# Production Of Viruses in Cell Culture

- Tissues are dissociated into a single-cell suspension by mechanical disruption, followed by treatment with a proteolytic enzyme.

- Cells are cultured in a plastic flask in Minimum Essential Media (MEM). As the cells divide, they cover the plastic surface.
- Epithelial and fibroblastic cells attach to the surface of the plastic and form a monolayer

# Virus Inoculation

Isolation is a long process and gold standard for viruses that can be cultured

- Adsorbed Technique

After the virus inoculation, there is an extra waiting time of 1 hour at 37ºC.

- No Adsorbed Technique

After the inoculation of the virus, a virus-producing medium is introduced without waiting time.

# Cytopathic Effect

- Some viruses kill the cells in which they replicate, and infected cells may eventually detach from the cell culture plate.
- The changes become visible and are called cytopathic effects

- Cell cytoplasm
- Cell nucleus

# Types of CPE

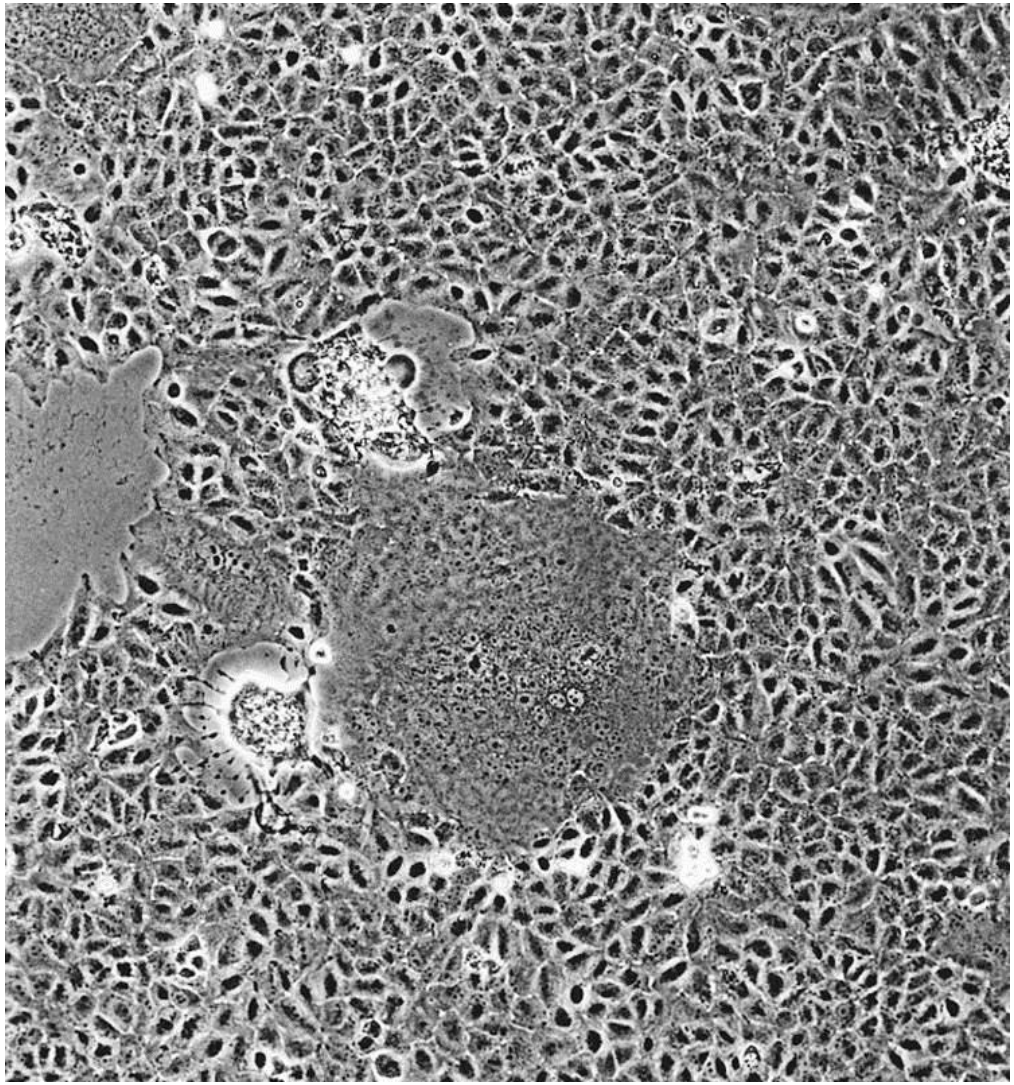
Cytoplasm,

- Rounding,
- Granulation,
- Vacuolization,
- The formation of syncytium (giant cell)
- Formation of inclusion body

In the nucleus,

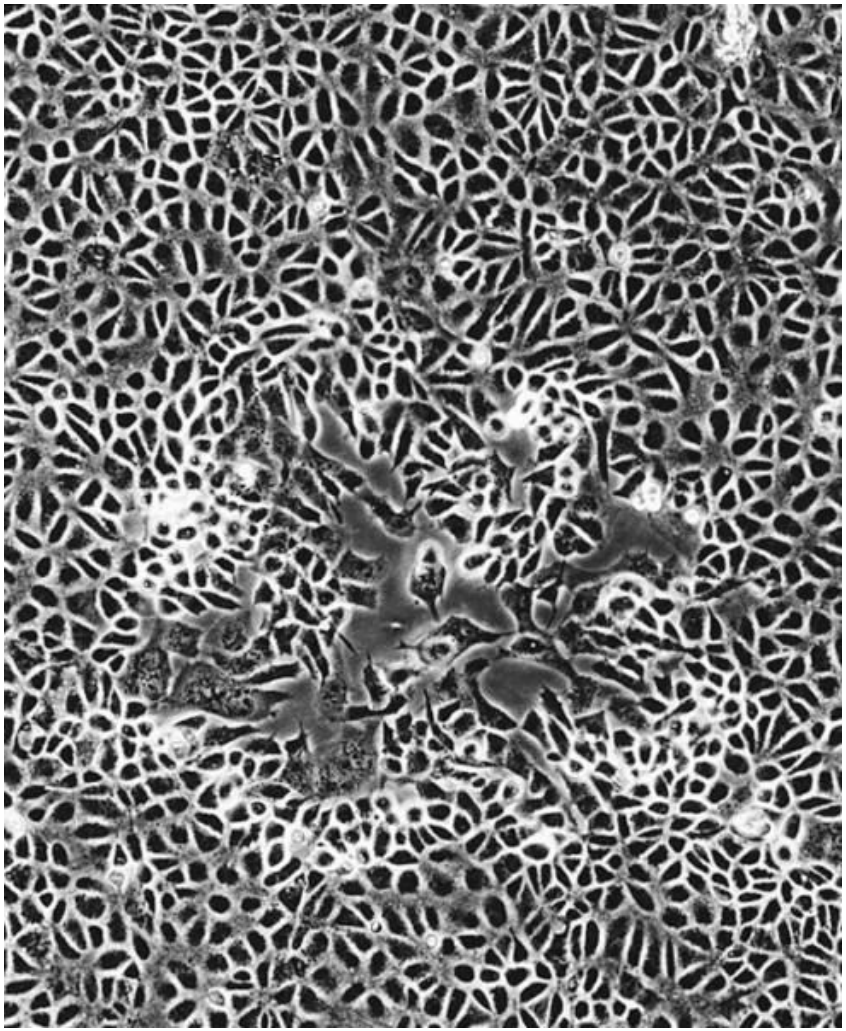
- pyknosis,
- Karyorekzis,
- Nuokeoli grow,
- Inclusion Bodies,

# CPE: Human Lung Carcinoma (A549) HK Measles

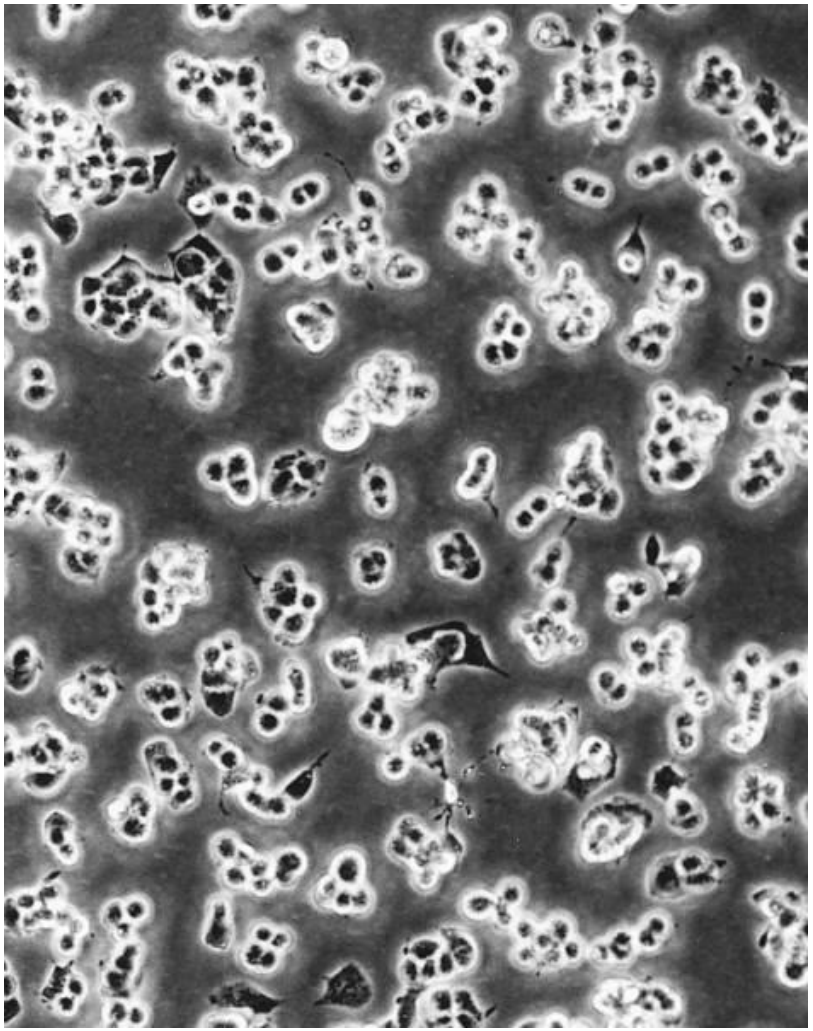


Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 2-4

# CPE: Monkey Kidney (BSC40) HK de vaccinia



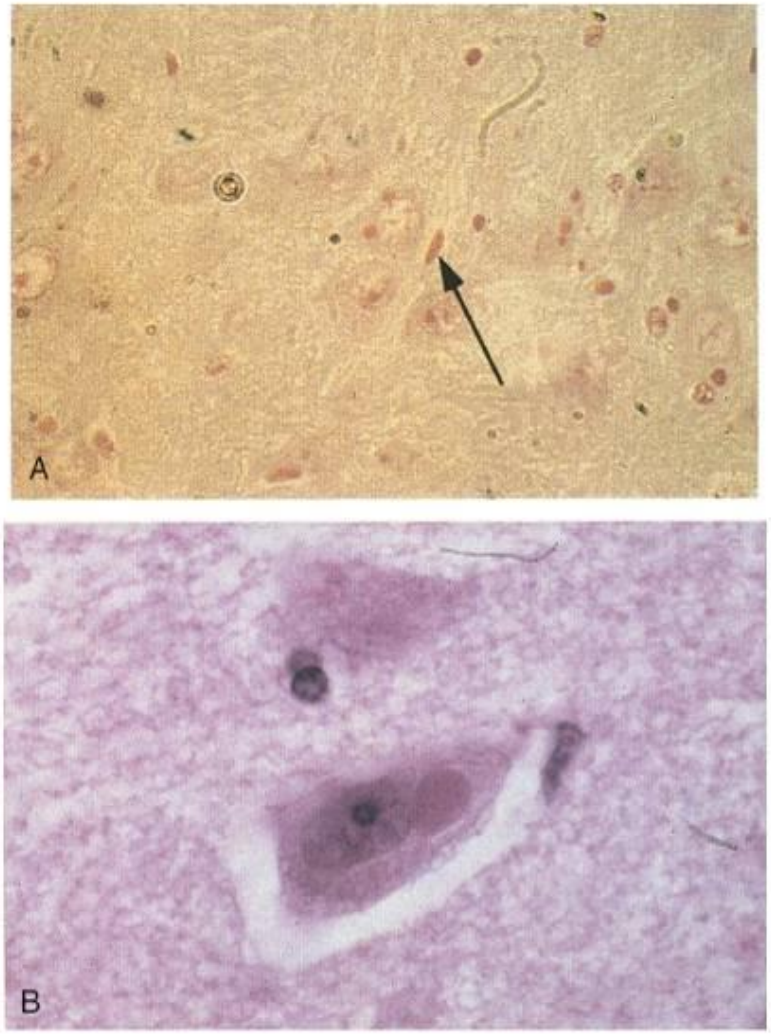
Low dose infection



High dose, 48 saat

Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 2-4

# Inclusion Bodies

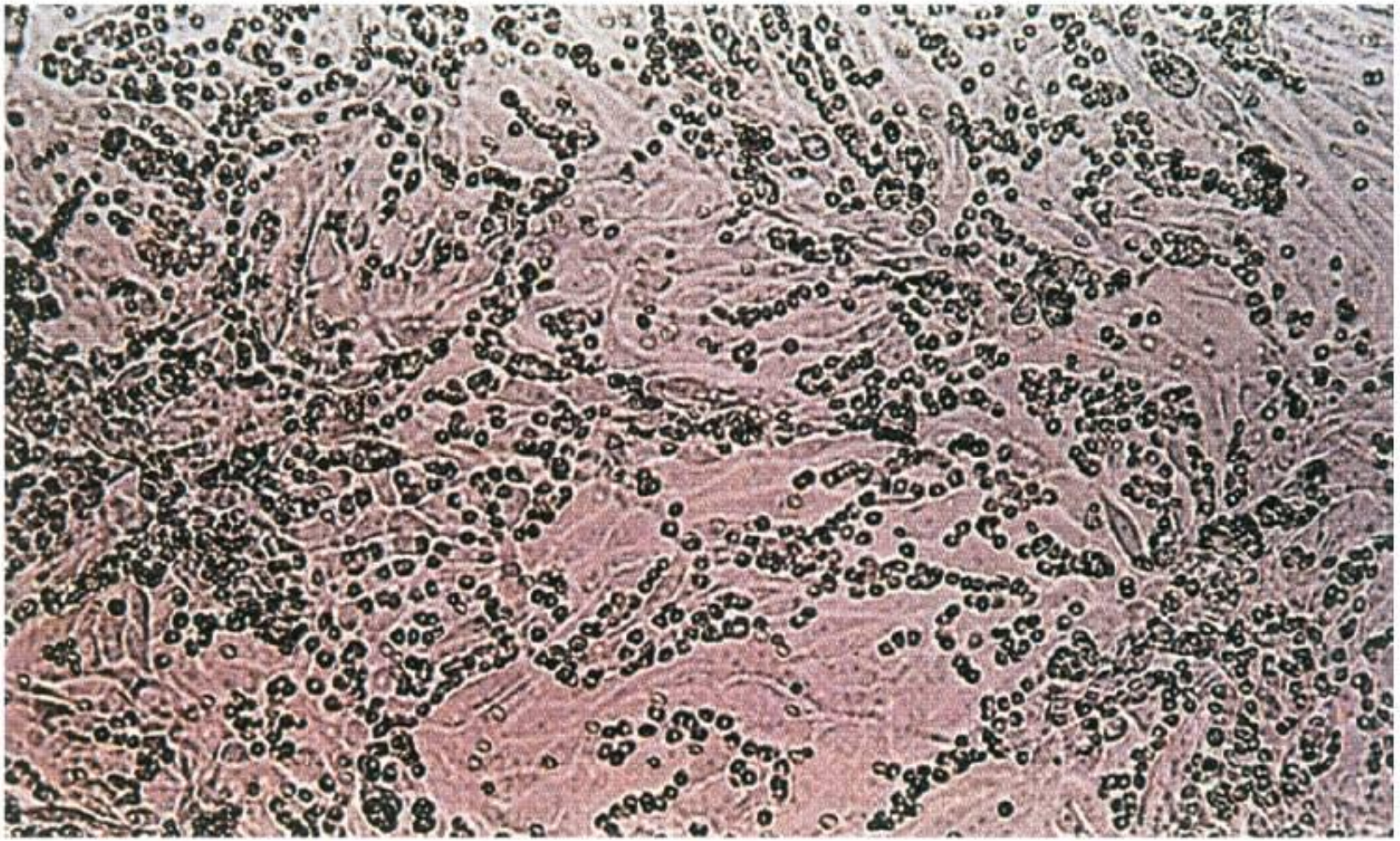



From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005, Fig. 51-3.

# Identification of non-CPE viruses in cell culture

- Electron microscopy
- Immunohistochemical (IFT, IPX) techniques
- the hemadsorption
- interference
- Metabolic inhibition

# Hemadsorption-1



From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005, Fig. 51-5.

## Interferon

"Interferons are proteins that are synthesized and secreted by the cell in the presence of different stimuli and protect the other cells from active virus infection".

DeSomer and Cocito 1968

# TYPES OF INTERFERON

TYPE I

**Interferon-alpha** (leukocyte interferon, about 20 related proteins)

- leukocytes, etc

**Interferon-beta** (fibroblast interferon)

- fibroblasts, epithelial cells, etc

TYPE II

**Interferon-gamma** (immune interferon)

- certain activated T-cells, NK cells

# IFN is also induced by other agents

- Virus DNA (active) and RNA (active and inactive)
- Rickettsia
- Bacteria (self-gram-negative)
- Live / inactive mycoplasma
- protozoa
- Nucleic acids are inherent. dsRNA