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 mμ 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 mμ 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 mμ 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 mμ, and a more recent determination by Wildy *et al.* (3) who used the negative contrast method gave an average value of 45.3 mμ. 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 mμ 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 mμ.

It is clear from our recent work on sectioned specimens that there is indeed a layer of material of width about 5 mμ around each particle, the over-all diameter being therefore about 38 mμ. 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.

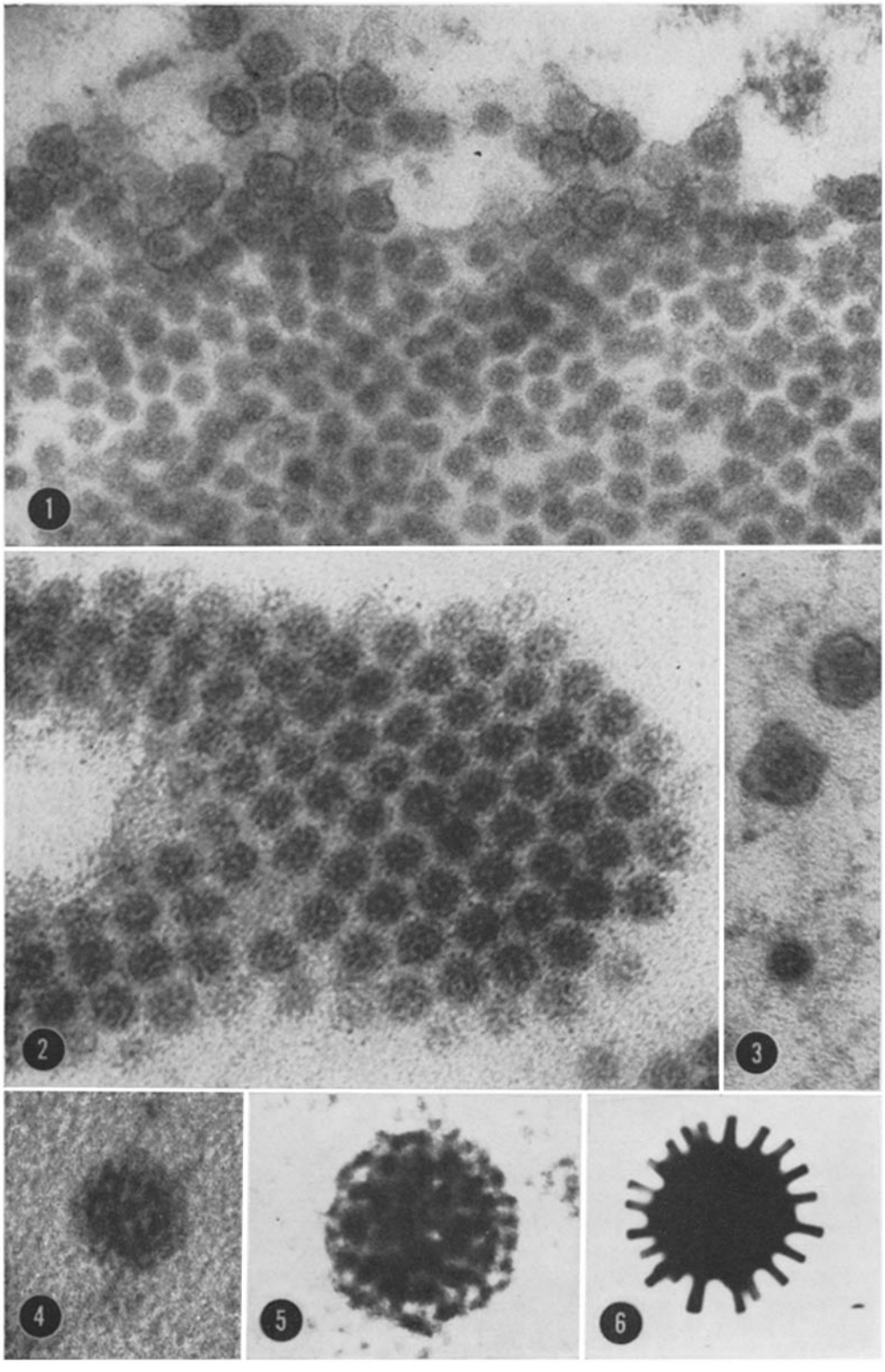
Further indication that the size of the particles is greater than 28 mμ has been obtained by measurements on particles arranged in crystalline formation. In Fig. 2 is illustrated a group of particles in close-packed hexagonal array. The average centre to centre separation between particles is 36 mμ, 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 mμ 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 mμ 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 mμ 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 mμ. 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 mμ. The filaments have an appreciably smaller diameter, the mean value being 38 mμ.

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

FIGURE 1

Portion of a large aggregate of 38 mμ polyoma virus particles; each particle consists of a dense core of diameter 28 mμ surrounded by a layer of less dense material. At the edge of the aggregate are several larger particles of diameter approximately 60 mμ which appear to form by the addition of a membrane to the 38 mμ particles. Lead hydroxide staining. × 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 mμ. Lead hydroxide staining. × 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. × 225,000.

FIGURE 4

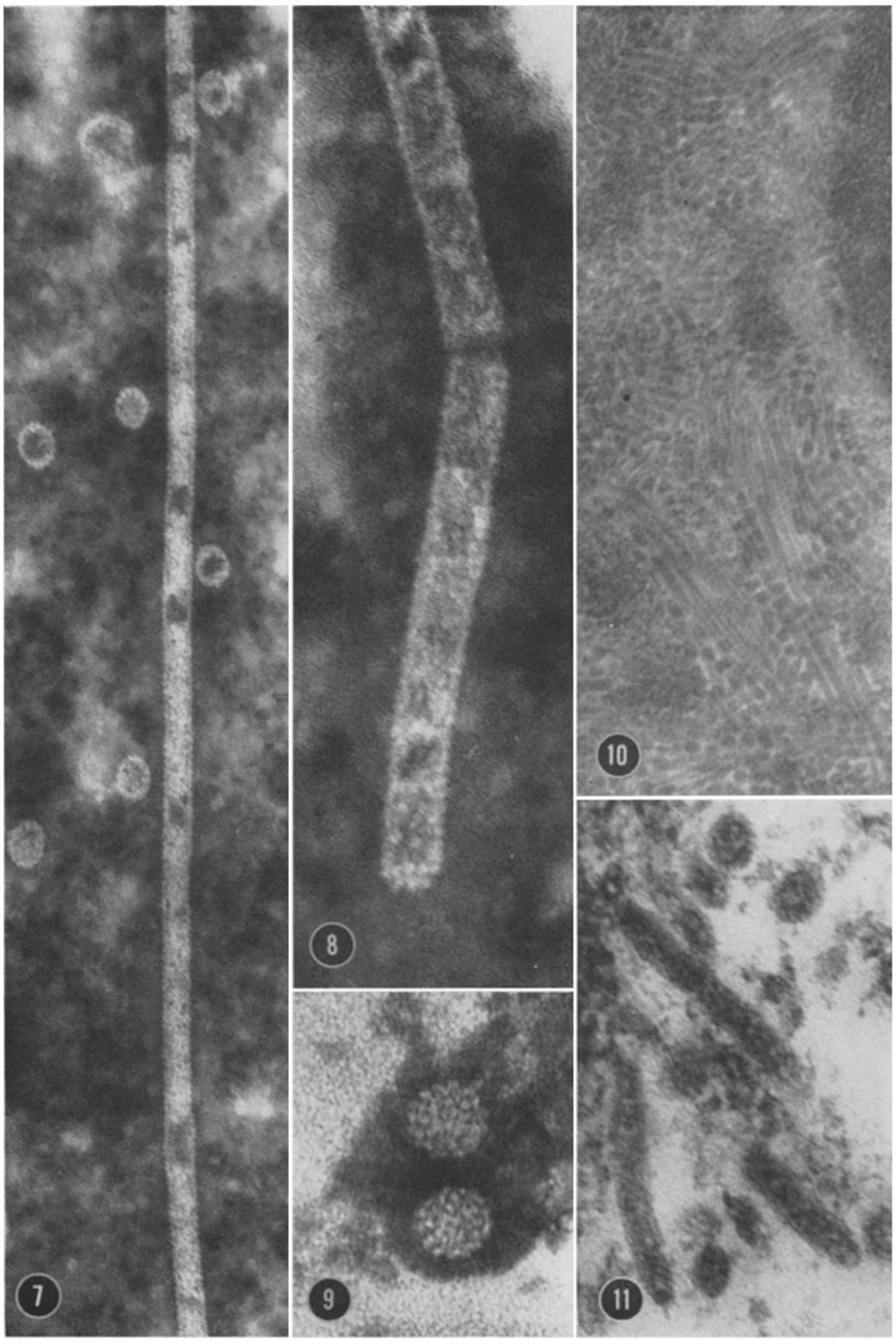
Higher magnification picture of 38 mμ particle stained with uranyl acetate, showing peripheral layer of low density. × 480,000.

FIGURE 5

38 mμ 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. × 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 mμ and a capsid of width 5 mμ as measured in sections, but larger particles of diameter about 60 mμ 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.

REFERENCES

1. HOWATSON, A. F., MCCULLOCH, E. A., ALMEIDA, J. D., SIMINOVITCH, L., AXELRAD, A. A., and HAM, A. W., Studies *in vitro, in vivo* and by electron microscope of a virus recovered from a C3H mouse mammary tumor: Relationship to polyoma virus, *J. Nat. Cancer Inst.*, 1960, **24,** 1131.
2. HOWATSON, A. F., and ALMEIDA, J. D., An electron microscope study of polyoma virus in hamster kidney, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 753.
3. WILDY, P., STOKER, M. G. P., MACPHERSON, I. A., and HORNE, R. W., The fine structure of polyoma virus, *Virology*, 1960, **11,** 444.
4. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 727.
5. GESSLER, A. C., BENDER, C. E., and PARKINSON, M. C., A new and rapid method for isolating viruses by selective fluorocarbon deproteinization, *Tr. New York Acad. Sc.*, 1956, **18,** 701.
6. BRENNER, S., and HORNE, R. W., A negative staining method for high resolution electron microscopy of viruses, *Biochim. et Biophysica Acta*, 1959, **34,** 103–110.
7. BERNHARD, W., FEBVRE, H. L., and CRAMER, R., Mise en evidence au microscope électronique d'un virus dans des cellules infectées *in vitro* par l'agent du polyome, *Compt. rend. Acad. s c*, 1959, **249,** 483.
8. BANFIELD, W. G., DAWE, C. J., and BRINDLEY D. C., Intracellular and extracellular particles

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. × 120,000.

FIGURE 8

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

FIGURE 9

Negative stained spherical particles at the same magnification as the filament in Fig. 8. × 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. × 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. × 200,000.

in tissue cultures inoculated with parotid tumor agent (polyoma virus), *J. Nat. Cancer Inst.*, 1959, **23,** 1123.

9. DMOCHOWSKI, L., GREY, C. E., and MAGEE, L. A., Studies on a virus ("Polyoma") inducing multiple tumors in animals, *Proc. Soc. Exp. Biol. and Med.*, 1959, **102,** 575.
10. KAHLER, H., ROWE, W. P., LLOYD, B. J., and HARTLEY, J. W., Electron microscopy of mouse parotid tumor (polyoma) virus, *J. Nat. Cancer Inst.*, 1959, **22,** 647.
11. DOURMASHKIN, R. R., and NEGRONI, G., Identification with the electron microscope of particles associated with polyoma virus in induced parotid gland tumours of C3H mice, *Exp. Cell Research*, 1959, **18,** 573.