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Fine Structure of Reovirus Type 2

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ABSTRACT

LOH, PHILIP C. (University of Hawaii, Honolulu), H. R. HOHL, and M. SOERGEL. Fine structure of reovirus type 2. J. Bacteriol. **89**:1140–1144. 1965.—The fine structure reovirus type 2 was studied by electron microscopy with the negative-staining method. The virus has a mean diameter of 772 A and shows evidence of icosahedral shape and 5:3:2 symmetry. The particle is composed of a core, an inner layer, and a capsid composed of 92 elongated hollow capsomeres. These capsomeres have mean dimensions of 116 A × 110 A and a central hole 48 A in diameter. In size and architecture, reovirus type 2 is very similar to the other members (reoviruses types 1 and 3) of this group of animal viruses.

The negative-staining technique of Brenner and Horne (1959) has been used to study the electron microscopic fine structure of a number of viruses; some of the recent results have been reviewed by Horne and Wildy (1961). Although several reports have appeared describing the size and structure of reovirus types 1 and 3 (Jordan and Mayor, 1962; Vasquez and Tournier, 1962; Gomatos et al., 1962) by the negative-staining technique, none has appeared describing reovirus type 2. The present communication describes the fine structure of partially purified reovirus 2 by the negative-staining method.

MATERIALS AND METHODS

Virus. Reovirus type 2 (strain D-5) obtained from L. Rosen of the Pacific Research Section, National Institutes of Health, Honolulu, Hawaii, was grown on a continuous line of green monkey kidney cells (BSC-1), with medium 199 supplemented with 2% fetal calf serum. After 3 to 5 days of incubation at 37 C, the virus was harvested in the following manner. Infected cell cultures were frozen and thawed five times successively at −20 C and room temperature, respectively), sonic-treated for 3 min at 20 kc, and the resulting brei was removed by centrifugation at 2,000 rev/min for 10 min. The supernatant fluid was concentrated by sedimentation in a Spinco model L centrifuge at 160,000 × *g* for 1.5 hr, and the pellet was resuspended in 0.1 volume of 98% Medium 199 plus 2% fetal calf serum, and stored at −20 C.

Virus assay. Virus titration was performed by the immunofluorescent plaque technique (Spendlove et al., 1963). The titer in human amnion cells (continuous line RA) of the seed virus was 7.7×10^7 immunofluorescent plaque units per milliliter.

Electron microscopy. In preparation for electron microscopy, 10 ml of the seed virus, after ultracentrifugation (160,000 × *g* for 1.5 hr) and resuspension in phosphate-buffered saline (*p*H 7.2), was treated for 1 hr at 37 C with 0.04% trypsin (Difco, 1:250). After enzyme treatment and slow centrifugation (2,000 rev/min for 15 min), the virus was once more sedimented (160,000 × *g* for 1.5 hr), washed once with phosphate-buffered saline (*p*H 7.2), and concentrated by ultracentrifugation. The final pellet was resuspended in 0.3 ml of triple-distilled water and used immediately.

A loopful of the concentrated virus suspension was added to a carbonized Formvar-coated grid, and after a brief interval a loopful of the negative-staining solution, consisting of 2% phosphotungstic acid (PTA) containing 0.4% sucrose at *p*H 6.0, was added. Excess fluid was removed by touching the surface with a fragment of filter paper, and the thin film of liquid was allowed to dry at room temperature. Grids were examined in a Hitachi HU-11 electron microscope.

RESULTS

Reovirus type 2 particles exhibiting a characteristic and regular appearance can be seen in Fig. 1. Little or no cellular debris was present, and a uniformity of appearance was observed in all the enzyme-treated preparations. In over 100 randomly selected particles, it was found that the diameter measured 773 A ± 28 A (Table 1). Most of these particles appeared to be in a good state of preservation, and many showed a marked hexagonal outline. In some cases, it was possible to see projections at the periphery of the particles which appeared to be profiles of hollow capsomeres. Many of the particles showed less density than the phosphotungstate, and these have been interpreted to be intact virions (82%). Those in which the centers were filled with phosphotungstate have been interpreted as being

empty capsids (18%). In no instance were outer envelopes or filamentous forms observed.

A few structures which could represent incomplete or damaged virus particles were present in these preparations. Spaces in the "empty" capsids normally occupied by the core were easily measured, since they became filled with phosphotungstate acid, and, consequently, were well delineated. In most instances, the shape of the core region is hexagonal. The size of the space normally occupied by the core was found to be 459 A ± 20 A (Table 1).

In most of the empty capsids, an inner layer structure measuring 32.5 A ± 2 A in diameter was observed, and it appeared to be in close association with the capsomere bases. This layer of very dense material was clearly visible between the capsomeres and the empty space of the core, and, in a few instances, was found free of capsomeres (Fig. 2). It was not possible to determine the precise structure of this inner layer in greater detail.

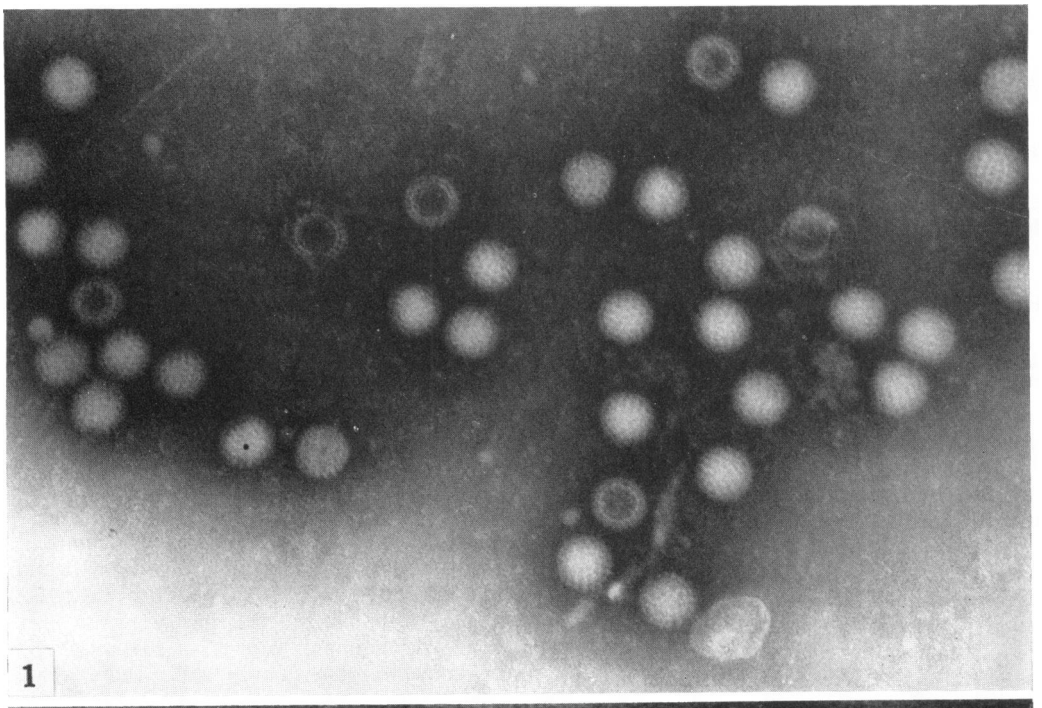
Examination of the capsid of the hexagonal complete particle appeared to indicate that there are four capsomeres composing each facet edge of the particle. Each edge of the hexagon measured at the periphery was estimated to be 401 A ± 33 A (Table 1). Capsomeres with both hexagonal and pentagonal profiles could be detected (Fig. 4 and 5). Fivefold axes of virus symmetry are indicated by the lines drawn. It can be seen that between any two pentagonal capsomeres there are located two morphological subunits, each being surrounded by six equally disposed subunits. Based on this finding and on the fact that there are four capsomeres per facet edge, and with use of the formula $10(n - 1)^2 + 2$ (Horne and Wildy, 1961), where n is the number of capsomeres per facet edge, the total number of capsomeres composing the capsid of reovirus type 2 would be 92, of which 12 are positioned in axes of fivefold symmetry. If these 12 subunits are each made up of 5 chemical subunits, and the remaining 80, of 6 chemical subunits, we would obtain a figure of 540 chemical subunits, again a multiple of 60, in accordance with the predictions of Crick and Watson (1956) for icosahedral symmetry.

Electron microscope studies of herpes simplex (Wildy, Russell, and Horne, 1960*a*), polyoma virus (Wildy et al., 1960*b*), GAL virus (Macpherson et al., 1961), and adenovirus types 5 and 7 (Wilcox, Ginsberg, and Anderson, 1963; Dales, 1962) have shown them to possess hollow capsomeres. As has been determined for reovirus types 1 and 3 (Jordan and Mayor, 1962; Vasquez and Tournier, 1962; Gomatos et al., 1962), the present investigation reveals that reovirus type 2 has capsomeres of the hollow type. The diameter of the hole was estimated to be 48 A ± 5 A. At the periphery of certain virus particles, it is possible to see distinct penetrations with PTA, which resulted in clear divisions between the capsomeres (Fig. 3). Measurement of these capsomeres gives a length of 116 A ± 12 A and a width of 110 A ± 7 A.

DISCUSSION

The interpretation of the structure of capsids and their subunits as revealed in negative-stained preparations has been discussed by Wildy et al. (1960*a*) and Williams, Kass, and Knight (1960). From the present study, it would appear that the structure of reovirus type 2 is composed of 92 capsomeres which are arranged in accordance with icosahedral symmetry. This is in agreement with what has been found for the other serotypes of reoviruses (1 and 3). Furthermore, the size of reovirus type 2 is compatible with those reported for reoviruses types 1 and 3 (Jordan and Mayor, 1962; Gomatos et al., 1962). Table 1 summarizes the measurements of the size and various structures of reovirus type 2 made in the present study and of those for serotypes 1 and 3, as reported by various investigators. From the table it appears that there is some variation in the sizes reported for the other reoviruses. The variation in size even within the same serotype could well be due to the type of suspending medium used in the preparation for electron microscopy (Gomatos et al., 1962).

Recently, in a reinterpretation of the arrangement of the capsomeres of reovirus type 3, Vasquez and Tournier (1964) concluded that the capsid is not formed from hexagonally or pentagonally faced capsomeres as was previously reported by them (1962) and others (Jordan and Mayor, 1962; Gomatos et al., 1962), but from morphological units composed by shared structural units. Whether the capsid of reovirus type 2 is composed of such shared structural units is at present difficult to ascertain. The capsomeres appear to be of the hollow type, and are very closely packed. This packing arrangement seems to prevent efficient penetration of the negative stain, thereby hindering clear distinction of the spacings between adjacent capsomeres. This phenomenon has been observed by Jordan and Mayor (1962) for reovirus type 1. They suggested that there is some connection between adjacent capsomeres which prevented penetration of the phosphotungstate ion. This problem of the arrangement of the capsomeres and their subunits needs further clarification, and, therefore, a more detailed analysis of intact and disrupted viral preparations is presently being conducted.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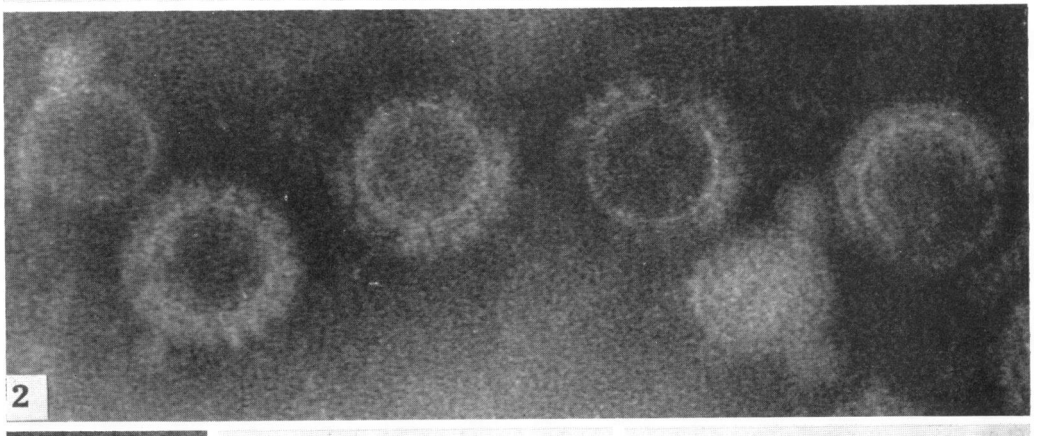
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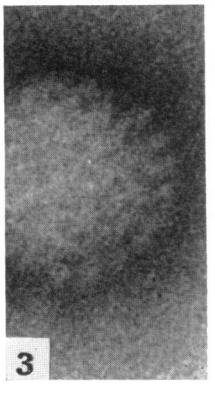
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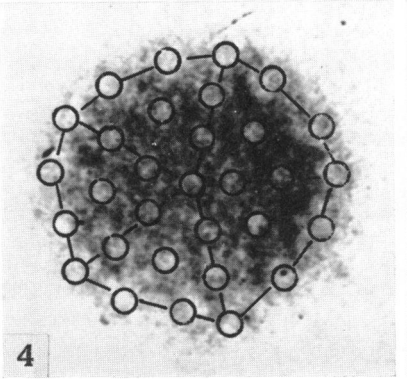
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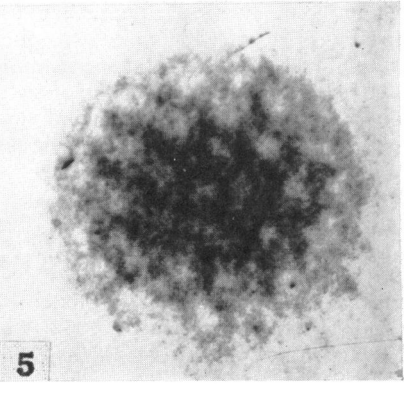
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TABLE 1. *Morphology of reovirus*

Structure	Reovirus 1			Reovirus 2	Reovirus 3	
	Jordan and Mayor, 1962	Sabin, 1959	Rhim et al., 1961	Loh et al., 1962	Vasquez and Tournier, 1962	Gomatos et al., 1962
Size	600	750	750	772 ± 28 (143) *	595 ± 25 (100)	760
Core	ND†	ND	ND	459 ± 20 (27)	325 ± 15 (50)	ND
Inner layer	ND	ND	ND	32 ± 2 (11)	ND	ND
Facet edge of capsid	ND	ND	ND	401 ± 33 (23)	240 1 (100)	ND
Hole of capsomere	ND	ND	ND	48 ± 5 (20)	40 ± 15 (55)	30–40
Length of capsomere	ND	ND	ND	116 ± 12 (20)	100 (100)	ND
Width of capsomere	ND	ND	ND	110 ± 7 (12)	80 (100)	75

* Sizes are given in Angstrom units; number of measurements is given in parentheses.
† ND = not done.

One important feature seen in the present study is the appearance of an inner layer of electron-dense material (32 A) which is located between the core and the bases of the capsomeres. Although no measurements were made by Vasquez and Tournier (1962), they reported the presence of this inner layer in their preparations of reovirus type 3. This inner layer has also been reported for other viruses by Horne (1963). In the present study it was not possible to observe the fine structure of this inner layer, but there is the impression that the capsomeres are "anchored" to this inner layer. Such a layer could correspond to the "building block" structure proposed by Klug and Caspar (1960). In conclusion, it would appear from the present study that reovirus type 2 is very similar in architecture and size to serotypes 1 and 3, and fits into the icosahedral series for animal viruses.

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FIG. 1. *Negative (phosphotungstate) stain of reovirus type 2.* ×*108,000.*

FIG. 2. *Coreless forms of reovirus type 2. Some of the empty capsids appear to have part of their capsomeres removed, leaving an inner layer.* ×*324,000.*

FIG. 3. *Virus particle showing the capsomeres at the edge penetrated with phosphotungstic acid.* ×*410,000.*

FIG. 4 and 5. *Capsid showing triangular arrangement of hollow capsomeres, shown with reversed contrast. Fivefold axes of virus symmetry are indicated by the lines drawn. One capsomere is surrounded by five equally spaced neighbors and the others are surrounded by six. Figure 5 represents an unretouched photograph of the same virus particle.* ×*550,000.*

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