JOURNAL OF BACTERIOLOGY, Mar., 1966
Vol. 91, No. 3
Copyright © 1966 American Society for Microbiology
Printed in U.S.A.

Fine Structure of a Virus in Cultured Lymphoblasts from Burkitt Lymphoma

KLAUS HUMMELER,[1] GERTRUDE HENLE, AND WERNER HENLE[2]

Virus Laboratories, Children's Hospital of Philadelphia, and the School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Received for publication 1 November 1965

The geographic distribution of Burkitt's lymphoma in Africa suggested that the neoplasm may be caused by an infectious agent which is transmitted by an arthropod vector (D. Burkitt, Brit. Med. J. **2**:1023, 1962). It was of interest, therefore, when electron microscopic studies revealed the presence of virus particles in five lines of lymphoblasts isolated from such tumors and maintained in continuous culture (Epstein et al., Wistar Inst. Symp. Monograph No. 4, p. 69, 1965; Stewart et al., J. Natl. Cancer Inst. **34**:319, 1965; Rabson et al., Intern. J. Cancer, *in press*). Depending upon the line, from about 0.5 to nearly 10% of the cells in thin sections were found to harbor the agent. The infected cells showed varying degrees of morphological alterations, and virus was also found associated with cellular debris. The virus particles were often hexagonal in shape with a single limiting membrane. Many of these structures appeared to be empty, but in some a nucleoid was evident. Occasionally particles were seen with a double membrane surrounding the dense nucleoid. These observations made it likely that the virus belongs to the herpes group, but, thus far, attempts to transmit it to a variety of hosts have failed (Epstein et al., J. Exptl. Med. **121**:761, 1965).

Since thin sections provide only limited morphological detail, more precise information as to the fine structure and symmetry of the virus was sought by the negative-contrast method. Because of the relative paucity of virus particles, it was thought to be difficult and cumbersome to separate them from disrupted cells in sufficiently pure and concentrated suspension for a meaningful study. For this reason, the method of negative staining of cell-associated virus particles described by Parsons (J. Cell Biol. **16**:620, 1963) was utilized. The EB3 line of lymphoblasts was used because it was noted to contain the greatest number of infected cells, i.e., from 5 to 10% of the total population (Epstein, *personal communication*). The cultures were grown on medium no. 1629 supplemented by 10% fetal calf serum. Lots of about 6×10^6 cells were sedimented in conical centrifuge tubes at $600 \times g$ for 10 min, and, after removal of the supernatant fluid, a dissecting needle was introduced into the pellets and withdrawn. The cell material adhering to the needle was floated off onto the surface of a solution of 4% phosphotungstate and 0.4% sucrose at *p*H 7.0 (PTA). The PTA solution was contained in the well of a depression slide, which was illuminated from below and viewed under a dissecting microscope. The floating cell material was touched lightly with a carbon-coated specimen grid, and the excess fluid was removed by pressing the grid gently onto filter paper. The grid, while still moist, was inserted into a Siemens Elmiskop I electron microscope. The specimens were viewed and photographed at a magnification of 40,000 ×.

Representative virus particles found in the preparations are shown in Fig. 1 to 7. The particles were most frequently single, but occasionally groups of them were seen. These groups were enclosed in a membranous structure as demonstrated in Fig. 1, which also shows most of the

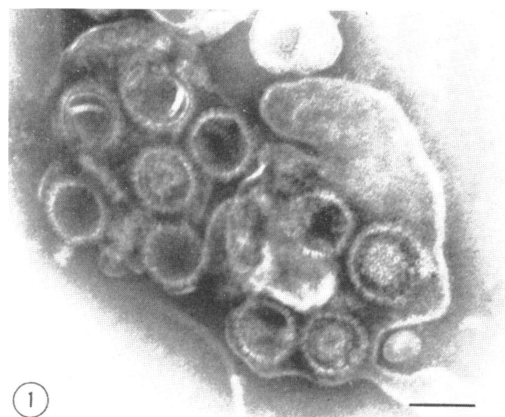

FIG. 1. *Two groups of virus particles, each enclosed in a membranous structure. Bar represents 0.1 μ.*

[1] Career Development Award K3-Hd-22708.
[2] Career Award 5-K6-AI-22,683.

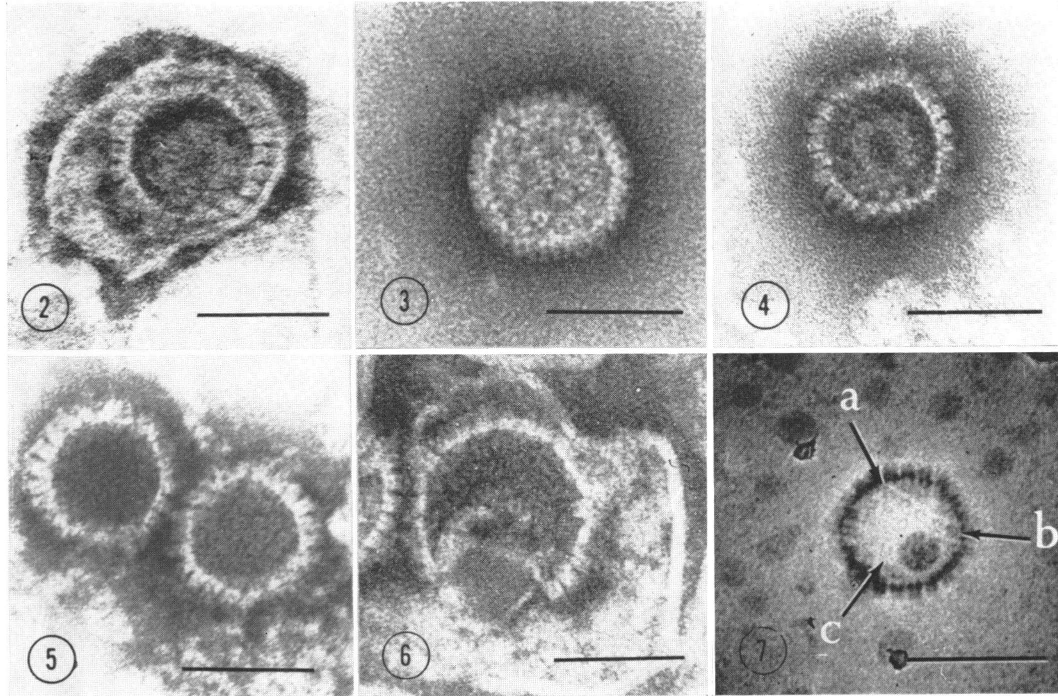

FIG. 2. *Virus particle enclosed in an envelope representing the second limiting membrane. Bar represents 0.1 μ.*
FIG. 3. *Virus particle showing least penetration of phosphotungstate with capsomers in disarrangement. Bar represents 0.1 μ.*
FIG. 4. *Virus particle with ringlike arrangement of capsomers. Bar represents 0.1 μ.*
FIG. 5. *Two empty virus capsids. Bar represents 0.1 μ.*
FIG. 6. *Disintegrated virus capsid. Bar represents 0.1 μ.*
FIG. 7. *Empty capsid with orderly arrangement of capsomers showing fivefold symmetry. Reverse negative image. Bar represents 0.1 μ.*

features observed with individual particles. It is noteworthy that not a single "complete" virus particle was found.

The negative-contrast preparations showed that PTA penetrated the capsid readily, indicating the complete or partial absence of a core (Wildy et al., Virology **12**:204, 1960). The capsids revealed, as a rule, disarrangement of the capsomers. This was true also for capsids which were enclosed by an envelope (Fig. 2). Various degrees of incompleteness are demonstrated in Fig. 3 to 6. Although the capsid in Fig. 3 showed minimal penetration of PTA, the capsomers, nevertheless, were disarranged, preventing a reliable evaluation of the basic symmetry of their arrangement. In Fig. 4 the capsomers formed a ringlike structure, and they were barely visible in the capsids in Fig. 5, denoting the complete absence of a core. A disintegrating capsid is shown in Fig. 6.

The particles appeared to be polygonal, mostly hexagonal, in shape. Their overall diameter was 1,100 A, and that of the central region, 750 A. The capsomers, clearly visible on the periphery of the capsid, measured 125 A in length, with a central hole of 40 to 50 A in width. The mean spacing distance between them was 125 A as measured from center to center. When adequately resolved, the capsomers at the circumference were found to number 23 or 24.

Although the absence of a core obscured the arrangement of the capsomers, in a few capsids an orderly arrangement was faintly discernible. Such a particle is presented in Fig. 7 in a reverse negative image. It shows a triangular facet, indicated by a, b, and c, with five capsomers on its base (a–b), suggesting a fivefold symmetry and a total number of 162 subunits (Horne and Wildy, Virology **15**:348, 1961).

On the basis of the morphological findings presented, the virus found in Burkitt lymphoma cells is clearly a member of the herpes group, confirming findings in thin sections (Epstein et al., Wistar Inst. Symp. Monograph No. 4, p. 69, 1965; Stewart et al., J. Natl. Cancer Inst. **34**:319,

1965; Rabson et al., Intern. J. Cancer, *in press*). Although similar to herpes simplex virus, several features seem to relate it more closely to cytomegalovirus, which belongs in this group on the basis of its size and configuration as described by K. O. Smith and L. Rasmussen (J. Bacteriol. **85**:1319, 1963). These authors observed not a single "complete" capsid among a much larger number of virus particles than that obtainable by the method used here. They calculated the ratio of infectious to noninfectious particles to be in the order of 1 in 10^6 to 10^7. A similar ratio appears to prevail among the virus particles in the lymphoma cell lines, which could well explain the up to now unsuccessful attempts at isolation of this agent in a variety of host systems. Application of direct and indirect immunofluorescence techniques to Burkitt lymphoma cells has ruled out herpes simplex and varicella viruses, and possibly also cytomegalovirus (G. Henle and W. Henle, J. Bacteriol. **91**:1248, 1966). It is conceivable that the described virus particles may thus turn out to be another hitherto unknown member of the herpes group.

Addendum in Proof

Recently, several complete virus particles were seen which confirmed the fivefold symmetry.

This investigation was supported by Public Health Service grants AI-04911 and CA-04568 from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute, respectively.