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Studies on the Structure of Poliovirus

By

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With 3 Figures

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Observations on the structure of poliomyelitis virus have been reported by several investigators (1—3). *Finch* and *Klug* (1) on the basis of x-ray diffraction studies suggested that the virus protein shell is made up of 60 subunits and has an icosahedral (5:3:2) symmetry. From the subunit spacing and particle symmetry they concluded that the diameter of the individual particles is approximately 300 Å. The subunit size measured as centre-to-centre distance was 60—65 Å. *Horne* and *Nagington* (2) on the basis of their electron micrographs further supported these interpretations. *Mayor* (3) in his study with type 1 poliovirus, on the basis of a micrograph, suggested that the virus capsid is composed of 32 subunits.

This paper presents some electron microscope observations using the negative staining technique (4) and the rotation technique (5, 6).

Materials and Methods

Poliovirus type 1 (Mahoney) strain used in these investigations was kindly supplied by Dr. *J. D. van Ramshorst* of the State Institute for Public Health, Utrecht. The medium 199 containing the purified virus was centrifuged at high speed (105,000 *g* for 3 hours in a Spinco model L) and the resulting pellet was resuspended in distilled water. This virus was diluted to an appropriate concentration and mixed with an equal quantity of 2% phosphotungstate (PTA) (phosphotungstic acid dissolved in distilled water and adjusted to pH 7.0 with potassium hydroxide). Two to three drops of 0.3% bovine serum albumin solution in distilled water were added to this mixture, which was then sprayed onto a piece of 80 Å thick carbon film mounted on a sheet of mica. The carbon film was separated from the mica sheet by floating it on distilled

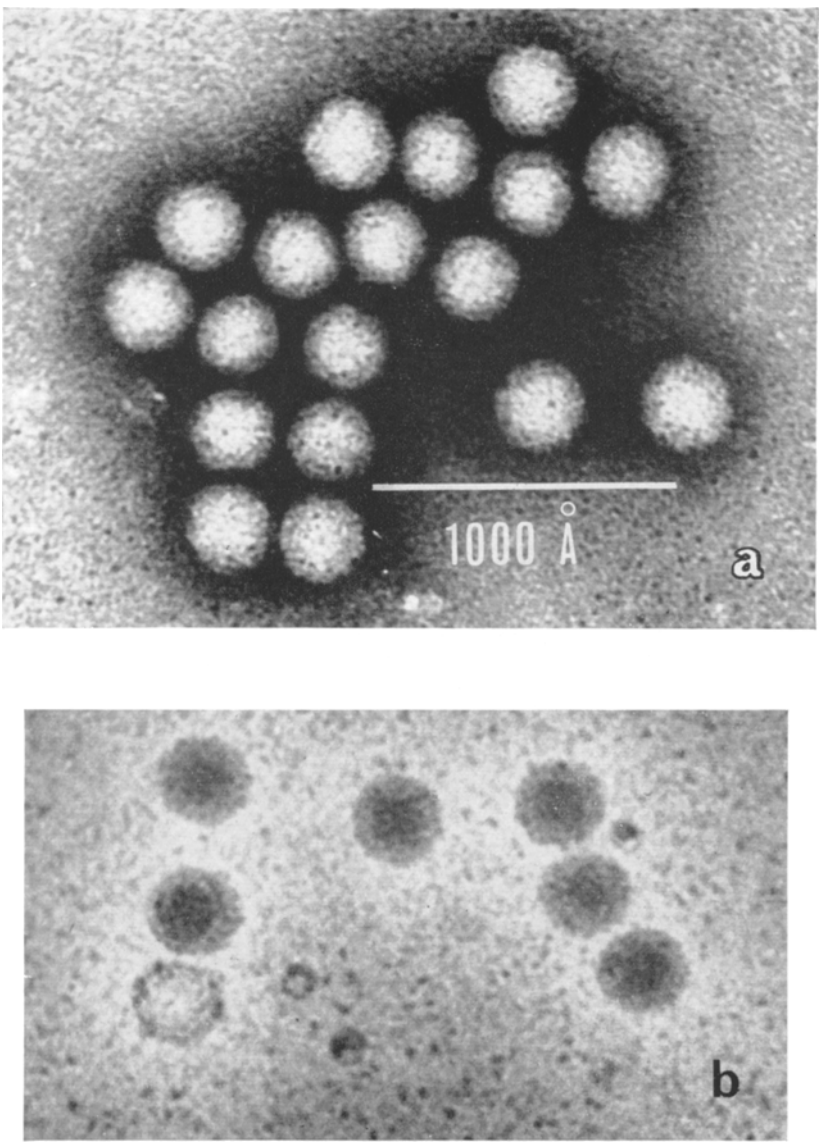

Fig. 1. Poliovirus type 1 (Mahoney) strain in negatively stained preparations.

Fig. 1a. Normal electron micrograph. Some of the particles show evidence of substructure. In several particles 3 subunits on one edge (in the periphery) can be seen.

Fig. 1b. Another field, printed in reversed contrast, showing an empty particle. The nucleic-acid-containing particle on the left (centre) is shown also in Fig. 2.

water containing 10% acetone and was mounted on copper grids. The grids were immediately examined in a Siemens Elmiskop I electron microscope at 80 kV. Micrographs were taken at an instrumental magnification of 80,000 times.

Results and Discussion

An electron micrograph of negatively stained particles from one of the typical fields is presented in Fig. 1. The particles exhibit a regular appearance and their size corresponds, in general, to what has been reported by

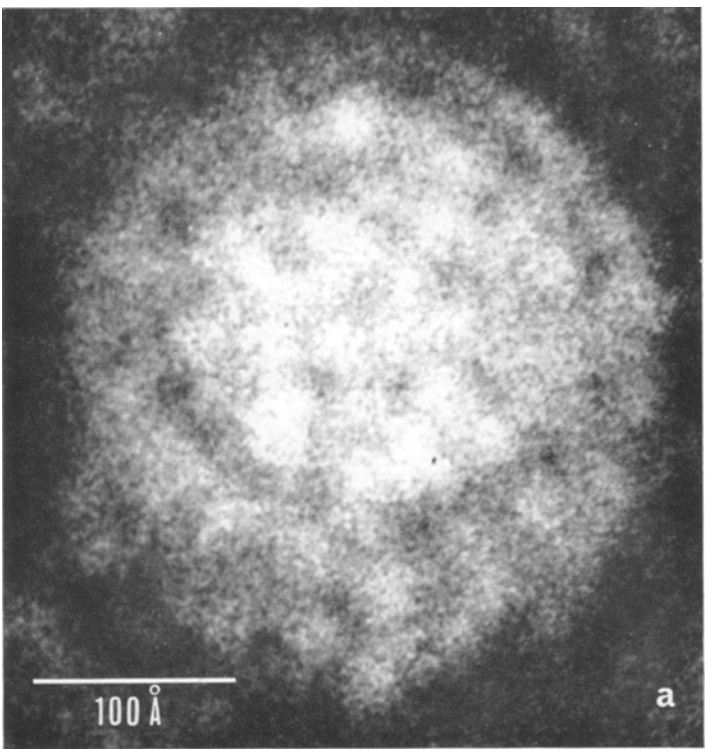

Fig. 2a. Electron micrograph of a nucleic-acid-containing particle of poliovirus type 1, showing a pattern of 6 subunits with one in the centre. The corresponding subunits in the model below are marked as 1—7.

earlier investigators. The diameter ranges between 275—300 Å. The photograph in Fig. 1b is printed in reversed contrast and also shows an empty particle. Such particles have also been reported earlier for this (2) and for other viruses (7). Some of the particles in the micrographs presented here appear more or less rounded while others show a clear hexagonal profile. These differences are presumably due to their different degrees of immer-

sion in PTA and due to differences in orientation. Some evidence of substructure can be seen in several of the particles.

One of the nucleic-acid-containing particles seen in Fig. 1b was highly magnified and is shown in Fig. 2a. Models containing 42 and 32 capsomeres respectively are shown in Fig. 2b and 3b. A pattern of 6 subunits with one in the centre (marked in the model in Fig. 2b as 1—7) can be seen in Fig. 2a. These appear to be approximately 50—60 Å in diameter and their

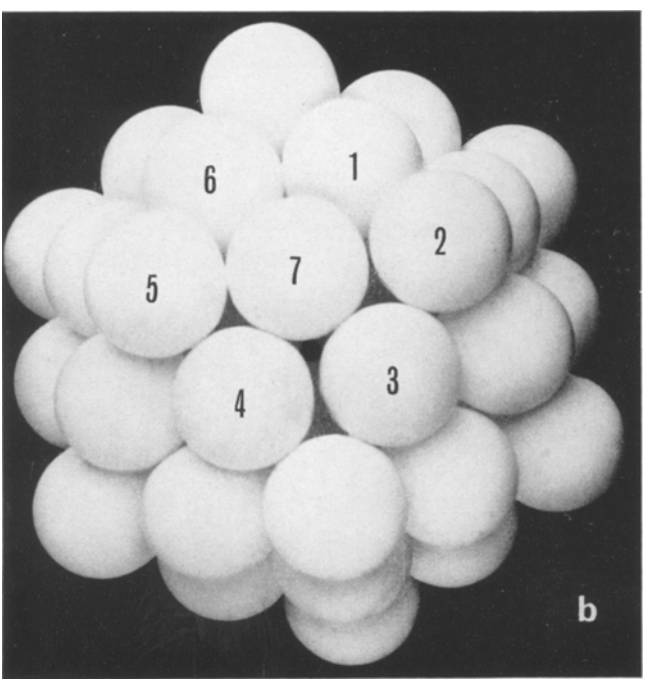

Fig. 2b. Photograph of a model of an icosahedron (with 5:3:2 symmetry) constructed from 42 table tennis balls each representing a capsomere. The photograph represents the model in 3-fold symmetry. Three subunits on each edge in the periphery and a pattern of 6 subunits with one in the centre can be seen.

centre-to-centre distances are also about the same. Several other subunits on the periphery of the particle can also be discerned. It should be pointed out that only those subunits which have most of them or a greater part of them exposed in the model in Fig. 2b are also the ones discernible in Fig. 2a. The reason for this seems understandable since one would expect to see only those subunits which either completely overlap or which are not overlapped at all or have very little overlapping. The profile and the appearance of the particle in Fig. 2a appear to resemble quite well the picture of the 42 capsomere model in Fig. 2b. Each edge

of the particle shows indication of 3 subunits as also apparent in the model. A comparison of Fig. 2a with the 32 capsomere model in Fig. 3b shows little similarity between the two. Both the models in Fig. 2b and 3b are shown in 3-fold symmetry, since it is only in this symmetry position that the particle would show a pattern of 6 subunits with one in the centre, and since the actual micrograph shows such a pattern, a comparison with the model would only be justified using this symmetry position. It is also known that the 42 subunit model is most stable in 3-fold symmetry since

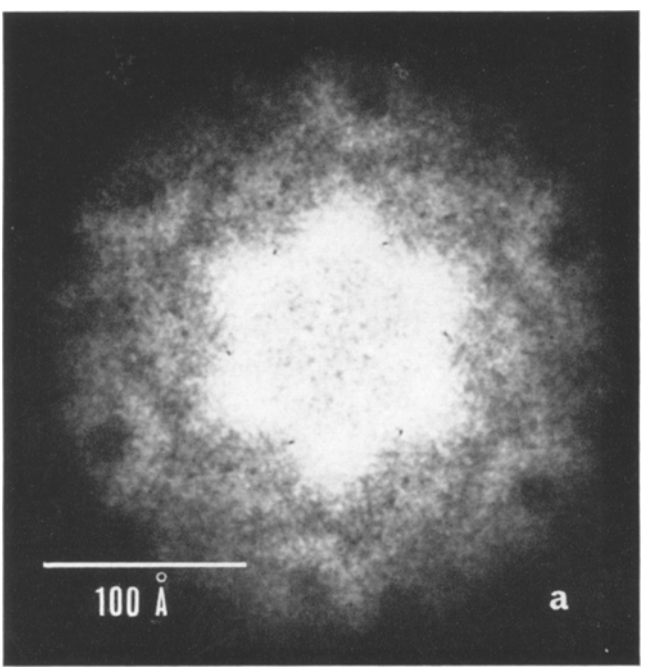

Fig. 3a. Same particle as in Fig. 2a but photographed by the rotation method (rotated 6/6), with the centre of the photograph in Fig. 2a as the centre of rotation. Some of the peripheral subunits are reinforced but the 6-subunit pattern as in the original has disappeared since the centre of rotation for the photograph was different from the central subunit in Fig. 2a.

it can rest well on any one of the triangular faces and that it shows a distinct hexagonal profile in this orientation (7).

A 6/6 rotation of the same particle as in Fig. 2a, made according to the rotation technique described and discussed earlier (5, 6), is presented in Fig. 3a. The centre of rotation used to make this photograph can be compared to the centre in the model in Fig. 2b and hence only some of those peripheral units which are also seen clearly in the model (Fig. 2b) are reinforced in Fig. 3a. The central subunits, as would be expected,

cannot be seen anymore, since the centre of the 6-subunit pattern and the centre of the particle (as apparent in the model in Fig. 2b) are different and apart; and since the micrograph in Fig. 3a has been made by taking the centre of the particle (Fig. 2a) as the axis of rotation.

The micrographs presented here provide evidence contrary to *Mayor*'s (3) interpretation that the poliovirus particle contains 32 subunits similar to that shown for turnip yellow mosaic virus (8—10). The single micro-

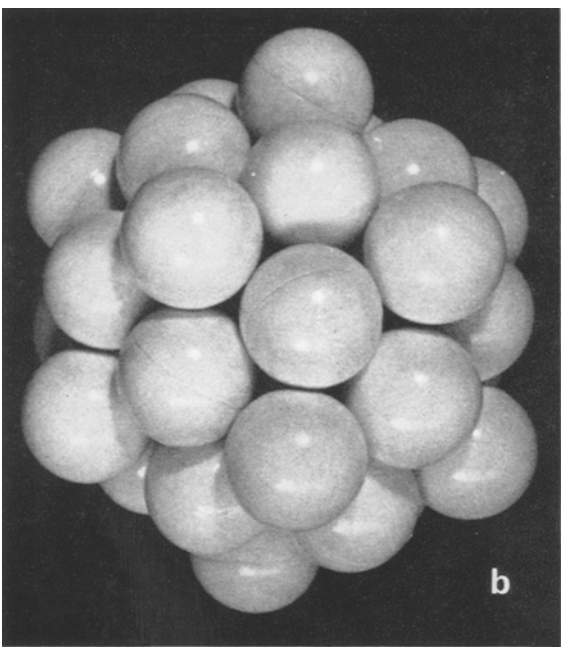

Fig. 3b. Photograph of a model of an icosahedron (with 5:3:2 symmetry) constructed from 32 table tennis balls each representing a capsomere. The photograph represents the model in 3-fold symmetry. The subunits in the periphery cannot be discerned so well, and the central subunit in the 6-subunit pattern lies more or less in the centre of the particle. On this basis, this model can be distinguished from Fig. 2b.

graph presented by *Mayor* does not appear very typical, and also does not exclude a 42 subunit structure.

According to *Caspar* and *Klug* (11) a particle with icosahedral symmetry and having 42 morphological units could have 30 hexamers and 12 pentamers. This would make a total of 240 structural units, or polypeptides. It may also imply the presence of 2 different kinds of polypeptides, one representing the hexamer and the other the pentamer. The fact that it might very well be true, is supported by recent indications of *Maizel* (12), *Summers* et al. (13), and *Rueckert* (14), who presented evidence

for more than one protein component and hence more than one type of peptide chain. *Maizel* (12) reported a molecular weight of 27,000 for the structural unit of poliovirus and *Boeyé*'s (15) data yielded a molecular weight of 42,000. The reasons for these differences are not clear. It is not known whether these determinations really represent monomers since these values are based on sedimentation equilibrium runs.

Schaffer and *Schwerdt* (16) found a molecular weight of 6.8×10^6 for the virus particle containing 25% RNA and 75% protein. This would give a molecular weight of about 5×10^6 for the protein. If the virus particle has 42 morphological subunits consisting of 30 hexamers and 12 pentamers and a total of 240 structural units, it would suggest an approximate molecular weight of 20,000—21,000 for the structural units. Since data on the molecular weight of the peptides (which appear to be of more than one kind) of poliovirus protein are still a matter of controversy, the question of the total number cannot be settled finally yet.

Summary

Electron micrographs of poliovirus using negative staining technique and rotation technique suggest that the virus probably has 42 morphological subunits and 240 structural units or polypeptides. The structural units may also represent two different types of proteins.

Acknowledgements

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