

## OBSERVATIONS ON THE FINE STRUCTURE OF POLYOMA VIRUS

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Study of thin sections of cells of hamster kidney infected with polyoma virus (1, 2) revealed numerous spherical particles of apparent diameter about 28  $m\mu$  that are thought to be the infective units, and in addition two other types of structures that are closely associated with the spherical units. These are larger spherical particles of diameter approximately 60  $m\mu$  which in some specimens are found in variable but always relatively small numbers in the cytoplasm of infected cells, and filamentous or tubular elements which are present in the nuclei of a small percentage of infected cells.

In the present note more detailed information about the fine structure of the spherical and filamentous elements obtained by thin sectioning and negative staining procedures is presented. The appearances of particles and filaments observed by these two different methods are compared and discussed in terms of a model for the small spherical particles proposed by Wildy *et al.* (3):

### MATERIALS AND METHODS

Specimens for thin sectioning were obtained from kidneys of hamsters killed at intervals from 2 to 6 days

after infection as newborns with a preparation of polyoma virus. The specimens were fixed in buffered osmium tetroxide, dehydrated in increasing concentrations of ethanol, and embedded in methacrylate. To enhance contrast some specimens were stained by one of two methods: (a) uranyl acetate was added to the 70 per cent solution of ethanol used in the first stage of dehydration at a concentration of 3 per cent, or (b) sections were floated on a solution of lead hydroxide for 15 minutes before being examined (4). When very high contrast was desired both methods were applied to the same specimen.

For the negative staining method, partially purified preparations of virus were obtained from kidneys of 5-day-old hamsters injected at birth with a potent polyoma virus preparation, by subjecting a homogenate of the kidneys to several cycles of fluorocarbon extraction (5). The partially purified virus preparation was treated with phosphotungstic acid by the method of Brenner and Horne (6). Specimens were examined in a Siemens Elmiskop I electron microscope.

### OBSERVATIONS AND DISCUSSION

There are several reports describing the size and appearance of particles identified as polyoma virus

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when viewed in sections of infected cells grown *in vitro* (1, 7, 8, 9), lesions induced in hamster kidney (1, 2), and tumors induced in mice (1, 9) by the virus. These reports all agree in attributing a diameter of 27 to 30  $m\mu$  to the great majority of the particles seen. However, measurements by Kahler *et al.* (10) on shadowed preparations of purified virus gave an average diameter of 44  $m\mu$ , and a more recent determination by Wildy *et al.* (3) who used the negative contrast method gave an average value of 45.3  $m\mu$ . It has been suggested (2) that part of the discrepancy in size between the values obtained by thin sectioning and other methods may be due to the presence round the particles of a layer of material that is not visible after the usual procedures of fixation and embedding. Wildy *et al.* (3) make what is essentially the same suggestion; namely, that what is seen in sections is not the whole particle but only the core, which from their work is estimated to be 27.3  $m\mu$  in diameter. Further, Dourmashkin *et al.* (11) report the presence of a thin, closely applied membrane round some of the particles observed by them in sections of infected cells of mouse tumors induced by polyoma virus, and give as the size range of the particles 30 to 40  $m\mu$ .

It is clear from our recent work on sectioned specimens that there is indeed a layer of material of width about 5  $m\mu$  around each particle, the over-all diameter being therefore about 38  $m\mu$ . The layer is very difficult to discern unless heavy metal staining is employed. Fig. 1 is from a section treated with lead hydroxide. In the lower half of the figure, many particles may be seen in which the central dense core is surrounded by a distinct but less dense layer. This outer layer is also visible after uranium staining. This is illustrated in Fig. 4 which shows at higher magnification a single particle stained by the uranyl acetate method.

Further indication that the size of the particles is greater than 28  $m\mu$  has been obtained by measurements on particles arranged in crystalline formation. In Fig. 2 is illustrated a group of particles in close-packed hexagonal array. The average centre to centre separation between particles is 36  $m\mu$ , a value slightly smaller than that obtained by direct measurement on isolated particles. This difference, however, may be due to compression during sectioning.

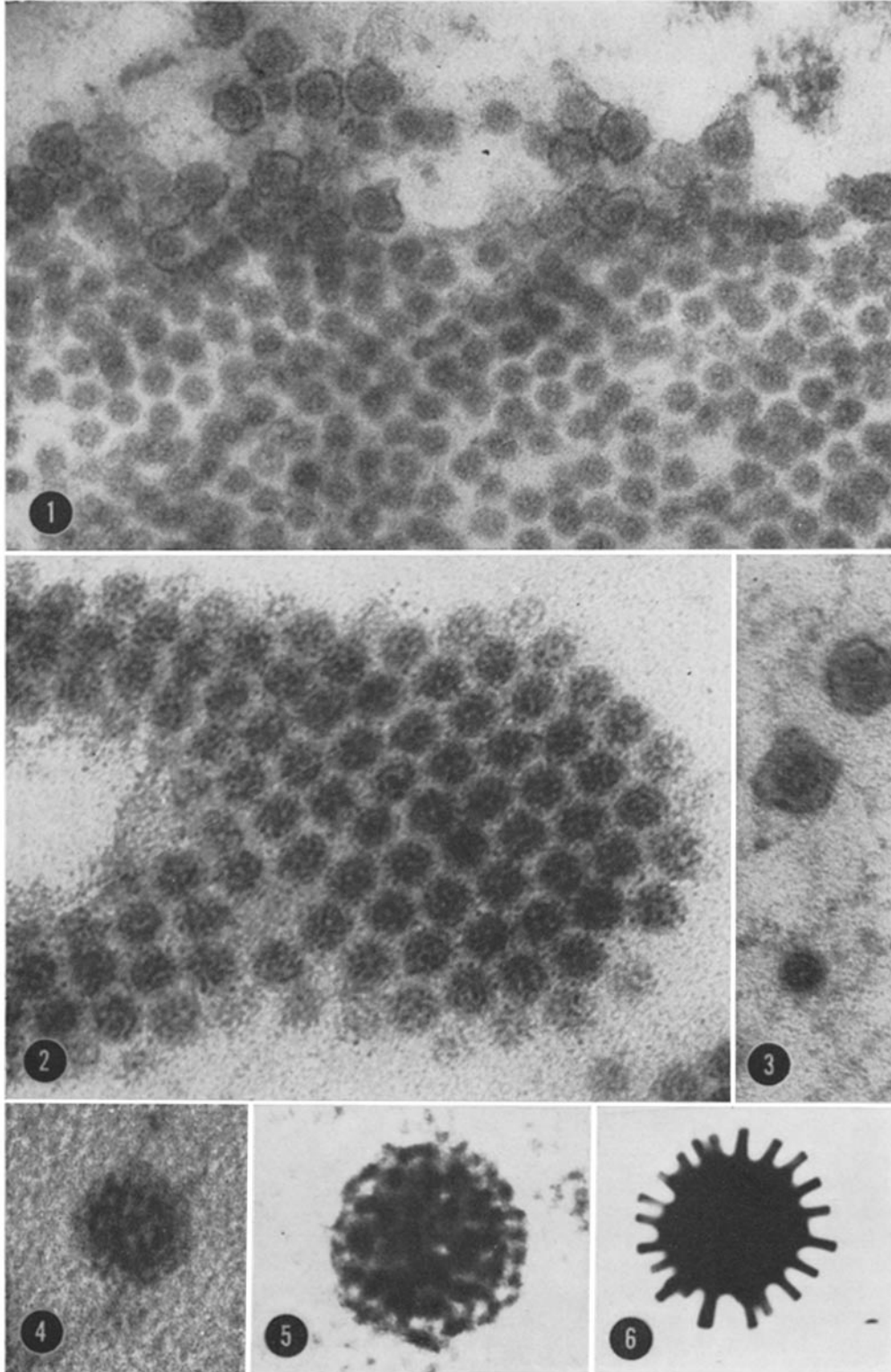
When specimens are treated by a combination of the two staining methods the particles appear as shown in Fig. 5. The core is very dense and, relative to the total diameter, somewhat larger than in

unstained or singly stained particles. The outer layer can be clearly distinguished from the core, and appears to consist of some 20 dense units separated by clear spaces. It seemed probable that these units of substructure, revealed in the outer layer of the sectioned particles, were the same as the subunits described by Wildy *et al.* (3) in purified polyoma virus particles treated by the negative contrast method. These authors concluded from their studies that the polyoma virus particle is covered by a protein coat or "capsid" consisting of 42 subunits or capsomeres arranged in 5:3:2 axial symmetry. The capsomeres are described as "elongated cylinders or prisms which stick out like the prickles of a hedgehog." In order to test whether the substructures seen in sectioned particles are related to the capsomeres described by Wildy *et al.*, a model was constructed after the one proposed by them. Fig. 6 is a photograph of a shadow cast by the model when placed in a parallel beam of light.

The number and distribution of the peripheral units seen in the shadow depends on the orientation of the model. By suitably orientating the model it should be possible to simulate the appearance of any virus particle in a section when viewed in the electron microscope. An example of the degree of correspondence that can be obtained may be seen by comparing Figs. 5 and 6. The units comprising the outer layer of the particle are shorter and flatter than those of the model, but the number and distribution are in good agreement. Thus the appearance of the particles in sections can be explained in terms of the model proposed by Wildy *et al.*

The uniformity in size and appearance of the 38  $m\mu$  particles is in striking contrast to the irregular appearance of the larger particles. Fig. 1 shows an area where these particles seem to be forming at the edge of a large cytoplasmic aggregate of 38  $m\mu$  particles. It seems clear that the mode of formation of the large particles is by addition round the small particles of a dense membrane. Possibly because of the presence of the membrane, detail within the particles is poor, but comparison of typical large and small particles in Fig. 3 shows that the nucleoid of the large particle is similar in size and appearance to the core of the small particle.

The filamentous or tubular elements are found within nuclei infected with polyoma virus, usually in close association with 38  $m\mu$  particles. Occasionally they are present in large numbers



and may be arranged roughly parallel to one another in groups or bundles (Fig. 10). Some of these cylindrical elements appear hollow, the centre being less dense than the periphery. In others, however, the centre is dense, and the appearance of individual filaments in sections stained with heavy metal (Fig. 11) suggests that they have the same basic structure as the spherical particles, namely a dense central core, in this case cylindrical, surrounded by a narrow, less dense sheath. The diameter of the filament is, however, somewhat smaller than that of the spherical particles, the mean value being  $32\text{ m}\mu$ . The resemblance between filaments and particles is more strikingly shown in partially purified virus preparations that have been treated by the negative staining method. Fig. 7 shows a long filament together with several spherical particles. The surface layer of the filament is composed of units that appear to be identical with the capsomeres of the

spherical particles. In some of the spherical particles, and at intervals along the filaments, the phosphotungstic acid has apparently penetrated the outer coat and dense patches are apparent. In negative stained preparations we find, in agreement with the value reported by Wildy *et al.*, that the average diameter of the spherical particles is approximately  $45\text{ m}\mu$ . The filaments have an appreciably smaller diameter, the mean value being  $38\text{ m}\mu$ .

Fig. 8 shows part of another filament at higher magnification and Fig. 9 shows two spherical particles at the same magnification for comparison of the surface structures. The filament terminates in a hemispherical cap, and at one point in its length there is a definite transverse break. There is some indication of segmentation of the filament into portions about the size of the spherical particles, suggesting that the filaments may represent a stage in the development of these particles. It is

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FIGURE 1

Portion of a large aggregate of  $38\text{ m}\mu$  polyoma virus particles; each particle consists of a dense core of diameter  $28\text{ m}\mu$  surrounded by a layer of less dense material. At the edge of the aggregate are several larger particles of diameter approximately  $60\text{ m}\mu$  which appear to form by the addition of a membrane to the  $38\text{ m}\mu$  particles. Lead hydroxide staining.  $\times 160,000$ .

FIGURE 2

Part of an aggregate in which the particles are arranged in regular crystalline array. The average centre to centre spacing is  $36\text{ m}\mu$ . Lead hydroxide staining.  $\times 240,000$

FIGURE 3

Two large and one small particle. The nucleoid of the large particle is similar to the core of the small particle. Uranyl acetate staining.  $\times 225,000$ .

FIGURE 4

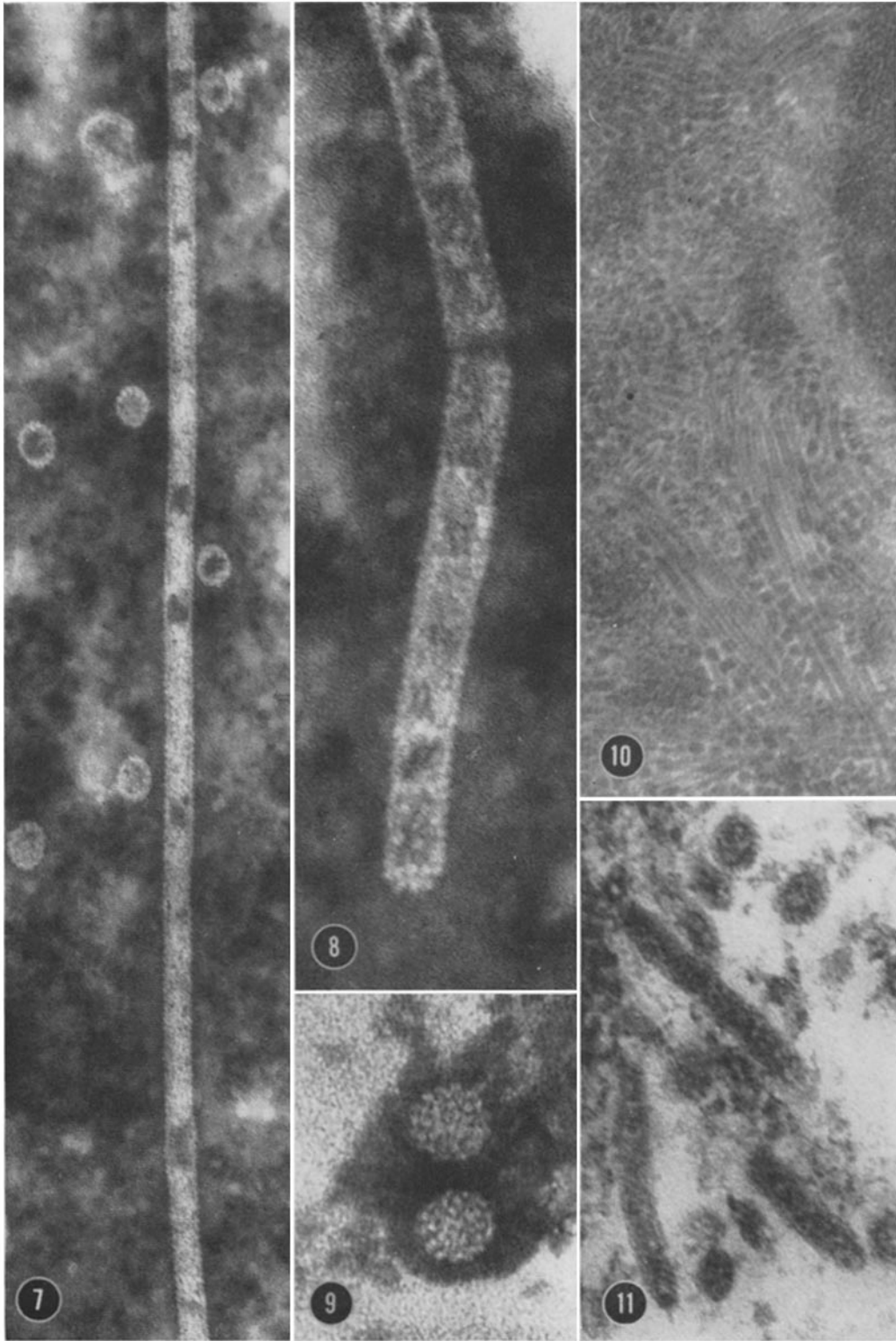
Higher magnification picture of  $38\text{ m}\mu$  particle stained with uranyl acetate, showing peripheral layer of low density.  $\times 480,000$ .

FIGURE 5

$38\text{ m}\mu$  particle stained with uranyl acetate followed by lead hydroxide, shown at high magnification. The peripheral layer is composed of about 20 dense units separated by clear spaces.  $\times 800,000$ .

FIGURE 6

Shadow cast by model of virus particle orientated in such a way that the number and distribution of the peripheral units are in closest agreement with those of the sectioned particle in Fig. 5.



interesting to note that if a portion of a solid cylinder of length equal to the diameter of the cylinder is converted into a sphere, the diameter of the sphere is greater than that of the cylinder by 14.5 per cent. This is close to the amount by which the measured diameter of the spherical particles exceeds that of the filaments. An alternative interpretation, however, is that the filaments are another, perhaps aberrant, form of the virus in which growth in length rather than formation of spherical particles has occurred.

#### SUMMARY

The fine structure of polyoma virus was examined in thin sections of infected cells and in partially purified preparations of the virus treated by the negative contrast method. The virus occurs mainly in the form of spherical particles consisting of a core of diameter  $28 \mu$  and a capsid of width  $5 \mu$  as measured in sections, but larger particles of diameter about  $60 \mu$  and long tubules or filaments are also seen. The surface of the filaments is composed of units similar to the capsomeres of the small spherical particles.

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#### FIGURE 7

Partially purified virus preparation stained by the negative contrast method. The surface of the long filamentous element is composed of subunits similar to the capsomeres of the spherical particles.  $\times 120,000$ .

#### FIGURE 8

Part of another filament showing the surface structure in more detail. Negative contrast.  $\times 250,000$ .

#### FIGURE 9

Negative stained spherical particles at the same magnification as the filament in Fig. 8.  $\times 250,000$ .

#### FIGURE 10

Part of the nucleus of a cell infected with polyoma virus showing the appearance of filaments at low magnification in a thin section.  $\times 62,000$ .

#### FIGURE 11

Group of filaments in a section through an infected nucleus at high magnification, showing central dense cylindrical core and narrow, less dense sheath surrounding the core. Lead hydroxide staining.  $\times 200,000$ .

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