Morphology of Echovirus 22

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Purified preparations of echovirus 22 were examined in the electron microscope. The virus was found to possess 32 capsomers arranged at the vertices of either a pentakis dodecahedron or a rhombic triacontahedron. The size of the virions ranges from 22×10^{-3} to $32 \times 10^{-3} \mu\text{m}$ with a mean of $27 \times 10^{-3} \mu\text{m}$ and a mode of $28 \times 10^{-3} \mu\text{m}$.

The picornavirus group was delineated in part upon the basis of the size of the virion. The virions of each serotype are expected to be between 15×10^{-3} and $30 \times 10^{-3} \mu\text{m}$ in diameter (3). The methods used historically to determine the size of prototypes of these viruses are legion. Jamison and Mayor (1) have examined the size of several picornaviruses by directly measuring virions in electron micrographs of purified virus preparations. They tentatively suggested two groups of picornaviruses based upon the size of the virion. This note represents an extension of the effort to examine the variability in size of the virion of members of the picornavirus group.

A stock echovirus 22 (Harris strain) was obtained from the Research Reference Reagents Branch (RRRB) of the National Institutes of Health, Bethesda, Md. Passages of virus in African green monkey kidney cells were made as needed. Passages 2 through 5 carried out in this laboratory were utilized for the experiments described. Samples were titrated and the identity of this virus was then confirmed by neutralization with reference horse antiserum (echovirus 22, Harris strain) obtained from the RRRB.

Stock virus suspensions were purified by centrifugation in preformed CsCl density gradients at 36,000 rev/min for 4.5 hr in a Spinco model L-2 ultracentrifuge. Virus bands were pooled and dialyzed overnight. Multiple passages of echovirus 22 were purified by this procedure.

Specimens were prepared for electron microscopy by simply placing a drop of virus-containing dialysate onto a parlodium-covered copper microscope grid. After 2 min, the grids were washed in a stream of distilled water, blotted, and then a drop of 2% aqueous phosphotungstic acid (PTA, pH 7.0) was added. After 20 sec, the PTA was removed by blotting.

Specimens were examined in the Phillips 200 electron microscope at an instrumental magnifi-

cation of approximately 35,800. Micrographs were obtained and the diameters of selected virions were determined by direct measurement of the images on the photographic plates by using a measuring ocular equipped with a metric scale. Criteria used to select virions for measurement have been explained elsewhere (1). Immediately after completion of a series of electron micrographs, the electron microscope was calibrated by using a carbon grating replica (2,160 lines/mm; Earnest Fullam, Inc., Schenectady, N.Y.).

The data demonstrated in Fig. 1 were obtained from one experiment and are representative of all data gathered during the course of many different experiments. Figure 1 is drawn from the measurement of 198 individually selected virus particles. It can be seen that the size of the particle varies from 22×10^{-3} to $32 \times 10^{-3} \mu\text{m}$. Further, the frequency distribution of the particles is normal ($\rho = 1.57 \times 10^{-3} \mu\text{m}$) with a mean of $27 \times 10^{-3} \mu\text{m}$ and a mode of $28 \times 10^{-3} \mu\text{m}$. The size of the virions was not observed to vary with passage number or sample within a particular passage.

These measurements indicate that echovirus 22 is significantly larger than other picornaviruses studied by similar methods. Jamison and Mayor (1) indicate these virions can be divided into at least two groups based on the size of the virion. Group I has a mean diameter of 23×10^{-3} to $24 \times 10^{-3} \mu\text{m}$ and group II has a diameter of $21 \times 10^{-3} \mu\text{m}$. The value of delineating groups of picornaviruses by using the diameter of the virion as determined by measurement of negatively stained virions as a criterion is polemic. The diameters of those viruses which have been studied by this method (Fig. 1; reference 1) are all within the limits set by the panel for picornaviruses (15×10^{-3} to $30 \times 10^{-3} \mu\text{m}$; reference 3). At present, no correlation of size and biological properties can be drawn.

Photomicrographs of selected individual par-

ticles were examined in attempts to discern the morphology of the echovirus 22 virion. Reversals of the photomicrographs were prepared by the usual photographic techniques to enhance con-

FIG. 1. Data obtained in a single experiment by measurement of the diameters of 198 selected virions.

trast. Figure 2 demonstrates such reversals. It can be seen that capsomeres or morphological subunits are easily discernible and spread regularly across the particle. If we assume each capsomere to be the vertex of a regular polyhedron, the most obvious features of the viral topography are rhombi which can be linked with other rhombi along the surface of the polyhedron (Fig. 2b and d). Figures 2a and b demonstrate a particle where two fivefold axes of symmetry can clearly be seen to form opposite vertexes of a rhombus. Data such as these indicate the echovirus 22 virion is (assuming icosahedral symmetry) an icosadeltahedron of the class $T = 3$, either a pentakis dodecahedron or a rhombic triacontahedron, and probably possesses 32 morphological subunits. A schematic diagram of a rhombic triacontahedron (marked for emphasis) is illustrated by Fig. 3. It is apparent that the particles (Fig. 2) closely resemble the model (Fig. 3).

Mayor (2) indicates that those picornaviruses studied (poliovirus 1, echoviruses 4, 11, and 24) would seem to possess icosahedral symmetry and can probably best be described as rhombic triacontahedra. She favors the interpretation of the picornavirus particle as a rhombic triaconta-

FIG. 2. Photographic reversals of electron micrographs of two echovirus 22 virions, selected to show the arrangement of morphological subunits. (a) An echovirus virion as seen at a magnification of $\sim 630,000$. (b) The same particle as illustrated in (a), only marked to emphasize interlocking rhombi. Two fivefold axes of symmetry at opposite vertices of a rhombus are marked in black. (c) An echovirus virion as seen at a magnification of $\sim 556,000$. (d) The same particle as illustrated in (c), only marked to emphasize interlocking rhombi.

FIG. 3. *A model of a rhombic triacontahedron marked to emphasize three interlocking rhombi*

hedron rather than the related pentakis dodecahedron because of "the constant finding that most particles appear to display a central rhombus." Echovirus 22 does display morphology consistent with the interpretation of morphological subunits as the vertices of interlocking rhombi, the solid being represented as either a rhombic triacontahedron or a pentakis dodecahedron (Fig. 2). However, examination of these and other particles seen in the electron micrographs only very infrequently reveals the presence of a central rhombus such as would be seen when viewing a rhombic triacontahedron along the twofold axis

of symmetry which Mayor considers "the most likely orientation for a stable equilibrium in this polyhedron." The particles demonstrated in Fig. 2 would seem to be represented more closely by the model of a pentakis dodecahedron when viewed along a fivefold axis of symmetry. In addition, were the particle illustrated in Fig. 2c and d to be a rhombic triacontahedron, one would probably visualize capsomeres behind the peripheral rhombus marked. One does not. It must be noted that one may consider a pentakis dodecahedron as a rhombic triacontahedron, the faces of which have been bisected such that the rhombi form two equilateral triangles with alternate vertices at different planes. It is indeed difficult to visualize the difference between these two closely related figures at the level of the image observed in the electron microscope. However, for the reasons noted, I believe the topography of the echovirus 22 virion to be more consistent with that of the pentakis dodecahedron than the related rhombic triacontahedron.

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