## The Occurrence of Intranuclear Crystals in Living HeLa Cells Infected with Adenoviruses.*

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In a recent study of the sequential changes produced by adenoviruses in HeLa cells, Boyer *et al.* (1) reported the presence of deoxyribonucleic acid (DNA) containing crystals after infection with adenoviruses type 3 and type 4. These findings confirm observations reported by other investigators (2–5). In order to exclude the possibility that the crystals might have resulted from fixation or other processes involved in preparing the material for light and electron microscope studies, living cultures were examined under the phase and interference microscopes.

In this study, two series of tissue cultures of HeLa cells were grown on coverslips. One was infected with adenovirus type 3 and the other with adenovirus type 5. As reported previously (1), none of the control cultures showed any crystals, but the cells infected with the adenoviruses revealed the presence of well defined crystals. These crystals were located predominantly in the nuclei and to a lesser extent in the cytoplasm.

The appearance of the crystals in fresh, unfixed, and unstained cells as seen under the interference microscope is illustrated in the accompanying photographs. Figs. 1 and 2 are photographs of cultures infected with adenovirus type 3; Fig. 3 shows cells infected with adenovirus type 5. In Fig. 1, numerous crystalline structures can be seen within the cell labelled *A*. The contrasting picture of two unaffected HeLa cells, resembling those observed in uninfected control cultures, is shown by the cells labelled *B*. Fig. 2 demonstrates two other cells from the same culture; in these cells, the crystals surround a central intranuclear mass, which in stained preparations also contains DNA as demonstrated by the Feulgen reaction (1). Figs. 1 and 2 also demonstrate the variation in size and shape shown by crystals present in living HeLa cells infected with adenovirus type 3. In the interference image, the crystals appear yellow when the surrounding nucleoplasm is purplish-blue.

The appearance of the crystals found in HeLa cells infected with adenovirus type 5 is shown in Fig. 3. Here, in the living cells, as well as in stained preparations the crystals have a characteristic bar-like shape which can be easily distinguished from the crystals observed after infection with adenovirus type 3. Another significant difference between the crystals characteristic of adenovirus type 3 and of adenovirus type 5 infection is their chemical composition. While the crystals of cells infected with adenovirus type 3 show all gradations—from Feulgen negative to strongly Feulgen positive—indicating varying concentrations of DNA, the great majority of crystals in cells infected with the type 5 virus do not give a positive Feulgen reaction. Whether there is a complete absence of DNA in such crystals or whether the concentration of DNA is too low to be detected by the Feulgen reaction cannot

* This investigation was supported in part by research grant C-1814 from the National Institutes of Health, United States Public Health Service, and in part by the Office of The Surgeon General, Department of the Army, under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board.

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§ *Received for publication, January 9, 1957.*

be decided at this time. The different chemical constitution of the two types of crystals is also reflected in their affinity for basophilic dyes (hematoxylin) and acidophilic dyes (eosin and fast green). The crystals which develop after adenovirus type 3 infection vary from acidophilia to basophilia, while nearly all crystals which form following infection with adenovirus type 5 are acidophilic, suggesting the presence of strongly basic groups of proteins.

The observation that the crystals are not only present in fixed preparations, but also in living cultures of HeLa cells infected with adenoviruses lends special significance to these crystalline structures. Whether they represent the infectious virus itself, whether they are a special stage in the formation of the virus, or whether they are products of interaction between virus and host cell cannot be decided at present. Whatever they may turn out to be, the possibility that they represent fixation artifacts can be excluded and their occurrence in living cells should stimulate further investigation of their role in the infectious process.

## Bibliography

1. Boyer, G. S., Leuchtenberger, C., and Ginsberg, H. S., *J. Exp. Med.*, 1957, **105**, 195.
2. Harford, C. G., Hamlin, A., and Parker, E., *Tr. Assn. Am. Physn.*, 1955, **68**, 82.
3. Kjellen, L., Lagermalm, G., Svedmyr, A. M., and Thorsson, K. G., *Nature*, 1955, **175**, 505.
4. Morgan, C., Howe, C., Rose, H. M., and Moore, D. H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 351.
5. Harford, C., Hamlin, A., Parker, E., and van Ravenswaay, T., *J. Exp. Med.*, 1956, **104**, 443.

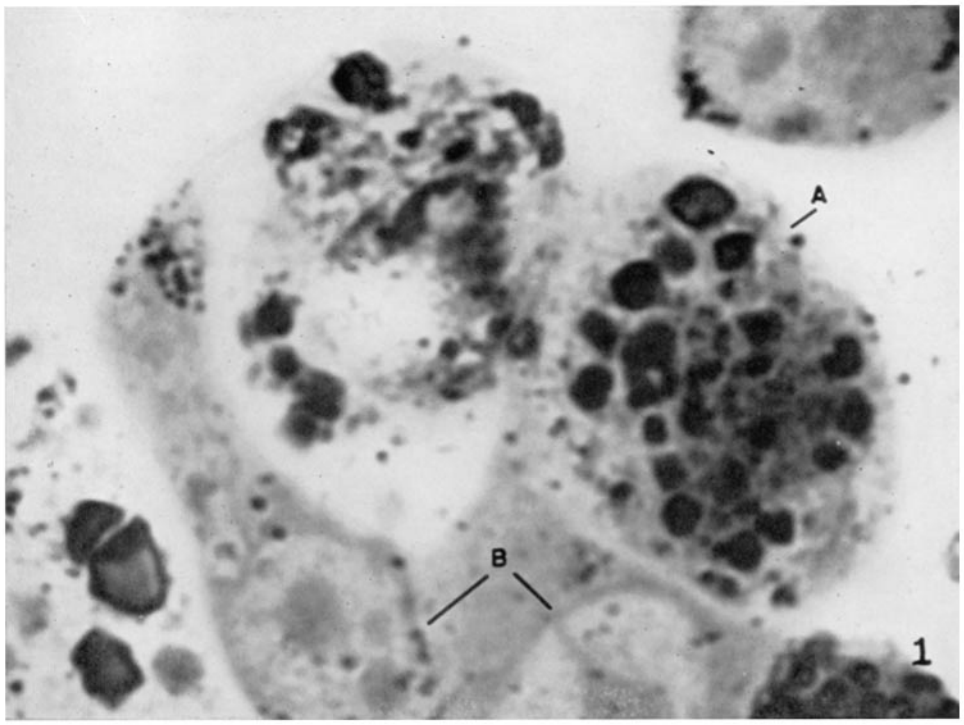
## Explanation of Plates

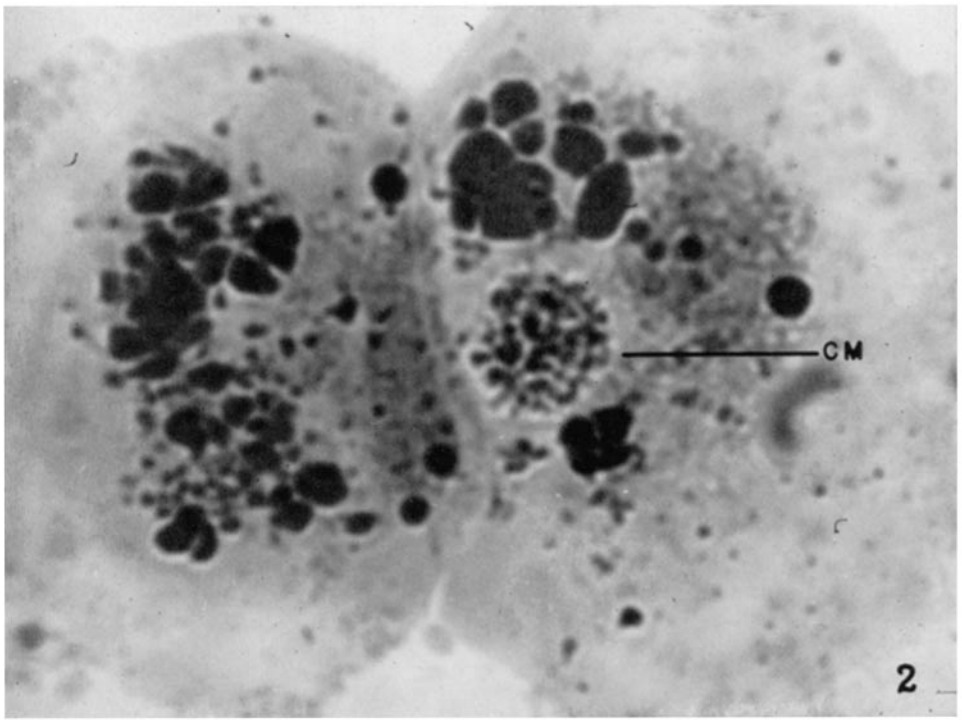
### Plate 103

HeLa cells 63 hours after infection with adenovirus type 3. Fresh preparation unfixed and unstained. Appearance under Baker interference microscope (40x objective). X 1600 (approximately).

FIG. 1. Cell labelled *A* contains many crystals typical of type 3 infection; cells labelled *B* are apparently unaffected, and resemble uninfected HeLa cells.

FIG. 2. Two infected HeLa cells showing crystals. Cell on right contains central mass (CM).



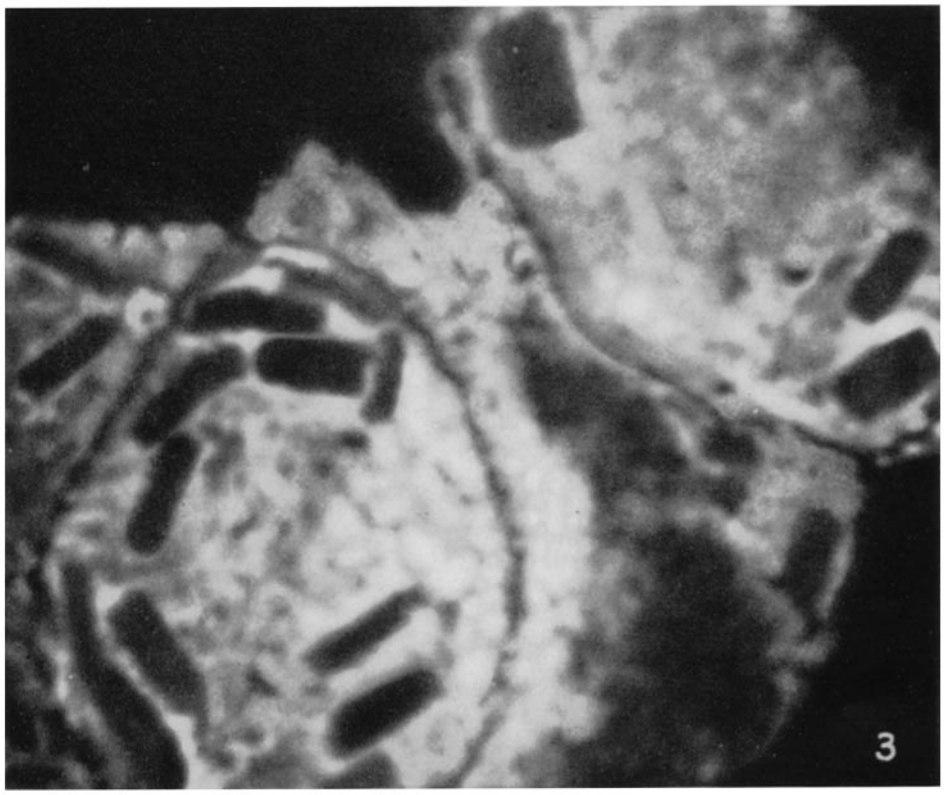




(Leuchtenberger and Boyer: Intranuclear crystals in HeLa cells)

PLATE 104

HeLa cells 36 hours after infection with adenovirus type 5. Fresh preparation unfixed and unstained. Appearance under Baker interference microscope (40x objective). × 3000 (approximately).

FIG. 3. Note bar-like crystals characteristic of infection with adenovirus type 5.




(Leuchtenberger and Boyer: Intranuclear crystals in HeLa cells)