Acta Medica Okayama

Volume 19, *Issue* 6 1965 *Article* 3

DECEMBER 1965

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Abstract

Adenovirus type 12 is of human origin and it shows carcinogenic activity in experimental animals. With negatively stained particles of this virus electron microscopic observations were carried out. As the result it was demonstrated that its capsid, like other adenoviruses, is an icosahedron and each capsomere is of a hexagonal shape with a hollow in its center, each of which is surrounded by 6 adjacent capsomeres and is composed of numerous small subunit-like-particles.

*PMID: 4223616 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 19. 287—292 (1965)

FINE STRUCTURE OF NEGATIVELY STAINED ADENOVIRUS TYPE 12

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Received for publication, October 12, 1965

Virus particles have a genetically fixed structure specific to the kind of viruses, and this structure is dependent on capsid, and it is also determined by the symmetrical arrangement of the capsomeres that are subunits of the capsid. The study on such fine structures of virus particles has made a rapid advance since BRENNER and HORNE[1] introduced the negative staining technique for electron microscopic observations of virus particles. According to the studies on the structure of adenovirus conducted with this technique in various types it is said that the virus particle is an icosahedron consisted of 252-capsomeres.

There are reports by ALMEIDA *et al.*[2] and MAYOR *et al.*[3] about the morphology of adenovirus type 12 and they have demonstrated that the structure is the same as that of other types of viruses. The present experiment was conducted with the purpose to observe still finer structures of adenovirus type 12 particles.

MATERIALS AND METHODS

The culture cells used were HeLa cells. At first cell suspension at the concentrations of $10-15\times10^4$ cells/ml were prepared, and 1 ml of this suspension in a culture tube was incubated at 37°C. Within 2—3 days culture the cell number increased to $20-30\times10^4$ cells/ml. Then the virus was inoculated. The growth medium for cells was composed of 20% calf serum plus YLE solution, and the YLE solution without serum served as the maintenance medium. The virus sample used was adenovirus type 12 (Huie strain) passaged by HeLa cells in our laboratory for a long time, and infected with the virus, 10^{2-3} $TCID_{50}$ /ml.

When the culture is continued under these conditions, on about the third day after inoculation the virus infected cells show specific cytopathogenic effects (CPE), and this CPE is seen in as much as 70—80% of the entire cells 5—7 days after inoculation. At this stage the virus is purified from the infected material for electron microscopic observation. In other words, the virus material is repeatedly frozen and thawed 30 times, subjected to ultracentrifugation at

100,000 × g, and the pellets thus obtained are resuspended in 1% phosphotungstic acid (PTA) (pH 7.0) for 15-minutes. Then the stained material is mounted on collodion membrane coated grids and observed in the electron microscope, Hitachi, HU-11 at the instrumental magnification of 50,000.

RESULTS AND DISCUSSION

The negative staining method employed for the elucidation of fine structures of the virus particles enables us to observe th fine structure by virtue of contrast produced by the electron dense substance of PTA that has penetrated into interspaces of the material under observation.

By this means it is not only possible to see the size and the shape of the virus particle as a whole but also it enables us to observe readily and directly the arrangement of capsomeres that are structural subunits making up the virus protein shell as white capsomeres against the black background of PTA.

Representative pictures of the virus particles observed from different angles are as shown in Figures 1 to 3.

Each one of the pictures on the left side shows a virion of adenovirus type 12 surrounded by PTA, the middle picture is of particle with superimposed orientation, and the picture on the right is that of 252-capsomere model in orientations similar to the virus particles. The capsid in each figure presents a hexagon from a plane view while it is an icosahedron at the three dimensional view, It is to be noted that each plane surface is of a right triangle on each edge of which are 6 capsomeres, and with the exception of the ones at apices all others are each surrounded by 6 capsomeres (Fig. 1). Consequently, when a common base of two right triangles is viewed side-wise at the equator, these two surfaces form a diamond shape (Fig. 2), and it is also possible to observe five triangles adjacent to each other when viewed from their common apex (Fig. 3). These findings verify that the virion in the picture is an icosahedron consisted of twenty equal surfaces each of which is a right triangle.

These are negative stain pictures of complete virus particles and most of the purified virus particles show such a structure but sometimes there are particles with incomplete structure. Possibly due to insufficient purification of virus particles, occasionally there can be recognized tissue debris in the vicinity of particles in (Fig. 4), a picture showing aside from the array of white capsomeres, there can be seen a white core with stronger contrast as if it is about to emigrate

Figs. 1—3 Show the virus particles observed in three different views. The pictures on the middle are of particles with superimposed orientation, and the pictures on the right are those of 252-capsomere models in orientations similar to the pictures of virus particles on the left side. × 450, 000

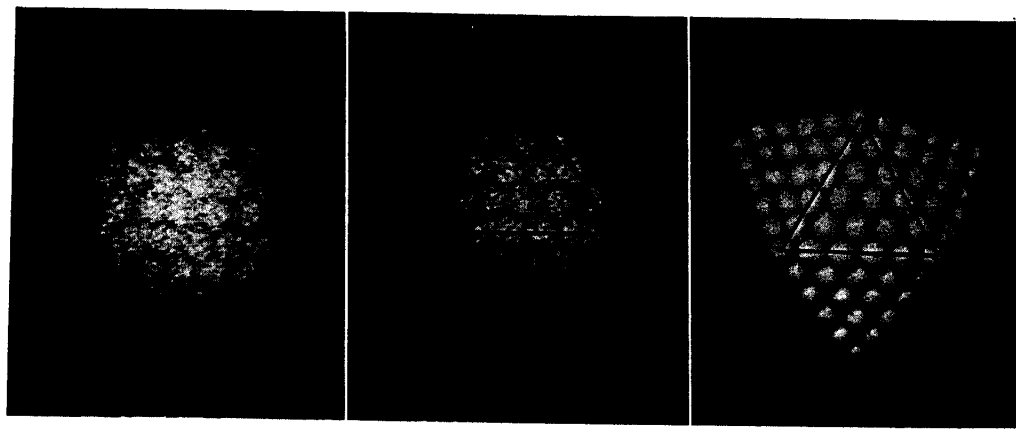


Fig. 1

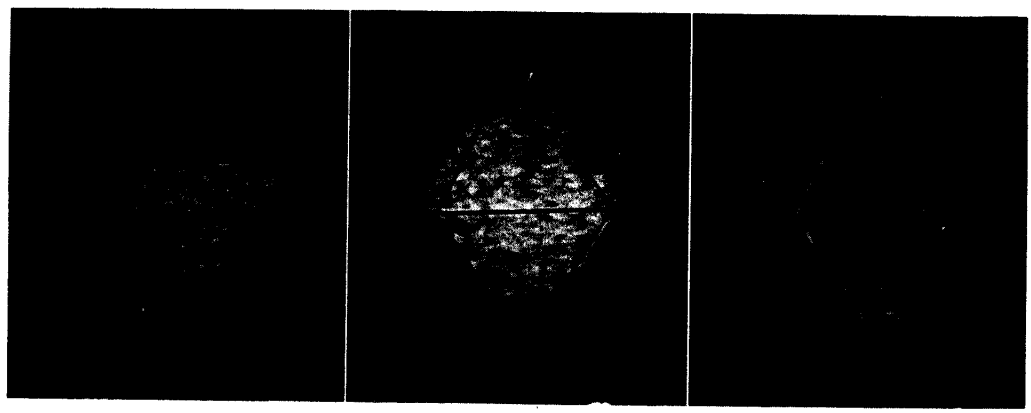


Fig. 2

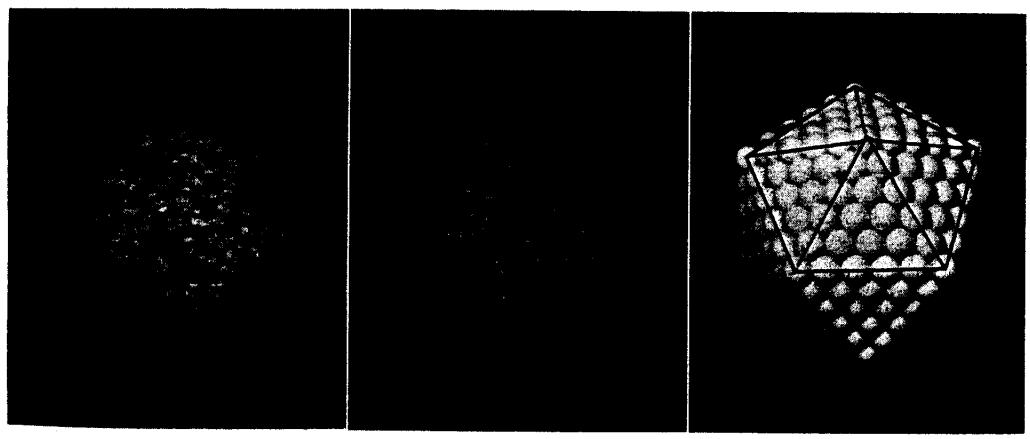
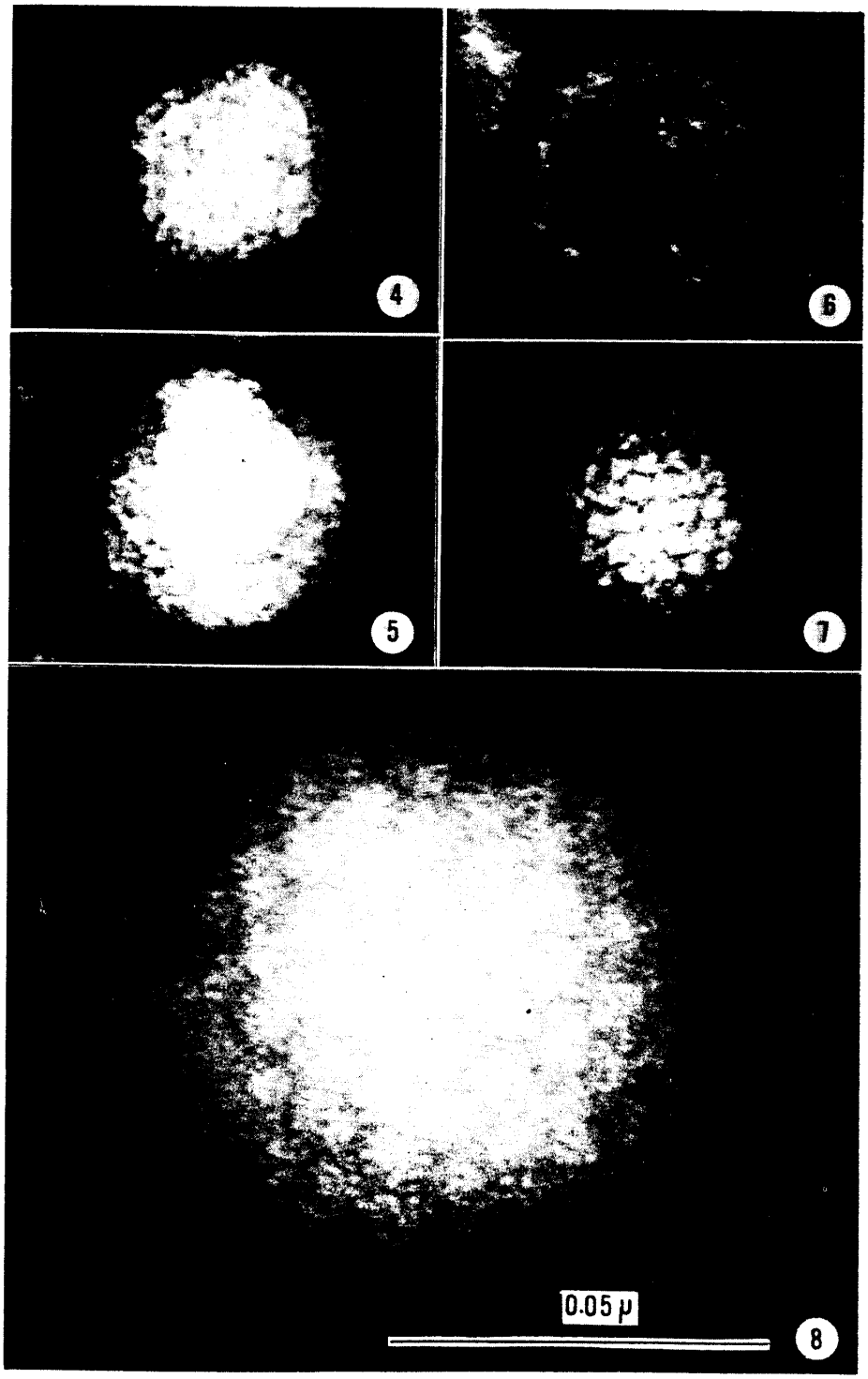


Fig. 3


4
6
5
7
0.05 μ
8

from the virion (Fig. 5), and sometimes empty shells without core in which PTA penetrates deep into the virion (Fig. 6).

As regards capsomeres themselves, each capsomere has a round hollow in its center, and probably because being surrounded by six other capsomeres, it gives somewhat a hexagonal appearance (Fig. 7). Fig. 8 shows a virion observed at a higher magnification, in which each capsomere seems to be an aggregation of numerous finer granules of a stronger contrast.

It is not possible to describe precisely about the structural arragement of these finer granules. However, they seem to have a close connection to chemical structural units in some way.

Generally, it is thought that capsomeres which are the structural units of a virus as observed in electron microscope would be composed of still finer subunits. According to HORNE *et al.*[4], as in the case of poliovirus, there are observed subunits smaller than capsomeres, but the structural arrangement of capsomeres themselves is sometimes not distinct. According to ALMEIDA *et al.* and MAYOR *et al.* who observed fine structures of negatively stained adenovirus type 12 in the electron microscope, they seem to have not yet recognized the presence of subunits with still finer structure.

In comparing the results obtained in the present observations on the negatively stained pictures of adenovirus type 12 with those of others, it seems that differences in the electron micrographs arise even with identical materials because of the difference in the degree of washings of virion during the purification process, the difference in the intensity of negative staining, and the difference in the developmental stage of virion itself.

SUMMARY

Adenovirus type 12 is of human origin and it shows carcinogenic activity in experimental animals. With negatively stained particles of this virus electron microscopic observations were carried out. As the result it was demonstrated that its capsid, like other adenoviruses, is an icosahedron and each capsomere is of a hexagonal shape with a hollow in its center, each of which is surrounded by 6 adjacent capsomeres and is composed of numerous small subunit-like-particles.

Figs. 4—6 Show the virus particles with incomplete structure in different stages of development. The virus particle with tissue debris in the vicinity of it in Fig. 4 and the one with a round white core in Fig. 5 are seen. The particle in Fig. 6 shows an empty shell without core. Fig. 7 shows that each capsomere of virus particle has a round hollow in its center, and shape of each capsomere shows a hexagonal appearance. ×450,000

Fig. 8 Shows an aggregation of numerous finer granules on capsomeres at a higher magnification of virus particles. ×1057,000

ACKNOWLEDGEMENT

The author expresses his thanks to Dr. Yoshiro YABE for supplying him with adenovirus type 12 (Huie strain), to Mr. Nobuo HAYASHI for his assistance in taking electron micrographs, and to Prof. Sakae MURAKAMI for his advices and guidance throughout this work.

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