REDUPLICATION OF NUCLEAR MEMBRANES IN TISSUE-CULTURE CELLS INFECTED WITH GUINEA-PIG CYTOMEGALOVIRUS

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Previous reports on the ultrastructure of virus-infected cells have established that viruses and the membranes of the cells may interact during virus replication and virus release.[1-3] Even though the cell and the virus are the only variants, it seems that the viral pathogenicity controls the cell reaction, as in the formation of a "coat" around virus particles and the elaboration of new membranes. Morgan and colleagues [1-3] have described nuclear-membrane reduplication in adenoviruses and herpes simplex virus infection. Similarly, multiple nuclear membranes and their reduplication have been observed in human and murine cytomegalovirus infections.[4-6] Stern and Friedman [4] and McGavran and Smith [7] have suggested that the membrane limiting the clusters of virus particles at the nuclear periphery are derived from the nuclear membranes. This report will describe another example of nuclear-membrane reduplication and its relationship to the virus particles within the nucleus of guinea-pig primary skin-muscle cells infected with guinea-pig cytomegalovirus. A possible function of these membranes will be suggested.

MATERIALS AND METHODS

Guinea-pig primary embryonic skin muscle in tissue culture was infected with guinea-pig cytomegalovirus, in the manner previously described.[8] The cells were fixed between the second and eighth day of virus infection, using a double fixation with glutaraldehyde and osmic acid, as previously reported.[9] The specimens were embedded in Araldite Fluka (Durcupan). Thin sections stained with lead citrate and uranyl acetate were examined in a Phillips 200 electron microscope.

OBSERVATIONS

Guinea-pig embryonic skin-muscle tissue-culture cells infected with guinea-pig cytomegalovirus undergo pathologic changes in both cytoplasm and nucleus. The nucleus contains a central inclusion, virus particles in various stages of development, and tubular structures. In addition, prominent multilamellated structures are present between the

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nuclear envelope and the intranuclear inclusion. These multilamellated structures are seen only in infected cells and are usually found in those cells having an intranuclear inclusion. They have never been observed in the center of the cell and are usually located at or near the nuclear membranes. These membranous structures are present in 2 different forms. One is needle-like in shape and the second is spherical or oval, resembling onion peel in structure. The needle-like structure (Fig. 1–3) appears to be composed of 4 parallel membranes. The inner membranes are more prominent and appear to be thicker. These 4 membranes form a tubular space occupied by amorphous material, apparently more electron-dense than the nucleoplasm, with which it seems to have continuity. The 2 thin external membranes are separated from the inner ones by a space 350–450 Å wide. One end of these needle-shaped structures appears to be continuous with the nuclear membranes, from which they arise perpendicularly or obliquely. When these structures are near virus particles, the outer thin membrane closest to the particles widens, balloons out, and encircles them (Fig. 2). This portion of widened membrane seems to join with both outer thin membranes of the needle-shaped structure. As virus particles pass through the membrane, it thickens at the site of contact. More difficult to recognize are the needle-shaped structures cut in cross section, as in Fig. 2, where two brief tracts of the thickened inner membranes are marked by Arrow A. The outer thin membrane appears to form a limiting vacuole that includes the virus particles. Clusters of virus particles sometimes appear disposed along membranes (Fig. 2) or are limited by a thin membrane in very close relation to the inner nuclear membrane (Fig. 3). In some instances a segment of needle-shaped structure appears to pass through the vacuole-limiting membrane (Fig. 3). This would suggest that in these cases the clusters of virus particles derive their limiting membrane from the outer thin membrane of the needle-shaped structures. More commonly, clusters of cytomegalovirus particles acquire a limiting membrane from the nuclear envelope as they approach the nuclear periphery. An example is seen in Fig. 4, where an invagination, probably from the inner nuclear membrane, surrounds a group of such particles.

The spherical or oval-shaped bodies are similarly located at the nuclear periphery, where their development can be observed. The nuclear membranes appear to twist around and curl up several times, forming ovoid bodies of different size (Fig. 5–7). These structures vary in the number of stratified membranes but appear to be in multiples of 2. The stratified membranes appear to maintain continuity as they reflect upon themselves. The stratification process seems to develop the spherical or oval-shaped bodies on the nuclear side of the nuclear envelope rather

than on its cytoplasmic side. The multilamellated body (Fig. 8) is almost completely separated from the nuclear envelope with only a brief tract of a double membrane interposed between the body and the nuclear membranes. This ovoid, almost rectangular-shaped structure is composed of 4 layers, with the 2 middle ones thicker than the outer 2. Only in a few instances were virus particles found near these spherical or oval-shaped bodies.

Discussion

Guinea-pig embryonic tissue culture infected with guinea-pig cytomegalovirus (CMV) simultaneously forms 2 types of multilamellated structures within the nucleus as the intranuclear inclusion develops. The first type is a needle-like structure frequently seen in close association with virus particles. The second type, spherical or oval and layered like onion peel in structure, is rarely found near nuclear virus particles. The two structures are similar in that (1) their inner lamellas appear thicker than their outer lamellas, (2) they appear to arise from the nuclear envelope, and (3) they are found within the nuclei of infected cells.

Morgan and his associates described reduplication and multiplication of nuclear membranes in HeLa cells infected with herpes simplex virus and 9 of the first 10 types of adenoviruses.[2,3] It is interesting that the reduplication of membranes in guinea-pig CMV infection appears as multiples of 2, as Morgan *et al.* described in Type 10 adenovirus infection.

Reduplication or multiplication of nuclear membranes has also been described in both human and murine CMV-infected cells.[4–6] The needle-like multilamellated structure described in guinea-pig CMV-infected cells is different from those seen in the human and murine CMV infection. The former structures not only represent a basic and important type of cellular response to infection by viral agents, as postulated by Gregg and Morgan,[2] but we think they have a direct relationship to virus release from the nucleus. These needle-like structures appear to "reach out" toward a group of nuclear virus particles. After a group of virus particles has been surrounded by a thin membrane, the needle-like structure appears to retract toward the nuclear envelope. Thus the needle-like structure becomes shorter and disappears, as the cluster of virus particles comes closer to the nuclear envelope. The human, murine, and guinea-pig strains of CMV and the herpes simplex virus have all been demonstrated by electron microscopy to acquire a second coat as the nuclear virus particles pass through the inner nuclear membrane.[3,6–9] Those virus particles present within the membranes of these mutilamellated needle-like structures are seen to have already acquired their second

coat. This would suggest these are morphologically "mature" virus particles. The needle-like structures provide a method for the release of the mature virus particles into the cytoplasm. Kasten, Wright, and McAllister described unusual needle-like crystals or folds in or over the nucleus of human CMV-infected cells observed by phase-contrast and time-lapse cinematography.[10] The relationship of these 2 types of needle-like structures is not yet known.

Most commonly, an invagination of the inner nuclear membrane occurs to allow release of guinea-pig CMV from the nucleus, a mechanism similar to that described above for the needle-like structure and also to the method of human CMV release suggested by McGavran and Smith.[7]

The ovoid multilamellated structure present in guinea-pig CMV is similar to a "nuclear membrane which has assumed a multiple appearance," described in both murine CMV infection [6] and adenovirus.[1] These ovoid bodies are present during the various stages of virus development but are rarely in direct association with virus particles. It seems, therefore, that they are not connected with virus maturation. We are unable to offer a hypothesis for the function of these structures.

It is particularly interesting that the nuclear-membrane reduplication and multiplication described thus far have been in association with DNA viruses that proliferate within the nucleus. The multiplication or reduplication of these nuclear membranes appear to result from the type of virus infecting the cell rather than the host-cell response because these changes have occurred in different tissue-culture cell lines and also in studies in vivo on murine CMV-infected mouse liver.

The reduplication of nuclear membranes in adenovirus infection may be associated with the burst type of release of large crystals of virus particles from the cell nucleus since adenovirus particles do not acquire a coat, as does the herpesviridae group.[11] Herpes simplex virus that has acquired a coat from these membranes has an antigenicity different from that of nuclear virus particles without a coat.[12] Further studies will be necessary to determine if those murine, guinea pig, and human CMV particles that have a coat also differ in antigenicity from the nuclear virus particles without the coat.

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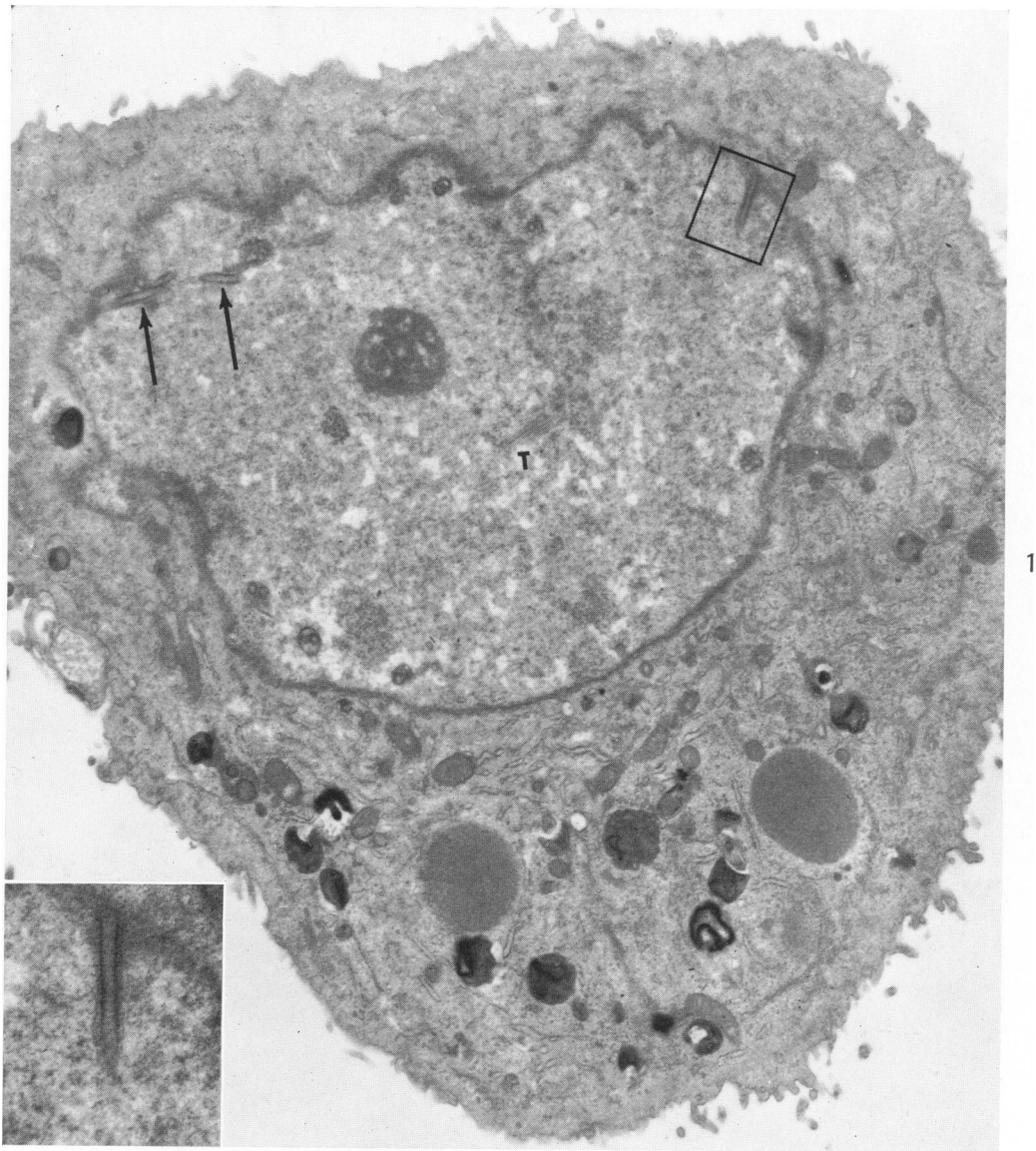
[*Illustrations follow*]

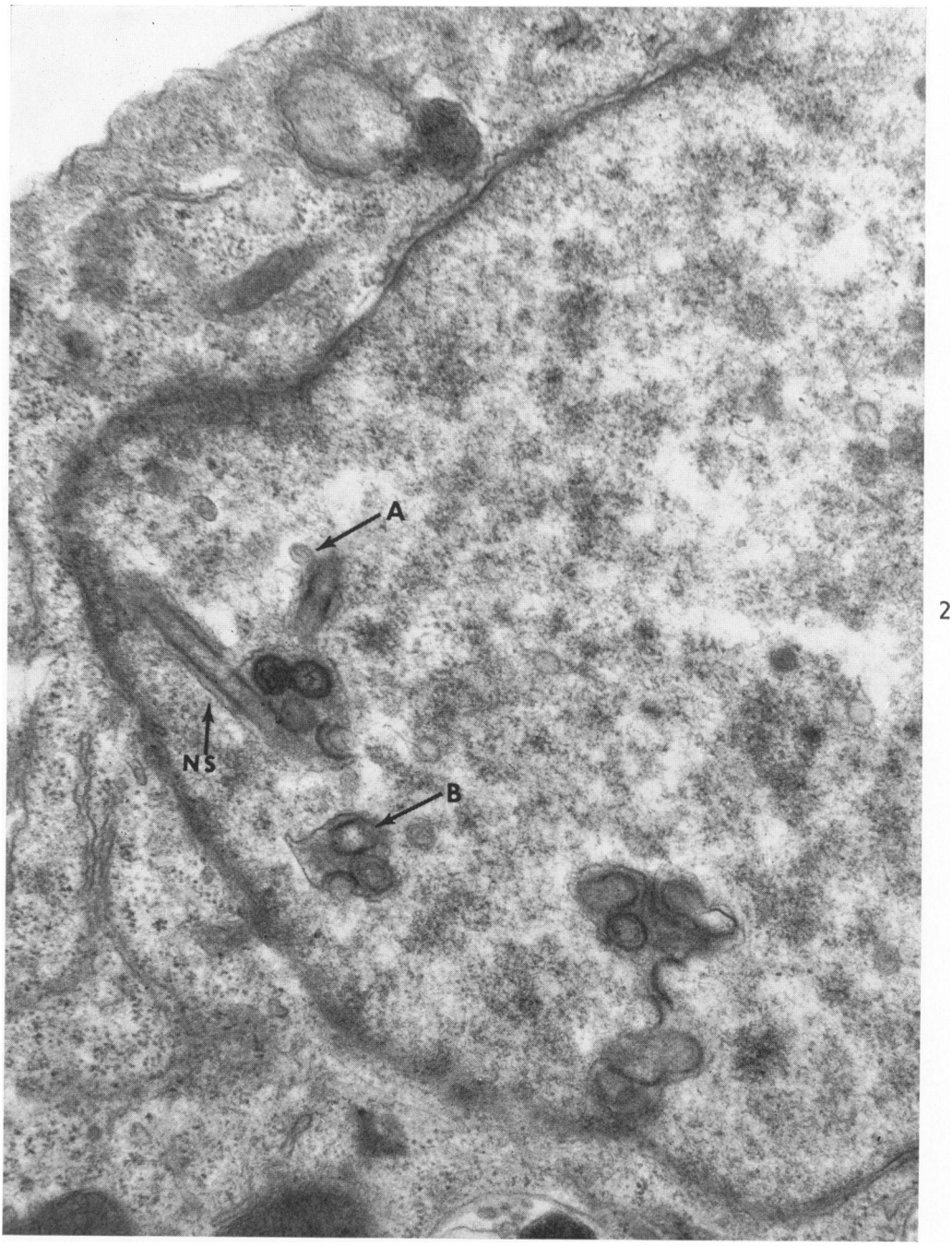
Legends for Figures

FIG. 1. Guinea-pig embryonic skin-muscle cell infected with GPCMV. Clusters of virus particles are present along nuclear periphery, around or inside membrane-limited vacuoles. Aggregate of tubular structures (T) is located at center of nucleus. At top left and right portion of nuclear membrane are multilamellated tubular structures (arrows and insert). Those at left are in close relation to virus particles. × 11,400. Inset shows continuity of nuclear membranes with those of lamellated needle-shaped structure. This one appears to be composed of 2 inner, parallel thick membranes, limiting space occupied by amorphous material, and by 2 outer thin membranes. One end seems to continue with nuclear membranes; other end appears to open toward cytoplasm. × 30,700.



1

FIG. 2. Composition of needle-shaped structure (NS) is more distinct. Even with some discontinuity, it appears to be derived from nuclear membranes. Two inner and outer membranes are parallel to each other, and outer ones are prominent enough to enable one to follow their course. Thin membrane encircles group of mature virus particles, thickening where virus particle contacts it. At Arrow A virus particle is in contact with outer thin membrane of needle-like structure. At Arrow B thin outer membrane of needle-like structure in tangential section is widened. Two virus particles appear to be entering this structure. Cluster of virus particles (lower right) is disposed along tortuous, discontinued membrane which differs in thickness throughout its course. At top of cluster is what appears to be thin membrane. × 27,000.



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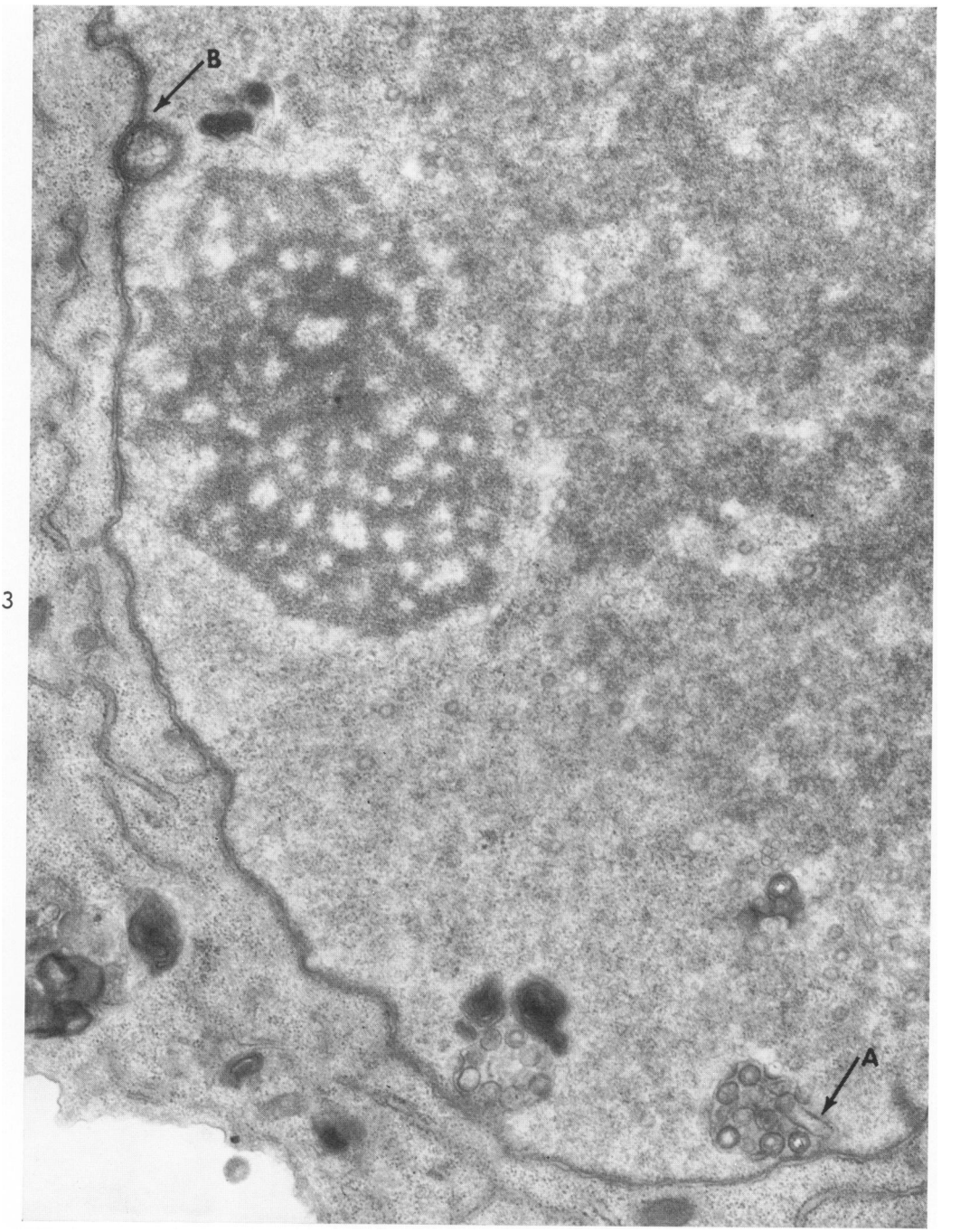

FIG. 3. Two clumps of virus particles limited by thin membrane in close relation to inner nuclear membrane appear at bottom. Short segment of needle-shaped structure (Arrow A) passes through vacuole-limiting membrane. At Arrow B is what may be beginning of nuclear-membrane reduplication, with new membranes assuming spherical shape delimiting portion of nucleoplasm. × 29,700.

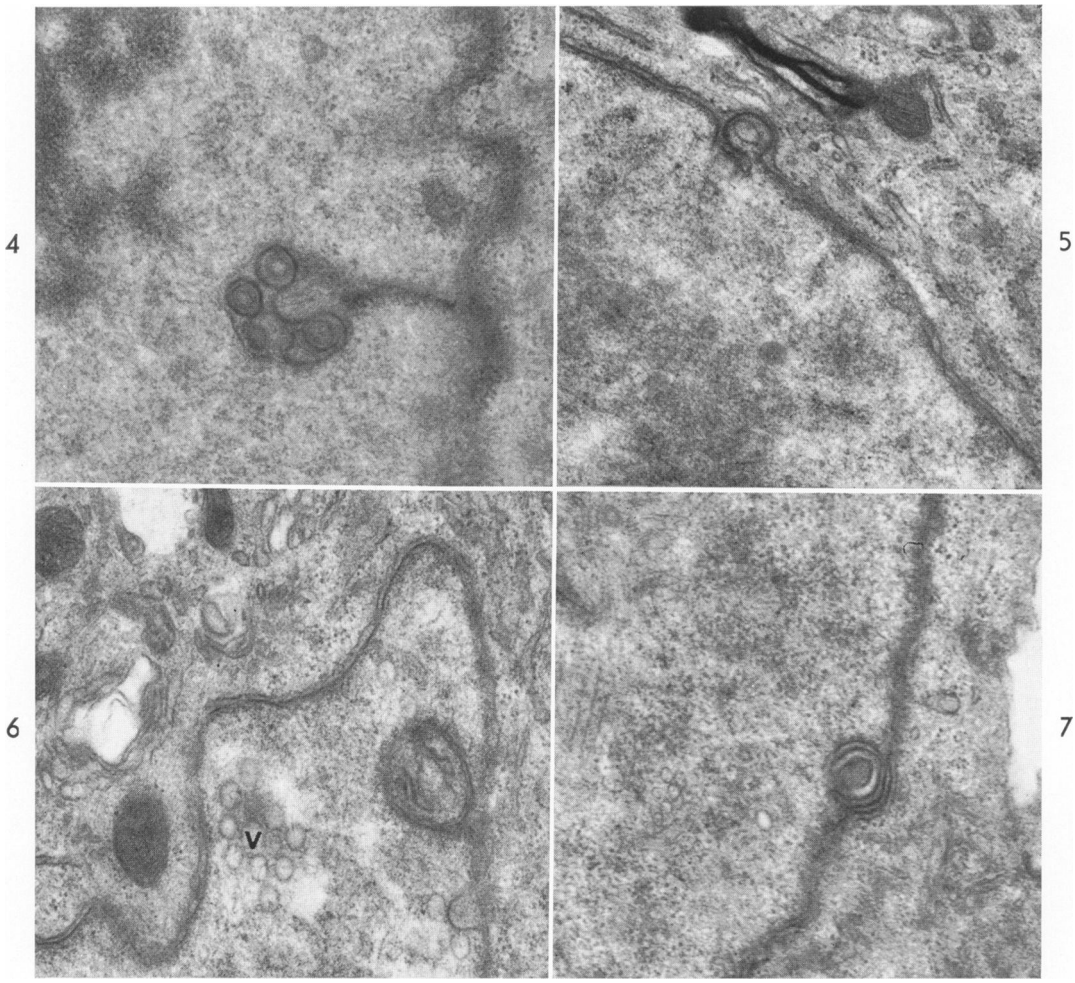

FIG. 4. Limiting membrane of group of mature virus particles appears to be in continuity with nuclear envelope by means of invagination of inner nuclear membrane. × 35,000.

FIG. 5 and 6. Two different stages of development of nuclear-membrane reduplication. FIG. 5. Nuclear envelope twists around, forming ovoid body composed of stratification of several lamellas, usually in multiples of 2. FIG. 6. Group of single-ring virus particles (V) occupies rarefied area of nucleus. × 43,000.

FIG. 7. Well-developed body, still attached to nuclear membranes, segregates some nucleoplasm in center of body. No virus is noted in vicinity of these early stages. Membranes are usually continuous where they reflect upon themselves. Reduplicated membranes are more numerous on cytoplasmic side of body. × 25,800.

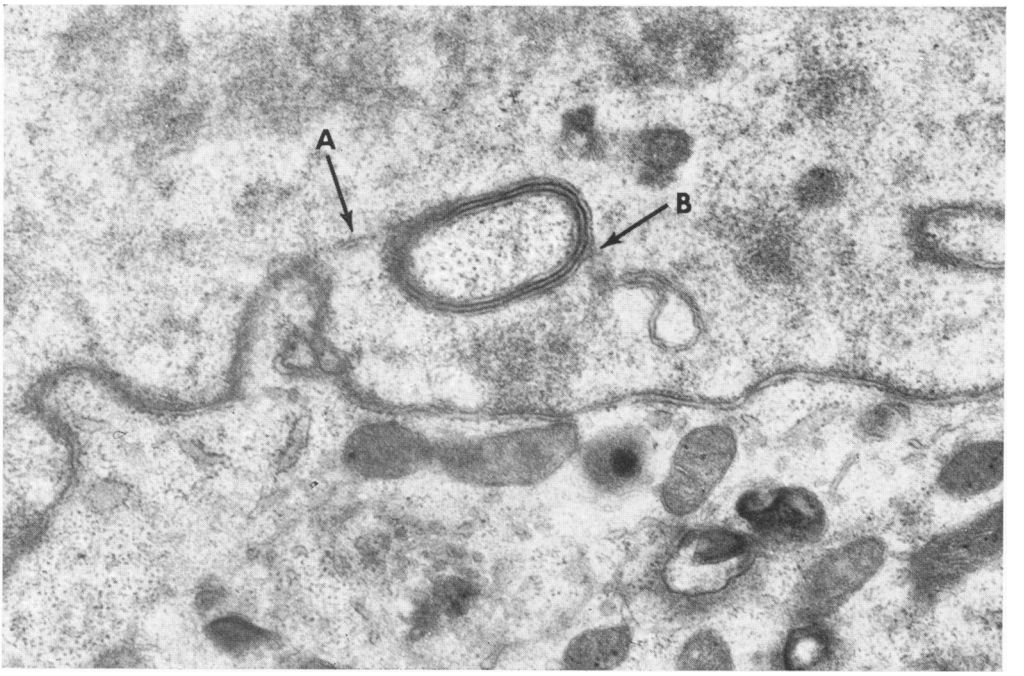

FIG. 8. Ovoid multilamellated body of greater dimensions is almost completely separated from nuclear envelope and is deeper in nucleoplasm and is composed of 4 layers of lamellas. Two middle lamellas appear to be thicker than the other 2. Brief tract of double membrane is interposed between nuclear envelope and multilamellated body (Arrow A). Nearby is another double-membrane-limited vacuole that seems likely to be derived from 2 outermost membranes (Arrow B) of ovoid body. × 32,000.