Biology

Biology: is a natural science concerned with the study of life and living organisms, including their structure, function, growth , evolution, distribution, and taxonomy**.**

How to Define Life

There are some characteristics that distinguish living and non-living organism**.**

1. **Organization**: Being structurally composed of one or more of cells which is the basic units of life. Organisms are organized from atoms up to cells. The matter is structured in an ordered way .Atoms are arranged into molecules, then into macromolecules, which make up organelles, which work together to form cells. Beyond this, cells are organized in higher levels to form entire multicellular organisms.
2. **Homeostasis**: Regulation of the internal environment to maintain a constant state. Stable internal conditions of pH, temperature, water balance, etc. for example, sweating to reduce temperature.
3. **Metabolism**: Refers to the sum of the total chemical processes that occur in a cell or organism that are necessary for life.These processes can be classified into anabolic and catabolic processes.
4. **Growth and Development**: Growth means that organism increases in size and number. Development refers to all changes that occur during life.
5. **Adaptation**: The ability to change over time in response to the environment. This ability is fundamental to the process of evolution and is determined by the organism's heredity.
6. **Response to stimuli**: Organisms respond to stimuli (Temperature, Water, Food Supplies, etc.) in order to survive & reproduce.
7. **Reproduction**: The ability to produce new individual organisms, either asexually from a single parent organism, or sexually from two parent organisms.

Water is essential for life:

Water is the most abundant molecule in cells, accounting for 70% or more of total cell mass. The total amount of water in our body is found in three main locations: within our cells (two-thirds of the water), in the space between our cells and in our blood (one-third of the water).

Water serves a number of essential functions in the Body: Water is the primary building block of cells.

- It regulates our internal body temperature by sweating and respiration.
- The carbohydrates and proteins that our bodies use as food are metabolized and transported by water in the bloodstream.
- Water is used to flush waste and toxins from the body via urine.
- Forms saliva.
- Lubricates joints.

Water has a number of important properties essential for life.

- Water(H_2O) is a polar molecule because of a slightly negative charge at the oxygen end and a slightly positive charge at the hydrogen end. Water molecules can form hydrogen bonds with each other. Polar substances are hydrophilic (water loving). Nonpolar substances are hydrophobic (water hating) and are repelled by water.
- **Solvent-** It is a very good solvent. Molecules such as salts, sugars, amino acids dissolve readily in water (once dissolved they can be transported e.g. glucose in the blood).
- **Water has a high specific heat capacity**. This means that water does not change temperature easily. This minimises fluctuations in temperature inside cells.
- **Latent heat of vaporization-** Water requires a lot of energy to change state from a liquid to a gas, providing a cooling mechanism (sweating). As water evaporates it extracts heats from the surrounding area, cooling the organism.
- **Density**- The solid state of water (ice) is less dense than the liquid state.
- **Cohesion and adhesion**Water molecules due to hydrogen bonds stick together, to other biologically important polar molecules.

PH scale: A scale ranging from 0 to 14 pH units, reflecting the concentration of hydrogen ions in solution. A solution with a pH of 7.0 is neutral. Solutions with a lower pH value (<7.0) are increasingly acidic, and those with a higher pH value (>7.0) are increasingly alkaline.

pH in living systems	
Compartment	**pH**
Gastric_acid	1
Lysosomes	4.5
Human_skin	5.5
Urine	6.0
Cytosol	7.2
Cerebrospinal_fluid (CSF)	7.5
Blood	7.34–7.45
Mitochondrial_matrix	7.5
Pancreas secretions	8.1

Buffers

A buffer is a substance that helps minimize the change in the pH of a solution when acids or bases are added. This is important because, most of the chemical processes that occur in living organisms are highly sensitive to pH, and drastic changes in pH can cause some serious trouble.

Prokaryotic and Eukaryotic Cells

Cells are of two types, eukaryotic, which contain a nucleus, and prokaryotic, which do not. Prokaryotes are single-celled organisms, while eukaryotes can be either single-celled or multicellular. The distinction between prokaryotes and eukaryotes is considered to be the most important distinction among groups of organisms. Eukaryotic cells contain membrane-bound organelles, such as the nucleus, while prokaryotic cells do not.

Comparison between prokaryotic and eukaryotic cells.

Similarities:

- They both have DNA as their genetic material.
- They are both membrane bound.
- They both have ribosomes.
- They have similar basic metabolism.

Differences:

1. Eukaryotes have a nucleus, while prokaryotes do not.

2. Eukaryotes have membrane-bound organelles, while prokaryotes do not.

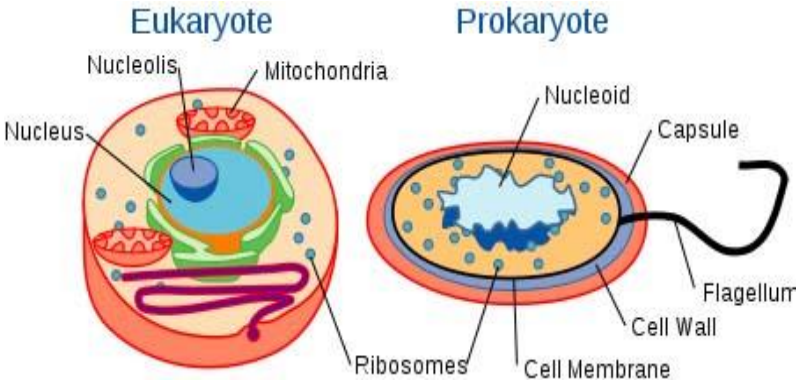
3. Eukaryotic cells are, on average, ten times the size of prokaryotic cells.

4. The DNA of eukaryotes is much more complex and therefore much more extensive than the DNA of prokaryotes.

5. Prokaryotes have a cell wall composed of peptidoglycan, a single large polymer of amino acids and sugar . Many types of eukaryotic cells also have cell walls, but none made of peptidoglycan.

6. The DNA of prokaryotes floats freely around the cell; the DNA of eukaryotes is held within its nucleus and associated with histones (proteins).

7. Eukaryotes undergo mitosis; prokaryotes divide by binary fission (simple cell division).



Comparison of features of prokaryotic and eukaryotic cells		
	Prokaryotes	**Eukaryotes**
Typical organisms	bacteria	plants, animals
Typical size	~ 1–5 µm	~ 10–100 µm
Type of nucleus	nucleoid region; no true nucleus	true nucleus with double membrane
DNA	circular (usually)	linear molecules (chromosomes) with histone and non-histone proteins
RNA/protein synthesis	coupled in the cytoplasm	RNA synthesis in the nucleus protein synthesis in the cytoplasm
Ribosomes	50S and 30S	60S and 40S
Cytoplasmic structure	very few structures	highly structured by endomembranes and a cytoskeleton
Cell movement	flagella made of flagellin	flagella and cilia containing microtubules; lamellipodia and filopodia containing actin
Mitochondria	none	one to several thousand
Chloroplasts	none	in algae and plants
Organization	usually single cells	single cells, higher multicellular organisms with specialized cells
Cell division	binary fission (simple division)	mitosis and meiosis
Chromosomes	single chromosome	more than one chromosome

Cell biology

Cell biology: is a branch of biology that studies cells – their physiological properties, their structure, the organelles they contain, interactions with their environment, their life cycle, division, death and cell function.

The cell: is the basic structural, functional and biological unit of all known living organisms. Cells consist of cytoplasm enclosed within a membrane, which contains many biomolecules such as proteins and nucleic acids. Organisms can be classified as unicellular (consisting of a single cell; including bacteria) or multicellular (including plants and human).

All human cells are multicellular, they are eukaryotic cells. Human cells are surrounded by plasma membrane and it contains the nucleus and organelles that are membrane bound. Most of the cells size range between 1 and 100 micrometers and are visible only with help of microscope. Trillions of cells are found in the human body. There are many different types of cells.

Parts of the human cell are as follows:

- ***Cell membrane*** - forms the outer covering of the cell, and is semi-permeable.
- ***Cytoplasm*** - is a gel-like matrix where all the other cell organelles are suspended inside the cell.
- ***Nucleus*** - contains the hereditary material DNA and directs the activities of the cell.

The Molecular Composition of Cells

Cells are composed of water, inorganic ions, and carbon-containing (organic) molecules. Water is the most abundant molecule in cells, accounting for 70% or more of total cell mass. Consequently, the interactions between water and the other constituents of cells are of central importance in biological chemistry.

Organic Molecules:

Carbohydrates:

A carbohydrate is a compound containing the elements carbon, hydrogen and oxygen in which the ratio of hydrogen to oxygen is the same as in water – two hydrogen's to one oxygen. The basic building blocks of carbohydrate molecules are the monosaccharides –glucose (the major nutrients of cells), fructose, and galactose. Two monosaccharides can form a covalent bond between them to form a disaccharide sugar. There are three kinds of disaccharides:

Sucrose is a compound containing a glucose joined to a fructose. Sucrose is commonly called table sugar. Maltose is a disaccharide containing two glucose molecules held together by a covalent bond. Lactose is a sugar found in milk formed by the combination of glucose and galactose.

When many monosaccharide molecules are joined together with covalent bonds, we have a polysaccharide, examples: Glycogen and Starch. Glycogen is a polysaccharide containing many hundreds of monosaccharide subunits. Glycogen is a food stored in the body for energy, found in the liver and skeletal muscles.

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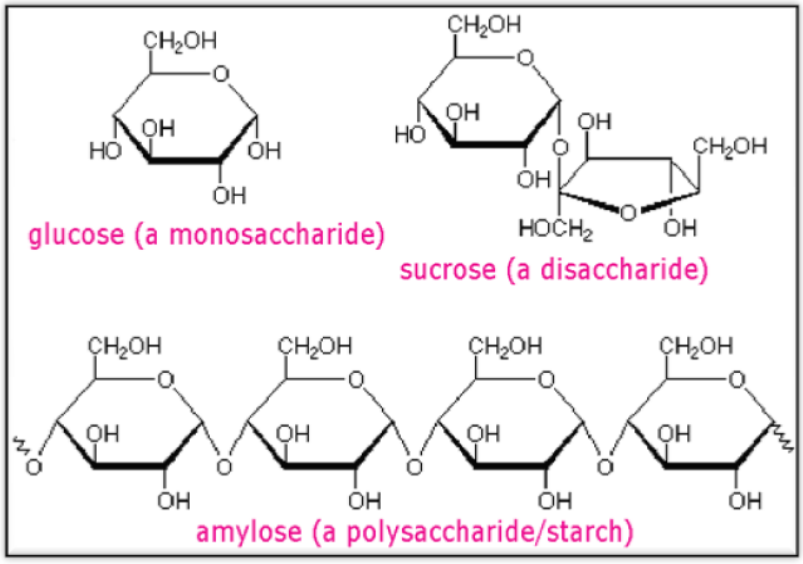

Lipids: Lipids are large molecules that do not dissolve in water. They contain carbon, hydrogen and oxygen. Three important lipids in the body are fats, phospholipids and steroids.

1. **Fats**- are lipid molecules formed from two building blocks, glycerol and three fatty acids. **Saturated fat** - fatty acids contain all hydrogens possible. There are no double bonds in the fatty acid portion of the molecule. Solids at room temperature. **Unsaturated fat** - more hydrogens can be added to the fatty acid molecules. There is at least one double bond in the fatty acid portion of the molecule. Liquids at room temperature.

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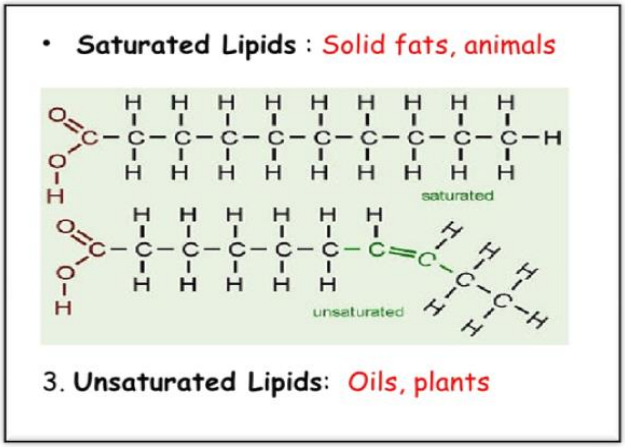
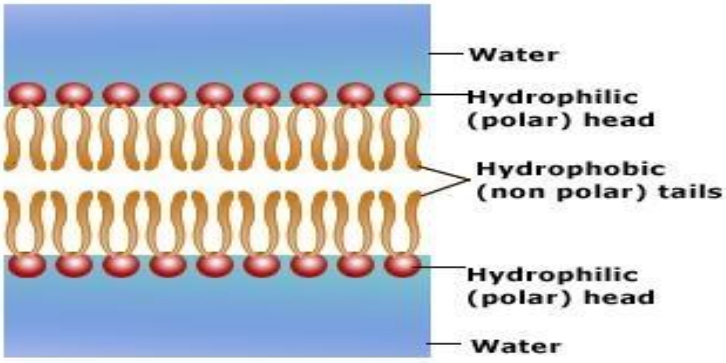


2. **Phospholipids**- The phospholipid molecule is similar to a fat except that the third fatty acid is replaced by a phosphate group. The phosphate end of the molecule will dissolve in water and is said to be hydrophilic ("likes water"). The fatty acid end of the molecule repels water and is called hydrophobic ("fears water"). Phospholipids are a major component of the membranes surrounding the cells of all organisms.



3. **Steroids** - differ greatly from fats and phospholipids in structure; they are complex ring compounds. Like fats and phospholipids they do not dissolve in water. Examples of steroids - cholesterol, vitamin D, cortisone, estrogen.

The roles of lipids:

1- Storing energy.

2-Lipids are the major components of cell membrane.

3-Lipid play roles in cell signaling both as steroid hormones (estrogen &testosterone) and as a message molecules that convey signals from cell surface receptors to targets within the cell.

Lipid storage disorders

Lipid storage disorders are a group of inherited metabolic disorders in which harmful amounts of lipids (fats) accumulate in some of the body's cells and tissues. People with these disorders either do not produce enough of one of

the enzymes needed to metabolize lipids or they produce enzymes that do not work properly. Over time, this excessive storage of fats can cause permanent cellular and tissue damage, particularly in the brain, peripheral nervous system, liver, spleen and bone marrow.

Proteins: Proteins are very large, complex molecules composed of the elements carbon, hydrogen, oxygen and nitrogen. Other elements are found in proteins in very small amounts. Protein molecules are constructed from building blocks called amino acids. There are twenty different kinds of amino acids. As amino acids are joined to each other with special covalent peptide bonds, the protein molecule grows larger and its shape becomes more and more complex.

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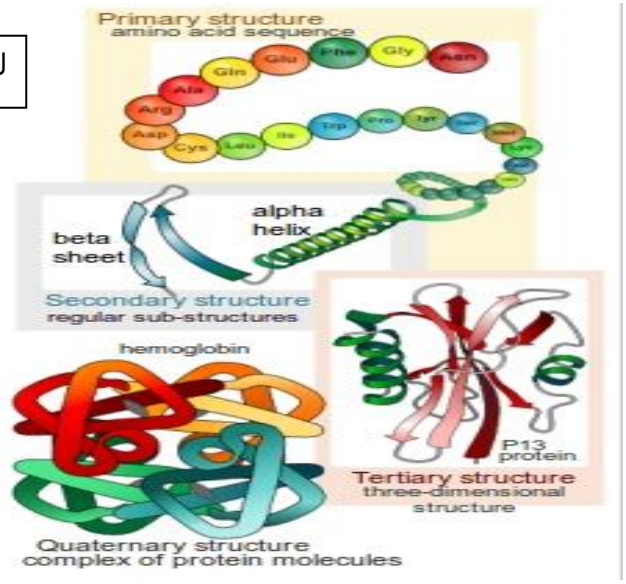


Proteins carry out a wide range of functions in the body:

1-Collagen and **keratin** are structural proteins. Collagen holds the tissues together throughout the body and strengthens ligaments and tendons. Keratin is a protein that toughens and waterproofs the skin.

2-The proteins **actin** and **myosin** permit our **muscles** to contract.

3- Enzymes are a special class of proteins that assist other chemicals to react with each other.

4- Many **hormones** that regulate body functions are proteins.

5-Hemoglobin is a blood protein that transports oxygen and carbon dioxide throughout the body

6-Antibodies are proteins in the blood and body fluids that help to fight infections.

Enzymes: Enzymes are proteins and can occur in the body in very small amounts. All the same, enzymes catalyze all processes in the body, enabling organisms to build up chemical substances such as other proteins, carbohydrates or fats that are necessary for life. All enzymes are proteins, but not all proteins are enzymes. If a protein can catalyze a biochemical reaction, it is an enzyme.

Hormones: Are chemical messengers that are secreted directly into the blood, which carries them to organs and tissues of the body to exert their functions. There are many types of hormones that act on different aspects of bodily functions and processes. Some of these include:

1-Development and growth

2-Metabolism of food items

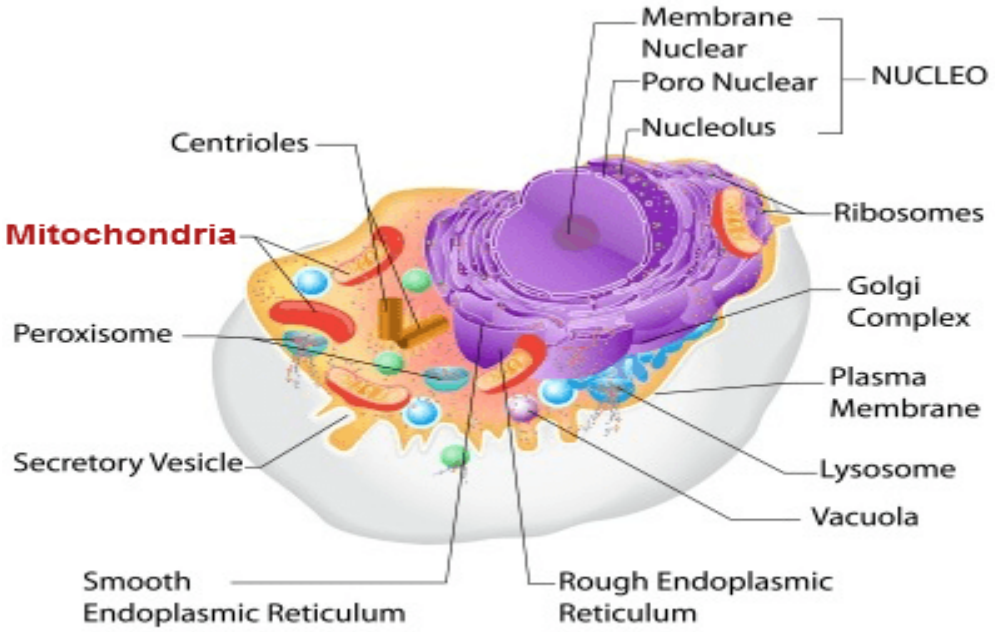
3-Sexual function and reproductive growth.

4-Health maintenance of body temperature.

Hormones are secreted from the endocrine glands in the body. The glands are ductless, so hormones are secreted directly into the blood stream rather than by way of ducts. The glands secrete hormone in microscopic amounts. Even a very slight excess of hormone secretion can lead to disease states, as can the slightest deficiency in a hormone.

Structure of the cell

A cell is the basic unit of structure and function in a living organism. When cells divide, the hereditary information they contain, as DNA, is passed from cell to cell. The term protoplasm includes the**" living part**" of the cell. It can be differentiated into **cytoplasm** and the **nucleus**.



Structure of typical human cell

The Cytoplasm

Cytoplasm is homogenous, clear jelly-like materials that fill the cells. The cytoplasm consists of cytosol and the cellular organelles except the nucleus. The cytoplasm plays a mechanical role,i.e. to maintain the shape, the consistency of the cell and to provide suspension to the organelles. In other words cytoplasm is the home of the cytoskeleton, a network of cytoplasmic filaments that are responsible for the movement of the cell and give the cell its shape.

Cellular Organelles:

Endoplasmic reticulum

The endoplasmic reticulum (ER) is a network of disk-like tubules, sacks and vesicles found in eukaryotic cells. Its main function is to operate as a transport system. It consists of lipid bi-layers which contain embedded proteins. This system of membrane is continuous with the double membrane that surrounds the cells' nucleus. The ER is often makes up more than 10% of cells' total volume. The ER is generally divided into two major sections: the **rough endoplasmic reticulum (RER)** and **smooth endoplasmic reticulum (SER).**

Rough Endoplasmic Reticulum (RER)

The term rough endoplasmic reticulum is based on the morphologic appearance of attached ribosomes, which are absent in smooth endoplasmic reticulum. Another morphologic distinction is the organization of the rough endoplasmic reticulum is interconnected flattened sacs (called **cisternae**), whereas the smooth endoplasmic reticulum forms a tubular network. Rough endoplasmic reticulum branches out and expands as protein synthesis increases, providing more surface area for ribosome to spread out and create more proteins.

The ER works in conjunction with the Golgi apparatus, to target the newly synthesized proteins to their proper locations. Most proteins produced by ribosomes of the rough endoplasmic reticulum are destined for secretion out of the cell. Once a protein is synthesized on a ribosome. It is enclosed within a vesicle, a small, membrane-bound "**bubble".** The vesicles travels to Golgi body, within the Golgi body, the proteins within the vesicle are further modified before they are exported from the cell. Cells that specialized in protein secretion contain large amounts of RER. For instance, cells of pancreas that produce the protein **insulin** have abundant rough endoplasmic reticulum.

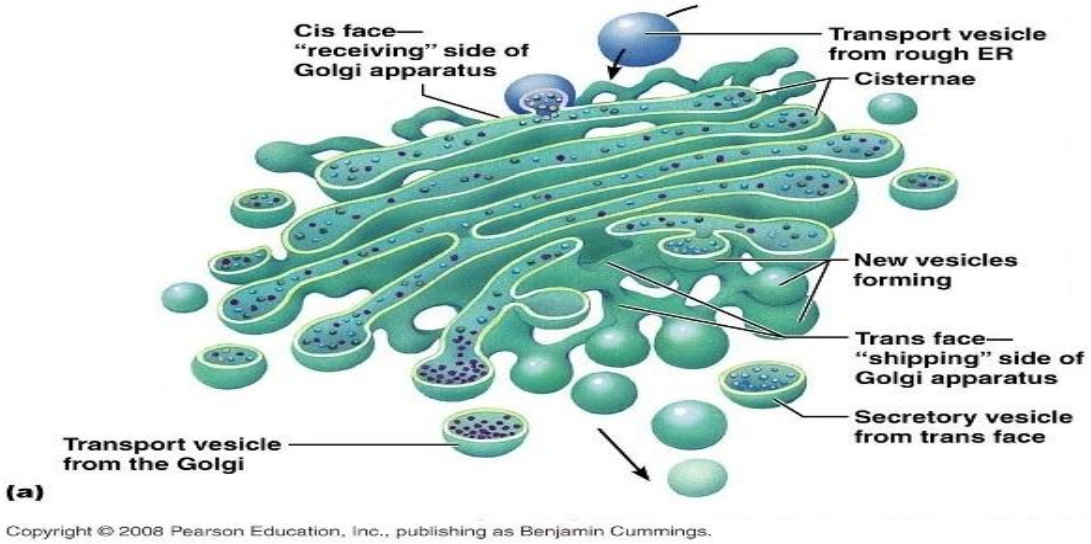
Smooth endoplasmic reticulum (SER)

Smooth endoplasmic reticulum has a few different functions in the cell. The SER does not have ribosomes and is the site of lipid metabolism. They provide surface area for the action of enzymes and storage space. These enzymes are used in the synthesis of carbohydrates and lipids. In liver cells the smooth ER produces enzymes that help to detoxify certain compounds. For, instance, liver cells remove alcohol and drugs from the bloodstream. In muscles the smooth ER assists in the contraction of muscle cells. Similarly, cells of the ovaries and testes, which produce the lipid-containing hormones estrogen and testosterone, contain large amounts of SER.

Another function of SER is the control the movement of newly synthesized proteins and lipids to their proper location in the cell or to the membrane to be sent outside the cell. This is done by a process called budding, where small vesicles of SER are pinched off to carry the proteins and lipids to their new location, various functions of endoplasmic reticulum makes it an important organelle for maintaining normal cell.

Golgi apparatus

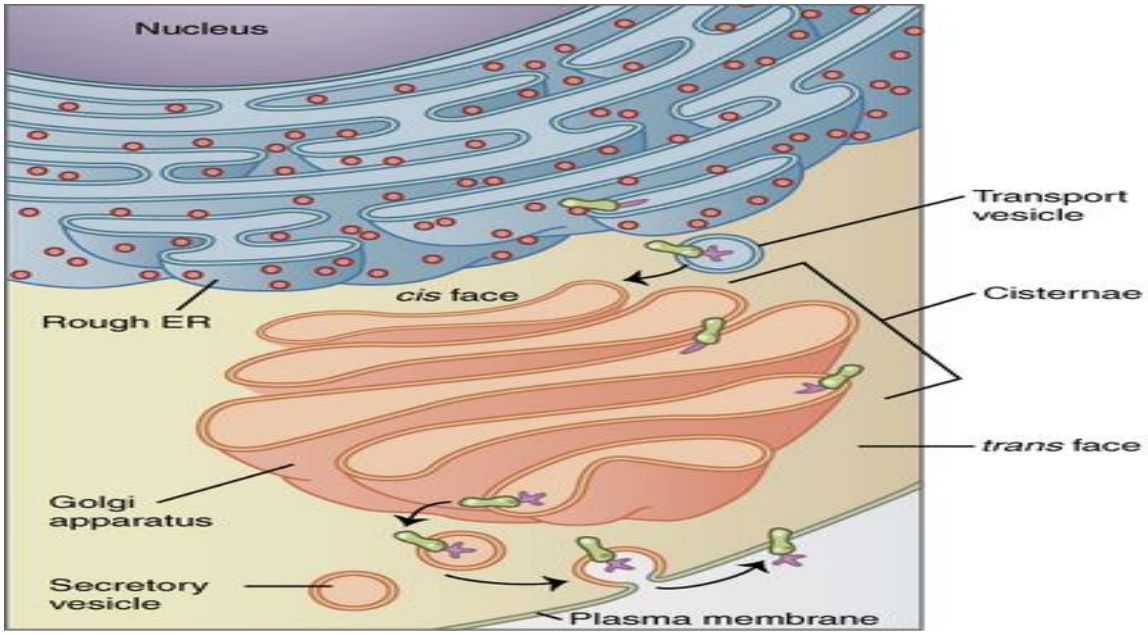
The Golgi apparatus found universally in human cells, it is typically composed of a series of five to eight cup-shaped, membrane-covered sacs called cisternae that look something like a stack of deflated balloons. The apparatus has three primary structures, a Golgi cisternae, Golgi vesicles and Golgi vacuoles. All the three structures are bound by a single unit membrane. It is ***cis* face** is the side facing the ER, while the *trans* face is directed towards the plasma membrane.



Function:

1. Golgi apparatus is responsible for handing the macromolecules that are required for proper cell functioning. It processes and packages these macromolecules for use within the cell or for secretion.
2. Golgi apparatus modifies proteins that it receives from the RER.
3. Transport lipids to vital parts of the cell and creates lysosomes.
4. Some of modifications made inside the Golgi complex include;

   Attaching polysaccharides to proteins to form gluco-proteins, cutting proteins into smaller active fragments, incorporating phosphates on to protein molecules and addition of a sulfate group to molecules.
5. Other function of Golgi apparatus include the production of glucosaminoglycans which go on to form parts of connective tissues.

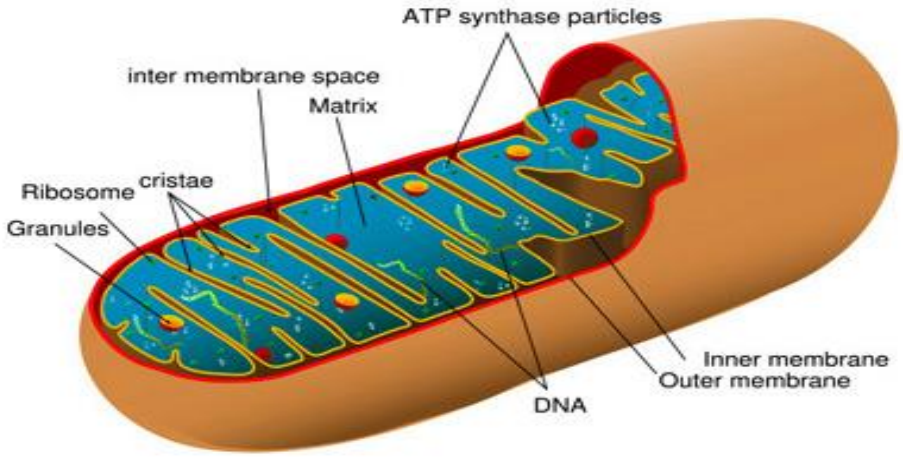


Mitochondria

The mitochondria are filamentous or granular cytoplasmic organelles found in all eukaryotic cells, there distribution in cell varies. They tend to accumulate in parts of cytoplasm where metabolic activity is more intense, such as the apical ends of ciliated cells, around the base of the flagellum or flagella , or at the base of ion-transferring cells. The cardiac muscle, mitochondria surround the contractile elements. The mitochondria have lipoprotein framework which contains many enzymes and co-enzymes required for energy metabolism and called **power house of the cell.**

The mitochondria are bound by double unit membrane. The two membrane are separated by wide peri-mitochondrial space or outer chamber. The outer membrane is smooth, relatively simple phospho-lipid bilayer, containing protein structure called **porins** which allows the passage of molecules up to 10 kilo-Daltons, nutrient molecules, ATP,ADP etc., can pass through the outer membrane. The matrix contains the enzymes that are responsible for the citric acid cycle reaction (Krebs cycle). Mitochondria contain the biochemical machinery involved in cellular respiration which take energy from breakdown of glucose and energy-rich ATP molecule which used a source of energy in metabolic reaction in the rest

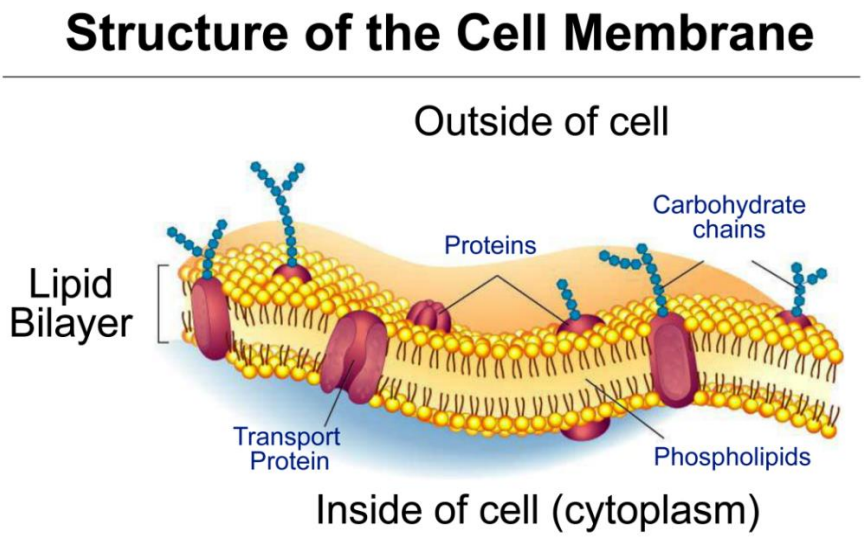
cell. Hence, mitochondria contain the enzymes required for the citric acid cycle, ATP synthesis and the oxidation of fatty acids. Mitochondria possess genetic material and ribosomes. Mitochondria DNA is circular and employs characteristic variants of the strand eukaryotic genetic code.



Mitochondria

Plasma Membrane

Structure of the Plasma Membrane
The cell membrane (or plasma membrane) surrounds all living cells. It controls how substances can move in and out of the cell and is responsible for many other properties of the cell as well. The membranes that surround the nucleus and other organelles are almost identical to the cell membrane. Membranes are composed of phospholipids, proteins and carbohydrates arranged in a fluid mosaic structure, as shown in this diagram.



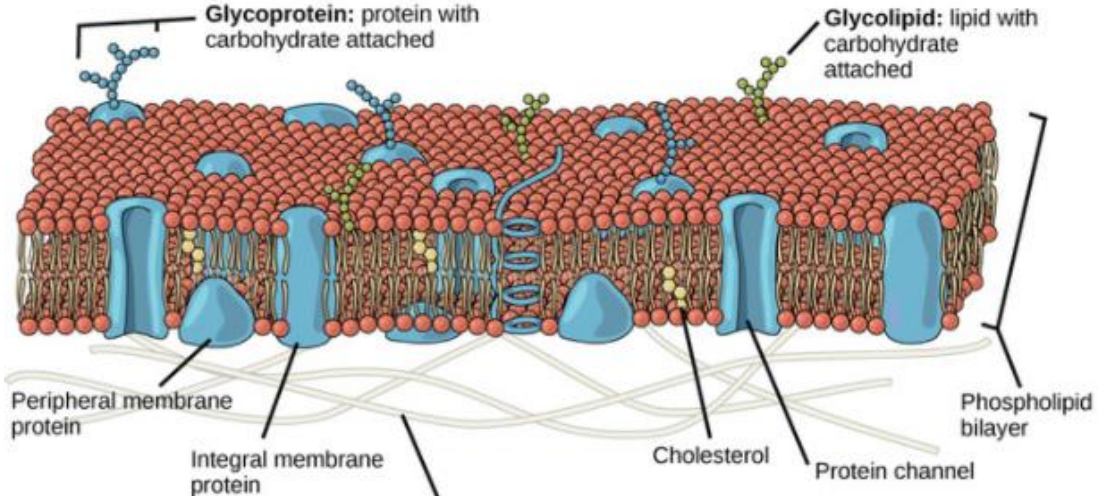
The phospholipids form a thin, flexible sheet, while the proteins "float" in the phospholipid sheet like icebergs, and the carbohydrates extend out from the proteins.

The phospholipids are arranged in a bilayer, with their polar, hydrophilic phosphate heads facing outwards, and their non-polar, hydrophobic fatty acid tails facing each other in the middle of the bilayer. The lipid bilayer is semi-permeable, allowing only certain molecules to diffuse across the membrane. Different kinds of membranes can contain phospholipids with different fatty acids, affecting the strength and flexibility of the membrane. Human cell membranes also contain cholesterol linking the fatty acids together and so strengthening the membrane.

The proteins usually span from one side of the phospholipid bilayer to the other (integral proteins), but can also sit on one of the surfaces (peripheral proteins). Proteins comprise about 50% of the mass of membranes, and are responsible for most of the membrane's properties.

Proteins found in plasma membrane serve different functions:

- **Channel Proteins** - form small openings for molecules to diffuse through the membrane.
- **Carrier Proteins**- binding site on protein surface "grabs" certain molecules and pulls them into the cell.
- **Receptor Proteins** - molecular triggers that set off cell responses (such as release of hormones or opening of channel proteins)
- **Cell Recognition Proteins**, to identify cells to the body's immune system.
- **Enzymatic Proteins** - carry out metabolic reactions**.**



The carbohydrates are found on the outer surface of all eukaryotic cell membranes, and are attached to the membrane proteins or sometimes to the phospholipids. Proteins with carbohydrates attached are called glycoproteins, while phospholipids with carbohydrates attached are called glycolipids. The carbohydrates are short polysaccharides composed of a variety of different monosaccharaides, and form a cell coat outside the cell membrane. The cell coat is involved in protection and cell recognition, and antigens such as the ABO antigens on blood cells are usually cell-surface glycoproteins.

<u>Function</u>

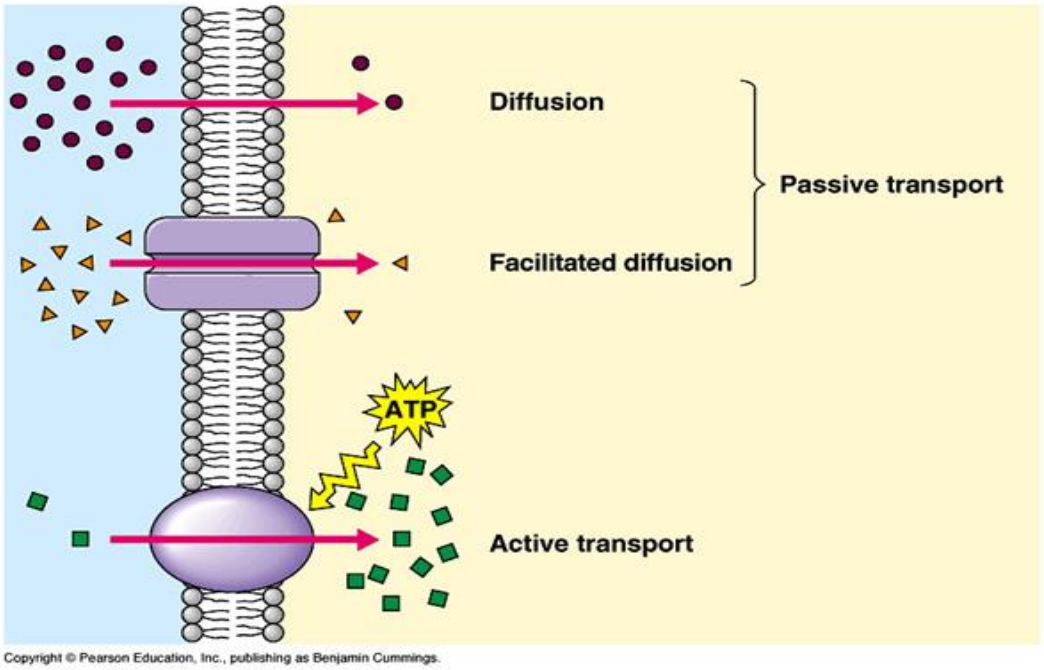
- Isolate the cytoplasm from the external environment
- Regulate the exchange of substances
- Communicate with other cells
- Identification
- The cell membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell.

Movement across Cell Membranes

The cell membrane is selectively permeable and able to regulate what enters and exits the cell, thus facilitating the transport of materials needed for survival. The movement of substances across the membrane can be either **"passive",** occurring without the input of cellular energy, or **"active**", requiring the cell to expend energy in transporting it. There are two ways in which substances can enter or leave a cell:

1) **Passive ways**:
a) Simple Diffusion
b) Facilitated Diffusion
c) Osmosis (water only)

2) **Active ways**:
a) Active Transport
b) Vesicle Transport



1) Passive ways:

a- Simple Diffusion

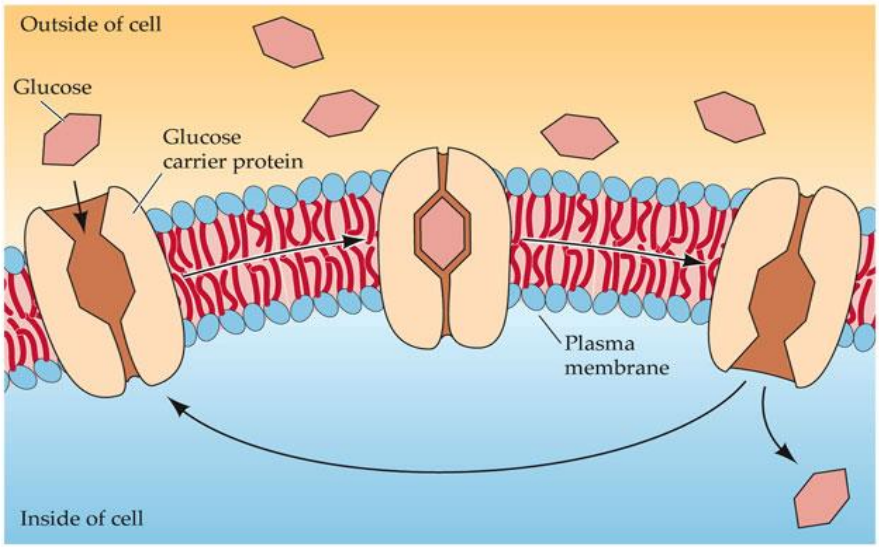
Diffusion is the net passive movement of particles (atoms, ions or molecules) from a region in which they are in higher concentration to regions of lower concentration (down a concentration gradient). It continues until the concentration of substances is uniform throughout.

An example: gas exchange for respiration — oxygen from blood to tissue cells, carbon dioxide in opposite direction.

b- Facilitated Diffusion

This is the movement of specific molecules down a concentration gradient, passing through the membrane via a specific carrier protein. Each carrier has its own shape and only allows one molecule (or one group of closely related molecules) to pass through. Selection is by size; shape; and charge. Common molecules entering/leaving cells this way include glucose (GLU Transporter) and amino-acids. It is passive and requires no energy from the cell.

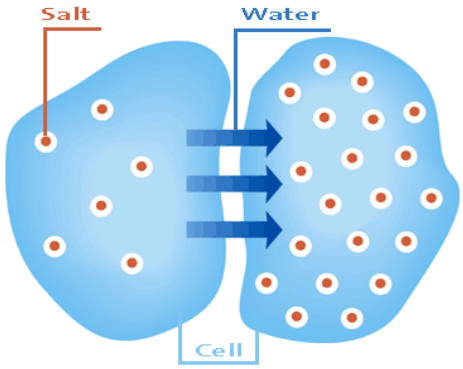
Facilitated Diffusion



c-Osmosis

Osmosis is a special example of diffusion. It is the diffusion of water through a partially permeable membrane from a more dilute solution to a more concentrated solution – down the water potential gradient).

Note: diffusion and osmosis are both passive, i.e. energy from ATP is **not** used.



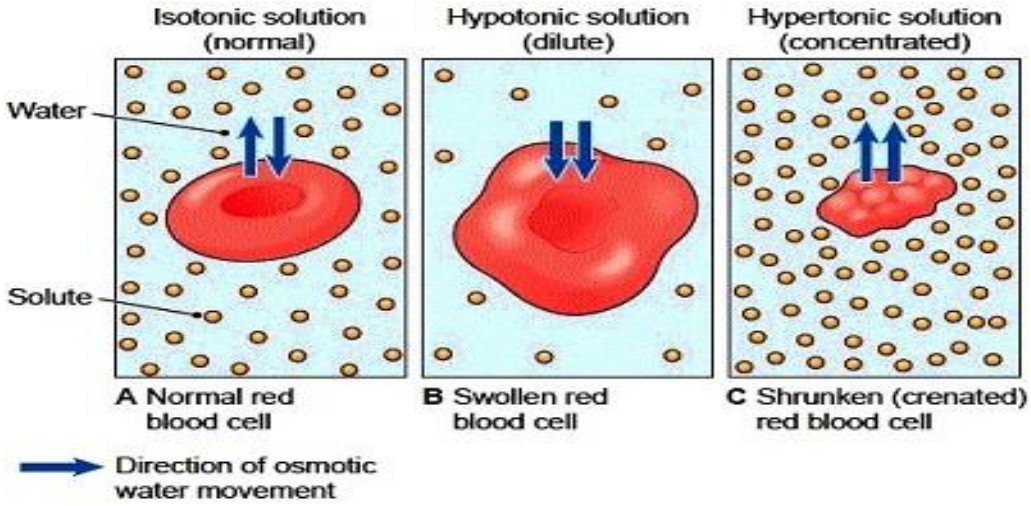
The Effects of Osmosis

When an human cell (for example, the red blood cell) is placed in a medium, which is a water solution , the possible consequences are listed below:

1- If the water concentration inside the cell is the same as that in the surrounding medium (the medium is an isotonic solution) there will exist a dynamic equilibrium between the number of molecules of water entering and leaving the cell and so the cell will retain its original size.

2- If the water concentration of the cell is lower than that of the medium (the medium is a **hypotonic solution**) surrounding the cell then osmosis will result in the cell gaining water. The water molecules are free to pass across the cell membrane in both directions, but more water molecules will enter the cell than will diffuse out with the result that water enters the cell, which will then swell up and could possibly burst.

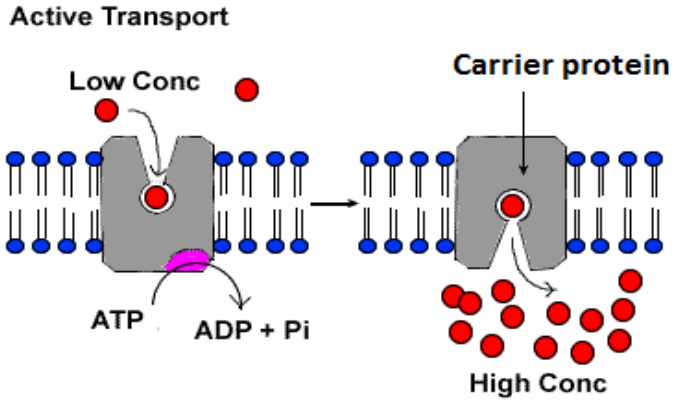
3- If the water concentration inside the cell is higher than that of the medium (the medium is a **hypertonic solution**) the number of water molecules diffusing out will be more than that entering and the cell will shrink due to osmosis.



2) Active ways

a. Active Transport

Active transport is the energy-demanding transfer of a substance across a cell membrane against its concentration gradient, from lower concentration to higher concentration. Special proteins within the cell membrane act as specific protein 'carriers'. The energy for active transport comes from ATP generated by respiration (in mitochondria).An example: Sodium/potassium pump in cell membranes (especially nerve cells); uptake of glucose by epithelial cells in the villi of the small intestine. There are two types of active transport: **primary active transport** that uses adenosine triphosphate (ATP), and **secondary active transport** that uses an electrochemical gradient.



b. Vesicle Transport

Some molecules or particles are just too large to pass through the plasma membrane or to move through a transport protein. So cells use two other active transport processes to move these macromolecules (large molecules) into or out of the cell. Vesicles or other bodies in the cytoplasm move macromolecules or large particles across the plasma membrane. There are two types of transport, **endocytosis** and **exocytosis** . Both processes are active transport processes, requiring energy.

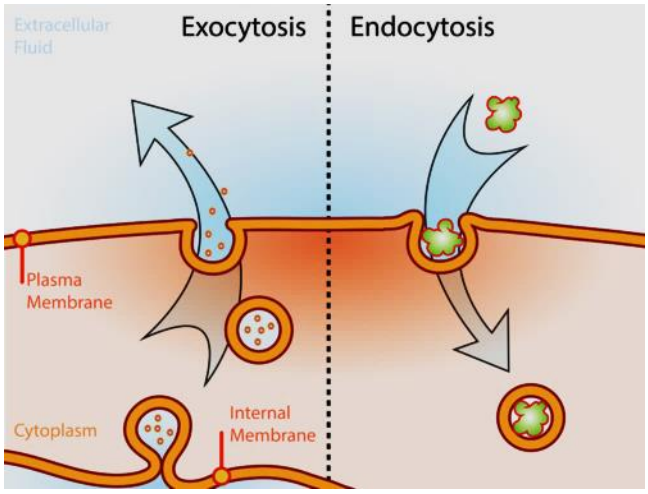
- **Endocytosis** is the process of capturing a substance or particle from outside the cell by engulfing it with the cell membrane. The membrane folds over the

substance and it becomes completely enclosed by the membrane. At this point a membrane-bound sac, or vesicle, pinches off and moves the substance into the cytosol. There are two main kinds of endocytosis:

1-Pinocytosis (‘cell drinking’) This is the uptake of large molecules (DNA, protein) from solution, by a form of endocytosis – the vesicles formed are minute and short-lived. In humans, this process occurs primarily for absorption of fat droplets.

2-Phagocytosis (‘cell eating’) This is the uptake of solid particles by a cell e.g., Phagocytes engulfing bacteria.

- **Exocytosis** describes the process of vesicles fusing with the plasma membrane and releasing their contents to the outside of the cell. Exocytosis occurs when a cell produces substances for export, such as a protein, or when the cell is getting rid of a waste product or a toxin. Newly made membrane proteins and membrane lipids are moved by exocytosis.



Cell membrane specialization.

The lateral of the cell membrane can show form "intercellular junction".
Function of these junctions:
1-They are the sites of adhesion between adjacent cell,
2-They prevent the flow of materials through the intercellular
3- They help in the cellular communication.

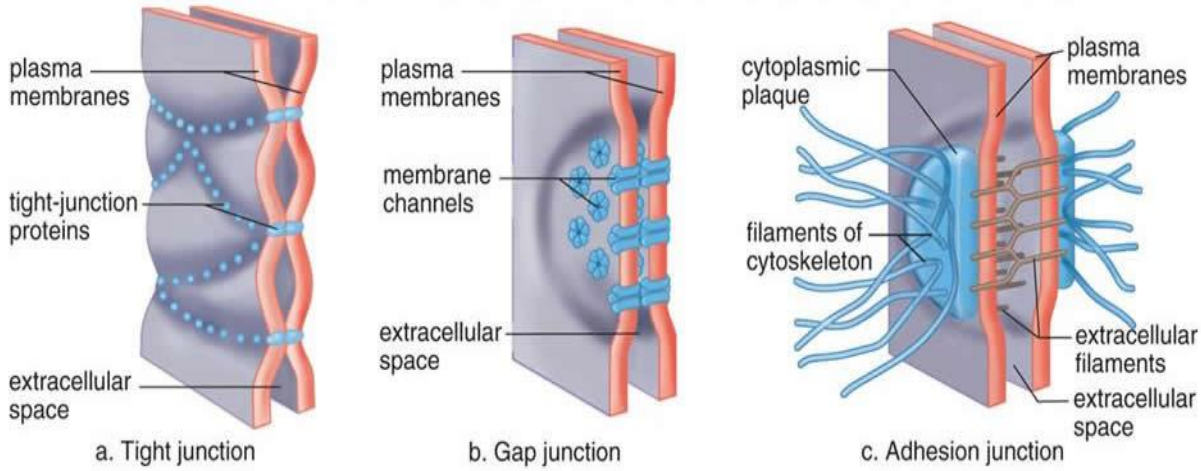
There are three types of junctions.

1-Adhesion junctions (desmosomes):

In this type, the internal cytoplasmic plaques firmly attached to the cytoskeleton with intercellular filaments. Bladder, adhesion junction hold cell together.(Desmosomes are **intercellular junctions that provide strong adhesion between cells**. Because they also link intracellularly to the intermediate filament cytoskeleton they form the adhesive bonds in a network that gives mechanical strength to tissues).

2- Tight junctions:
Adjacent cells are even more closely by tight junctions in which plasma membrane proteins actually attach to each other producing a zipper like fastening. These junctions between cells form an impermeable prevent the flow of materials in intercellular space. e.g., in the kidneys the urine stays within kidney tubules because the cells are joined by tight junctions.

3- Gap junctions:
It allow to communicate, and is formed when two in identical plasma membrane channels joins. The channel of each cell is lined by six plasma membrane proteins (hexamears).Gap junction are important in heart muscle and smooth muscle because they permit a flow of ions that is required for the cells to contact.



Apical modification of plasma membrane:

1-**Microvilli:**
Fingers like extensions of plasma membrane that are particularly abundant on the surface of the cells, involved in the absorption, such as the epithelial cells lining the intestine.

2-**Stereocilia:** from actin filament in sensing cells.
Specialized form of microvilli. The stereocilia of auditory hair cells, are responsible for hearing by detecting sound vibrations.

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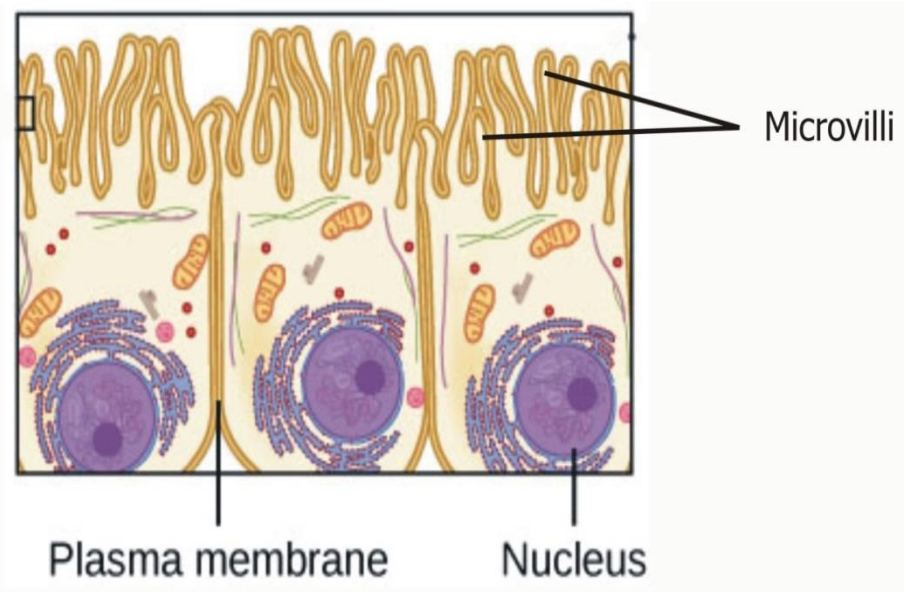


Molecular Biology

Molecular biology concerns the molecular basis of biological activity between the various systems of a cell, including the interactions between the different types of DNA, RNA and proteins and their biosynthesis, and studies how these interactions are regulated.

Definitions:

Genetics: Study of inherited variations.

Gene: A sequence of DNA that instructs a cell to produce a particular protein.

Allele: Alternate forms of a gene that occur at a given locus in chromosome.

Homozygous: having two identical alleles of a gene (TT or tt).

Heterozygous: having two different alleles of a gene (Tt).

Phenotype: The expression of a gene in traits or symptoms.

Genotype: The alleles combinations in an individual that cause particular traits or disorders.

The anatomy of gene

Although there is no such thing as a 'typical' gene, there are certain basic requirements for any gene to function. The most obvious is that the gene has to encode the information for the particular protein (or RNA molecule). The double-stranded DNA molecule has the potential to store genetic information in either strand, although in most organisms only one strand is used to encode any particular gene. There is the potential for confusion with the nomenclature of the two DNA strands, which may be called coding/non-coding, sense/antisense, plus/minus, transcribed/non-transcribed, or template/non-template. A site for starting transcription is required, and this encompasses a region that binds RNA polymerase known as the **promoter** (P), and a specific start point for transcription (TC). A stop site for transcription (tC) is also required. From TC start to tC stop is sometimes called the **transcriptional unit**, that is, the DNA region that is copied into RNA. Within this transcriptional unit there may be regulatory sites for translation, namely a start site (TL) and a stop signal (tL). Other sequences involved in the control of gene expression may be present either upstream or downstream from the gene itself.

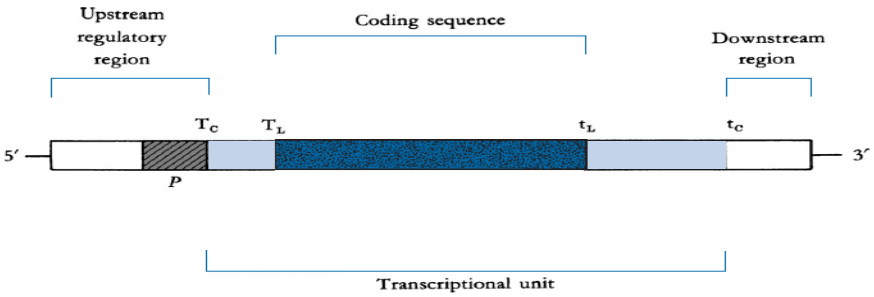


Fig. 2.6 Gene organisation. The transcriptional unit produces the RNA molecule and is defined by the transcription start site (T_C) and stop site (t_C). Within the transcriptional unit lies the coding sequence, from the translation start site (T_L) to the stop site (t_L). The upstream regulatory region may have controlling elements such as enhancers or operators in addition to the promoter (P), which is the RNA polymerase binding site.

Gene structure in prokaryotes

In prokaryotic cells such as bacteria, genes are usually found grouped together in **operons**. The operon is a cluster of genes that are (often coding for enzymes in a metabolic pathway) and that are under the control of a single promoter/regulatory region. Perhaps the best known example of this arrangement is the *lac* operon (Fig. 2.7), which encodes for the enzymes responsible for lactose catabolism. The fact that structural genes in prokaryotes are often grouped together means that the transcribed mRNA may contain information for more than one protein. Such a molecule is known as a **polycistronic** mRNA, with the term **cistron** equating to the 'gene' as we have defined it (*i.e.* encoding one protein). Thus, much of the genetic information in bacteria is expressed *via* polycistronic mRNAs whose synthesis is regulated in accordance with the needs of the cell at any given time. This system is flexible and efficient, and it enables the cell to adapt quickly to changing environmental conditions.

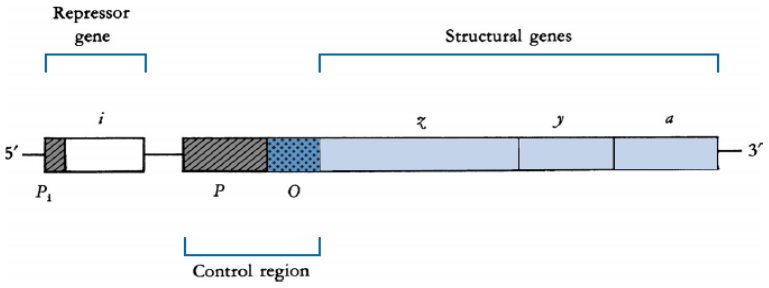


Fig. 2.7 The *lac* operon. The structural genes *lacZ*, *lacY*, and *lacA* (noted as z, y, and a) encode ß-galactosidase, galactoside permease, and a transacetylase, respectively. The cluster is controlled by a promoter (*P*) and an operator region (*O*). The operator is the binding site for the repressor protein, encoded by the *lacI* gene (*i*). The repressor gene lies outside the operon itself and is controlled by its own promoter, P_i.

Gene structure in eukaryotes

A major defining feature of eukaryotic cells is the presence of a membrane-bound nucleus, within which the DNA is stored in the form of chromosomes. Transcription therefore occurs within the nucleus and is separated from the site of translation, which is in the cytoplasm. Gene structure and function in eukaryotes are more complex than in prokaryotes. Eukaryotic genes contained 'extra' pieces of DNA that did not appear in the mRNA that the gene encoded. These sequences are known as intervening sequences or **introns**, with the sequences that will make up the mRNA being called **exons**.

DNA Replication :

- Replication of DNA is semiconservative .
- During replication the 2 complementary strand unwind and each single strand serve as template directing the synthesis of a new complementary strand .
- The new result of replication is thus 2 progeny DNA molecules identical to the parental double helix . A site where DNA is locally opened called replication fork .

Enzymes involved in DNA replication and their function :

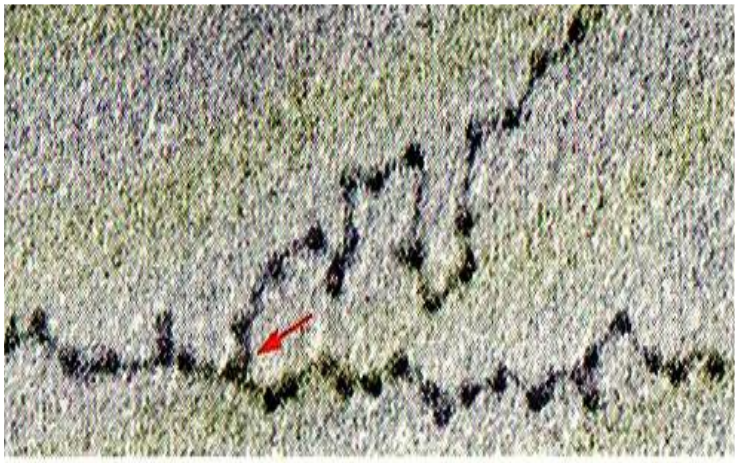
1. **Helicase** : unwinds parental double helix .
2. **Binding Proteins** : stabilize separate strands .
3. **Primase** : adds short RNA Primer to template strand .
4. **DNA Polymerase** :

❖ Adding bases to the new DNA chain , added Bases in (5'→3') direction only and DNA is antiparallel, the new strand is synthesized continuously in (5'→3') direction called Leading strand, while the other daughter strand is synthesized discontinuously by short segments of DNA called Okazaki Fragments (5'→3') that are joined together by ligase and called Lagging strand .

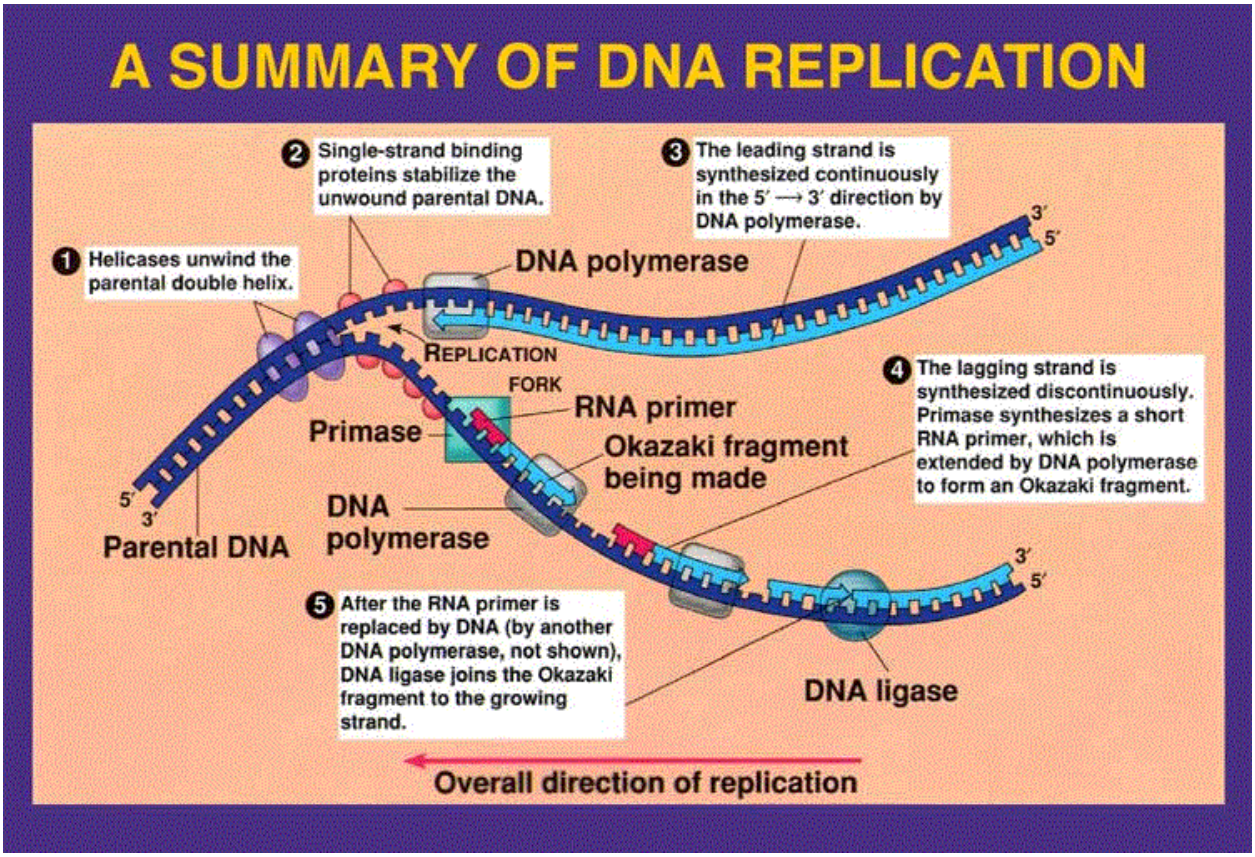
❖ Proofreading activity checks and replaces incorrect bases .

❖ Removing RNA primer .

5. **Ligase**: joins okazaki fragments and seals other nicks in sugar phosphate backbone .



Electron micrograph of a eukaryote replicating fork demonstrating the presence of histone- protein containing nucleosomes on both branches.



Genome:

A genome is an organism's complete set of deoxyribonucleic acid (DNA), a chemical compound that contains the genetic instructions needed to develop and direct the activities of every organism. The human genome contains approximately 3.2 billion of base pairs, which reside in the 23 pairs of chromosomes **(**22 autosome

pairs + 2 sex chromosomes) in the haploid genome. Human genome comprise of around 30,000 - 40,000 genes. Only about 3% of human genome codes for proteins while 40-50% is repetitive DNA and others is unknown function.

The flow of genetic information

It is a remarkable fact that an organism's characteristics are encoded by a four-letter alphabet, defining a language of three-letter words. The letters of this alphabet are the nitrogenous bases adenine (A), guanine (G), cytosine (C), and thymine (T). So how do these bases enable cells to function? The expression of genetic information is achieved ultimately *via* proteins, particularly the enzymes that catalyse the reactions of metabolism. Proteins are condensation heteropolymers synthesized from amino acids, of which 20 are used in natural proteins. Given that a protein may consist of several hundred amino acid residues.

The flow of genetic information is unidirectional, from DNA to protein, with **messenger RNA** (**mRNA**) as an intermediate. The copying of DNA-encoded genetic information into RNA is known as **transcription** (TC), with the further conversion into protein being termed **translation** (TL). This concept of information flow is known as the **Central Dogma** of molecular biology and is an underlying theme in all studies of gene expression.

Transcription and translation

These two processes are the critical steps involved in producing functional proteins in the cell. Transcription involves synthesis of an RNA from the DNA template provided by the non-coding strand of the transcriptional unit in question. The enzyme responsible is **RNA polymerase.** In prokaryotes there is a single RNA polymerase enzyme, but in eukaryotes there are three types of RNA polymerase (I, II, and III). These synthesize ribosomal, messenger, and transfer/5S ribosomal RNAs, respectively. When the RNA molecule is released, it may be immediately available for translation (as in prokaryotes) or it may be processed and exported to the cytoplasm (as in eukaryotes) before translation occurs. Translation requires an mRNA molecule, a supply of charged tRNAs (tRNA molecules with their associated amino acid residues), and ribosomes (composed of rRNA and ribosomal proteins). The ribosomes are the sites where protein synthesis occurs; in prokaryotes, ribosomes are composed of three rRNAs and some 52 different ribosomal proteins. The ribosome is a complex structure that essentially acts as hold the mRNA in place so that the codons may be matched up with the appropriate **anticodon** on the tRNA, thus ensuring that

the correct amino acid is inserted into the growing polypeptide chain. The mRNA molecule is translated in a 5'→3' direction.

Transcription (RNA synthesis) :

Transfer of genetic information from DNA by the synthesis of a complementary **RNA** molecule under the direction of **RNA polymerase** .

Transcription occurs in three stages :

1- Initiation :

Transcription begins when **transcription factors** help **RNA polymerase** bind to the **promoter** (which is a special sequence that signals the start of the gene).

Transcription factor regulate which genes are transcriped in a particular cell type .

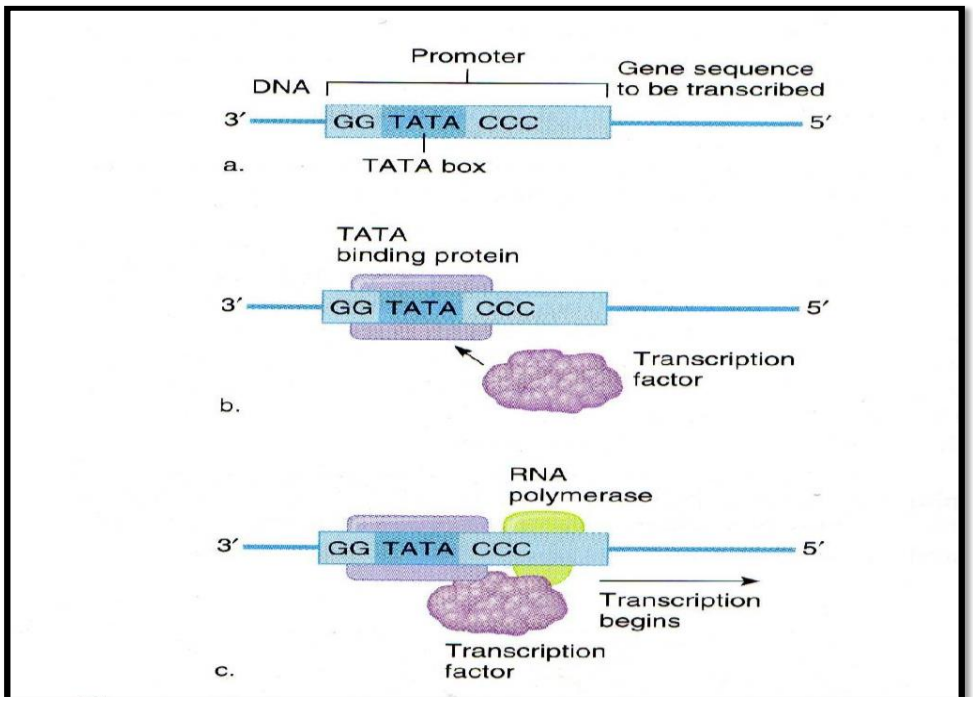


Figure: Setting the stage for transcription to begin: a- Proteins that initiate recognize specific sequences in the promoter region of a gene. b-A binding protein recognizes the TATA region and binds to the DNA. This allows other transcription factors to bind. c- the bound transcription factors form a pocket that allows RNA-polymerase to bind and begin making RNA.

2- Elongation :

RNA polymerase unwind the DNA double helix locally, RNA polymerase then adds RNA nucleotides to a growing chain, in a sequence complementary to the DNA template strand . RNA is transcribed from the **template strand** of DNA .

The other DNA strand is called the **coding strand** .

3- Termination :

A termination sequence in the gene signals the end of transcription .

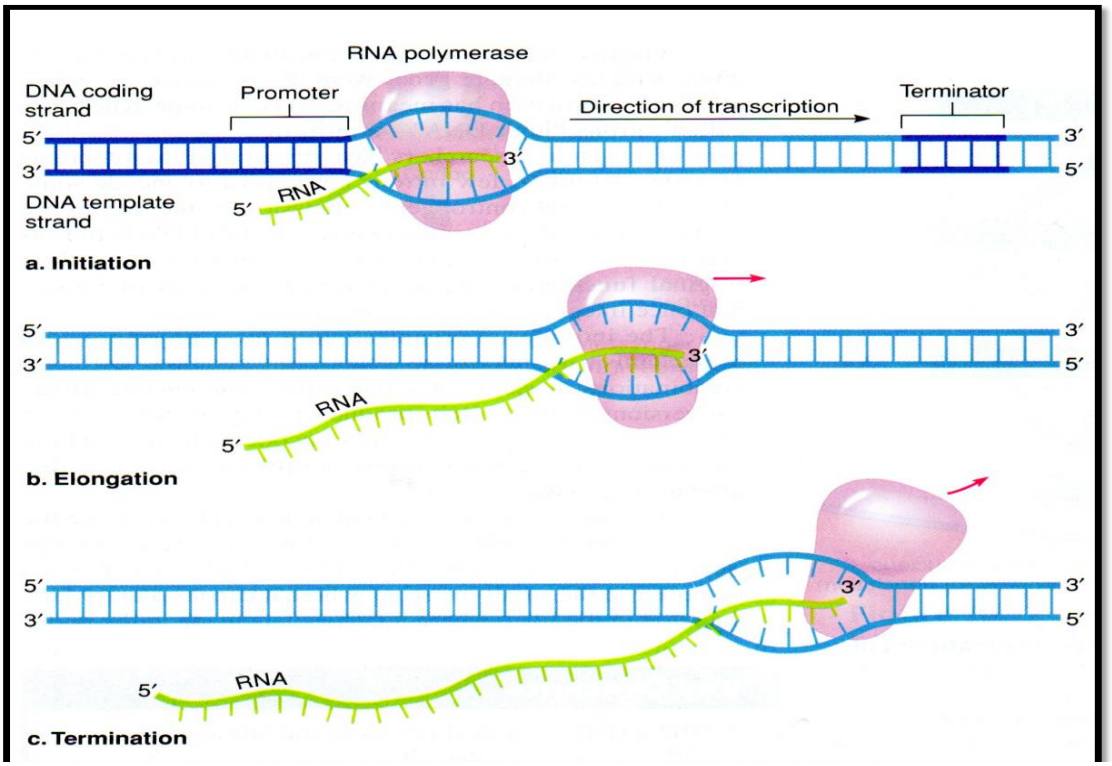


Figure: Transcription: Initiation is the control point that determines which genes are transcribed. RNA nucleotides are added during elongation. A termination sequence in the gene signals the end transcription.

mRNA Processing in Eukaryotes

In the nucleus a gene composed of **Exons** (coding sequences) and **Introns** (non coding sequences). Both of these are transcriped to pre- mRNA or primary transcripts (primary mRNA). Pre mRNA undergo 3 major modification prior to their transport to the cytoplasm for translation :

1- 7-Methyl guanosine caps are added to the 5' ends of the primary transcripts.

2- Poly (A) tails (a series of adenine molecules) are added to the 3 ends of the transcripts . It is important for RNA stability and translation of polypeptide .

3- Processing involves **spliceosomes** consist of snRNAs and protein subunits in the nucleus to remove the intron and **splice together the exon into mRNA.**

Mature mRNA transmits to the cytoplasm where it directs protein synthesis.

Transcription and RNA Processing occur in the nucleus while translation occurs in the cytoplasm.

Translation (Protein Synthesis)

The Process which the genetic information (which is stored in the sequence of nucleotides in an mRNA molecule) is translated , according to the specification of the genetic code into the sequence of amino acids in the polypeptide gene product. A ribosome has two subunits (each composed of rRNA and various proteins) small subunit and large subunit . Eukaryotic ribosome (80 S) has small subunit (40 S) and large subunit (60 S) .

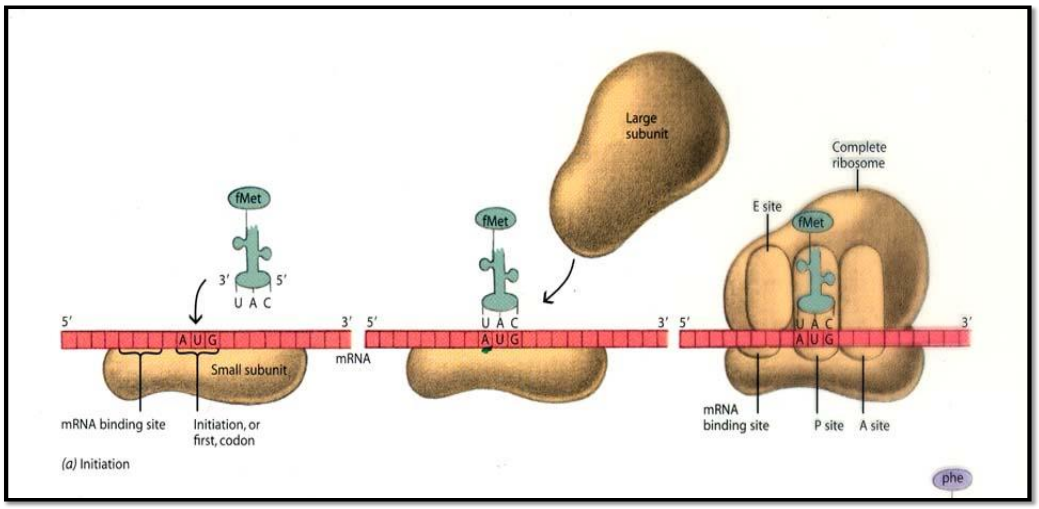
Protein synthesis consist of 3 phases :

1- Initiation Phase :

A small ribosomal subunit binds to mRNA : an initiator tRNA with the anticodon UAC pairs with the start codon AUG.

The large ribosomal subunit completes the ribosome , initiator tRNA carry methionine occupies the P-site. The A-site is ready for the second tRNA

The small and large subunit together form two tRNA binding sites P(Peptidyl) site and A (Aminoacyl) site .



2- Elongation Phase : Elongation consist of 3 steps :

1st A second charged tRNA with an anticodon complementary to the second codon on mRNA binds to A-site .

2nd- Peptide bond formation : Peptidyl transferase (part of the large ribosomal subunit) can catalyze formation of peptide bond between the amino acids carried by the two tRNAs . This bond forming reaction connects the methionine at the P-site to the amino acid carried by the tRNA at the A-site . It also disconnects methionine from the initiation tRNA as a result the tRNA at the A-site now carries two amino acids .

3rd- Translocation :

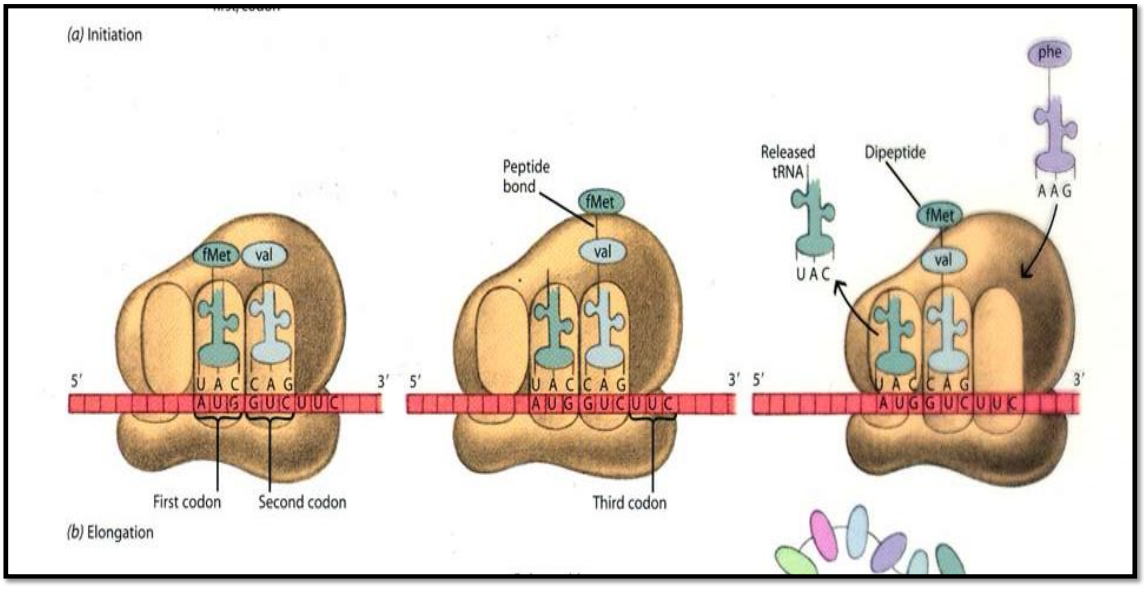
Three concerted movements occur , collectively called translocation :

1- Uncharged -tRNA leave the P-site .

2- The dipeptide-tRNA in the A-site moves to the P-site .

3- The ribosome moves a long the mRNA by three nucleotides (codon) to place the next codon in the A-site .

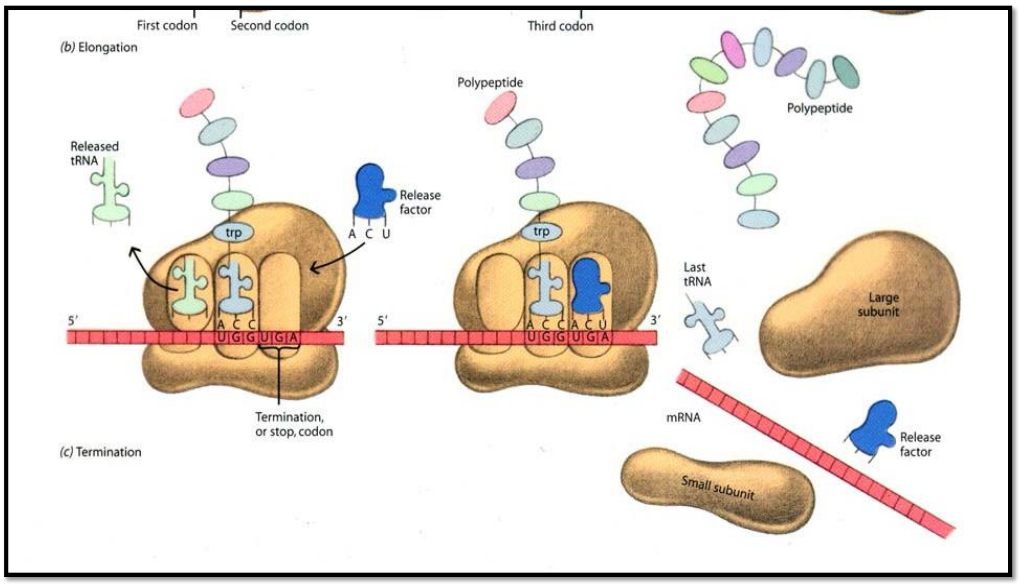
The empty A-site now receives another tRNA whose identity is determined by the next codon in the mRNA and the (peptide bond formation and translocation) occurs once again .



3- Termination Phase:

Termination of protein synthesis occurs when one of 3 stop codons (UAG , UAA , UGA) appears in A-site of the ribosome . A protein called release factor

recognize stop codons and hydrolysis the bond between the last tRNA at the P-site and the **polypeptide** releasing them . The ribosomal subunits dissociate .
The resultant **polypeptide chain** may be enzyme , hormone , antibody , or structural proteins .



Features of the genetic code :

1- Genetic code is **Triplet** : AAA code for lysine .

2- **Unambiguous** : each Triplet codon has only one meaning .

3- The genetic code has start and stop signals . There is one **start signals** (**AUG**) . and there is 3 **stop signals** (**UAA** , **UAG** , **UGA**) .

4- **Universal** : the code is the same and stable in all living organisms.

5- The genetic code is **nonoverlapping** and **degenerate.**

Nucleic Acids

Introduction

Nucleic acids, and DNA in particular, are key macromolecules for the continuity of life. DNA bears the hereditary information that's passed on from parents to children, providing instructions for how (and when) to make the many proteins needed to build and maintain functioning cells, tissues, and organisms.

Roles of DNA and RNA in cells

Nucleic acids, macromolecules made out of units called nucleotides, come in two naturally occurring varieties: **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**. DNA is the genetic material found in living organisms, all the way from single-celled bacteria to multicellular mammals like you and me. Some viruses use RNA, not DNA, as their genetic material, but aren't technically considered to be alive (since they cannot reproduce without help from a host).

DNA in cells

In eukaryotes, such as plants and animals, DNA is found in the **nucleus**, a specialized, membrane-bound move in the cell, as well as in certain other types of organelles (such as mitochondria and the chloroplasts of plants). In prokaryotes, such as bacteria, the DNA is not enclosed in a membranous envelope, although it's located in a specialized cell region called the **nucleoid**.

In eukaryotes, DNA is typically broken up into a number of very long, linear pieces called **chromosomes**, while in prokaryotes such as bacteria, chromosomes are much smaller and often circular (ring-shaped). A chromosome may contain tens of thousands of **genes**, each providing instructions on how to make a particular product needed by the cell.

From DNA to RNA to proteins

Many genes encode protein products, meaning that they specify the sequence of amino acids used to build a particular protein. Before this information can be used for protein synthesis, however, an RNA copy (transcript) of the gene must first be made. This type of RNA is called a **messenger RNA (mRNA)**, as it serves as a messenger between DNA and the ribosomes,

molecular machines that read mRNA sequences and use them to build proteins. This progression from DNA to RNA to protein is called the **"central dogma"** of molecular biology. Importantly, not all genes encode protein products. For instance, some genes specify **ribosomal RNAs (rRNAs)**, which serve as structural components of ribosomes, or **transfer RNAs (tRNAs)**, cloverleaf-shaped RNA molecules that bring amino acids to the ribosome for protein synthesis. Still other RNA molecules, such as tiny **microRNAs (miRNAs)**, act as regulators of other genes, and new types of non-protein-coding RNAs are being discovered all the time.

Nucleotides

DNA and RNA are polymers (in the case of DNA, often very long polymers), and are made up of monomers known as **nucleotides**. When these monomers combine, the resulting chain is called a **polynucleotide** (*poly-* = "many").

Each nucleotide is made up of three parts: a nitrogen-containing ring structure called a **nitrogenous base**, a **five-carbon sugar**, and at least one **phosphate group**. The sugar molecule has a central position in the nucleotide, with the base attached to one of its carbons and the phosphate group (or groups) attached to another. Let's look at each part of a nucleotide in turn.

Bases include the pyrimidine bases (cytosine, thymine in DNA, and uracil in RNA, one ring) and the purine bases (adenine and guanine, two rings). The phosphate group is attached to the 5' carbon. The 2' carbon bears a hydroxyl group in ribose, but no hydroxyl (just hydrogen) in deoxyribose.

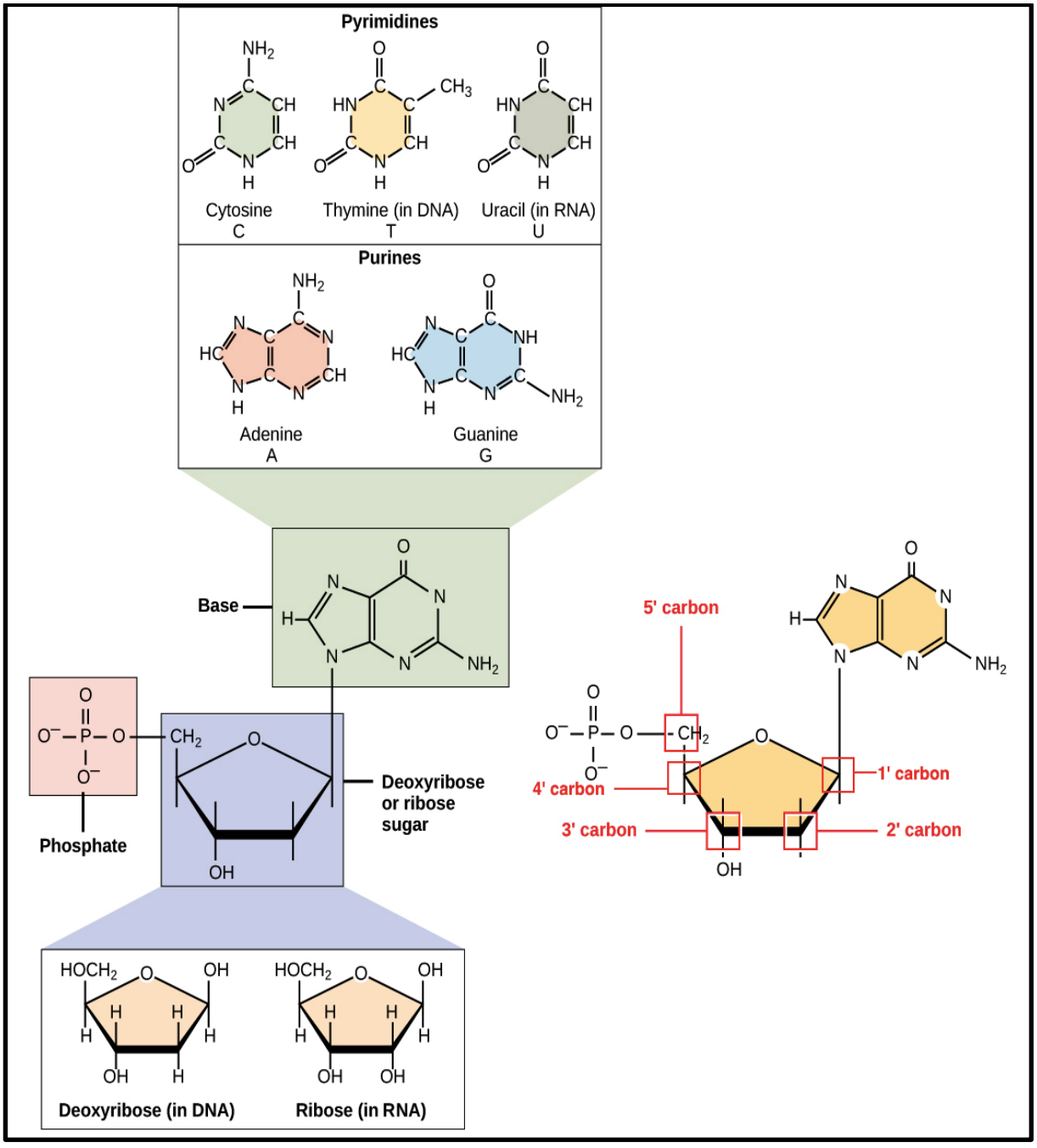


Image of the components of DNA and RNA, including the sugar (deoxyribose or ribose), phosphate group, and nitrogenous base.

Nitrogenous bases

The nitrogenous bases of nucleotides are organic (carbon-based) molecules made up of nitrogen-containing ring structures.

Each nucleotide in DNA contains one of four possible nitrogenous bases: adenine (A), guanine (G) cytosine (C), and thymine (T). Adenine and guanine are **purines**, meaning that their structures contain two fused carbon-nitrogen rings. Cytosine and thymine, in contrast, are **pyrimidines** and have a single carbon-nitrogen ring. RNA nucleotides may also bear adenine, guanine and cytosine bases, but instead of thymine they have another pyrimidine base called uracil (U). As shown in the figure above, each base has a unique structure, with its own set of functional groups attached to the ring structure.

In molecular biology shorthand, the nitrogenous bases are often just referred to by their one-letter symbols, A, T, G, C, and U. DNA contains A, T, G, and C, while RNA contains A, U, G, and C (that is, U is swapped in for T).

Sugars

In addition to having slightly different sets of bases, DNA and RNA nucleotides also have slightly different sugars. The five-carbon sugar in DNA is called **deoxyribose**, while in RNA, the sugar is **ribose**. These two are very similar in structure, with just one difference: the second carbon of ribose bears a hydroxyl group, while the equivalent carbon of deoxyribose has a hydrogen instead. The carbon atoms of a nucleotide's sugar molecule are numbered as 1′, 2′, 3′, 4′, and 5′ (1′ is read as "one prime"), as shown in the figure above. In a nucleotide, the sugar occupies a central position, with the base attached to its 1′ carbon and the phosphate group (or groups) attached to its 5′ carbon.

Phosphate

Nucleotides may have a single phosphate group, or a chain of up to three phosphate groups, attached to the 5' carbon of the sugar. Some chemistry sources use the term "nucleotide" only for the single-phosphate case, but in molecular biology, the broader definition is generally accepted^11start superscript, 1, end superscript

In a cell, a nucleotide about to be added to the end of a polynucleotide chain will bear a series of three phosphate groups. When the nucleotide joins the growing DNA or RNA chain, it loses two phosphate groups. So, in a chain of DNA or RNA, each nucleotide has just one phosphate group.

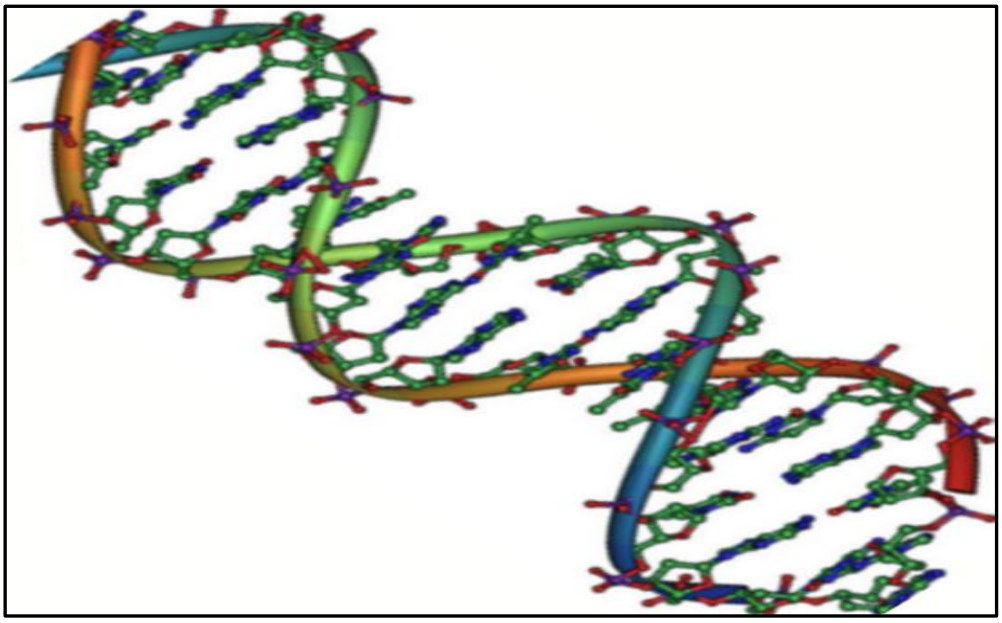
Polynucleotide chains

A consequence of the structure of nucleotides is that a polynucleotide chain has **directionality** – that is, it has two ends that are different from each other. At the **5’ end**, or beginning, of the chain, the 5’ phosphate group of the first nucleotide in the chain sticks out. At the other end, called the **3’ end**, the 3’ hydroxyl of the last nucleotide added to the chain is exposed. DNA sequences are usually written in the 5' to 3' direction, meaning that the nucleotide at the 5' end comes first and the nucleotide at the 3' end comes last.

As new nucleotides are added to a strand of DNA or RNA, the strand grows at its 3’ end, with the 5′ phosphate of an incoming nucleotide attaching to the hydroxyl group at the 3’ end of the chain. This makes a chain with each sugar joined to its neighbors by a set of bonds called a **phosphodiester linkage**.

Properties of DNA

Deoxyribonucleic acid, or DNA, chains are typically found in a **double helix**, a structure in which two matching (complementary) chains are stuck together, as shown in the diagram at left. The sugars and phosphates lie on the outside of the helix, forming the backbone of the DNA; this portion of the molecule is sometimes called the **sugar-phosphate** backbone. The nitrogenous bases extend into the interior, like the steps of a staircase, in pairs; the bases of a pair are bound to each other by hydrogen bonds.

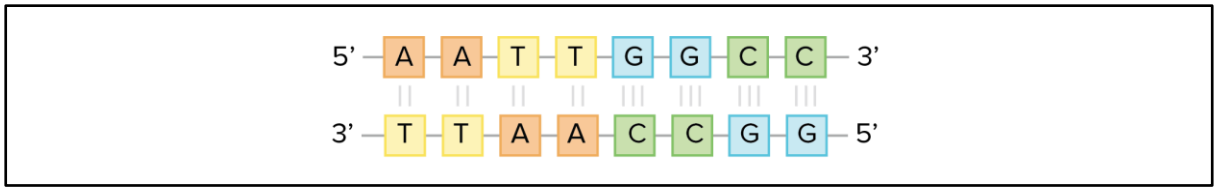


Structural model of a DNA double helix

The two strands of the helix run in opposite directions, meaning that the 5′ end of one strand is paired up with the 3′ end of its matching strand. (This is referred to as **antiparallel** orientation and is important for the copying of DNA.)

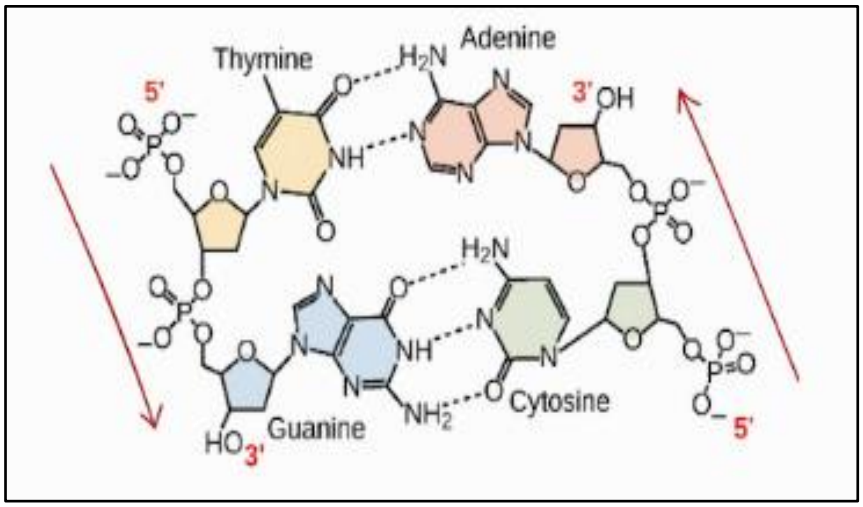
So, can any two bases decide to get together and form a pair in the double helix? The answer is a definite no. Because of the sizes and functional groups of the bases, base pairing is highly specific: A can only pair with T, and G can only pair with C, as shown below. This means that the two strands of a DNA double helix have a very predictable relationship to each other.

For instance, if you know that the sequence of one strand is 5'-AATTGGCC-3', the complementary strand must have the sequence 3'-TTAACCGG-5'. This allows each base to match up with its partner:



These two strands are complementary, with each base in one sticking to its partner on the other. The A-T pairs are connected by two hydrogen bonds, while the G-C pairs are connected by three hydrogen bonds.

When two DNA sequences match in this way, such that they can stick to each other in an antiparallel fashion and form a helix, they are said to be **complementary**.



Hydrogen bonding between complementary bases holds DNA strands together in a double helix of antiparallel strands. Thymine forms two hydrogen bonds with adenine, and guanine forms three hydrogen bonds with cytosine.

Properties of RNA

Ribonucleic acid (RNA), unlike DNA, is usually single-stranded. A nucleotide in an RNA chain will contain ribose (the five-carbon sugar), one of the four nitrogenous bases (A, U, G, or C), and a phosphate group. Here, we'll take a look at four major types of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and regulatory RNAs.

Messenger RNA (mRNA)

Messenger RNA (**mRNA**) is an intermediate between a protein-coding gene and its protein product. If a cell needs to make a particular protein, the gene encoding the protein will be turned "on," meaning an RNA-polymerizing enzyme will come and make an RNA copy, or transcript, of the gene's DNA sequence. The transcript carries the same information as the DNA sequence of its gene. However, in the RNA molecule, the base T is replaced with U. For instance, if a DNA coding strand has the sequence 5'-AATTGCGC-3', the sequence of the corresponding RNA will be 5'-AAUUGCGC-3'.

Once an mRNA has been produced, it will associate with a ribosome, a molecular machine that specializes in assembling proteins out of amino acids. The ribosome uses the information in the mRNA to make a protein of a specific sequence, "reading out" the mRNA's nucleotides in groups of three (called **codons**) and adding a particular amino acid for each codon.

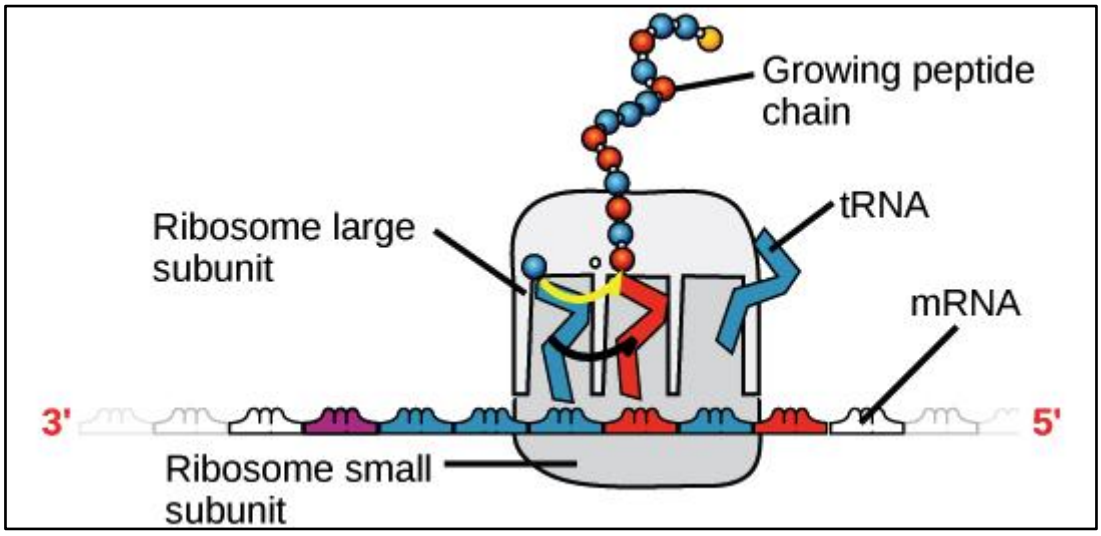
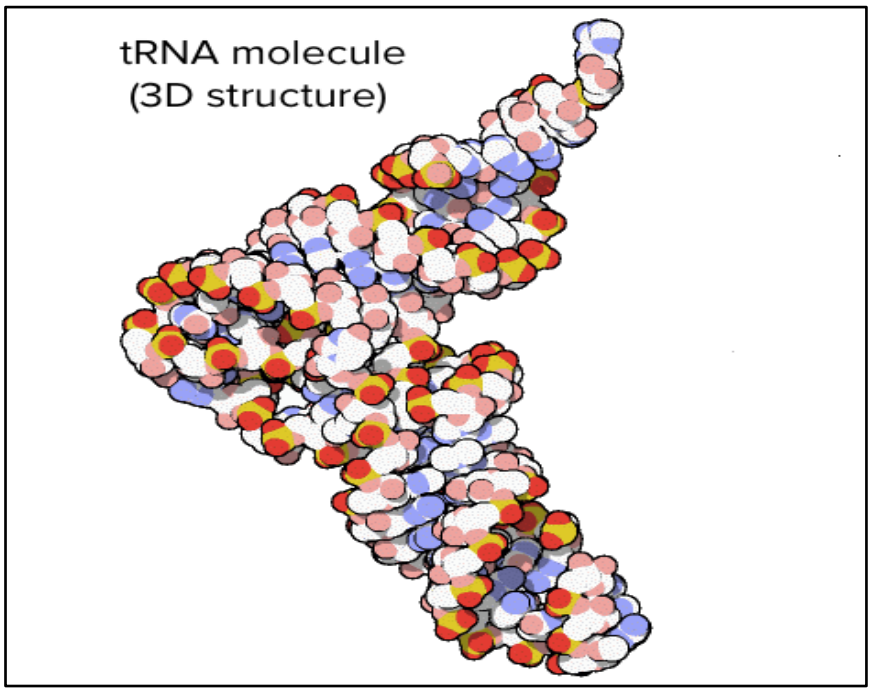


Image of a ribosome (made of proteins and rRNA) bound to an mRNA, with tRNAs bringing amino acids to be added to the growing chain. The tRNA that binds, and thus the amino acid that's added, at a given moment is determined by the sequence of the mRNA that is being "read" at that time.

Ribosomal RNA (rRNA) and transfer RNA (tRNA)

Ribosomal RNA (**rRNA**) is a major component of ribosomes, where it helps mRNA bind in the right spot so its sequence information can be read out. Some rRNAs also act as enzymes, meaning that they help accelerate (catalyze) chemical reactions – in this case, the formation of bonds that link amino acids to form a protein. RNAs that act as enzymes are known as **ribozymes**.

Transfer RNAs (**tRNAs**) are also involved in protein synthesis, but their job is to act as carriers – to bring amino acids to the ribosome, ensuring that the amino acid added to the chain is the one specified by the mRNA. Transfer RNAs consist of a single strand of RNA, but this strand has complementary segments that stick together to make double-stranded regions. This base-pairing creates a complex 3D structure important to the function of the molecule.



Structure of a tRNA. The overall molecule has a shape somewhat like an L.

Regulatory RNA (miRNAs and siRNAs)

Some types of non-coding RNAs (RNAs that do not encode proteins) help regulate the expression of other genes. Such RNAs may be called regulatory RNAs. For example, **microRNAs** (**miRNAs**) and **small interfering RNAs siRNAs** are small regulatory RNA molecules about 22 nucleotides long. They bind to specific mRNA molecules (with partly or fully complementary sequences) and reduce their stability or interfere with their translation, providing a way for the cell to decrease or fine-tune levels of these mRNAs.

These are just some examples out of many types of noncoding and regulatory RNAs. Scientists are still discovering new varieties of noncoding RNA.

Summary: Features of DNA and RNA

	DNA	RNA
Function	Repository of genetic information	Involved in protein synthesis and gene regulation; carrier of genetic information in some viruses
Sugar	Deoxyribose	Ribose
Structure	Double helix	Usually single-stranded
Bases	C, T, A, G	C, U, A, G

Although RNA transcripts are not made up of two separate strands, RNA can sometimes fold back on itself to form double-stranded regions and complex 3D structures. We will see examples of RNA folding when we look at transfer RNA (tRNA) and protein translation. In addition, some viruses have genomes made of double-stranded RNA.

Immunology

Immune system:

- Instead of being localized at a specific organ, the human immune system is actually spread out among many different areas of the body.
- It uses a variety of different defense mechanisms and specialized cells to catch any pathogens that might enter the body.
- A pathogen is any agent, living or non-living that can bring harm to the cells of our body. One of the main functions of the immune system is to be able to differentiate between its own cells and foreign pathogens.
- This can be done because the cells of the body contain unique macromolecules that are used by the immune system to distinguish them from the pathogens.
- On the contrary, the pathogens (such as bacterial cells) actually contain their own unique macromolecules that can be used by the immune system to seek them out and kill them off.
- Any substance that can be used to initiate a set of immune defense mechanisms is known as **an antigen**. Our immune system can be divided into two – the
- **Innate (non-specific) immune system**
- **Acquired (specific) immune system.**
- The innate immune system is responsible for carrying out antigen-independent defense mechanisms immediately following infection.
- It is the primary line of defense against pathogens and uses not only physical barriers against the pathogens but also the process of inflammation.
- The acquired immune system however is specific as to what it attacks (required antigens) and takes several days to actually kick in. Unlike the innate immune system, the adapted immune system has "memory" and consists of two subdivisions.
- One is the cell-mediated immunity (involves T-lymphocytes) and the other is the antibody mediated immunity (involves B-lymphocytes).

Oral immune system

- Immunity is the sum of all naturally occurring defense mechanisms that protect humans from infectious and other disease.
- There are two types of resistance mechanisms: non-specific (innate) and specific (acquired). The body's immune system has several components, all of which are active in the oral cavity.
- The soft and hard tissues of the oral cavity are under protection by both nonspecific and specific immune factors.
- The function of these protective factors is to:

1. Limit the microbial colonization of the oral surfaces.
2. Prevent the penetration of noxious substances through the surfaces and ensuing damage to the underlying tissues.

- Salivary glands secrete organic molecules that can be categorized into five major groups: amylase, mucins, phospho-proteins, glycoproteins, and immunoglobulins.
- Non-specific immune factors These are factors present in saliva that include lysozyme, the lactoperoxidase system, lactoferrin, high molecular weight glycoproteins and other salivary components that may act as bacterial agglutinins.
- Unlike antibodies, these nonspecific factors lack any aspect of immunological memory and are not subject to specific stimulation.
- Several of the non-specific immune factors may interact with the specific salivary immune factors which are immunoglobulins, resulting in a mutual amplification of their respective activities.

Lysozyme (LZ):

- It comes from minor and major salivary glands, gingival crevicular fluid and salivary leukocytes.
- It is present in newborn babies at level equal to those of adults and may exert antimicrobial function already before tooth emergence.
- It has the ability to hydrolyze the specific bonds in exposed bacterial cell walls causing cell lysis and death (muramidase activity).
- LZ has been proposed as a lytic factor for bacteria to which immunoglobulins have bound.
- LZ and other antibacterial systems in saliva exclude susceptible invading pathogens, which are not adapted to oral conditions.
- This may be the most important action of the salivary antibacterial systems. Lysozyme, as a strongly cationic protein, can activate bacterial autolysins, which can also destroy the cell walls.

Peroxidase system:

- Peroxidase in saliva is produced in acinar cells of parotid and submandibular glands but not in minor salivary glands.
- Peroxidase enzymes catalyze the reaction of bacterial metabolic product, hydrogen peroxide (H_2O_2) with salivary thiocyanate (SCN-) originates from serum to produce hypothiocyanite (OSCN-).
- This system protects human host protein and cells from hydrogen peroxide toxicity, it is highly toxic to bacterial enzymes required energy production and the bacterial activity is inhibited.

- The more hypothiocyanite present in saliva, the less can dental plaque produce acids after stimulation with glucose.
- Peroxidase systems are effective against a variety of microorganisms, such as mutans streptococci, lactobacilli, yeasts, many anaerobes and also some viruses.

Lactoferrin:

- It has antibacterial activity.
- LF is secreted by serous cells of major and minor salivary glands, also polymorphonuclear leukocytes are rich in LF.
- Ferric iron (Fe3-) is an essential microbial nutrient.
- Lactoferrin is iron-binding glycoprotein making ferric iron unavailable for microbial use.
- This phenomenon is known as nutritional immunity. Lactoferrin in its unbounded states has direct bactericidal effect on some microorganisms including mutans streptococci.

Mucins:

- They are salivary agglutinins of acinar cell origin and able to agglutinate bacteria.
- They are of high molecular weight and contain more than 40% carbohydrates.
- It inhibits the adhesion of bacterial cells to soft tissue surfaces by interacting with adhesins.
- Mucins also interact with hard tissue surfaces and mediate specific bacterial adhesion to the tooth surface.

Statherin:

- Although proteases of oral microflora are able to degrade statherin, it seems that concentrations of statherin remain high enough to exert its action as long as saliva remains in the mouth before swallowing.
- It is present in both submandibular and parotid saliva.
- The key activity is to prevent precipitation of calcium phosphate in ductal saliva and oral fluid to maintain supersaturation, to prevent the formation of ductal stones and calcium phosphate crystal growth on tooth surfaces.
- Statherin is also known to promote the adhesion of Actinomyces viscosus to tooth surfaces.

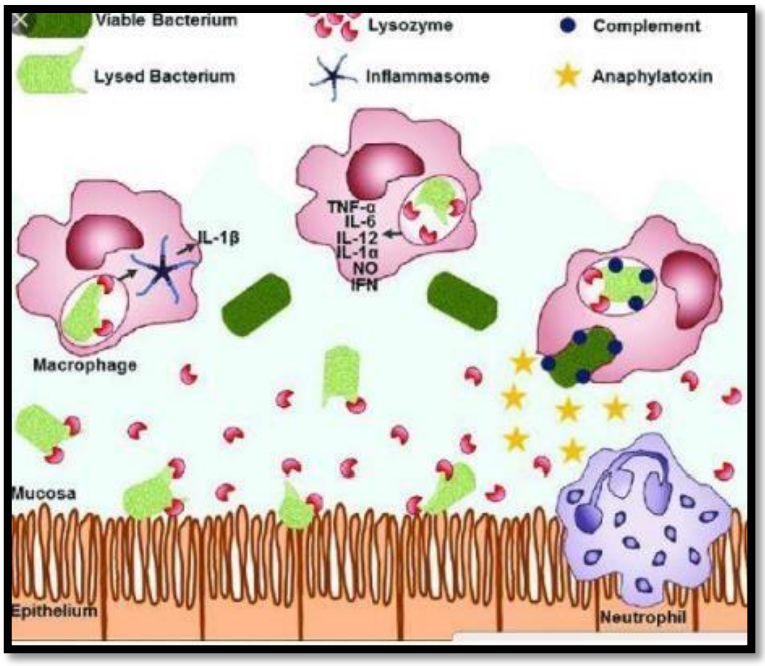
Prolin rich protein (PRPs)

- Like statherin are needed to inhibit spontaneous precipitation of calcium phosphate salts in the salivary glands and their secretion.

- PRPs are readily adsorbed from saliva to hydroxyapatite surfaces and they are present in initially formed acquired enamel pellicle.
- The multifunctional properties of PRPs, like statherins, are shown by their ability to promote selectively the attachment of bacteria (A. viscosus and Streptococcus gordonii) to apatitic surfaces.
- The modulation of bacterial adhesion is mediated by the carboxy-terminal region of the molecule, which is not able to bind to tooth surfaces.
- They constitute as much as 25–30% of all proteins in saliva, form a complex group with a large number of genetic variants.

Histatins:

- They have a broad antimicrobial spectrum against bacteria as well as oral yeasts.
- The histatins are synthesized in the parotid and submandibular glands, which means that they are practically always present in whole saliva.
- It modulates the precipitation of behavior of calcium phosphate.



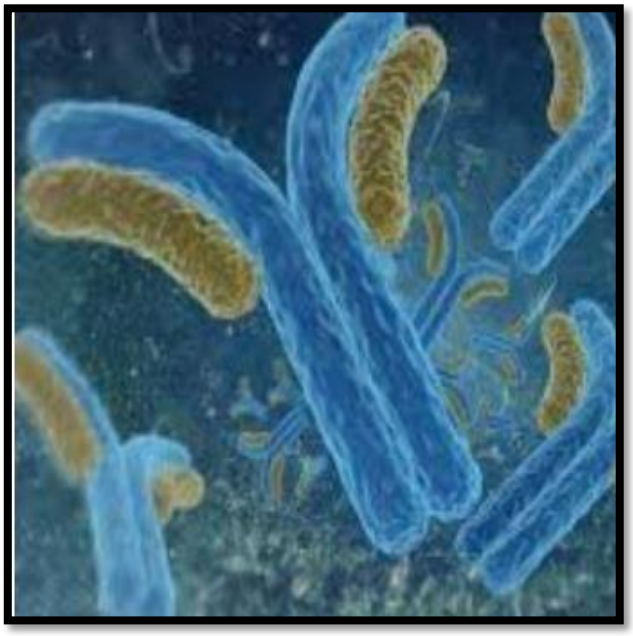
Some immune component

Specific immune factors

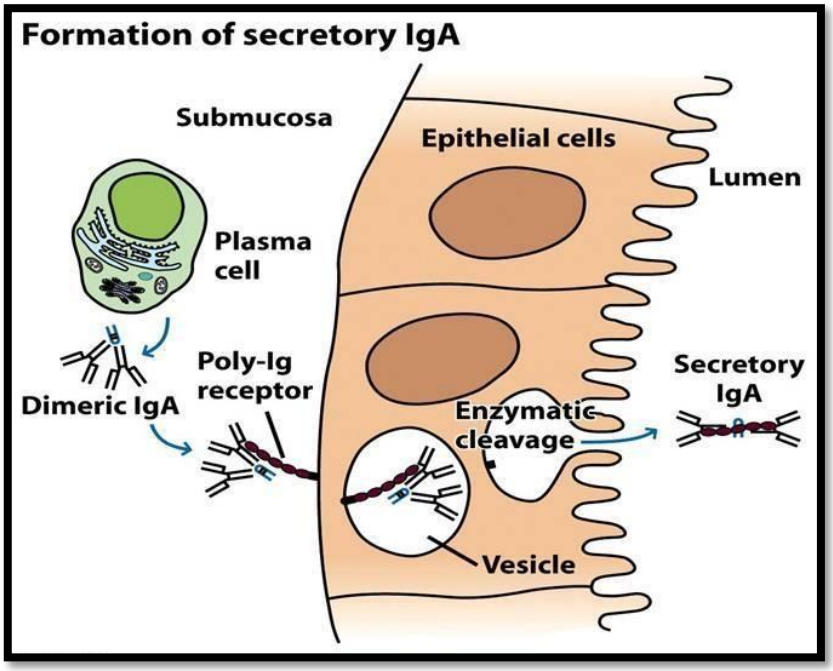
- These factors include immunoglobulins may be directed at specific bacterial molecules which may be important in the biological activity of the target organisms.
- The proportion of different Ig classes present in saliva are IgA > IgG > IgM > IgD > IgE.

Immunoglobulin IgA:

- Salivary IgA is produced by plasma cells located in the major and minor salivary glands.
- It is the predominant salivary immunoglobulin which under normal condition is the only immunoglobulin secreted in saliva.
- All exocrine gland secretions are rich in IgA. In individuals with gingivitis or periodontitis, the inflammation in the periodontal tissues will result in the transudation of serum proteins which include IgG, IgA, IgM and complement factors.
- IgA present in saliva differs from serum IgA with molecular structure. While serum IgA occurs mainly in the monomeric form, IgA in saliva composed of dimeric associated with J chain and secretory component (SC).
- This complex known as secretory IgA (S-IgA). The ability of secretory IgA to inhibit adherence of bacteria to dental enamel appears to be related to its ability to bind to surface adhesins of bacteria as well as to neutralize their negative surface charge.
- On the other hand, IgA has been shown to bind to mutanse streptococci facilitating bacterial aggregation and removal from the oral cavity.
- Secretory IgA molecules are multivalent antibodies, and can prevent the adverse effects of bacterial toxins and enzymes.
- Secretory IgA initiates the inflammatory process. It prevents both bacteria and viruses from adhering to mucous membranes. IgA deficiency is the most common type of immunoglobulin deficiency and is seen in autoimmune diseases such as rheumatoid arthritis and lupus.



Salivary IgA



Immunoglobulin IgG:

- It is the predominant immunoglobulin in blood, lymph, peritoneal fluid and cerebrospinal fluid.
- It accounts for approximately 75% of the total serum immunoglobulins in normal adults and is the most abundant antibody produced during secondary humoral immune response in the blood.

- IgG is the only class of immunoglobulin that can cross the placenta in humans, and it is responsible for protection of the new born during the first months of life.
- The direct exposure of the tooth surfaces to gingival fluid, dental plaque may, in addition to salivary immunoglobulins, be exposed to significant amounts of serum immunoglobulins.
- It is therefore believed that serum IgG has the potential to modulate the oral colonization by plaque-forming bacteria, especially during tooth eruption.
- A significant proportion of the immunnoglobulin molecules, particularly IgG, that become incorporated in dental plaque occur as fragments as a result of proteolytic degradation by enzymes excreted by plaque bacteria.
- Immunoglobulin G is effective against bacteria, viruses, and fungi.

Effects of IgA and IgG response in relations to protection of tooth surfaces:

1. Inhibition of bacterial adherence by:
a. Blockage of bacterial adhesins.
b. Bacterial agglutination.
2. Inhibition of bacterial enzymes.
3. IgA has anti-inflammatory activity in gingiva, while IgG has the property of induction of inflammation in gingival tissues and opsonization of bacteria thus facilitating bacterial phagocytosis and killing.

Immunoglobulin IgM

- It normally exists as a pentamer in serum but can also occur as a monomer.
- It has an extra domain on the mui chain (CH4) and another protein covalently bound via S-S called J-chain.
- This chain helps it to polymerize to the pentamer form.
- It is the first Ig to be made by fetus in most species and new B cells when stimulated by Ags.
- It is the 3rd most abundant Ig in serum.
- It is a good complement fixing Ig leading to lyses of microorganisms
- It is also a good agglutinating Ig, hence clumping microorganisms for eventual elimination from the body.
- It is also able to bind some cells via Fc receptors.
- B cells have surface IgMs , which exists as monomers and lacks J chain but have an extra 20 amino acid at the C-terminal that anchors it to the cell membrane.

Immunoglobulin IgD

- It exists as monomers.
- It is found in low levels in serum and its role in serum is uncertain
- It is found primarily on B cells surface and serves as a receptor for Ag.

- It does not fix complement.

Immunoglobulin IgE

- It occurs as a monomer and has an extra domain in the constant region.
- It is the least common serum Ig, but it binds very tightly to Fc receptors on basophils and mast cells even before interacting with Ags.
- It is involved in allergic reactions because it binds to basophils and mast cells.
- It plays a role in parasitic helminthic diseases. Serum levels rise in these diseases.
- Eosinophils have Fc receptors for IgEs and when eosinophoils bind to IgEs coated helminthes death of the parasite results.

Immunization of dental caries:

- Immunization is a process of exposing the host to an antigen in order to stimulate antibodies.
- Different types of antigen can be used as the whole bacterial cells, glucosyltransferase enzyme, cell wall protein and others.

Different routes of immunization have been used to achieve caries immunity:

- The classical parenteral route of immunization (subcutaneous) by selected antigen to stimulate serum IgG and to a lesser extent IgM and IgA.
- Immunoglobulins reach the oral cavity by transduction with crevicular fluid.
- Stimulation of S-IgA antibody response in saliva by repeated injection of salivary gland with antigen vaccine.
- Passive immunization with orally applied antibody.
- Protection against dental caries by immunization would be achieved by immune components from serum by IgA antibodies in salivary secretions or by a combined effect of serum and salivary components.

Oral vaccine design:

- Oral immunization focused on the development of a vaccine that could induce the production of sIgA (mainly salivary and other secretion with poor serum level) effective in production against caries.
- Vaccination leads to migration of antigen sensitized precursor B-cells from GALT (gut associated lymphoid tissues) to salivary glands.

- The precursor B cell expanded colony and mature in to plasma cells under the influence of T-cells in addition mucosal associated lymphoid tissue (MALT) may also induces IgA production.
- Secretory IgA enhances agglutination of bacteria, neutralizes toxins, enzymes viruses and inhibits bacterial adhesion.
- Mucosal immunity and SIgA acts on luminal surfaces of most epithelial lining of conjunctiva, nasopharynx, oropharynx, GIT, RT, UGT and the duct of exocrine glands.
- The principle Ab in the mucosal immunity mostly dimeric IgA, While peripheral immunity (IgM and IgG) WHICH protects the peripheral anatomical sites and paranchimal organs is mediated by when the pathogen manage to escape from the mucosal barrier Abs work in conjunction of complement system leading to neutralization, opsonization, agglutination and destruction of the cell by phagocytosis.
- IgA is the major Ab produced by B-cells mostly released in to the GIT fluids saliva tears and other secretions.
- deficiency of IgA occur in generalized B- cell deficiency, crowns disease a topic allergy, steroids treatment and may increase or decreased in dental caries.

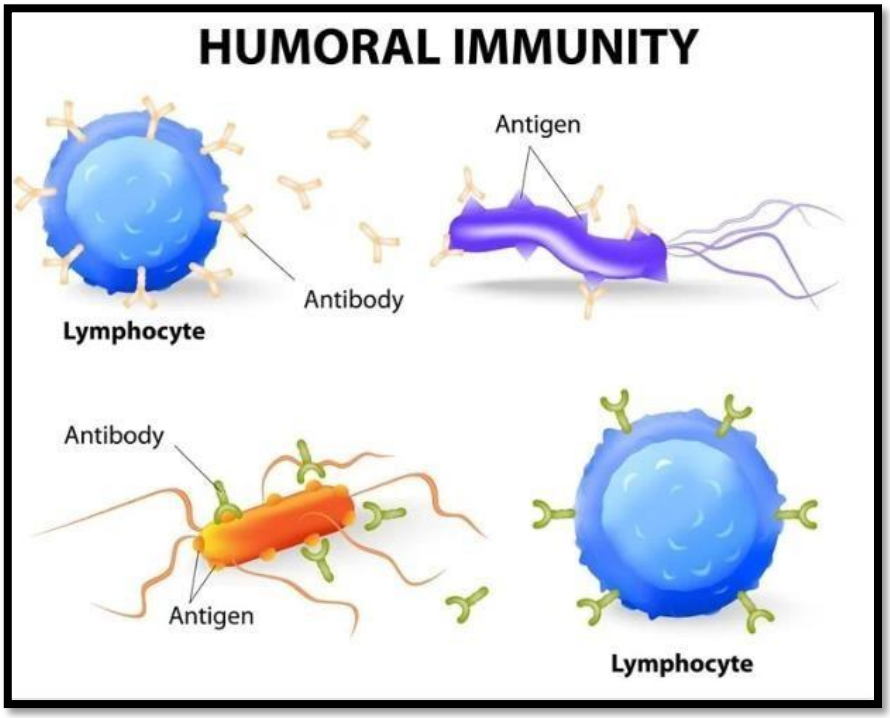
Humoral vs Cell-mediated Immunity

- The innate/general resistance system and the adaptive system are the two main subsystems of the immune system.
- To generate an efficient immune response, the innate and adaptive systems constantly interact with one another.
- The innate immune system, also known as general resistance, consists of several defensive mechanisms that are constantly active and serve as the first line of defense against pathogenic substances.
- These responses, however, are not limited to a single pathogenic agent. Innate immune cells are selective for conserved molecular patterns seen on all microorganisms.
- In contrast to the innate immune system, the adaptive immune system's responses are tailored to the specific pathogen. This response will take longer than the natural response to manifest.
- Adaptive immunity contains specialized immune cells and antibodies that target and eliminate foreign invaders while also remembering what those substances look like and creating a new immune response to prevent sickness in the future.

- Adaptive immunity can last a few weeks or months, or it might endure a long period, even for the rest of a person's life.
- Humoral immunity and cell-mediated immunity are two forms of adaptive immune responses that allow the human body to protect itself against dangerous agents including bacteria, viruses, and poisons, in a targeted manner.
- While there is some overlap between these immune response arms - both rely on lymphoid cell functions, there are also some significant differences.

Humoral immunity

- When foreign material - antigens - is recognized in the body, the body responds with an antibody-mediated reaction.
- Extracellular intruders, such as bacteria, are commonly found in this foreign material.
- B cell lymphocytes, a type of immune cell that makes antibodies after detecting a specific antigen, are principally responsible for this method.
- Lymphocytes known as naive B cells circulate throughout the body via the lymphatic system.
- These cells produce antigen-specific molecules that are necessary for detecting infectious pathogens in the human body.
- When naive B cells in the lymphatic system come into contact with an antigen, they begin the differentiation process that results in the formation of memory B cells and effector B cells.
- Memory B cells and effector B cells produce the same antigen-specific molecules as their parent naive B cell during this development.
- The activated memory B cells express these antigen-specific molecules on their surface with the help of T cell lymphocytes, which are activated by MHC class II receptors that recognize microbial-associated antigens.
- The effector B cells secrete these molecules in the blood to bind the antigen of interest.



Cell-mediated immunity

- Cell-mediated immunity, unlike humoral immunity, does not rely on antibodies to perform adaptive immunological activities.
- Mature T cells, macrophages, and the production of cytokines in response to an antigen are the main drivers of cell-mediated immunity.
- To recognize intracellular target antigens, T cells that participate in cell-mediated immunity rely on antigen-presenting cells that have membrane-bound MHC class I proteins.
- The maturation and differentiation of naive T cells into helper or killer T cells are dependent on the binding specificity of MHC proteins to external antigens.
- Cell-mediated immunity is activated when cells in the body are infected by a virus, bacterium, or fungus (intracellular invaders).
- T lymphocytes can detect malignant cells with the help of MHC class I proteins.
- Helper T cells, killer T cells, and macrophages are the three main kinds of lymphocytes involved in cell-mediated immunity.
- When a "helper" T cell encounters an antigen-presenting cell in the body, it releases cytokines, which are signaling proteins.

- These cytokines cause "killer" T lymphocytes and macrophages to flock to the antigen-presenting cell in an attempt to eliminate it.

Humoral vs cell-mediated

- B cells activate humoral immunity, whereas T cells activate cell-mediated immunity.
- The major difference between humoral and cell-mediated immunity is that humoral immunity produces antigen-specific antibodies, whereas cell-mediated immunity does not.
- T lymphocytes, on the other hand, kill infected cells by triggering apoptosis.
- Humoral immunity develops quickly, whereas cell-mediated immunity takes longer.
- Extracellular microorganisms and their poisons are targeted by humoral immunity. Intracellular microorganisms (such as bacteria) and tumor cells are targets of cell-mediated immunity.
- The Ig, CD40, CD21, and Fc receptors are the humoral immunity's accessory receptors.
- The accessory receptors of cell-mediated immunity are CD2, CD3, CD4, CD8, CD28, and integrins.
- Humoral immunity recognizes the unprocessed antigens. In humoral immunity, plasma B cells release antibodies.
- Cytokines are released by T-cells.
- Tumor cells and transplants are immune to humoral immunity. Tumor cells and transplants are both affected by cell-mediated immunity.

Significance of humoral and cell-mediated immune response

- T-cell responses, which are part of cell-mediated immunity, play a vital role in controlling viral infections.
- T-cells do this through developing effector activities such as the generation of chemokines and cytokines, which can have direct and indirect antiviral effects, as well as assisting in the overall immune response regulation.

- Certain effector T-cells can kill virus-infected cells via cell-to-cell contact, providing an important mechanism of killing the host's cells, which serve as offspring virus generation sites.
- Immune responses mediated by cells are not only beneficial during the acute phase of viral infections, but they also help to create long-term immunological memory.
- Humoral immunity is extremely important in both health and disease, and it can be both useful and harmful.
- Antibody-mediated protection against pathogens induced by vaccines or infections is crucial in host defense, but pathogen-specific antibodies can also promote infectious processes or drive pathology.
- Loss of immunological tolerance is linked to the generation of self-reactive antibodies, which can aggravate the condition, and loss of growth control can lead to a variety of B cell malignancies.

PROTOZOA

General Features

Single-cell eukaryotic microorganisms belonging to kingdom protista are classified as **Protozoa** (Greek *Protos: first*; *zoon: animal*).

- The single protozoal cell performs all functions.
- Most of the protozoa are completely nonpathogenic but few may cause major diseases such as malaria, leishmaniasis, and sleeping sickness.
- Protozoa like *Cryptosporidium parvum* and *Toxoplasma gondii* are being recognized as opportunistic pathogens in patients a infected with human immunodeficiency virus (HIV) and in those undergoing immunosuppressive therapy.
- Protozoa exhibit wide range of size (1–150 µm),

Structure

The typical protozoan cell is bounded by a trilaminar unit membrane, supported by a sheet of contractile fibrils enabling the cell to move and change in shape

Cytoplasm

It has 2 portions:

- **Ectoplasm**: Outer homogeneous part that serves as the organ for locomotion and for engulfment of food by producing **pseudopodia** is called as the ectoplasm. It also helps in respiration, discharging waste material, and in providing a protective covering of cell.
- **Endoplasm**: The inner granular portion of cytoplasm that contains nucleus is called endoplasm. The endoplasm shows number of structures—the Golgi bodies, endoplasmic reticulum, food vacuoles, and contractile vacuoles. Contractile vacuoles serve to regulate the osmotic pressure.

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Nucleus

The nucleus is usually single but may be double or multiple; some species having as many as hundred nuclei in a single cell.

- The nucleus contains one or more nucleoli or a central Karyosome

-The chromatin may be distributed along periphery (peripheral chromatin) or as condensed mass around the karyosome

Reproduction

Reproduction can be:

* Asexual reproduction
* Sexual reproduction.

Reproduction usually occurs asexually in protozoans; however, sexual reproduction occurs in cillates and sporozoas.

Amebiasis

Entamoeba histolytica

Amebiasis
(Amebic Dysentery)

Causal agent: ***Entamoeba histolytica*** *is well recognized as a pathogenic amoeba.*

Geographic Distribution: Worldwide, with higher incidence of amebiasis in developing countries.

In industrialized countries, risk groups include male homosexuals, travelers and recent immigrants.

Morphology

E. histolytica occurs in 3 forms.

-Trophozoite

-Precyst

-Cyst.

Trophozoite

Trophozoite is the vegetative or growing stage of the parasite. It is the only form present in tissues.

-It is irregular in shape and varies in size from 12–60 µm; average being 20 µm.

-It is large and actively motile in freshly-passed dysenteric stool, while smaller in convalescents and carriers.

-The parasite, as it occurs free in the lumen as a commensal is generally smaller in size, about 15–20 µm and has been called the **minuta form**.

-**Cytoplasm:** Outer ectoplasm is clear, transparent, and refractile. Inner endoplasm is finely granular, having a **ground glass appearance**. The endoplasm contains nucleus, food vacuoles, erythrocytes, occasionally leucocytes, and tissue debris.

Pseudopodia are finger-like projections formed by sudden jerky movements of ectoplasm in one direction, followed by the streaming in of the whole endoplasm.

-Typical amoeboid motility is a **crawling** or **gliding** movement and not a free swimming one. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube.

-Pseudopodia formation and motility are inhibited at low temperatures.

-Nucleus is spherical 4–6 μm in size and contains central karoyosome, surrounded by clear halo and anchored to the nuclear membrane by fine radiating fibrils called the linin network, giving a cartwheel appearance. The nucleus is not clearly seen in the living trophozoites, but can be clearly demonstrated in preparations stained with iron-hemotoxylin.

-The nuclear membrane is lined by a rim of chromatin distributed evenly as small granules.

The trophozoites from acute dysenteric stools often contain phagocytosed erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other commensal intestinal amoebae.

-The trophozoites divide by **binary fission** in every 8 hours.

-Trophozoites survive up to 5 hours at 37°C and are killed by drying, heat, and chemical sterilization. Therefore, the infection is not transmitted by trophozoites . Even if live trophozoites from freshy-passed stools are ingested, they are rapidly destroyed in stomach and cannot initiate infection.

Precystic Stage

Trophozoites undergo encystment in the intestinal lumen.

Encystment does not occur in the tissues nor in feces outside the body.

-Before encystment, the trophozoite extrudes its food vacuoles and becomes round or oval, about 10–20μm in size. This is the precystic stage of the parasite

-It contains a **large glycogen vacuole** and two **chromatid bars**.

-It then secretes a highly retractile cyst wall around it and becomes cyst..

Cystic Stage

The cyst is spherical in shape about 10–20 μm in size.

-The early cyst contains a single nucleus and two other structures—a mass of glycogen and 1–4 *chromatoid bodies or chromidial bars*, which are cigar-shaped refractile rods with rounded ends .The chromatoid bodies are so called because they stain with hematoxylin,like chromatin.

-As the cyst matures, the glycogen mass and chromidial bars disappear and the nucleus undergoes 2 successive mitotic divisions to form 2 and then 4 nuclei.

The mature cyst is, thus **quadrinucleate**

The cyst wall is a highly refractile membrane, which makes it highly resistant to gastric juice and unfavorable environmental conditions.

-The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations.

-With iron hemotoxylin stain, nuclear chromatin and chromatoid bodies appear deep blue or black, while the glycogen mass appears unstained.

-When stained with iodine, the glycogen mass appears golden brown, the nuclear chromatin and karysome bright yellow, and the chromatoid bodies appear as clear space, being unstained.

Life Cycle

E. histolytica passes its life cycle only in 1 host-man

Infective form: Mature quadrinucleate cyst passed in feces of convalscents and carriers. The cysts can remain viable under moist conditions for about 10 days.

Mode of transmission:

Man acquires infection by swallowing food and water contaminated with cysts.

-As the cyst wall is resistant to action of gastric juice, the cysts pass through the stomach undamaged and enter the small intestine.

-**Excystation**: When the cyst reaches caecum or lower part of the ileum, due to the alkaline medium, the cyst wall is damaged by trypsin, leading to excystation.

-The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall, through which **quadrinucleate amoeba** is liberated. This stage is called the **metacyst**

Metacystic trophozoites: The nuclei in the metacyst immediately undergo division to form **8 nuclei**, each of which gets surrounded by its own cytoplasm to become **8 small amoebulae** or **metacystic trophozoites**.

-If exystation takes place in the small intestine, the metacystic trophozoites do not colonize there, but are carried to the caecum.

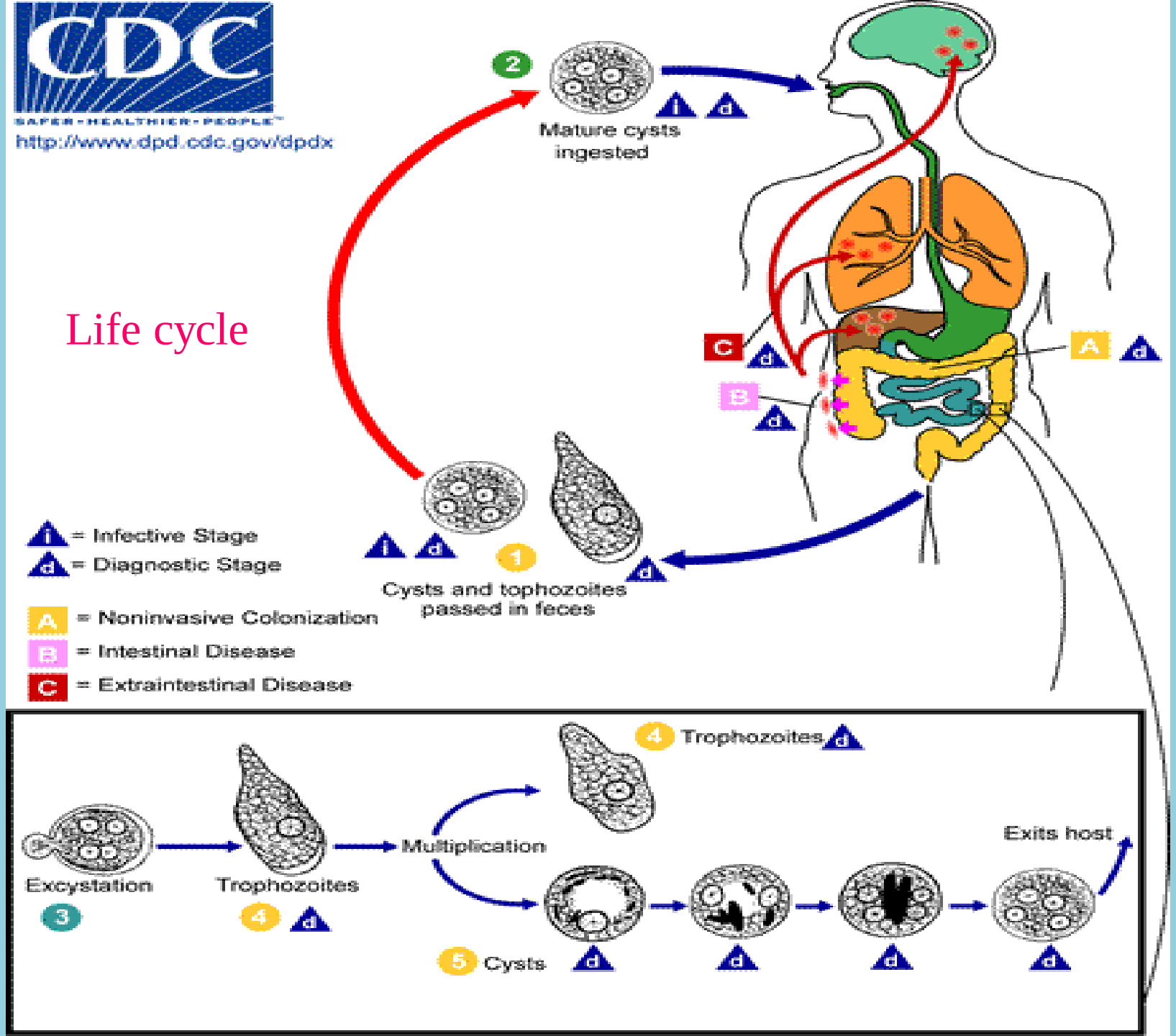
-The optimal habitat for the metacystic trophozoite is the submucosal tissue of caecum and colon, where they lodge in the glandular crypts and grow by binary fission

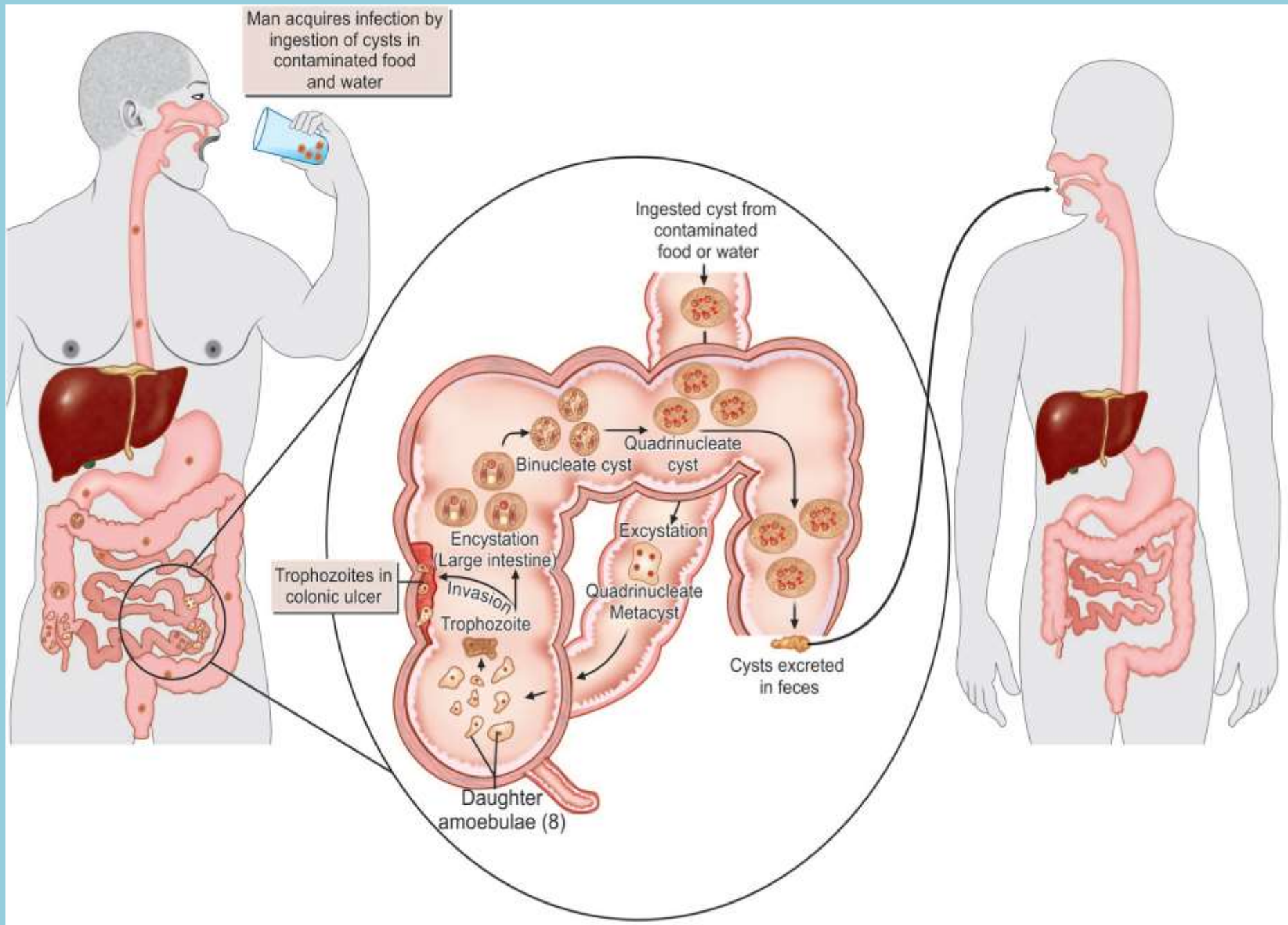
-Some develop into precystic forms and cysts, which are passed in feces to repeat the cycle.

-The entire life cycle is, thus completed in one host.

In most of the cases, *E. histolytica* remains as a commensal in the large intestine without causing any ill effects. Such persons become carriers or asymptomatic cyst passers and are responsible for maintenance and spread of infection in the community. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are the characteristics of amoebiasis.

Life cycle


CDC
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx
2
Mature cysts ingested
i
d
C
d
A
d
B
d
i
d
1
Cysts and tophozoites passed in feces
d
i = Infective Stage
d = Diagnostic Stage
A = Noninvasive Colonization
B = Intestinal Disease
C = Extraintestinal Disease
4 Trophozoites d
Exits host
Excystation
3
Trophozoites
4
d
Multiplication
5 Cysts
d
d
d
d



LIFE CYCLE OF ENTAMOEBA HISTOLYTICA

Pathogenesis and Clinical Features

E. histolytica causes intestinal and extraintestinal amoebiasis.

Incubation period is highly variable. On an average, it ranges from 4 days to 4 months.

Amoebiasis can present in different forms and degree of severity, depending on the organ affected and the extent of damage caused.

Intestinal Amoebiasis

The lumen-dwelling amoebae do not cause any illnesss.

They cause disease only when they invade the intestinal tissues. This happens only in about 10% of cases of infection,

the remaining 90% being asymptomatic.

-Not all strains of *E. histolytica* are pathogenic or invasive. Differentiation between pathogenic and nonpathogenic strains can be made by susceptibility to complement-mediated lysis and phagocytic activity or by the use of genetic markers or monoclonal antibodies and zymodeme analysis.

-The metacystic trophozoites penetrate the columnar

epithelial cells in the **crypts of Liberkühn** in the colon.

-Penetration of the amoeba is facilitated by the motility of

the trophozoites and the tissue lytic enzyme, **histolysin**,

which damages the mucosal epithelium. Amoebic **lectin** another virulence factor mediates adherence

Mucosal penetration by the amoeba produces discrete ulcers with pinhead center and raised edges.

Sometimes, the invasion remains superficial and heals spontaneously. More often, the amoeba penetrates to submucosal layer and multiplies rapidly, causing lytic necrosis and thus forming an abscess. The abscess breaks down to form an ulcer.

Amoebic ulcer

is the typical lesion seen in intestinal amoebiasis
The ulcers are **multiple** and are confined to the colon, being most numerous in the **caecum** and next in the **sigmoidorectal region**.
The intervening mucous membrane between the ulcers remains healthy.

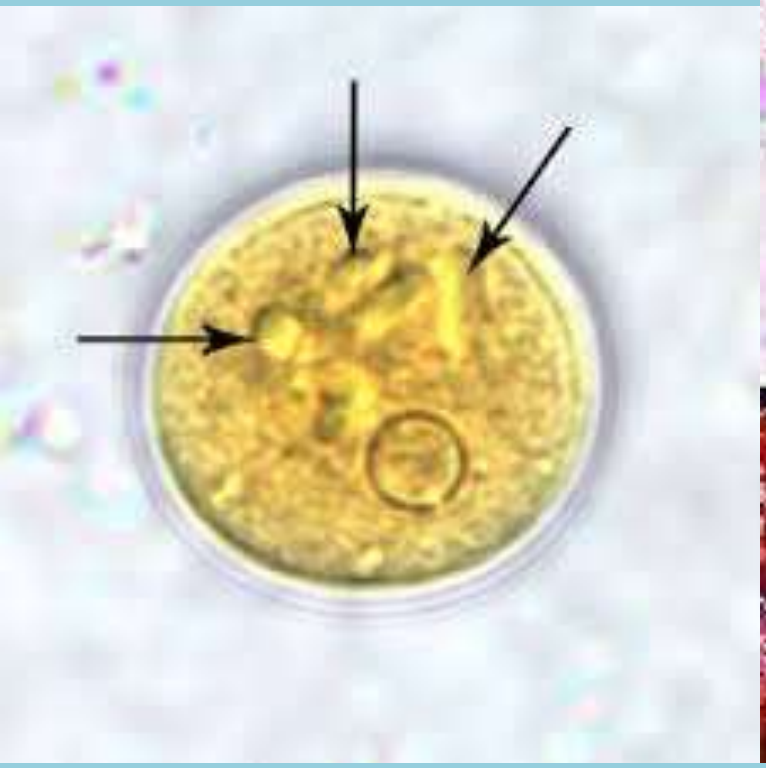
Ulcers appear initially on the mucosa as raised nodules with pouting edges. They later break down discharging brownish necrotic material containing large numbers of trophozoites.

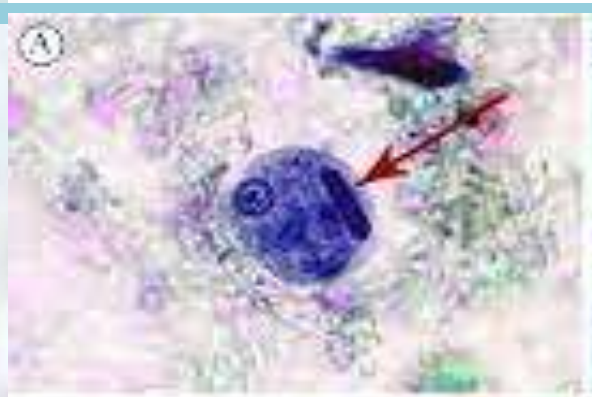
-The typical amoebic ulcer is **flask-shaped** in cross section, with mouth and neck being narrow and base large and rounded.

-Multiple ulcers may coalesce to form large necrotic lesions with ragged and undermined edges and are covered with brownish slough.

The ulcers generally do not extend deeper than submucosal layer, but amoebae spread laterally in the submucosa causing extensive undermining and patchy mucosal loss. Amoebae are seen at the periphery of the lesions and extending into the surrounding healthy tissues. Occasionally, the ulcers may involve the muscular and serous coats of the colon, causing perforation and peritonitis. Blood vessel erosion may cause hemorrhage.

-The superficial lesions generally heal without scarring, but the deep ulcers form scars which may lead to strictures, partial obstruction, and thickening of the gut wall.




A


B


C


D

Clinical Features of Intestinal Amoebiasis

The clinical picture covers a wide spectrum from noninvasive carrier state to fulminant colitis.

-The incubation period is highly variable from 1–4 months.

-The clinical course is characterized by prolonged latency, relapses and intermissions.

-The typical manifestation of intestinal amoebiasis is amoebic dysentery. This may resemble bacillary dysentery, but can be differentiated on clinical and laboratory grounds. Compared to bacillary dysentery , it is usually insidious in onset and the abdominal tenderness is less and localized

The stools are large, foul-smelling, and brownish black, often with blood streaked mucus intermingled with feces.

The RBCs in stools are clumped and reddish-brown in color. Cellular exudate is scanty. Charcot-Leyden crystals are often present.

E.histolytica trophozoites can be seen containing ingested erythrocytes.

- The patient is usually afebrile and nontoxic.

-In fulminant colitis, there is confluent ulceration and necrosis of colon. The patient is febrile and toxic.

-Intestinal amoebiasis does not always result in dysentery. Quite often, there may be only diarrhea or vague abdominal symptoms popularly called '**uncomfortable belly**' or '**growling abdomen**.'

-Chronic involvement of the caecum causes a condition simulating appendicitis

Extraintestinal Amoebiasis

Hepatic Amoebiasis

Hepatic involvement is the most common extraintestinal complication of amoebiasis. Although trophozoites reach the liver in most cases of amoebic dysentery, only in a small proportion do they manage to lodge and multiply there.

In the tropics, about 2–10% of the individuals infected with *E.histolytica* suffer from hepatic complications.

-The history of amoebic dysentery is absent in more than

50% of cases.

-Several patients with amoebic colitis develop an enlarged tender liver without detectable impairment of liver function or fever. This acute hepatic involvement (**amoebic hepatitis**) may be due to repeated invasion by amoebae from an active colonic infection or to toxic substances from the colon reaching the liver.

It is probable that liver damage may not be caused directly by the amoebae, but by lysosomal enzymes and cytokines from the inflammatory cells surrounding the trophozoites.

In about 5–10% of persons with intestinal amoebiasis , **liver abscesses** may ensue .

The center of the abscess contains **thick chocolate brown pus (anchovy sauce pus),** which is liquefied necrotic liver tissue. It is bacteriologically sterile and free of amoeba. At the periphery, there is almost normal liver tissue, which contains invading amoeba.

-Liver abscess may be multiple or more often solitary , usually located in the upper right lobe of the liver.

Jaundice develops only when lesions are multiple or when they press on the biliary tract.

Untreated abscesses tend to rupture into the adjacent tissues through the diaphragm into the lung or pleural cavity, pericardium, peritoneal cavity, stomach, intestine,

or inferior vena cava or externally through abdominal wall and skin.

-The incidence of liver abscess is less common in women and rare in children under 10 years of age.

A. Laboratory diagnosis of *Entamoeba histolytica*

Intestinal amoebiasis

Stool examination
- Microscopy
- Macroscopy
- Iodine stained preparation
- Trichome stained preparations to demonstrate trophozoite or cyst

Stool culture
Media used
- Boeck and Drbohlav
- NIH polygenic
- Craig's
- Nelson's
- Robinson's

Mucosal scrapings
Wet mount
Stained preparation

Serodiagnosis
- IHA
- ELISA
- Latex agglutination test

Molecular diagnosis
- DNA probe

B. Laboratory diagnosis of amoebic liver abscess

Amoebic liver abscess

Microscopy
of pus or aspirate

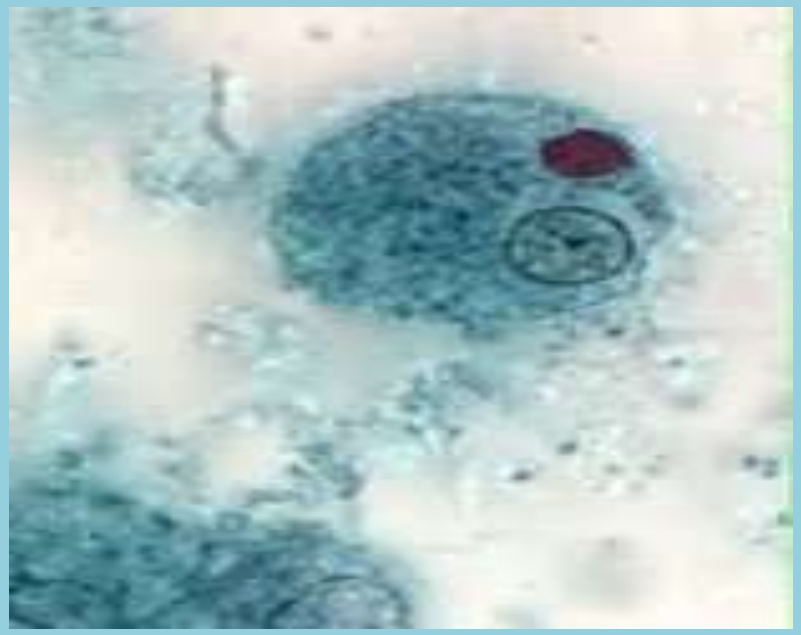
Histopatholoigical examination
of pus or aspirate

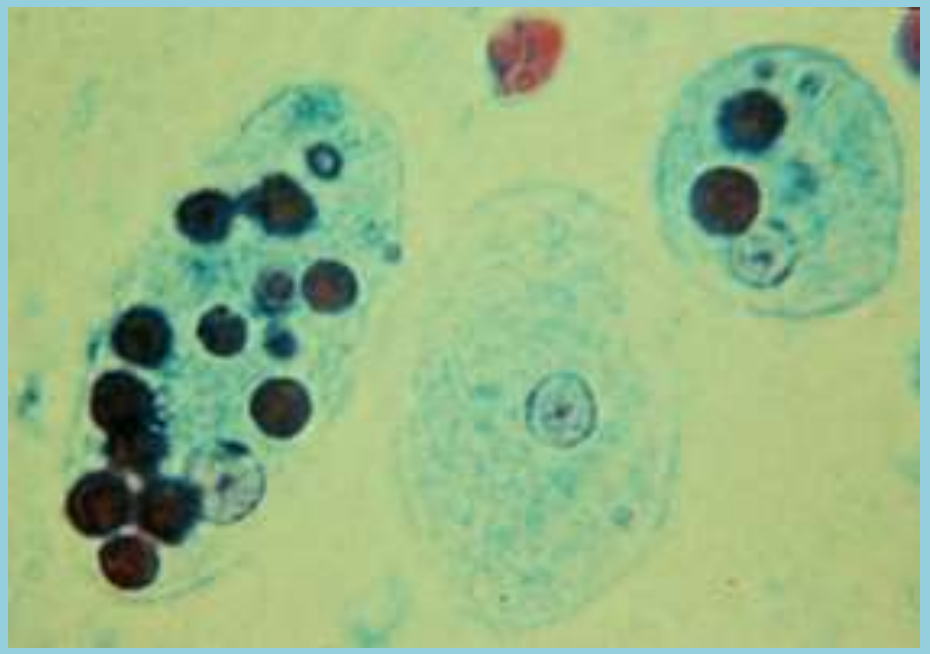
Serodiagnosis
- IHA
- ELISA
- Latex agglutination test

Radiological examination
- X-ray
- USG
- CT scan
- MRI

Stool examination

Trophozoites of *Entamoeba histolytica* with ingested erythrocytes (trichrome stain)



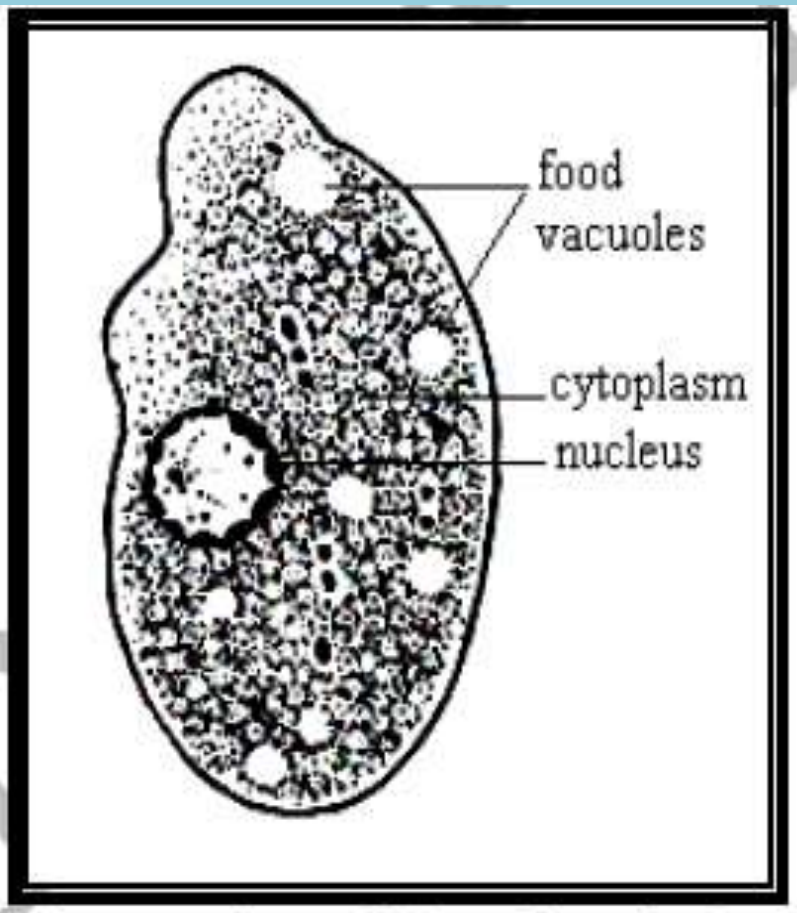


The ingested erythrocytes appear as dark inclusions.

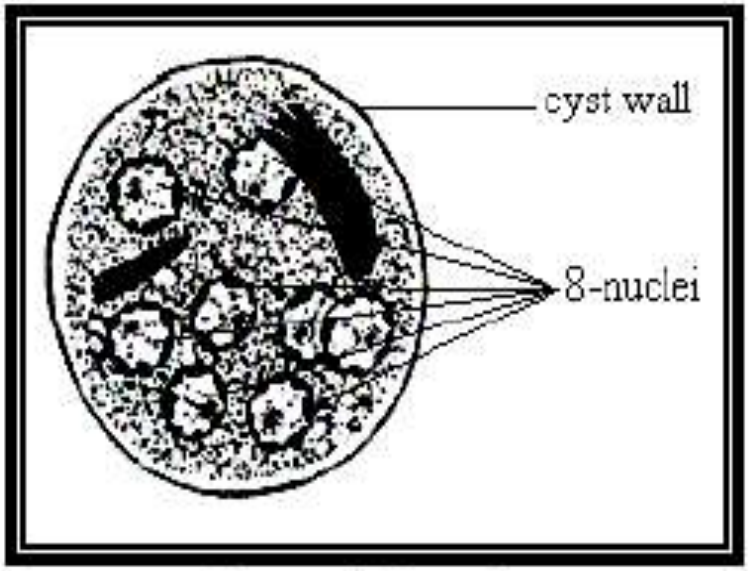
Erythrophagocytosis is the only morphologic characteristic that can be *E. dispar* from the nonpathogenic *E. histolytica* used to differentiate

- **Treatment:** Metronidazole (Flagyll)**.**

- ***Entamoeba coli***
- It has a cosmopolitan distribution, two stages trophozoite and cyst. The diameter of the trophozoite is (15-50µ), it has a spherical shape, the ectoplasm couldn't recognize from the endoplasm, the food vacuoles contain bacteria and other enteric microbes. The nuclear membrane studded from the inner surface with large irregular chromatin granules with eccentric and large karyosome. The trophozoite has a sluggish movement, shortly extended pseudopodia.
- The mature cyst has a diameter of (10-35µ), 8 nuclei, the chromatoid bodies have an irregular sharp ended (splinter-like). It lives in the lumen of the caecum and lower level of the large intestine.



***Entamoeba coli* (trophozoite).**

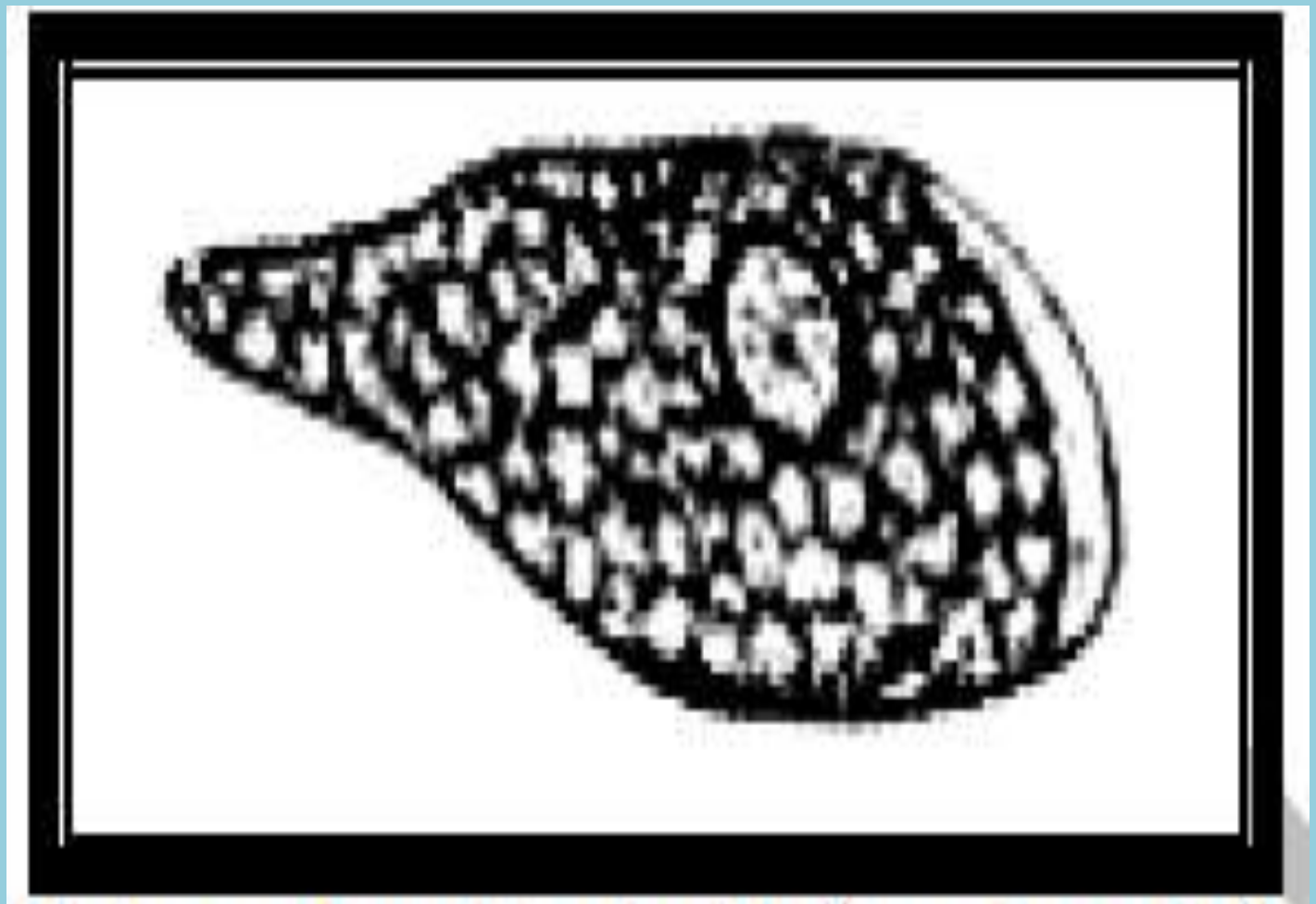


***Entamoeba coli* (cyst).**

- The life cycle is similar to that of ***E. histolytica***, except that the trophozoite in this example doesn't attack the mucosa of the intestine, so that it is described as non-pathogenic (commensal) ameba. Its presence is evidence that the host has ingested fecal material.

Entamoeba gingivalis

- It is a parasite of the mouth of man and other mammals, including several species of monkeys and of dogs and cats. It lives in/on the teeth, gums, and sometimes tonsils, only the trophozoite stage has been described which is measure (5-35µ) in diameter. In most respects it closely resembles ***E. histolytica***, with a few to several fingerlike pseudopodia, finely granular endoplasm, and clear ectoplasm. The nucleus contains a small karyosome that is central or slightly eccentric in position. Endocytotic vacuoles are often numerous and the parasite will ingest bacteria, leukocytes and erythrocytes although it is not itself invasive. No cysts are formed and transmission is entirely by oral to oral contact. Multiple samplings reveal the parasite to colonize the oral cavity of nearly all adult humans.



Entamoeba gingivalis (trophozoite).

- Diagnosis: by demonstration of trophozoites in materials removed from gingival margin or from between the teeth or cavities of decayed teeth. The presence of this amoeba in the mouth suggests the need for better oral hygiene

Intestinal, Oral, and Genital Flagellates

By

Assit. Prof. Dr. Marwa M. Ali

- Parasitic protozoa, which possess whip-like flagella as their organs of locomotion are called as flagellates and classified as
- — Phylum: Sarcomastigophora
- Subphylum: Mastigophora
- Class: Zoomastigophora (mastix: whip)

-Depending on their habitat, they can be considered under:

-Lumen-dwelling flagellates: Flagellates found in the alimentary tract and urogenital tract .

-Hemoflagellates: Flagellates found in blood and tissues

- Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical diseases(*Giardia lamblia)*, which can cause diarrhea and (*Trichomonas vaginalis)*, which can produce vaginitis and urethritis.

Giardia Lamblia

- **Habitat:**

G. lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine.

Morphology :

It exists in 2 forms:

-Trophozoite (or vegetative form)

- Cyst (or cystic form).

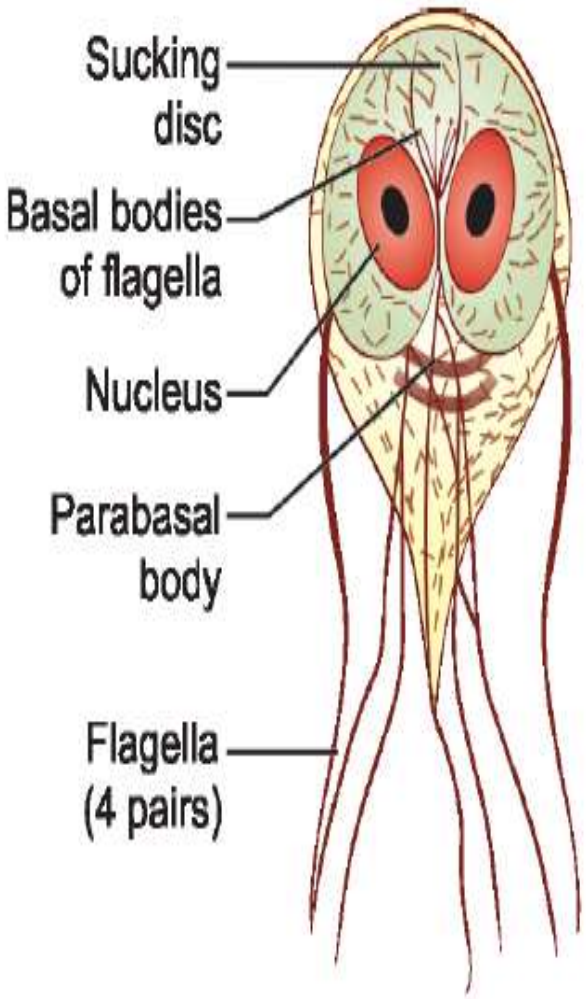
Trophozoite

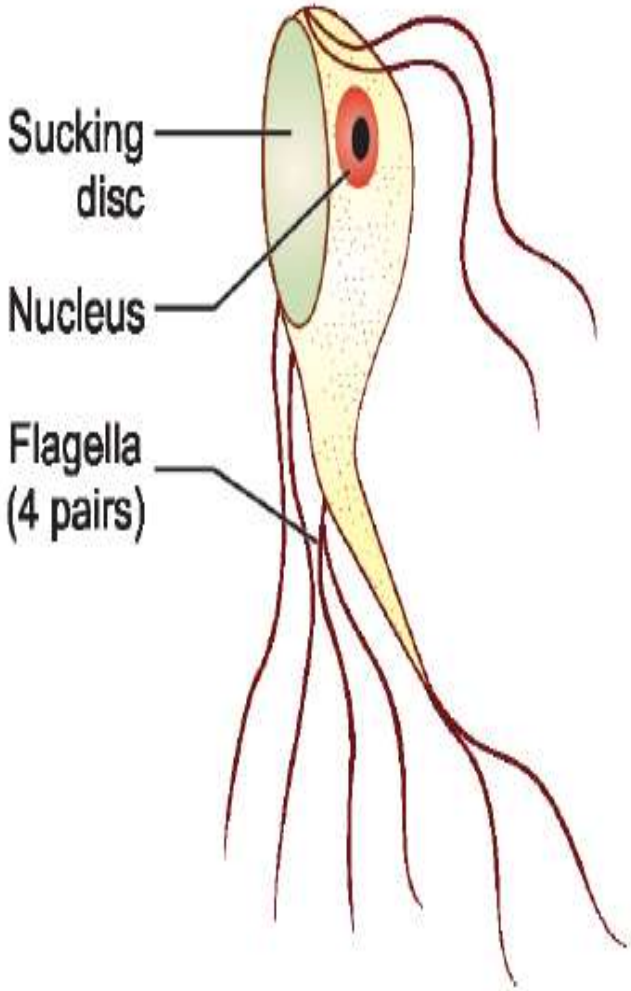
- The trophozoite is in the shape of a tennis racket (heart shaped or pyriform shaped) and is rounded anteriorly and pointed posteriorly
- It measures 15 µm x 9 µm wide and 4 µm thick.
- Dorsally, it is convex and ventrally, it has a concave sucking disc, which helps in its attachment to the intestinal mucosa.
- It is bilaterally symmetrical and possesses: 1 pair of nuclei and 4 pairs of flagella
- Blepharoplast, from which the flagella arise (4 pairs)
- 1 pair of axostyles, running along the midline
- Two sausage shaped parabasal or median bodies, lying transversely posterior to the sucking disc.
- The trophozoite is motile, with a slow oscillation about its long axis, often resembling falling leaf

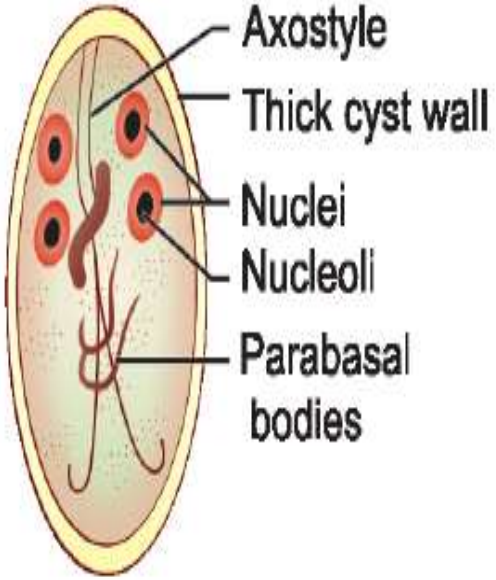
Cyst

It is the infective form of the parasite

- The cyst is small and oval, measuring 12 µm x 8 µm and is surrounded by a hyaline cyst wall.
- Its internal structure includes 2 pairs of nuclei grouped at one end. A young cyst contains 1 pair of nuclei.
- The axostyle lies diagonally, forming a dividing line within cyst wall.
- Remnants of the flagella and the sucking disc may be seen in the young cyst.


Sucking disc
Basal bodies of flagella
Nucleus
Parabasal body
Flagella (4 pairs)


Sucking disc
Nucleus
Flagella (4 pairs)


Axostyle
Thick cyst wall
Nuclei
Nucleoli
Parabasal bodies

Life Cycle

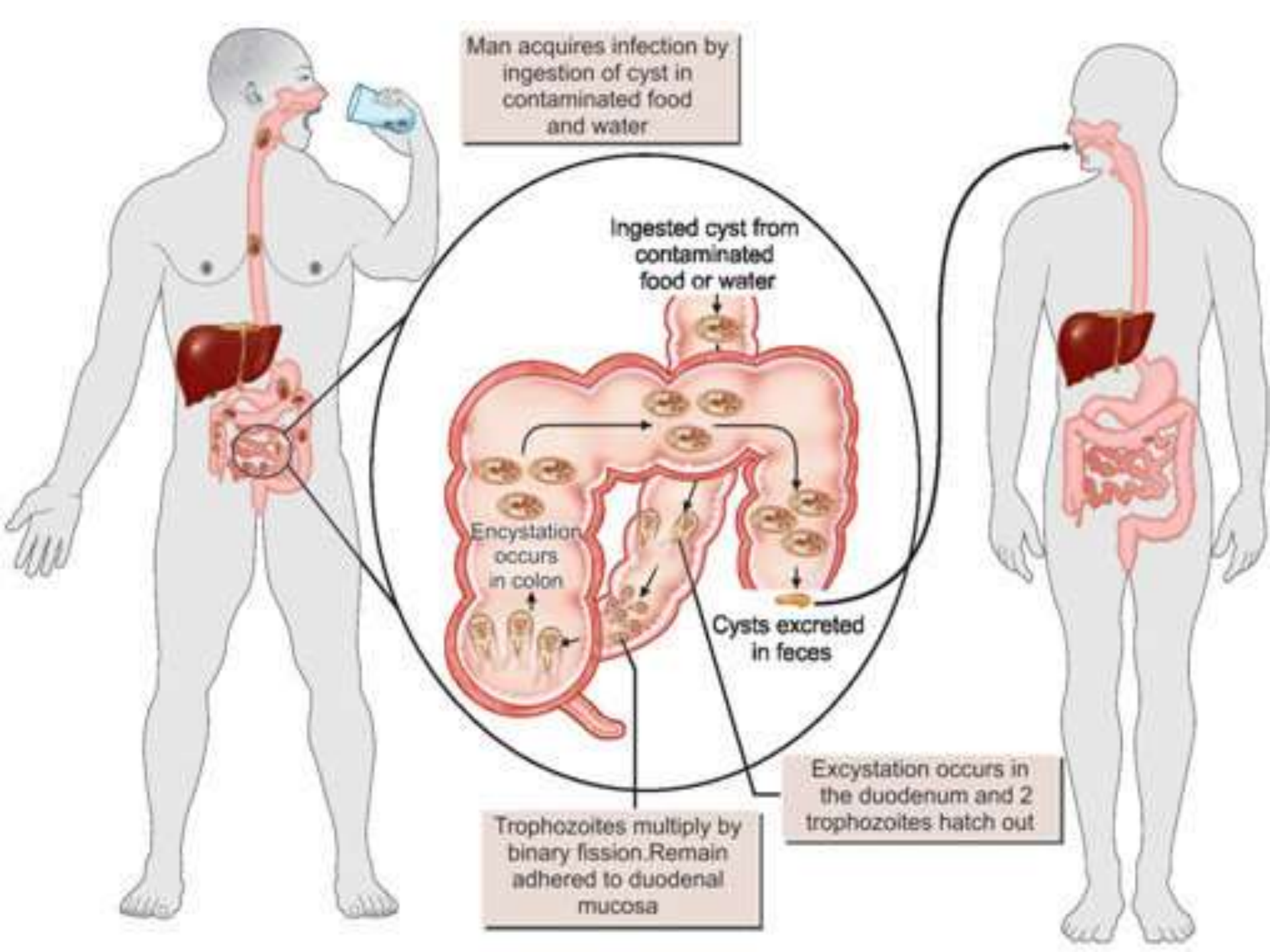
- Giardia passes its life cycle in 1 host.

Infective form: Mature cyst.

Mode of transmission:

- Man acquires infection by ingestion of cysts in contaminated water and food.
- Direct person to person transmission may also occur in children, male homosexuals, and mentally ill persons. .
- Within half an hour of ingestion, the cyst hatches out into two trophozoites , which multiply successively by binary fission and colonize in the duodenum

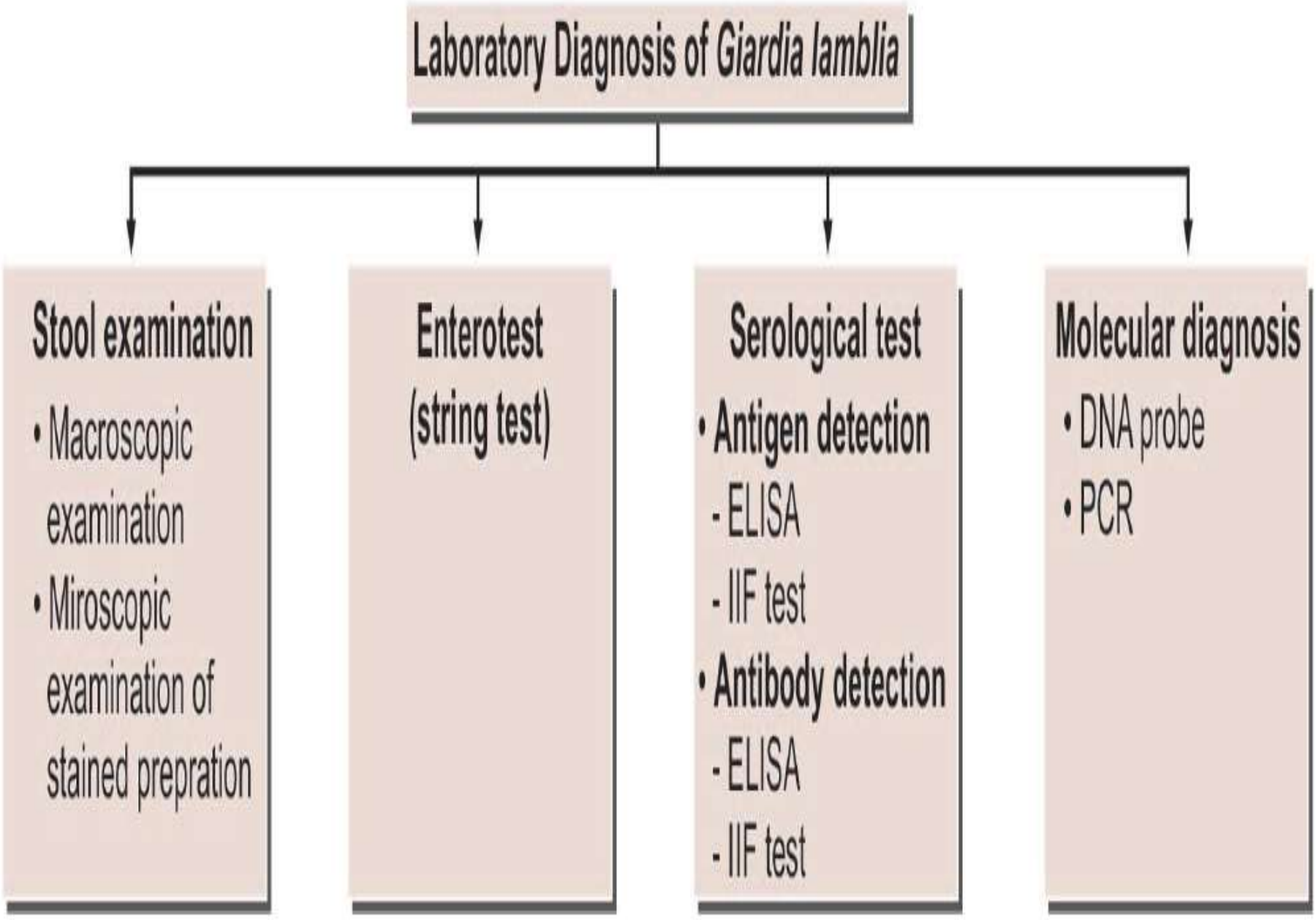
- The trophozoites live in the duodenum and upper part of jejunum, feeding by pinocytosis.
- During unfavorable conditions, encystment occurs usually ir colon
- Cysts are passed in stool and remain viable in soil and water for several weeks.
- There may be 200,000 cysts passed per gram of feces.
- Infective dose is 10–100 cysts.


Man acquires infection by ingestion of cyst in contaminated food and water
Ingested cyst from contaminated food or water
Encystation occurs in colon
Cysts excreted in feces
Excystation occurs in the duodenum and 2 trophozoites hatch out
Trophozoites multiply by binary fission.Remain adhered to duodenal mucosa

Pathogenicity and Clinical Features

- *G. lamblia* is typically seen within the crypts of duodenal and jejunal mucosa. It does not invade the tissue, but remains tightly adhered to intestinal epithelium by means of the sucking disc.
- They may cause abnormalities of villous architecture by cell apoptosis and increased lymphatic infiltration of lamina propria.
- Variant specific surface proteins (VSSP) of giardia play an important role in virulence and infectivity of the parasite.
- Often they are asymptomatic, but in some cases, Giardia may lead to mucus diarrhea, fat malabsorption (steatorrhea), dull epigastric pain, and flatulence. The stool contains excess mucus and fat but no blood.

- Children may develop chronic diarrhea, malabsorption of fat, vitamin A, protein, sugars like xylose disaccharides, weight loss, and sprue - like syndrome.
- Occassionally, Giardia may colonize the gall bladder, causing biliary colic and jaundice.
- Incubation period is variable, but is usually about 2 weeks.

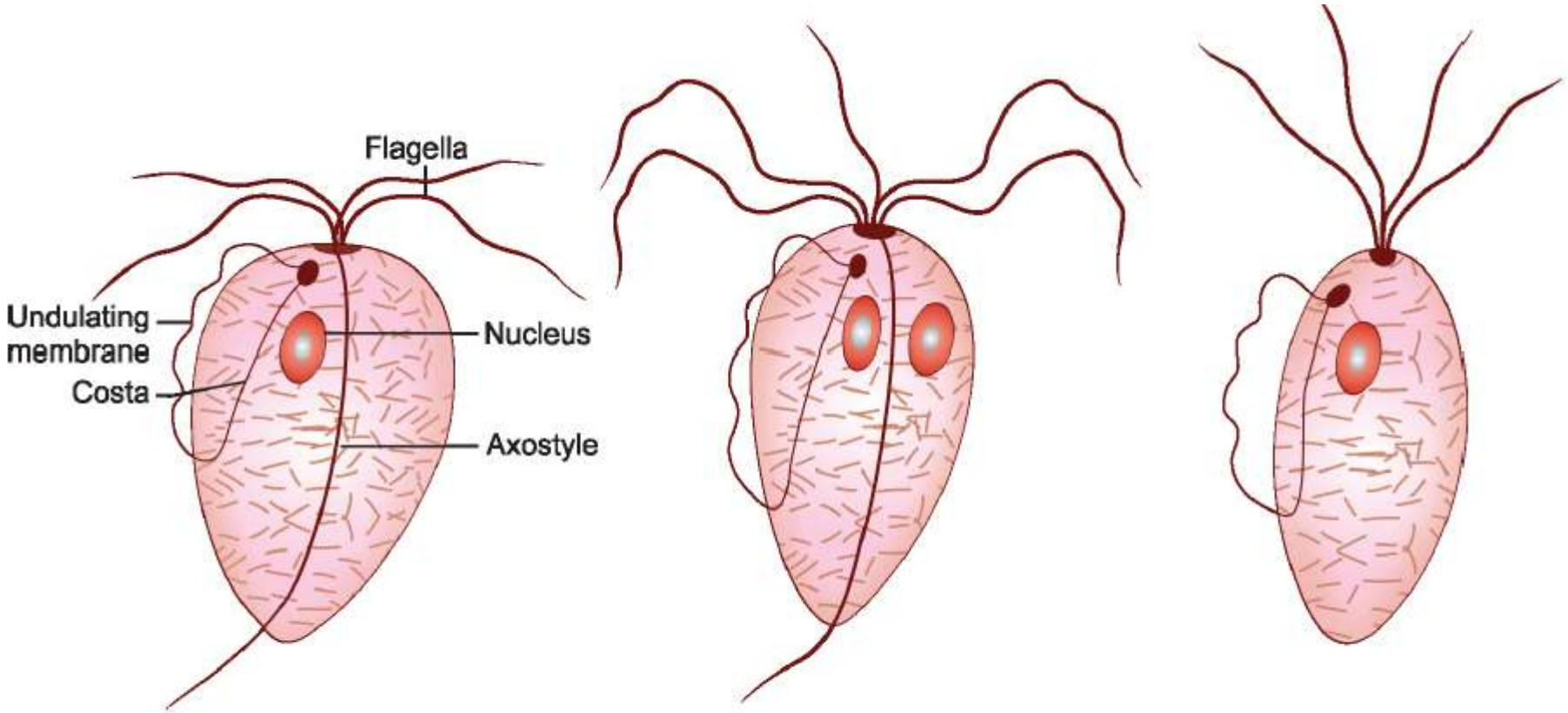

Laboratory Diagnosis of Giardia lamblia
Stool examination
• Macroscopic examination
• Miroscopic examination of stained prepration
Enterotest (string test)
Serological test
• Antigen detection
- ELISA
- IIF test
• Antibody detection
- ELISA
- IIF test
Molecular diagnosis
• DNA probe
• PCR

Trichomonas

- Trichomonas differs from other flagellates, as they exist only in trophozoite stage. Cystic stage is not seen.

Genus Trichomonas has 3 species, which occur in humans

- *T. vaginalis*
- *T. hominis*
- *T. tenax*



Trichomonas species. A. *T. vaginalis*; B. *T. hominis*; C. *T. tenax*

Trichomonas Vaginalis

Prevalence of trichomoniasis

- varies from 5% patients at hospitals to 75% in sexual workers.

Morphology

- It is pear-shaped or ovoid and measures 10–30 µm in length and 5–10 µm in breadth with a short undulating membrane reaching up to the middle of the body
- It has four anterior flagella and fifth running along the outer margin of the undulating membrane, which is supported at its base by a flexible rod, costa.
- A prominent axostyle runs throughout the length of the body and projects posteriorly like a tail.
- It is motile with a rapid jerky or twitching type movement

Habitat

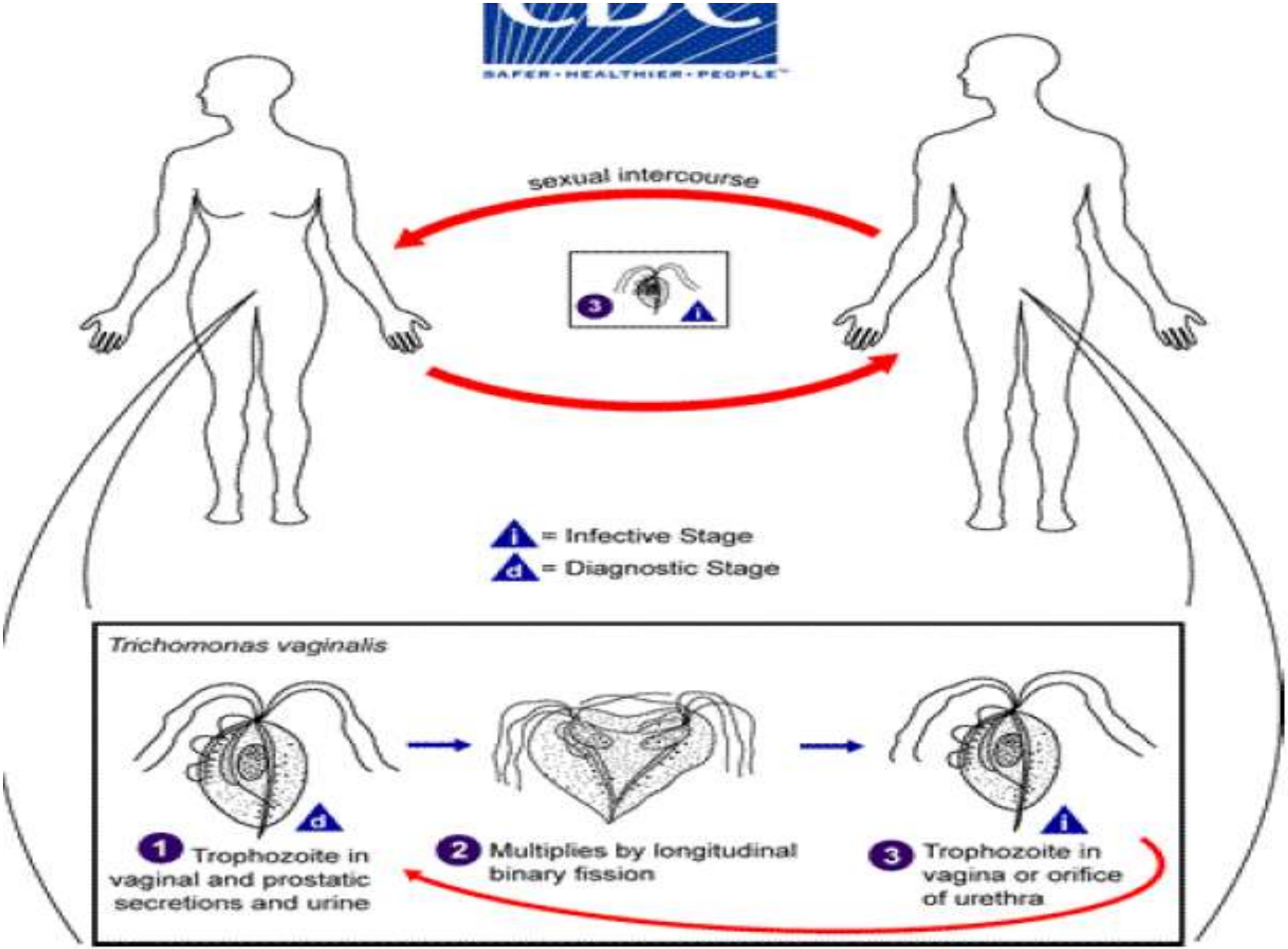
- In females, it lives in vagina and cervix and may also be found in Bartholin's glands, urethra, and urinary bladder.
- In males, it occurs mainly in the anterior urethra, but may also be found in the prostate and preputial sac.

Life Cycle Life cycle of *T. vaginalis*

- is completed in a single host either male or female.

Mode of transmission:

- The trophozoite cannot survive outside and so infection has to be transmitted directly from person to-person.
- Sexual transmission is the usual mode of infection.
- Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhea, syphillis, or human immunodeficiency virus (HIV).
- Babies may get infected during birth.
- Fomites such as towels have been implicated in transmission
- Trophozoites divide by binary fission.
- As cysts are not formed, the trophozite itself is the infective form.
- Incubation period is roughly 10 days.


SAFER · HEALTHIER · PEOPLE™
sexual intercourse
3
i
i = Infective Stage
d = Diagnostic Stage
Trichomonas vaginalis
d
1 Trophozoite in vaginal and prostatic secretions and urine
2 Multiplies by longitudinal binary fission
i
3 Trophozoite in vagina or orifice of urethra

Trichomonas has following virulence factors:

- Protein liquids and proteases: They help in adherence of trophozoites to epithelial cells of the genitor-urinary tract.
- Lactic and acetic acid: it lower the pH of the vaginal fluid. The low pH of vaginal secretion is cytotoxic to epithelial cells
- Enzymes cysteine proteases: it is responsible for haemolytic activity of the parasite.

Pathogenesis

- *T. vaginalis* particularly infects squamous epithelium and not columnar epithelium.
- It secretes cystine proteases, lactic acid, and acetic acid, which disrupt the glycogen levels and lower the pH of the vaginal fluid.
- It is an obligate parasite and cannot live without close association with the vaginal, urethral, or prostatic tissues.
- Parasite causes petechial hemorrhage (strawberry mucosa), metaplastic changes, and desquamation of the vaginal epithelium.
- Intracellular oedema and so called chicken-like epithelium, is the most characteristic feature of trichomoniasis.

Clinical Features

- Infection is often asymptomatic, particularly in males, although some may develop urethritis, epididymitis, and prostatitis.
- In females, it may produce severe pruritic vaginitis with an offensive, yellowish green, often frothy discharge, dysuria,. Cervical erosion is common. Endometritis and pyosalpingitis are infrequent complications.
- Rarely, neonatal pneumonia and conjunctivitis have been reported in infants born to infected mothers.
- The incubation period of trichomoniasis is 4 days to 4 weeks.

Laboratory Diagnosis

1)Microscopic examination

- Vaginal or urethral discharge is examined microscopically in saline wet mount preparation for characteristic jerky and twitching motility and shape. In males, trophozoites may be found in urine or prostatic secretions.

-Fixed smears may be stained with Giemsa stains.

-Direct fluroscent antibody (DFA) is another method of detection of parasite and is more sensitive than the wet mount.

2)Culture

- Culture is recommended when direct microscopy is negative and is considered as a 'gold standard' as well as the most sensitive (95%) method for the diagnosis of T. vaginalis infection.
- It grows best at 35°–37°C under anaerobic conditions. The optimal pH for growth is 5.5–6.0.
-

3)Molecular method

DNA hybridization and PCR are also highly sensitive (97%) and specific (98%) tests for the diagnosis of trichomoniasis.

Trichomonas tenax

T. tenax, is a harmless commensal which lives in mouth in the periodontal pockets, carious tooth cavities, and less often in tonsillar crypts.

- It is smaller (5–10 µm) than *T. vaginalis*.
- It is transmitted by kissing, through salivary droplets, and fomites. There are sporadic reports of its involvement in respiratory infections and thoracic abscesses.
- Better oral hygiene rapidly eliminates the infection and no therapy is indicated.

Trichomonas hominis

- *T. hominis* measures 8–12 µm, pyriform-shaped, and carries 5 anterior flagella and an undulating membrane that extends the full length of the body.
- It is a very harmless commensal of the caecum.
- Microscopic examination of stool will reveal motile trophozoite of T. *hominis*.
- Transmission occurs in trophic form by fecal oral route.

Leishmania

By

Assit. Prof. Dr. Marwa M. Ali

- All members of the genus Leishmania are obligate intracellular parasites that pass their life cycle in 2hosts—the mammalian host and the insect vector, female sandfly.
- In humans and other mammalian hosts, they multiply within macrophages, in which they occur exclusively in the amastigote form, having an ovoid body containing a nucleus and kinetoplast.
- In the sandfly, they occur in the promastigote form, with a spindle shaped body and a single flagellum arising from anterior end.
- Leishmaniasis has an immense geographical distribution in the tropics and subtropics of the world, extending through most of the Central and South America, part of North America, central and Southeast Asia, India, China, the Mediterranean region, and Africa.
- The disease affects the low socioeconomic group of people. Overcrowding, poor ventilation, and collection of organic material inside house facilitate its transmission.

- Across the tropics, 3 different diseases are caused by various species of genus Leishmania. These are:

❑ Visceral leishmaniasis: The species *L. donovani* complex infecting internal organs (liver, spleen, and bone marrow) of human is the causative parasite.

❑ Cutaneous leishmaniasis: The species *L. tropica* complex, *L. aethiopica*, *L. major* and *L. mexicana* complex are the causative parasite.

❑ Mucocutaneous leishmaniasis : It is caused by the *L. braziliensis* complex .

Classification

- The genus Leishmania includes a number of different varieties and subspecies, which differ in several features such as antigenic structure, isoenzymes, and other biochemical characteristics, growth properties, host specificity, etc
- Leishmania species can also be classified on the basis of geographical distribution.

Table 5.6: Classification of *Leishmania* based on Geographical Distribution

Old world leishmaniasis	New world leishmaniasis
Leishmania donovani	*Leishmania braziliensis complex*
Leishmania infantum	*Leishmania mexicana complex*
Leishmania tropica	*Leishmania chagasi*
Leishmania major	*Leishmania peruviana*
Leishmania aethiopica	

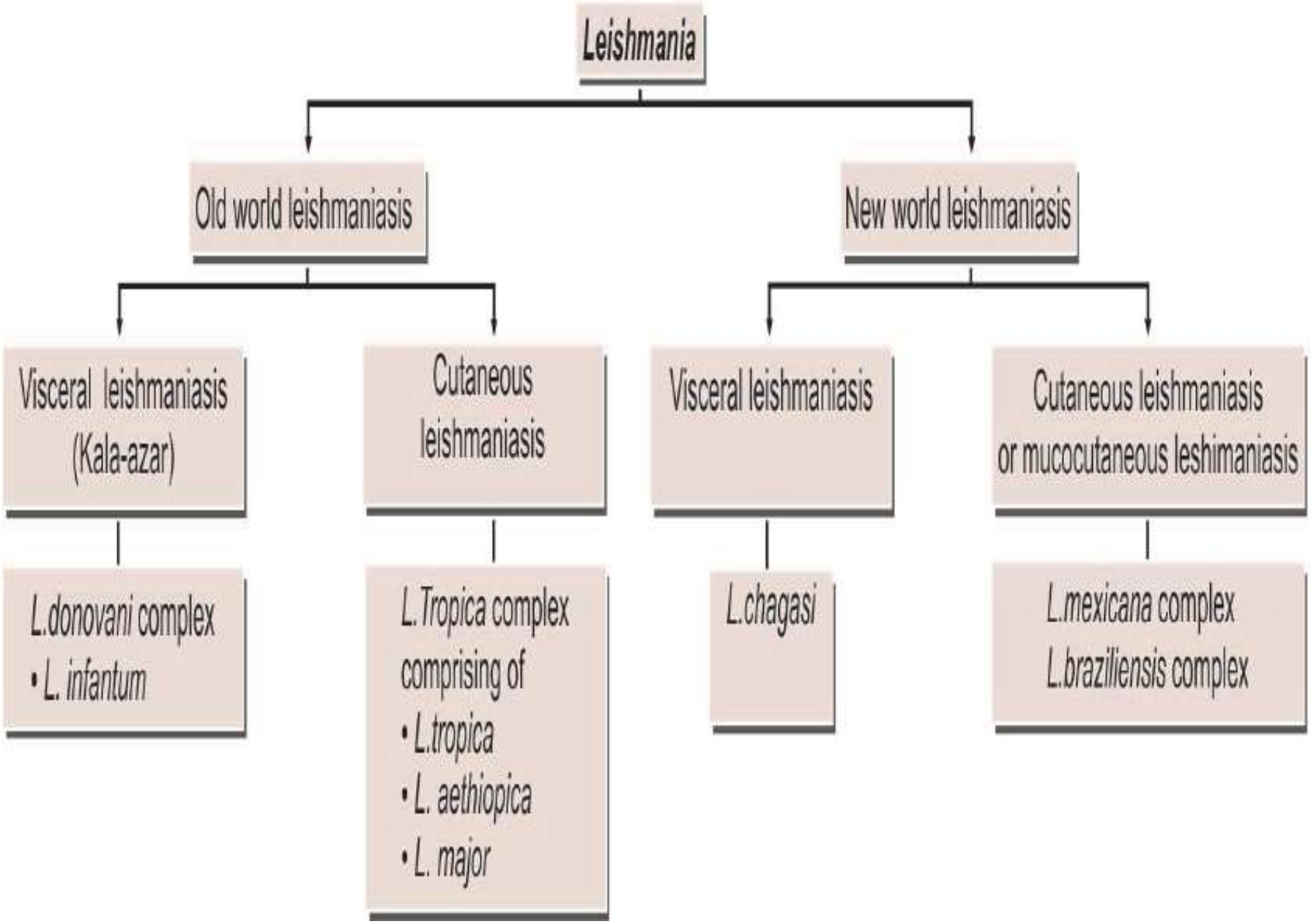
Note: The vector for old world leishmaniasis is sandfly of the genus *Phlebotomus* and for new world leishmaniasis is sandfly of the genera *Lutzomyra* and *Psychodopygus*.

Old World Leishmaniasis

Leishmania donovani causes visceral leishmaniasis or Kala-azar. It also causes the condition, Post Kala-azar Dermal Leishmaniasis (PKDL)

Habitat

The amastigote (LD body) of *L. donovani* is found in the reticuloendothelial system. They are found mostly within the macrophages in the spleen, liver, bone marrow and less often in other locations such as skin, intestinal mucosa, and mesenteric lymph nodes.


Leishmania
Old world leishmaniasis
New world leishmaniasis
Visceral leishmaniasis (Kala-azar)
Cutaneous leishmaniasis
Visceral leishmaniasis
Cutaneous leishmaniasis or mucocutaneous leshimaniasis
L.donovani complex
• L. infantum
L.Tropica complex comprising of
• L.tropica
• L. aethiopica
• L. major
L.chagasi
L.mexicana complex
L.braziliensis complex

- Morphology

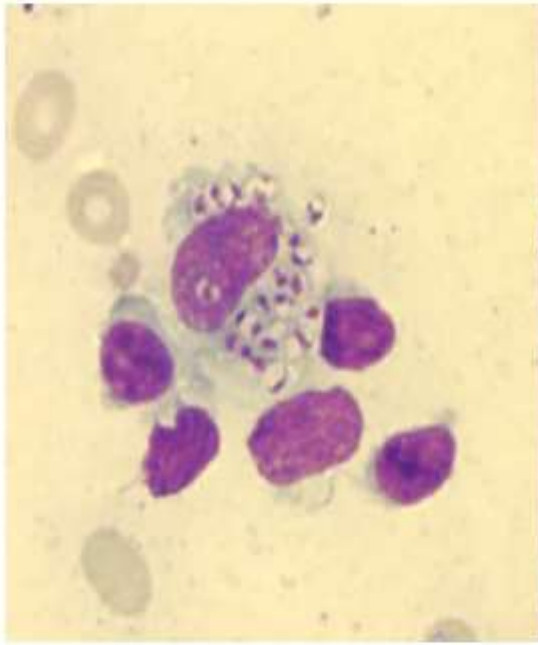
The parasite exists in 2 forms:

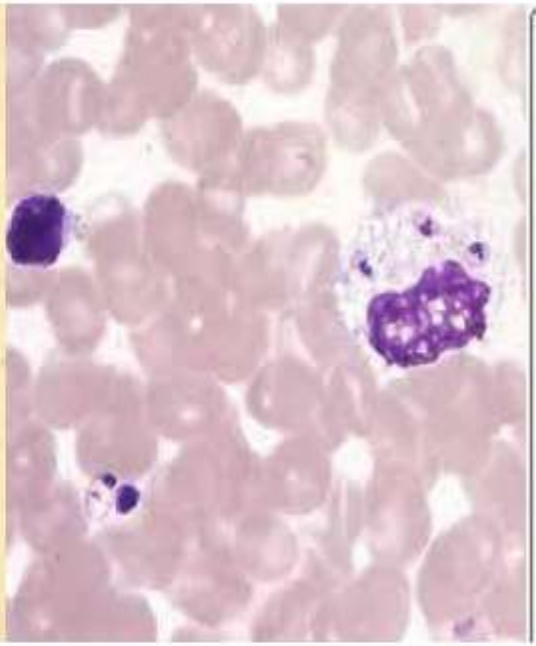
- Amastigote form: in humans and other mammals.
- Promastigote form: in the sandfly and in artificial culture.

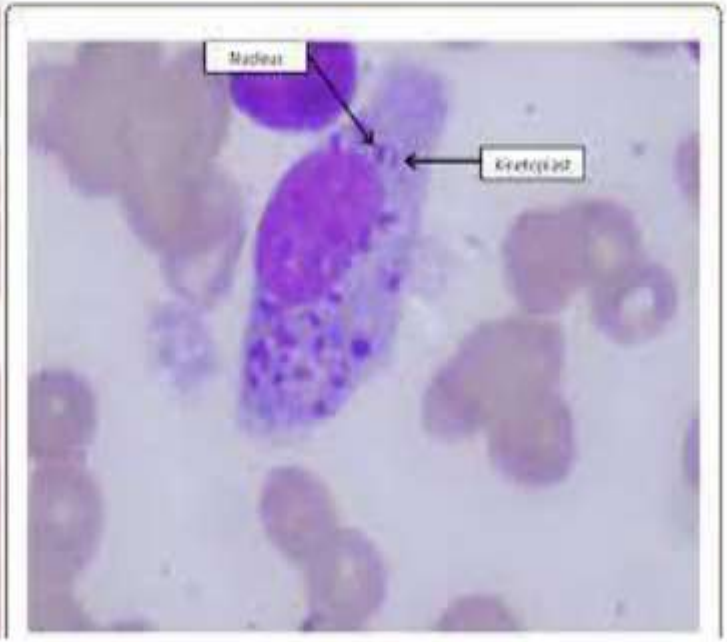
Amastigote

- The amastigote form (LD body) is an ovoid or rounded cell,about 2–4 µm in size .
- ıt is typically intracellular, being found inside macrophages, monocytes, neutrophils, or endothelial cells.
- They are also known as **LD bodies**.
- Smears stained with Leishman, Giemsa, or Wright's stain show a pale blue cytoplasm enclosed by a limiting membrane.
- The large oval nucleus is stained red. Lying at the right angles to nucleus, is the red or purple stained **kinetoplast**.
- In well stained preparations, the kineloplast can be seen consisting of a **parabasal body** and a dot like **blepharoplast** with a delicate thread connecting the two. The axoneme arising from the blepharoplasty extends to the anterior tip of the cell.
- Alongside the kinetoplast a clear unstained vacuole can be seen.
- Flagellum is absent.

Amastigote stage





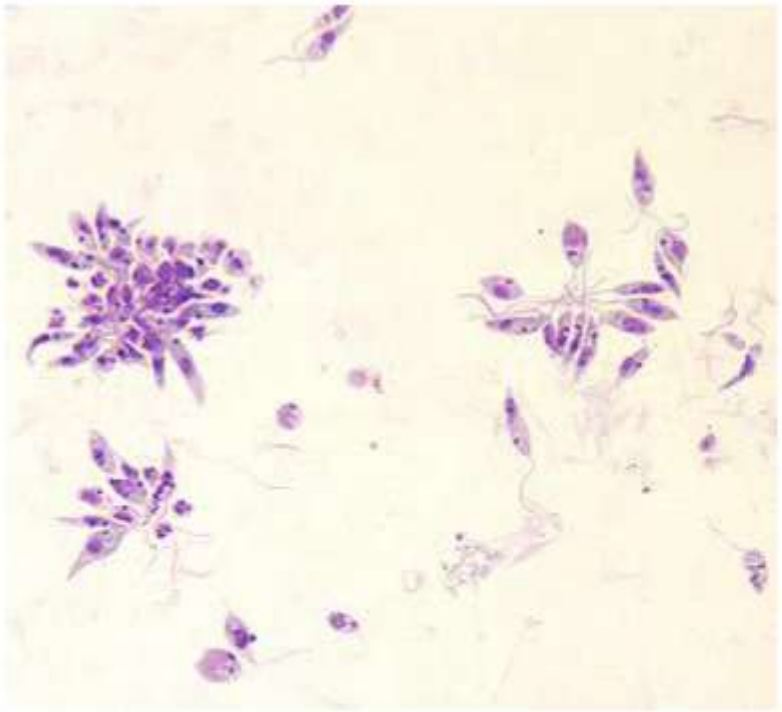


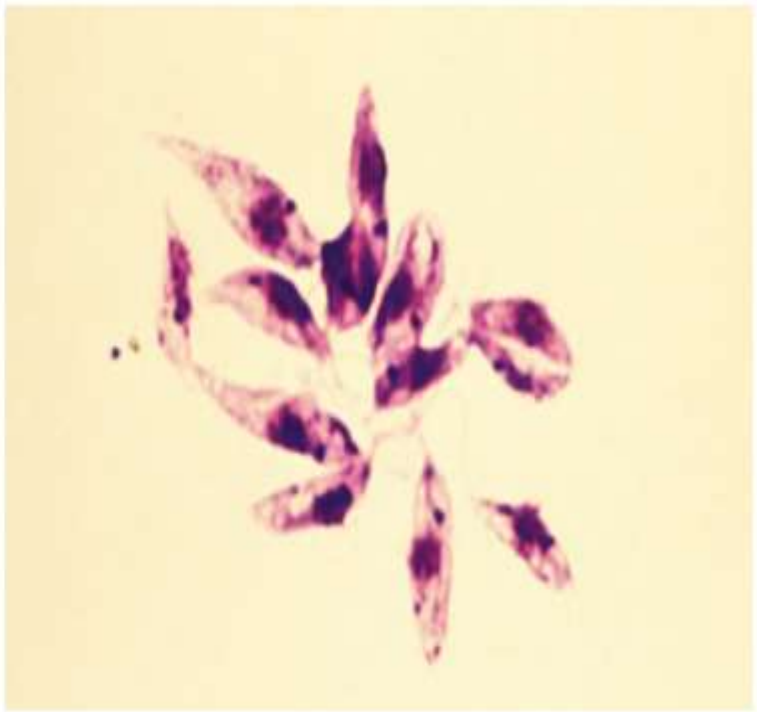
Promastigote

- It is a flagellar stage and is present in insect vector, sandfly and in cultures.
- The promastigotes, which are intitially short, oval or pear shaped forms, subsequently become long spindle shaped cells.

- A single nucleus is situated at the center. The kinetoplast lies transversely near the anterior end.
- The flagellum is single, delicate.
- Giemsa or Leishman stained films show pale blue cytoplasm with a pink nucleus and bright red kinetoplast.
- A vacuole is present near the root of the flagellum.
- There is no undulating membrane.
- Promastigote forms, which develop in artificial cultures, have the same morphology as in the sandfly

promastigote





Life Cycle

L. donovani completes its life cycle in 2 hosts :

- Definitive host: Man, dog, and other mammals.
- Vector: Female sandfly (Phlebotomus species).

Infective form: Promastigote form present in midgut of female sandfly

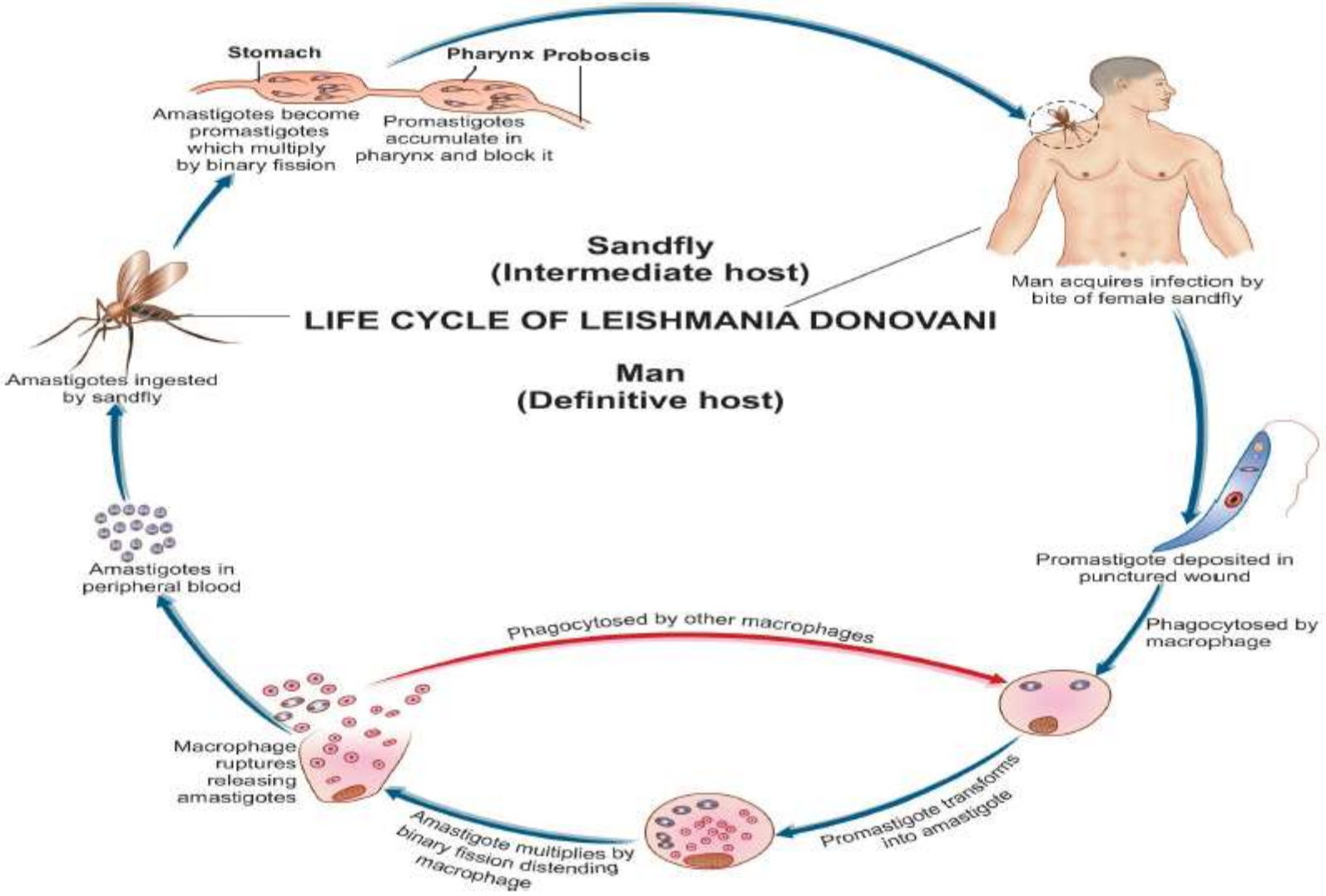
Mode of transmission:

- Humans acquire by bite of an infected female sandfly.
- It can also be transmitted vertically from mother to fetus, by blood transfusion, and accidental inoculation in the laboratory.

Incubation period: Usually 2–6 months, occasionally it may be as short as 10 days or as long as 2 years.

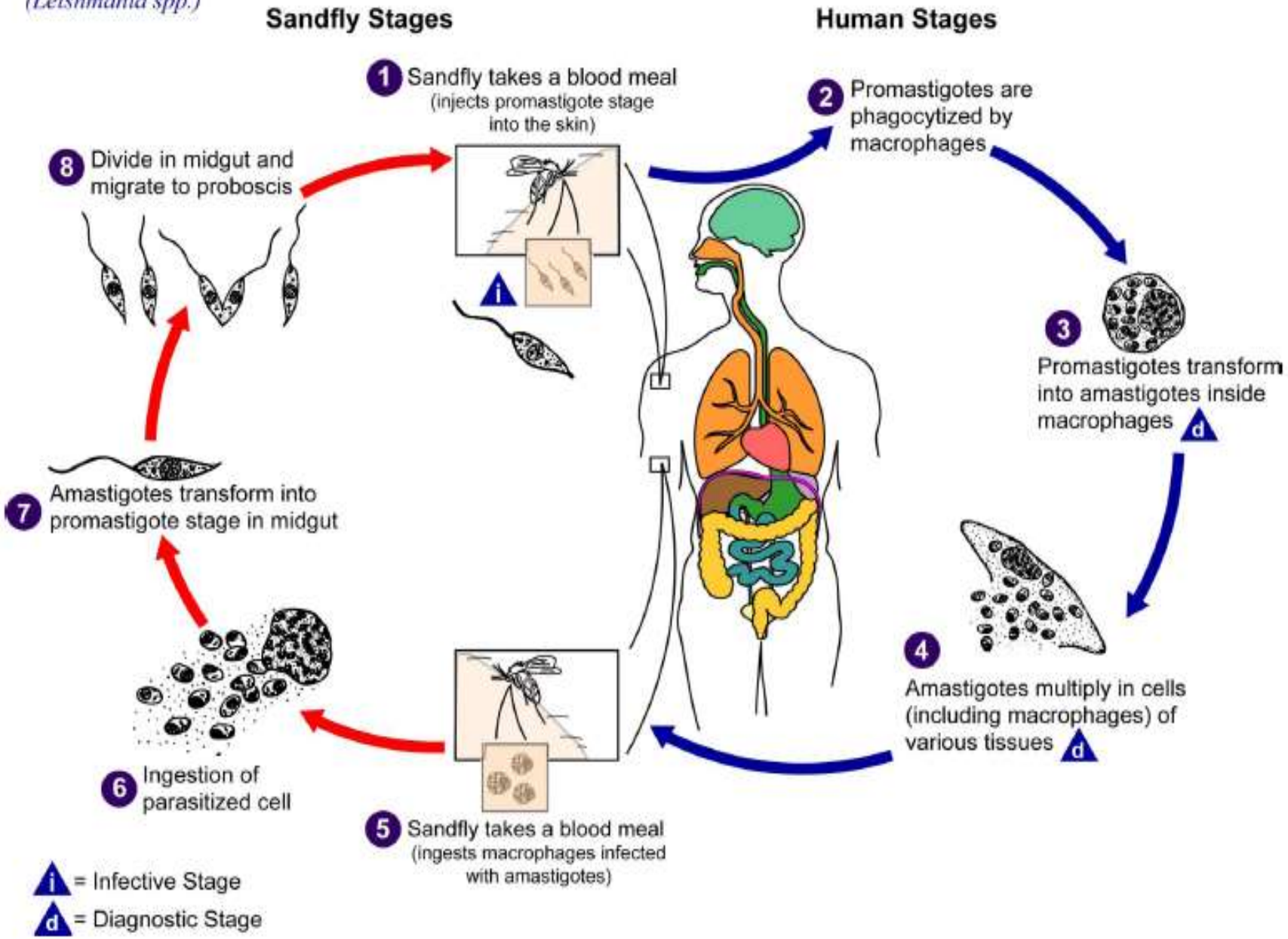
- The sand fly regurgitates the promastigotes in the wound caused by its proboscis.
- These are engulfed by the cells of reticuloendothelial system (macrophages, monocytes, and polymorphonuclear leucocytes) and change into amastigote (LD body) within the cells .
- The amastigote multiplies by binary fission, producing numerous daughter cells that distend the macrophage and rupture it. The liberated daughter cells are in turn , phagocytosed by other macrophages and histiocytes.
- Small number of LD bodies can be found in peripheral blood inside neutrophils or monocytes

- When a vector sandfly feeds on an infected person, the amastigotes present in peripheral blood and tissue fluids enter the insect along with its blood meal. In the midgut (stomach) of the sand fly, the amastigote elongates and develops into the promastigote form.
- The promastigote multiples by longitudinal binary fission and reaches enormous numbers. They may be seen as **large rosettes** with their flagella entangled.
- In the sand fly, they migrate from the midgut to the pharynx and hypostome, where they accumulate and block the passage.
- Such **blocked sandflies** have difficulty in sucking blood. When they bite a person and attempt to suck blood, plugs of adherent parasites may get dislodged from the pharynx and they are deposited in the punctured wound. It takes about 10 days for the promastigotes to reach adequate numbers after ingestion of the amastigotes, so as to block the buccal cavity and pharynx of the sandfly. This is, therefore, the duration of **extrinsic incubation period**.


Stomach
Pharynx
Proboscis
Amastigotes become promastigotes which multiply by binary fission
Promastigotes accumulate in pharynx and block it
Sandfly
(Intermediate host)
LIFE CYCLE OF LEISHMANIA DONOVANI
Man
(Definitive host)
Man acquires infection by bite of female sandfly
Amastigotes ingested by sandfly
Promastigote deposited in punctured wound
Amastigotes in peripheral blood
Phagocytosed by other macrophages
Phagocytosed by macrophage
Macrophage ruptures releasing amastigotes
Amastigote multiplies by binary fission distending macrophage
Promastigote transforms into amastigote

Leishmaniasis

(Leishmania spp.)



- This period is also synchronous with the gonadotropic cycle of the vector, so that amastigotes ingested during a single blood meal, are ready to be transmitted when the sandfly takes the next blood meal after its eggs have been laid

Pathogenicity

L. donovani causes visceral leishmaniasis or kala-azar.

- Kala-azar is a reticuloendotheliosis resulting from the invasion of reticuloendothelial system by *L. donovani*.

- The parasitized macrophages disseminate the infection to all parts of the body.

- In the spleen, liver, and bone marrow particularly, the amastigotes multiply enormously in the fixed macrophages to produce a 'blockade' of the reticuloendothelial system. This leads to a marked proliferation and destruction of reticuloendothelial tissue in these organs.

Kala -azar

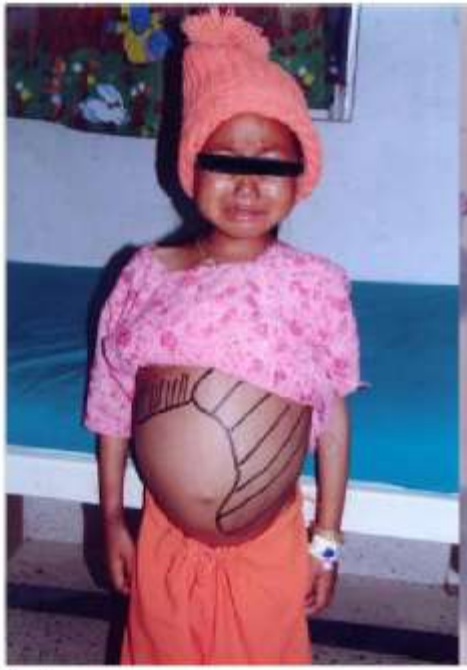


Fig. 1

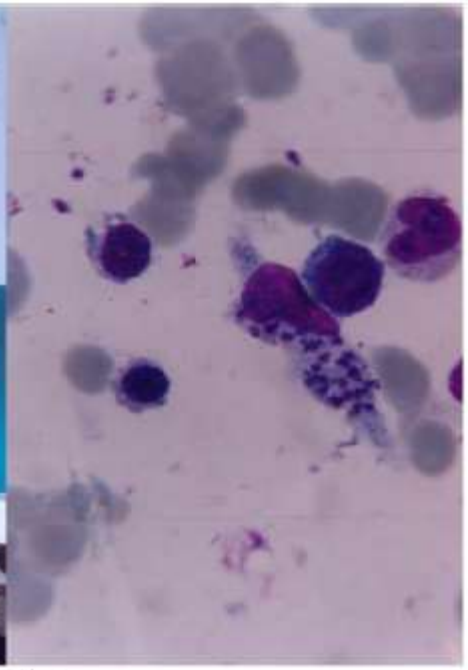


Fig. 2

Spleen:

- The spleen is the most affected organ. It is grossly enlarged and the capsule is thickened due to perisplenitis.
- Spleen is soft and friable and cuts easily due to absence of fibrosis.
- The cut section is red or chocolate in color due to the dilated and engorged vascular spaces.
- The trabeculae are thin and atrophic.
- Microscopically, the reticulum cells are greatly increased in numbers and are loaded with LD bodies.
- Lymphocytic infiltration is scanty, but plasma cells are numerous

Liver:

- The liver is enlarged ?
- The Küpffer cells and vascular endothelial cells are heavily parasitized, but hepatocytes are not affected.
- Liver function is, therefore, not seriously affected, although prothrombin production is commonly decreased.
- The sinusoidal capillaries are dilated and engorged.
- Some degree of fatty degeneration is seen. The cut surface may show a 'nutmeg' appearance

Bone marrow:

- The bone marrow is heavily infiltrated with parasitized macrophages, which may crowd the hematopoietic tissues.
- Peripheral lymph nodes and lymphoid tissues of the nasopharynx and intestine are hypertrophic, although this is not seen in Indian cases.
- Severe anemia with hemoglobin levels of 5–10 g/dL may occur in Kala-azar, as a result of infiltration of the bone marrow as well as by the increased destruction of erythrocytes due to hypersplenism. Autoantibodies to red cells may contribute to hemolysis.
- Leucopenia with marked neutropenia and thrombocytopenia are frequently seen. Antibodies against WBCs and platelets suggest an autoimmune basis for the pancytopenia observed in Kala-azar

Causes of anemia in Kala-azar

- Splenic sequestration of RBCs
- Decreased erythropoesis due to replacement of bone marrow with parasitized macrophages.
- Autoimmune hemolysis
- Hemorrhage

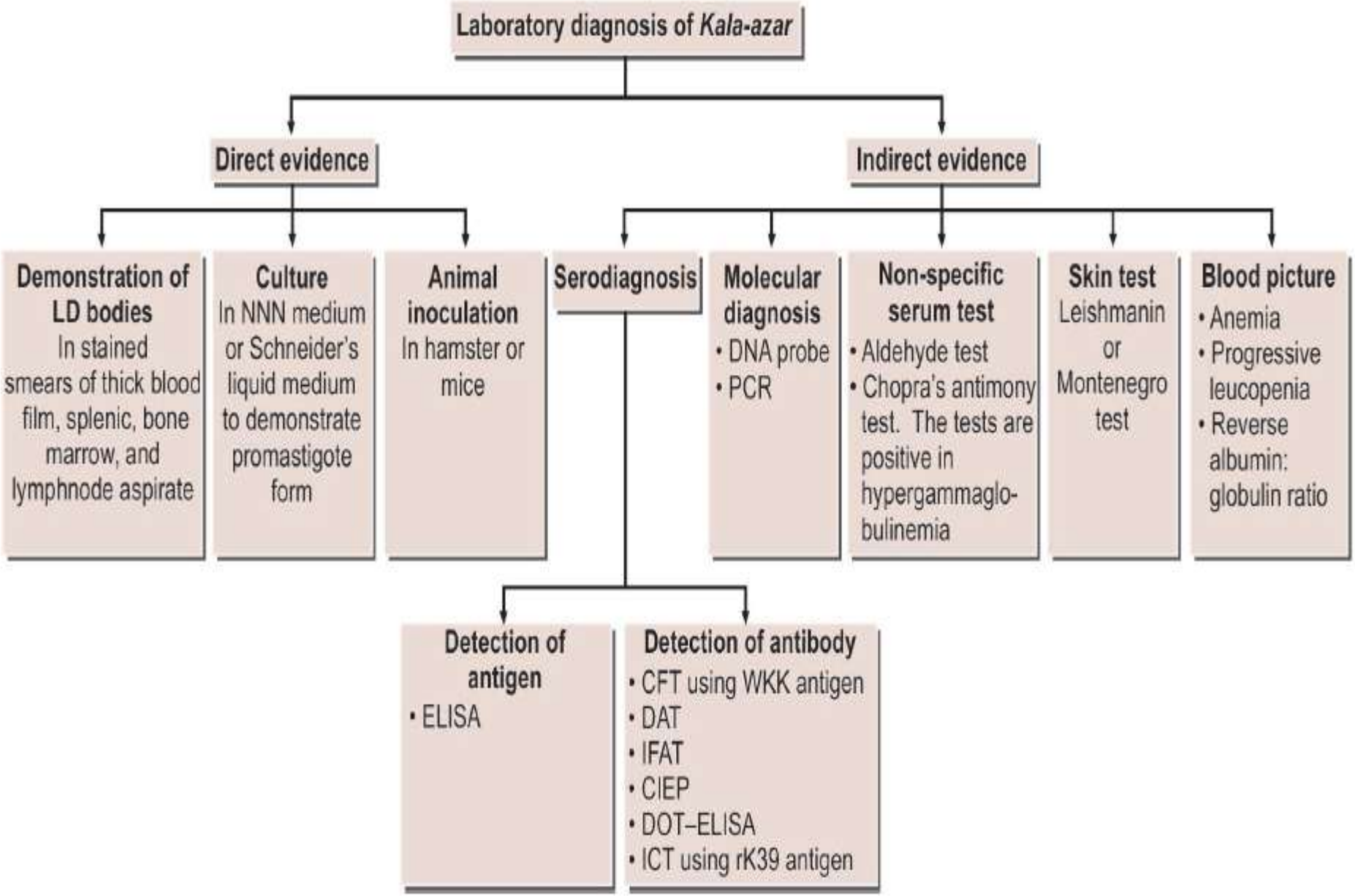
Clinical Features of Kala-Azar

- The onset is typically insidious. The clinical illness begins with fever, which may be continuous, remittent, or irregular.
- Splenomegaly starts early and is progressive and massive
- Hepatomegaly and lymphadenopathy also occur but are not so prominent.
- Skin becomes dry, rough, and darkly pigmented (hence, the name Kala-azar).
- The hair become thin and brittle.
- Cachexia with marked anemia, emaciation, and loss of weight is seen.
- Epistaxis and bleeding from gums are common.
- Most untreated patients die in about 2 years, due to some intercurrent disease such as dysentery, diarrhea, and tuberculosis

Immunity

- The most important immunological feature in Kala-azar is the marked suppression of cell mediated immunity to leishmanial antigens. This makes unrestricted intracellular multiplication of the parasite possible. Cellular responses to tuberculin and other antigens are also suppressed and may be regained some 6 weeks after recovery from the disease.

- In contrast, there is an overproduction of immunoglobulins, both specific anti-leishmanial antibodies as well as nonspecific polyclonal IgG and IgM. Circulating immune complexes are demonstrable in serum.

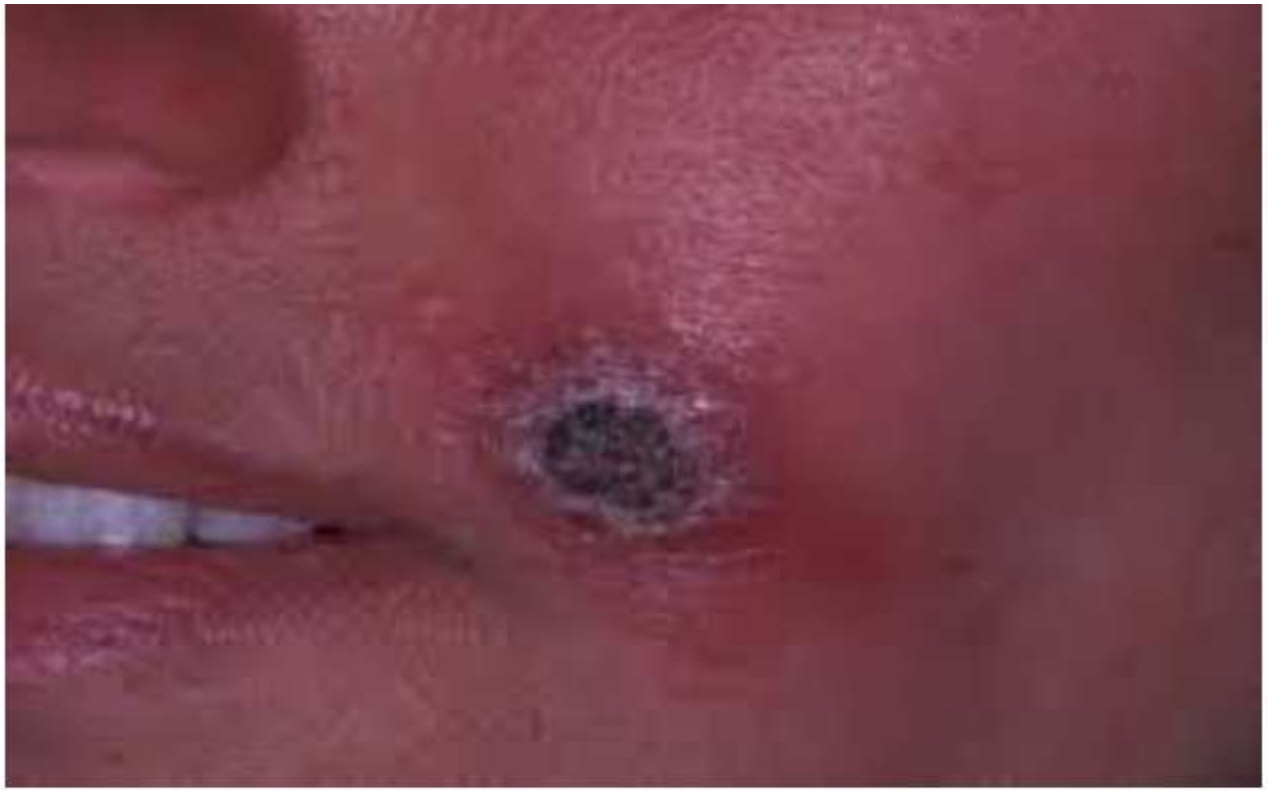

Laboratory diagnosis of *Kala-azar*
Direct evidence
Indirect evidence
Demonstration of LD bodies
In stained smears of thick blood film, splenic, bone marrow, and lymphnode aspirate
Culture
In NNN medium or Schneider's liquid medium to demonstrate promastigote form
Animal inoculation
In hamster or mice
Serodiagnosis
Molecular diagnosis
• DNA probe
• PCR
Non-specific serum test
• Aldehyde test
• Chopra's antimony test. The tests are positive in hypergammaglo-bulinemia
Skin test
Leishmanin or Montenegro test
Blood picture
• Anemia
• Progressive leucopenia
• Reverse albumin: globulin ratio
Detection of antigen
• ELISA
Detection of antibody
• CFT using WKK antigen
• DAT
• IFAT
• CIEP
• DOT–ELISA
• ICT using rK39 antigen

Leishmania Tropica Complex

It includes 3 species:

- *Leishmania tropica*
- *Leishmania major*
- *Leishmania aethiopica*

- All these species cause old world cutaneous leishmaniasis. The disease is also known as oriental sore, Delhi boil or Bagdad boil



Baghdad boil

Habitat

- *L. tropica* causing cutanaeous leishmaniasis (old world cutaneous leishmaniasis) are essentially the parasite of skin.

- The amastigote forms occur in the reticuloendothelial cells of the skin, whereas promastigote forms are seen in sandfly vector.

Morphology

- Morphology of *L. tropica* complex is indistinguishable from that of *L. donovani.*

- They are ingested by sandflies feeding near the skin lesions.
- In the midgut of the sandfly, the amastigotes develop into promastigotes, which replicate profusely.
- These are in turn transmitted to the skin of persons bitten by sandflies in the skin, the promastigotes are phagocytosed by mononuclear cells, in which they become amastigotes and multiply.
- However, they remain confined to the skin, without being transported to the internal organs, as is the case in visceral leishmaniasis.

Incubation period:

- Incubation period varies from 2–8 months.

Pathology

- Amastigote forms are found in histiocytes and endothelial cells. There is an inflammatory granulomatous reaction with infiltration of lymphocyte and plasma cells. Early lesions are papular, followed by ulceration necrosis. Papule and ulcer are the main pathological lesions. They heal over months to years, leaving scars

Laboratory Diagnosis

- **Microscopy**

❖ Smear is made from the material obtained from the indurated edge of nodule or sore and stained by Giemsa or Leishman stain.

❖ Amastigotes are found in large numbers inside the macrophages.

❖ Definitive diagnosis is made by demonstration of amastigote in the smear collected from the lesion.

- **Culture**

❖Promastigote forms can be isolated by culture of the aspirate material in NNN medium.

- **Skin Test**

❖Leishmanin skin test is helpful. Positive leishmanin test in children under 10 years of age from endemic areas is highly suggestive of the disease. The skin test is negative in diffuse cutaneous leishmaniasis.

❖Serology These are of limited value as the patient shows no detectable levels of circulating antibodies

- **New World Leishmaniasis**

(*L. braziliensis* complex and *L. mexicana* complex)

Habitat

These occur as intracellular parasite. The amastigote form is seen inside the macrophages of skin and mucous membrane of the nose and buccal cavity. The promastigote form occurs in vector species Lutzomyra.

Morphology

Morphology of amastigote and promastigote forms of both the parasites is same as that of the other 2 species of Leishmania.

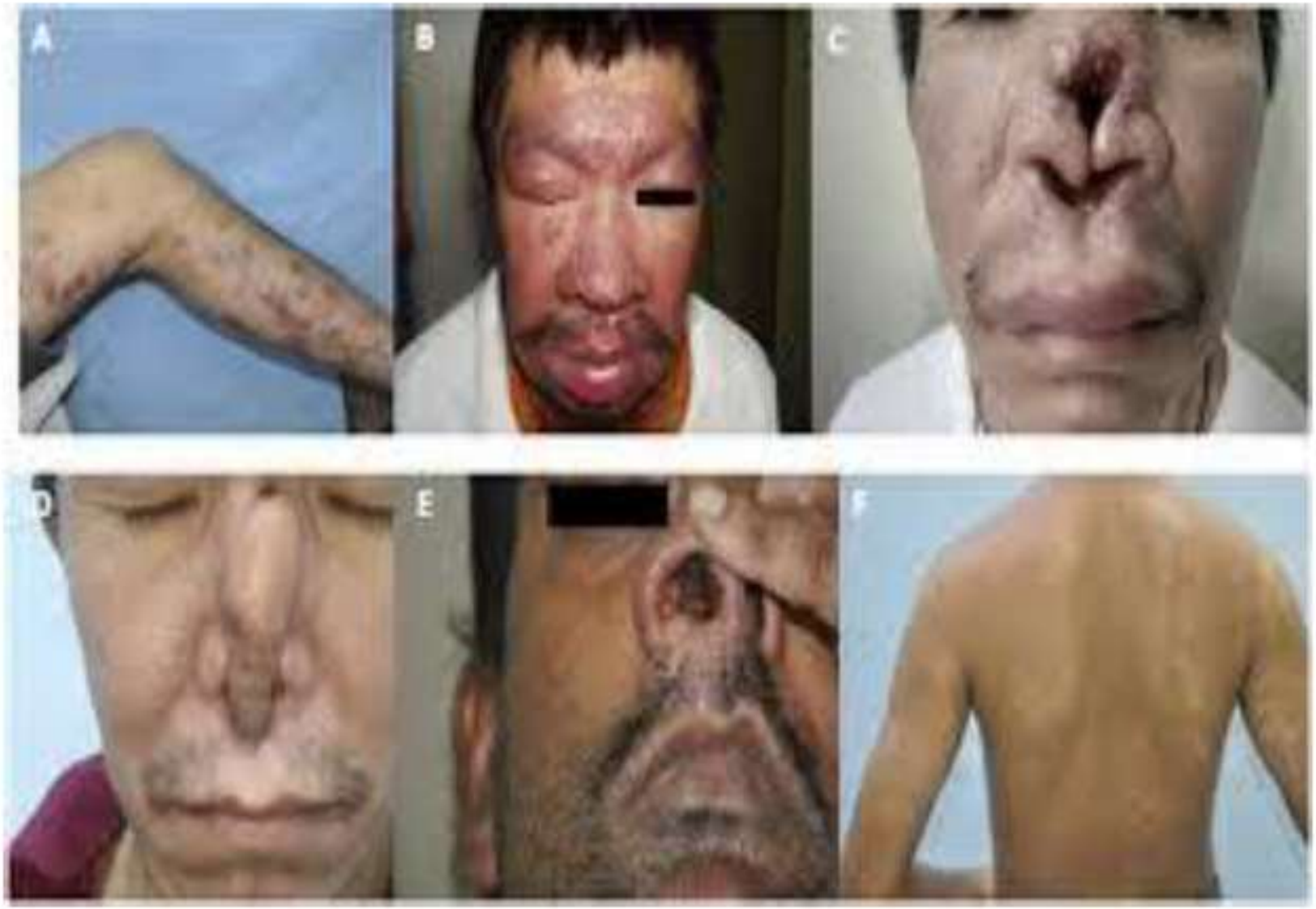
Life Cycle

The life cycle of Leishmania species causing the new world cutaneous and mucocutaneous leishmaniasis is similar to that of *L. donovani* except:

- Amastigotes are found in the reticuloendothelial cells and lymphoid tissues of skin, but not in the internal organs.
- The infection is transmitted to man from animals by bite of sandfly vectors of genus Lutzomiya.
- Direct transmission and autoinfection also occurs man to-man.

Clinical Features

- *L. mexicana* complex leads to cutaneous leishmaniasis which closely resembles the old world cutaneous leishmaniasis. However a specific lesion of caused by *L. mexicana* is chiclero ulcer which is characterized by ulcerations in pinna.
- Chiclero ulcer is also called as self healing sore of Mexico.
- *L. brazilensis* complex causes both mucocutaneous leishmaniasis and cutaneous leishmaniasis.
- *L. braziliensis* causes the most severe and destructive form of cutaneous lesion. It involves the nose, mouth, and larynx.
- The patient experiences a nodule at the site of sandfly bite with symptoms consistent with oriental sore.
- Subsequent mucocutaneous involvement leads to nodules inside the nose, perforation of the nasal septum, and enlargement of the nose and lips (espundia).
- If the larynx is involved, the voice changes as well.
- Ulcerated lesions may lead to scarring and tissue destruction that can be disfiguring.
- The disease occurs predominantly in Bolivia, Brazil, and Peru.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