Investigation on Intranuclear Paracrystalline Inclusions
Induced by Adenovirus 5 in KB Cells*

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Intranuclear paracrystalline bodies have been observed in cells infected with adenovirus type 5 (Leuchtenberger & Boyer, 1957; Morgan et al. 1957, 1960). The present study reports the attempts to isolate these paracrystalline proteins from KB cell nuclei and discusses their nature and possible role.

KB cells were grown as monolayers in 1 l. bottles (5 x 10^7 to 6 x 10^7 cells/bottle) in Eagle's basal medium supplemented with 10 % calf serum. The cells were infected at a multiplicity of 100/infecting cell units/cell (Warocquier, Ménard & Samaille, 1966) and the virus was allowed to grow for 48 hr. The paracrystalline bodies were extracted directly from infected KB cells by homogenizing them in 1 ~oo (W/V) citric acid (Mirsky & Pollister, 1946) or 0.15 ~oo (v/v) Cemulsol (Zalta et al. 1962) using a Dounce homogenizer. The suspension was centrifuged at 1000 g for 10 min. and the pellet examined in the electron microscope, after fixation in glutaraldehyde + osmium tetroxide and embedding in Araldite (Richardson, Jarett & Finke, 1960). Sections were stained in uranyl acetate + lead hydroxide.

We have, by these two methods, selectively extracted the paracrystals from the nuclei: the paracrystals have completely disappeared, whereas the virus particles are still present within the nucleus, and the nuclei are morphologically well preserved. The cytoplasmic supernatant containing the paracrystalline inclusions was cleared of cytoplasmic organelles and membranes by centrifuging at 45,000 g for 2 hr: the pellet thus obtained contained few virus particles but no crystalline structure.

We observed a relationship between the paracrystalline inclusions and the virus particles. There are structures, similar to virus DNA-nucleoid, in close association with the paracrystal; and, besides many nucleoids next to the paracrystal, partially coated nucleoids and entirely encapsidated virus particles can be observed (Fig. 1). From this observation, it is suggested that the intranuclear inclusions induced by adenovirus 5 could perhaps constitute a reserve of structural proteins for the biosynthesis of the virus capsids. This hypothesis is supported by the fact that we were unable to find in the citric and Cemulsol extracts anything else but the structural proteins of adenovirus and by the results of immunofluorescence staining, carried out as described by Hayashi & Russell (1968); a rat antiserum to adenovirus type 5 and a rabbit antiserum to hexons showed large fluorescent polyhedral intranuclear inclusions corresponding to paracrystals. The paracrystalline inclusions probably correspond to a peculiar steric arrangement of virus material produced in large excess by the infected cell. The crystalline array would be due to the natural tendency of the virus morphological subunits to assemble. It is also possible that the inclusions are a side product and that the true precursors of virus are directly assembled or go through structures not detected here.

The intranuclear paracrystalline inclusion has been defined by Torpier & Petitprez (1968) as an aggregate of rectilinear tubular protein fibres in parallel disposition. The fine structure of the tubules exhibited on cross-sections at high magnification leads us to propose the model

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Fig. 1. Ultrathin section of KB cell infected by adenovirus type 5. The nucleus appears swollen and multilobed and contains, besides many densely stained virus particles, very large paracrystalline inclusion bodies. These paracrystals fill about 50 to 70% of the nuclear volume ($\times 9500$). Cross (CS) and longitudinal (LS) sections of the paracrystals are presented at higher magnification ($\times 60,000$).
presented on Fig. 2. Since the major components of the paracrystals seem to be adenovirus material, i.e. capsid subunits, or precursors or side products of the virus protein synthesis, it is tempting to assume that the tubular subunits correspond to virus capsid subunits (hexon, penton and fibres), as suggested by the size of these morphological subunits. However, further studies are required to confirm this hypothesis.

Fig. 2. An ultrastructural model for the tubular fibre of the paracrystal is presented and compared with electron micrograph at high resolution of cross-section of a paracrystal. In cross-section, some tubules exhibit clear evidence of being constituted of regularly arranged subunits (indicated by arrows). These subunits possibly correspond to virus capsid subunits, hexon, penton and fibres. Scale represents 1000 Å.

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REFERENCES


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