Virus Cultivation: Cell Culture
MIC 204

Dr Shilpa Kaistha

Department of Microbiology

IBSBT/CSJMU Kanpur

Tissue Culture

- Cultivation of organ or tissue for virus propagation
- Three broad types:
  - Organ Culture: Part of the organ grown in in vitro conditions
    - Tracheal ring culture for coronovirus isolation
  - Explant Culture: Fragments of tissue grown as explants in plasma clot
- Rarely used in virology
  - Cell culture: cells are separated from tissue and grown under controlled conditions in suitable growth media. Based on origin and chromosome property these are of 3 types
    - Primary cell culture
    - Secondary cell culture
    - Immortalized cell lines

Cell Culture for Virus Cultivation

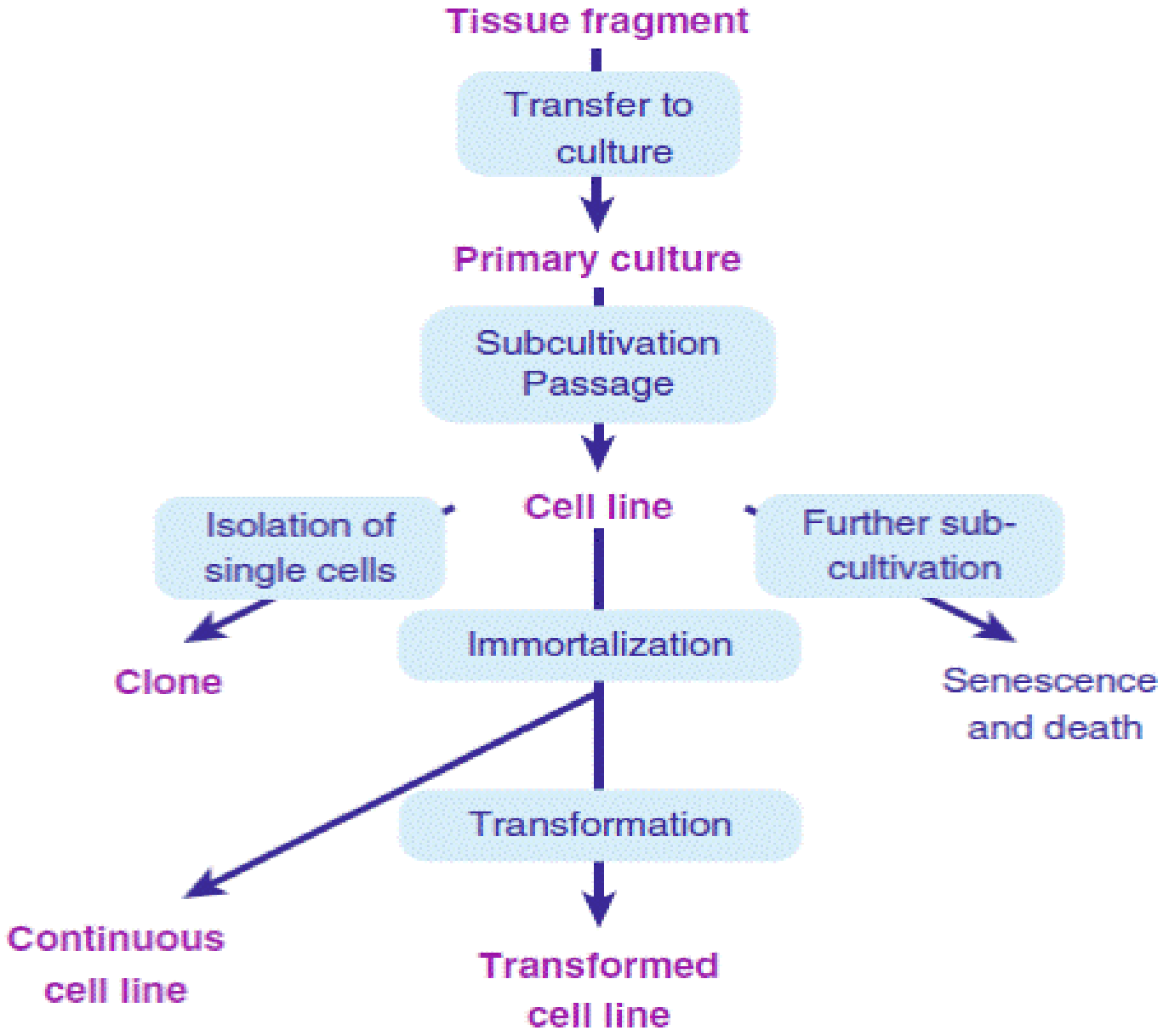
- Used isolated cells that are cultured in vitro
- Cell Culture Media developed
  - **Use of cell culture media**: The culture medium is the most important component of the culture environment, because it provides the necessary nutrients, growth factors, and hormones for cell growth, as well as regulating the pH and the osmotic pressure of the culture.

  - Discovery and use of antibiotics and antifungals to control contamination
  - Discovery of proteolytic enzymes (eg trypsin) that can free surrounding tissue without injuring cells
  - Disposable tissue culture flasks, etc

Cell Culture media

Component	Function
Carbon source (glucose/glutamine)	Source of energy
Amino acid	Building blocks of protein
Vitamins	Promote cell survival and growth
Balanced salt solution	An isotonic mixture of ions to maintain optimum osmotic pressure within the cells and provide essential metal ions to act as cofactors for enzymatic reactions, cell adhesion etc.
Phenol red dye	pH indicator. The color of phenol red changes from orange/red at pH 7–7.4 to yellow at acidic (lower) pH and purple at basic (higher) pH.
Bicarbonate /HEPES buffer	It is used to maintain a balanced pH in the media

https://en.wikipedia.org/wiki/Cell_culture



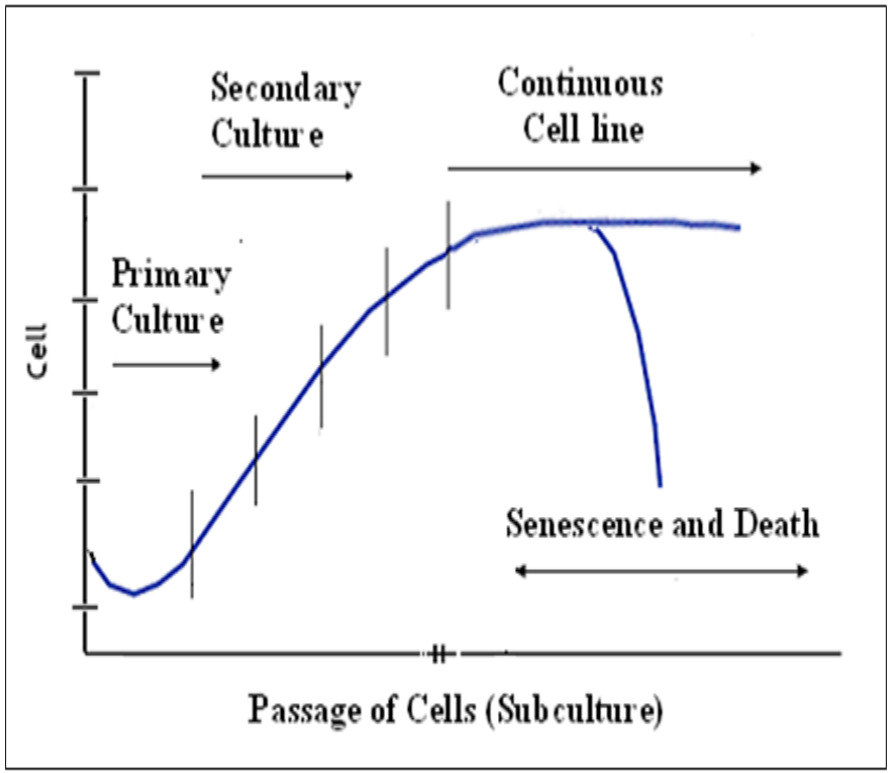
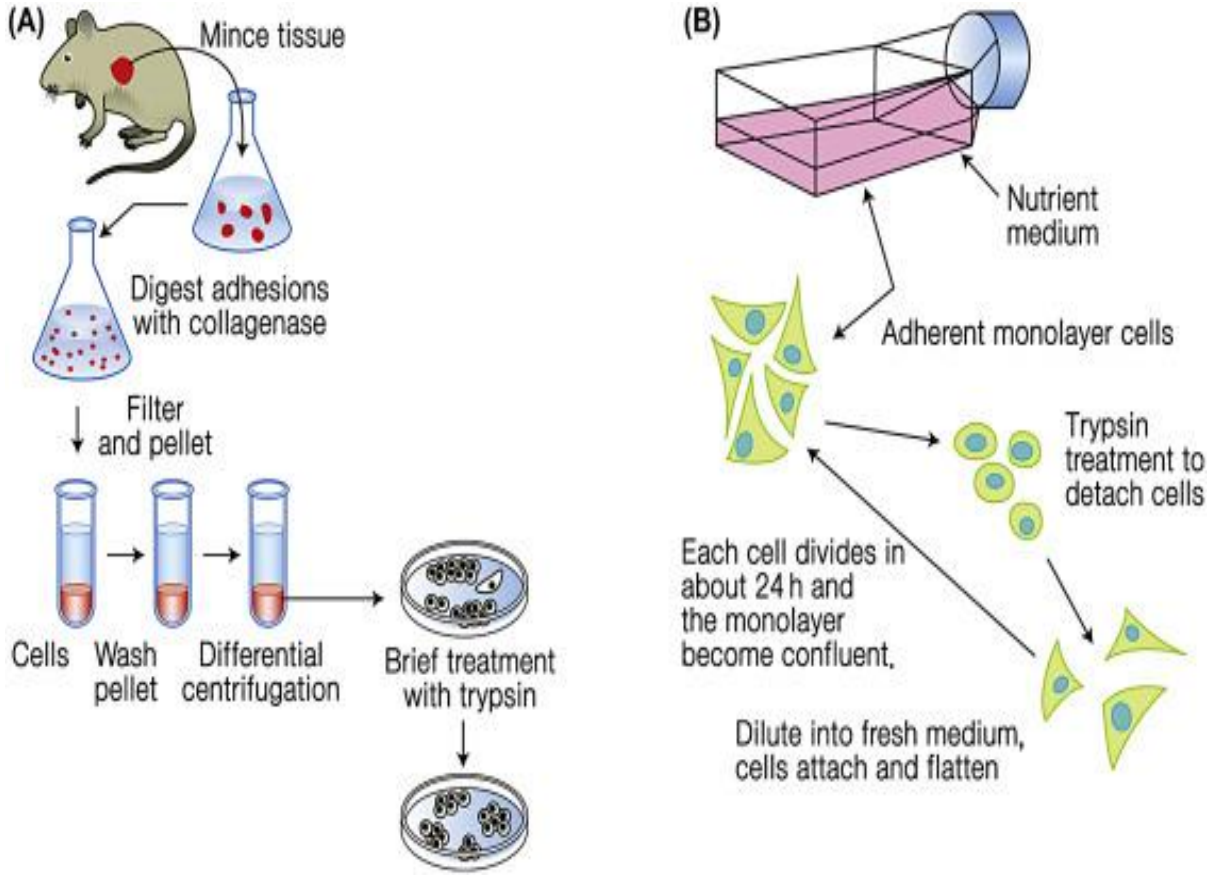


Figure 1: The growth kinetics of cells for generation of continuous cell lines from primary cell culture

Adv. Anim. Vet. Sci. 2 (4S): 1 – 10.

Primary Cell Line



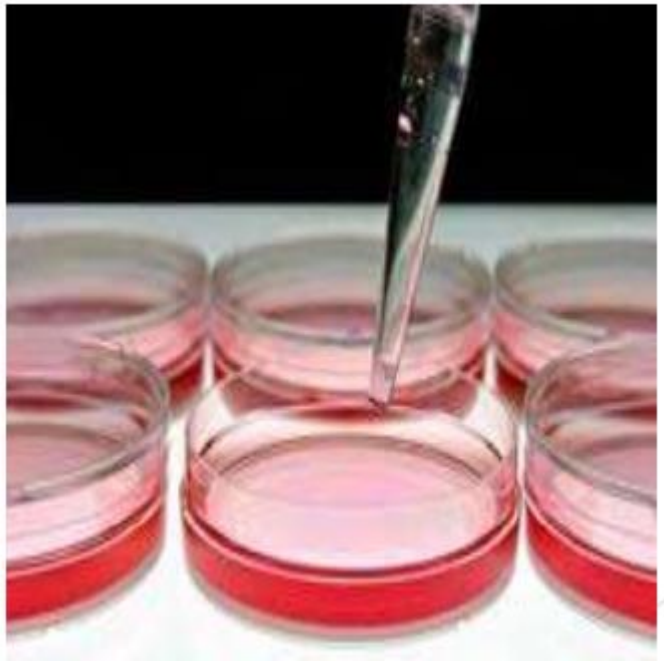
https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/animal-viruses

- Isolated directly from a human donor or animal tissue
- Undergo very limited handling or manipulation to preserve their original characteristics and functions
- Mortal
- Contact inhibition
- Anchorage dependent: adherent cells
- Suspension cells
- Eg. Human embryonic kidney primary cell culture, chick embryo cell culture

Types of cell culture

SUSPENSION

ADHERENT

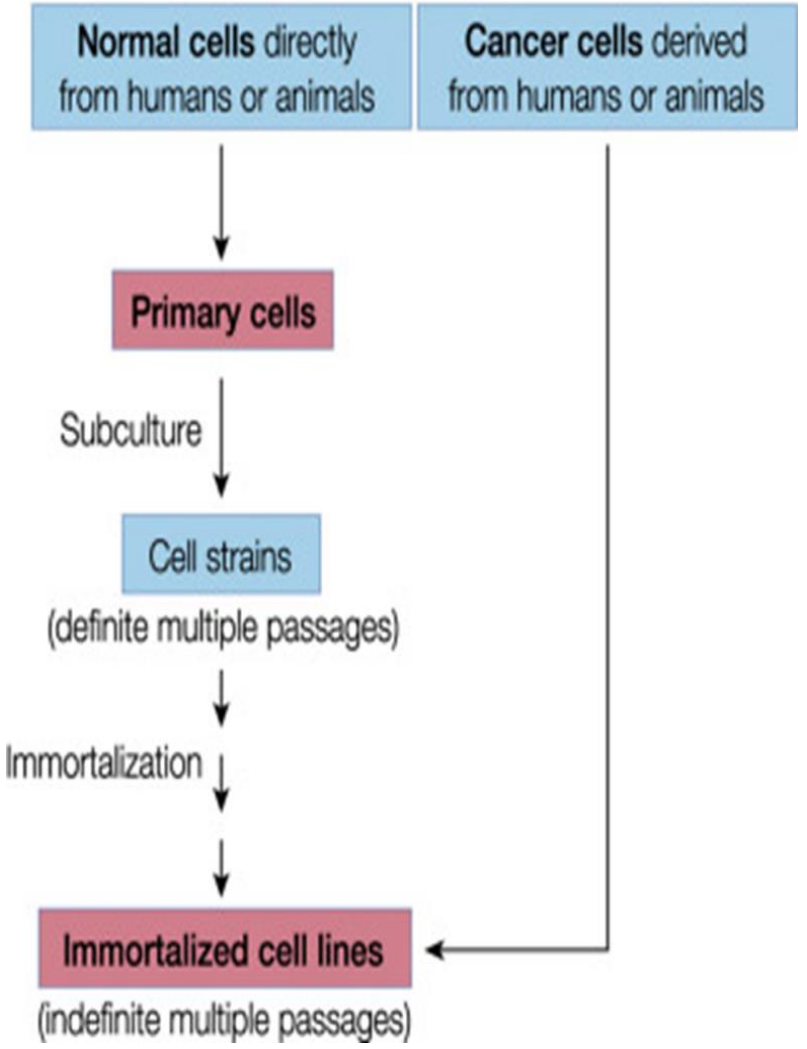


Diploid cell line (Semi continuous cell lines)

- Diploid containing same number of chromosomes as parent cells
- Can be subcultured up to 50 times using serial transfer before cell strain is lost
- Used for the production of fastidious viruses eg.
- Used for the production of viral vaccines
- Eg. Rhesus embryo diploid cell strain

Continuous/Immortalized culture

- 2 types of continuous cell culture
  - Finite:/ secondary cell culture: Senescence after 30 /finite number of divisions
  - Infinite/transformed cell line: When a finite cell line undergoes transformation and acquires ability to divide indefinitely, it is known as continuous cell line
- Process is known as cell transformation
- Occur spontaneously or can be chemically induced or virally induced
- Properties
  - Immortal
  - Rapid growth
  - No contact inhibition
  - Homogenous
  - Suspension or monolayer
  - Anueploidy: abnormal chromosome morphology or number
- Hep 2 cells, HeLa cells, Monkey kidney cell line (Vero cells)
- BHK-21 (Baby Hamster Kidney cell lines



	Primary Cells	Immortalized Cells
Origin	Isolated directly from donor	Isolated from tumors or intentionally immortalized with viruses
Lifespan	Limited	Unlimited lifespan in culture
***In Vivo* Model**	Yes	No
Function	Closely resembles cell function	May lack functions or characteristics of normal cells
Maintenance	Not considered primary cells following passage or culture	Can be maintained to provide consistent experimental results
Donor Characteristics	Available	Not available
Use Cases	Immunology, inflammation, vaccination or other biological experiments requiring cells with a close match to *in vivo* function	Studying tumor cells; If the cell of interest is not available or it is impractical to use primary cells

https://cellero.com/

	Primary Cells	Immortalized Cells
Origin	Isolated directly from donor	Isolated from tumors or intentionally immortalized with viruses
Lifespan	Limited	Unlimited lifespan in culture
***In Vivo* Model**	Yes	No
Function	Closely resembles cell function	May lack functions or characteristics of normal cells
Maintenance	Not considered primary cells following passage or culture	Can be maintained to provide consistent experimental results
Donor Characteristics	Available	Not available
Use Cases	Immunology, inflammation, vaccination or other biological experiments requiring cells with a close match to *in vivo* function	Studying tumor cells; If the cell of interest is not available or it is impractical to use primary cells

https://cellero.com/

Table 48.3 Some cell cultures in common use

a. Primary cell cultures	
1. Rhesus monkey kidney cell culture	
2. Human amnion cell culture	
3.Chick embryo fibroblast cell culture	
b. Diploid cell strains	
1.WI-38	Human embryonic lung cell strain
2.HL-8	Rhesus embryo cell strain
c. Continuouscell lines	
1. HeLa	Human carcinoma of cervix cell line
2.HEP-2	Human epithelioma of larynx cell line
3. KB	Human carcinoma of nasopharynx cell line
4. McCoy	Human synovial carcinoma cell line
5. Detroit-6	Sternal marrow cell line
6. Chang C/I/L/K	Human conjunctiva (C) Intestine (I), Liver (L) and Kidney (K) cell lines
7. Vero	Vervet monkey kidney cell line Baby hamster kidney cell line

Ananthnarayan and Panickar, Medical Microbiology 7th edition

Virus Infection

- Confluent cell lines are suspended in suitable media with required virus. Virulent virus will cause cell lysis and virus particles are released in to the surrounding media.
- Newly produced virions infect adjacent cells. This will result in localized area of cell destruction and lysis resulting in plaque formation
- Viral infection detected by the observing changes in the cell culture monolayer, known as cytopathic effect (CFE)

Susceptible cell lines:

Influenza virus- MDCK cells, Vero cells.

Paramyxoviruses- DF-1 cells, Vero cells.

Adenoviruses- HEK cells, HuH7 cells.

Herpesviruses- LMH cells.

Respiratory syncytia virus- Hep2 cells, Vero cells.

https://nptel.ac.in/courses/102/103/102103039/

Viral CPE

- ***Cell Death***
- **Total destruction/Complete CPE**
  - Most severe type of CPE.
  - All cells in the monolayer shrink rapidly, become dense in a process known as pyknosis, and detach from the glass within three days. Eg Enterovirus, Zika Virus
- **Subtotal destruction/Incomplete CPE**
  - Detachment of most but not all cells.
  - Togavirus, picorna virus, paramyxovirus
- **Focal Destruction/CPE**
  - Localized attack- spread due to direct cell to cell transfer of virus rather than diffusion through extracellular media
  - Characteristics effects: rounding of cells,
  - Herpes virus, Pox Virus

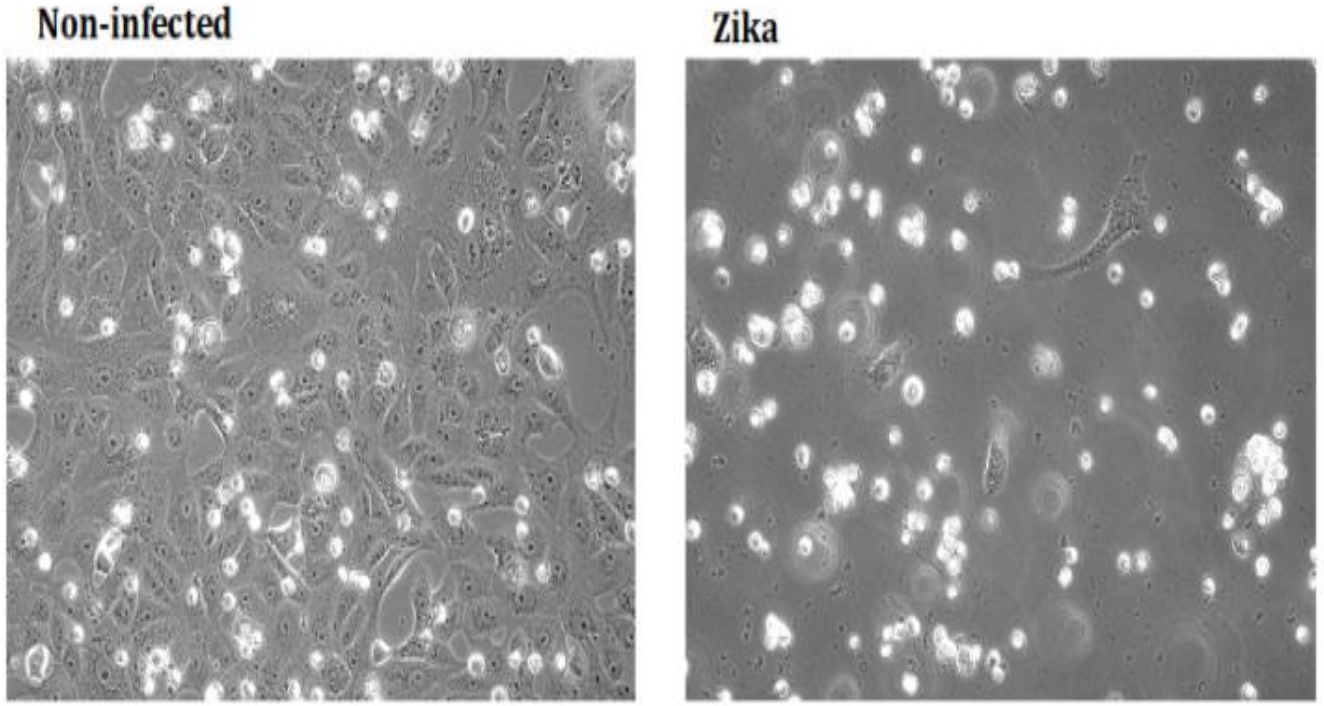
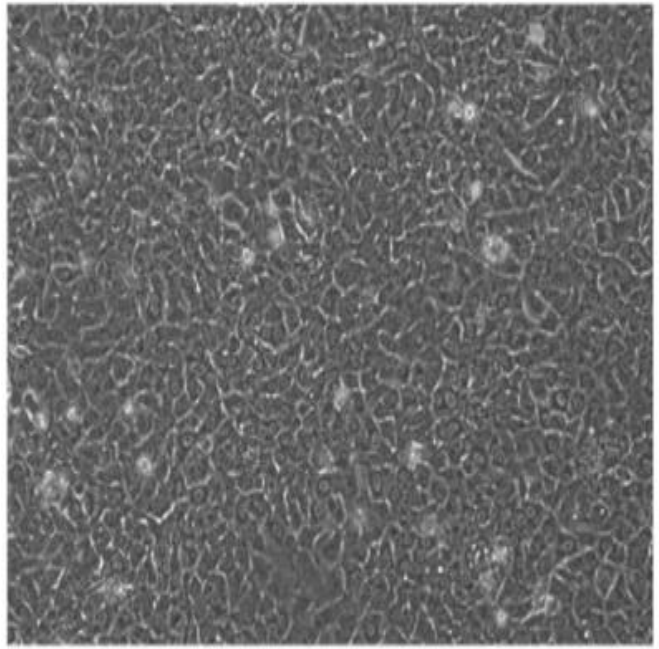


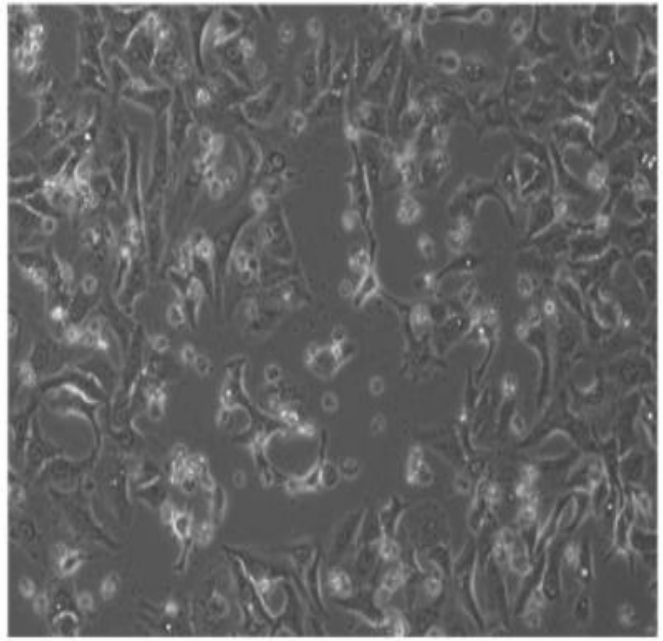
Figure 1 | Vero cells were infected with Zika virus. After 72 h of infection, a clear CPE is evident by the presence of cell debri as a result of cell death.

Viral CPE

- ***Morphological Alterations***
  - Nuclear shrinking (pyknosis), cell membrane perforation- Picorna Virus
  - Perforation of nuclear membrane- alpha virus, herpes virus
  - Cytoplasm vacuole/ Foamy degeration- Polyoma virus, Flavivirus, retrovirus
  - Swelling and clumping: Cells enlarge and form cluster of cells- grape like morphology Adenovirus
  - Syncytia formation- paramyxovirus, coronavirus
  - Margination and chromosome breaking- Herpes virus
  - Rounding up and detachment of cultured cells-herpes virus, rhanbdovirus, adenovirus, picorna virus



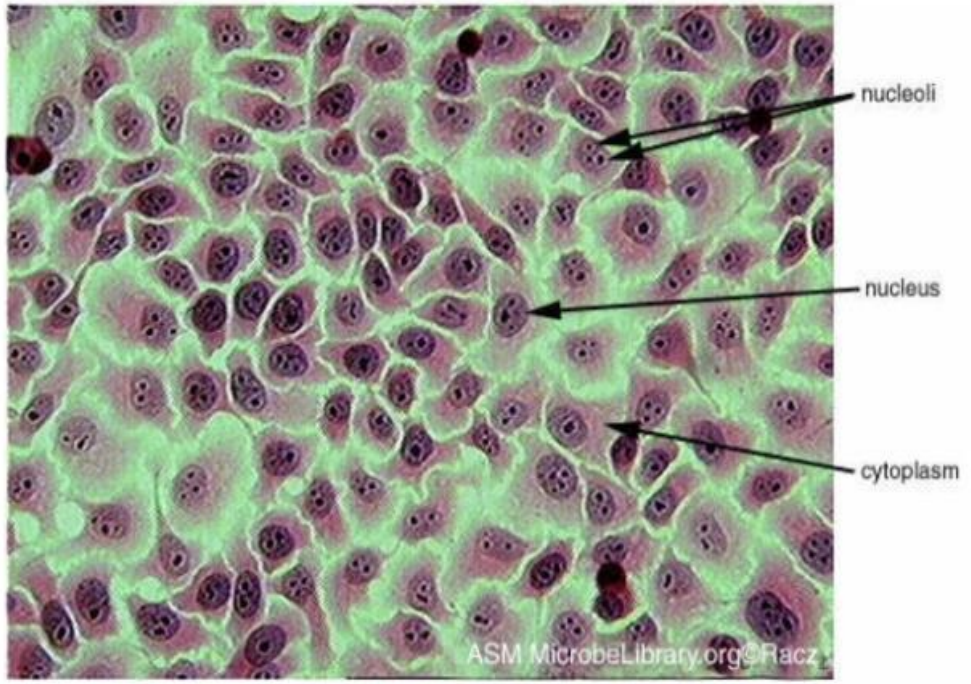
Normal human epithelial cells

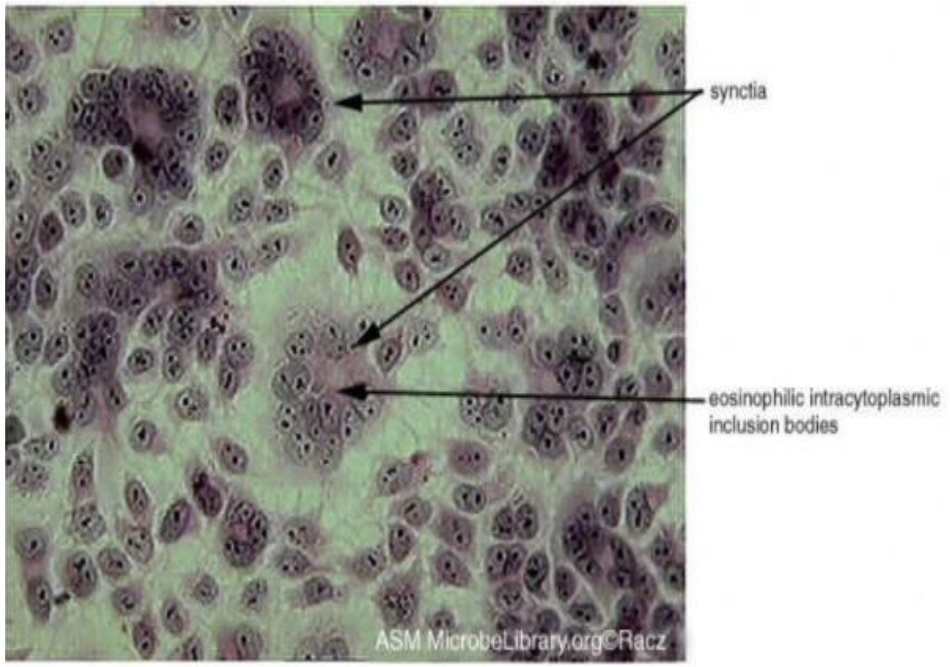


Adenovirus infected human epithelial cells

https://nptel.ac.in/courses/102/103/102103039/

Synctia formation with inclusion bodies





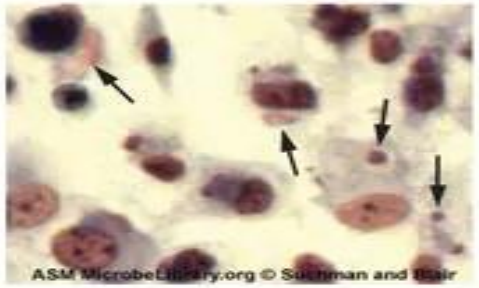
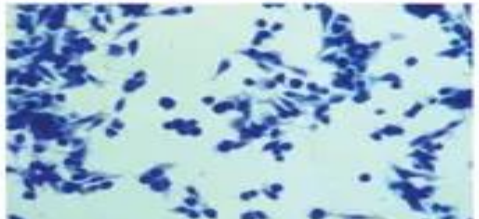
Vaccinia virus infection of Vero cells
https://www.asmscience.org/content/education

Viral Inclusion Bodies

- Insoluble structures in the cell seen by staining procedure
- Indicate areas of host cell wherein virus synthesis and assembly occurs
- Vary – single, multiple; small or large; round or irregularly shaped
- Virion in nucleus, clumps of ribosomes: Adenovirus
- Virion in cytoplasm:
  - Rabies virus (Negri bodies)
  - Pox virus- Guarnieri bodies

- Chromatin clumps in Nucleus: Herpes virus

Cytopathic Effects of Specific Viruses

Virus	Cytopathic Effect	Example
Paramyxovirus	Syncytium and faint basophilic cytoplasmic inclusion bodies	
Poxvirus	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	
Herpesvirus	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	
Adenovirus	Cell enlargement, rounding, and distinctive "grape-like" clusters	

https://medizzy.com/feed/755933

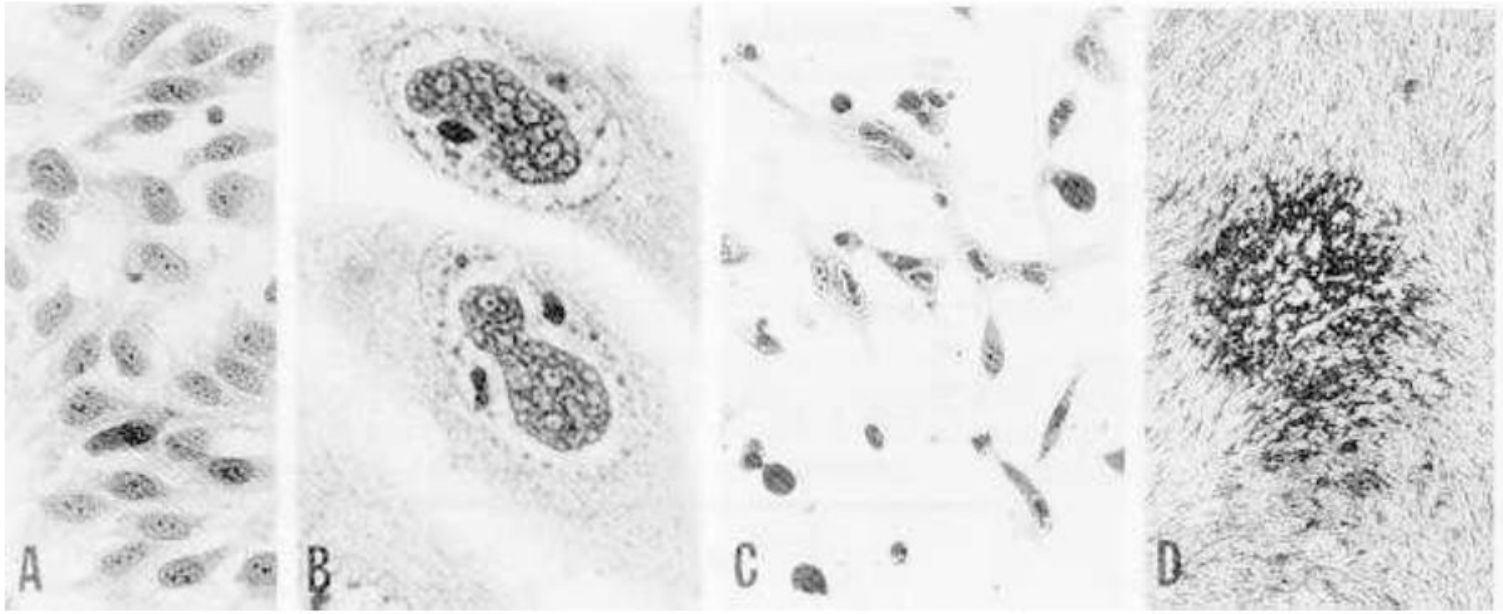


Figure 44-1 Development and progression of viral cytopathology

Human embryo skin muscle cells were infected with human cytomegalovirus and stained at selected times to demonstrate (A) uninfected cells, (B) late virus cytopathic effects (nuclear inclusions, cell enlargement), (C) cell degeneration, and (D) a focus of infected cells in a cell monolayer (i.e., a plaque), hematoxylin and eosin stain. (A, × 255; B, × 900; C, × 225; D, × 20.)

From: Chapter 44, Effects on Cells

Medical Microbiology. 4th edition.
Baron S, editor.
Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

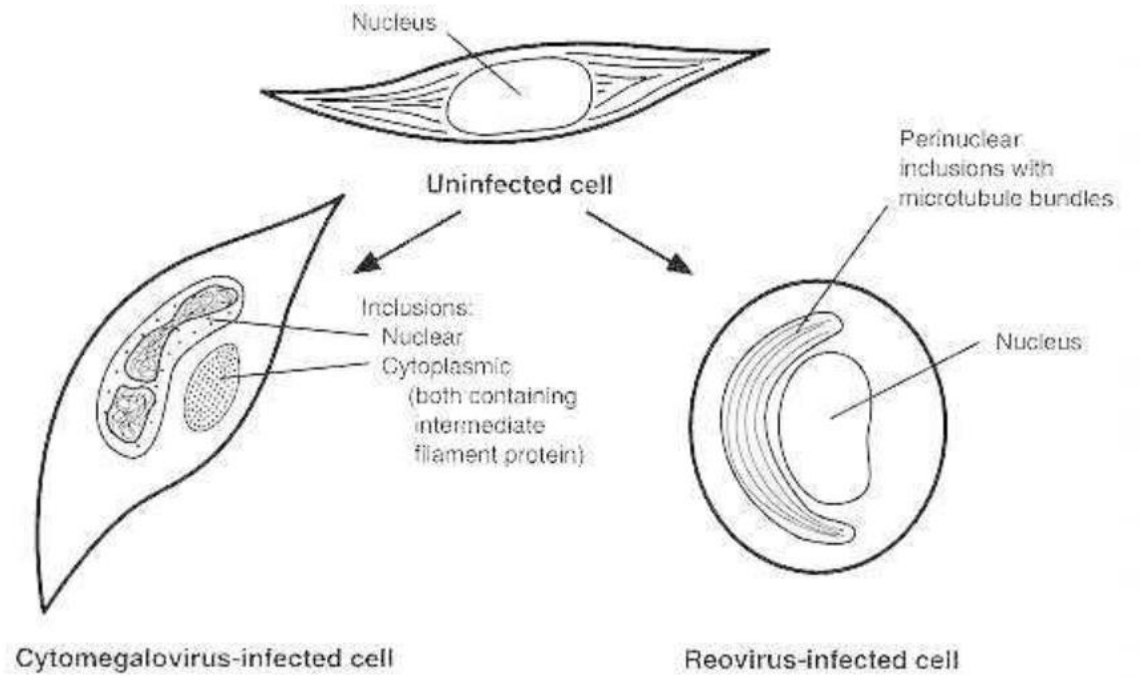


Figure 44-4 Alteration of cytoskeleton organization by virus infection

Normal cells have networks of microtubules, and intermediate filaments throughout the cytoplasm. Infection with reovirus causes a perinuclear aggregation of microtubules, and infection with cytomegalovirus causes a modification of intermediate filaments proteins, including their relocation into the nuclear and cytoplasmic inclusion bodies.

From: Chapter 44, Effects on Cells

Medical Microbiology. 4th edition.
Baron S, editor.
Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

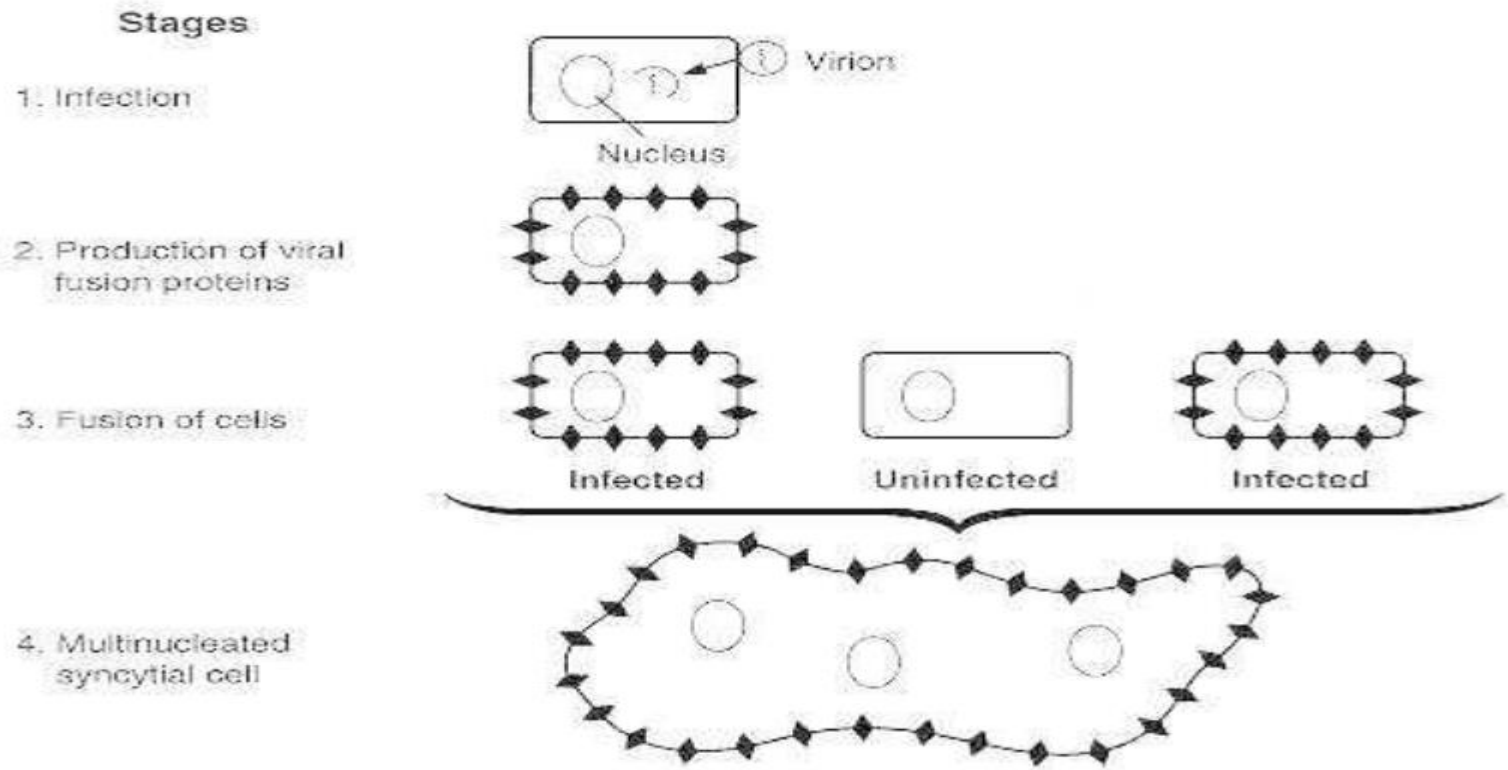
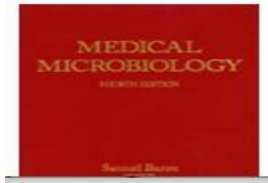


Figure 44-2 Formation of multinucleated cells

The figure represents the cytopathology of measles virus-induced syncytia.

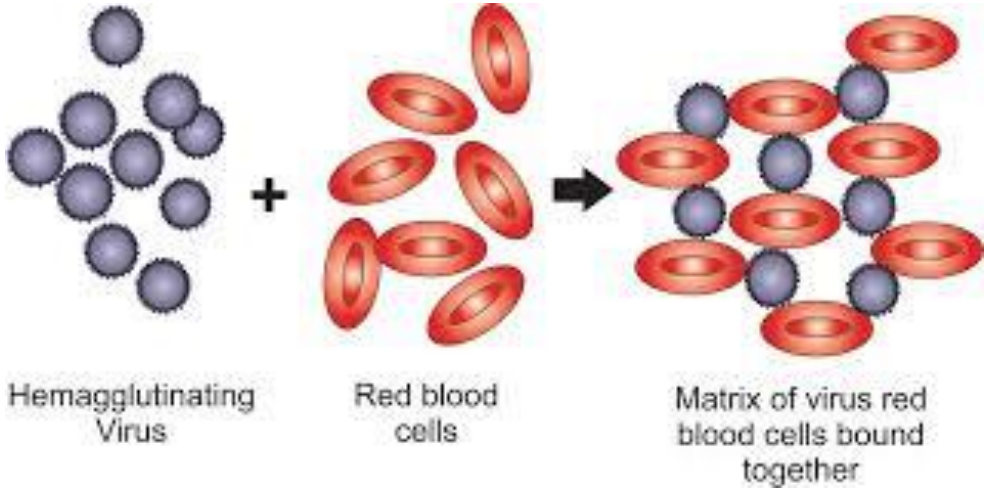
From: Chapter 44, Effects on Cells

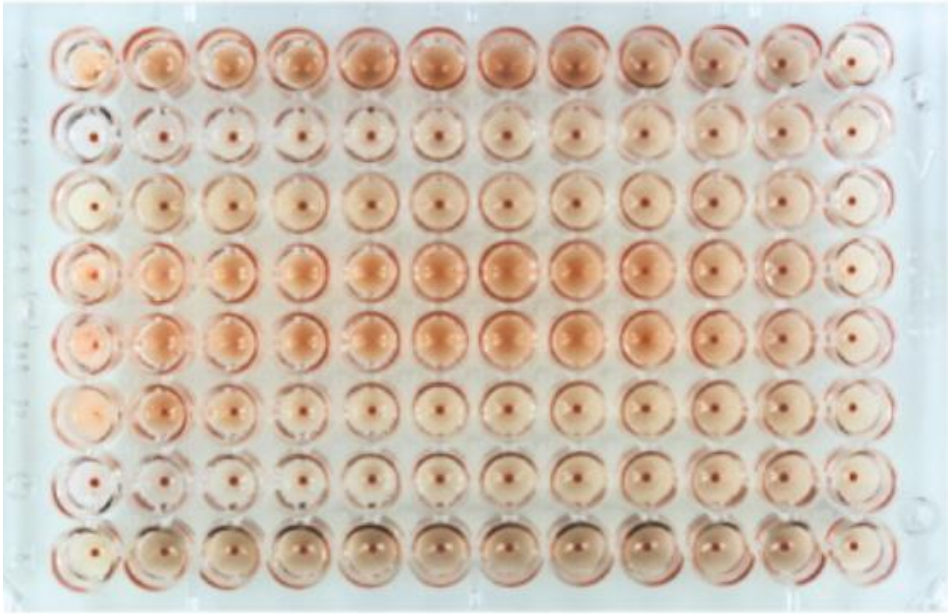


Medical Microbiology. 4th edition.
Baron S, editor.
Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

Hemadsorption

- Viruses like Influenza and Parainfluenza virus express Haemagglutin on their surface
- Causes RBC adsorption
- Virus multiple in cells and cause adsorption and finally hemagglutination





https://link.springer.com/protocol/10.1007/978-1-0716-0346-8_1

Viral Transformation

- **Oncogenic RNA viruses**
  - Retroviruses contain retroposons t into host genome using Reverse transcriptase and integrase enzymes
- **Oncogenic DNA viruses**
  - Do not contain Rt but contain genes that act early to induce expression of host cell genes responsible for cell cycle regulation
  - Eg. Adenovirus, Herpes virus
- **Acute transforming viruses**
  - Produce tumor in infected animals in a few weeks.
  - Can transform cells in culture
  - Viral oncogene is altered and when inserted in host acts as a defective proto-oncogene or changes the proto-oncogene to dysregulate cell growth
  - Eg SV40 T antigen
- **Slow Transforming viruses**
  - Takes months to produce tumors/cancer
  - Do not contain oncogenes but insert near an cellular oncogene to alter its function
- **Transactivating Viruses**
  - Contain extra genes that influence uncontrolled growth
  - Insert genes at cis acting sites

Virus associated with Human Cancers

Virus	Cancer
Epstein Barr Virus	Burkitt's Lymphoma
Human papilloma Virus	Cervical Cancer
Hepatitis B virus	Liver cancer/Hepatocellular carcinoma
Human T cell lymphotrophic virus	Adult T cell leukemia
Human Herpes Virus 8	Kaposi Sarcoma

Chromosomal abberations

Chromosome damage may be caused directly by the virus particle or indirectly by events occurring during synthesis of new viral macromolecules (RNA, DNA, protein).

The chromosome damage (Fig. 44-5) may or may not be faithfully repaired, and in either case, it may or may not be compatible with survival of the infected cell.

When the cell survives, the virus genome may persist within the cell, possibly leading to continued instability of cellular genomic material or to altered expression of cellular genes (e.g., cellular oncogenes).

Virus-induced genomic instability appears to be associated with accumulation of mutations and related to the process of cell immortalization and oncogenic transformation.

Eg. cytomegalo virus, Retroviruses, Hepadna virus

Figure 44-5 Chromosomal aberrations resulting from cytomegalovirus infection of human peripheral blood lymphocytes

From: Chapter 44, Effects on Cells

Medical Microbiology. 4th edition.
Baron S, editor.
Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

Disrupt Metabolic machinery

Virus	Metabolic Changes	Mechanism	References
Adenovirus (ADWT)	Increased glycolysis for nucleotide synthesis and increased glutaminolysis and reductive carboxylation	E4ORF1-mediated activation of cellular MYC	Fisher et al., Proc Soc Exp Biol Med, 1957; Thai et al, Cell Met, 2014; Thai and Thaker et al., Nature Comm, 2015
Human cytomegalovirus (HCMV)	Increased glycolysis and nucleotide synthesis; greater increases in TCA cycle and lipid biosynthesis; increased anaplerotic use of glutamine	CAMPKK-AMPK signaling necessary; increased GLUT4 translocation to plasma membrane; ?	Munger et al., Nat Biotech, 2008; Chambers et al., J Virol, 2010; Vastag et al., PLoS Pathog, 2011; Terry et al., PNAS, 2012; Yu et al., J Virol, 2011
Herpesvirus-1 (HSV-1)	Increased glycolysis and anaplerotic influx to TCA cycle through pyruvate carboxylase; upregulation of pyrimidine nucleotide synthesis	Increased PFK-1 activity; GOT2 activity ?	Vastag et al., PLoS Pathog, 2011; Abrantes et al., Biochem Biophys, 2012; Grady et al., PNAS 2013
Influenza A virus	Increased glycolysis	?	Ritter et al., BMC Syst Biol, 2010
Dengue virus (DENV)	Increased glycolysis and increased fatty acid biosynthesis	DENV NS3 recruits FAS to sites of viral replication; ?	Fontaine et al., J Virol, 2015; Heaton et al., PNAS, 2010
Zika virus (ZIKV)	Increased glucose incorporation into TCA cycle intermediates (HFF-1) and into PPP (C6/36 mosquito cells)	?	Thaker et al., Cell Met, 2019
Vaccinia virus (VACV)	Increased glutaminolysis; *De novo* fatty acid biosynthesis and ß-oxidation	VACV protein C16 stabilizes HIF-1α; ?	Fontaine et al., J Virol, 2014; Greseth et al., Plos Path, 2014; Mazzon et al., PNAS, 2013

Metabolic Inhibition as CPE

- Normal cell culture, media turns from red to yellow due to acid production. In the presence of viral infection, when metabolism is affected, there is no change in color of media

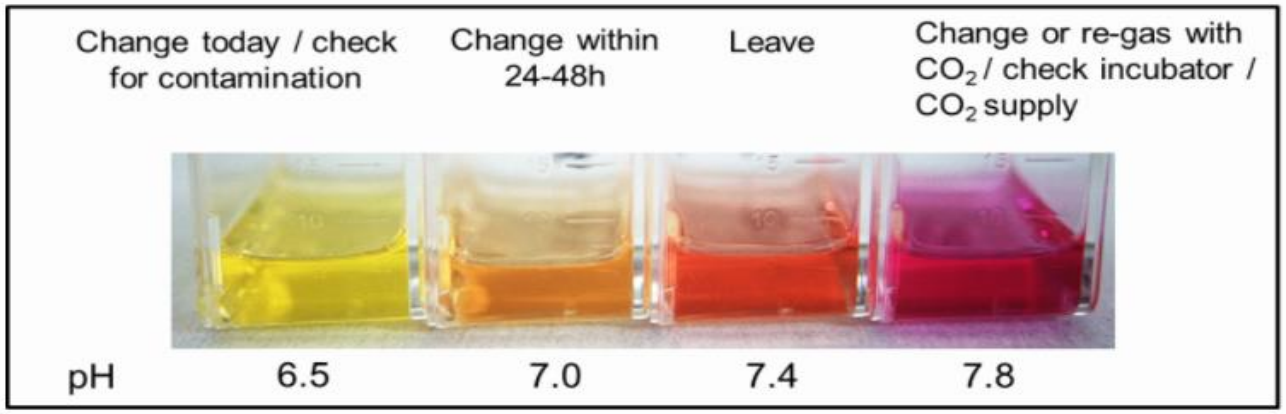
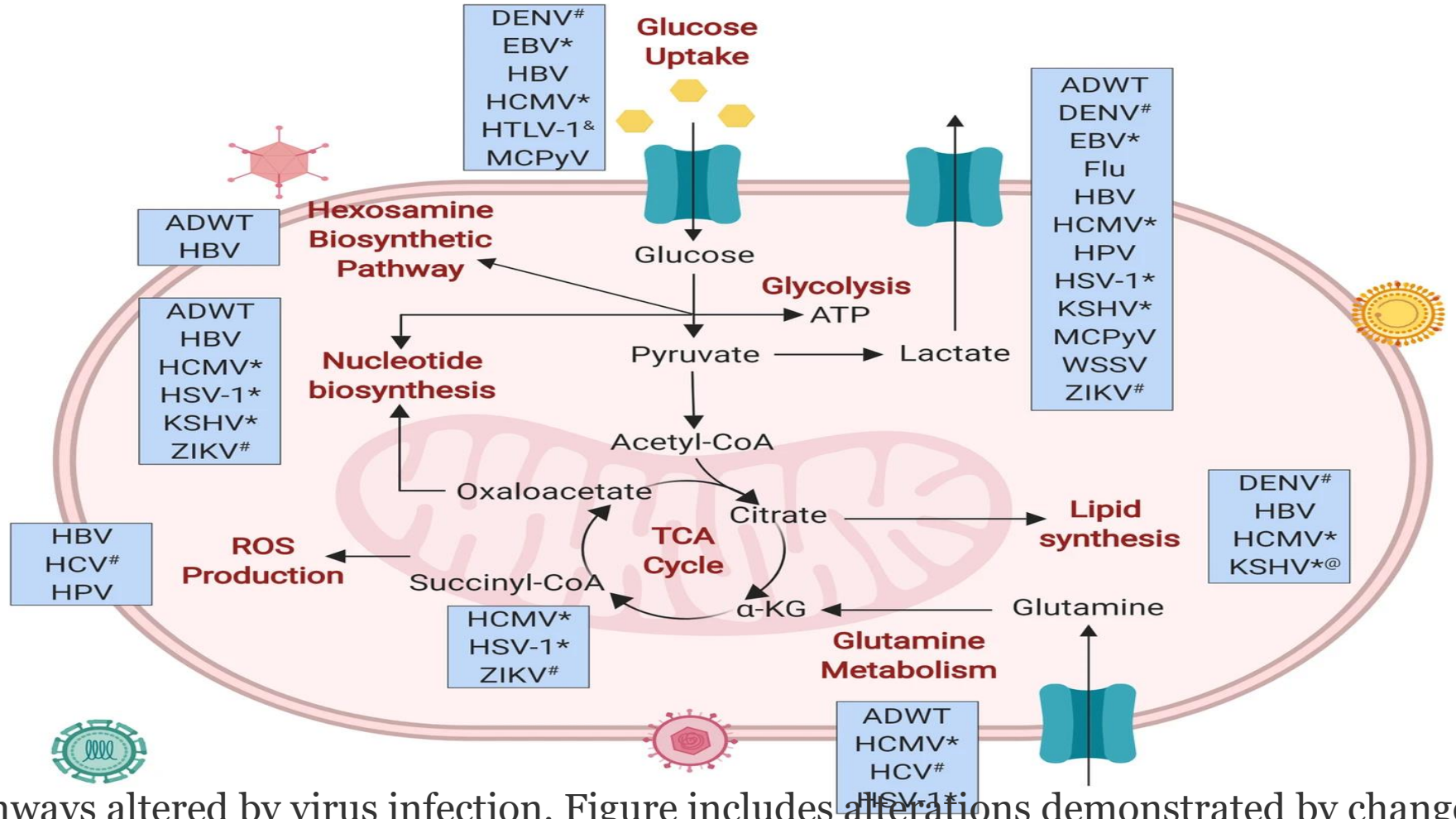


Figure 1. Cell culture media are generally supplemented with Phenol Red to act as a pH indicator. The colour of the medium helps advise if cultures are at physiological pH and can assist with troubleshooting.

https://www.phe-culturecollections.org.uk/

Viral hijacking of cellular metabolism. *BMC Biol* 17, 59 (2019).



Metabolic pathways altered by virus infection. Figure includes alterations demonstrated by changes in metabolite levels, flux, and tracing. *Herpesvirus family; #Flavivirus family; &virus downregulates this metabolic activity; @KSHV upregulates lipid synthesis but downregulates cholesterol synthesis. Created with BioRender.com

Summary

- Viral infection can be studied in vivo (natural hosts) or in vitro (embryonated eggs/ cell or tissue culture)
- Viral infection causes changes in cell structure, metabolism and physiology
- Changes are refered to as cytopatheic effects
- CPE can be used as an indicator for qualitative and quantitative analysis of viral infection

- Thank you