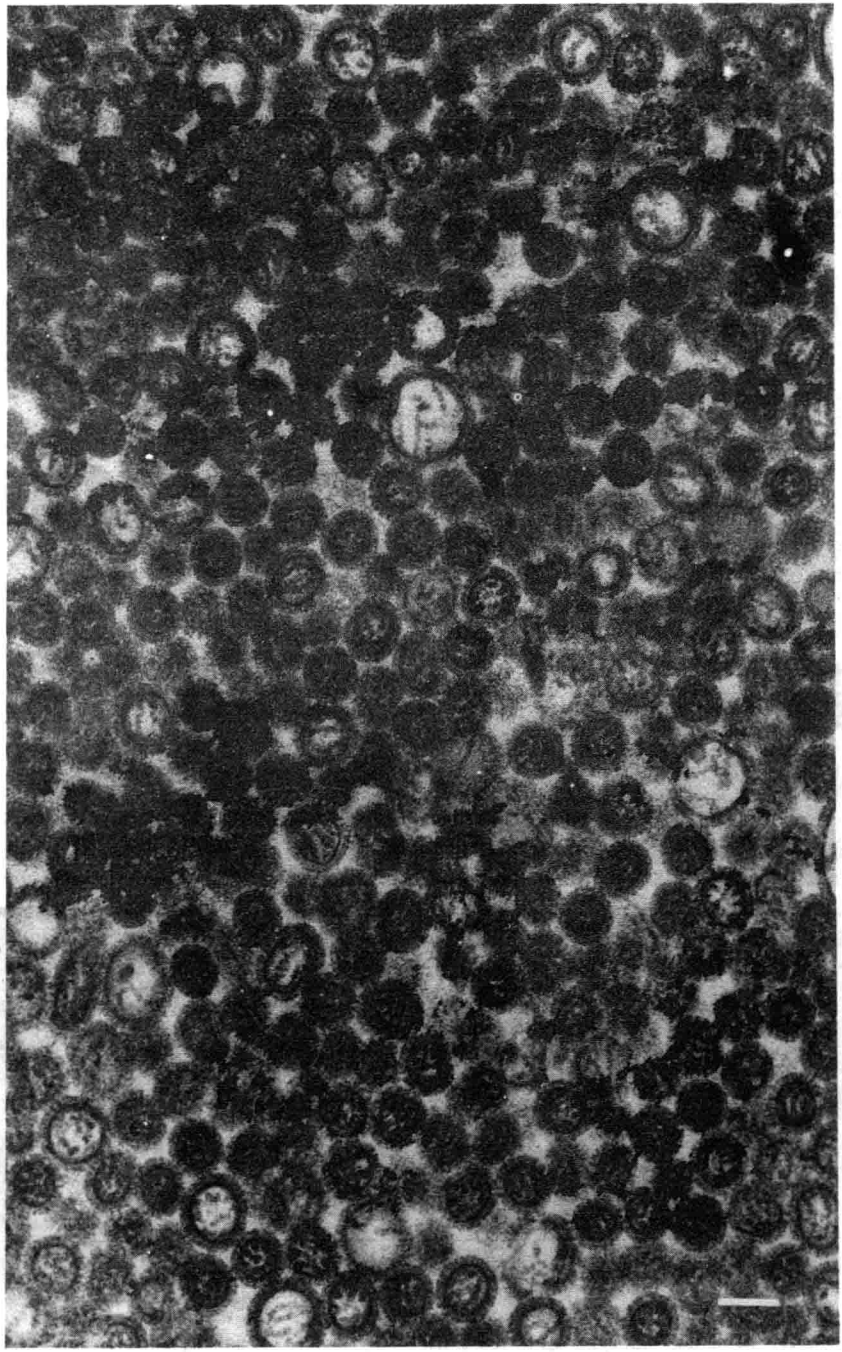

FIG. 4.

mined from the ratio of infectious particles to hemagglutinating units. Depending on the strain of virus and the technique used to determine hemagglutination (Hirst and Pickels, 1942; Salk, 1944; Levine *et al.*, 1953; Drescher, 1957), one hemagglutinating unit represents approximately 10^7 to 10^8 virions (Seto *et al.*, 1961; see also review by Hoyle, 1968). Highly infectious virus (called "standard virus") when titrated in embryonated eggs gives an infectivity titer to hemagglutinating unit ratio of about 10^6. With strains of virus which can be assayed by plaque production, standard virus produces 10^4 to 10^6 plaques per hemagglutinating unit.

Influenza virus is usually grown in cell cultures or in the chorioallantoic membrane of the chick embryo. The virus is liberated into the culture medium or the chorioallantoic fluid and must be separated from contaminating cell components before it can be used for biochemical studies. Since many of the currently used purification procedures employ rate-zonal and/or isopycnic centrifugation procedures, purified virions would be expected to be homogeneous in size and density. Figure 4 shows a thin section from a pellet of purified virus of the B/Lee strain. The observed variation in the diameter of the particles depends partially on where relative to its center each particle was sectioned; however, it exceeds that expected for virions of uniform size. Empty shells, usually larger in diameter than filled particles, are also seen. Analysis of the band width obtained when virus is centrifuged to equilibrium in sucrose also indicates some heterogeneity in density. However, no difference in particle morphology was observed when fractions from different positions in a band of A_0/WSN virus was examined in the electron microscope (I. T. Schulze, unpublished observations).

Since the RNA of influenza virus constitutes a small fraction of the particle mass, multiploid particles (particles with more than one genome) or partial diploids (particles with extra copies of some but not all genes) would not be expected to vary in size or density so long as the RNA is contained within a single nucleocapsid and envelope. However, some large particles are found within most virus populations. Hirst has reported that there can be a 2-fold variation in the diameter of particles within virus preparations from cells infected with two mutant strains and has observed heterozygous particles within such populations (Gotlieb

FIG. 4. Morphology of purified virus as revealed by thin sectioning. B/Lee virus, grown in the allantoic sacs of chick embryos was purified as described by Pons and Hirst (1968a). Sections, cut from a pellet of virus obtained by centrifugation, were stained as described by Schulze (1972). Since particles are randomly oriented within the pellet, some are cut near the surface whereas others are cut near their centers. × 70,000; marker = 1000 Å. Modified from Schulze *et al.* (1970).

and Hirst, 1954; Hirst, 1962). Such particles could arise from errors in packaging. Alternatively, they could arise by fusion of the membranes of two particles after their liberation from the cell. Particles can, in fact, be induced to fuse by treating them with phospholipase C after their glycoprotein spikes have been removed (I. T. Schulze, unpublished observations). Nermut and Frank (1971) have suggested that some of the pleomorphic forms observed in aged preparations result from coalescence of two or more virions.

C. Size of the Virus Particle

Based on measurements from electron micrographs, the diameter of the majority of virions within a preparation varies around 1000 Å. Diameters appear to vary somewhat from strain to strain; however, the values obtained also depend on the techniques used. For example, freeze-drying and shadowing of A_2/Singapore virus gave a mean diameter of 1015 Å while negative staining alone gave a mean diameter of 1270 Å (Nermut and Frank, 1971). With the A_0/WSN strain, diameters calculated from glutaraldehyde-fixed particles were smaller than those obtained from unfixed particles when both were negatively stained. The lower of these two values agreed with those obtained from sections through pellets (Schulze, 1972). The increase in diameter seen in the absence of fixation is presumably due to flattening of the virion on the grid.

The first measurements of the diameter of the virion were made by ultrafiltration (Elford *et al.*, 1936). A range of 800 Å to 1200 Å was obtained for the two strains of virus then in existence. All subsequent isolates fall within the range. It should be pointed out, however, that this variation indicates a 3-fold difference in the volume of a spherical particle.

Approximately 0.8–1.1% of the dry weight of influenza virus is RNA, 4.6–6% is carbohydrate, 20–24% is lipid, and the remainder of the virion (70–75%) is protein. Based on particle counts and protein determinations by the method of Lowry *et al.* (1951),[3] an average virion has been estimated by Reimer *et al.* (1966) to contain 4.2×10^{-16} gm (2.5×10^8 daltons) of protein. If 70% of the virion is protein, the dry weight of the particle is approximately 6×10^{-16} gm, a value which agrees well with that determined earlier by Ada *et al.* (1958). Hoyle (1968) has estimated the water content of hydrated A_0/DSP particles to be

[3] It should be pointed out that this technique, although very sensitive, may be inaccurate if the amino acid composition of the virus differs substantially from that of the protein standard, or if nonprotein components of the virus preparation alter the color reaction.