South Valley University Botany and microbiology Department Faculty of Science

Virology and Bacterial metabolism

4-th Chemistry and Microbiology

Part (1) VIROLOGY

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رؤية الكلية:

التميز في تعليم العلوم الاساسية والبحث العلمي للمساهمة في التنمية المستدامة.

رسالة الكلية:

تقديم تعليم مميز في مجالات العلوم الاساسية وانتاج بحوث علمية تطبيقية للمساهمة في التنمية المستدامة من خلال اعداد خريجين متميزين طبقا للمعايير الاكاديمية القومية وتوفير خدمات مجتمعية وبيئية تلبي طموحات جنوب الوادى وبناء الشراكات المجتمعية الفاعلة.

Contents

VIROLOGY

General Properties of Viruses

A) Physical Properties:

1- The Size of Virus:

The size of virus is measured by nanometer (nm) which is equal to 10^{-9} meter. Also, it is measured by the Angstrom (A°) which is 1/24 of nm. The size can be measured by several methods:

a) E.M.

Shadow casting:

Several types of heavy metals such as gold or chromium are evaporated under vacuum. The virus under investigation is exposed to the vapors, so that the atoms of the metal will cast on the near surface of the virus at an oblique angle. The casted particles can be easily detected by the microscope as an opaque due to the presence of the metal atoms.

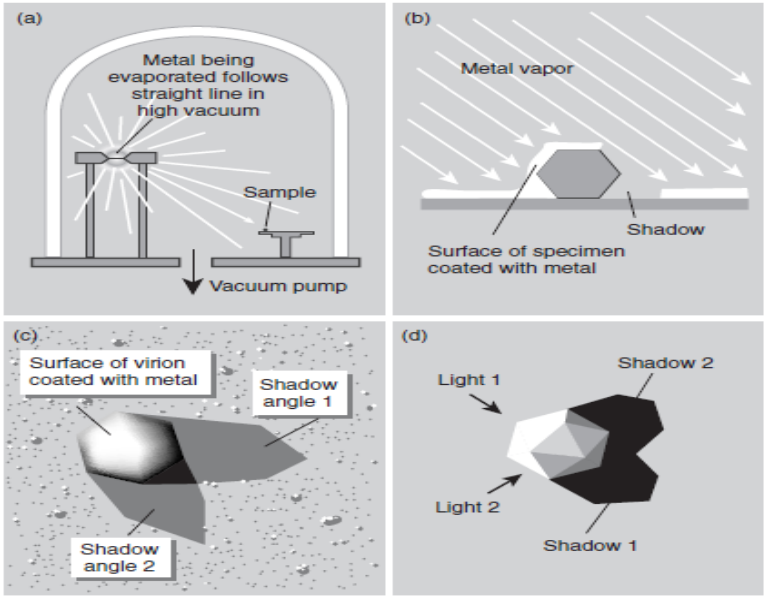


Figure (1): Shadowing specimens for viewing in the electron microscope. a: A sample of heavy metal is vaporized in a vacuum chamber. b: Any object in the path will cast a shadow. c: a double-

shadowed virus in the electron microscope. d: An icosahedral model is placed in two light beams to show the equivalence of the shadows.

Negative staining:

The virus under investigation is mixed with a salt of sodium phosphtungestate which will inhibit the passage of the rays of EM but the virus particle will allow the passage of the rays of EM.

Positive staining:

Potassium phosphotungestate is used. This method is useful in staining thin sections.

b) Ultrafiltration:

By using a filter made from cellulose acetate membrane.

The virus preparation is passed through a series of filters (membranes) of different known pore sizes. The approximate size of the virus can be determined by the filter (membrane) which allows the virus to pass and that which holds it back.

c) Ultracentrifugation:

It depends on the rate of sedimentation of the suspended particles.

- The virus suspension is added in nitrocellulose tubes containing dense solution.
- High speed centrifugation (10000-30000 rpm is needed while bacteria need only 1000-3000 rpm) and cooling are used so virus particles migrate through the dense solution and settle in a zone of fluid of equal density.
- Calculate their migration distance which is a function of their molecular weight and the size of the virus can be determined according to the sedimentation coefficient.

This method is known as density gradient centrifugation in which 2 procedures are used.

1-Rate zonal centrifugation: in which sucrose solution is used.

2-Equilibrium density centrifugation: in which cesium chloride is used.

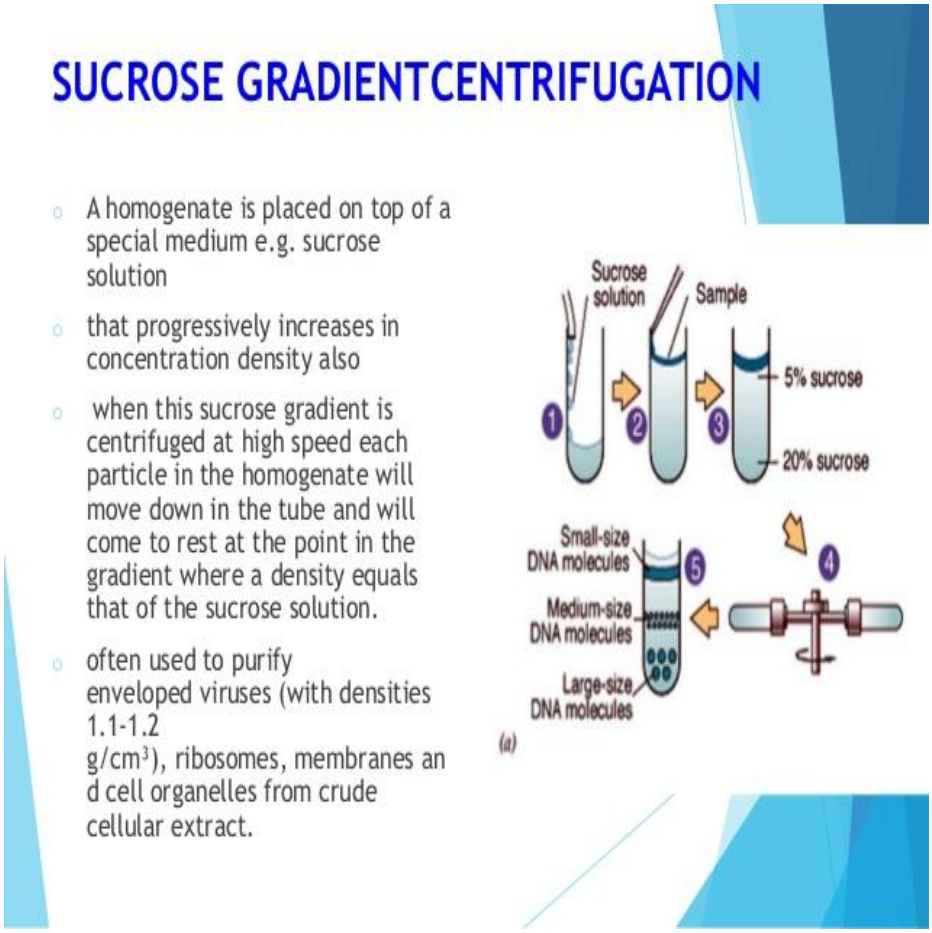


Figure (2): Sucrose gradient centrifugation to determine the size of virus.

2- Shape of the Viruses:

Most viruses are spherical in shape; some are brick-shaped as pox or long filament as influenza virus.

Plant viruses are rod shaped. Bacterial viruses are sperm-shaped with polyhedral head. The shape of the viruses can be determined by E.M., cryo E.M. and X-crystallography.

B) Chemical Composition of Viruses:

- All viruses contain protein coat and nucleic acid (either DNA or RNA).
- The nucleic acids are built up from nucleotide units.
- Each nucleotide consists of:

(1) A molecule of pentose sugar either ribose or deoxyribose.

(2) A molecule of phosphoric acid.

(3) A base which may be adenine, guanine, cytosine and either thymine (in DNA) or uracil (in RNA viruses).

- Some viruses may contain other chemical components as lipid envelope. The lipids of viruses have been fractionated into phospholipids, cholesterol and neutral fat.

C) Structure of Viruses:

1) Nucleic Acid (NA):

This may be either DNA or RNA. Most of DNA viruses are double stranded except parvo viruses which are single stranded, while most RNA viruses are single stranded except reoviruses and birnaviruses.

All viruses contain one copy of their genome (haploid) except retoviruses which have two copies of their genome (diploid).

Functions:

1-It is the infectious part of the virus. Loss of NA core leads to loss of infectivity.

2-It carries the genetic information for viral replication, virulence and antigenic specificity.

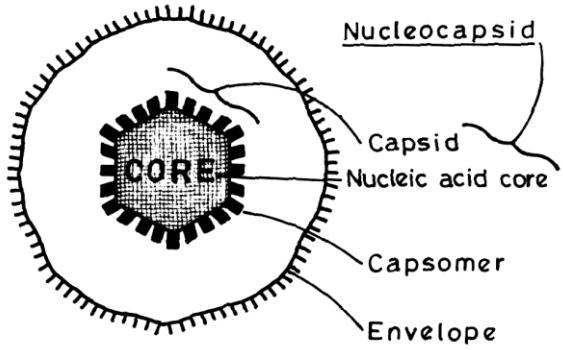


Figure (3): The structure of a complete enveloped viral particle.

2) Capsid:

Structure:

It consists of small protein subunits called capsomers which are the morphological subunits of the capsid consisting of identical or different protein molecules which can be seen by electron microscope.

Functions:

1- It protects the viral genome (DNA or RNA) against inactivation by nucleases.

2- Arrangement of capsomers determines the structural symmetry of the virion.

There are 3 forms of virus symmetry:

A) Cubical symmetry → the capsid is an icosahedra and the virus resemble a crystal e.g. herpes and adeno viruses. Icosahedron is a geometric form, with 20 triangular faces and 12 corners.

B) Helical symmetry → the capsid is helical in structure e.g. myxo viruses

C) Complex symmetry → the capsid exhibits both cubic & helical symmetry (the capsid is complicated in structure) → e.g. pox viruses.

3- It participates in the attachment of virions to susceptible host cells.

4- It determines the antigenicity of the virion. Antibodies formed against protein coat antigens neutralize virus infectivity.

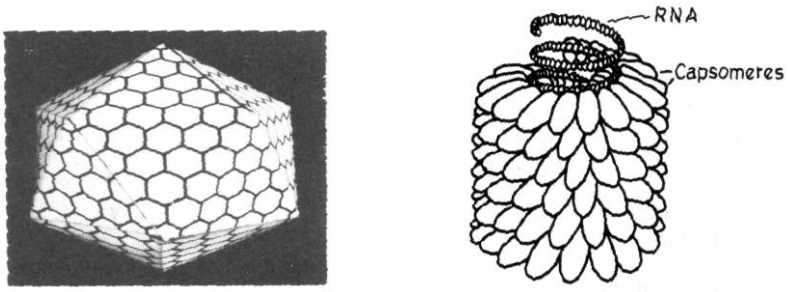


Figure (5): Icosahedral viral symmetry and Helical viral symmetry.

Nucleoprotein:

The capsid together with the NA form what is called nucleoprotein.

3) Envelope:

*It is a lipid or lipoprotein coat enclosing the capsid derived mostly from the host nuclear membrane (all DNA viruses except pox virus) or from the cytoplasmic membrane (all RNA viruses).

*Viral envelope contains glycoprotein which is virus encoded. It is responsible for the interaction with the cellular receptors and represents an important viral antigen.

*Enveloped viruses are sensitive to ether due to their lipid content. Treatment with ether will result in loss of infectivity.

*Non enveloped viruses are more stable at hostile environmental conditions so transmitted often by feco oral route. While enveloped viruses are less stable so transmitted by parentral, sexual or respiratory routes.

*Only seven families of animal viruses exist as naked nucleocapsid, others are surrounded by lipid or lipoprotein envelope.

D) Reaction to physical and chemical agents:

(1) Temperature:

Most viruses are heat labile and inactivated if incubated for 1/2 hour at 56°C except some heat resistant virus as serum hepatitis. Preservation at 4°C is sufficient for only several days, while preservation for month, or years must be at -70° or lower in liquid nitrogen. Some viruses are sensitive to repeated freezing and thawing.

(2) Radiation:

Visible light is destructive to many viruses, also UV rays destroy them much more rapidly. Ionizing radiation (x-rays or γ-rays) causes breaks in the nucleic acid and therefore inactivates it.

(3) PH:

Viruses remain viable at PH values 6.5-7.5, but high acidity or alkalinity destroys many viruses except enteroviruses which can resist acidic environment (e.g. in the stomach).

(4) Chemical Agents:

These are important as disinfectants and in the preparation of inactivated vaccines.

Chemical agents include;

a) Oxidizing agent e.g. chlorine, iodine.
b) Alkylating agents e.g. fomaldehyde, glutaraldehyde.
c) Protein denaturants e.g. alcohol, and phenol.
d) N.A denaturants e.g. B propriolactone.
e) Detergents e.g. non ionic detergent and anionic detergent as SDS which solubilize viral envelope.
f) Ether and chloroform: Enveloped virus are inactivated by ether and chloroform while, non enveloped viruses are resistant to ether and chloroform.

(5) Antibiotics:

Have no effect on viruses i.e. viruses are resistant to antibiotics because they have no metabolic activity.

Classification of Viruses

Classification of viruses is mainly based upon:

1- Nucleic Acid Type, strandedness and method of replication.
2- Size and morphology including symmetry.
3- Susceptibility to physical & chemical agents especially ether.
4- Immunogic properties.
5- Natural method of transmission.
6- Host tissue and cell Tropism.

ICTV classification:

(The International Committee on Taxonomy of Viruses)

Viral classification starts at the level of order and follows as thus:

Order (-virales)
Family (-viridae)
Subfamily (-virinae)
Genus (-virus)
Species

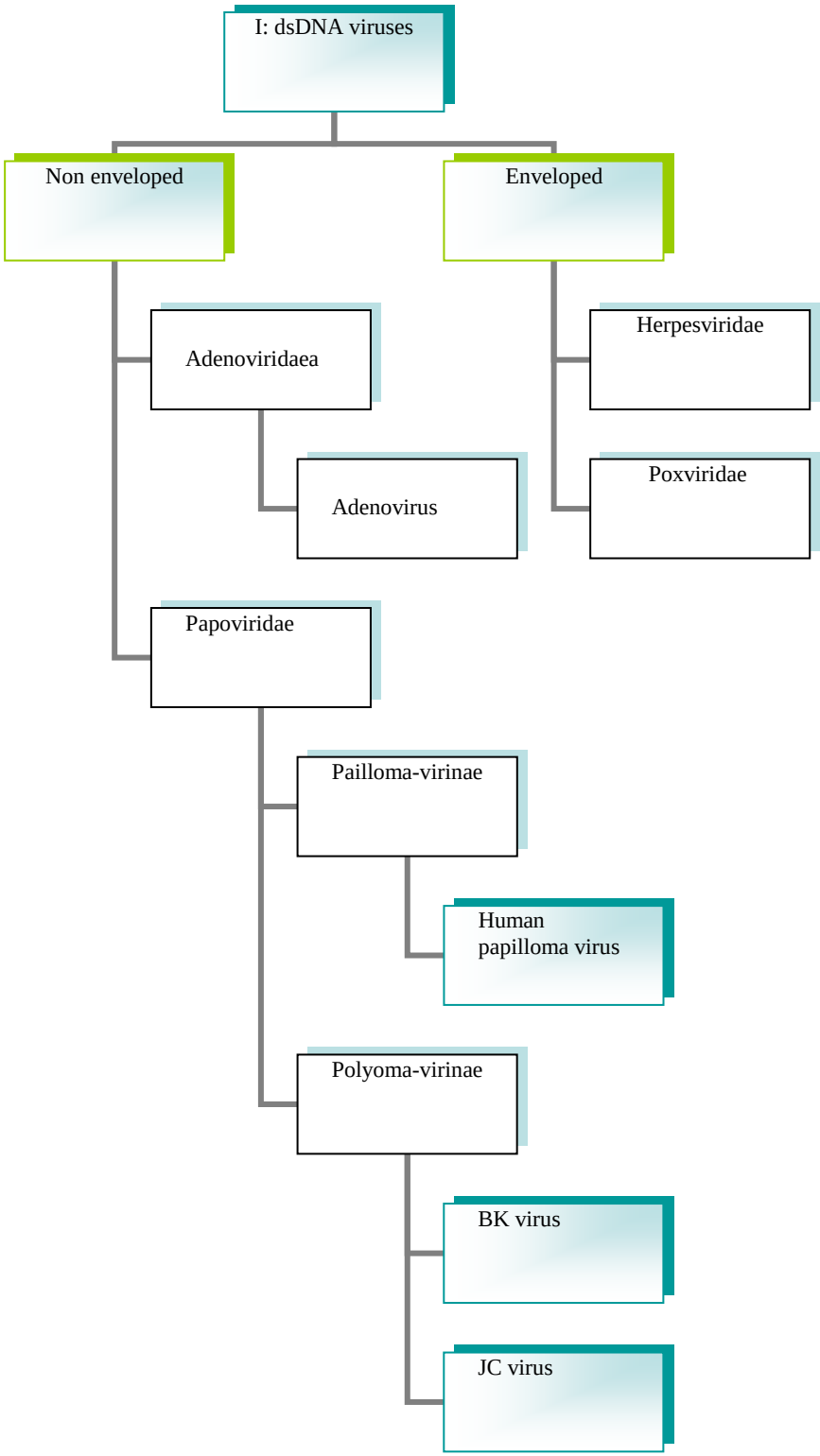
So far, six orders have been established by the ICTV: Caudovirales, Herpesvirales, Mononegavirales, Nidovirales, Picornavirales and Tymovirales.

These orders span viruses with varying host ranges.

Baltimore classification:

is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication.

- I: dsDNA viruses.
- II: (+) sense ssDNA viruses.
- III: dsRNA viruses.
- IV: (+) sense ssRNA viruses.
- V: (−) sense ssRNA viruses.
- VI: (+) sense ssRNA-RT viruses RNA with DNA intermediate in life-cycle.
- VII: dsDNA-RT viruses.

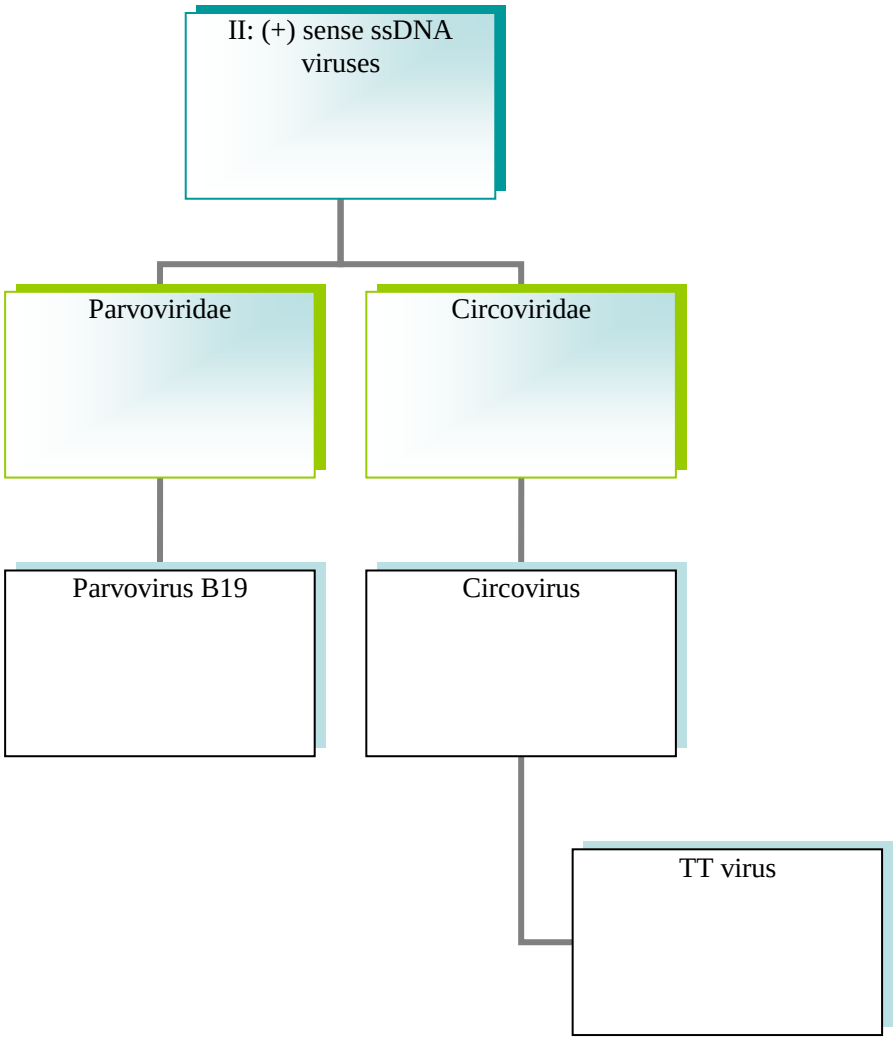

I: dsDNA viruses
Non enveloped
Enveloped
Adenoviridaea
Herpesviridae
Adenovirus
Poxviridae
Papoviridae
Pailloma-virinae
Human
papilloma virus
Polyoma-virinae
BK virus
JC virus

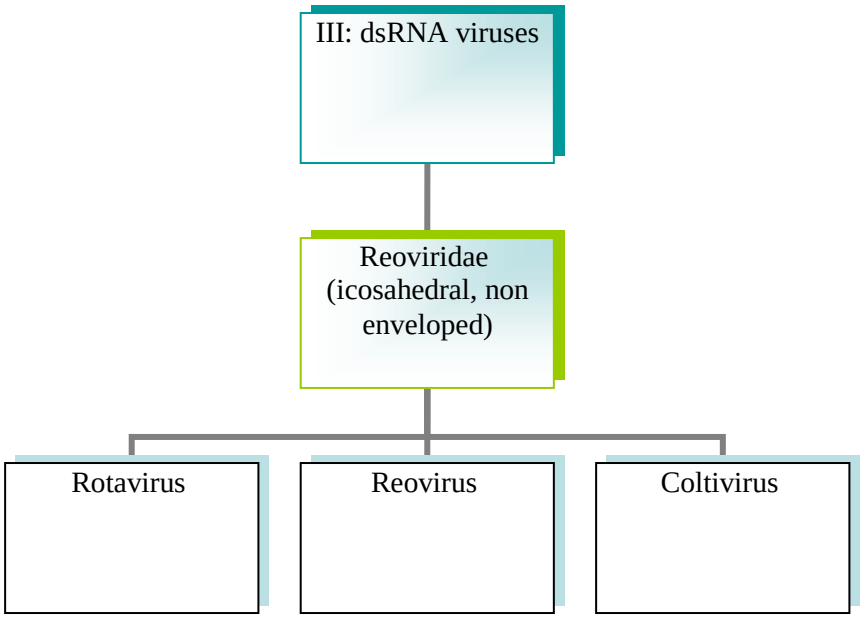
Herpesviridae

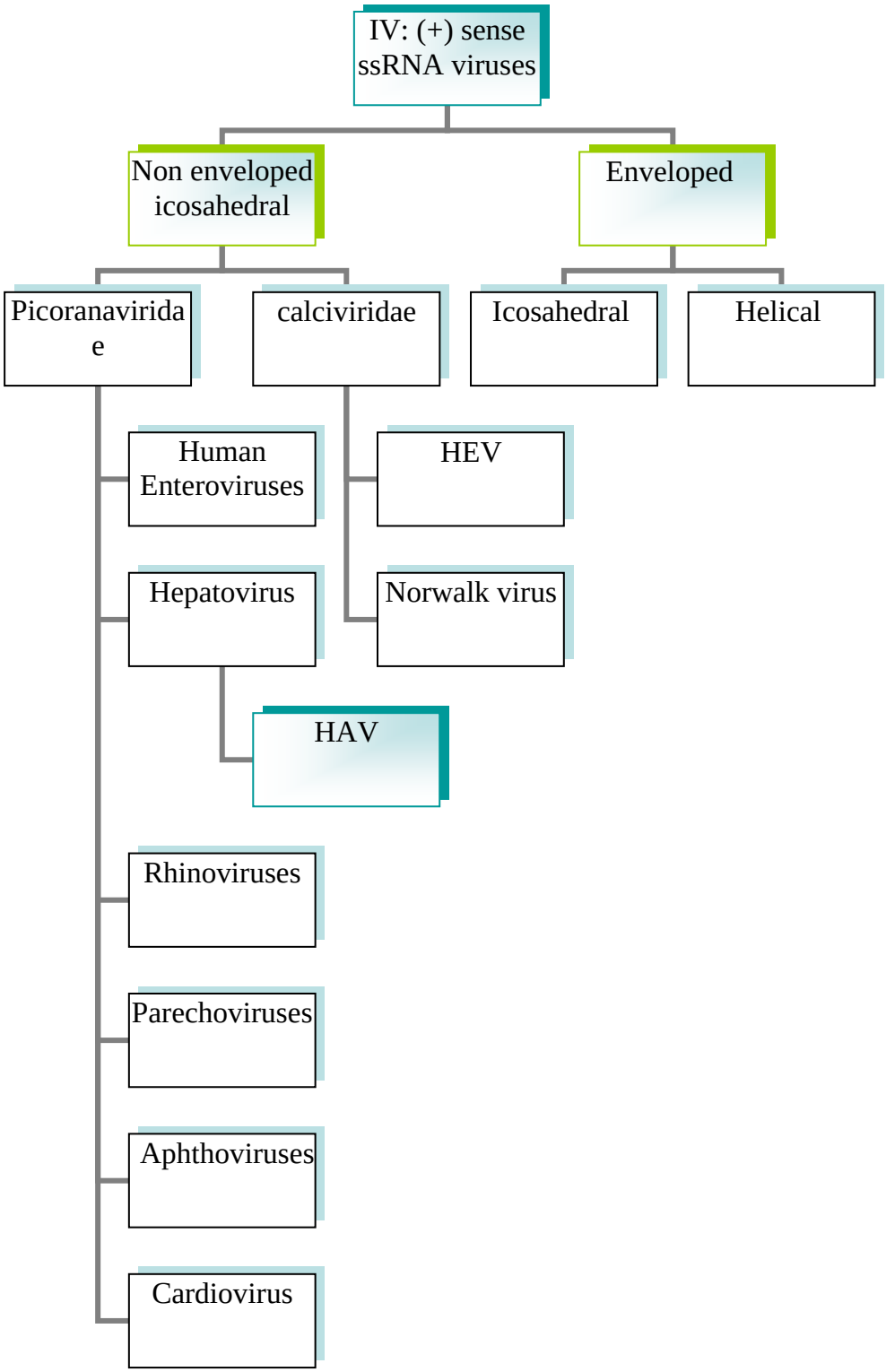
Alphaherpesvirinae	Betaherpesvirinae	Gammaherpesvirinae
(Herpes simplex group)	(Cytomegalovirus group)	(Lymphoproliferative group)
1-HSV 1	1- human CMV	1-EBV
2-HSV 2	2-human herpes virus 6	2-human herpes virus 8
3-VZ virus	3- human herpes virus 7	

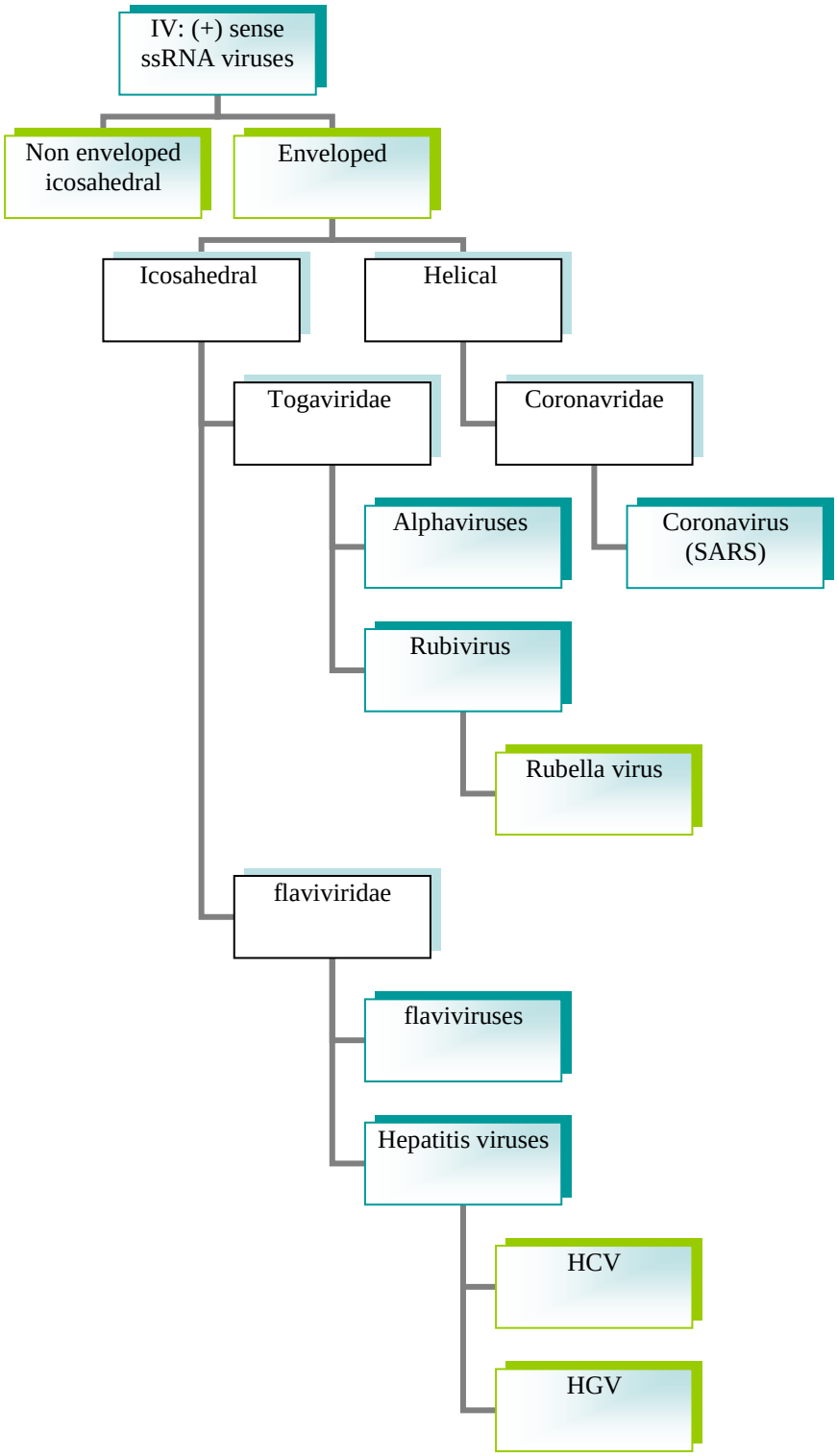
Poxviridae

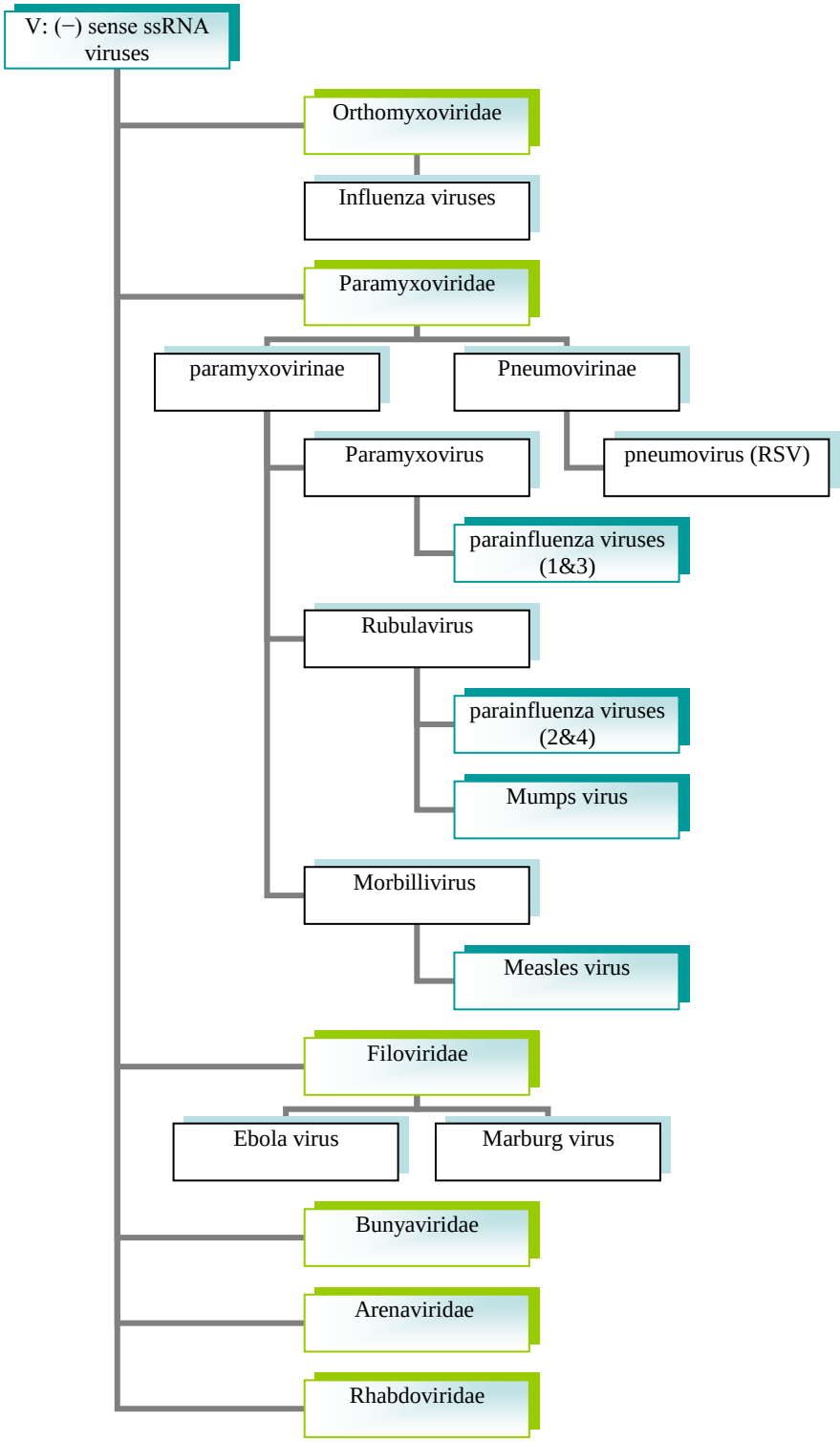
1- Mollscum contagiosum virus.
2- Vaccinia virus.
3- Variola virus.
4- Cow pox virus.
5- Monkey pox v.

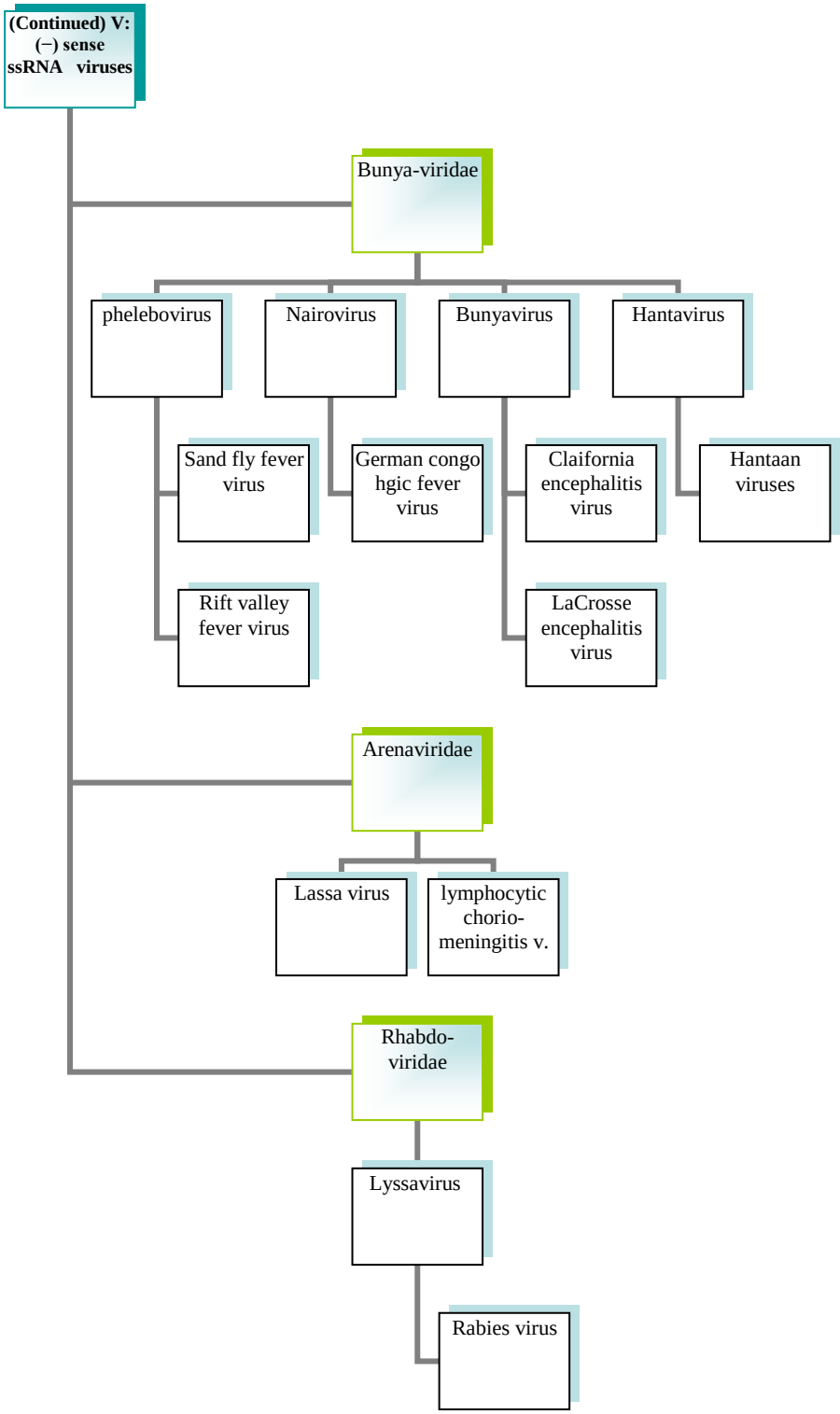

II: (+) sense ssDNA viruses
Parvoviridae
Circoviridae
Parvovirus B19
Circovirus
TT virus


III: dsRNA viruses
Reoviridae (icosahedral, non enveloped)
Rotavirus
Reovirus
Coltivirus

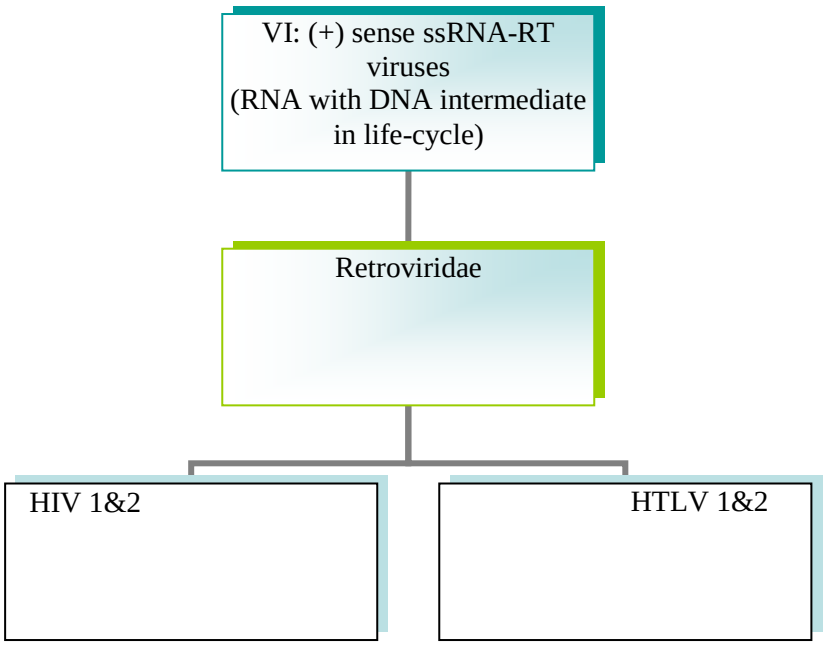

IV: (+) sense ssRNA viruses
Non enveloped icosahedral
Enveloped
Picoranaviridae
calciviridae
Icosahedral
Helical
Human Enteroviruses
HEV
Hepatovirus
Norwalk virus
HAV
Rhinoviruses
Parechoviruses
Aphthoviruses
Cardiovirus

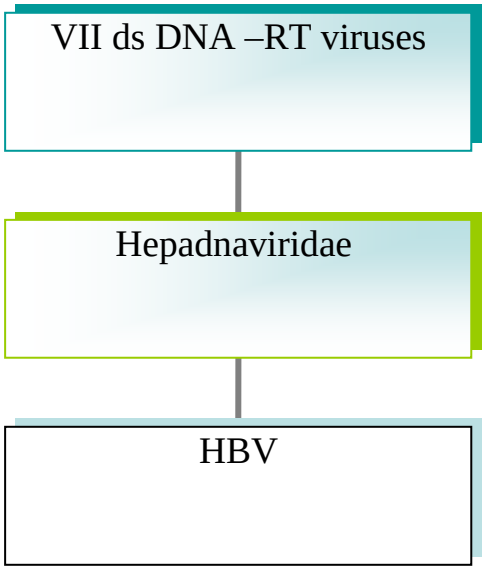

IV: (+) sense ssRNA viruses
Non enveloped icosahedral
Enveloped
Icosahedral
Helical
Togaviridae
Coronavridae
Alphaviruses
Coronavirus (SARS)
Rubivirus
Rubella virus
flaviviridae
flaviviruses
Hepatitis viruses
HCV
HGV


V: (−) sense ssRNA viruses
Orthomyxoviridae
Influenza viruses
Paramyxoviridae
paramyxovirinae
Pneumovirinae
Paramyxovirus
pneumovirus (RSV)
parainfluenza viruses (1&3)
Rubulavirus
parainfluenza viruses (2&4)
Mumps virus
Morbillivirus
Measles virus
Filoviridae
Ebola virus
Marburg virus
Bunyaviridae
Arenaviridae
Rhabdoviridae


(Continued) V: (−) sense ssRNA viruses
Bunya-viridae
phelebovirus
Nairovirus
Bunyavirus
Hantavirus
Sand fly fever virus
German congo hgic fever virus
Claifornia encephalitis virus
Hantaan viruses
Rift valley fever virus
LaCrosse encephalitis virus
Arenaviridae
Lassa virus
lymphocytic chorio-meningitis v.
Rhabdo-viridae
Lyssavirus
Rabies virus

NB: (−) sense ssRNA viruses are enveloped helical viruses.
Other enveloped helical (−) sense ssRNA viruses: HDV & astroviruses but they have not been classified in certain family till now.





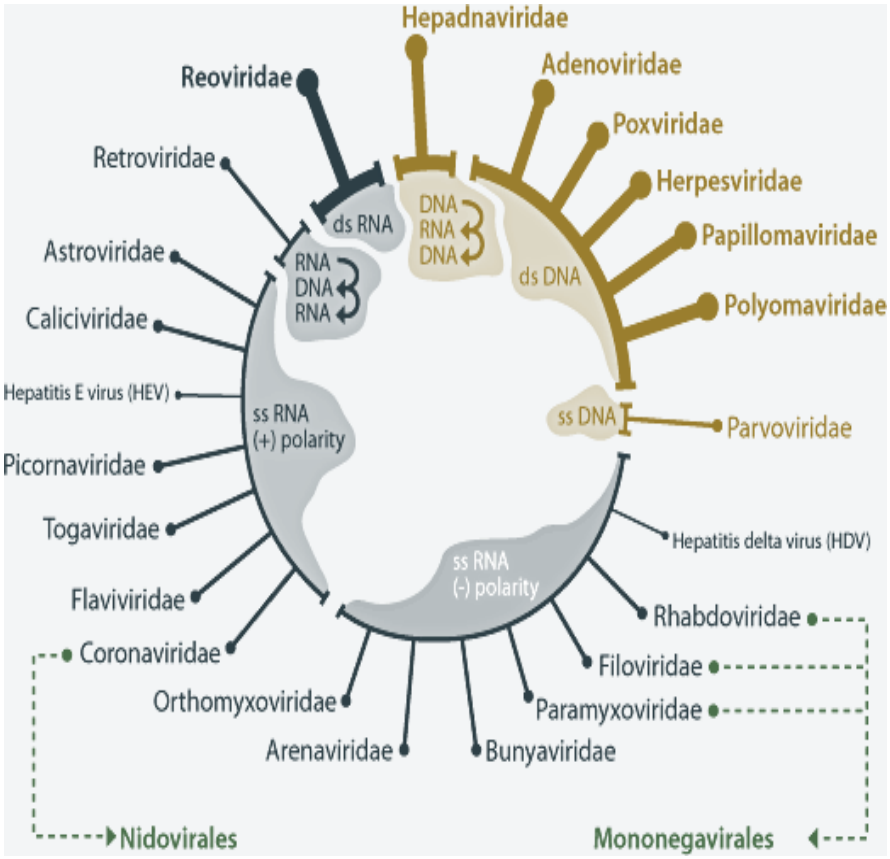


Figure (6): Types of viruses according to the type of nucleic acid.

Notes:
Hepatitis viruses are a heterogeneous group belonging to different families in which:
HAV → picornaviridae.
HBV→ hepadnaviridae.
HCV & HGV→ flaviviridae.
HDV→ is a defective delta virus.
HEV→ calciviridae.
Rubella is a toga virus but not transmitted by arthropods.
Hantaviruses are bunyaviruses but not transmitted by insects. They are transmitted by inhalation of rodent extra.

Pathogenesis of Viral Infections

Pathogenesis of viral infection refers to the interaction of viruses with the host cells that result in disease production. It includes the following phases:

a) Virus entry into the host.
b) Primary replication.
c) Virus spread through the host.
d) Localization of the virus in target organs.
e) Cell injury and clinical illness.
f) Release of the virus from the host.
g) Host defenses against viral infections.

a) Virus entry into the host:

The most common routes of infection include:

1- The respiratory route:

Certain viruses attack the respiratory tract as adeno, ortho, paramyxo and rhinoviruses. Other viruses as pox initiate infection after inhalation but cause generalized infection usually without respiratory symptoms.

2- The alimentary route:

There are certain viruses which initiate infection through the alimentary tract e.g. Herpes, FMD may infect oral mucosa.

3- The skin route:

By injury or trauma: e.g. rabies which is transmitted by bites of animals whose saliva contain rabies virus.

By inoculation: as in contaminated vaccines or blood transfusion as serum hepatitis and AIDS.

By arthropods: By mechanical transmission as papilloma and pox viruses or by biological transmission as most arboviruse (Toga and some Reo Viruses).

b) Primary replication of the virus:

Viruses usually replicate at the primary site of entry as the initial dose is usually insufficient to establish infection.

c) Virus spread through the host:

Some viruses (e.g. influenza virus) cause no further systemic spread, they spread locally over the epithelial cell surface with no invasion of the underlying tissues or distant spread.

Many other viruses produce diseases at sites distant from their point of entry their spread occurs by one of the following routes:

(1) Spread by Lymphatics:

The virus pass from the exposed surfaces (Skin, mucus membranes ... etc) into the local lymph nodes or adsorbed to lymphocytes or taken up by macrophages. The virus is carried by lymphatic vessels which discharge it into the general circulation e.g. pox viruses.

(2) Spread by blood Vessels:

The virus gain access to the circulation by several ways:

- By lymphatic pathways via the thoracic duct or by phagocytosis then enter the circulation or
- By direct involvement of vascular endothelial cells near the site of entry.

(3) Spread of the virus by nerves:

Certain viruses travel along nerves to CNS e.g. rabies or pseudorabies, polio virus and herpes zoster.

d) Localization of the virus in target organs (Tropism):

*Tropism: It is the affinity of the virus to certain organs or tissues where virus multiplication chiefly occurs and main pathological lesions are found. Such organs are called target organs.

*The factors which determine such localization or tropism i.e. the specificity of certain viruses to certain tissues may be due to:

1) The presence of specific cell receptors.

2) The presence of specific metabolites and enzymes needed by the virus.
3) The presence of some environmental factors as temperature, e.g. Pox virus tends to locate in the skin due to its lower temperature than internal organs.

*The virus tropism can be altered during the course of laboratory multiplication by passage in different hosts or by passage through different routes. The virus then losses its capacity to grow in certain organs or its capacity to be virulent for the natural host, this alteration is called virus variation.

*Viral diseases can be classified clinically according to the tropic nature of the virus into:

a) Dermotropic viruses: which have the affinity to multiply in the skin e.g. pox.
b) Pneumotropic viruses: which affect the respiratory tract as parainfluenza, and adeno viruses.
c) Viscerotropic viruses: which affect viscera (internal organs) as hepatitis viruses.
d) Neurotropic viruses: which affect CNS as rabies virus.
e) Pantropic viruses: which show no selective properties to certain types of cells as herpes viruses.

e) Cell injury and clinical illness:

Clinically the outcome of viral infection may be one of the following:

1- Abortive infection:

It is the infection in which no progeny viruses are produced. This occurs due to:

* Infected cells lack enzymes, promoters, transcription factors or other compounds needed for viral replication. These cells are known as non permissive cells.

* Infected cells can support viral replication but the infecting virus itself is genetically defective or unable to replicate in such type of cells.

* Cell death as a result of viral infection before viral replication has been completed.

2- Subclinical or inapparent infection:

These are infections that do not give overt signs of their presence. It may be discovered accidentally when screening or pass unnoticed and the patient recovers from infection or develops one form of persistent viral infections. E.g. in patients infected with HCV, usually the initial infection is subclinical or inapparent infection then a small percent of these patients have spontaneous viral clearance while the majority pass to chronic HCV infection.

3- Clinically apparent infection that may be

a- Acute viral infection:

Following an incubation period of variable duration depending on the nature of infecting virus, clinical signs of infection are detectable this may be followed by complete recovery or the patient passes to persistent viral infection.

b- Persistent viral infections:

Mechanisms that help persistence of viral infection:

1. Integration of viral genome into host cell DNA e.g. retroviruses.
2. Rapid antigenic variations.
3. Spread from one cell to another without extracellular phase (e.g. CMV).
4. Immuonosupression (e.g. AIDs).
5. Formation of virus-antibody complex that remains infectious.

Persistent viral infections may be one of the following:

a- Chronic infections: are those in which replicating virus can be continuously detected usually with low or mild symptoms e.g. HBV chronic carrier.

b- Latent infections: are those in which the virus is hidden (no replication) with periodic activation. The viral sequences are detectable by molecular techniques in lesions containing the virus. E.g. Herpes simplex virus.

c- Slow infections: are those with long incubation period up to 20 years e.g. HIV and subacute sclerosing pan encephalopathy.

At the cell level the outcome of viral infection may be one of the following:

1- Abortive infection.

2- Productive infection: in which the host cell is not killed and the progeny virions are produced and released.

3- Latent infection: in which there is persistence of viral genome in the host cell with or without its incorporation into the host cell genome. There is no production of progeny virions. Such latent infections can be activated later on leading to productive infection.

4- Lytic infection: in which viral infection results in host cell death and release of progeny virions. cell death occurs due to:

- Cell lysis or
- Expression of viral genes with toxic proteins production that shut off host cell functions or
- being a target of the immune system

Mechanism of Disease Production:

1- Cytopathogenesis:

Cell invasion may result in abortive infection, productive infection, latent infection or cell death (lytic infection) through cell lysis, toxic protein production or being a target of the immune system.

2- Oncogenic effect:

Incorporation of the viral nucleic acid into the cellular genome leads to transformation. Transformed cells are characterized by increased rate of growth, loss of contact inhibition and alteration of its antigenic specificity due to expression of the viral antigens.

3- Immunopathogenesis:

Cell damage, lymphokines production, immune complexes deposition and delayed type hypersensitivity result in localized immune response and more cell damage. 1FN and other lymphokines produce general symptoms (fever, malaise, headache.....).

f) Release of the virus from the host (virus shedding):

The last stage in pathogenesis of viral infections is shedding of the virus into the environment. Shedding of the virus usually occurs from surfaces involved in virus entry and it represents the time at which an infected individual is infectious to others. In some viral infections as rabies human represents the last host so no shedding occurs.

a) Local infections:

In these infections the virus remain localized at the sites of entry in the skin, conjunctiva or mucous surfaces, release of the virus occurs from such surfaces.

b) Generalized infections:

In these infections release of the virus depends on the target organs. E.g.

Excretion in saliva and sputum: e.g. rabies and mumps.

Excretion in feces: Viruses which resist the acidity, bile salts and enzymes of lower GIT are excreted in the faeces of infected individual as enteroviruse, infectious hepatitis and rota viruses.

Excretion in urine: e.g. CMV, mumps and rubella.

g) Host defenses against viral infections:

(Discussed in details in immunology book)

Interference Phenomenon

It is known that concurrent (mixed) infection even of a single cell with two different viruses may occur. In these conditions the following outcomes are possible:

1) Dual Infection:

Two viruses can enter the same cell, replicate independently and produce their own characteristic type of infection.

2) Recombination:

It occurs when two fairly closely related viruses infect the same cell, the progeny particles, may be produced with either the identical characters of each parent or with genetic properties of both parents.

3) Exaltation:

In some mixed infection one virus may enhance the growth of the second virus.

4) Interference:

It is one of the biological properties of viruses, it is a cellular phenomenon. It is occur when one virus enters into a cell and modifies it, so that multiplication of a second virus is inhibited, the 1st virus is called interfering virus and the 2nd one is called the suppressed virus.

Mechanisms of Viral Interference:

1) One virus may inhibit the ability of the 2nd to adsorb to the cell, either by blocking its receptors (Retro and enteroviruses) or by destroying its receptors (orthomyxoviruses).
2) The cell metabolites or enzymes necessary for viral growth are taken by or are under the direction of the 1st virus.
3) The 1st virus may stimulate the infected cell to produce an inhibitor (interferon) that prevents the replication of the 2nd virus.

Types of Interference:

1) Auto-interference:

It is the ability of virus to interfere with its own replication. It occurs when a cell is infected with one type of virus at a high multiplication.

2) Heterologous interference:

It occurs between unrelated types of virus.

3) Homologous interference:

It occurs between related viruses.

Diagnosis of Viral Infection

Collection and Handling of Samples:

In viral diseases the optimum time for collection of such samples, is as early as possible in the course of the disease. The type of material to be obtained is usually controlled by the nature of the disease suspected.

Because most of the viruses are very sensitive, collected samples must be handled with care in order to preserve the present virus as well as to prevent the spreading of infection to other locality; therefore samples are protected from dehydration as well as from heat by putting the material in closed containers which can be preserved over crushed ice.

Samples for pathological examination are transported after fixation with formalin. Also, blood samples for virus isolation are transported to the laboratory under cooling conditions and also obtained for detection of antibodies in the serum.

Preparation of the Samples for Virus Work:

The samples must be rendered bacteria and fungus free by addition of antibiotics and antifungal. The pathological materials are prepared by making a 10-20% suspension in physiological saline or Hank's salt solution and centrifuged; the supernatant is used for virus isolation.

Purification of Viruses

Aim of Purification:

1- Isolation of the virus in pure form and preparation of a pure culture of the virus.

2- Preparation of viral antigens in pure form to be used in diagnosis, Production of immune sera, and vaccine production.

3- To study the morphological characters of the virus or its physical properties.

4- For chemical analysis of virus.

It is better to determine the pH stability range of the virus and optimum temperature before the technique.

Methods:

(1) Liberation of Virus from Cells:

It is done by ultrasonication, homogenization or repeated freezing and thawing.

(2) Physical Methods:

a) Differential centrifugation that consists of low and high speed centrifugation to deposit firstly the large contaminating particles and then the virions.

b) Density gradient centrifugation (as discussed before).

(3) Chemical Methods:

1) **Precipitation:** By ammonium sulfate or by cold ethyl, methyl or butyl alcohol.

2) **Chromatography:** In chromatography the virus is allowed to pass through a column of gel under suitable ionic conditions and according to the electric charge on the surface of the virus and impurities, each can be eluted with solutions of different ionic strengths.

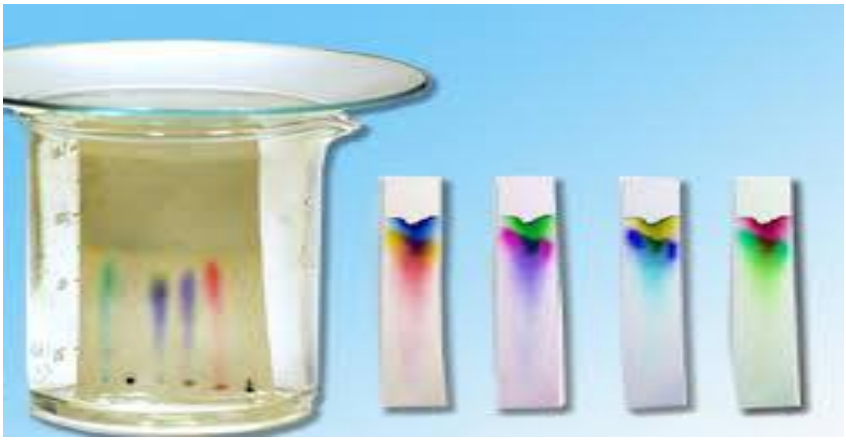
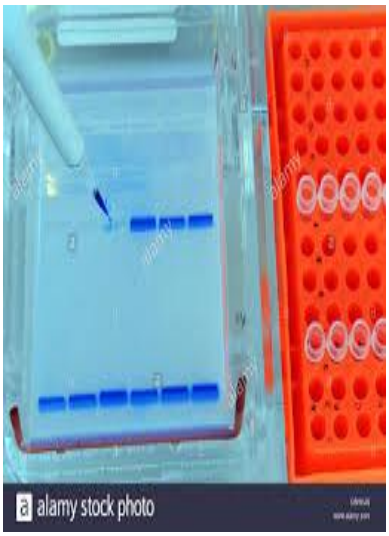


Figure (7): Chromatography with silica gel.

3) **Electrophoresis:** The particles are separated according to the number and distribution of charges on their surfaces and the speed of migration of the particles.



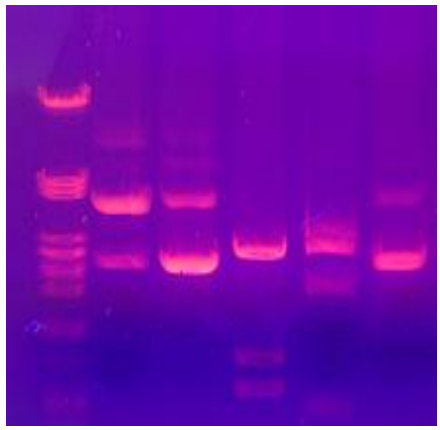


Figure (8): Gel electrophoresis for purification of the viruses.

(4) Biological Methods:

E.g. HA-elution for myxovirus (Adsorption):

By mixing virus with washed RBCs at 0-4°C the virus will attach the surface of RBCs which are carried to the bottom of the tube as it is centrifuged. The virus can be eluted from RBCs at 37°C by the aid of

nuraminidase enzyme and the RBCs are removed by centrifugation, while the virus remains in the supernatant fluid.

(5) Immunological Methods:

By using specific antibodies to adsorb the virus then treat it to be eluted with suitable enzyme.

Figure (9): Attachment of specific antibodies to specific antigen.

The laboratory procedures used in diagnosis of viral diseases include:

I. Direct methods by:

A -Detection of viruses in clinical specimens.

B -Detection of viral components in clinical specimens:

1-viral proteins. 2-viral nucleic acid.

C -Detection of cytopathic effects in the cells of clinical specimens.

D -Isolation of viruses.

II. Indirect methods by:

Serologic detection of antiviral antibodies.

I- Direct methods:

A- Detection of viruses in clinical specimens:

a- Light microscope: This can be used to visualize some large viruses e.g. poxviruses in which elementary bodies can be seen in skin lesions (papules and vesicles). Inclusion bodies can also be seen under the light microscope in several viral infections. In rabies, intracytoplasmic inclusions called "Negri bodies" can be detected in nerve cells.

b- Electron microscope: is used to demonstrate virus particles in vesicular fluid or tissue extracts treated with special stains. It is only successful if large numbers of particles (10^9/ml) are present.

c- Immunoelectron microscopy (IBM): Addition of specific antisera to the clinical material leads to aggregation of virus so it can be detected more readily than separate virus particles e.g. diagnosis of hepatitis A virus and rotavirus in stool samples.

d- Immuno fluorescence microscopy: Detection of virus in smears from lesions using fluorescein labeled specific antisera e.g. diagnosis of rabies in brain smears.

B -Detection of viral components in clinical specimens:

1- Detection of viral proteins:

Can be done by:

a- Using specific antibodies to detect viral antigens via any of the following techniques:

- Solid phase immunoassays i.e. radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA).
- Complement fixation test (CFT).
- Immunoflouresence (IF).
- Latex agglutination.

c- Detection of the pattern of protein electrophoresis.

d- Detection of viral enzymes e.g. detection of RT in HIV infection.

e- Heamagglutination and heamadsorption.

2- Detection of viral nucleic acid:

Can be done by:

a- Nucleic acid hybridization:

Principle:

Using DNA probes which is a single strand of the nucleic acid of the virus in question that will hybridize with its complementary strand in the specimen. Probes are labeled and can be easily detected.

Probes are labeled by:

- Radioactive material.
- Chemically modified nucleotide.

Types:

- In situ hybridization to detect virus nucleic acid in tissue samples.
- Blotting to detect virus nucleic acid extracted from pathologic specimens. It is of two types:

Southern blot for DNA viruses.

Northen blot for RNA viruses.

Advantages:

- It can be used to detect and quantitate the virus.
- It can be used to detect slowly replicating and non replicating (latent) viral infections.

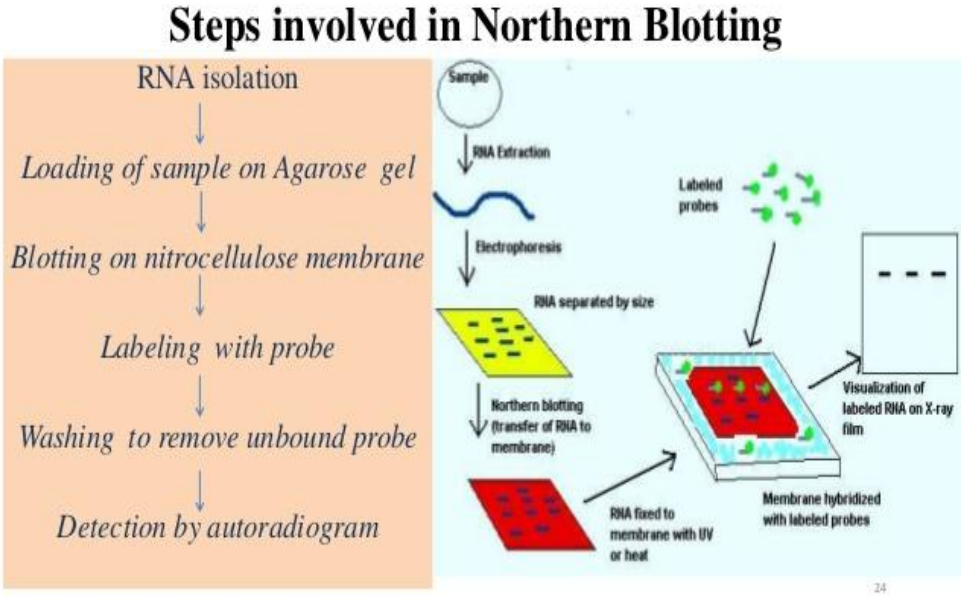


Figure (10): detection of viral nucleic acid.

b- Polymerase chain reaction (PCR): it is a technique that involves amplification of a short sequence of a target DNA or RNA (which may be in low concentration e.g. one copy leading to accumulation of large amounts of that sequence, so it can be easily detected.

c- Electrophoresis pattern of restriction endonuclease fragments produced from DNA viruses e.g. HSV.

d- Electrophoresis pattern of separated segments of segmented RNA viruses e.g. influenza virus.

C- Detection of cytopathic effects in the cells of clinical specimens:

It is a rapid method for diagnosis of viral infection by detection of cytopathic effects in the cells in which they replicate. These effects occur in the host cells as well as in cell cultures. These effects include:

(1) Cell lysis:

Accumulation of large amount of capsid protein and N.A (viral particles) may cause a general inhibition of both host cell and viral synthetic activities. Death of the cell is followed by lysis and release of large numbers of virions e.g. adeno viruses.

(2) Changes in cell morphology: e.g.

(a) Cell rounding:

The cells rounded up and aggregates in grape like clusters e.g. adeno viruses.

(b) Cell fusion:

Formation of multinuclealed giant cells (syncytia formation) by fusion of infected cell to each other, e.g. paramyxo and herpes viruses.

(3) Cell transformation:

Certain tumor viruses cause cells to change morphology and to multiply at a faster rate than uninfected cells. As a result transformed cells develop foci.

(4) Inclusion bodies:

It was found that one half of the viral diseases of man and animals are associated with the presence of abnormal bodies known as inclusion bodies. However some viruses do not produce cytopathic effects e.g. rubella virus

The nature of the inclusions varies with the virus concerned, they may represent:

- Aggregations of mature virions or
- Unassembled viral subunit. (NA. and proteins) or
- It may be remnant of viral multiplication or
- It may be due to virus induced changes in cell structure.

They may be single or multiple, large or small, rounded or irregular, esinophilic or basophilic.

The site of inclusion bodies is variable in different viral infections. They may be:

- Intracytoplasmic e.g. Negri bodies in Rabies or
- Intranuclear as in case of herpes viruses or ton's bodies in yellow fever or
- Intranuclear and intracytoplasmic as in case of measles.

D -Isolation of viruses:

Isolation of virus from clinical specimens is done by inoculation on tissue culture, chick embryo or laboratory animals according to the virus in question.

1) Emberyonated Hen's Egg Inoculation:

Advantages:

(1) Cheap and easily maintained.
(2) Easily manipulated.
(3) Easily identified and labeled.
(4) Generally free from natural factors of defense.

Routes of Egg Inoculation:

There are various methods depending on the type of the virus and its affinity.

a) Yolk sac inoculation:

- The embryos used at 5-8 days old.
- This route used for isolation of some toga and herpes viruses.

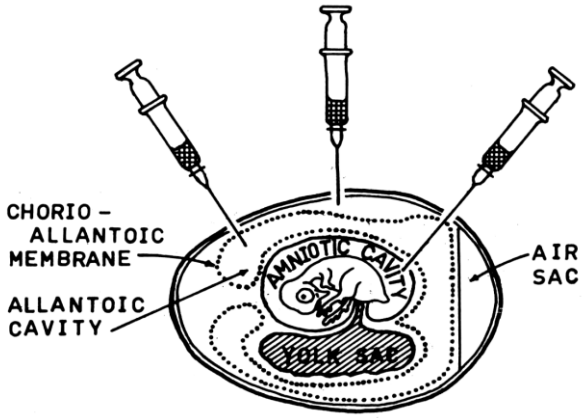


Figure (11): Routes of inoculation of chick embryo for viral isolation.

b) Allantoic cavity inoculation (AC):

- The embryo used at 9-11 days old.
- This route used for isolation of influenza viruses.

c) Chorioallantoic membrane inoculation (CAM):

- The embryo used at 11-13 days old.
- This route used for isolation of Herpes and Pox viruses.

Pocks Lesion:

They are white opaque areas visible macroscobically on the transparent chorioallantoic membrane that result from infection of cells by viral agents which cause proliferation and death of these infected cells.

The lesions vary in size and shape according to the nature of the infecting virus. Some may be circular, raised and dome-shaped; others may have a central depression.

Intracytoplasmic or intranuclear inclusion bodies are readily detected in stained sections of pocks lesion. Pocks lesions are also valuable for titration of viruses; each infectious virus unit produces a pock.

d) Amniotic cavity inoculation:

- The embryo used at 10-14 days old.

- Used for influenza A, B &C and mumps-virus.

2) Animal Inoculation:

The type of animal as well as the route of inoculation depends on the type of the virus. Laboratory animals used include mice, G. Pigs and Rabbits. Different routes were used as intracerebral, subcutaneous, intramuscular…etc. The white suckling mouse (1-3 days old) is the most widely used; it is susceptible to encephalitis viruses by the intracerebral route.

Animal inoculation is used mainly for:

- Primary isolation of new viruses.
- Study of pathogenesis of viral diseases.
- Study of oncogenic effect of some viruses.

3) Tissue Culture:

Advantages:

This system of cultivation has several advantages over animal and embryonated hen's egg inoculation because:

- It is easier.
- It is more economic.
- The cells have no or seldom to have antibodies or other inhibitors to interfere with the growth of the virus.
- The effect of the virus on cell cultures can often be detected in a very short time.

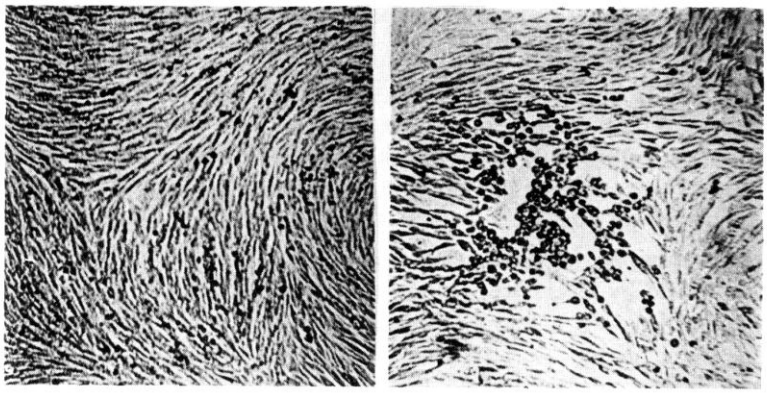


Figure (12): To the left, uninfected tissue culture monolayer. To the right infected tissue culture showing CPE, cells are lysed and fall off the glass.

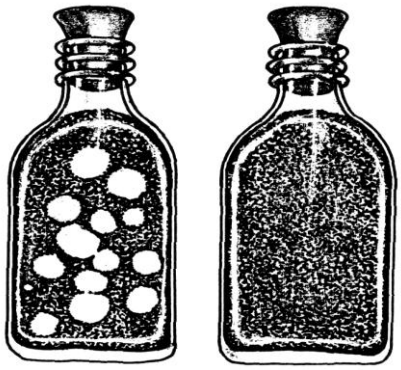


Figure (13): To the right, a bottle of uninfected tissue culture monolayer, to the left a bottle of infected tissue culture showing plaques.

Preparation of cell culture:

Under sterile conditions; the tissues are placed in balanced salt solution then fragmented and disaggregated by trypsin 0.25%. This is followed by centrifugation to obtain the cellular deposit which is washed and placed in enriched media containing proteins, amino acids, serum, vitamins and antibiotics then incubated.

The separated cells attach to the surface of the culture vessels and continue to divide, during division, the cell contact to each other till they fulfill the empty areas on the surface of the vessel and form a confluent single layer of cell (mono layer) (Fixed cells). Also a suspension form can be prepared.

Types of Cell Culture:

1) Primary cell culture:

*Obtained directly from animals or human tissues e.g. monkey kidney.

*Such cells can only divide for several passages (4-6) and then degenerate.

*It is of two forms:

- Mono-cell layer form which is used mainly for diagnosis of viral infection.
- Suspension form which is used for vaccine production and for diagnosis of viral infection.

2) Secondary cell culture:

*This type of culture is prepared by subculture of the primary cell culture.

*It continues to divide for a limited number (20 times).

*It is for diagnosis of viral infection.

3) Diploid cell culture:

*Such cells are derived from human or animal fetal tissue usually fibroblasts.

*They have the normal diploid number of chromosomes.

*It can be used for vaccine production.

*This type of cells is able to grow for many months (not more than 1 year), generally die after 50-70 subcultures.

*E.g. human embryo lung tissue.

4) Continuous cell lines:

*These are the most widely used for diagnostic work.

*They are derived either from a tumor or from normal cells, which after repeated cultures, have become transformed, so that they behave like tumor-derived cells i.e., they can divide indefinitely.

*They are aneuploid, i.e., they have an abnormal number of chromosomes.

*Such cells cannot be used to prepare vaccines for human use because of their malignant characteristics.

*E.g. VERO and Hela cells which are derived from carcinoma of the cervix.

Value or Uses of Cell Culture:

1) Isolation and identification of unknown viruses.
2) Titration o f viruses.
3) Preparation of some viral antigens.
4) Preparation of viral vaccines (except continuous cell lines).
5) It is used in some serological tests as IF.

Recognition of Virus Growth in Cell Cultures:

1) Cytopathic effects:

Many viruses kill the cells in which they replicate, some times with characteristic appearances or cytopathic effects (CPE). These effects occur in the host as well as in cell cultures and are often useful in the diagnostic laboratory. These include:

(a) Cell lysis.
(b) Changes in cell morphology: e.g. Cell fusion and Cell rounding.
(c) Cell transformation.
(d) Plaque Formation.

Some viruses may be detected macroscopically by their ability to form plaques in monolayer cells under solid medium. A plaque is a focus of virus infected cells which doesn't take up the vital stain (neutral red) and thus appears as a clear area against a background of stained viable cells.

(e) Inclusion bodies.

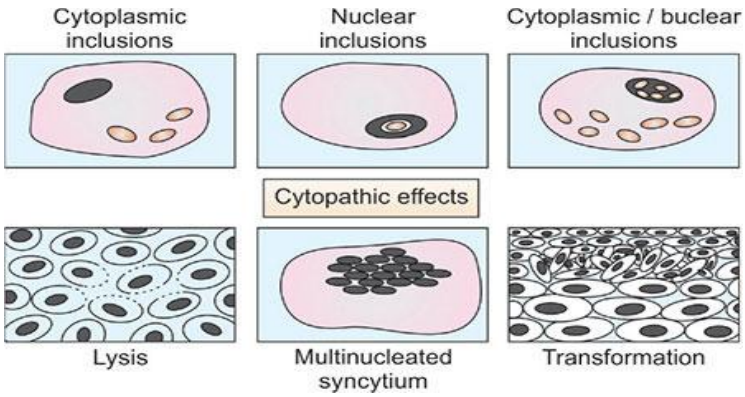


Figure (14): Cytopathic effects caused by viruses.

2- Haemadsorption:

When RBCs are added to infected cells they will appear as rosettes or clumps on the areas where the virus is growing. This is useful in haemagglutinating viruses e.g. influenza virus.

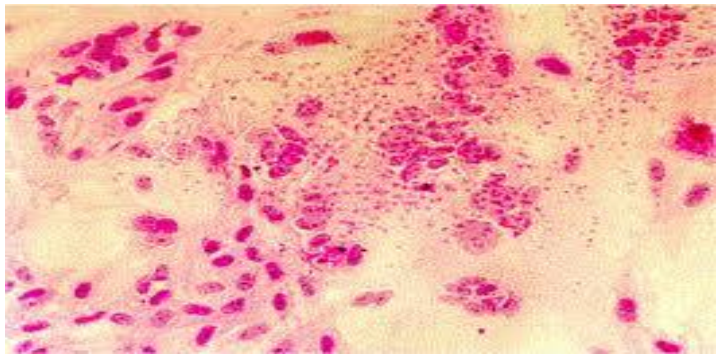


Figure (15): Clumps formed by RBCs.

3- Direct immuonoflouresence (IF):

Infected cell sheets on cover slips or microtiter plates are treated with fluorescein labeled specific antibody and examined for positive fluorescence.

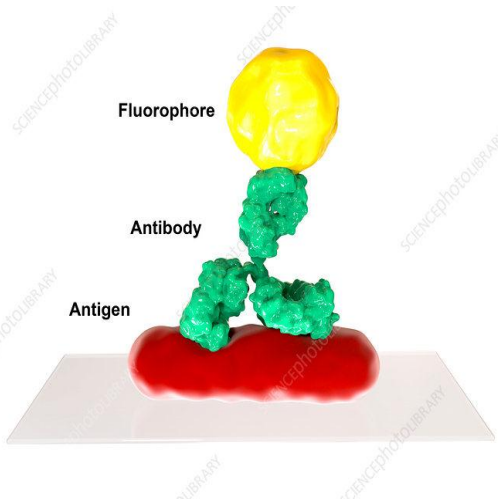


Figure (16): Direct immuonoflouresence between antigen and antibody.

4- Interference:

In some viruses which do not produce CPE their growth can be proved by their ability to interfere with the growth of another CPE producing virus, e.g. rubella (do not produce CPE) interfere with echo virus type II.

5- Detection of viral antigens by serology:

Soluble antigens which diffuse in the nutrient medium or those released after freezing and thawing of tissue culture cells, can be detected by any serologic method including:

1- Complement fixation:

This is an antigen antibody reaction that occurs in the presence of a third component known as the complement. The antigen unites with its specific antibody and the resulting complex fixes the complement.

Indicator system formed of sheep RBCs coated with their antibody is used. If there is antigen antibody reaction complement is fixed and no heamolysis of RBCs occurs. If the sample is negative i.e. no antigen antibody reaction occurred, the complement is free and causes heamolysis of RBCs.

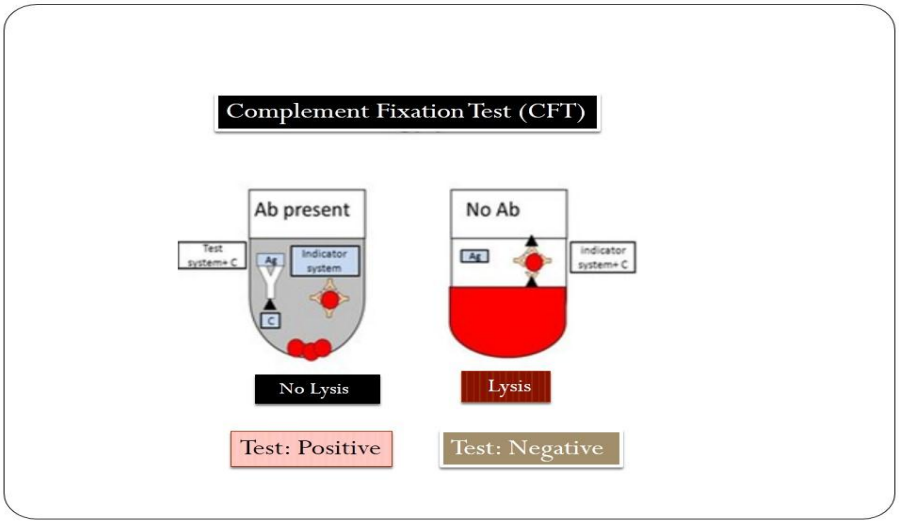


Figure (17): Complement fixation test to detect viruses.

2- **Haemagglutination**:

Some viruses e.g. influenza, mumps, adeno and yellow fever virus can cause agglutination of RBCs (passive heamagglutination test). This test is used for detection and titration of heamagglutinatig viruses in cultures.

3- Solid phase immuonoassyays:

a- radioimmuonoassy (RIA):

This test measures radioactive labeled antigens. It has been greatly replaced by ELISA due to hazards of radiation.

b- ELISA:

To measure antigen; double antibody technique is used. A known antibody is fixed to the solid phase. The test material containing antigen is added and the excess is washed. A specific known antibody labeled with enzyme is added. After washing a substrate is added and the enzyme activity is measured colorurimetrically and related to antigen concentration.

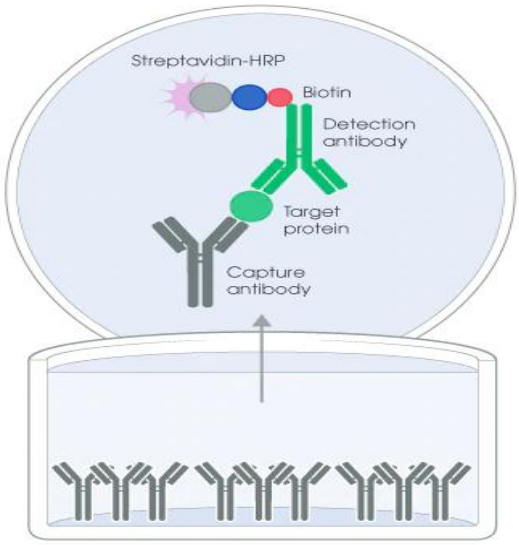


Figure (18): ElISA test for detecting antigens.

4- Direct immuonoflouresence (IF):

It is an antigen antibody reactions in which we use fluorescein labeled antibodies. Fluorescein is a dye which emits greenish fluorescence under ultraviolet light (UV), and it can be tagged to immunoglobulin molecules.

In this test, fluorescein labeled antibody is layered over the antigen fixed on a slide. The site where the antibody adheres to its specific antigen will be seen as apple green fluorescence.

6- Neutralization tests:

Neutralization of the effects of virus on tissue culture by specific antisera can be used to identify the type of isolated virus (see below).

II. Indirect methods by:

Serologic detection of antiviral antibodies:

Serologic diagnosis of virus infections can be established by detecting a rising antibody titer to the virus or by detection of sero conversion (patient who was previously sero negative turns positive due to primary infection). The first sample should be collected early after disease onset, the second 1-2 weeks later.

If paired sera are not available or rapid diagnosis is needed, as in diagnosis of rubella in early pregnancy; detection of IgM antibodies to the virus is resorted to. The detection of IgM in a single serum sample indicates recent infection.

The presence of IgM antibodies to any virus e.g. CMV or rubella- in the newborn serum indicates infection in utero (IgM does not cross the placenta).

Serologic diagnosis is of value to:

- Determine the type of infecting virus.
- Diagnose viruses difficult to isolate or slow viral infections.
- Differentiate acute or chronic infection (by antibody type; IgM is the main antibody in acute infection).
- Differentiate active or convalescent infection (by detection of rising antibody titer in active infection).
- Differentiate primary infection and reinfection:

Criteria for diagnosing Primary Infection:

1. A significant rise in titre of IgG/total antibody between acute and convalescent sera (a four-fold or greater increase in titre).
2. Presence of IgM.
3. Seroconversion.

Criteria for diagnosing re-infection/re-activation:

It is often very difficult to differentiate re-infection/re-activation from a primary infection. Under most circumstances, it is not important to differentiate between a primary infection and re-infection. However, it is very important under certain situations, such as rubella infection in the first trimester of pregnancy where primary infection is associated with a high risk of fetal damage whereas re-infection is not.

In general, a sharp large rise in antibody titres is found in re-infection whereas IgM is usually low or absent in cases of re-infection/re-activation.

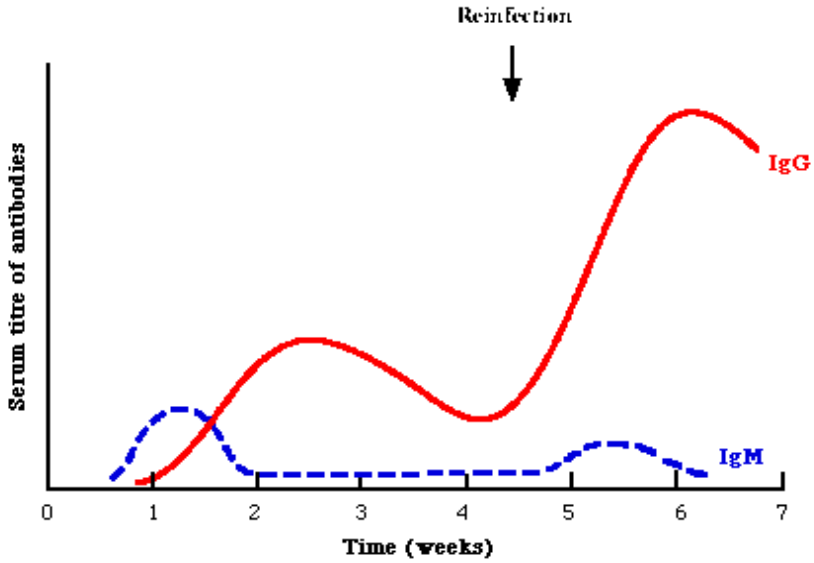


Figure (19): Serologic diagnosis.

Serological events following primary infection and reinfection. Note that in reinfection, IgM may be absent or only present transiently at a low level.

Limitations of serological diagnosis:

1. Long length of time required for diagnosis for paired acute and convalescent sera.
2. Mild local infections such as HSV genitalis may not produce a detectable humoral immune response.
3. Extensive antigenic cross-reactivity between related viruses e.g. HSV and VZV, Japanese B encephalitis and Dengue, may lead to false positive results.

4. Immunocompromised patients often give a reduced or absent humoral immune response.
5. Patients with infectious mononucleosis and those with connective tissue diseases such as SLE may react non-specifically giving a false positive result.
6. Patients given blood or blood products may give a false positive result due to the transfer of antibody.

The tests used for serologic diagnosis of viral infections include:

1- Virus neutralization test:

When viruses are added to appropriate cells growing in tissue culture; they will cause cell destruction called CPE such virus infectivity can be inhibited or neutralized by virus specific antibodies.

this test can be done by mixing a known virus with the serum and after incubation for some time the mixture is added to tissue culture cells if serum contains the specific antibody it will neutralize the virus and no CPE will occur if it does not contain the specific antibody the virus will retain its infectivity and will cause CPE in cells of tissue culture.

This test is used for:

- Diagnosis of viral infection by detection of antibodies in patient serum using a known virus.
- Identification of a virus isolated from patient using a known antibody.

2- Haemagglutination inhibition (HI) tests:

Some viruses e.g. influenza, mumps, adeno and yellow fever virus can cause agglutination of RBCs (passive heamagglutination test). Inhibition of this heamagglutination can be used for detection of antibodies in serum samples.

HI inhibition test is done by mixing a known virus with the serum sample and incubation for some time then RBCs suspension is added; if the serum contains antibody specific to the added virus, it will combine

with the attachment sites on the virus and prevent its agglutinating action on red cells.

3- Solid phase immuonoassays:

a- Radioimmunoassay:

This test measures radioactive labeled antibodies. It has been replaced by ELISA which is safer due to the risk of radiation when performing RIA.

b- ELISA:

It does not require specialized equipments and no hazards of radioactivity. This method depends on the conjugation of an enzyme to either antigen or antibody then the enzyme activity on a substrate is used as a quantitative measure. Solid phase ELISA is the most widely used.

To measure the antibody, indirect method is used. A known antigen is fixed to a solid phase (plastic cup or microplate) then incubated with the test serum dilution, wash to remove excess antibody is done and then re-incubated with antigloubin labeled with a suitable enzyme e.g. horseradish peroxidase. The labeled antigloubin will attach to the antibody bound to the fixed antigen. After washing the enzyme activity is measured by adding a specific substrate and measuring the degree of color change.

4- **Complement fixation.** (see above)

5- Indirect immunofluorescence:

It is an antigen antibody reactions in which we use fluorescein labeled antibodies. Fluorescein is a dye which emits greenish fluorescence under ultraviolet light (UV), and it can be tagged to immunoglobulin molecules.

In this test, patient serum (antibody) is layered over the antigen fixed on a slide a time is given for reaction to occur and the excess is washed. If the antibody is present it will adhere to its specific antigen and this can be detected by adding fluorescein labeled antigloubin that will be seen as apple green fluorescence under UV rays.

NB: serologic tests that depend on change in color as an end result are ELISA and IF.

Assay of Viral Infectivity (Virus Titration or quantitation):

Definition:

It is quantitative determination of viral activity; the concentration of virus in a sample which can produce disease, lesions, or recognizable effect on host.

Virus titer:

It is the number of infectious units per mL of sample.

Value of virus titration:

1. Determination of infectious doses.
2. Study of virus multiplication.
3. Determination of vaccine doses.
4. Determination of virus concentration in various materials.

Types of viral infectivity assays:

I- Quantitative assay:

In which the exact number of infectious virus particles in the sample is determined. This can be done by:

A- Physical methods:

1- Quantitative Polymerase Chain Reaction (qPCR):

Quantification by qPCR relies on serial dilutions of standards of known concentration being analyzed in parallel with the unknown samples for calibration and reference.

Disadvantages:

- Detects both infectious and non infectious particles.
- Viral sequence variations may reduce its accuracy.

2- Solid phase immunoassays:

ELISA:

To measure the amount of the antigen (virus); double antibody technique is used. A known antibody is fixed to the solid phase. The test material containing antigen is added and the excess is washed. A specific known antibody labeled with enzyme is added. After washing a

substrate is added and the enzyme activity is measured colorurimetrically and related to antigen concentration.

RIA:

Radioactive iodine labeled antigen competes with non labeled antigen in the sample for a fixed amount of specific antibody in a limited time.

The more the antigen in the test sample, the less chance for labeled antigen to combine with antibody molecules thus by measuring the quantity of labeled antigen bound to the antibody, a measure of antigen in the test sample can be obtained.

To perform a radioimmunoassay, a known quantity of an antigen is made radioactive. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two bind to one another. Then, a sample containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen to compete with the radiolabeled antigen ("hot") for antibody binding sites.

As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured using a gamma counter. Using known standards, a binding curve can then be generated which allows to measure the amount of antigen in the sample.

3- Hemagglutination assay:

It is a quantification assay specific for heamagglutinating viruses e.g. influenza virus. In this assay, dilutions of the sample are incubated with a 1% erythrocyte solution for one hour and the virus dilution at which agglutination first occurs is visually determined.

It is an easy and rapid method but it measures both infectious and non infectious virus particles.

4- Electron microscope:

Virus particles can be counted directly using the E.M by caparison with standard suspension of latex particles of similar size.

Disadvantages:

- Concentrated preparation of the virus is needed.
- It does not distinguish infectious from non infectious particles.

B- Biological methods:

1- Plaque assay:

Plaque-based assays are the standard method used to determine virus concentration in terms of infectious dose.

Viral plaque assays determine the number of plaque forming units (pfu) in a virus sample, which is one measure of virus quantity. This assay uses a confluent monolayer of cells which is infected with the virus at varying dilutions and covered with a semi-solid medium, such as agar or carboxymethyl cellulose, to prevent the virus infection from spreading indiscriminately.

A viral plaque is formed when a virus infects a cell within the fixed cell monolayer. The virus infected cell will lyse and spread the infection to adjacent cells where the infection-to-lysis cycle is repeated.

The infected cell area will create a plaque (an area of infection surrounded by uninfected cells) which can be seen macroscopically or with microscope. Plaque formation can take 3 – 14 days, depending on the virus being analyzed. Plaques are generally counted manually and the results, in combination with the dilution factor used to prepare the plate, are used to calculate the number of plaque forming units per sample unit volume (pfu/mL). The pfu/mL result represents the number of infective particles within the sample and is based on the assumption that each plaque formed is representative of one infective virus particle.

<u>Advantages:</u>

- CPE of infected cells within a plaque can be distinguished from non infected cells of tissue culture with or without staining.
- Can be counted macroscopically.

2- Pocks lesions:

Certain viruses e.g. herpes, vaccinia and pox viruses produce pocks lesions on CAM when inoculated in emberyonated eggs. These lesions can be used for virus titration.

II- Quantal assay:

It does not quantify the number of infectious virus particles in the inoculum but, rather, their presence in a given amount of a given sample. It is performed by inoculating serial dilutions of virus into suitable culture. Time is allowed for virus to replicate and destroy cultured cells or infect them. Titer is expressed as reciprocal of the highest dilution that kills/infects 50% of subjects in test system. The following end points are used:

- TCID50 (tissue culture infectivity dose 50) is the reciprocal of the highest dilution that infects or kill 50% of the inoculated cultures.
- LD50 (lethal dose 50) is the reciprocal of the highest dilution that causes death of 50% of the inoculated laboratory animals.
- ELD50 (embryo lethal dose 50) this term is used when the virus is inoculated in emberyonated eggs.

NB: Preservation of viruses:

Viruses can be preserved by the following methods:

1- Low temperature:

Viruses will retain its infectivity for several days if kept at 4 ºC in refrigerator repeated freezing and thawing is avoided.

Preservation for months or years must be at -70 ºC or lower in deep freezer or in liquid nitrogen at -169 ºC.

The containers may be screw capped vials or rubber stoppered vials or heat sealed glass ampoules. The cell associated viruses need dimethyl sulfoxide (DMSO) or glycerol to retain its infectivity when frozen at -70 ºC.

2- chemical agents:

10% serum or 10% DMSO must be added to cell associated viruses during its preservation also glycerol stabilize the outside of cells and some viruses so it added at 50% concentration in pox viruses preservation for vaccine preparation.

3- lypholyzation:

It means rapid deep freezing of the virus suspension followed by rapid dehydration. The lypholyzed virus can be stored at ordinary temperature or at 4 ºC. Addition of 10% fetal bovine serum or casein during lypholyzation is desirable. This method is widely used for preservation of live vaccines. The lypholyzed vaccines are reconstituted with sterile water when needed.

Treatment of Viral Infections

Few antiviral drugs are in clinical use because of:

1. Lack of selective toxicity since viruses are obligate intracellular parasites, it is difficult to find an antiviral drug that selectively inhibits the virus without affecting the cell.
2. Many rounds of virus replication occur during the incubation period and the virus has spread before symptoms appear making drugs are relatively ineffective.
3. Some viruses are latent within cells and no current antiviral can kill them.
4. Emergence of drug resistant mutants e.g. influenza virus and HIV.

The general idea behind modern antiviral drug design is to identify viral proteins, or parts of proteins, that can be disabled. These "targets" should generally be as unlike any proteins or parts of proteins in humans as possible, to reduce the likelihood of side effects. The targets should also be common across many strains of a virus, or even among different species of virus in the same family, so a single drug will have broad effectiveness. Antiviral drugs include:

I- Drugs that approaches different stages of virus life cycle:

A -Attachment:

This phase of replication can be inhibited in two ways:

a) Using agents which mimic the V.A.P. (virus associated protein) and bind to the cellular receptor, e.g.:

- anti-receptor antibodies
- V.A.P. anti-idiotypic antibodies

- natural ligands of the receptor, e.g. epidermal growth factor/Vaccinia virus
- Synthetic ligands, e.g. synthetic peptides resembling the receptor-binding domain of the V.A.P. itself.

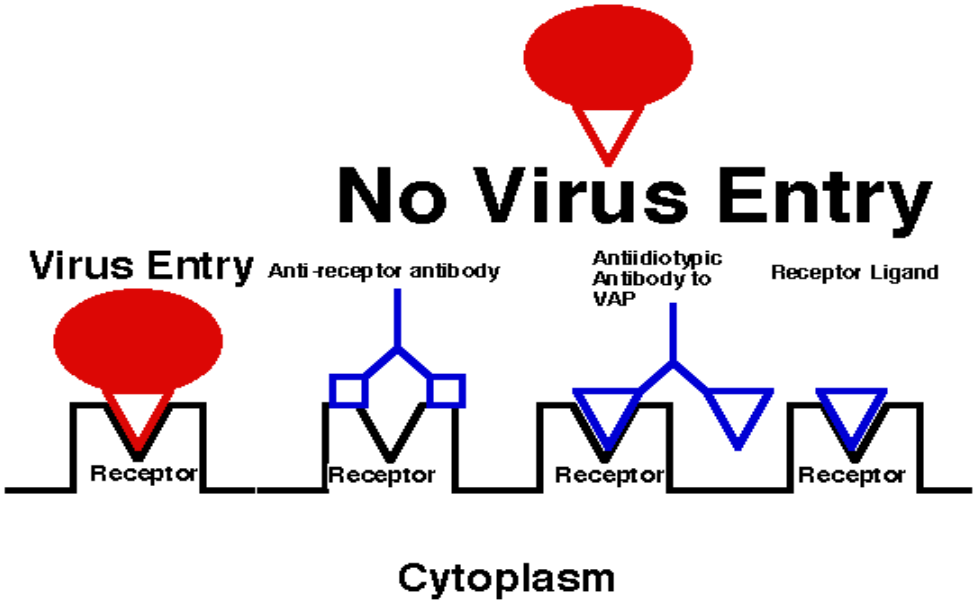


Figure (20): Using agents which mimic the V.A.P.

b) Agents which mimic the receptor and bind to the V.A.P:

- anti-V.A.P. antibodies (a natural component of the antibody response to virus infection/vaccination)
- receptor anti-idiotypic antibodies
- Synthetic receptor mimics, e.g. sialic acid derivatives/influenza virus.

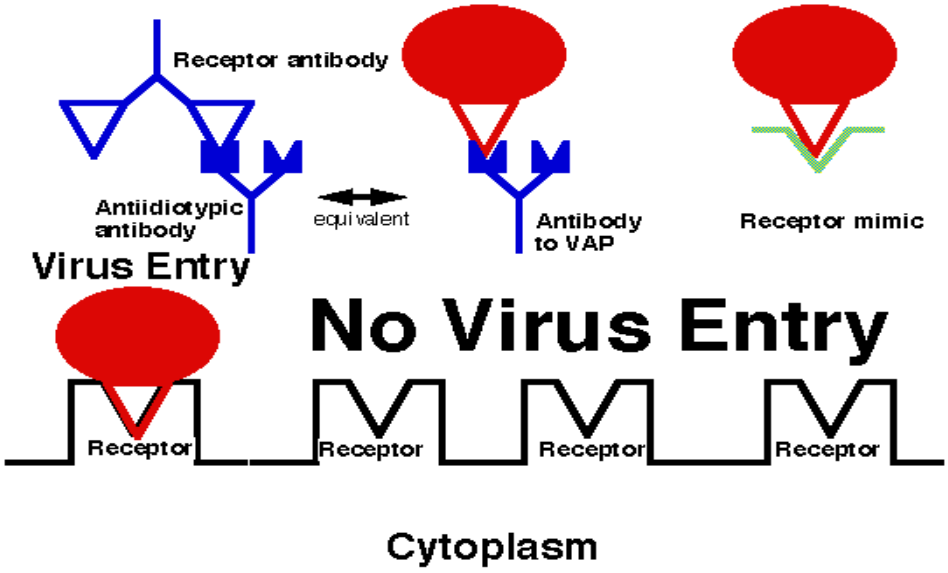


Figure (21): Agents which mimic the receptor and bind to the V.A.P.

There are considerable problems with clinical use of any of these substances. The cost of synthetic peptides is prohibitive when the amounts required for clinically effective whole body doses; the generation of anti-idiotypic antibodies is a complex, poorly understood process; the pharmacokinetics of many of these synthetic compounds is very poor.

B- Penetration / Uncoating:

It is difficult to specifically target these stages of the life cycle as relatively little is known about them. Uncoating in particular is largely mediated by cellular enzymes, although like penetration, is often influenced by one or more virus proteins.

Pleconaril is a broad spectrum anti-picorna virus agent. It is orally bioavailable and reduces peak viral titers by more than 99%. It is a small cyclic drug which binds to a canyon pore of the virus so it blocks attachment and uncoating of the viral particle.

Amantadine and **rimantadine** are active against influenza A viruses. The action of these closely related agents is complex and incompletely understood, but they are believed to block cellular membrane ion channels.

- The target for both drugs is the matrix protein (M2).
- Drug-treated cells are unable to lower the pH of the endosomal compartment (a function normally controlled by the M2 gene product), a process which is essential to induce conformational changes in the HA protein to permit membrane fusion.

Enfuvirtide (fuzeon) it is an agent that used for treatment of HIV infection. It inhibits fusion of the viral envelope with the cell membrane. It binds to gp41 on the viral envelope, thereby blocking the entry of HIV into the cell. It must be administered by injection and it is quite expensive.

C- Genome Replication (nucleic acid synthesis):

Many viruses have evolved their own specific enzymatic mechanisms to preferentially replicate virus nucleic acids at the expense of cellular molecules; however there is often sufficient specificity in virus enzymes to provide a target for a specific antiviral agent, and this method has produced the majority of the specific antiviral drugs currently in use.

Genome replication inhibitors include:

I- nucleoside/nucleotide analogues:

NB. Nucleosides are glycosylamines consisting of a nucleobase bound to a ribose or deoxyribose sugar via a beta-glycosidic linkage. Examples of nucleosides include cytidine, uridine, adenosine, guanosine, thymidine and inosine.

NB. Nucleotides: they differ from nucleoside analogues in having an attached phosphate group. They are molecules that, when joined together, make up the structural units of RNA and DNA. In addition,

nucleotides participate in cellular signaling (cyclic guanosine monophosphate and cyclic adenosine monophosphate), and are incorporated into important cofactors of enzymatic reactions (e.g. coenzyme A). Nucleotide derivatives such as the nucleoside triphosphates play central roles in metabolism as they serve as sources of chemical energy (e.g. adenosine triphosphate).
The majority of genome replication inhibitors function as polymerase substrate (i.e. nucleoside/nucleotide) analogues.

Nucleoside/nucleotide analogues are in fact **pro-drugs**, since they need to be phosphorylated before becoming effective. This is the key to their selectivity. They include:

Acyclovir:

Mechanism of action:

It is a guanosine analogue that inhibits viral DNA polymerase and terminates viral DNA chain growth by incorporation into the growing chain. It is effective mainly against HSV-2 & HSV-2, EB & VZ viruses are also susceptible but to lesser extent. CMV is resistant.

Selective toxicity: is due to

* It is phosphorylated by HSV thymidine kinase 200 times more efficiently than by cellular enzymes.

* The cell DNA polymerase is less sensitive to it than the viral DNA polymerase.

Resistance:

Develops due to mutation in thymidine kinase gene or in viral DNA polymerase.

Therapeutic uses:

*It is used topically for treatment of herpetic mucocutaneous infections e.g. herpetic corneal ulcers and for herpetic skin lesions.

*Parenteral administration is used in treatment of serious systemic infections e.g. herpes virus encephalitis.

*It is effective in prevention of systemic infection by HSV-1 or varicella-zoster virus in immunocompromised patients.

Gancyclovir:

It is a guanosine analogue that differs from acyclovir by single carboxyl side chain this structural difference makes it 50 times more effective against CMV than acyclovir.

Acyclovir is not effective in CMV infection due to lack of the gene for thymidine kinase required for drug phosphorylation.

Gancyclovir is specifically phosphorylated by a CMV-encoded kinase encoded by gene UL97.

Gancyclovir is used for treatment of CMV infection in immuonocomprimised patients e.g. retinitis caused by CMV in AIDS patients.

Nucleoside/nucleotide RT inhibitors of HIV:

- **Zidovudine (AZT):** Is a thymidine analogue that inhibits the replication of HIV by inhibiting viral RT. AZT reduces morbidity and mortality. It causes bone marrow depression and it is very expensive.
- **Dideoxyinosine (ddI) and dideoxycytidine (ddC):** They also inhibit HIV as described for AZT; however, they are les toxic.

- **Stavudine (d4T) and Lamivudine (3TC)** are used in HIV patients intolerant or resistant to AZT or ddI.

4- **Ribavirin**:

Mechanism of action:

It is a synthetic nucleotide which is effective against many DNA and RNA viruses. It acts by interfering with synthesis of mRNA.

Therapeutic uses:

- It is used as aerosol preparations for treatment of respiratory syncytial virus infections in children.
- Influenza B infection.
- Hepatitis C virus infection.

II- Non nucleoside/nucleotide analogues:

Many other drugs that inhibit virus genome replication function in a way other than being nucleoside/nucleotide analogues. This group of drugs inhibits viral enzymes essential for replication. E.g.

- Non-nucleoside RT inhibitors of HIV: e.g. Nevirapine, efavirenz.
- Integrase inhibitors (e.g. raltegravir), which splices the synthesized DNA into the host cell genome.it is used in treatment of HIV infection.
- Protease inhibitors:

Some viruses include an enzyme known as a protease that cuts viral protein chains apart so they can be assembled into their final configuration.

Saquinavir, ritonavir, indinavir, lopinavir and nelfinavir (Viracept) are inhibitors of the protease encoded by HIV. The main side effect of this group is the deposition of fat in the back of the neck "buffalo hump" and in other parts of the body.

- **Foscarnet** inhibits the DNA polymerase of all herpes viruses and HIV. It is used for treatment of acyclovir resistant herpes virus infections and CMV retinitis.

D- Assembly:

Rifampicin acts at the assembly phase. It inhibits late stages of assembly of pox virus

E- Release phase:

The final stage in the life cycle of a virus is the release of completed viruses from the host cell, and this step has also been targeted by antiviral drug developers. Two drugs named zanamivir(Relenza) and oseltamivir (Tamiflu) that have been recently introduced to treat influenza prevent the release of viral particles by blocking a molecule named neuraminidase that is found on the surface of flu viruses, and also seems to be constant across a wide range of flu strains.

II- Drugs that act by immune system stimulation:

A second strategy for fighting viruses involves encouraging the body's immune system to attack them, rather than attacking them directly. Some antivirals of this sort do not focus on a specific pathogen, instead stimulating the immune system to attack a range of pathogens.

The best-known of this class of drugs are interferons, which inhibit viral synthesis in infected cells. One form of human interferon named "interferon alpha" is well-established as part of the standard treatment for hepatitis B and C, and other interferons are also being investigated as treatments for various diseases.

A more specific approach is to synthesize antibodies, protein molecules that can bind to a pathogen and mark it for attack by other

elements of the immune system. Once researchers identify a particular target on the pathogen, they can synthesize quantities of identical "monoclonal" antibodies to link up that target. Monoclonal antibody therapy is now used to fight respiratory syncytial virus in babies, and antibodies purified from infected individuals are also used as a treatment for hepatitis B.

III- Viral vaccines for prevention of viral diseases (are discussed with each type).

Interferon

Definition:

It is a host-coded protein that inhibits viral replication and produced by living cells of cell culture, embryonated eggs or in laboratory animals in response to viral infection or other inducers (mitogens, bacteria, dsRNA). It is the 1st line of defense against viral infection.

General Properties of Interferon:

1) It is a small protein containing all amino acids but without N.A with low molecular weight of about 25,000-45,000 dalton (1/24 of hydrogen atom).
2) It is thermo stable at 4°C and resists heating at 50°C for 1 hr.
3) It is active through wide range of pH from 2-10.
4) Non toxic, weakly antigenic and can not be neutralized by specific antiserum against the virus used to make it.
5) It is inactivated by proteolytic enzymes as trypsin and can be concentrated by precipitation with ammonium sulphate.
6) Produced after a short latent period from infection and has a short life span.
7) Species specific i.e. IFN produced by guinea-pig is ineffective in mouse or human cells but not specific in action i.e. IFN induced by one virus is effective against others.

Types of Interferon:

Type I interferon: include two subtypes

1) IFN-α produced by leukocytes.

2) IFN-β produced by fibroblasts.

Also both IFN-α and IFN-β are produced from virus infected cells.

Type II interferon:

It includes IFN-γ (immune interferon). It is produced by:

- TH1 and CD8 T cells.
- NK cells.
-

	IFN-α	**IFN-β**	**IFN-γ**
type	I	I	II
Produced by	Leukocytes and virus infected cells.	Fibroblasts and virus infected cells.	T-lymphocytes, TH1 and CD8 T cells and NK cells.
No of genes encoding it	> 20	1	1
Inducing agents	Viruses and dsRNA	Viruses and dsRNA	mitogens
Stability at PH 2	stable	stable	labile
Introns in gene	-	-	+
Location of gene	Chromosome 9	Chromosome 9	Chromosome 12
IFN receptor	IFNAR	IFNAR	IFNGR
glycosylation	-	+	+

Mechanism of Interferon Action:

1- Type I IFN:

- **Inhibition of viral replication:**

IFN do not kill viruses, the mechanism of action is complex and indirect. Viruses attach by specific receptors to a cell which is stimulated to produce interferon molecules. These diffuse out from the cell and attach to receptors on the neighboring cells and induce the formation of protein called translation inhibitory protein (TIP) (anti-viral protein) which bind to cellular ribosomes and alter them in such away normal cell function to continue but prevent the synthesis of virus directed protein as enzymes and protein coat for progeny virus, so new virus is not formed (inhibit replication).

- **Increased expression of MHC-1:**

This leads to better presentation and killing of virus infected cells by cytotoxic T lymphocytes.

- **Activation of NK cells:** to kill virus infected cells**.**
- **Antiproliferative action.**

2- Type II IFN:

- It is the principle macrophage activating cytokine.
- It increases expression of MHC-I and MHC-II and co stimulatory molecules on the surface of APCs.
- It increases cytotoxic action of NK cells.
- It increases production of TH1 and inhibits proliferation of TH2.

Factors Affecting Production of Interferon:

1) Factors related to the virus:

a) Type of the virus: All viruses have the ability to produce interferon but to variable degrees e.g. adenoviruses are the weakest for producing interferon.

b) Virulence of the virus: High virulent viruses produce low amounts of interferon while, low virulent viruses produce high amounts of interferon.

c) Viability of the virus particles: Inactivated viruses are usually better inducer than live viruses.

2) Factors related to the infected cells:

a) Age of the cell: Young cells are poor interferon producers.

b) Type of the cell: Tumor or cancer cells are less interferon producers than normal cells.

3) Temperature:

Viruses with a low optimal temperature for growth (35°C) usually produce large amounts of IFN. Viruses which replicate best at higher temperature (40°C) tend to induce little of interferon.

(4) Other Factors:

All factors which are unfavorable for virus multiplication leading to high interferon production.

Comparison between interferon and antibodies.

	Interferon	**Antibodies**
1- Produced from	Viral infected cells	B-Lymphocytes
2- Produced after	3-6 hrs	Few days
3- Time of production	50 hours	Several days or months
4- Specificity	Cell specific	Viral specific
5- Effect of heat	Thermostable	thermolabile
6- Antigenicity	Poor antigenic	Antigenic
7- Penetrate living	yes	No

cells		

Biological Significance of Interferon:

1- The production of interferon can occur *in vivo* as well as *in vitro*.

2- The formation of interferon is not only associated with virus but also with other inducers (mitogens, bacteria and dsRNA).

3- Interferon play an important role in non immune defense as in patients with hypogammaglobulinemia, they can recover from viral infection.

4- The protection by interferon develops rapidly before antibody formation.

5- IFN Produced by any type of cells, while antibody is produced by B-lymphocyte.

6- IFN shows no viral specificity as antibody but cell specific.

7- IFN can penetrate living cells and therefore prevent intracellular viral multiplication.

8- IFN manufactured early and appears in blood stream a few hrs after infection.

All these criteria make interferon an ideal first line non specific defense mechanism.

Therapeutic Uses of Interferon:

I- It is an ideal therapeutic agent in certain virus infection due to:

1) Its broad antiviral spectrum.
2) Its lack of toxicity for the host.
3) Its poor antigenicity.
4) Its high activity.

E.g.

- Chronic hepatitis B infection.
- Chronic hepatitis C infection.
- Papilloma virus infection.
- Topical in herpetic keratoconjunctivitis.

II- type I IFN has antiproliferative action and stimulates macrophages and NK cells so used in cancer therapy e.g. the use of IFN-α in treatment of non Hodgkin's lymphoma, hairy cell leukemia, Kaposi sarcoma, melanoma and chronic myeloid leukemia.

III- IFN-β is used in treatment of multiple sclerosis.

IV- IFN-γ is used in patients with chronic granulomatous diseases to reduce the infection.

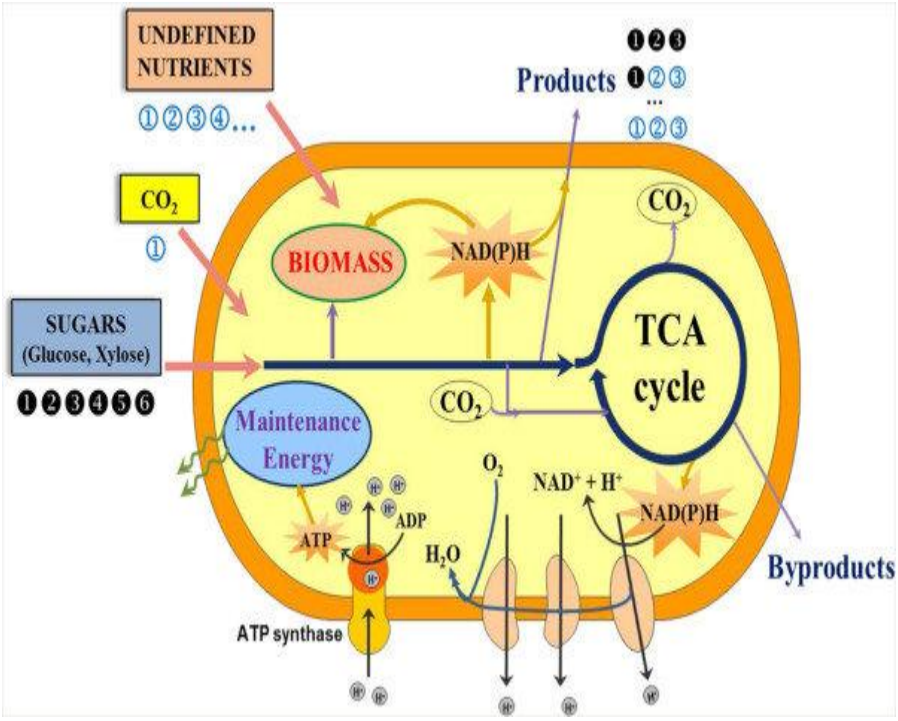
Disadvantages of Interferon*:*

1) Difficult to be produced in large amounts.
2) Effective for short period.
3) Can not be used to block viral synthesis which has been initiated within the cell, therefore interferon can be used for prophylaxis than for cure.
4) Rapidly diluted by body fluids and inactivated by proteolytic enzymes, therefore it is better to be used in localized infection.
5) Has side effects on GIT, CNS, and can cause bone marrow suppression.

Assay of Interferon:

By virus plaque reduction test, by exposing the cells to preparations containing interferon for a period of 12-24 hrs and then challenging the cell with a standard amount of virus known to produce a certain number of plaque. The titer of interferon preparation is the reciprocal of that dilution which reduces this number by 50%.

Part (2): Bacterial Metabolism



Prepared by:

Dr. Rokaia Elamary

CONTENTS

Bacterial growth, nutrition and adaptation

Bacteria is divided into two categories on the bases of their effect on the surrounding environment:

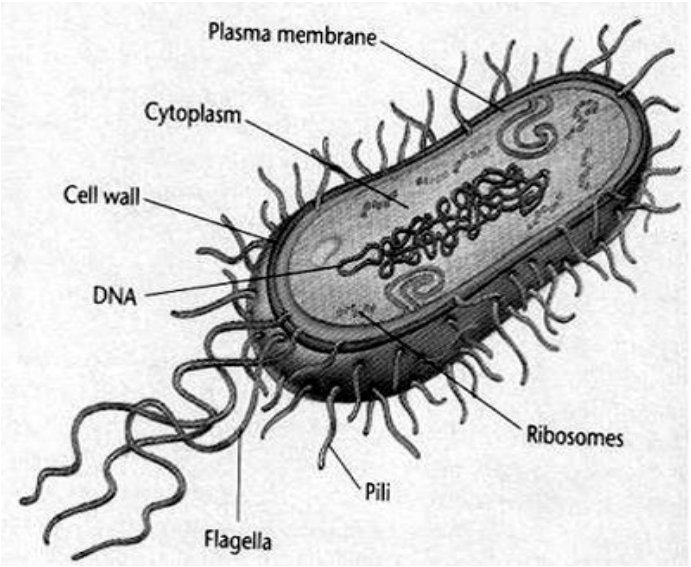
(1) Disease- causing bacteria represented by a small group of pathogenic bacteria.

(2) Useful and harmless bacteria that could be divided again into the following:

- Antibiotic producers (for disease control).
- Enzyme producers (for the production of biodetergents, insecticides, biodegradable plastics, bacteria used for biomining or leaching metals from low- grade ores, bacteria used in food industry and other biochemical reactions).

In general, the aim of studying bacteria is to minimize the harmful effects and maximize the useful activities of these organisms.

The bacterial cell



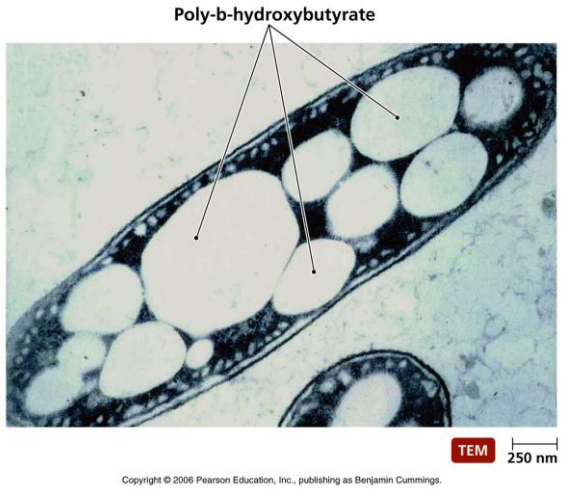
The simple diagram above presents the constituents of the bacterial cell (cross section). The bacterial cell wall followed by plasma membrane are enclosing the cytoplasm with different constituents that were studied previously.

Important storage granules in the bacterial cell

(1) Poly- ß-hydroxybutyrate (PHB's):

- A linear polymer that some species accumulate when nutrients decrease.
- PHB acts as a reservoir of carbon and energy.

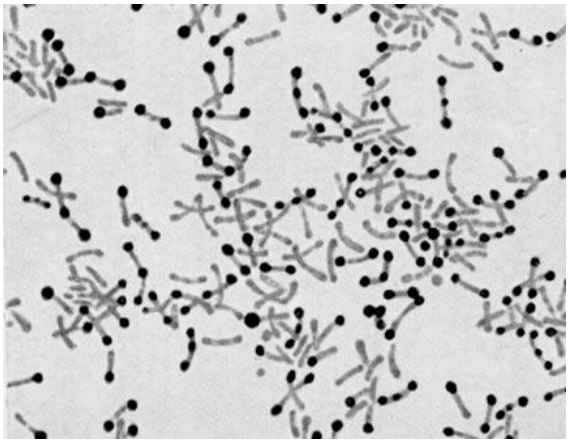
- Enzymes for the synthesis and de- polymerization are present on the surface of the PHB's.
- PHB's are the basis of the biodegradable plastics (Biopol).
- Other polymer "poly- ß- hydroxyalkanoates, PHA's" occur in some bacteria (e. g. PHA's in *Pseudomonas oleovorans*).



(2) Polyphosphates:

- Occur in most types of bacteria.
- Act as reservoirs of phosphate- energy metabolism- sequester cations storage.

- When stained, give different colour from the stain (metachromacy)- so called metachromatic granules.

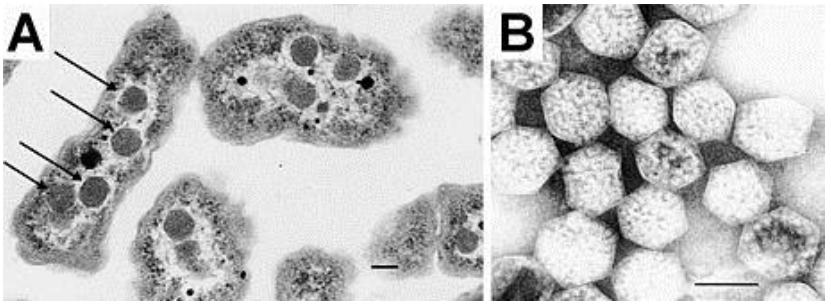


(3) Gas vacuoles:

- Occur in photosynthetic, aquatic bacteria.
- Consists of clusters of gas- filled vesicles with protein walls (about 70 nm in diameter each).
- Affects buoyancy of free- floating cells in the aquatic environments, that in turn affects the quantity of received light.

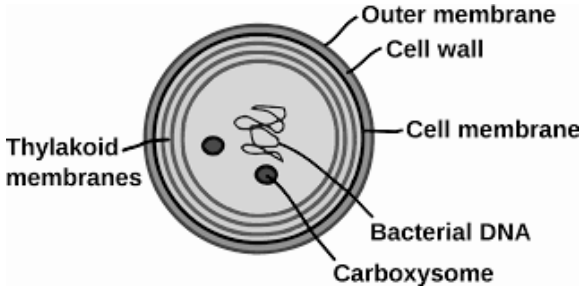
(4) Carboxysomes:

- Intracellular bodies (100- 150 nm in diameter) generally found in autotrophic bacteria.
- It is a membranous sac containing many copies of the enzyme "Ru Bis CO" involved in carbon dioxide fixation.



(5) Thylakoids:

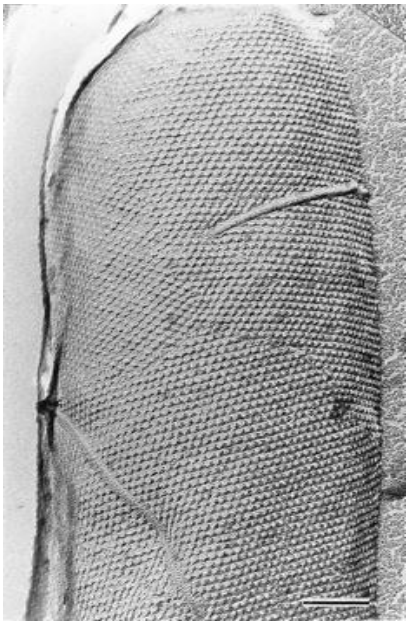
- Intracellular flattened sacs occur generally in Cyanobacteria, close to the cell envelope.
- Contain chlorophylls and enzymes for photosynthesis and respiration (sites of photosynthesis and respiration).
- There are chlorosomes or *Chlorobium* vesicles found in the order Chlorobiineae similar to thylakoids.



(6) S- layers:

- The outermost layer of the cell.
- Consists of separating patterns of protein or glycogen subunits arranged in squares or hexagons.

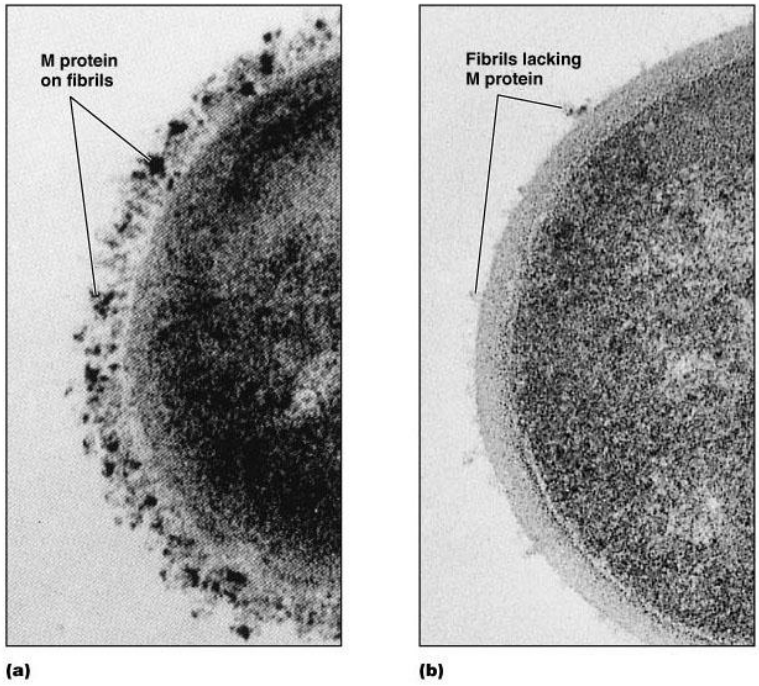
- In Gram –ve bacteria, it covers the outer membrane but, in some archaebacteria, it is the cell wall (overlying cytoplasmic membrane).
- Double S- layers occur in strains of *Aquaspirillum* and *Bacillus* and in one strain occur on both sides of the cell wall.



(7) M proteins:

- A thin layer on the cell wall in certain virulent strains of *Streptococcus*.

- It makes cells less susceptible to phagocytosis, thus contributing to cell virulence.



Bacterial growth

Adaptation to the nutritional and physical environment

Unlike plant and animal cells, most bacteria are exposed to a constantly changing physical and chemical environment. Within limits, bacteria can react to changes in their environment through changes in patterns of structural

proteins, transport proteins, toxins, enzymes, etc., which adapt them to a particular ecological situation. For example, *Vibrio cholerae* does not produce the cholera toxin that causes diarrhea unless it is in the human intestinal tract. *Bacillus subtilis* does not make the enzymes for tryptophan biosynthesis if it can find tryptophan in its environment. If *E. coli* is fed on glucose and lactose together, it will use the glucose first because it takes two less enzymes to use glucose than it does to use lactose. The bacterium *Neisseria gonorrhoeae* will develop a sophisticated iron gathering and transport system if it senses that iron is in short supply in its environment.

Bacteria have developed sophisticated mechanisms for the regulation of both catabolic and anabolic pathways. Generally, bacteria do not synthesize degradative (catabolic) enzymes unless the substrates for these enzymes are present in their environment. For example, synthesis of enzymes that degrade lactose would be wasteful unless the substrate for

these enzymes (lactose) is available in the environment. Similarly, bacteria have developed diverse mechanisms for the control of biosynthetic (anabolic) pathways. Bacterial cells shut down biosynthetic pathways when the end product of the pathway is not needed or is readily obtained by uptake from the environment. For example, if a bacterium could find a preformed amino acid like tryptophan in its environment, it would make sense to shut down its own pathway of tryptophan biosynthesis, and thereby conserve energy. However, in real bacterial life, the control mechanisms for all these metabolic pathways must be reversible, since the environment can change quickly.

Some of the common mechanisms by which bacterial cells regulate and control their metabolic activities are discussed below. These mechanisms have been observed or described in the bacterium, *Escherichia coli*, and they are mostly untested and unproved to exist in many other bacteria or

prokaryotes (although, whenever they are looked for, they are often found).

Many prokaryotes are able to convert any given carbon source into biosynthetic building blocks—e.g., amino acids, purines, pyrimidines, lipids, sugars, and enzyme cofactors. The amount and activity of each enzyme in these biosynthetic pathways are carefully regulated so that the cell produces only as much of any compound as is needed at any time.

During the process of evolution, some bacteria have lost genes that encode certain biosynthetic reactions and are hence likely to require nutritional supplements. For example, *Mycoplasma*, whose DNA content is about one-quarter the size of that of *E. coli*, has many nutritional requirements and has even lost the ability to make a cell wall.

Biosynthesis and nutrition

Nutritional requirements of cells

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. In the laboratory, bacteria are grown in culture media which are designed to provide all the essential nutrients in solution for bacterial growth.

The major elements

At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a

structural or functional role in the cells. (See the following Table for the general physiological functions of the elements).

Trace Elements

Trace elements are metal ions required by certain cells in such small amounts that it is difficult to detect (measure) them, and it is not necessary to add them to culture media as nutrients. Trace elements are required in such small amounts that they are present as "contaminants" of the water or other media components. As metal ions, the trace elements usually act as cofactors for essential enzymatic reactions in the cell. One organism's trace element may be another's required element and vice-versa, but the usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, and Mo.

Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S^o, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Carbon and energy sources for bacterial growth

In order to grow in nature or in the laboratory, a bacterium must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature, and pH. Sometimes bacteria are referred to as individuals or groups based on their patterns of growth under various chemical (nutritional) or physical conditions. For example, phototrophs are organisms that use light as an energy source; anaerobes are organisms that grow without oxygen; thermophiles are organisms that grow at high temperatures.

All living organisms require a source of energy. Organisms that use radiant energy (light) are called **phototrophs**. Organisms that use (oxidize) an organic form of carbon are called **heterotrophs** or **chemo(hetero)trophs**. Organisms that oxidize inorganic compounds are called **lithotrophs**.

The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-

hydrogen bond) or by CO_2. Organisms that use organic carbon are **heterotrophs** and organisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**.

Thus, on the basis of carbon and energy sources for growth four major nutritional types of prokaryotes may be defined. Almost all eukaryotes are either photoautotrophic (e.g. plants and algae) or heterotrophic (e.g. animals, protozoa, fungi).

Major nutritional types of prokaryotes

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Lithotrophy is unique to prokaryotes and photoheterotrophy, common in the Purple and Green Bacteria, occurs only in a very few eukaryotic algae. Phototrophy has not been found in

the Archaea, except for nonphotosynthetic light-driven ATP synthesis in the extreme halophiles.

Growth Factors

This simplified scheme for use of carbon, either organic carbon or CO_2, ignores the possibility that an organism, whether it is an autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called growth factors.

Growth factors are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories.

1. **purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)

2. **amino acids**: required for the synthesis of proteins

3. **vitamins**: needed as coenzymes and functional groups of certain enzymes

Some bacteria (e.g *E. coli*) do not require any growth factors: they can synthesize all essential purines, pyrimidines, amino acids and vitamins, starting with their carbon source, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require purines, pyrimidines, vitamins and several amino acids in order to grow. These compounds must be added in advance to culture media that are used to grow these bacteria. The growth factors are not metabolized directly as sources of carbon or energy, rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**. Thus, a strain of *E. coli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph and would be designated *E. coli****trp-*****.**

Some vitamins that are frequently required by certain bacteria as growth factors are listed in the following Table. The function(s) of these vitamins in essential enzymatic reactions gives a clue why, if the cell cannot make the vitamin, it must be provided exogenously in order for growth to occur.

Common vitamins required in the nutrition of certain bacteria.

Vitamin	Coenzyme form	Function
p-Aminobenzoic acid (PABA)	-	Precursor for the biosynthesis of folic acid
Folic acid	Tetrahydrofolate	Transfer of one-carbon units and required for synthesis of thymine, purine bases, serine, methionine and pantothenate
Biotin	Biotin	Biosynthetic reactions that require CO_2 fixation
Lipoic acid	Lipoamide	Transfer of acyl groups in oxidation of keto acids
Mercaptoethane-sulfonic acid	Coenzyme M	CH_4 production by methanogens
Nicotinic acid	NAD (nicotinamide adenine dinucleotide) and NADP	Electron carrier in dehydrogenation reactions
Pantothenic acid	Coenzyme A and the Acyl Carrier Protein (ACP)	Oxidation of keto acids and acyl group carriers in metabolism
Pyridoxine (B_6)	Pyridoxal phosphate	Transamination, deamination, decarboxylation and racemation of amino acids
Riboflavin (B_2)	FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide)	Oxidoreduction reactions
Thiamine (B_1)	Thiamine pyrophosphate (TPP)	Decarboxylation of keto acids and transaminase reactions
Vitamin B_{12}	Cobalamine coupled to adenine nucleoside	Transfer of methyl groups
Vitamin K	Quinones and napthoquinones	Electron transport processes

Factors affecting bacterial growth

Nutritional requirements

Bacteria differ dramatically with respect to the conditions that are necessary for their optimal growth. Carbon is the element required in the greatest amount by bacteria since hydrogen and oxygen can be obtained from water, which is a prerequisite for bacterial growth. Also required is a source of energy to fuel the metabolism of the bacterium. Therefore, the fundamental nutritional needs are the carbon source and the energy source.

In addition to carbon, bacteria need energy, which is almost always obtained by the transfer of an electron from an electron donor to an electron acceptor.

There are three basic sources of energy: light, inorganic compounds, and organic compounds. Phototrophic bacteria use photosynthesis to generate cellular energy in the form of adenosine triphosphate (ATP) from light energy. Chemotrophs obtain their energy from chemicals (organic

and inorganic compounds); chemolithotrophs obtain their energy from reactions with inorganic salts; and chemoheterotrophs obtain their carbon and energy from organic compounds (the energy source may also serve as the carbon source in these organisms).

In most cases, cellular energy is generated by means of electron- transfer reactions, in which electrons move from an organic or inorganic donor molecule to an acceptor molecule via a pathway that conserves the energy released during the transfer of electrons by trapping it in a form that the cell can use for its chemical or physical work. The primary form of energy that is captured from the transfer of electrons is ATP.

Many bacteria can use a large number of compounds as carbon and energy sources, whereas other bacteria are highly restricted in their metabolic capabilities. While carbohydrates are a common energy source for eukaryotes, these molecules are metabolized by only a limited number of bacterial species, since most bacteria do not possess the necessary

enzymes to metabolize these often complex molecules. Many species of bacteria instead depend on other energy sources, such as amino acids, fats, or other compounds. Other compounds of significance to bacteria include phosphate, sulfate, and nitrogen. Low levels of phosphate in many environments, particularly in water, can be a limiting factor for the growth of bacteria, since many bacteria cannot synthesize phosphate. Most bacteria can convert sulfate or sulfide to the organic form needed for protein synthesis. The capability of a living organism to incorporate nitrogen from ammonia is widespread in nature, and bacteria differ in their ability to convert other forms of nitrogen, such as nitrate in the soil or dinitrogen gas (N_2) in the atmosphere, into cell material.

A particularly important nutrient of bacteria is iron, an abundant element in the Earth's crust. Iron is a component of heme proteins, such as hemoglobin in red blood cells and cytochromes in electron transfer chains, as well as many

other iron- containing proteins involved in electron- transfer reactions. Iron is needed for the growth of almost all organisms. In aerobic environments at neutral pH values, ferrous iron (iron in the +2 state) is oxidized to ferric iron (iron in the +3 state), which is virtually insoluble in water and unable to enter cells. Many bacteria synthesize and secrete chemicals called siderophores that bind very tightly to iron and make it soluble in water. The bacteria then take up these iron- siderophore complexes and remove the iron for their synthetic tasks. The ability to acquire iron in this way is particularly important to pathogenic (disease- causing) bacteria, which must compete with their host for iron. In anaerobic environments, iron can exist in the more soluble ferrous state and is readily available to bacteria.

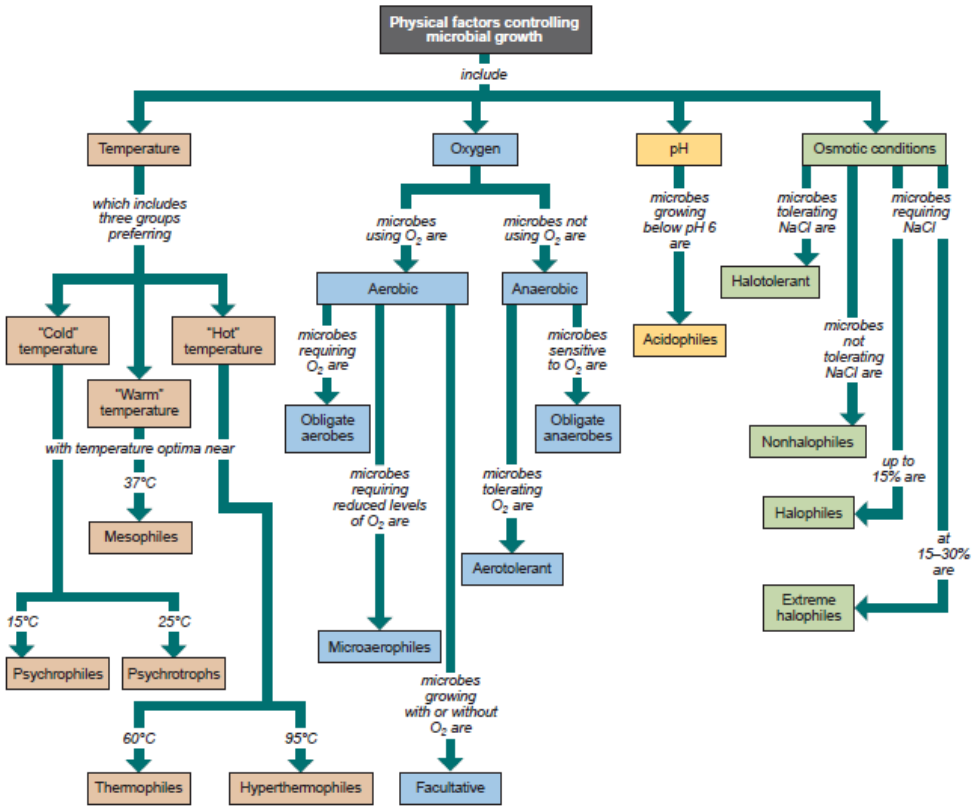
Some bacteria are obligate parasites and grow only within a living host cell. *Rickettsia* and *Chlamydia*, for example, grow in eukaryotic cells, and *Bdellovibrio* grow in bacterial cells. *Treponema pallidum* is difficult, if not impossible, to grow in

culture, probably because it requires low oxygen tension and low oxidation- reduction levels, which are provided by the presence of animal cells, rather than any specific nutrient. Because some bacteria may thrive only as animal or plant parasites or only in a rich source of nutrients such as milk, they likely do not thrive as free bacteria in nature. Many bacteria from natural environments exist in a consortium with other bacteria and are difficult to isolate and culture separately from the other members of that partnership.

The bacterial cell growth and reproduction (that ends with two daughter cells) require every atom and molecule in the parent cell to be duplicated. Insertion of these molecules into its correct place will follow and, finally, the growing structure will become a mature daughter cell.

Bacterial cells are made up of macromolecules such as proteins, nucleic acids, polysaccharides and lipids. These molecules are always accompanied with some low- molecular weight compounds. These compounds may become part of

the new generation of macromolecules or take part in the energy metabolism of the cell.



Culture media

Since the time of Pasteur and Koch, microbiologists have been growing bacterial and other microbial species in laboratory cultures. Today, many of the media used in the medical diagnostic bacteriology laboratory have their origins

in the first Golden Age of Microbiology. These early media often contain blood or serum to mimic the environment in the human body.

For any bacterium, to be propagated for any purpose, it is necessary to provide the appropriate biochemical and biophysical environment. The biochemical (nutritional) environment is made available as a **culture medium**, and depending upon the special needs of particular bacteria (as well as particular investigators) a large variety and types of culture media have been developed with different purposes and uses. Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties.

The manner in which bacteria are cultivated, and the purpose of culture media, varies widely. **Liquid media** are used for growth of pure batch cultures, while solidified media are used widely for the isolation of pure cultures, for estimating viable

bacterial populations, and a variety of other purposes. The usual gelling agent for solid or **semisolid medium** is **agar**, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100 degrees and remains liquid until cooled to 40 degrees, the temperature at which it gels) and because it cannot be metabolized by most bacteria. Hence as a medium component it is relatively inert; it simply holds (gels) nutrients that are in aqueous solution.

For the isolation and identification of microorganisms, two types of culture media are commonly used. A chemically undefined medium, or **complex medium** contains nutrients in which the exact components or their quantity is not completely known. For example, in nutrient broth or nutrient agar media it is not known precisely what carbon and energy sources or other growth factors are present because complex media typically contain animal or plant digests (e.g., beef extract, soybean extract) or yeast extracts of an undefined nature. Complex media are commonly used in the teaching

laboratory because the purpose is simply to grow microbes and not be concerned about what specific nutrients are needed to accomplish this action.

The second type of medium is a chemically defined or **synthetic medium**. In this medium, the precise chemical composition and amount of all components are known. This medium is used when trying to determine an organism's specific growth requirements. Culture media can be devised to select for or differentiate between microbial species and the basic ingredients of the growth media can be modified to provide fast and critical information about the organism causing an infection or disease.

5.2 Composition of a Complex and a Synthetic Growth Medium

Ingredient	Nutrient Supplied	Amount
A. Complex Agar Medium		
Peptone	Amino acids, peptides	5.0 g
Beef extract	Vitamins, minerals, other nutrients	3.0 g
Sodium chloride (NaCl)	Sodium and chloride ions	8.0 g
Agar		15.0 g
Water		1.0 liter
B. Synthetic Broth Medium		
Glucose	Simple sugar	5.0 g
Ammonium phosphate ($(NH_4)_2HPO_4$)	Nitrogen, phosphate	1.0 g
Sodium chloride (NaCl)	Sodium and chloride ions	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	Magnesium ions, sulphur	0.2 g
Potassium phosphate (K_2HPO_4)	Potassium ions, phosphate	1.0 g
Water		1.0 liter

A Comparison of Special Culture Media

Name	Components	Uses	Examples
Selective medium	Growth stimulants Growth inhibitors	Selecting certain microbes out of a mixture	Mannitol salt agar for staphylococci
Differential medium	Dyes Growth stimulants Growth inhibitors	Distinguishing different microbes in a mixture	MacConkey agar for gram-negative bacteria
Enriched medium	Growth stimulants	Cultivating fastidious microbes	Blood agar for streptococci; chocolate agar for *Neisseria* species

A **selective medium** contains ingredients to inhibit the growth of certain microbes in a mixture while allowing the growth of others. The basic growth medium may contain extra salt (NaCl) or a dye to inhibit the growth of intolerant

or sensitive organisms but permits the growth of those species or pathogens one wants to isolate.

Another modification to a basic growth medium is the addition of one or more compounds that allow one to differentiate between very similar species based on specific biochemical or physiological properties. This **differential medium** contains in the culture plate specific chemicals to indicate which species possess and which lack a particular biochemical process. Such indicators make it easy to distinguish visually colonies of one organism from colonies of other similar organisms on the same culture plate.

Although many microorganisms grow well in nutrient broth and nutrient agar, certain so-called fastidious organisms (require complex nutrition) may require an **enriched medium** containing special nutrients.

As mentioned above, many of the *Bacteria* and *Archaea* are impossible to cultivate in any laboratory culture medium yet devised. In fact, less than 1% of the species in natural water

and soil samples can be cultured. So, it is impossible to estimate accurately microbial diversity in an environment based solely on culturability. Such nonculturable organisms are said to be in a **VBNC** (**viable but non-culturable**) state. Procedures for identifying VBNC organisms include direct microscopic examination and, most commonly, amplification of diagnostic gene sequences or 16S rRNA sequences.

Microbiologists believe that part of the reason for non-cuturability of these organisms may be due to their presence in a "foreign" environment. In other words, most species have adapted to their own familiar and specific environment and a complex or synthetic medium is not their typical home. Therefore, these species go into a type of dormancy state and do not divide; that is, they are viable, but not culturable. Studies on VBNC *Bacteria* and *Archaea* present a vast and as yet unexplored field, which is important not only for detection of human pathogens, but also to reveal the diversity of these domains.

Major organic compounds of living organisms

A typical prokaryotic cell is about 70% water. If all the water is evaporated, the predominant "dry weight" remaining consists of **organic compounds**, which are those compounds related to or having a carbon basis: the carbohydrates, lipids, proteins, and nucleic acids. Except for the lipids, each class represents a **polymer** (*poly* = many; *mer* = part) built from a very large number of building blocks called **monomers** (*mono* = one).

Bacteria in the living world

Bacteria have the property of living in extreme weathers like extreme cold and extreme heat. They are able to live long because they become inactive for a long period of time. Bacteria play an important role in the environment. Many dead materials are decomposed by bacteria. If there were no bacteria, the environment would have been polluted and full of harmful microorganisms. They degrade the dead organic matter and convert it into energy and nutrients. For example, they decompose trees and get their food from them.

Organic carbon present in the environment in the form of dead organism and photosynthetic bacteria is able to eat up all the carbon dioxide from the atmosphere if there are no decomposers on earth. It can be imagined that if carbon dioxide is reduced from the atmosphere, there would have been no photosynthesis in plants and as a result no food would have been produced by plants. Decomposers or

bacteria help in cycling of minerals like carbon, nitrogen and sulfur. They are also helpful in making drugs, antibodies and hormones. Nitrogen is the most important component of plants. Plants totally rely on nitrogen for their health and growth and they cannot inhale it directly from the environment. They are dependent on soil for the supply of nitrogen. Through the process of nitrogen fixation is very important because it makes nitrogen from the atmosphere available to plants. This process takes place through bacteria such as *Rhizobium* and cyanobacteria. These species of bacteria convert the atmospheric nitrogen into nitrates and nitrites which is the part of their metabolism and make it available to the plants. Some plants have modified themselves so well that they are now able to store the bacteria into their tissues. Using soil rich with beneficent bacteria can increase the productivity, growth and health of the plants. If carbon dioxide is the useful component of plants, similarly oxygen is also necessary for them because plants need

oxygen in the process of respiration. They take oxygen by digesting the sugars present in them and use it for their growth. They absorb oxygen from the roots and if the roots will be able to absorb nutrients and energy from the soil only then they will be able to provide large amount of oxygen to the plants.

Most bacteria are free- living but some are living in association with other organisms such as animals, plants or other microorganisms. They normally occur as members of mixed communities that may include fungi, algae, protozoa,…etc. These communities are found in various habitats such as surfaces of plants and animals, in water and soil and inside the animal, plant and human bodies. Those microorganisms related to a specific habitat are known collectively as the **microflora** of this habitat. Therefore, e. g. in human body, there are microflora of skin, microflora of intestine and so on.

The microflora of each habitat have to compete with other microorganisms for nutrients, oxygen and many other factors. This competition may be encouraging (synergistic) or inhibitory (antagonistic) for each specific organism. For example, an antibiotic- producing organism is able to eliminate other organisms in a phenomenon known as **antagonism**.

Balanced communities.

If the habitat remains undisturbed, there will be a stable community of different organisms in which various beneficial and antagonistic interactions reach a balance status. Therefore, any pathogenic organism, for example, cannot be established in such a community because it will lack the ability to compete with those already established in this habitat.

Transient communities.

In contrast to mixed stable communities of microorganisms, there are some transient or temporary communities in which

some microorganisms will predominate. For example, in cholera, the patient intestine will be dominated by the casual organism, *Vibrio cholera*. Another example in lakes, reservoirs and stagnant water bodies, the conditions are favourable for the proliferation of certain organisms such as cyanobacteria and some eukaryotic organisms. The results are visible layers of these organisms ate at or near the surface of water known as bloom. The formation of bloom is stimulated by excess of nutrients such as nitrogen leached from agricultural fertilizers and by thermal stratification of water. The death and decomposition of bloom organisms due to changes in the favourable conditions may cause severe depletion of oxygen in water and, sometimes, asphyxiation of fish and other aquatic organisms.

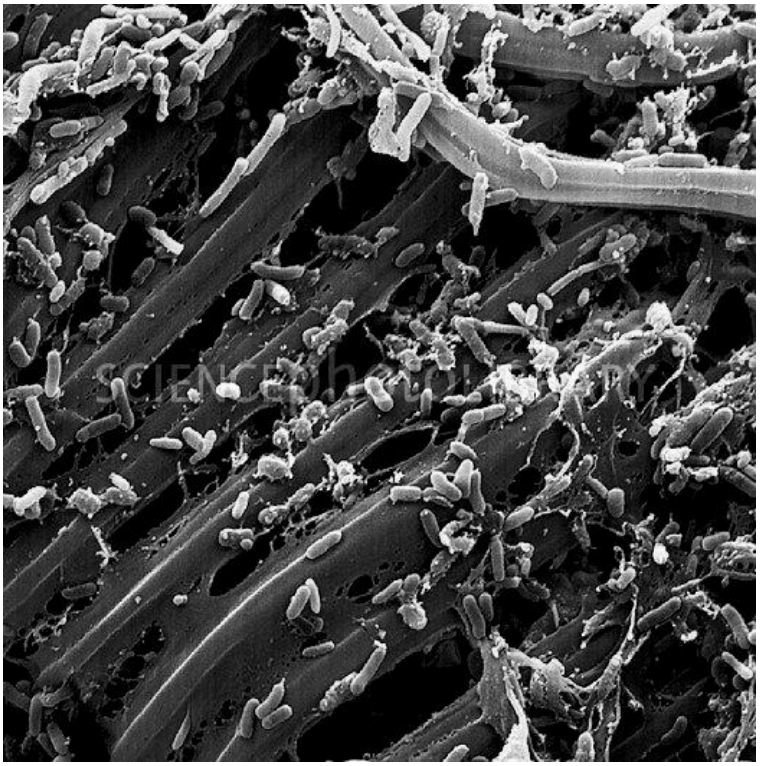
Bloom formation can be inhibited by the circulation of water (pumping) to avoid water stratification or by the addition of certain chemicals (anti- cyanobacterial) such as dichlone (dichloronaphthoquinone).

Saprophytes, predators, parasites and symbionts

1- Saprophytes.

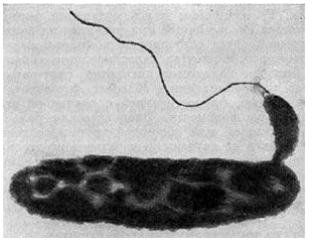
Bacterial saprophytes feed on dead organic matter. They are considered extremely important in soil biology, as they break down dead and decaying organic matter into simple substances that can be taken up and recycled by plants. The term "saprophytes" is usually used to refer to saprophytic fungi or bacteria. Therefore, these heterotrophic organisms are very important for the cycles of matters such as carbon, nitrogen.

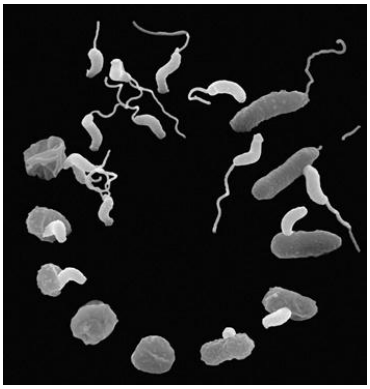
Some saprophytes can use only soluble compounds and others can assimilate complex polymers and compounds by secreting extracellular enzymes. Therefore, a team of saprophytic organisms is working together in the polymers and compounds breakdown process for complete degradation.



Saprophytic bacteria on cucumber in compost

2- <u>Predators</u>.





A true predator (*Bdellovibrio*) is represented in the Figure. *Bdellovibrio* require specific species of gram negative bacteria to grow. The cells are small and highly motile. When they encounter a susceptible host cell, they attach themselves to the outer surface of the cell, bore a hole in the cell wall and push themselves into the periplasmic space (the space between the outer membrane and cytoplasmic membrane of gram negative bacteria). Once there, the cell is killed and its contents consumed. *Bdellovibrio* reproduces within the cell and once the contents of the victim are consumed, the daughter cells will burst out of the shell of the dead cell and head off to find other targets.

Members of the order Myxobacterales are Gram negative rods that live in soil on dung and decaying vegetation. Most species obtain their nutrients from victims of other microorganisms. They release enzymes to digest other fungi and bacteria and live on their soluble products. Therefore, they are not typical predators because predators usually ingest their victims and then digest them (such as in *Bdellovibrio*).

3- <u>Parasites</u>.

A parasite is an organism that lives on or inside another living organism (the host) and benefits from the host in some way, usually by obtaining nutrients. The host may suffer varying degrees of damage ranging from slight inconvenience to death.

Parasitism may be facultative (the alternative way of living for some free- living bacteria) or obligatory. The latter organisms cannot be cultured in the laboratory unless they are grown on specialized preparations of

living cells from the host. Some references classify parasitism as one of the symbiotic relationships as discussed below.

A parasite that cause disease or death of the host is called pathogen. Therefore, not all parasites are pathogens and *vise versa*.

Bacteria	Disease
Vibrio cholerae	Cholera
Salmonella typhii	Typhoid
Diplococcus pneumoniae	Pneumonia
Mycobacterium tuberculosis	Tuberculosis

Some pathogenic bacteria

4- <u>Symbionts</u>.

Symbiosis means any stable physical association between different organisms (symbionts). Thus, there are at least three types of relationships based on the quality of the relationship for each member of the symbiotic association.

1. <u>Mutualism</u>. Both members of the association benefit. For plant, one classic mutualistic association is

Rhizobia which are soil bacteria that fix nitrogen (diazotrophs) after becoming established inside root nodules of legumes. Rhizobia require a plant host; they cannot independently fix nitrogen. Root nodule symbiosis enables nitrogen- fixing bacteria to convert atmospheric nitrogen into a form that is directly available for plant growth. Biological nitrogen fixation provides a built- in supply of nitrogen fertilizer for many legume crops such as peas, beans and clover.

Root nodules from bur clover, *Medicago* spp.

2. Commensalism. There is no apparent benefit or harm to either member of the association. A problem with commensal relationships is that if you look at one long enough and hard enough, you often discover that at least one member is being helped or harmed during the association. Consider our relationship with *Staphylococcus epidermidis*, a consistent inhabitant of the skin of humans. Probably, the bacterium produces lactic acid that protects the skin from colonization by harmful microbes that are less acid tolerant. But it has been suggested that other metabolites that are produced by the bacteria are an important cause of body odours (good or bad, depending on your personal point of view) and possibly associated with certain skin cancers. "Commensalism" best works when the relationship between two organisms is unknown and not obvious.

3. Parasitism. In biology, the term parasite refers to an organism that grows, feeds and is sheltered on or in a

different organism while contributing nothing to the survival of its host. In microbiology, the mode of existence of a parasite implies that the parasite is capable of causing damage to the host. This type of a symbiotic association draws our attention because a parasite may become pathogenic if the damage to the host results in disease. Some parasitic bacteria live as normal flora of humans while waiting for an opportunity to cause disease. Other non-indigenous parasites generally always cause disease if they associate with a non- immune host. Parasitology, actually a branch of microbiology, refers to the scientific study of parasitism but somehow it developed into a discipline that deals with eukaryotic parasites exclusively.

Questions

1- **What are the PHB's ? What it is used for? and what is its benefits to the bacterial cell?**

2- **Why metachromatic granules are very important to the bacterial cell? Why it was named 'metachromatic"?**

3- **How can gas vacuoles help the bacteria in the aquatic environment?**

4- **Explain the importance of carboxysomes?**

5- **What are the storage bodies involved in the bacterial photosynthesis?**

6- **Of what materials the S- layers made? What its importance in the bacterial cell?**

7- **M- proteins play an important role in the bacterial virulence. Explain?**

8- **What are the differences between balanced and transient bacterial communities?**

9- **Compare between the bacterial saprophytes, predators and parasites giving an example on each relationship?**

10- **What is symbiosis? How can you divide different symbiotic relationships and on what bases?**

11- Explain the differences between different symbioses?

12- Why parasitism is sometimes considered as a kind of symbiosis?

13- What is parasitology?

Bacterial metabolism

The most abundant organic compounds in organisms are:

- Protein
- Nucleic Acids
- Carbohydrates
- Lipids

These compounds are called macromolecules and its synthesis starts from simple molecules. Metabolism: the sum of all chemical reactions within a living organism including building up molecules (anabolism or biosynthesis) and breaking down molecules (catabolism).

The life-support processes of even the most structurally simple organism involve a large number of complex biochemical reactions. Most, although not all, of the biochemical processes of bacteria also occur in eukaryotic microbes and in the cells of multicellular organisms, including humans.

However, the reactions that are unique to bacteria are fascinating because they allow microorganisms to do things we cannot do. For example, some bacteria can live on cellulose, whereas others can live on petroleum.

Through their metabolism, bacteria recycle elements after other organisms have used them. As discussed above, other bacteria can live on diets of many inorganic substances such as carbon dioxide, iron, sulfur, hydrogen gas, and ammonia.

Catabolic and Anabolic Reactions

The term **metabolism** refers to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy.

In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism,** the breakdown of complex organic compounds into simpler

ones. These reactions are called catabolic, or degradative reactions. Catabolic reactions are generally hydrolytic reactions (reactions that use water and in which chemical bonds are broken), and they are exergonic (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water.

The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism,** the building of complex organic molecules from simpler ones. These reactions are called anabolic, or biosynthetic, reactions. Anabolic processes often involve dehydration synthesis reactions (reactions that release water), and they are endergonic (consume more energy than they produce). Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions generate the materials for cell growth.

Catabolic reactions provide building blocks for anabolic reactions and furnish the energy needed to drive anabolic

reactions. This coupling of energy-requiring and energy-releasing reactions is made possible through the molecule adenosine triphosphate (ATP) that stores energy derived from catabolic reactions and releases it later to drive anabolic reactions and perform other cellular work.

A molecule of ATP consists of an adenine, a ribose, and three phosphate groups. When the terminal phosphate group is split from ATP, adenosine diphosphate (ADP) is formed, and energy is released to drive anabolic reactions. Using P to represent a phosphate group (Pi represents inorganic phosphate, which is not bound to any other molecule), we write this reaction as follows:

ATP =ADP + Pi + energy

Then, the energy from catabolic reactions is used to combine ADP and a Pi to resynthesize ATP:

ADP + Pi + energy =ATP

Thus, anabolic reactions are coupled to ATP breakdown, and catabolic reactions are coupled to ATP synthesis.

The chemical composition of a living cell is constantly changing: some molecules are broken down while others are being synthesized. This balanced flow of chemicals and energy maintains the life of a cell.

Only part of the energy released in catabolism is actually available for cellular functions because part of the energy is lost to the environment as heat. Because the cell must use energy to maintain life, it has a continuous need for new external sources of energy. Metabolic activity (reactions) also involves enzymes.

Enzymes

Cell's **metabolic pathways** (sequences of chemical reactions) are determined by its enzymes, which are in turn determined by the cell's genetic makeup.

Enzyme components

Apoenzyme: protein portion of an enzyme

Cofactor: non-protein component of an enzyme (Example: iron, zinc, magnesium, calcium)

Coenzyme: is a cofactor that is an organic molecule (Example: vitamins)

Holoenzyme: apoenzyme and cofactor

Although some enzymes consist entirely of proteins, most consist of both a protein portion, called an apoenzyme, and a nonprotein component, called a cofactor. Ions of iron, zinc, magnesium, or calcium are examples of cofactors. If the cofactor is an organic molecule, it is called a coenzyme. Apoenzymes are inactive by themselves; they must be activated by cofactors. Together, the apoenzyme and cofactor form a holoenzyme, or whole, active enzyme. If the cofactor is removed, the apoenzyme will not function.

Coenzymes may assist the enzyme by accepting atoms removed from the substrate or by donating atoms required by the substrate. Some coenzymes act as electron carriers, removing electrons from the substrate and donating them to

other molecules in subsequent reactions. Many coenzymes are derived from vitamins. Two of the most important coenzymes in cellular metabolism are nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Both compounds contain derivatives of the B vitamin niacin (nicotinic acid), and both function as electron carriers. Whereas NAD is primarily involved in catabolic (energy- yielding) reactions, NADP is primarily involved in anabolic (energy- requiring) reactions.

The flavin coenzymes, such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), contain derivatives of the B vitamin riboflavin and are also electron carriers.

Another important coenzyme, coenzyme A (CoA), contains a derivative of pantothenic acid, another B vitamin. This coenzyme plays an important role in the synthesis and breakdown of fats and in a series of oxidizing reactions called the Krebs cycle.

As noted earlier, some cofactors are metal ions, including iron, copper, magnesium, manganese, zinc, calcium, and cobalt. Such cofactors may help catalyze a reaction by forming a bridge between the enzyme and a substrate. For example, magnesium (Mg^{2+}) is required by many phosphorylating enzymes (enzymes that transfer a phosphate group from ATP to another substrate). The Mg^{2+} can form a link between the enzyme and the ATP molecule. Most trace elements required by living cells are probably used in some such way to activate cellular enzymes.

Factors influencing Enzyme Action

Terminology:

Optimum: the environmental state where the enzyme functions the most efficiently

Maximum: the maximum environmental limit in which the enzyme can function

Minimum: the minimum environmental limit in which the enzyme can function

Inhibitors:

Competitive inhibitors**:** are agents that fill the active site of an enzyme. They compete with the substrate

Non-competitive inhibitors**:** These agents do not compete with the substrate for the enzyme's active site, but rather a different region of the enzyme**.** This is known as allosteric inhibition.

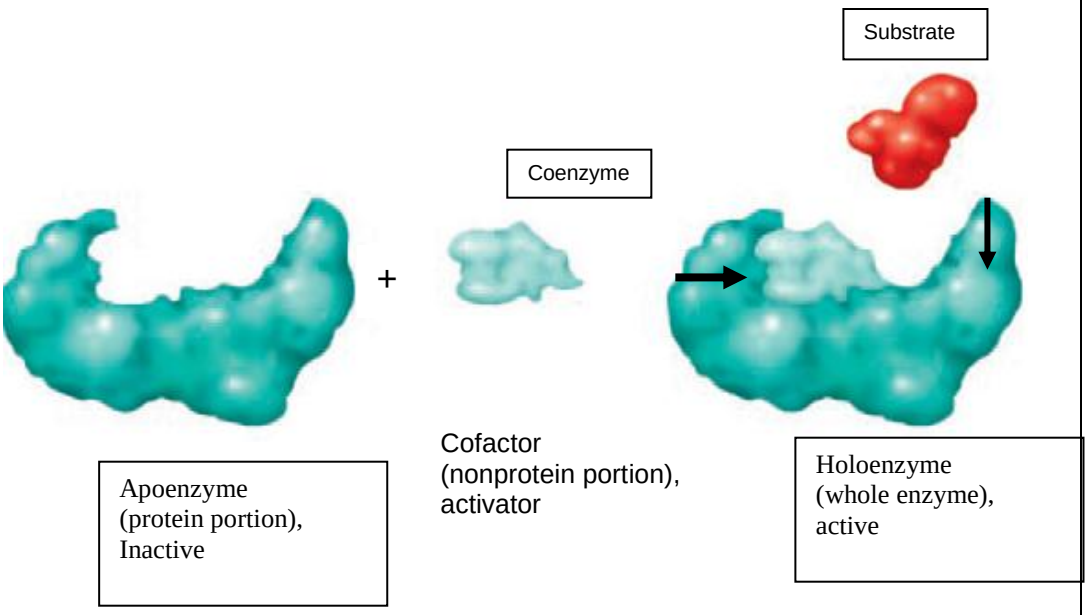
Enzymes and Chemical Reactions

Substances that can speed up a chemical reaction without being permanently altered themselves are called **catalysts.** In living cells, **enzymes** serve as biological catalysts. As catalysts, enzymes are specific. Each acts on a specific substance, called the enzyme's **substrate** (or substrates, when there are two or more reactants), and each catalyzes only one reaction. For example, sucrose (table sugar) is the substrate of the enzyme sucrase, which catalyzes the hydrolysis of sucrose to glucose and fructose. As catalysts, enzymes typically accelerate chemical reactions. The three-

dimensional enzyme molecule has an active site, a region that interacts with a specific chemical substance (see the Figure below).

The enzyme orients the substrate into a position that increases the probability of a reaction. The **enzyme–substrate complex** formed by the temporary binding of enzyme and reactants enables the collisions to be more effective and lowers the activation energy of the reaction.

An enzyme's ability to accelerate a reaction without the need for an increase in temperature is crucial to living systems because a significant temperature increase would destroy cellular proteins. The crucial function of enzymes, therefore, is to speed up biochemical reactions at a temperature that is compatible with the normal functioning of the cell.



Components of a holoenzyme. Many enzymes require both an apoenzyme (protein portion) and a cofactor (nonprotein portion) to become active. The cofactor can be a metal ion, or if it is an organic molecule, it is called a coenzyme (as shown here). The apoenzyme and cofactor together make up the holoenzyme, or whole enzyme. The substrate is the reactant acted upon by the enzyme.

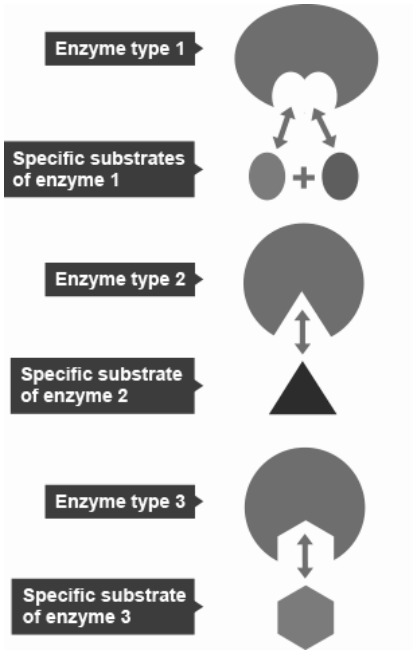
Enzyme Specificity and Efficiency

The specificity of enzymes is made possible by their structures. Enzymes are generally large globular proteins that range in molecular weight from about 10,000 to several million. Each of the thousands of known enzymes has a characteristic three dimensional shape with a specific surface configuration as a result of its primary, secondary, and tertiary structures. The unique configuration of each enzyme

enables it to “find” the correct substrate from among the large number of diverse molecules in the cell.

Enzymes are extremely efficient. Under optimum conditions, they can catalyze reactions at rates 108 to 1010 times (up to 10 billion times) higher than those of comparable reactions without enzymes. The turnover number (maximum number of substrate molecules an enzyme molecule converts to product each second) is generally between 1 and 10,000 and can be as high as 500,000. For example, the enzyme DNA polymerase I, which participates in the synthesis of DNA, has a turnover number of 15, whereas the enzyme lactate dehydrogenase, which removes hydrogen atoms from lactic acid, has a turnover number of 1000.

Many enzymes exist in the cell in both active and inactive forms. The rate at which enzymes switch between these two forms is determined by the cellular environment.



Naming Enzymes

The names of enzymes usually end in -ase. All enzymes can be grouped into six classes, according to the type of chemical reaction they catalyze. Enzymes within each of the major classes are named according to the more specific types of reactions they assist. For example, the class called oxidoreductases is involved with oxidation- reduction reactions (described shortly). Enzymes in the oxidoreductase class that remove hydrogen from a substrate are called dehydrogenases; those that add molecular oxygen (O_2) are

called oxidases. Dehydrogenase and oxidase enzymes have even more specific names, such as lactate dehydrogenase and cytochrome oxidase, depending on the specific substrates on which they act.

CLASSIFICATION OF ENZYMES

Group of Enzyme	Reaction Catalysed	Examples
1. Oxldoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl etc.) from one substrate to another.	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid synthetase

The mechanism of enzymatic action

Enzymes lower the activation energy of chemical reactions. The general sequence of events in enzyme action is as follows:

1- The surface of the substrate contacts a specific region of the surface of the enzyme molecule, called the active site.

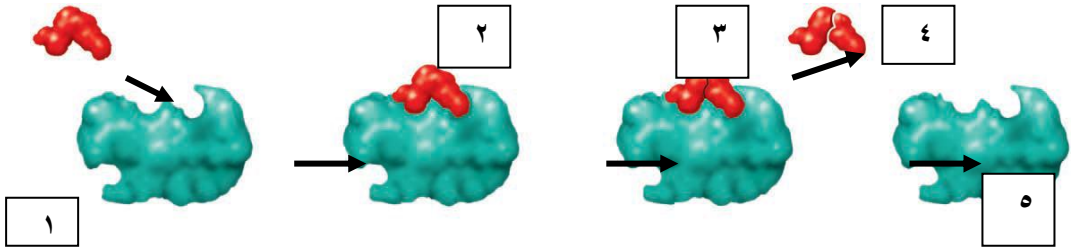
2- A temporary intermediate compound forms (enzyme–substrate complex).

3- The substrate molecule is transformed by the rearrangement of existing atoms, the breakdown of the substrate molecule, or in combination with another substrate molecule.

4- The transformed substrate molecules (the reaction products) are released from the enzyme molecule because they no longer fit in the active site.

5- The unchanged enzyme is now free to react with other substrate molecules (see the Figure below).

As a result of these events, an enzyme speeds up a chemical reaction. As mentioned earlier. Enzymes have specificity for particular substrates. For example, a specific enzyme may be able to hydrolyze a peptide bond only between two specific amino acids.



1= Substrate insertion into receptor site, 2= enzyme- substrate complex, 3= separation of product, 4+ product, 5= free enzyme.

Other enzymes can hydrolyze starch but not cellulose; even though both starch and cellulose are polysaccharides composed of glucose subunits, the orientations of the subunits in the two polysaccharides differ. Enzymes have this specificity because the three- dimensional shape of the active site fits the substrate somewhat as a lock fits with its key. However, the active site and substrate are flexible, and they change shape somewhat as they meet to fit together more tightly. The substrate is usually much smaller than the enzyme.

A certain compound can be a substrate for several different enzymes that catalyze different reactions, so the fate of a compound depends on the enzyme that acts on it. At least

four different enzymes can act on glucose 6-phosphate, a molecule important in cell metabolism, and each reaction will yield a different product.

Enzymes are subject to various cellular controls. Two primary types are the control of enzyme synthesis and the control of enzyme activity (how much enzyme is present versus how active it is).

Several factors influence the activity of an enzyme. Among the more important are temperature, pH, substrate concentration, and the presence or absence of inhibitors**.**

Questions

1- **Define the two main processes of bacterial metabolism**

2- **What are the components of an enzyme?**

3- **Discuss the factors that influence enzyme action?**

4- **Write on enzyme specificity and efficiency?**

5- **What are the general steps of enzyme action?**

6- **Explain, with drawing, the action of an enzyme on a substrate?**

7- **Why the enzyme is considered a reusable compound?**

8- **What are the factors that influence enzyme activity?**

Pathways of Energy Production

Most of a cell's energy is produced from carbohydrate catabolism. Glucose is the most commonly used carbohydrate. To produce energy in the form of ATP from glucose, microbes use two general processes:

(1) Respiration: glucose is completely broken down

An ATP generating process in which molecules are broken down and the final electron acceptor is an inorganic molecule.

In aerobic respiration:

The final electron acceptor is oxygen (final products: carbon dioxide and water and a high yield of ATP, 38 total).

In anaerobic respiration:

The final electron acceptor is inorganic molecule other than oxygen (much smaller yield of ATP).

(2) Fermentation: glucose is partially broken down (fermentation does not require oxygen, final electron acceptor

is organic molecule). It produces only small amounts of ATP (total 2 ATP). Much of the original glucose energy remains in the chemical bonds of the organic end- products (Example: ethanol or lactic acid).

Metabolic processes in bacteria are quite diverse. Bacteria have evolved different ways to access energy available in the natural environment so that they can use it to stay alive and perform a variety of functions. Bacterial metabolism is also affected by other organisms. Humans, for example, rely on bacteria in their gut to breakdown food into components which their bodies can access.

One aspect of bacterial metabolism involves the collection of energy. One of the processes available to bacteria is familiar to humans: respiration. However, unlike humans, bacteria can use gases other than oxygen in their respiration processes, and some bacteria are even capable of surviving in anaerobic environments as well as environments which contain air. This

is a rather remarkable adaptation which allows the bacteria to survive in harsh environments as circumstances change.

Many bacteria are heterotrophs, using organic materials for energy just like humans do. The organisms can access the molecules inside the materials in a variety of ways. One technique they use is fermentation, in which materials are broken down into usable components. Some bacteria can also photosynthesize, using light for energy as long as they have access to nutrients, and others are capable of surviving on inorganic materials. Known as lithotrophs or autotrophs, these bacteria can survive in extremely harsh environments.

An important part of the metabolism of any organism involves the mobilization of chemical energy to derive biosynthesis (because many reactions require the activation of their reactants to an increased energy state).

One of the main usual chemical energy forms used is adenosine triphosphate (ATP). Other energy- rich compounds

are NAD (nicotinamide adenine dinucleotide), NADP, FAD (flavine adenine dinucleotide).

Energy Production

Nutrient molecules, like all molecules, have energy associated with the electrons that form bonds between their atoms. When it is spread throughout the molecule, this energy is difficult for the cell to use. Various reactions in catabolic pathways, however, concentrate the energy into the bonds of ATP, which serves as a convenient energy carrier. ATP is generally referred to as having "high-energy" bonds. Actually, a better term is probably unstable bonds. Although the amount of energy in these bonds is not exceptionally large, it can be released quickly and easily. The "high energy" unstable bonds of ATP provide the cell with readily available energy for anabolic reactions.

Before discussing the catabolic pathways, we will consider two general aspects of energy production: the concept of oxidation reduction and the mechanisms of ATP generation.

Oxidation-Reduction Reactions

Oxidation is the removal of electrons (e^-) from an atom or molecule, a reaction that often produces energy. An example of an oxidation in which molecule A loses an electron to molecule B. Molecule A has undergone oxidation (meaning that it has lost one or more electrons), whereas molecule B has undergone reduction (meaning that it has gained one or more electrons).

Oxidation and reduction reactions are always coupled; in other words, each time one substance is oxidized, another is simultaneously reduced. The pairing of these reactions is called **oxidation-reduction** or a **redox reaction.**

In many cellular oxidations, electrons and protons (hydrogen ions, H^+) are removed at the same time; this is equivalent to

the removal of hydrogen atoms, because a hydrogen atom is made up of one proton and one electron.

Because most biological oxidations involve the loss of hydrogen atoms, they are also called **dehydrogenation** reactions. For example, An organic molecule is oxidized by the loss of two hydrogen atoms, and a molecule of NAD^+ is reduced. The coenzyme NAD^+ assists enzymes by accepting hydrogen atoms removed from the substrate, in this case the organic molecule. NAD^+ accepts two electrons and one proton. One proton (H^+) is left over and is released into the surrounding medium. The reduced coenzyme, NADH, contains more energy than NAD^+. This energy can be used to generate ATP in later reactions.

An important point to remember about biological oxidation reduction reactions is that cells use them in catabolism to extract energy from nutrient molecules. Cells take nutrients, some of which serve as energy sources, and degrade them from highly reduced compounds (with many hydrogen

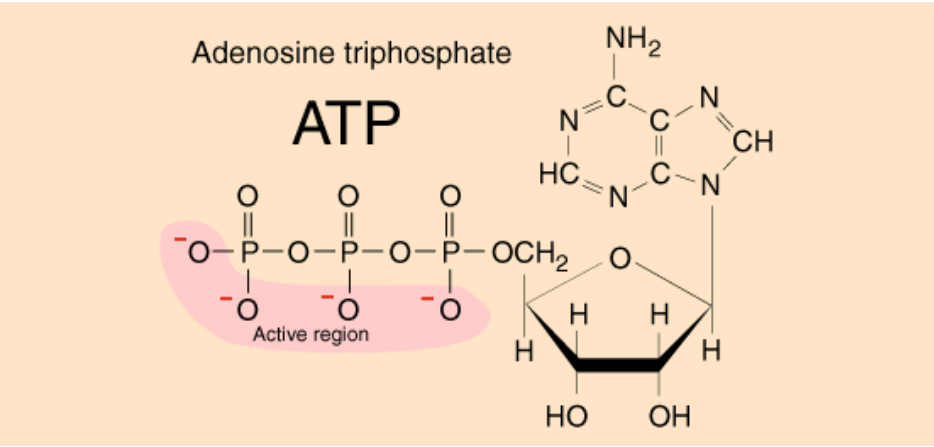
atoms) to highly oxidized compounds. For example, when a cell oxidizes a molecule of glucose (C6H12O6) to CO2 and H2O, the energy in the glucose molecule is removed in a stepwise manner and ultimately is trapped by ATP, which can then serve as an energy source for energy-requiring reactions. Compounds such as glucose that have many hydrogen atoms are highly reduced compounds, containing a large amount of potential energy. Thus, glucose is a valuable nutrient for organisms.

ATP and its role in metabolism:

Much of the energy released during oxidation-reduction reactions is trapped within the cell by the formation of ATP. Specifically, a phosphate group, P, is added to ADP with the input of energy to form ATP:

- All biosynthetic pathways require the participation of ATP.

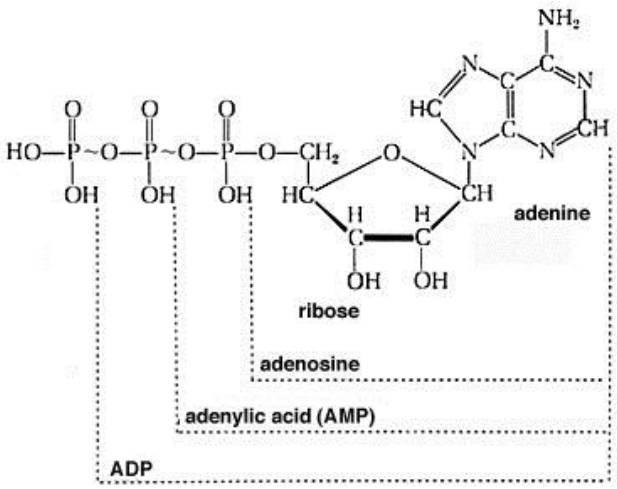
ATP structure illustrating that it is a derivative of adenosine monophosphate (AMP) to which additional two phosphate groups are attached.



The active region involve two active (and highly reactive) bonds between phosphorus and oxygen. Therefore, ATP is able to donate phosphate groups to metabolic intermediates converting them into the activated form.

During catabolism, useful energy is temporarily conserved in the "high energy bond" of ATP. No matter what form of energy a cell uses as its primary source, the energy is ultimately transformed and conserved as ATP- the universal currency of energy exchange in biological systems. When

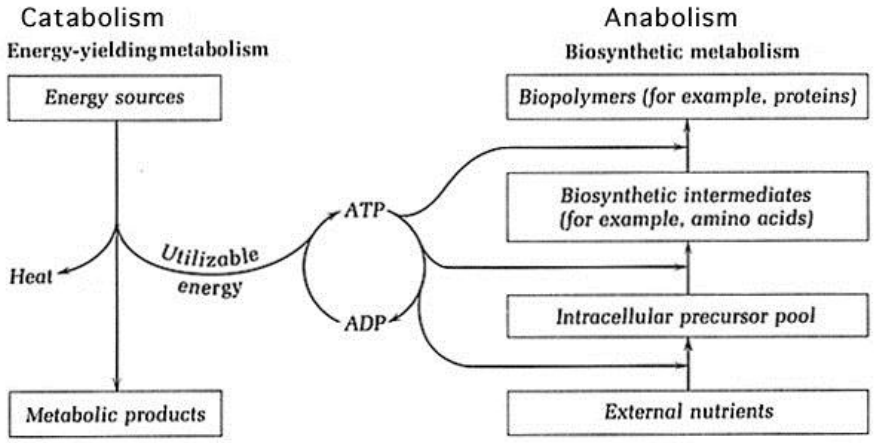
energy is required during anabolism, it may be spent as the high energy bond of ATP which has a value of about 8 kcal per mole. Hence, the conversion of ADP to ATP requires 8 kcal of energy, and the hydrolysis of ATP to ADP releases 8 kcal.



A more detailed structure of ATP. ATP is derived from the nucleotide adenosine monophosphate (AMP) or adenylic acid, to which two additional phosphate groups are attached through pyrophosphate bonds (~P). These two bonds are energy- rich in the sense that their hydrolysis yields more energy than a corresponding covalent bond. ATP acts as a coenzyme in energetic coupling reactions wherein one or both of the terminal phosphate groups is removed from the ATP molecule with the bond energy being used to transfer part of the ATP to another molecule to activate its role in metabolism. For example:

Glucose+ ATP-----> Glucose-P + ADP or Amino Acid + ATP----->AMP-Amino Acid + PPi.

The role of ATP in metabolism is illustrated in the following Figure.



The relationship between catabolism and anabolism in a cell is represented in the following Figure. During catabolism, energy is changed from one form to another, such energy transformations are never completely efficient, i.e., some energy is lost in the form of heat. The efficiency of a catabolic sequence of reactions is the amount of energy made available to the cell (for anabolism) divided by the total amount of energy released during the reactions.

ATP Synthesis in Prokaryotes

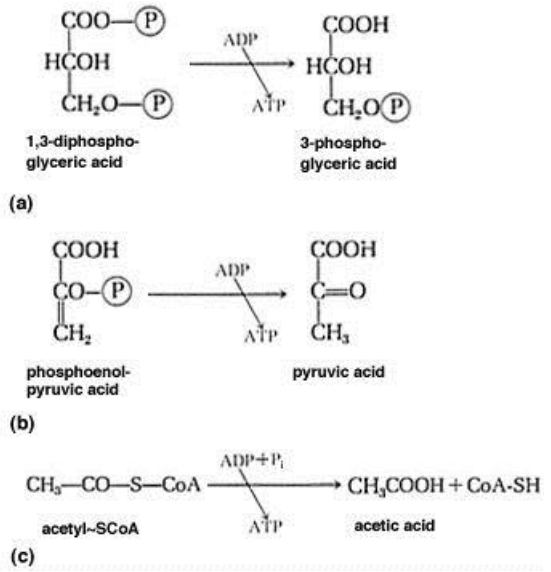
The objective of a catabolic pathway is to make ATP: to transform either chemical energy or electromagnetic (light) energy into the chemical energy contained within the high-energy bonds of ATP. Cells fundamentally can produce ATP by substrate level phosphorylation, oxidative phosphorylation and photophosphorylation.

Substrate level phosphorylation (SLP) is The simplest, oldest and least-evolved way to make ATP. In a substrate level phosphorylation, ATP is made during the conversion of an organic molecule from one form to another. Energy released during the conversion is partially conserved during the synthesis of the high energy bond of ATP. SLP occurs during fermentations and respiration (the TCA cycle), and even during some lithotrophic transformations of inorganic substrates.

In **oxidative phosphorylation,** electrons are transferred from organic compounds to one group of electron carriers (usually

to NAD^+ and FAD). Then, the electrons are passed through a series of different electron carriers to molecules of oxygen (O2) or other oxidized inorganic and organic molecules. This process occurs in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotes. The sequence of electron carriers used in oxidative phosphorylation is called an **electron transport chain (system)**.

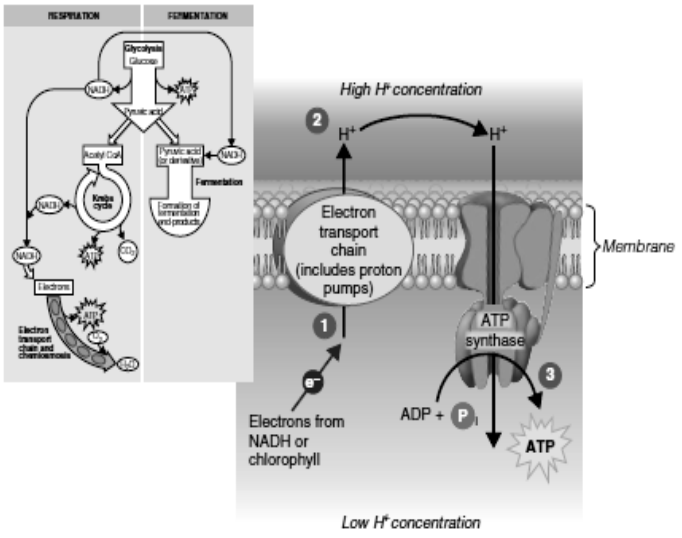
The transfer of electrons from one electron carrier to the next releases energy, some of which is used to generate ATP from ADP through a process called chemiosmosis.



In the above Figure, Three examples of substrate level phosphorylation. (a) and (b) are the two substrate level phosphorylations that occur during the Embden- Meyerhof pathway, but they occur in all other fermentation pathways which have an Embden- Meyerhof component. (c) is a substrate level phosphorylation found in *Clostridium* and *Bifidobacterium*. These are two anaerobic (fermentative) bacteria who learned how to make one more ATP from glycolysis beyond the ı of pyruvate.

Figure 5.15 Chemiosmosis. An overview of the mechanism of chemiosmosis. The membrane shown could be a prokaryotic plasma membrane, a eukaryotic mitochondrial membrane, or a photosynthetic thylakoid. The numbered steps are described in the text.

Q What is the proton motive force?



The third mechanism of phosphorylation, **photophosphorylation,** occurs only in photosynthetic cells, which contain light-trapping pigments such as chlorophylls. In photosynthesis, organic molecules, especially sugars, are synthesized with the energy of light from the energy-poor building blocks carbon dioxide and water.

Photophosphorylation starts this process by converting light energy to the chemical energy of ATP and NADPH, which, in turn, are used to synthesize organic molecules. As in oxidative phosphorylation, an electron transport chain is involved.

Both of the previous types are Electron Transport Phosphorylation (ETP) mechanisms that are much more complicated than SLP. ETP takes place during respiration, photosynthesis, lithotrophy and possibly other types of bacterial metabolism. ETP requires that electrons removed from substrates be dumped into an electron transport system (ETS) contained within a membrane. The electrons are

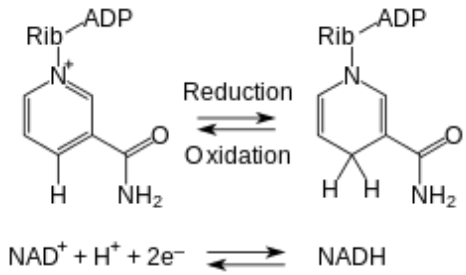
transferred through the ETS to some final electron acceptor in the membrane (like O_2 in aerobic respiration), while their traverse through the ETS results in the extrusion of protons and the establishment of a proton motive force (pmf) across the membrane. An essential component of the membrane for synthesis of ATP is a membrane- bound ATPase (ATP synthetase) enzyme. The ATPase enzyme transports protons, thereby utilizing the pmf (protons) during the synthesis of ATP. The idea in electron transport phosphorylation is to drive electrons through an ETS in the membrane, establish a pmf, and use the pmf to synthesize ATP. Obviously, ETP take a lot more "gear" than SLP, in the form of membranes, electron transport systems, ATPase enzymes, etc.

NAD

Another coenzyme commonly involved in energy- producing metabolism, derived from the vitamin niacin, is the pyridine nucleotide, NAD (Nicotinamide Adenine Dinucleotide). The basis for chemical transformations of energy usually involves

oxidation/ reduction reactions. For a biochemical to become oxidized, electrons must be removed by an oxidizing agent. The oxidizing agent is an electron acceptor that becomes reduced in the reaction. During the reaction, the oxidizing agent is converted to a reducing agent that can add its electrons to another chemical, thereby reducing it, and reoxidizing itself. The molecule that usually functions as the electron carrier in these types of coupled oxidation- reduction reactions in biological systems is NAD and its phosphorylated derivative, NADP.

NAD or NADP can become alternately oxidized or reduced by the loss or gain of two electrons. The oxidized form of NAD is symbolized NAD; the reduced form is symbolized as NADH, $NADH_2$ or $NADH + H^+$.

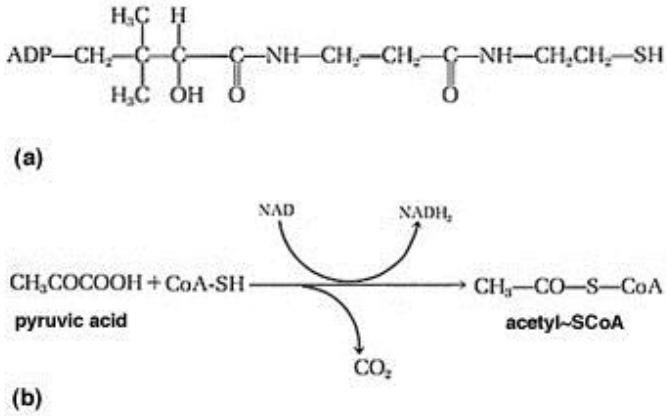


Many bacterial protein toxins including the cholera toxin, pertussis toxin and diphtheria toxin, exert their enzymatic activity using NAD as a co- substrate. The toxins are referred to as ADP- ribosylation toxins, because they cleave NAD into nicotinamide plus ADP- ribose (ADPR) and then transfer the ADPR to some host molecule. For example, the diphtheria toxin transfers ADPR to elongation factor 2, irreversibly inactivating its role in chain elongation during protein synthesis. Thus, the biological activity of the diphtheria toxin is to inhibit protein synthesis in eukaryotic cells.

Coenzyme A

Coenzyme A is another coenzyme frequently involved in energy- generating metabolism of procaryotes. Coenzyme A is involved in a type of ATP-generating reaction seen in some fermentative bacteria and in all respiratory organisms. The reaction occurs in association with the oxidation of keto acids such as pyruvic acid and alpha ketoglutaric acid. These

substrates are central to glycolysis and the TCA cycle, respectively, and they are direct or indirect precursors of several essential macromolecules in a cell. The oxidations of pyruvate and alpha ketoglutarate, involving Coenzyme A, NAD, a dehydrogenation reaction and a decarboxylation reaction, are two of the most important, and complex, reactions in metabolism.



The Structure of Coenzyme A. a) CoA- SH is a derivative of ADP. The molecule shown here attached to ADP is pantothenic acid, which carries a terminal thiol (-S) group. (b) the oxidation of the keto acid, pyruvic acid, to acetyl~ SCoA. This is the reaction that enters two carbons from pyruvate into the TCA cycle.

In the oxidation of keto acids, coenzyme A (CoA or CoASH) becomes attached through a thioester linkage (~S) to the

carboxyl group of the oxidized product. Part of the energy released in the oxidation is conserved in the thioester bond. This bond energy can be subsequently used to synthesize ATP, as in the case of the clostridia that convert acetyl~SCoA +ADP + Pi--------> acetic acid + CoASH + ATP. Or in the case of respiratory organisms, the thioester bond energy is expended when acetyl~SCoA condenses with oxalacetate in order to drive the TCA cycle into its oxidative branch.

Questions

1- **What are the main two processes for producing energy from glucose in bacteria?**

2- **Explain the main differences between auto- and heterotrophy in bacteria?**

3- **Discuss in brief the role of ATP in bacterial metabolism?**

4- **Explain, with drawing, the relation between catabolism and anabolism illustrating the role of ATP?**

5- **Write on one compound involved in energy processes in bacteria other than ATP?**

6- **Define the main processes for ATP synthesis in the bacterial cell?**

7- **Discuss the role of plasma membrane in the synthesis of ATP?**

Diversity of metabolism in prokaryotes

Heterotrophic Types of Metabolism

Heterotrophy (i.e. chemoheterotrophy) is the use of an organic compound as a source of carbon and energy. It is the complete metabolism package. The cell oxidizes organic molecules in order to produce energy (catabolism) and then uses the energy to synthesize cellular material from these the organic molecules (anabolism). Animals are familiar with heterotrophic metabolism. Many Bacteria (except few Archaea) are heterotrophs, particularly those that live in associations with animals. Heterotrophic bacteria are the masters of decomposition and biodegradation in the environment.

Carbohydrate Catabolism

Most microorganisms oxidize carbohydrates as their primary source of cellular energy. **Carbohydrate catabolism,** the breakdown of carbohydrate molecules to produce energy, is

therefore of great importance in cell metabolism. Glucose is the most common carbohydrate energy source used by cells. Microorganisms can also catabolize various lipids and proteins for energy production.

To produce energy from glucose, microorganisms use two general processes: cellular respiration and fermentation. (In discussing cellular respiration, we frequently refer to the process simply as respiration.

Both cellular respiration and fermentation usually start with the same first step, glycolysis, but follow different subsequent pathways. Before examining the details of glycolysis, respiration, and fermentation, we will first look at a general overview of the processes.

The respiration of glucose typically occurs in three principal stages: glycolysis, the Krebs cycle, and the electron transport chain (system).

1- Glycolysis is the oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH.

2- The Krebs cycle is the oxidation of acetyl CoA (a derivative of pyruvic acid) to carbon dioxide, with the production of some ATP, energy-containing NADH, and another reduced electron carrier, FADH2 (the reduced form of flavin adenine dinucleotide).

3- In the electron transport chain (system), NADH and FADH2 are oxidized, contributing the electrons they have carried from the substrates to a "cascade" of oxidation reduction reactions involving a series of additional electron carriers. Energy from these reactions is used to generate a considerable amount of ATP.

In respiration, most of the ATP is generated in the third step. Because respiration involves a long series of oxidation-reduction reactions, the entire process can be thought of as involving a flow of electrons from the energy-rich glucose molecule to the relatively energy-poor CO2 and H2O molecules. Glycolysis and the Krebs cycle generate a small

amount of ATP and also supply the electrons that generate a great deal of ATP at the electron transport chain stage.

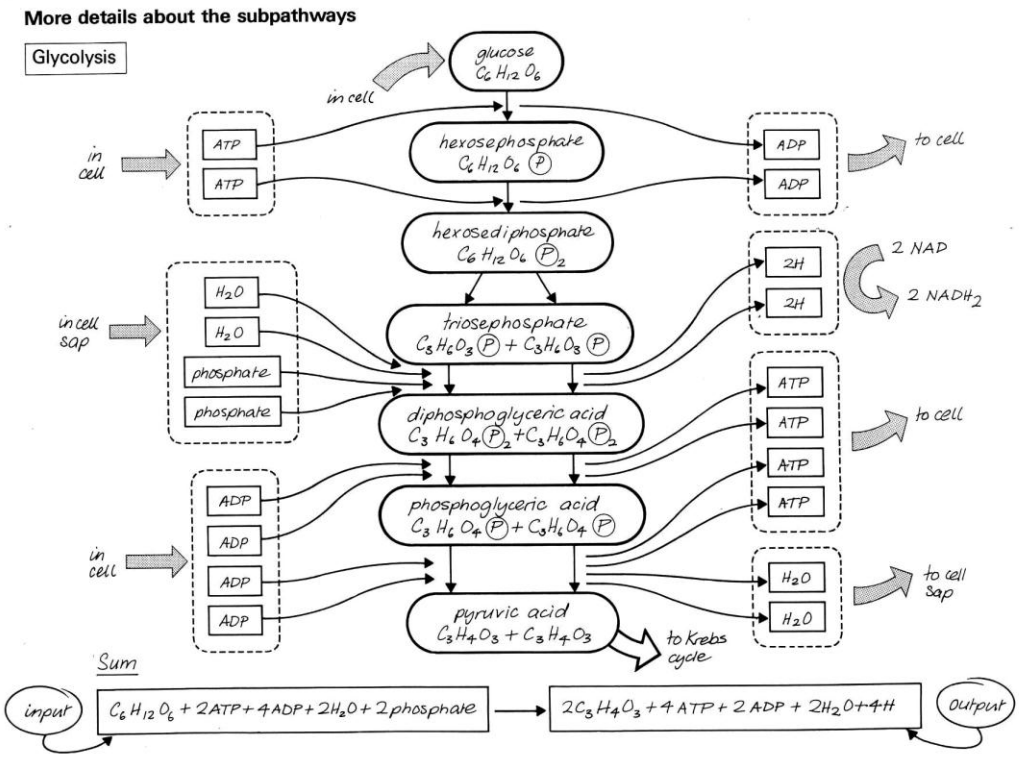
Typically, the initial stage of fermentation is also glycolysis. However, once glycolysis has taken place, the pyruvic acid is converted into one or more different products, depending on the type of cell. These products might include alcohol (ethanol) and lactic acid. Unlike respiration, there is no Krebs cycle or electron transport chain in fermentation. Accordingly, the ATP yield, which comes only from glycolysis, is much lower.

The Embden- Meyerhof Pathway (glycolysis)

Glycolysis**,** the oxidation of glucose to pyruvic acid, is usually the first stage in carbohydrate catabolism. Most microorganisms use this pathway; in fact, it occurs in most living cells. Glycolysis is also called the *Embden-Meyerhof pathway*. The word *glycolysis* means splitting of sugar, and this is exactly what happens. The enzymes of glycolysis catalyze the splitting of glucose, a six-carbon sugar, into two

three-carbon sugars. These sugars are then oxidized, releasing energy, and their atoms are rearranged to form two molecules of pyruvic acid. During glycolysis NAD^+ is reduced to NADH, and there is a net production of two ATP molecules by substrate-level phosphorylation.

Glycolysis does not require oxygen; it can occur whether oxygen is present or not. This pathway is a series of ten chemical reactions, each catalyzed by a different enzyme. Because two molecules of ATP were needed to get glycolysis started and four molecules of ATP are generated by the process, there is a net gain of two molecules of ATP for each molecule of glucose that is oxidized.



Alternatives to glycolysis

Many bacteria have another pathway in addition to glycolysis for the oxidation of glucose. The most common alternative is the pentose phosphate pathway; another alternative is the Entner- Doudoroff pathway.

The Pentose Phosphate Pathway

The pentose phosphate pathway (or hexose monophosphate shunt) operates simultaneously with glycolysis and provides a

means for the breakdown of five-carbon sugars (pentoses) as well as glucose. A key feature of this pathway is that it produces important intermediate pentoses used in the synthesis of (1) nucleic acids, (2) glucose from carbon dioxide in photosynthesis, and (3) certain amino acids. The pathway is an important producer of the reduced coenzyme NADPH from NADP. The pentose phosphate pathway yields a net gain of only one molecule of ATP for each molecule of glucose oxidized.

Bacteria that use the pentose phosphate pathway include *Bacillus subtilis*, *E. coli*, *Leuconostoc* and *Enterococcus faecalis* .

The Entner-Doudoroff Pathway

From each molecule of glucose, the Entner-Doudoroff pathway produces two molecules of NADPH and one molecule of ATP for use in cellular biosynthetic reactions. Bacteria that have the enzymes for the Entner-Doudoroff pathway can metabolize glucose without either glycolysis or

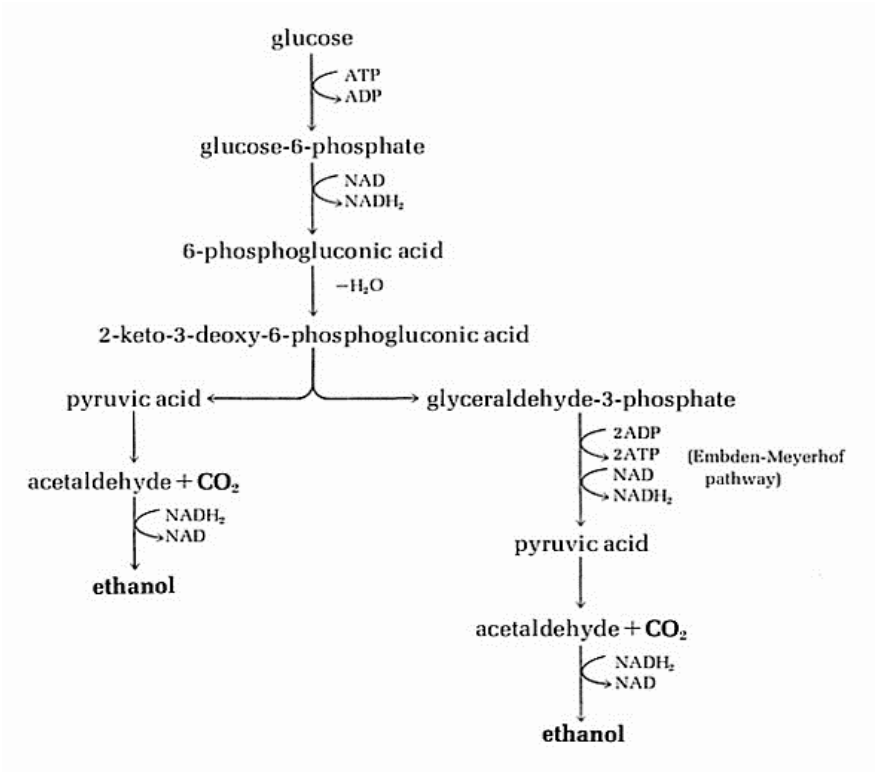
the pentose phosphate pathway. The Entner-Doudoroff pathway is found in some gram-negative bacteria, including *Rhizobium, Pseudomonas* and *Agrobacterium*; it is generally not found among gram-positive bacteria. Tests for the ability to oxidize glucose by this pathway are sometimes used to identify *Pseudomonas* in the clinical laboratory.

Only a few bacteria, most notably *Zymomonas*, employ the Entner- Doudoroff pathway as a strictly fermentative way of life. However, many bacteria, especially those grouped around the pseudomonads, use the pathway as a way to degrade carbohydrates for respiratory metabolism. The E- D pathway yields 2 pyruvic acid from glucose (same as the E-M pathway) but the net energy yield is one mole of ATP per mole of glucose utilized. In the E-D pathway, glucose phosphate is oxidized to 2-keto-3-deoxy-6-phosphogluconic acid (KDPG) which is cleaved by KDPG aldolase to pyruvate and GAP. The latter is oxidized to pyruvate by E- M enzymes wherein 2 ATP are produced by substrate level

phosphorylations. Pyruvic acid from either branch of the pathway is reduced to ethanol and CO_2, in the same manner as yeast, by the "yeast- like bacterium", *Zymomonas*. Thus, the overall reaction is:

Glucose ---------->2 ethanol +2 CO_2, and a net gain of 1 ATP.

Zymomonas is a bacterium that lives on the surfaces of plants, including the succulent Maguey cactus which is indigenous to Mexico. Just as grapes are crushed and fermented by resident yeast to wine, so may the Maguey flesh be crushed and allowed to ferment with *Zymomonas*, which gives rise to "cactus beer" or "pulque", as it is known in Mexico. Many cultures around the world prepare their native fermented brews with *Zymomonas* in deference to the yeast, *Saccharomyces*, although they may not have a choice in the matter. *Zymomonas* has potential advantageous over yeast for the industrial production of alcohol.



The Entner- Doudoroff Pathway of Fermentation.

Respiration

After glucose has been broken down to pyruvic acid, the pyruvic acid can be channeled into the next step of either fermentation or cellular respiration.

Cellular respiration, or simply **respiration,** is defined as an ATP-generating process in which molecules are oxidized and the final electron acceptor is (almost always) an inorganic

molecule. An essential feature of respiration is the operation of an electron transport chain.

There are two types of respiration, depending on whether an organism is an **aerobe,** which uses oxygen, or an **anaerobe,** which does not use oxygen and may even be killed by it. In **aerobic respiration,** the final electron acceptor is O2; in **anaerobic respiration,** the final electron acceptor is an inorganic molecule other than O2 or, rarely, an organic molecule.

Aerobic Respiration

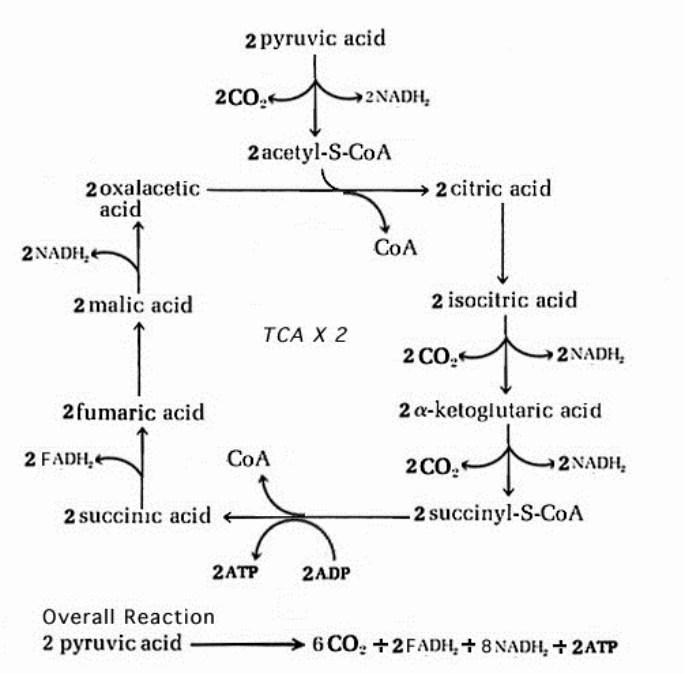
The **Krebs cycle,** also called the tricarboxylic acid (TCA) cycle or citric acid cycle, is a series of biochemical reactions in which the large amount of potential chemical energy stored in acetyl CoA is released step by step. In this cycle, a series of oxidations and reductions transfer that potential energy, in the form of electrons, to electron carrier coenzymes, chiefly NAD. The pyruvic acid derivatives are oxidized; the coenzymes are reduced.

Pyruvic acid, the product of glycolysis, cannot enter the Krebs cycle directly. In a preparatory step, it must lose one molecule of CO2 and become a two-carbon compound. This process is called **decarboxylation.** The two-carbon compound, called an acetyl group, attaches to coenzyme A through a high-energy bond; the resulting complex is known as acetyl coenzyme A (acetyl CoA).

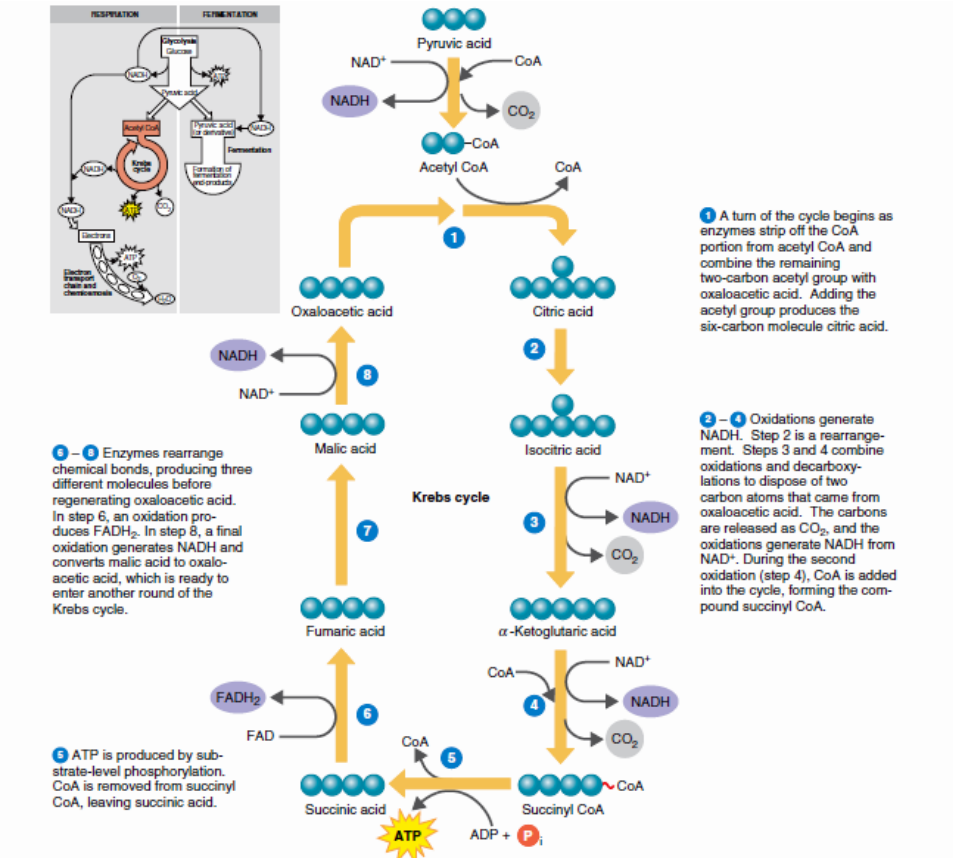
During this reaction, pyruvic acid is also oxidized and NAD is reduced to NADH. Remember that the oxidation of one glucose molecule produces two molecules of pyruvic acid, so for each molecule of glucose, two molecules of CO2 are released in this preparatory step, two molecules of NADH are produced, and two molecules of acetyl CoA are formed.

Once the pyruvic acid has undergone decarboxylation and its derivative (the acetyl group) has attached to CoA, the resulting acetyl CoA is ready to enter the Krebs cycle. As acetyl CoA enters the Krebs cycle, CoA detaches from the acetyl group. The two-carbon acetyl group combines with a

four carbon compound (oxaloacetic acid) to form the six-carbon citric acid. This reaction energy is obtained from cleavage of the high-energy bond between the acetyl group and CoA. The formation of citric acid is thus the first step in the Krebs cycle. Keep in mind that each reaction is catalyzed by a specific enzyme. The chemical reactions of the Krebs cycle fall into several general categories; one of these is decarboxylation. For example, in step 3 isocitric acid, a six-carbon compound, is decarboxylated to the five-carbon compound called α-ketoglutaric acid. Another decarboxylation takes place in step 4. Because one decarboxylation has taken place in the preparatory step and two in the Krebs cycle, all three carbon atoms in pyruvic acid are eventually released as CO2 by the Krebs cycle. This represents the conversion to CO2 of all six carbon atoms contained in the original glucose molecule.



The tricarboxylic acid (TCA) or Kreb's cycle



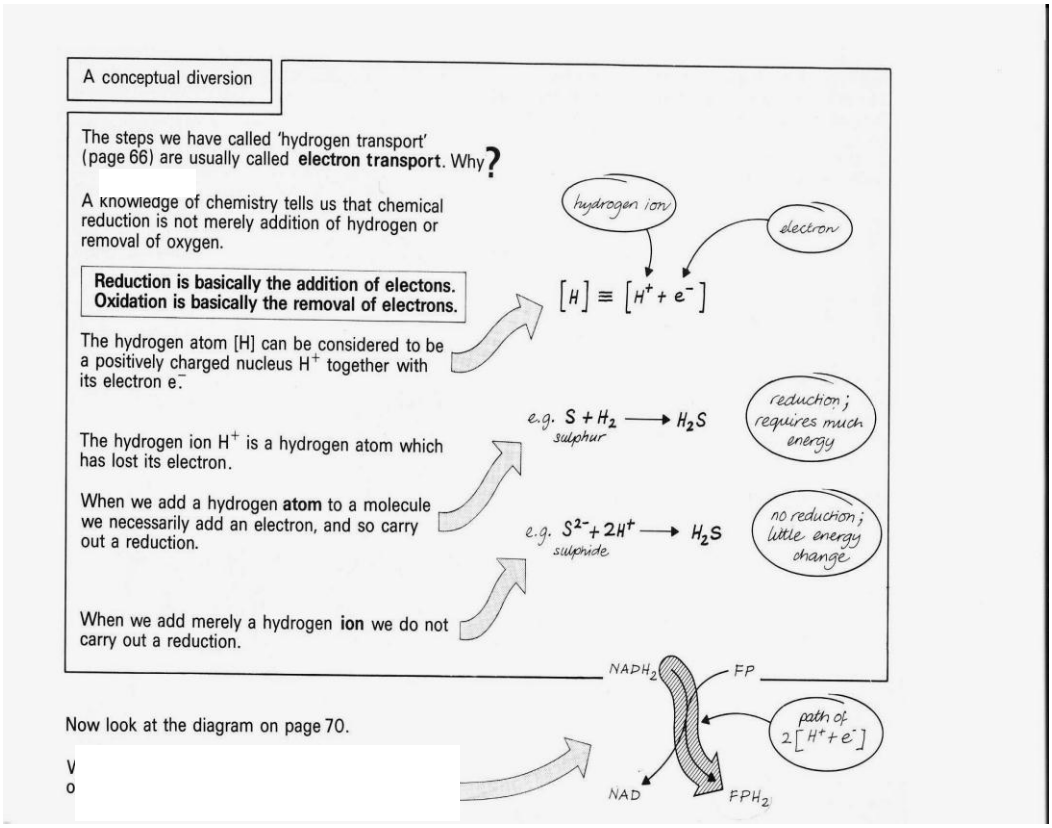
Another general category of Krebs cycle reactions is oxidation-reduction. For example, in step 3, two hydrogen atoms are lost during the conversion of the six-carbon isocitric acid to a five-carbon compound (the six-carbon compound is oxidized). Hydrogen atoms are also released in the Krebs cycle in steps 4, 6, and 8 and are picked up by the coenzymes NAD^+ and FAD. Because NAD^+ picks up two

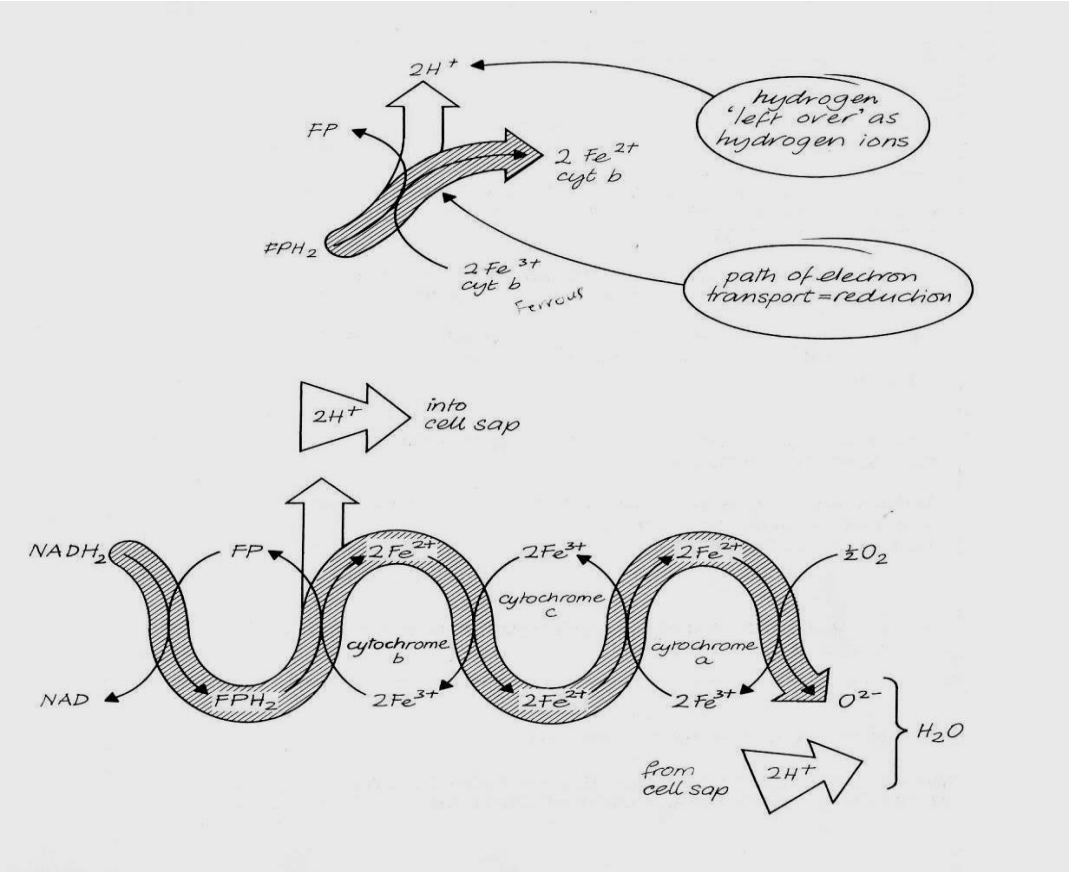
electrons but only one additional proton, its reduced form is represented as NADH; however, FAD picks up two complete hydrogen atoms and is reduced to FADH2. Respirations result in the complete oxidation of the substrate by an outside (exogenous) electron acceptor. In addition to a pathway of glycolysis, four essential structural or metabolic components are needed:

1. **The tricarboxylic acid (TCA) cycle** (also known as the citric acid cycle or the Kreb's cycle): when an organic compound is utilized as a substrate, the TCA cycle is used for the complete oxidation of the substrate. The end product that always results from the complete oxidation of an organic compound is CO_2.

2. A membrane and an associated electron transport system (ETS). The ETS is a sequence of electron carriers in the plasma membrane that transports electrons taken from the substrate through the chain of carriers to a final electron acceptor. The electrons enter the ETS at a very low redox

potential (E'o) and exit at a relatively high redox potential. This drop in potential releases energy that can be harvested by the cells in the process of ATP synthesis by the mechanisms of electron transport phosphorylation. The operation of the ETS establishes a proton motive force (pmf) due to the formation of a proton gradient across the membrane.





Electron transport system (ETS) or chain ends with oxygen as the final electron acceptor (aerobic respiration)

3. An outside electron acceptor ("outside", meaning it is not internal or indogenous to the pathway, as is pyruvate in a fermentation). For aerobic respiration the electron acceptor is O_2, of course. Molecular oxygen is reduced to H_2O in the last step of the electron transport system. But in the bacterial processes of anaerobic respiration, the final electron

acceptors may be SO_4 or S or NO_3 or NO_2 or certain other inorganic compounds, or even an organic compound, such as fumarate.

4. A transmembranous ATPase enzyme (ATP synthetase).

This enzyme utilizes the proton motive force established on the membrane (by the operation of the ETS) to synthesize ATP in the process of electron transport phosphorylation. The reaction catalyzed by the ATPase enzyme is as follows:

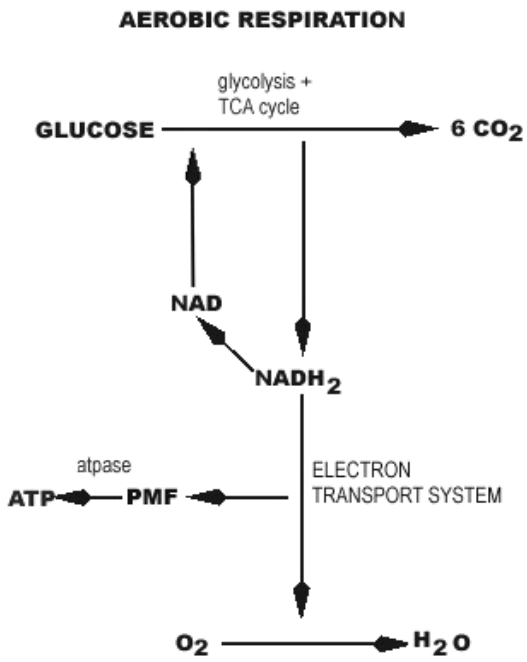
$$ADP + Pi + 2\ H^+ \text{<----------> } ATP.$$

It is important to appreciate the reversibility of this reaction in order to account for how a fermentative bacterium, without an ETS, could establish a necessary pmf on the membrane for transport or flagellar rotation. If such an organism has a transmembranous ATPase, it could produce ATP by SLP, and subsequently the ATPase could hydrolyze the ATP, thereby releasing protons to the outside of the membrane.

The diagram below of aerobic respiration integrates these metabolic processes into a scheme that represents the overall

process of respiratory metabolism. A substrate such as glucose is completely oxidized to CO_2 by the combined pathways of glycolysis and the TCA cycle. Electrons removed from the glucose by NAD are fed into the ETS in the membrane. As the electrons traverse the ETS, a pmf becomes established across the membrane. The electrons eventually reduce an outside electron acceptor, O_2, and reduce it to H_2O. The pmf on the membrane is used by the ATPase enzyme to synthesize ATP by a process referred to as "oxidative phosphorylation".

The TCA cycle (including the steps leading into it) accounts for the complete oxidation of the substrate and provides 10 pairs of electrons (from glucose) for transit through the ETS. For every pair of electrons put into the ETS, 2 or 3 ATP may be produced, so a huge amount of ATP is produced in a respiration, compared to a fermentation.



Model of aerobic respiration. Glucose is oxidized to CO_2 via the TCA cycle. Most electrons are removed from the glucose by NAD and donated to the electron transport system in the cell membrane. The ultimate electron acceptor is O_2 which becomes reduced to H_2O. As a result of the electron transport process, pmf is established on the membrane. pmf drives the synthesis of ATP during the process of oxidative phosphorylation.

Glucose is dissimilated in a pathway of glycolysis to the intermediate, pyruvate, and it is the pyruvate that is moved into the TCA cycle, eventually becoming oxidized to 3 CO_2. Since 2 pyruvate are formed from one glucose, the cycle must turn twice for every molecule of glucose oxidized to 6 CO_2.

Initially, pyruvate is oxidized and decarboxylated in a complex reaction involving NAD, Coenzyme A, and pyruvate dehydrogenase (pyruvate decarboxylase), forming the most central molecule in metabolism, Acetyl CoA.. Acetyl CoA condenses with the 4C-compound, oxalacetic acid, to form the first stable intermediate of the TCA cycle, 6C- citric acid (citrate), a tricarboxylic acid. Citrate is isomerized to isocitrate, which is oxidized and decarboxylated forming alpha- ketoglutarate (akg). Alpha ketoglutarate dehydrogenase uses CoA and NAD to oxidize akg to succinyl CoA in a reaction analogous to the pyruvate dehydrogenase reaction above. Succinyl CoA is converted to succinate during a substrate level phosphorylation yielding high energy GTP (equivalent to ATP). This completes the decarboxylation of pyruvate forming 3 CO_2. The remaining three steps in the cycle complete the oxidation of succinate and regenerate the oxalacetate necessary to drive the cycle. During the oxidation of pyruvic acid to 3 CO_2 by one turn of

the TCA cycle, 4 $NADH_2$, 1 $FADH_2$ and one ATP (actually GTP) are produced. Since the TCA cycle is an important amphibolic pathway, several intermediates of the cycle may be withdrawn for anabolic (biosynthetic) pathways (See the Figure).

The overall reaction for the aerobic respiration of glucose is

Glucose + 6 O_2----------> 6 CO_2+ 6 H_2O + 688 kcal (total)

which can be written

Glucose ----------> 6 CO_2+ 10 $NADH_2$+ 2 $FADH_2$+ 4 ATP

(2$NADH_2$ from glycolysis, 8$NADH_2$ from two turns of TCA, 2 $FADH_2$ from two turns of TCA; 2ATP (net) from glycolysis, 2 ATP (GTP) from two turns of TCA)

In *E. coli*, 2 ATP are produced for each pair of electrons that are introduced into the ETS by $NADH_2$. One ATP is produced from a pair of electrons introduced by $FADH_2$. Hence, the equation can be rewritten:

Glucose + 6 O_2----------> 6 CO_2+ 6 H_2O + 20 ATP (ETP) + 2 ATP (ETP) + 4 ATP (SLP) + 688 kcal (total)

Since a total of 26 ATP is formed during the release of 688 kcal of energy, the efficiency of this respiration is 26x 8/ 688 or about 30 percent. In *Pseudomonas* (or mitochondria), due to the exact nature of the ETS, 3 ATP are produced for each pair of electrons that are introduced into the ETS by $NADH_2$ and 2 ATP are produced from a pair of electrons introduced by $FADH_2$. Hence, the overall reaction in *Pseudomonas*, using the same dissimilatory pathways as *E. coli*, is

Glucose + 6 O_2----------> 6 CO_2+ 6 H_2O + 38 ATP + 688 kcal (total) giving a corresponding efficiency is about 45 percent.

Respiration in some procaryotes is possible using electron acceptors other than oxygen (O_2). This type of respiration in the absence of oxygen is referred to as anaerobic respiration. Although anaerobic respiration is more complicated than the

foregoing statement, in its simplest form it represents the substitution or use of some compound other than O_2 as a final electron acceptor in the electron transport chain. Electron acceptors used by procaryotes for respiration or methanogenesis (an analogous type of energy generation in archaea) are described in the Table below.

Electron acceptors for respiration and methanogenesis in procaryotes

electron acceptor	reduced end product	name of process	organism
O_2	H_2O	aerobic respiration	*Escherichia,Streptomyces*
NO_3	NO_2, N_2O or N_2	anaerobic respiration: denitrification	*Bacillus,Pseudomonas*
SO_4	S or H_2S	anaerobic respiration: sulfate reduction	*Desulfovibrio*
fumarate	succinate	anaerobic respiration: using an organic e-acceptor	*Escherichia*
CO_2	CH_4	methanogenesis	*Methanococcus*

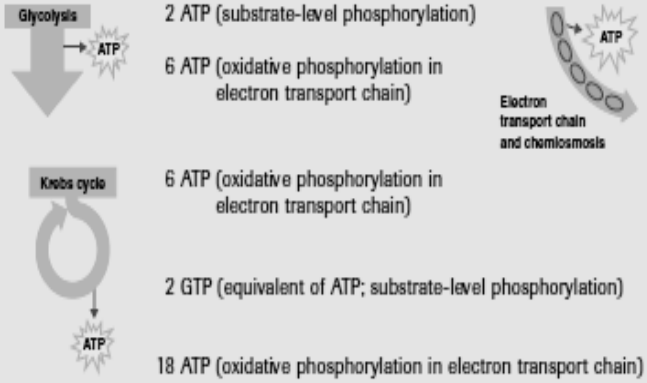
A Summary of Aerobic Respiration

The electron transport chain regenerates NAD^+ and FAD, which can be used again in glycolysis and the Krebs cycle.

The various electron transfers in the electron transport chain generate about 34 molecules of ATP from each molecule of glucose oxidized: approximately three from each of the ten molecules of NADH (a total of 30), and approximately two from each of the two molecules of FADH2 (a total of four). To arrive at the total number of ATP molecules generated for each molecule of glucose, the 34 from chemiosmosis are added to those generated by oxidation in glycolysis and the Krebs cycle. In aerobic respiration among prokaryotes, a total of 38 molecules of ATP can be generated from one molecule of glucose. Note that four of those ATPs come from substrate-level phosphorylation in glycolysis and the Krebs cycle. The following Table provides a detailed accounting of the ATP yield during prokaryotic aerobic respiration.

P Yield during Prokaryotic Aerobic Respiration of One Glucose Molecule

Source	ATP Yield (Method)
Glycolysis	
1. Oxidation of glucose to pyruvic acid	2 ATP (substrate-level phosphorylation)
2. Production of 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Preparatory Step	
1. Formation of acetyl CoA produces 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Krebs Cycle	
1. Oxidation of succinyl CoA to succinic acid	2 GTP (equivalent of ATP; substrate-level phosphorylation)
2. Production of 6 NADH	18 ATP (oxidative phosphorylation in electron transport chain)
3. Production of 2 FADH	4 ATP (oxidative phosphorylation in electron transport chain)
	Total: 38 ATP



Anaerobic Respiration

In anaerobic respiration, the final electron acceptor is an inorganic substance other than oxygen (O2). Some bacteria, such as *Pseudomonas* and *Bacillus*, can use a nitrate ion (NO3) as a final electron acceptor; the nitrate ion is reduced to a nitrite ion (NO2), nitrous oxide (N2O), or nitrogen gas (N2). Other bacteria,

such as *Desulfovibrio*, use sulfate (SO4) as the final electron acceptor to form hydrogen sulfide (H2S). Still other bacteria

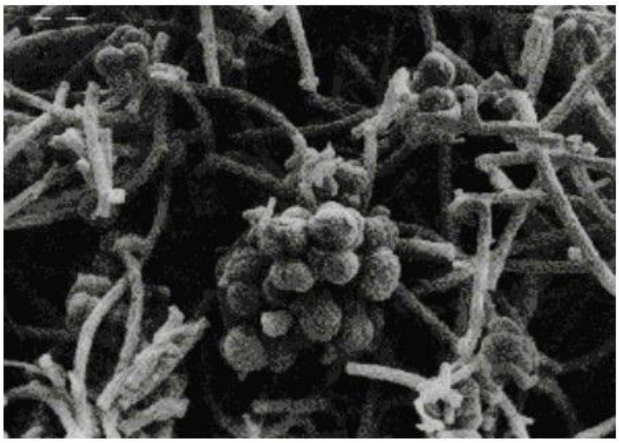
use carbonate (CO3) to form methane (CH4). Anaerobic respiration by bacteria using nitrate and sulfate as final acceptors is essential for the nitrogen and sulfur cycles that occur in nature. The amount of ATP generated in anaerobic respiration varies with the organism and the pathway. Because only part of the Krebs cycle operates under anaerobic

conditions, and because not all the carriers in the electron transport chain participate in anaerobic respiration, the ATP yield is never as high as in aerobic respiration. Accordingly, anaerobes tend to grow more slowly than aerobes.

Feature	Aerobic respiration	Anaerobic respiration
Oxygen requirement	Yes, always	No, never
Waste products	Carbon dioxide and water	Carbon dioxide and ethanol
Efficiency in releasing energy from glucose	Very efficient (most of glucose's energy is released)	Less efficient (some energy locked in ethanol is not released)
Some energy released as heat	Yes	Yes, but less than for aerobic respiration

Biological methanogenesis

Biological methanogenesis is the primary source of methane (natural gas) on the planet. Methane is preserved as a fossil fuel (until we use it all up) because it is produced and stored under anaerobic conditions, and oxygen is needed to oxidize the CH_4 molecule. Methanogenesis is not really a form of anaerobic respiration, but it is a type of energy- generating metabolism that requires an outside electron acceptor in the form of CO_2. Methane is a significant greenhouse gas because it is naturally produced in fairly quantities and it absorbs up to 15 times more heat than carbon dioxide.



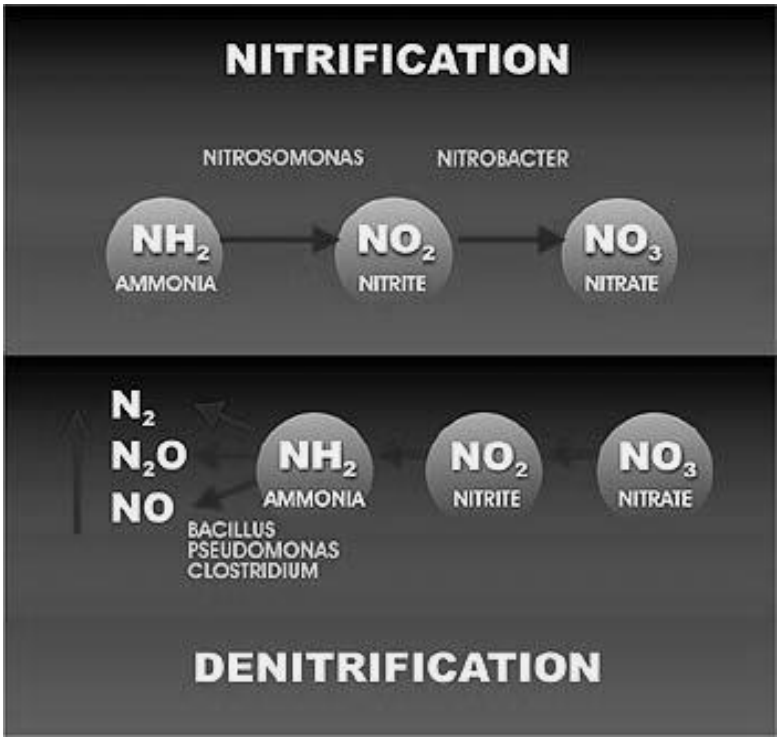
Various types of methanogenic bacteria

Denitrification and other anaerobic respiring bacteria

Denitrification is an important process in agriculture because it removes NO_3 from the soil. NO_3 is a major source of nitrogen fertilizer in agriculture. Almost one- third the cost of some types of agriculture is in nitrate fertilizers The use of nitrate as a respiratory electron acceptor is usually an alternative to the use of oxygen. Therefore, soil bacteria such as *Pseudomonas* will use O_2 as an electron acceptor if it is available, and disregard NO_3. This is the rationale in maintaining well- aerated soils by the agricultural practices of plowing and tilling. *E. coli* will utilize NO_3 (as well as fumarate) as a respiratory electron acceptor and so it is able to respire in the anaerobic intestinal habitat.

Among the products of denitrification, N_2O is of a major concern because it is a greenhouse gas with 300- times the heat absorbing capacity of CO_2. Denitrifying bacteria that respire using N_2O as an electron acceptor yield N_2 and

therefore provide a sink for the N_2O. although this does not ameliorate denintrification of the soil.



Sulfate reduction is not an alternative to the use of O_2 as an electron acceptor. It is an obligatory process that occurs only under anaerobic conditions. Methanogens and sulfate reducers may share habitat, especially in the anaerobic sediments of eutrophic lakes where they crank out methane and hydrogen sulfide at a surprising rate.

Anaerobic respiring bacteria and methanogens play an essential role in the biological cycles of carbon, nitrogen and sulfur. In general, they convert oxidized forms of the elements to a more reduced state. The lithotrophic procaryotes metabolize the reduced forms of nitrogen and sulfur to a more oxidized state in order to produce energy. The methanotrophic bacteria, which uniquely posses the enzyme methane monooxygenase, can oxidize methane as a source of energy.

Fermentation

After glucose has been broken down into pyruvic acid, the pyruvic acid can be completely broken down in respiration, as previously described, or it can be converted to an organic product in fermentation, whereupon NAD and NADP are regenerated and can enter another round of glycolysis. Fermentation can be defined in several ways, but we define it here as a process that:

1. Releases energy from sugars or other organic molecules, such as amino acids, organic acids, purines, and pyrimidines.

2. Does not require oxygen (but sometimes can occur in its presence).

3. Does not require the use of the Krebs cycle or an electron transport chain;

4. Uses an organic molecule as the final electron acceptor.

5. Produces only small amounts of ATP (only one or two ATP molecules for each molecule of starting material) because much of the original energy in glucose remains in the chemical bonds of the organic end-products, such as lactic acid or ethanol.

During fermentation, electrons are transferred (along with protons) from reduced coenzymes (NADH, NADPH) to pyruvic acid or its derivatives. Those final electron acceptors are reduced to the end-products.

An essential function of the second stage of fermentation is to ensure a steady supply of NAD and NADP so that glycolysis

can continue. In fermentation, ATP is generated only during glycolysis.

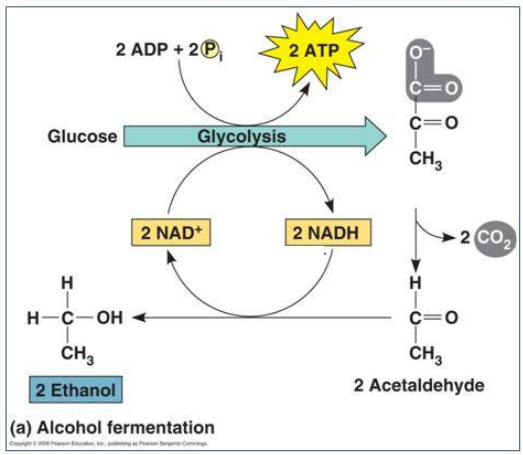
Microorganisms can ferment various substrates; the end-products depend on the particular microorganism, the substrate, and the enzymes that are present and active. Chemical analyses of these end-products are useful in identifying microorganisms. The following are two of the more important processes: lactic acid fermentation and alcohol ermentation.

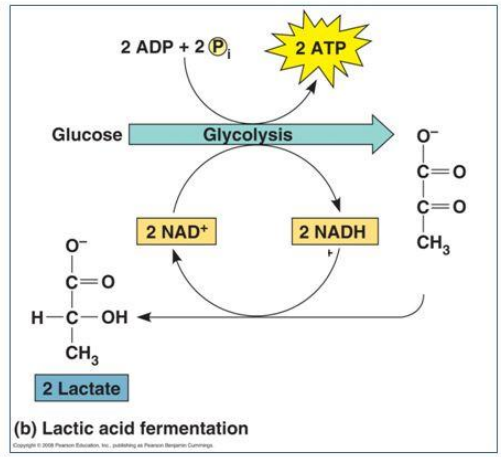
Lactic Acid Fermentation

During glycolysis, which is the first phase of **lactic acid fermentation,** a molecule of glucose is oxidized to two molecules of pyruvic acid. This oxidation generates the energy that is used to form the two molecules of ATP. In the next step, the two molecules of pyruvic acid are reduced by two molecules of NADH to form two molecules of lactic acid.

Two Types of Fermentation:

both regenerate NAD+ so that glycolysis can continue





ALCOHOL FERMENTATION
- in yeast cells and some bacteria
- produces ethyl alcohol and CO_2

LACTIC ACID FERMENTATION
- in vertebrate muscle cells and some bacteria
- produces lactic acid

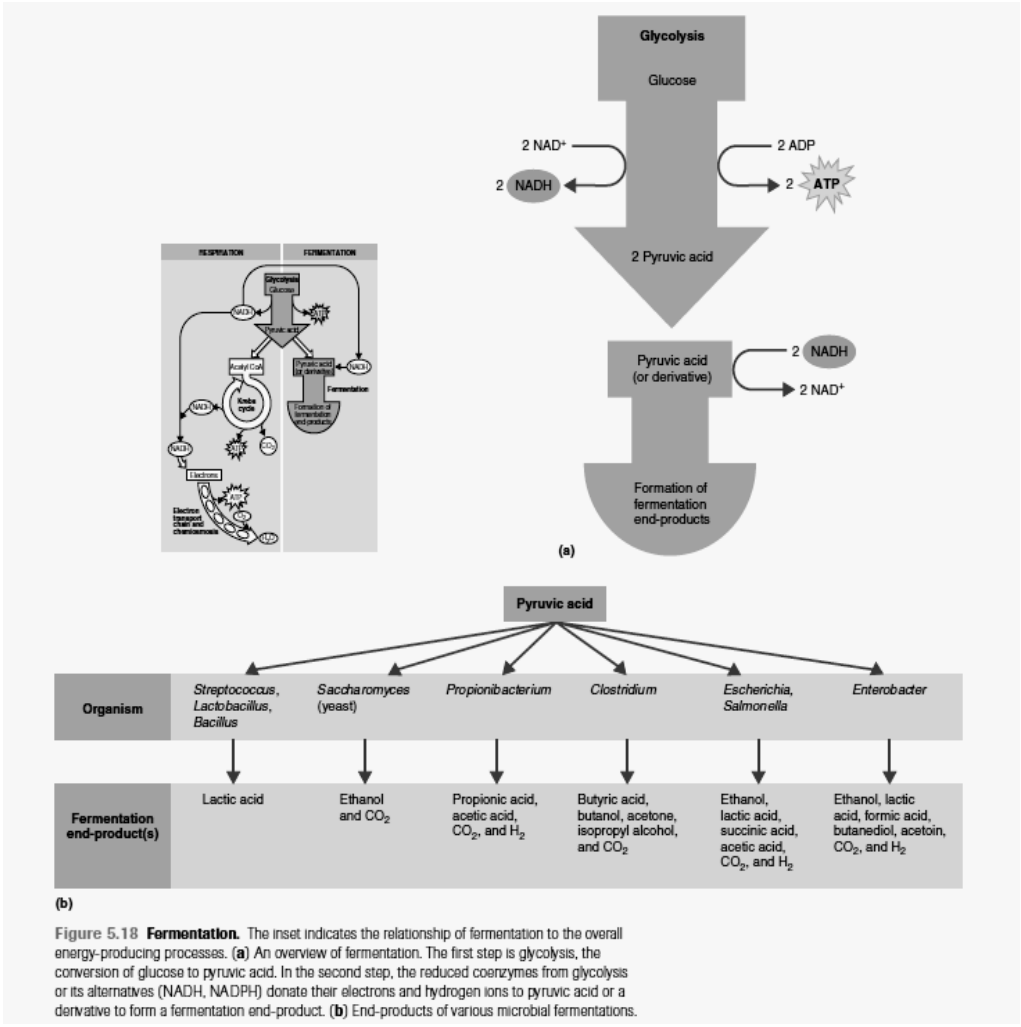


Figure 5.18 **Fermentation.** The inset indicates the relationship of fermentation to the overall energy-producing processes. (**a**) An overview of fermentation. The first step is glycolysis, the conversion of glucose to pyruvic acid. In the second step, the reduced coenzymes from glycolysis or its alternatives (NADH, NADPH) donate their electrons and hydrogen ions to pyruvic acid or a derivative to form a fermentation end-product. (**b**) End-products of various microbial fermentations.

Because lactic acid is the end-product of the reaction, it undergoes no further oxidation, and most of the energy produced by the reaction remains stored in the lactic acid. Thus, this fermentation yields only a small amount of energy. Two important genera of lactic acid bacteria are

Streptococcus and *Lactobacillus*. Because these microbes produce only lactic acid, they are referred to as **homolactic** (or *homofermentative*). Lactic acid fermentation can result in food spoilage. However, the process can also produce yogurt from milk, sauerkraut from fresh cabbage, and pickles from cucumbers.

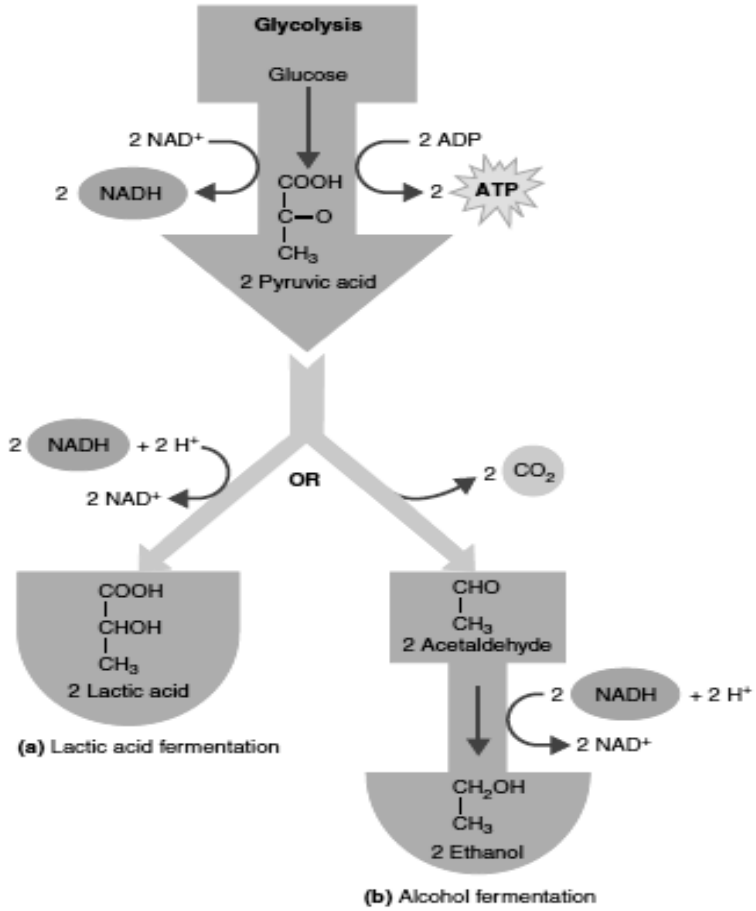
Alcohol Fermentation

Alcohol fermentation also begins with the glycolysis of a molecule of glucose to yield two molecules of pyruvic acid and two molecules of ATP. In the next reaction, the two molecules of pyruvic acid are converted to two molecules of acetaldehyde and two molecules of CO2. The two molecules of acetaldehyde are next reduced by two molecules of NADH to form two molecules of ethanol. Again, alcohol fermentation is a low-energy-yield process because most of the energy contained in the initial glucose molecule remains in the ethanol, the end-product.

Alcohol fermentation is carried out by a number of bacteria and yeasts. The ethanol and carbon dioxide produced by the yeast *Saccharomyces* are waste products for yeast cells but are useful to humans. Ethanol made by yeasts is the alcohol in alcoholic beverages, and carbon dioxide made by yeasts causes bread dough to rise.

Organisms that produce lactic acid as well as other acids or alcohols are known as **heterolactic** (or *heterofermentative*) and often use the pentose phosphate pathway.

The following Table lists some of the various microbial fermentations used by industry to convert inexpensive raw materials into useful end-products. The next Table provides a summarized comparison of aerobic respiration, anaerobic respiration, and fermentation.



Types of fermentation

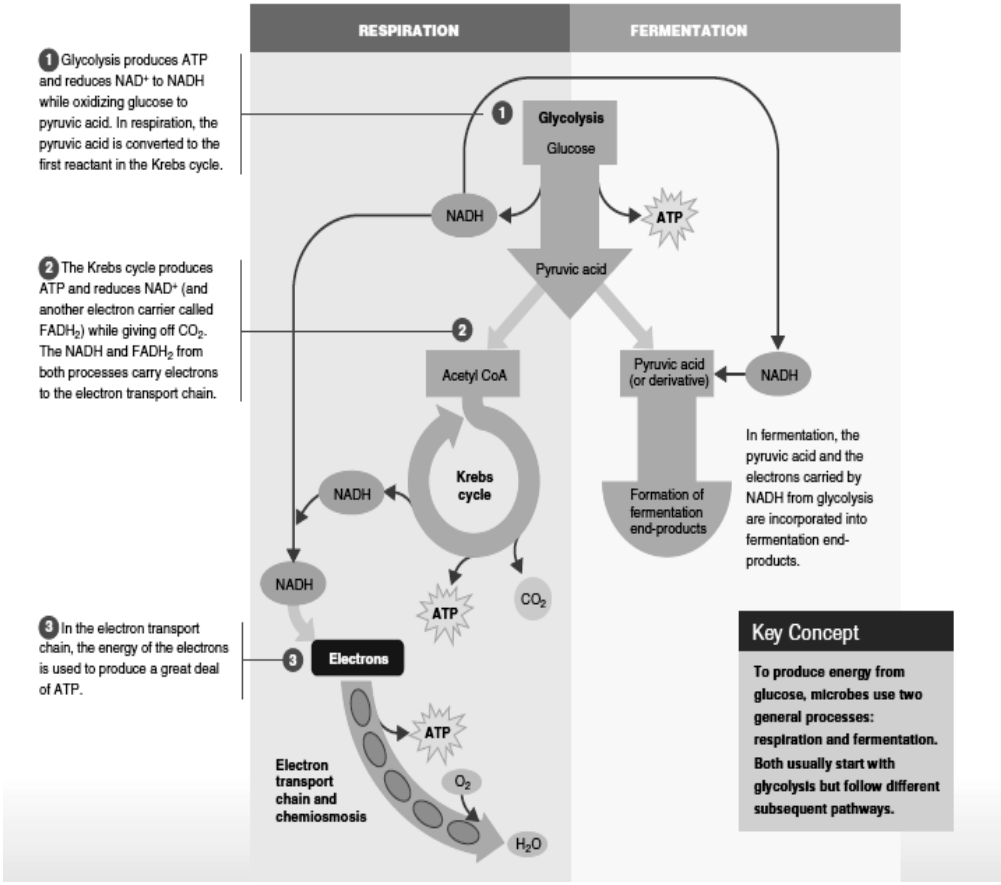
1 ome Industrial Uses for Different Types of Fermentations*

Fermentation End-Product(s)	Industrial or Commercial Use	Starting Material	Microorganism
Ethanol	Beer	Malt extract	*Saccharomyces cerevisiae* (yeast, a fungus)
	Wine	Grape or other fruit juices	*Saccharomyces cerevisiae* (yeast)
	Fuel	Agricultural wastes	*Saccharomyces cerevisiae* (yeast)
Acetic Acid	Vinegar	Ethanol	*Acetobacter*
Lactic Acid	Cheese, yogurt	Milk	*Lactobacillus, Streptococcus*
	Rye bread	Grain, sugar	*Lactobacillus delbruckii*
	Sauerkraut	Cabbage	*Lactobacillus plantarum*
	Summer sausage	Meat	*Pediococcus*
Propionic Acid and Carbon Dioxide	Swiss cheese	Lactic acid	*Propionibacterium freudenreichii*
Acetone and Butanol	Pharmaceutical, industrial uses	Molasses	*Clostridium acetobutylicum*
Glycerol	Pharmaceutical, industrial uses	Molasses	*Saccharomyces cerevisiae* (yeast)
Citric Acid	Flavoring	Molasses	*Aspergillus* (fungus)
Methane	Fuel	Acetic acid	*Methanosarcina*
Sorbose	Vitamin C (ascorbic acid)	Sorbitol	*Gluconobacter*

*Unless otherwise noted, the microorganisms listed are bacteria.

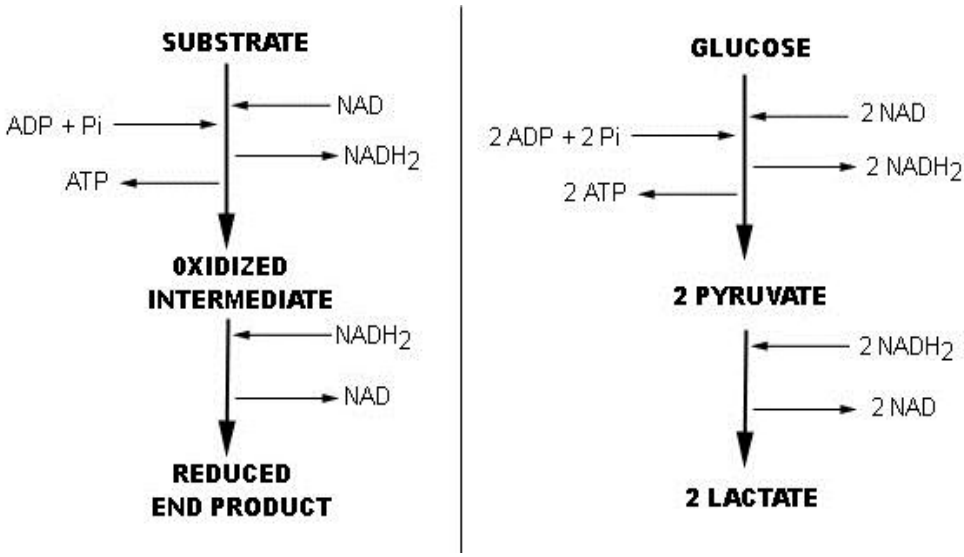
1 erobic Respiration, Anaerobic Respiration, and Fermentation Compared

Energy-Producing Process	Growth Conditions	Final Hydrogen (Electron) Acceptor	Type of Phosphorylation Used to Generate ATP	ATP Molecules Produced per Glucose Molecule
Aerobic Respiration	Aerobic	Molecular oxygen (O_2)	Substrate-level and oxidative	36 (eukaryotes) 38 (prokaryotes)
Anaerobic Respiration	Anaerobic	Usually an inorganic substance (such as NO_3^-, SO_4^{2-}, or CO_3^{2-}) but not molecular oxygen (O_2)	Substrate-level and oxidative	Variable (fewer than 38 but more than 2)
Fermentation	Aerobic or anaerobic	An organic molecule	Substrate-level	2



Fermentation is an ancient mode of metabolism, and it must have evolved with the appearance of organic material on the planet. Fermentation is metabolism in which energy is derived from the partial oxidation of an organic compound using organic intermediates as electron donors and electron

acceptors. No outside (exogenous) electron acceptors are involved; no membrane or electron transport system is required; all ATP is produced by substrate level phosphorylation. By definition, fermentation may be as simple as two steps illustrated in the following model. Indeed, some amino acid fermentations by the clostridia are this simple. But the pathways of fermentation are more complex, usually involving several preliminary steps to prime the energy source for oxidation and substrate level phosphorylations.



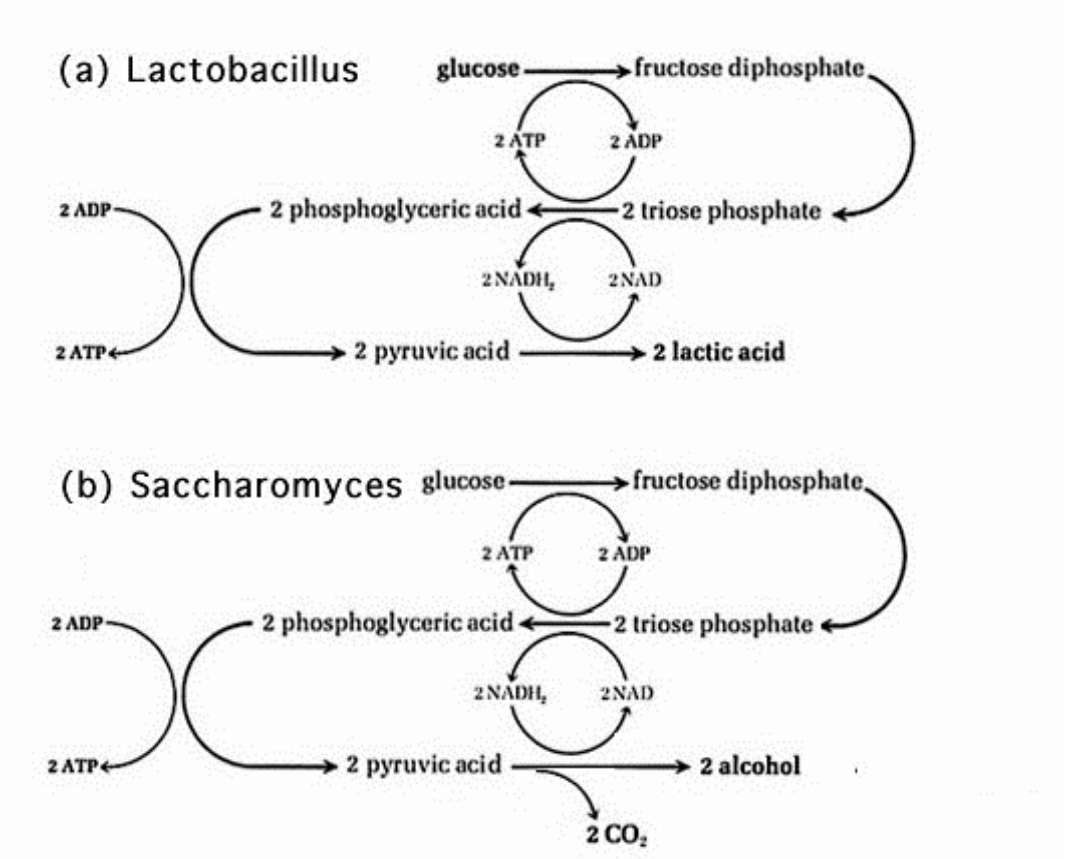
Model fermentation. **Left**: The substrate is oxidized to an organic intermediate; the usual oxidizing agent is NAD. Some of the energy released by the oxidation is conserved during the synthesis of ATP by the process of substrate level phosphorylation. Finally, the oxidized intermediate is reduced to end products. Note that $NADH_2$ is the reducing agent, thereby balancing its redox ability to drive the energy-producing reactions. **Right**: In lactic fermentation by *Lactobacillus*, the substrate (glucose) is oxidized to pyruvate, and pyruvate becomes reduced to lactic acid. Redox balance is maintained by coupling oxidations to reductions within the pathway. For example, in lactic acid fermentation via the Embden-Meyerhof pathway, the oxidation of glyceraldehyde phosphate to phosphoglyceric acid is coupled to the reduction of pyruvic acid to lactic acid.

In biochemistry, fermentation pathways start with glucose. This is because it is the simplest molecule, requiring the fewest catalytic steps, to enter into a pathway of glycolysis and central metabolism. In procaryotes there exist three major pathways of glycolysis (the dissimilation of sugars): the classic Embden- Meyerhof pathway, which is also used by most eucaryotes, including yeast (*Saccharomyces*): the phosphoketolase or heterolactic pathway related to the hexose- pentose shunt; and the Entner- Doudoroff pathway. Whether or not a bacterium is a fermenter, it will likely dissimilate sugars through one or more of these pathways.

As mentioned earlier, the pathway of glycolysis is operated by *Saccharomyces* to produce ethanol and CO_2, by the "homolactic" acid bacteria to produce lactic acid, and by many other bacteria "heterolactic" to produce a variety of fatty acids, alcohols and gases. Some end products of Embden- Meyerhof fermentations are essential components of foods and beverages, and some are useful fuels and industrial solvents. Diagnostic microbiologists use bacterial fermentation profiles (e.g. testing an organism's ability to ferment certain sugars, or examining an organism's array of end products) in order to identify them, down to the genus level.

The first three steps of the EM pathway prime (phosphorylate) and rearrange the hexose for cleavage into 2 trioses (glyceraldehyde- phosphate). Fructose 1,6-diphosphate aldolase is the key (cleavage) enzyme. Each triose molecule is oxidized and phosphorylated followed by two substrate level phosphorylations that yield 4 ATP during

the pathway to pyruvate. Lactic acid bacteria reduce the pyruvate to lactic acid (lactate); yeast reduce the pyruvate to alcohol (ethanol) and CO_2 as shown in the Figure below The oxidation of glucose to lactate yields a total of 56 kcal per mole of glucose. Since the cells harvest 2 ATP (16 kcal) as useful energy, the efficiency of the lactate fermentation is about 29 percent (16/ 56). Alcohol fermentations have a similar efficiency.



(a) The Embden Meyerhof pathway of lactic acid fermentation in lactic acid bacteria (*Lactobacillus*) and (b) the Embden- Meyerhof pathway of alcohol fermentation in yeast (*Saccharomyces*). The pathways yield two moles of end products and two moles of ATP per mole of glucose fermented. The steps in the breakdown of glucose to pyruvate are identical. The difference between the pathways is the manner of reducing pyruvic acid, thereby giving rise to different end products.