PHARMACEUTICAL MICROBIOLOGY

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OBJECTIVES

- History of Virology
- Structure of Viruses
- General Characteristics of Viruses
- Cultivation of Viruses
- Effect of Chemical and Physical Factors on Viruses

Virology

- Viruses are tiny infectious agents that can cause diseases in the organism. They are more primitive than bacteria. They are obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants and animals.

- Ultramicroscopic size, ranging from 20 nm up to 450 nm (diameter) (Bacteria 1000-2000 nm).

- Viruses can not be seen with light microscopes; they can be only seen with electron microscopes.

History of Virology

- Viruses were too small to be seen with the first microscopes. The cause of viral infections was unknown for years.

- Although Edward Jenner (1798) and Louis Pasteur (1885) developed the first vaccines to protect against viral infections, they did not know that viruses existed.

- Louis Pasteur first proposed the term virus.

History of Virology

- 1892- Ivanovski and Beijerinck showed that a disease in tobacco was caused by a virus.
- 1890s- Loeffler and Frosch discovered an animal virus that causes foot and mouth disease in cattle.
- 1931- viruses were first grown in embryonated chick egg.
- 1983- HIV was isolated and identified by researchers at the Pasteur Institute in France.

General Characteristics of Viruses

- Viruses are not made out of cells.
- Basic structure consists of a protective protein shell (**capsid**) surrounding nucleic acid core. Some virus strains have an extra membrane (lipid) surrounding it called an **envelope**.
- Viruses do not have organelles and ribosomes.
- Viruses are inactive macromolecules outside the host cell, they are only active inside the host cells.

General Characteristics of Viruses

- Viruses can not reproduce unless they invade a specific host cell and use its genetic and metabolic machinery to make and release new viruses.

- They can not synthesize proteins, because they don’t have ribosomes and must use the ribosomes of host cells to translate viral messenger RNA into viral proteins.

General Characteristics of Viruses

- They don’t have enzymes for most metabolic processes.

- Viruses can not generate or store energy in the form of adenosine triphosphate (ATP), but have to derive their energy, and all other metabolic functions, from the host cell.

- They also use the host cell’s basic building materials, such as amino acids, nucleotides, and lipids.

Cultivation of Viruses

- Viruses can't be grown in culture media or on agar plates alone, there must be living cells to support their replication.

- Viruses can only reproduce inside host cells. There are three common methods for cultivation of viruses.

  - ✓ Tissue cultures
  - ✓ Embryonated eggs
  - ✓ Experimental animals

Cultivation of Viruses

Live animals such as monkeys, mice, rabbits, guinea pigs are widely used for cultivating virus. After the animal is inoculated with the virus, the animal is:

- ✓ observed for signs of disease
- ✓ visible lesions
- ✓ killed (infected tissues can be examined for virus)

Cultivation of Viruses

The signs of viral growth in embryonated eggs:

- ✓ death of the embryo
- ✓ defects in embryonic development
- ✓ damage in membranes (discrete opaque spots called pocks)

Cultivation of Viruses

Cytopathic effect is an observable morphological change that occurs in host cells because of viral replication.

- ✓ changes in size and shape
- ✓ cytoplasmic inclusion bodies
- ✓ cell lysis
- ✓ alter DNA
- ✓ cells fuse to form multinucleated cells
- ✓ transform cells into cancerous cells

General Characteristics of Viruses

- Antibiotics block metabolic pathways in bacteria (inhibit cell wall formation; protein synthesis).
- Viruses use host cell metabolic pathways.
- Viruses do not possess a cell wall and so are not affected by antibiotics.
- Antibiotics are not used to treat viral diseases because they are ineffective and may harm helpful bacteria (normal human flora).

General Characteristics of Viruses

- Interferons (IFNs) are a group of signaling proteins made and released by host cells in response to the presence of several pathogens (such as viruses, bacteria, parasites and tumor cells).

- IFNs belong to the large class of proteins known as cytokines; molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens.

General Characteristics of Viruses

Viral-infected cells secrete interferons to warn neighboring cells. Interferons enter neighboring cells - produce proteins that block viral reproduction and degrade viral RNA.

Viruses contain antigenic parts which produce strong immunity.

Surface proteins of the viral envelope or viral capsid proteins are good antigenic components.

Structure of Viruses

- Viruses composed of **nucleic acid** either **DNA or RNA** (but **never both**), **surrounded by a protein coat** called the **capsid**.
- The **capsid** is composed of small structural units called **capsomeres.**
- The capsid **protects nucleic acid from inactivation by the outer physical conditions.**
- Some viruses have additional **lipoprotein envelope, composed of virally coded protein and host lipid.** The viral envelope is covered with **glycoprotein spikes.**

Structure of Viruses

- While the genomes of all known cells are comprised of double-stranded (ds) DNA, the genomes of viruses can be comprised of **single-stranded (ss) or double-stranded DNA or RNA.**

- Nucleic acids can be either:
  - ✓ **single-stranded** (ss) or **double-stranded** (ds)
  - ✓ **linear** or **circular**
  - ✓ **unsegmented** (single molecule) or **segmented** (two or more molecules)

Structure of Viruses

DNA Genomes

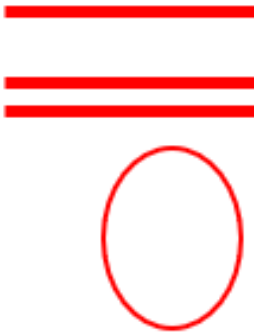
ss, linear ——————Parvoviruses

ds, linear

ss, circular

ds, circular

RNA Genomes



ss, linear

ds, linear ——————Reoviruses

ss, circular

Structure of Viruses

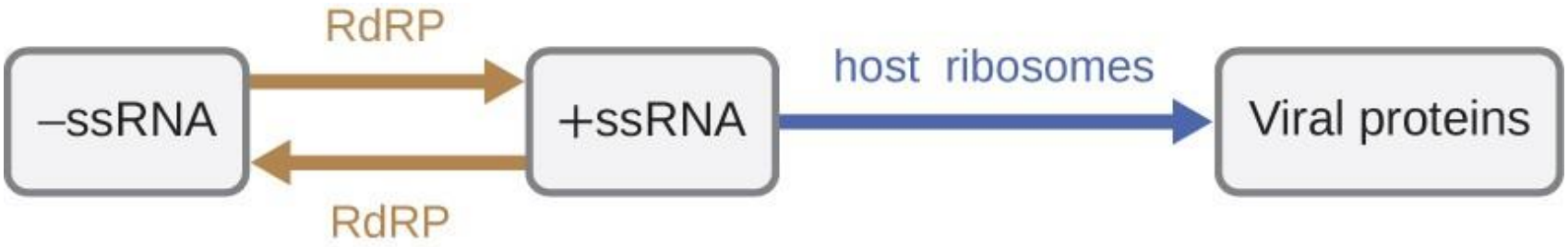
All DNA viruses consist of double-stranded DNA except for the **Parvoviruses**, which have **single-stranded DNA genome.**

All **RNA** viruses are single-stranded except **Reovirus** which is **double-stranded**.

Structure of Viruses

ssRNA may be positive (+) or negative (-):

- ✓ (+) RNA genome can serve as mRNA and directly translated into protein
- ✓ (-) RNA genome can not serve as mRNA and can not be directly translated into protein.



RdRP = viral RNA-dependent RNA polymerase

+ssRNA = positive (+) single strand

–ssRNA = negative (–) single-strand RNA

Structure of Viruses

- The genomes of viruses can be unsegmented (single molecule) or segmented (two or more molecules).

- Segmented genomes are much more common amongst RNA viruses than DNA viruses (Genome segmentation is rare among DNA viruses).

- If a virus has a segmented genome and if two variants of that virus infect a single cell, progeny virions can contain some segments from one parent and some segments from the other one.

Structure of Viruses

- ✓ Viral genomes are surrounded by protein shells known as **capsids**.
- ✓ The capsid is composed of small structural units called **capsomeres**.
- ✓ Subunits called **protomers** aggregate to form capsomeres.

Structure of Viruses

The functions of the capsid:

- ✓ it protects the nucleic acid from digestion by enzymes and inactivation by the outer conditions
- ✓ contains special sites on its surface that allow the virion to attach to a host cell.
- ✓ provides proteins that enable the virion to penetrate the host cell membrane and, in some cases, to inject the infectious nucleic acid into the cell’s cytoplasm.
- ✓ the capsid is antigenic.

Structure of Viruses

- The number and arrangement of the capsomeres are useful in identification and classification.

- Depending on the arrangement of capsomeres, they can be grouped into three groups :

  - ✓ Icosahedral Symmetry
  - ✓ Helical Symmetry
  - ✓ Complex

Structure of Viruses

Icosahedral (Cubic) Symmetry

- An icosahedron is defined as being made up of 20 equilateral triangular faces arranged around the surface of a sphere.

- **DNA Viruses:** Parvoviridae, Papovaviridae, Adenoviridae, Herpetoviridae

- **RNA Viruses:** Picornaviridae, Reoviridae, Togaviridae

Structure of Viruses

Helical Symmetry

- The virus particle is elongated or pleomorphic (not spherical), and nucleic acid is spiral.
- Capsomeres are arranged round the nucleic acid.
- Helical symmetric capsids are only found in RNA viruses.
- All helical symmetric viruses that cause disease in the human are enveloped.

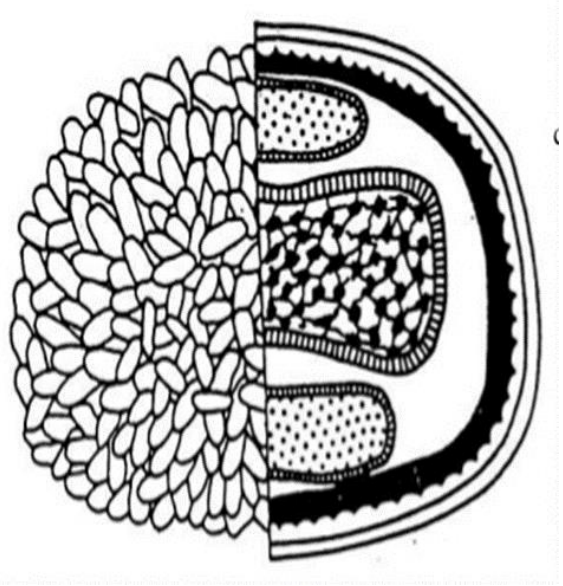
RNA Viruses: Orthomyxoviridae (Influenza), Paramyxoviridae (bovine respiratory syncytial virus), Rhabdoviridae (Rabies).

Structure of Viruses

Capsids of Complex Symmetry

Some viruses do not fit into the category of having helical or icosahedral capsids. They compose more complex structures.

Poxviridae, Arenaviridae, Coronoviridae



Structure of Viruses

- The **capsid** is the protein shell that encloses the **nucleic acid**; with its enclosed nucleic acid, it is called the **nucleocapsid**.

(Nucleocapsid = genome + capsid)

- Naked viruses consist of only a nucleocapsid.
- Many types of virus have a lipid-glycoprotein membrane (**envelope**) surrounding the nucleocapsid. It is derived from the plasma membrane of the host cell.
- **Virion:** complete virus particle-it is the form in which the virus moves between cells or hosts.

Structure of Viruses

- **Viral envelope** is made up of a **lipid** bilayer and is comprised of **host-cell lipids.** It also contains **virally encoded proteins,** often **glycoproteins** which are trans-membrane proteins.

- These viral proteins serve many purposes, such as **binding to receptors on the host cell, playing a role in membrane fusion and cell entry, etc.**

Structure of Viruses

There are two types of proteins.

- **Peplomer** is a glycoprotein spike on a viral capsid or viral envelope. These protrusions will only bind to certain receptors on the host cell; they are essential for both host specificity and viral infectivity.

- Influenza virus has two kinds of peplomers: triangular, spike-shaped "**haemagglutinin**" and mushroom-shaped "**neuraminidase**".

Structure of Viruses

- The second group of proteins are called **matrix proteins.**
- Matrix proteins are **located at the base of the glycoprotein** protrusions.
- These proteins bind to the lipid layer of the envelope with hydrophobic bonds.
- Matrix proteins play an important role in **budding virus particles**.

Structure of Viruses

Naked (non-enveloped) viruses are released from the infected cell by the disruption of the cellular membrane (often by apoptosis= programmed cell death initiated by virus or host).

Enveloped viruses exit their host cell by budding from cellular membrane. Enveloped viruses acquire their lipid envelope from the membranes of host cells.

Structure of Viruses

- **Enveloped** viruses are **sensitive to ether, chloroform, bile salts** due to their lipid content (**Non-enveloped** viruses are **resistant**).

- **Glycoproteins** enable enveloped viruses to **bind to specific receptors on the membrane of host cells**.

- Envelope glycoproteins are **antigenic** molecules.

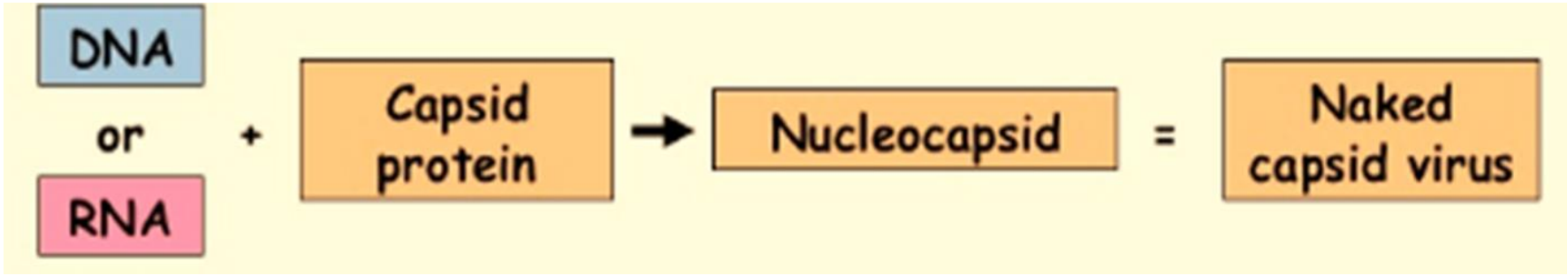
Structure of Viruses

- **Rabies virus** glycoproteins bind to the neuronal nicotinic acetylcholine receptors **(neurotoxic effect).**

- **Influenza virus** is an enveloped virus with two important surface **glycoproteins** called **hemagglutinin** and **neuraminidase.**

- **Neuraminidase** is important during virus entry, by **degrading the mucus barrier of the respiratory tract** and allowing virus to reach cells.

Structure of Viruses



Viral Enzymes

Some viruses contain enzymes that are encoded by viral genes. These enzymes function in viral replication.

Polymerases

- **DNA polymerase** (found in DNA viruses and catalyzes the synthesis of **DNA from DNA**)
- **RNA polymerase** (All negative ss-RNA viruses have RNA dependent RNA polymerase inside virions (it transforms negative single stranded **RNA into mRNA**). This enzyme is not found in human or animal cells.
- **Reverse transcriptase** (catalyzes the transcription of retrovirus **RNA into DNA**). This enzyme is found in Retroviruses.

Inclusion Bodies

- Inclusion bodies are nuclear or cytoplasmic aggregates of stainable substances, usually proteins.
- They typically represent sites of viral multiplication in a bacterium or a eukaryotic cell and usually consist of viral capsid proteins.
- Structures with distinct size, shape, location and staining properties.

Inclusion Bodies

- Can be seen under light microscope after staining.
- May be seen in cytoplasm or nucleus.
- Generally acidophilic in nature. Pink in color on staining with Giemsa.
- Some may be basophilic as well.
- Helps in diagnosis.
- For example, **Negri bodies** are used for the diagnosis of **Rabies** (intracytoplasmic inclusion bodies).

Viral Interference

Viral interference is a phenomenon for which a cell infected by a virus becomes resistant toward a second outcoming infection by a superinfectant virus.

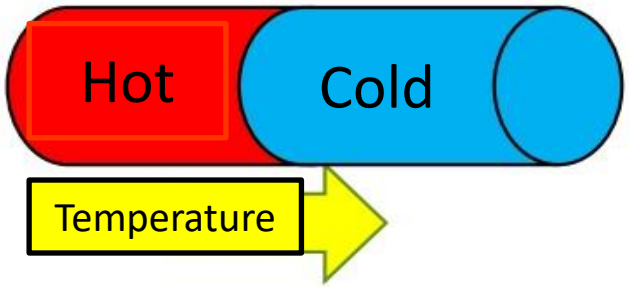
Effects of Physical and Chemical Factors on Viruses

Temperature

- Viruses are generally sensitive to high temperatures. Temperature is one of the most important factors affecting virus survival, as it can affect the state of viral proteins and virus RNA or DNA.

- Generally, as temperature rises, virus survival decreases.

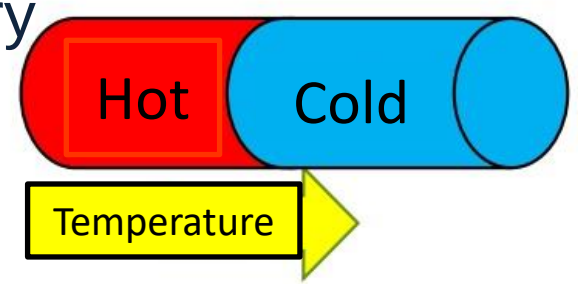
- Maintaining temperatures above 60°C for 60 min is generally enough to inactivate most viruses.



Effects of Physical and Chemical Factors on Viruses

Temperature

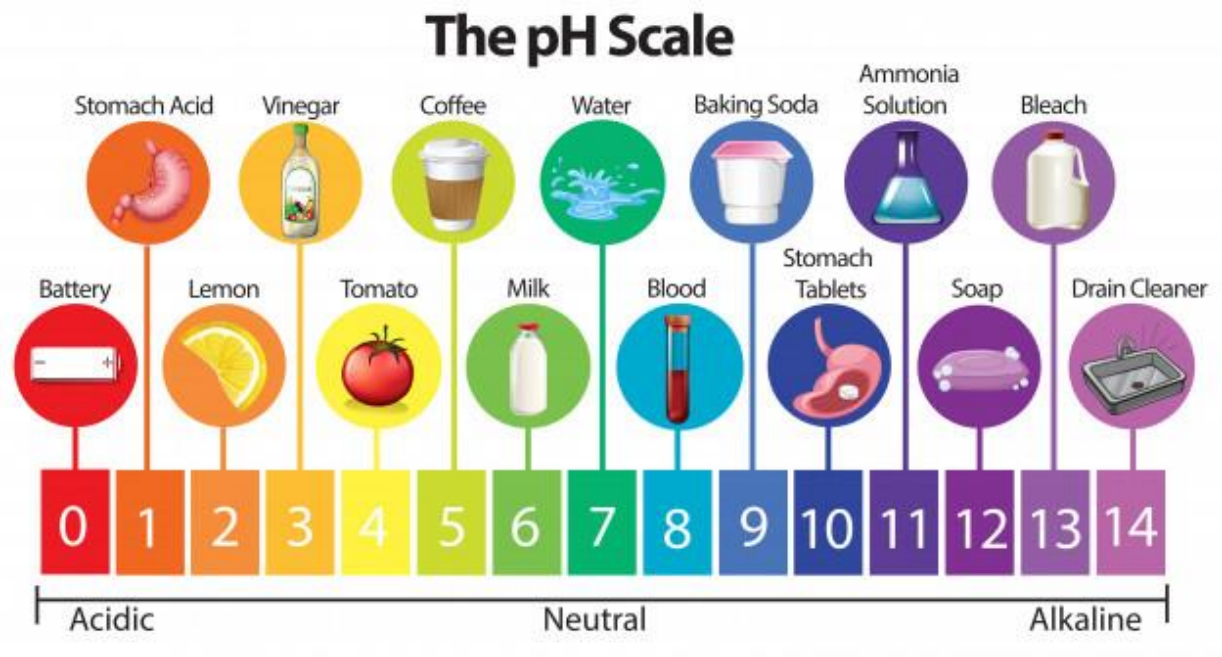
- Surface proteins are denatured within a few minutes at these temperatures; hence the virion is no longer capable of normal cellular attachment.
- Envelope viruses are less resistant to heat due to the lipid structure of envelope.
- Many viruses can be kept for months at refrigerator temperatures and stored for years at very low temperatures between -70 ^{0}C and -196 ^{0}C.



Effects of Physical and Chemical Factors on Viruses

pH

- Most viruses prefer neutral pH and an isotonic environment.
- Viruses can survive between pH 5-9. They are inactivated at acidic and alkaline pH values.



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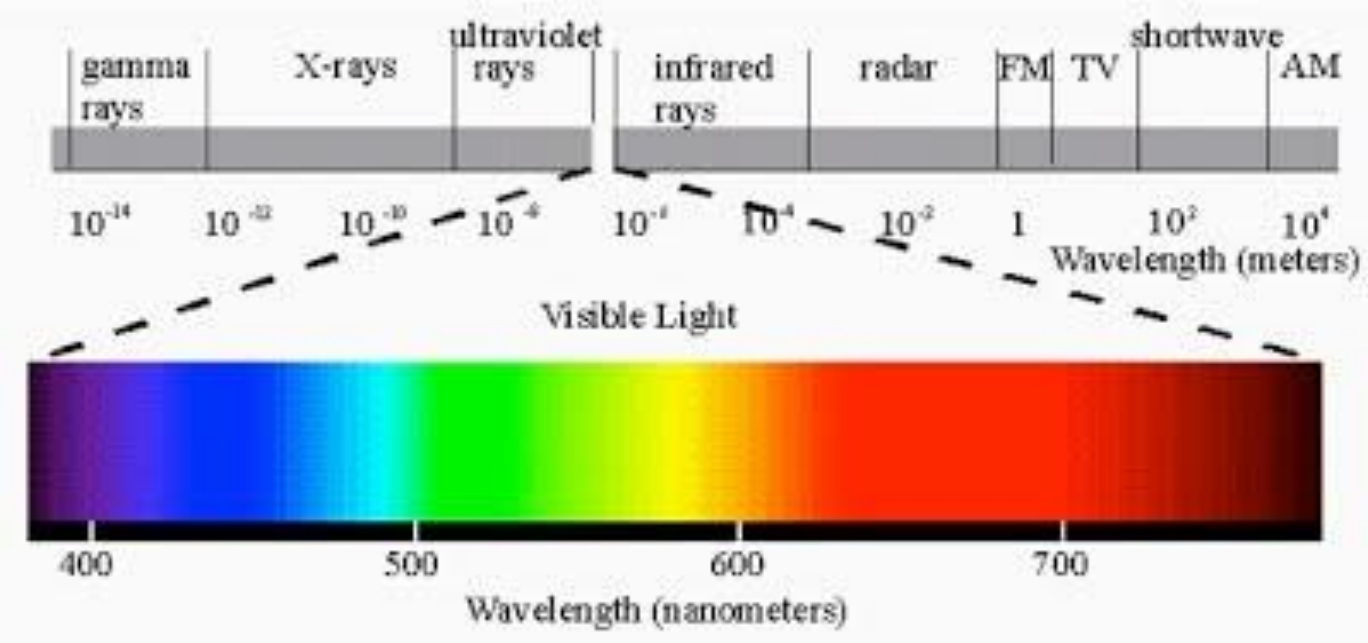
Effects of Physical and Chemical Factors on Viruses

Rays

Viruses are inactivated by UV rays, Ionizing radiation (X-rays, gamma rays).



https://sites.google.com/site/frinanostream/tutorials/spectrophotometry

Effects of Physical and Chemical Factors on Viruses

Lipid Solvents

- Enveloped viruses are sensitive to ether, chloroform, bile salts due to their lipid content (Non-enveloped viruses are resistant).

- Enveloped viruses usually fail to establish infection in the gastrointestinal tract, because these are destroyed by bile secreted in the gastrointestinal tract.

Effects of Physical and Chemical Factors on Viruses

Antiseptics and Disinfectants

- Most of the antiseptic and disinfectants that are effective on bacteria demonstrate limited activity against viruses.

- Enveloped viruses are much more susceptible to drying and disinfectants because disinfectants and drying removes envelope.

- When envelope is removed, virus is no longer infectious. (Attachment to host cell is mediated by virion proteins located in the envelope).

Effects of Physical and Chemical Factors on Viruses

Antiseptics and Disinfectants

- Chlorine and iodine compounds, oxidizing agents, some aldehydes (glutaraldehyde) and strong acidic or alkaline agents are active against most viruses.

- Formaldehyde is used for vaccine preparation. It inactivates viral nucleic acid (which is responsible from infectivity). It is also used to kill unwanted viruses and bacteria that might contaminate the vaccine during production.

Effects of Physical and Chemical Factors on Viruses

Most Resistant

- **Prions**
- **Endospores of bacteria**
- **Mycobacteria**
- **Cysts of protozoa**
- **Vegetative protozoa**
- **Gram negative bacteria**
- **Fungi, including most fungal spores**
- **Viruses without envelopes**
- **Gram positive bacteria**
- **Viruses with lipid envelopes**

Least Resistant