STRUCTURE AND DEVELOPMENT OF VIRUSES OBSERVED IN THE ELECTRON MICROSCOPE

III. Influenza Virus*

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Plates 9 to 18

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Electron microscopic studies of influenza virus have been reported periodically for more than a decade, but the knowledge thus gained concerning the structure and especially the development of this group of viral agents is still rudimentary.

Early work by Taylor, Sharp, and associates (1–3) disclosed that strains of swine, type A (PR8) and type B (Lee) virus, isolated by centrifugation from the chorioallantoic fluid of infected chicken embryos, were composed of spherical or ovoid particulate units, with average diameters of approximately 78, 78, and 97 mμ, respectively. These investigators also observed that the viral particles apparently possessed an "internal differentiation in the structure, marked by a single region of relatively high density in the individual particles." Mosley and Wyckoff (4) first described elongated or filamentous forms in preparations of the PR8, Weiss, and Lee strains of virus, noted that these rod-like structures frequently appeared to be partly segmented into spherical particles having the same diameter, and suggested that there was a significant relationship between the two forms. It has since been shown that both filamentous and spherical forms can usually be demonstrated in preparations of influenza virus from infected chorioallantoic fluids, regardless of the strain employed, although filaments are especially numerous in recently isolated A strains (5). Moreover, filaments have been seen in tissue cultures of infected chorioallantoic membrane (6), as well as in thin sections of infected membrane and mouse lung (7–9). In connection with these latter observations, it has been pointed out that the viral particles appear to develop solely from the surface of cells and that the filaments often show segmentation into spheres. Consequently, it is scarcely surprising that "the most general interpretation is that the filaments represent an intermediate stage in the multiplication of virus and that the viral par-

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ticles arise by segmentation of the long forms" (10). Emphasis must be placed, however, on the technical aspects of the work from which this interpretation derives. In studies of virus recovered by centrifugation from chorioallantoic fluid, the usual procedure has been to examine air-dried preparations; when tissue sections were used, the embedding plastic was removed. The bulk of support for the foregoing hypothesis is therefore based on results obtained by methods which since have been shown to cause variable but often extreme distortion of both viral and tissue components (11, 12).

In the current study, ultrathin sections of tissue infected with influenza virus were examined with the embedding substance in place. It will be shown that the virus does indeed exist in both spherical and rod-shaped forms, but that these are probably distinct entities. The two viral forms will be demonstrated to possess characteristic internal structure and to exist only at the surface of cells which otherwise exhibit no recognizable morphologic abnormalities. Lastly, an hypothesis will be advanced concerning the probable mode of viral development in a particular cell-virus system.

Materials and Methods

The PR8, Lee, and A/Persian Gulf/2/52[1] strains of virus were used. Eleven-day-old chicken embryos were inoculated by the allantoic route with approximately 1000 EID_{50} of virus in the form of infected chorioallantoic fluid and then were incubated at 35°C. Six to 44 hours after infection the chorioallantoic membranes were removed and immediately fixed in 1.0 per cent osmium tetroxide buffered at pH 7.4, according to the method of Palade (13). Small pieces were excised from the fixed membranes, dehydrated in graded dilutions of ethyl alcohol, and embedded in methacrylate. Sections were cut on a Porter-Blum (14) type microtome, floated onto the surface of a 30 per cent acetone–70 per cent water mixture, and picked up directly on formvar-coated grids. The sections were examined in an RCA type EMU-2 E electron microscope.

RESULTS

The findings were similar with all three strains of virus and those obtained with a single strain—PR8—will therefore be described almost exclusively in order to maintain continuity of presentation. Both rod-like and spherical forms presumed to be viral particles were observed at the surface of entodermal cells of the chorioallantoic membrane.

Fig. 1 shows parts of two entodermal cells. The intervening cell walls pass diagonally through the upper left portion of the field and have variable orientation to the plane of section. Numerous dense viral particles are present at the surface of the upper cell. Projecting from the lower cell are non-specific cytoplasmic extensions which are generally larger than the virus and vary in shape, with occasional branching. When transected at the appropriate level their interiors can be seen

[1] The A/Persian Gulf/2/52 strain of virus was kindly supplied by Dr. A. Isaacs, National Institute for Medical Research, London, England.

to be continuous with the cytoplasm of the cell. Scattered among them are a few obliquely sectioned viral rods.

Fig. 2 illustrates part of another entodermal cell at higher magnification. The nucleus of an adjacent cell occupies the upper left portion of the field. The irregularly distended intracellular space passing in a vertical direction through the figure is bounded by the cell walls, which are thickened and more dense in one area near the center of the illustration. Lining the free surface of the cell are numerous, moderately dense particles, presumed to be influenza virus. The particles near the top (illustrated at higher magnification in Fig. 9) are spherical, whereas the remainder are rod-like.[2] Some of these short rods appear to be attached by a pedicle or stalk to the sharply defined cell wall. Near the spheres the wall is less clearly demarcated. A relatively uniform distance separates the spheres as well as those rods occupying the middle third of the field. Serial sections revealed that rods which were centrally placed with respect to the surfaces of the section had the greatest density to the electron beam. Rods whose axial centers were nearer one or the other surface showed lower density, since less of their substance was contained within the section. Such observations indicated that adjacent rods differing in density (most evident in the lower third of Fig. 2) lay at varying levels. Superimposition would then account for their inconstant spacing. The nuclear and cytoplasmic components illustrated in this and the remaining figures appear to have been unaffected by the virus and cannot be differentiated from those observed in normal cells.

Longer rods were encountered on the free surfaces of other cells, as shown in Fig. 3. Superimposition in this relatively thick section probably accounts for the fact that many of the rods appear to be contiguous. A cytoplasmic extension with several rods attached to it is present in the lower third of the field. The inset in the upper left corner illustrates a considerably thinner section viewed at higher magnification. At the left margin a viral particle with a poorly defined central body lies adjacent to the tip of a short cytoplasmic extension. Near the center of the inset a characteristic cytoplasmic extension has been cut across. Serial sections have revealed numerous such extensions which seemed to be detached from the host cell but which in reality joined the main body of cytoplasm at another level. Attached to this cytoplasmic extension are ten viral particles. The limiting membranes of at least two of these particles are continuous with the cell wall, apparently permitting communication between their interiors and the cytoplasm of the host cell. It is evident that the level of the section relative to these short rod-like forms will determine whether the site of attachment is revealed. The inset in the lower right corner of the figure shows a single short rod at a magnification sufficient to demonstrate more clearly the continuity of the membrane with the cell wall. This rod contains a rather poorly defined internal body. Study of sufficiently thin sections revealed some short rods which contained either such a circumscribed, ellipsoidal body or multiple granules, but the majority did not exhibit well defined internal structures.

Fig. 4 illustrates still longer rods projecting from the surface of an entodermal

[2] Hereafter, for the sake of brevity, the spherical forms of virus will be referred to as spheres, and the rod-like forms as rods.

cell. Several appear to be attached to the host cell by narrow stalks. In the lower portion of the field, one rod lies nearly parallel to the cell wall, an orientation not infrequently observed.

Fig. 5 shows part of another entodermal cell. The irregularly shaped, granular intracytoplasmic bodies of variable size, possessing thin, serrated membranes and located near the free surface were repeatedly encountered in both infected and uninfected cells. They do not resemble mitochondria and their nature remains obscure. Scattered along the cellular surface are rods of variable length, resembling in morphology and manner of attachment those illustrated in Figs. 2 and 3. In addition, there are several characteristic cytoplasmic extensions which are easily distinguishable from the rods. Near the bottom of the field are two bundles of much longer rods, which undoubtedly correspond to the so called filamentous forms of the virus. The lower bundle is transected nearly parallel to its long axis, the other obliquely. In the former, one rod exhibits a constriction near its distal end. Adjacent rods are separated by a remarkably uniform distance. The inset shows at higher magnification two bundles which have been cross-sectioned. The ellipsoidal shape of the rods probably reflects compression by the microtome knife. Their interiors reveal no consistent structure and exhibit a density to the electron beam approximating that of the methacrylate. Each rod possesses a dense, sharply defined limiting membrane, averaging 30 A in thickness. Occasionally, as revealed by several rods, there is a suggestion of a second limiting membrane. The majority, however, have an amorphous external coat with an indistinct margin. Again, the rods exhibit a relatively constant spacing. In these and in other sections the diameter of most of the rods, as measured from the dense limiting membrane, was between 30 and 35 mμ, although a considerable range (from 20 to 40 mμ) was encountered. The distance separating the rods was generally 20 to 25 mμ. Assuming that this separation resulted from the presence of an enveloping structure, the limits of which were not defined by the electron microscope, the actual diameter of the rods can be assumed to approximate 50 to 60 mμ.

Fig. 6 shows a viral bundle, approximately 2.8 μ in length, extending from the surface of the cell. Individual rods pass for variable distances through the field before moving out of the plane of section. As previously noted, such rods are separated by a relatively constant distance, and each shows a poorly defined outer coat, a dense, sharply defined limiting membrane and an interior devoid of structure. Near the center of the field a cytoplasmic extension with two attached rods has been cross-sectioned at some distance from its site of attachment. On the left and closer to the cell are two ellipsoidal cytoplasmic extensions; these exhibit membranes of variable definition, indicating their irregularity of shape and oblique position within the section. Short rods are also present on the surface of the host cell. Although the short rods differ from the long rods, in that they appear to possess a poorly defined internal structure, both forms when central to the plane of section show a dense limiting membrane, an amorphous outer coat, similar diameters, and nearly identical spacing.

Fig. 7 illustrates five consecutive serial sections of the surface of one cell, the fifth micrograph showing only the lower part of the field. Three cross-sectioned rods (indicated by *A* in section I) maintain nearly the same position relative to

the cell, whereas a bundle of rods cut obliquely (indicated by *B*) passes through the plane of each section at progressively greater distances from the cell.[3] When the long axis of a rod is eccentric to the plane of section, the rod lacks density and its obliquely transected limiting membrane is therefore poorly defined, as illustrated by *a* in section I, *g* in section II, and *d* in section III. The short rods exhibit a similar phenomenon (previously mentioned in the discussion of Fig. 2), as demonstrated by rods *e* and *f*, sections I and II, and rods *h* to *k*, sections III to V. If the chord of a curved rod is parallel to the plane of section, two of its segments may appear at the same level, as shown by *b* and *g*, sections I and II, respectively. As might be expected, the limiting membrane encloses the distal end of the rod (*b* and *c*, sections II and IV, respectively); it may enclose the proximal end as well (*a* and *l*, section II and IV), presumably after detachment. In the lower right corner of the plate, the upper portions of sections I to IV have been printed as a composite picture. This permits reconstruction of rods *a* to *e* and *g* from component parts and illustrates their spatial relationships in two dimensions. Rod *d* is discontinuous, probably reflecting displacement of a segment contained in section II or III.

Fig. 7 a illustrates two consecutive serial sections of spherical viral particles at the surface of a cell. Comparison reveals that the spheres appear less dense when they are eccentric to the plane of section. Those which are only slightly eccentric continue to exhibit a sharply defined membrane with little reduction in diameter. When the plane of section is sufficiently removed from the center of the sphere, there is loss in definition of the limiting membrane and the consequent decrease in density renders the particle nearly invisible. Thus, in Fig. 7a the spherical particles indicated by capital letters A–I lie close to the center of the plane of section, whereas those designated a–i in the adjacent section are the same spheres cut eccentrically. Particles J–M occupy nearly an equal thickness of both sections. Particles N–S appear in only one of the sections.

Fig. 8 shows numerous viral particles at the cell surface. Near the bottom of the field, particles are seen which resemble the short rods previously illustrated; those lacking a membrane either proximal or distal to the cell surface are characteristic of rods cut obliquely. That portion of the cell wall passing in a nearly vertical direction through the field is sharply defined, though discontinuous. On the free surface to its left can be seen spherical and ellipsoidal particles. Those particles centrally placed within the section exhibit a spherical internal body, averaging 20 to 22 mμ in diameter, separated by a zone of lesser density from a sharply defined limiting membrane 30 A thick and 40 to 45 mμ in diameter. Each sphere is enveloped by poorly demarcated material of low density to the electron beam. If the relatively uniform distance separating the spheres reflects the presence of some viral structure not visible in thin sections, then the diameter of the spheres can be calculated in the same manner as for the rods. Since the spacing generally measures 20 to 22 mμ, the diameter of the spheres approximates 60 to 70 mμ. In Fig. 8, as in Fig. 7 a, the particles on the surface of the cell lie at

[3] Employing the cell wall for reference on the assumption that it is perpendicular to the plane of section, one can demonstrate that the point at which a rod passes out of the plane of one section coincides with the point at which it enters the next. This observation, also made on other serial sections, fails to support the hypothesis advanced by Williams and Kallman (15) that tissue is missing between adjacent sections.

different levels within the section. Because the section is thin, a majority exhibit sharply defined limiting membranes. In contrast, the particles just beneath the cell wall either possess no well defined structure, or show incomplete limiting membranes, despite the fact that several are moderately dense. At the top of the illustration the cell wall is indistinct, suggesting that it has been cut obliquely. Unlike the particles at the bottom of the field, however, a majority possess intact limiting membranes, indicating that they are spheres rather than obliquely sectioned rods. If they were cross-sectioned rods, they should exhibit characteristic spacing.

Fig. 9 illustrates the upper portion of Fig. 2. Spherical forms of the virus are present on the free surface, which passes horizontally. Those particles closest to the cell are incomplete, in that they lack structure on the side contiguous to the cell wall. This is an extremely thin section and the material comprising the outer coat of the viral spheres is not well visualized, since it exhibits a density only slightly exceeding that of the methacrylate.

In thick sections the superimposition of adjacent viral structure and excessive scattering of electrons by the methacrylate prevent adequate resolution. Between these two extremes sections can be found which, while permitting high resolution, provide maximal contrast without excessive superimposition. Figs. 10 and 11 illustrate such sections. The cell wall passes horizontally across each field. The outer coat of the viral spheres is revealed as a zone of material separated from the limiting membrane by an area of lesser density and possessing a poorly defined peripheral border. The original prints reveal fine radial striae passing through this zone. Superimposition probably accounts for the apparent presence of the cell wall within some of the viral particles. In the left third of each field can be seen ellipsoidal forms and obliquely sectioned short rods. Near the center of Fig. 11 one viral sphere exhibits a relatively well defined inner body, measuring 22 mμ in diameter. Inspection of this and similar micrographs revealed that the inner bodies possess a central core of different density. The clear definition of internal structure exhibited by this viral particle may reflect its central location within the plane of section. If the thickness of the section were slightly less than the diameter of the limiting membrane, two opposite segments of the sphere would then be cut away leaving little to overlie the internal body. The limiting membrane would appear more dense and slightly thicker than in ultrathin sections. Comparison with Fig. 9 suggests such to be the case. Random distribution of the particles would result in relatively few lying at the same level. Thus superimposition of component parts probably accounts for the fact that most of the spheres illustrated in Figs. 10 and 11 show poorly defined internal structures. The possibility, however, that some completed spherical forms of the virus lack a circumscribed central body cannot be ruled out.

DISCUSSION

The identification of influenza virus has been based on the absence of similar particles at the surface of normal cells, as well as of cells infected with fowl pox, vaccinia, and herpes simplex viruses. Although investigators who employed thick sections have encountered difficulty in distinguishing viral rods from non-specific cytoplasmic extensions (9), thin sections such as those

described herein have revealed that the latter were almost invariably of greater diameter, tended to curve more abruptly, did not possess an outer coat, frequently exhibited a thicker membrane of lesser density, and usually contained cytoplasmic components. Measurement of viral size was complicated by the diffuse outer boundary of the external coat and it is suggested that the relatively constant distance separating the particles resulted from the presence of structure not revealed by the microscope. Measurements made to include this spacing showed that the majority of viral rods were 50 to 60 mμ in diameter, whereas the spheres were 60 to 70 mμ.

As was previously noted in the case of herpes simplex (11), considerable distortion, characterized by flattening and fragmentation, appeared to result from the surface forces which accompany drying a suspension of virus or drying a section after immersion in solvent to remove the embedding plastic. We have found that such dried preparations of influenza virus frequently contained segmented rods, an observation which has led several investigators to suggest that the spheres were actually formed from the rods by a process of segmentation (4, 6, 7, 9, 10, 16). Examination of thin sections from which the methacrylate had not been removed occasionally showed constriction of a rod (as in Fig. 5), which could be interpreted as evidence of segmentation, but multiple constrictions of a single rod were rarely encountered. Moreover, numerous spheres were present on the cell surface without rods adjacent to them, and when both viral forms occurred within the same area, the rods were rarely interposed between the spheres and the host cell. These observations indicate that segmentation of the rods did not represent the primary manner in which the spheres were formed.

The entodermal cells of the chorioallantoic membrane did not show characteristic and reproducible changes in either nuclei or adjacent cytoplasm when the virus inoculum was sufficiently dilute to avoid direct cytotoxic effects. Virus was produced at the surface without apparent morphologic alteration of the remainder of the host cell. These observations do not exclude the possibility that components of the virus may be synthesized in the nucleus or the depths of the cytoplasm, but they do establish beyond reasonable doubt that the integration of components into typical viral particles takes place only at the periphery of the infected cell. In this connection, Watson and Coons (17) demonstrated that multiplication of influenza virus in chicken embryos was characterized cytologically by a diffuse type of immunospecific staining with fluorescent antibody, which was first detectable in the nuclei and later in the cytoplasm of infected cells. They suggested the possible role of S ("soluble") antigen in this type of staining. Liu (18) subsequently examined this suggestion by experiments wherein the occurrence of influenzal antigens in epithelial cells of the respiratory tract of infected ferrets was studied by means of specifically absorbed sera. The results

clearly showed that the nuclei contained only S antigen and that V ("viral") antigen was concentrated at the free border of the cells. It is not known whether the S antigen is actually formed in the nucleus or reaches it by diffusion from the cytoplasm. Since Liu also found, as described by Francis and Stuart-Harris (19), that epithelial cells of the nasal mucosa in ferrets underwent degeneration and desquamation following infection, it seems reasonable to conclude that development of the virus at the cell periphery represents a basic mechanism which operates in the same manner, regardless of the capacity of the infectious agent to induce cytopathogenic effects. Attention may be drawn here to the conflicting evidence of Harford *et al.* (20), who described intracytoplasmic "inclusion bodies" in the bronchial epithelial cells of mice after inoculation of mouse-adapted as well as unadapted type A influenza virus. Although the particles comprising these bodies were not uniform in size and exhibited no internal structure, it was suggested that they were viral particles. No virus was demonstrable at the free surface of the cells. Extensive examination in our laboratory of bronchial epithelium from mice infected with influenza virus has revealed similar bodies, but it should be emphasized that the cells of chicken embryo chorioallantoic membranes infected with vaccinia, fowl pox, and herpes simplex viruses contain intracytoplasmic structures which are indistinguishable from them morphologically. There is little doubt that such bodies are associated with cellular injury and do not represent an "inclusion body" composed of viral particles.

On the basis of the data obtained thus far, the following hypothesis can be advanced to explain the development of influenza virus. The rods form by a process of extrusion from the cell wall, their interiors appearing to be continuous with the cytoplasm of the host cell at early stages of development. Although a few short rods contain a poorly defined body or aggregate of granules, the majority exhibit no definite internal structure. Amorphous material of low density to the electron beam coats the limiting membrane. As the rods lengthen, a segment adjacent to the cell constricts into a stalk. Rods differing in length and each possessing a complete limiting membrane detach either singly or in bundles. The interiors of the longer rods are composed of homogeneous material approximating the embedding plastic in electron density. In addition, spherical bodies of moderate density differentiate at or just beneath the cell surface. As these particles move out through the cell wall each acquires an internal body, a sharply defined, dense membrane, and an envelope of less dense material. However, it should be emphasized that it has been difficult to determine actual stages of viral growth at specific intervals. Although only short rods were seen 6 and 8 hours after infection, an insufficient number of infected cells have been encountered

thus far to state with certainty that these forms were representative of the total viral population. At subsequent intervals after infection, forms believed to represent a variety of stages were observed at the surface of different cells and even at different parts of the surface of the same cell.

The fact that two forms of the same virus exhibit such different shape and internal structure raises the question whether both are capable of initiating infection. Donald and Isaacs (21) have shown that the longer rod-like or filamentous forms of virus may be disintegrated by ultrasonic vibration with a concomitant rise in hemagglutinin titer but no increase in infectivity. This suggests either that the filaments are non-infectious, or that each of these relatively large morphologic units contains a single infective locus. The first of these possibilities seems to be incompatible with the hypothesis that influenza virus develops primarily by the segmentation of filaments, since, if the filamentous forms were completely non-infectious, segmentation would give rise only to subunits devoid of infective properties. The second possibility is likewise at variance with the segmentation hypothesis, which must include the assumption that the filaments are more or less uniformly composed of infectious material. If this were the case, mechanical disintegration should, within limits, cause a demonstrable increase in the number of infective units, in contrast to the finding that no increase in infectivity occurs. Further information bearing on this subject has been presented recently by Burnet (22), who reported that the filamentous forms of virus were readily destroyed either by hypotonic salt solutions or by surface-active agents, at concentrations closely approximating those necessary to cause lysis of fowl erythrocytes. Damage to the filaments, as evidenced by the appearance of distorted, beaded, and angulated forms, preceded their dissolution by osmotic forces. The filaments could also be destroyed by shaking with ether, as was first noted by Hoyle (23), but their destruction by this procedure or by exposure in water did not reduce appreciably the infectivity titer of the starting material, which unquestionably contained both spherical and filamentous forms of virus. These observations, when viewed together with the results of the studies described herein, lead us to the belief that the spherical form of the virus is the elemental infectious unit and that the filamentous form not only develops in a somewhat different manner, but also is largely or completely non-infective. If one assumes, for the sake of argument, that this view may be correct, then the demonstrated lack of internal structure in the filaments suggests the possibility that infectivity of the spherical elements is related to the material of which their inner bodies are composed. Nucleic acids may constitute part of this material and it is clearly important that a comparison of the nucleic acid content of the two forms of virus be undertaken.

SUMMARY

Rods and spheres believed to represent viral particles were observed at the free surface of entodermal cells of the chorioallantoic membrane 6 to 44 hours after infection. Although occasional short rods revealed poorly defined internal bodies, the majority, as well as all the longer rods (filaments), exhibited no visible internal structure. The spheres presumed to lie central to the plane of section contained an inner body 20 to 22 mμ in diameter. Both forms possessed a dense, sharply defined limiting membrane 30 A thick and a diffuse external coat of lesser density. Where superimposition within the section was minimal, the viral particles were separated by a relatively constant distance. Measured to include this spacing, on the assumption that it reflected the presence of a component of the outer coat, the diameters of a majority of the rods were 50 to 60 mμ, whereas the spheres averaged 60 to 70 mμ. The rods appeared to form by a process of extrusion from the cell wall and became detached either singly or in bundles of variable length. The spheres seemed to differentiate at the cell surface and to acquire the inner body, limiting membrane, and outer coat as they migrated through the membrane of the host cell. No characteristic changes were seen in the nuclei or adjacent cytoplasm, and recognizable viral particles were never encountered in these areas of the cell. No support was obtained for the assumption that the spheres developed primarily by segmentation of the rods. It is suggested that the spherical form of the virus is the elemental infectious unit and that the filamentous form is largely or completely non-infective.

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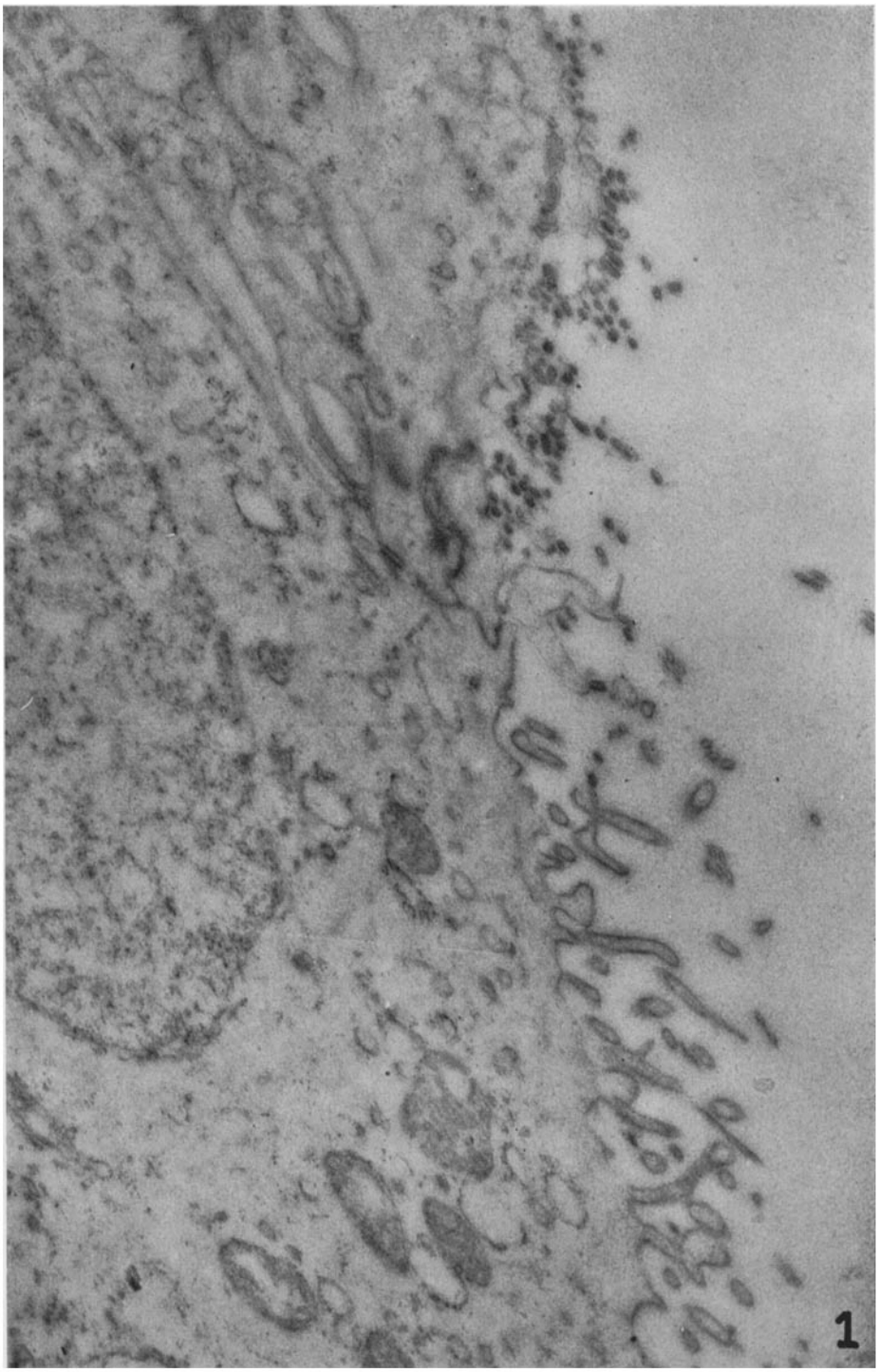
EXPLANATION OF PLATES

PLATE 9

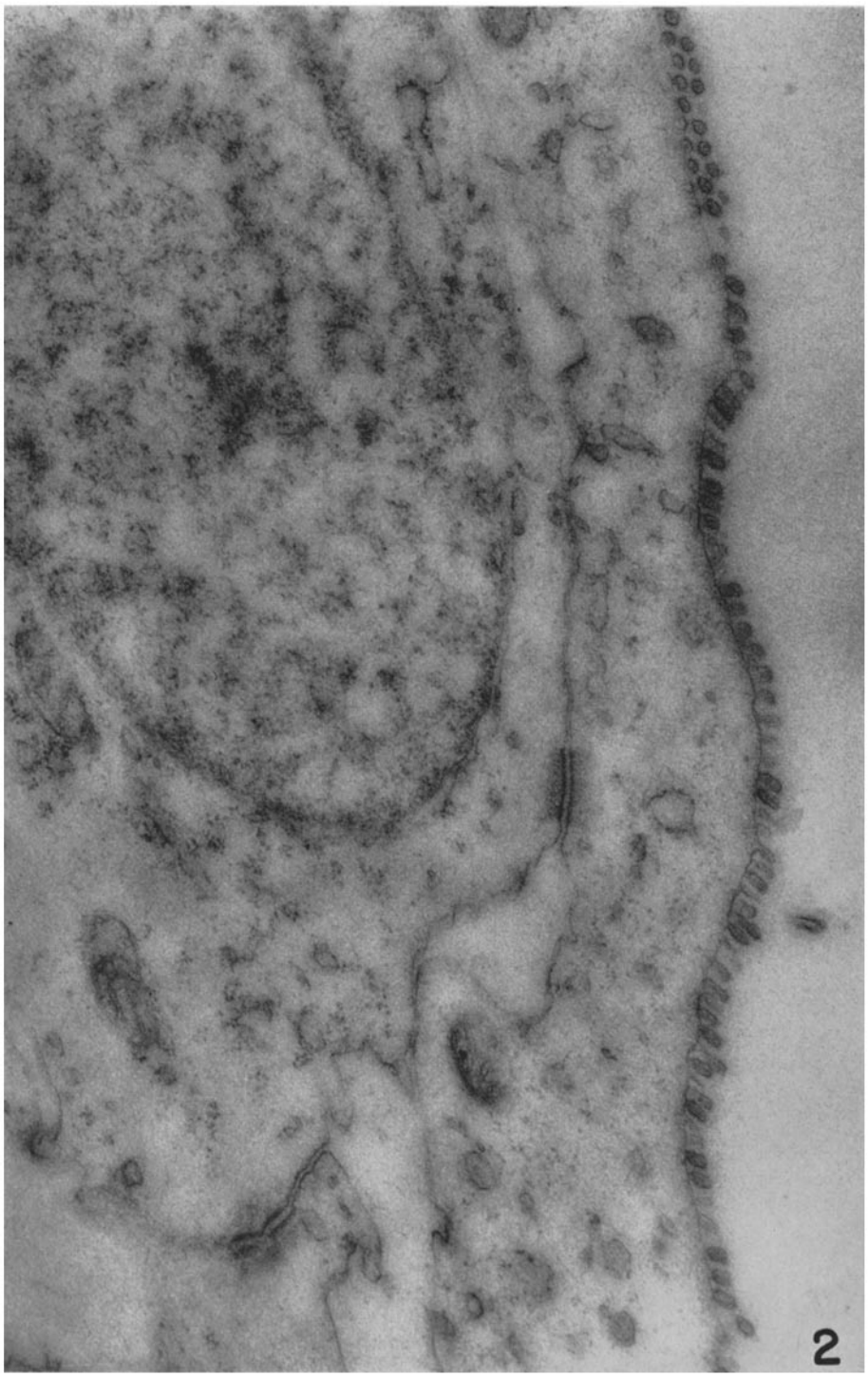
FIG. 1. Part of two entodermal cells. At the surface of the upper cell are characteristic dense particles of influenza virus. Non-specific, irregularly shaped cytoplasmic extensions project from the lower cell. Those transected longitudinally are clearly protrusions of the cytoplasm, whereas others sectioned obliquely presumably communicate with the cell at a different level. Interspersed among the cytoplasmic extensions are a few viral rods which can be distinguished even at this magnification by the fact that they are narrower and more uniform in diameter. × 26,000.



(Morgan *et al.*: Structure and development of viruses. III)

PLATE 10

FIG. 2. Near the right border of the illustration is part of an entodermal cell with viral particles on its free surface. The variable density of the short rods reflects their position, those of lesser density being only partially contained within the section. Near the top of the field are spherical viral particles lying adjacent to the cell wall. × 43,000.



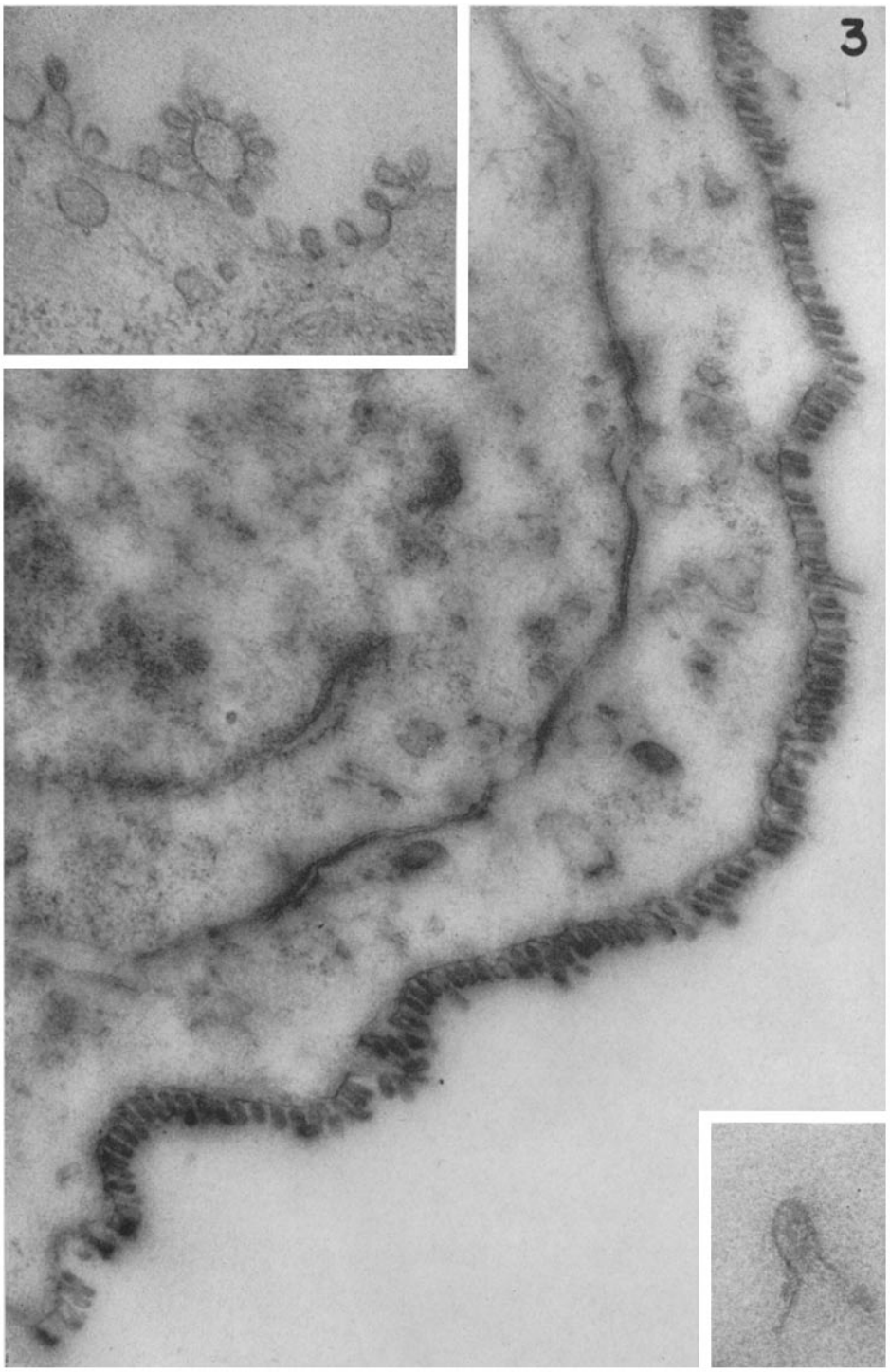
(Morgan *et al.*: Structure and development of viruses. III)

PLATE 11

FIG. 3. Part of an entodermal cell with somewhat longer viral rods projecting from its surface. In the lower third of the field several of these rods appear to be attached to a cytoplasmic extension. The relatively great density of a majority of the rods reflects the fact that this is a rather thick section and their apparent contiguity in some areas is probably the result of superimposition. × 40,000.

The inset at the upper left corner shows at higher magnification the surface of another entodermal cell. On the left a cytoplasmic extension sectioned longitudinally has a viral particle at its tip. Near the center of the field is a cross-sectioned cytoplasmic extension to which are attached 10 short rods, several appearing to have membranes continuous with the cell wall. × 65,000.

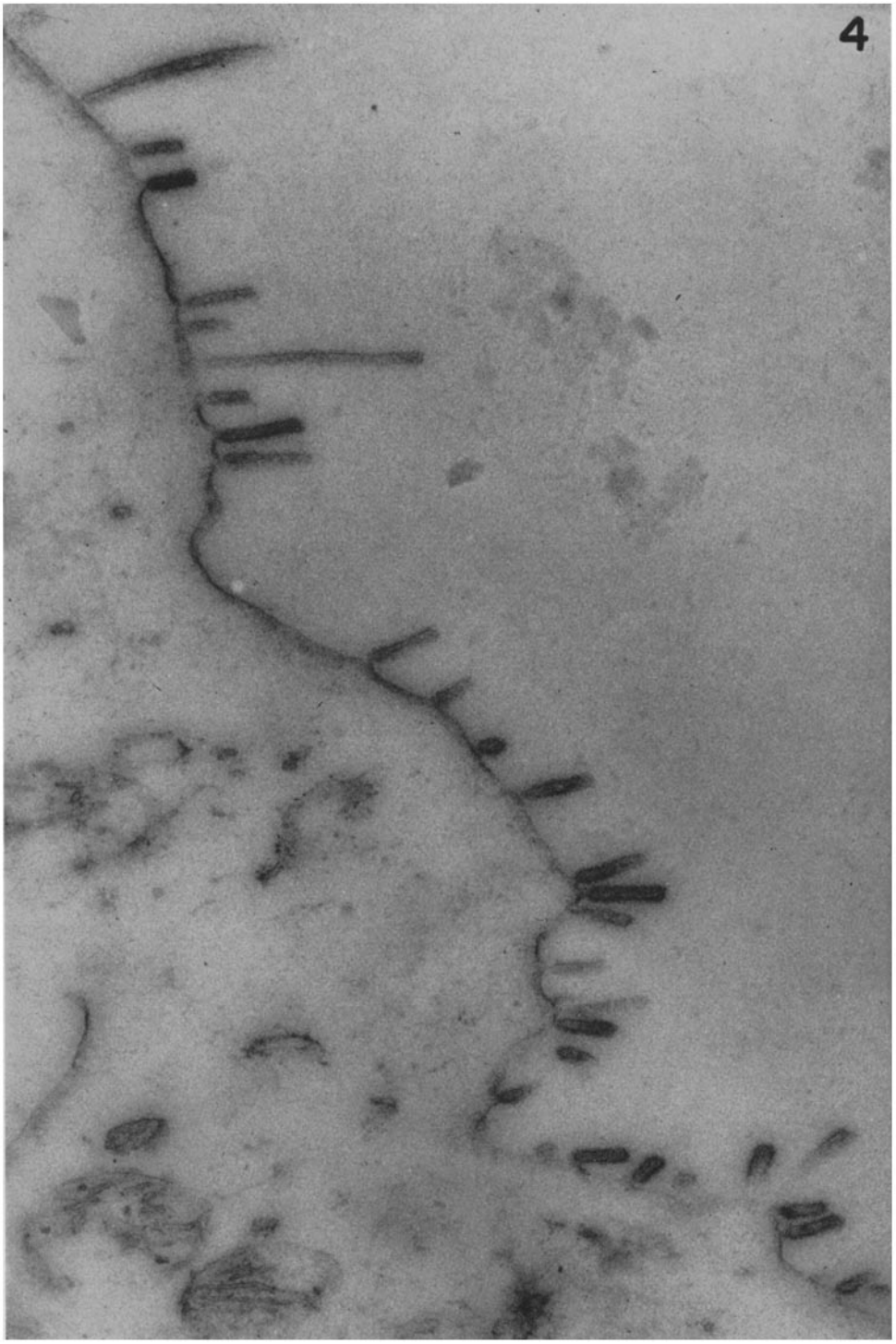
The inset at the lower right corner illustrates an unusually thin section of a short rod containing a poorly defined internal body. Only a fragment of cell wall on the left is perpendicular to the plane of section. The continuity of this wall with the membrane of the virus is clearly shown. × 157,000.



(Morgan *et al.*: Structure and development of viruses. III)

PLATE 12

FIG. 4. Viral rods of variable length projecting from the surface of the host cell. Several appear to be attached to the cell wall by a thin stalk. Near the lower border of the field the rods show differences in their orientation with respect to the surface of the cell. × 47,000.

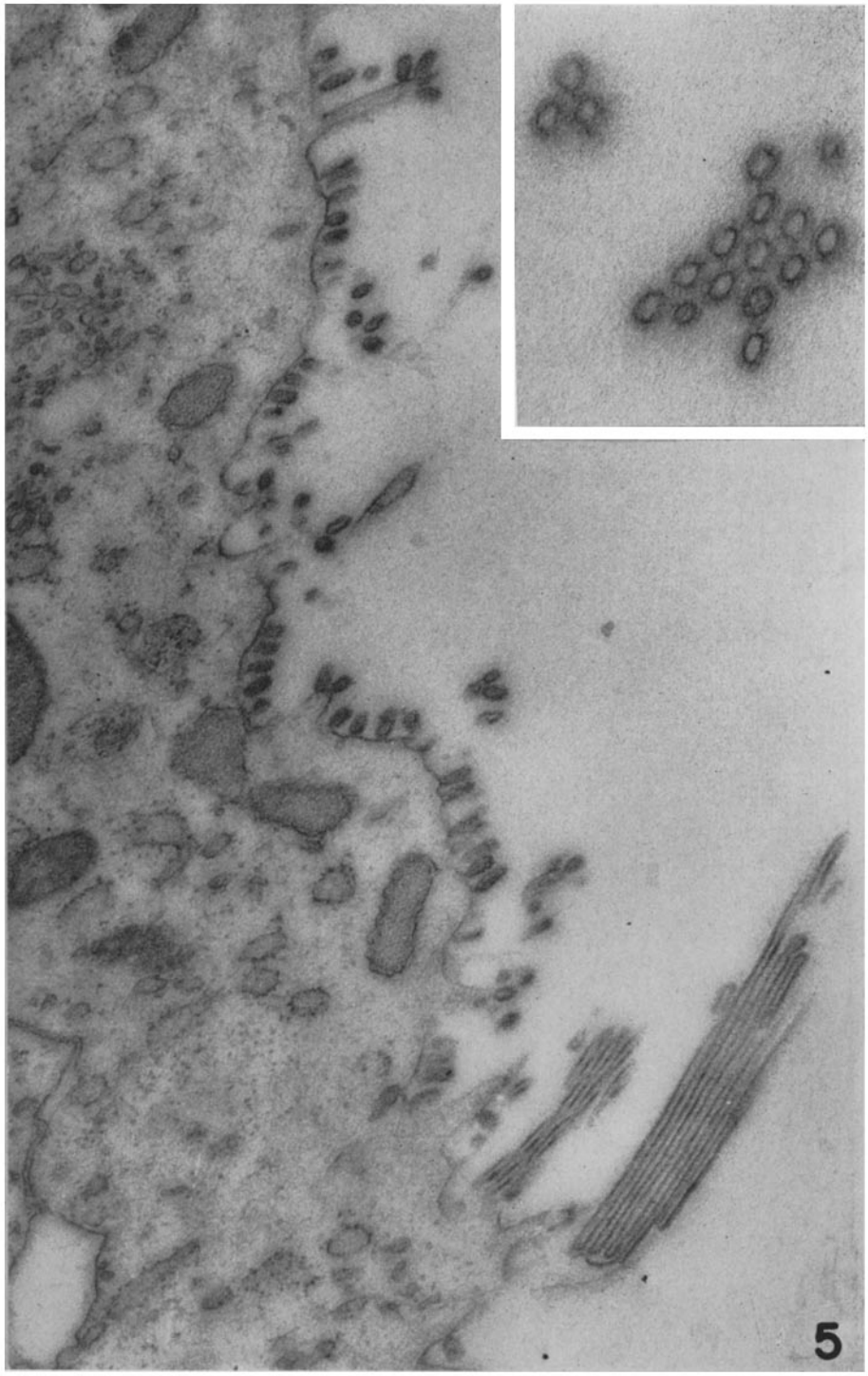


(Morgan *et al.*: Structure and development of viruses. III)

PLATE 13

FIG. 5. Rods believed to be at different stages of development. In the lower right corner and nearly parallel to the plane of section lies a bundle of rods. The rods are separated by amorphous material of low density to the electron beam. An adjacent bundle has been cut obliquely. Without serial sections it is impossible to tell at this magnification whether the spherical forms are actually spheres or whether they represent short rods in cross-section. It is likewise impossible to determine whether those particles which seem to lie at a distance from the host cell have been released or whether they are attached to extensions of the cell not contained within the plane of section. × 43,000.

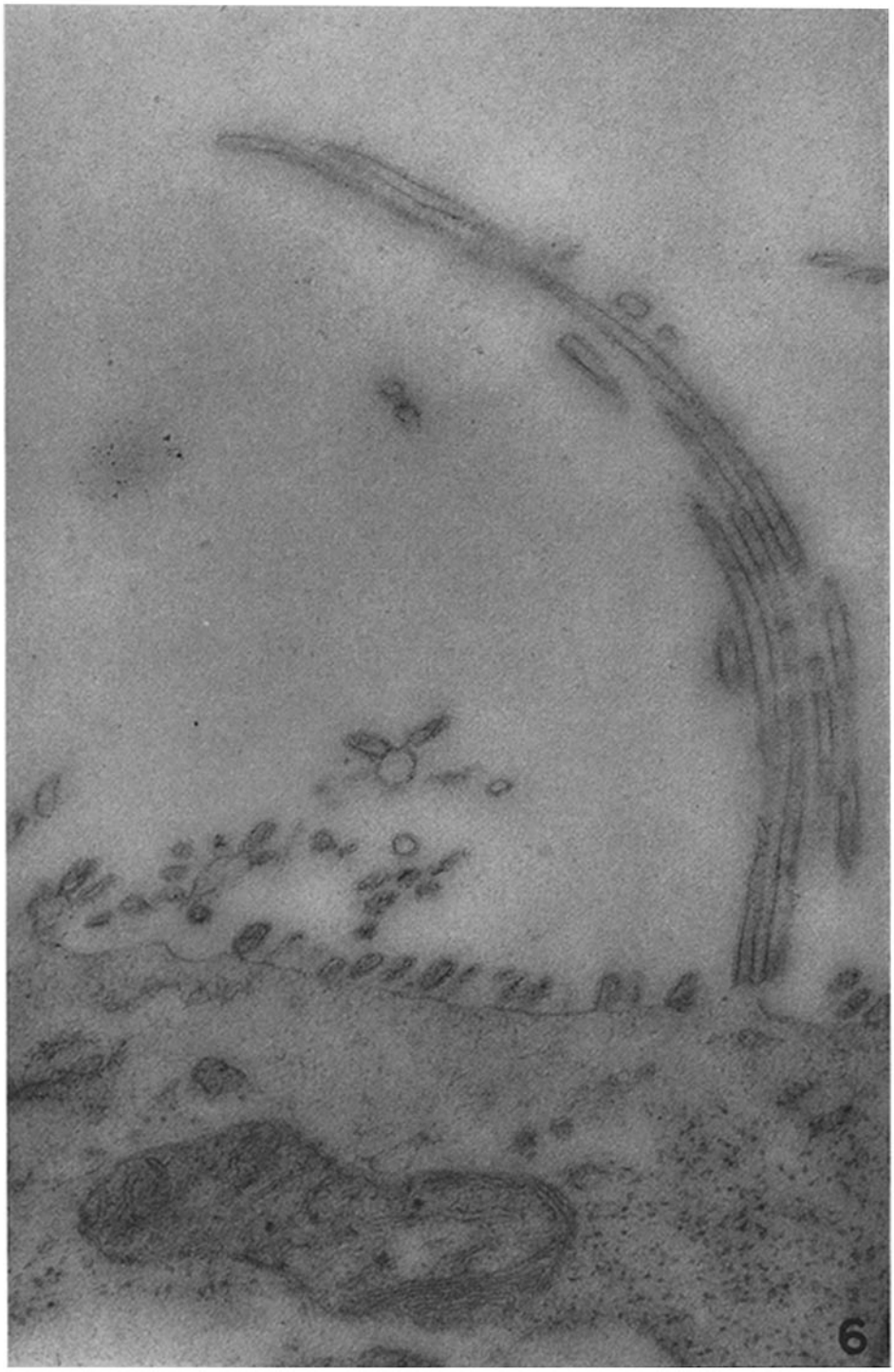
The inset illustrates at higher magnification two bundles of rods in cross-section. The rods exhibit interiors of low density to the electron beam, sharply defined limiting membranes, and amorphous external coats. They are separated by a relatively constant distance. × 103,000.



(Morgan *et al.*: Structure and development of viruses. III)

PLATE 14

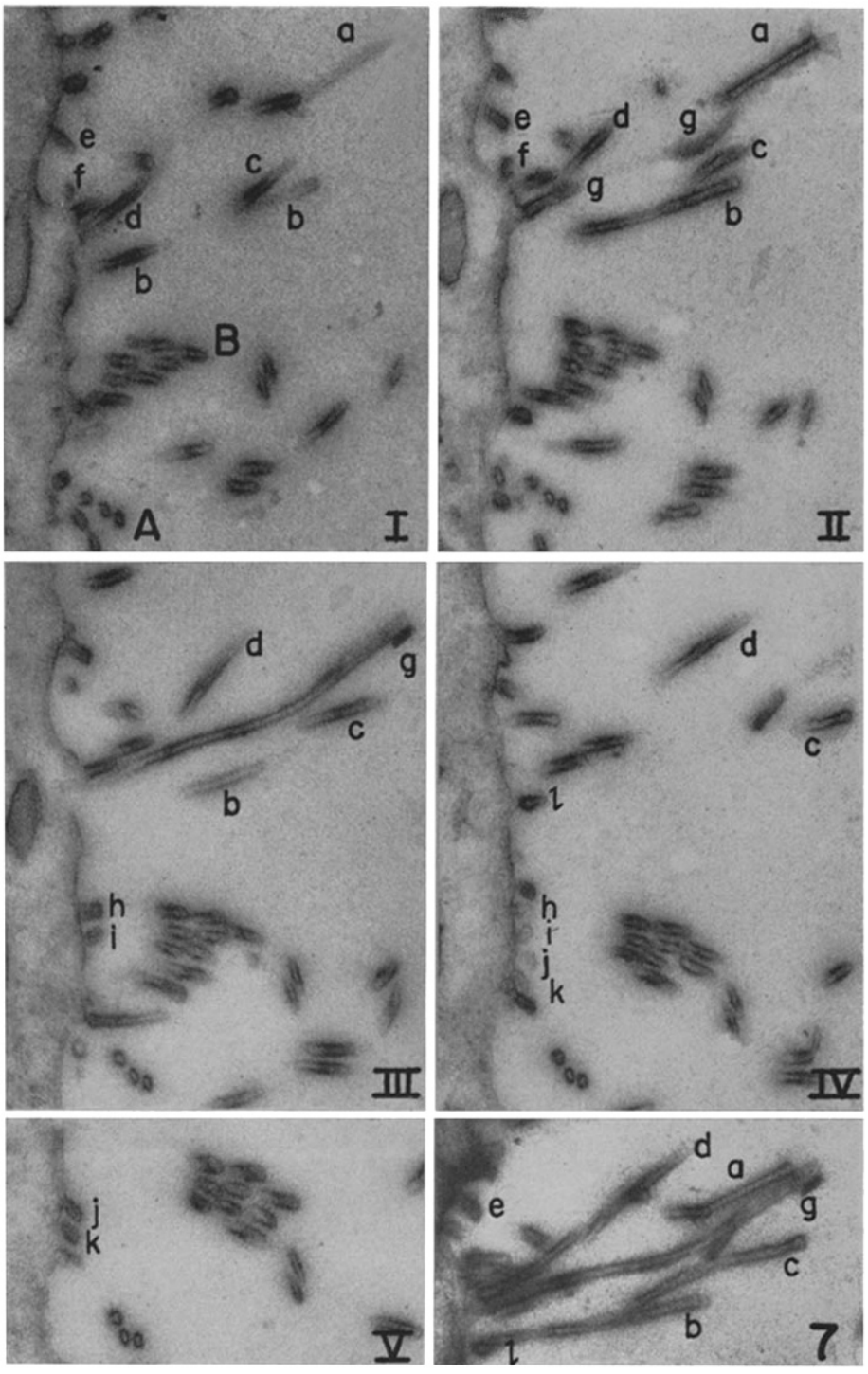
FIG. 6. Part of an entodermal cell from the surface of which projects a bundle of long, curved rods. The rods are slightly oblique to the plane of section and exhibit characteristic spacing. The intact membrane enclosing one particle in the upper third suggests that it is a completed short rod carried out during growth of adjacent rods. (That the peripheral coat of the virus has an adherent property is suggested by the persistence of bundles in sections immersed for brief periods in amyl acetate and subjected to the disruptive surface forces which accompany drying.) Near the center of the field, two short rods are attached to a characteristic cross-sectioned cytoplasmic extension, which probably communicates with the host cell at a different level. Cytoplasmic extensions sectioned obliquely are evident in the left third of the illustration. The cytoplasm of the host cell appears to be unaffected by the virus. × 60,000.



(Morgan *et al.*: Structure and development of viruses. III)

PLATE 15

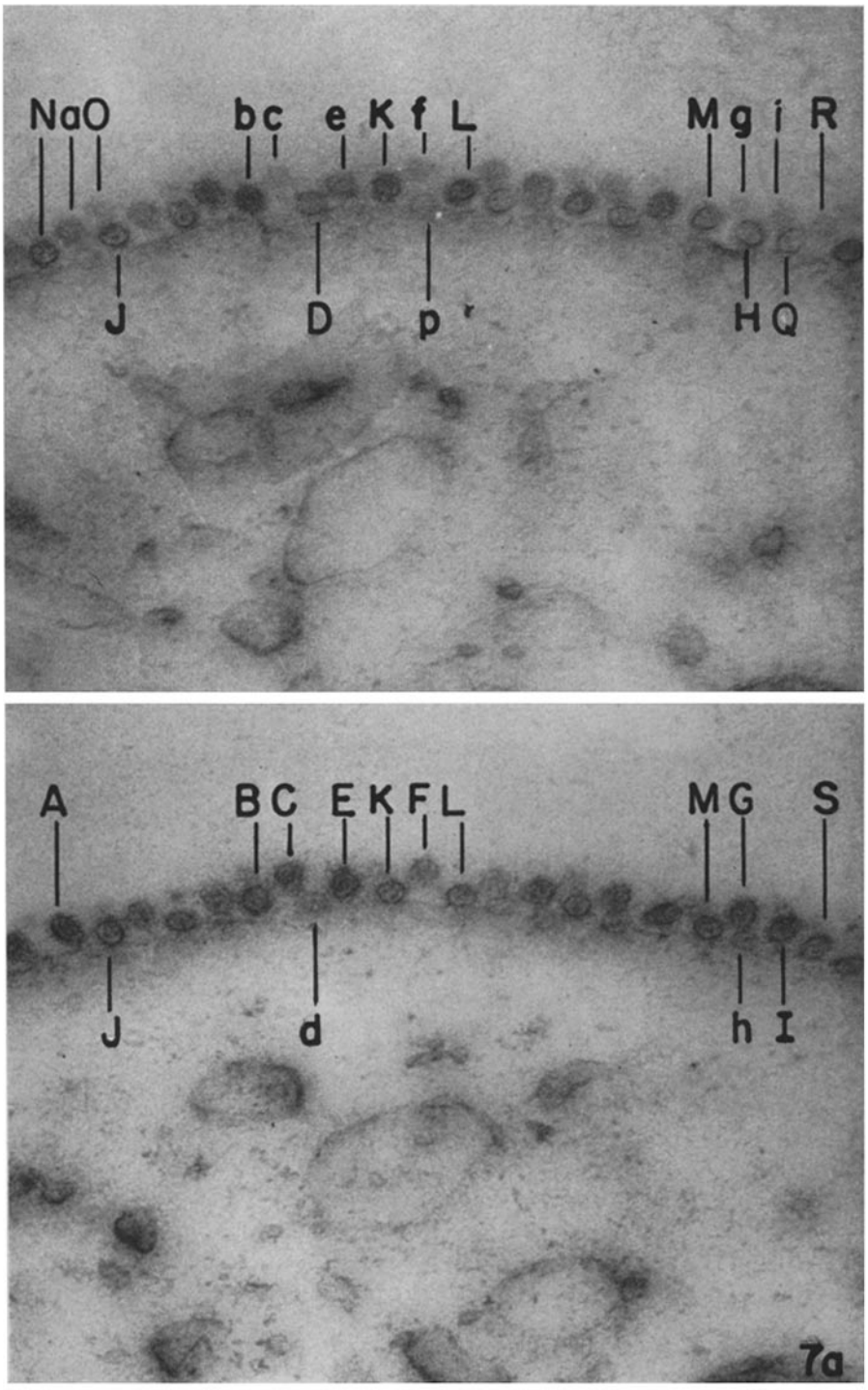
FIG. 7. Five consecutive serial sections of viral rods, the fifth illustrating only the lower half of the field. Bundle *A* is nearly perpendicular and bundle *B* is oblique to the plane of section. Comparison of the short rods, *e*, *f*, and *h* to *k* in adjacent sections clearly reveals that their density reflects the level at which they lie within the plane of section. Rod *a*, present in sections I and II, possesses a complete limiting membrane and appears to have been released. The terminal segment of rod *g* (section III) was probably damaged during preparation of the specimen. In the lower right corner a composite print of the upper portion of the first four serial sections provides a reconstruction in two dimensions of rods *a* to *e* and *g*. A segment of rod *d* has been displaced, probably by impact of the microtome knife. It is evident that individual rods have variable orientation with respect to the cell surface. × 45,000.



(Morgan *et al.*: Structure and development of viruses. III)

PLATE 16

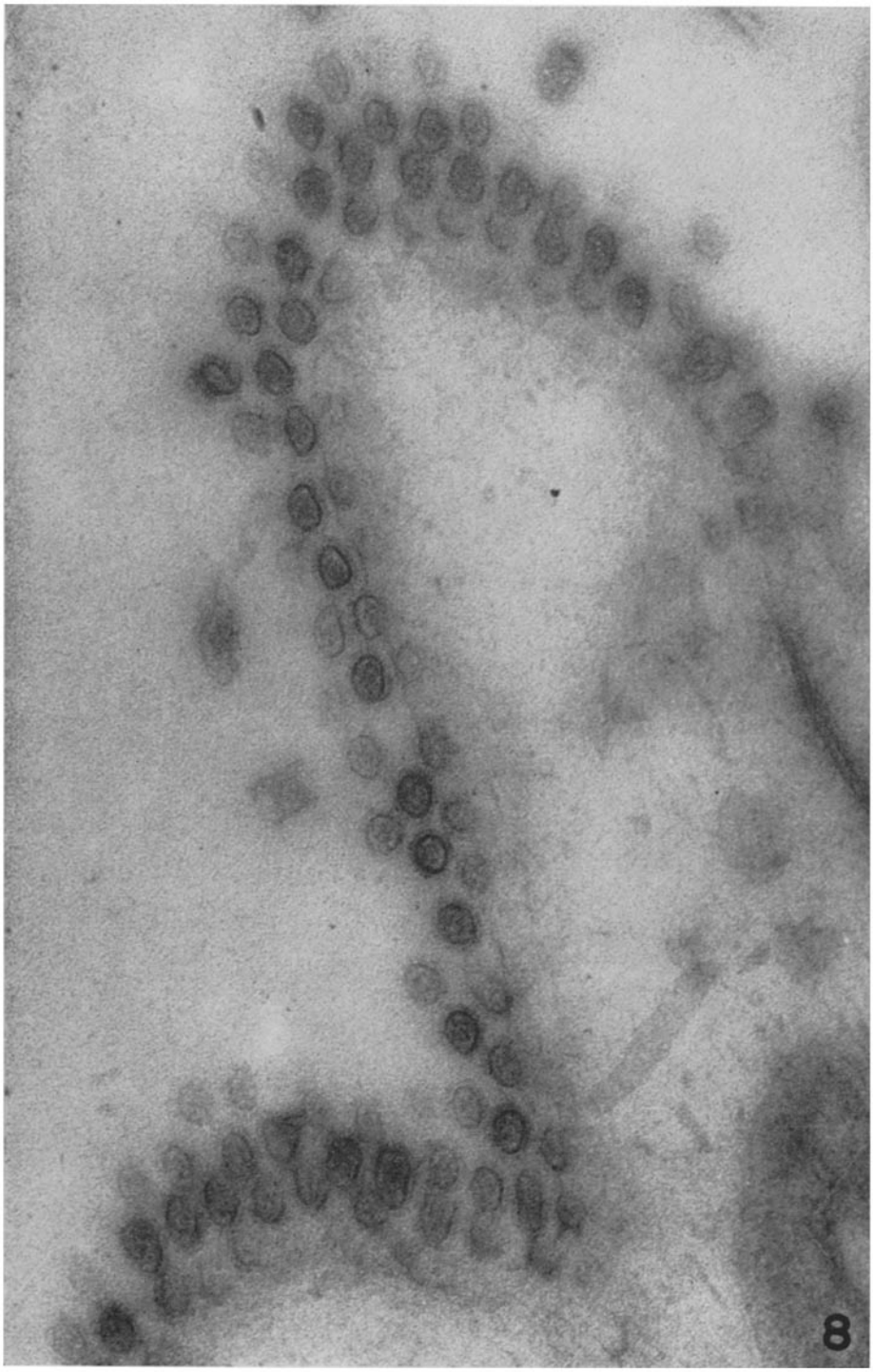
FIG. 7 a. Two consecutive serial sections of viral spheres. Three groups of particles have been labelled. Particles close to the center of the planes of section (*A* to *M*) are dense and exhibit a well-defined membrane, whereas those which are eccentric (a to i) are less dense and show no sharply delineated structure. Particles N to S appear in only one section. Although the remaining particles have not been labelled, their relationships can be readily ascertained. × 70,000.



(Morgan *et al.*: Structure and development of viruses. III)

PLATE 17

FIG. 8. Spherical and rod-like forms of the virus at the surface of an entodermal cell. Near the lower border can be seen ellipsoidal particles which lack a membrane at one end and have the appearance of short rods sectioned obliquely. Characteristic spheres lie adjacent to that portion of the cell wall which passes in a nearly vertical direction through the field. Those assumed to occupy the full thickness of the section exhibit typical spacing and possess an inner body, a dense limiting membrane, and an amorphous coat. Several particles just beneath the cell wall show incomplete limiting membranes. In the upper third of the field, where the cell wall is oblique to the plane of section, the majority of particles appear to be spheres. If they were cross-sectioned rods, the characteristic spacing should be present. × 107,000



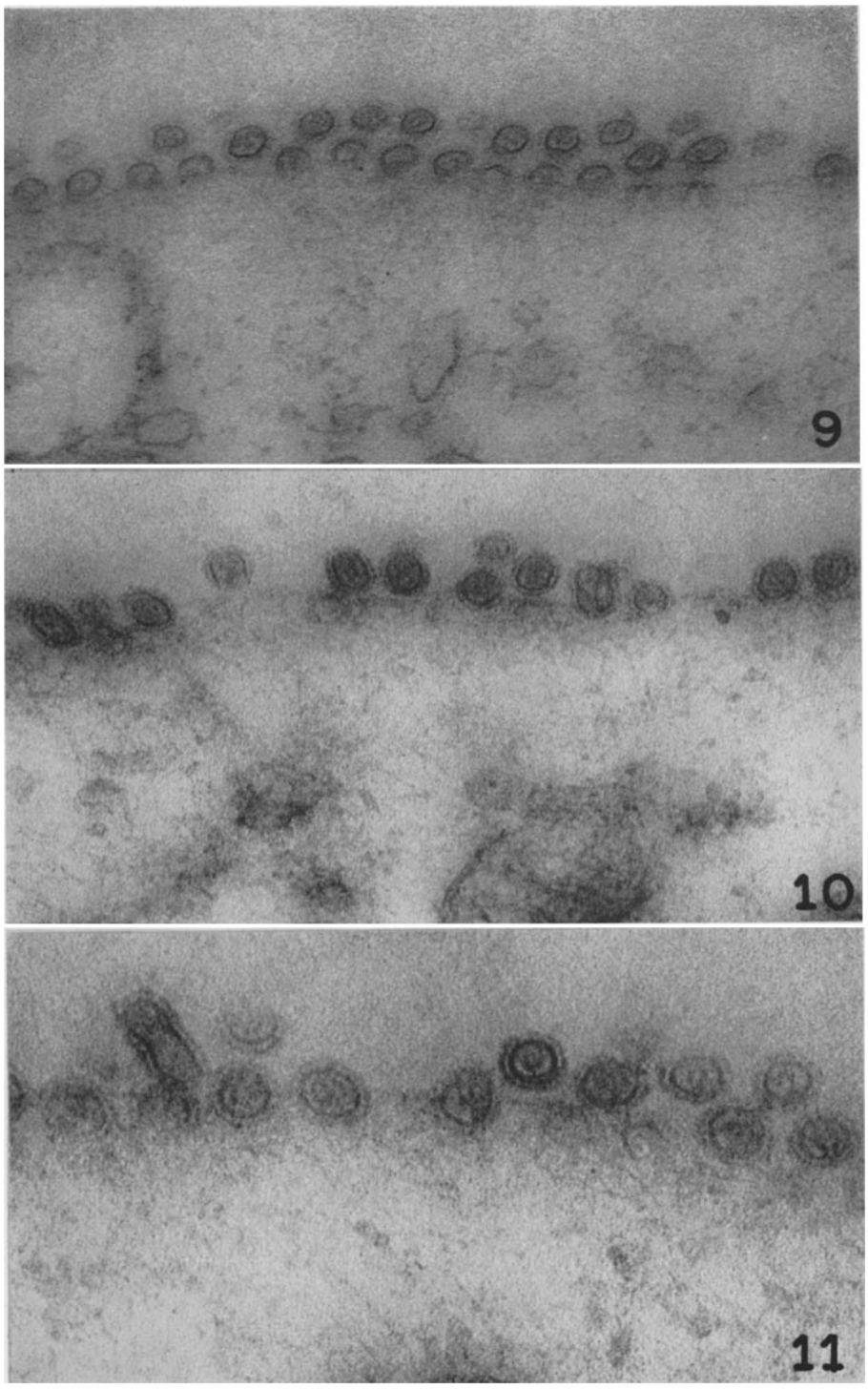
(Morgan *et al.*: Structure and development of viruses. III)

PLATE 18

FIG. 9. The upper portion of the field illustrated in Fig. 2. The spheres nearest the host cell exhibit incomplete membranes on the side adjacent to the cell wall and are believed to represent a stage of development. Adjacent to them, but farther from the host cell, are characteristic, completed spheres. × 95,000.

FIG. 10. A somewhat thicker section which permits better visualization of the material coating the short rods and spheres. This peripheral zone of material has a diffuse but recognizable margin and is separated from the limiting membrane by an area of lesser density. × 95,000.

FIG. 11. A section similar in thickness to that shown in Fig. 10. The structure of one sphere can be clearly visualized. This viral particle is probably central to the plane of section, whereas the others are eccentric. The cell wall appears to pass through several of the spheres, probably because of superimposition within the section. In the left third of the field an obliquely sectioned rod exhibits a moderately dense, but amorphous interior. × 136,000.



(Morgan *et al.*: Structure and development of viruses. III)