Observations on the Fine Structure of Type 5 Adenovirus. By M. A. EPSTEIN. (*From the Bland-Sutton Institute of Pathology, The Middlesex Hospital, London, England.*)

Since the first observation with the electron microscope of intranuclear virus-like particles in sections of tissue culture cells infected with adenovirus (1), numerous later studies of similar material have established certain morphological features of this agent (2–5). The particles have been reported to be round or ellipsoid and an inner body and outer limiting membrane have been described (3–5). On the other hand particles dried from suspension and examined as whole mounts appeared polyhedral in shape (6, 7), but some workers consider that this may merely represent an artefact caused by the drying (5).

With the exception of a few specimens fixed with formalin or formalin followed by osmium (5), all the work on sectioned adenovirus has been concerned with material fixed with buffered osmium, either alone (8) or with dichromate added (9). Now it has recently been shown that potassium permanganate fixation for electron microscopy (10) is of especial value when applied to viruses; not only has it revealed important structures in both vaccinia virus (11, 12) and the Rous virus (13), but it has consistently given outstandingly good morphological preservation of virus particles throughout the material to which it was applied.

It was considered, therefore, that useful information might be obtained regarding the fine structure of a virus of the adeno group by the use of permanganate fixation in conjunction with thin sectioning techniques for electron microscopy. The present brief note reports the preliminary results which have been obtained.

Materials and Methods

Suspensions of type 5 adenovirus have been prepared from infected HeLa cell cultures by treatment with a fluorocarbon (14); the fluorocarbon was applied 3 times using methods described elsewhere (12). Pellets were then obtained from the suspensions by high-speed centrifugation and samples were taken from their virus-containing zones for permanganate fixation, dehydration, and embedding in *n*-butyl methacrylate exactly as in earlier work (12). Sections were cut with a diamond knife (15) attached (16) to a Porter-Blum microtome and were mounted, after flattening by the method of Sotelo (17), on carbon-coated grids (18) for examination in a Philips electron microscope, type EM100.

OBSERVATIONS

Samples from the virus-containing zones of the pellets were found to be composed of uniform particles about 60 mμ in diameter unaccompanied by any other recognisable formed structures (Fig. 1); biological tests combined with the morphological studies have established the viral nature of the particles.

At higher magnification it was found that the particles were hexagonal in outline and varying amounts of individual particles were present in a given section. Those particles which were cut centrally and which, in consequence, had a substantial segment included, could be seen to contain a central dense nucleoid surrounded by a less dense outer zone or viroplasm (Fig. 2); a fine outer limiting membrane was also observed (Fig. 2).

In very thin sections examined at high magnification the hexagonal profile of the particles was particularly clear (Figs. 3 and 4) and their measurements could be taken with relative ease. The dense nucleoids were found to have a diameter of about 35 mμ whilst the outer part of the virus, of low density, formed a zone about 12 mμ across (Fig. 3), the whole particle being thus about 60 mμ in diameter. When observed in such sections, the limiting membranes around the particles were tenuous or even indistinct and the nucleoids appeared to consist of two structurally differentiated elements (Figs. 3 and 4). There was a diffuse dense material and a thread-like component; the latter often seemed as if rolled into a ball in a haphazard manner (Fig. 3), but in favourably oriented sections of extreme thinness it could be seen to be arranged in parallel curving array (Fig. 4). The apparent structure of the virus as seen in thin sections is shown diagrammatically in Fig. 5.

DISCUSSION AND CONCLUSIONS

The present work has shown that methacrylate-embedded adenovirus has a hexagonal outline when observed in thin sections with the electron microscope. This confirms the earlier findings from whole mount preparations of this virus air-dried from suspension (6, 7) and disposes of the view that in such material the shape might have been altered in the course of drying (5). That the virus has hitherto been found to be round when examined in methacrylate sections (1–5) could have been due either to inferior preservation by the fixatives used as compared to permanganate, or to the

fact that the particles studied were almost exclusively intracellular; it might be that only free extracellular particles possess a hexagonal profile. Since the particles described here have shown this feature irrespective of their orientation to the plane of sectioning, the virus must be a polyhedron in shape; it has already been suggested from results obtained with whole mounts that the form of polyhedron involved might be an icosahedron (6), and that this is so has been established quite recently by the use of newly introduced techniques (19).

The insubstantial appearance of the outer membrane of the virus which has been found on examining the thinnest sections (Figs. 3 and 4) suggests that the outer zone or viroplasm of this agent extends to the surface without being covered by a series of molecular layers of different composition. Such a covering probably explains the situation in the case of other viruses studied after permanganate fixation in which very definite double outer limiting membranes have been found (12, 13), the term membrane being used in the sense which has recently been discussed elsewhere (20). On the other hand, in view of the line of slight surface electron density discernible in the thicker sections of adenovirus (Fig. 2), it might be that the material of the viroplasm shows some special molecular orientation in its outermost layers. The appearances in question could also result from the deposition of traces of contaminating material on the outside of the particles during extraction, or from minor changes in the surface layers brought about during preparation.

No surface structure has been detected in the sectioned virus particles examined in the present work although it is very evident in similar material prepared by the new air-dried whole mount methods of Brenner and Horne (19). This difference could result either from a failure of the present techniques to reveal such surface structure or from the fact that the virus is in actuality smooth, the underlying structural organisation being made manifest by some procedure in the preparation method which Brenner and Horne have used (19).

The size of the virus particle reported here agrees well with all previous findings (1–5) in which material has been examined without air drying and possible accompanying distortion, and the diameter of the inner body or nucleoid also shows good agreement. The apparent structural differentiation observed here in the nucleoid has not previously been described; although so far no information is available concerning the significance of this finding, it is tempting to regard it as a reflection of the presence of more than one component within this region of the virus

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Explanation of Plate 253

PLATE 253

All the figures, apart from the last one, are electron micrographs of pellets of purified adenovirus.

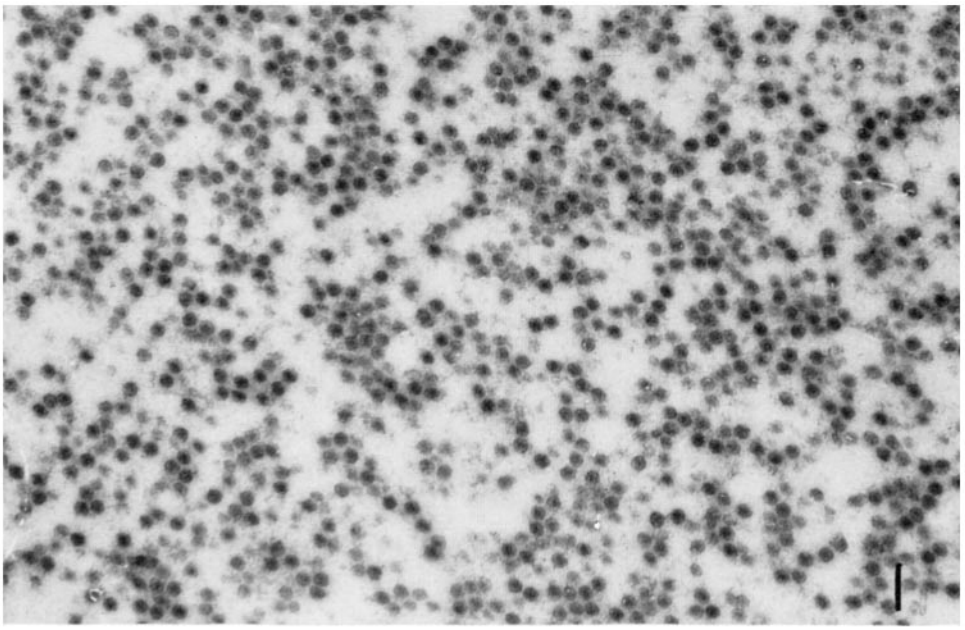
FIG. 1. Survey picture of a section cut through a representative region of a virus-containing zone of a pellet. Uniform particles about 60 mμ in diameter fill the field; no other recognisable formed elements are present. × 25,000.

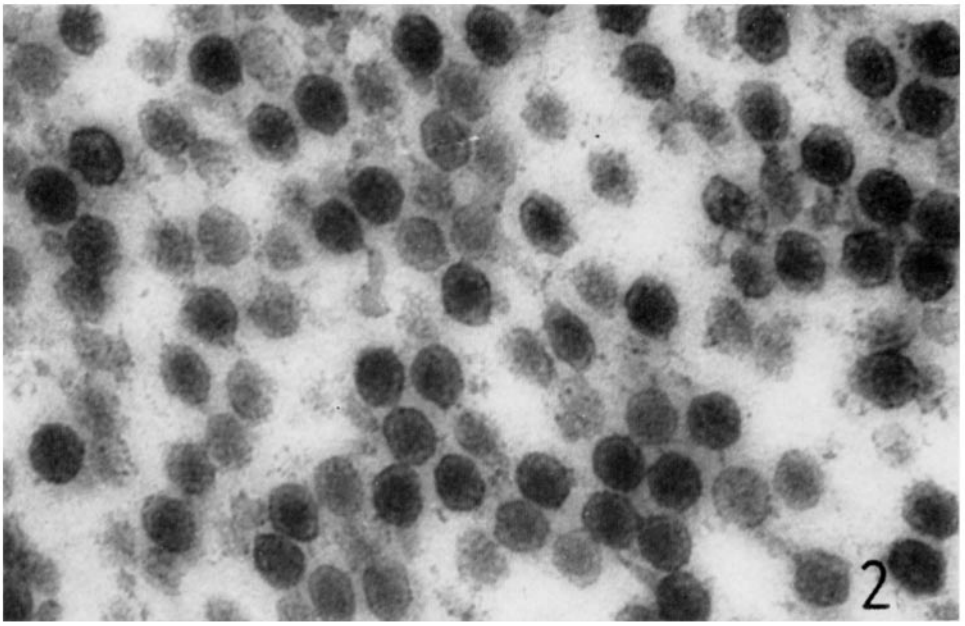
FIG. 2. Detail of a small area of a section cut through the virus-containing zone of a pellet. The individual particles are hexagonal in outline and are bounded by a very fine membrane. Particles cut centrally and having a substantial segment included in the section can be seen to possess a central dense nucleoid surrounded by a less dense outer zone or viroplasm. × 102,000.

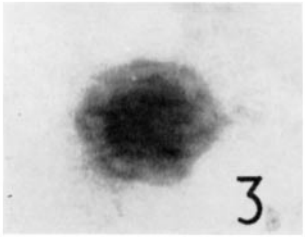
FIG. 3. Section through an individual adenovirus particle. The hexagonal profile, central dense nucleoid surrounded by a less dense viroplasm, and tenuous outer limiting membrane can be seen. The nucleoid seems to consist of diffuse material together with a thread-like component rolled into a ball in a haphazard manner. × 300,000.

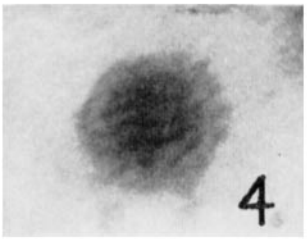
FIG. 4. Very thin section through an individual adenovirus particle showing the structure of the nucleoid with its diffuse dense material and thread-like component arranged here in parallel curving array. × 300,000.

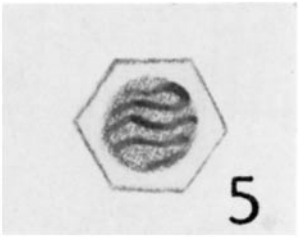
FIG. 5. Diagram to show the assumed fine structure of an adenovirus particle deduced from its appearance in electron micrographs of thin sections such as that of Fig. 4. × 300,000.











(Epstein: Type 5 adenovirus)