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Electron Microscopy of Herpes Simplex Virus

II. Sequence of Development

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Examination of infected cells at sequential intervals after infection revealed that the first viral forms to appear were capsids enclosing cores of low density. Not until the 6th hr were dense cores encountered, and at approximately the same time enveloped virus was seen. Envelopment occurred most frequently in close proximity to the nuclear surface, although the process was also encountered within the nuclear matrix and in the cytoplasm. There was often extensive proliferation of the nuclear membrane. Envelopment of the virus by budding from the cell surface was not observed. It was concluded that enveloped virus consitutes the infectious particle and that the unenveloped capsid is unstable outside the cell. Nevertheless, it is likely that capsids enclosing infectious nucleic acid can pass directly from one cell to another after fusion has taken place.

Herpes simplex virus has been extensively studied in sectioned cells (8, 11, 16, 22, 23, 25, 34, 35, 37, 41), and there is general agreement as to the manner in which the viral particles are formed. Nevertheless, before investigating the effects of a blocking agent (hydroxyurea) and before the application of ferritin-conjugated antibodies, it seemed advisable to re-examine in detail the process of development at sequential stages of infection in a manner analogous to the studies by Stoker et al. (40), Watson et al. (44), and Siminoff and Menefee (36). The present communication illustrates and describes the results of this investigation and reports several unusual types of viral particles.

MATERIALS AND METHODS

Cells and culture media. A line of stable human amnion cells (FL) was used. Eagle's minimal essential medium (MEM) with Hanks' balanced salt solution was obtained from Grand Island Biological Co., Grand Island, N.Y., and was supplemented with 10% calf serum. For infectivity titrations, the medium was supplemented with 5% calf serum.

Virus. The Miyama strain of herpes simplex virus was used. Of the three variants, which can be differentiated by their cytopathic effects (26), the −GCr and +GCr variants were employed for the present experiments. The results with both strains of virus were similar. High-titer virus stocks were prepared from heavily infected FL cells 48 to 72 hr after infection, and the supernatant fluid obtained after low-speed centrifugation was used for inoculation.

Virus infection and titration. Monolayers of FL cells were prepared in screw-cap test tubes or in 30-ml plastic bottles (Falcon Plastic, Los Angeles, Calif.). The former contained 3×10^5 to 6×10^5 cells; the latter, 10^6 to 2×10^6. The cultures were infected with the virus at multiplicities of 8 to 30 plaque-forming units (PFU). After 1 hr at 37 C, the monolayers were washed three times with MEM, and medium supplemented with 5% calf serum was added. In time-sequence studies, the cultures were removed at intervals for assay of cell-associated virus and for electron microscopy. For the former, the culture fluid was replaced with 2 ml of fresh MEM and the cultures were frozen and kept at −20 C. The cultures were later thawed and sonically treated. Viral infectivity titration was performed by the end-point method (expressed as $TCID_{50}$) or by the microplaque technique reported by Farnham (12).

Preparation for electron microscopy. The cells were treated as described in the preceding paper.

RESULTS

Characteristic viral particles. The earliest signs of infection, which were detected at 4.5 hr, consisted of irregular condensation and beginning margination of the nuclear chromatin (Fig. 1). Only rarely were viral particles encountered at this stage. By the 5th hr, scattered viral particles with capsids and cores of low density were observed. In an extensive study of many nuclei at

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this stage of infection, about 100 viral particles were found, and all exhibited cores of low density. Partially formed or incomplete virus of the type illustrated in Fig. 3 (see also the upper particle in Fig. 2) was rarely encountered.

Capsids enclosing electron-dense cores were first noted at 6 hr (Fig. 4, arrow). In addition, there were empty particles and clusters of irregularly shaped granules (Fig. 5). Crystallization occurred, but only at late stages of infection (Fig. 6).

Bizarre viral forms. Although the types of viral particles illustrated above constituted the majority, there were also bizarre forms. Figure 7 illustrates an intranuclear crystal composed of capsids enclosing a variety of internal components. Some are filled with electron-dense material; others contain oval cores of differing density or short rods; still others exhibit multiple, small, internal granules. A considerable proportion are empty. The inset of Fig. 8 shows another unusual form of the virus, in which dense, tadpole-shaped structures lie free or are partially enclosed within the capsid. Occasionally, crystals containing many of these structures were encountered. Figure 8 illustrates an example. Numerous viral particles within the crystalline matrix appear either to be malformed or to be in the process of disintegration. To the right, dense filaments seem to project into the crystal, and some lie between the capsids. Figure 9 shows two contiguous crystals composed of capsids with strikingly different cores. Scattered dense filaments are evident. In the upper part of Fig. 10, there are distorted, interwoven membranes, which presumably resulted from aberrant capsid synthesis. A collection of granules occupies the lower portion of the field. Occasionally, tubules (Fig. 11) and spirals (inset) were seen within infected nuclei, and in rare instances bundles of filaments were encountered. The former are reminiscent of intranuclear structures associated with adenovirus (24).

Envelopment. Figure 12 illustrates part of a reduplicated nuclear membrane extending into the nuclear matrix near several capsids. The cytoplasm occupies the right margin of the field. In Fig. 13, double membranes enclose rows of capsids within a nucleus. Figure 14 shows a similar phenomenon, but some membranes appear to have fused, thus forming one or more thickened lamellae. Such a process can result in the formation of extraordinary concentric lamellae (Fig. 15), among which are generally scattered capsids or enveloped virus. Not infrequently, enveloped virus is also found between nuclear membranes (Fig. 16). Reduplication of the nuclear membrane may remain localized at the nuclear margin, extending neither inward to the nuclear matrix nor outward to cytoplasm (Fig. 17). At first glance, such membranes appear to resemble the reduplicated membranes observed in association with adenovirus (15), but fusion is a distinctive phenomenon. As will become plain shortly, fusion does not occur between paired membranes, but back to back as it were, between the contiguous membranes of two pairs.

Figures 18 and 19 show proliferation of fused nuclear membranes outward into the cytoplasm. It is noteworthy that in both instances there are few viral particles in the vicinity. Figure 20 illustrates the site of fusion of cytoplasmic membranes, probably rough endoplasmic reticulum. The fusion is not occurring between paired membranes enclosing the cisternae (see arrows) but rather, as mentioned above, between the external aspect of adjacent pairs.

Occasionally, virus was encountered in the process of budding into cytoplasmic vacuoles (Fig. 21 and 22). In this situation, the membranes were generally thin and devoid of ribosomes.

Figures 23 and 24 illustrate cytoplasm at advanced stages of infection. The former shows numerous enveloped particles aggregated within large vacuoles, many of which intercommunicate. The irregular, jagged surface of the cell (at the upper left) suggests that vacuoles have ruptured, with extrusion of their contents into the extracellular space. The inset (taken from the cytoplasm of another cell) illustrates the two characteristic types of enveloped particles, one with a zone of low density separating the capsid from the envelope and the other with dense material between these structures. In both cases, the envelope is enclosed by a thin membrane, so that each particle lies within a walled vacuole. It will be noted that almost all enveloped viral particles belong to one of these two types. In Fig. 24, the vacuoles are small, generally enclosing one, or occasionally two, particles. There are double capsid forms. Unenveloped capsids are also evident. It should be emphasized at this point that intracytoplasmic virus, whether enveloped or not, almost invariably exhibits a dense core. Figure 25 illustrates the two characteristic types of virus on the surface of an infected cell. Figure 26 shows intracytoplasmic capsids with dense cores which differ in size and shape. At the left, three enveloped viral particles appear to lie within channels of the endoplasmic reticulum. Figure 27 illustrates part of a nucleus (at the bottom) containing capsids. Close to the nuclear membrane (on the left), virus appears to be in process of envelopment. Lamellae are evident along part of the surface of the cell (at the top).

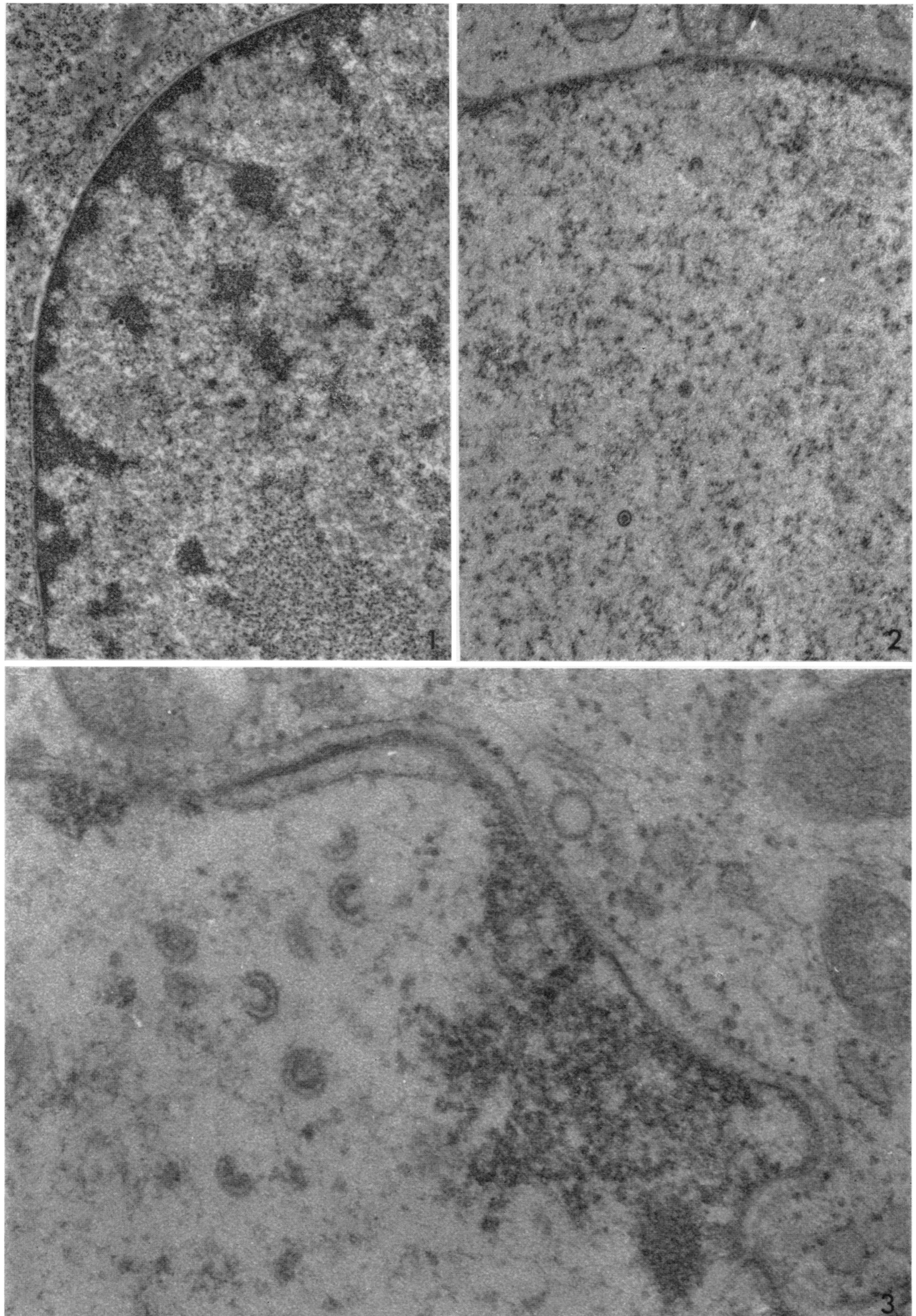

FIG. 1. *Irregular condensation and margination of nuclear chromatin at 4.5 hr. No virus is visible.* × *18,000.*
FIG. 2. *First viral particles composed of capsids and cores of low density, encountered at 5 hr.* × *30,000.*
FIG. 3. *Partially formed cores and capsids of the type encountered at early stages of infection. Note that these incomplete forms are not oriented with respect to any structure suggestive of a template.* × *74,000.*

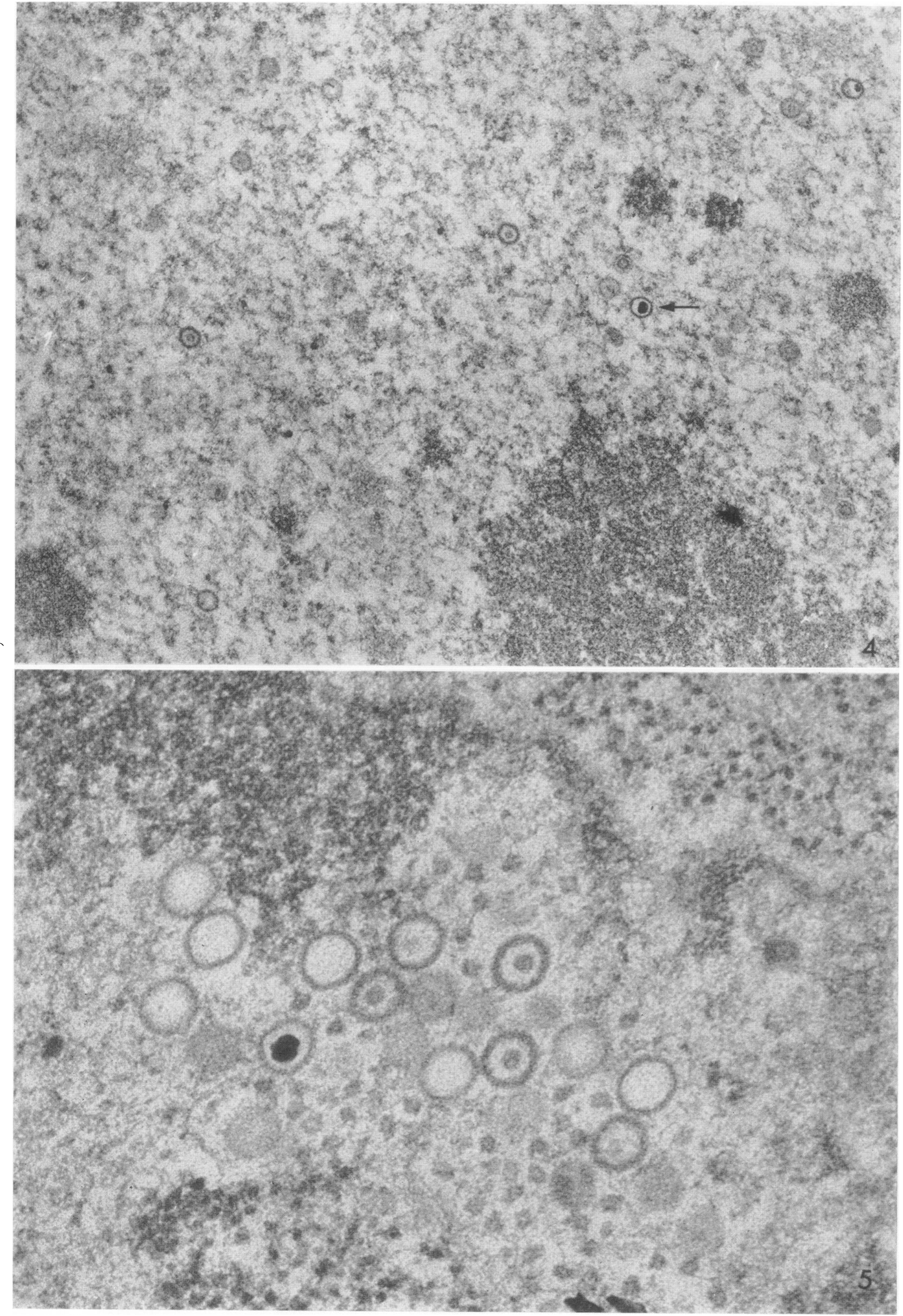

FIG. 4. *First dense core form (arrow), observed at 6 hr. It is characteristic of this stage that the viral particles tend to be scattered, suggesting that if differentiation occurs at circumscribed foci the virus is rapidly dispersed.* × *35,000.*

FIG. 5. *Characteristic cores and capsids adjacent to granules, the nature of which is uncertain.* × *90,000.*

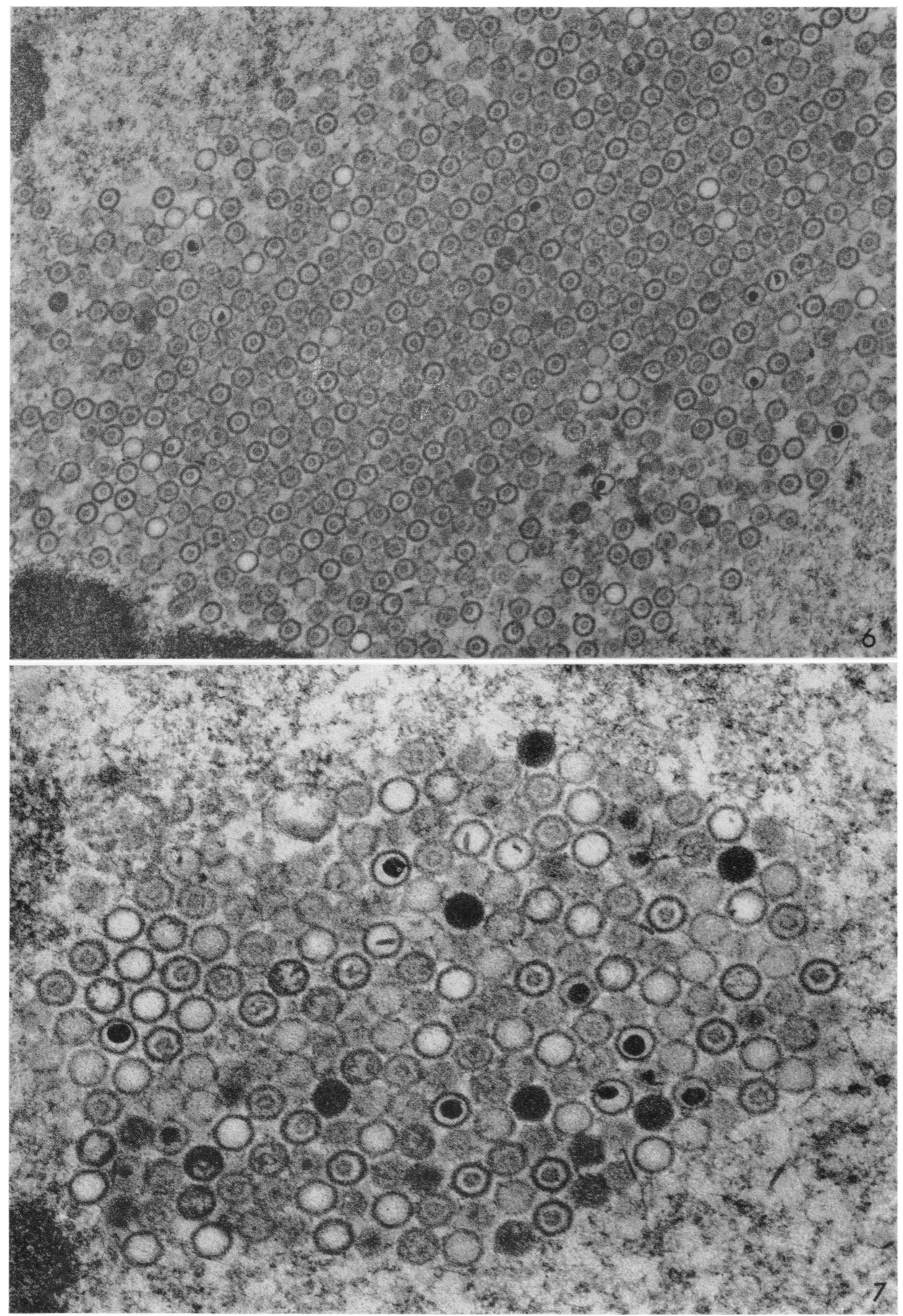

FIG. 6. *Typical intranuclear crystal composed of capsids and cores. Note the small proportion of dense cores. The margins of the crystal appear to be undergoing dissolution.* × 35,000.

FIG. 7. *Pleomorphic cores with capsids composing a crystal. Many of the capsids are malformed or only partially formed.* × 55,000.

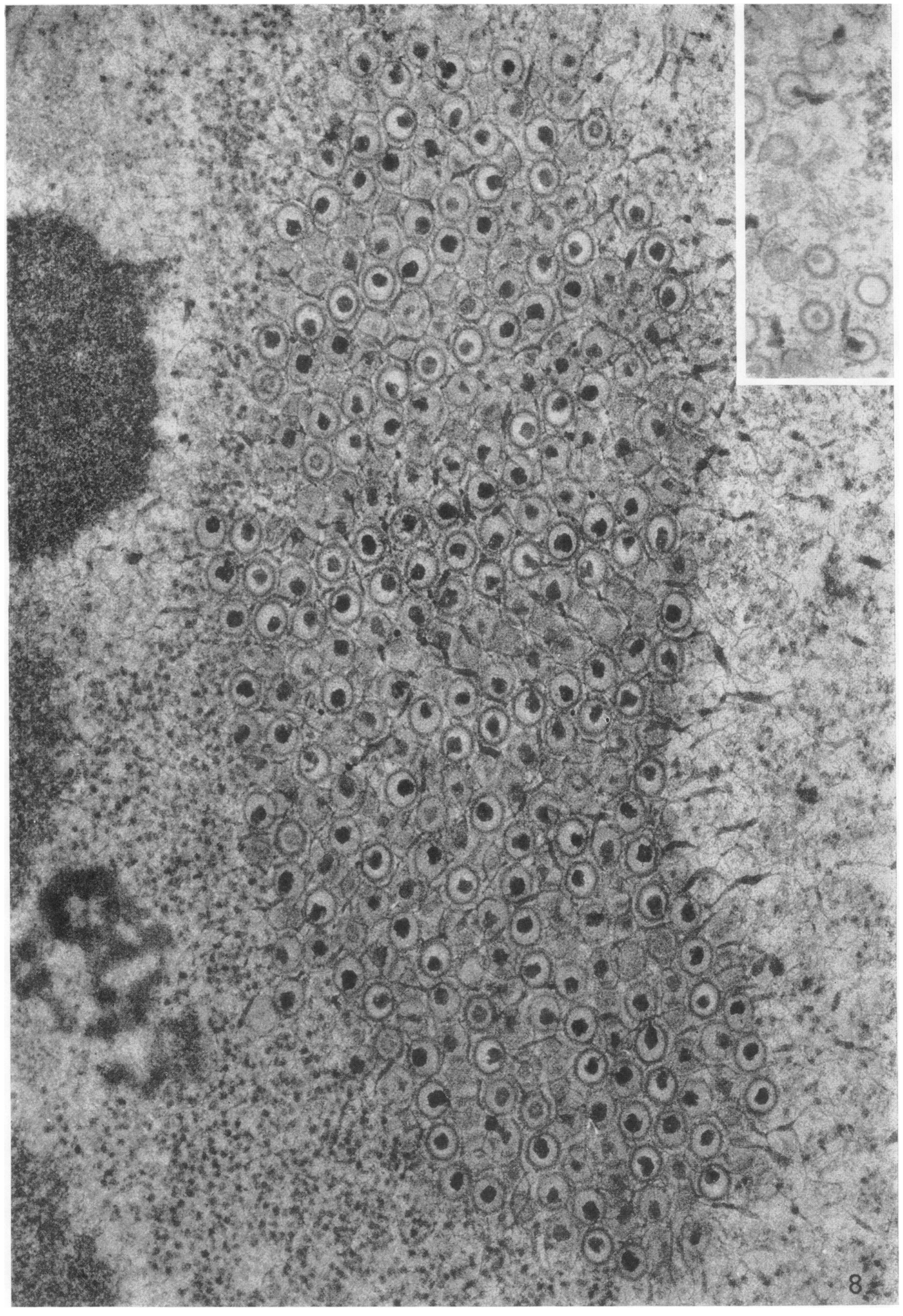

FIG. 8. *Dense, elongated filaments projecting into, and lying within, a crystal. As in the preceding micrograph, many of the capsids are malformed. Inset: tadpole-like structures partially enclosed by capsids.* × *54,000.*

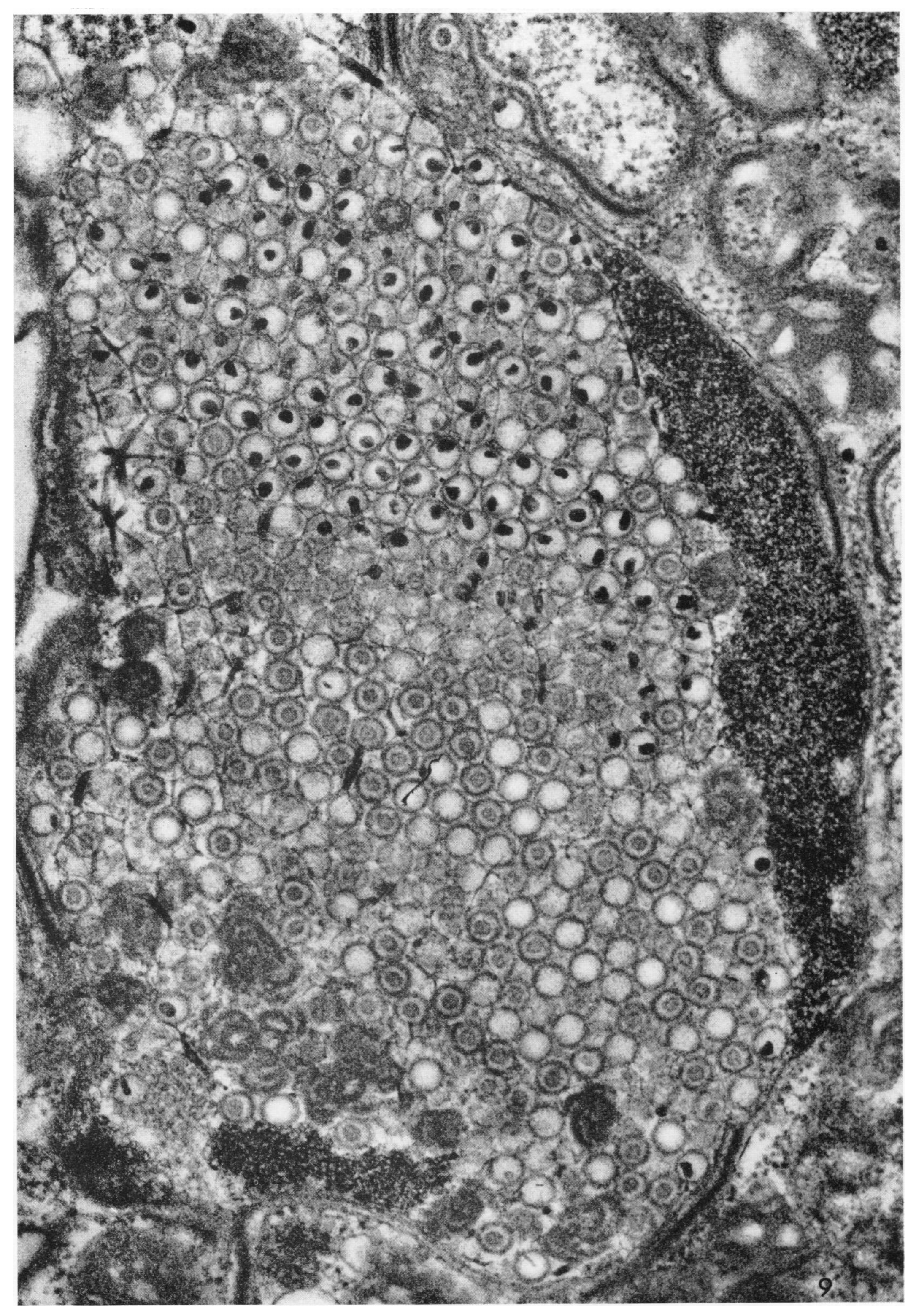

FIG. 9. *Two crystals composed of capsids containing cores of differing structure.* × 55,000.

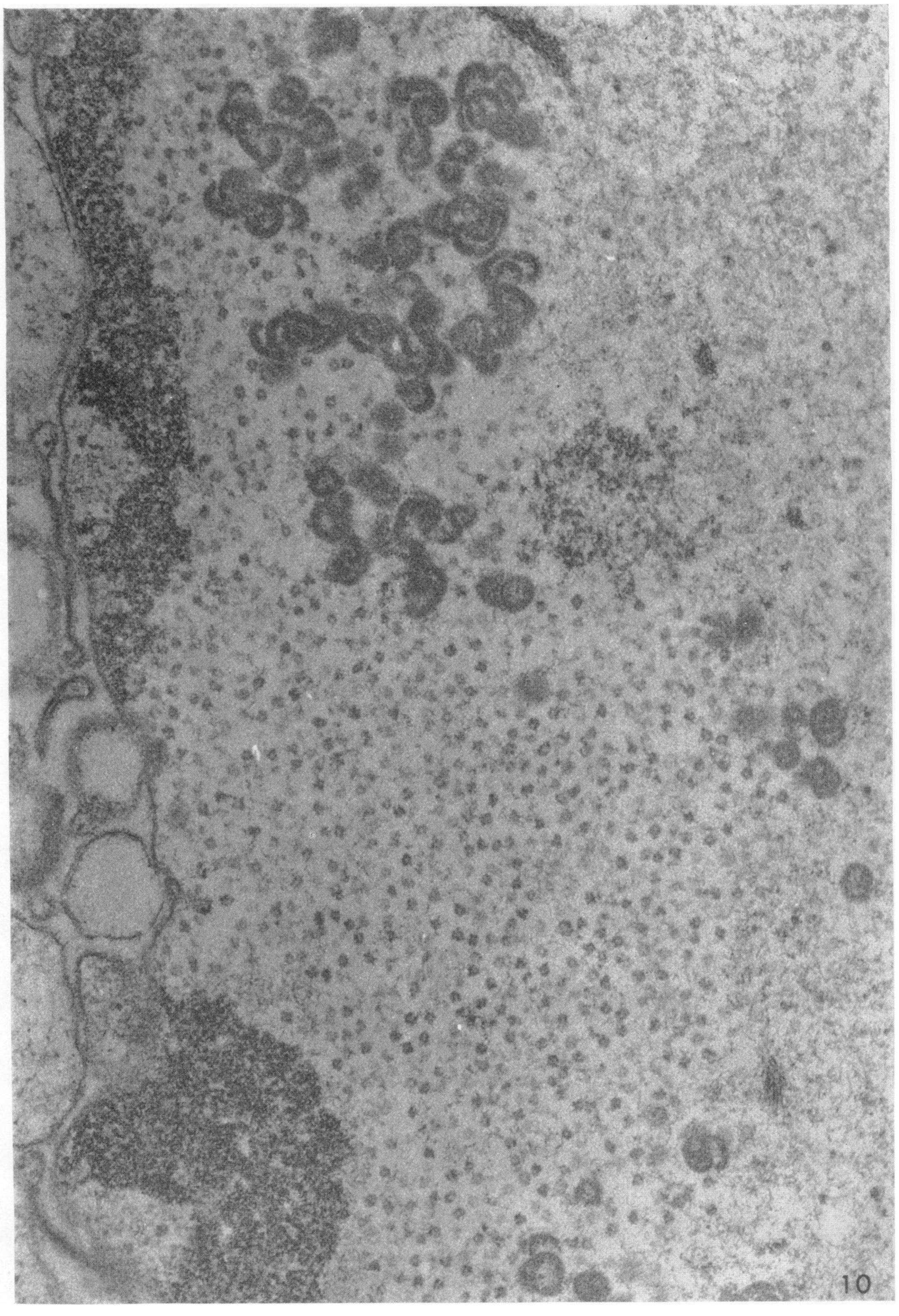

FIG. 10. *Interwoven membranes encountered late in the course of infection and thought to be an aberrant form of the capsid.* × *55,000.*

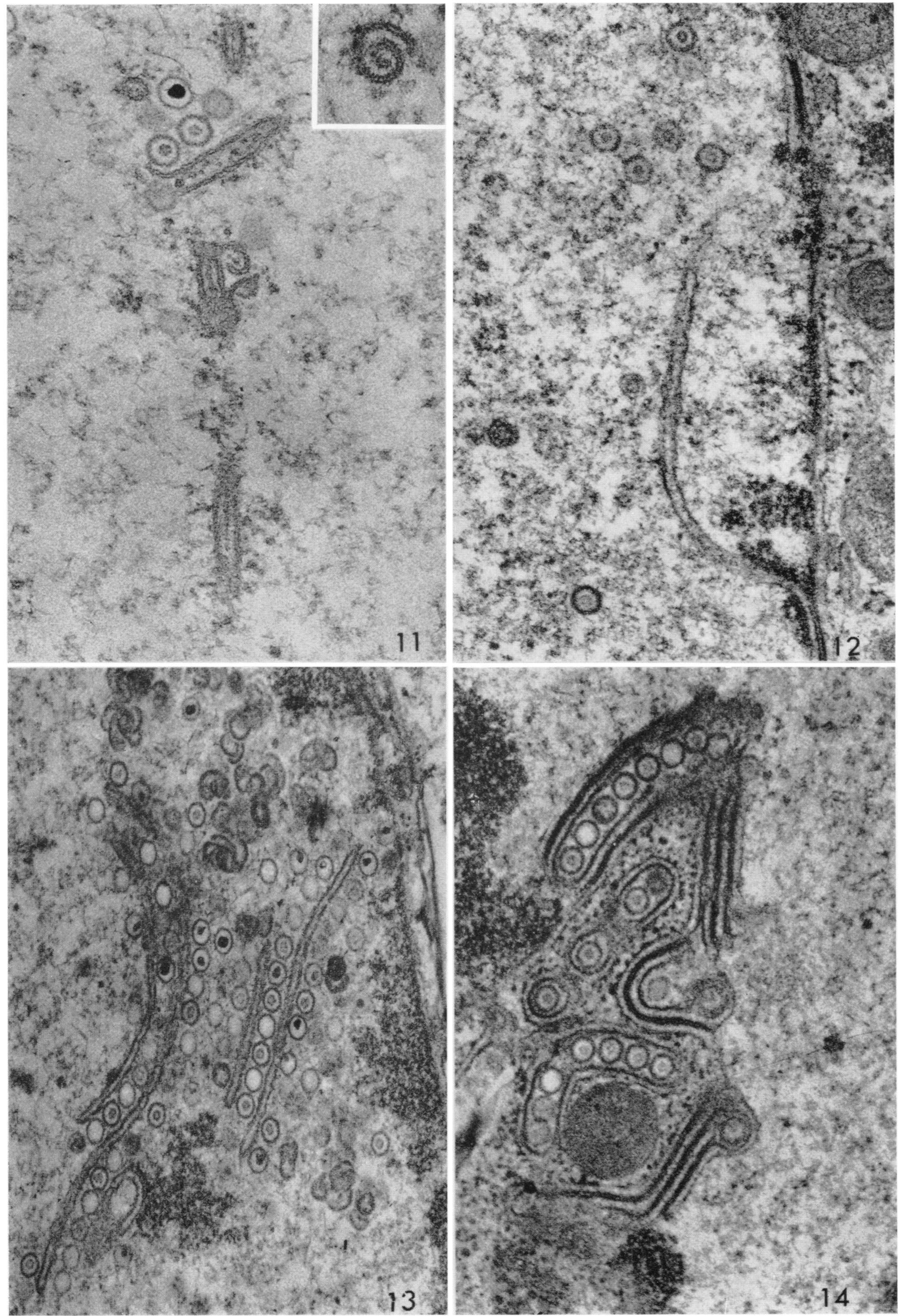

FIG. 11. *Intranuclear tubules.* × *55,000. Inset: spiral.* × *70,000.*
FIG. 12. *Extension of a reduplicated nuclear membrane into the nuclear matrix.* × *55,000.*
FIG. 13. *Capsids arrayed on double membranes within a nucleus.* × *30,000.*
FIG. 14. *Characteristic intranuclear membranes enclosing capsids.* × *40,000.*

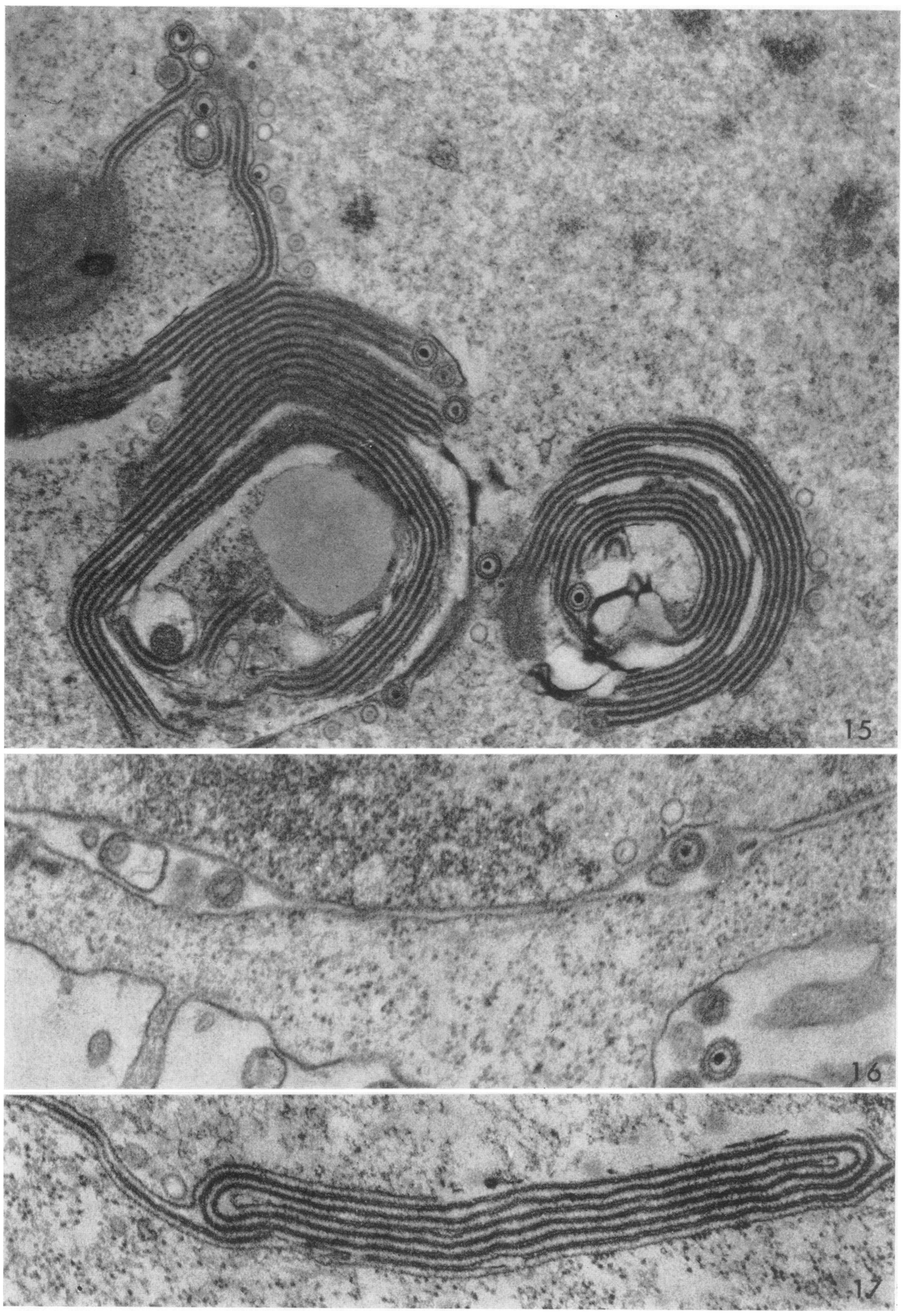

FIG. 15. *An unusually large array of intranuclear, fused membranes associated with capsids and enveloped virus.* × *28,000.*

FIG. 16. *Enveloped virus between the nuclear and cytoplasmic membrane.* × *32,000.*

FIG. 17. *Multiple fused membranes at the margin of a nucleus.* × *40,000.*

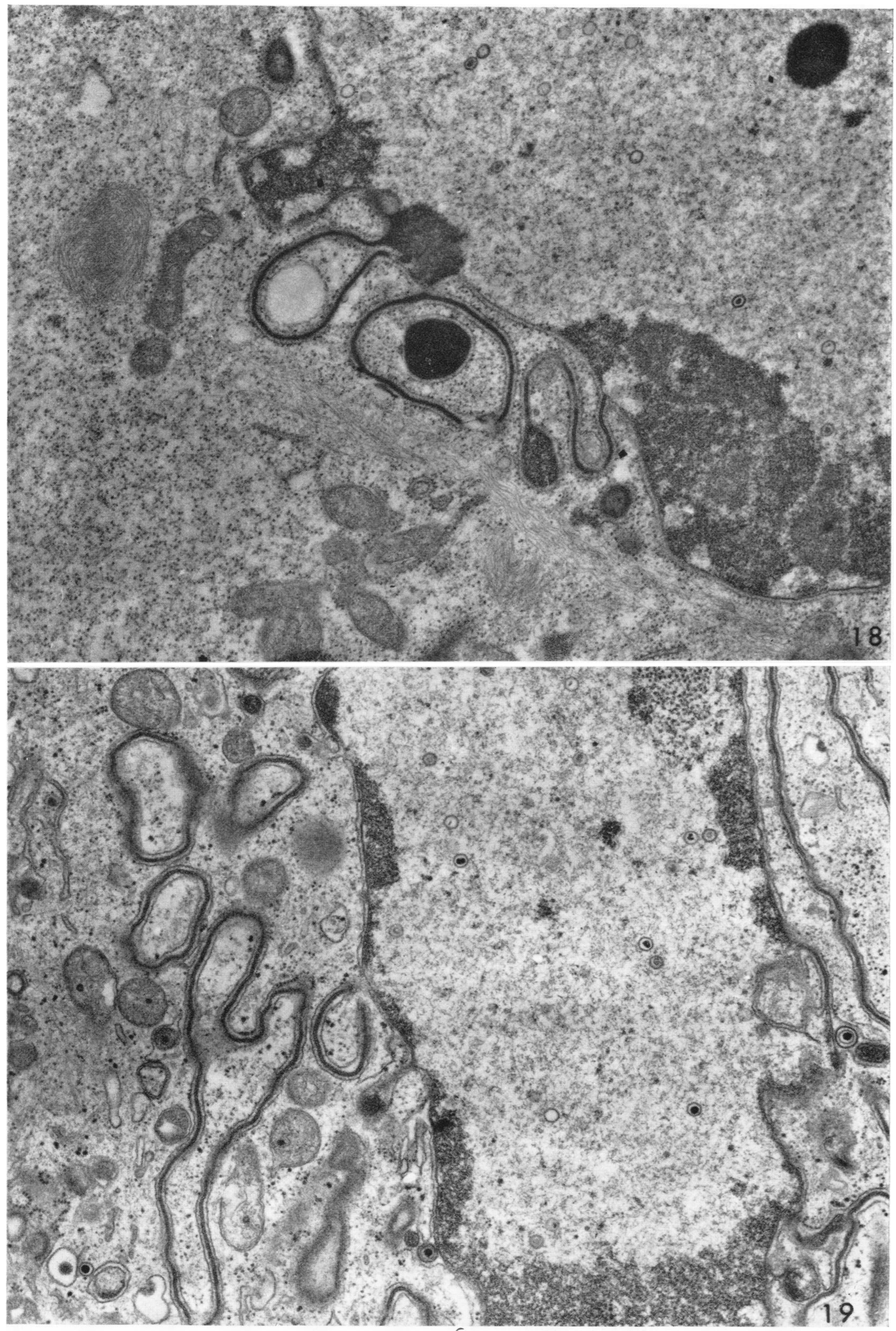

FIG. 18. *Fused nuclear membranes extending into the cytoplasm toward the left.* × *22,000.*

FIG. 19. *Fused nuclear membranes, many of which have detached and lie free with the cytoplasm at the left and right margins of the field.* × *22,000.*

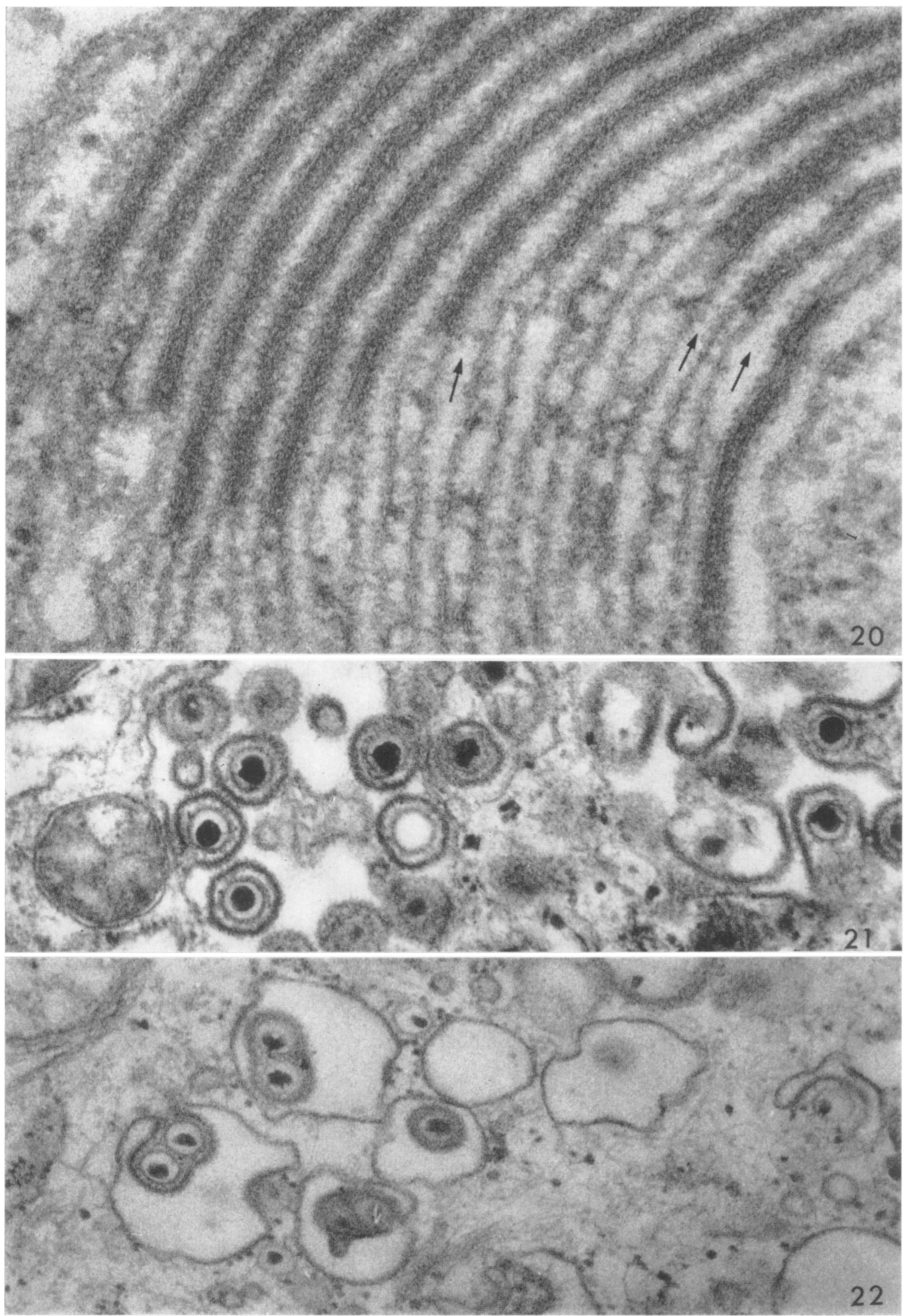

FIG. 20. *Zone of transition between discrete and fused cytoplasmic membranes. The arrows indicate interior channels of the endoplasmic reticulum.* × *200,000.*
FIG. 21. *Capsids budding (and thus becoming enveloped) into cytoplasmic vacuoles.* × *95,000.*
FIG. 22. *Another example of budding. Note two twin capsid forms on the left.* × *60,000.*

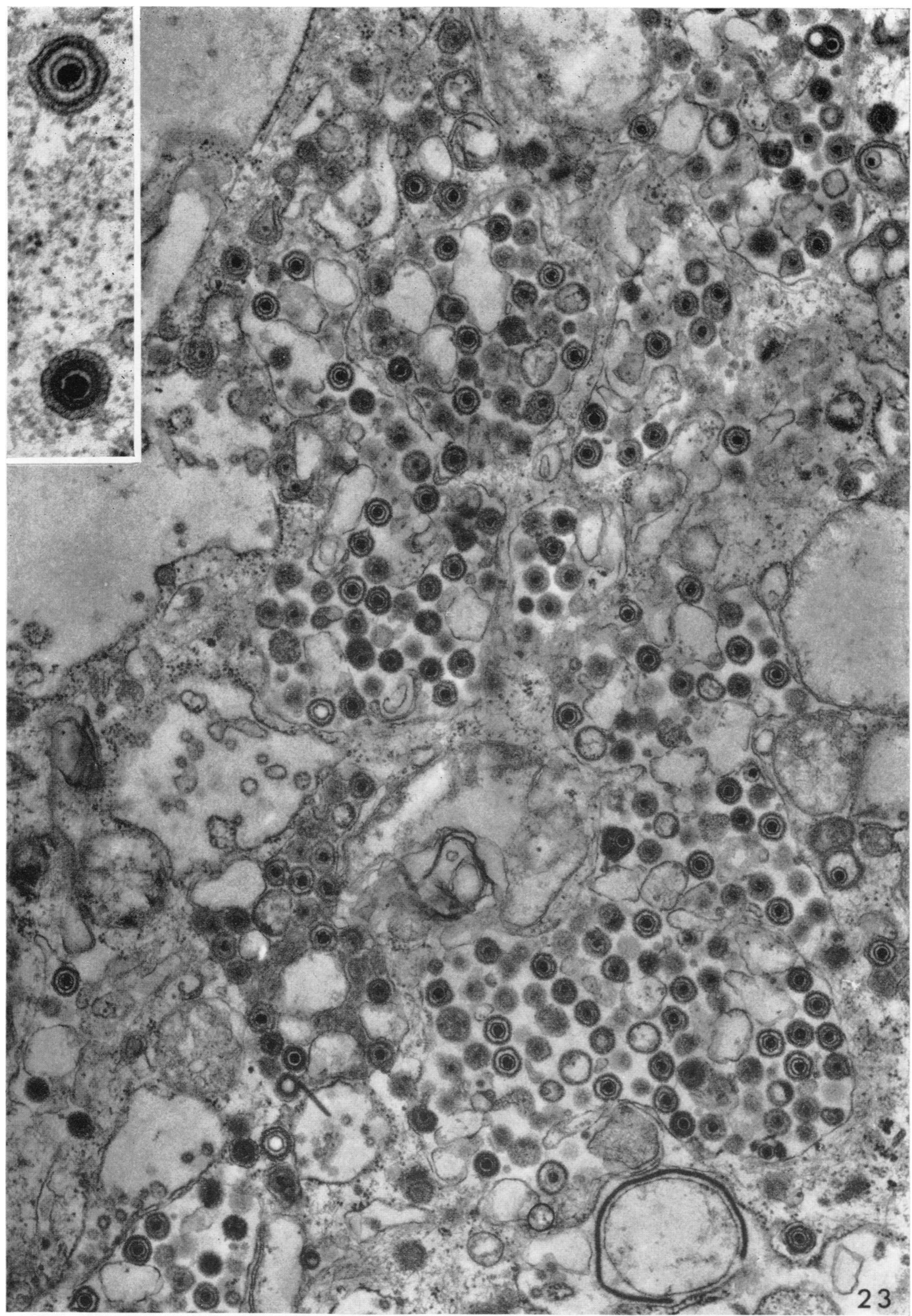

FIG. 23. *Intracytoplasmic virus, the majority of which lies within large vacuoles. The irregular margin of the cell at the upper left suggests that one or several vacuoles have ruptured with release of virus.* × *28,000. Inset: the two types of virus most commonly encountered. In the upper viral particle, a clear zone separates the capsid from the envelope; in the lower, a dense zone separates the capsid from the envelope. Both are enclosed within a third (cytoplasmic) membrane, which is shed upon release.* × *58,000.*

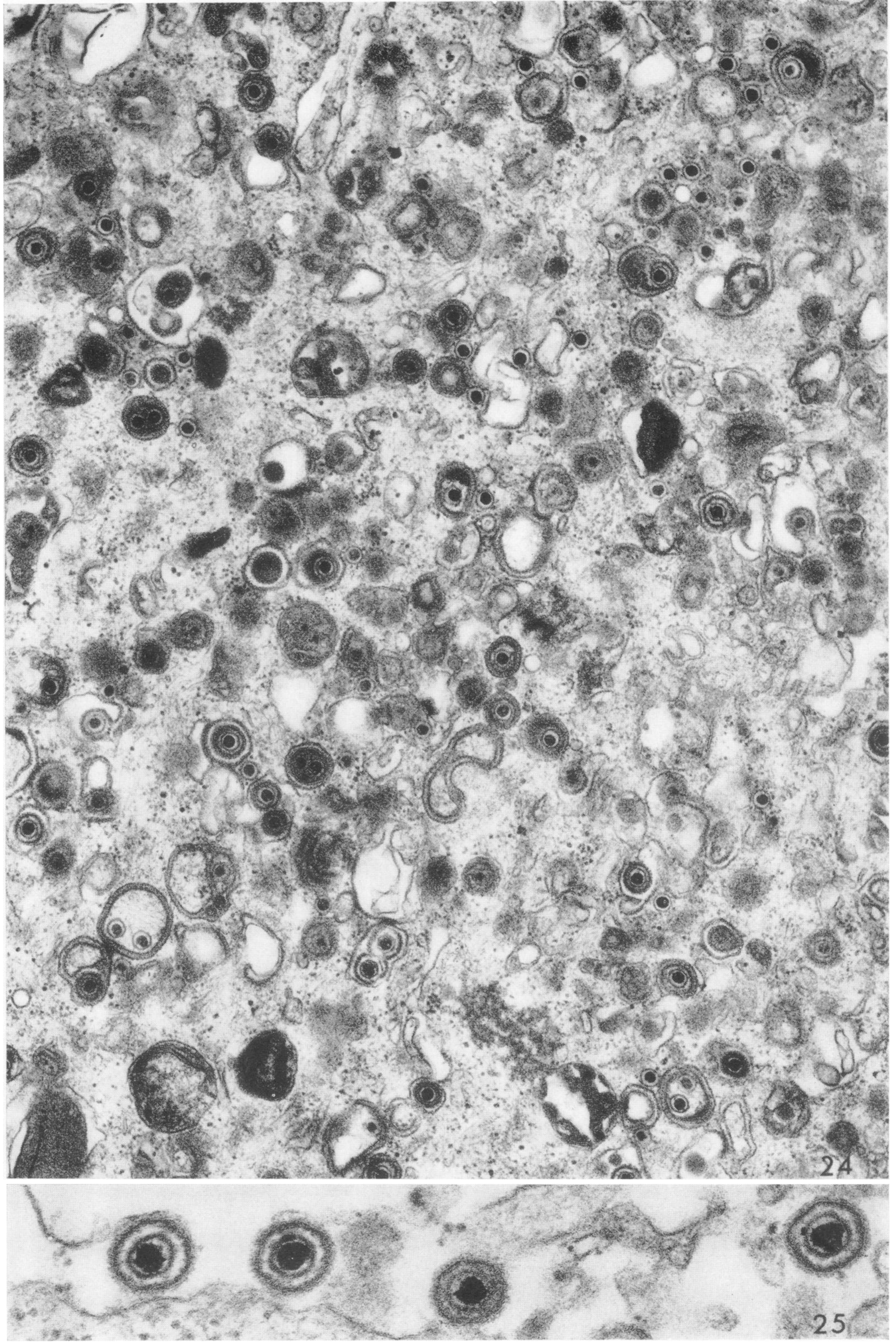

FIG. 24. *Cytoplasm containing virus. No more than two particles lie within a given vacuole. Note the twin capsid forms and, near the right margin in the mid-third, a capsid budding into a vacuole. Free capsids are also present. Compare the proportion of dense cores seen here with those generally observed in the nucleus (Fig. 6 and 7).* × *28,000.*

FIG. 25. *Extracellular virus of the types shown in the inset to Fig. 23. The virus lies on the surface of an infected cell and is therefore not in the process of digestion.* × *75,000.*

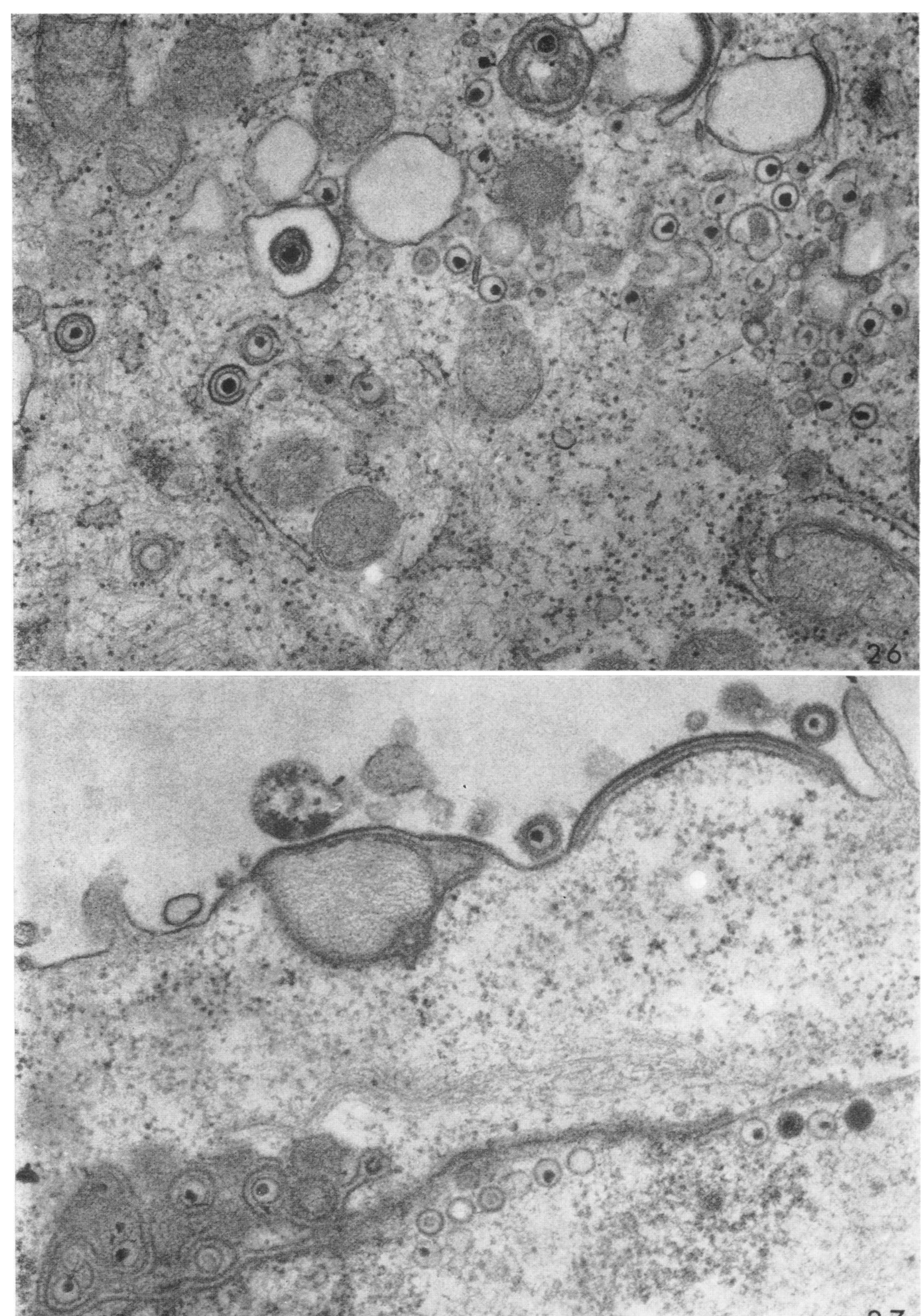

FIG. 26. *An unusual micrograph showing enveloped virus within rough endoplasmic reticulum at the left.* × *40,000.*

FIG. 27. *Capsids within the nucleus (at the bottom) and in the process of envelopment at the nuclear margin (lower left). The surface of the cell has been altered.* × *42,000.*

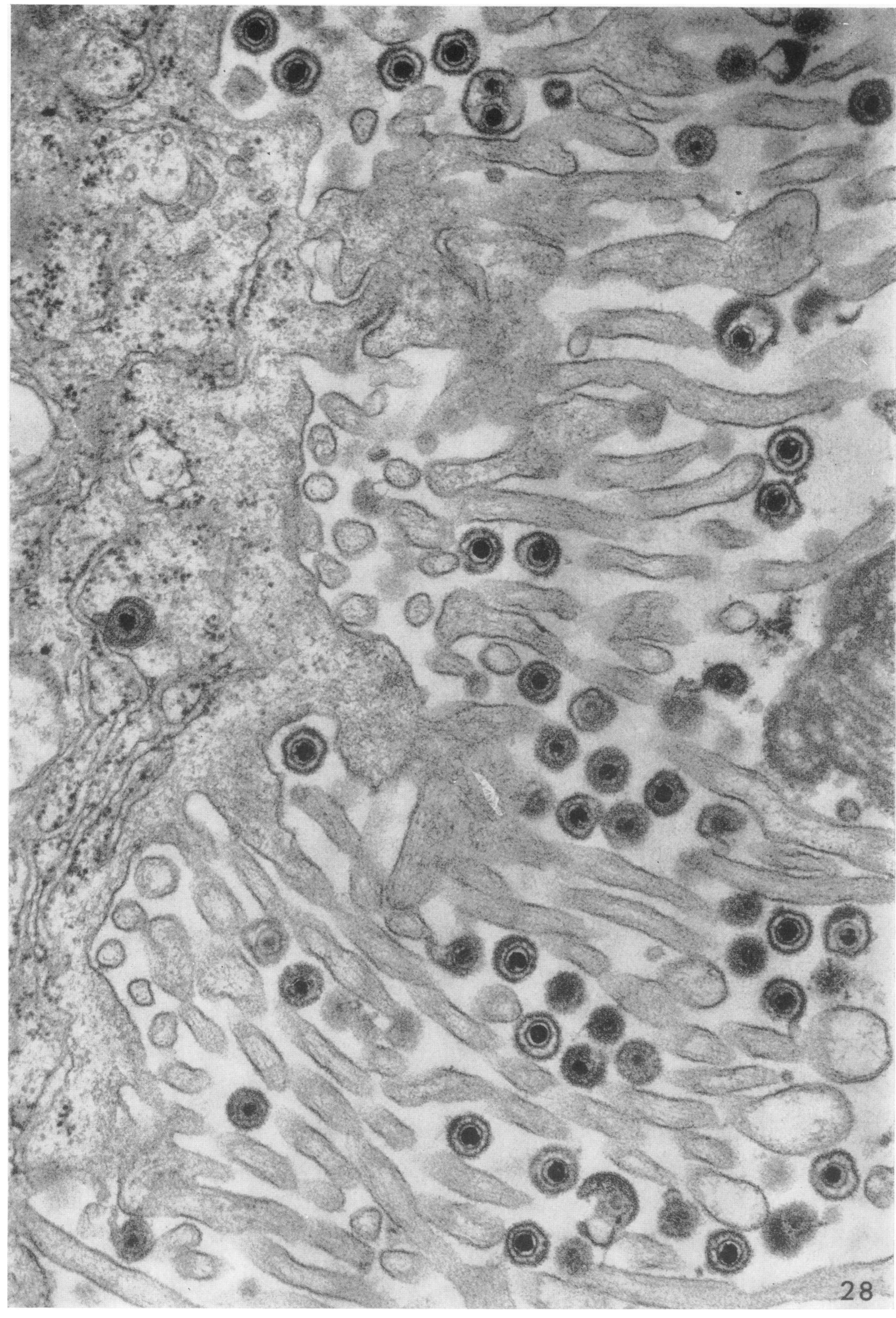

FIG. 28. *Virus scattered between cytoplasmic projections from an infected cell. The envelopes of some particles appear to be undergoing digestion at random sites.* × *38,000.*

In Fig. 28, released viral particles are lodged between cytoplasmic processes of an infected cell, the cytoplasm of which occupies the right portion of the field. Close examination of the viral envelopes reveals that some appear to be in the process of disintegration. It should be pointed out, however, in contrast to the virus shown in the preceding paper, that damaged segments are oriented at random with respect to the cell surface.

Unfortunately, time-sequence study is clear-cut only during the initial stages of viral synthesis, for by about the 8th hr not only are many stages visible at one time within any given cell but asynchrony has become apparent when different cells are compared. Accordingly, it was decided to summarize in a rougly quantitative manner the forms of virus observed at intervals after infection, as shown in Fig. 29. Although cores enclosed by capsids were first seen at 4 hr, they were also encountered as late as 48 hr after infection. At 6 hr, dense cores with capsids were observed, and at approximately the same time particles with envelopes appeared. The latter accumulated within the cytoplasm as infection progressed, whereas the former maintained roughly the same numbers. These results are in general agreement with those of Watson et al. (44), Siminoff and Menefee (36), and Stoker et al. (40), although the last group reported that capsids appeared somewhat later. Figure 29 also shows that crystals and aberrant forms of the virus did not appear until late in the the course of infection.

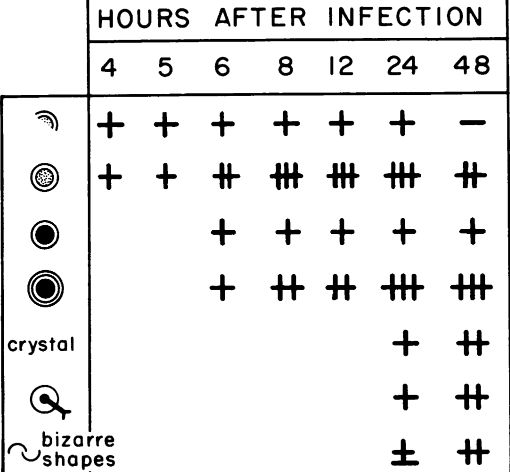

FIG. 29. *Chart summarizing the relative numbers of different viral forms encountered at sequential intervals after infection.*

DISCUSSION

Formation of the core and capsid can be better understood when examined in conjuction with data derived from blocking experiments. Accordingly,discussion of this aspect of development will be deferred until the conclusion of the third paper in this series. It should be mentioned, however, that bizarre forms of the core were not uncommon and have been observed previously in studies of herpes simplex virus (34, 36, 41), as well as of other members of this group (2, 3, 6, 7, 18, 30–32, 42, 45). Tadpole-like structures have also been encountered in studies of Lucké adenocarcinoma virus (13) and cytomegalovirus (19, 30). In view of the fact that such cores were not observed at early stages of infection in the present study and were rarely seen within enveloped particles, one is led to conclude that the bizarre forms are aberrant and result from asynchronous or otherwise defective mechanisms of assembly. Another peculiar arrangement of the virus is illustrated by Fig. 10. The capsid-like material is interwoven and does not enclose cores. Although it was suggested previously (25) that such particles were in the process of formation, their presence only at terminal stages of infection suggests that they are examples of aberrant capsid differentiation in the absence of core formation.

There are differing explanations of the manner in which the viral envelope is formed. Morgan et al. (25) and Shipkey et al. (34) stressed the role of the nuclear membrane, whereas Epstein (8) and Siminoff and Menefee (36), while observing enveloped virus in nuclei, believed that the process of envelopment most commonly occurred within cytoplasmic vacuoles or by budding at the cellular surface. The present study supports the concept that envelopment occurs in the nucleus and cytoplasm. However, budding of the virus at the cell surface could not be confirmed.

The nuclear membranes can proliferate in such a manner as to remain localized at the surface of the nucleus, project inward into the nuclear matrix, or extend outward into the cytoplasm. In the last instance, they not infrequently become detached. Although proliferation of nuclear membranes has been observed in previous studies of herpes simplex virus (11, 25, 34, 35, 41, and 44), their extension into the cytoplasm has been more difficult to document (25, 34). Protrusion of nuclear membranes so as to form vesicles in the cytoplasm has also been seen in pseudorabies virus (14), cytomegalovirus (28), and infectious bovine rhinotracheitis (2) virus. [In this regard, it is remarkable that Achong and Epstein (1) and Epstein et al. (9) noted membranes, which were indistinguishable from those illustrated by Fig.

18 and 19, in their studies of biopsies and cultured cells from Burkitt lymphomas, although they were unable to demonstrate viral particles.] The complex question of membrane fusion will be discussed in a later report.

Envelopment of the virus must occur very rapidly, since stages in the process, whether in the cytoplasm or nucleus, were seen infrequently. The majority of particles seemed to acquire an envelope in the immediate vicinity of the nuclear membrane as they passed from the nucleus into the cytoplasm. The observation that there are two types of envelopes—one thick and closely applied to the capsid, the other thin and separated from the capsid by a zone of low density—must mean that there are two distinct types of membranes capable of enveloping the virus. The possibility could not be excluded that some envelopes derive from the endoplasmic reticulum, since virus was occasionally observed within channels lined by membranes with associated ribosomes. Studies of other members of this group of viruses have shown enveloped forms of the virus in the nucleus, as in the case of varicellazoster virus (42), cytomegalovirus (20, 21, 27), and the Lucké virus (13), and in the cytoplasm, as in studies of herpes B virus (5), pseudorabies virus (14), rhinopneumonitis virus (6), cytomegalovirus (20), and the virus associated with the Lucké renal adenorenal carcinoma of frogs (18). The suggestion, advanced in an earlier report (25), that unenveloped capsids necessarily disintegrate within the cytoplasm, was erroneous.

Of particular interest is the fact that no report of herpes simplex virus or any other virus in this group illustrates or describes release of virus from the cell by the process of budding from the cell surface suggested by Epstein (8) and by Siminoff and Menefee (36). Two alternative explanations for Epstein's findings may be advanced, namely, that either he happened to have a unique strain of virus or that he was led into an erroneous interpretation of his micrographs. One can suggest that several of his micrographs, which are very similar to those illustrating the initial paper of this series, show virus gaining entry. Regarding the report by Epstein and Holt (10) of adenosine triphosphatase at the surface of extracellular virus, it can be argued that the enzyme on the surface of the host cells may have coated the virus in a fortuitous manner during the process of release. Close inspection of the published micrographs leaves considerable doubt as to whether the stained material is an intrinsic part of the envelope of the virus. The micrographs by Siminoff and Menefee (36) are difficult to interpret, since several are reproduced at low magnification. One particle in their Fig. 20 may have been in the act of budding. We are inclined, however, to maintain that virus is released by the process of "reverse phagocytosis" originally proposed (25). A similar phenomenon has been reported in studies of pseudorabies virus (14) and the Lucké virus (18). Of interest is an unusual situation pertaining to the Lucké virus within the epithelium of renal tubules (13, 18, 45), for under these circumstances extracellular virus was found to possess a protective coat enclosing the envelope. Disruption of the cell with release of intact cytoplasmic vacuoles containing virus could account for this, but to date such a manner of egress has not been described.

One problem of considerable significance has proved difficult to solve conclusively, i.e., whether the core enclosed by a capsid but devoid of an envelope is infectious. This question was initially posed by Pirie (29). Watson and Wildy (43) suggested that the envelope was not necessary for infection, although they gave no supporting data. Subsequently, in a study of the sequential stages of development correlated with negative staining of purified virus and determination of infectivity in their cultures, Watson, Wildy, and Russell (44) concluded that "both naked [cores enclosed by capsids but devoid of envelopes] and enveloped particles are infective." Smith (38), however, separated capsids from the enveloped forms by cesium chloride density gradient centrifugation, and, although admittedly this procedure is damaging to the virus, his results leave little room for argument. A fraction containing more than 99% unenveloped capsids had a particle-PFU ratio of 8,000, whereas fractions with 70 to 82% enveloped virus showed a particle-PFU ratio of 10 to 12. Our results are in complete agreement with his conclusion, namely, " that the envelope plays an essential role in herpes virus infection." Among the thousands of extracellular particles encountered during intensive study over a period of 3 years (the time necessary to assemble the data published herein), almost all possessed envelopes. We are led to conclude, therefore, that the unenveloped capsid is unstable outside the cell and that the enveloped capsids are the infectious or complete virus.

The foregoing hypothesis is consistent with the possibility that the unenveloped capsid can transmit infection to neighboring cells that have become fused to the host cell. Black and Melnick (4) noted the formation of plaques when cultures of kidney epithelial cells were infected with herpes B virus and incubated in the presence of antibody. They further observed that virus from initially infected cells would pass directly to neighboring cells and that "several cycles may have occurred in this manner before any infectious virus was

released into the medium." Two explanations of these data are possible. Infection of adjacent cells was caused either by fully infectious, stable virus, which was not released into the medium, or by an unstable form of the virus. Stoker (39) re-examined the problem using herpes simplex virus in HeLa cells. He reported that when cells were disrupted by freezing, only 1 in 130 (0.8%) released infective virus, despite the fact that 43.6% of the intact cells could give rise to plaques, i.e., infect adjacent cells in the culture. He concluded that "these results raise the possibility that vegetative herpes virus can spread from cell to cell." If the assumption is correct that the unenveloped capsid is unstable in the extracellular environment but may possess infectious nucleic acid, then it follows that this form of the virus accounts for the spread of infection from one cell to the next in the absence of otherwise demonstrably infectious particles. The possible mechanism of cell fusion, which would permit the spread of unenveloped capsids directly to adjacent cells, will be discussed in paper IV.

As noted in the description of Fig. 28, the envelopes of the extracellular virus frequently appear to be in the process of disintegration. It seems not unreasonable to assume that the enzyme believed capable of digesting the envelope of virus at the moment of entry into uninfected cells diffuses into the tissue culture media in sufficient quantity to digest the viral envelopes of newly released particles. This would explain the "motheaten" appearance of such envelopes (*see* Fig. 12 of the preceding paper) and also account for the relative instability of the virus, noted by so many investigators. For example, Scott et al. (33) reported the half-life to be 1.5 hr at 37 C.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Achong, B. G., and M. A. Epstein. 1966. Fine structure of the Burkitt tumor. J. Natl. Cancer Inst. **36**:877–897.
2. Armstrong, J. A., H. G. Pereinra, and C. H. Andrewes. 1961. Observations on the virus of infectious bovine rhinotracheitis, and its affinity with the herpes virus group. Virology **14**:276–285.
3. Becker, P., J. L. Melnick, and H. D. Mayor. 1965. A morphologic comparison between the developmental stages of herpes zoster and human cytomegalovirus. Exptl. Mol. Pathol. **4**:11–23.
4. Black, F. L., and J. L. Melnick. 1955. Microepidemiology of poliomyelitis and herpes-B infections. Spread of the viruses within tissue cultures. J. Immunol. **74**:236–242.
5. Buthala, D. A., and J. Mathews. 1963. Studies on herpes virus simiae (B-virus). Intracellular development. Cornell Vet. **53**:494–505.
6. Darlington, R. W., and C. James. 1966. Biological and morphological aspects of the growth of equine abortion virus. J. Bacteriol. **92**:250–257.
7. Donnellan, W. L., S. Chantra-Umporn, and J. M. Kidd. 1966. The cytomegalic inclusion cell. An electron microscopic study. Arch. Pathol. **82**:336–348.
8. Epstein, M. A. 1962. Observations on the mode of release of herpes virus from infected HeLa cells. J. Cell Biol. **12**:589–597.
9. Epstein, M. A., B. G. Achong, Y. M. Barr, B. Zajac, G. Henle, and W. Henle. 1966. Morphological and virological investigations on cultured Burkitt tumor lymphoblasts (strain Raji). J. Natl. Cancer Inst. **37**:547–559.
10. Epstein, M. A., and S. J. Holt. 1963. Electron microscopic observations on the surface adenosine triphosphatase-like enzymes of HeLa cells infected with herpes virus. J. Cell Biol. **19**: 337–347.
11. Falke, D., R. Siegert, and W. Vogell. 1959. Elektronenmikroskopische Befunde zur Frage der Doppelmembrandbildung des Herpes-simplex-virus. Arch. Ges. Virusforsch. **9**:484–496.
12. Farnham, A. E. 1958. The formation of microscopic plaques by herpes simplex virus in HeLa cells. Virology **6**:317–327.
13. Fawcett, D. W. 1957. Electron microscope observations on intracellular virus-like particles associated with the cells of the Lucké renal adenocarcinoma. J. Biophys. Biochem. Cytol. **2**:725–742.
14. Felluga, B. 1963. Electron microscopic observations on pseudorabies virus development in a line of pig kidney cells. Ann. Selavo **5**:412–424
15. Gregg, M., and C. Morgan. 1959. Reduplication of nuclear membranes in HeLa cells infected with adenovirus. J. Biophys. Biochem. Cytol. **6**:539–540.
16. Holmes, I. H., and D. H. Watson. 1963. An electron microscope study of the attachment and penetration of herpes virus in BHK21 cells. Virology **21**:112–123.
17. Hummeler, K., G. Henle, and W. Henle. 1966. Fine structure of a virus in cultured lymphoblasts from Burkitt lymphoma. J. Bacteriol. **91**:1366–1368.
18. Lunger, P. D., R. W. Darlington, and A. Granoff. 1965. Cell-virus relationships in the Lucké renal adenocarcinoma: an ultrastructure study. Ann. N.Y. Acad. Sci. **126**:289–314.
19. Luse, S. A., and M. G. Smith. 1958. Electron microscopy of salivary gland viruses. J. Exptl. Med. **107**:623–632.
20. McGavran, M. H., and M. G. Smith. 1965. Ultrastructural, cytochemical, and microchemi-

cal observations on cytomegalovirus (salivary gland virus) infection of human cells in tissue culture. Exptl. Mol. Path. **4**:1-10.

21. Middelkamp, J. N., G. Patrizi, and C. A. Reed. 1967. Light and electron microscopic studies of the guinea pig cytomegalovirus. J. Ultrastruct. Res. **18**:85-101.
22. Morgan, C., S. A. Ellison, H. M. Rose, and D. H. Moore. 1954. Internal structure in virus particles. Nature **173**:208-209.
23. Morgan, C., S. A. Ellison, H. M. Rose, and D. H. Moore. 1954. Structure and development of viruses as observed in the electron microscope. I. Herpes simplex virus. J. Exptl. Med. **100**: 195-202.
24. Morgan, C., C. Howe, H. M. Rose, and D. H. Moore. 1956. Structure and development of viruses observed in the electron microscope. IV. Viruses of the RI-APC group. J. Biophys. Biochem. Cytol. **2**:351-360.
25. Morgan, C., H. M. Rose, M. Holden, and E. P. Jones. 1959. Electron microscopic observations on the development of herpes simplex virus. J. Exptl. Med. **110**:643-656.
26. Nii, S., and J. Kamahora. 1961. Cytopathic changes induced by herpes simplex virus. Biken's J. **4**:51-58.
27. Patrizi, G., J. N. Middelkamp, J. C. Herweg, and H. K. Thornton. 1965. Human cytomegalovirus: electron microscopy of a primary viral isolate. J. Lab. Clin. Med. **65**:825-838.
28. Patrizi, G., J. N. Middelkamp, and C. A. Reed. 1967. Reduplication of nuclear membranes in tissue-culture cells infected with guinea-pig cytomegalovirus. Am. J. Pathol. **50**:779-790.
29. Pirie, N. W. 1957. Material in virus preparations not necessary for manifestations of characteristic properties. Ciba Foundation Symp. Nature Viruses, p. 56-68.
30. Ruebner, B. H., T. Hirano, R. J. Slusser, and D. N. Medearis. 1965. Human cytomegalovirus infection. Electron microscopic and histochemical changes in cultures of human fibroblasts. Am. J. Pathol. **46**:477-496.
31. Ruebner, B. H., T. Hirano, R. Slusser, J. Obsorn, and D. N. Medearis, Jr. 1966. Cytomegalovirus infection. Viral ultrastructure with particular reference to the relationship of lysosomes to cytoplasmic inclusions. Am. J. Pathol. **48**: 971-989.
32. Ruebner, B. H., K. Miyai, R. J. Slusser, P. Wedemeyer, and D. N. Medearis. 1964. Mouse cytomegalovirus infection. An electron microscopic study of hepatic parenchymal cells. Am. J. Pathol. **44**:799-821.
33. Scott, T. F. McN., D. L. McLeod, and T. Tokumaru. 1961. A biologic comparison of two strains of herpes virus hominis. J. Immunol. **86**:1-12.
34. Shipkey, F. H., R. A. Erlandson, R. B. Bailey, V. I. Babcock, and C. M. Southam. 1967. Virus biographies. II. Growth of herpes simplex virus in tissue culture. Exptl. Mol. Pathol. **6**: 39-67.
35. Siegert, R., and D. Falke. 1966. Elektronenmikroskopische Untersuchungen über die Entwicklung des Herpesvirus hominis in Kulturzellen. Arch. Ges. Virusforsch. **19**:230-249.
36. Siminoff, P., and M. G. Menefee. 1966. Normal and 5-bromo-deoxyuridine-inhibited development of herpes simplex virus. An electron microscope study. Exptl. Cell Res. **44**:241-255.
37. Sirtori, C., and M. Basisio-Bestetti. 1967. Nuclear changes in KB tumor cells infected with herpes simplex virus. Cancer Res. **27**:367-376.
38. Smith, K. O. 1964. Relationship between the envelope and the infectivity of herpes simplex virus. Proc. Soc. Exptl. Biol. Med. **115**:814-816.
39. Stoker, M. G. P. 1958. Mode of intercellular transfer of herpes virus. Nature **182**:1525-1526.
40. Stoker, M. G. P., K. M. Smith, and R. W. Ross. 1958. Electron microscopic studies of HeLa cells infected with herpes virus. J. Gen. Microbiol. **19**:244-249.
41. Swanson, J. L., J. E. Craighead, and E. S. Reynolds. 1966. Electron microscopic observations on herpes virus hominis (herpes simplex virus) encephalitis in man. Lab. Invest. **15**:1966-1981.
42. Tournier, P., F. Cathala, and W. Bernhard. 1957. Ultrastructure et développement intracellulaire du virus de la varicelle observé au microscope électronique. Presse Méd. **52**:1230-1235.
43. Watson, D. H., and P. Wildy. 1963. Some serological properties of herpes virus particles studied with the electron microscope. Virology **21**:100-111.
44. Watson, D. H., P. Wildy, and W. C. Russell. 1964. Quantitative electron microscopic studies on the growth of herpes virus: the techniques of negative staining and ultramicrotomy. Virology **24**:523-538.
45. Zambernard, J., and M. Mizell. 1965. Viral particles of the frog renal adenocarcinoma: causative agent or passenger virus? I. Fine structure of primary tumors and subsequent intraocular transplants. Ann. N.Y. Acad. Sci. **126**:127-145.