# *Microbial Metabolism*

4th & 5th Lectures

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# Objectives of the lectures

1-define microbial metabolism
2- discuss the importance of transport systems in bacterial cell
3- determine the main metabolic pathways
4-clarify the importance of metabolism in microbial life and in the diagnosis of infectious diseases
5- discuss the controlling on metabolic pathways
6-how to detect microbial metabolism

# Bacterial transport systems

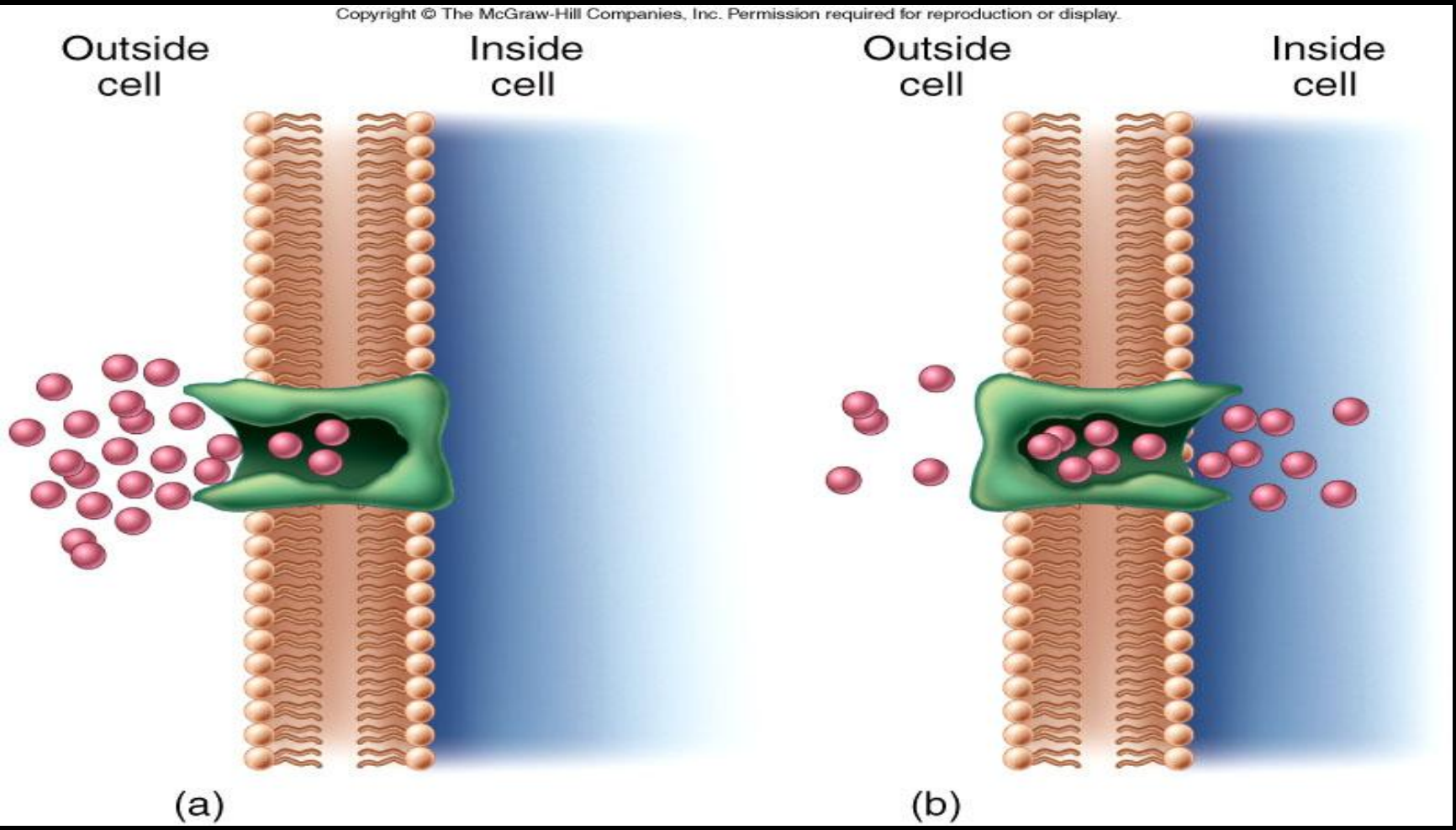
## FUNCTION OF CYTOPLASMIC MEMBRANE

The major functions of the cytoplasmic membrane are
(1) selective permeability and transport of solutes;
(2) Electron transport and oxidative phosphorylation, in aerobic species;
(3) excretion of hydrolytic exoenzymes;
(4) bearing the enzymes and carrier molecules that function in the biosynthesis of DNA, cell wall polymers, and membrane lipids;
and (5) bearing the receptors and other proteins of the chemotactic and other sensory transduction systems.

## Permeability and Transport

The cytoplasmic membrane forms a hydrophobic barrier impermeable to most hydrophilic molecules. However, several mechanisms **(transport systems) exist that enable the cell to transport nutrients into and waste products out of the cell.**
There are three general transport mechanisms involved in membrane transport: **passive transport, active transport, and group translocation**

*Facilitated Diffusion :* is passive diffusion of a substrate against a concentration gradient ,does not required energy .(e.g.glycerol)



## PASSIVE TRANSPORT

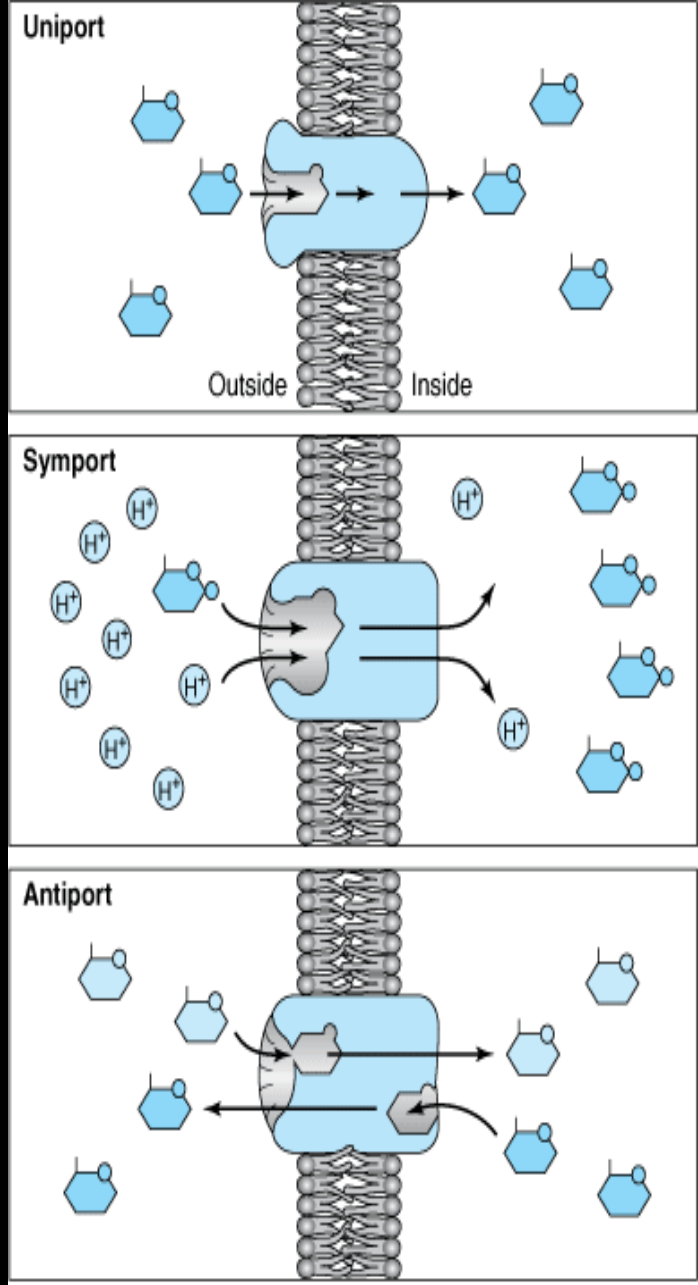
This mechanism relies on diffusion, uses no energy, and operates only when the solute is at higher concentration outside than inside the cell. Simple diffusion accounts for the entry of very few nutrients including dissolved oxygen, carbon dioxide, and water itself. Simple diffusion provides neither speed nor selectivity. Facilitated diffusion also uses no energy so the solute never achieves an internal concentration greater than what exists outside the cell. However, facilitated diffusion is selective. Channel proteins form selective channels that facilitate the passage of specific molecules. Facilitated diffusion is common in eukaryotic microorganisms (eg, yeast), but is rare in prokaryotes. Glycerol is one of the few compounds that enters prokaryotic cells by facilitated diffusion.

## ACTIVE TRANSPORT

Many nutrients are concentrated more than a thousand fold as a result of active transport. There are two types of active transport mechanisms depending upon the source of energy employed:
ion-coupled transport and ATP-binding cassette (ABC) transport.

### 1- Ion-Coupled Transport (CHEMIOSMOTIC DRIVEN TRANSPORTER

These systems move a molecule across the cell membrane at the expense of a previously established ion gradient such as proton-motive or sodium-motive force.
There are three basic types: uniport, symport, and antiport
Ion-coupled transport is particularly common in aerobic organisms, which have an easier time generating an ion-motive force than do anaerobes.
1-Uniporters catalyze the transport of a substrate independent of any coupled ion. 2-Symporters catalyze the simultaneous transport of two substrates in the same direction by a single carrier; for example, an H+ gradient can permit symport of an oppositely charged ion (eg, glycine) or a neutral molecule (eg, galactose). 3-Antiporters catalyze the simultaneous transport of two like-charged compounds in opposite directions by a common carrier (eg, H+:Na+). Approximately 40% of the substrates transported by *Escherichia coli utilize this mechanism.*

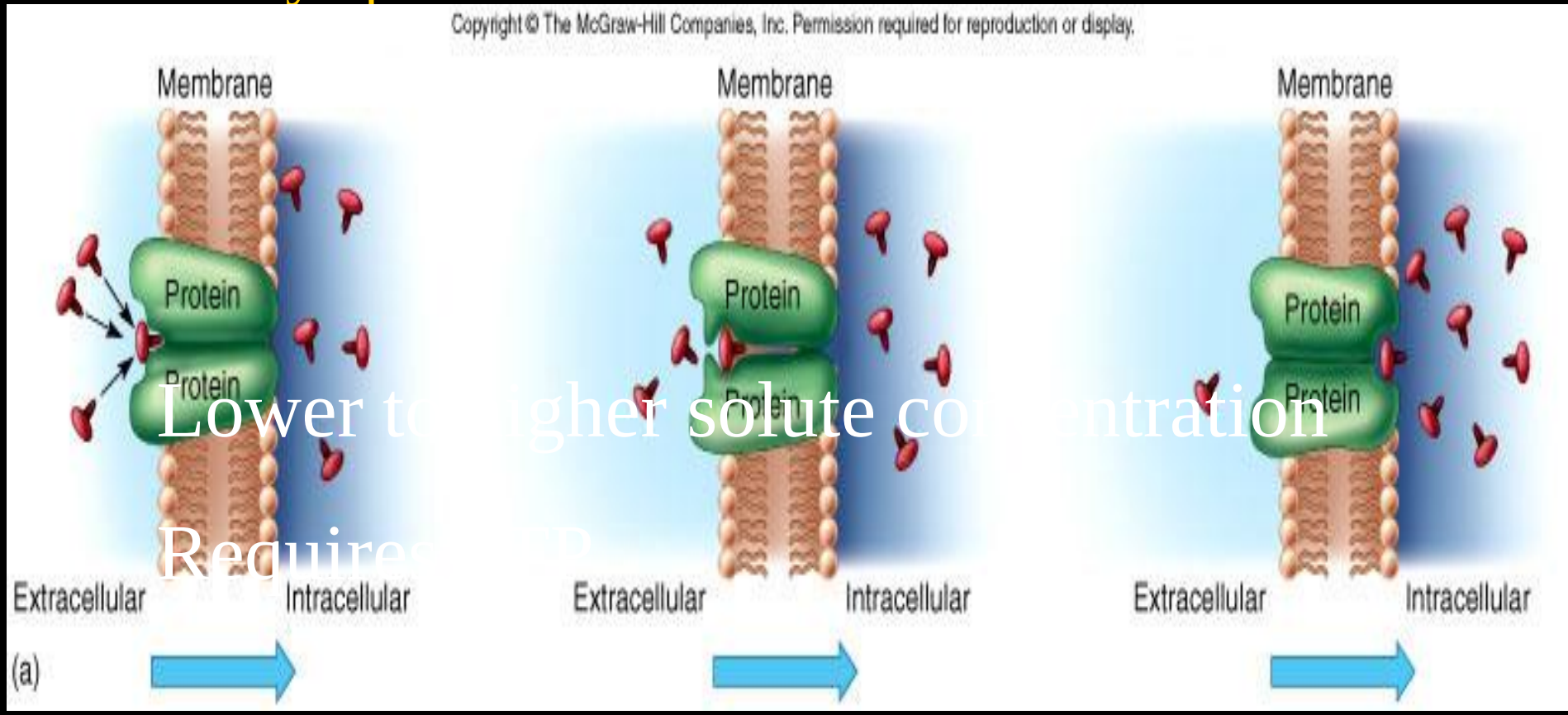



Source: Brooks GF, Butel JS, Morse SA: *Jawetz, Melnick, & Adelberg's Medical Microbiology*, 24th Edition: http://www.accessmedicine.com

# *Active Transport*

**1-binding protein dependent transporters** (shock-sensitive): many nutrients is facilitated by specific binding protein

**2-chemiosmotic driven transporters** : move amolecule across the cytoplasmic membrane.




Lower to higher solute concentration

Requires ATP

## 2- ABC Transport (BINDING PROTEIN DEPENDENT TRANSPORTERS)

**This mechanism employs ATP directly to transport solutes into the cell. In gram-negative bacteria, the transport of many nutrients is facilitated by specific binding proteins located in the periplasmic space; in gram-positive cells the binding proteins are attached to the outer surface of the cell membrane. These proteins function by transferring the bound substrate to a membrane-bound protein complex. Hydrolysis of ATP is then triggered, and the energy is used to open the membrane pore and allow the unidirectional movement of the substrate into the cell. Approximately 40% of the substrates transported by *E coli utilize this mechanism.***

## GROUP TRANSLOCATION

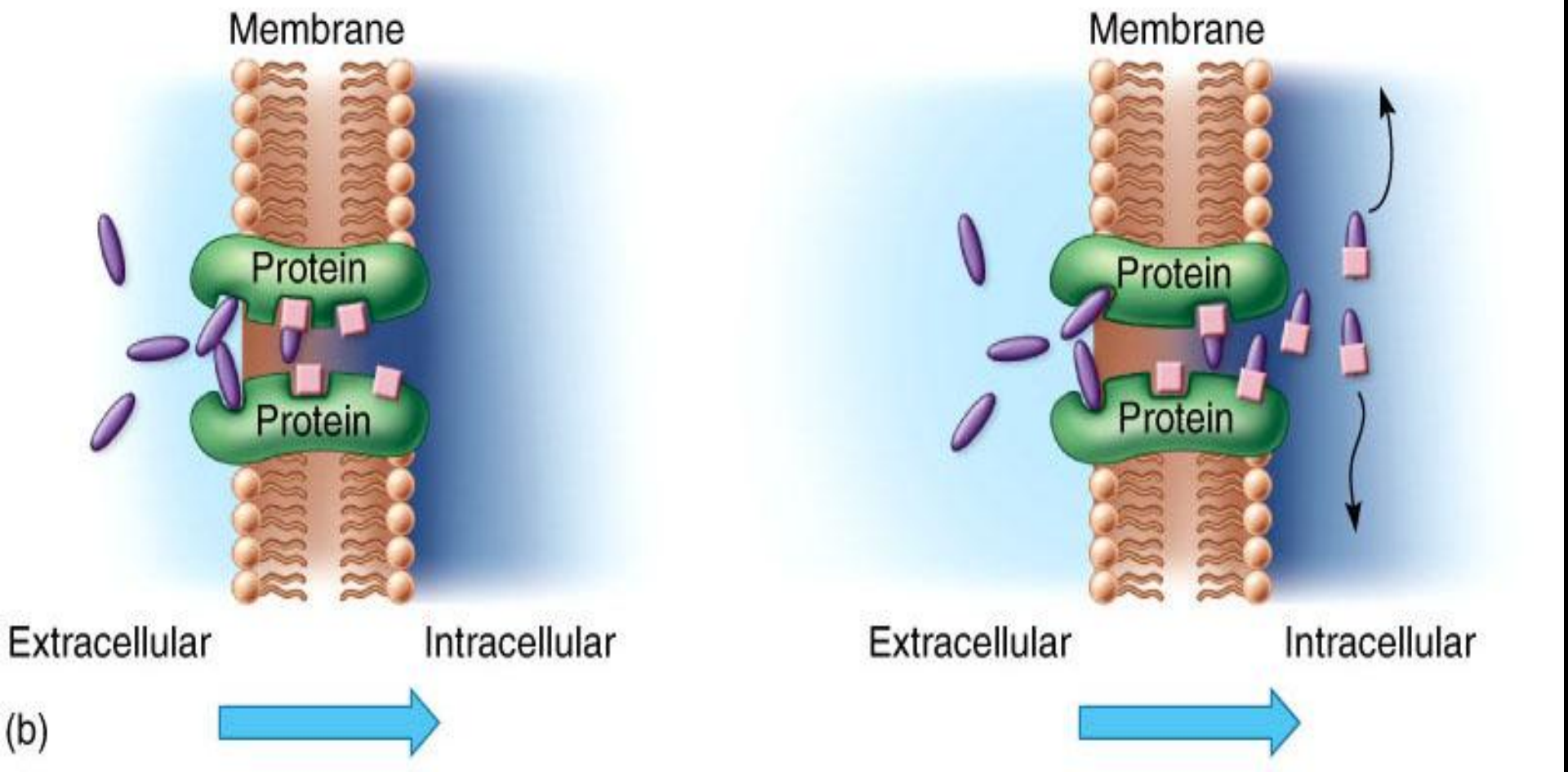
**In addition to true transport, in which a solute is moved across the membrane without change in structure, bacteria use a process called group translocation (vectorial metabolism) to effect the net uptake of certain sugars (eg, glucose and mannose), the substrate becoming phosphorylated during the transport process. In a strict sense, group translocation is not active transport because no concentration gradient is involved. This process allows bacteria to utilize their energy resources efficiently by coupling transport with metabolism. In this process, a membrane carrier protein is first phosphorylated in the cytoplasm at the expense of phosphoenolpyruvate; the phosphorylated carrier protein then binds the free sugar at theexterior membrane face and transports it into the cytoplasm, releasing it as sugar-phosphate. Such systems of sugar transport are called phosphotransferase systems . Phosphotransferase systems are also involved in movement towards these carbon sources (chemotaxis) and in the regulation of several other metabolic pathways (catabolite repression).**

# *Group Translocation*

## (vectorial metabolism)

## phosphotransferase system( glucose and mannose)





## SPECIAL TRANSPORT PROCESSES

Iron (Fe) is an essential nutrient for the growth of almost all bacteria. Under anaerobic conditions, Fe is generally in the +2 oxidation state and soluble. However, under aerobic conditions, Fe is generally in the +3 oxidation state and insoluble. The internal compartments of animals contain virtually no free Fe; it is sequestered in complexes with such proteins as **transferrin and lactoferrin. Some bacteria solve this problem by secreting siderophores—compounds that chelate Fe and** promote its transport as a soluble complex. One major group of siderophores consists of derivatives of hydroxamic acid (–CONH2OH), which chelate Fe3+ very strongly. The iron-hydroxamate complex is actively transported into the cell by the cooperative action of a group of proteins that span the outer membrane, periplasm, and inner membrane. The iron is released, and the hydroxamate can exit the cell and be used again for iron transport. Some pathogenic bacteria use a fundamentally different mechanism involving specific receptors that bind host transferrin and lactoferrin (as well as other iron-containing host proteins). The Fe is removed and transported into the cell by an energydependent process.

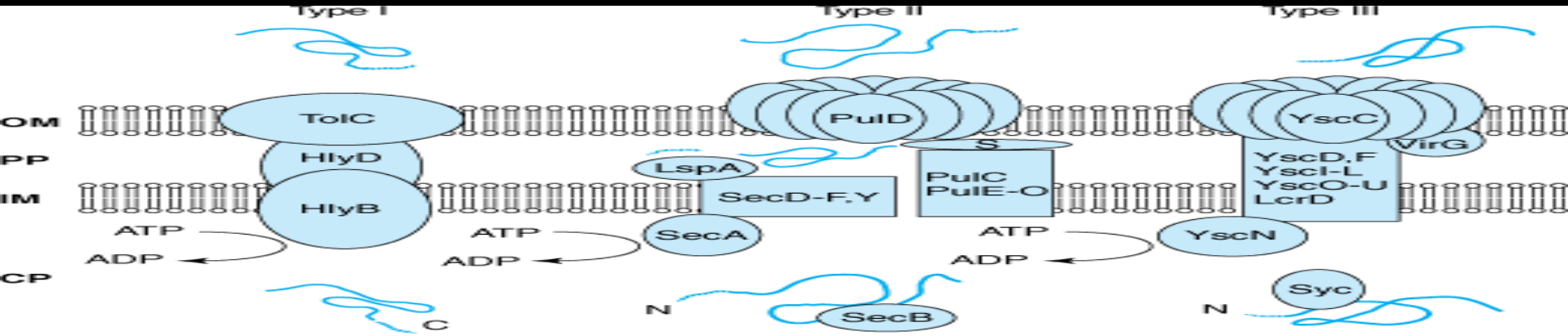
## Electron Transport and Oxidative Phosphorylation

The cytochromes and other enzymes and components of the respiratory chain, including certain dehydrogenases, are located in the cell membrane. The bacterial cell membrane is thus a functional analog of the mitochondrial membrane—a relationship which has been taken by many biologists to support the theory that mitochondria have evolved from symbiotic bacteria

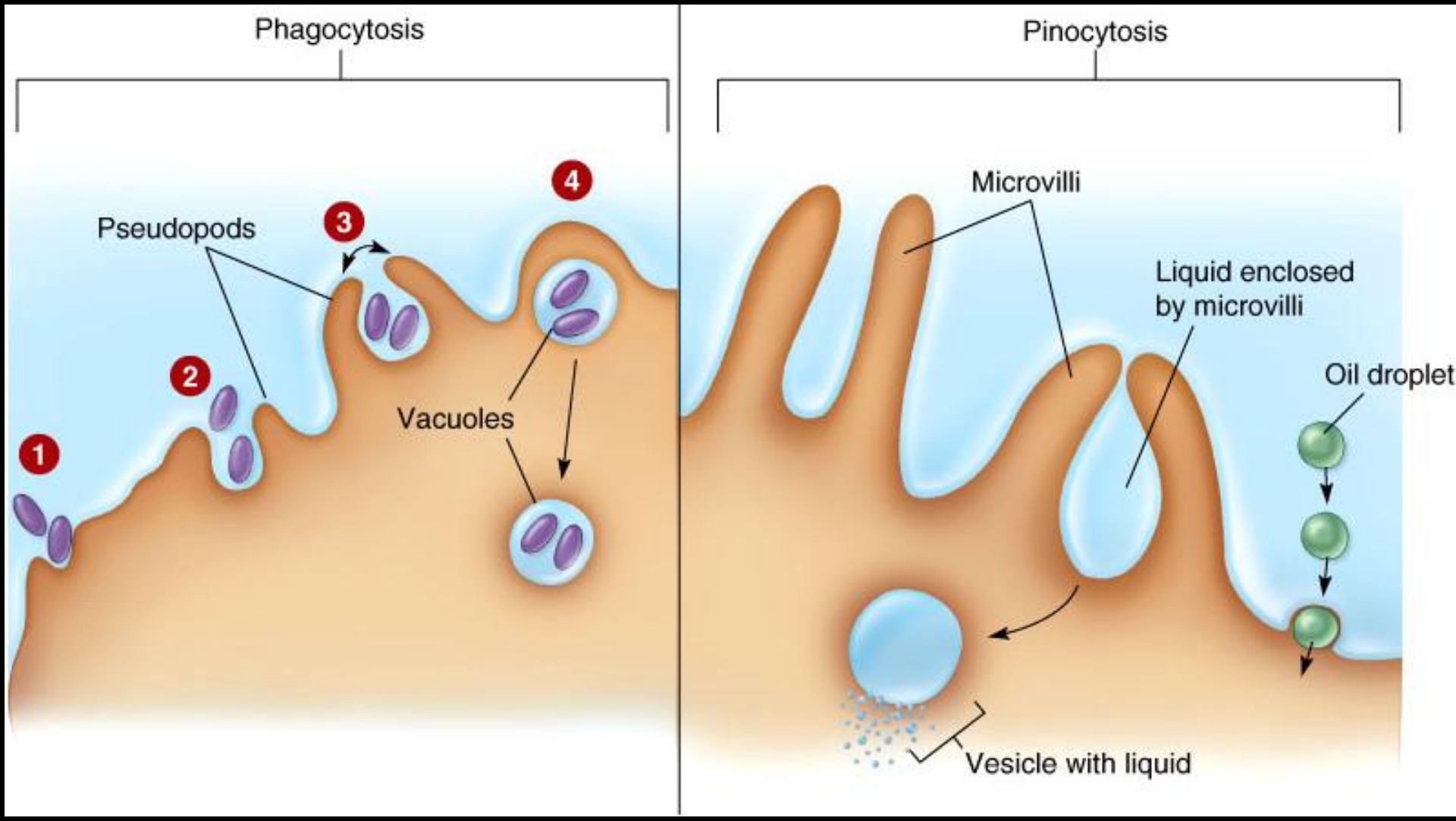
# Excretion of Hydrolytic Exoenzymes and Pathogenicity Proteins

**All organisms that rely on macromolecular organic polymers as a source of nutrients (eg, proteins, polysaccharides, lipids) excrete hydrolytic enzymes that degrade the polymers to subunits small enough to penetrate the cell membrane. Higher animals secrete such enzymes into the lumen of the digestive tract; bacteria (both gram-positive and gram-negative) secrete them directly into the external medium or into the periplasmic space between the peptidoglycan layer and the outer membrane of the cell wall in the case of gram-negative bacteria**

**In gram-positive bacteria, proteins are secreted directly, but proteins secreted by gram-negative bacteria must traverse the outer membrane as well. Five pathways of protein secretion have been described in gram-negative bacteria: the type I, type II, type III, type IV, and type V secretion systems. A schematic overview of the type I, type II, and type III systems is presented in Figure BELOW Proteins secreted by the type I and type III pathways traverse the inner membrane (IM) and outer membrane (OM) in one step, whereas proteins secreted by the type II and type V pathways cross the IM and OM in separate steps. Proteins secreted by the type II and type V pathways are synthesized on cytoplasmic ribosomes as preproteins containing an extra leader or signal sequence of 15 to 40 amino acids—most commonly about 30 amino acids—at the amino terminal and require the sec system for transport across the IM. In *E coli, the sec pathway comprises a number of IM* proteins (SecD to SecF, SecY), a cell membrane-associated ATPase (SecA) that provides energy for export, a chaperone (SecB) that binds to the preprotein, and the periplasmic signal peptidase. Following translocation, the leader sequence is cleaved off by the membrane-bound signal peptidase and the mature protein is released into the periplasmic space. In contrast, proteins secreted by the type I and type III systems do not have a leader sequence and are exported intact.**

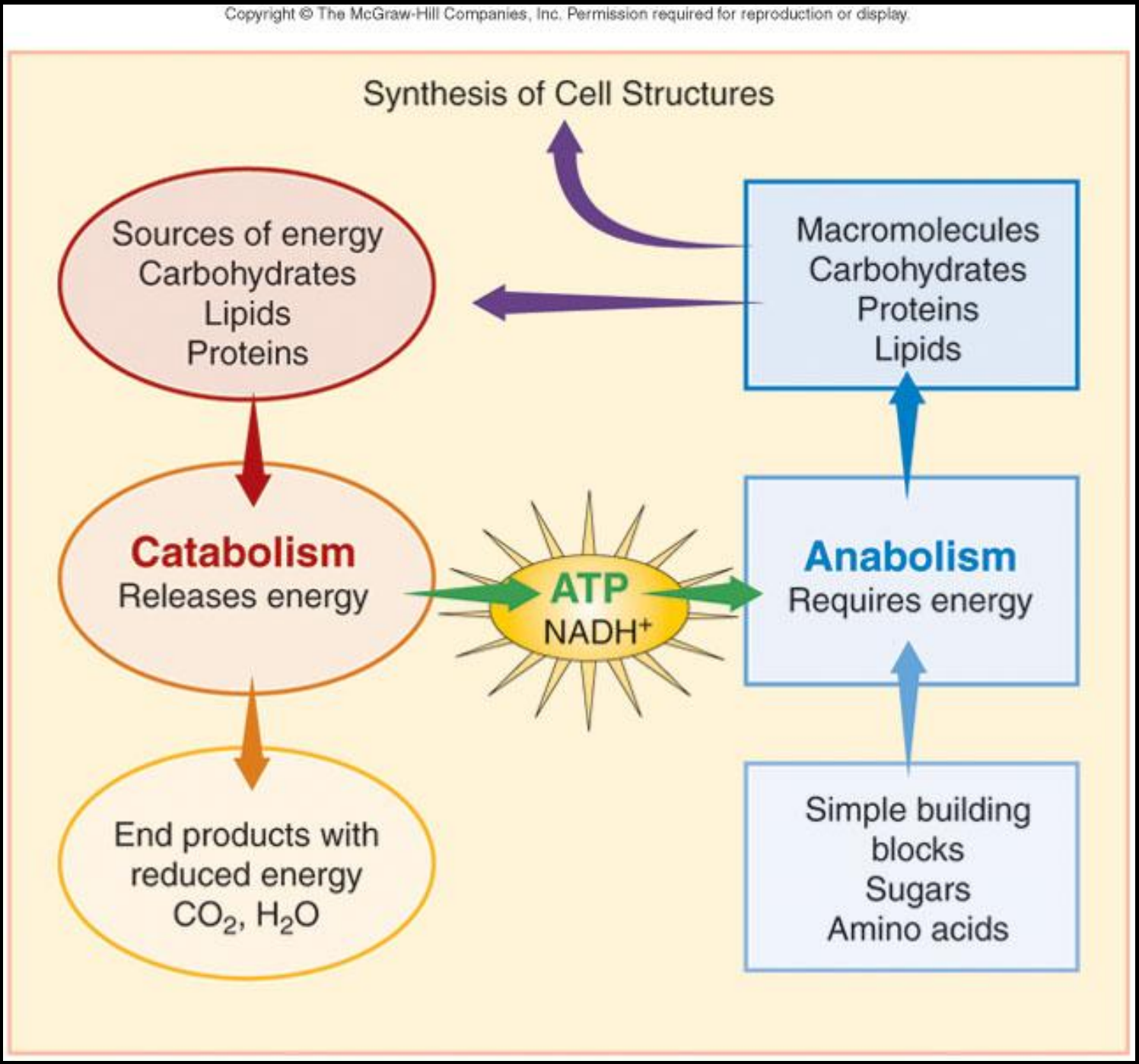


# *Phagocytosis & Endocytosis*



# *Metabolism - The sum total of all chemical reactions & physical workings occurring in a cell*

- 1. Catabolism ( Catabolic )
  - breakdown of complex organic molecules into simpler compounds
  - releases **ENERGY**
- 2. Anabolism ( Anabolic )
  - the building of complex organic molecules from simpler ones
  - requires **ENERGY**



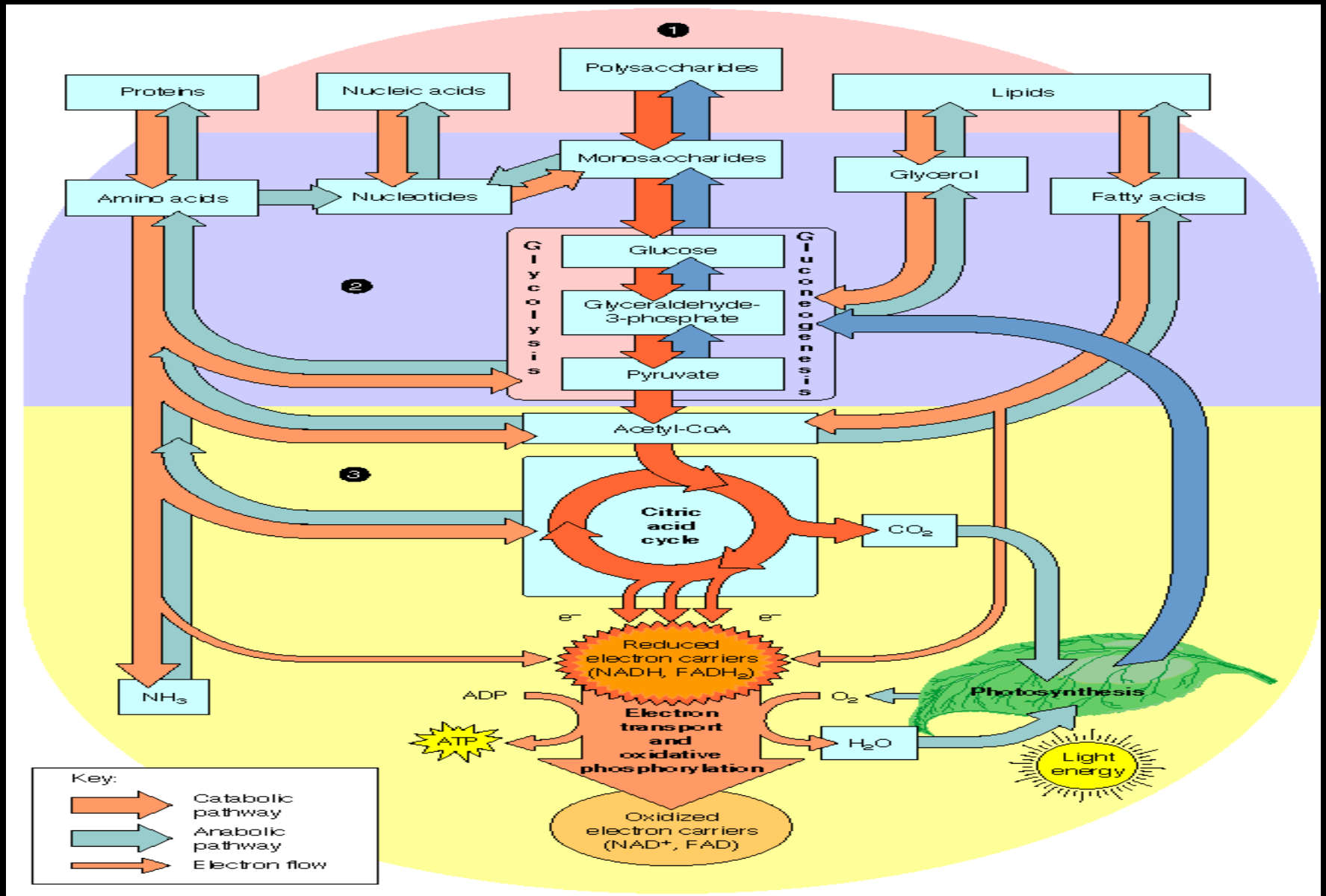
# *What are nutrients that bacteria want?*

C → Sugar, Lipid → Energy, Biosynthesis

N → Protein → Biosynthesis

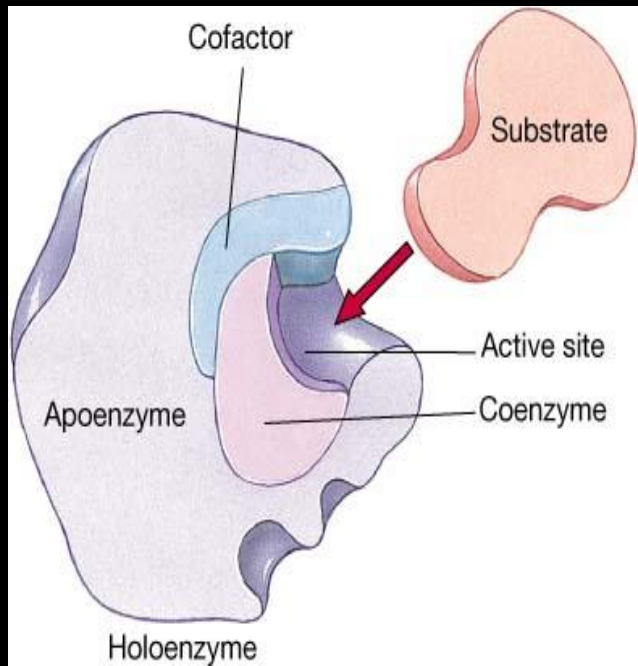
O → Air → Energy

# Overview of Metabolism



# *Enzymes - catalysts that speed up and direct chemical reactions*

- Simple enzymes – consist of protein alone
- Conjugated enzymes or holoenzymes – contain protein and nonprotein molecules
  - apoenzyme –protein portion
  - cofactors – nonprotein portion
    - metallic cofactors – iron, copper, magnesium
    - coenzymes -organic molecules - vitamins
- A. Enzymes are substrate specific
  - Lipases Lipids
  - Sucrases Sucrose
  - Ureases Urea
  - Proteases Proteins
  - DNases DNA



# Enzyme Components

2 Parts

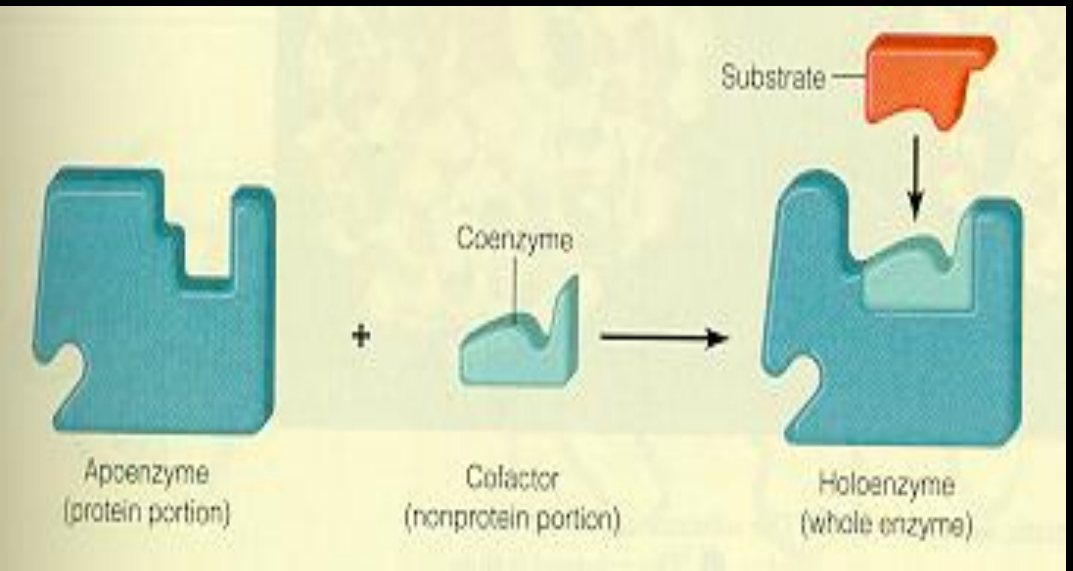
1. Apoenzyme - protein portion

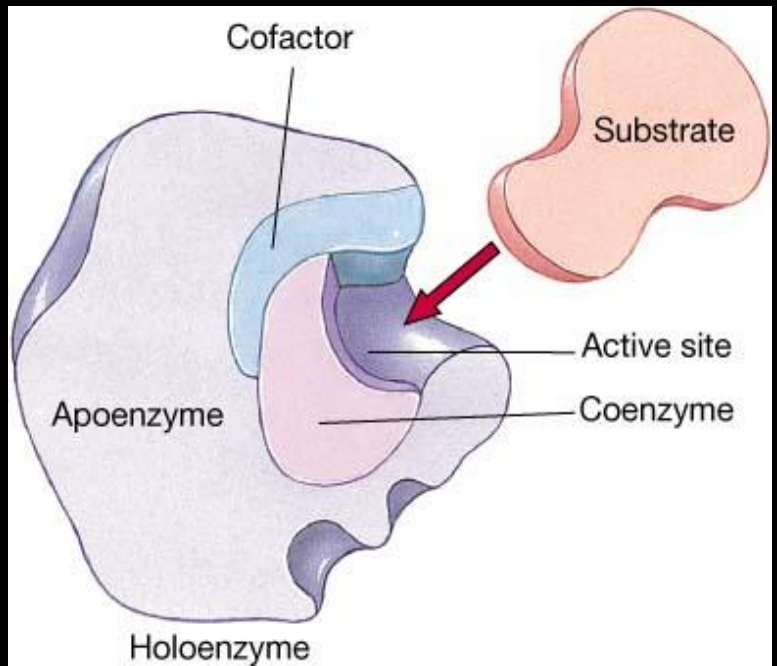
2. Coenzyme (cofactor) - non-protein

* metallic cofactors – iron, copper, magnesium

* coenzymes -organic molecules - vitamins

## Holoenzyme - whole enzyme



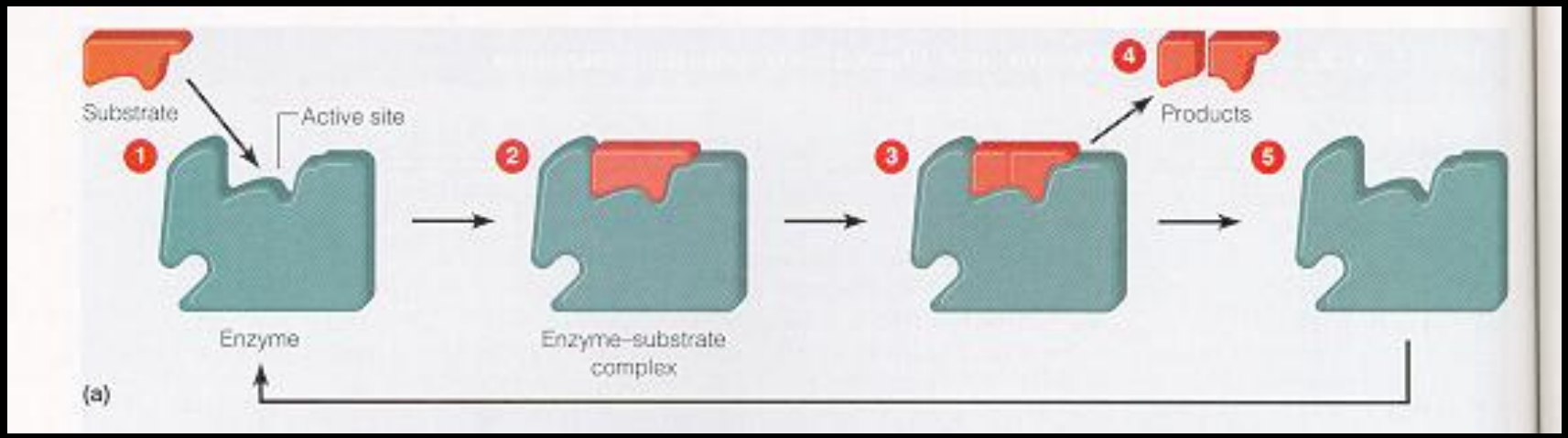



# *Types of Enzymes*

**Constitutive enzymes** – always present, always produced in equal amounts or at equal rates, regardless of amount of substrate; enzymes involved in glucose metabolism

**Induced enzymes** – not constantly present, produced only when substrate is present, prevents cell from wasting resources

Enzyme Specificity can be explained by the Lock and Key Theory

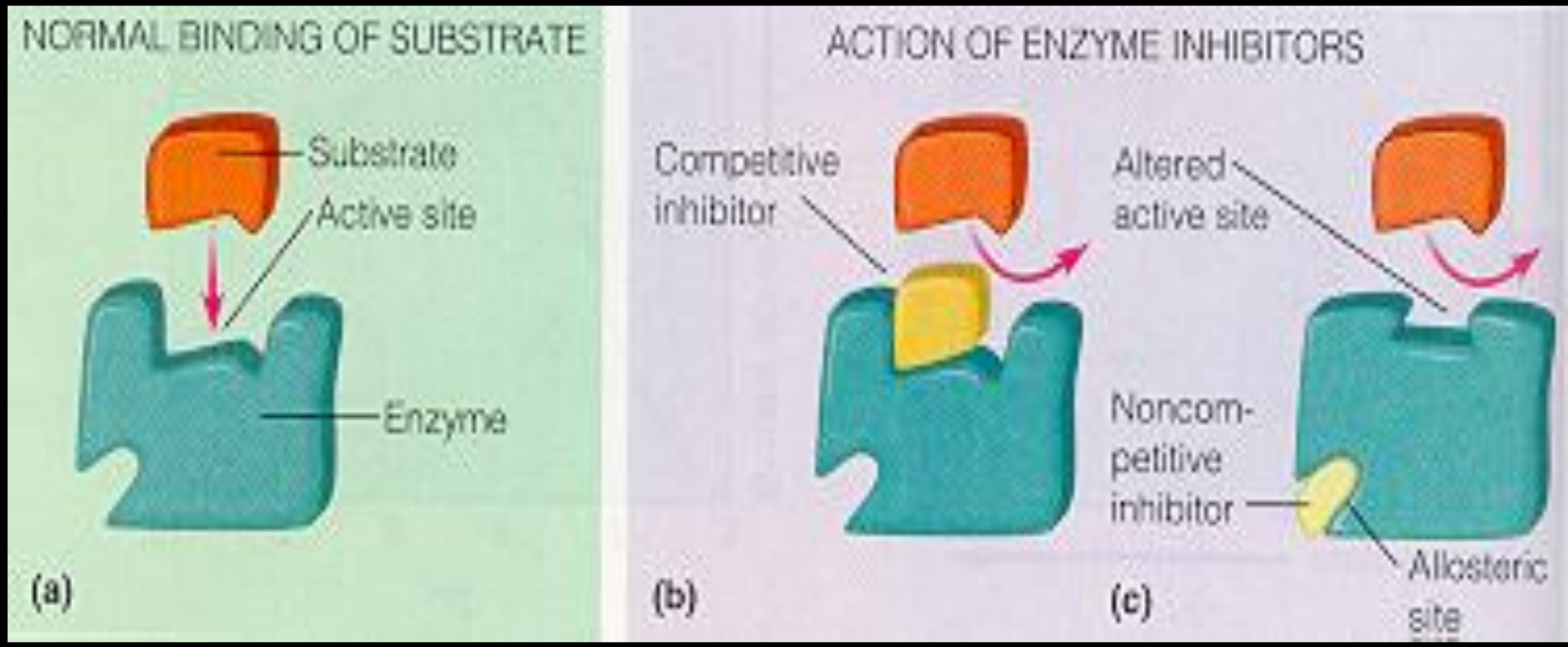



E + S -----> ES ------> E + P

Inhibitors can effect enzymatic activity

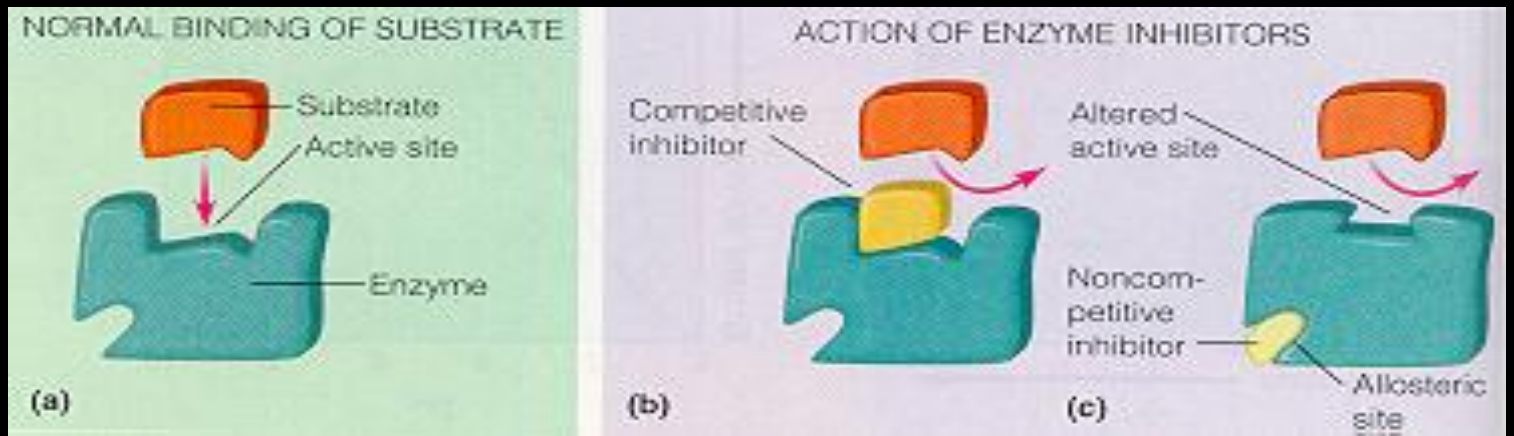
1. Competitive Inhibitors

2. Noncompetitive Inhibitors

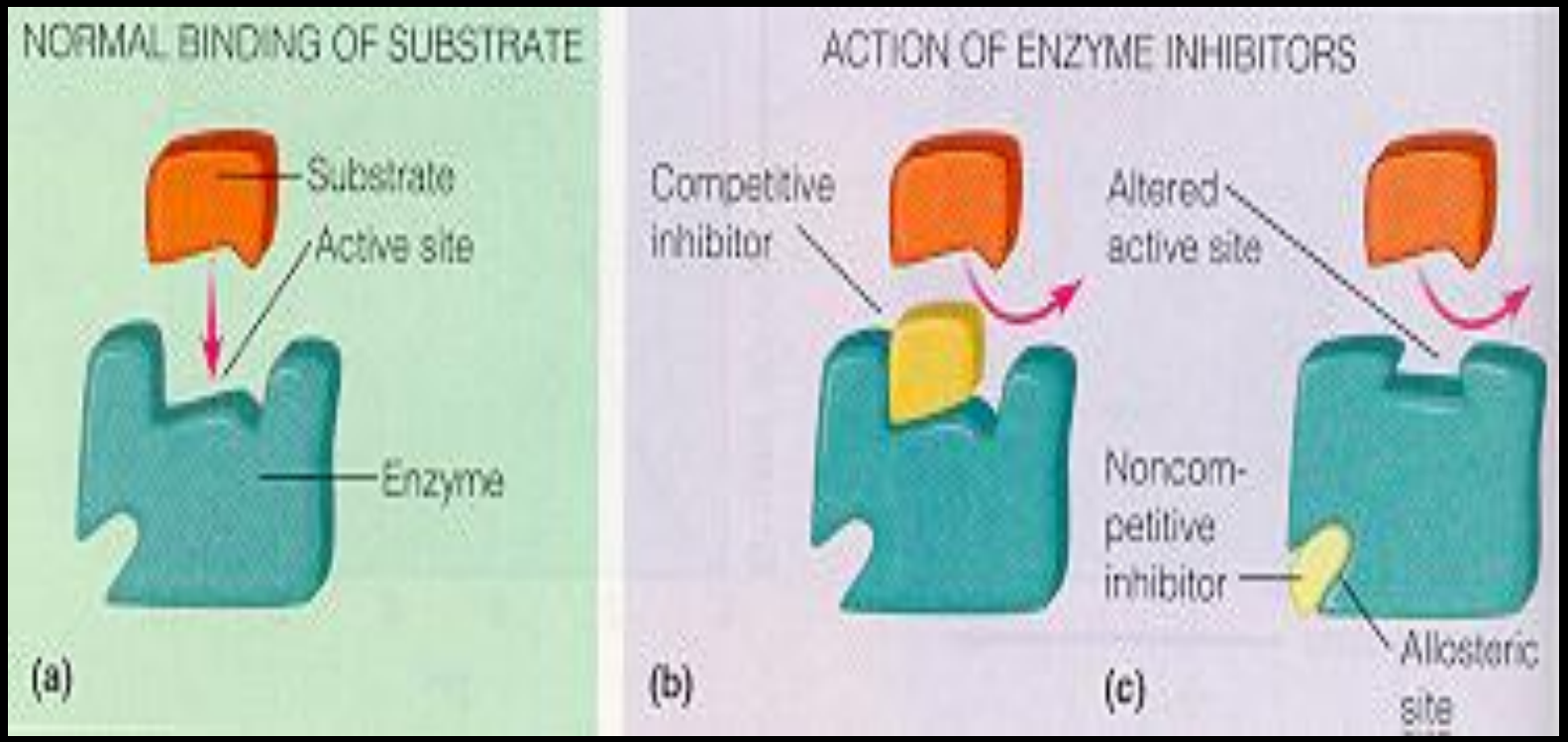


# *Competitive Inhibitors -compete for the active site*

- 1. Penicillin
  - competes for the active site on the enzyme involved in the synthesis of the pentaglycine crossbridge
- 2. Sulfanilamide (Sulfa Drugs)
  - competes for the active site on the enzyme that converts PABA into Folic Acid
    - Folic Acid - required for the synthesis of DNA and RNA



# Non-competitive Inhibitors - attach to an allosteric site



# Control of enzyme activity

1-Competitive inhibition – substance that resembles normal substrate competes with substrate for active site

2-Feedback inhibition – concentration of product at the end of a pathway blocks the action of a key enzyme

3-Feedback repression – inhibits at the genetic level by controlling synthesis of key enzymes

4-Enzyme induction – enzymes are made only when suitable substrates are present

# *Energy Production*

- 1. Oxidation
  - refers to the loss of Hydrogens and or electrons
- 2. Reduction
  - the gain of Hydrogens and or electrons
- Reduction and Oxidation (redox reaction)
  - Reduction and oxidation always occur together. In a reduction-oxidation reaction (redox reaction), one substance gets reduced, and another substance gets oxidized. The thing that gets oxidized is called the electron donor, and the thing that gets reduced is called the electron acceptor.

# ROLE OF METABOLISM IN BIOSYNTHESIS & GROWTH

Microbial growth requires the polymerization of biochemical building blocks into proteins, nucleic acids, polysaccharides, and lipids. The building blocks must come preformed in the growth medium or must be synthesized by the growing cells. Additional biosynthetic demands are placed by the requirement for coenzymes that participate in enzymatic catalysis.

Biosynthetic polymerization reactions demand the transfer of anhydride bonds from ATP. Growth demands a source of metabolic energy for the synthesis of anhydride bonds and for the maintenance of transmembrane gradients of ions and metabolites.

**The biosynthetic origins of building blocks and coenzymes can be traced to relatively few precursors, called focal metabolites.**

**focal metabolites**

**glucose 6-phosphate,** phosphoenolpyruvate, oxaloacetate, and -ketoglutarate give rise to most biosynthetic end products. Microbial metabolism

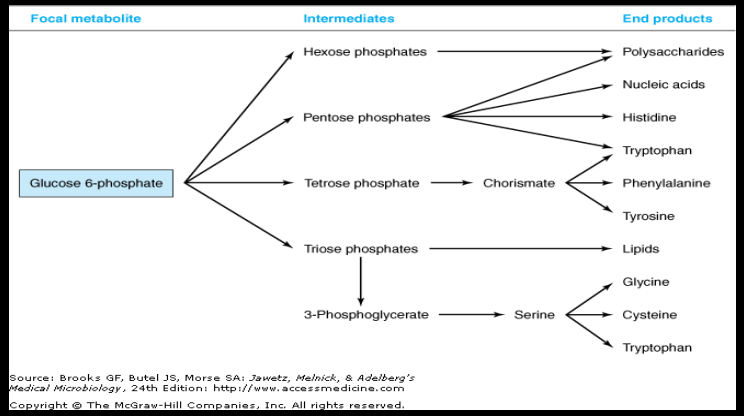
can be divided into four general categories:

(1) pathways for the interconversion of focal metabolites,

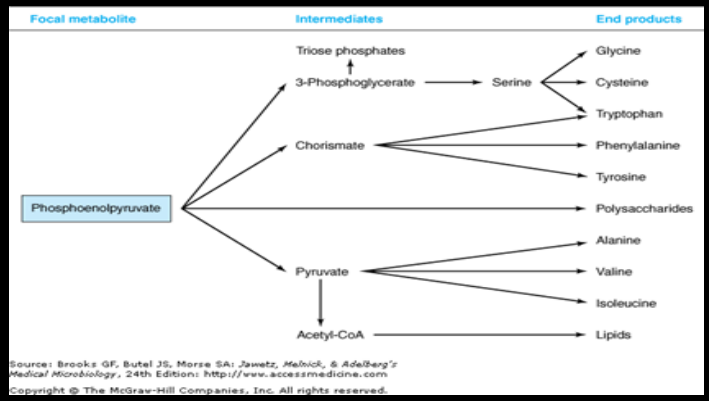
(2) (2) assimilatorypathways for the formation of focal metabolites,

(3) (3) biosynthetic sequences for the conversion of focal metabolites to end products,

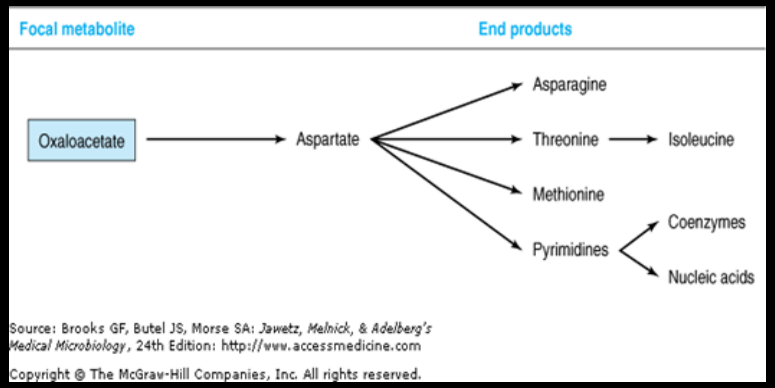
(4) and (4) pathways that yield metabolic energy for growth and maintenance.



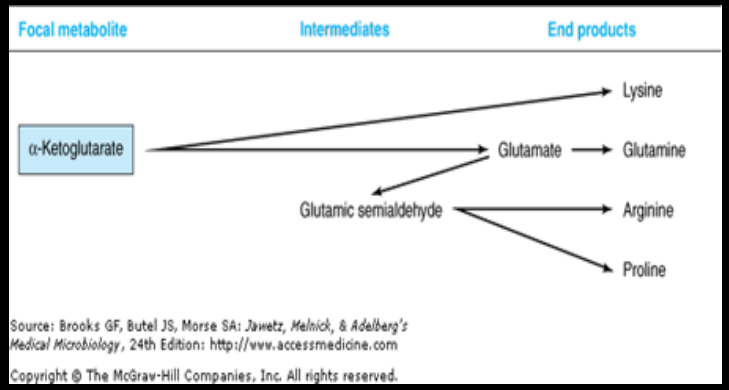

**Biosynthetic end products formed from glucose 6-phosphate. Carbohydrate phosphate esters of varying chain length serve as intermediates in the biosynthetic pathways.**




# Biosynthetic end products formed from phosphoenolpyruvate




- Biosynthetic end products formed from oxaloacetate. The end products aspartate, threonine, and pyrimidines serve as intermediates in the synthesis of additional compounds.




- Biosynthetic end products formed from -ketoglutarate.

# *Carbohydrate Catabolism*

- Microorganisms oxidize carbohydrates as their primary source of energy
- Glucose - most common energy source
- Energy obtained from Glucose by:
  - Respiration
  - Fermentation

# *Carbon Source*

The most Carbon source of bacteria is “Glucose”

- The major carbohydrate-metabolizing pathway are

1. Embden–Meyerhof–Parnas (EMP) pathway, glycolysis
2. Entner–Doudoroff (ED) pathway
3. Pentose phosphate (PP) pathway
4. Tricarboxylic acid cycle, TCA cycle, Kreb’s cycle, citric acid cycle

# *Aerobic Cellular Respiration*

- Electrons released by oxidation are passed down an Electron Transport System with oxygen being the Final Electron Acceptor

- General Equation:

- Glucose + oxygen----> Carbon dioxide + water
- 
- ATP

# *Aerobic Cellular Respiration*

- 4 subpathways

- 1. Glycolysis
- 2. Transition Reaction
- 3. Kreb's Cycle
- 4. Electron Transport System

# *Anaerobic Respiration*

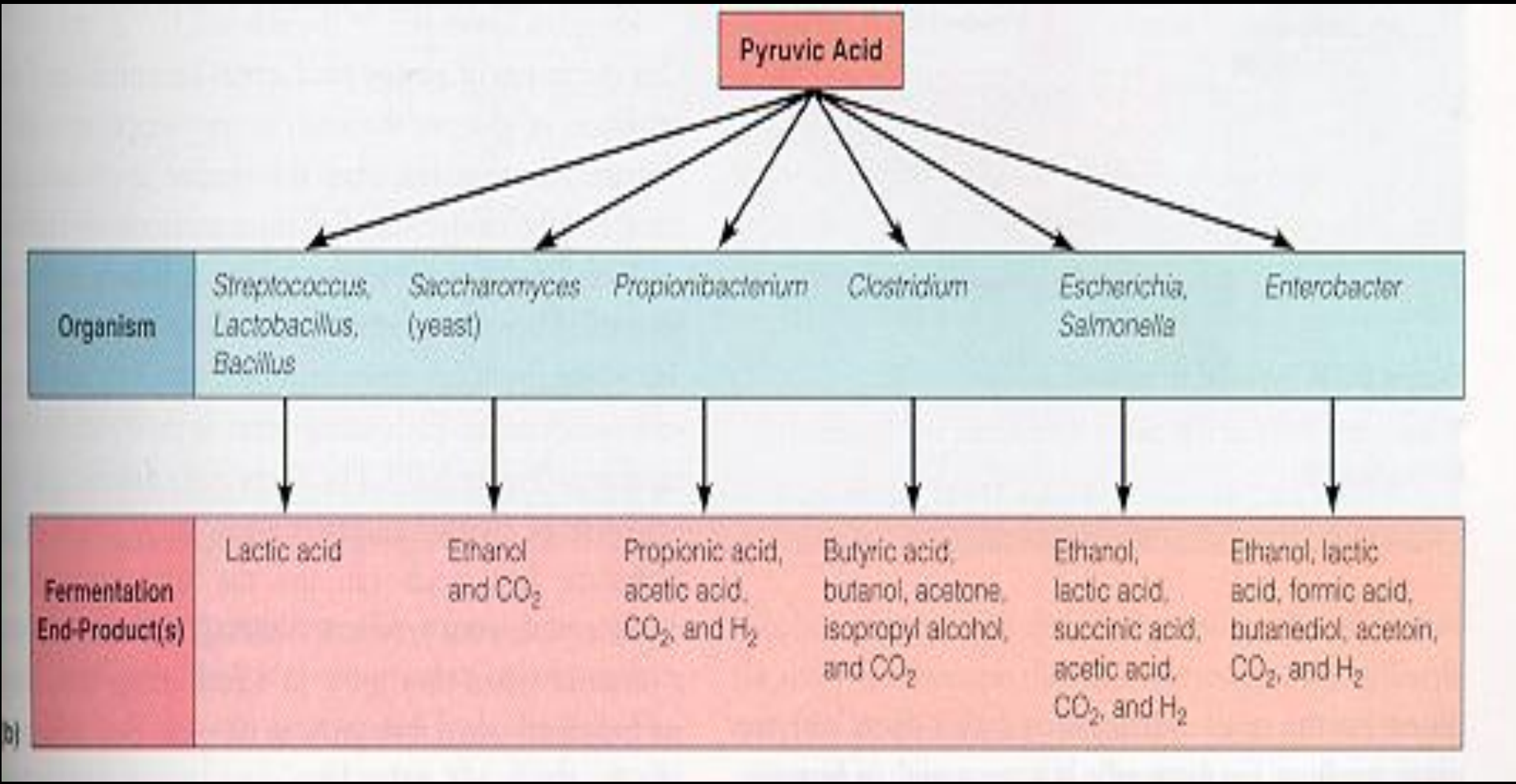
- Electrons released by oxidation are passed down an E.T.S., but oxygen **is not** the final electron acceptor

- Nitrate ($NO_3$-) ----> Nitrite ($NO_2$-)
- Sulfate ($SO^2_4$-) ----> Hydrogen Sulfide ($H_2S$)
- Carbonate ($CO^2_4$-) -----> Methane ($CH_4$)

# *Fermentation*

- Incomplete oxidation of glucose or other carbohydrates in the absence of oxygen
- Uses organic compounds as terminal electron acceptors
- Yields a small amount of ATP
- Production of ethyl alcohol by yeasts acting on glucose
- Formation of acid, gas & other products by the action of various bacteria on pyruvic acid
- **Glycolysis - plus an additional step**

# Fermentation End Products



# *1. Lactic Acid Fermenation*

- Food Spoilage
- Food Production
- 2 Genera:
  - *Streptococcus*
  - *Lactobacillus*

# *2. Alcohol Fermentation*

- Alcoholic Beverages
- Bread dough to rise
- *Saccharomyces cerevisiae* (Yeast)

# *3. Mixed - Acid Fermentation*

- End products - "FALSE"
- *Escherichia coli* and other enterics

# *4-Propionic Acid Fermentation*

- End Products:
  - Propionic acid
  - $CO_2$
- Propionibacterium sp.

# *Photosynthesis - conversion of light energy from the sun into chemical energy by* **lithotrophs**

- Chemical energy is used to reduce $CO_2$ to sugar ($CH_2O$)
- Carbon Fixation - recycling of carbon in the environment (Life as we known is dependant on this)
- Photosynthesis by lithotrophs
  - Green Plants
  - Algae
  - Cyanobacteria

- $6\ CO_2 + 6\ H_2O + \text{sunlight} \text{ -----> } C_6H_{12}O_6 + 6\ O_2$
- 2 Parts:
  - 1. Light Reaction
  - 2. Dark Reaction

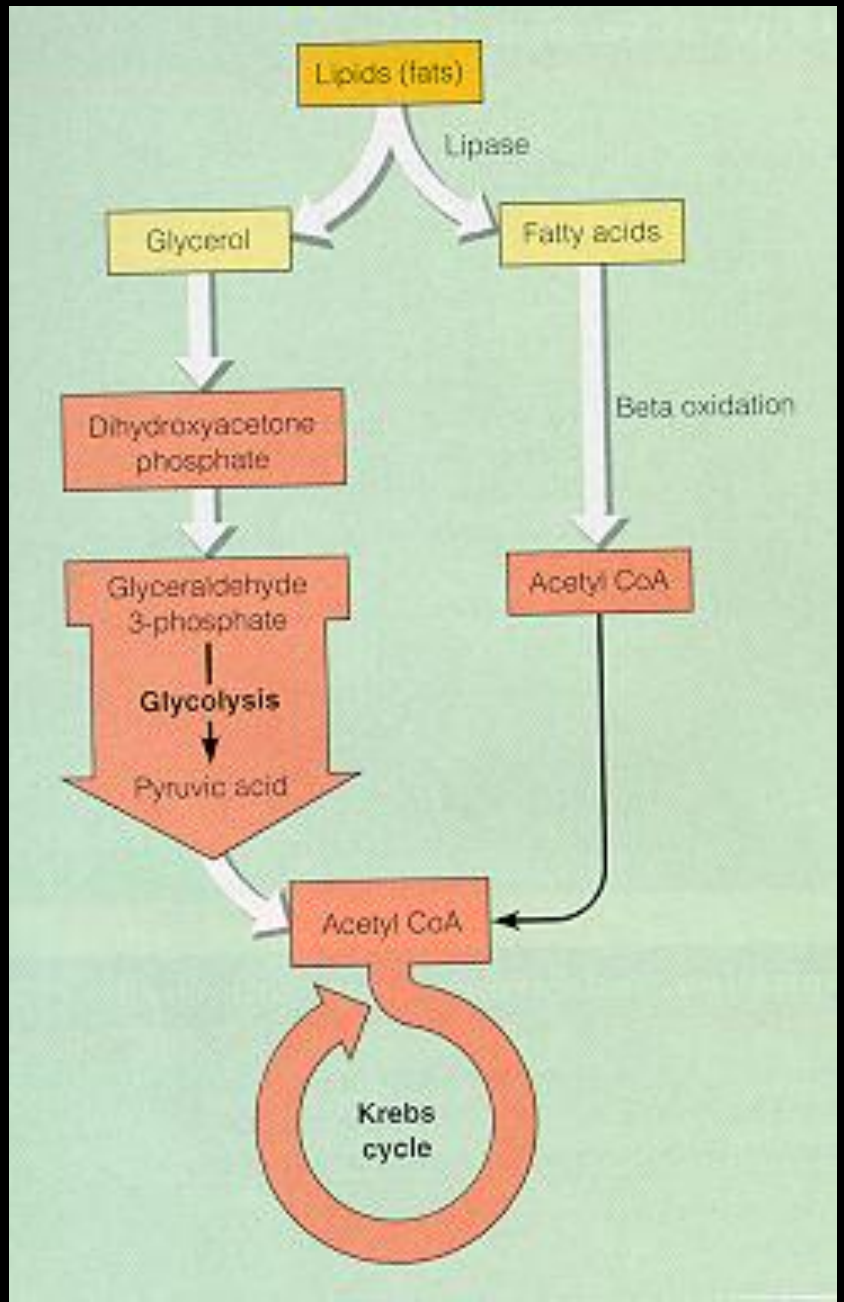
# *Lipid Metabolism*

- Lipids are essential to the structure and function of membranes
- Lipids also function as energy reserves, which can be mobilized as sources of carbon
- 90% of this lipid is “triacyglycerol”

triacyglycerol $\xrightarrow{\text{lipase}}$ glycerol + 3 fatty acids

- The major fatty acid metabolism is “β-oxidation”

# Lipid Catabolism

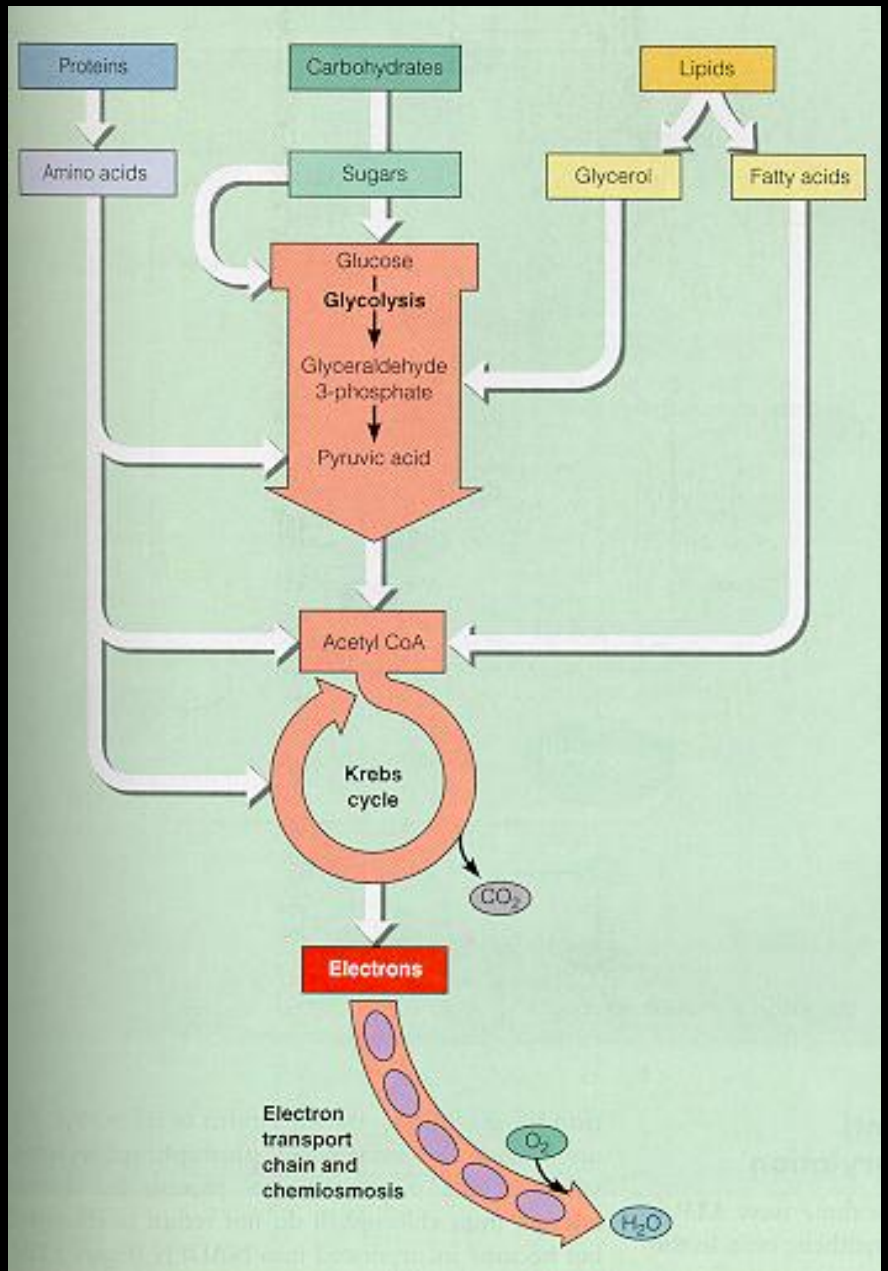


# *Nitrogen Metabolism*

- **Nitrogen** is an essential element of biological molecules, such as amino acids, nucleotides, proteins, and DNA

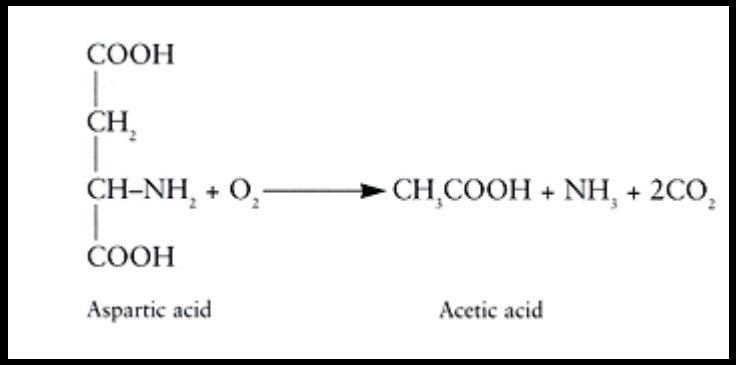
- Bacteria vary widely in their ability to utilize various sources of nitrogen for synthesis of proteins

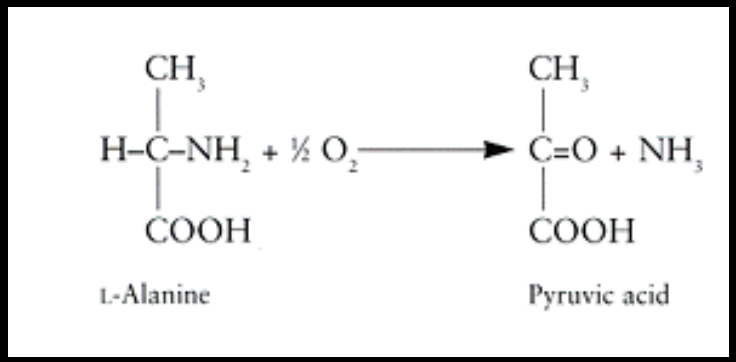
# Protein Catabolism



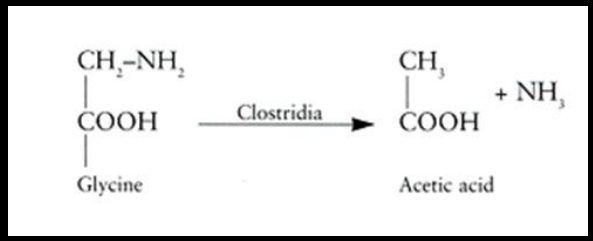
# *Pathways Involved in Nitrogen Utilization*

1. Protein Digestion – by proteinase and peptidase
2. Oxidative Deamination

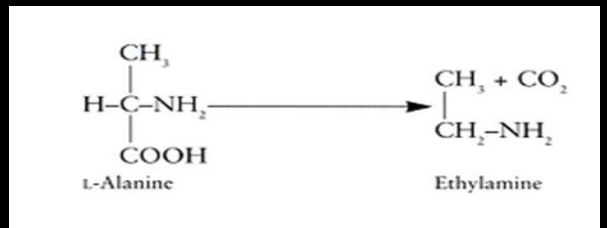


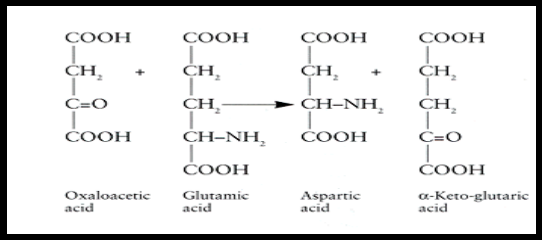

3. Reductive Deamination




4. Decarboxylation

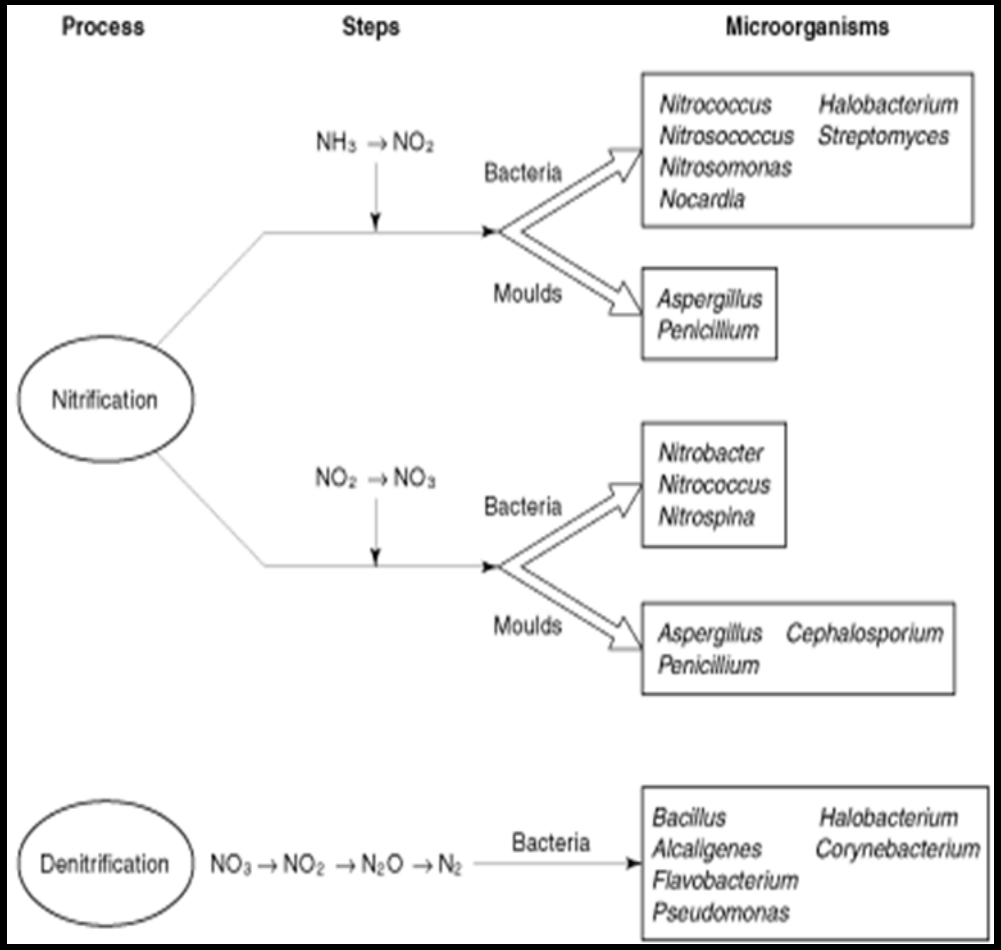


# 5. Transamination Reactions




# 6. Nitrification

# 7. Denitrification



# *Benefits of studying metabolism of bacteria in food microbology*

1. **Can prolong shelf life of food product by control or block enzyme of the pathway (Chemical Preservatives)**
2. **Can be used to detection contamination by looking at metabolic activity**
3. **Used for diagnosis of microorganisms by biochemical tests**
4. **Used for study a kinetics of microorganisms**

# *Chemical Preservative*

1. Benzoic Acid

   Inhibit to enzyme in Glycolysis and TCA pathway

2. Sorbic Acid

   Inhibition by sorbic acid may cause cell death, slowing of growth

# *Metabolic Activity Test*

1. Adenosine Triphosphate Assay

   - ATP bioluminescence has been applied for determining microbial quality of both raw and finished food products

2. Catalase Test

This test has been applied in monitoring microbial contamination of raw materials, food samples from in-plant production lines and finished food products.

3. Electrical Impedance Test

The most common application of impedance test is for estimating the aerobic plate count of food samples

4. Microcalorimetry

This technique has been applied in the study of microbial spoilage of ground beef and canned foods, and estimation of bacteria in milk and meat products.

5. Pyruvate Estimation

Pyruvate estimation has been applied mainly to determine the adequacy of sanitation practices in milk production and the bacteriological quality of raw and pasteurized milk