VOLUME IV

THE CELL

Biochemistry, Physiology, Morphology

Edited by

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PREFACE

The excellent reception accorded the first volume has been very encouraging. It is most rewarding for the contributors to realize that this treatise is filling a real need for a synthesis of up-to-date knowledge in the many active fields of research on the cell.

The chapters in these volumes deal with many different types of cells. Of course what one would desire is to have presented cells of the whole animate creation. "We ought not to hesitate nor to be abashed," said Aristotle, "but boldly to enter upon our researches concerning animals of every sort and kind, knowing that in not one of them is Nature or Beauty lacking." Selection, however, was necessary so that not all areas could be covered.

J. BRACHET
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July, 1960

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CHAPTER 1

Viruses

By RENÉ THOMAS

I. Introduction

This chapter is by no means an attempt to review recent developments in virus research; the bibliography is far from extensive and, except for a few references added in proof, it ends at the beginning of 1958, when the paper was written. Our purpose is rather to outline some of the main research lines in the field of virology in connection with their bearing on

general biology, and, in addition, to provide a minimal background of general information. Such important questions as, for instance, the relationships between cancers and viruses will not be treated here (see Oberling, Le Breton, and co-workers, in Volume V). Much information and stimulating discussions will be found in Luria's "General Virology" (1953) and recent review (1958). Most of the facts with which we shall deal are known from work on a very limited number of viral "species." A number of these facts have been demonstrated for the first time and most clearly for bacterial viruses. Partly for this reason, but also for personal ones, the present account will be biased towards bacterial viruses.

We first have to define our material, which is not an easy task in the case of viruses. The old definition based on the *size* of viruses is obviously insufficient by itself. It could hardly be expected that the limits of visibility with the light microscope or the filterability through bacterial filters could give more than an arbitrary, although often convenient, borderline between viruses and cells. In fact, some bacterial species and other microorganisms are smaller than the large viruses. Many bacteria of normal size can, under certain conditions, give rise to submicroscopic stages which can be reconverted into normal bacteria. On the other hand, some viruses (e.g., poxviruses) can be visualized with the light microscope after suitable coloration.

The most outstanding character common to all viruses is probably that they are *obligatory intracellular parasites of specific hosts.* This property is insufficient, however, to define a virus, since some small bacteria and other organisms are also intracellular parasites which have in many cases not yet been grown on acellular medium. On the other hand it is conceivable, although admittedly dubious, that further progress in the isolation of subcellular structures could finally enable us to grow viruses without the presence of intact cells.[1]

The relations between a virus and its host cell are more intimate than any other parasite-host relationship and should be situated at a different level of integration: increasing knowledge of the chemical composition of viruses shows that virus particles lack energy-yielding and synthetic enzyme systems, and in most or all cases lack one of the two classes of nucleic acids. It may be inferred that the need for a specific intracellular medium has here a deep significance and means more than just a group of complex nutritional requirements. When we look at viruses from this point of view the difficulty is no longer to distinguish them from cells, but rather from cell constituents. Viruses are, indeed, frequently com-

[1] Bacterial protoplasts, on which phages grow readily, could hardly be considered as subcellular units, since they differ from ordinary bacterial cells only by the absence of cell wall constituents.

pared to "normal" cell constituents, from which they are believed by many to have arisen.

The distinction between viruses and cell constituents is not always an easy one to make and is probably sometimes meaningless. Especially interesting are the cases of the "kappa" factor in *Paramecium* (see Beale, 1954) and of the "génoïde" in *Drosophila* (see L'Héritier, 1957). The value of *pathogenicity* as a criterion is far from absolute, since many viruses multiply, at least in some hosts, without any harmful effect. As for the *transmissibility,* those normal cell constituents to which viruses are usually paralleled are also endowed with genetic continuity. In the case of these cell constituents, the transmission is achieved, in principle, from mother cell to daughter cells (although sexuality and related phenomena involve transfers that are sometimes very similar to contagion). On the contrary, viruses typically infect cells *from outside.* They are found not only in the cells, but also as extracellular, infectious but metabolically inactive particles. It is frequently the case, however, at least for bacterial viruses, that the viral genetic material associates with the host genome and adopts its rhythm of division. The disease is then carried as the inherited potentiality to produce virus particles along with immunity against infection from outside (provirus). If for some reason the ability to produce mature virus particles disappears, the viral genetic material may become hardly distinguishable from a host genetic marker conferring immunity (Jacob and Wollman, 1957).

Recent developments of outstanding importance indicate that, at least in the case of bacteriophages, virus particles are functionally differentiated into a genetic moiety which is introduced into the host cell and a coat which remains outside (Hershey and Chase, 1952). The coat is involved in the processes of fixation onto the cell and of injection of the genetic material. Once introduced in the cell, the latter—if not reduced to a provirus—turns to its own advantage the energy-yielding and synthetic systems of the host cell. It induces the synthesis of a number of nonhost substances, part of which will appear in the new viral particles. Recent evidence indicates that infection may be carried out, in the case of tobacco mosaic virus and of some animal viruses, using isolated genetic material (rather pure nucleic acid preparations).

For short, many viruses may be characterized as follows. They occur as submicroscopic infective particles, metabolically inert by themselves and lacking part of the fundamental constituents of a cell. These particles are able to invade specific host cells. This process involves, often and perhaps always, some kind of dissociation of the viral constituents. The viral genetic material then turns to its advantage the host cell synthetic abilities, and more virus is finally produced. Many viral forms,

however, associate in a more or less symbiotic way with the host cell. The occurrence of mature particles and of infection from outside then becomes facultative and, in some cases, really exceptional. Such viral forms may be hard to distinguish from "normal" cell constituents.

To conclude this introduction, let us quote the definitions recently proposed by two outstanding virologists. ". . . whatever mechanisms future discovery may reveal, it seems now a fruitful unifying view to consider viruses as genetically specific cell constituents containing coded DNA or RNA which can, as one of their genetic functions, determine their own incorporation into specific vehicles for transmission to other cells" (Luria, 1958). "Viruses are complex organized infectious entities which can reproduce from their genetic material only" (Lwoff, 1959).

II. Properties of Virus Particles

A. Detection and Titration

The most obvious manifestation of viruses, in general, lies in the production of abnormalities in the host. This is the way that viruses usually are discovered. These symptoms will not be described in this paper, except for the important distinction between local and "systemic" (generalized) infections.

Local lesions are of special interest for the direct evaluation of the number of infectious units. This evaluation can be made for bacteriophages by plating an appropriate dilution of the virus preparation with an excess of sensitive bacteria on agar medium. After bacterial growth the phage particles that were present at the moment of plating can be scored as "plaques," discrete areas of growth inhibition. For animal viruses, the evaluation is often made by counting the number of lesions on an appropriate membrane, e.g., the chorioallantoic membrane of the chick embryo (Beveridge and Burnet, 1946). A more recent procedure is to count the number of plaques on a layer of tissue culture cells (Dulbecco, 1952). For plant viruses the determination is usually carried out by rubbing serial dilutions of the virus preparation onto susceptible plant leaves and scoring the number of lesions.

Generalized infections also are used, especially for animal virus titrations. The procedure followed is to determine what dilution of a virus preparation can give positive responses (end-point titration), or to count the number of positive and negative responses in groups of hosts, each group being treated with a different dilution of the virus preparation.

The method of plaque counts, when applied with dilutions of the same virus preparation, shows usually that the number of lesions varies

as a linear function of the virus concentration. This observation indicates that a single material particle is enough to induce a lesion. This conclusion is confirmed by the fact that the distribution of the numbers of plaques produced by aliquots of a single suspension agrees with the distribution (Poisson) expected for independently distributed particles. Again the same conclusion comes out from the analysis of the frequency of positive responses as a function of dilution.

Each positive effect can thus be related to a single particle. Every virus particle however, for various reasons, will not succeed in establishing infection, so that the measured titer of infectious units will usually be proportional, rather than equal, to the real concentration of virus particles. The factor is frequently close to unity for bacteriophages, but it is often of the order of only 10^{-6} for plant viruses.

B. Morphology and Chemical Nature

When a viral particle is detected by any property other than the effects on the host, its identification needs special caution. The difficulties are similar to those encountered in proving that a given biological activity—for instance, enzymatic or transforming activity—is associated with a given molecule. Moreover it is known that different kinds of particles related to the same virus may be infectious. For instance, crystals of tobacco mosaic virus (TMV), TMV rods and various degradation forms of it, including ribonucleic acid (RNA) preparations, have been observed to be infectious. Particles homogeneous in size, shape, and properties have, however, been isolated in a number of cases and have been found to be always associated with a given viral disease, but lacking in the noninfected host. As their number is proportional, and in some cases almost equal, to the number of infective units, it is quite reasonable to call them virus particles.

Viruses are detected as individual particles either by optical methods (essentially electron microscopy) or by autoradiography of radioactive preparations (see page 27). A number of other physical techniques (ultrafiltration, sedimentation, diffusion, sedimentation equilibrium, light scattering, electrophoresis) have given valuable information about size and/or shape of virus particles. Some of them are essential tools for isolation and purification of viruses.

1. Bacterial Viruses (Bacteriophages, Phages)

The known bacteriophages appear as tadpole-shaped particles (Figs. 1 and 2), whose morphology is remarkably homogeneous for a given species. The head is more or less spherical or polyhedral, from about 100 mμ in diameter for the large ones down to about 20 mμ or less for the smallest.

FIG. 1. Particles of T2 bacteriophages. Magnification: ×60,000. Electron micrograph by Dr. R. C. Williams.

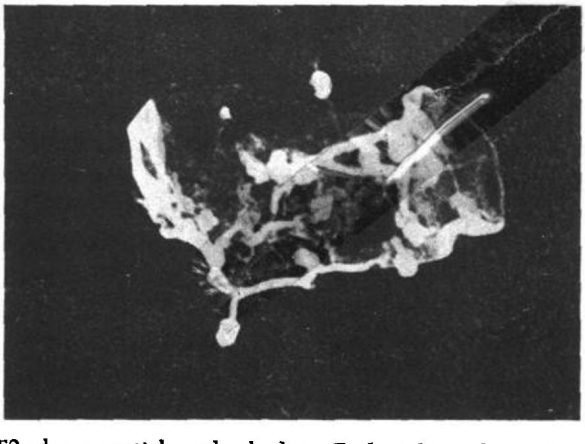

FIG. 2. T2 phage particles adsorbed on *Escherichia coli* membrane. Magnification: ×40,000. Electron micrograph by Dr. R. C. Williams.

The length and width of the tail vary depending on the type. The number of infective particles, as given by plaque counts, is in the best preparations close to the number of particles counted with the electron microscope (Luria *et al.*, 1951; Kellenberger and Arber, 1957).

Like other viruses, bacteriophages contain proteins and nucleic acid. Their nucleic acid is deoxyribonucleic acid (DNA) and is present in unusually high proportion, about 50%. Osmotic shock experiments (Anderson, 1949, 1953; Herriot, 1951) with phage T2 from *Escherichia coli* yield "ghosts" with normal morphology but empty heads, which are noninfectious (Fig. 3). During this treatment DNA is released from the phage particles. It is now sensitive to deoxyribonuclease (DNAase), unlike the DNA of intact phage particles. Most of the phage proteins sediment with the ghosts. The intact phage particle can thus be described in a simplified way as a DNA core enclosed in a protein membrane to which the tail is attached.

The most extensively studied phage, T2, contains 2×10^{-10} μg. of DNA (if it were all in a single molecule, it would correspond to a molecular weight of 1.2×10^8) whereas the small ϕX174 contains only about 70 times less DNA (corresponding to a molecular weight of only about 1.7×10^6) (Sinsheimer, 1957). The structure of most phage DNAs seems to be similar to other DNA preparations (X-ray diffraction pattern; four different nucleotides with the molar ratios: adenine $\approx$ thymine and cytosine $\approx$ guanine). It was found recently, however, that the small phage X174 contains a single-stranded DNA (Sinsheimer, 1959a, b). The so-called T-even phages of *E. coli* contain instead of cytosine, hydroxymethylcytosine (HMC) (Wyatt and Cohen, 1953); in part of the HMC nucleotides (hydroxymethyl deoxycytidylic acid) the nitrogen base is linked to one or two molecules of glucose (Sinsheimer, 1954, 1956; Volkin, 1954; Streisinger and Weigle, 1956).

The protein shell of phages can be dissociated by several methods, including freezing and thawing (Williams and Fraser, 1956). It is then possible to distinguish head membranes, intact tails, and tails degraded in various ways. The tail includes a central core surrounded by two protein sheaths, a proximal one and a distal one. The distal sheath is involved in the process of fixation on bacterial receptors, and, at least in T2, can be dissociated into several (four?) threads. The head membrane and the tail tip, at least, are antigenic, and the blockage of the latter by antiserum prevents phage adsorption. An enzyme, active on cell wall constituents, has been demonstrated in some cases (Kozinski and Opara, 1956; Kozloff *et al.*, 1957). In T2 this enzyme has the specificity of a lysozyme (Koch and Dreyer, 1958).

Besides external constituents and DNA, phage particles contain some

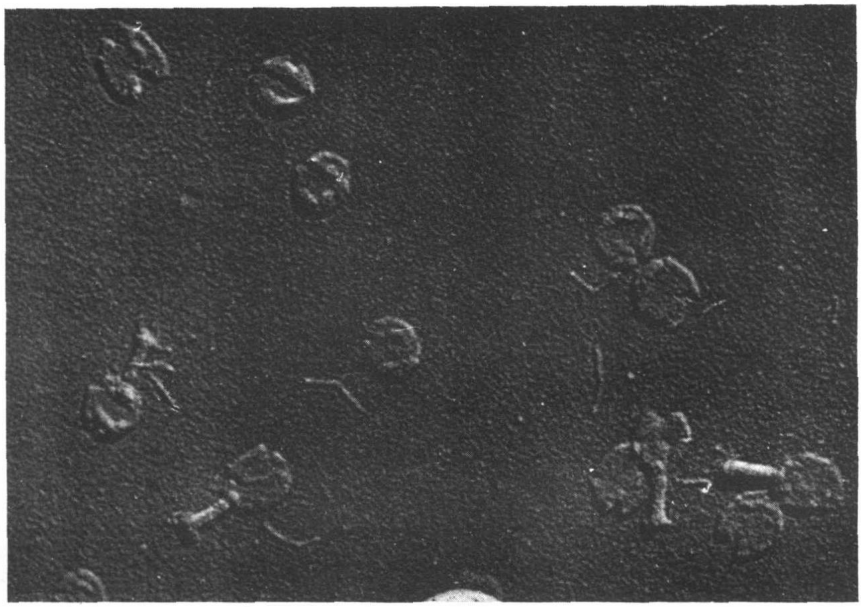

FIG. 3. T2 ghosts, head membranes, and tail fibers. Magnification: ×72,000. Electron micrograph by Dr. R. C. Williams.

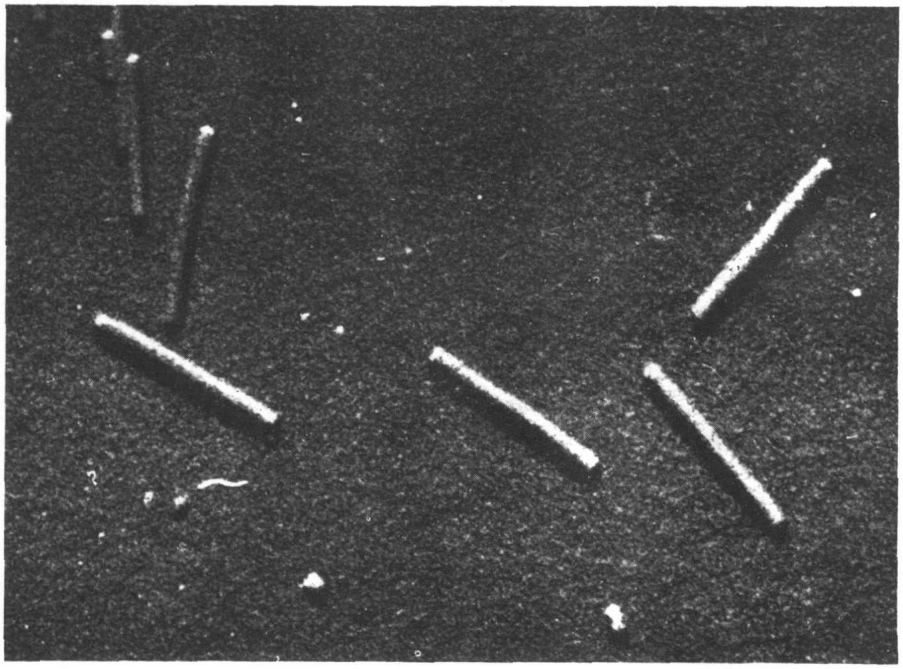

FIG. 4. Tobacco mosaic virus particles. Magnification: ×90,000. Electron micrograph by Dr. R. C. Williams.

minor internal constituents, which were investigated by Hershey (1955, 1957b) in order to determine whether DNA is associated with some other substances in phage "chromosome." Fractionation of labeled phage enabled him to characterize a "nonsedimentable" protein (in opposition to the other proteins, constituting the ghost), a peptide and two low molecular weight substances, which were later identified as polyamines (purescine and spermidine) by Ames *et al.* (1958).

Work of Volkin and Astrachan (1956b) excludes the presence of ribonucleic acid (RNA) in significant amounts.

Summary. Bacteriophages are, typically, tadpole-shaped particles containing DNA and proteins in about equal amounts. The DNA is enclosed in a shell (head membrane and tail) including several different proteins.

2. *Plant Viruses*

Plant viruses are either elongated rods [e.g., tobacco mosaic virus (TMV), 300×15 mμ] or small spheres, frequently about 20 mμ in diameter (e.g., turnip yellow mosaic virus). Figures 4 and 7 show both types of plant viruses (TMV and tomato bushy stunt virus) at the same magnification. In the case of rod-shaped viruses, various lengths are usually found in electron microscopic preparations. There is reason to think that they occur through fragmentation of particles of standard length or of their di- or trimers. Both types of plant viruses tend to crystallize. Some can be precipitated from purified suspensions into very regular crystals. The elongated type gives paracrystals, with parallel orientation of the rods, but without a regular three-dimentional network.

Plant viruses seem to be composed exclusively of ribonucleic acid and proteins. The relative amount of these two constituents is highly variable (6% RNA in TMV, 38% in turnip yellow mosaic virus), but the absolute amount of RNA per virus particle is rather uniform. The total RNA of a TMV particle corresponds to a molecular weight of about 2.5×10^6; it is now generally considered a single molecule rather than a group of subunits (Schramm and Gierer, 1957).

The protein moiety of TMV has been extensively studied and found to be composed of approximately 2300 probably identical polypeptide chains (Fraenkel-Conrat and Singer, 1954; Braunitzer, 1954, 1955; Schramm and Anderer, 1955). These polypeptide chains have a molecular weight of about 17,000. The polypeptide chains of different TMV strains have different terminal amino acid sequences (Nice and Fraenkel-Conrat, 1955).

As a first approximation, a TMV particle could be visualized as a central RNA string surrounded with a protein envelope made from a helical aggregation of the above-mentioned polypeptide molecules

Schramm *et al.*, 1955; Franklin, 1955a; Hart, 1955b, 1956). Very suggestive is the fact that viral particles partly degraded by alkali or by detergents appear on electron microscope pictures as fragments of rods with normal diameter, threaded on a central string (Fig. 5) which disappears following ribonuclease (RNAase) digestion. Some recent results, however, suggest that the above-described picture is oversimplified. Franklin *et al.* (1957) conclude from X-ray diffraction studies that, in intact virus particles, RNA is not really arranged as a central core, but rather as a coaxial coiled strand, with protein inside as well as outside (see also Ginoza, 1958). Schramm *et al.* (1955) already had observed that the central string of alkali-degraded TMV contains not only RNA, but also about 10 % protein. Pirie (1956) showed that following heat denaturation TMV proteins are not entirely coagulated and that part of them remain associated with the RNA.

Interestingly enough, TMV proteins in some conditions spontaneously reaggregate into hollow rods of various lengths (Schramm and Zillig, 1955; Franklin, 1955b). When RNA from various sources—including Grünberg-Manago and Ochoa's (1955) synthetic polynucleotides (Hart and Smith, 1956)—is present, the protein reaggregates around the RNA into virus-like rods with RNA again protected against RNAase (Fig. 6). The important investigations concerning infectivity after reaggregation of TMV will be discussed below (page 21).

Less is known about the small spherical plant viruses (Fig. 7). It seems clear, however, that, here too, RNA is enclosed in a protein sheath (Markham, 1953).

Summary. Plant viruses are elongated rods, or more or less spherical particles. In both cases a ribonucleic core is protected by a protein sheath made of many, possibly identical, polypeptide molecules.

3. *Animal Viruses*

The size, shape, and composition of animal viruses are quite variable. Viruses of similar biological behavior are usually found to occur as morphologically similar particles ("elementary bodies") of similar composition. Elementary bodies are frequently more or less spherical or brick-shaped (Figs. 8–10)—about 450 mμ in diameter for psittacosis, 230 mμ for vaccinia (Fig. 10), 80 mμ for influenza (Fig. 8), 37 mμ for poliomyelitis. In some viruses filaments are found in addition to the spheres, but there are reasons to believe that they are noninfectious. Insect viruses which multiply in the cell nucleus are rods (about 280 × 40 mμ for polyhedrosis of silkworm). Cytoplasmic insect viruses are spherical or polyhedral. Some of them are as small as 12–15 mμ in diameter. A number of insect viruses, but not all (Smith, 1958), are found in intracellular inclusions, nuclear

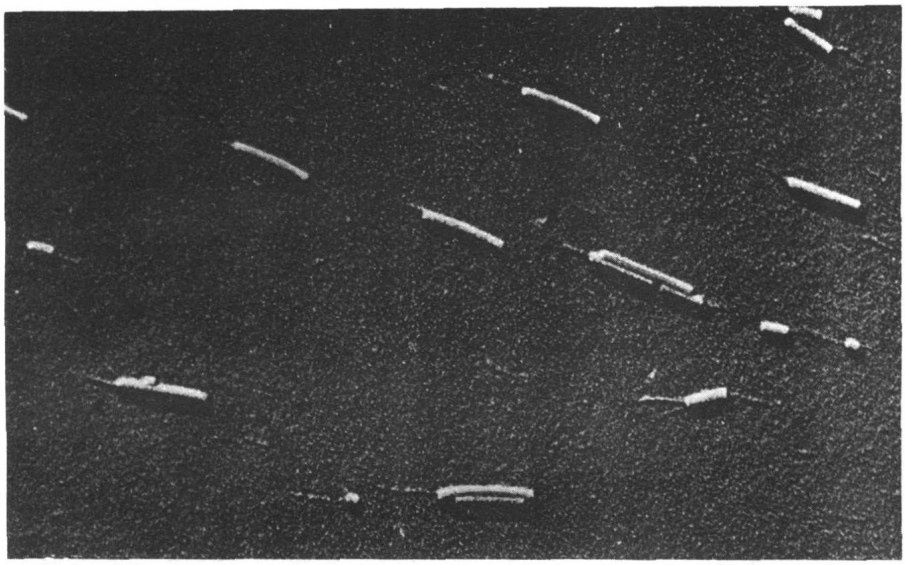

FIG. 5. Tobacco mosaic virus, degraded by detergent to show ribonucleic acid cores. Magnification: ×38,333. Electron micrograph by Dr. R. C. Williams.

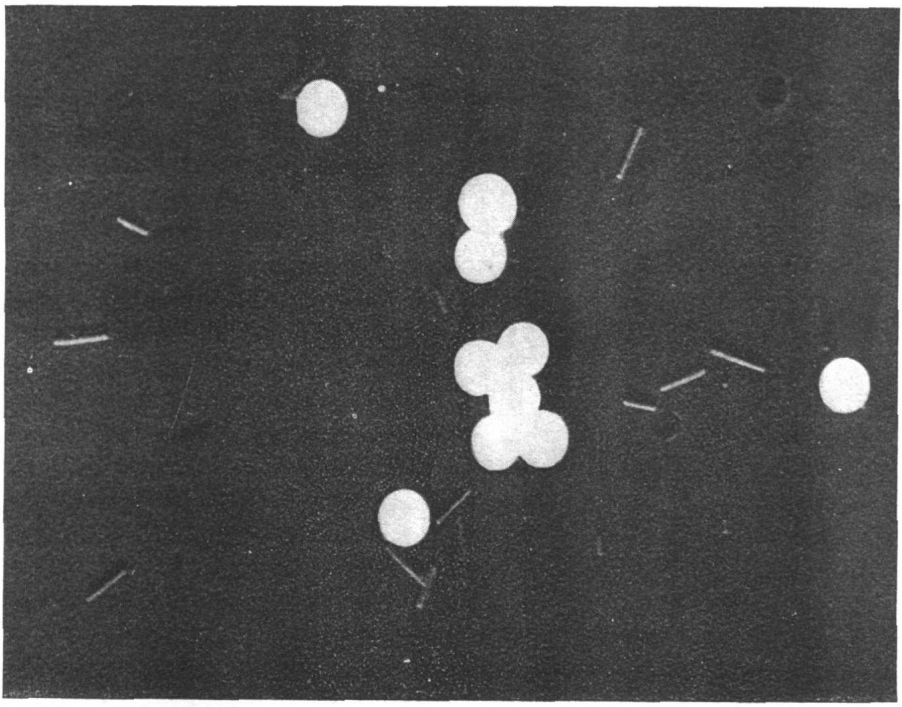

FIG. 6. Reconstituted tobacco mosaic virus containing both protein and nucleic acid. Magnification: ×27,275. Electron micrograph by Dr. R. C. Williams.