Ministry of Higher Education

And Scientific Research

University of Baghdad

College of Science

Department of Biology

Theoretical Microbial Physiology

المرحلة الثالثة- الدراستين الصباحية والمسائية

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LEC 1

Microbial cell:

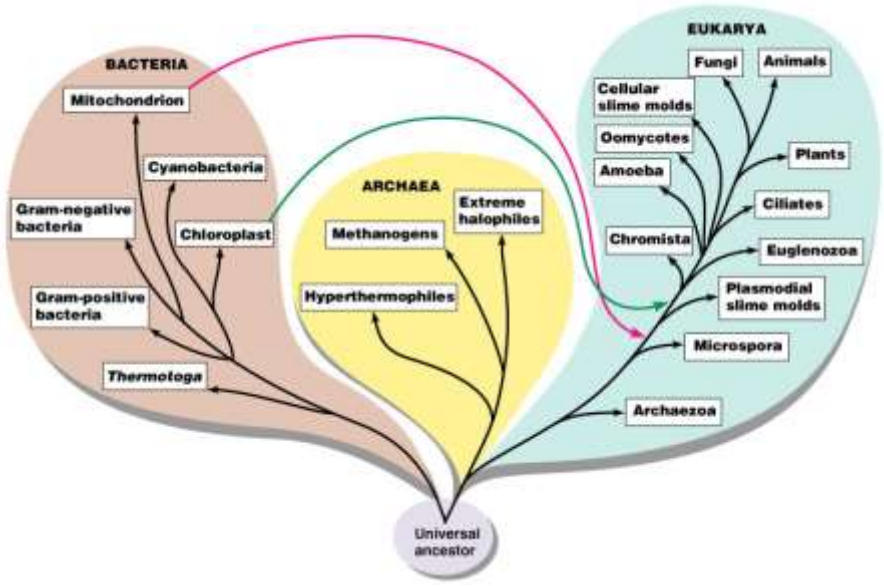
The distinction between **prokaryotes** and **eukaryotes** is considered to be the most important distinction among groups of organisms. Eukaryotic cells contain membrane-bound organelles, such as the nucleus, while prokaryotic cells do not. Differences in cellular structure of prokaryotes and eukaryotes include the presence of mitochondria and chloroplasts, the cell wall, and the structure of chromosomal DNA.

Prokaryotes were the only form of life on Earth for millions of years until more complicated eukaryotic cells came into being through the process of evolution.

Characters	Eukaryote	Prokaryote
Nucleus	Present	Absent
Number of chromosomes	More than one	One--but not true chromosome: Plasmids
Cell Type	Usually multicellular	Usually unicellular (some cyanobacteria may be multicellular)
True Membrane bound nucleus	Present	Absent
Example	Animals and Plants	Bacteria and Archaea
Genetic Recombination	Meiosis and fusion of gametes	Partial, un-directional transfers DNA
Lysosomes and peroxisomes	Present	Absent
Microtubules	Present	Absent or rare
Endoplasmic reticulum	Present	Absent
Mitochondria	Present	Absent
Cytoskeleton	Present	May be absent
DNA wrapping on proteins.	Eukaryotes wrap their DNA around proteins called histones.	Multiple proteins act together to fold and condense prokaryotic DNA.
Ribosomes	Larger	Smaller
Vesicles	Present	Present
Golgi apparatus	Present	Absent
Chloroplasts	Present (in plants)	Absent; chlorophyll scattered in the cytoplasm

Flagella	Microscopic in size; membrane bound; usually arranged as nine doublets surrounding two singlets	Submicroscopic in size, composed of only one fiber
Permeability of Nuclear Membrane	Selective	not present
Plasma membrane with steroid	Yes	Usually no
Cell wall	Only in plant cells and fungi (chemically simpler)	Usually chemically complexed
Vacuoles	Present	Present
Cell size	10-100um	1-10um

Phylogenetic Tree of Life (3 Domains)



Difference between Eubacteria and Archaebacteria

Monera can be classified into three major groups: the eubacteria (True bacteria), cyanobacteria (blue green algae) and archaebacteria (ancient bacteria). The eubacteria are the commonly encountered bacteria in soil, water and living in or on larger organisms, and include the Gram positive and Gram negative bacteria.

The archaebacteria grow in unusual environments such as salt brines, hot springs and in the ocean depths. They are a group of most primitive prokaryotes which are believed to

have evolved immediately after the evolution of the first life. They are of three types:- methanogens, Halophiles and thermoacidophilies.

No	Character	Eubacteria	Archaebacteria
1.	Habitat	Present everywhere, both harmful and help relationship with human.	Mostly inhabit in extreme environmental conditions, no infect human.
2	Cell wall	Peptidoglycan with muramic acid.	Variety of types, no muramic acid.
3	Membrane lipids	Ester linked, straight - chained fatty acids are present containing L-glycerol phosphate.	Ether linked branched aliphatic chains are present containing D-glycerol phosphate.
4	tRNA	Thymine present in most tRNAs N-formylmethionine (f met) carried by initiator tRNA	No thymine in TΨC arm of tRNA methionine (met) carried by initiator tRNA
5	Intron	Introns are absent	Introns are present
6	Antibiotic sensitivity	Yes	No

Characteristic	Archaea	Bacteria	Eukarya
Membrane lipids with branched hydrocarbons	✔		
Chromosomes are circular	✔	✔	
Lacks nuclear envelopes	✔	✔	
Lacks membrane bound organelles	✔	✔	
Methionine is the initiator amino acid for protein synthesis	✔		✔
Lack peptidoglycan in the cell wall	✔		✔
Growth not inhibited by streptomycin and chloramphenicol	✔		✔
Histones are associated with DNA	✔		✔
Contains several types of RNA polymerase	✔		✔

Microbial cell Morphology and Fine Structure

A- Morphology of microbial cells:

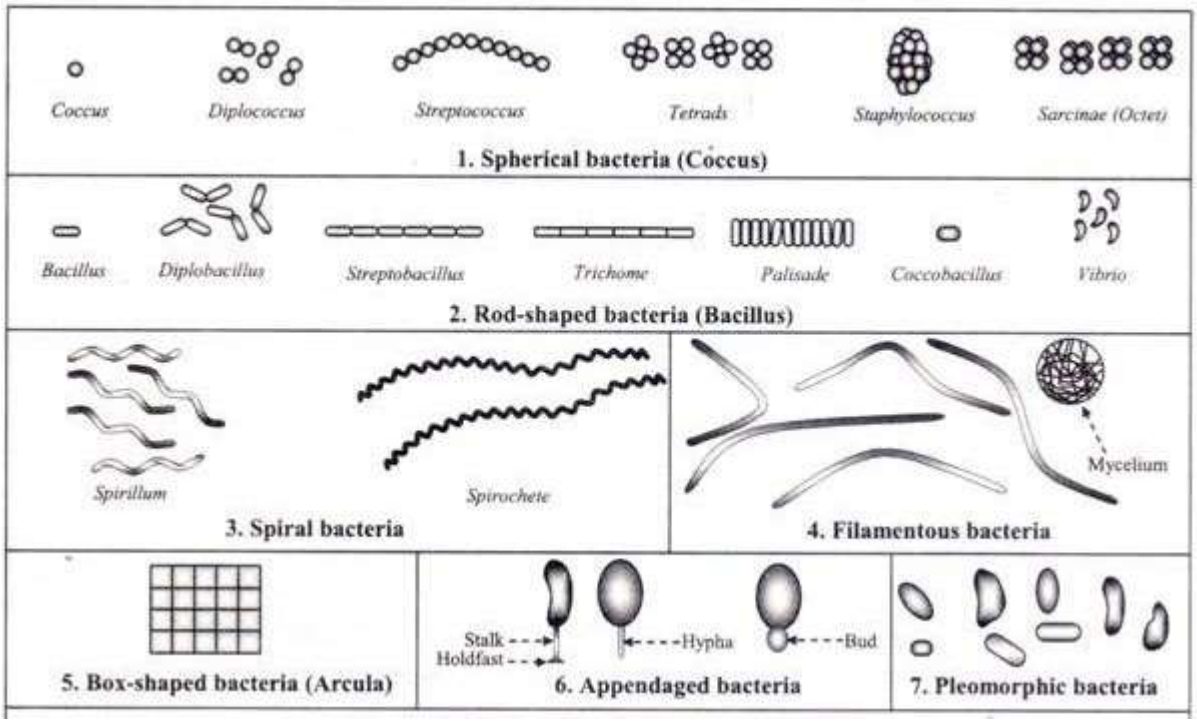
Morphology includes the size, shape and arrangement of microbial cell. However, these features vary with species of microorganisms.

1- Size, Shape and Arrangement of Bacterial Cells:

The three basic bacterial shapes are coccus (spherical), bacillus (rod-shaped), and spiral (twisted), however pleomorphic bacteria can assume several shapes.

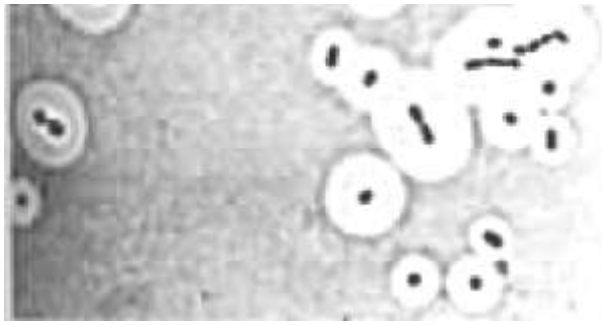
- **Cocci** (or coccus for a single cell) are round cells, sometimes slightly flattened when they are adjacent to one another.
- **Bacilli** (or bacillus for a single cell) are rod-shaped bacteria.
- **Spirilla** (or spirillum for a single cell) are curved bacteria which can range from a gently curved shape to a corkscrew-like spiral. Many spirilla are rigid and capable of movement. A special group of spirilla known as spirochetes are long, slender, and flexible.

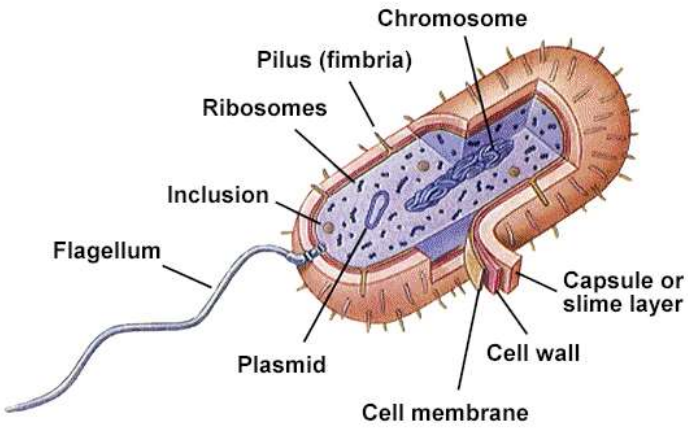
Average size of bacteria is 0.5 -2.0 um in diameter. Shapes of Bacteria are Coccus (Chain = Streptoccus, Cluster = Staphylococcus), Bacillus (Chain = Streptobacillus), Coccobacillus, Vibrio = curved, Spirillum, Spirochete, Square.



B- Structure of Microbial cell

1- Capsule or Slime Layer: Some bacterial cells produce a capsule or a slime layer called **Glycocalyx** of material external to the cell. Capsules are composed of either polysaccharides or polymers of amino acids called polypeptides. The capsule of *Streptococcus pneumoniae* type III is composed of glucose and glucuronic acid in alternating β-1, 3- and β-1, 4- linkages. This capsular, is responsible for the virulence of the pneumococcus, *Bacillus anthracis*, which is a virulence factor for this organism, adhere bacteria to surface (S. mutans and enamel of teeth), prevents Phagocytosis (Complement can't penetrate sugars), avoid the killing effects of lysosomal enzymes, and serve as antigenic determinants.. **(K-antigen)**





2- Flagella: Many microorganisms are motile—that is, able to move from place to place in a concerted manner—especially in an aqueous environment. In the case of bacteria, this motility is accomplished by means of simple strands of protein (flagellin) woven into helical organelles called flagella. The bacterial flagellum is attached at the cell surface by means of a basal body. The basal body contains a motor that turns the flagellum, which propels the organism through the liquid environment.

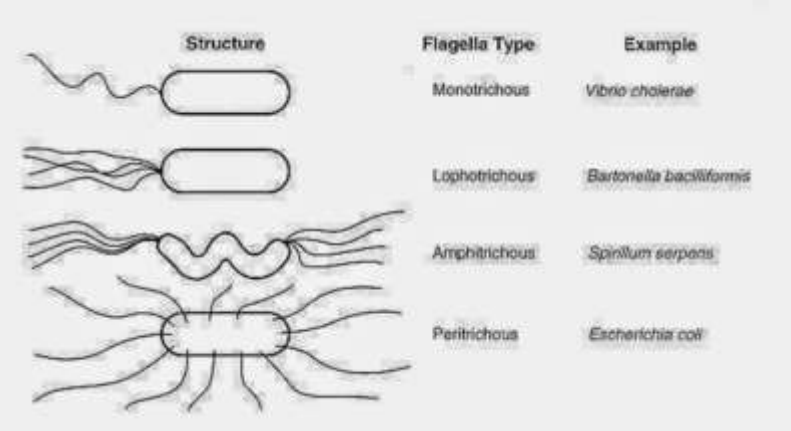
Spirochaetial movement is show several types of movements such as flexing, spinning, swarming (Proteus species) and creeping as they are flexible and helical bacteria lack flagella. Periplasmic flagella or axial fibrils or endoflagella cause the rotation of the opposite direction.

Gliding movement: such as species of cyanobacteria (cytophaga) and mycoplasma in contact with a solid surface.

Chemotaxis: movement of bacteria towards chemical attraction and away from chemical repellants.

Arrangement basis for classification

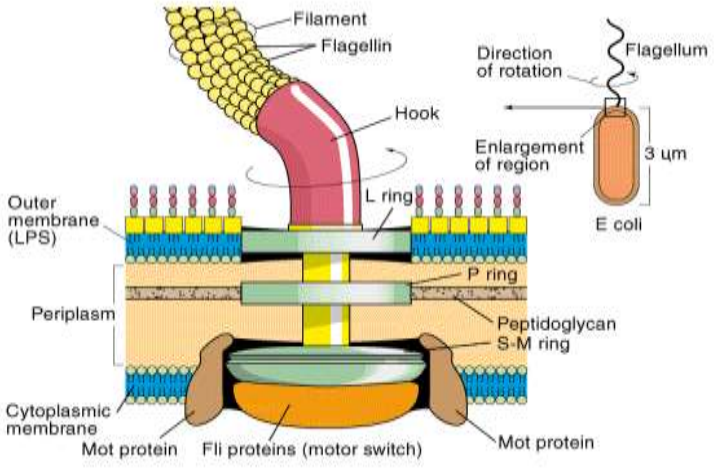
- **Atrichous:** lack the flagella ex: Lactobacillus.
- **Monotrichous**; is one flagella (Vibrio) or more one (Cephalotrichous: Pseudomonas)
- **Lophotrichous**; tuft at one end
- **Amphitrichous**; both ends
- **Peritrichous**; all around bacteria like Proteus vulgaris



antigenic determinants (H-antigen), observed during bacterial infection.

Structure of Flagella

The bacterial flagellum is made up of the protein flagellin. Its shape is a 20-nanometer-thick hollow tube. It is helical and has a sharp bend just outside the outer membrane; this "**hook**" allows the axis of the helix to point directly away from the cell. A shaft runs between the hook and the **basal body**, passing through protein rings in the cell's membrane that act as bearings. Gram-positive organisms have two of



these basal body rings, one in the peptidoglycan layer and one in the plasma membrane. Gram-negative organisms have four such rings: the L ring associates with the lipopolysaccharides, the P ring associates with peptidoglycan layer, the M ring is embedded in the plasma membrane, and the S ring is directly attached to the plasma membrane. The filament ends with a capping protein.

The basal body is anchored in the cytoplasmic membrane and cell wall. There are presences of *rings* which are surrounded by a pair of proteins called **Mot.** These proteins actually drive the flagellar motor causing rotation of the filament. Another set of proteins called **Fli proteins** in *hair* function as the motor switch, reversing rotation of the flagella in response to intra-cellular signals.

3- Pili or Fimbriae. Many bacteria possess external structures (protein) that are shorter and more rigid than flagella. These structures have been termed pili (from Latin meaning "hair") or fimbriae (from Latin meaning "fringe"). These appendages also appear to arise from a basal body or granule located either within the cytoplasmic membrane or in the cytoplasm immediately beneath the membrane. Generalized or common pili play a role in cellular adhesion to surfaces or to host cells/colonization & antigenic determinants, while the sex pili which join the other bacterial cell for transfer of genome.

Some of the differences between fimbriae and pili are as follows:

No	**Characteristics**	**Fimbriae**	**Pili**
1	Definition	Fimbriae are tiny bristle-like fibers arising from the surface of bacterial cells.	Pili are hair like microfibers that are thick tubular structure made up of pilin.
2	Length	Shorter than pili	Longer than fimbriae.
3	Diameter	Thin	Thicker than fimbriae.
4	Number	No. of fimbriae are 200-400 per cell.	No of pili is less1-10 per cell.
5	Made up of	Fimbrillin protein.	Pilin protein.
6	Rigidity	Less rigid.	More rigid than fimbriae.
7	Found in	Both gram positive and gram negative bacteria.	Only gram negative bacteria.

8	Formation	Is governed by bacterial genes in the nucleoid region.	Is governed by plasmid genes.
9	Function	- Responsible for cell to surface attachment. Specialized for attachment that enables the cell to adhere the surfaces of other bacteria; and agglutinate the blood cells (Antigenic properties). Also, metabolic activity: fim+ cells possess higher rate of metabolic activity than the fim-cells. - Do not function in active motility. - No receptors of other.	- Responsible for bacterial conjugation (F-pili) to exchange of genetic information from F+ to F-. Two basic functions of pili. They are gene transfer and attachment. - Type IV pili shows twitching type of motility. - Serve as receptor for certain viruses.
10	Examples	*Salmonella typhimurium, Shigella dysenteriae.* *Shigella dysenteriae* uses its fimbriae to attach to the intestine and then produces a toxin that causes diarrhea.	*Escherichia coli, Neisseria gonorrhoeae.* *Neisseria gonorrhoeae*, the cause of gonorrhea, uses pili to attach to the urogenital and cervical epithelium when it causes disease.

LEC 2

4- S-layers

They are Proteins form the outer most cell envelope component of a broad spectrum of bacteria and archaea. The well-defined arrangement of functional groups on S-layer lattices allows the binding of molecules and particles in defined regular arrays. S-layers also represent templates for the formation of inorganic nanocrystal super lattices composed of CdS, Au, Ni, Pt or Pd.

The S-layer may protect bacteria from harmful enzymes or changes in pH. It may contribute to virulence by protecting the bacterium against complement attack and phagocytosis. The S-layer can function as an adhesion, enabling the bacterium to adhere to host cell membranes and environmental surfaces in order to colonize. Many of the cell-associated protein adhesions used by pathogens are components of the S-layer.

5- Cell Wall (outer membrane)

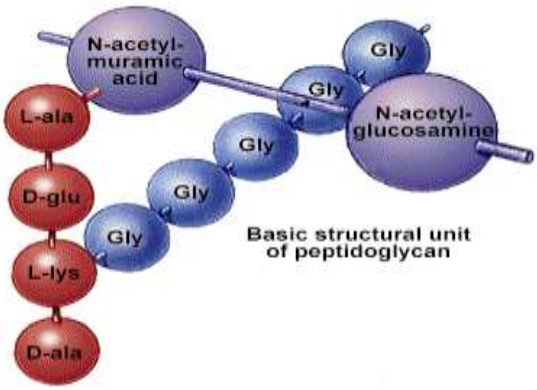
Layers of the cell envelope lying between the cytoplasmic membrane and capsule are the 'cell wall'. It is external to cytoplasmic membrane. A bacterial cell wall is prokaryotic. Mycoplasmas have no cell wall.

Functions: The cell wall is mostly rigid but also exhibit tensile strength. The strength depends on peptidoglycan, murein or mucopeptide which is the main component. The cell wall maintains the bacterial shape and protects the cell from lysis. The cell wall plays an important part in cell division. During cell division, a transverse partition is formed from the cell wall by its inward growth. Many antibacterial agents inhibit cell wall synthesis. Differential Gram-staining character leading to classification into Gram-positive and Gram-negative bacteria reside in the cell-wall. Peptidoglycan layer is common component, others are special components.

- Bacterial cell wall contains a special polymer called Peptidoglycan. Its basic structure is a carbohydrate backbone of alternating units of N-acetyl glucosamine and N-acetyl muramic acid.

- These are cross-linked with oligopeptides contain both D- and L-amino acids.

- Teichoic acid-Lipoteichoic acids: found **only in Gram-positive** bacteria, composed of Glycerol,



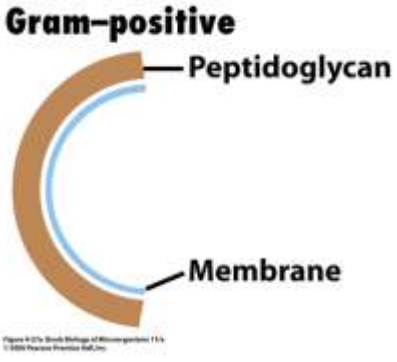
Phosphates, & Ribitol, play role in attachment for Phages; in growth of bacterial cell by regulating the activity of enzyme autolysin; acid prevent the extensive breakdown and they also store phosphorus.

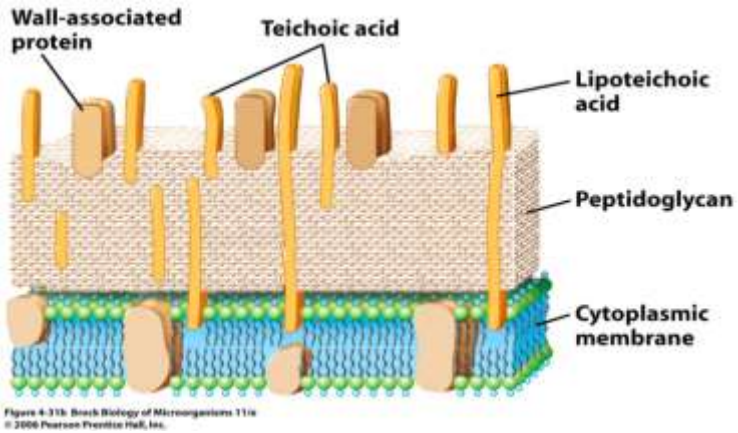
- Lipopolysaccharides: Lipopolysaccharides (LPS) found **only in Gram-negative bacteria.**

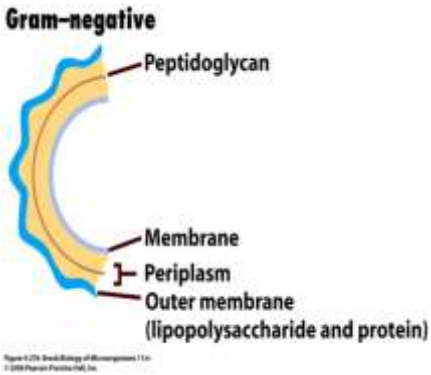
A- Gram-positive bacteria have a thick layer of peptidoglycan, many sheets external to the cytoplasmic membrane like Lipoteichoic acids, stained blue, *Staphyloccocus*, Streptocooci, *Bacillus*. (Protoplasts-L-form: Lysozyme affects which loss Most Cell wall).

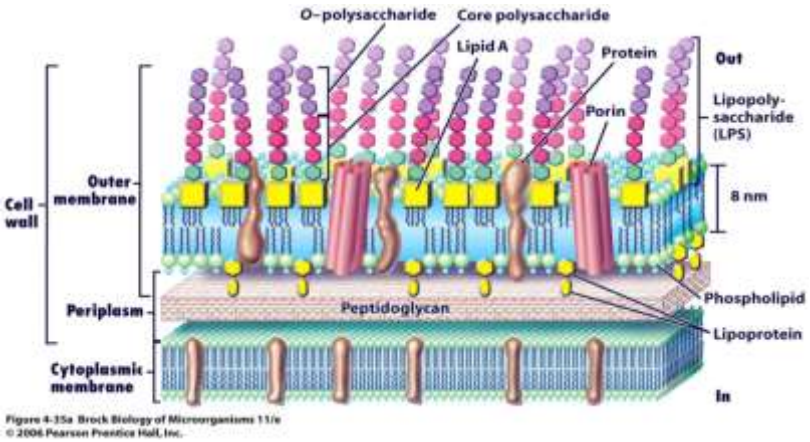
B- Gram-negative bacteria contain lipopolysaccharide (LPS) attached to the outer membrane... source of the O-antigen and endotoxin reaction, stained purpel/Red. Enteric bacteria group, *Esch. coli*, *Klebsiella*, *Salmmonella*, *Pseudomonas*, (*Spheroplasts)

- **LPS** structures are composed of **lipid A**, which binds to the outer membrane. Endotoxic portion of the molecule; Causing Toxic Shock, High Fever, Sepsis
- The **polysaccharide** moiety appears on the cell surface, serving as an antigenic determinant **O antigen**- Host cells develop during bacterial infection (Anti-O AB)
- **Matrix protein**: outer membrane responsible for transport of ions, nutrients and waste across the membrane. Control the cell plasma contents, also act as receptor sites for bacteriophages and bacteriocins.









A typical cell wall

A few bacteria are able to live or exist without a cell wall. The mycoplasmas are a group of bacteria that lack a cell wall. Mycoplasmas have sterol-like molecules incorporated into their membranes and they are usually inhabitants of osmotically-protected environments. *Mycoplasma pneumoniae* is the cause of primary atypical bacterial pneumonia, known in the vernacular as "walking pneumonia". For obvious reasons, penicillin is ineffective in treatment of this type of pneumonia. Sometimes, under the pressure of antibiotic therapy, pathogenic bacteria can revert to cell wall-less forms (called **spheroplasts** or **protoplasts**) and persist or survive in osmotically-protected tissues. When the antibiotic is withdrawn from therapy the organisms may regrow their cell walls and reinfect unprotected tissues.

L –forms are cell wall deficient forms of bacteria. They develop spontaneously or in the presence of penicillin, lysozyme or other agents that interfere with cell wall synthesis. They require a solid medium with right osmotic strength. They were named after the name Lister Institute of London, where discovered .Clinical significance: Latent infection / chronic infection, and resistance to cell wall active agents.

Cytoplasmic Membrane

The cytoplasmic membrane is a highly selective permeability barrier constructed of lipids (lipid matrix responsible for fluidity) and proteins that forms a bilayer with

hydrophilic exteriors and a hydrophobic interior. The major function of the cytoplasmic membrane is to act as a permeability barrier, preventing leakage of cytoplasmic metabolites into the environment, transport of nutrients into and out of the cell. Its lack the sterols except in mycoplasma.

Function of plasma membrane:

1- Transport nutrients by permease.

2- It consists of enzymes of biosynthetic pathways, such as peptidoglycan, LPS…etc.

3- Attachment site for bacterial chromosome and plasmid DNA.

4- Inner membrane invaginates to form mesosomes that site for respiratory activity.

5- It provides permeability barrier, thus prevent the escape of cellular materials outside the cell, and show selective permeability.

6- Cytoplasmic Granules or Inclusions

Poly β hydroxyl-butyrate (PHB): source of carbon and energy, it have single-layered membrane.

Inorganic granules include Sulfer granules (photosynthesis bacteria) and Meta chromatic granules or polyphosphate, volutin granules in *Corynebacterium* and *Mycobacterium* (source of building blocks for nucleic acid and ATP).

Lipid inclusion: source for energy of *Bacillus, Mycobacterium, Spirillum*.

Magnetosomes: found in magnetotactic bacteria.

Gas vesicles: found in floating forms prokaryote in lakes and sea. These vesicles not found in flagellar microorganisms, and provide buoyancy and keep the cell floating form.

8- Ribosomes

The cytoplasm of all cells has a fine granular appearance observed in many electron micrographs. Tiny particles called ribosomes are responsible for this look. Ribosomes contain approximately 65% RNA and 35% protein. The ribosome orchestrates the

polymerization of amino acids into proteins (i.e., protein synthesis). At higher magnification under the electron microscope the ribosome particles are spherical. In properly prepared specimens the ribosomes are observed as collections or chains held together on a single messenger RNA (mRNA) molecule and are referred to as **polyribosomes** or simply polysomes. The more or less spherical ribosome particle, when examined by sucrose gradient sedimentation, has been found to have a sved berg coefficient of 70S. (A Svedberg unit denotes the rate of sedimentation of a macromolecule in a centrifugal field and is related to the molecular size of that macromolecule.) The prokaryotic ribosome may be separated into two lower-molecular-weight components: one of 50S and another of 30S. Only the complete 70S particle functions in polypeptide synthesis. By comparison, the ribosomes of eukaryotic cells are associated with the endoplasmic reticulum, are larger (80S), and are composed of 40S and 60S subunits. The function of both 70S and 80S ribosomes in protein synthesis is identical. Curiously, eukaryotic mitochondria characteristically display 70S ribosomes—not the 80s particles that you would expect—because mitochondria probably evolved from endosymbiotic prokaryotic cells, a hypothesis supported by extensive analyses comparing bacterial and mitochondrial genomes.

9- Mesosomes

It is folded membranous elaborations (could be in the form of vesicles, tubules, lamellae) that occur irregularly at the inward side of the cell membrane of the prokaryotic organisms like certain cyanobacteria, nitrogen-fixing bacteria (e.g *Azotobacter*), sporulating bacteria, etc. It is to be noted here that not all the Prokaryotic cells have mesosomes.

The major function of mesosomes is to increase the surface area of the plasma membrane. This drastic increase in the surface area of the membrane mainly helps the cell to carry out cellular respiration more efficiently. Also, it thought to aid in photosynthesis, cell secretions/enzymes, electron transport, cell division, cell wall formation, DNA replication. Though not separate or independent cell structures, Mesosomes can be chemically induced in bacteria.

Mesosomes are of two types:

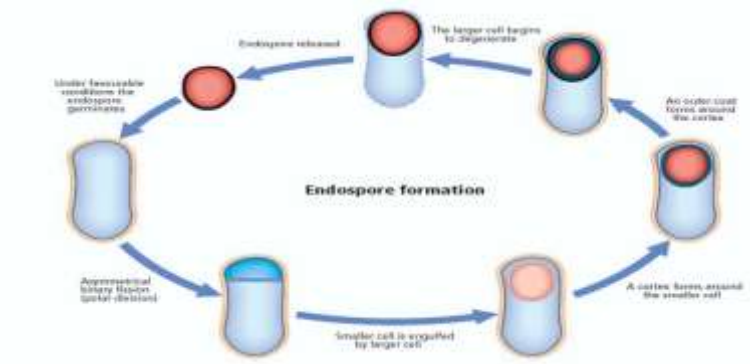
1. **Septal** mesosomes that extend from the plasma membrane towards the centre in the cell cytoplasm and are associated with nuclear material)

2. **Lateral** mesosomes that are located at the periphery and need not be associated with nucleus.

10- Endospore Formation

The process of sporulation begins when vegetative (actively growing cells) exhaust their source of nutrients; begin of forming endospores, common in nature. Spore forming Bacteria are very resistant to lysozyme, heat, radiation, drying and can remain dormant for hundreds of years in nature. Once conditions are again favorable for growth, the spores can germinate and return to the vegetative state. Aerobic Bacillus group & Anaerobic Clostridium develop Endospore formation. Both are widely distributed in nature intestinal -human and animals.

The cortex contains dipicolinic acid (DCA) connection with Ca ions calicium dipicolinate

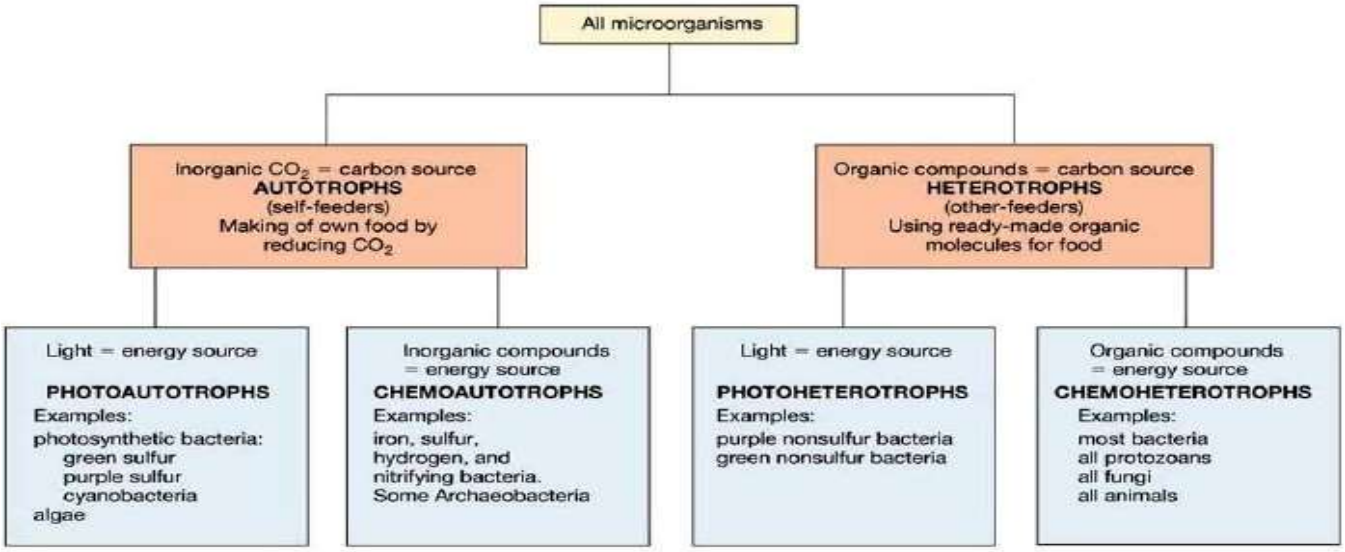


that to form (15%).

LEC 3

Requirements for bacterial growth

1- Microbial Nutrition & Energy



Microorganisms **need to acquire energy** in order to survive because:

1- To maintain the structural integrity of the cell by repairing any damage to its constituents.

2- To synthesis new cellular components such as nucleic acids, polysaccharides and enzymes.

3- To transport certain substances into the cell from its surroundings.

4- For the cell to grow and multiply.

Major* Nutritional Types of Microorganisms

Major Nutritional Types	Energy source	Hydrogen/ electron	carbon source	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy) (photoautotrophs)	Light energy	Inorganic hydrogen/electron (H/e–) donor	CO_2 carbon source	Algae Purple and green sulfur bacteria Cyanobacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy) (Photoheterotrophs)	Light energy	Organic H/e– donor	Organic carbon source	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy) (Chemoautotrophs)	Chemical energy source (inorganic)	Inorganic H/e– donor	CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy) (Chemoheterotrophs)	Chemical energy source (organic)	Organic H/e– donor	Organic carbon source	Protozoa, Fungi, Most nonphotosynthetic bacteria (including most pathogens)

- **Saprophytic bacteria**/ Nonpathogenic that take energy by fermentation/respiration, found in nature in decaying material of soil, water, vegetation and circulation of minerals.

A- Major Elements of nutrient

At an elementary level, the nutritional requirements of a bacterium such as E. coli are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn (required in large amount and lack of which can limit growth). These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells.

Table: Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material

Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes (Nitrogen: can be limiting, nitrogen-fixing bacteria (*Rhizobium*) very important in food chain.
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S^o, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain non heme iron-proteins and a cofactor for some enzymatic reactions (Iron, may be limiting factor in bacterial survival)

B- Minor Elements of nutrient (Trace Elements)

In addition to the four major elements (CNPS) the microorganisms also need a large number of metals in trace quantities. These trace elements are known to be essential for functioning of various enzymes in the microbial cells. Apart from the trace elements the microbes may also need some growth factors (vitamins) which cannot be synthesized from single carbon or nitrogen sources and which must be supplied in small amount to these organisms to allow their proper growth and development. Trace elements: potassium, magnesium, calcium, iron, copper, molybdenum, and zinc are needed as **cofactors for enzymes**.

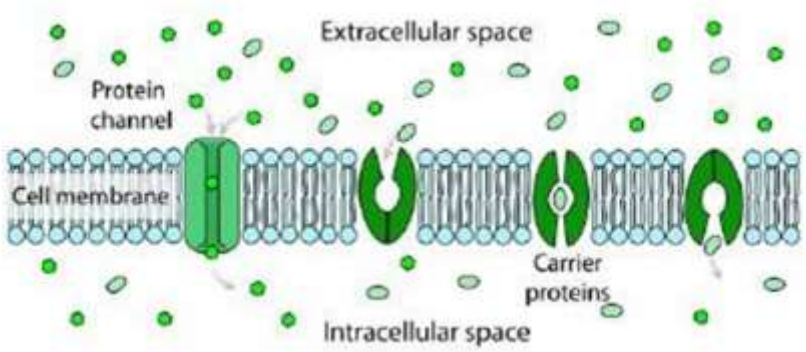
- There are Two types of trace elements:

1- Essential growth factors - purines or pyrimidines or amino acids required after mutation of any bacteria

2- Accessory growth factors – X and V factors for the good growth of H. influenzae.

Up take of nutrients by the microbial cell

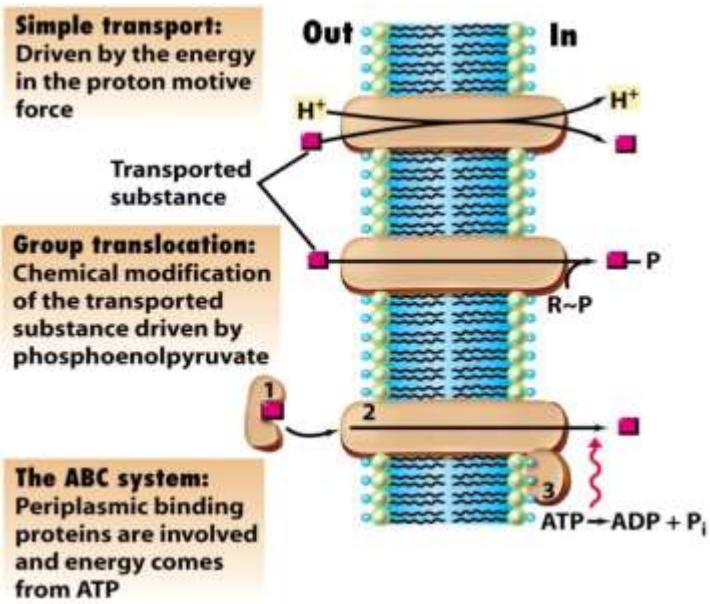
I- Passive transport: (Permeability absorption- most microorganisms): is a movement of molecules from a more crowded (high conc.) to a less crowded (low conc.) area without the use of energy.



- Simple diffusion: very small molecules ex: glycerol, H_2O, O_2 and CO_2.

- Facilitated diffusion: using carrier molecules called permease embedded in the plasma membrane. Each permease is specific for the particular molecule being transported, increases with the concentration gradient much more rapidly. The carrier subsequently changes back to its original shape and is ready to pick up another molecule (process reversible).

II- Active transport: is a movement of molecules from a less crowded (low conc.) to a more crowded (high conc.) area with the use of energy.



- Membrane bound transport systems (simple)

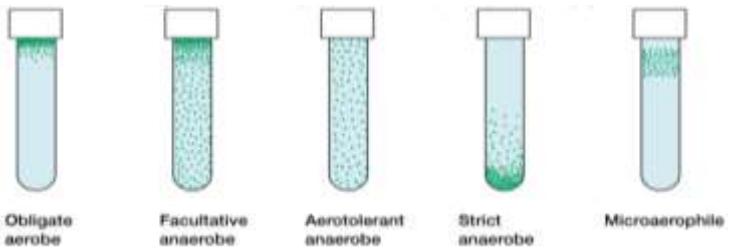
- Group translocation: sugar outside cell membrane with Phosphoenolpyruvate and Transferase enzyme System (PTS) due to sugar-phosphate and pyruvate (inside).

- Binding protein transport system (ABC): carrying the solute across the space binding protein utilizes ATP.

III- Iron uptake: is difficult by extreme insolubility, transport by siderophores. There are two types of siderophore either hydroxamates (ferrichrome) or phenolates-catecholates (enterobactin). Fe+3 ⟶ Fe+2

IV- Endocytosis

2- Oxygen



Most of the microorganisms need molecular oxygen for respiration. In these, the oxygen serves as terminal electron acceptor and, such organisms are referred to as '**obligate aerobes'** (*M. tuberculosis*, *P.aeruginosa)*. As opposed to this there are a few organisms, which do not use molecular oxygen as terminal electron acceptor. These microbes are called '**obligate anaerobes'** (Mostly found in intestinal tract (95-99%)- Clostridia, Mouth &Vagina(90%)) lack both catalase and superoxide diamutase. Aerobes, which can grow in the absence of oxygen, are called '**facultative anaerobes' (**Most human pathogens & normal flora.. Staphylococci, streptococci, E.coli) and the anaerobes which can grow in the presence of oxygen are referred to as '**facultative aerobes (aerotolerant anaerobic)** like *Lactobacillus plantarum* and *Peptococcus anaerobius* (do not contain catalase or superoxide diamutase) as a result of their ability to produce high levels of an

enzyme (NADH oxidase) that reduce oxygen to water according to the reaction. In addition to these major classes, there are organisms, which grow best at reduced oxygen pressure but are obligate aerobes and these are called '**Microaerophilic'** (Neisseria spp).

- Several toxic forms of oxygen can be formed in the cell as the result of respiration, but enzymes are present that can neutralize most of them. Hydrogen peroxide is one of those forms that can be neutralized by catalase.

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- The acidity or alkalinity of an environment can greatly affect microbial growth. Organisms that grow best at low pH are called acidophiles (< 5 pH, ex: Lactobacilli); those that grow best at high pH are called alkaliphiles (Vibriod). Most organisms grow best between pH 6 and 7.2 (**Neutrophilic** bacteria: most human-animal commensals & pathogens).
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- **Mesophilic** Bacteria (20-40C)..Most human commensal & pathogens.
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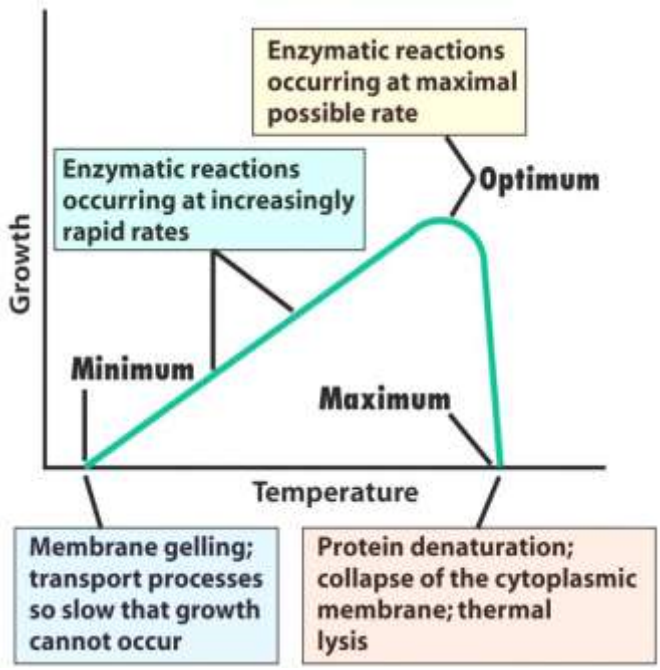
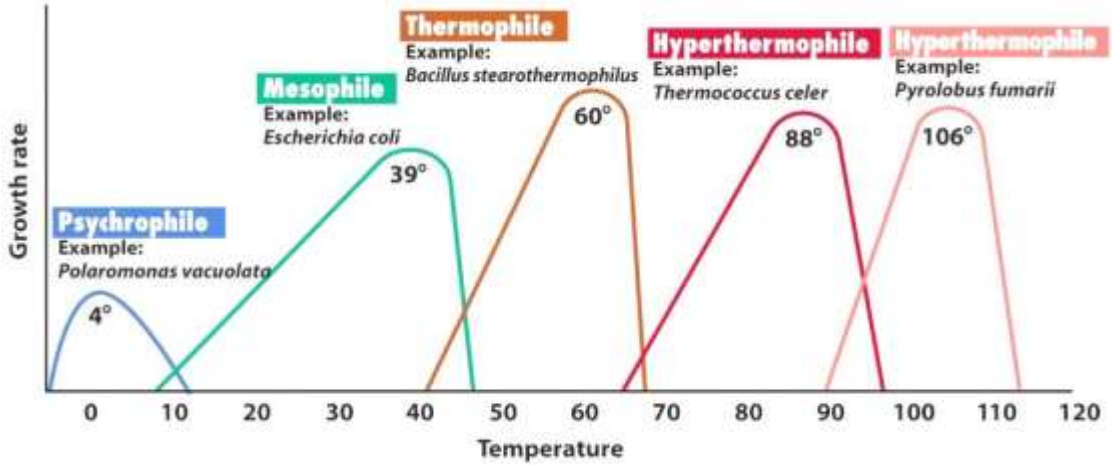
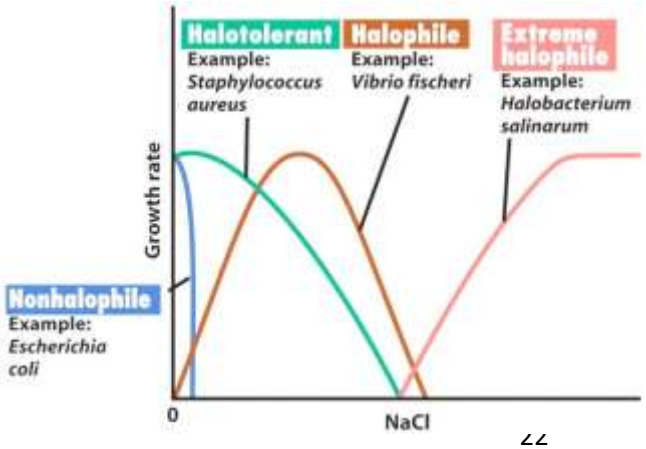


- Microorganisms can be grouped by the temperature ranges they require.



5- Salinity (Ionic strength and Osmotic pressure)

Some microorganisms (halophiles) have evolved to grow best at reduced water potential, and some (extreme halophiles) even require high levels of salts for growth; those requiring high osmotic pressures are called osmophilic. Osmolality is regulated by the active transport of K+ ions into the cell; internal ionic strength is kept constant by



excretion of the positively charged organic polyamine putrescine.

LEC 4

Microbial Cultivation

Cultivation is the process of propagating organisms by providing the proper environmental conditions (nutrient, temperature, pH, oxygen, etc).

Culture Media: Nutrients (carbohydrates & proteins, blood, minerals). Types of media:

- Defined media: exact chemical composition is known (Basal media – Nutrient agar).
- Complex Media: Contain multiple organic nutrients from partial digestion of yeast, beef, soy, or milk. Exact chemical composition not known (our nutrient media are example)
- Selective media: contains substances that favor or inhibit growth of particular class of micro-organisms. For example, Trypticase (TSA media) have no glucose thereby selecting for organisms that can meet their carbon requirements from other sources. Media with antibiotics integrated would be another. Sometimes selective substance can be placed on surface, as with antibiotic disks. Like MacConkeys agar (Bile salts+ Lactose+neutral red dye for Gram-ve bacteria ex: *E.coli*, other enteric bacteria).
- Differential media: Allow us to see visible changes in colonies depending on how they use some element of the media. Use of red blood cells in blood agar is example or SS agar (For Isolation of *Salmonella, Shigella, V. cholera* from stool specimens).
- Anaerobic media: "stab cultures" into any type of agar; or media with reducing agents that eliminate free oxygen

Types of media based on composition (chemical composition)

1- Synthetic

2- Complex media

Types of media based on consistency (Physical nature)

1- Solid or agar media, 2- Liquid or broth media, 3- Semi solid media

Maintenance and Preservation of Pure Cultures

The following points highlight the top four methods used for maintenance and preservation of pure cultures.

1. Refrigeration:

Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped.

Thus their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

2. Paraffin Method:

This is a simple and most economical method of maintaining pure cultures of bacteria and fungi. In this method, sterile liquid paraffin in poured over the slant (slope) of culture and stored upright at room temperature.

The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture is preserved for several years.

3. Cryopreservation:

Cryopreservation (i.e., freezing in liquid nitrogen at-196°C) helps survival of pure cultures for long storage times. In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol that prevent the formation of ice crystals and promote cell survival.

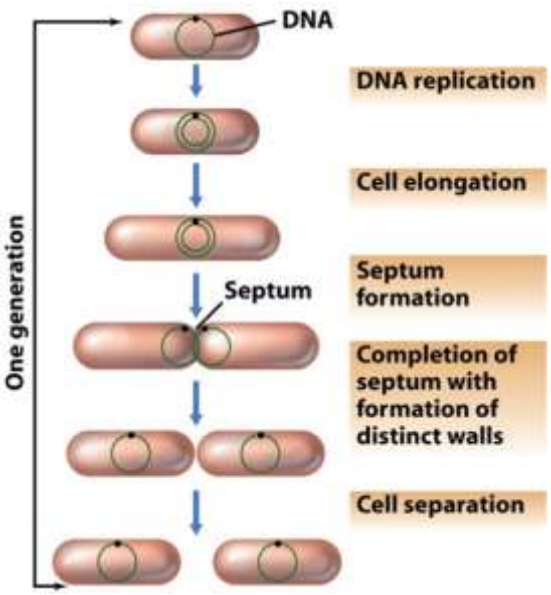
4. Lyophilisation (Freeze-Drying):

In this method, the culture is rapidly frozen at a very low temperature (-70°C) and then dehydrated by vacuum. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years.

Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze- drying method is the most frequently used technique by culture collection centres.

Microbial growth

Growth is an orderly increase in the quantity of cellular constituents. It depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves increase in cell mass and number of ribosome, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division. This asexual process of reproduction is called **binary fission**. The prokaryotic cell cycle: most prokaryotes reproduce by binary fission, although some prokaryotes reproduce by budding, fragmentation, and other means.



A "**batch culture**" is a closed system in broth medium in which no additional nutrient is added after inoculation of the broth. Typically, a batch culture passes through four distinct stages:

- Lag stage, logarithmic (exponential) growth, stationary stage and death stage.

1. Lag Phase (Tooling up). Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity. The **length of the lag phase** is apparently dependent on a wide variety of factors including the nature of media, size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

2. **Exponential (log) Phase**. The exponential phase of growth is a pattern of **balanced growth** wherein all the cells are dividing regularly by binary fission, and are growing

by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation (n = number of generations). If nutrient levels or other environmental conditions change, unbalanced growth results occurred. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached.

Unbalanced growth is readily observed in two types of experiments: **shift-up**, where a culture is transferred from a nutritionally poor medium to a richer one, there is a lag while the cells first construct new ribosomes to enhance their capacity for protein synthesis. This is followed by increases in protein and DNA synthesis. Finally, the expected rise in reproductive rate takes place.

In a **shift-down** experiment, where a culture is transferred from a rich media to a poor one, there a lag in growth because cells need time to make the enzyme required for the biosynthesis of unavailable nutrients. The cell division and DNA replication continue after the shift-down, but net protein and RNA synthesis slow. The cells become smaller and reorganize metabolically until they are able to grow again.

3. **Stationary Phase**. Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or toxic waste products; 3. exhaustion of space, in this case called a lack of "biological space".

There are two alternative hypotheses for cell number constant with loss of their viability:

Starving cells that show an exponential decline in density have reversibly lost their ability to reproduce and the microbes are temporarily unable to grow, these cells called **viable but nonculturable (VBNC)** is thought to be the result of a genetic response triggered in starving stationary phase cells. VBNC microbes could cause a public health threat, as many assays that test for food and drinking water safety are culture-based.

[2]**Programmed cell death** is genetically programmed to survive by a fraction of the microbial population programmed to commit suicide. Dead cell represent the source of nutrients to enable the eventual growth of those cells in the population that did not initiate suicide.

4. **Death Phase**. If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed.). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

A "**continuous culture**" is an open system in which fresh media is continuously added to the culture at a constant rate, and old broth is removed at the same rate.

- Two types of this culture: **chemostats** and **turbidostats**.

1- Chemostats is providing an essential nutrient (amino acid) in limiting quantities at the same rate as media containing microorganisms is removed and the growth rate is determined by the rate which new media is fed into the growth chamber.

2- Turbidostats has a photocell that measures the turbidity of the culture in the growth vessels. The flow rate of media is automatically regulated to maintain cell density.

Character	Turbidostat	Chemostat
Dilution rate	Different	Constant
Culture media	Contain all nutrient in excess	limited
Operator	Best at high dilution rated	Stable and effective at lower dilution rated

Continuous culture systems are very useful because:

- provide a constant supply of cells in exponential phase and growing at a known rate.

- Study of microbial growth at very low nutrient levels close to those present in natural environments.

- Essential for research and food and industrial microbiology.

LEC 5

Environmental Factors Affecting Growth

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, and such factors as pH, temperature, and aeration must be carefully controlled. The microbial growth also is greatly affected by the chemical and physical nature of their surroundings. An understanding of environmental influences aids in the control of microbial growth and the study of the ecological distribution of microorganisms.

1. Nutrients

All microorganisms require the following nutrients to grow, repair themselves, and to replicate:

a- Carbon, nitrogen, sulfur, phosphorus, and other macro mineral nutrients.

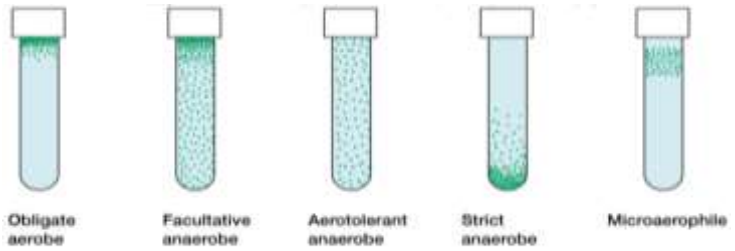
b- Various trace elements (minor elements).

c- In addition, some microorganisms require various as well as additional growth factors (e.g., specific amino acids).

d- Although we are concerned with ways microorganisms satisfy their own nutritional needs, we can note that in satisfying such needs, they also help recycle elements in the environment.

e- Fastidious microorganisms whose nutritional needs are unusually complex are termed fastidious.

2- Oxygen



Most of the microorganisms need molecular oxygen for respiration. In these, the oxygen serves as terminal electron acceptor and, such organisms are referred to as '**obligate aerobes'** (*M. tuberculosis*, *P.aeruginosa)*. As opposed to this there are a few organisms, which do not use molecular oxygen as terminal electron acceptor. These microbes are called '**obligate anaerobes'** (Mostly found in intestinal tract (95-99%)- Clostridia, Mouth

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Oxygen exclusion from culture in obligate anaerobes by: reducing agents such as sodium thioglycolate can be added to liquid cultures, sealed with a layer of petrolatum and paraffin, placed in a container from which the oxygen is removed by chemical means.

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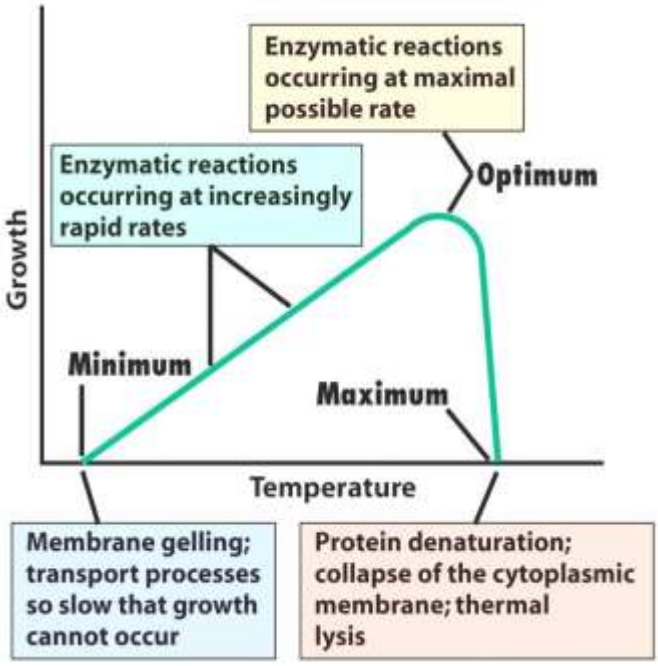

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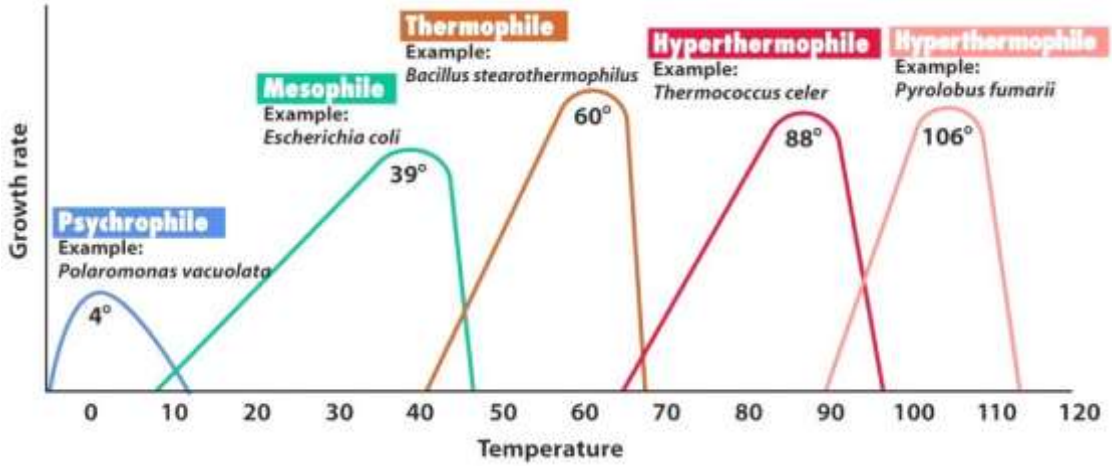

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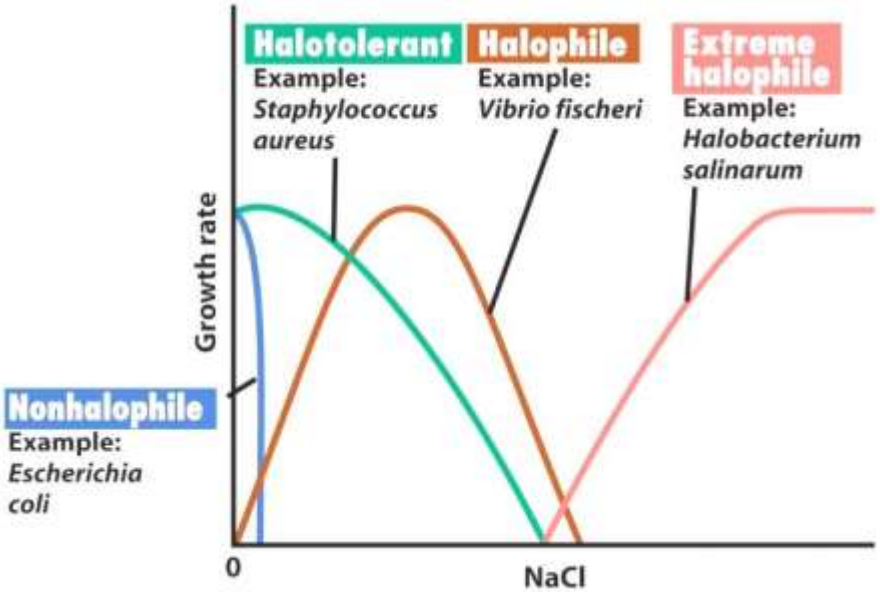

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Microbial Bioenergetics

Metabolism, (from the Greek term *metabollein*), It is the total of all chemical reaction that occur in cell. Metabolisms entails are thousands of different reaction, most of them falls into of two general categories:

Anabolism[Greek, *ana*, up],sometimes also termed as biosynthesis, the reactions that consume energy in order to build large molecules and cellular structures from smaller and simpler one,theyare**endergonic reactions**.It involve a series of stapes **(1)** conversion of the organism's carbon source into aset of small molecules called **precursor metabolites**; **(2)** synthesis of monomers and other building blocks(i.e., amino acids, nucleotides, simple carbohydrates, and simple lipids) from precursor metabolites; **(3)** synthesis of macromolecules (i.e., proteins, nucleic acids, complex carbohydrates, and complex lipids); and **(4)**assembly of macromolecules into cellular structures.

Catabolism [Greek, *cata*, down, and ballein to throw], is the opposite or complement of anabolism that involve the breakdown of relatively large complex organic molecules into smaller and simpler molecules and often release energy, they are**exergonic reactions**

Considerable metabolic diversity exist in the microbial world. However, there are several biochemical principles common to all types of metabolism. These are;**(1)** the use of ATP to store energy captured during exergonic reactions so it is can be used to derive endergonic reaction; **(2)** the organization of metabolic reactions into pathways and cycles;**(3)** the catalysis of metabolic reactions by enzymes; and **(4)** the importance of oxidation – reduction reactions in energy conservation.

Adenosine Triphosphate (Metabolic money)

In what way do cells extract energy from electrons of fuel compounds? To answer this question, we must look more closely to the chief energy carrier and powerhouse molecule**Adenosin triphosphate(ATP)**, which also been described as metabolic money because it can be earned, banked, saved, spend, and exchanged. This molecule provides connection between energy yielding reactions (catabolism) and other cellular activities that require energy. Some clues to its energy storage behavior lie in its unique molecular structure. The molecular structure of **ATP** first deduced by Lohman in 1930 and confirmed by Alexander Todd *et, al*., in 1948, it is three - part molecule, consist of; nitrogen base (adenine) linked to a 5 carbon sugar (ribose), with a chain of three phosphate groups are esterifies to the 5 – position of ribose moiety (**figure – 1**).The two terminal phosphoryl groups are highly energized, when **ATP** was utilize in anabolic processes the phosphoanhydride bonds that link these groups together are break down or hydrolyzes almost completely to produce **adenosine diphosphate (ADP),orthophosphate (Pi),** andlarge amount of energy is released.

Adenosine ~~PPP~~ + ~~H_2O~~Adenosine ~~—PP~~(ADP)+ Pi + ~~energy~~ (- 7.3 Kcal)

Adenosine — P — P+ H_2O ⟶ Adenosine — P (AMP) + Pi + energy (- 7.3Kcal)

Adenosine —P + H_2O ⟶ Adenosine+ P + energy (-3.7 Kcal)

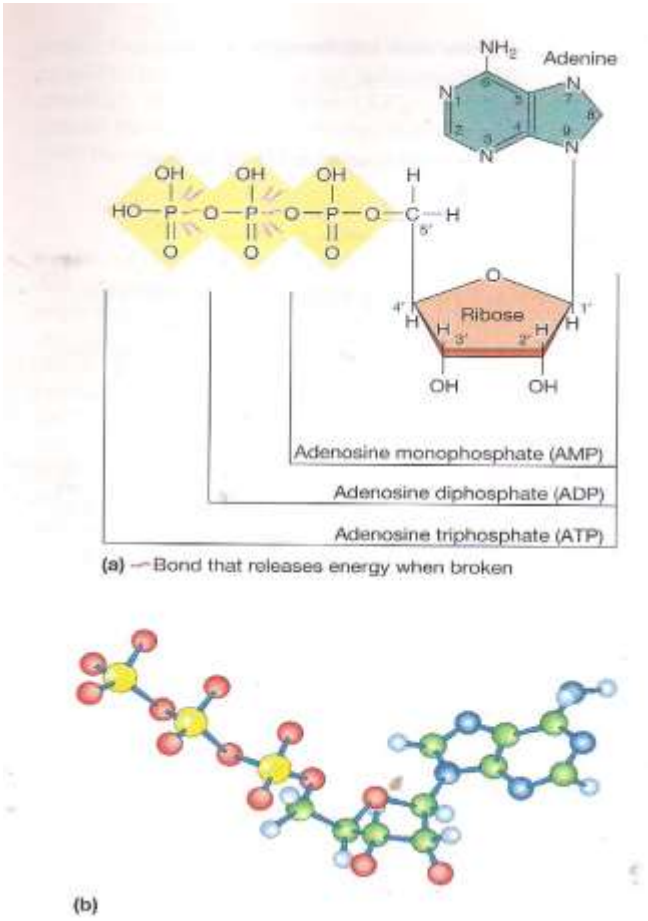


Figure -1: Adenosine Triphosphate and Adenosine Diphosphate

(a) Structure of ATP, ADP, and AMP
(b) A molecular model of ATP

The trapping of energy in form of **ATP** is called **Phosphorylation,** there are three types of phosphorylationsprocesses followed by living cells:

Photophosphorylation: It occurs in the presence of light in photosynthetic cells with assistant of photopigments (chlorophylls and carotenoids),which trap photoenergy from solar radiation that exited and release electrons from photopigments, these electrons pass through a series of electron carriers and generates phosphate bonds in form of **ATP**.

Oxidative phosphorylation: Is the processes in which **ATP** is formed as a result of the transfer of electrons(**e^-**)that collected by certain electron carriers like **NAD**, **NADP**, and **FAD,** and passed into **electron transport chain(ETC),**locatedin the inner membrane of mitochondria in eukaryotic cells and in plasma membrane in prokaryotic cells,finally the **e^-** combined with oxygen, which act as final electron acceptor, the subsequent passage of electrons though electron carriers release energy used to generate ATP from ADP**..** One molecule of **NAD**generates three molecules of **ATP** when it enters the **ETC,** however one molecule of **FAD** generates only two molecules of **ATP**, this is due to the fact that **FAD** enters **ETC** later than **NAD.**

Substrate level phosphotylation: Is a type of metabolic reaction that results in generation of **ATP** or guanosine triphosphate (**GTP**) by direct transfer and donation of a phosphoryl group to ADP or GDP from phosphorylated reactive intermediate, for example:

GTP + ADT ⟶ ATP + GDP

Oxidation – Reduction Reactions

The release of energy from an energy source in biosystemnormally involves biological oxidation, fundamentally is the removal of electrons from various substrates (**electron donor**), which thereby oxidized, and does not necessarily involves oxygen. In living cells the removed **e^-** cannot be remain ina free state there must be immediately transferred to other compounds or molecules (**electron acceptor),** which thereby reduced. So oxidation is the loss of **e^-**, and reduction is gain of **e^-** , an acceptor is oxidizing agent and the donor is reducing agent. By convention such reaction is written with the donor to the right of the acceptor and the number (**n**) of electrons (**e^-**) transferred.

Acceptor + e^- ⟶ donor

Oxidation – reduction are always coupled so termed **redox reaction**. The equilibrium constant for such reaction is called the **standered reduction potential(*E*°)** and is a measure of the tendency of the donor to loss electrons. The reference standered for the reduction potential is the hydrogen system with an***E*°**(the reduction potential at pH 7.0) of – 0.42 volts or – 420 millivolts.

In most cells oxidation of organic substrates is accomplished by removal of hydrogen (**dehydrogenation**), hydrogen atom provides one proton **(H^+)** and one electron **(e^-)**. In living cells enzymes involved in dehydrogenation processes are **dehydrogenase enzymes**, these enzymes like other enzymes are highly specific for their respective substrates, and all of these include coenzymes, which are less restricted; one coenzyme may act in several different other apoenzymes and reactions. Dehydrogenises coenzymes (**electron carriers**), resemble shutter that are alternately loaded and unloaded, repeatedly accepting and releasing e- and H to facilitate the transfer of redox energy. Most of coenzymes transfer both e- and H. The most common electron carriers are:

NAD; nicotinamide adenine dinucleotide phosphate

NADP; nicotinamide adenine dinucleotide phosphate

FAD; flavin adenine dinucleotide

The essential portion of **NAD** and **NADP** is nicotainamide (Niacin, Vitamin B3), the pyridine ring of this group accepts substrates **H** or $\mathbf{e^-}$, being reduced to **NADH** and **NADPH**, the reduced form in turn transfer the accepted hydrogen to second acceptor that is at low energy level and being reoxidizedto **NAD** and **NADP**(**figure -2**).

FAD, contain riboflavin (Vitamin B2), so far all vitamins are analogous part of coenzymes, thus is common to several dehydrogenises, when reduced by substrates hydrogen represented as **FADH**, the coenzyme very commonly reoxidized to **FAD** by transfer of hydrogen to an iron – bearing respiratory pigments of cytochrom system.

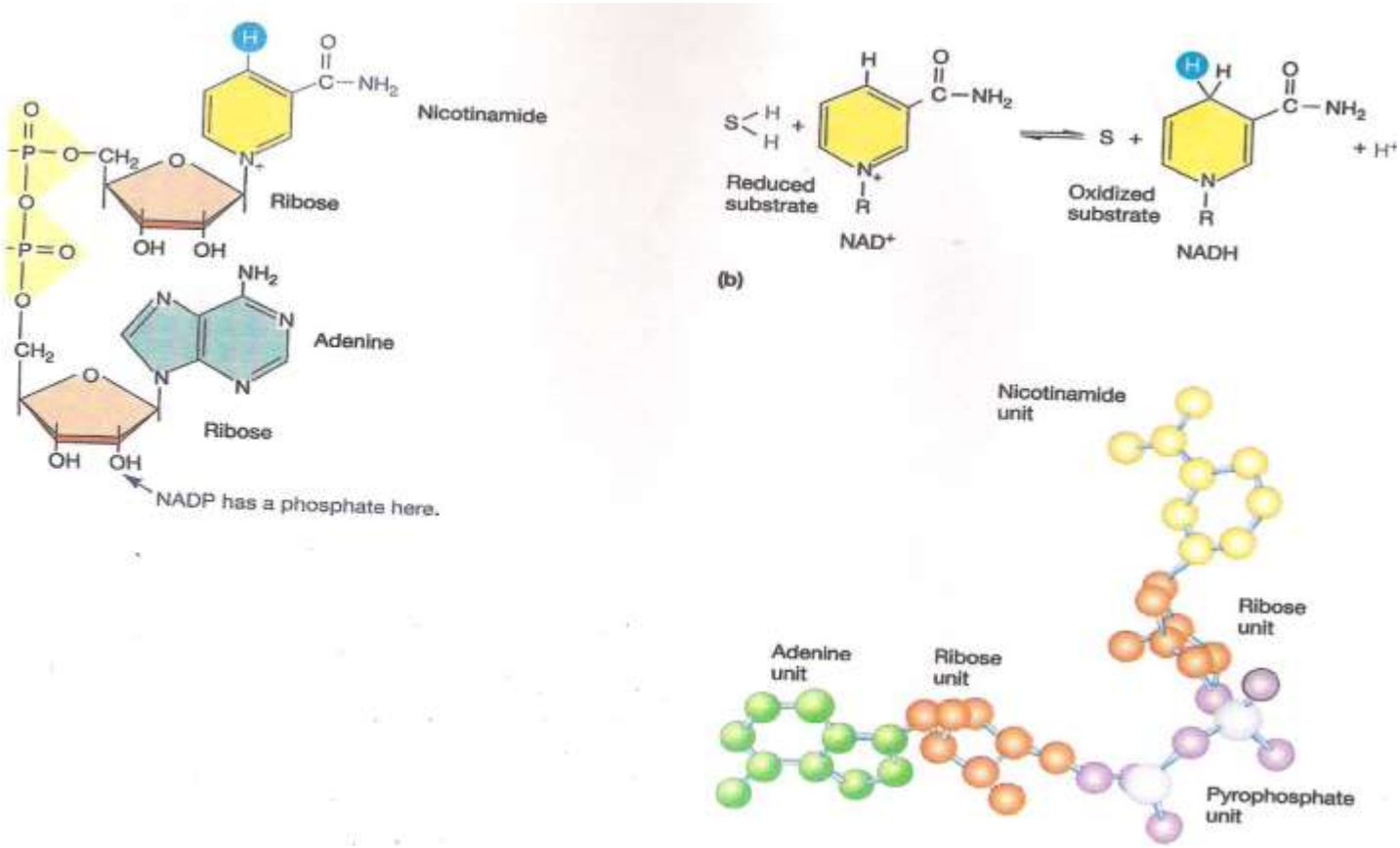


Figure – 2 : The structure and functions of respiratory coenzymes

(a) The structure of NAD and NADP, NAD differ from NAD in having an extra phosphate on one of its ribose sugar unit.

(b) NAD can accept electron and a hydrogen from a reduced substrate (SH_2). These are carried on the nicitinamide ring.

The Thermodynamics and Bioenergetics

Energy may be most simply defined as the capacity to do work, the energy in living cells which is termed as bioenergy, mostly trapped as**ATP,** and is used to do cellular works like biosynthesis, transport, growth, and motility.The flow of energy through organisms at cellular and molecular level termed **Bioenergetics**; that concern with how organism extract energy from surrounding environment and how this energy used to fuel the myriad of life. To understand the basicconcepts of bioenergetics some principles of **thermodynamics lows**(is the study of energy transformation, that describe the relationships between thermal energy, or heat and other forms of energy, and how energy affects matter). These principles facilitate a perception of how an energy – producing and energy – utilizing reaction occurs within the same cell and how the organism is able to accomplish various work functions.

The first law of thermodynamics:state the energy neither created nor destroyed, but converted from one form to another, that the total amount of energy in system remains constant, which confirm the principle of energy conservation, such as,theplants do not produce energy but transform light energy tochemical energy. The first low alone cannot explain why energy released by a reaction and absorbed by other?. Scientific explanation for this phenomenon requires some knowledge of the **second thermodynamics low,** which is about the quality of energy, it state that as energy is transferred or transformed, more and more of it is wasted. The second low also states that there is a natural tendency of any isolated system to degenerate into more disorder state. There are number of ways to state the second low, at a very microscopic level in isolated system any natural process in that system progresses in the direction of increasing disorder, or entropy of system. In fact any discussion of energy must begin with definition of **Entropy(S);** simply is the measure of disorder combined to system thermal change, may be also considered as a measure of a system thermal energy that is available. The chemical reactions that cause a larger increase in **(S)** value[Where the difference between the products and precursor entropies **(Δ S)** is greater than zero], are favored and occur spontaneously with release of energy (**exergonic reactions**), reactions that cause a decrease in **S** value **[Δ S $\leq$ 0]** require input of energy (**endergonic reaction**), thus cannotoccurs spontaneously. So how can we determine which reaction occur spontaneously and which one require an input of energy?. The concept of **Gibbs Free EnergyG(**is a portion of system energy that is able to perform work in certain temperature and pressure), provides a useful function for measuring spontaneity of a system. Energetically favored reaction are those in which a larger amount or free energy is released (**i.e.** they have larger negative Δ **G**). The coal of the cell to harness the **- Δ G.** The first and the second laws can be combined into useful equation, relating the change in energy that can occur in chemical reactions and other processes.

$$\Delta G = \Delta H - T\Delta S$$

ΔG; is a measure for the change of system free energy

ΔH ; refers to the change in enthalpy (the change of heat content)

T; refers to the temperature in Kelvin (°C + 273)

ΔS; is the change in entropy occurring during the reaction.

According to the equation , when entropy decreases and enthalpy increases, ΔG is +ve and not spontaneous, and it does not matter what the temperature of system is. Temperature comes into play when the entropy and enthalpy both increase or both decrease.

Chemical reactions classified as **exergonic** and **endergonic** based on free energy, an exergonic reaction proceeds with a net release of energy - Δ **G,** the magnitude of Δ G for an exergonic reaction is the maximum amount of work that the reaction can perform, for the overall reaction of cellular respiration,

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

ΔG = - 686Kcal / mole, which mean for each mole (180g) of glucose broken down by respiration, 686Kcal of energy are made available to do work in the cell, that the products have 686Kcal less free energy than the reactants.

An endergonic reaction is one that absorbs free energy from its surroundings, so **ΔG** is positive and never proceeds spontaneously that require input of energy. For conversionof $CO_2$and water to sugar, Δ G = + 686Kcal/ .

$$6\,CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6$$

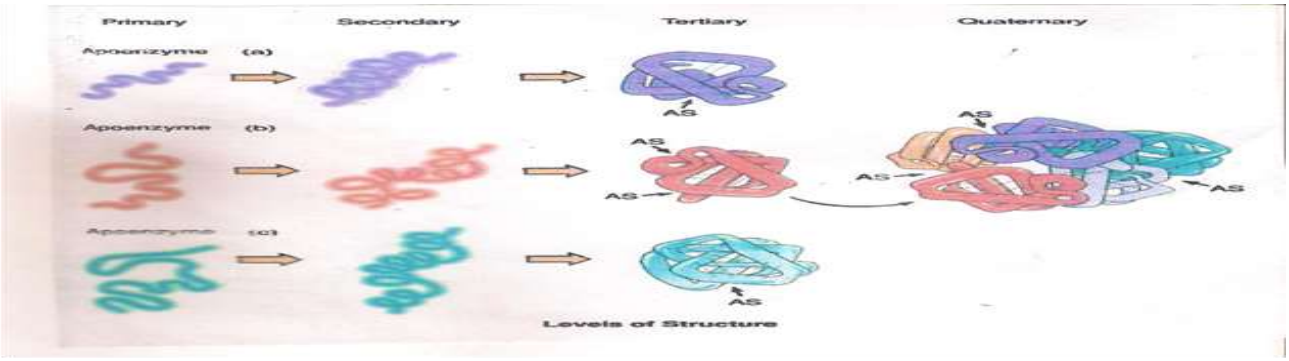
Lec(7)

Microbial Enzymes

Chemical reactions of life are organized and complex, but even when they are highly organized cannot proceed without the tool of life, **Enzymes,** are biological catalysts , designed to do the work of life , each enzyme has one specific job to do. They are proteins act as catalysts, speed up a chemical reaction without being altered during the reaction, this means enzymes can be recycled and do not need to be made in large amount. This due to enzymes lower the **activation energy** (the amount of energy necessary to push the reactants over an energy barrier so that the rection can proceed) needed to derive biochemical reactions in normal cellular temperature, in this manner cellular proteins are not damaged by excess heat for reaction.

Enzymes structure

Enzymes are globular proteins ranging in size from 1300 to millions Dalton, with a thousands of amino acids are bounded together with covalent bonds called peptide bonds, linked as polypeptide. Like all proteins an enzyme exhibits levels of molecular complexity includes **primary**, **secondary**, and **tertiary** structures, the larger enzymes exhibits **quaternary** organization (**figure – 1**). The first three structural levels arise from polypeptide chains folding process and. this folding cause the surface of apoenzyme acquire distinct tree dimintional forms (**3D)** known as a " **native conformation**". The enzymes native conformation which is essential for functional activity maintained and stabilized by **hydrogen** and **bisulfide bonds,** disruption (breakage) of these bonds lead to the loss of enzyme function known as **denaturation. 3D** conformation relates to enzymes specificity cause the surface feature of the tertiary structure provides a unique and specific site usually a crevices or groove called the **active site** or **catalytic site,** which is small porion (include a few amino acids) compared to the overall size of the protein molecule, used for attachment and accepting of substrate that certain enzyme acted on.



(Figure-1): Leveles of Enzymes Structur

Cofactors and Coenzymes

Many enzymes are composed only of protein, sometimes referred as **simple enzymes.** However some enzymes consist of protein (**apoenzyme)** and nonprotein component (**prosthetic groups**). non protein portions are required for participation in the catalytic reaction. The complete enzyme consisting of apoenzyme and its prosthetic group called the **holoenzyme**.. Prosthetic groups fall into two major categories: **Cofactors** and **Coenzymes**.

Cofactors: are commonly metal ions, bind permanently or reversibly to the enzyme, so most of trace elements used by living cells are primarily used as cofactors to serve as structural or functional role, such elements include magnesium, manganese, zinc, iron, copper, cobalt, and selenium. For example $\mathbf{Mg^{2+}}$ needed for DNA polymerace to form a growing DNA molecule. Metal cofactors participate in precise enzymic functions, in general help to form a metal – bridge between enzyme and substrate that assists to bring active site and substrates closely together, or as an electron acceptor/donor in redox reactions. In addition to these roles metals also bind directly to the enzyme to stabilize it in the active conformation or perhaps to induce the formation of active site.

Coenzymes: are small organic compounds work in conjugation with apoenzyme, are present in cells in resonabley constant concentration and play a dynamic role in metabolism, often derived of vitamins. Many important coenzymes like Coenzyme A, NAD, NADP, and FAD are derived from vitamin B complex, so vitamins are clearly important nutrients required as growth factors in majority of living cells, in that vitamins deficiencies prevent the complete holoenzymes from forming, which compromises both the chemical reactions and structure or function dependent upon that reaction. The general action of coenzymes is transmission of functional groups within substrates that act as carrier of these groups so they are loosely attached to the apoenzyme and can dissociate from the protein after products have been formed, and some of them used in generation of energy throughout catabolic pathways.

Classification of Enzymes

Enzymes can be classified in different ways, which based on many criteria, the most common and followed classification categories are: (**1**) **Location of enzymes;** In the basement of enzymes locationin and site of activity, enzymes generally divided into two main groups, **Exoenzymes,** these type of enzymes after their initial biosynthesis inside cells are transported extracellularly where they break down large food molecules and harmful chemicals like; cellulose, amylase, and pinicillinas. **Endoenzymes**, these enzymes are retained intracellular and function there, most metabolic pathways enzymes are of this variety. (**2**) **Presence in the cell**, enzymes are not all produced in equal amounts or at equal rates, according to this criteria, two types of enzymes figured in microbial cells, **Constitutive enzymes,** are always present relatively in constant amounts regardless of the amount of their substrates, such as the enzymes involved in utilizing of glucose, and, **Inducible enzymes,** these

enzymes not constantly present in cells ,they are selectively synthesis depending on metabolic requirements**,** and when their specific substrates they acted on are present. This property of selective synthesis prevent cells from wasting energy and it is an important metabolic control that ceasing non demand pathways**.** (**3**) **Systematic classification,** Despite the large number and bewildering diversity of enzymes present in cells, they are clssifide as belonging to one of **six classes** (includes other subclasses according to the type of reaction catalyzed), recommended in 1973 by international union of biochemistery and molecular biology (**IUBMB**), in this system the arbitrary name is end with the suffix ***ase,*** and usually enzymes named using the profix that related to the type of chemical reaction they catalyze.

The systematic major classes of enzymes

Oxidoreductase Oxidation – reduction reactions, **Lactate dehydrogenase**

Pyruvate + NADH + H ⟶ **Lactate + NAD^+**

Transferase Involves transfer of groups among substrates, **Aspartate carbomyltransferas**

Aspartate + carbomylphosphate ⟶ **cabomylaspartate + phosphate**

Hydrolase Cleave bonds in molecules with addition of water**, glucose -6- phosphate**

Glucose- 6 –P + H_2O ⟶ **glucose + Pi**

Lyase Removal or addition of double bounds, **Fumarate hydratase**

L – malate ⟶ **Fumarate + H_2O**

Isomerase Reaction involving isomerizations, change substrates into other isomeric form

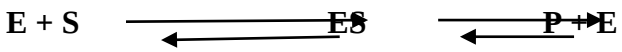
Alanine racemase : L – alanine ⟶ **D - alanine**

Ligase Involve Joining of two molecules with input of ATP, and removal of water

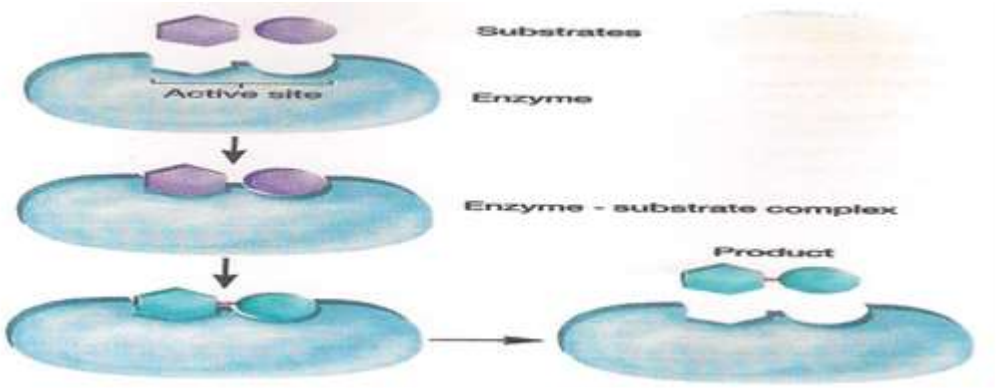
Glutamine synthetase: Glutamine + NH_3 + ATP ⟶ **glutamine + ADP +Pi**

The Mechanism of Enzyme Reactions

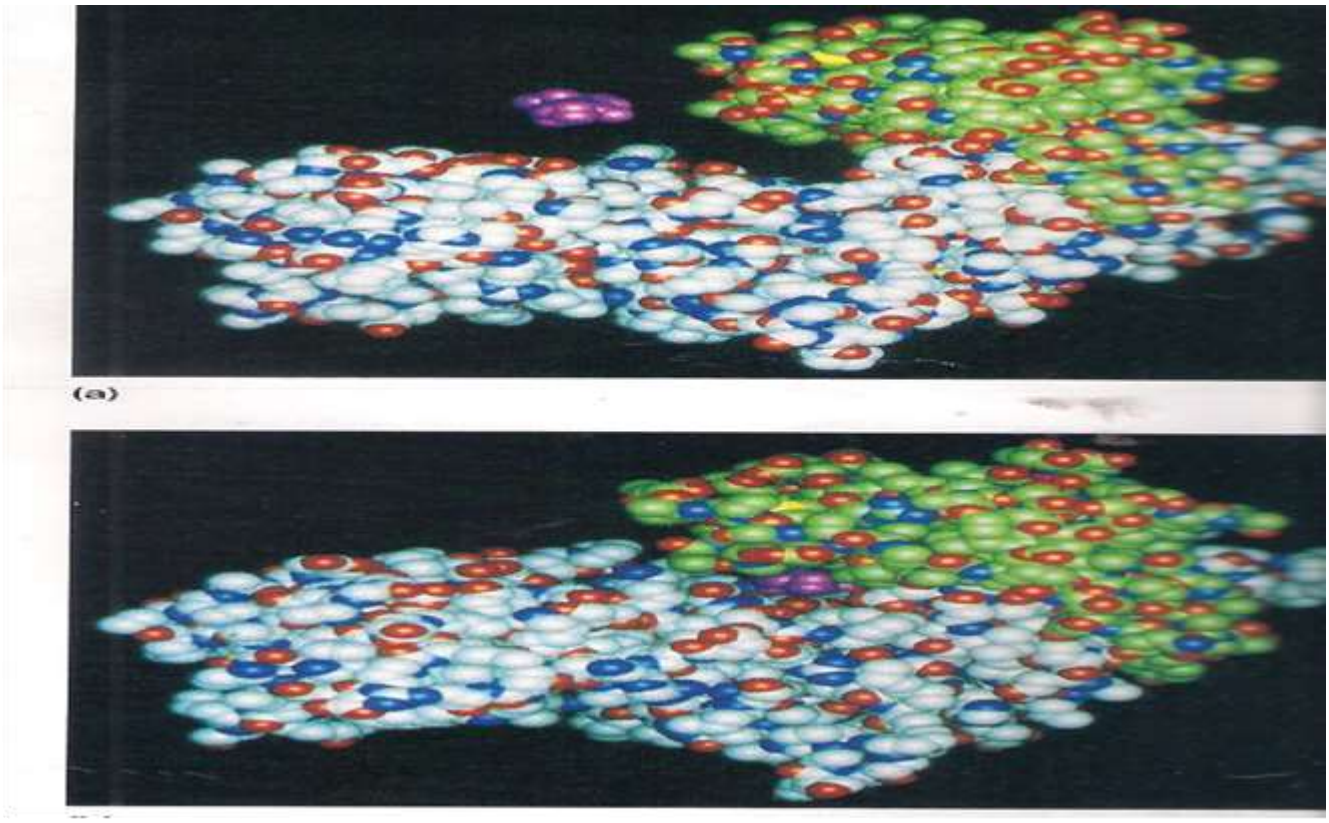
It is important to keep in mind that enzymes speed up chemical reactions without altering the energy barrier or equilibrium constant. If the reaction is endergonic, the presence of enzyme will not shift its equilibrium so that more product can be formed. Enzymes simply speed up the rate at which a reaction speeds toward its final equilibrium. How do enzymes catalyze reactions?. First it must bind the substrate to form the **enzyme – substrate complex** (**ES)** " **transition state complex**"



which resemble both substrate and product and falls between the process of conversion of substrate to product, that many molecular changes occurs in this level like , breakagr, formation or rearrangement of bonds, and developments of other changes. This state doesn't take place if not supplied with amount of energy equivalent to the **activation energy** which required to attain the transition state which falls at the top of energy barrier that has to be overcome and to complet the reaction. Presence of Enzyme accelerat the reaction by lowering the activation energy, therefore more substrate molecules will have sufficient energy to come together even though the equilibrium constant is unchanged **.** In fact enzymes bring substrates together at their **active site** to form the **ES.** An enzyme can interact with the substrate that acted on in two general ways, it may be rigid and shaped to precisely fit the substrate so the correct substrate binds specifically and is positioned properly in actine site for reaction. This mechanism referred to as **Lock – and – key model** (**figure - 2**). An enzyme also may be change shape when it binds the substrate so that the active site surround and precisely fit the substrate, this has been called **Induced fit model** (**figure - 3).** The formation of an **ES complex** can lower the activation energy in many ways, in most cases, substrates are held in the active site by weak interactions, such as **hydrogen binds** and **ionic bonds**, as this interaction achieved the enzyme may put stress on bonds that must be broken, making it easier for the reaction to reach the transition state, and the **R** groups at the active site amino acids residues may create a microenvironment that is conducive to specific reaction.



(Figure-2): Lock – and – Key Model of Enzyme Function



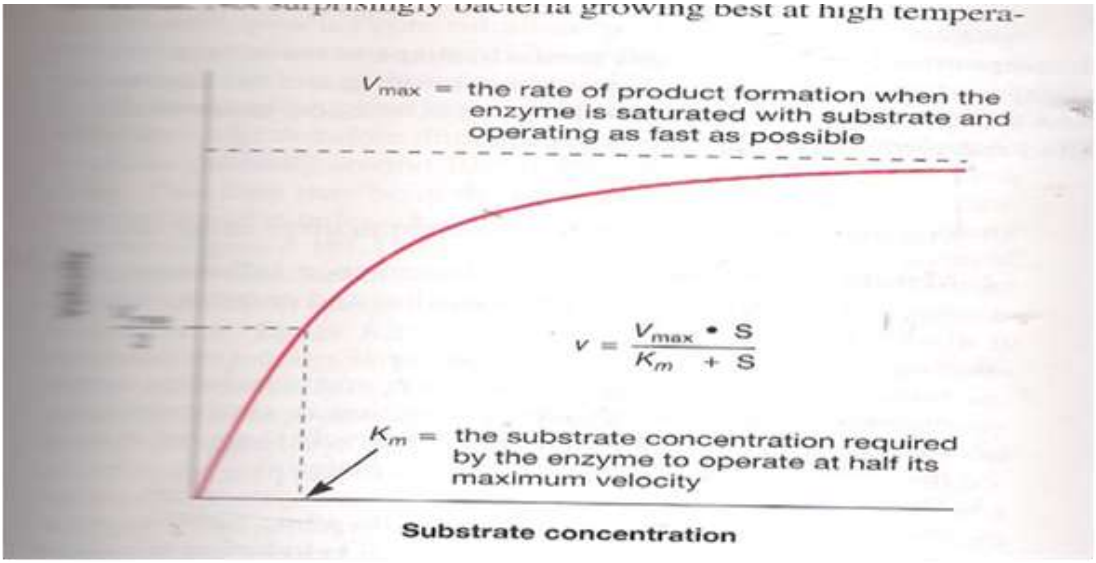
(Figure-3): The Induced Fit Model of Enzyme Function

The effect of Environment on enzymes activity

Enzymes activity varies greatly with the change in environmental factors. One of the most important being the substrate concentration. Substrate concentration (**S**) usually low within cells, at very low concentration , an enzyme make product slowly because it seldom contract substrate molecules, if more substrate molecules are present, the enzyme binds substrate more often, and the reaction velocity (**V**), usually expressed in term of the rate of product formation, greater than at a lower substrate concentration, the initial reaction velocity (**V°**), is proportional to substrate concentration so its in 1st order reaction thus, if more substrate molecules present, an enzyme binds more often, under initial velocity that increase with substrate concentration. Eventually further increase in substrate concentration does not affected the reaction rate and the latter become constant "Zero order reaction" because the available enzyme molecules are binding substrate and converting it to the product as rapidly as possible. That is the enzyme is saturated with substrate and operating at maximal velocity ($\mathbf{V_{max}}$). The resulting substrate concentration curve is hyperbola. It is useful to know the substrate concentration an enzyme need to function adequately.

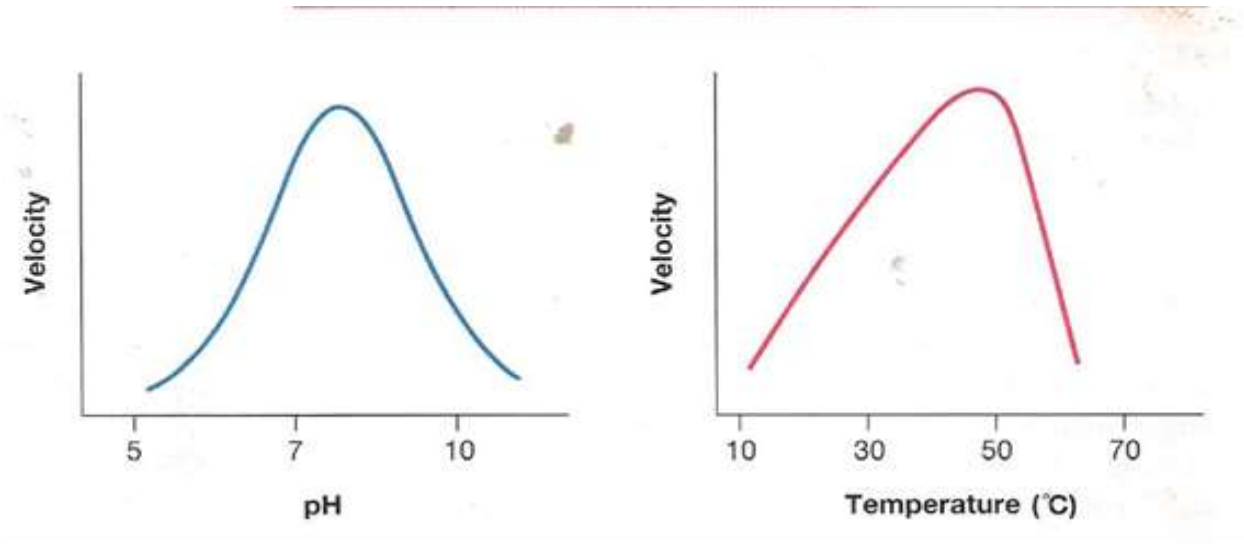
Usually the **Michaelis – Menten** (**Km**), the substrate concentration required for the enzyme to achieve half maximal velocity, is used as a measure of the apparent affinity of an enzyme for its substrate. The lower **Km** value, the lower substrate concentration at which an enzyme catalyzes its reaction, enzyme with a low Km value said to have a high affinity for their substrate. **Km** value is not fixed value but varies according to substrate, temperature, and pH (**figure – 4**).

When the reciprocal valu es of **S** and **V** are used in plotting of **V** versus **S,** a straight line curve is obtain as represented by **Lineweaver – Burk**, used as accurate determination of required substrate concentration for a certain enzyme.



(Figure-4): Michaelis – Menten Kinetics of Enzymes

Enzymes activity also affected by general environmental conditions, such as with alteration in **Temperature** and **pH**(**figure - 5**). Each enzyme works best at certain optimal conditions which favor the most active conformation for the enzyme molecule. Temperature has a major impact on reaction rate, each enzyme has temperature optima for maximum activity. If temperature rises to much above optimum, thermal agitation begins to disrupt the weak bonds that stabilize the protein active conformation and the enzymes structure disrupted and its activity los. This phenomenon known as **denaturation.** Enzymes likewise function most rapidly at specific pH optimum, when the pH deviated to greatly form an enzyme optimum activity slow and the enzyme may be damaged. The temperature and pH optima of a microorganism's enzymes often reflect the temperature and pH of its habitat.

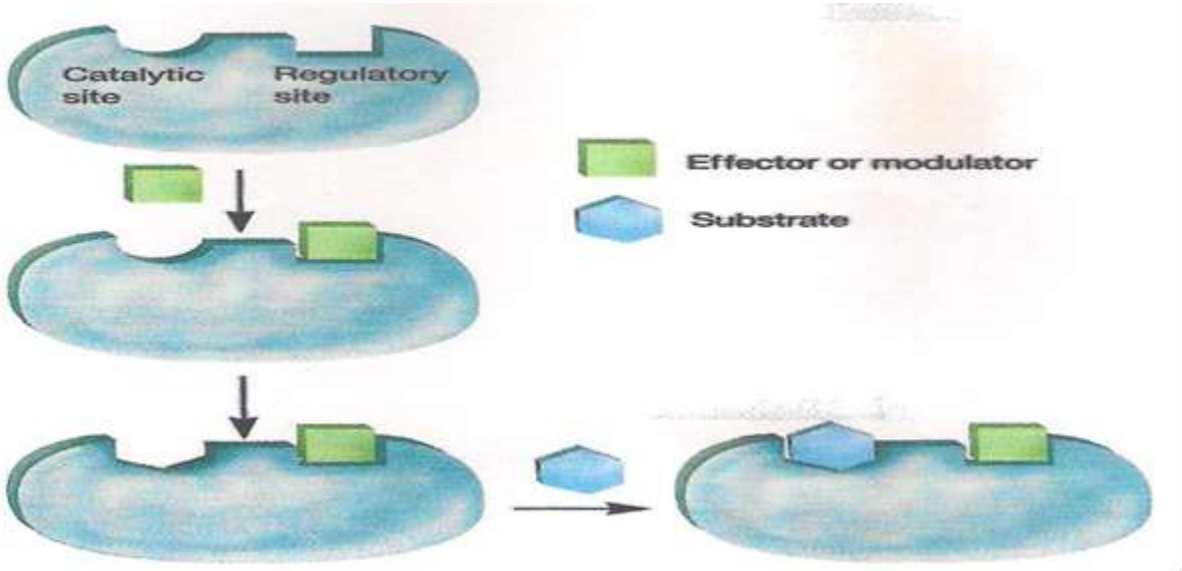


(Figure-5): The Effect of pH and Temperature on Enzymes Activity

Allosteric Enzymes

Allosteric is derived from the Greek root ***allo***, meaning "the other". These are regulatory enzymes, their catalytic activity depends upon non – covalent binding of specific small molecules as ligands at a unique region of the enzyme quite different and away from the active site known as **regulatory site, or allosteric site.** Ligands binding can be either activators or inhibitors (**figure - 6**). Most allosterically regulated enzymes are constructed of two or more polypeptide chain each subunit has its own active site, the allosteric site are often located where subunits join. The ligands that bined at the allosteric site are called **allosteric effectors** or **modulators,** binding of modulator to an enzyme alter the **3D** conformational structure of the active site and thus affected the affinity for substrate, activator

stabilizes the conformation that has functional active site, while the binding of an inhibitor stabilize the inactive form of the enzyme, so most of allosteric enzymes are key enzymes play a major role in balancing the flow of traffic between anabolic and catabolic pathways. For example, ATP binds to several catabolic enzymes allosterically, inhibioting their activity by lowering their affinity for substrate. the substrate saturation curve of these enzymes is often sigmoidal rather than hyperbolic.



(Figure-6): Allosteric Regulation

Enzymes Inhibitors

An enzymes inhibitor is a molecule that binds to an enzyme and decreases its activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance. As many drugs and pharmaceutical compounds are enzyme inhibitors, and much of our basic knowledge of metabolic pathways was determined by using of inhibitors of specific enzymes. Inhibitors compounds can be bind to the free enzyme or to the ES complex and affect the velocity of reaction. Several classes of inhibitors are defined based on their reaction kinetics:

Types of enzymes inhibitors:

1. **Reversible inhibitors**

Reversabile inhibitors attach to enzymes with non – covalent interactions such as hydrogen bonds, hydrophobic interactions, and ionic bonds. Multiple weak bonds between the inhibitors and the active site combine to produce strong and specific binding ,reversabile inhibitors generally do not undergo chemical reactions when bound to enzyme and can be easly removed by dilution and dialysis.

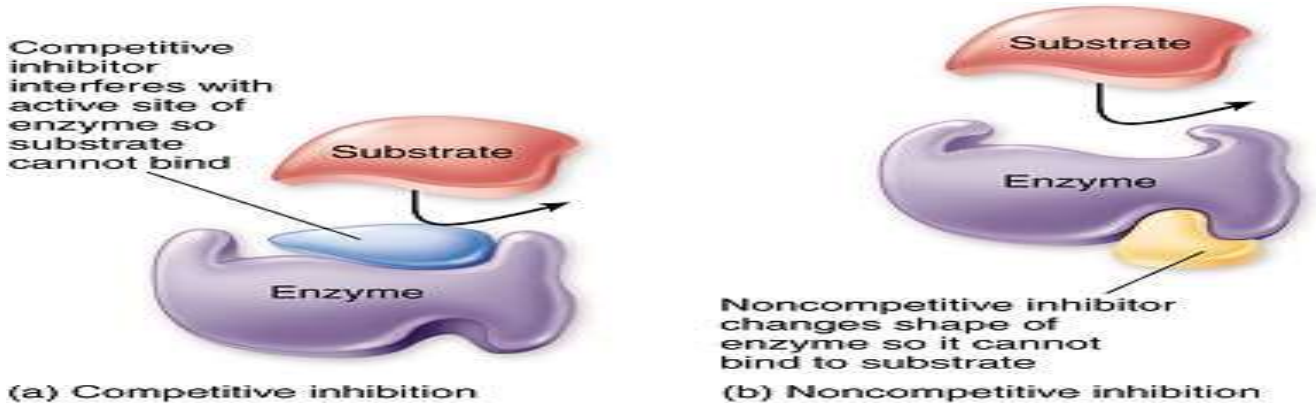
There are two main kindes of reversible enzyme inhibitors, they are classified according to the effect of the varying the concentration of the enzyme's substrate on the inhibitor.

•Competitive Inhibitors:

A competitive inhibitor is any compound which closely resembles the chemical structure and molecular geometry of the substrate that competes with the substrate for binding to free enzyme. The inhibitor may interact with the enzyme at the active site, but no reaction takes place, or inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site (figure – 7 a).

•Non competitive Inhibitors:

A noncompetitive inhibitor is a substance that interacts with the enyzme, but usually not at the active site, reacts either remote from or very close to the active site. The net effect of a non competitive inhibitor is reduction the activity and the enzymic reaction not proceed efficiently, but does not affected the binding of substrate. This inhibition model are not influenced by concentrations of the substrate as is the case for competive inhibitor, and are depends only on the concentration of inhibitors (figure – 7 b).



(Figure -7) Enzymes reversible inhibitors

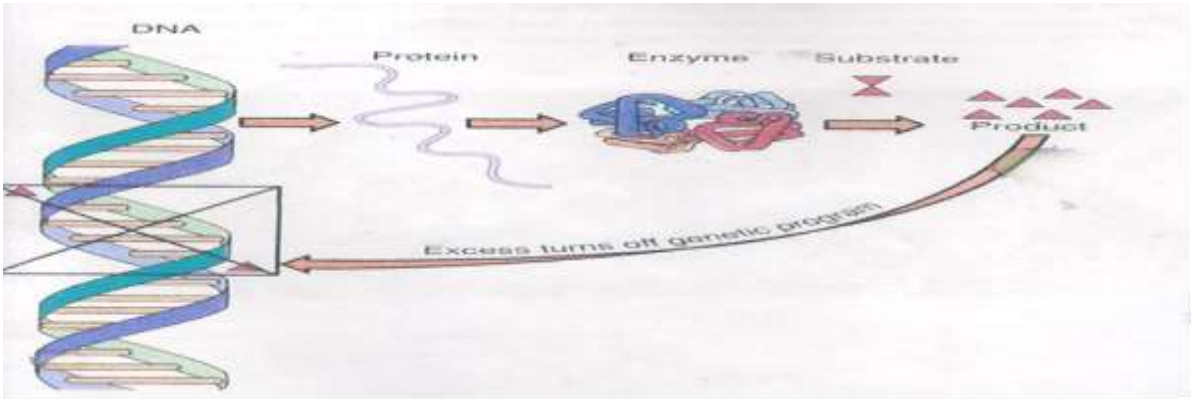
2. **Irreversible inhibitors**

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

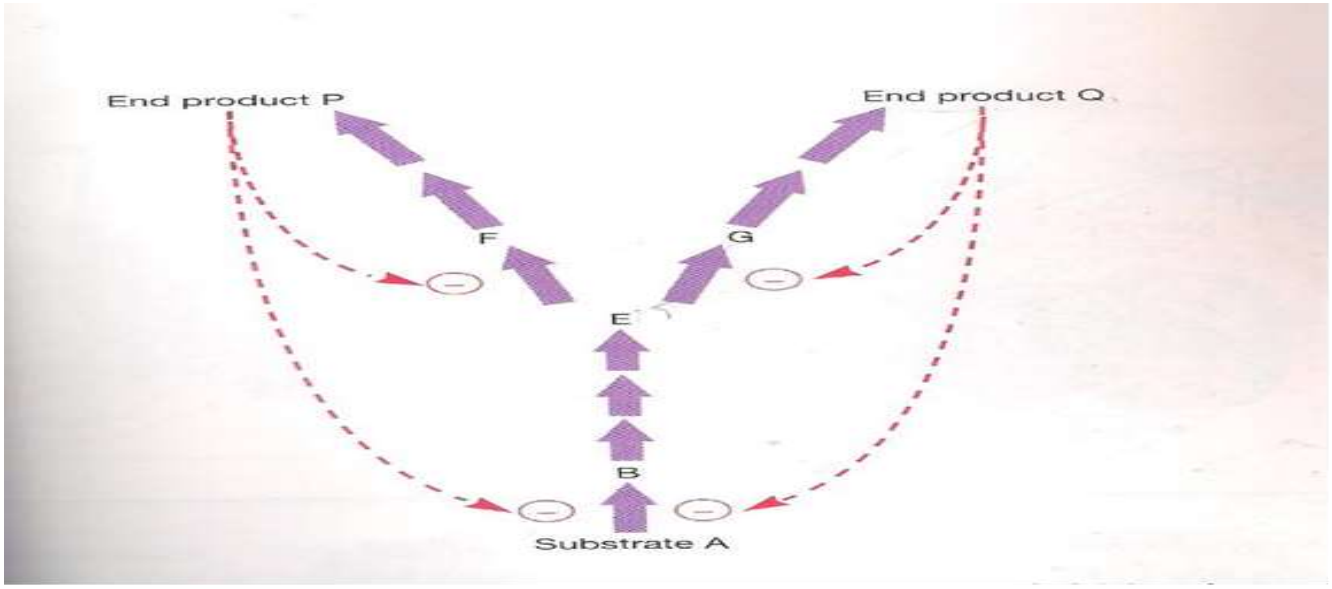
Since many enzymes contain sulfhydral (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor. Heavy metals such as Ag^{+}, Hg^{2+}, Pb^{2+} have strong affinities for -SH groups. Nerve gases such as diisopropylfluorophosphate (DFP) inhibit the active site of acetylcholine esterase by reacting with the hydroxyl group of serine to make an ester. Oxalic and citric acid inhibit blood clotting by forming complexes with calcium ions necessary for the enzyme metal ion activator.

Regulation of Metabolic Pathways

The physiological integration of enzymes into metabolic pathways, which are proceed in highly organized manner, so its necessary to regulate the activity of enzymes participates in pathways, most of regulatory behavior in cells related to the key enzyme **(Pacemaker),** usually are allosteric enzyme, which responed to various regulation signals, and control the flwo of metabolic pathways as cellular demands. Cells regulate these enzyme in two procedures : **long – term regulation,** occurs by change the rate of de novo synthesis genetically, at either transcriptional or translational level (**figure -8**). **Short – term regulation** of an enzyme occurs through modulation of the activity of key enzymes by activators and inhibitors. This is reffered to as **feedback inhibition(figure - 9).** The end product of pathway is feed back to reaction system that fits to allosteric site of key enzyme, by this fitness enzyme distorted and can no longer bind to its substrates, this attachment does not denature the enzyme and its quite reversible.



(Figure-8): Long- Term Regulation of Enzymes



(Figure-9): Short-Term Regulation of Enzymes (Feed Back Inhibition)

(Lec. 8)

Microbial Metabolism

Catabolic Pathways

Catabolism is the set of metabolic pathways that breaks down complex compounds to simpler units, often supply material needed for biosynthesis, including precursor metabolites, and reducing power.

Precursor metabolites serve as starting molecules for biosynthetic pathways. Reducing power is used the carbon skeleton provided by the precursor metabolites, as they are transformed into amino acids, nucleotides, and other small molecules needed for synthesis of macromolecules .

Pathways that function both catabolically and anabolically are called **amphibolic** pathways (Greek *amphi*, on both side).

The central catabolic (**amphibolic)** pathways that take place in microbial cells are:

Glycolysis (glucose breaking down) and **Tricarboxylic acid cycle (TCA)** . Many of these reactions are freely reversible and can be used to synthesize or degrade molecules depending on the nutrient available and the need of microbe.

Glycolysis

Microorganisms employ several metabolic pathways to catabolize glucose (fuel molecule) and other sugars. Because of this metabolic diversity their metabolism is often confusing. To avoid confusion as much as possible, the ways in which microorganism degrade sugars to **pyruvate** and similar intermediates are introduced by focusing on only three routes: **(1) Embden-meyerhof pathway**, **(2) Pentose phosphate pathway**, and **(3) Entner –Doudoroff pathway** .
These three pathways collectively termed **glycolytic pathways** or **glycolysis** [Greek *glyco*, sweet, and *lysis*, a loosening].

Glycolysis is the most important type of mechanism by which organism obtains energy from organic compound in the absence of molecular oxygen. As it occurs in the absence of oxygen, there, it is also called anaerobic fermentation .

The Embden-Meyerhof pathway (EMP)

The sequence anaerobic reactions from glucose to pyruvate is called Embden-Meyerhof pathway, because the complete pathway were elucidated by Gustav Embden (who gave the manner of cleavage of fructose 1, 6- diphosphate and pattern of subsequent steps) and Otto Meyerhof (who confirmed Embden's work and studied the energetic of glycolysis) .It is an important amphibolic pathway that

provides several essential precursor metabolites, found in all major groups of organisms, andt occurs in the cytoplasmic matrix of prokaryotes .

The pathway as a whole may be divided in to two phases **(figure - 1)**:

The six - carbon phase 6C (preliminary phase), in this phase energy is consumed, as glucose is phosphorylated twice, and is converted to **fructose 1,6- biphosphate**. This phase consumes two **ATP** molecules for each glucose and " primes the pump" by adding phosphates to each end of the sugar. In essence, the organism invest some of its **ATP** so that more can be made later in the pathway.

The three – carbon phase 3C (energy conserving phase), begins when the enzyme **fructose 1,6-biphosphate aldolase** catalyze the cleavage of fructose 1,6-biphosphate into two halves (**3C**), each with a phosphate group . One of the products, **dihydroxyacetone phosphate**, is immediately converted to **glyceraldehyde-3-phosphate (G3P)** . This yields two molecules of G3P, which are converted to **pyruvate** in a five-steps. Because dihydroxyacetone phosphate can be easily changed to G3P , both halves of fructose 1,6-biphosphate are used in the three 3C phase. First, G3P is oxidized with **NAD** as electron acceptor (to form **NADH**), and a phosphate **(P_i)** is simultaneously incorporated to give a high energy molecule called 1,3-biphosphoglycerate. The high – energy phosphate on carbon one is subsequently donated to **ADP** to produce **ATP**. This synthesis of **ATP** is called **substrate-level phosphorylation** because **ADP** phosphorylation is coupled with the exergonic breakdown of a high energy bond.

A somewhat similar process generate a second **ATP** by substrate – level phosphorylation. The phosphate group on 3- phosphoglycerate shifts to carbon two, and 2- phosphoglycerate is dehydrated to form a second high – energy molecule, phosphoenolpyruvate. This molecule donates its phosphate to **ADP** forming a second **ATP** and **pyruvate,** the final product of the pathway.

The EMP degrade one glucose to two **pyruvates** by the sequence of reactions , and **ATP** , **NADH** are also produced. The yields of **ATP** and **NADH** may be calculated by considering the two phase separately.

The Pentose Phosphate Pathway (PPP)

The **Pentose phosphate pathway,** also called **Hexose monophosphate shunt or Phosphogluconate pathway ,** a second glycolysis pathway , may be used at the same time as either the EMP or the Entener- Doudoroff pathway. It can operate either aerobically or anaerobically, is important in both biosynthesis and catabolism and is the major source for NADPH require for anabolic processes. There are two distinct phases in PPP; oxidative phase, the linear portion of pathway carries out oxidation and decarboxylation of G-6-P, producing **5C** sugar ribulose – 5 – phosphate. The pathway begins with the oxidation of G- 6-P to 6- phosphogluconate followed by the oxidation of 6-phosphogluconate to the ribulose 5- phosphate, **CO_2** and **NADHs** are generated during these oxidations (**figure -2**) . The overall reaction for this phase is:

$$\mathbf{3\ G\text{-}6\text{-}P + 6\ NADP + H2O \longrightarrow 2\ ribulose\ 5\text{-}\ phosphate + 3CO_2 + 6\ NADPH + 6H^+}$$

Ribulose 5- phosphate is then converted to a mixture of three, four, five, six, and seven – carbon sugar phosphate in a series of non – oxidative reactions (non – oxidative phase) . Two enzymes play a central role in non – oxidative subsequent transformations:

Transketolase and **Transaldolase**, catalyze transfer of 2-C and 3-C molecular fragments respectively , in each case from a ketose donor to an aldose acceptor. These enzymes creat a reversible link between PPP and EMP.

The overall result is that three G-6-P are converted to:

$$\mathbf{3\ G\text{-}6\text{-}P + 6NADP + 3H_2O \longrightarrow 2\ F\text{-}6\text{-}P + G3P + 3CO_2 + 6\ NADHP + H^+}$$

These intermediates are used in two ways. The fructose 6- phosphate can be changed back to G-6-P, while G3P is converted to pyruvate by enzymes of the EMP . Alternatively two G3P may combine to form fructose 1,6- biphosphate.

The PPP is a good example of an **amphibolic** pathway as it has several catabolic and anabolic functions that are summarized as follow:

1. **NADPH** from the pentose phosphate pathway serves as : a source of electrons for the reduction of molecules during biosynthesis

2. The pathway produce two important precursor metabolites : Erythrose 4 –phosphate, which is used to synthesis aromatic amino acids and vitamin B_6(pyridoxal), and ribose 5 –phosphate, which is a major component of nucleic acids. Note that when a microorganism growing on pentose carbon source, the pathway in turn can function biosynthetically to supply hexose sugars (*e,g.* ,glucose needed for biosynthesis of peptidoglycan).

3. Intermediates in the pathway may be use to produce ATP, G3P and F-6-P from the pathway can enter the EMP and can be converted to pyruvate, as ATP is produced by substrate – level phosphorylation. Pyruvate may be oxidized in TCA to provide more energy.

The Entner – Doudoroff Pathway (ED pathway)

Although the Embden – Meyerhof pathway is the most common rout for conversion of **hexoses** to **pyruvate**, the **Entner – Doudoroff pathway** for sugars breaking down is used by only prokaryotes, like some soil microbes, such as *Pseudomonas*, *Rhizobium*, *Azotobacter*, and a few other gram-negative bacteria. Very few gram-positive bacteria have this pathway, with the intestinal bacterium *Enterococcus faecalis* being a rare exception.

The **ED pathway** catabolize glucose to pyruvic acid using enzymes distinct either from those used in EMP or PPP. The pathway begins with the same reaction as the PPP, the formation of g-6- p, which is then converted to 6- phosphogluconate **(figure – 3)**

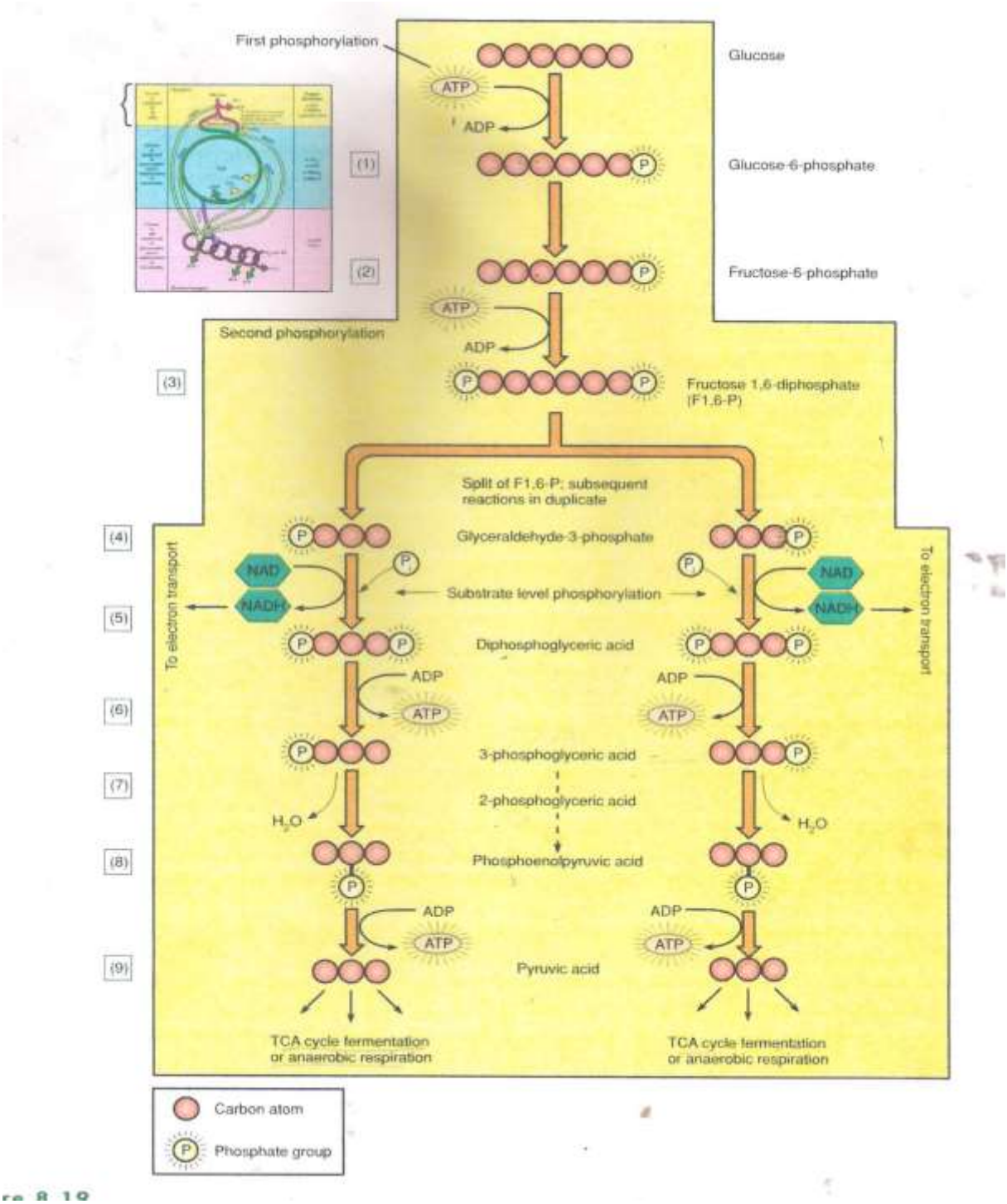
Instead of being further oxidized, 6-phosphogluconate is dehydrated to form 2-keto-3-deoxy-6-phosphogluconate or **KDPG**, the key intermediate in this pathway. **KDPG** is then cleaved by **KDPG aldolase** to pyruvate and G3P. The G3P is converted to pyruvate in the EMP. If the Entner – Doudoroff pathway degrades glucose to pyruvate in this way, a net yield 1 **ATP** for every one glucose molecule processed, as well as 1 **NADH** and **NADPH.**

The Tricarboxylic Acid Cycle (TCA Cycle)

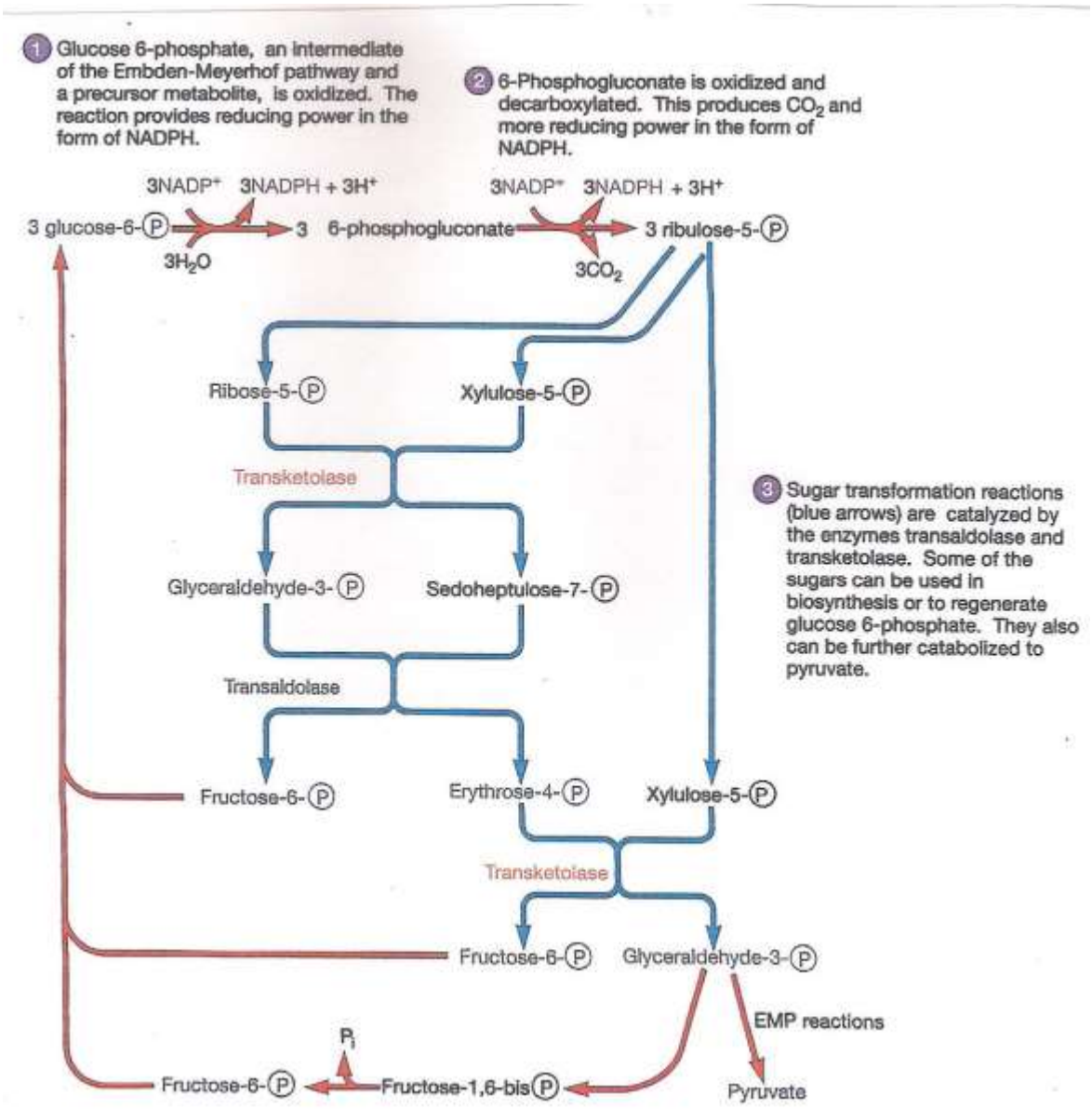
The TCA also known as the **citric acid cycle** or **Krebs cycle,** is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetate derived from carbohydrates, fats and proteins in to CO_2 and chemical energy in the form of ATP. In the glycolytic pathways, the energy captured by the oxidation of glucose to pyruvate is limited to no more than two **ATP** generated by substrate- level phosphorylation, so during the aerobic respiration, the catabolic process continues by oxidizing pyruvate to three **CO_2** through TCA cycle**.** The first step of this process employs a multienzyme system called **pyruvate dehydrogenase complex**. It oxidize, cleaves and decarboxylate pyruvate to form two carbon- molecule, **acetyl-coenzyme A** (**acetyl-CoA**), is the starting point for the cycle (figure - 4).

Acetyl-coA is energy – rich thiol links acetic acid to **coenzyme A**. **Acetyl-coA** then enter the **tricarboxylic acid cycle (TCA).** In the first reaction **acetyl-coA** is condensed with a 4C intermediate, **oxaloacetate**, to form **citrate,** a molecule with 6C. Citrate is rearranged to give isocitrate, a more readily oxidized. Isocitrate is subsequently oxidized and decarboxylated twice to yield -ketoglutarate (**five carbons**), and then succinyl- CoA (**four carbons**), a molecule with a high - energy bond.
At this point two **NADH** molecules have been formed and two carbons lost from the cycle as **CO_2.** The cycle continues when succinyl-CoA is converted to succinate. This involves breaking the high – energy bond in succinyl – CoA and using the energy released to form one **GTP** by **substrate-level phosphorylation**. **GTP** is also high – energy molecule, and it is functionally equivalent to **ATP**. Two oxidation steps follow, yielding one **FADH** and one **NADH**. The last oxidation step regenerates oxaloacetate, and as long as there is a supply of **acetyl-CoA** the cycle can repeat itself. Inspection of figure – 4 shows that the TCA cycle generates two **CO_2** molecules, three **NADH** molecules, one **FADH**, and one **GTP** for each acetyl-CoA molecule oxidized.

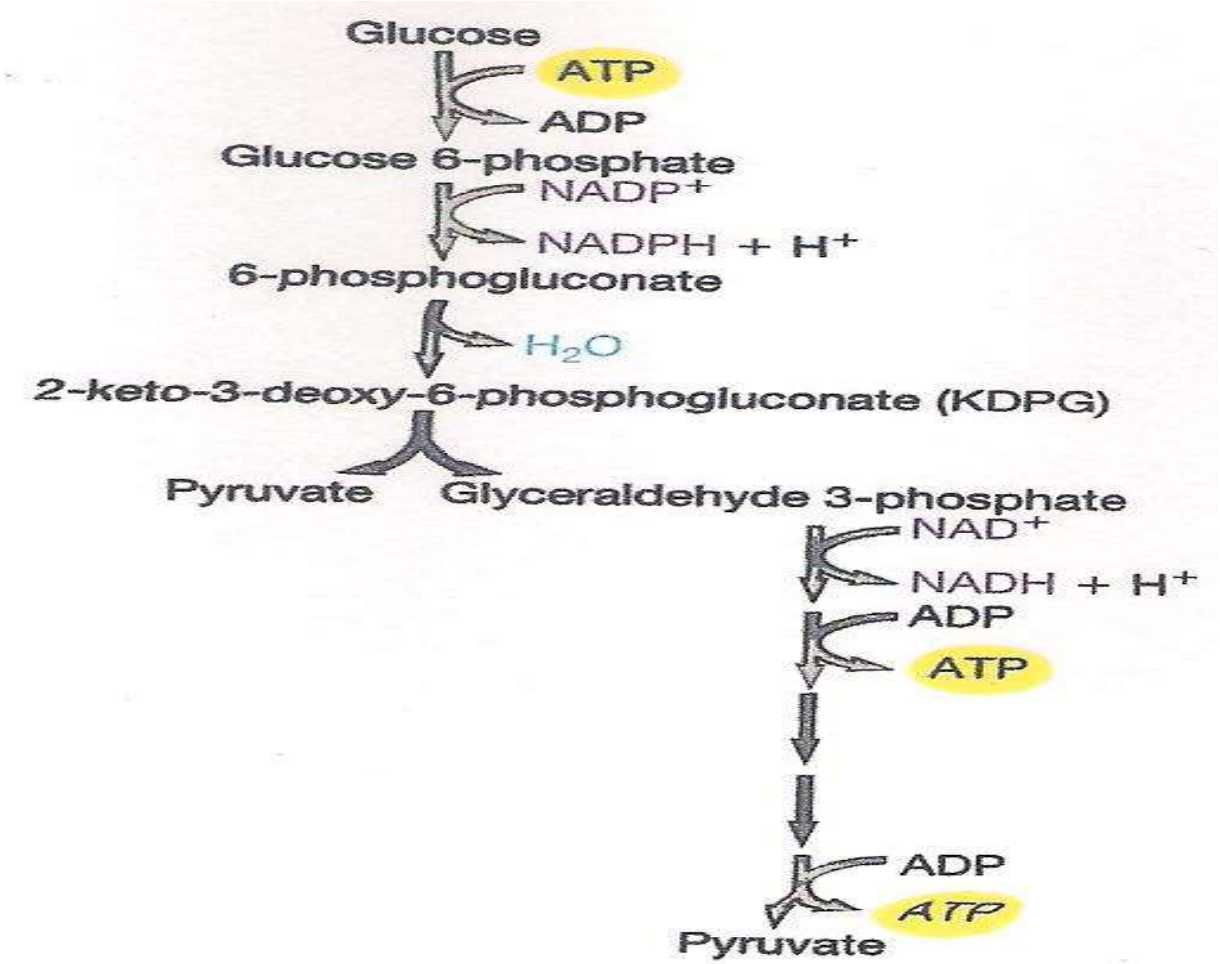
TCA cycle enzymes are widely distributed among microorganisms. In prokaryotes, they are located in the cytoplasmic matrix. In eukaryotes, they are found in the mitochondrial matrix. The complete cycle appears to be functional in many aerobic bacteria, free-living protests, and fungi. This is not surprising because the cycle is such an important source of energy. Even those microorganisms that lack the complete TCA cycle usually have most of the cycle enzymes, because the TCA cycle is also a key source of carbon skeletons for use in biosynthesis.



(Figure -1): The Embden – Meyerhof Pathway (EMP)



(Figure-2) The Pentose Phosphate Pathway (PPP)



(Figure-3) The Entner – Doudoroff Pathway (ED pathway)

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Respiration

Aerobic Respiration

Aerobic respiration is a series of enzymes – catalyzed reaction in which electrons are transferred from fuel molecule such as glucose to oxygen as a final electron acceptor . This pathway is the principal energy - yielding scheme for aerobic microorganisms, and it provides both ATP and metabolic intermediates for many other pathways in the cell, including those of protein, lipid, and carbohydrate synthesis.

Aerobic respiration in microorganisms can be summarized by an equation:

Glucose ($C_6H_{12}O_6$) + 6O_2 +38ADP + 38 P_i ⟶ 6CO_2 + 6H_2O + 38ATP

The Respiratory Chain: (Electron Transport System)

We now come to energy chain, which is the final "processing mill" for electron and hydrogen, and the major generator of **ATP**. Overall, the electron transport system (**ETS**) consists of a chain of special redox carriers that receive electrons from reduced carriers (**NADH, $FADH_2$**) generated by TCA cycle and glycolysis, and shuttle them in a sequential and orderly fashion. The flow of electrons down this chain is highly energetic and gives off **ATP** at various points. At its end, an enzyme catalyzes the final acceptances of electrons and hydrogen by oxygen, producing water. Some variety exists from one organism to another, but the principal compounds that carry out this complex reaction are:
NADH dehydrogenase, **flavoproteins**, **coenzyme Q** (**ubiquinone**), and **cytochromes**. These complex compounds contain a metal that readily facilitates receiving and donating electrons (being reduced and oxidized).
The highly compartmentalized structure of the respiratory chain is an important factor in its function. Not in that the electron transport carriers and enzymes are embedded in the inner mitochondrial membrane in eukaryotes. Bacteria carry them in the cell membrane (**figure–1**) .

Elements of electron Transport (the energy cascade):

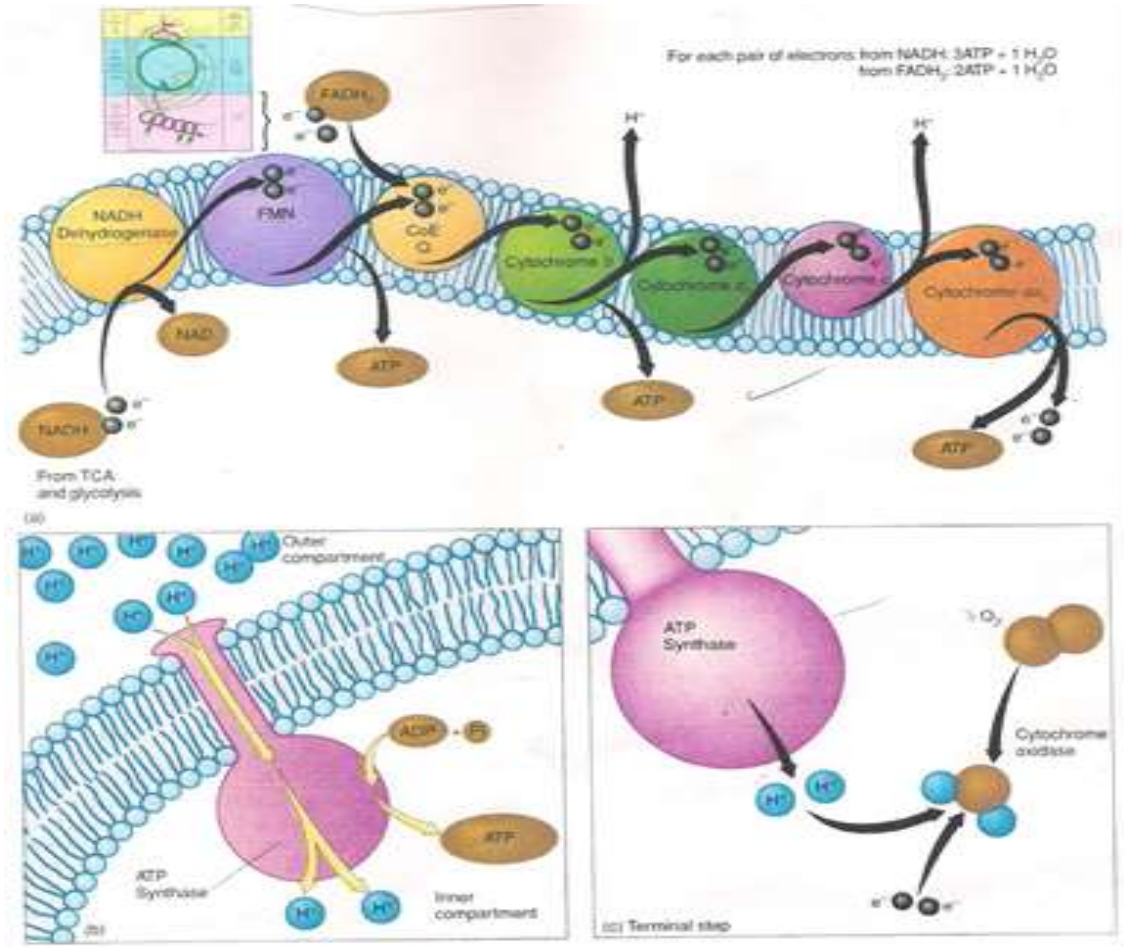
The principal questions about the electron transport system are :

* **How are the electrons passed from one carrier to another in the series?**
* **How is this coupled to ATP synthesis?**
* **Where and how is oxygen utilized?**

Although the biochemical details of this process are rather complicated, the basic reaction consists of a number of redox reactions now it's familiar. In general the seven carrier compounds and their enzymes are arranged in linear sequence and are reduced and oxidized in turn.

The sequence of electron carriers in the respiratory chain of most aerobic organisms is: (**1**) **NADH dehydrogenase**, (**2**) **flavin mononucleotide (FMN)**, (**3**) **coenzyme Q**, (**4**) **cytochrom *b*,** (**5**) **cytochrom c_1**, (**6**) **cytochrom *c*,** and (**7**) **cytochrom aa_3 (figure – 1).**.
Conveyance of the **NADHs** from TCA cycle and glycolysis to the first carrier sets in motion the remaining six steps. With each redox exchange, the energy level of the reactant is lessened. The released energy is captured and used by the **ATP synthase complex** station near the **ETS**.

Each **NADH** that enters electron transport gives rise to **3 ATPs.** This coupling of **ATP** to electron transport is termed **oxidative phosphorylation.** Since **$FADH_2$** from the TCA cycle is metabolically equivalent to **FMN**, it releases only enough energy to synthesize **2 ATPs**.



(Figure-1) Electron Transport System in Prokaryotes

The Theory of ATP formation by Oxidative Phosphorylation

What biochemical processes are involved in the coupling electron transport to the production of ATP? As we know that in eukaryotes, the component of electron transport is embedded in a precise sequence on mitochondrial membrane. This stations them essentially between two compartments: the inner mitochondrial matrix, and the outer mitochondrial membrane and cytoplasm. According to a widely accepted concept called the ***chemiosmotic hypothesis***, as the electron transport carriers shuttle electrons, they actively pump hydrogen ions (**protons**) into the outer compartment of the mitochondrion. This process set up a concentration gradient of hydrogen ions called the **proton motive force** (**PMF**). The **PMF** also generates a difference in charge between the outer membrane compartment **(+)** and the inner membrane compartment **(-)**. The potential energy inherent in such a separation of charge can be harnessed to produce ATP (**figure–1a,b**). Although the exact mechanism is not completely understood, it appears that special pores penetrating the ATP synthase complex transport hydrogen ions (**protons**) from outside compartment back into the mitochondrial matrix. This process releases sufficient free energy for the synthesis of **ATP** from an **ADP** and $\mathbf{p_i}$

Bacterial **ATP** synthesis occurs by means of this same overall process. However, bacteria have the **ETS** stationed in the cell membrane and the direction of the proton movement is from the cytoplasm to the priplasmic space. In both cell types, the chemiosmotic theory has been supported by test showing that the oxidative phosphorylation is blocked if the mitochondrial or bacterial cell membranes are disrupted.

Yield of ATPs from oxidative phosphorylation

The total of five **NADHs** (**4** from TCA cycle and **one** from glycolysis) can be used to synthesize:

15 ATPs from ETS (5 ×3 pre electron pair)
And

15 × 2 = 30 ATPs per glucose

The single $FADH_2$ producing during the TCA cycle results in :

2 × 2 = 4 ATPs per glucose

Summary of aerobic respiration

(1) The total yield of ATP is **40 molecules: 4** from glycolysis, **2** from TCA cycle, and **34** from electron transport.
However, since **2 ATPs** were expended in early glycolysis, these have a maximum of **38 ATPs**. The actual number may be lower in certain eukaryotic cell and bacteria.

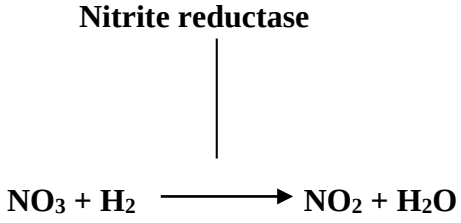
(2) Six carbon dioxide molecules are generated during TCA cycle.

(3) Six oxygen molecules are consumed during electron transport.

(4) Six water molecules are reduced in electron transport and one in glycolysis, but one is send in the TCA cycle, this leaves a net of **6**.

Anaerobic respiration

Some bacteria have evolved an anaerobic respiratory system that function like aerobic cytochrome system except that it utilizes oxygen containing salts, rather than free oxygen, as the final electron acceptor. Of these, the nitrate (NO_3) and nitrate (NO_2) reduction system are best known. The reaction in species such as *Escherichia coli* is represented as:

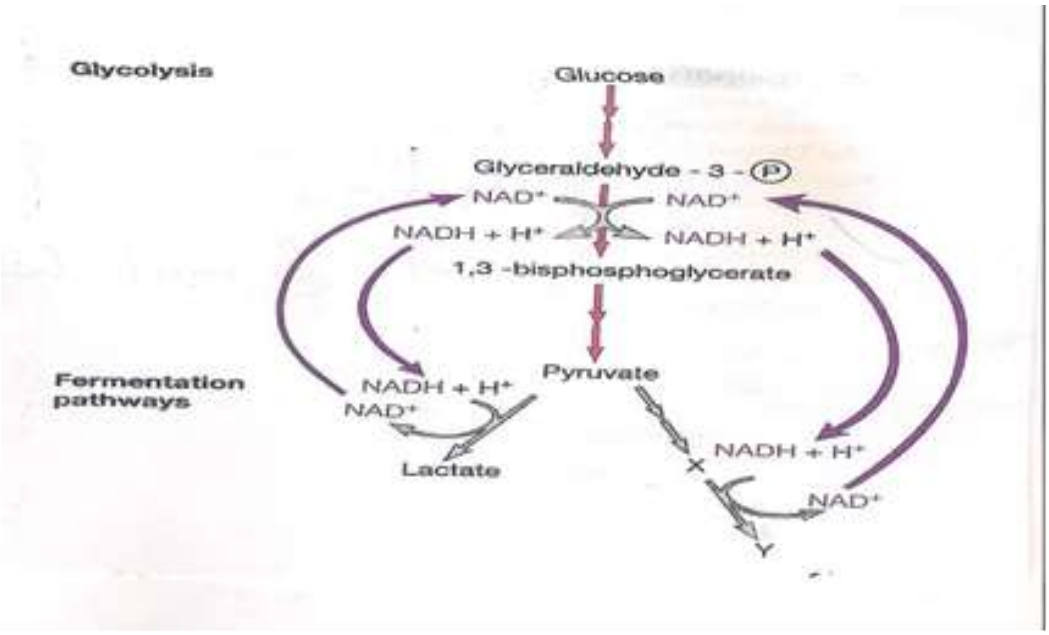


Reduction here is the removal of oxygen from nitrate. A test for this reaction is one of the battery of physiological tests in identifying bacteria.

Some species of *Pseudomonas* and *Bacillus* possess enzymes that can further reduce nitrate to nitric oxide (**NO**), nitros oxide (N_2O), and even nitrogen gas (N_2). This process called **denitrification**, is very important step in recycling nitrogen in the biosphere. Other oxygen containing nutrients reduced anaerobically by various bacteria are carbonates and sulfates. Non of anaerobic pathways produce as much **ATP** as aerobic respirati

Fermentation

Some chemoorganotrophic microbes do not respire because either they lack electron transport chain or they repress the synthesis of electron transport chain component under anoxic conditions, making anaerobic respiration impossible. Yet **NADH** produced by glycolysis pathway must still be oxidized back to **NAD**. If **NAD** is not regenerated, oxidation of glyceraldehyde 3- phosphate will cease and glycolysis will stop. Many microorganisms solve this problem by slowing or stopping **pyruvate dehydrogenase** activity and using pyruvate or one of its derivatives as an electron acceptor for reoxidation of **NADH** in **fermentation** process (**figure – 2**).



(Figure-2) Reoxidation of NADH during Fermentation

There are many kinds of fermentation, and they often characteristic of particular microbial groups. Three unifying themes should be kept in mind when microbial fermentation are examined:

There are many kinds of fermentation, and they often characteristic of particular microbial groups. Three unifying themes should be kept in mind when microbial fermentation are examined:

(1) **NADH** is oxidized to **NAD**

(2) The electron acceptor is often either **pyruvate** or **pyruvate derivatives.**

(3) **Oxidative phosphorylation** cannot operate.

(4) **ATP** is generated exclusively by **Substrate level phosphorylation**, and oxygen not participate.

Major pathways for fermentation of sugars

Alcoholic fermentation; Yeasts**,** many fungi, protests, and some bacteria ferment sugar to ethanol and CO_2 in process called Alcoholic fermentation. Pyruvate decarboxylated to acetaldehyde, which then reduced to ethanol by alcohol dehydrogenase with NADH as electron donor (**figure – 3, No.2**).

Lactic acid fermentation; Lactate is acommon end product of fermentation. Some organisms, collectively cald the lactic acid bacteria (LAB) are subdivided
according to their fermentation products;
homolactic fermentative species produce, single end product lactic acid, whereas the **heterolactic fermentative** species produce other compounds, mostly ethanol and carbon dioxide, along with lactate. These differences are due to the employment of different pathways for glucose oxidation: homolactic fermenters directly reduce almost all their pyruvate to lactate with the enzyme lactate dehydrogenase (**figure-3, No1).**
Heterolactic fermenters employ more complicated pathway for pyruvate reduction and generate substantial amount of products other than lactate, the overall reaction:

glucose $\longrightarrow$ **CO_2 + lactate + ethanol**

Alcoholic and lactic acid fermentations are quite useful. Alcoholic by yeasts produces alcoholic beverages; CO_2 from this fermentation causes bread to rise. Lactic acid fermentation can spoil foods, but also is used to make yogurt, sauerkraut, cheese, and pickles.

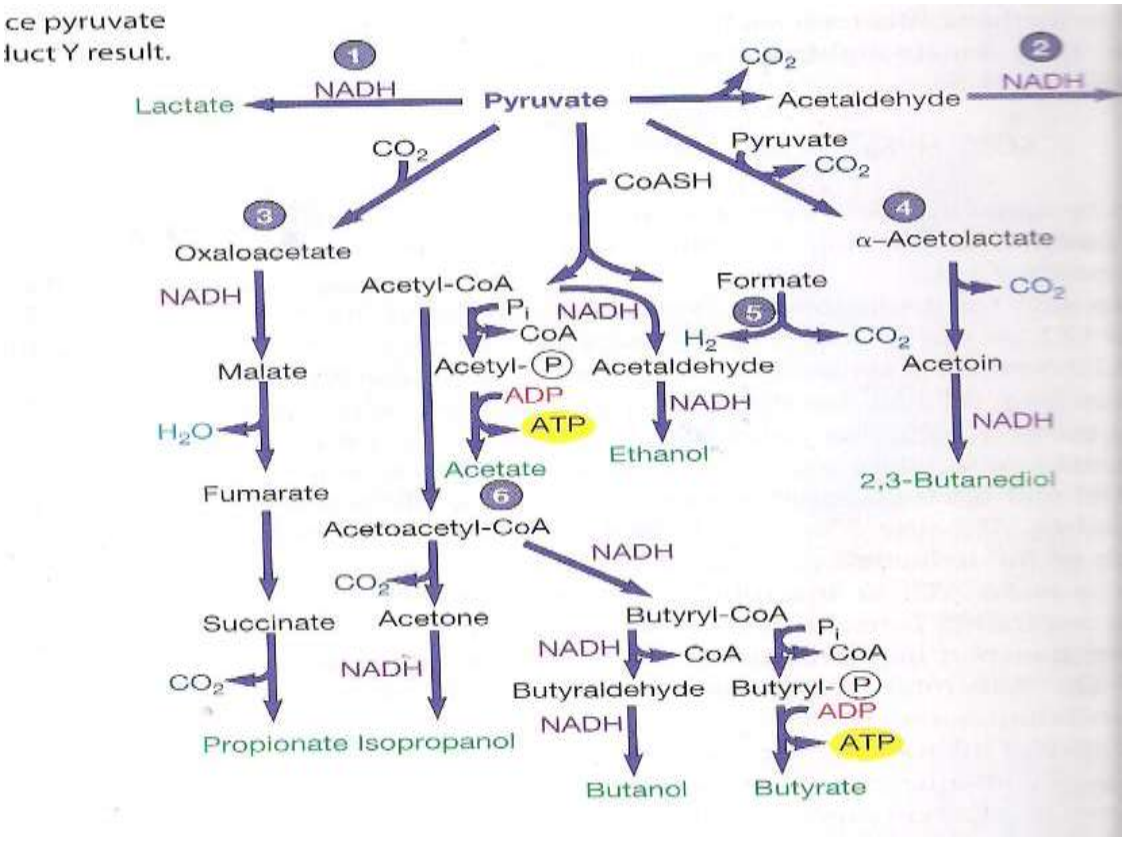
Propionate fermentation; Propionate is a major end product of various fermentation, and many bacteria convert glucose to a mixture of propionate, acetate and CO_2.

Mixed acid fermentation; is carried out by the facultative anaerobic enterobacteria, members of genera *Salmonella*, *Escherichia*, *Shigella*, *Citrobacter* and *Proteus* ferment glucose to a mixture of acids (acetate, lactate, succinate and formic acid), with excretion of ethanol, but not butanediol. The butanediol fermenters such as *Klebsiella*, *Enterobacter*, *Serratia*, *Erwinia*, and *Hafnia* produce fewer acids but considerable amount of 2,3-butanidiol. pyruvate is converted to acetone, which is then reduced to 2,3– butanediol with NADH (**figure-3, No4**) . This difference is the basis of diagnostic key used to differentiate members of enterobacteria.

Formic acid fermentation; many bacteria especially members of the family Enterobacteriaceae , can metabolize pyruvate to formic acid and other products (**figure-3, No.5**). Formic acid may be converted to H_2 and CO_2 by formic acid hydrogenlyase (a combination of at least two enzymes).

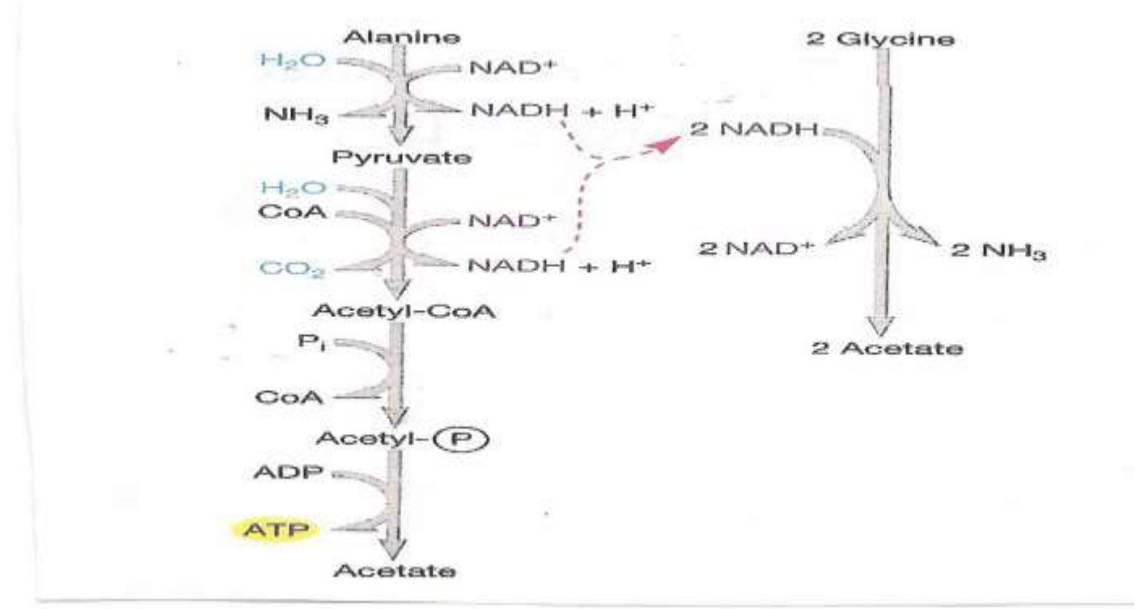
HCOOH ⟶ CO2 +H2

Butarate and acetone – butanol fermentation; Butyrate and butanol are typical fermentation end products of a number of clostridial species (**figure-3, No.6**).



(Figure – 3) Major pathways for fermentation of sugars in prokaryotic cell

Some members of the genus clostridium ferment mixture of amino acids, such as **proteolytic clostridia** , ***C. sporogenes*** and ***C. botulinum***, they carry out the **Stickland reaction ,** in which one amino acid is oxidized and other amino acid act as $\mathbf{e^-}$ acceptor (**Figure-4**). Show the way in which the alanine is oxidized and glycine reduced to produce **acetate**, $\mathbf{CO_2}$, and $\mathbf{NH_3}$, some **ATP** is formed from **acetyl phosphate** by **substrate - level phosphorylation** , and the fermentation is quite useful for growing in anoxic, protain rich environment. The **stickland reaction** is used to oxidized several amino acids: alanine, leucine, isoleucine, valine, phenylalanine, tryptophane, and histidine. Certain bacterial strains also ferment amino acids (alanine, arginine, glutamine, therionine,and glycine) by other mechanisms. In addition to sugars and amino acids, some organic acids such as **acetate, propionate,** and **citrate** are fermented. Some of these fermentations are of great practice importance, for example **citrate** can be converted to **diacetyle** and give flavor to fermented milk



(Figure-4) The Stickland Reaction

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PHOTOSYNTHESIS

Microorganisms derive energy not only from the oxidation of inorganic and organic compounds, but also from light energy, which they capture and use to synthesize **ATP** and **reducing power** (e.g., **NADPH**). They harvest the energy of sunlight and use it to power the synthesis of **ATP**. The conversion of solar energy to chemical energy is called **Photosynthesis.** The general reaction of photosynthesis can be summarized as follow:

$$6\,CO_2 + 12\,H_2X \longrightarrow C_6H_{12}O_6 + 12\,X + H_2O$$

(X depends on the source of electron for reducing power and can be a number of different compounds) .

Usually a phototrophic organism reduces and incorporates **CO_2**. Photosynthesis is one of the most significant metabolic processes on Earth because almost all our energy is ultimately derived from solar energy. It provides photosynthetic organisms with the **ATP** and reducing power necessary to synthesize the organic material required for growth. In turn these organisms serve as the base of most food chains in the biosphere. One type of photosynthesis is also responsible for replenishing our supply of O_2, a remarkable process carried out by a variety of organisms, both eukaryotic and prokaryotic. Although photosynthesis is performed differently by different organisms, the process always involves two reactions :

- **Light - dependent reactions;** are those reactions used to derive energy from sunlight.

- **Dark - dependent reactions;** the reactions which use that energy to fix **CO_2.**

The Role Of Photosynthetic Pigments

Photosynthetic organisms are highly visible in their natural habitat because they possess photopigments, **Chlorophylls** and **Carotenoids**. Colored pigments, used to capture light energy, these pigments vary in color because they absorb different wavelengths of light.
The primary light – absorbing pigments are **chlorophylls**. Most photosynthetic organisms, including an important group of bacteria called **cyanobacteria**, use **chlorophyll a**. A different group of photosynthetic bacteria, the purple and green bacteria, use **bacteriochlorophyll**. This absorbs wavelengths not absorbed by **chlorophyll a**, enabling the green and purple bacteria to grow in habitat where other photosynthetic organisms can not.

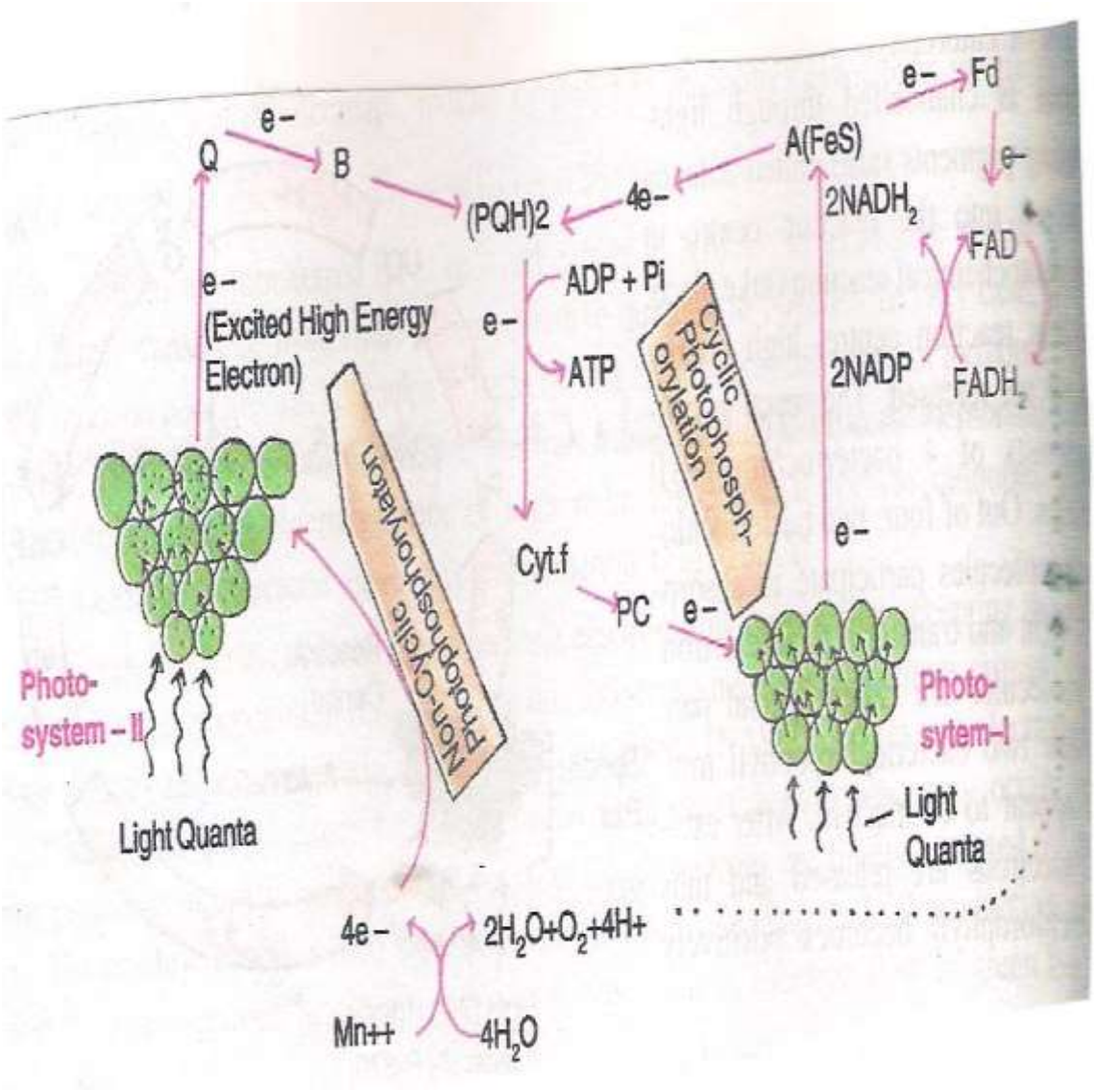
The **carotenoids** are accessory pigments that increase the efficiency of light utilization; they do this by absorbing wavelengths of lights not absorbed by **chlorophylls** and transferring that energy to **chlorophyll**.

Chlorophyll and other light absorbing pigments are organized in protein complex called **Photosystem** located in special photosynthetic membrane. In **cyanobacteria**, these are found in stacked membrane called **Thylacoids.** Plants and algae also have thylacoids, in the stroma of their **chloroplasts**, the photosynthetic pigments in purple and green bacteria are either contained within **cytoplasmic membrane** or within structure called **Chlorosom**e attached to the membrane.

Photophosphorylation

Within the photosystem there are two components that work together to harvest energy, the **antenna complex**, is composed of hundreds of light – gathering pigments (**chlorophyll** and **accessory pigments**), this complex act as a funnel, capturing the energy of light and then transferring it to a **reaction-center chlorophyll**. When this chlorophyll absorbs that energy, one of its **electron** become excited, or raised to higher energy level, in a manner similar to **oxidative phosphorylation**. The high energy electron is sequentially pass along series of electron carriers in membrane embedded electron transport chain, the flow of e^- dawn an ETC that leads to the ultimate reduction of NADH to NADHP. In addition this results in translocation of protons (H^+) across membrane, generating **PMF. ATP synthase** permits the flow of protons back across the membrane and uses the energy to generate **ATP.** The high energy electron from reaction – center chlorophyll that passes along the **ETS** may or may not be returned to their original source.

In **cyclic photophosphorylation (a cyclical pathway)**, the chlorophyll molecule regains the lost e- from water , In **non – cyclical photophosphorylation**, **PMF** is produced, but in addition, high energy electrons are drawn off to generate reducing power, electrons must still be returned to chlorophyll, but they must come from a different source, such as hydrogen sulfide.
(**figure-1**).



(Figur-1) Cyclical and non - cyclical Photosynthesis in Bacteria

Electron Source

Photosynthetic organisms require electron source to generate reducing power in form of **NADPH**, the compound used to obtain electrons dictates wither or not oxygen (**O_2**) is evolved during photosynthesis.

Oxygenic Photosynthesis

When plants, algae, and cyanobacteria use **non – cyclical photophosphorylation** to make reducing power, they use water as electrons source that are returned to the chlorophyll. When electron extracted from water proton and ($\mathbf{O_2}$) are released. It is because of oxygenic photophosphorylation that obligate aerobes, including humans and other animals are able to inhabit earth.

Anoxyginic Photosynthesis

Anoxygenic photosynthesis such as green and purple bacteria, lack the sophisticated photosystem that the oxygenic photosynthetic organisms us to generate **NADPH**. Instead they must use alternative mechanism such as hydrogen sulfide, hydrogen gas, and reducing organic molecules. Because water not used as electron source, anoxygenic phototrophes do not evolve ($\mathbf{O_2}$).

ATP and **NADPH** which produced from photophosphorylation processes have short life spam so they must recycled by producer cells quickly because they can not store in cells as storage compounds, the cells overcome this situation by autofix of $\mathbf{CO_2}$ to high energy storage compounds .

Fixation of CO_2 by Autotrophs

Autotrophs use $\mathbf{CO_2}$ us their sole or principle carbon source and the reduction and incorporation of $\mathbf{CO_2}$ requires much energy. Many autotrophs obtain energy by trapping light during photosynthesis, but some derive energy from the oxidation of reduced electrons donors. Autotrophic $\mathbf{CO_2}$ fixation is crucial to life on Earth because it provides the organic matter on which heterotrophs depend.

Four different $\mathbf{CO_2}$ **– fixation** pathways have been identified in microorganisms. Most autotrophs use the **Calvin cycle**, which is also called the **Calvin – Benson cycle** or the **Reductive Pentose Phosphate Cycle**. The Calvin cycle is found in photosynthetic euocaryotes and most photosynthetic bacteria. It is absent in some obligatory anaerobes and microaerophilic bacteria.

The Calvin Cycle

The Calvin cycle is also called the reductive pentose phosphate cycle because it is essentially the reverse of the pentose phosphate pathway. Thus many of the reactions are similar, in particular the sugar transformation. The reactions of the Calvin cycle occur in the chloroplast stroma of eucaryotic microbial autotrophs. In cyanobacteria, some nitrifying bacteria, and thiobacilli (sulfur oxidizing chemolithtrophs), the Calvin cycle is associated with inclusion bodies called **carboxysomes.** These are polyhedral structures that contain the enzymes critical to the Calvin cycle and may be the site of $\mathbf{CO_2}$ fixation.

The Calvin cycle is divided into three phases: **Carboxylation phase**, **Reduction phase,** and **Regeneration phase** (**figure – 2**).
During the carboxylation phase, the enzyme **ribulose 1,5-biphosphate carboxylase** also called **ribulose biphosphate carboxylase / oxygenase**
(Rubisco), catalyzes the addition of **CO_2** to the 5- carbon molecule ribulose 1,5 – biphosphate **(RuBP)**, forming a six – carbon intermediate that rapidly and spontaneously split into two molecules of 3 – phosphoglycerate (**PGA**). Note that **PGA** is an intermediate of the **EMP**, and in the reduction phase, **PGA** is reduced to glyceraldehyde 3 – phosphate by two reactions that are essentially the reverse of two **EMP** reactions. The difference is that the Calvin cycle enzyme glyceraldehyde 3 – phosphate dehydrogenase uses **NADPH** rather than **NADH**. Finally, in the regeneration phase, **RuBP** is regenerated, so that the cycle can repeat. In addition, this phase produces carbohydrates such as glyceraldehyde 3 – phosphate, fructose 6 – phosphate, and glucose 6 – phosphate, all of which are precursor metabolites. This portion of cycle is similar to the pentose phosphate pathway and involves the **transketolase** and **transaldolase reactions** .

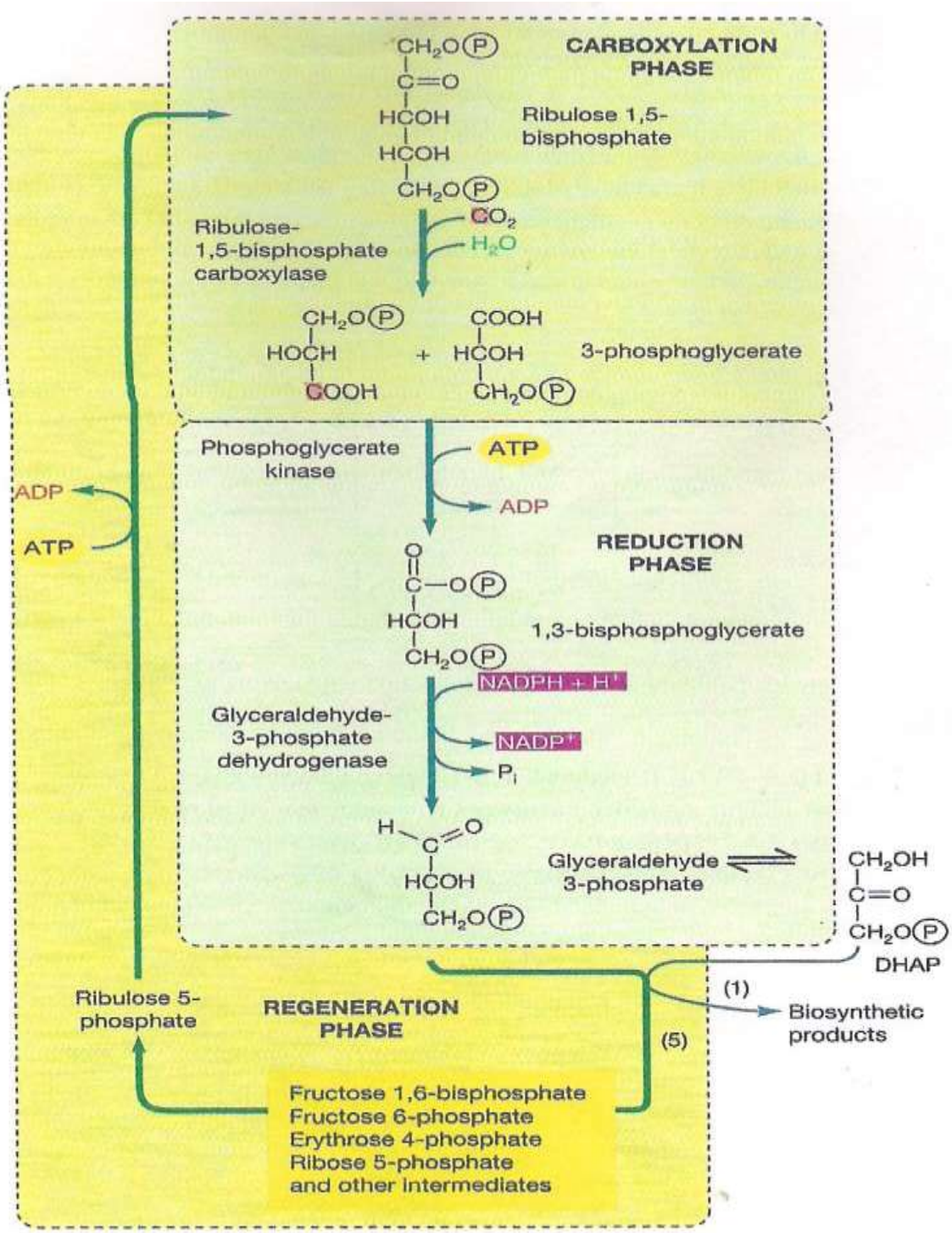
To synthesize fructose 6 – phosphate or glucose 6 – phosphate from **CO_2**, the cycle must operate six times to yield the desired hexose's and reform the six **RuBP** molecules .

$$\textbf{6 RuBP + 6 } CO_2 \longrightarrow \textbf{12 PGA} \longrightarrow \textbf{6 RuBP + fructose 6 P}$$

The incorporation of one **CO_2** into organic material requires three **ATPs** and two **NADPH**. The formation of glucose from **CO_2** may be summarized by the following equation:

$$\textbf{6 } CO_2 \textbf{ + 18 ATP + 12 NADPH + 12 H+ + 12 } H_2O \longrightarrow \textbf{glucose + 18ADP +18Pi + 12NADP}$$

The precursor metabolites formed in Calvin cycle can then be used to synthesize other precursor metabolites and essential molecules.



(Figure - 2) The Calvin Cycle

Anabolic Pathways – Synthesizing Subunit from Precursor Molecules

While prokaryotes as a group are highly diverse with respect to the compounds they use for energy, they are remarkably similar when comparing their biosynthetic processes. Using precursor metabolites, reducing power in form of **NADPH**, and **ATP**, cells synthesize the necessary subunits using specific anabolic pathways. Organisms that lack one or more enzymes in a given pathway must have the end product of that pathway provided from an external source. This is why fastidious bacteria such as the lactic acid bacteria require many different growth factors. Once the subunits are either synthesized or supplied, they can be assembled to make macromolecules. Various different macromolecules can then be joined to form the structures that make up the cell.

- **Lipid Synthesis**

Synthesis of lipids in microorganisms can be viewed as having two essential components – **Fatty acid** synthesis and **Glycerol** synthesis.
Synthesis starts with the transfer of the acetyl group of **acetyl-CoA** to a carrier protein called **acyl carrier protein (ACP).** This carrier serves to hold the fatty acid chain as it elongated by progressively adding 2- carbon units. When the newly synthesized fatty acid reaches its required length, usually **14**, **16**, or **18** carbons long, it is released from **ACP**. The glycerol components is synthesized from **glyceraldehyde 3 – phosphate .**

- **Amino Acids Synthesis**

Proteins are composed of various combinations of **20** different amino acids. Amino acids can be grouped into structurally related families that share common pathway of biosynthesis. Some are synthesized from precursor metabolites formed during **glycolysis**, while other are derived from compounds of the **TCA cycle** .

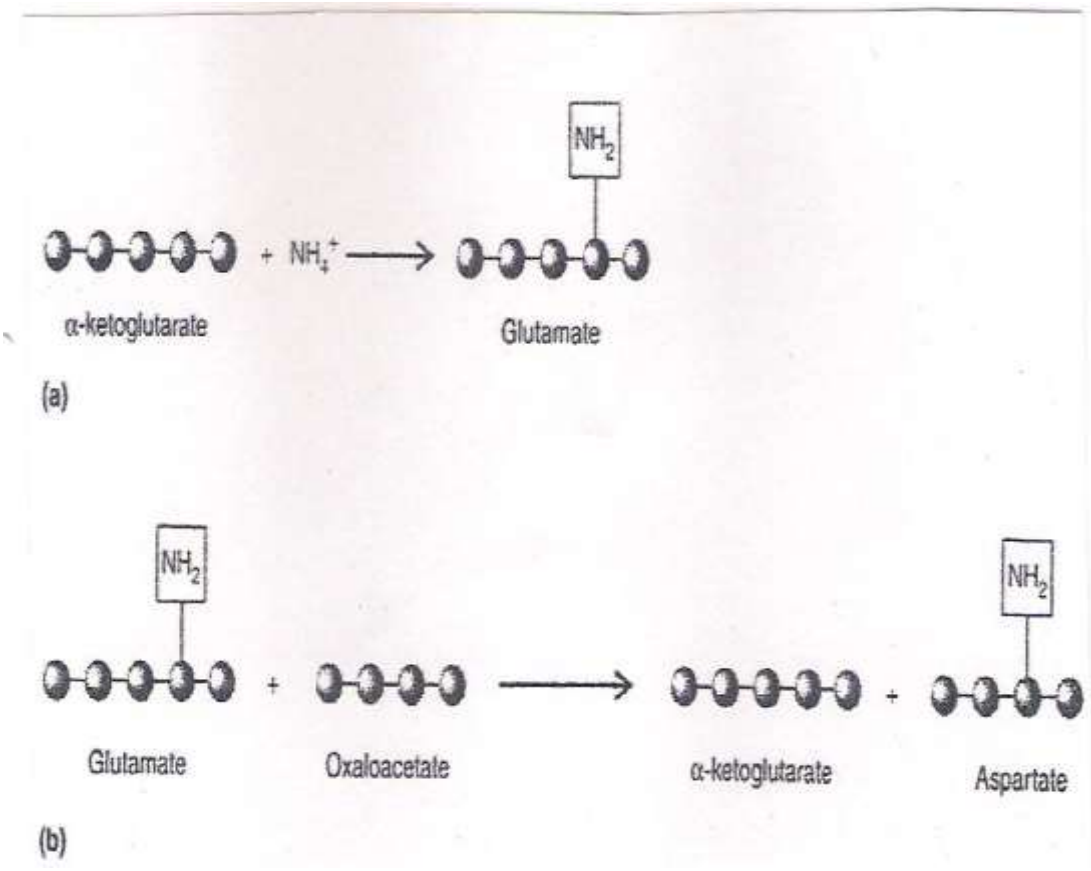
Branched pathways are used to synthesize families of amino acids. These are controlled at key points by **allosteric enzymes**, regulating the flow of certain branches as well as the common initial steps of the pathway. The amino acids that are the end product of the various branches serve as feedback inhibitors.

Glutamate

Although all amino acids are necessary for protein synthesis, glutamate is especially important because it is used to form many other amino acids. In addition, its synthesis provides a mechanism for bacteria to incorporate nitrogen into organic material.

Bacteria that synthesize glutamate use single – step reaction that incorporates ammonia into precursor metabolites **α - ketoglutarate**, produced in the **TCA cycle** (**Figure – 3a**). Once glutamate has been produced, its amino group can be transferred to other carbon compounds to produce amino acids such as aspartate (**Figure – 3 b**).

This transfer of amino group, **a transamination** , regenerates α - ketoglutatate from glutamate. The α- ketoglutarate can then be used again to incorporate more ammonia.



(Figure-3) Glutamate synthesis in Microbial cell

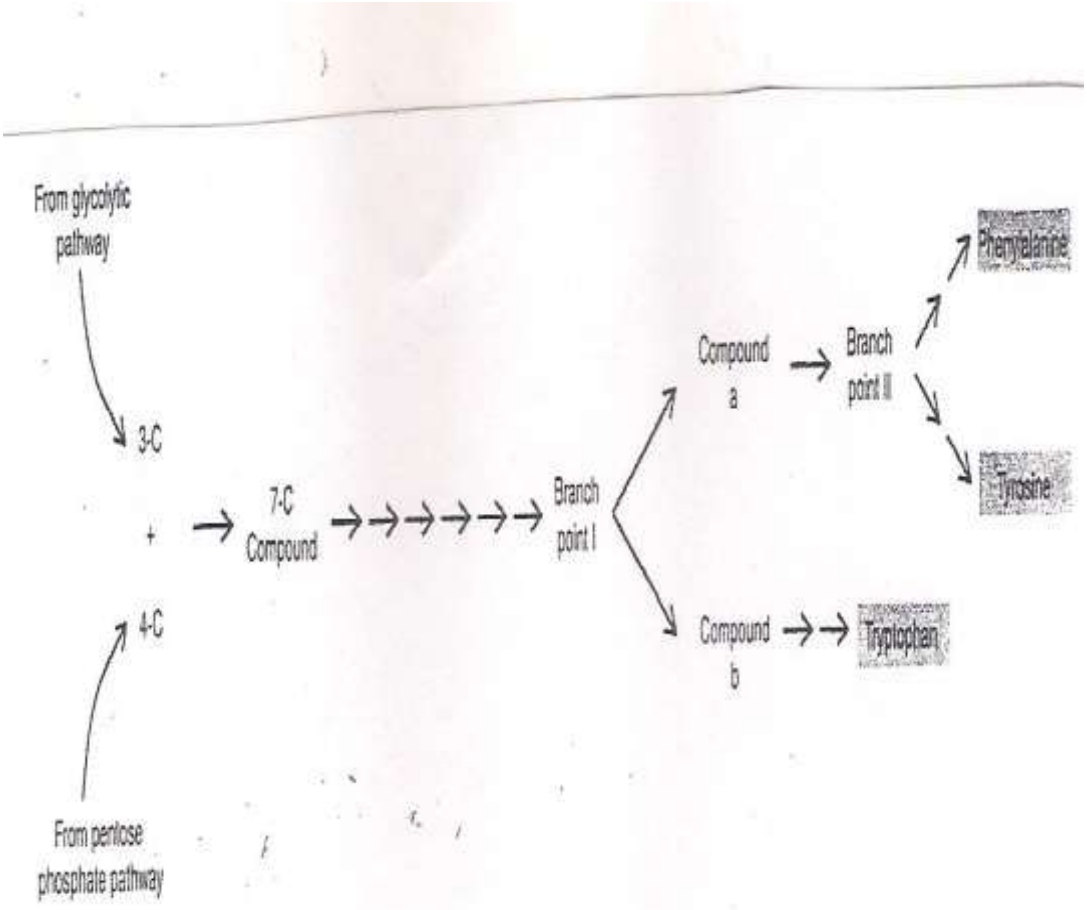
The synthesis of aromatic amino acids such as tyrosine, phenylalanine, and tryptophane requires multistep branching pathway (**Figure – 4**).
This serves as an excellent illustration of many important features of the regulation of amino acids synthesis.

The pathway begins with the formation of 7 – carbon compound, resulting from the joining of two precursor metabolites, **erythrose 4 – phosphate** (**4-carbon**) and **phosphoenolpyruvate** (**3-carbon**). These precursors originate in the **PPP** and **EMP**, respectively. The 7-catbon compound is converted through a series of steps until a branch point is reached. At this juncture two options are possible. If synthesis proceeds in one direction, tryptophan is produced. In the other direction, another branch point is reached; from there, rather tyrosine or phenylalanine can be made.

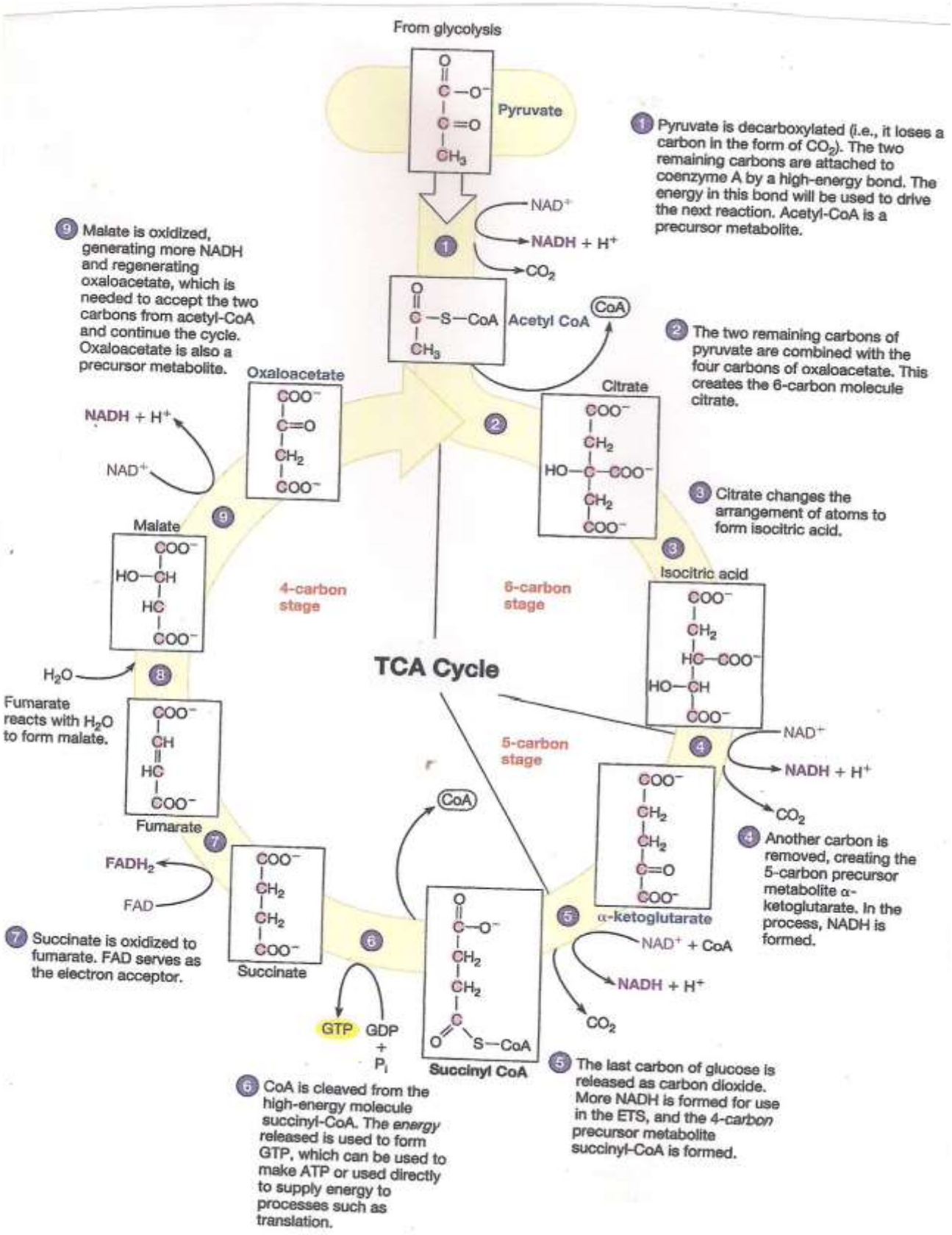
When a given amino acid is provided to a cell, it would be a waste of carbon, energy, and reducing power for that cell continue synthesizing it. But when only one product of branched pathway is present, how does the cell control synthesis**?** In the pathway for aromatic amino acid biosynthesis, this partly occurs by regulating the enzymes at the branch points. Tryptophan acts as

feedback inhibitor of the enzymes that directs the branch to its synthesis; this sends the pathway to the steps leading to the synthesis of the other amino acids, tyrosine and phenylalanine. Likewise, these two amino acids each inhibit the first enzyme of the branch leading to their synthesis.

In addition, the three amino acids each control the first step of the full pathway, the formation of 7-carbon compound. Three different enzymes can catalyze this step; each has the same active site, but they have different allosteric sites. Each aromatic amino acid acts as a feedback inhibitor for one of the enzymes. If all three amino acids are present in the environment, then very little of the 7-carbon compound will be synthesized. If only one or two of those amino acids are present, then proportionally more of the compound will be synthesized.



(Figure -4) Biosynthesis of Aromatic Amino Acids



(Figure – 4): The Tricarboxylic Acid Cycle (TCA)