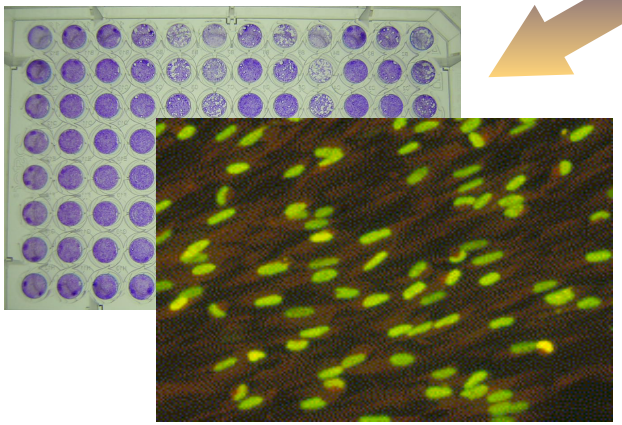
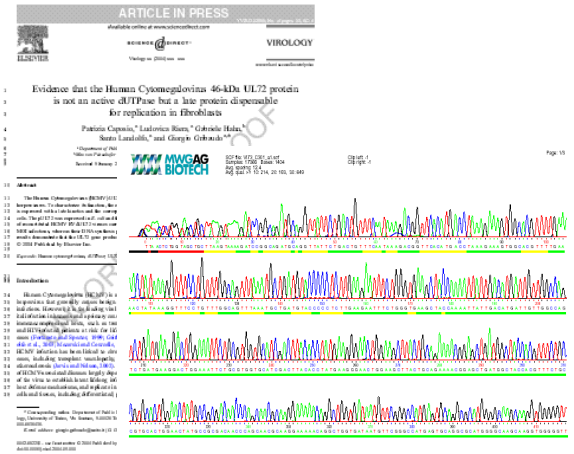
VIROLOGY

Virus cultivation and assay 1

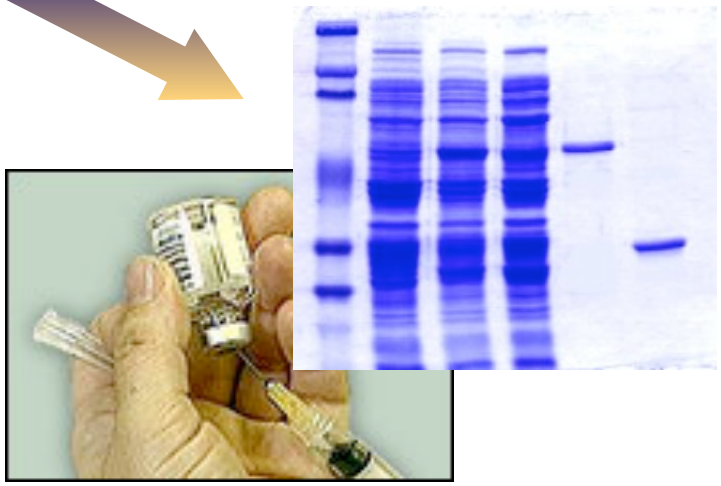
Why do we need to grow viruses?



Diagnosis of infections



Research

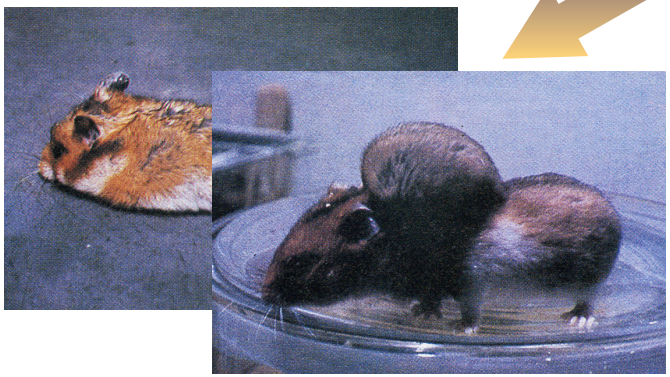


Production of antigens

Virus cultivation: critical issues

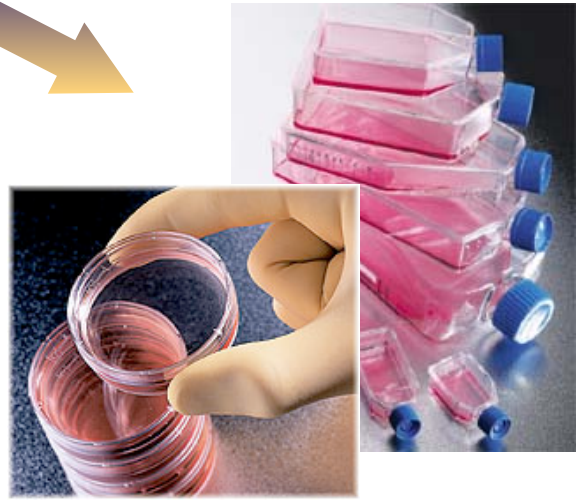
- Viruses cannot grow outside a living cell
- The range of cell types in which many of them replicate is limited
- A few cannot be grown in the lab at all

Development of host systems suitable for virus cultivation



Laboratory animals

Embryonated chicken eggs

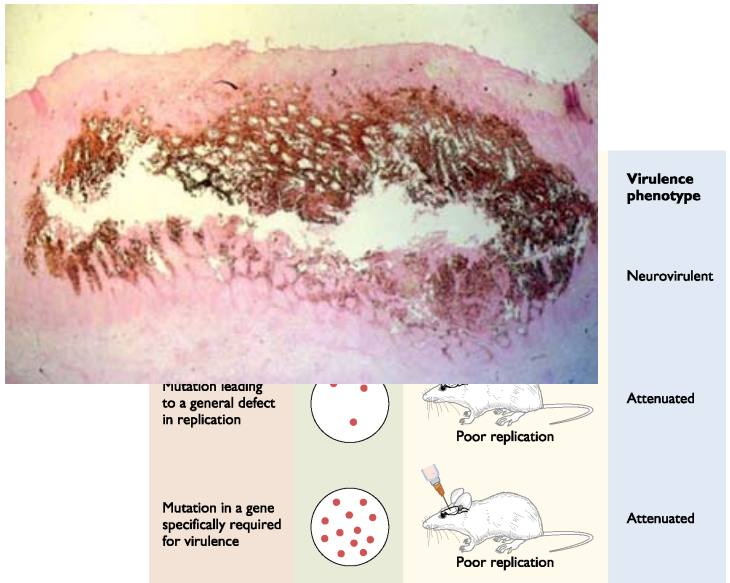


Cell cultures

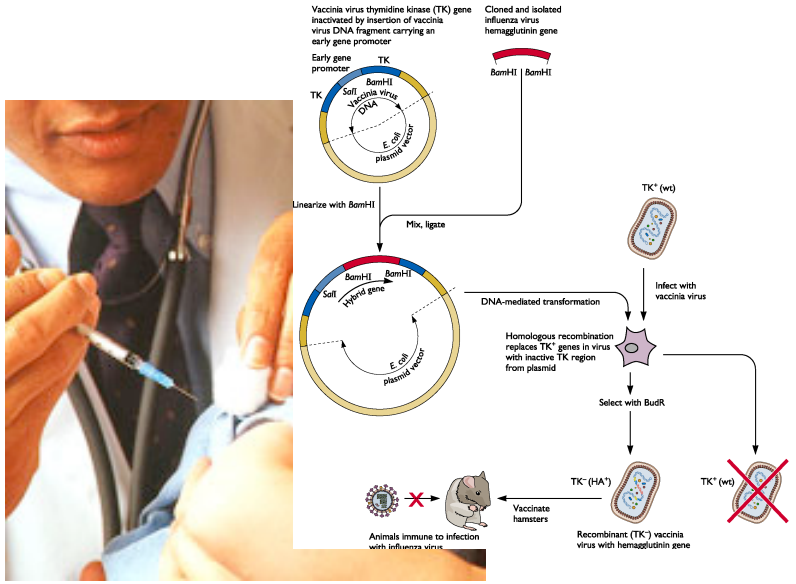
Virus cultivation: Laboratory animals

- Historically the only way to study viruses was from aninal to animal
- Animal models of human infections
- Some viruses can be studied only in this way

Laboratory animals in Virology

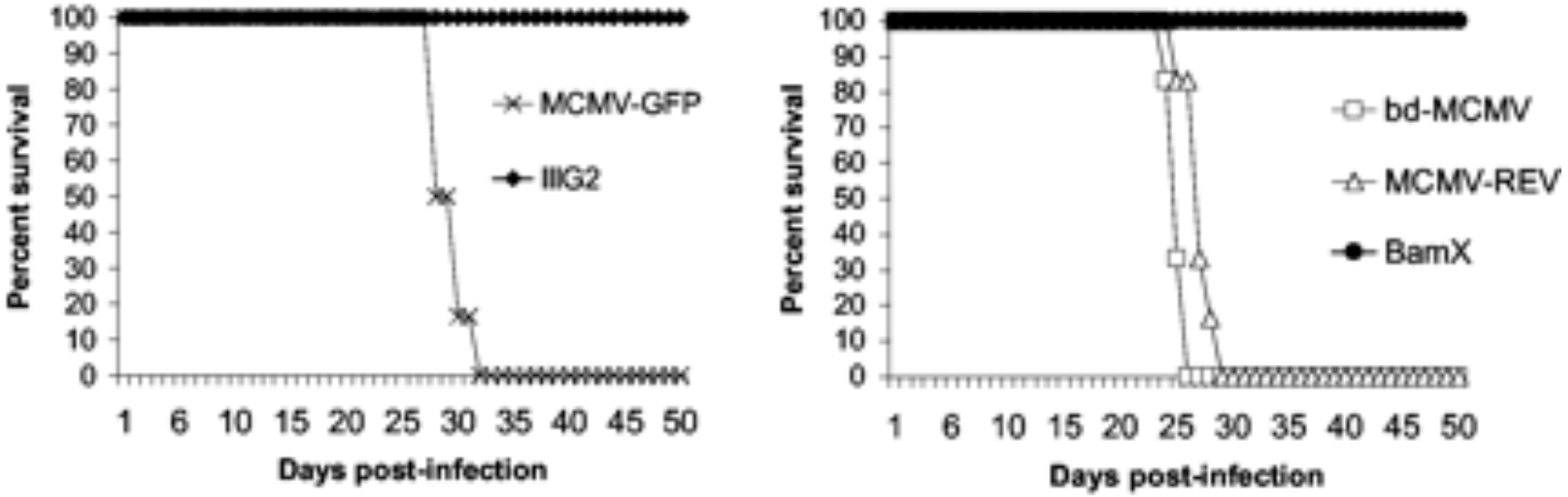


Pathogenesis studies



Vaccine development

Laboratory animals give unique insight into virus pathogenesis



From Lembo et al., J. Virol., 78, 2004

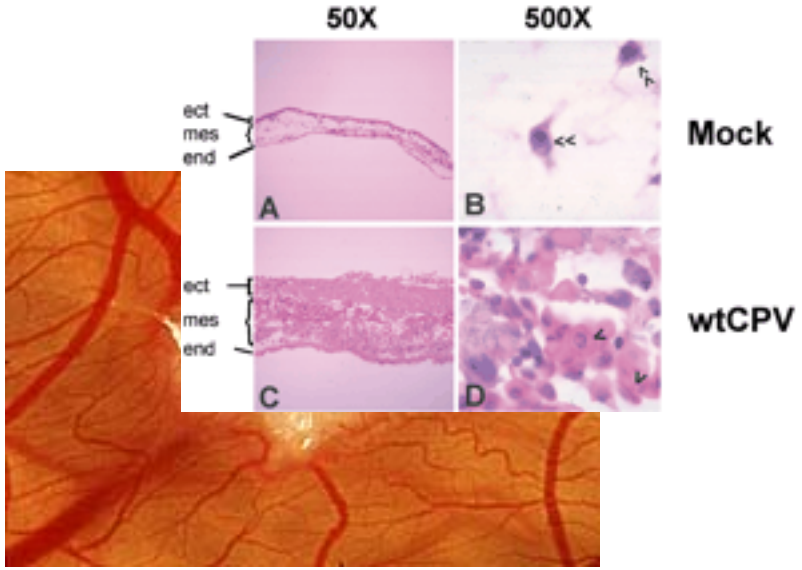
The first revolution in Animal Virology:

1932 - Introduction of methods for cultivating viruses in fertilized chicken eggs

E.W. Goodpasture

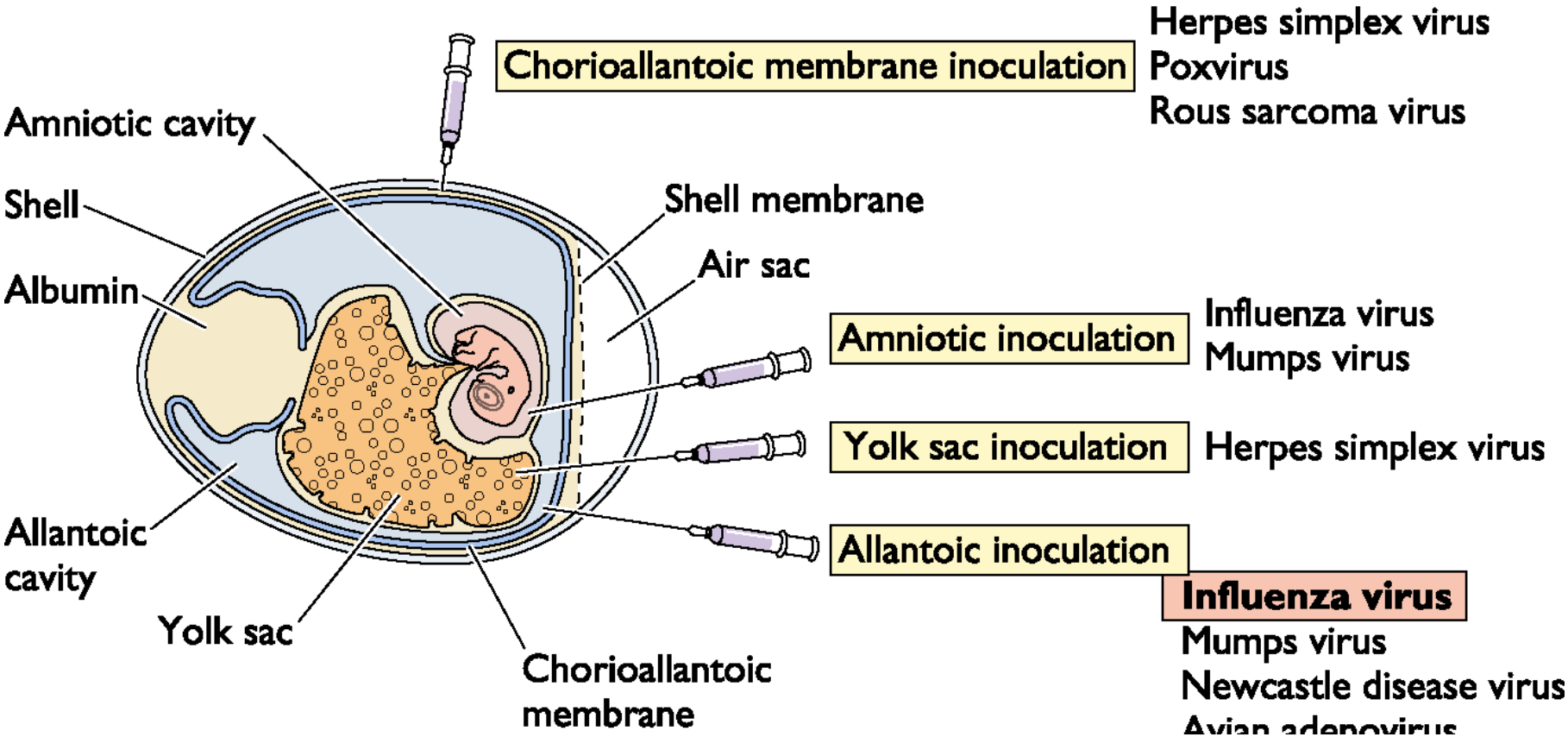
Virus cultivation: Embryonated eggs

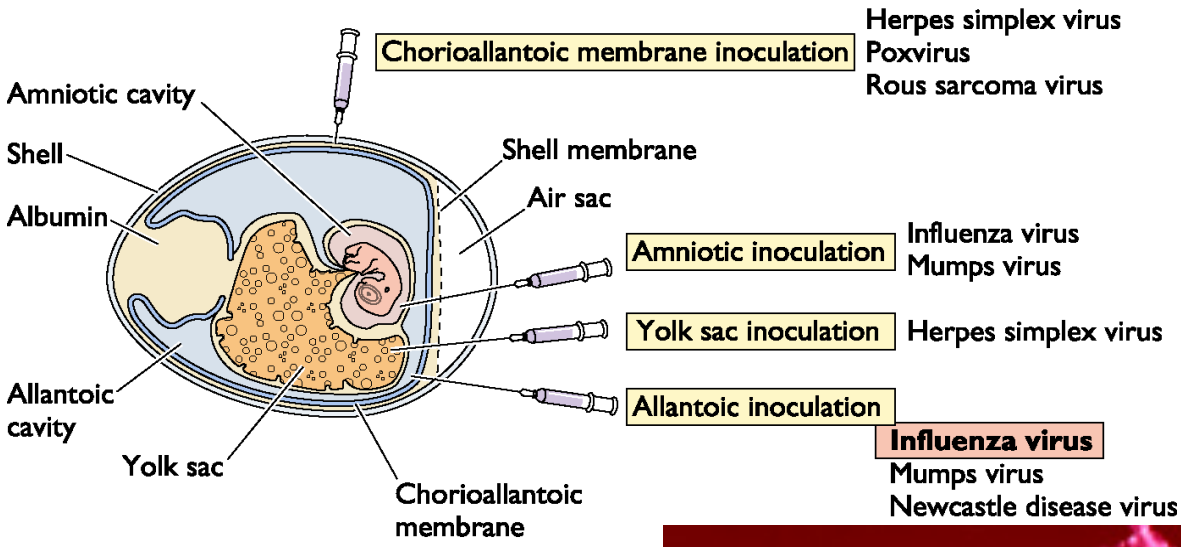
Vaccine production

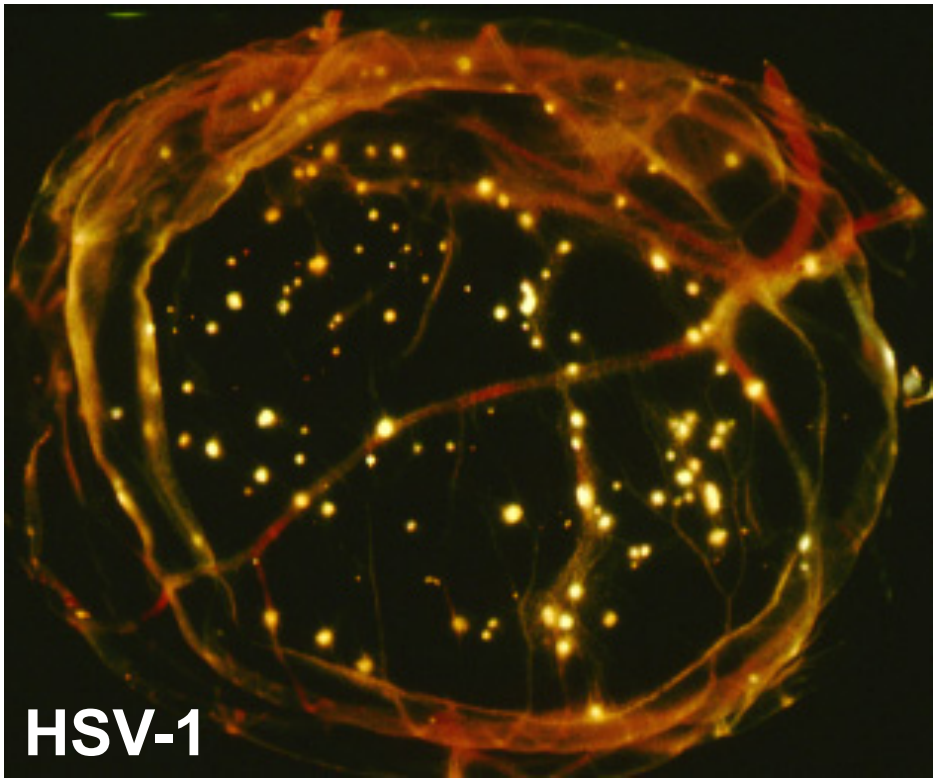


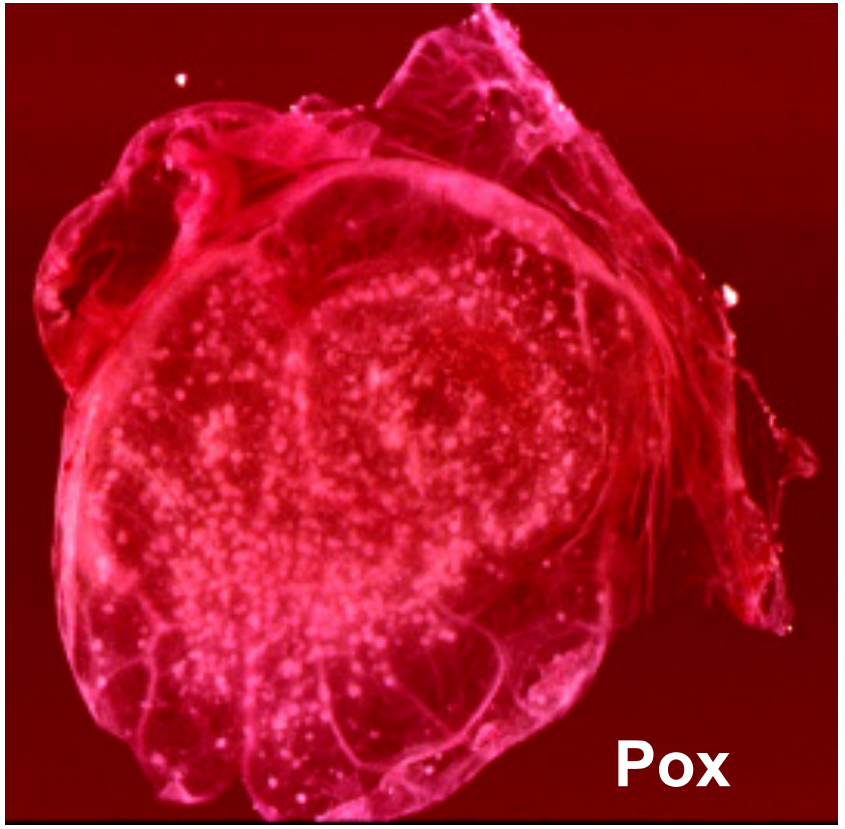
Pathogenesis studies

Embryonated eggs: inoculation



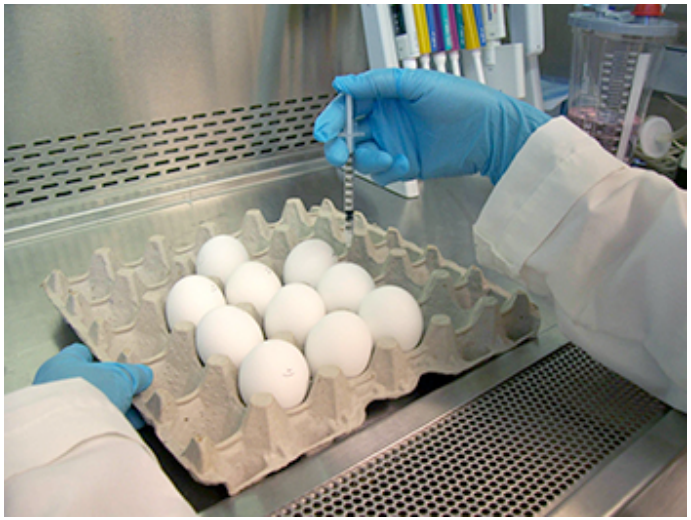

Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Yolk sac
Chorioallantoic membrane


HSV-1

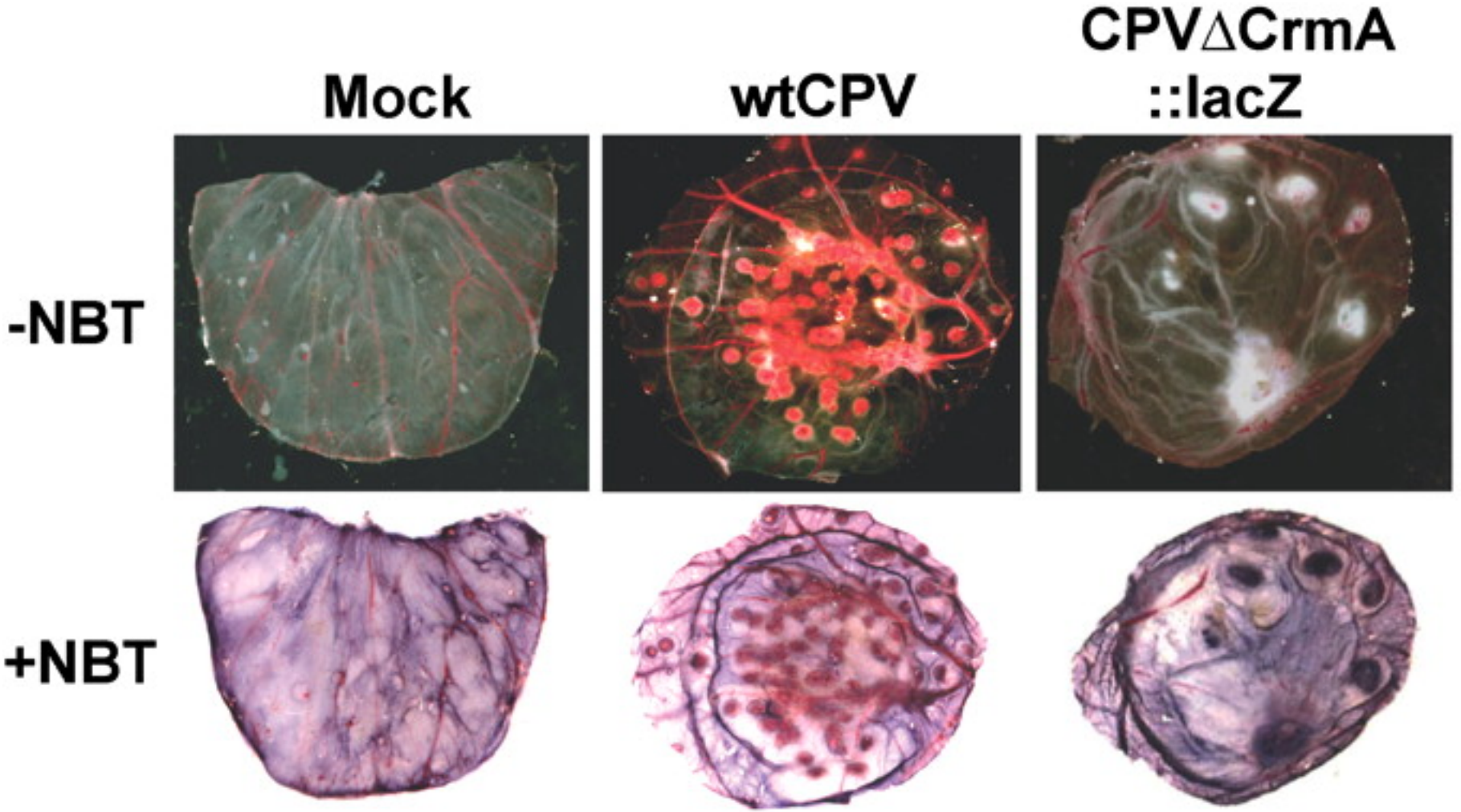

Pox

Egg-Based Flu Vaccines

The most common way that flu vaccines are made is using an egg-based manufacturing process



Embryonated eggs: CAM's pocks



The second revolution in Animal Virology:

1949 - The development of methods for cultivating viruses in *in vitro* cell cultures

J. F. Enders

T. H. Weller

F. C.Robbins

The Nobel Prize in Physiology or Medicine 1954

"for their discovery of the ability of poliomyelitis viruses to grow in cultures of various types of tissue"

JOHN F. ENDERS, FREDERICK C. ROBBINS,
THOMAS H. WELLER

The cultivation of the poliomyelitis viruses in tissue culture

Nobel Lecture, December 11, 1954

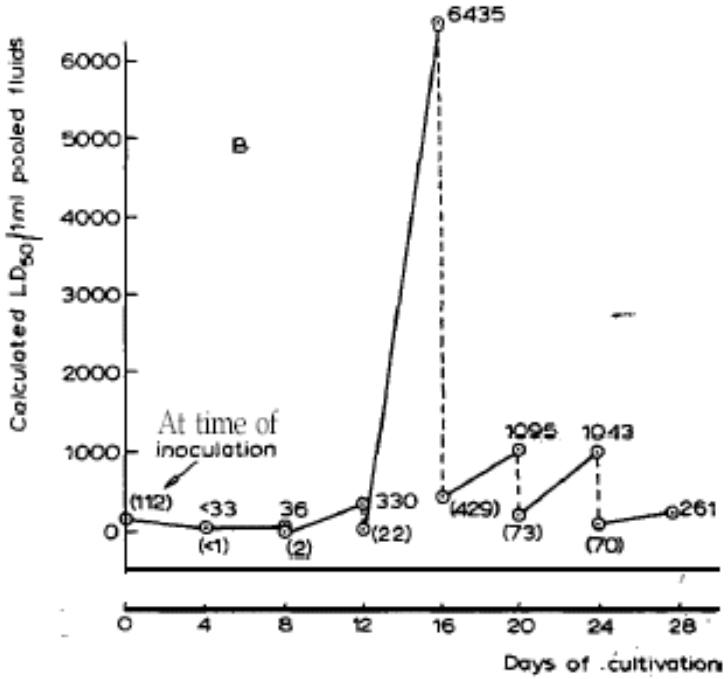
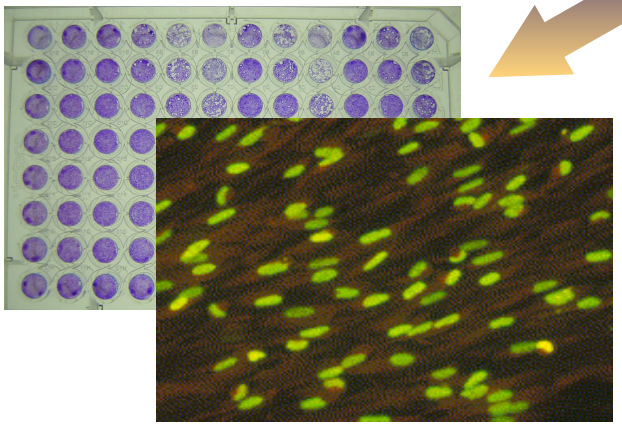
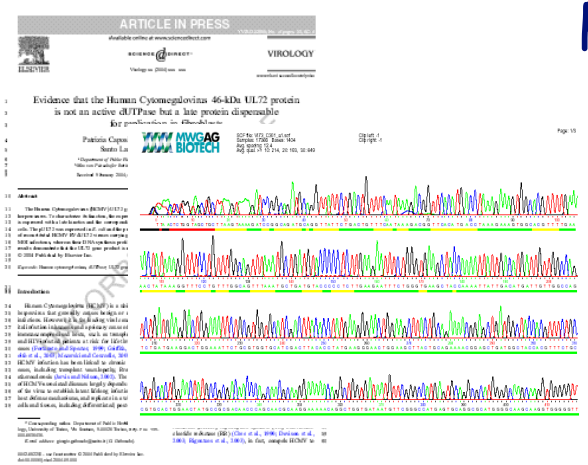


Fig. 1. Mouse infectivity of pools of fluids removed at four-day intervals from suspended cell cultures of human embryonic skin-muscle tissue inoculated with Lansing mouse-brain virus. (From *J. Immunol.*, 69 (1952) 652.)

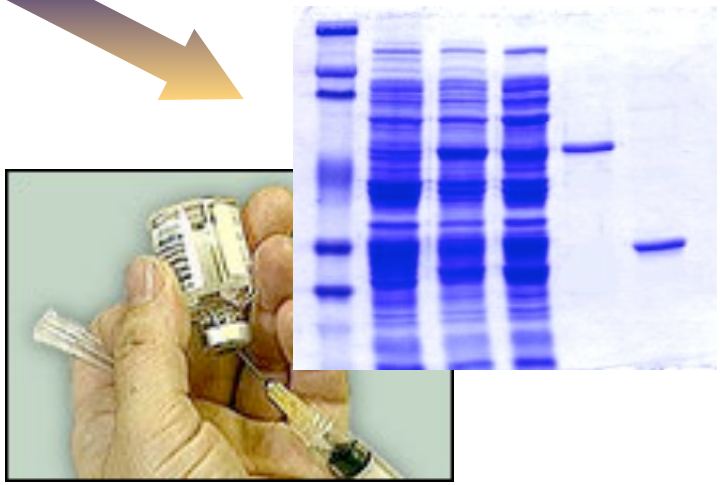
Virus cultivation: Cell cultures



Diagnosis of infections

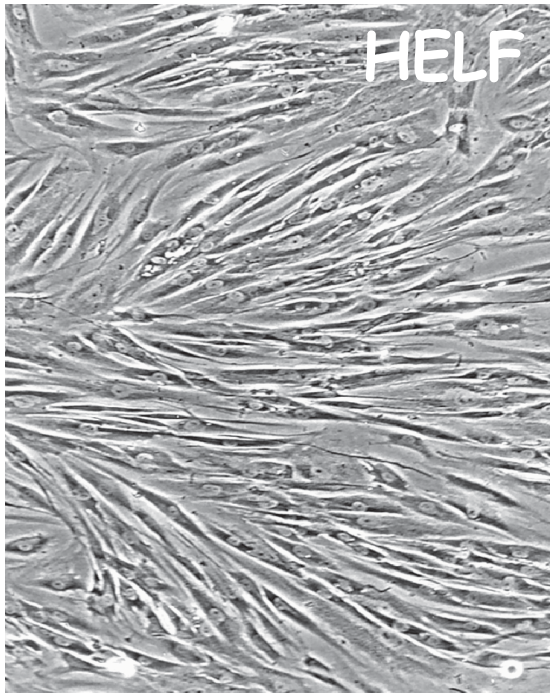


Research

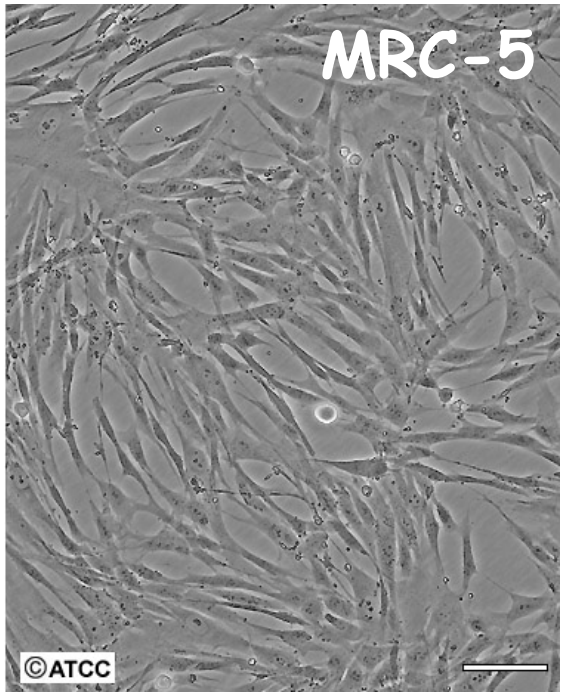


Production of antigens

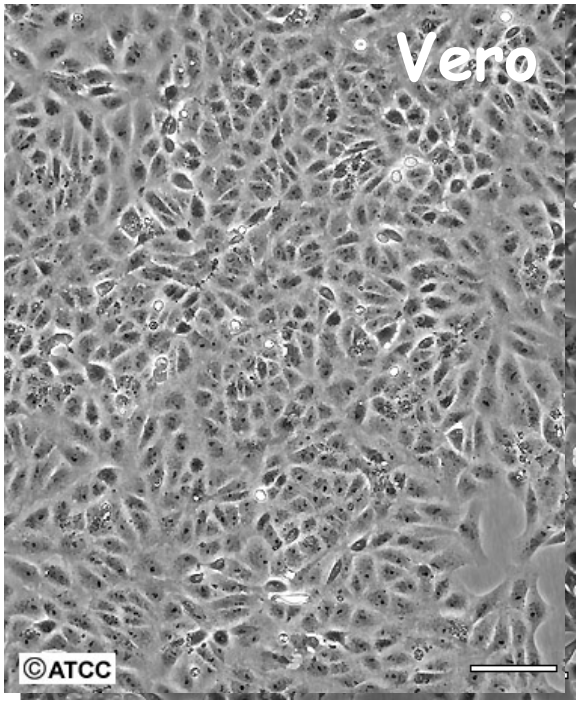
Cell cultures in Virology



Primary cell cultures



Diploid cell strain



Continuous cell lines

Culture type	Examples	Virus supported
Primary	Monkey kidney	Influenza virus, Paramyxovirus, Enterovirus
	Rabbit kidney	HSV
	Human embryonic kidney	Adenovirus, Enterovirus
Diploid	HELF, MRC-5	CMV, HSV, VZV, Adenovirus, RSV, Rhinovirus, Enterovirus
Continuous	Hep-2	RSV, HSV, Adenovirus, Paramyxovirus, Enterovirus
	A459	HSV, Adenovirus, Enterovirus
	MDCK	Influenza virus
	LLC-MK2	Enterovirus, Paramyxovirus
	RD	Enteroviruses, HSV
	BGMK	Coxsackievirus
	Vero	HSV, Paramyxovirus, Coxsackievirus

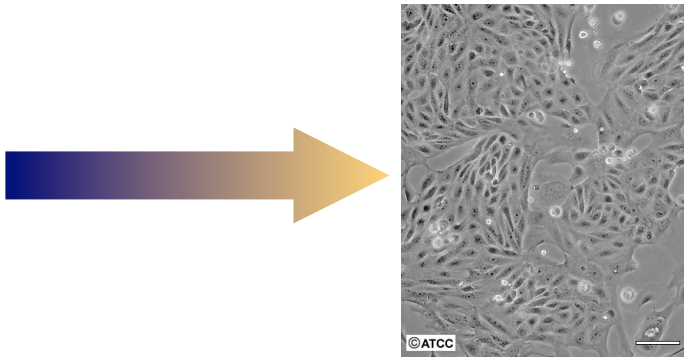
www.viromed.com

	Adenovirus	Coxsackie A	Coxsackie B	Cytomegalovirus	Echovirus	Herpes simplex	Influenza A,B	Measles	Mumps	Parainfluenza	Polio	Rhinovirus	RSV	Rubella	Varicella zoster
PRIMARY ANIMAL CELL CULTURES															
Rabbit kidney						•									
Rhesus monkey kidney		•	•		•		•	•	•	•	•				
SERIALLY PROPAGATED CELL CULTURES															
A549	•					•									•
BGMK			•								•				
H292															
HEK293	•														
HeLa	•		•			•					•		•		
HeLa 229															
HEp-2	•		•			•					•		•		
LLC-MK2		•	•		•		•		•	•	•				
McCoy															
MDCK							•								
Mink lung						•									
MRC-5	•	•		•	•	•					•	•	•		•
RD		•				•					•				
SF	•	•			•	•					•				•
Vero			•			•			•		•				
WI-38	•	•			•	•					•	•	•		•

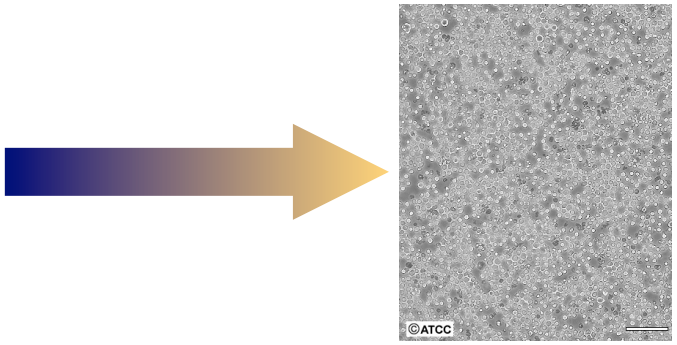
How to chose the appropriate cell culture system?

- The aim of the experiment
- Limitations in the in vitro host range
- Ease of alternative possibile procedures

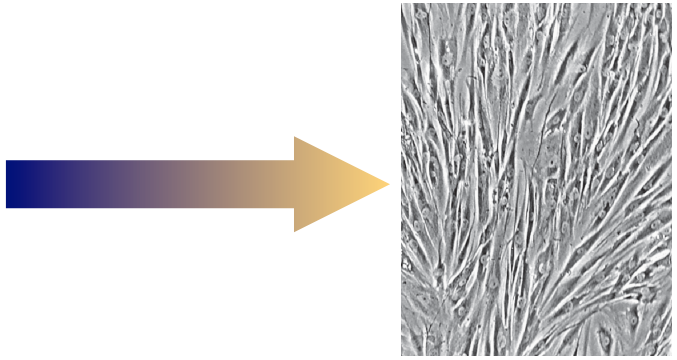
How to chose the appropriate cell culture system?



Adherent cell line



Suspension cell line

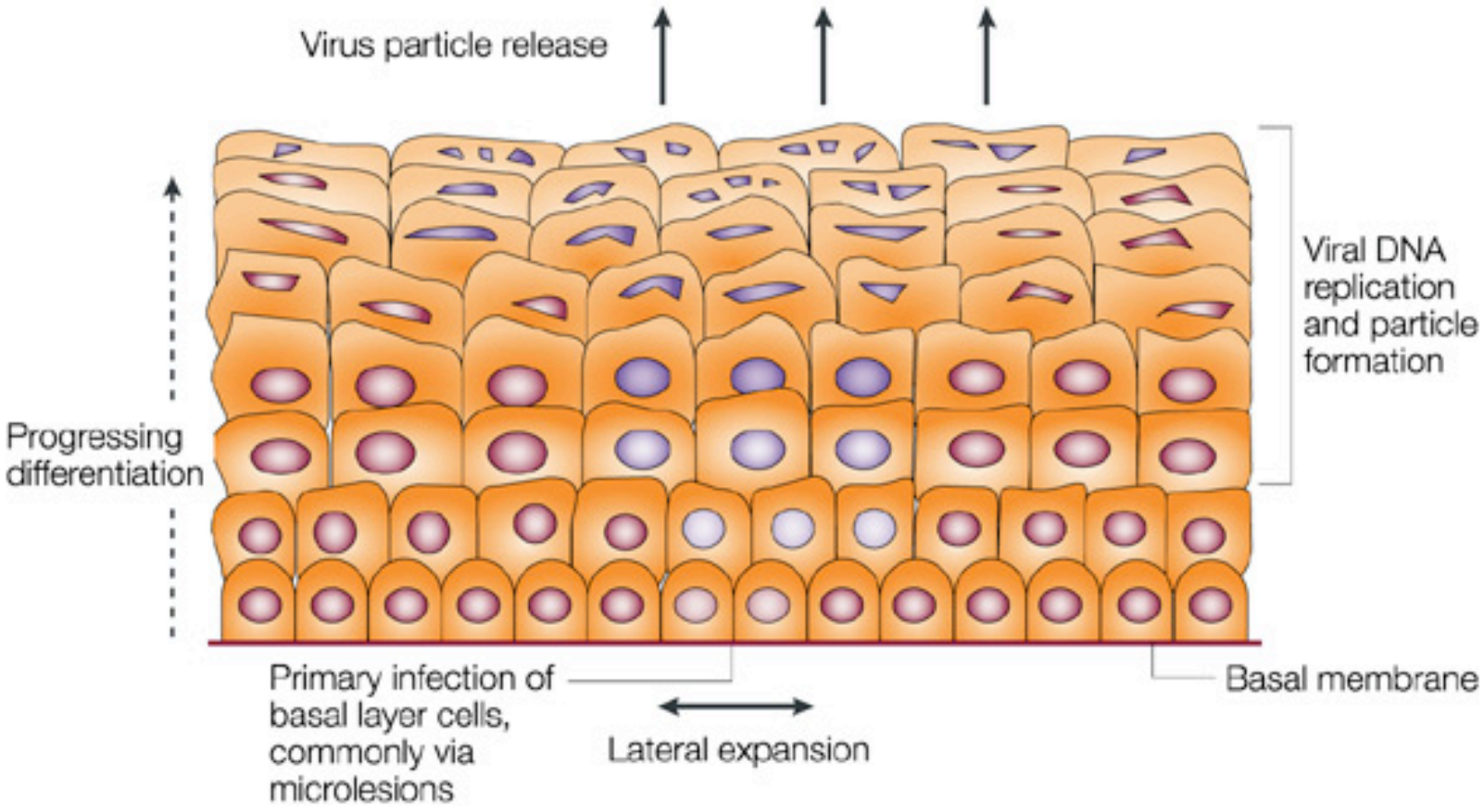


Primary or diploid cell strain

How to study the viruses that have proved difficult to propagate in cell culture?

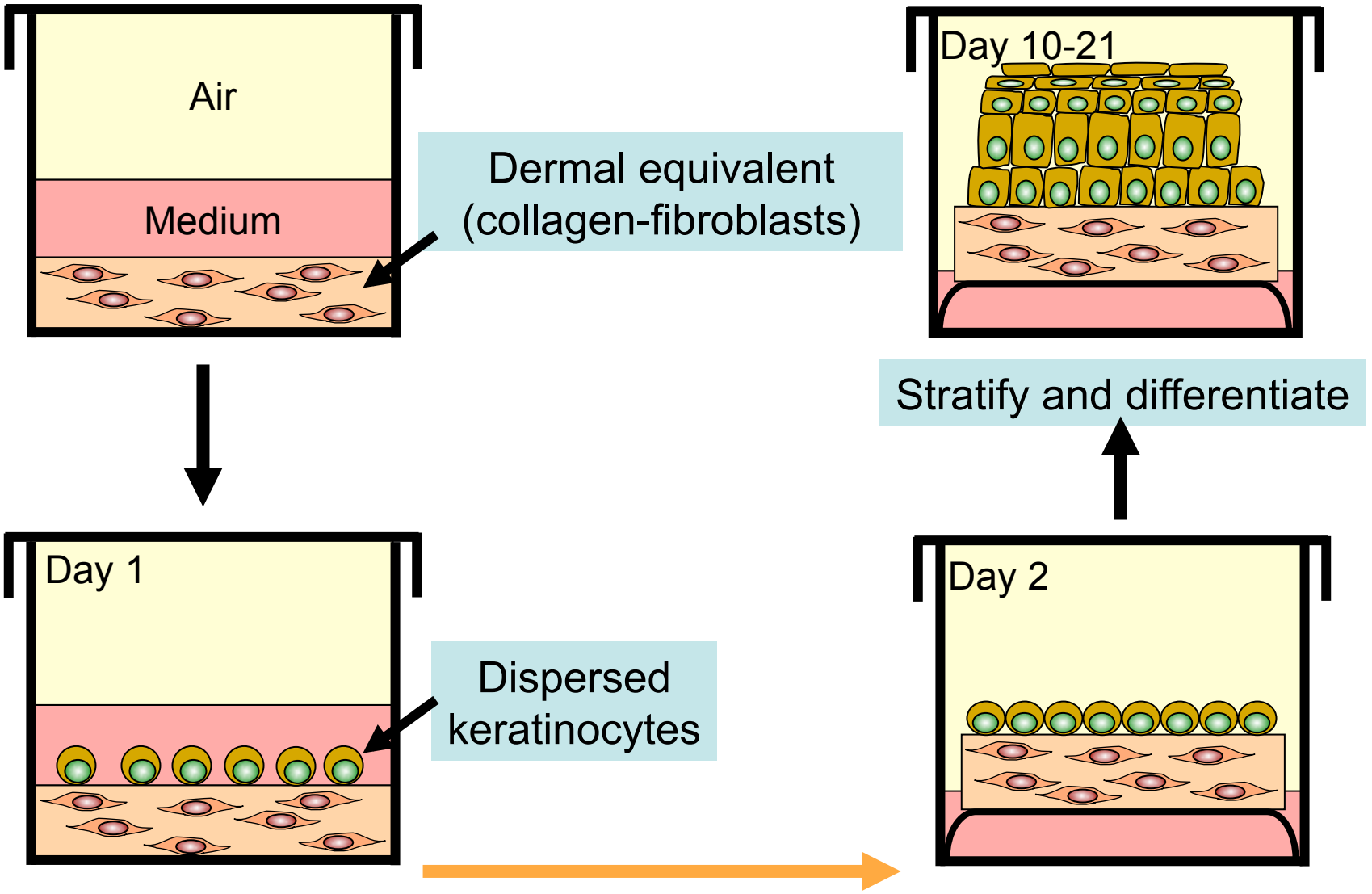
An example:the organotypic culture system approach to study in vitro the HPV replicative cycle

Replication cycle of papillomavirus

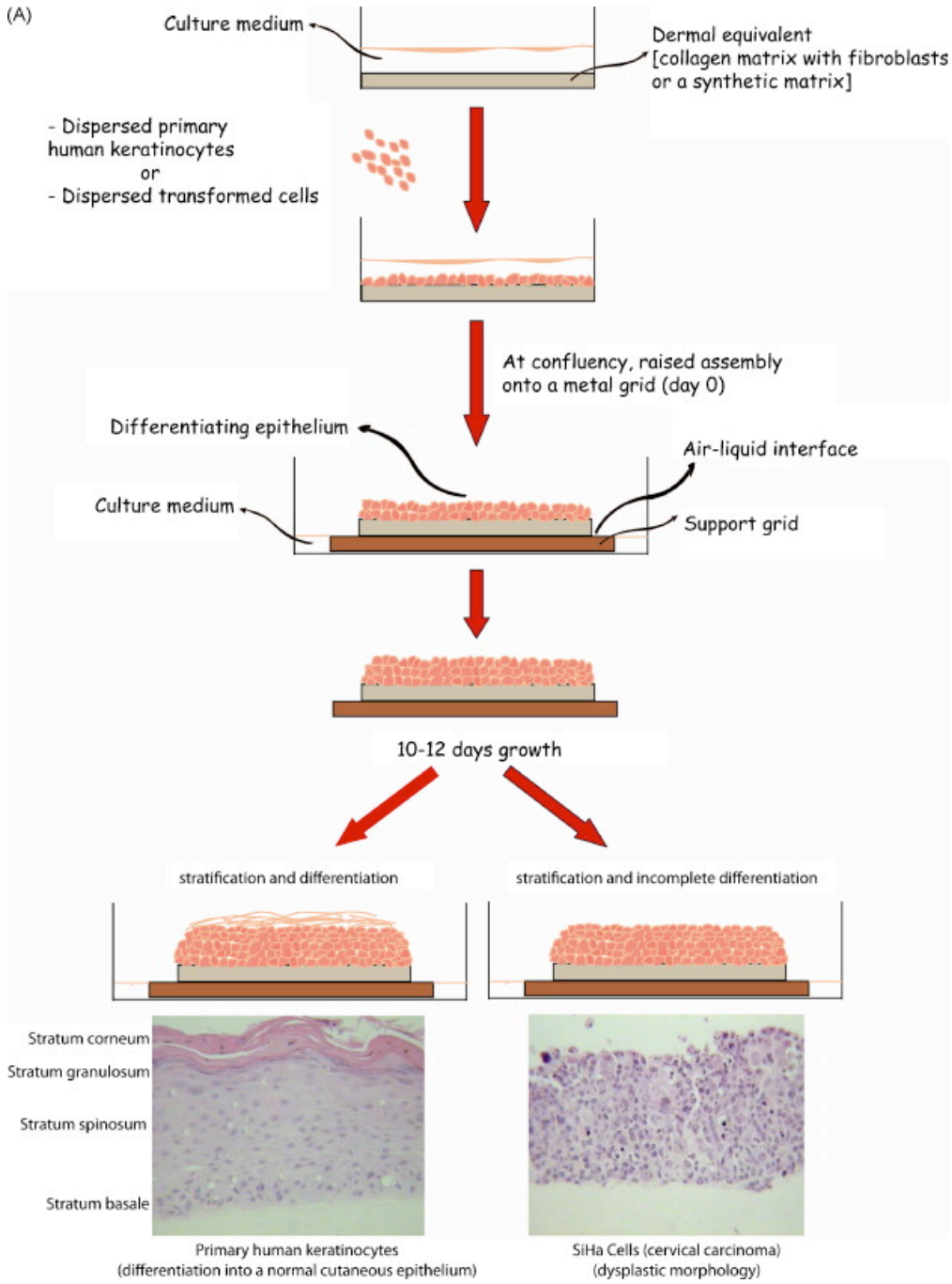


Nature Reviews | Cancer

Organotypic epithelial raft cultures

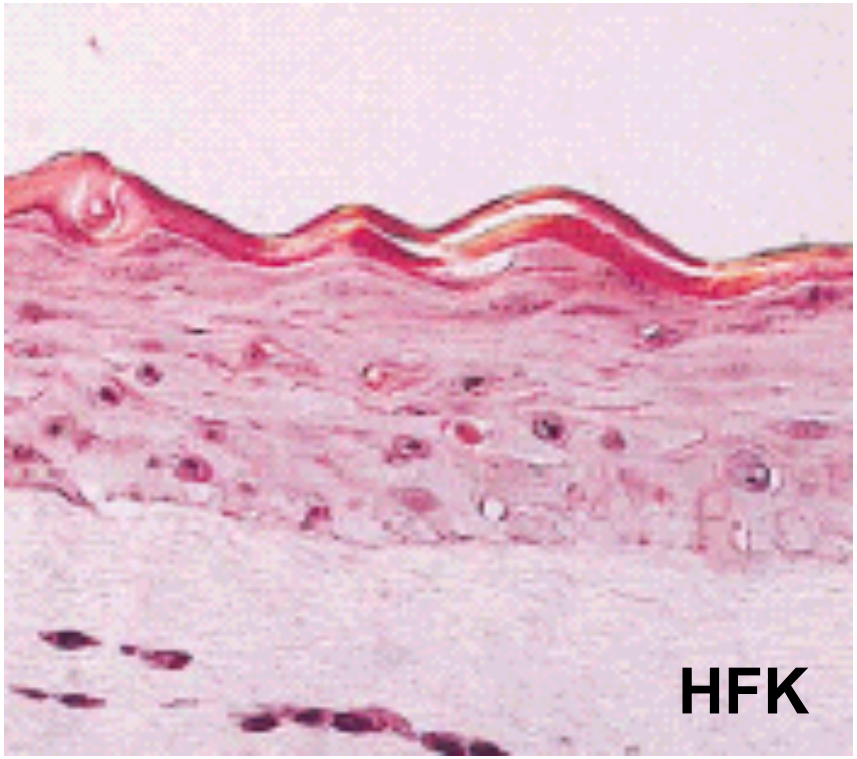


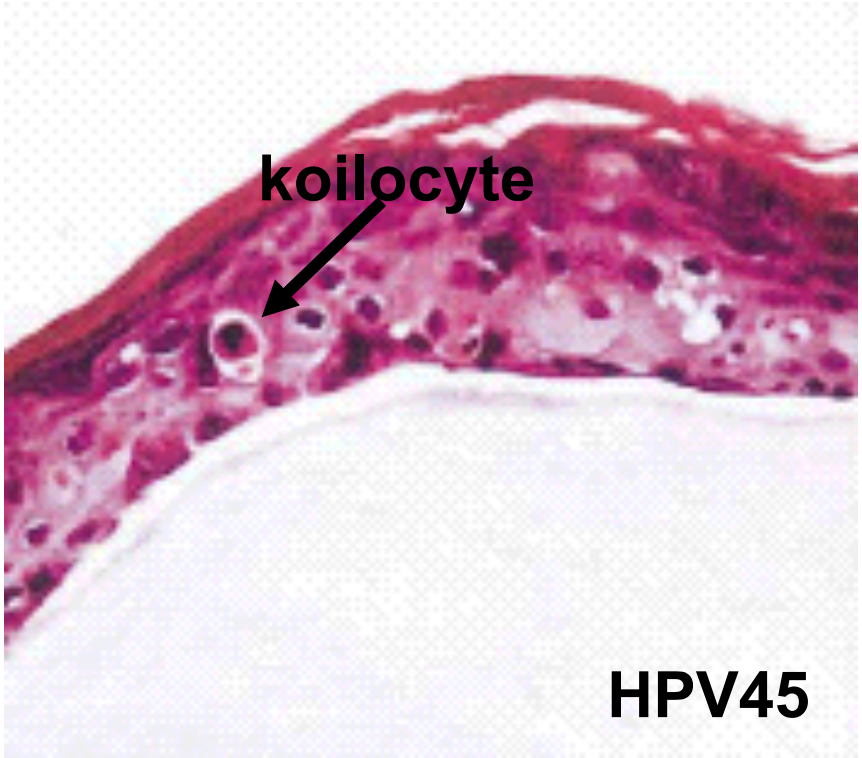
Preparation of organotypic epithelial raft cultures from dispersed cells or tissue biopsy explants.



Andrei et al., Antivir. Res. 85, 431-449, 2010

HPV growth in raft cultures

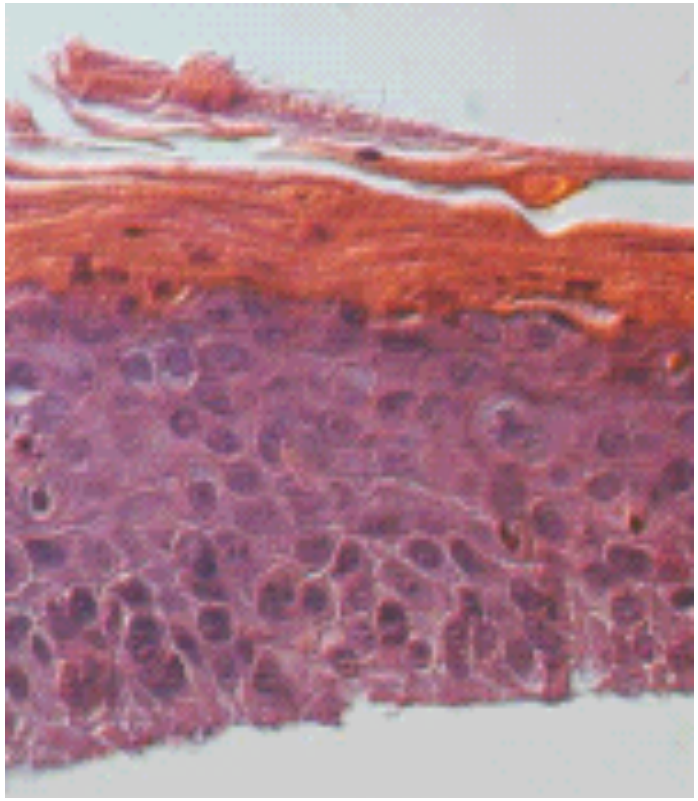


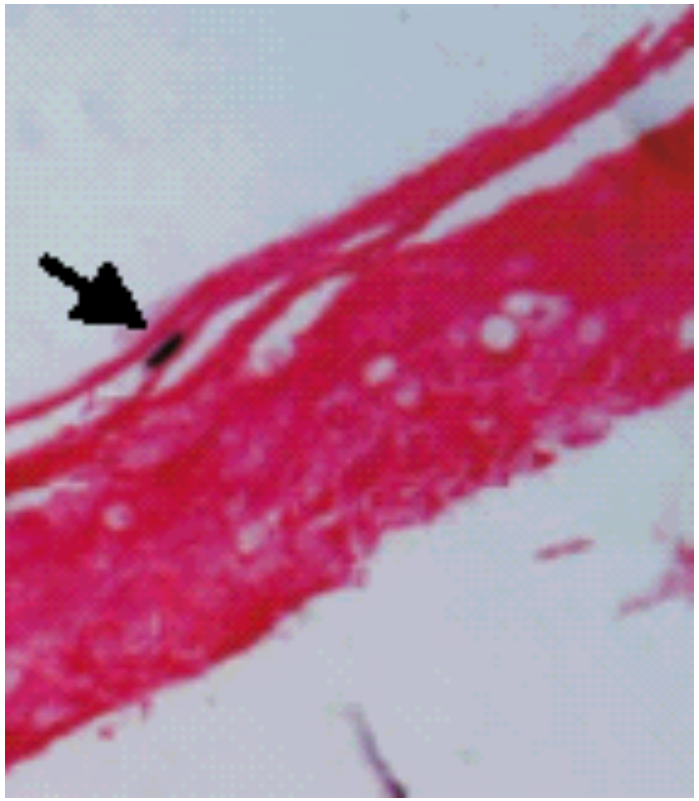


Morphology of HPV45 cell line grown in raft culture

From McLaughlin-Drubin et al.,Virology 312, 2003

HPV growth in raft cultures

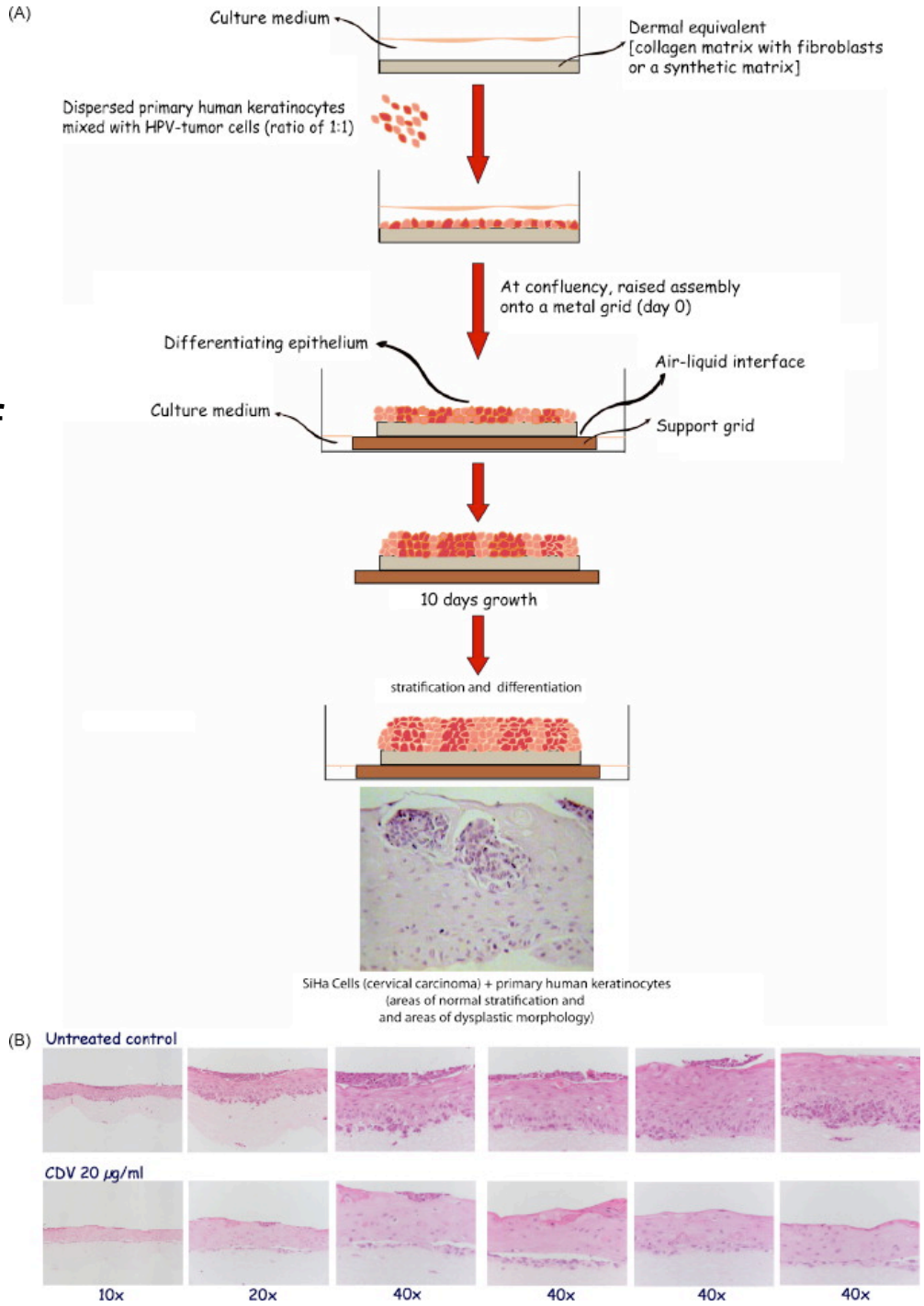




Positive HPV16 L1 staining in a fully stratified and differentiated epithelial raft culture tissue

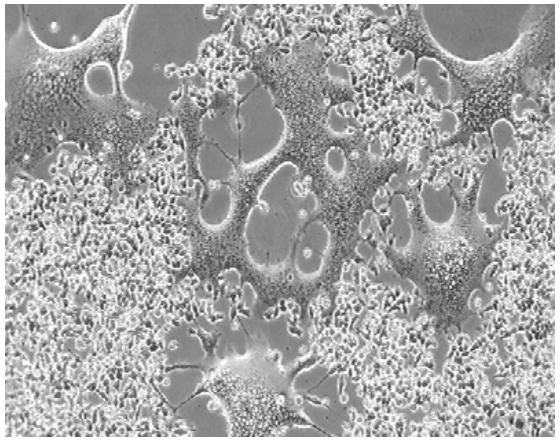
From McLaughlin-Drubin et al.,Virology 322, 2004

Co-cultures of primary human keratinocytes and HPV-positive cells to evaluate the selectivity of anti-HPV agents

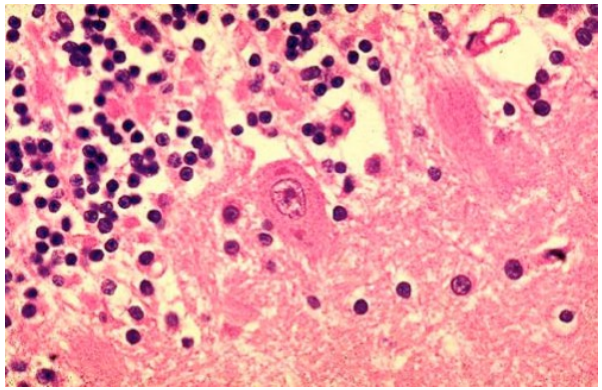


Andrei et al., Antivir. Res. 85, 431-449, 2010

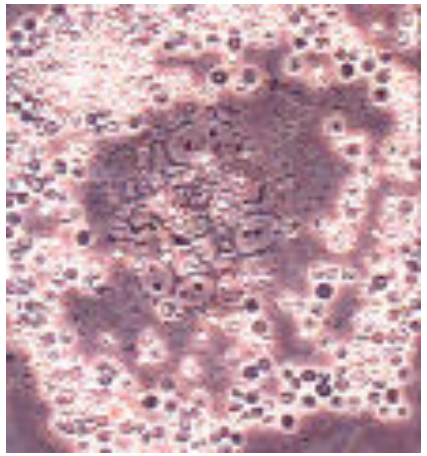
Recognition of Viral Growth in Culture



Cytopathic effect



Inclusion bodies



Hemadsorption

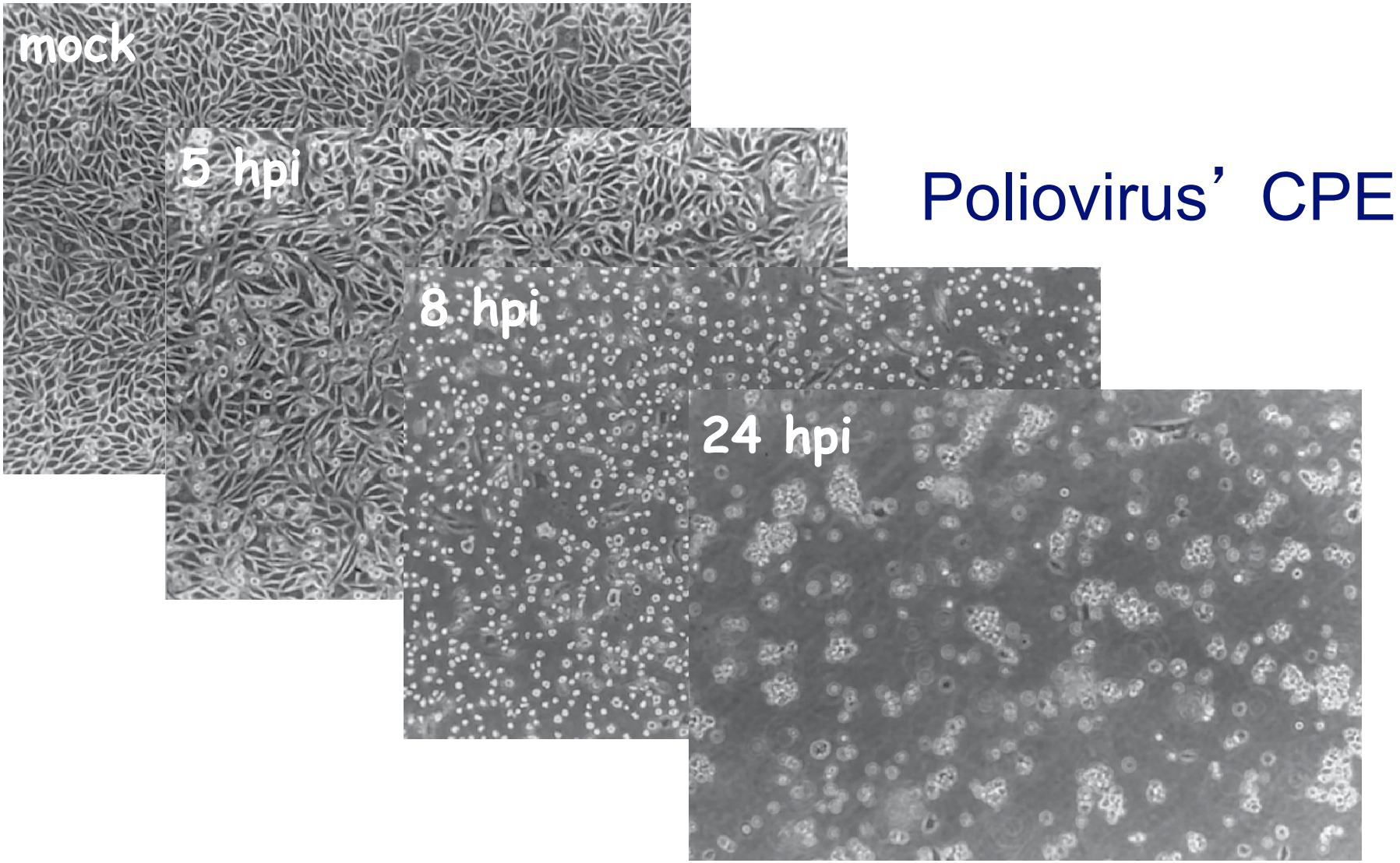
Virus cultivation: The Cytopathic Effect

The simplest and most widely used criterion for infection

Examples of CPE

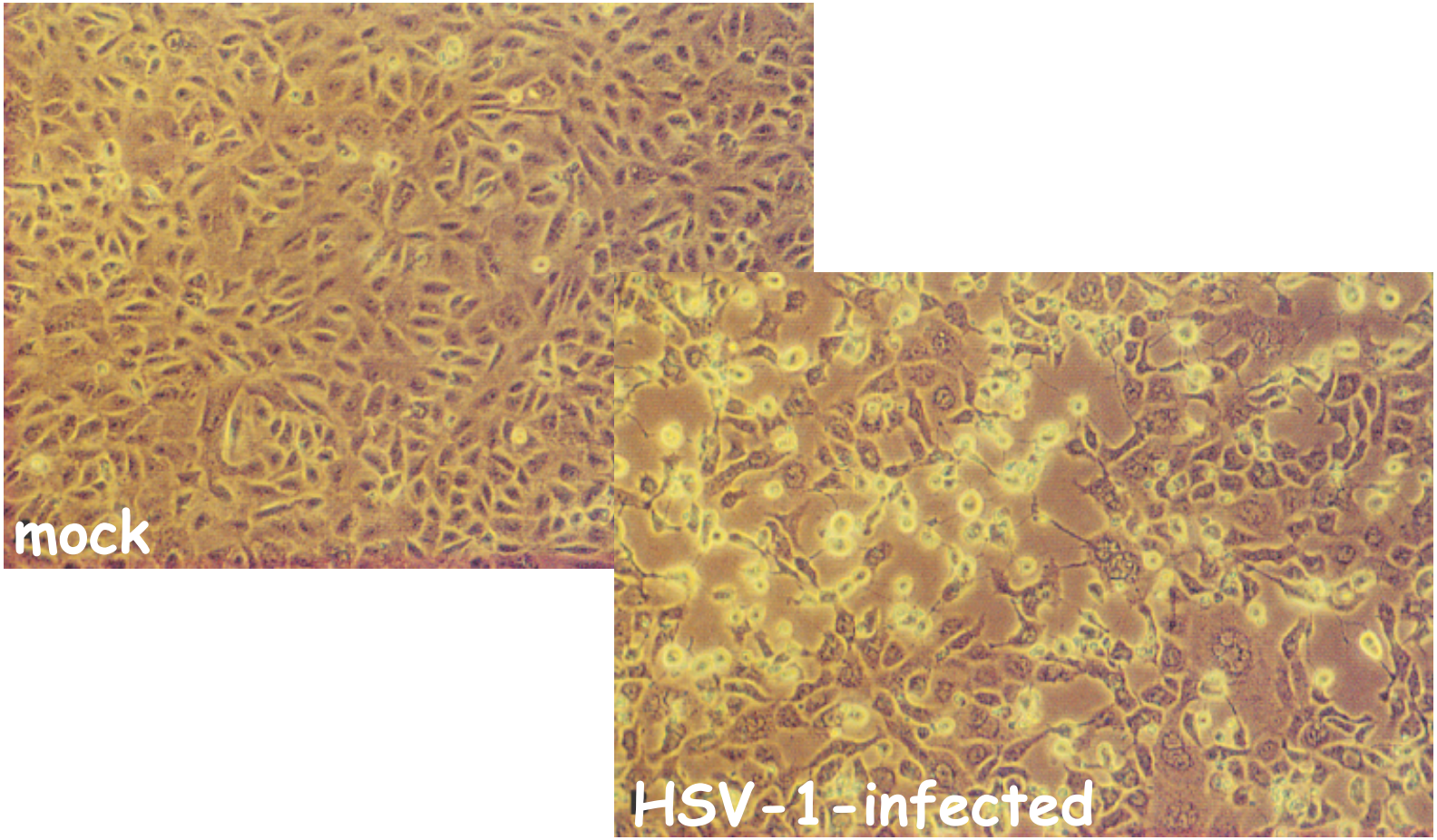
Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Papovaviruses
Syncytia (cell fusion)	Paramyxoviruses, coronaviruses
Margination and breaking of chromosomes	Herpesviruses
Rounding up and detachment of tissue culture cells	Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses

Viral CPE: cell rounding, detachment and lysis

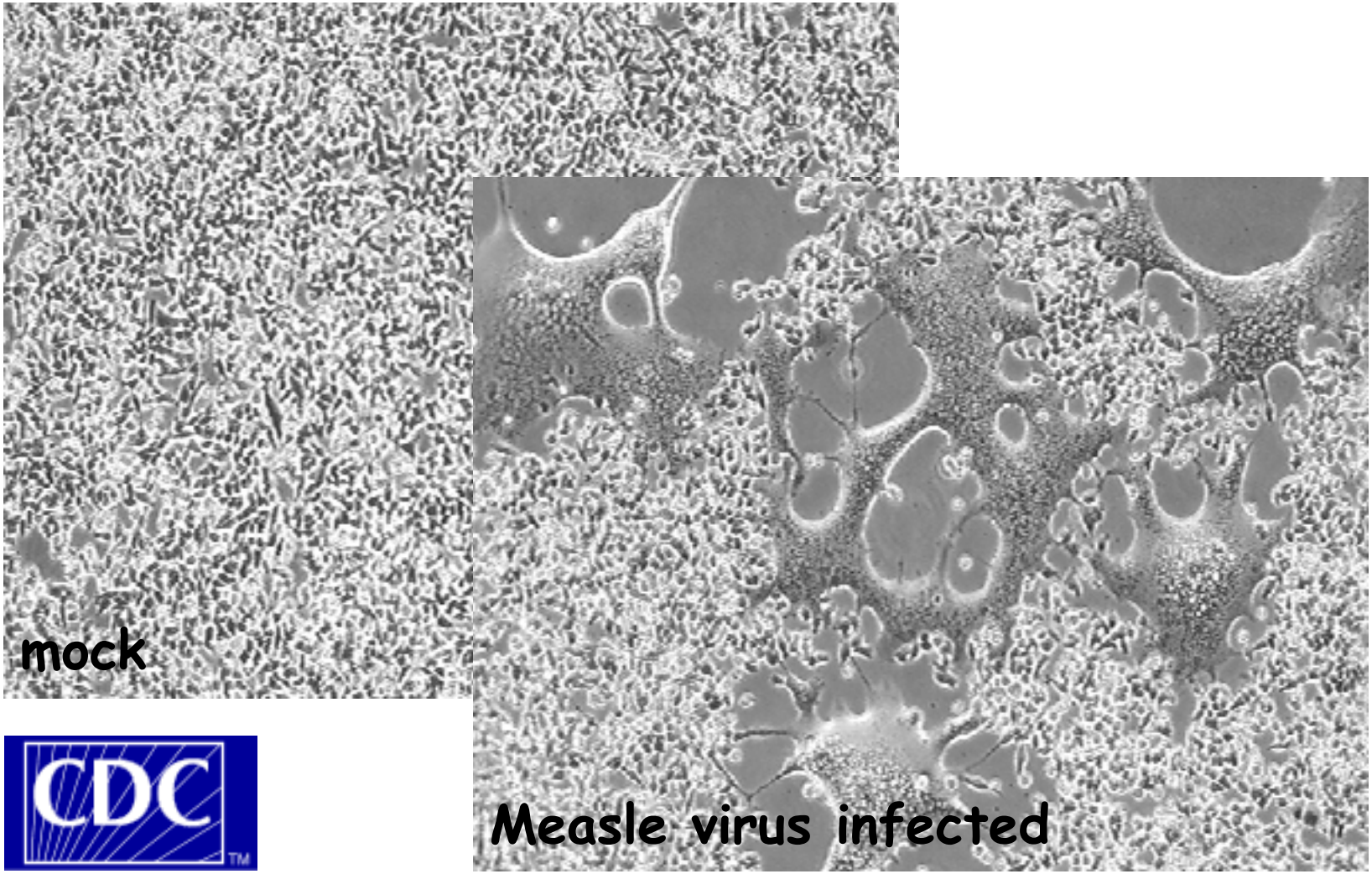


Poliovirus' CPE

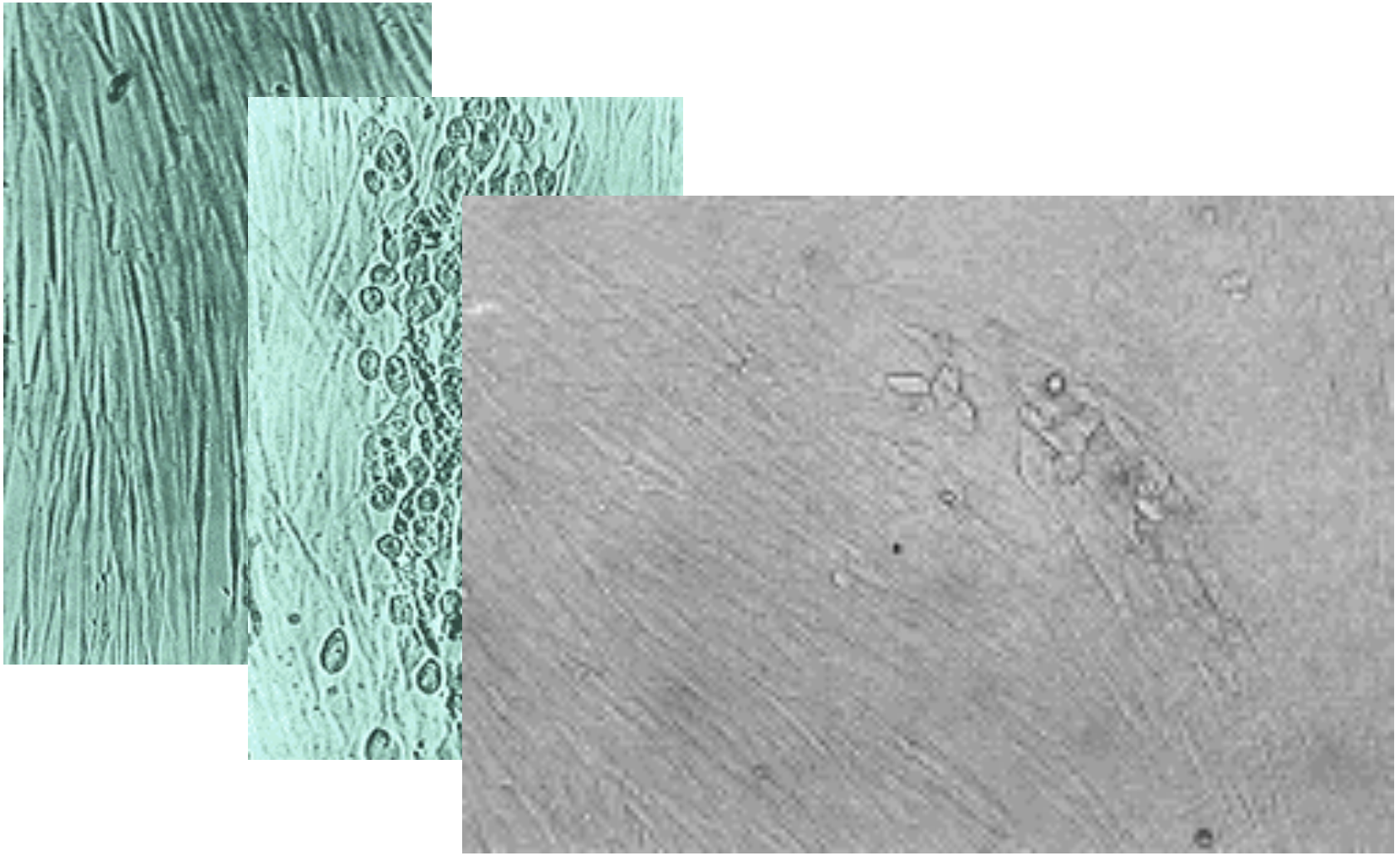
Herpes Simplex Virus CPE in Vero cells



Measle Virus CPE in B95a cells

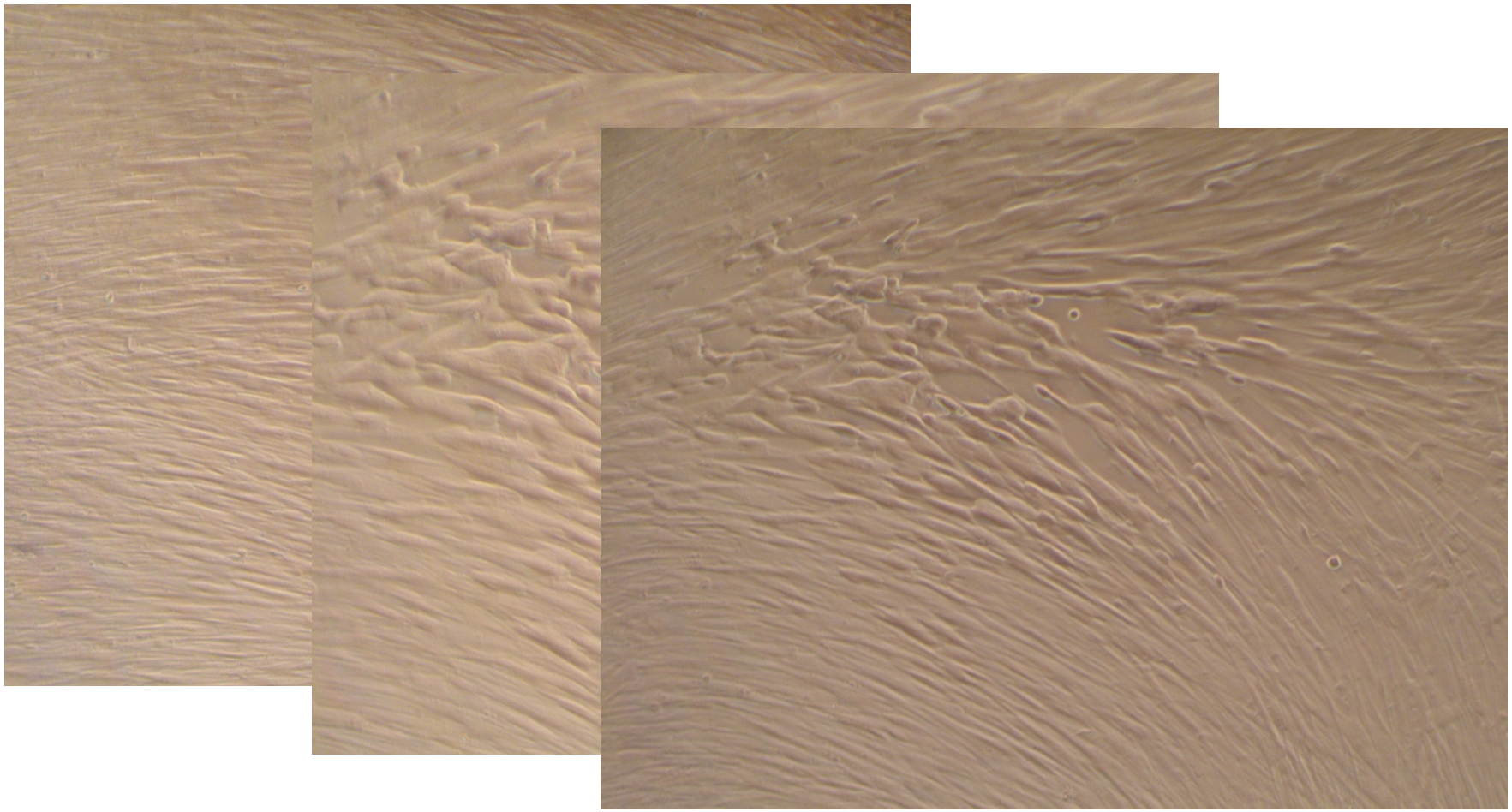


CPE: cell rounding and size increase

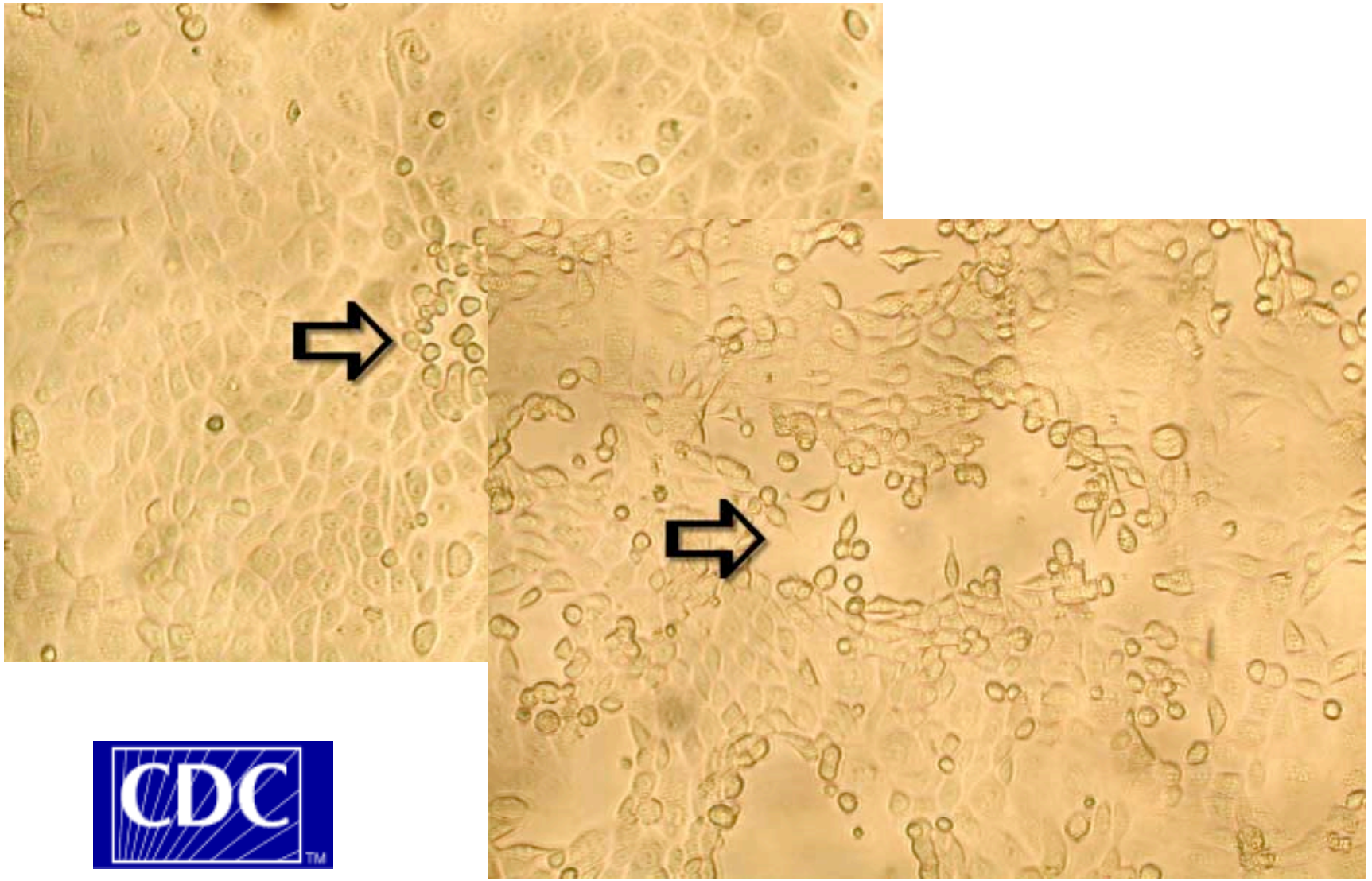


HCMV cytopathic effect on a fibroblast monolayer

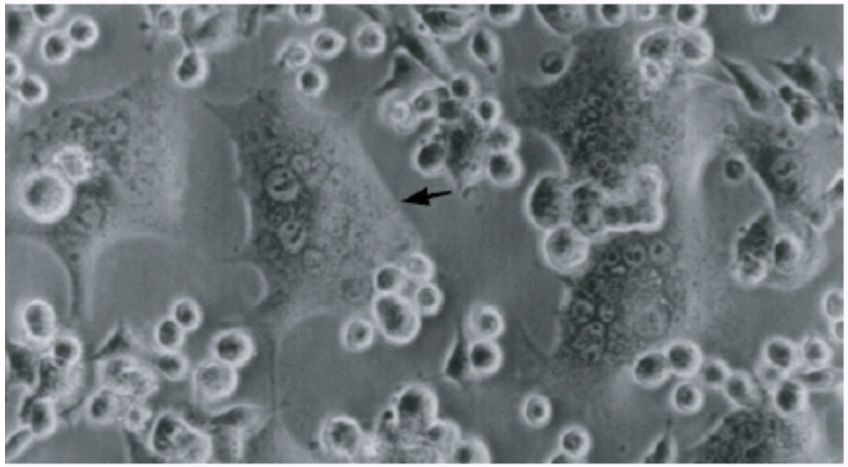
HCMV cytopathic effect on a fibroblast monolayer

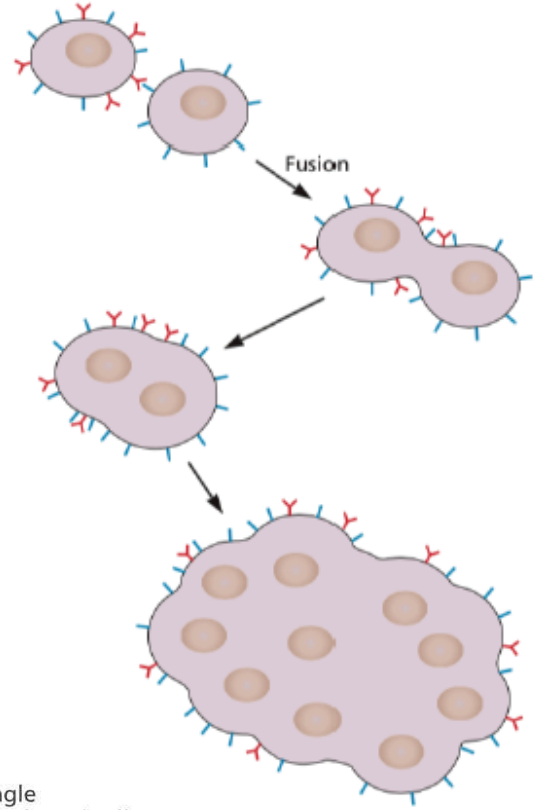


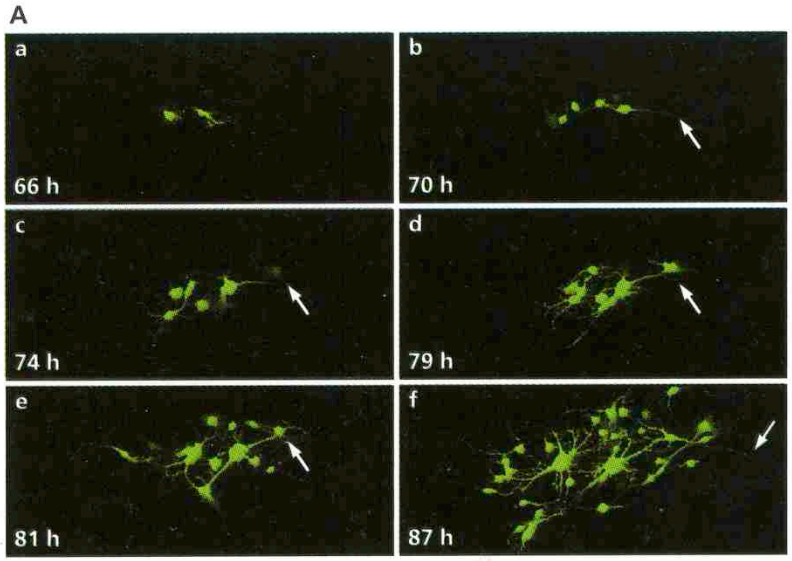
Human metapneumovirus CPE

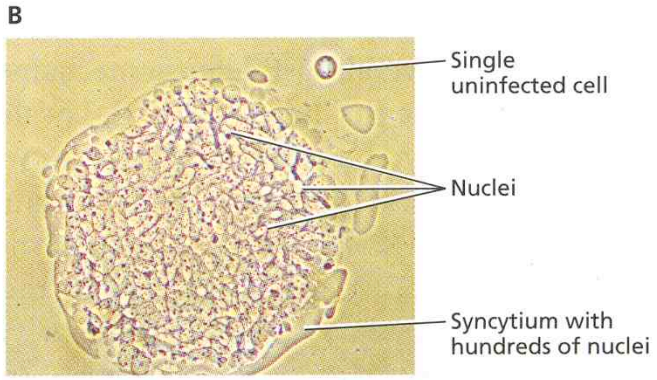


Virus CPE: syncythium formation

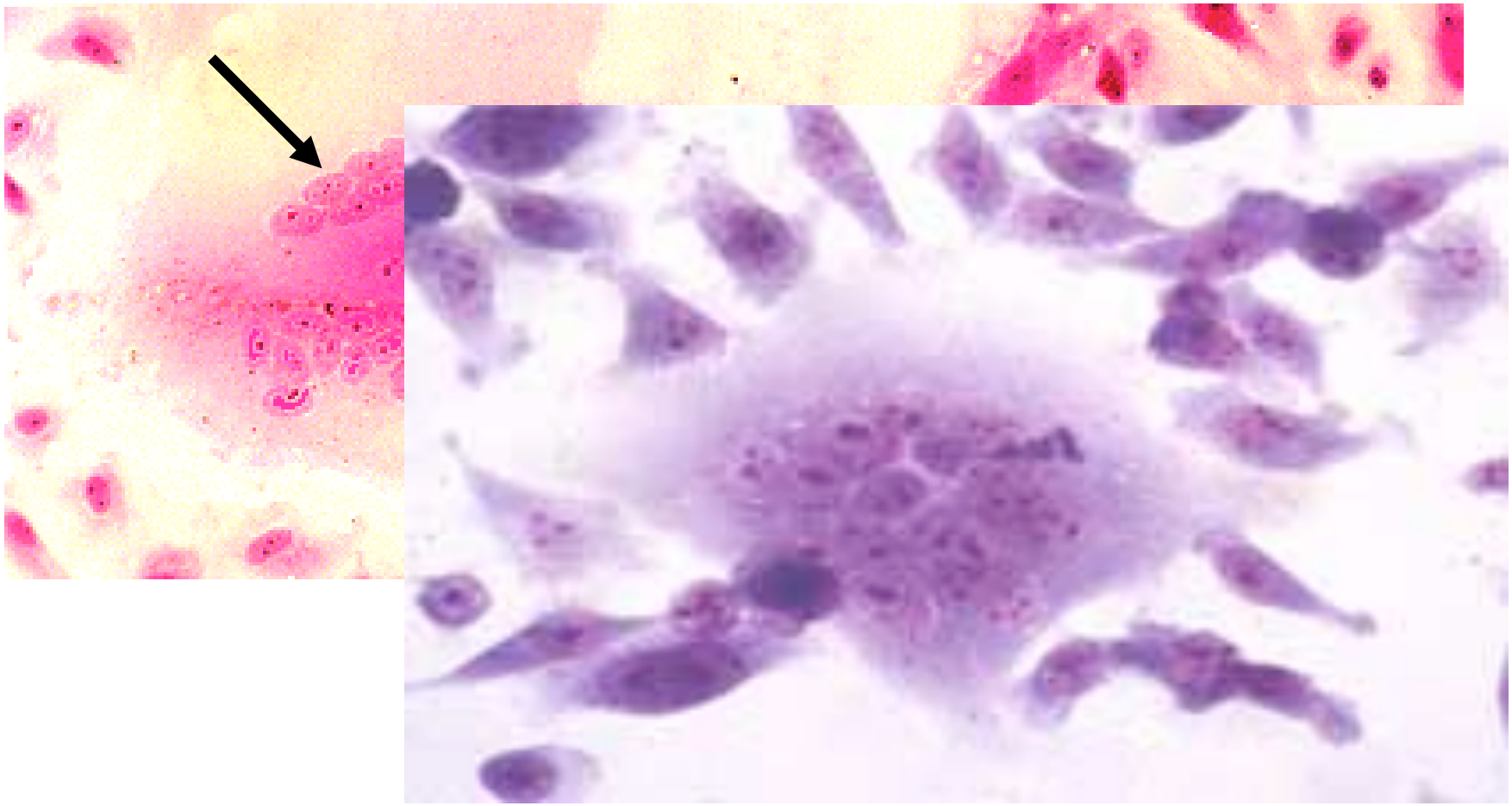






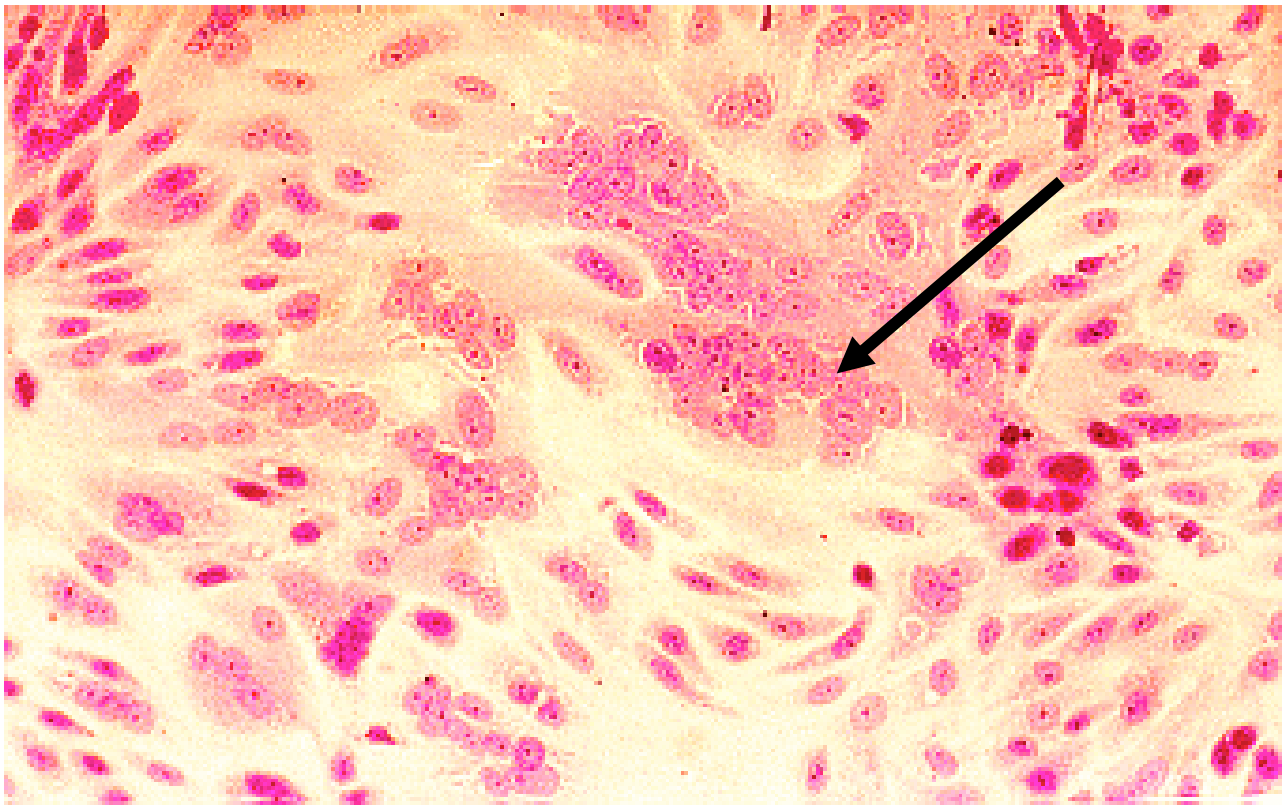


Virus CPE: syncythium formation



Formation of giant multinuclear cells (syncytium) by measles virus infection

Virus CPE: syncythium formation

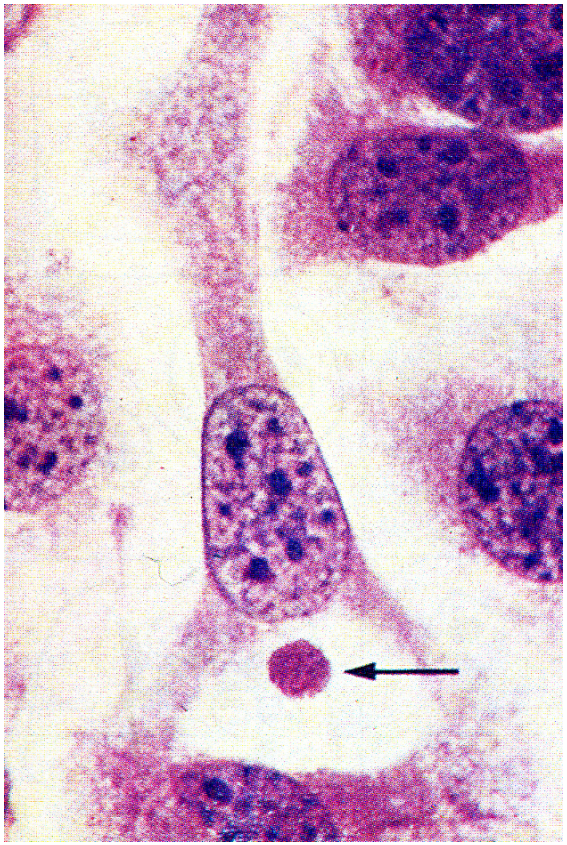


Formation of giant multinuclear cells (syncytium) by RSV infection

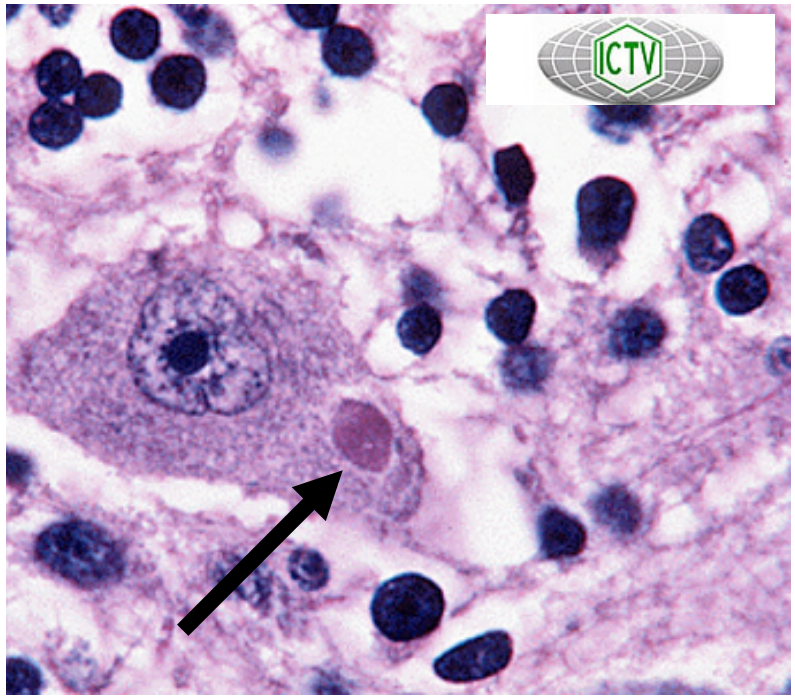
Inclusion bodies

Inclusion bodies	
Virions in nucleus	Adenoviruses
Virions in the cytoplasm (Negri bodies)	Rabies virus
"Factories" in the cytoplasm (Guarnieri bodies)	Poxviruses
Clumps of ribosomes in virions	Arenaviruses
Clumps of chromatin in nucleus	Herpesvir

Inclusion bodies: Pox and Rhabdo

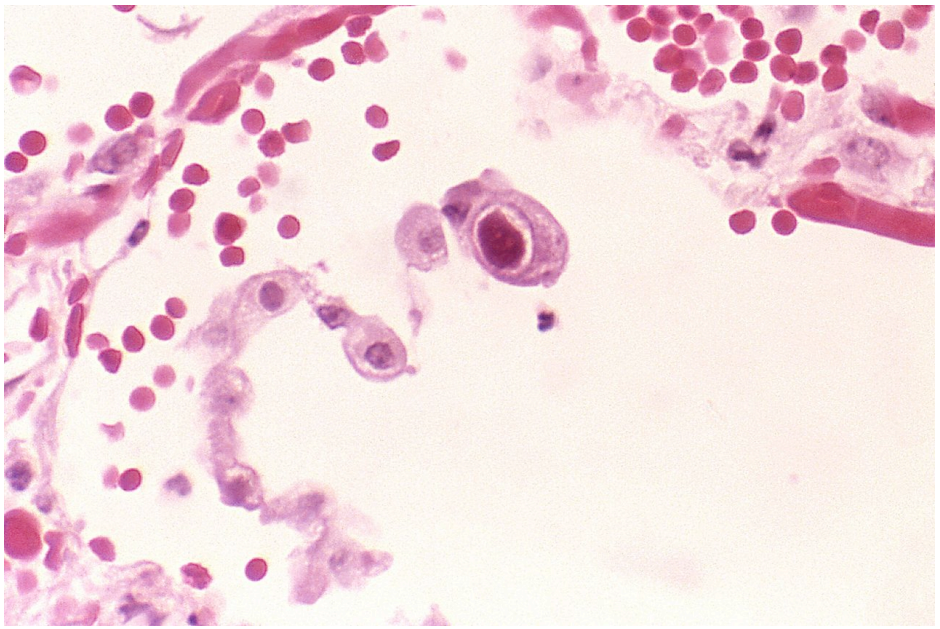


Monkey kidney cell with Guarnieri body in the cytoplasm

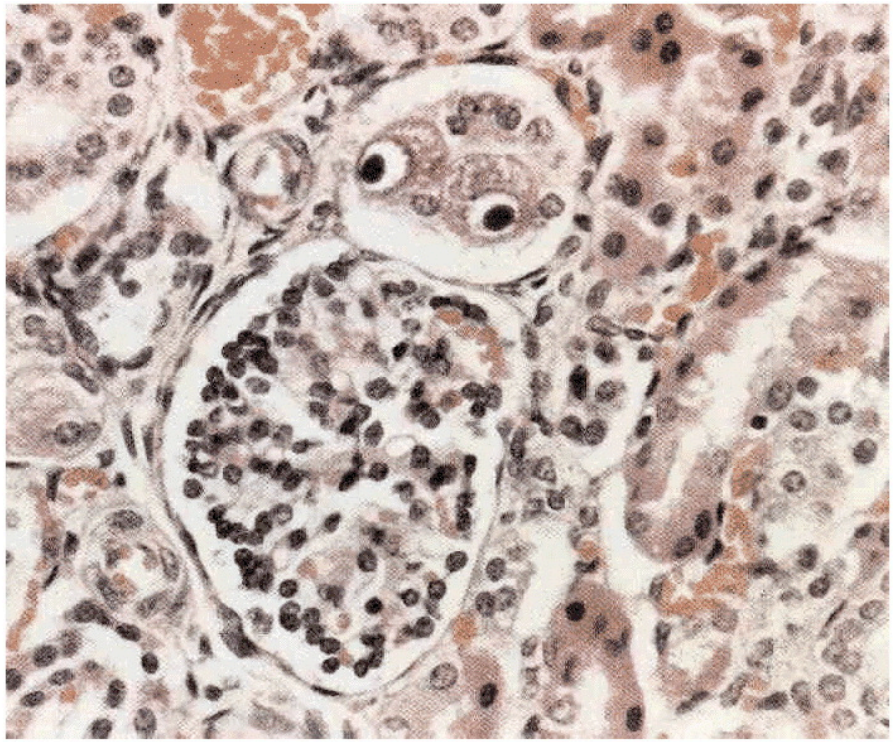


Purkinje cell with Negri body in the cytoplasm

Inclusion bodies: HCMV



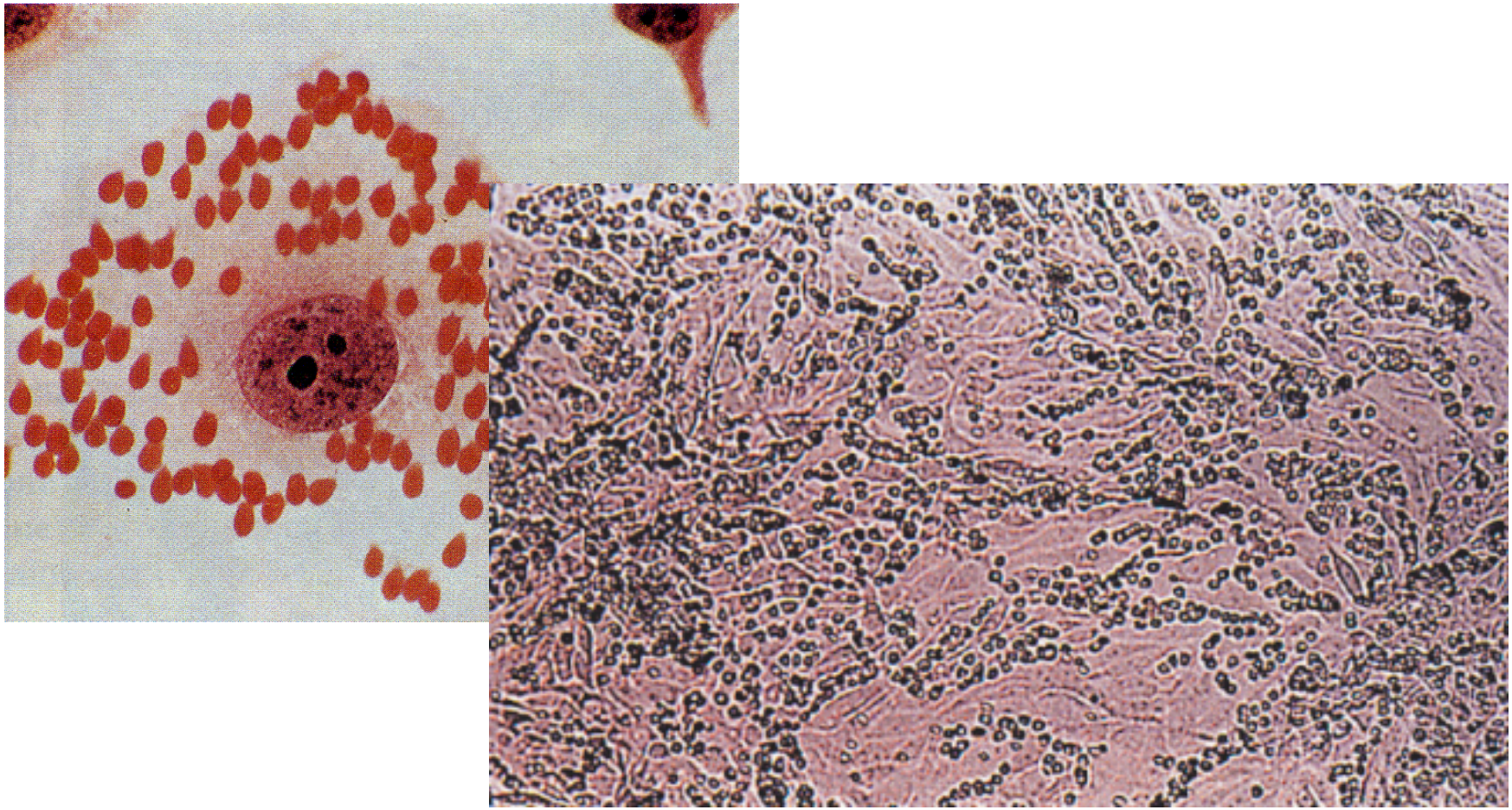
Human Cytomegalovirus infection of a lung pneumocyte, showing owl's eye appearance of a large cell at center.



The photomicrograph shows a section of kidney taken at autopsy from a three-month-old boy who died of disseminated HCMV infection contracted in utero. A single periglomerular renal tubule contains large, intranuclear viral inclusion bodies typical of those found in cells infected with cytomegalovirus. Such inclusion bodies are commonly seen at autopsy or in biopsy specimens from the kidneys, lungs, and other organs in cases of congenital or acquired cytomegalovirus infection.

Herriot R, Gray ES. N Engl J Med 1994;331:649-649.

Hemadsorption



Red blood cells attach specifically to virus-infected cells