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NOTES

Structure of Virus Particles Extracted from a Burkitt Lymphoma Cell Line

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Burkitt lymphoma has been the object of intense interest in recent years because of epidemiological evidence suggesting that it may be caused by an arthropod-borne virus (B. Burkitt, p. 615, *in* Viruses, Nucleic Acids, and Cancer, Symp. Fundamental Cancer Res., 17th, The Williams & Wilkins Co., Baltimore, 1963). The presence of particle resembling herpesvirus in cultured Burkitt lymphoma cells (M. A. Epstein et al., J. Exptl. Med. **121**:761, 1965; S. Stewart et al., J. Natl. Cancer Inst. **34**:319, 1965; A. Rabson et al., Intern. J. Cancer **1**:89, 1966; I. Toshima et al., Cancer Res., *in press*) further suggests viral involvement. The structure and symmetry of the virus particles in the EB3 cell line derived from Burkitt lymphoma has been demonstrated by the negative-staining technique (K. Hummeler, G. Henle, and W. Henle, J. Bacteriol. **91**:1366, 1966; I. Toplin and G. Schidlovsky, Science **152**:1084, 1966). However, the total number of capsomeres of the virus has not been unequivocally established.

We recently derived a clonally selected Burkitt lymphoma cell line, P3HR-1, which produces much more herpes-type virus than the P3J parent culture or other clonally derived lines (Y. Hinuma et al., *in preparation*). The fine structure and symmetry of the capsid of virus particles extracted from the P3HR-1 cells have been studied by electron microscopy by use of the negative-staining technique.

Procedures for cultivation of P3HR-1 cells and for extraction of the virus particles will be reported elsewhere (Y. Hinuma et al., J. Virol., *in press*). Briefly, the packed cells (3×10^8 to 3×10^9) were suspended in distilled water (1 to 5 ml) and disrupted with sonic oscillation. The virus particles were sedimented at 53,000 $\times$ *g* for 60 min after removal of cell debris by centrifugation at 3,000 $\times$ *g* for 20 min. In some studies, this differential centrifugation was repeated once. The final pellet was suspended in 0.5 to 2 ml of distilled water. Usually the samples were further diluted from 1:2 to 1:10 with distilled water before examination. For negative staining, the viral sample was mixed with an equal volume of 2% phosphotungstic acid (PTA), *p*H 7.0, containing 0.2% sucrose. One drop of this mixture was placed on a carbon-coated collodion-covered grid, and the excess fluid was drained with filter paper. The specimens were examined immediately and photographed with a Hitachi HU-11A electron microscope at a basic magnification of 30,000 times.

Representative virus particles in the preparations are shown in Fig. 1, 2, 3, 6 and 7. The virus was most frequently viewed as single particles, but occasionally groups of them were seen, as demonstrated in Fig. 1 and 2. Particles of different appearance, i.e., "full," "empty," and "partially empty" capsids, are illustrated. Relatively few isolated particles were contained within an envelope (Fig. 3). The envelopes varied considerably in size, shape, and structural integrity.

The particles appeared to be polygonal; most were hexagonal in shape. This was particularly evident when the capsid was partially filled with PTA (Fig. 1 and 2). The overall diameter of the capsid was approximately 1,100 A, and that of the central region was approximately 820 A. The capsids had symmetrically arranged capsomeres projecting from their surfaces. The capsomeres appeared to be hollow, elongated, polygonal prisms; they were approximately 130 A in length and approximately 100 A in width. These were separated from each other by approximately 30 A at their base, this distance increasing peripherally. Most of the capsomeres appeared to be hexagonal in cross section, but a few appeared to be pentagonal both in situ in intact capsids and in the disintegrated ones (Fig. 4 and 5). The axial hole in the center of the capsomere was approximately 40 A in diameter, but it is not possible to tell whether these are circular or polygonal in cross-section. When adequately resolved, the capsomeres at the circumference were found to number 23 to 25.

Many of the capsomeres were surrounded by

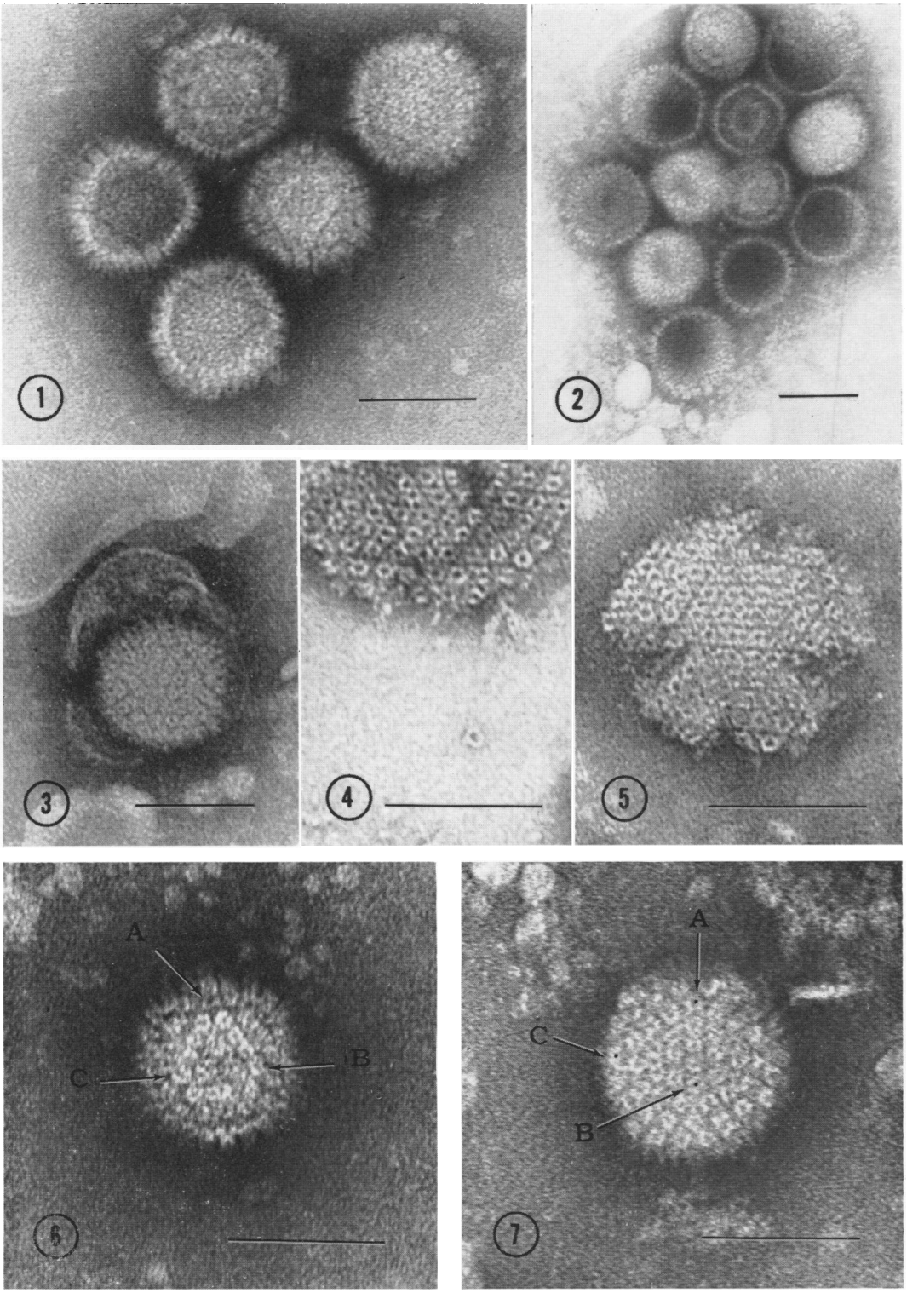

FIG. 1–7. *Electron micrographs of negatively stained virus particles in the P3HR-1 Burkitt lymphoma cell line. The bar in each figure represents 1,000 A.*

FIG. 1 and 2. *Two groups of unenveloped virus particles of different appearance.*

FIG. 3. *Full capsid enclosed in an envelope. Hexagonal packing of capsomers is seen on the surface of capsid.*

FIG. 4 and 5. *Two disintegrated capsids. Polygonal cross-section of capsomeres and hexagonal packing of them are clearly visible. A free-lying capsomere released by disintegration of capsid is seen in the lower part of Fig. 4.*

FIG. 6 and 7. *Two capsids showing an equilateral triangular facet, indicated by A, B, and C, with five capsomeres on its base (A-B), respectively. Four capsomeres (A and B in each figure) are seen surrounded by five neighbors and situated on an axis of fivefold symmetry, respectively.*

six neighbors, but a few of the capsomeres were surrounded by five others (Fig. 6 and 7), suggesting that they were situated on axes of fivefold symmetry. Between these points of fivefold symmetry, three capsomeres, each surrounded by six others, were seen. Equilateral triangular facets indicating a regular polyhedron were a characteristic feature of many of the particles (Fig. 1, 6, and 7). The symmetrical arrangement of the capsomeres appeared to retain their configuration after disruption of the capsid (Fig. 4 and 5).

The evidence derived from these electron micrographs strongly suggests that the particles in the P3HR-1 cell line consist of icosahedral capsids with 162 capsomeres, i.e., 5 capsomeres on an edge (R. Horne and P. Wildy, Virology **15**:348, 1961).

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