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NOTES

Electron Microscopy of the Development of Rubella Virus in BHK-21 Cells

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Consideration of the morphology of rubella virus has varied widely, but recent evidence indicates that the virion is spherical with a diameter of 42 to 75 nm (4, 8, 9; T. Hobbins and K. Smith, Bacteriol. Proc., p. 181, 1968). However, the mode of maturation of the virus has not been described; consequently, classification has remained in doubt. The capacity of BHK-21 cells grown in suspension to support the production of high titers of rubella virus (12) and its hemagglutinin (7) prompted us to explore rubella morphogenesis in this system.

The conditions for culture of BHK-21 13S cells in suspension using serum-free medium, the method of infection (rubella strain RA 27/3(13) adapted to BHK-21 cells by approximately 38 passages), and the techniques for assay of infectivity and hemagglutinin have been described (7). The development of infectivity and hemagglutinin is shown in Fig. 1. Cells were taken from suspension cultures 4 hr after infection and at daily intervals for 7 days. They were centrifuged (630 × *g* for 5 min) and the undisturbed cell pellet was fixed for 30 min in glutaraldehyde; it was then postfixed in osmium tetroxide and embedded in an Araldite-Epon mixture. Sections were stained with lead citrate and uranyl acetate.

Virus particles were observed budding from intracytoplasmic and marginal membranes for the first time in cells harvested 2 days postinoculation, at which time cell morphology appeared normal (Fig. 2, 3). The largest number of particles associated with cells occurred on day 4. This and later harvests contained increasing numbers of degenerating cells and increasing amounts of cell debris intermixed with virus particles. Virus budding from marginal membranes was more prominent than budding from intracytoplasmic membranes into cisternae of the endoplasmic reticulum (Fig. 4, 5). No morphological change preceded virus budding. Free particles accumulated within cisternae and within protected intercellular spaces. Nuclear changes were not evident, nor were crystalline inclusions (9, 10).

Virus particles were round or oval and consisted of an electron-dense core (mean diameter, 28 nm) surrounded by a rather electron-lucid halo (Fig. 6). Virion diameter was 61 nm (range 56 to 73 nm; mean from measurement of 100 particles; calibration via 54,864 line per inch diffraction grating replica). These particles were never observed in control BKH-21/13S cells, nor in BHK-21 cells (American Type Culture Collection CCL 10) used extensively in this laboratory for other virus studies. The latent virus of this cell

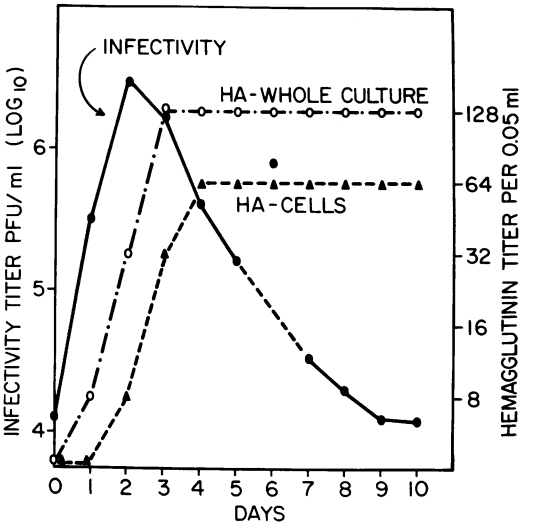

FIG. 1. *Growth curve of rubella virus in BHK-21 cells maintained in suspension in serum-free medium. The details of the infection of these cells and the virus assay have been described (7). Infectivity of whole-culture samples was determined in BHK-21/WI-2 cell monolayers. HA, hemagglutinin.*

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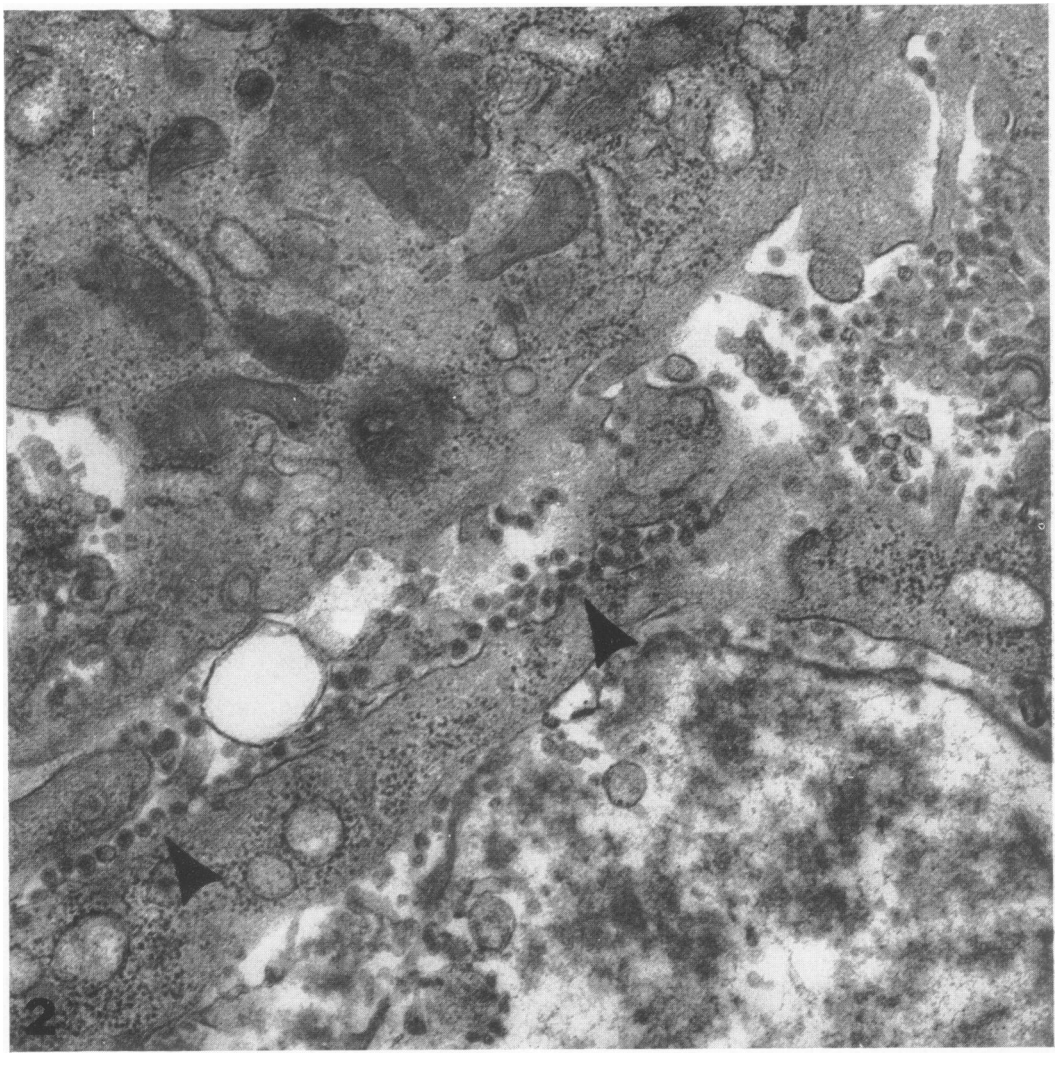

FIG. 2. *BHK-21 cell culture 4 days after infection with rubella virus. Virions are budding from marginal membranes and are accumulating within intercellular space. These virus particles were first seen 3 days after rubella infection, reached their greatest numbers by day 4, and were associated with cell destruction in later harvests. They were never seen in control cells.* × *40,000.*

line was observed, however, in moderate numbers in infected and control cultures (1, 3). This latent virus is 85 to 100 nm in diameter and morphologically distinguishable from the 61-nm virus described (Fig. 7).

The appearance of particles with uniform morphology in rubella-infected BHK-21 cells coincident with the development of infectivity suggests that the particles were the virus inoculated. These observations support the observations of Holmes and Warburton (8), who found identical particles extracellularly in the supernatant fluid of cultures of BHK-21/WI-2 cells infected with two other rubella virus strains (58459/P from Australia, and "Putnam" from the U.S.). Moreover, recent negative-contrast observations of rubella virus, especially those of Hobbins and Smith (Bacteriol. Proc., p. 181, 1968), are entirely consonant with the rubella morphology reported here.

The maturation of rubella by budding from membranes generally resembles that of several other ribonucleic acid (RNA) viruses: murine leukemia viruses (5), avian leukosis viruses (13), infectious bronchitis viruses (2), and some larger

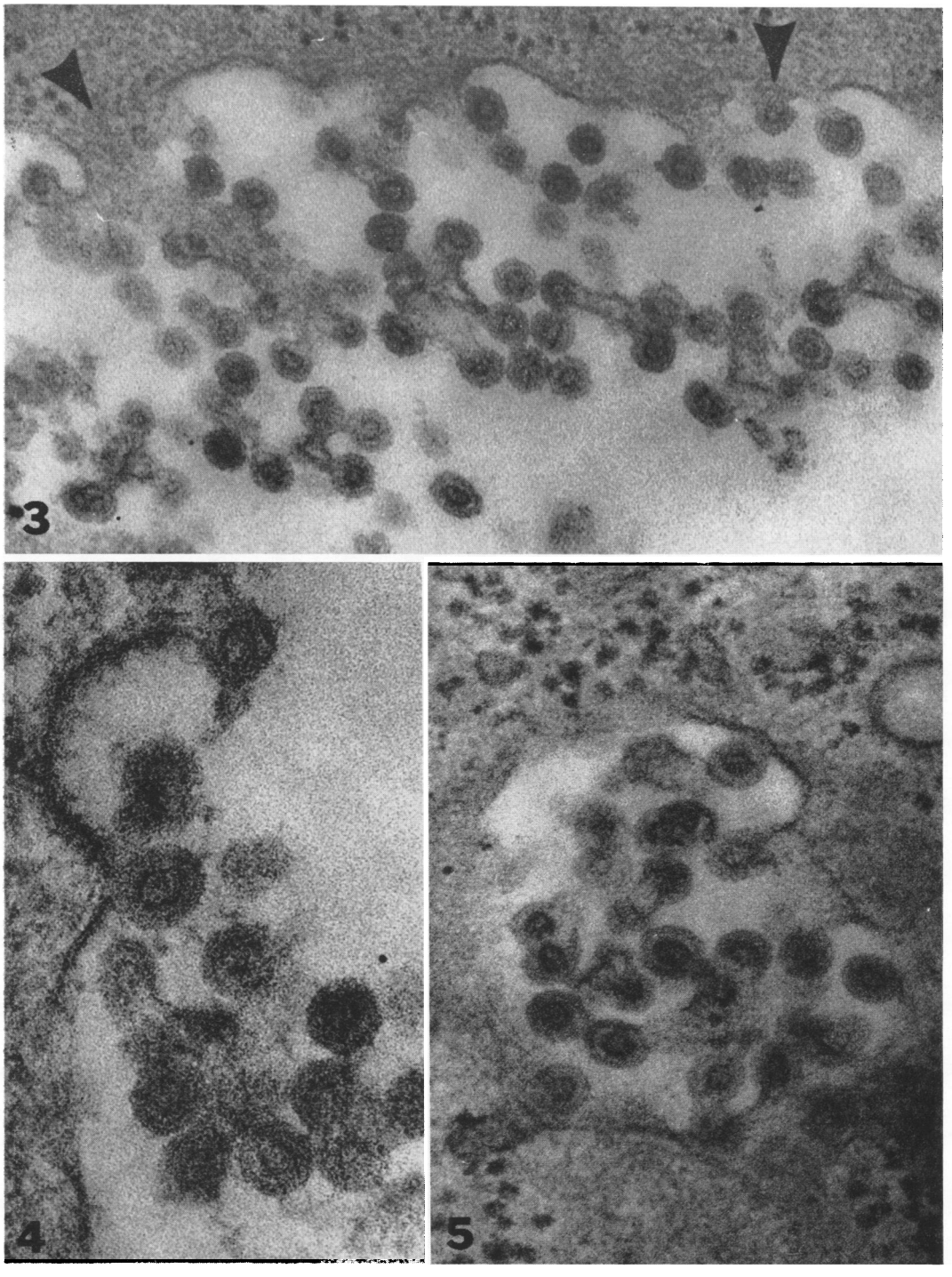

FIG. 3. *Rubella virions budding from marginal membrane of BHK-21 cell individually and in groups associated with cell extrusions (arrows).* × *108,000.*

FIG. 4. *Higher magnification of rubella virions budding from marginal membrane. Membrane appears contiguous with outer layer of virus particle.* × *178,000.*

FIG. 5. *Rubella virus budding into and accumulating within a cytoplasmic vacuole. The number of particles observed within such cisternae were fewer than those found in association with marginal membranes.* × *148,000.*

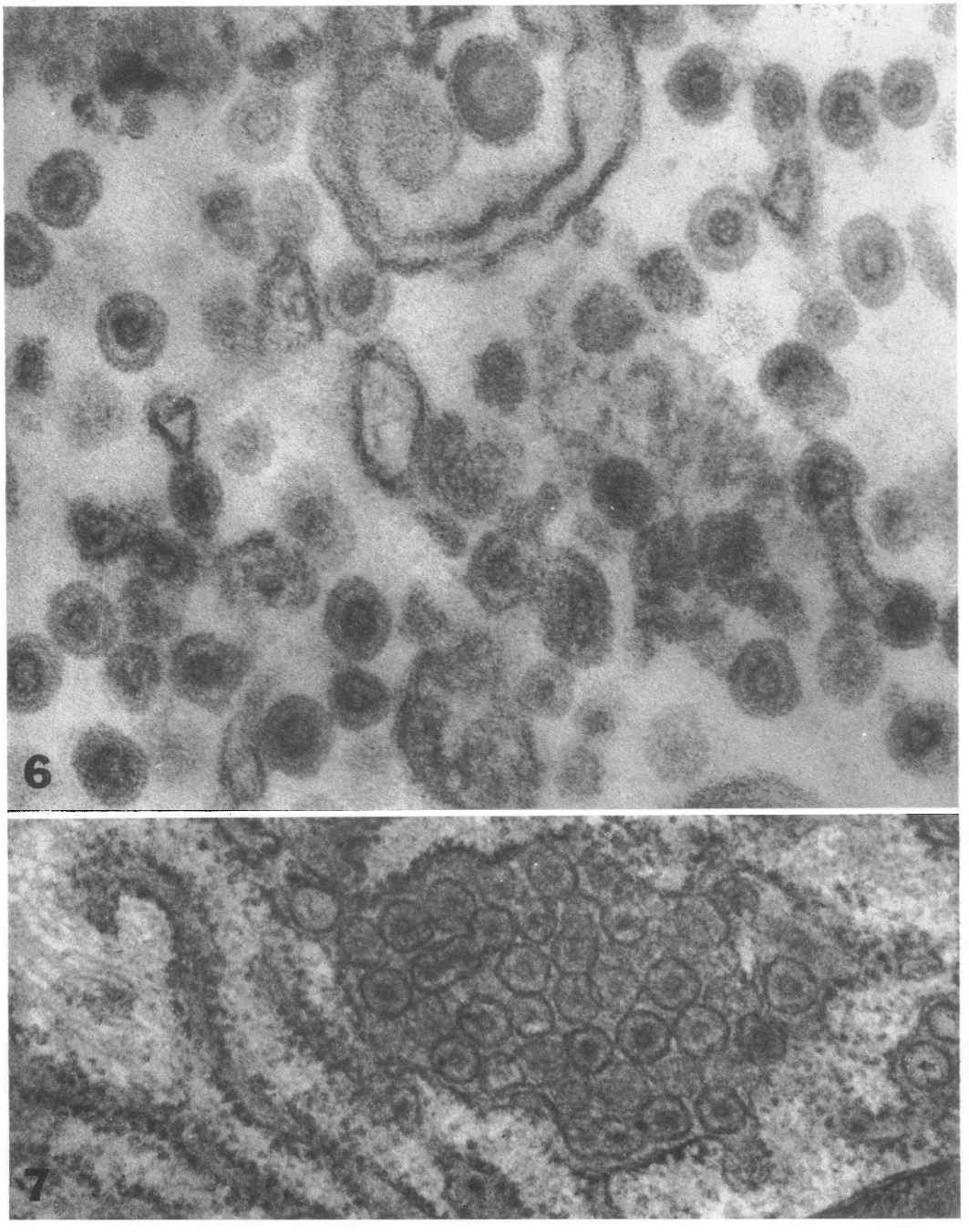

FIG. 6. *Extracellular virions associated with cell debris. Virion diameter was 61 nm and the rather electron-dense core was 28 nm in diameter.* × *192,000.*

FIG. 7. *Latent virus of BHK-21 cells within the cisterna of the endoplasmic reticulum of an uninfected cell. These 85 to 100-nm particles have been found in many BHK cell clones. They do not appear to interfere with the replication of other viruses, including rubella; both viruses were observed in some cells.* × *65,000.*

arboviruses [e.g., California group arboviruses (11)]. In size and fine structure, rubella resembles lactic dehydrogenase virus (6). However, virion size, morphology, mode of maturation, and biological properties taken together are not identical to those of any other known virus and do not serve to place rubella satisfactorily within present classification schemes. Consideration of rubella as a member of the "arbovirus group," a family with remarkably heterogeneous physical properties, does little at this time toward establishment of meaningful taxonomy for small, ether-sensitive, viruses (8; D. Carver and P. Marcus, Bacteriol. Proc., p. 181, 1968).

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