Electron Microscopic and Histochemical Studies of an Unusual Crystalline Protein Occurring in Cells Infected by Type 5 Adenovirus. Preliminary Observations.*

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In the course of studies which are designed to yield information concerning the physical and chemical nature of the adenoviruses, as well as the manner of their development in infected cells, it has recently been discovered that type 5 adenovirus exhibits certain remarkable features. Earlier electron microscopic observations of types 3, 4, and 7 adenovirus in thin sections of HeLa cells showed that the viral particles develop in the nucleus and frequently exhibit crystalline arrangement in a cubic body-centered lattice (1, 2). Histochemical studies demonstrated that these crystals are strongly Feulgen-positive, thus indicating that the virus contains deoxyribonucleic acid (3). Type 5 resembles types 3, 4, and 7 with respect to size, shape, internal structure, development in cell nuclei, and formation of Feulgen-positive crystals. However, a large proportion of cells which are infected by type 5 virus, as revealed by the electron microscope, also contain extraordinary crystals that are not composed of viral particles. These crystals often exceed 30 μ in length and are readily visible in the light microscope. Histochemical procedures show that they are composed of protein and are devoid of nucleic acid.

Type 5 adenovirus was supplied by Dr. Maurice Hilleman, Walter Reed Army Institute of Research. The virus was propagated in the HEp2 line of cells, originally derived from human laryngeal carcinoma by Dr. Alice Moore, Sloan-Kettering Institute, and obtained by us from Mrs. Joan B. Daniels, Massachusetts State Diagnostic Laboratory. Cell cultures were maintained in a modified Hanks-Eagle solution containing 10 per cent horse serum. The methods of preparing specimens for histochemical study in the light microscope and for electron microscopic examination have been previously described (1, 3).

Fig. 1 illustrates part of a nucleus containing an irregularly shaped crystal with smooth faces. Two aggregates of characteristic viral particles are present in the nuclear matrix adjacent to the crystal. The inset at the lower right is a photograph obtained by light microscopy of the same nucleus in a contiguous thick section stained with the Feulgen technique. The viral aggregates are Feulgen-positive and therefore appear as two semiopaque regions. The crystal separating them is Feulgen-negative and hence is unstained.

Fig. 2 shows the interior of a nucleus containing numerous scattered viral particles. A six-sided crystal, devoid of

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virus, is present near the lower border. Fig. 3 illustrates a nucleus containing three crystals composed of virus. The lattice of these viral crystals appears to be identical with that encountered in studies of cells infected with types 3, 4, and 7 adenovirus.

Fig. 4 illustrates part of a protein crystal and reveals the molecular lattice. At the lower left corner are several viral particles. The variation in density of these particles is characteristic of adenovirus (1). In Fig. 5 an intranuclear crystal is oriented with respect to the plane of section in such a manner that the molecules form a linear pattern spaced at 400 A. Viral particles are scattered on the periphery. It is impossible to determine whether two viral particles near the lower left corner are incorporated within the crystal or reflect superimposition in the section. Examination of many such crystals has shown that, although virus is occasionally encountered within the crystalline matrix, the majority do not contain recognizable particles.

It is evident from preliminary observations that these crystals, unlike the viral crystals, are generally elongated. In cross-section (Fig. 4) the molecules are seen as points of density arranged in a regular lattice, whereas in longitudinal or oblique sections (Fig. 5) superimposition results in the appearance of parallel bands.[1]

[1] An identical phenomenon was observed in sections of the polyhedra associated with *Bombyx mori* L. (silkworm) and *Porthetria dispar* L. (gipsy moth) insect viruses. It is important to point out, however, that the intranuclear polyhedra, in contrast to the protein crystals associated with type 5 adenovirus, exhibit a lattice with closer molecular spacing and enclose numerous viral particles (4).

The crystals develop only in nuclei containing viral particles. In cells with disrupted nuclei, crystals may be encountered in the cytoplasm, and in disintegrating cells they are observed in the process of being released.

The crystals in sections of cells fixed in osmium tetroxide, as well as those in smears fixed in formalin, methanol, or Carnoy's solution are uniformly unstained after the Feulgen reaction and after exposure to 0.025 per cent azure B bromide solution at pH 4.0, indicating the probable absence of both DNA and RNA. They show a moderately intense coloration with the xanthoproteic test for aromatic amino acid residues and are strongly colored by a modified cytochemical Millon reaction (5), indicative of the presence of tyrosine residues in a protein. The presence of amino groups, principally of lysine residues in the protein of the crystal, is revealed by the binding of naphthol yellow S (flavianic acid) which stains chiefly the basic groups of lysine, arginine, and histidine residues (6). The crystals are only slightly colored after the Sakaguchi reaction for arginine, differing markedly in this respect from nucleohistone in the nucleus. They also fail to stain with the periodic acid-Schiff method.

The protein crystals in HEp2 cells are also seen in HeLa cells infected by type 5 adenovirus. They have not been found in cultures of normal HeLa and HEp2 cells, nor have they been associated thus far with infection by other viruses. Their existence under these circumstances makes it almost certain that they are specifically related to the effect of the virus upon the cell and that they represent either (*a*) a cellular product which is distinct from any component of the virus, (*b*) an abnor-

mal protein related immunochemically to the virus, or (*c*) the actual protein component of the virus which has not been combined with DNA to form complete viral particles. Of these possibilities the first seems unlikely, but the decision must await the results of chemical and immunologic studies. In any event, the crystals presumably result from some peculiarity or abnormality in development of the virus, since electron microscopic examination has failed to disclose them in cells infected by types 1 and 2, as well as types 3, 4, and 7.[2] It is attractive to postulate that the crystalline protein is related to, or even identical with, the protein of the virus, and that it may represent a counterpart of the non-infective proteins of plant viruses, such as those of tobacco mosaic and turnip yellow mosaic disease, which lack nucleic acids (7–11).

[2] The protein crystals conceivably may be associated with one or more of the 12 remaining antigenic types of adenovirus which have not yet been studied, and an investigation of this possibility has already been started.

Bibliography

1. Morgan, C., Howe, C., Rose, H. M., and Moore, D. H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 351.
2. Low, B. W., and Pinnock, P. R., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 483.
3. Bloch, D. P., Morgan, C., Godman, G. C., Howe, C., and Rose, H. M., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 1.
4. Morgan, C., Bergold, G. H., Moore, D. H., and Rose, H. M., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 187.
5. Rasch, E. M., and Swift, H., *J. Histochem. and Cytochem.*, 1953, **1,** 392.
6. Deitch, A. D., *Lab. Inv.*, 1955, **4,** 324.
7. Markham, R., Matthews, R. E. F., and Smith, K. M., *Nature*, 1948, **162,** 88.
8. Takahashi, W. N., and Ishii, M., *Nature*, 1952, **169,** 419.
9. Jeener, R., and Lemoine, P., *Nature*, 1953, **171,** 935.
10. Commoner, B., Yamada, M., Rodenberg, S. D., Wang, T., and Basler, E., Jr., *Science*, 1953, **118,** 529.
11. Bawden, F. C., and Pirie, N. W., *J. Gen. Microbiol.*, 1956, **14,** 460.
12. Morgan, C., Moore, D. H., and Rose, H. M., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 21.

Explanation of Plates

Plate 165

Fig. 1. Part of a nucleus containing two aggregates of viral particles separated by a crystal. The knife has passed diagonally across the field imposing scratches and periodic banding on the crystal (12). The nuclear membrane traverses the right and lower borders. × 11,000.

The inset shows the same nucleus in a contiguous, thick, Feulgen-stained section photographed in the light microscope. The viral aggregates are Feulgen-positive. The unstained crystal is Feulgen-negative. The dense granules in the lower right are osmiophilic.

Fig. 2. A six-sided intranuclear crystal with adjacent viral particles oriented at random. When viewed at sufficient magnification the molecular array is clearly visible, revealing that the crystal has been sectioned nearly at right angles to the long axis. × 20,000.

Fig. 3. Three crystals composed of viral particles. The parallel dense zones indicate particles central to the plane of section and reflect orientation of the crystalline lattice (1). × 15,000.

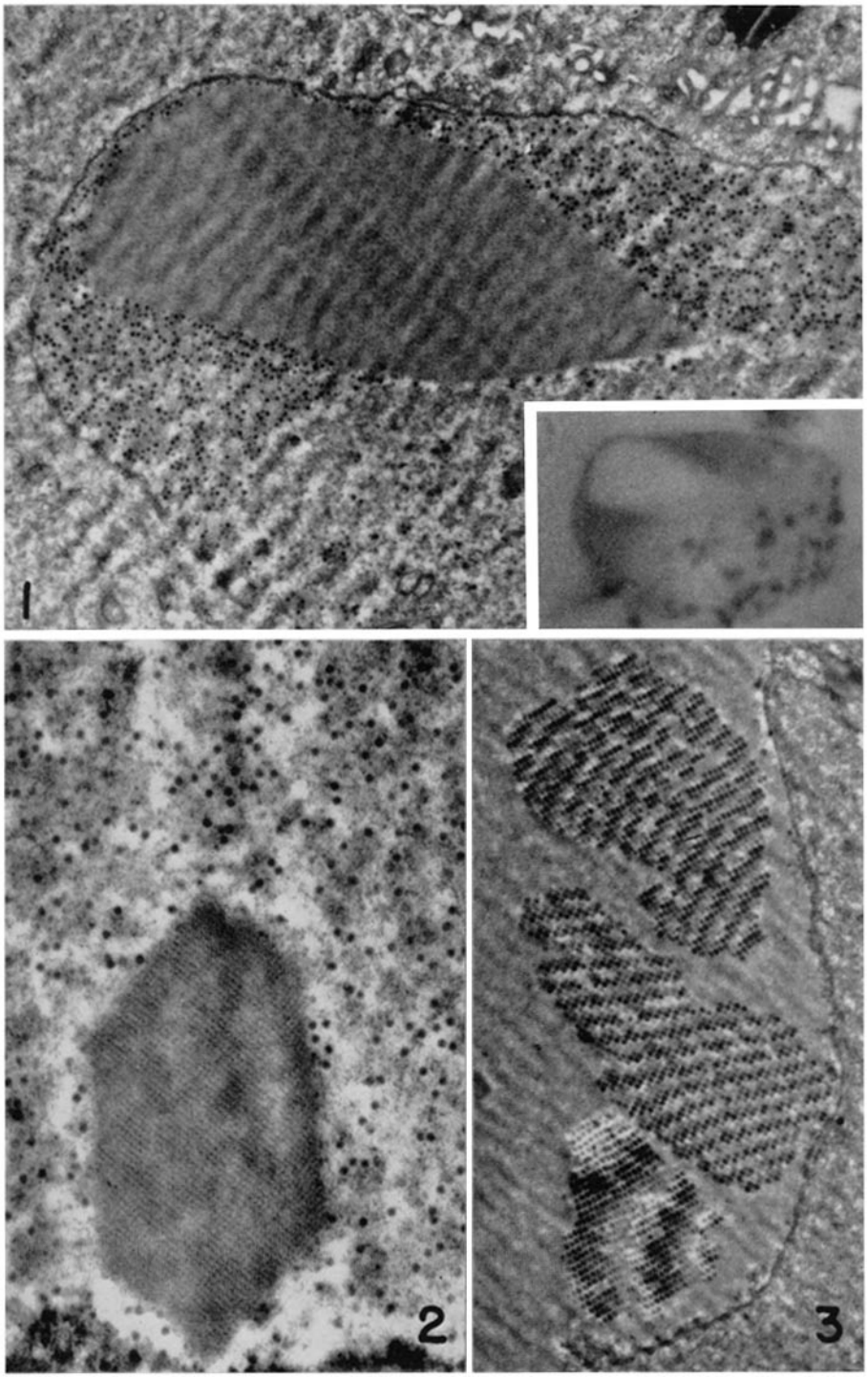

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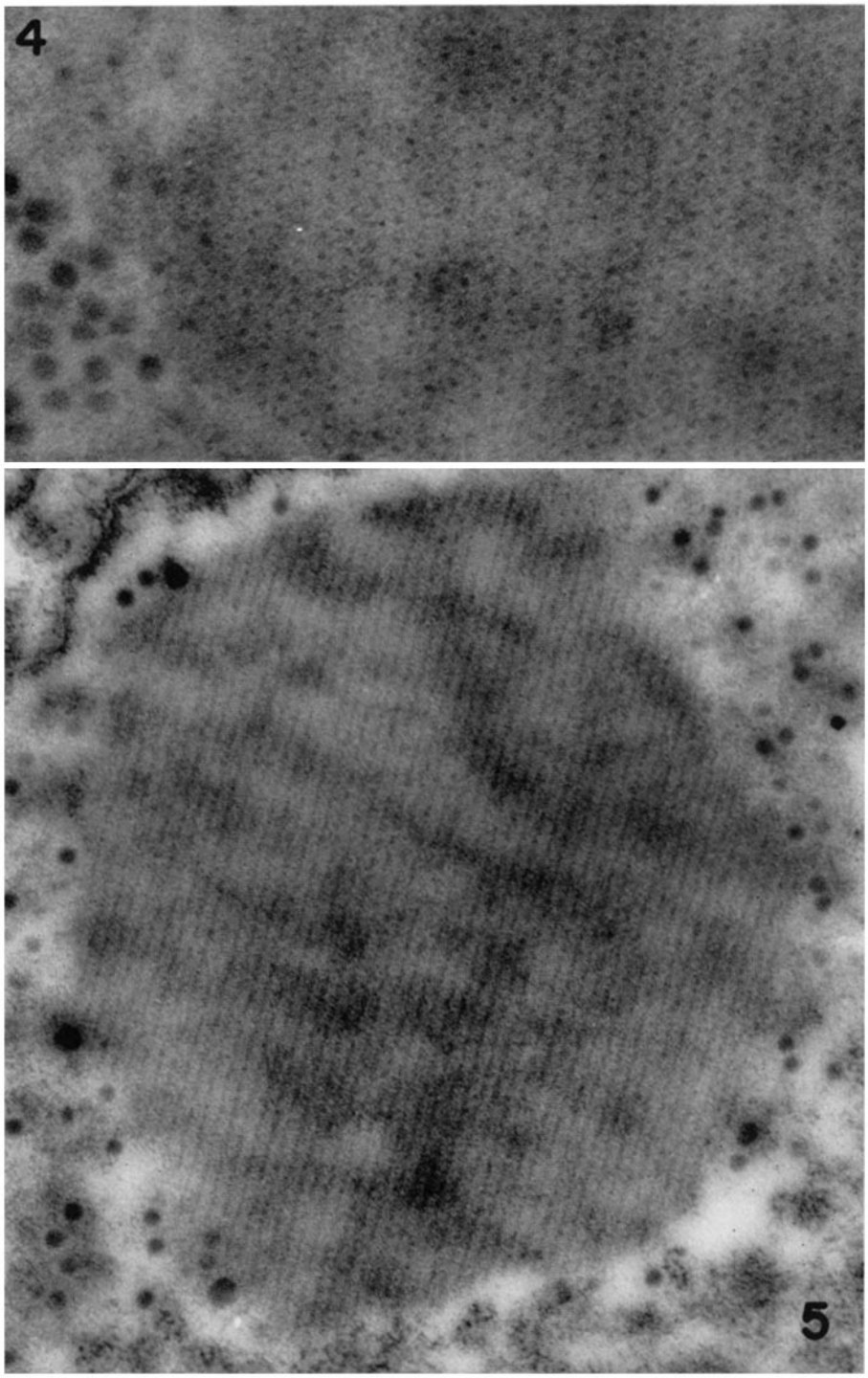
PLATE 166

FIG. 4. Part of a crystal in cross-section. The points of increased density reflect the pattern of the crystalline lattice. Characteristic viral particles are visible in the nuclear matrix at the lower left corner. × 75,000.

FIG. 5. An intranuclear crystal sectioned at such an angle that superimposition of the molecules has resulted in a linear pattern. The lines exhibit an average spacing of 400 A. Viral particles are present at the periphery. × 48,000.



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