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## **Synthesis of Infective Poliovirus in BSC-1 Monkey Cells Enucleated with Cytochalasin B**

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## Synthesis of Infective Poliovirus in BSC-1 Monkey Cells Enucleated with Cytochalasin B

**Abstract.** *BSC-1 monkey kidney cells were enucleated by two cycles of centrifugation in the presence of cytochalasin B. The resulting populations contained 99.5 percent enucleated cells. After infection, newly synthesized poliovirus was recovered from the enucleated populations. Final virus yield per enucleated cell was about one-fifth the yield per infected untreated cell.*

Cytochalasin B, produced by the fungus *Helminthosporium dematiodeum*, causes profound reversible changes in the form of vertebrate cells in culture (1). A technique has been described for enucleating cells by centrifugation in the presence of cytochalasin B (2). With a modification of the technique, we enucleated more than 99 percent of the cells in a monolayer of BSC-1 monkey cells. Such enucleated monolayers apparently retain the capacity to support biosynthetic activities, since infectious poliovirus could be recovered from them when they were infected after enucleation.

In early experiments with enucleated cell fragments prepared by microsurgery (3, 4), synthesis of poliovirus capsid antigen in these bits of human cell cytoplasm was detected by fluorescence microscopy after incubation with fluorescein-conjugated antibody to poliovirus (3). RNA synthesis in the presence of actinomycin—that is, viral RNA replication (5, 6)—was detected by radioautography with [<sup>3</sup>H]uridine. No newly synthesized infectious poliovirus could be recovered from enucleate fragments

in those studies, because cells containing nuclei were always present in large numbers during the infection.

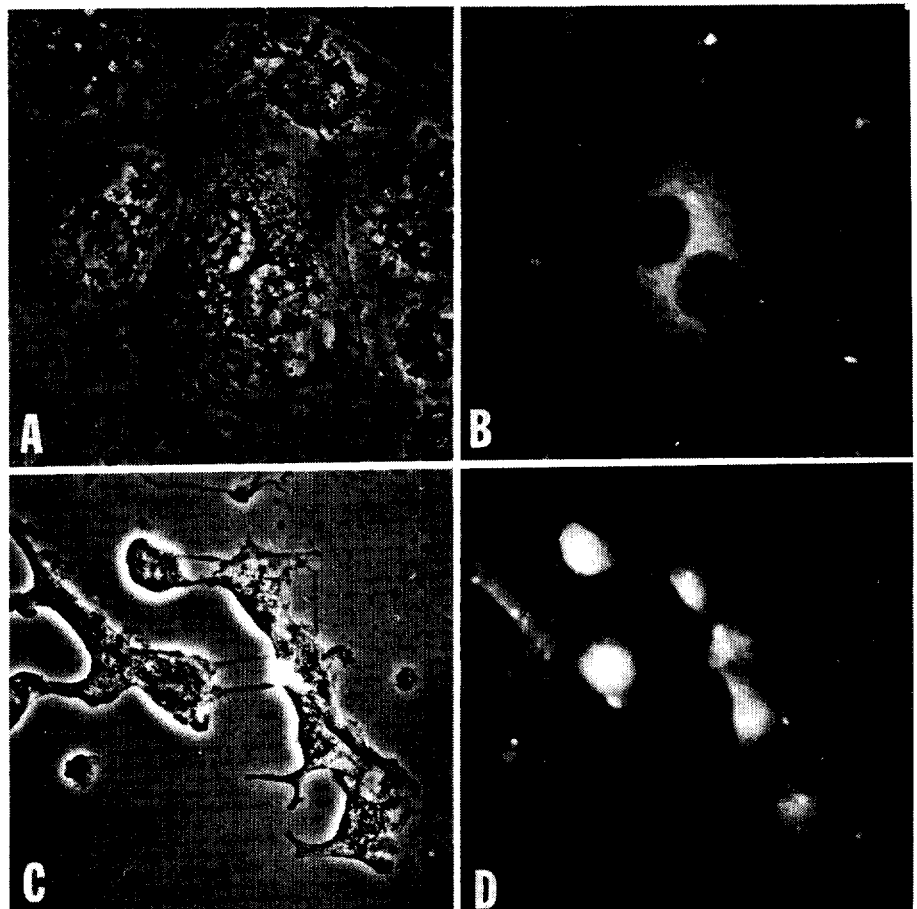
For these experiments, BSC-1 epithelial monkey kidney cells were grown on 12-mm circular glass cover slips in Dulbecco's modification of Eagle's medium supplemented with 10 percent calf serum. While the cells remained on cover slips, their nuclei were drawn off en masse by transferring the cover slips to centrifuge tubes with medium containing cytochalasin B (10  $\mu$ g/ml), and simultaneously centrifuging the cover slips at the bottom of Corex test tubes at 5000 rev/min for 50 minutes at 37°C (2). After enucleation the cells were washed free of cytochalasin B with normal medium. By 1 hour after centrifugation they recovered from the effects of the drug and appeared normal

with regard to general shape and morphology (7). One cycle of centrifugation in the presence of cytochalasin B removed nuclei from 95 percent of the cells on the cover slip.

Once the cover-slip cultures recovered fully from the effects of the treatment, they were subjected to a second cycle of enucleation. Approximately 95 percent of the cells that retained their nuclei in the first cycle lost them in the second cycle. Enucleated cells were not visibly damaged by this second treatment. In three experiments, an average of 99.5 percent of the cells on the twice-treated cover slips were enucleated.

Neither 50 minutes in the presence of cytochalasin B nor centrifugation in the absence of cytochalasin B removed nuclei from the cells. We have not carried out more than two sequential enucleations on any cover slip.

Cover slips, either enucleated or untreated, were placed in separate 9.6-cm<sup>2</sup> petri dishes and infected for 30 minutes with 0.1 ml of type 1 poliovirus at a multiplicity of 100 plaque-forming units (PFU) per cell. Unadsorbed virus was removed by three washes with serum-free medium. One milliliter of medium



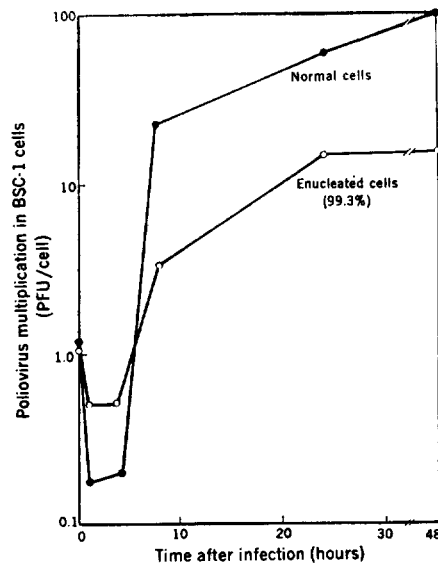
**Fig. 1.** Poliovirus antigen detected by fluorescence in the cytoplasm of infected BSC-1 monkey cells. Cells on cover slips were fixed in acetone : methanol (3 : 1), stained with monkey antiserum to poliovirus type 1, and counterstained with horse antiserum to monkey immunoglobulin G. Cover slips were mounted in glycerol : phosphate-buffered saline free of Ca<sup>2+</sup> and Mg<sup>2+</sup> (1 : 1). (A) Untreated cells 4 hours after infection are shown with phase contrast illumination ( $\times 400$ ). (B) The same field as in (A) is shown with dark-field ultraviolet illumination. Fluorescent cytoplasm denotes cells in which poliovirus capsid antigen has been synthesized. By 4 hours after infection, 24 percent of the untreated cells and 1 percent of the enucleated cells were fluorescent. (C) Enucleated cells 8 hours after infection are shown with phase contrast illumination ( $\times 400$ ). (D) The same field as in (C) is shown with dark-field ultraviolet illumination. Fluorescence fills the cytoplasm of infected enucleated cells. By 8 hours after infection, 95 percent of the untreated cells and 50 percent of the enucleated cells were fluorescent.

Fig. 2. Titer of poliovirus in infected cover slips of untreated and enucleated BSC-1 cells. Cultures were infected at a multiplicity of 100 PFU per cell for 30 minutes. At times thereafter, dishes with a cover slip in 1 ml of medium were frozen to  $-190^{\circ}\text{C}$  and thawed. The thawed medium was sonicated and titered for plaque-forming units on BSC-1 monolayers (8).

with 10 percent calf serum was then added, and the infection was allowed to proceed at  $37^{\circ}\text{C}$ . Untreated BSC-1 cell cultures responded with a typical cytopathic effect: 4 hours after infection, cells began to come off the cover slip and lyse. Poliovirus had similar cytopathic effect on the enucleated cells, but it was delayed until about 8 hours after infection (Fig. 1).

At different times after infection, cover slips and medium were frozen to  $-190^{\circ}\text{C}$  for later plaque titration of total infectious virus (Fig. 2). Other infected and control cover slips were fixed in acetone:methanol (3:1) immediately after infection and 4 and 8 hours afterward. These were stained with monkey antibody to poliovirus type 1, counterstained with fluorescein-conjugated equine antibody to monkey gamma globulin, and examined for fluorescence under dark-field ultraviolet illumination at  $\times 400$  magnification. Cells with fluorescent cytoplasm were considered to contain newly synthesized viral capsid antigen (Fig. 1). Newly synthesized poliovirus was detected in enucleate populations both by immunoassay (Fig. 1) and plaque assay (Fig. 2).

Only half of the enucleated cells contained detectable amounts of viral capsid antigen 8 hours after infection, at which time more than 90 percent of the untreated cells had synthesized detect-



able antigen. The number of infectious viruses per cell increased about 30-fold in the enucleates, as compared with a 300-fold increase in infected untreated cells (Fig. 2). Thus, enucleates supported capsid synthesis and virus growth less efficiently than did cells with nuclei. Cover-slip cultures subjected to cytochalasin B for 1 hour but not centrifuged, or cultures centrifuged in the absence of the drug, permitted virus growth at a rate indistinguishable from that of untreated cells.

The final yield of poliovirus per fluorescent enucleate cell was about one-fifth the final yield of poliovirus per fluorescent untreated cell (Fig. 2). A reduced yield was expected because some cytoplasm was lost from each cell during enucleation (2), and because both infected and uninfected enucleated cells shrank in size with time in culture (Fig. 1). The number of enucleates never fell by more than 50 percent in

the first 12 hours after enucleation. The yield of poliovirus was high enough to exclude the possibility that infectious virus was released only from the small minority of nucleate cells present on treated cover slips (8).

Enucleated cell fragments made from L cells by cytochalasin B treatment have been used to show that the host cell nucleus is not necessary for uncoating of vaccinia virus and establishment of viral DNA synthesis (9). These data show that monkey cell nuclei are not necessary for uncoating, translation, and replication of poliovirus RNA, and for the assembly of infectious virus.

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8. The time course of synthesis of whole virus in both untreated and enucleated BSC-1 cells is slower than that reported for HeLa cells infected at similar multiplicities (6). This is probably because monolayers, rather than cells in spinner culture, were infected here.
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