South Valley University | Botany and microbiology Department | Faculty of Science

ENZYMOLOGY and VIROLOGY

3-rd chemistry and Microbiology

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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:

Enzymology

Enzymology is the study of enzymes, their kinetics, structure, and function, as well as their relation to each other.

Enzyme from the Greek ένζυμο, *énsymo*, which means ***én*** ("in") and ***simo*** ("yeast").

- ✧ Biocatalysts
- ✧ Biological middlemen
- ✧ Organic catalysts
- ✧ Enzyme proteins regulate metabolic reaction rates.
- ✧ i.e., they control metabolism molecules that accelerate or catalyze chemical reactions (A--->B) in cells by breaking old covalent bonds and forming new covalent bonds.
- ✧ A biological catalyst… but, different from a chemical catalyst.
- ✧ Enzymes have a complex structure act only up a specific substrate do not change the direction of reactions.
- ✧ Enzymes convert substrates to products without changing themselves

catalysis***** = acceleration of the rate of a chemical reaction by a catalyst.

Some important dates in early Enzyme History

1836 Berzelius coined the term catalysis (Gk: to dissolve).

1878 Kuhne used the word enzyme (Gk: in yeast) to indicate the catalysis taking place in the biological systems.

1883 Buchner isolated enzyme system from cell-free extract of yeast. He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol.

1898 Ducleaux - uses suffix "ASE" for enzyme naming

1926 James sumner first achieved the isolation and crystallization of enzyme urease from jack bean

Enzyme Parts List

The activity of an enzyme depends, at the minimum, on a specific protein chain. In many cases, the enzyme consists of the protein and a combination of one or more parts called **cofactors**. This enzyme complex is usually simply referred to simply as the enzyme.

Apoenzyme: The polypeptide or protein part of the enzyme is called the **apoenzyme** and may be inactive in its original synthesized structure. The inactive form of the apoenzyme is known as a **proenzyme or zymogen**. The proenzyme may contain several extra amino acids in the protein which are removed and allow the final specific tertiary structure to be formed before it is activated as an apoenzyme.

Active site: All enzymes possess an area in their molecular organization where substrate materials bind themselves in order to undergo chemical change. This binding site is called the **active site** of an enzyme. An enzyme may have one or more active sites. In addition to the active sites, an enzyme may also have **regulatory sites**, to which regulatory substances bind and regulate the activity of the enzyme.

Cofactors: Some enzymes do not need any additional components to show full activity. However, others require non-protein molecules called **cofactors** to be bound for activity. Cofactors can be either inorganic (*e.g.*, metal ions and iron-sulfur clusters) or organic compounds (e.g. flavin and heme). Organic cofactors can be either **prosthetic groups**, which are tightly bound to an enzyme, or **coenzymes**, which are released from the enzyme's active site during the reaction. Coenzymes include NADH, NADPH and ATP. These molecules act to transfer chemical groups between enzymes. An example of an enzyme that contains a cofactor is **carbonic anhydrase**.

Enzymes that require a cofactor but do not have one bound are called ***apoenzymes*** or ***apoproteins***. An apoenzyme together with its cofactor(s) is called a ***holoenzyme*** (this is the active form). Most cofactors are not covalently attached to an enzyme but are very tightly bound. However, organic prosthetic groups can be covalently bound (*e.g.*, thiamine pyrophosphate in the enzyme pyruvate dehydrogenase). The term "**holoenzyme**" can also be applied to enzymes that contain multiple protein subunits, such as the DNA polymerases, here the holoenzyme is the complete complex containing all the subunits needed for the activity.

Coenzyme: Coenzymes are small organic molecules that transport chemical groups from one enzyme to another. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins, this is when these compounds cannot be made in the body and must be acquired from the diet. The chemical groups carried include the hydride ion (H^-) carried by NAD or $NADP^+$, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosylmethionine.

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 700 enzymes are known to use the coenzyme NADH.

Coenzymes are usually regenerated and their concentrations maintained at a steady level inside the cell: for example, NADPH is regenerated through the pentose phosphate pathway and S-adenosylmethionine by methionine adenosyltransferase.

Another type of cofactor is an inorganic metal ion called a **metal ion activator**. The inorganic metal ions may be bonded through coordinate covalent bonds. The major reason for the nutritional requirement for minerals is to supply such metal ions as Zn^{+2}, Mg^{+2}, Mn^{+2}, Fe^{+2}, Cu^{+2}, K^{+1}, and Na^{+1} for use in enzymes as cofactors.

Final Enzyme (Apoenzyme + Cofactor = Holoenzyme): The type of association between the cofactor and the apoenzymes varies. In some cases, the bonds are rather loose and both come together only during a reaction. In other

cases, they are firmly bound together by covalent bonds. The activating role of a cofactor is to either: activate the protein by changing its geometric shape, or by actually participating in the overall reaction.

The overall enzyme contains a specific geometric shape called the **active site** where the reaction takes place. The molecule acted upon is called the **substrate**.

Enzyme Nomenclature and Classification

Enzymes are commonly named by adding a suffix "-ase" to the root name of the substrate molecule it is acting upon. For example, **Lipase** catalyzes the hydrolysis of a lipid triglyceride. **Sucrase** catalyzes the hydrolysis of sucrose into glucose and fructose.

A few enzymes discovered before this naming system was devised are known by common names. Examples are pepsin, trypsin, and chymotrypsin which catalyzes the hydrolysis of proteins.

The latest systematic nomenclature system known as the **International Enzyme Commission (IEC)** system is based upon the type of reaction catalyzed. There are six broad groups of enzymes in this system as shown in the table on the left.

For example, when using this system, "urease" becomes "urea amidohydrolase." Do not be overly concerned about enzyme names, but be able to recognize a substance as an enzyme by its "-ase" ending. Some types of reactions that are being catalyzed will be self-evident

Specificity

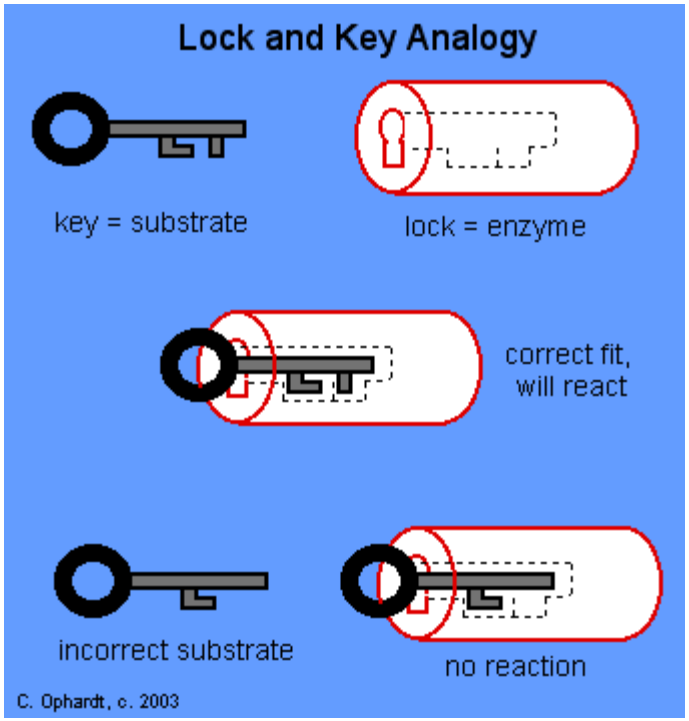
Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity.

Some enzymes that produce secondary metabolites are described as promiscuous, as they can act on a relatively broad range of different substrates. It has been suggested that this broad substrate specificity is important for the evolution of new biosynthetic pathways.

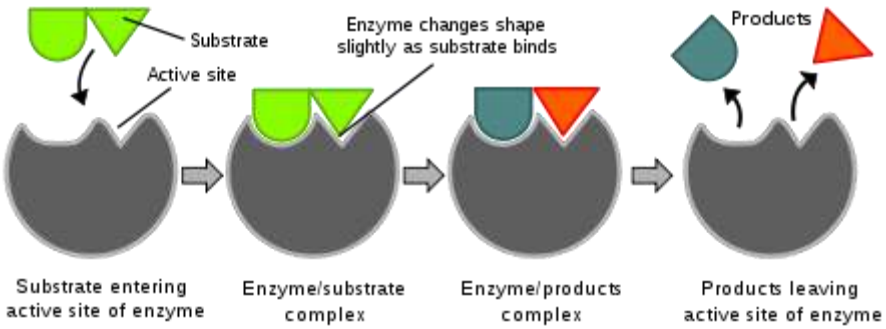
Lock and key" model

Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. However, while this model explains enzyme specificity, it fails to explain the stabilization of

the transition state that enzymes achieve. The "lock and key" model has proven inaccurate, and the induced fit model is the most currently accepted enzyme-substrate-coenzyme figure.



Induced fit" model



Diagrams to show the induced fit hypothesis of enzyme action.

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continually reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side chains which make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge are determined.

Enzyme Kinetics: Basic Enzyme Reactions

Enzymes are catalysts and increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products.

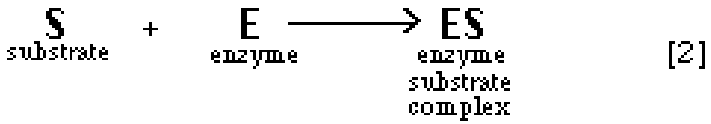
The basic enzymatic reaction can be represented as follows

$$S + E \longrightarrow P + E \qquad [1]$$

where E represents the enzyme catalyzing the reaction, S is the substrate, the substance is changed, and P is the product of the reaction.

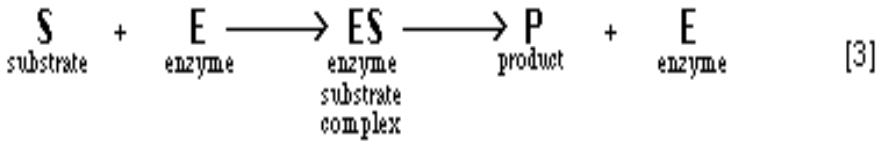
Enzyme Kinetics: The Enzyme Substrate Complex

A theory to explain the catalytic action of enzymes was proposed by the Swedish chemist Savante Arrhenius in 1888. He proposed that the substrate and enzyme formed some intermediate substance which is known as the enzyme-substrate complex. The reaction can be represented as:



[2]

If this reaction is combined with the original reaction equation [1], the following results:



[3]

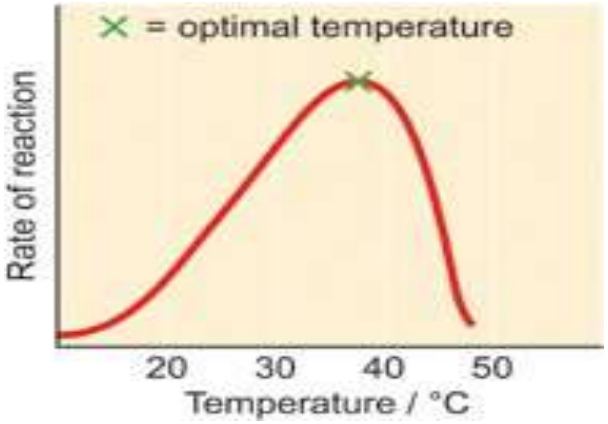
The existence of an intermediate enzyme-substrate complex has been demonstrated in the laboratory, for example, using catalase and a hydrogen peroxide derivative.

Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect

the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

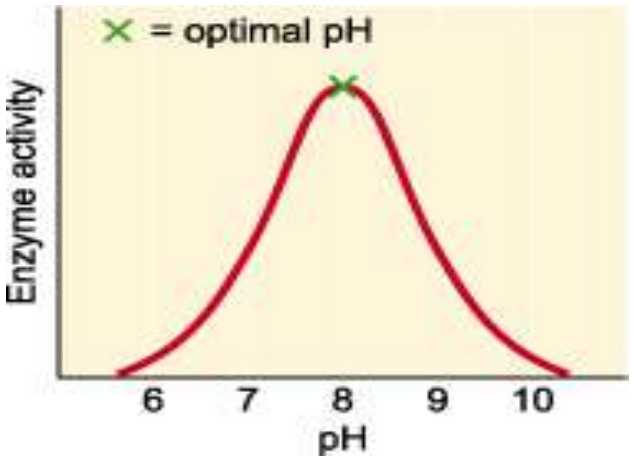
Temperature



As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 ^{o}C) for the enzymes in human cells.

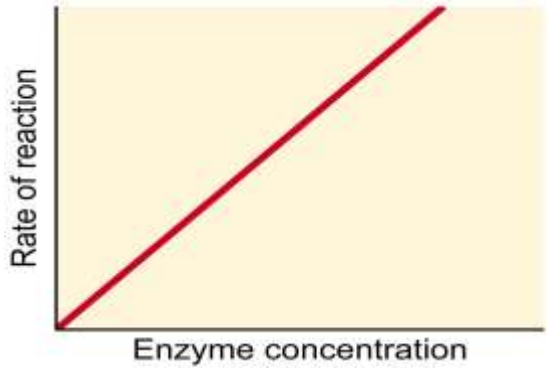
Above this temperature, the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

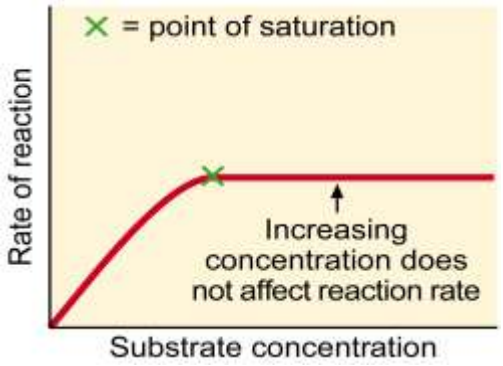
pH



Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.

Concentration of enzymes and substrate





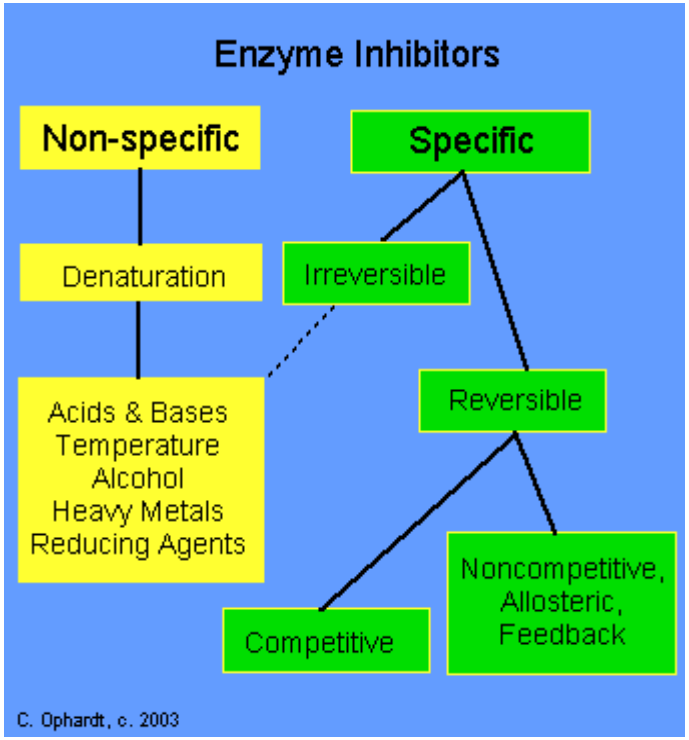
The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is

because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate. (See graph). Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration. (See graph).

Enzyme Inhibitors

Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. There are a variety of types of inhibitors including: nonspecific, irreversible, reversible - competitive and noncompetitive. Poisons and drugs are examples of enzyme inhibitors.



Nonspecific Inhibitors:

A nonspecific inhibition affects all enzymes in the same way. Non-specific methods of inhibition include any physical or chemical changes which ultimately **denature** the protein portion of the enzyme and are therefore irreversible.

Temperature: Usually, the reaction rate increases with temperature, but with enzyme reactions, a point is reached when the reaction rate decreases with increasing temperature. At high temperatures the protein part of the enzyme begins to denature, thus inhibiting the reaction.

Acids and Bases: Enzyme activity is also controlled by pH. As the pH is decreased or increased, the nature of the various acid and amine groups on side chains is altered with resulting changes in the overall shape structure of the enzyme.

Specific Inhibitors:

Specific Inhibitors exert their effects upon a single enzyme. Most poisons work by specific inhibition of enzymes. Many drugs also work by inhibiting enzymes in bacteria, viruses, or cancerous cells and will be discussed later.

Competitive Inhibitors:

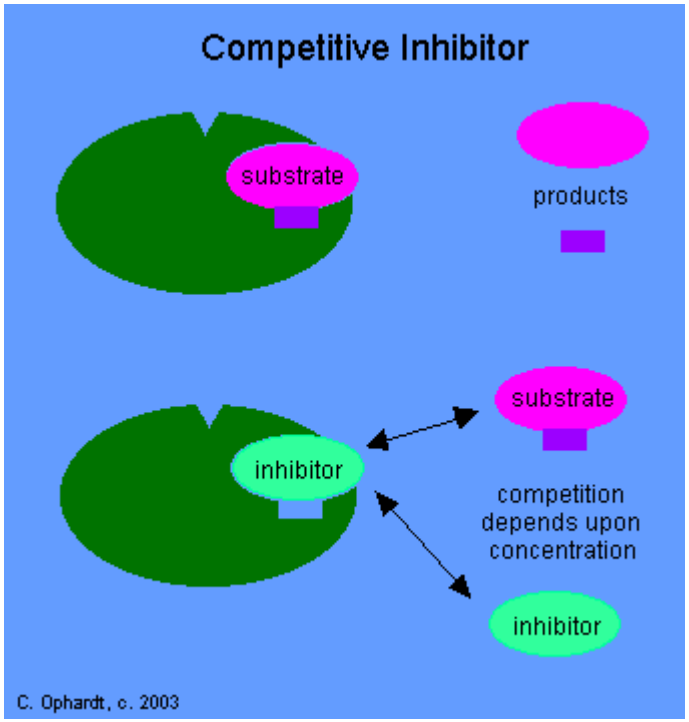
A competitive inhibitor is any compound which closely resembles the chemical structure and molecular geometry of the substrate. The inhibitor competes for the same active site as the substrate molecule. The inhibitor may interact with the enzyme at the active site, but no reaction takes place. The inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site.

Example: Ethanol is metabolized in the body by oxidation to acetaldehyde, which is in turn further oxidized to acetic acid by aldehyde oxidase enzymes. Normally, the second reaction is rapid so that acetaldehyde does not accumulate in the body.

A drug, **disulfiram (Antabuse) inhibits** the aldehyde oxidase which causes the accumulation of acetaldehyde with subsequent unpleasant side-effects of nausea and vomiting. This drug is sometimes used to help people overcome the drinking habit.

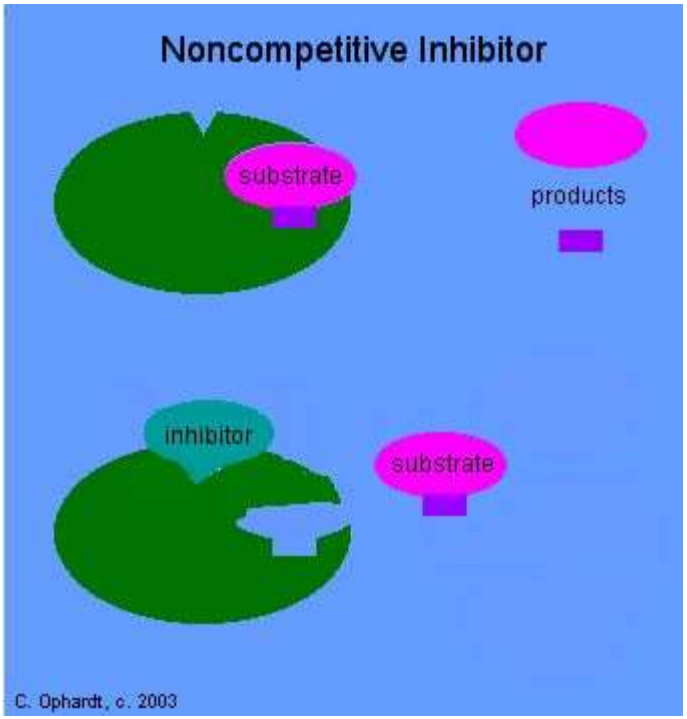
Methanol poisoning occurs because methanol is oxidized to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the

oxidation of methanol. Ethanol is oxidized in preference to methanol and consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.



Non competitive Inhibitors:

A noncompetitive inhibitor is a substance that interacts with the enyzme, but usually not at the active site. The noncompetitive inhibitor reacts either remote from or very close to the active site. The net effect of a non competitive inhibitor is to change the shape of the enzyme and thus the active site, so that the substrate can no longer interact with the enzyme to give a reaction. Non competitive inhibitors are usually reversible, but are not influenced by concentrations of the substrate as is the case for a reversible competitive inhibitor. See the graphic.



Irreversible Inhibitors form strong covalent bonds with an enzyme. These inhibitors may act at, near, or remote from the active site. Consequently, they may not be displaced by the addition of excess substrate. In any case, the basic structure of the enzyme is modified to the degree that it ceases to work.

Since many enzymes contain sulfhydral (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor. Heavy metals such as Ag^{+}, Hg^{2+}, Pb^{2+} have strong affinities for -SH groups.

Nerve gases such as diisopropylfluorophosphate (DFP) inhibit the active site of acetylcholine esterase by reacting with the hydroxyl group of serine to make an ester.

Oxalic and citric acid inhibit blood clotting by forming complexes with calcium ions necessary for the enzyme metal ion activator.

Classification of Enzymes

Enzymes Are Classified into six functional Classes (EC number Classification) by the International Union of Biochemists (I.U.B.).

on the Basis of the Types of Reactions That They Catalyze

- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

Principle of the international classification

Each enzyme has **classification number**

consisting of four digits:

Example, **EC:** (**2.7.1.1**) **HEXOKINASE**

- **EC: (2.7.1.1) these components indicate the following groups of enzymes:**

- **2. IS CLASS (TRANSFERASE)**

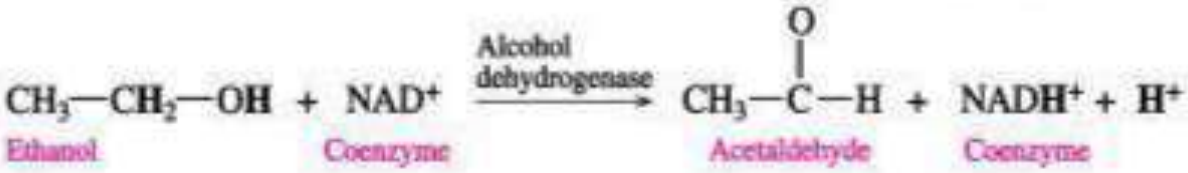
- **7. IS SUBCLASS (TRANSFER OF PHOSPHATE)**

- **1. IS SUB-SUB CLASS (ALCOHOL IS PHOSPHATE ACCEPTOR)**
- **1. SPECIFIC NAME**

  ATP,D-HEXOSE-6-PHOSPHOTRANSFERASE (Hexokinase)

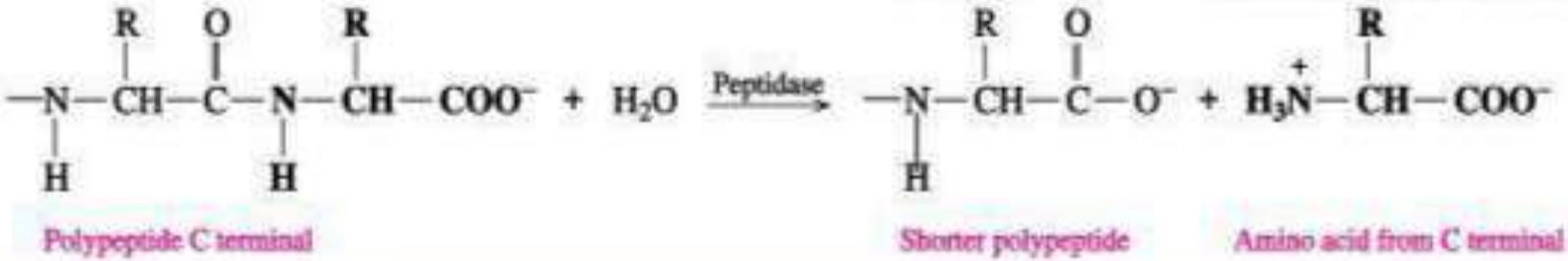
Oxidoreductases, Transferases and Hydrolases

Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds



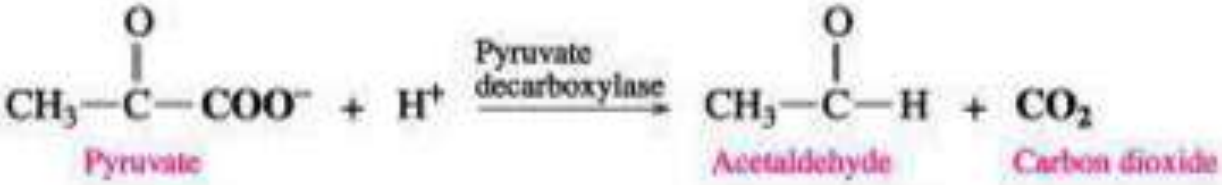
2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups

3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose



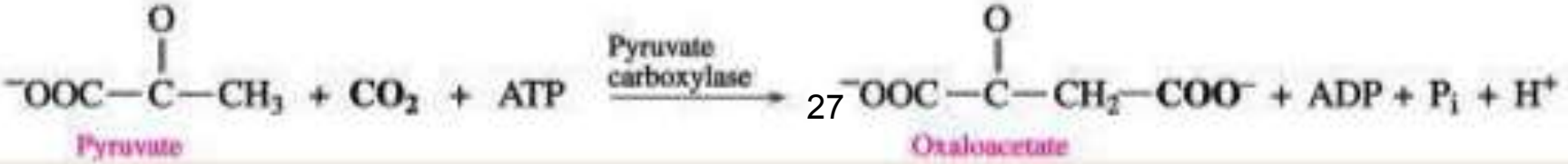
Lyases, Isomerases and Ligases

Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3



5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers

6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO_2



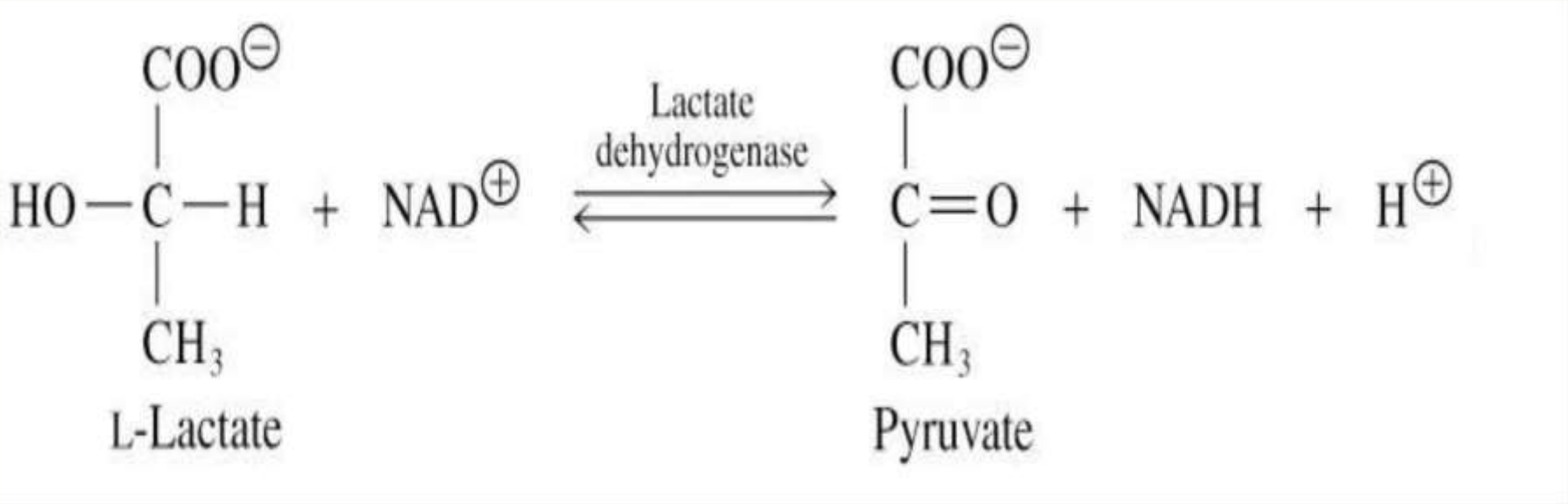
EC 1. Oxidoreductases

- **Biochemical Activity:**
  - **Catalyse Oxidation/Reduction Reactions**

    Act on many chemical groupings to add or remove hydrogen atoms.
- **Examples:**
  - **Lactate dehydrogenase.**
  - **Glucose Oxidase.**
  - **Peroxidase.**
  - **Catalase.**
  - **Phenylalanine hydroxylase.**

1. Oxidoreductases

- Catalyze oxidation-reduction reactions



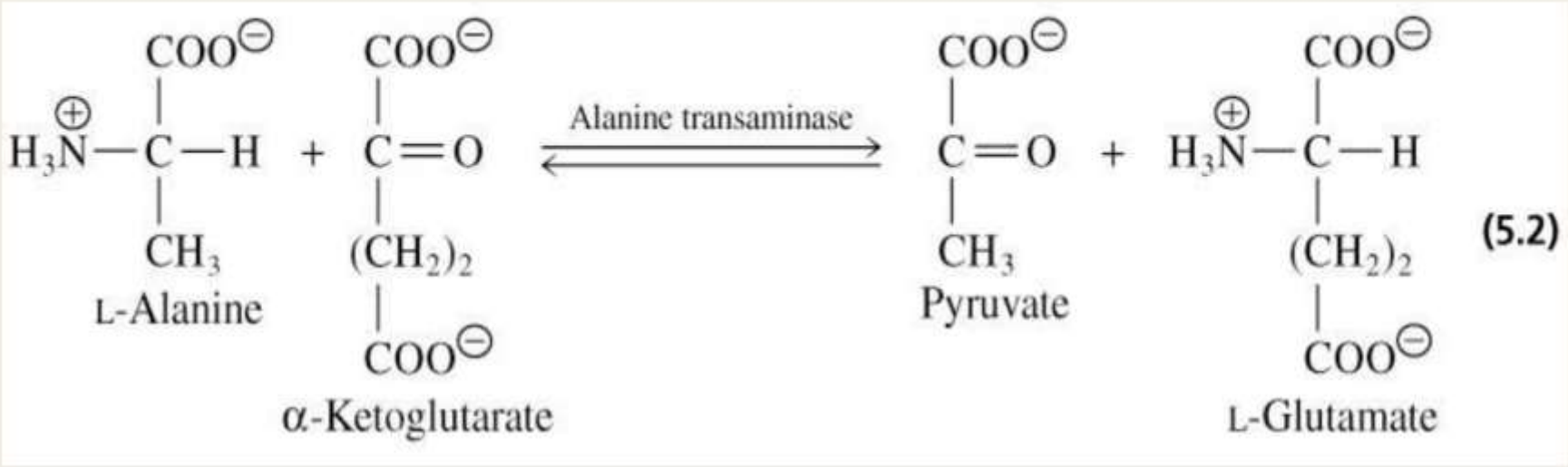
- oxidases
- peroxidases
- dehydrogenases

EC 2. Transferases

- **Biochemical Activity:**
  - **Transfer a functional groups (e.g. methyl or phosphate) between donor and acceptor molecules.**
- **Examples:**
  - **Transaminases (ALT & AST).**
  - **Phosphotransferases (Kinases).**
  - **Transmethylases.**
  - **Transpeptidases.**
  - **Transacylases.**

2. Transferases

- Catalyze group transfer reactions

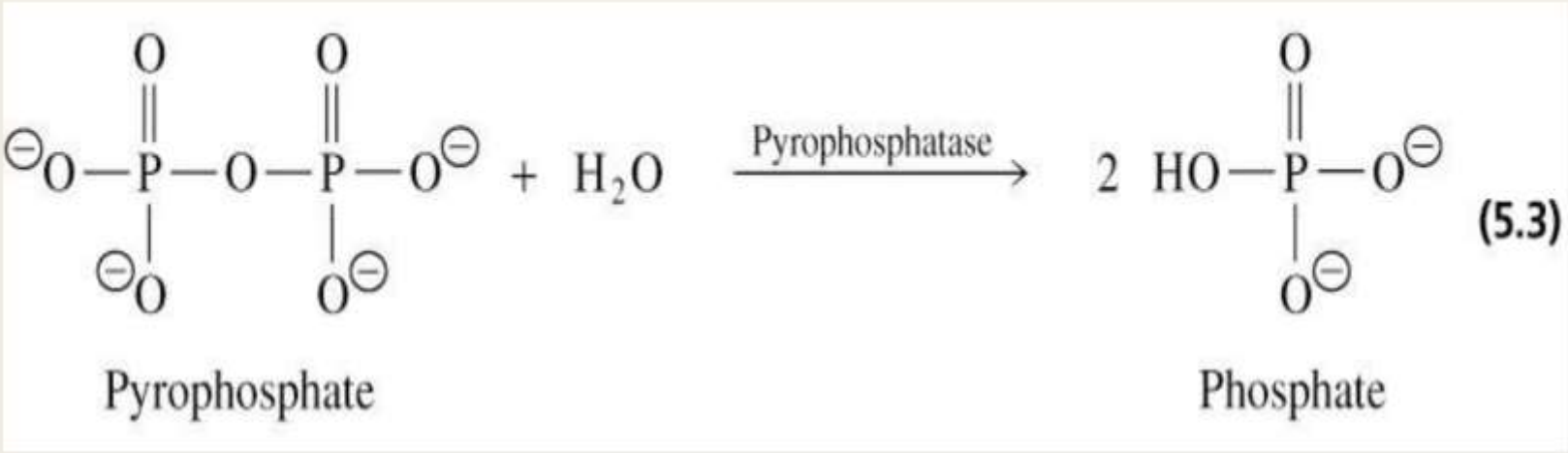


EC 3. Hydrolases

- **Biochemical Activity:**
  - **Catalyse the hydrolysis of various bonds Add water across a bond.**
- **Examples:**
  - **Protein hydrolyzing enzymes (Peptidases).**
  - **Carbohydrases (Amylase, Maltase, Lactase).**
  - **Lipid hydrolyzing enzymes (Lipase).**
  - **Deaminases.**
  - **Phosphatases.**

3. Hydrolases

- Catalyze hydrolysis reactions where water is the acceptor of the transferred group



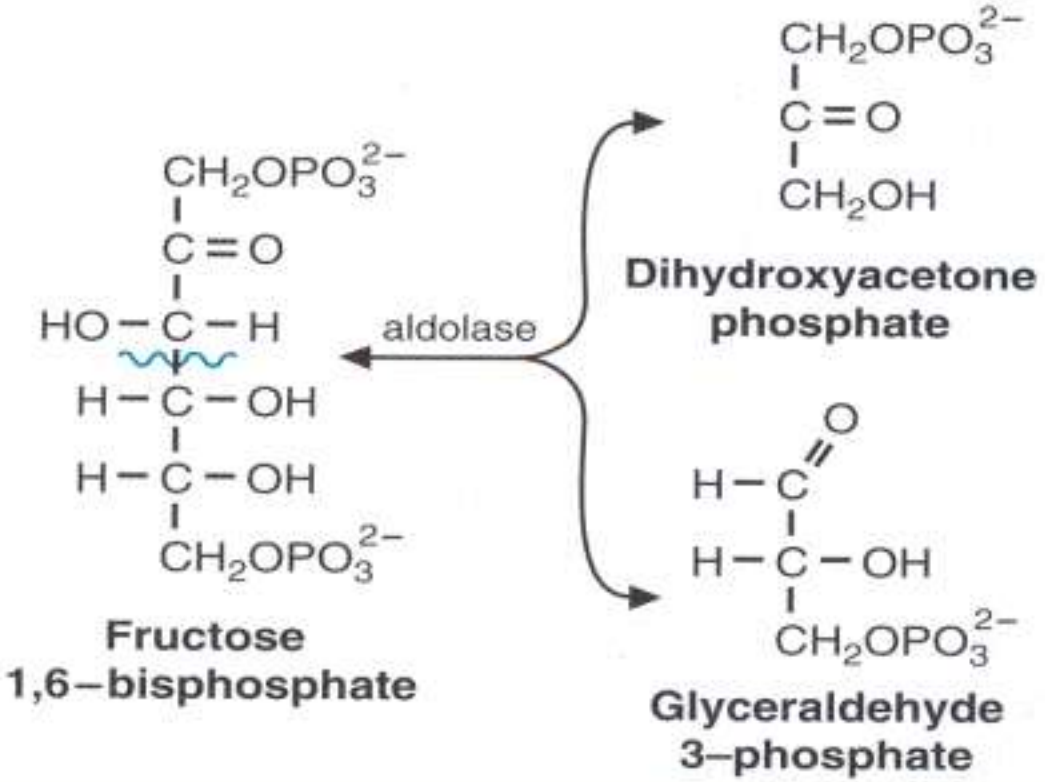
(5.3)

- esterases
- peptidases
- glycosidases

EC 4. Lyases

- **Biochemical Activity:**
  - **Cleave various bonds by means other than hydrolysis and oxidation.**
  - **Add Water, Ammonia or Carbon dioxide across double bonds, or remove these elements to produce double bonds.**
- **Examples:**
  - **Fumarase.**
  - **Carbonic anhydrase.**

4. Lyases

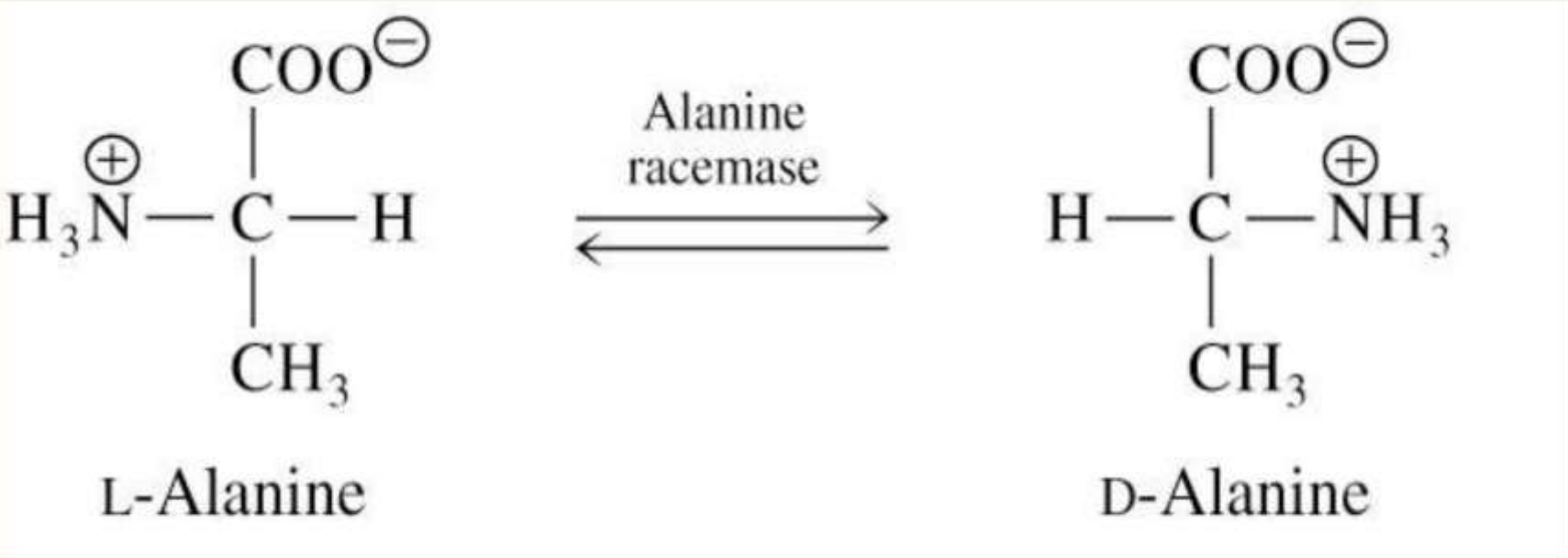

CH2OPO3 2−
C=O
HO−C−H
H−C−OH
H−C−OH
CH2OPO3 2−
Fructose
1,6−bisphosphate
aldolase
CH2OPO3 2−
C=O
CH2OH
Dihydroxyacetone
phosphate
O
H−C
H−C−OH
CH2OPO3 2−
Glyceraldehyde
3−phosphate

EC 5. Isomerases

- **Biochemical Activity:**
  - **Catalyse isomerization changes within a single molecule.**
  - **Carry out many kinds of isomerization:**
    - **L to D isomerizations.**
    - **Mutase reactions (Shifts of chemical groups).**
- **Examples:**
  - **Isomerase.**
  - **Mutase.**

5. Isomerases

- Catalyze isomerization reactions



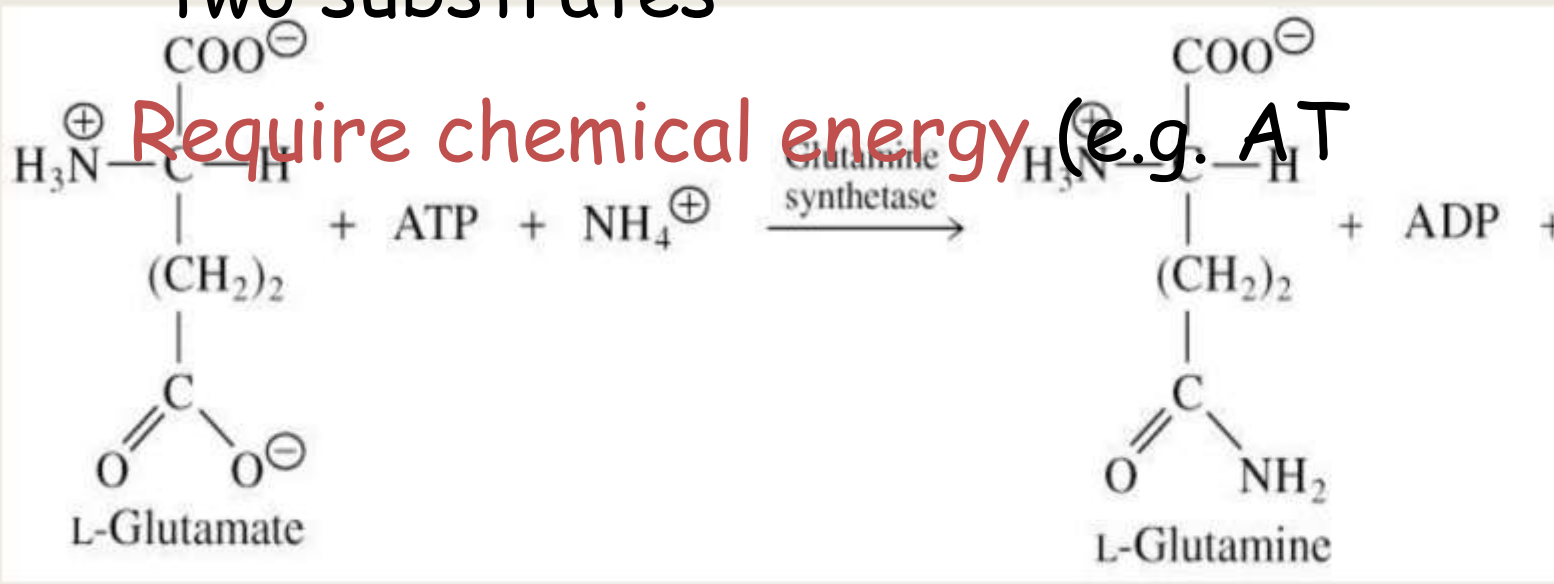
EC 6. Ligases

- **Biochemical Activity:**
  - **Join two molecules with covalent bonds**

    Catalyse reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP.
- **Examples:**
  - **Acetyl~CoA Carboxylase.**
  - **Glutamine synthetase**

6. Ligases (synthetases)

- Catalyze ligation, or joining of two substrates



Require chemical energy (e.g. AT

Isozymes

Isozymes were first described by R. L. Hunter and Clement Markert (1957) who defined them as *different variants of the same enzyme having identical functions and present in the same individual*. This definition encompasses (1) enzyme variants that are the product of different genes and thus represent different loci (described as *isozymes*) and (2) enzymes that are the product of different alleles of the same gene (described as *allozymes*).

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains *identical* to the original, then it is likely that one or the other will be lost as mutations accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of expression, then the two variants may both be favoured by natural selection and become specialised to different functions. For example, they may be expressed at different stages of development or in different tissues.

Allozymes may result from point mutations or from insertion-deletion (indel) events that affect the coding sequence of the gene. As with any other new mutations, there are three things that may happen to a new allozyme:

- It is most likely that the new allele will be non-functional—in which case it will probably result in low fitness and be removed from the population by natural selection.
- Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from the active site), then the mutation may be selectively neutral and subject to genetic drift.
- In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyse a slightly different chemical reaction, in which case the

mutation may cause an increase in fitness, and be favoured by natural selection.

Examples

An example of an isozyme is glucokinase, a variant of hexokinase which is not inhibited by glucose 6-phosphate. Its different regulatory features and lower affinity for glucose (compared to other hexokinases), allow it to serve different functions in cells of specific organs, such as control of insulin release by the beta cells of the pancreas, or initiation of glycogen synthesis by liver cells. Both these processes must only occur when glucose is abundant.

1.) The enzyme lactate dehydrogenase is a tetramer made of two different sub-units, the H-form and the M-form. These combine in different combinations depending on the tissue:

2.) Isoenzymes of creatine phosphokinase: Creatine kinase (CK) or creatine phosphokinase (CPK) catalyses the interconversion of phospho creatine to creatine . CPK exists in 3 isoenzymes. Each isoenzymes is a dimer of 2 subunits M (muscle), B (brain) or both

3.) Isoenzymes of alkaline phosphatase: Six isoenzymes have been identified. The enzyme is a monomer, the isoenzymes are due to the differences in the carbohydrate content (sialic acid residues). The most important ALP isoenzymes are α_1-ALP, α_2-heat labile ALP, α_2-heat stable ALP, pre-β ALP and γ-ALP. Increase in α_2-heat labile ALP suggests hepatitis whereas pre-β ALP indicates bone diseases.

Distinguishing isozymes

Isozymes (and allozymes) are variants of the same enzyme. Unless they are identical in their biochemical properties, for example their substrates and enzyme kinetics, they may be distinguished by a biochemical assay. However, such differences are usually subtle, particularly between *allozymes* which are often neutral variants. This subtlety is to be expected, because two enzymes that differ significantly in their function are unlikely to have been identified as *isozymes*.

While isozymes may be almost identical in function, they may differ in other ways. In particular, amino acid substitutions that change the electric charge of the enzyme are simple to identify by gel electrophoresis, and this forms the basis for the use of isozymes as molecular markers. To identify isozymes, a crude protein extract is made by grinding animal or plant tissue with an extraction buffer, and the components of extract are separated according to their charge by gel electrophoresis. Historically, this has usually been done using gels made from potato starch, but acrylamide gels provide better resolution.

All the proteins from the tissue are present in the gel, so that individual enzymes must be identified using an assay that links their function to a staining reaction. For example, detection can be based on the localised precipitation of soluble indicator dyes such as tetrazolium salts which become insoluble when they are reduced by cofactors such as NAD or NADP, which generated in zones of enzyme activity. This assay method requires that the enzymes are still functional after separation (native gel electrophoresis), and provides the greatest challenge to using isozymes as a laboratory technique.

Isoenzymes differ in kinetics (they have different K_M and V_{max} values).

Isozymes and allozymes as molecular markers

Population genetics is essentially a study of the causes and effects of genetic variation within and between populations, and in the past, isozymes have been amongst the most widely used molecular markers for this purpose. Although they have now been largely superseded by more informative DNA-based approaches (such as direct DNA sequencing, single nucleotide polymorphisms and microsatellites), they are still among the quickest and cheapest marker systems to develop, and remain (as of 2005) an excellent choice for projects that only need to identify low levels of genetic variation, e.g. quantifying mating systems.

Other major examples

- The cytochrome P450 isozymes play important roles in metabolism and steroidogenesis.
- The multiple forms of phosphodiesterase also play major roles in various biological processes. Although more than one form of these enzymes have been found in individual cells, these isoforms of the enzyme are unequally distributed in the various cells of an organism. From the clinical standpoint they have been found to be selectively activated and inhibited, an observation which has led to their use in therapy.

Enzymatic Component of Antioxidants System

Superoxide Dismutase (SOD-E.C.1.15.1.1)

SODs are the representative of metalloproteins that catalyze the dismutation of superoxide radicle into O_2 and H_2O_2 under stress conditions; hence regarded as the first line of defense. Based upon specific location and affinity to bind with metal cofactor, they are typically classified as Fe-SOD (chloroplast), CuZn-SOD (plastid and cytosol) and Mn-SOD (mitochondria) isoform. The elevated level of SOD slows the rate of conversion of superoxide radicle (O_2^-) into caustic hydroxyl radicle

Catalase (CAT-E.C.1.11.1.6)

CAT is first identified and characterized encoded by the nuclear gene. It is a heme-containing tetrameric protein responsible for the cellular level of H_2O_2 into O_2 and H_2O by dismutation reaction especially in peroxisome and glycosomes due to the presence of oxidase enzyme. These cellular compartments are the main hub to carry out energetic-metabolic pathways (photorespiration and β-oxidation of fatty acids) and generate H_2O_2a higher rate. High turnover rate and not essentiality of reducing elements represented CAT as an effective detoxifying agent of H_2O_2 in an energy-efficient way.

Ascorbate Peroxidase (APX-E.C.1.1.11.1)

APX is also a heme-containing enzyme and exists in different isoforms based mainly depend upon location *viz*; sAPX (stroma of chloroplasts), tAPX (thylakoid), gmAPX (membrane of glyoxisome), cAPX (cytosol). APX catalyzes the reduction of hydrogen peroxide into water molecules using ascorbate as a reducing agent; the first step of the Ascorbate-Glutathione cycle. CAT and APX act simultaneously in different locations of the cellular compartment, but widely distributed APX are considered as a more efficient scavenger in stress conditions than CAT due to high affinity toward H_2O_2.

Dehydroascorbate Reductase (DHAR-EC.1.8.5.1)

DHAR, chemically a monomeric enzyme with thiol in side-chain reduces dehydroascorbate to regenerates ascorbate. The reduction reaction is initiated by accepting an electron from reduced glutathione as a reducing substrate. Oxidation-reduction of redox biology facilitates the excessive accumulation of ascorbate in the apoplast and symplast of cells, consequently provide stress tolerance by maintaining redox homeostasis.

Monodehydroascorbate Reductase (MDHAR-E.C.1.6.5.4)

Flavin adenine dinucleotide is a major constituent of enzyme MDHAR usually dispersed in the chloroplast as well as cytosol. These enzymes are highly specific for monodehydroascorbate (MDHA) and catalyze reversible reaction to regenerate ascorbate using NADPH as the electron donor to increase the pool size of ascorbate. Direct and indirect, MDHAR interconnection with APX is important in scavenging of H_2O_2 along with AsA level and thus maintaining redox state under oxidative stress.

Guaiacol Peroxidase (GPX-E.C.1.11.1.7)

GPX is a heme-containing monomeric enzyme located at intracellular or/and extracellular to restrict H_2O_2 formation. It assists many processes such as cell wall lignification, ethylene biosynthesis and also helps in wound healing in the plant; hence regarded as a "Stress Enzyme". In such a process, GPX exploits H_2O_2 to oxidize the substrate by using guaiacol/pyrogallol as a reducing substrate and consume a left-over portion of peroxide in a constructive manner.

Glutathione Reductase (GR-E.C.1.6.4.2)

GR belongs to the class oxidoreductase that transfers electron transfers electrons from NADPH to glutathione disulfide (GSSG) to generate reduced glutathione (GSH). In a series of the reaction catalyzed by MDHAR, DHAR and APX, GSH is paid to remove hydrogen peroxide. In a plant cell, a high ratio of GSH/GSSG is

crucial for providing tolerance under stress. The other important enzymatic antioxidants include Glutathione S-Transferases (GST), Methionine Sulfoxide Reductase (MSR), Glutaredoxin (GRX), Thioredoxin (TRX) and Peroxiredoxins (PRXs).

Industrial use of enzymes

Enzymes are used in the food, agricultural, cosmetic, and pharmaceutical industries to control and speed up reactions in order to quickly and accurately obtain a valuable final product**.** Enzymes are crucial to making cheese, brewing beer, baking bread, extracting fruit juice, tanning leather, and much more. The industrial uses of enzymes are also increasing since they are being used in the production of biofuels and biopolymers. The enzymes can be harvested from microbial sources or can be made synthetically. Yeast and E. coli are commonly engineered to overexpress an enzyme of interest. This type of enzyme engineering is a powerful way to obtain large amounts of enzyme for biocatalysis in order to replace traditional chemical processes.

Industrial uses of enzymes: Examples

Breweries wouldn't be able to brew our beer without enzymes and the yeast that contain them. One of the first steps of the brewing process involves sprouting grain and breaking that starch into maltose and glucose sugar molecules via amylase enzymes. Yeast then consume these simple sugars and produce alcohol and carbon dioxide via glycolysis and alcoholic fermentation. These processes together require a whopping 12 enzymes! Using the whole yeast organism is much more efficient that trying to recreate this process with synthetic enzymes**.** The alcoholic fermentation process takes two pyruvate molecules from glycolysis and converts them to ethanol via pyruvate dehydrogenase and alcohol dehydrogenase. The production of cheese follows a similar process, but instead requires bacteria to perform glycolysis to convert the sugars in milk to the lactic acid that gives cheese and yogurt its exceptional flavor.

Enzymes are transforming the non-food industrial sectors to improve processes and decrease energy usage. For example acrylamide is made from acrylonitrile using nitrile hydratase. The organism *Rhodococcus rhodochrous* J1 was directed to overexpress the enzyme nitrile hydratase. This enzyme efficiently converts acrylonitrile into acrylamide under mild conditions and offers an improvement over more traditional techniques.

The conventional method of producing glycolic acid involved reacing formaldehyde with carbon monoxide over an acid catalyst at high temperature and pressure. Enzymes have offered a more mild alternative. E. Coli can be made to overexpress nitrilase, which, when combined with other enzymes such as lactoaldehyde reductase and lactoaldehyde dehydrogenase in a chain reaction provides an easier method for glycolic acid production.

Enzyme engineering

If whole organisms can't be used for an industrial process then it might require a particular enzyme structure and orientation. This is difficult to accomplish with traditional harvesting or chemical synthesis methods. Many times a specific enantiomer is required to improve the efficiency of a reaction, and it can be difficult to find a high proportion of a specific molecule in nature. However, with new directed evolution technologies it has become possible to develop designer enzymes by forcing mutations in the enzyme production processes of bacteria or yeast**.** These mutations sometimes produce an organism that is particularly useful for producing enzymes in industry**.** This process can improve organism and enzyme stability, substrate specificity, and enantioselectivity. Most industrial processes demand that an enzyme be highly specific to the substrate, and there is always room for improvement to the process.

Other industrial application of enzymes in industry

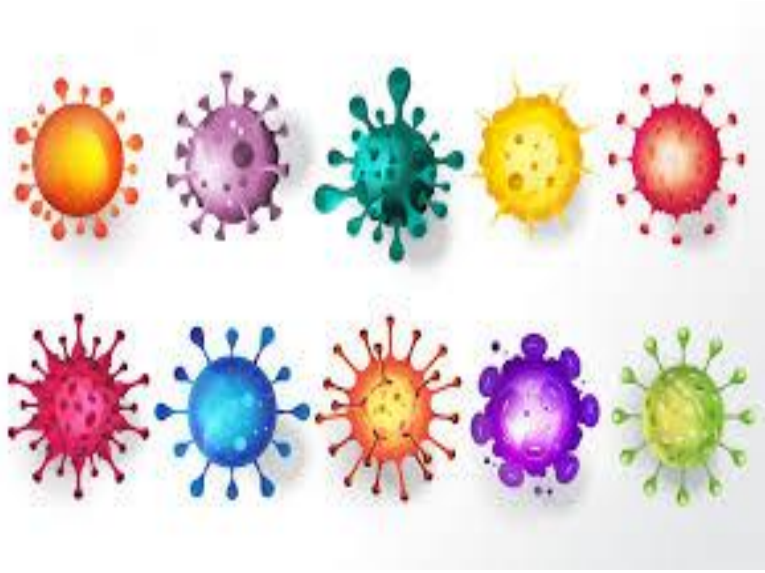
Other industrial application of enzymes in industry include lipase, polyphenol oxidases, lignin peroxidase, horseradish peroxidase, amylase, nitrite reductase**,** and urease. Many of these enzymes are used for biosensors because of the specific affinity between a substrate and its enzyme. Others, such as horseradish peroxidase, are used for chemical detection of biomarkers in tissue.

Of course, we can't overlook the importance of enzymes in the food industry. Purified enzymes are essential for brewing beer, baking bread, making cheese, and extracting fruit juice. Cheesemaking is an age old tradition that requires surprisingly few ingredients: milk, bacteria, rennet, and salt. The bacterial culture is the source of flavor and texture. Rennet is an enzyme that breaks down the milk protein casein to form the cheese curd. The enzyme is naturally found in the stomach's of milk

drinking/producing animals, but fermentation-produced chymosin is sourced from plant, fungal, and microbial sources for industrial cheese-making purposes.

The quest for green technology is driving innovation in both the production of specific enzymes and in the use of enzymes already available. Whether it is in the form of using enzymes to make a new use of an old renewable energy source, or simply eliminating the need for extreme temperatures and pressures to synthesize a product, enzymes are an increasingly important component of green energy technologies. Our ability to create designer enzymes will push these molecules to the forefront of many industrial processes including food, drugs, cosmetics, plastics, and much more in the immediate future.

Virology



Viruses constitute a group of infectious agents which are characterized by their ability to produce several diseases in man, animals and plants.

Definition of Viruses:

Viruses are very small particles, ultramicroscopic, so they are not seen by ordinary microscope. They are obligate intracellular agents which can replicate only in the living susceptible cells because they depend on cell metabolites in their growth.

Virion: Complete virus particle.

VIRUS HISTORY

In 1884 C. Chamberland, in Pasteur's lab, discovered that if you passed a liquid containing bacteria through an unglazed PORCELAIN tube, the bacteria were **COMPLETELY RETAINED** and the solution that passed through (the **FILTRATE**) was sterile. The advantages of this tool were immediately apparent, for with it one could sterilize solutions containing heat-sensitive components by filtration through sterile porcelain tubes into sterile containers. By carefully controlling the components of the porcelain tubes you could **CONTROL THE PORE SIZE** and selectively remove larger organisms while letting smaller ones pass through.

This type of filtration immediately became one means of testing the Germ Theory, since if you passed an infected sample through a filter that would hold back all microbes, the filtrate should not induce the disease in a new host if a microbe was responsible. You could then begin to devise ways of growing the suspected pathogen. However, in 1892 D. IWANOWSKI applied this test to a filtrate of plants suffering from **TOBACCO MOSAIC DISEASE** with shocking results; the filtrate was FULLY CAPABLE of producing the **ORIGINAL DISEASE** in new hosts. When

repeated, filtrations produced the same results and nothing could be seen in the filtrates using the most powerful microscopes, nor could anything be cultivated from the filtrates, Iwanowski and associates concluded that they had discovered a new pathogenic life-form which they called by the unimaginative, but functional, name of "**FILTERABLE VIRUS**". We now know that viruses range in size from 20 nm (10^{-9} meters) to 250 nm.

By the early 1900 diseases like foot-mouth-disease in cattle, some cancers (in animals) and yellow fever in humans had been demonstrated to be caused by filterable viruses. The scientific community knew that it had a new group of dangerous pathogens to contend with. The term "**VIRUSES**" became permanently associated with this life form. You have previously seen that bacterial viruses or bacteriophage (phage) were discovered in 1915 & 1917. Viruses, however were not "seen" until the electron microscope was developed in the late 1930s. This site contains electron micrographs of many bacteriophages.

We now know that viruses exist that attack perhaps every form of cellular life on this planet. I haven't seen references to thermophilic phage, but I would be surprised if they didn't exist. We are discovering new viruses all the time and most virologists feel we have only scratched the surface of viral variety. For example, when sea water is concentrated and examined under the electron microscope it teems with **VIRUS-LIKE PARTICLES** and we have no idea what they are or where they come from or what their hosts are?

The nature of viruses became even more confusing when it was observed in 1935 that they could be **CRYSTALLIZED** like inorganic salts (table salt) and protein molecules. This observation started a spirited, but rather barren, argument as to whether viruses are really "alive" or a "form of

life". People have argued that viruses are like salt crystals that grow and reproduce (sort of). In my view this discussion is a waste of time by people who need to "get-a-life". Viruses clearly **REPLICATE** their genetic material, which like that of all other life forms, is composed of nucleic acid polymers. Viruses have one major characteristic in common: they are **OBLIGATE INTRACELLULAR PARASITES**. Viruses are **UNABLE** to grow and reproduce **OUTSIDE OF A LIVING CELL**. Therefore their survival is absolutely dependent upon the continued survival of their hosts. This poses an interesting dilemma for pathogens that often as not kill their hosts, wouldn't you say?

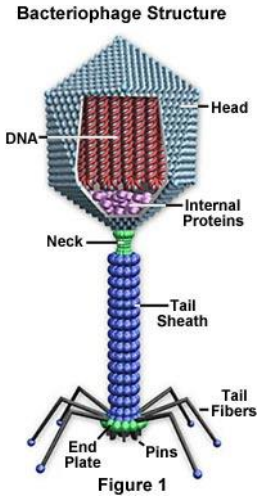


Figure 1

T-EVEN PHAGE. This is a large bacteriophage. It happens to be one of the most complex viruses. Not all phage are large; some are composed of only 7 genes. This is an *E. coli* phage and it has been studied intensely and much is known about it.

The intracellular nature of viruses presents a challenge for the investigator who must not only grow the virus but also be able to cultivate the virus' host cell. With plant and bacterial viruses it was possible to extract sufficient virus from an infected host to do analysis on it. These studies showed that viruses were mainly **COMPOSED OF PROTEIN AND**

NUCLEIC ACID. With multicellular eukaryotic viruses the field of virus investigation could only move as rapidly as the advancements in eukaryotic **TISSUE CULTURING**. The first breakthrough in this problem came with the discovery in 1931 that the fertilized hen's eggs could serve as a "petri dish" for some viruses. This capacity led to the first use of artificially cultivated viruses for vaccine production. Even today many viruses are grown on eggs because they are relatively inexpensive and because the techniques are so well established.

Introduction

A **virus** is a microscopic particle that can infect the cells of a biological organism. Viruses can only replicate themselves by infecting a host cell and therefore cannot reproduce on their own. At the most basic level, viruses consist of genetic material contained within a protective protein coat called a capsid. They infect a wide variety of organisms: both eukaryotes (animals, yeasts, fungi and plants) and prokaryotes (bacteria). A virus that infects bacteria is known as a *bacteriophage*, often shortened to *phage*. The study of viruses is known as virology, and those who study viruses are known as virologists. The word virus comes from the Latin, *poison* (syn. *venenum*).

It has been argued extensively whether viruses are living organisms. Most virologists consider them non-living, as they do not meet all the criteria of the generally accepted definition of life. They are similar to obligate intracellular parasites as they lack the means for self-reproduction outside a host cell, but unlike parasites, viruses are generally not considered to be true living organisms. A definitive answer is still elusive because some organisms considered to be living exhibit characteristics of both living and non-living particles, as viruses do. For those who consider viruses

living, viruses are an exception to the cell theory proposed by Theodor Schwann, as viruses are not made up of cells.

Viruses are smaller and less complex than bacteria. As science became aware of the role of the viruses in human disease, the techniques of bacteriology were modified to accommodate the viruses and the discipline of virology grew up within bacteriology. Because of this, we will begin this unit on viruses with bacteriophages, the viruses that infect bacterial cells. Animal viruses will be dealt with separately. But the lessons learned from the replication events of the bacteriophages will be directly applied to understanding the replication of viruses such as Herpes and HIV.

Viruses are the cause of many diseases in humans ranging from AIDS and cancer to the common cold. Microbiologists have developed vaccines for many viral diseases, but haven not been as successful in discovery of treatments for the diseases. It is the opposite in bacteriology, at least since the discovery of antibiotics. We have generally been able to treat bacterial disease, but besides the toxoid vaccines, vaccination against bacterial diseases has been hit-and-miss.

Size

To put viral size into perspective, a medium sized virion next to a flea is roughly equivalent to a human next to a mountain twice the size of Mount Everest. Some filoviruses have a total length of up to 1400 nm, however their capsid diameters are only about 80 nm. The majority of viruses which have been studied have a capsid diameter between 10 and 300 nanometres. While most viruses are unable to be seen with a light microscope, some are as large or larger than the smallest bacteria and can be seen under high optical magnification. More commonly, both scanning

and transmission electron microscopes are used to visualise virus particles.

A notable exception to the normal viral size range is the recently discovered mimivirus, with a diameter of 750 nm which is larger than a *Mycoplasma* bacterium. They also hold the record for the largest viral genome size, possessing about 1000 genes (some bacteria only possess 400) on a genome approximately 1.2 megabases in length. Their large genome also contains many genes which are conserved in both prokaryotic and eukaryotic genes. The discovery of the virus has led many scientists to reconsider the controversial boundary between living organisms and viruses, which are currently considered as mere mobile genetic elements.

The important differences between viruses and other unicellular organisms:

1- Viruses contain one type of nucleic acid (NA), either DNA or RNA.

2- The NA of viruses presents centrally and covered by a protein coat which acts as a protective agent.

3- Viruses have no metabolic activity of their own as well as they lack enzyme systems and other constituents needed for independent growth and multiplication.

4- They lack Ribosome and transfer RNA.

5- They multiply by special replication cycle i.e. reproduced solely from their N.A by a complicated process of biosynthesis.

6- Viruses grow only in living tissue, not in artificial media.

7- Viruses are resistant to antibiotics, because they are metabolically inert (lack metabolic enzymes).

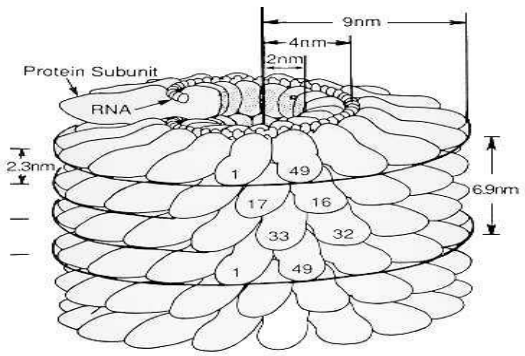
8- Most viruses are susceptible or sensitive to interferon.

9- Some viruses are able to induce latent infections by the integration of their NA with the DNA of the infected cells.

Morphology

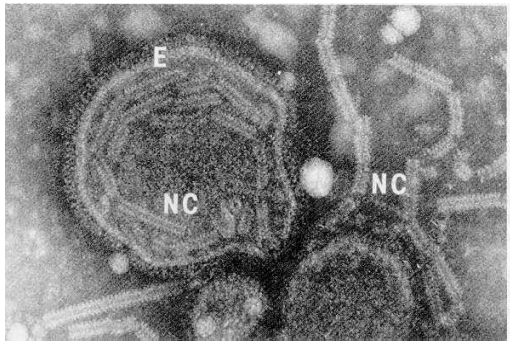
Helical Symmetry

In the replication of viruses with helical symmetry, identical protein subunits (protomers) self-assemble into a helical array surrounding the nucleic acid, which follows a similar spiral path. Such nucleocapsids form rigid, highly elongated rods or flexible filaments; in either case, details of the capsid structure are often discernible by electron microscopy. In addition to classification as flexible or rigid and as naked or enveloped, helical nucleocapsids are characterized by length, width, pitch of the helix, and number of protomers per helical turn. The most extensively studied helical virus is tobacco mosaic virus (Fig. 3). Many important structural features of this plant virus have been detected by x-ray diffraction studies. Figure 4 shows Sendai virus, an enveloped virus with helical nucleocapsid symmetry, a member of the paramyxovirus family.



The helical structure of the rigid tobacco mosaic virus rod.

About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). Each turn contains a nonintegral number of subunits (16-1/3), producing a pitch of 2.3 nm. The RNA (2x1O6 Da) is sandwiched internally between adjacent turns of capsid protein, forming a RNA helix of the same pitch, 8 nm in diameter, that extends the length of virus, with three nucleotide bases in contact with each subunit. Some 2,130 protomers per virion cover and protect the RNA. The complete virus is 300 nm long and 18 nm in diameter with a hollow cylindrical core 4 nm in diameter.



Fragments of flexible helical nucleocapsids (NC) of Sendai virus, a paramyxovirus, are seen either within the protective envelope (E) or free, after rupture of the envelope.

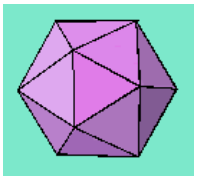
The intact nucleocapsid is about 1,000 nm long and 17 nm in diameter; its pitch (helical period) is about 5 nm. (x200,000) (courtesy of A. Kalica, National Institutes of Health.)

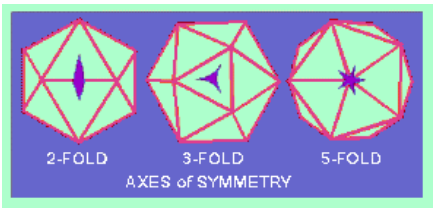
An ICOSAHEDRON

Is composed of 20 facets, each an equilateral triangle, and 12 vertices, and because of the axes of rotational symmetry is said to have **5:3:2 symmetry**

Axes of Symmetry

There are, in fact, six 5-fold axes of symmetry passing through the vertices, ten 3-fold axes extending through each face and fifteen 2-fold axes passing through the edges of an icosahedron.





In an attempt to clarify the **terminology for virus components,** Caspar *et al.* (1962) made a number of proposals which were generally accepted. Briefly, the proposals are as follows:

1. The **CAPSID** denotes the protein shell that encloses the nucleic acid. It is built of structure units.
2. **STRUCTURE UNITS** are the smallest functional equivalent building units of the capsid.
3. **CAPSOMERS** are morphological units seen on the surface of particles and represent clusters of structure units.
4. The capsid together with its enclosed nucleic acid is called the **NUCLEOCAPSID**.
5. The nucleocapsid may be invested in an **ENVELOPE** which may contain material of host cell as well as viral origin.
6. The **VIRION** is the complete infective virus particle

General Features of Viruses

1. small size

cannot be viewed with a light microscope

pass through filters that retain bacteria

range of sizxe = 0.1-0.3 micrometers

2. characteristic shapes - spherical (complex), helical, rod or polyhedral, sometimes with tails or envelopes. Most common polyhedron is the icosahedron which as 20 triangular faces.

3. obligate intracellular parasites Viruses do not contain within their coats the machinery for replication. For this they depend upon a host cell and this accounts for their existence as obligate intracellular parasites. Each virus can only infect certain species of cells. This refers to the virus **host range**.

4. **no built-in metabolic machinery** Viruses have no metabolic enzymes and cannot generate their own energy.

5. **no ribosomes** Viruses cannot synthesize their own proteins. For this they utilize host cell ribosomes during replication. Features 4 and 5 account for the obligate intracellular parasitism of viruses.

6. only one type of nucleic acid Viruses contain either DNA or RNA (never both) as their genetic material. The nucleic acid can be single-stranded or double stranded.

7. **do not grow in size** Unlike cells, viruses do not grow in size and mass leading to a division process. Rather viruses grow by separate synthesis and assembly of their components resulting in production of a "crop" of mature viruses.

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.

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.

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):

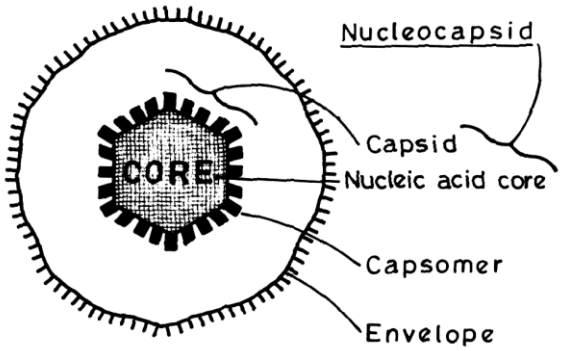
This may be either DNA or RNA. Most of DNA viruses are double stranded except parvo viruses which are single stranded, while most RNA viruses are single stranded except reoviruses and birnaviruses.

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.



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.

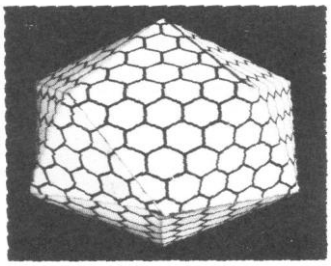
There are 3 forms of virus symmetry:

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.

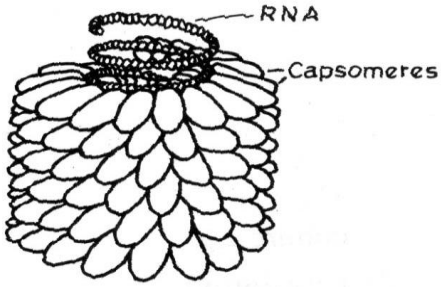
B) Helical symmetry → the capsid is helical in structure e.g. myxo viruses

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.



Icosahedral viral symmetry



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:

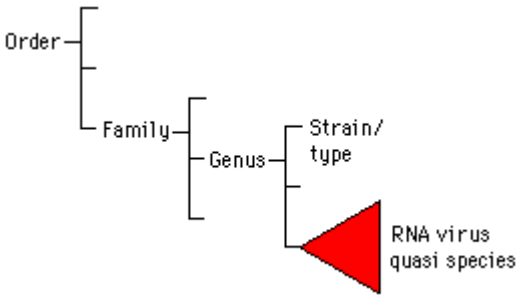
Have no effect on viruses i.e. viruses are resistant to antibiotics because they have no metabolic activity.

Viral Classification and Replication

How are viruses classified?

Hierarchical virus classification:

order - family - subfamily - genus - species - strain/type



At the moment classification is really only important from the level of families down. All families have the suffix **viridae** e.g.

- Poxviridae
- Herpesviridae
- Parvoviridae
- Retroviridae

Members of the family Picornaviridae are generally transmitted via the faecal/oral and airborne routes.

Genera have the suffix **virus**. Within the Picornaviridae there are 5 genera:

- enterovirus (alimentary tract) species e.g. poliovirus 1, 2, 3
- cardiovirus (neurotropic) species e.g. mengovirus
- rhinovirus (nasopharyngeal region) species e.g. Rhinovirus 1a

- apthovirus (cloven footed animals) species e.g. FMDV-C
- hepatovirus (liver) species e.g. Hepatitits A virus

The definition of `species' is the most important but difficult assignment to make with viruses. There is an element of subjectivity about it. Consider the lentivirus genus, it is known to contain many different species including the following:

- HIV-1, Human Immunodeficiency Virus 1
- HIV-2, Human Immunodeficiency Virus 2
- SIV, Simian Immunodeficiency Virus
- FIV, Feline Immunodeficiency Virus
- BIV, Bovine Immunodeficiency Virus
- Visna (sheep)
- EIAV (horses)
- CAEV (goats)

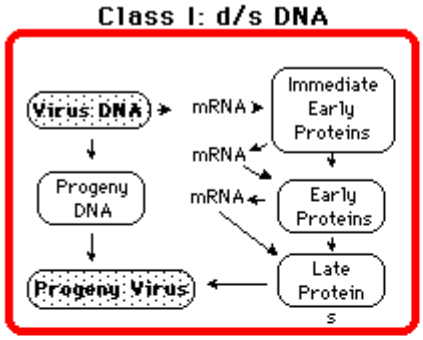
But there are viruses that are intermediate between HIV and SIV. Should these be a different species or included with HIV or SIV and if so which? HIV-1 has many different strains with different properties, some infect brain cells, others infect macrophage cells. When do strains diverge far enough to become separate species? HIV and similar retroviruses have been called quasispecies because any one individual although infected with a particular strain of HIV actually carries an enormous number (10^{15}) of different viral genome sequences. Such variation is extreme but is a feature of viruses with an RNA genome.

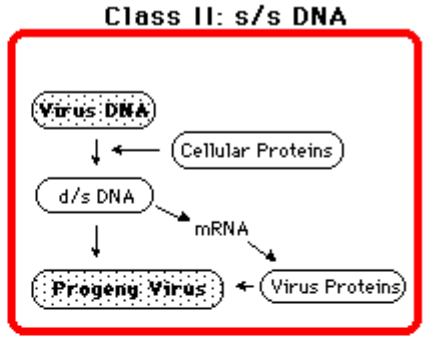
Basis of Taxonomic Classification.

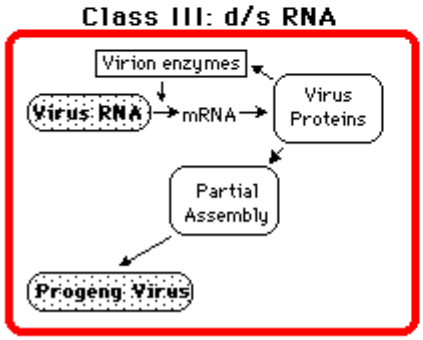
Features such as morphology (size, shape, enveloped/ unenveloped), physicochemical properties (molecular mass, pH, thermal, ionic stability), genome (RNA, DNA, segmented sequence, restriction map, modifications etc), macromolecules (protein composition and function), antigenic properties, biological properties (host range, transmission tropism etc) are all considered.

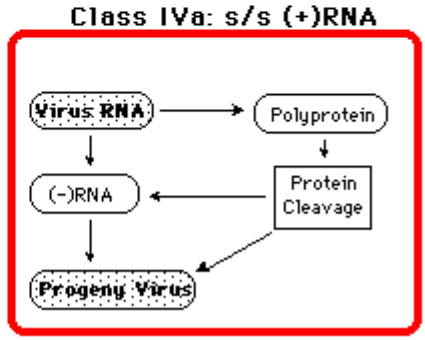
The Baltimore Classification

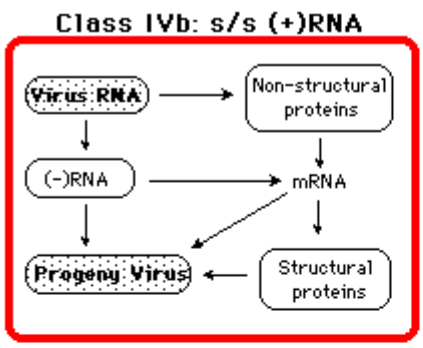
By convention the top strand of coding DNA written in the 5' - 3' direction is + sense. mRNA sequence is also + sense. The replication strategy of the virus depends on the nature of its genome. Viruses can be classified into seven (arbitrary) groups:

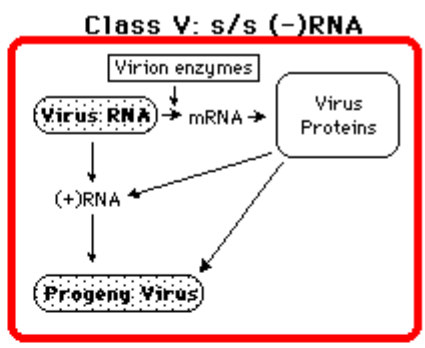

Class I: d/s DNA
Virus DNA
mRNA
Immediate Early Proteins
mRNA
Progeny DNA
mRNA
Early Proteins
Progeny Virus
Late Protein
s

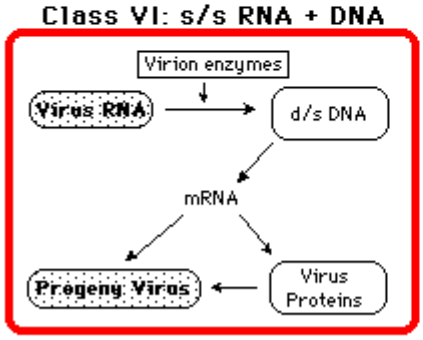

Class II: s/s DNA
Virus DNA
Cellular Proteins
d/s DNA
mRNA
Progeny Virus
Virus Proteins

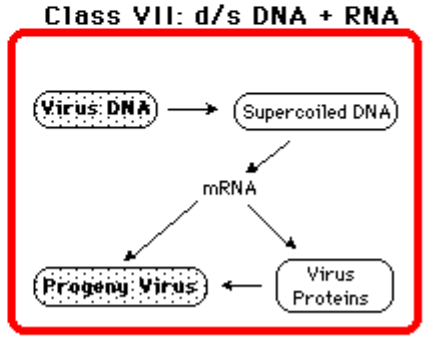

Class III: d/s RNA
Virion enzymes
Virus RNA
mRNA
Virus Proteins
Partial Assembly
Progeny Virus


Class IVa: s/s (+)RNA
Virus RNA
Polyprotein
(-)RNA
Protein Cleavage
Progeny Virus


Class IVb: s/s (+)RNA
Virus RNA
Non-structural proteins
(-)RNA
mRNA
Progeny Virus
Structural proteins


Class V: s/s (-)RNA
Virion enzymes
Virus RNA
mRNA
Virus Proteins
(+)RNA
Progeny Virus


Class VI: s/s RNA + DNA
Virion enzymes
Virus RNA
d/s DNA
mRNA
Progeny Virus
Virus Proteins


Class VII: d/s DNA + RNA
Virus DNA
Supercoiled DNA
mRNA
Progeny Virus
Virus Proteins

I: Double-stranded DNA (Adenoviruses; Herpesviruses; Poxviruses, etc)

Some replicate in the nucleus e.g. adenoviruses using cellular proteins. Poxviruses replicate in the cytoplasm and make their own enzymes for nucleic acid replication.

II: Single-stranded (+)sense DNA (Parvoviruses)
Replication occurs in the nucleus, involving the formation of a (-)sense strand, which serves as a template for (+)strand RNA and DNA synthesis.

III: Double-stranded RNA (Reoviruses; Birnaviruses)
These viruses have segmented genomes. Each genome segment is transcribed separately to produce monocistronic mRNAs.

IV: Single-stranded (+)sense RNA (Picornaviruses; Togaviruses, etc)

a) Polycistronic mRNA e.g. Picornaviruses; Hepatitis A. Genome RNA = mRNA. Means naked RNA is infectious, no virion particle associated polymerase. Translation results in the formation of a polyprotein product, which is subsequently cleaved to form the mature proteins.

b) Complex Transcription e.g. Togaviruses. Two or more rounds of translation are necessary to produce the genomic RNA.

V: Single-stranded (-)sense RNA (Orthomyxoviruses, Rhabdoviruses, etc)

Must have a virion particle RNA directed RNA polymerase.
a) Segmented e.g. Orthomyxoviruses. First step in replication is

transcription of the (-)sense RNA genome by the virion RNA-dependent RNA polymerase to produce monocistronic mRNAs, which also serve as the template for genome replication.
b) Non-segmented e.g. Rhabdoviruses. Replication occurs as above and monocistronic mRNAs are produced.

VI: Single-stranded (+)sense RNA with DNA intermediate in life-cycle (Retroviruses)

Genome is (+)sense but unique among viruses in that it is **DIPLOID**, and does not serve as mRNA, but as a template for reverse transcription.

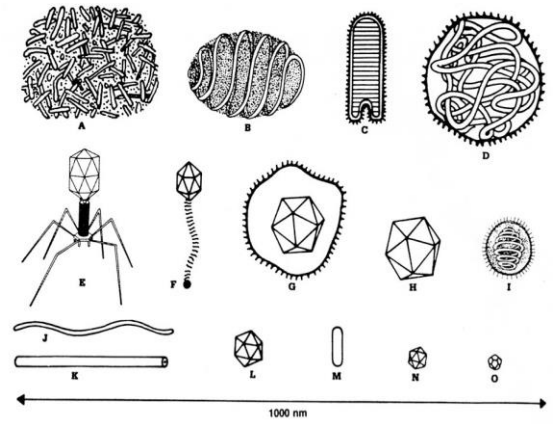
VII: Double-stranded DNA with RNA intermediate (Hepadnaviruses)
This group of viruses also relies on reverse transcription, but unlike the Retroviruses, this occurs inside the virus particle on maturation. On infection of a new cell, the first event to occur is repair of the gapped genome, followed by transcription.

Classification of Viruses according to host range.

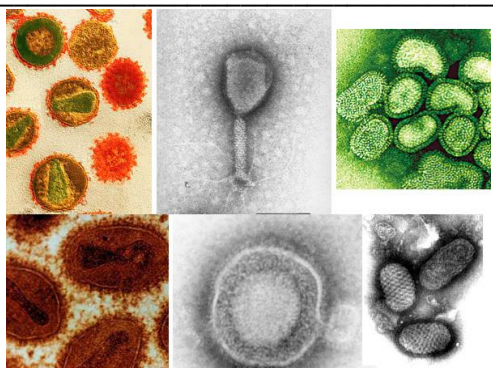
Viruses are classified on the basis of **host range** (see below), morphology (size, shape), type of nucleic acid (DNA, RNA, single-stranded, double-stranded, linear, circular, segmented, etc.) and occurrence of auxilliary structures such as tails or envelopes.

Host range refers to the type of cell in which the virus is able to replicate. In its **broadest sense** host range arranges viruses into four groups: bacterial viruses (bacteriophage), animal viruses, insect viruses (bacculoviruses) and plant viruses. However, viruses in archaea, protista, yeast, molds and fungi have also been described. In a **narrower sense**, host range may be defined by specific species that are infected by the

virus. Thus, each bacterial virus only infects certain species of bacteria; each animal virus only infects certain species of animals; and so on. In a more **limited sense**, when a virus infects a multicellular organism, it usually infects only a certain type of cell in the organism. Hence, the rhinoviruses which cause the common cold only infect cells of the upper respiratory tract, and the human immunodeficiency virus (HIV) only infects primarily a specific type of cell (CD4+ cells) of the human immune system.



Comparative size and shape of various groups of viruses representing diversity of form and host range. A. Smallpox virus B. Orf virus C. Rhabdovirus D. Paramyxovirus E. Bacteriophage T2 F. Flexuous-tailed bacteriophage G. Herpes virus H. Adenovirus I. Influenza virus J. Filamentous flexuous virus K. Tobacco mosaic virus L. Polyoma/papilloma virus M. Alflafa mosaic virus N. poliovirus O. Bacteriophage phiX174. Viruses have fundamentally three morphologies: 1. polygonal, the most common polygon being the icosahedron (E, F, G, H, L, N); 2. helical, wherein the capsomeres assemble as a helix enclosing the nucleic acid) (D, I, J, K, M; B is controversial); 3. complex, wherein the proteins are laid down in patches or layers (A). Some animal viruses have envelopes which enclose their nucleocapsid (D, G, I). The envelopes are embedded with viral proteins that secre their entry and exit in cells. Only bacteriophages have tails which are used for adsorption and penetration of their host cell.



Gallery of electron micrographs of viruses illustrating diversity in form and structure.

Clockwise: Human immunodeficiencyvirus (HIV); Aeromonas virus 31, Influenza virus, Orf virus, Herpes simplex virus (HSV), Smallopx virus.

Virus Multiplication

The One-Step Growth Curve:

The one-step growth curve is a representation of the overall change, with time, in the amount of infectious virus in a single cell that has been infected by a single virus particle. The one-step growth curve begins with the eclipse period, which is followed by a period of exponential growth

A. Eclipse period:

*It is the time elapsed from initial entry and disassembly of the parental virus to the assembly of the first progeny virion.

*In this period:

- The virus can not be detected in the host cells.
- The ability of the virus to infect other cells disappears.
- Active synthesis of virus components is occurring.

*For most of human viruses it ranges from 1-20 hrs.

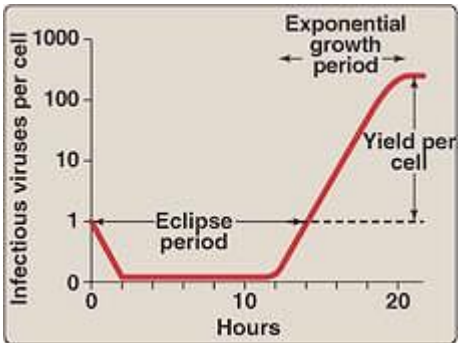
B. Exponential growth:

The number of progeny virus produced within the infected cell increases exponentially for a period of time, then reaches a plateau, after which no additional increase in virus yield occurs.

The maximum yield per cell is characteristic for each virus-cell system, and reflects the balance between:

1) The rate at which virus components continue to be synthesized and assembled into virions, and

2) The rate at which the cell loses the synthetic capacity and structural integrity needed to produce new virus particles. This may be from 8 to 72 hours or longer, with yields of 100 to 10,000 virions per cell.



One-step growth curve of a single cell infected with a single virus.

Virus Multiplication:

It is the ability of the virus to invade a susceptible host cell and multiply intracellularly within the host, with subsequent escape into the

external environment. The virus is an obligatory cell parasite. Unlike bacteria, viruses contain no metabolic enzymes for the synthesis of their constituents; therefore depend on the cellular pool to supply them with nucleotides, amino acids, enzyme and energy.

The interaction of the viruses with the host cell comprises a cycle which includes:
(1) Adsorption.
(2) Penetration and uncoating.
(3) Eclipse.
(4) Synthesis of new viral components.
(5) Assembly.
(6) Release of new virus from the cell.

(1) Adsorption (recognition and attachment):

It is the 1st step in virus replication. The essential features of adsorption process include: Collision, ionic attraction, and attachment.

A-Collision:

It is a random movement and meeting of the virus particles with the host cell and is dependent on the relative concentration of virus particles and cells and on environmental conditions as temperature, ionic condition (Presence of electrolytes) and the pH.

B-Ionic Attraction:

Both virus particles and cells are negatively charged at pH 7 a positive ions are therefore required as counter ions and this is met most efficiently by magnesium and sodium ions.

C-Attachment:

It involves the actual binding of virus particles to the surface of the host cells depending on the presence of specific receptors on both virus particles and cells. Absence of receptors is accompanied by failure of virus to adsorb to cells which may be an important factor in non susceptibility of host cells (i.e. the presence of receptors determines the cell tropism and viral pathogenicity).

Receptors for some viruses:

Virus	Receptor	Biological Function or Type of Molecule
Adeno-associated virus	Heparan sulfate	Glycosaminoglycan; part of extracellular matrix
Adenovirus (subgroups A, C-F)	CAR (Coxsackie and adenovirus receptor); also used by several coxsackie viruses	Immunoglobulin (Ig)-like
Epstein-Barr virus	CD21 (aka CR2, complement receptor 2)	Ig-like; complement receptor
Herpes simplex virus	Heparan sulfate. Also used by AAV, Dengue, others	Glycosaminoglycan; part of extracellular matrix
HIV-1	CD4. Also used by human herpesvirus 7.	Ig-like; role in helper T cell function.
Influenza virus	Sialic acid. Also used by reo-, corona- virus.	Carbohydrate
Measles virus	SLAM. Some vaccine strains can use CD46; this is also used by human herpesvirus 6.	T/B cell surface protein; involved in cellular activation
Poliovirus	Pvr (poliovirus receptor, aka CD155)	Ig-like
Rabies virus	Acetylcholine receptor, NCAM (CD56)	Neuronal receptor or adhesion molecule
Rhinovirus (major subtypes)	ICAM-1 (intercellular adhesion molecule 1); also used by some coxsackieviruses.	Ig-like; role in cell adhesion.
SV40	MHC-1 (major	Antigen presentation

	histocompatibility complex type 1)	
Vaccinia virus	EGF receptor (epidermal growth factor)	Growth factor receptor

(2) Penetration and uncoating:

Penetration: is the 2nd step in virus replication in which there is a transmission of the virus particle or the N.A from the surface of the infected cell to the interior of the cells. This may occur by one of the following methods:

1- Viropexis (Pinocytosis or endocytosis):

Occurs mainly in non enveloped viruses in which the virus particles are engulfed by the cell membrane and drown into the phagocytic vacuoles of the cell (endosomes). Release of the virus into the cytoplasm involves many mechanisms that are mainly facilitated by one or more of viral molecules. Failure of release of the virus from the endosome before fusion with lysosomes leads to degradation of the virus by lysosomal enzymes and abortion of infection.

2- Fusion:

The enveloped viruses undergo a preliminary fusion of their envelops with the plasma membrane prior to release of the nucleocapsid into the cytoplasm e.g. herpes viruses.

3- Direct penetration through the external plasma membrane:

E.g. bacteriophage:

Uncoating: means release of the infectious N.A from the viral coat in viruses penetrated by viropexis the process occur by the aid of lyzosomal enzymes which are present in the phagocytic vacuoles of the cytoplasm.

(3) Eclipse:

*It is the time elapsed from initial entry and disassembly of the parental virus to the assembly of the first progeny virion.

*In this period:

- The virus can not be detected in the host cells.
- The ability of the virus to infect other cells disappears.
- Active synthesis of virus components is occurring.

*For most of human viruses it ranges from 1-20 hrs.

(4) Synthesis of New Viral Components:

The site of viral synthesis varies according to the type of the N.A and structure of the virus.

Generally Most DNA viruses synthesize their DNA in the nucleus of the host cell, while the protein develops in the cytoplasm with the exception of pox virus which develops their DNA and protein in the cytoplasm.

On the other hand RNA viruses synthesize all viral components in the cytoplasm except orthomyxo and some of the paramyxoviruses and leukoviruses in which part of the cycle takes place in the nucleus.

It will be helpful to deal with the synthesis of new viral component in DNA and RNA viruses separately.

DNA Replication

The N.A of DNA viruses is replicated in the nucleus (except in pox virus is produced in the cytoplasm). In most DNA viruses the steps of replication involved in the production of new virions include:

1- Formation of Early Proteins:

a) Transcription of early genes: it is the part of viral genome transcribed prior to initiation of viral DNA synthesis to produce early mRNA by DNA dependent RNA polymerase (Transcriptase) which is a host cell enzyme.

b) Migration of this virus-induced m-RNA to the ribosomes in the cytoplasm where it is translated into virus-coded enzymes e.g. thymidine kinase and DNA polymerase and other "early proteins" which initiate and maintain the synthesis of new viral DNA.

2- Replication of Viral DNA:

By means of a virus coded DNA polymerase, other early proteins and host cell enzymes. The synthesis of new DNA usually begins 2-4 hours following infection and continues for some hours. (Adeno viruses do not begin for at least 10 hrs and continue for more than 8 hrs).

DNA enter the cell mostly as double stranded, separation of strands is followed by attraction of complementary bases A-T and G-C which are linked together by DNA polymerase forming double stranded DNA which also separate to form new copies.

NB: Single stranded DNA viruses utilize cell enzymes to synthesize complementary DNA strand to form ds DNA which is then transcribed to mRNA by the cellular DNA dependent UNA polymerase.

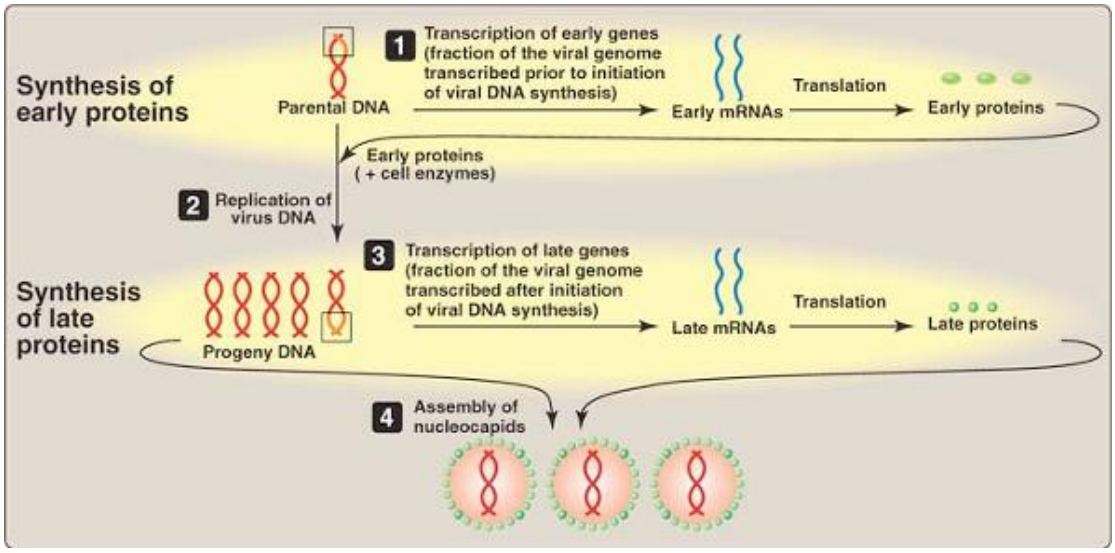
3- Formation of Late Protein:

By transcription of late genes which is the part of viral genome (both in progeny and parental DNA) transcribed after synthesis of viral DNA to form late mRNA, this is followed by translation of this late mRNA, into late proteins which include most of the structural proteins (capsid) for new progeny. The late proteins also regulate the production of additional early enzymes.

4- Maturation:

In most DNA viruses, it occurs in the nucleus except pox viruses in the cytoplasm.

Once NA and protein synthesis is initiated, assembly of the capsid takes place around the N.A molecule.



Replication of DNA

RNA Replication

The mRNA transcription varies according to the nature of the RNA in the virions.

1) ssRNA viruses of positive polarity (+ve sense) are sub-divided into two groups:

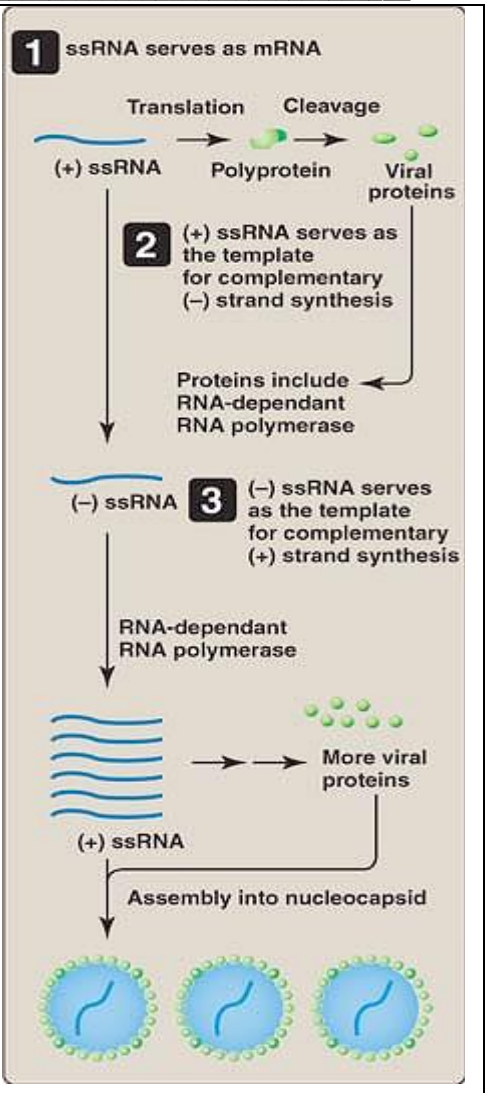
a) Polycistronic mRNA e.g. Picornaviruses; Hepatitis A.

Genome RNA = mRNA.

Translation results in the formation of a polyprotein product, which is subsequently cleaved to form the mature proteins.

b) Complex Transcription e.g. Togaviruses.

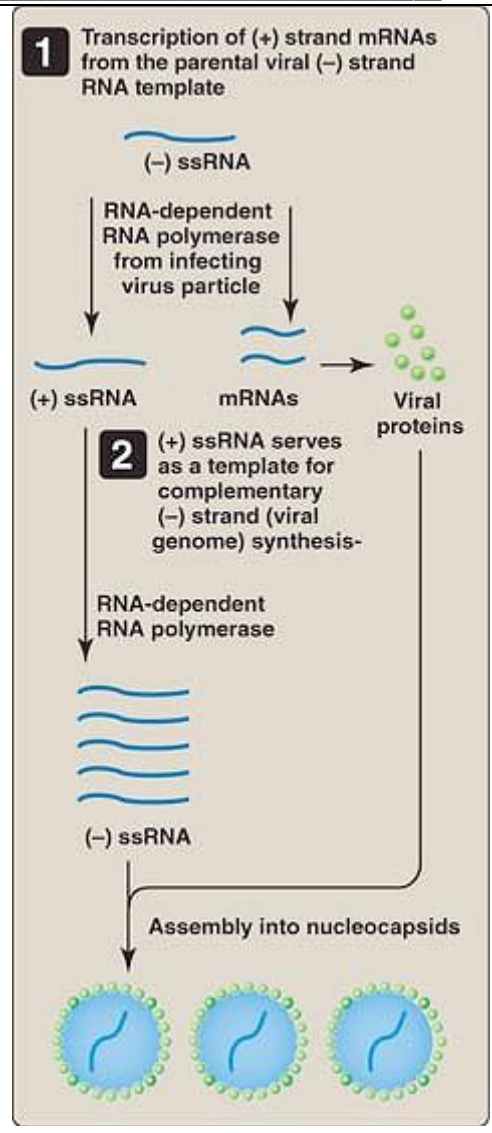
Two or more rounds of translation are necessary to produce the genomic RNA i.e. +ve sense ssRNA act as a template to form –ve sense ssRNA strand which in turn act as a template for synthesis of more +ve sense ssRNA .



Replication of +ve sense ssRNA

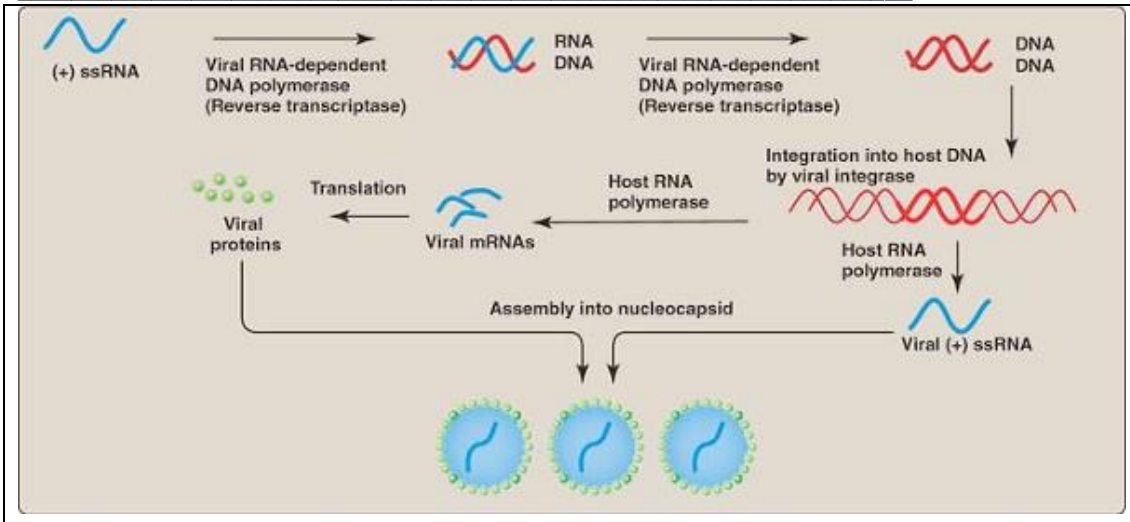
2- In ssRN A of negative polarity (-ve sense) must be transcribed by RNA dependent RNA polymerase which is present in the virus (this enzyme is not present in eukaryotic cells so they lack the ability to sensitize mRNA from RNA template) into complementary (positive sense) mRNA.

The genomic RNA is copied into DS RNA replicative intermediate. Its positive strand acts as a template for the synthesis of more negative strands for the progeny virus.



Replication of -ve sense ssRNA

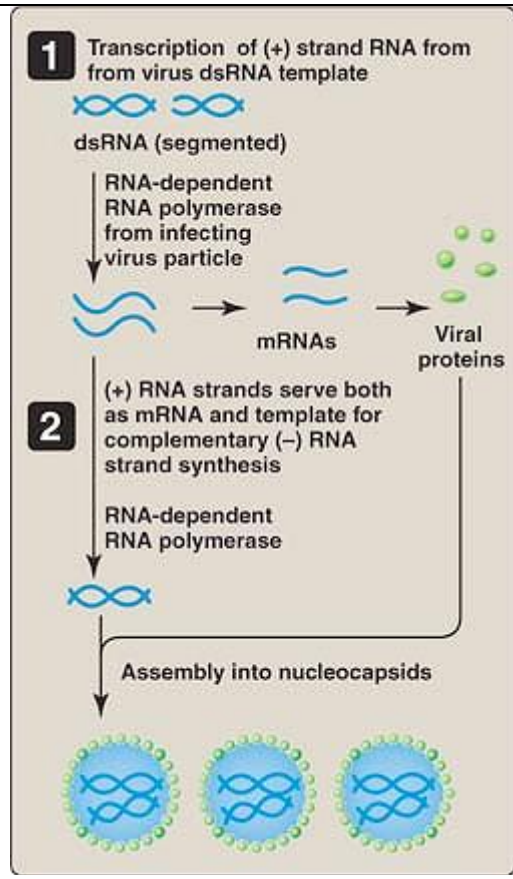
3- In ssRNA of retroviruses the parental viral RNA is transcribed by a virus-associated reverse transcriptase (RNA- dependent DNA polymerase) into an ssDNA copy, which form a DNA-RNA hybrid, the RNA strand is digested away and replaced by a DNA copy to give a dsDNA molecule. This is integrated into the chromosomal DNA of the host cell and is now termed proviral DNA. Viral mRNA is transcribed from the proviral DNA and viral proteins are synthesized.



(+) sense ssRNA genome that replicates via a DNA intermediate.

4- Ds RNA viruses (Reoviruses), the virus carry its own RNA dependent RNA polymerase; this enables the transcription of mRNA from one strand.

The mRNA is translated into viral proteins.



Ds RNA viruses

(5) Assembly:

Site of assembly:

It occurs in the nucleus in DNA viruses (except pox viruses) this requires transport of the virion proteins into the nucleus.

In RNA viruses and pox viruses it occurs in the cytoplasm.

Mechanism:

Capsid may be assembled as an empty structure (procapsid) to be later filled with NA (higher chance for errors). This occurs in icosahedral viruses. Capsid may be assembled around the genome from the start (less chance for error) this occurs in helical viruses.

Envelope acquisition occurs after association of the nucleocapsid with the host cell membranes either cell membrane or nuclear membrane to acquire the envelop from them this is determined by the type of viral genome. Most RNA viruses acquire their envelops from the cytoplasmic membrane while most DNA viruses acquire their envelops from the nuclear membrane.

(6) Release of Progeny Virions from the Cell:

Differ according to the type of virus:

1. Naked viruses:

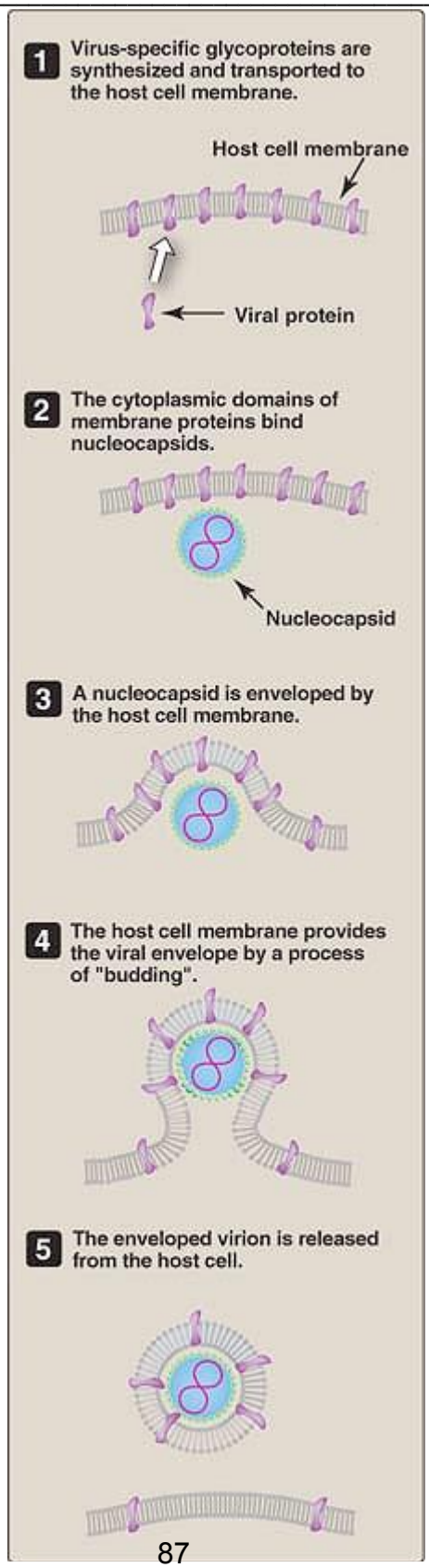
In naked (unenveloped) viruses, release of progeny is usually a passive event resulting from the disintegration of the dying cell and, therefore, may be at a relatively late time after infection.

2. Enveloped viruses:

The release of the enveloped viruses is by the budding process. A consequence of this mechanism of viral replication is that progeny viruses are released continuously.

3. cell-cell fusion (syncytia formation):

E.g. herpes virus, paramyxovirus.


1
Virus-specific glycoproteins are synthesized and transported to the host cell membrane.
Host cell membrane
Viral protein
2
The cytoplasmic domains of membrane proteins bind nucleocapsids.
Nucleocapsid
3
A nucleocapsid is enveloped by the host cell membrane.
4
The host cell membrane provides the viral envelope by a process of "budding".
5
The enveloped virion is released from the host cell.

Release of enveloped virus from a host cell by the process of budding.

Abnormal Replication of Viruses:

Sometimes viruses fail to replicate and their replication cycle may stop at any step during the replication cycle leading to abortive infection. This may be due to inability of the used cells for such replication (non permissive cells) or these viruses are defective and unable for replication.

During viral replication many defective rather than infectious particles are produced.

Viral Genetics:

The genetic studies of viruses are important for:

1-Mapping the location of genes on the viral genome (construction of genetic maps).

2-selection of appropriate mutants.

3-study of the function of gene products.

4-understanding of the recombinant DNA technology which provides an important tool for diagnosis, prevention and control of viral infection.

Mutation:

There are two principal types of virus mutants:

1-Point mutants in which there is a change in a single nucleotide base. The most important point mutants are the conditional lethal mutants.

2- Deletion in which a whole sequence or region of nucleic acid has been deleted.

Conditional-lethal Mutants:

Are mutants that are lethal under one set of conditions termed non permissive conditions but that yield normal infectious progeny under other conditions termed permissive conditions. They include:

1-Temperature-sensitive (ts):

The Ts mutants grow at low temperature (permissive) but not at high temperature (non permissive).

2-Host range (hr) mutants:

Host range mutants are able to grow and form plaques in one kind of cells (permissive), while abortive infection occur in another type (non permissive).

Plaque-size Mutants:

1-Small-plaque mutants:

Many virus strains give rise to spontaneous mutants that form smaller plaques than wild-type virus because their adsorption is inhibited by sulfated polysaccharides present in the agar.

2-Large-plaque mutants are also known.

Drug-resistant Mutants:
e.g. herpes virus mutants resistant to phosphor-mono-acetic acid
Enzyme-deficient Mutants:

Mutations that result in loss of the ability of viruses to encode several enzymes essential for their multiplication.

METHODS OF STUDY OF VIRUSES

Diagnosis of viral infections

Viruses can be studied in a number of direct and indirect ways and all these methods can be applied in a **diagnostic situation**, ie. is this patient infected with a particular virus? There are two approaches:

1. detection and demonstration of the virus itself; and
2. the study of the host's response to that virus

One of the earliest ways of detecting a virus was by inoculating a susceptible host (laboratory) animal with infectious material derived from a patient or sick animal and then observing that animal for signs of disease. Fertile hens eggs proved useful systems for a number of viruses (especially myxoviruses) and are still used for influenza.

Today, live animals are rarely used as **"in vitro" cell cultures** have largely replaced them.

In recent years "non-cultivable" viruses have been extensively studied by molecular techniques ("genetic engineering").

The structure of different viruses has been elucidated by a range of electron microscopy and x-ray crystallography techniques. Viruses amplified by growth in culture (or in a few special cases, directly from patient specimens without amplification) can be demonstrated by electron microscopy.

Viral antigens can be detected by a wide range of **serological techniques** utilising polyclonal or monoclonal antibodies. Techniques include

precipitation, agglutination, immunofluorescence, ELISA, complement fixation and radio immuno assays. These same techniques, utilising purified viral antigens, can be used to detect specific antibodies to those viruses in the patient's serum. Identification of different classes of antibodies (IgG and IgM) can aid in differentiating between a current infection and immunity.

Some viruses (eg. myxo- and paramyxoviruses, including influenza) have the property of **haemagglutination** (causing red blood cells to stick together) which can be used to detect and quantitate the virus (by haemagglutination) or specific antibodies to that virus (haemagglutination inhibition).

Similarly, **neutralisation** of viral infectivity by antibodies can be used to detect and quantify either virus or specific antibody to that virus.

Modern **molecular techniques** of both protein chemistry and nucleic acid biochemistry have greatly improved the specificity of virus diagnostic procedures. Methods include:-

- polyacrylamide gel electrophoresis (PAGE) of protein fragments
- western blotting, and identification of specific proteins with labelled probes
- polymerase chain reaction (PCR), to amplify specific segments of viral nucleic acid
- Southern blotting, and DNA hybridisation with labelled probes
- sequencing of portions of the viral genome
- restriction fragment length polymorphisms of viral nucleic acid

Applications

The application of sophisticated molecular technology has enabled the generation of diagnostic assays for viruses that have not yet been visualized or cultured. **Hepatitis C virus** is the prime example. This RNA virus has never been cultured, but portions of its genome were extracted from blood known to be infectious for hepatitis C. By means of adapted PCR techniques, the nucleotide sequence of the entire viral genome was eventually assembled. Knowing some gene sequences enabled biochemists to synthesise corresponding small portions of proteins (peptides). Some peptides were found to be major antigenic determinants of the virus and these peptides have now been incorporated into commercial ELISA tests designed todetect human antibodies to hepatitis C. The presence of antibodies has been shown to be associated with chronic hepatitis C infection and a high risk of transmitting hepatitis C in blood transfusions. As from 1993, blood transfusion services in South Africa routinely screen all blood donations for hepatitis C antibody.

Molecular biology methods have been used to compare degrees of relatedness of similar organisms and to build **phylogenetic trees** ("family trees" based on genomic similarities). The ability to detect and sequence portions of a viral genome permits genetic markers of specific sub-strains to be identified. This has led to the new science of **genetic epidemiology** (ie. disease tracing). For example, health authorities have been able to document the recent spread of the raccoon strain of **rabies** (as distinct from pre-existing skunk rabies) across the USA . On a global scale, the progression of different genetic strains (genotypes) of polio type 1 (not otherwise distinguishable) can now be followed from one country or continent to another, sometimes replacing pre-existing strains. The strain causing the last recorded polio outbreak in South Africa (Kwazulu, 1988)

was previously found in Zimbabwe and was probably imported from there.

ANIMAL VIRUSES

Replication of Animal Viruses

Outside its host cell a virus is an inert particle. However, when it encounters a host cell it becomes a highly efficient replicating machine. After attachment and gaining entry into its host cell, the virus subverts the biosynthetic and protein synthesizing abilities of the cell in order to replicate the viral nucleic acid, make viral proteins and arrange its escape from the cell. The process occurs in several stages and differs in its details among DNA-containing and RNA-containing viruses.

The Stages of Replication

1. The first stage in viral replication is called the **attachment (adsorption) stage**. Like bacteriophages, animal viruses attach to host cells by means of a complementary association between attachment sites on the surface of the virus and receptor sites on the host cell surface. This accounts for specificity of viruses for their host cells. Attachment sites on the viruses (usually called **virus receptors**) are distributed over the surface of the virus coat (capsid) or envelope, and are usually in the form of glycoproteins or proteins. Receptors on the host cell (called the **host cell receptors**) are generally glycoproteins imbedded into the cell membrane. Cells lacking receptors for a certain virus are resistant to it and cannot be infected. Attachment can be blocked by antibody molecules that bind to viral attachment sites or to host cell receptors. Since antibodies block the initial attachment of viruses to their host cells, the

presence of these antibodies in the host organism is the most important basis for immunization against viral infections.

2. The **penetration stage** follows attachment. Penetration of the virus occurs either by engulfment of the whole virus, or by fusion of the viral envelope with the cell membrane allowing only the nucleocapsid of the virus to enter the cell. Animal viruses generally do not "inject" their nucleic acid into host cells as do bacteriophages, although occasionally non enveloped viruses leave their capsid outside the cell while the genome passes into the cell.

3. Once the nucleocapsid gains entry into the host cell cytoplasm, the process of **uncoating** occurs. The viral nucleic acid is released from its coat. Uncoating processes are apparently quite variable and only poorly understood. Most viruses enter the host cell in an engulfment process called receptor mediated endocytosis and actually penetrate the cell contained in a membranous structure called an endosome. Acidification of the endosome is known to cause rearrangements in the virus coat proteins which probably allow extrusion of the viral core into the cytoplasm. Some antiviral drugs such as amantadine exert their antiviral effect my preventing uncoating of the viral nucleic acid.

4. Immediately following uncoating, the **viral synthesis stage** begins. Exactly how these events will unfold depends upon whether the infecting nucleic acid is DNA or RNA.

In DNA viruses, such as Herpes, the viral DNA is released into the nucleus of the host cell where it is transcribed into early mRNA for transport into the cytoplasm where it is translated into **early viral proteins**. The early viral proteins are concerned with replication of the

viral DNA, so they are transported back into the nucleus where they become involved in the synthesis of multiple copies of viral DNA. These copies of the viral genome are then templates for transcription into late mRNAs which are also transported back into the cytoplasm for translation into **late viral proteins**. The late proteins are structural proteins (e.g. coat, envelope proteins) or core proteins (certain enzymes) which are then transported back into the nucleus for the next stage of the replication cycle.

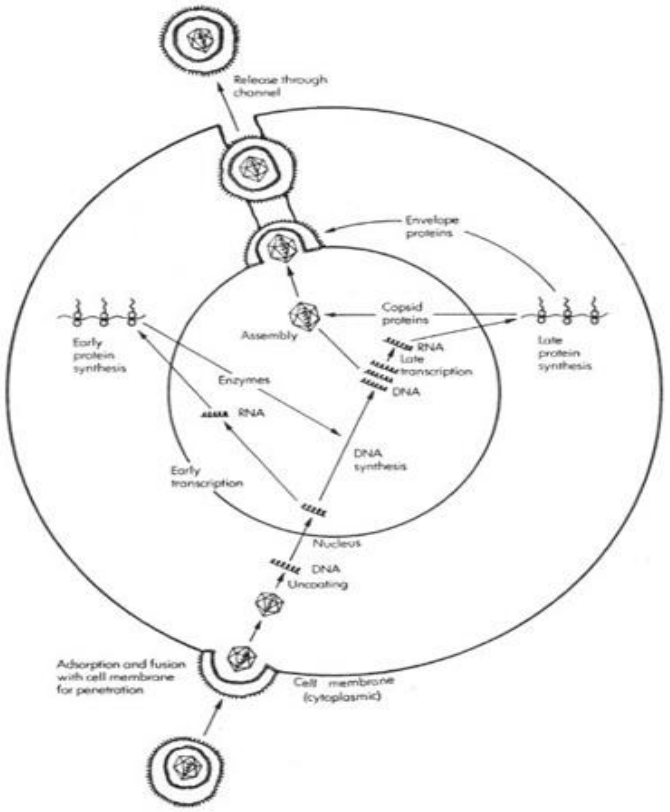
In the case of some RNA viruses (e.g. picornaviruses), the viral genome (RNA) stays in the cytoplasm where it mediates its own replication and translation into viral proteins. In other cases (e.g. orthomyxoviruses), the infectious viral RNA enters into the nucleus where it is replicated before transport back to the cytoplasm for translation into viral proteins.

5. Once the synthesis of the various viral components is complete, the **assembly stage** begins. The capsomere proteins enclose the nucleic acid to form the viral nucleocapsid. The process is called **encapsidation**. If the virus contains an envelope it will acquire that envelope and associated viral proteins in the next step.

6. The **release stage** is the final event in viral replication, and it results in the exit of the mature virions from their host cell. Virus maturation and release occurs over a considerable period of time. Some viruses are released from the cell without cell death, by **egestion**, whereas others are released when the cell dies and disintegrates. In the case of enveloped viruses, the nucleocapsid acquires its final envelope from the nuclear or cell membrane by a budding off process (**envelopment**) before **egress** (exit) out of the host cell. Whenever a virus acquires a membrane envelope, it always inserts specific viral proteins into the envelope which

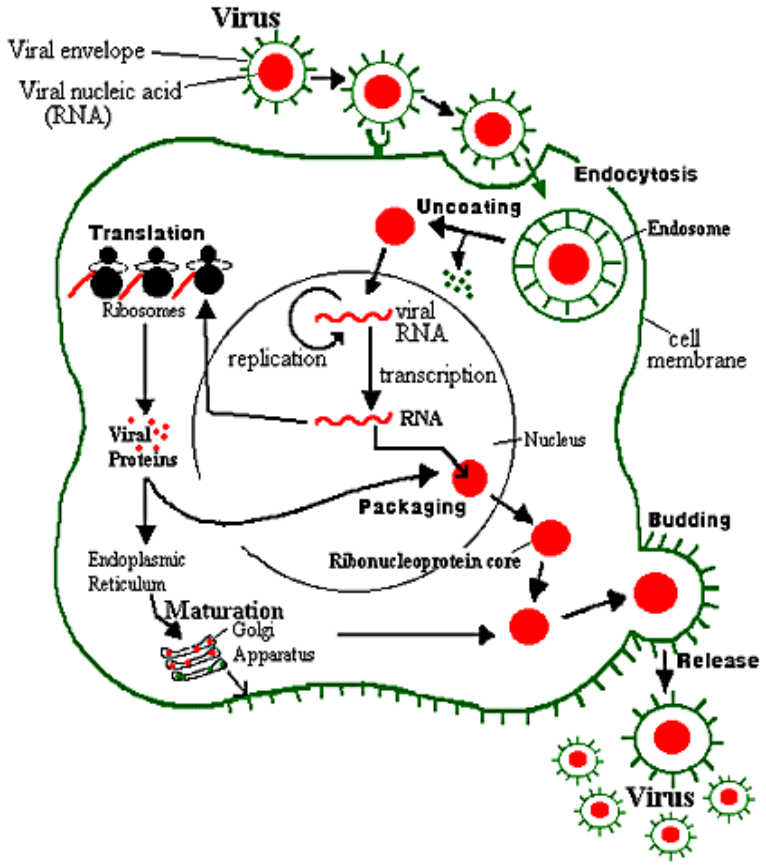
become unique viral antigens and which will be used by the virus to gain entry into a new host cell.

Below are illustrated the modes of replication of two viruses that conform to this model. Herpes simplex virus (HSV) is an enveloped, double stranded DNA virus; Influenza virus is an enveloped, single stranded (-) RNA virus that contains a segmented genome.



The replication cycle of Herpes Simplex virus. 1. Specific proteins in the viral envelope attach to host cell receptors on the cell membrane. 2. Penetration is achieved when the viral envelope fuses with the cell membrane releasing the nucleocapsid directly into the cytoplasm. 3. The virion is uncoated and the viral DNA is transported into the nucleus. 4. In the nucleus, the viral DNA is transcribed into early mRNAs which are transported to the cytoplasm for the translation of early proteins. These early proteins are brought back into the nucleus and participate in the replication of the virus DNA into many

copies. The viral DNA is then transcribed into the late mRNAs which exit to the cytoplasm for translation into the late (nucleocapsid and envelope) proteins. 5. The capsid proteins encapsidate the newly replicated genomes. The envelope proteins are imbedded in the nuclear membrane. 6. The nucleocapsids are enveloped by budding through the nuclear membrane, and the mature viruses are released from the cell through cytoplasmic channels.



The replication cycle of Influenza A Virus. The virus adsorbs to the cell surface by means of specific receptors. 2. The virus is taken up in a membrane enclosed endosome by the process of receptor mediated endocytosis. 3. Uncoating takes place in the endosome and the viral RNA (genome) is released into the cytoplasm. 4. The (-)RNA of the viral genome is transported into the nucleus where it is replicated and copied by a viral enzyme into (+)RNA which is both messenger RNA and serves as a templatee for more (-)RNA. The (+)RNA is transported into the cytoplasm for translation into early

and late viral proteins. 5. The viral core proteins are transported back into the nucleus to assemble as the capsid around the viral (-)RNA forming the "ribonucleoprotein core" or the genome-containing nucleocapsid of the virus. The viral envelope proteins assemble themselves in the cell membrane. 6. The nucleocapsid recognizes specific points on cell membrane where viral proteins have become inserted and buds off of the membrane to be released during enclosure in the viral envelope.

How Viruses Cause Disease

Their are several possible consequences to a cell that is infected by a virus, and ultimately this may determine the pathology of a disease caused by the virus.

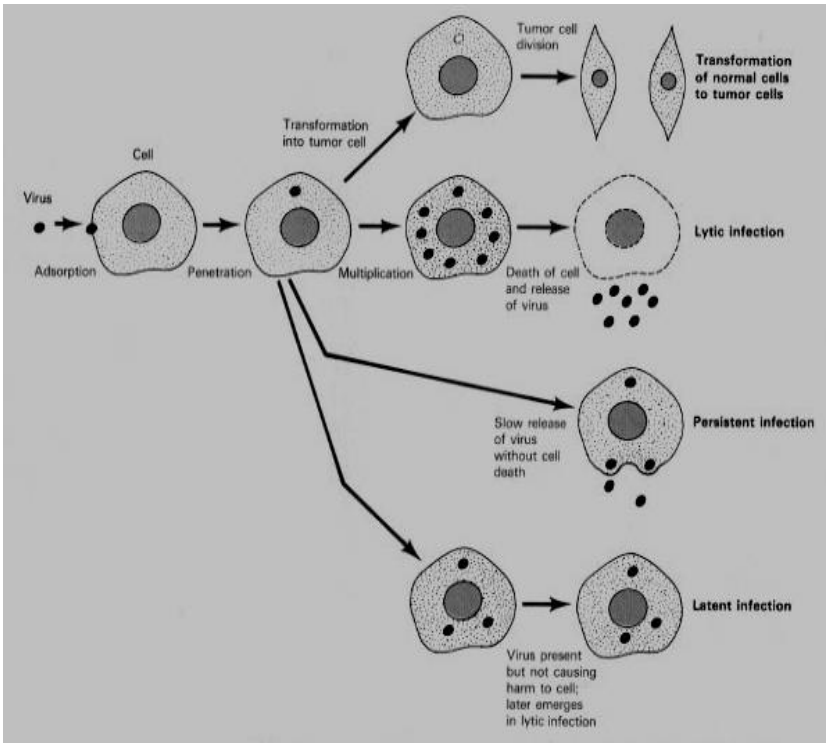
Lytic infections result in the destruction of the host cell. Lytic infections are caused by virulent viruses, which inherently bring about the death of the cells that they infect.

When enveloped viruses are formed by budding, the release of the viral particles may be slow and the host cell may not be lysed. Such infections may occur over relatively long periods of time and are thus referred to as **persistent infections**.

Viruses may also cause **latent infections**. The effect of a latent infection is that there is a delay between the infection by the virus and the appearance of symptoms. Fever blisters (cold sores) caused by herpes simplex type 1 result from a latent infection; they appear sporadically as the virus emerges from latency, usually triggered by some sort of stress in the host.

Some animal viruses have the potential to change a cell from a normal cell into a tumor cell, the hallmark of which is to grow without restraint. This process is called **transformation**. Viruses that are able to transform

normal cells into tumor cells are referred to as **oncogenic viruses** and their role in causing cancer in humans will be discussed later.



The possible effects that animal viruses may have on the cells that they infect.

The vast majority of viral infections in humans are inapparent or asymptomatic. Viral pathogenesis is the abnormal situation and it is of no particular value to the virus, although it typically results in the multiplication of the viruses that can be transmitted to other individuals. For pathogenic viruses, there are a number of critical stages in replication which determine the nature of the disease they produce.

The Stages of Viral Infections

1. Entry into the Host

The first stage in any virus infection, irrespective of whether the virus is pathogenic or not. In the case of pathogenic infections, the site of entry can influence the disease symptoms produced. Infection can occur via several portals of entry.

Skin - Most viruses which infect via the skin require a breach in the physical integrity of this effective barrier, e.g. cuts or abrasions. Some viruses employ vectors, e.g. ticks, mosquitos, etc to breach the skin.

Respiratory tract - The respiratory tract and all other mucosal surfaces possess sophisticated immune defense mechanisms, as well as non-specific inhibitory mechanisms (cilliated epithelium, mucus secretion, lower temperature, etc) which viruses must overcome. Nonetheless, this is the most common point of entry for most viral pathogens.

Gastrointestinal tract - a fairly protected mucosal surface, but some viruses (e.g. enteroviruses, including polioviruses) enter at this site.

Genitourinary tract - less protected than the GI, but less frequently exposed to extraneous viruses.

Conjunctiva - an exposed site and relatively unprotected.

2. Primary Replication

Having gained entry to a potential host, the virus must initiate an infection by entering a susceptible cell. Some viruses remain localized after primary infection, but others replicate at a primary site before dissemination and spread to a secondary site. Examples are given in the table below.

Localized Infections:		
Virus:	**Primary Replication:**	
Rhinoviruses	Upper respiratory tract	
Rotaviruses	Intestinal epithelium	
Papillomaviruses	Epidermis	
Systemic Infections:		
Virus:	**Primary Replication:**	**Secondary Replication:**
Enteroviruses (poliovirus)	Intestinal epithelium	Lymphoid tissues, CNS
Herpesvirus (HSV types 1 and 2)	Oropharynx or urogenital tract	Lymphoid cells, peripheral nervous system, CNS
Rabies virus	Mucle cells and connective tissue	CNS

3. Dissemination Stage

There are two main mechanisms for viral spread throughout the host: via the bloodstream and via the nervous system.

The virus may get into the bloodstream by direct inoculation - e.g. arthropod vectors, blood transfusion or I.V. drug abuse. The virus may travel free in the plasma (Togaviruses, Enteroviruses), or in association with red cells (Orbiviruses), platelets (HSV), lymphocytes (EBV, CMV) or monocytes (Lentiviruses). the presence of viruses in the bloodstream is referred to as a **viremia**. **Primary viremia** may be followed by more generalized **secondary viremia** as the virus reaches other target tissues or replicates directly in blood cells.

In some cases, spread to nervous system is preceded by primary viremia, as above. In other cases, spread occurs directly by contact with neurons at the primary site of infection. Once in peripheral nerves, the virus can spread to the CNS by axonal transport along neurons (e.g. HSV). Viruses

can cross synaptic junctions since these frequently contain virus receptors, allowing the virus to jump from one cell to another.

4. Tissue/Cell tropism

Tropism is the ability of a virus to replicate in particular cells or tissues. It is influenced partly by the route of infection but largely by the interaction of a virus attachment sites (virus receptors) with specific receptors on the surface of a cell. The interaction of the virus receptors with the host cell receptors may have a considerable effect on pathogenesis.

5. Host Immune Responses

There are several ways that the host immune responses may contribute to viral pathology. The mechanisms of cell mediated immunity are designed to kill cells which are infected with viruses. If the mechanisms of antibody mediated immunity result in the production of antibodies that cross-react with tissues, an autoimmune pathology may result.

6. Secondary Replication

This occurs in systemic infections when a virus reaches other tissues in which it is capable of replication. For example, polioviruses initiate infection in the GI where the produce an asymptomatic infection. However, when disseminated to neurons in the brain and spinal cord, where the virus replicates secondarily, the serious paralytic complication of poliomyelitis occurs. If a virus can be prevented from reaching tissues where secondary replication can occur, generally no disease results.

7. Direct Cell and Tissue Damage

Viruses may replicate widely throughout the body without any disease symptoms if they do not cause significant cell damage or death. Although retroviruses (e.g. HIV) do not generally cause cell death, being released from the cell by budding rather than by cell lysis, they cause persistent infections and may be passed vertically to offspring if they infect the germ line. Conversely, most other viruses, referred to as **virulent viruses**, ultimately damage or kiil their host cell by several mechanisms, including inhibition of synthesis of host cell macromolecules, damage to cell lysosomes, alterations of the cell membrane, development of inclusion bodies, and induction of chromosomal abberations.

8. Persistence versus Clearance

The eventual outcome of any virus infection depends on a balance between the ability of the virus to persist or remain latent (persistence) and the forces of the host to completely eliminate the virus (clearance).

Long term persistence is the continued survival of a critical number of virus infected cells sufficient to continue the infection without killing the host. It results from two main mechanisms:

a. Regulation of lytic potential. For viruses that do not kill their host cells, this is not usually a problem. But for lytic (virulent) viruses, there may be ways to down regulate their replicative and lytic potential so that they can persist in a state of latency without replication and damage to their host cell. This is the case with herpes viruses.

b. Evasion of immune surveillance. This may be due to several conditions that are properties of the host or the virus. Some viruses, such as influenza, can undergo antigenic shifts or antigenic drift that allows them to bypass a host immune response. Some viruses, e.g., measle, may

induce a form of immune tolerance such that the host is unable to undergo an effective immune response to the virus. Other viruses, such as HIV, may set up a direct attack against cells of the immune system such that the immune system is compromised in its ability to attack or eliminate the virus.

VIRAL DISEASES OF HUMANS

1) The Common Cold

The common cold is probably the most prevalent infectious disease that occurs in humans. It is estimated that there are up to a billion colds per year in the United States. Children have about 6 to 10 colds a year. This is due to lack of acquired immunity and because children are often in close contact with each other in daycare centers and schools. Adults average about 2 to 4 colds a year, although the range varies widely. Women, especially those 20 to 30 years of age, have more colds than men, possibly because of their closer contact with children. On average, people older than 60 have fewer than one cold a year.

Everyone is familiar with the symptoms of the common old, which are sore throat, cough, conjunctivitis and increased flow of mucus. Sneezing and coughing are common; fever is rare, except in young children. Usually, the infection is mild, lasting only a few days. However, it is a leading cause of doctor visits and missed days from school and work.

Viruses that Cause Colds

The common cold (rhinitis or coryza) is caused by several groups of viruses, although **rhinoviruses** have gotten the most attention. Other

cold-causing viruses include **adenoviruses**, **coronaviruses**, **respiratory syncytial virus (RSV)**, **parainfluenza** and **influenza viruses**. Rhinoviruses seldom produce serious illness, but others such as parainfluenza and RSV can produce severe respiratory illness in infants and young children.

Transmission of Colds

Quite a few studies have been done on the transmission of cold viruses, especially caused by Rhinoviruses. These viruses are usually transmitted by contact with an infected person's contaminated skin (e.g. hand) or a contaminated environmental surface, then touching your eyes or nose, which are the routes of inoculation.. Although colds can be spread by large particles expelled by coughing or sneezing at close range, the viruses apparently are not spread by kissing.

Colds occur at all times of the year although there are two peaks of increased incidence or "cold seasons": one is in April-May and the other in September-October.

Treatment of Colds

A cure for the common cold has been elusive. Most colds are self limiting and will go away within a few days. However, there are many treatments and over-the counter drugs and remedies available to relieve the symptoms of a cold. These undoubtedly represent a huge profitable market for the pharmaceutical industry and include the following:

Vaccines for the Common Cold

Vaccines are not forthcoming because colds are caused by over 200 different viruses, colds are not life-threatening, and there is too much money to be made off of the relief of symptoms.

2) INFLUENZA

Influenza is a disease caused by a member of the Orthomyxoviridae. Many features are common with those of the paramyxovirus infections of the respiratory tract.

CLINICAL FEATURES

Influenza is characterised by fever, myalgia, headache and pharyngitis. In addition there may be cough and in severe cases, prostration. There is usually ***not*** coryza (runny nose) which characterises common cold infections.

Infection may be very mild, even asymptomatic, moderate or very severe.

Source

The reservoir is acute infection in other human beings.

Spread

Is rapid via aerial droplets and fomites with inhalation into the pharynx or lower respiratory tract.

Incubation

Is short: 1-3 days. Rapid spread leads to epidemics

Complications

Tend to occur in the young, elderly, and persons with chronic cardio-pulmonary diseases

Consist of:

1. Pneumonia caused by influenza itself; and

2. Pneumonia caused by bacteria

- *Haemophilus influenzae*

- *Staphylococcus aureus*
- S*treptococcus pneuminiae*

3. Other viral superinfection, eg. Adenovirus.

Overall death rates increase in times of influenza epidemics.

LABORATORY DIAGNOSIS

A. *Viral Isolation*:

Respiratory secretions:

- direct aspirate
- gargle
- nasal washings

a) Rapid examination of cells by immunofluorescence.

b) Inoculation of cell cultures (or eggs).

B. *Serology*

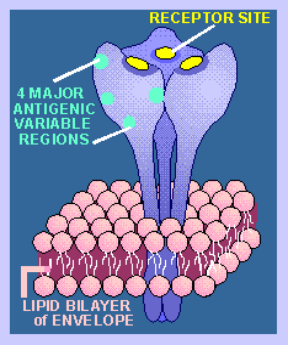
serum antibodies by haemagglutination inhibition

INFLUENZA VIRUSES

Diagramatic representation of the morphology of an influenza virion.

The virion is generally rounded but may be long and filamentous. A **single-stranded RNA** genome is closely associated with a helical nucleoprotein **(NP)**, and is present in **eight separate segments of ribonucleoprotein (RNP),** each of which has to be present for successful

replication. The segmented genome is enclosed within an outer lipoprotein **envelope**. An antigenic protein called the **matrix protein (MP 1)** lines the inside of the envelope and and is chemically bound to the RNP. The envelope carries two types of protruding spikes. One is a box-shaped protein, called the **neuraminidase (NA),** of which there are nine major antigenic types, and which has enzymic properties as the name implies.



The other type of envelope spike is a trimeric protein called the **haemagglutinin (HA)**

The haemagglutinin functions during attachment of the virus particle to the cell membrane, and can combine with specific receptors on a variety of cells including red blood cells.

The lipoprotein envelope makes the virion rather labile - susceptible to heat, drying, detergents and solvents.

Influenza Epidemiology

Localized epidemics of influenza occur every 2-3 years. Several world-wide pandemics have occurred in the last 400 years, the most disastrous of record being the 1918-1919 pandemic of "Spanish Flu", which killed

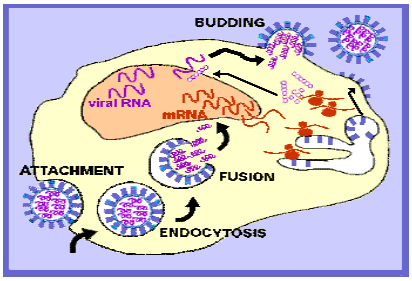
20 million world-wide and 500,000 in the U.S. These pandemics occur every 10-40 years.

A new **avian influenza** strain appeared in Hong-Kong in 1997, apparently jumping directly from the avian host to humans. The resulting strain A(H5N1), also called **avian influenza virus**, infected 18 people in Hong Kong and caused 6 deaths. Since 2003, more than 100 human H5N1 cases have been diagnosed in Thailand, Vietnam, Cambodia, and Indonesia. Of those cases, more than half have died as a result of the virus. Close contact with infected poultry has been the primary source for human infection. Though rare, there have been isolated reports of human-to-human transmission of the virus. Genetic studies confirm that the influenza A virus H5N1 mutates rapidly. Should it adapt to allow easy human-to-human transmission, a pandemic could ensue. At this time, it is uncertain whether the currently circulating H5N1 virus will lead to a global disease outbreak in humans.

Vaccines to protect humans against H5N1 viruses are currently under development.

REPLICATION

The Life Cycle of Influenza Virus



Receptor-bound viruses are taken into the cell by **endocytosis**. In the low pH environment of the endosome, RNP is released from MP1, and the

viral lipoprotein envelope **fuses** with the lipid-bilayer of the vesicle, releasing viral RNP into the cell cytoplasm, from where it is transported into the nucleus. New viral proteins are translated from transcribed messenger RNA (mRNA). New viral RNA is encased in the capsid protein, and together with new matrix protein is then transported to sites at the cell surface where envelope haemagglutinin and neuraminadase components have been incorporated into the cell membrane. Progeny virions are formed and released by **budding**.

The cell does not die (at least not initially).

Flu is one of a rare few viruses that has its **genome in separate segments** (eight). - This increases the potential for **recombinants** to form (by interchange of gene segments if two different viruses infect the same cell), and may contribute to the rapid development of new flu strains in nature - can also be duplicated in the laboratory (used for making **vaccine strains**). Avian and human strains recombining in pigs in the Far East may permit virulent human strains to evolve.

CLASSIFICATION of virus STRAINS

Is done on the basis of antigenicity of **NP** and **MP** into three main groups:

Influenza A -HA undergoes minor and occasional major changes - very important.

- NA some variation.

Influenza B) Undergoes relatively slow change in HA with time. Known only in man.

Influenza C) Uncommon strain, known only in man.

NOMENCLATURE

Influenza strains are named in the following way:				
A	**SINGAPORE**	**6**	**86**	**(H1N1)**
TYPE of influenza	**TOWN** where first isolated	**NUMBER** of isolates	**YEAR** of isolation	MAJOR **TYPE of HA and NA**

EPIDEMIOLOGY

Influenza A virus is essentially an avian virus that has "recently" crossed into mammals. Birds have the greatest number and range of influenza strains. Avian haemagglutinins sometimes appear in pig human and horse influenza strains.

Every now and then (10 - 15 years) a major new pandemic strain appears in man, with a totally new HA and sometimes a new NA as well **(antigenic shift).** This variant causes a major epidemic around the world (pandemic).

Over the subsequent years this strain undergoes minor changes **(antigenic drift)** every two to three years, probably driven by selective antibody pressure in the populations of humans infected.
See chart below indicating main pandemic strains in previous years.

Influenza A Evolution

1874 --- (H3N8)
1890 --- (H2N2) .....Pandemic
1902 --- (H3N2)
1918 --- (H1N1).....Pandemic
1933 --- (H1N1).....First strains isolated
1947 --- (H1N1).....Variation detected

1957 --- (H2N2)....."Asian" Flu pandemic

1968 --- (H3N2)....."Hong Kong" Flu pandemic

1976 --- (H1N1)....."Swine" Flu, non-epidemic

1977 --- (H1N1) + (H3N2)....."Russian" Flu epidemic

This **constant antigenic change** down the years means that new vaccines have to be made on a regular basis.

New influenza strains spread rapidly in children in schools and créches and in places where people crowd together. Influenza epidemics may cause economically significant absenteeism.

TREATMENT

Antibiotics are often prescribed - have no effect on virus but may prevent or cure **bacterial superinfection.** The drug **Amantadine** may prevent influenza if taken continuously by high-risk persons at the time of an epidemic, but is not used widely.

PREVENTION

Vaccines at best give about 70% protection.

They may sometimes not be effective against the most recently evolved strains because the rate of evolution outpaces the rate at which new vaccines can be manufactured.

Types of Vaccine

Killed Whole Virus

Rather pyrogenic, not used today.

Live Virus

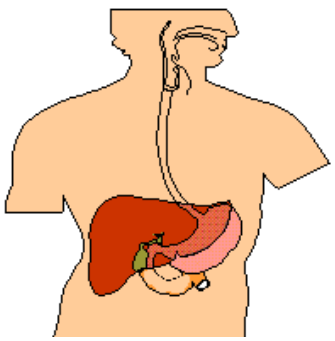
Attenuated strains were widely used in Russia but not elsewhere.

Virus Subunit

HA extracted from recombinant virus forms the basis of today's vaccines. For example, the **WHO Recommendation for Influenza Vaccine, 1995-1996**, contains two **A** strains and one **B** strain:-

A / Singapore / 6 / 86 (H1N1)
A / Johannesburg / 33 / 94 (H3N2)
B / Beijing / 84 / 93

Synthetic
Much research is being done to try and find a neutralising epitope that is more stable, and can therefore be used for a universal vaccine.



(3) VIRAL HEPATITIS

The term **VIRAL HEPATITIS** is usually used to describe infections caused by agents whose primary tissue tropism is the liver. To date, **at least five hepatitis viruses** have been recognized, and these have been named:- Hepatitis **A, B, C, D and E.**

Clinical Features

Hepatitis due to all these viruses presents clinically in a very similar fashion, especially during the acute phase of the illness. Thus a specific diagnosis can only be made in the laboratory.
The majority of infections are totally **asymptomatic**, but common clinical features include: *anorexia, nausea, vomiting, right upper quadrant pain* and *raised liver enzymes AST and ALT.*
Jaundice is the hall mark of infection, but tends to develop late. Anicteric cases are also very common.

Hepatitis A - "Infectious Hepatitis"

Caused by a picornavirus, **Enterovirus 72** This is a small, non-enveloped icosahedral particle, 27 nm in diameter, containing a **ssRNA genome**

Clinical Features

Incubation period 3-5 weeks (mean 28 days) Milder disease than Hepatitis B; asymptomatic infections are very common, especially in children. Adults, especially pregnant women, may develop more severe disease.

Although convalescence may be prolonged, there is **no chronic form** of the disease.

Pathogenesis

Virus enters via the gut; replicates in the alimentary tract and spreads to infect the liver, where it multiplies in hepatocytes.

Viraemia is transient. **Virus is excreted in the stools for two weeks preceding the onset of symptoms**.

Epidemiology

World-wide distribution; **endemic in most countries**. The incidence in first world countries is declining. There is an especially high incidence in developing countries and rural areas.
In rural areas of South Africa , the seroprevalence is 100%.

Transmission – Enteric

Large numbers of virus particles are excreted in stools, before the onset of symptoms.

1) Case-to-case, via faecal-oral route.
    Outbreaks in creches are very common.
2) Contamination of food or water with sewage
    Infected food handlers
    Shell fish grown in sewage-polluted water

Diagnosis

Virus cannot be cultured *in vitro* from clinical material, and diagnosis is made on the presence of **HAV-specific IgM** in the patient's blood.

Prevention

1) *Passive immunisation* -

Normal immunoglobulin given to:
    Travellers to third world countries
    Household contacts of acute cases

2) *Active Immunization*

Inactivated cell culture-derived vaccine has recently become available; not in general use

Hepatitis E

Recently identified cause of enterically transmitted non-A, non-B **(NANB)** hepatitis

Calicivirus

spherical, non enveloped, 27-34 nm particles containing a ssRNA genome.

Clinical Features

Incubation period 30-40 days Acute, self limiting hepatitis, no chronic carrier state

Age: predominantly young adults, 15-40 years

Complications

Fulminant hepatitis in pregnant women. Mortality rate is high (up to 40%).

Pathogenesis

Similar to hepatitis A; virus replicates in the gut initially, before invading the liver, and virus is shed in the stool prior to the onset of symptoms.

A large inoculum of virus is needed to establish infection.

Epidemiology

little is known yet. The incidence of infection appears to be low in first world countries.

1) Large outbreaks have been described in India, Mexico and North Africa where the source of infection is usually gross faecal contamination of drinking water supplies.

2) Case-to-case transmission to household contacts appears to be uncommon. This suggests that a large inoculum is needed to establish infection.

The incidence of infection in South Africa is unknown.

Diagnosis

No routine laboratory tests are available as yet. Virus cannot be cultured *in vitro*.

1) Calicivirus-like particles in the stool, by electron microscopy
2) Specific IgM in serum

3) PCR HEV-specific sequences in stool

PARENTERALLY TRANSMITTED HEPATITIS B, C, D and G

Hepatitis B

Hepadna virus

42nm Virions (also known as "Dane particles") contain a circular dsDNA genome

HBV Antigens

HBsAg = surface (coat) protein

produced in excess as small spheres and tubules

HBcAg = inner core protein

HBeAg = secreted protein; function unknown

Clinical Features

Incubation period 2 - 5 months

Insidious onset of symptoms. Tends to cause a more severe disease than Hepatitis A.

Asymptomatic infections occur frequently.

Pathogenesis

Infection is **parenterally transmitted**. The virus replicates in the liver and virus particles, as well as excess viral surface protein, are shed in large amounts into the blood. Viraemia is prolonged and the **blood of infected individuals is highly infectious**.

Complications

1) Persistant infection:-

Following acute infection, approximately 5% of infected individuals fail to eliminate the virus completely and become persistantly infected.

Those who are at particular risk include: babies, young children
immunocompromised patients males > females

The virus persists in the hepatocytes and **on-going liver damage** occurs because of the host immune response against the infected liver cells.

Chronic infection may take one of two forms: *Chronic persistent Hepatitis* - the virus persists, but there is minimal liver damage.

Chronic Active Hepatitis - There is aggressive destruction of liver tissue and rapid progression to cirrhosis or liver failure.

2) Patients who become persistently infected are at risk of developing **hepatocellular carcinoma (HCC).**

HBV is thought to play a role in the development of this malignancy because:

a) 80% of patients with HCC are carriers of hepatitis B.
b) Virus DNA can be identified in hepatocellular carcinoma cells.
c) Virus DNA can integrate into the host chromosome.

3) Fulminant Hepatitis

Rare; accounts for 1% of infections.

Epidemiology

Prevalence of disease in Africa

World-wide there are 450 million persistant carriers of hepatitis B, 50 million of which are in Africa. Carriage rates vary markedly in different areas. In South Africa, infection is much more common in rural communities than in the cities.

*Hepatitis B is **parenterally transmitted***

1) **Blood:**

- Blood transfusions, serum products,
- sharing of needles, razors
- Tattooing, acupuncture
- Renal dialysis
- Organ donation

2) **Sexual intercourse**

3) **Horizontal transmission** in children, families, 'close personal contact'. This is the major mode of transmission in South Africa where the majority of individuals become infected at between three and nine years of age.
Horizontal transmission also occurs in children's institutions and mental homes.

4) **Vertical transmission** - perinatal transmission from a carrier mother to her baby

- transplacental (rare)
- during delivery
- post natal , breast feeding , close contact

(This is the major mode of transmission in South East Asia)

Diagnosis: **Serology**

A. Acute infection with resolution

Viral antigens:

1) **Surface antigen (HBsAg)** is secreted in excess into the blood as 22 nm spheres and tubules. Its presence in serum indicates that virus replication is occurring in the liver.

2) **'e' antigen (HBeAg)** secreted protein is shed in small amounts into the blood. Its presence in serum indicates that a high level of viral replication is occurring in the liver.

3) **core** antigen (HBcAg) core protein is not found in blood.

Antibody response:

1) **Surface antibody** (anti-HBs) becomes detectable late in convalescence, and indicates immunity following infection. It remains detectable for life and is not found in chronic carriers (see below).
2) **e antibody** (anti-HBe) becomes detectable as viral replication falls. It indicates low infectivity in a carrier.

3) **Core IgM** rises early in infection and indicates recent infection
4) **Core IgG** rises soon after IgM, and remains present for life in both chronic carriers as well as those who clear the infection. Its presence indicates exposure to HBV.

Prevention

1) Active Immunization

Two types of **vaccine** are available:

Serum derived - prepared from HBsAg purified from the serum of HBV carriers

Recombinant HBsAg - made by genetic engineering in yeasts

Both vaccines are equally safe and effective. The administration of three doses induces protective levels of antibodies in 95% of vaccine recipients.

Universal immunization of infants was introduced in April 1995. Infants receive 3 doses at 6, 10 and 14 weeks of age.

Vaccine should be administered to people at high risk of infection with HBV:

1) Health care workers
2) Sexual partners of chronic carriers
3) Infants of HBV carrier mothers

2) Passive Antibody

Hepatitis B immune globulin should be administered to non immune individuals following single episode exposure to HBV-infected blood.

For example: needlestick injuries

Hepatitis C

The major cause of parenterally transmitted non A non B hepatitis. It eluded identification for many years. In 1989, the genome was cloned from the serum of an infected chimpanzee.

Virology

Putative **Togavirus** related to the Flavi and Pesti viruses.

Has a ssRNA genome

Does not grow in cell culture, but can infect Chimpanzees

Clinical Features

Incubation period 6-8 weeks Causes a milder form of acute hepatitis than does hepatitis B But 50% individuals develop chronic infection, following exposure.

Complications

1) Chronic liver disease

2) Hepatocellular carcinoma

Epidemiology

Incidence endemic world-wide; high incidence in Japan, Italy and Spain. In South Africa, 1% blood donors have antibodies.

Transmission

- Blood transfusions, blood products
- organ donation
- Intravenous drug abusers
- Community acquired: mechanism unclear. ?Vertical transmission
- sexual intercourse

Diagnosis

1) Serology

Reliable serological tests have only recently become available. HCV-specific IgG indicates exposure, not infectivity

2) PCR detects viral genome in patient's serum

Delta Agent

Defective virus which requires Hepatitis B as a helper virus in order to replicate. Infection therefore ***only occurs in patients who are already infected with Hepatitis B.***

Clinial Features

Increased severity of liver disease in Hepatitis B carriers.

Virology

virus particle 36 nm in diameter encapsulated with HBsAg, derived from HBV delta antigen is associated with virus particles ssRNA genome

Epidemiology

Identified in intra-venous drug abusers in Italy. Incidence in South Africa is unknown.

Hepatitis G (HGV)

A virus originally cloned from the serum of a surgeon with non-A, non-B, non-C hepatitis, has been called Hepatitis G virus. It was implicated as a cause of parenterally transmitted hepatitis, but is no longer believed to be a major agent of liver disease. It has been classified as a Flavivirus and is distantly related to HCV.

4) Severe Acute Respiratory Syndrome (SARS)

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a coronavirus, called SARS-associated Coronavirus (SARS-

CoV). SARS was first reported in Asia in February 2003. Over the next few months, the illness spread to more than two dozen countries in North America, South America, Europe, and Asia before the SARS global outbreak of 2003 was contained. Apparently civets, maintained in China as a source of exotic food, are the primary reservoir for the coronavirus associated with SARS.

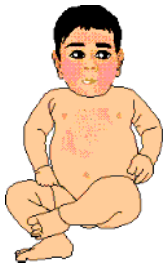
The Coronavirus associated with SARS

In general, SARS begins with a high fever (temperature greater than 100.4°F). Other symptoms may include headache, an overall feeling of discomfort, and body aches. Some people also have mild respiratory symptoms at the outset. About 10-20% of patients have diarrhea. After 2 to 7 days, SARS patients may develop a dry cough. Most patients develop pneumonia.

The main way that SARS seems to spread is by close person-to-person contact. The virus is transmitted most readily by respiratory droplets produced when an infected person coughs or sneeze and droplets are propelled a short distance (generally up to 3 feet) through the air and deposited on the mucous membranes of the mouth, nose, or eyes of persons who are nearby. The virus also can spread when a person touches a surface or object contaminated with infectious droplets and then touches his or her mouth, nose, or eye(s).

Because of its high mortality and ease of spread, SARS-CoV containment is a critical to its control during an outbreak.

5) Measles



Measles (**rubeola**) is one of the most infectious diseases known. Prior to widespread immunization, measles was a common childhood disease, with greater than 90% of infants and children infected by 12 years of age.

Measles Virus

Measles virus is an enveloped Ss (-)RNA virus, a member of the paramyxovirus family

Pathogenesis of Measles

The pathogenesis of measles resembles the general pattern for smallpox. The disease presents with cough, runny nose, fever, red eyes and white spots (**Koplick spots**) inside the mouth. This is followed 3 to 7 days later by a red blotchy skin rash, which spreads from the face to the rest of the body. The rash usually lasts 4 - 7 days but can persist for up to 3 weeks. Measles is frequently complicated by middle ear infection or diarrhea. The disease can be severe, with bronchopneumonia or brain inflammation (encephalitis) leading to death in approximately 2 of every 1,000 cases in developed countries. In the developing world, case-fatality rates often exceed 150 deaths per 1000 cases.

Transmission

Measles is spread by respiratory droplets or by direct contact with nasal or throat secretions of infected persons, and less commonly, by articles contaminated with nose and throat secretions.

Treatment

There is no specific antiviral therapy for measles, and the basic treatment consists of providing necessary supportive therapy such as hydration and antipyretics and treating complications such as pneumonia.

Prevention

Measles vaccine contains live, attenuated measles virus. It is available as a single-antigen preparation or combined with live, attenuated mumps or rubella vaccines, or both. Combined measles, mumps, and rubella (MMR) vaccine is recommended whenever one or more of the individual components are indicated.

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6) Mumps

Mumps is a disease involving the parotid and other salivary glands. The causative agent is an enveloped Ss (-)RNA virus in the Paramyxovirus family.

Mumps begins with sudden onset of fever and swelling and tenderness of the parotids, sometimes followed by other glands in the throat. The virus is localized here but may spread to the testes or ovaries, especially in adolescents and young adults, the thyroid gland, and occasionally the central nervous system.

Transmission

Mumps is spread by coughing and sneezing.

Treatment

No specific treatment is available for persons with mumps. Treatment is supportive.

Prevention

Mumps vaccine contains live, attenuated mumps virus.

7) Rubella (German Measles)

Rubella or **German measles** is caused by Rubivirus, a member of the Togavirus family of enveloped Ss (+)RNA viruses. Other togaviruses include Eastern Equine Encephalitis virus and Western Equine Encephalitis virus.

German measles (rubella) is distinct from measles (rubeola). The disease is similar to measles but milder, of shorter duration, and involving fewer complications.

The pattern of multiplication and dissemination of the virus is similar to measles. The disease is highly contagious, spread by nasal secretions. Rash appears 14-25 days following infection. Unlike measles the symptoms may be inapparent.

The rubella vaccine is available as a single antigen preparation, combined with mumps vaccine, or combined with measles and mumps vaccines (MMR). More than 95% of vaccinees 12 months of age or older develop permanent immunity with a single vaccination.

8) Herpes

Herpes viruses are a leading cause of human viral disease, second only to influenza and cold viruses. They cause overt disease such as cold sores and chickenpox, or they may remain latent for many years to be reactivated in later life, as in shingles.

The name *herpes* is derived from the Greek word *herpein* which means to creep. This reflects the spreading or creeping nature of the skin lesions caused by many herpes viruses.

There are 25 types of herpes viruses. Six types cause medical problems in humans.

Herpes Viruses Pathogenic for Humans

Pathogenesis of HSV-1 and HSV-2 Infections

The site of the initial infection is usually the oral or genital mucosa, depending on the way in which the person acquires the virus. It is often noted that HSV-1 infects above the waist and HSV-2 infects below the waist.

HSV-1 can set up a primary infection in the lips, move to the trigeminal ganglion where it can remain latent. The virus can subsequently reactivate, move to the original site of infection and result in cold sores.

HSV-1 is usually spread mouth to mouth (kissing or the use of utensils contaminated with saliva) or by transfer of infectious virus to the hands after which the virus may enter the body via any wound or through the eyes.

HSV-2 is normally spread sexually and is found in the anus, rectum and upper alimentary tract as well as the genital area. In addition, an infant can be infected at birth by a genitally-infected mother.

9) Human herpes virus 6 (HHV-6)

This herpes virus is found worldwide and is found in the saliva of the majority (90%) of adults. It infects almost all children by the age of two and the infection is life-long. It replicates in B and T lymphocytes in the oropharynx. It can set up a latent infection in T cells which can later be activated when the cells are stimulated to divide.
Human herpes virus-6 has two forms, HHV-6A and HHV-6B. The latter causes **exanthem subitum**, otherwise known as **roseola infantum**. This a common disease of young children. Symptoms include fever and sometimes upper respiratory tract infection and lymphadenopathy. The symptoms last a few days after an incubation period of around 14 days. The fever subsides leaving a macropapular rash on the trunk and neck that last a few days longer.

In adults, primary infection by HHV is associated with a mononucleosis. It has also been associated with a number of neurological disorders, including encephalitis and seizures. It has been postulated to play a role in multiple sclerosis and chronic fatigue immunodeficiency syndrome.

Human herpes virus (HHV-8)

This virus is also known as **Kaposi's sarcoma associated herpes virus (KSHV)** and is found in the saliva of many AIDS patients. It infects peripheral blood lymphocytes. The distribution of the virus may explain

why some populations of HIV-infected people come down with Kaposi's sarcoma while others do not.

10) Smallpox

Smallpox disease has been known throughout recorded history and has occurred in epidemics many times. Before vaccination about 95% of the population contracted some form of disease, one-quarter died, and many were left blind or disfigured.

Smallpox (variola) is caused by **variola** virus, a member of the Poxviridae family. Poxviruses are all large, ovoid, dsDNA viruses, just barely visible in the light microscope. Poxviruses are capable of causing skin lesions in a variety of animals including humans. **Vaccinia** is a laboratory strain of the virus used for vaccination against smallpox.

The variola virus emerged in human populations thousands of years ago. Except for laboratory stockpiles, the variola virus has been eliminated. However, in the aftermath of the events of September and October, 2001, there is heightened concern that the variola virus might be used as an agent of bioterrorism.

Pathogenesis of Smallpox

The incubation period for smallpox is 1-12 days before the symptoms of fever, headache and rash appear.

The virus enters through the respiratory tract, grows on mucous membranes, and spreads to regional lymph nodes where it multiplies before entering the bloodstream. Fever and other symptoms appear at this time. The virus invades internal organs, (heart, liver, kidney) and skin, producing the typical smallpox lesions. Death may result due to hemorrhage and generalized toxemia.

Epidemiology

Variola occurs in two predominant strains – **variola major**, which has a 30% or greater mortality rate, and **variola minor**, with a 1% mortality rate. Before the introduction of smallpox vaccination, almost everyone eventually developed smallpox, and either died of it or developed lifelong immunity. Smallpox is highly contagious. The infectious dose is unknown, but is believed to be only a few virus particles.

Generally, direct and fairly prolonged face-to-face contact is required to spread smallpox from one person to another. Smallpox also can be spread through direct contact with infected bodily fluids or contaminated objects such as bedding or clothing. Rarely, smallpox has been spread by virus carried in the air in enclosed settings such as buildings, buses, and trains. Humans are the only natural hosts of variola. Smallpox is not known to be transmitted by insects or animals.

Treatment and Prevention

There is no proven treatment for smallpox. Patients with smallpox may be helped by intravenous fluids, medicine to control fever or pain, and antibiotics for any secondary bacterial infections that may occur.

Immunity

Immunity to smallpox acquired from active infection is lifelong. Immunity that results from vaccination is probably only complete for 3-5 years. After this period, smallpox infection can occur but it is less severe. Maternal antibody provides infants with passive protection for 3-5 months following birth.

11) **Rabies**

Rhabdoviruses, which include rabies and vesicular stomatitis virus (VSV), are large, enveloped, bullet-shaped ss (-)RNA viruses. There are over 200 known rhabdoviruses that can infect mammals, fish, insects, arthropods and plants. However, rabies is the only rhabdovirus which can "naturally" infect humans.

Incidence of Rabies

Rabies is an ancient disease shown to be of viral etiology by Pasteur in the 1880's. For over a decade, Pasteur carried out the serial passage of rabies virus in rabbits, eventually succeeding in isolation of an attenuated preparation which was used to treat people bitten by rabid dogs.

Deaths due to rabies is rare in the United States (1 or 2 each year). This is primarily due to animal control and vaccination programs begun in the 1940's that have nearly eliminated domestic dogs as reservoirs of rabies in the U.S., and to the availability of effective rabies vaccines and immunoglobulins that can be used before or after exposure to the virus.

Unfortunately, on a global scale, there are at least 50,000 reported deaths per year due to rabies, which is a grossly underreported disease. Rabies is the tenth leading cause of death due to infectious disease on a world-wide basis. This is due to the reservoir of infected dogs in underdeveloped countries (and the lack of resources to accomplish control or mass vaccination of dogs), and the unavailability of post exposure prohylaxis in poor or remote regions.

Transmission

Transmission of rabies virus usually occurs when infected saliva of a host is passed to an uninfected animal. Most commonly this is through a bite

and virus-containing saliva of an infected animal. Other routes of transmission that have been documented include direct inoculation of mucous membranes (eye, nose, mouth) and aerosol transmission.

Symptoms

Early symptoms include flu-like signs (malaise, fever or headache) which last a few days. There may be abnormal sensation at the site of the infection progressing to hypersensitivity (to drafts, loud noises, bright lights, etc.), irritability, nervousness, hallucinations, insomnia and anxiety. Muscle spasms, salivation and perspiration are common. There is difficulty in swallowing and violent expulsion of fluids. The sight or sound of water can induce contraction of the throat muscles (hydrophobia). The acute period of disease lasts 2-10 days. When the virus invades the CNS, it produces severe encephalitis. Once clinical signs of rabies appear, the disease is almost always fatal. To date there are only six documented cases of human survival from clinical rabies.

Epidemiology

The principal source or reservoir of Rabies virus is wild mammals, especially skunks, bats, foxes, raccoons, coyotes and squirrels. Domestic animals such as cattle, dogs cats, horses, sheep or goats and swine may acquire the disease accidentally. Humans also acquire the disease accidentally and are a dead-end infection for the virus.

Globally, in terms of human disease, dogs represent the most important reservoir. Infection of humans usually follows bites by rabid animals and is almost invariably fatal, once signs of disease occur.

Treatment

There is no treatment for rabies after symptoms of the disease appear.

However, an extremely effective rabies vaccine regimen can provide immunity to rabies when administered after an exposure or for protection before exposure occurs .

Prevention

Because rabies is a fatal disease, the goal of public health is: 1. to prevent human exposure to rabies by education, and 2. to prevent the disease by anti-rabies treatment if exposure occurs. medical advice.

VIRUSES AND CANCER

The earliest relationship between cancer and viruses was demonstrated in the early 1900s, when **chicken leukemia** and **chicken sarcoma** were transferred to healthy animals by cell-free filtrates.

Transformation of Normal Cells into Tumor Cells

1. When activated, **oncogenes transform** normal cells into cancerous cells.

2. Viruses capable of producing **tumors** are called **oncogenic viruses**.

3. Several DNA viruses and retroviruses are oncogenic.

4. The genetic material of oncogenic viruses becomes integrated into the host cell's DNA.

5. **Transformed cells lose contact inhibition**, contain **virus-specific antigens** (TSTA and T antigen), exhibit **chromosomal abnormalities**, and can **produce tumors** when injected into susceptible animals.

DNA Oncogenic Viruses

1. **Oncogenic viruses** are found among the Adenoviridae, Herpesviridae, Poxviridae, and Papovaviridae.

2. The **EB virus**, a *Herpesvirus*, causes Burkitt's lymphoma and nasopharyngeal carcinoma. ***Hepadnavirus*** causes liver cancer.

RNA Oncogenic Viruses

1. Among the RNA viruses, only **retroviruses** seem to be oncogenic.

2. **HTLV-I and HTLV-2** have been associated with human leukemia and lymphoma.

3. The virus's **ability to produce tumors** is related to the **production of reverse transcriptase**. The DNA synthesized from the viral RNA becomes incorporated as a **provirus** into the host cell's DNA.

4. A **provirus** can remain **latent**, can **produce viruses**, or can **transform** the host cell.

THE IMMUNE SYSTEM AND CANCER

1. Cancer cells are normal cells that have undergone **transformation**, **divide uncontrollably**, and possess **tumor-associated antigens**.

2. The response of the immune system to cancer is called **immunological surveillance** which involves **cell-mediated immunity**.

3. **T_c cells** (**cytotoxic T cells**) recognize and lyse cancerous cells.

4. Cancer cells **can escape detection and destruction** by the immune system.

5. Cancer cells may (i) **suppress T cells** or (ii) **grow faster than the immune system can respond**.

Immunotherapy

1. **Tumor necrosis factor** (**TNF**) and other **cytokines** are being tested as cancer treatments.

2. **Immunotoxins** are chemical poisons linked to a **monoclonal antibody**; the antibody selectively locates the cancer cell for release of the poison.

3. A vaccine consisting of tumor antigens has been effective in controlling one type of cancer in poultry.

Latent Viral Infections

1. A **latent** viral infection is one in which the virus remains in the host cell for long periods without producing an infection.

2. Examples are **cold sores** and **shingles**.

Persistant Viral Infections

1. **Persistant** viral infections are disease processes that occur over a long period and are generally fatal.

2. Persistant viral infections are caused by conventional viruses; viruses accumulate over a long period.

TREATMENT/ PREVENTION OF VIRAL DISEASES

Major viral disease treatment factoids:

1. No viral disease has ever been CURED by medical treatment.

2. Viruses are not susceptible to ANTIBIOTICS. If a doctor tells you he/she is treating your VIRUS INFECTION with an antibiotic, he/she is either stupid or lying and you should seek more competent medical advice.

The obligate intracellular parasitic nature of viruses makes it very difficult to treat them. Because viral reproduction occurs ***only inside*** living cells and mostly uses the host cell's own metabolic machinery to generate new virions, it is a formidable task to find or develop drugs that are both (a) able to penetrate the cell's cytoplasmic membrane and (b) to selectively damage ONLY viral components. For example, drugs that disrupt a virus' protein or nucleic acid synthesis as well as the host's system are an unacceptable option. How then can viral infections be dealt with?

The classical, and still most effective, weapon in the war against viruses, immunization, is routed in the evolutionary struggle between the host and the viral pathogen. Vaccination, by arousing a host's evolved immunological defense against foreign antigens (e.g. specific viruses), usually prevents infection. Vaccination has eliminated smallpox as a human disease and we are now attempting to do the same thing to polio and measles. This "extermination strategy" works optimally on viruses that are limited to ***Homo sapiens***.

Many viruses however survive in non-hominid reservoirs (e.g. influenza &, hantavirus). In these cases the strategy depends on the reservoir(s) and mechanism(s) of dissemination to humans. The most common strategies used in this continuous battle are:

- Immunization of hosts with dead or attenuated virus (e.g. flu, polio) or with genetically engineered (G.E.) antigens.
- Immunization of hosts using cloned viral DNA shot directly into host cells.
    - Transcription and translation of the viral DNA produces enough viral antigen to immunize the host.
- Immunization of hosts using foods containing cloned viral genes that produce viral antigen(s).
    - Hosts ingesting the food/antigen become immune to the virus.
- Immunization of humans and alternate hosts (e.g. pets and vets against rabies).
- Minimize contact between humans and the natural reservoir(s) (e.g. Hantavirus & mice).
- Minimize vector/human contact (e.g. mosquito/yellow fever).

As our knowledge of virus genetics has grown we are developing molecular biological strategies for dealing with viruses. While none of these yet can be said to cure an established viral infection, they can, by minimizing the virus load in the infected individual, prevent or decrease the spread of the virus to new hosts (e.g. HIV from mother to fetus) and its damage to an infected host. These approaches include:

- The use of analogues that inhibit crucial viral enzymes.

- AZT and acyclovir specifically inhibit the replication of the genomes of HIV and Herpes viruses respectively.
- Protease inhibitors inhibit HIV proteases that are required to form a functional virion.
- Ribavirin blocks genome formation of several viruses.

- The use of agents that block infection
  - Amantadine blocks influenza penetration and uncoating.
  - Monoclonal antibodies bind virus particles in the blood which inactivates them and marks them for destruction by immune cells.
  - G.E. soluble receptors bind virions in the blood/serum and prevent them from reaching the cell-bound receptors.
    - Since all viruses require specific receptors, any virus can be treated this way as long as the receptor is known, cloned and produced in large quantities.
- The use of agents that stimulate or enhance the efficacy of the host's immune system.
  - Interferons (IL-2) that kills viruses and activates T-killer cells.
  - Cytokines that stimulate killer T-cell production.
  - Cytokines that stimulate antibody production.

Physicians are beginning to cautiously talk of a "CURE" for HIV through the use of combinations of the above treatments. The idea being that if the combined attack can lower the virion concentration sufficiently then the body's natural immunity can "clean up" the remaining virus and render the host virus free. Predictions about such an outcome are chancy, but they are not as totally unreasonable to consider as they were (in 1997).

RETROVIRUSES AND PRIONS

In addition to Viruses as described here above, there are two further forms of non-cellular infectious agent which have been discovered in recent years: Retroviruses and Prions .

RETROVIRUSES

Viruses occur in two forms: Proviruses and Retroviruses. The two forms are the same in terms of structural organisation - their difference is only in the detail of the biological process by which they control the host cell's reproductive processes. Retroviruses have been identified only in relatively recent times. The discovery and definition of Retroviruses is associated in substantial part with the name of Peter Duesberg.

Several expert virologists have asserted that the reproductive process employed specifically by Retroviruses implies a possible situation in which the genetic material of the Retrovirus can become incorporated with that of the host cell such that further multiplication of the joint genetic structure may occur without replication of the protein coat of the Retrovirus. This possibility would have the important implication that in the combined form the Retrovirus would be ***undetectable and ignored by the host's organism's immune system***. For understanding of the details of viral reproduction is such that we cannot properly assess the epistemological status of the view that Retroviruses might 'hide' in this manner. Should this view be treated as speculation, as empirical fact, or as something between these extremes?

An ability to trigger multiplication of a host's cells in a way unrelated to the host's needs is close to a definition of *cancer*; this correspondence

relates to modern virological theories which suggest that many forms of cancer may have their origins in the behaviour of some sorts of viruses.

PRIONS

Some neurological diseases are caused by ***pr***otein ***in***fectious particles (PRIONS). These include several animal and at least 3 human diseases. One of these diseases, KURU, infects its victims when they eat the brain tissue of their enemies (a questionable activity at best). The best studied of these diseases is scrapies in sheep. The disease entity seems to be composed completely of PROTEIN and to entirely lack any nucleic acid. This poses a major problem given the significant role of DNA and RNA in life. Three theories are currently being considered to explain prions.

1. That prions contain, as yet **undetected nucleic acid**. Extensive purification and testing using the most sensitive methods available have failed to demonstrate any nucleic acid in purified, infectious prions.
2. That an **unknown bacterium** that is hard to cultivate and that passes through filters is responsible. Again, there is no proof for such an organism.
3. That prions represent a **type of protein that is able to convert a "normal" protein into a "prion protein"**. This theory is currently the most popular and there is some evidence accumulating to suggest that it is valid. This theory says that when the prion protein gets into the brain of a victim it binds to a normal or pre-prion protein and somehow converts it into a prion; the new-prion then proceeds to convert other natural proteins. As the

number of prions increase destruction of the brain occurs, eventually killing the victim.

The **MAD COW** disease that was first detected in England and parts of Europe a few years ago is apparently a new prion disease and it has caused the use of beef in Britain to fall precipitously. Thousands of cattle have been slaughtered and their carcasses destroyed to prevent the spread of this disease. At least 3 farm workers have died from a disease with ***symptoms like those of the Mad Cow disease***.

Would you eat a hamburger made from British beef? In the summer of 1997 the FDA began allowing importation's of British beef back into the US.

ARE PRIONS VIRUSES?

When prion diseases—various degenerative diseases of the nervous system—were first discovered, they were commonly viewed as being caused by a "slow-acting" virus or viruses. However, work by several researchers over the past few decades has shown rather convincingly that the pathological agent of these disorders is not a virus but an infectious protein. While a small but significant contingent of scientists still believes that prion diseases are caused by viruses, in my opinion the available data fit best with a protein-only model. A summary of the most critical evidence in establishing this point follows:

1. Many years ago, it was observed that patients with prion disorders (such as Creutzfeldt-Jacob disease) do not exhibit the classical immune responses that the body initiates upon viral infection, including fever, leukocytosis, and humoral (that is, antibody-mediated) immunity.

2. Normally, viruses can be inactivated by a number of chemical treatments, including formalin or heat treatment. Indeed, this is how many viruses are inactivated to be used in the generation of vaccine preparations. However, such treatments were ineffective in reducing the infectivity of prions. Additionally, viruses contain either a DNA or an RNA genome; therefore, viruses can be inactivated by treatments that modify or damage DNA, such as ultraviolet light or ionizing radiation. These treatments have no effect on the infectivity of prions.

3. Conversely, treatments that modify or hydrolyze proteins do reduce prion infectivity. Therefore, a protein or proteins are essential for infectivity.

4. Probably the most important piece of evidence indicating that prions are not viruses is that prion infectivity copurifies with the prion protein—that is, prion infectivity is always associated with the purification of the prion protein. For example, the brain of a prion-infected mouse can be subjected to purification until only the prion protein remains at any detectable and significant level—all other proteins and nucleic acids can be removed. This purified preparation of prion protein can then be injected into the brain of another mouse, and that mouse will develop the prion disease. Furthermore, the degree of infectivity is associated with the concentration of the prion protein. As more prion protein is added, the injected mouse will develop the disease more quickly. In no case can infection be achieved with a preparation that lacks the prion protein.

Collectively, the current data strongly demonstrate that the infectious prion agent is an infectious protein, not a virus. It has not been ruled out that prions have a nonprotein component (such as a small molecule) that has not yet been identified. The manner in which a single protein

containing no nucleic acid can be infectious is an interesting story in itself, though I will not get into it here.

ARE THERE PRIONS IN OUR FUTURE?

This is a case of an Emerging Disease about which we understand too little to know whether to be ***scared-out-of-our-wits*** or just to be wary and concerned. The Press/TV/Tabloids find prions a good way to sell their services and they tend to hype it up for that purpose. However, there are scientists who are very concerned about the potential dangers of prions. The following are some points of information (tentative) to keep in mind:

1. There is still some serious debate within the scientific community as to the existence of prions and their role in diseases like the Mad Cow Disease.
2. Currently the preponderance of data support the idea of prions being "killer proteins".
3. Prions are very tough; they are not destroyed by autoclaving, cooking temperatures, most disinfectants or being buried in the soil for months.
4. They are slow acting, however there was a recent death in a young person after exposure only a couple of years previously.
5. No cases of Mad Cow Disease disease (in cattle) have been reported in the US, but Mad Cow-like diseases infect deer and elk in the US.
6. There is no treatment for prion diseases; it is a death sentence.
7. Prevention is uncertain. In UK they have killed and burned a significant percentage of the cattle. British beef can not be imported into most countries.

8. The disease seems to be spread by animals eating the remains of other animals, particularly of closely related species. However, it also seems to be spread by other means, yet unknown.
9. Feeding of animal parts to cattle and sheep is in the process of being banned in the US.

VIROIDS

Viroids are tiny strands of RNA, usually only a few hundred nucleotides long. Viroids can interfere with a plant's metabolism.

Generally speaking, where viroids come from and how they can disrupt the host cell are not known.

Plants fall victim to agents composed of NAKED RNA that are only 300 to 400 nucleotides long, called **VIROIDS**. The evidence is conclusive that viroids cause plant diseases, but the mechanism of pathogenicity is not known. So far NO HUMAN viroids have been discovered, but it is considered a real possibility that they exist.

Appendix

1.0 The Baltimore System for Virus Classification

By convention the top strand of coding DNA written in the 5' - 3' direction is + sense. mRNA sequence is also + sense. The replication strategy of the virus depends on the nature of its genome. Viruses can be classified into seven (arbitrary) groups:

I: Double-stranded DNA (Adenoviruses; Herpesviruses; Poxviruses, etc) Some replicate in the nucleus e.g. adenoviruses using cellular proteins. Poxviruses replicate in the cytoplasm and make their own enzymes for nucleic acid replication.

II: Single-stranded (+) sense DNA (Parvoviruses) Replication occurs in the nucleus, involving the formation of a (-) sense strand, which serves as a template for (+) strand RNA and DNA synthesis.

III: Double-stranded RNA (Reoviruses; Birnaviruses) These viruses have segmented genomes. Each genome segment is transcribed separately to produce monocistronic mRNAs.

I**V: Single-stranded (+) sense RNA** (Picornaviruses; Togaviruses, etc)

a) Polycistronic mRNA e.g. Picornaviruses; Hepatitis A. Genome RNA = mRNA. Means naked RNA is infectious, no virion particle associated polymerase. Translation results in the formation of a polyprotein product, which is subsequently cleaved to form the mature proteins.

b) Complex Transcription e.g. Togaviruses. Two or more rounds of translation are necessary to produce the genomic RNA.

V: Single-stranded (-) sense RNA (Orthomyxoviruses, Rhabdoviruses, etc)

Must have a virion particle RNA directed RNA polymerase.

a) Segmented e.g. Orthomyxoviruses. First step in replication is transcription of the (-)sense RNA genome by the virion RNA-dependent RNA polymerase to produce monocistronic mRNAs, which also serve as the template for genome replication.

b) Non-segmented e.g. Rhabdoviruses. Replication occurs as above and monocistronic mRNAs are produced.

VI: Single-stranded (+) sense RNA with DNA intermediate in life-cycle (Retroviruses)

Genome is (+) sense but unique among viruses in that it is diploid, and does not serve as mRNA, but as a template for reverse transcription.

VII: Double-stranded DNA with RNA intermediate (Hepadnaviruses) This group of viruses also relies on reverse transcription, but unlike the Retroviruses, this occurs inside the virus particle on maturation. On infection of a new cell, the first event to occur is repair of the gapped genome, followed by transcription.

1.1 List of important virus families that contain genera that infect humans and the symptoms that they cause

DNA- containing viruses

Adenoviridae

Human Adenoviruses - primarily respiratory and conjunctival infections

Astroviridae

Astrovirus - flulike symptoms

Herpesviridae

Herpes simplex virus type 1 - stomatitis; upper respiratory infections

Herpes simplex virus type 2 - genital infections

Varicella-zoster - chicken pox; herpes zoster; shingles ,

Human Cyotmegalovirus - jaundice; hepatosplenomegaly, brain damage, death

Epstein-Barr Virus - Burkitt lymphoma; nasopharyngeal carcinoma; infectious mononucleosis

Papovaviridae

Human papilloma viruses- benign tumors (warts); cervical cancer

Human polyoma viruses - progressive leukoencephalopathy (PML); transform cells

in tissue culture

Poxviridae

Orthopoxvirus

Variola - smallpox

Cowpox - vesicular lesions on skin

Unclassified Round-structured viruses

Norwalk agent "Noroviruses" - gastroenteritis

RNA - containing viruses

Arenaviridae

Lymphcytic choriomeningitis virus (LCM) - fatal meningitis

Lassa virus - hemorrthagic fever, frequently fatal

Bunyaviridae

Hanta virus

Coronaviridae

Human Coronavirus - SARS - severe acute respiratory syndrome

Filoviridae

Ebola - acut hemorrhagic fever almost 90% case mortality

Marburg - hemorrhagic fever, frequently fatal

Flaviviridae

Yellow Fever - hemorrhagic fever, hepatitis, nephritis

Dengue - fever, arthralgia, rash

West Nile - fever, arthralgia, rash

Hepatitis C virus - hepatitis

Orthomyxoviridae

Influenza virus type A - acute respiratory disease

Influenza virus type B - acute respiratory disease

Influenza virus type C - acute respiratory disease

Paramyxoviridae

Parainfluienza viruses - croup, common cold syndrome, mild respiratory disease

Mumps - parotitis, orchitis, meningoencephalitis

Measles - measles

Subacute sclerosing panencephalitis (SSPE) - chronic degeneration of CNS

Respiratory syncytial virus (RSV) - pneumonia and bronchiolitis in infants and children, common cold syndrome

Picornaviridae

Human Enteroviruses

Poliovirus - poliomyelitis

Coxsackie virus A - aseptic meningitis, paralysis, and common cold syndrome

Coxsackie virus B - aseptic meningitis, paralysis, , severe systemic illness of newborns

Hepatitis A virus - infectious hepatitis

Human Rhinoviruses - common cold, bronchitis, croup, bronchopneumonia

Reoviridae

Colorado Tick fever virus - encephalitis

Human Rotaviruses - diarrhea in infants

Retroviridae (RNA-tumor viruses)

Human immunodefficiency virus - acquired immune defficiency syndroime (AIDS)

Human T-lymphotrophic virus (HTLV) -

Rhabdoviridae

Rabies virus - encephalitis, usually fatal

Togaviridae

Eastern Equine Encephalitis virus - encephalitis

Western Equine Encephalitis virus - encephalitis

Rubella (Measles) - severe deformities of fetuses in first trimester of pregnancy.

Glossary of Virology:

(+)sense RNA (plus-sense RNA): A virus with a single-stranded RNA genome of the same polarity ('sense') as mRNA.

(-)sense RNA (minus-sense RNA): A virus with a single-stranded RNA genome of the opposite polarity ('sense') as mRNA.

Abortive Infection: When a virus infects a cell (or host), but cannot complete the full replication cycle, i.e. a non-productive infection.

Acute Infection: Relatively brief infections, i.e. a few days to a few weeks, following which the virus is usually eliminated completely from the body by the immune system.

'Arboviruses': A large a diverse group of viruses, taxonomically unrelated which are classically transmitted by arthropod vectors, e.g. mosquitoes, ticks, etc.

Assembly: The stage of replication during which all the structural components come together at one site in the cell and the basic structure of the virus particle is formed.

Attachment: The binding of a virus particle to a specific receptor on the surface of a host cell.

Capsid: A protein shell comprising the main structural unit of a virus particle.

Chronic Infection: The converse of acute infections, i.e. prolonged and stubborn. Caused by viruses which are able to persist in the body.

Complement fixation (CF): An assay for detecting the presence of antibodies reactive against a particular antigen, e.g. a virus.

Envelope: A lipid membrane enveloping a virus particle.

Fusion Protein: The protein(s) on the surface of a virus particle responsible for fusion of the virus envelope with cellular membranes.

Gene expression: An important stage of viral replication at which virus genetic information is expressed: one of the major control points in replication.

Genome replication: The stage of viral replication at which the virus genome is copied to form new progeny genomes.

Haemagglutination-inhibition: An assay used for certain types of viruses which are able to agglutinate red blood cells. Haemagglutination-inhibition records blocking of this process by antibodies, and thus, the presence of antibodies against the virus.

Latent Infection: Viruses which are able to down-regulate their gene expression can establish a truly latent state, i.e. with strictly limited gene expression and without ongoing virus replication. Latent virus infections typically persist for the entire life of the host.

Matrix Protein: A structural protein of a virus particle which underlies the envelope and links it to the core.

Maturation: The stage of viral replication at which a virus particle becomes infectious.

Molecular epidemiology: The use of nucleotide sequence information to study the diversity and distribution of virus populations.

mRNA: Messenger RNA, translated on ribosomes to produce proteins.

Neutralization: Blocking of virus infection by antibodies; also, an assay which measures this.

Nucleocapsid: The core of a virus particle consisting of the genome plus a complex of proteins.

Penetration: The stage of viral replication at which the virus genome enters the cell.

Persistent Infection: Infections in which ongoing virus replication occurs, but the virus adjusts its replication and pathogenicity so as to avoid killing host. They differ from chronic infections in that whereas in chronic infections, the virus is usually eventually cleared by the host (unless the infection proves fatal), in persistent infections, the virus may continue to be present and to replicate in the host for its entire lifetime.

Polyprotein: A long polypeptide encoding several mature proteins which are subsequently released by protease cleavage.

Receptor: A specific molecule on the surface of a cell which is used by a virus for attachment.

Release: The stage of viral replication at which virus particles escape the infected cell.

Tropism: The ability of a virus to infect specific cell or tissue types.

Uncoating: The stage of viral replication at which structural proteins are lost and the virus genome is exposed to the replication machinery.

Vector: An organism responsible for transmitting a pathogen from one host to another, e.g. a mosquito. (In molecular biology, a molecule used to clone nucleic acid sequences).

Virions: Structurally mature, extracellular virus particles.

Virus attachment protein: The protein on the surface of a virus particle responsible for binding the receptor.