RAMSADAY COLLEGE

Semester-3
Core Course

CC-3/GE3: MICROBIAL METABOLISM (THEORY)

MCB-G-CC-3-3-TH

Unit 2 Nutrient Uptake And Transport

THE COMMON FEATURES OF NUTRIENT UPTAKE BY BACTERIA:

Bacteria can only take in dissolved molecules. Uptake mechanisms are specific; that is, the necessary substances, and not others, are acquired.

❖They take carbon, oxygen, hydrogen, nitrogen, sulfur, and phosphorus as **macroelements or macronutrients** because they are required in relatively large amounts. **They are found in organic molecules such as proteins, lipids, nucleic acids, and carbohydrates.** Other macroelements are potassium, calcium, magnesium, and iron. **They exist as cations and generally are associated with and contribute to the activity and stability of molecules and cell structures such as enzymes and ribosomes. Thus they are important in many cellular processes, including protein synthesis and energy conservation.**

❖ Other elements are required in small amounts- in nature, they are ubiquitous and usually present in adequate amounts to support the growth of microbes. Microbiologists call these elements **micronutrients** or **trace elements.** The **micronutrients—manganese, zinc, cobalt, molybdenum, nickel, and copper—are needed by most cells. Micronutrients are part of certain enzymes, and they aid in catalysis of reactions and maintenance of protein structure.**

Some microbes are able to synthesize all the organic molecules they need from macroelements. However, some microbes are unable to synthesize certain molecules needed for survival. These molecules are called **growth factors, and they** must be obtained from the environment. There are three types of growth factors: amino acids, purines and pyrimidines, and vitamins.

Bacteria use several different transport mechanisms to uptake nutrients from their environment:

1) passive diffusion,
2) facilitated diffusion,
3) primary and secondary active transport, and
4) group translocation.

1) Passive Diffusion

➢**Passive diffusion, often called diffusion or simple diffusion, is the process by which molecules move from a region of higher concentration to one of lower concentration; that is, the molecules move down the concentration gradient.**

➢The rate of passive diffusion depends on the size of the concentration gradient between a cell's exterior and its interior. **A large concentration gradient is required for adequate nutrient uptake by passive diffusion (i.e., the external nutrient concentration must be high while the internal concentration is low).**

➢The rate of diffusion decreases as more nutrient accumulates in the cell, unless the nutrient is used immediately upon entry.

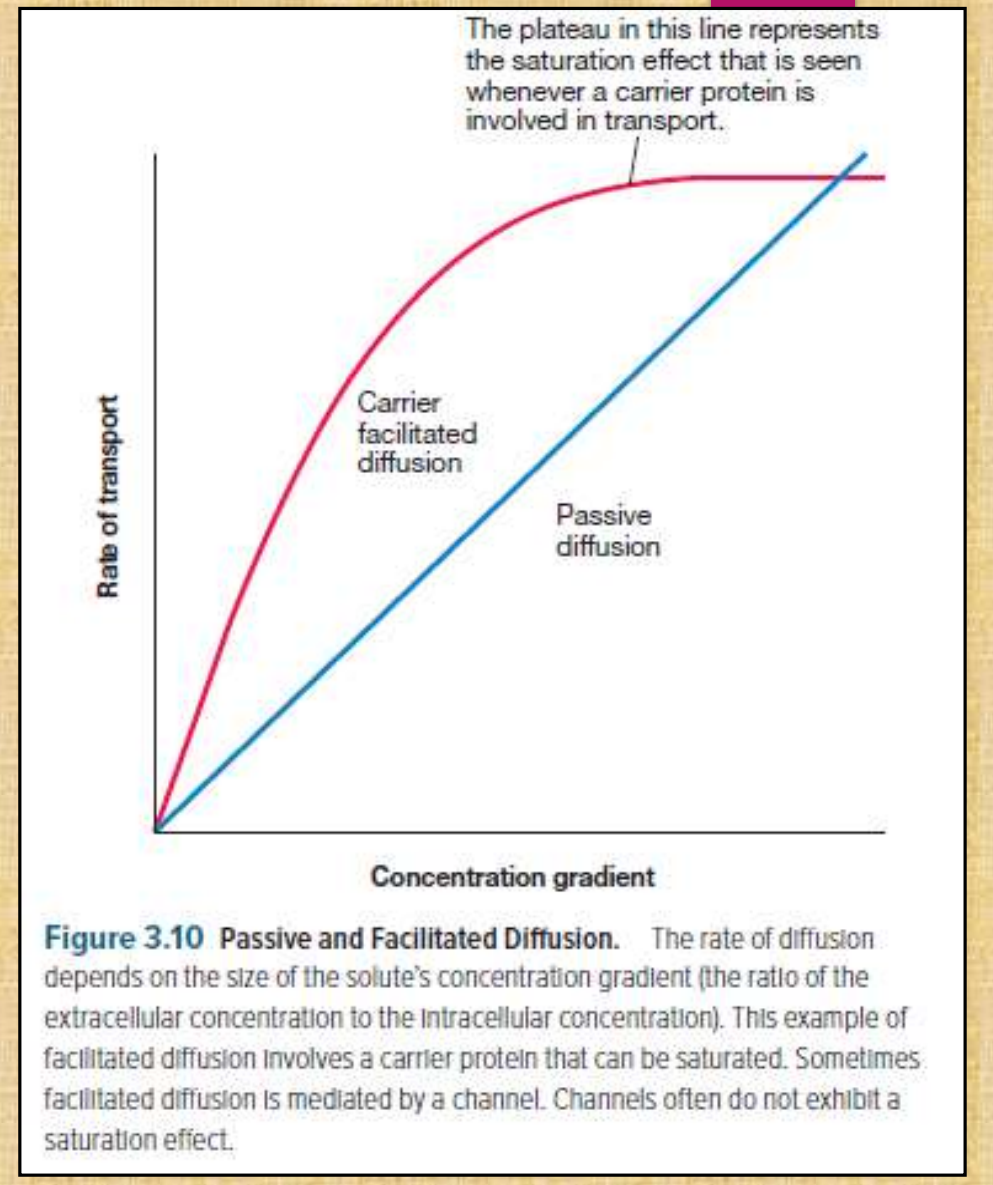


Figure 3.10 Passive and Facilitated Diffusion. The rate of diffusion depends on the size of the solute's concentration gradient (the ratio of the extracellular concentration to the intracellular concentration). This example of facilitated diffusion involves a carrier protein that can be saturated. Sometimes facilitated diffusion is mediated by a channel. Channels often do not exhibit a saturation effect.

Most substances cannot freely diffuse into a cell. However, **some gases, including O_2 and CO_2, easily cross the plasma membrane by passive diffusion. H_2O also moves across membranes by passive diffusion**. This is important because it allows the cell to adjust to differences in solute concentrations. **Larger molecules, ions, and polar substances must enter the cell by other mechanisms, all of which involve specialized proteins that are referred to as transport proteins.**

TRANSPORT PROTEINS:

Bacterial cells employ a variety of transport proteins in their uptake mechanisms. These important proteins are embedded in membranes and are classified into several types. The two major types are

a) channels and
b) carriers.

Channels are proteins that form pores in membranes through which substances can pass; they are often involved in facilitated diffusion. Channels show some specificity for the substances that pass through them, but this is considerably less than that shown by carriers, which are far more substrate specific.

Carriers are so named because they carry nutrients across the membrane.

2) Facilitated Diffusion:

✓During **facilitated diffusion, substances move** across the plasma membrane with the **assistance of transport proteins** that are either channels or carriers. The rate of facilitated diffusion increases with the concentration gradient much more rapidly and at lower concentrations of the diffusing molecule than that of passive diffusion (figure 3.10).

✓The resulting curve resembles an enzyme-substrate curve and is different from the linear response seen with passive diffusion.

✓**An example of channel-mediated facilitated diffusion is that involving aquaporins which transport water.** Aquaporins are members of the major intrinsic protein (MIP) family of proteins. MIPs facilitate diffusion of small polar molecules, and they are observed in virtually all organisms.

✓**Facilitated diffusion is truly diffusion, even though a transport protein is involved. A concentration gradient spanning the membrane drives the movement of molecules, and no metabolic energy input is required**

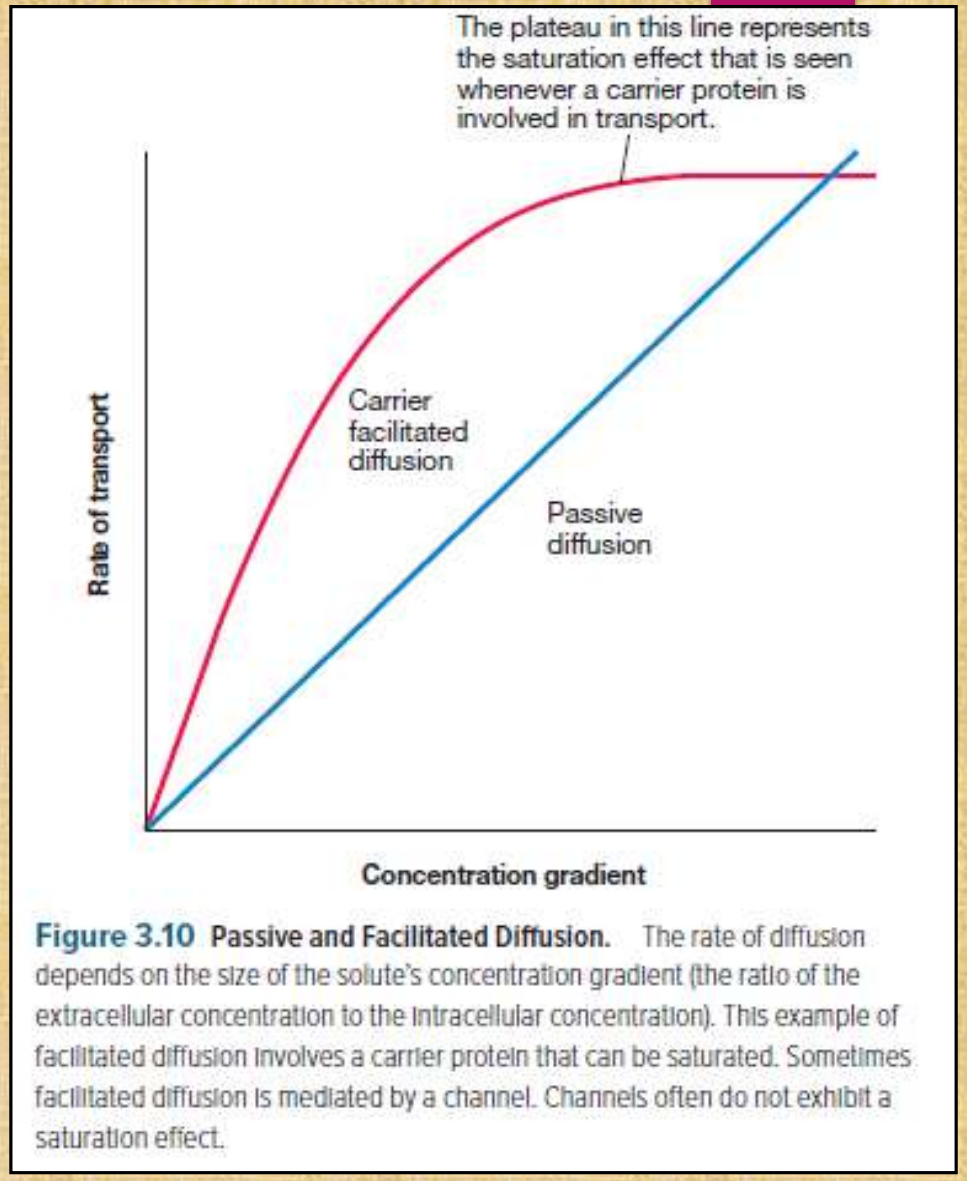


Figure 3.10 Passive and Facilitated Diffusion. The rate of diffusion depends on the size of the solute's concentration gradient (the ratio of the extracellular concentration to the intracellular concentration). This example of facilitated diffusion involves a carrier protein that can be saturated. Sometimes facilitated diffusion is mediated by a channel. Channels often do not exhibit a saturation effect.

If the concentration gradient disappears, net inward movement ceases. The gradient can be maintained by transforming the transported nutrient to another compound, as occurs when a nutrient is metabolized.

After the **solute molecule binds to the outside, the carrier is thought to change conformation and release the molecule on the cell interior (figure 3.11).**

The carrier subsequently changes back to its original shape and is ready to pick up another molecule. The net effect is that a hydrophilic molecule can enter the cell in response to its concentration gradient.

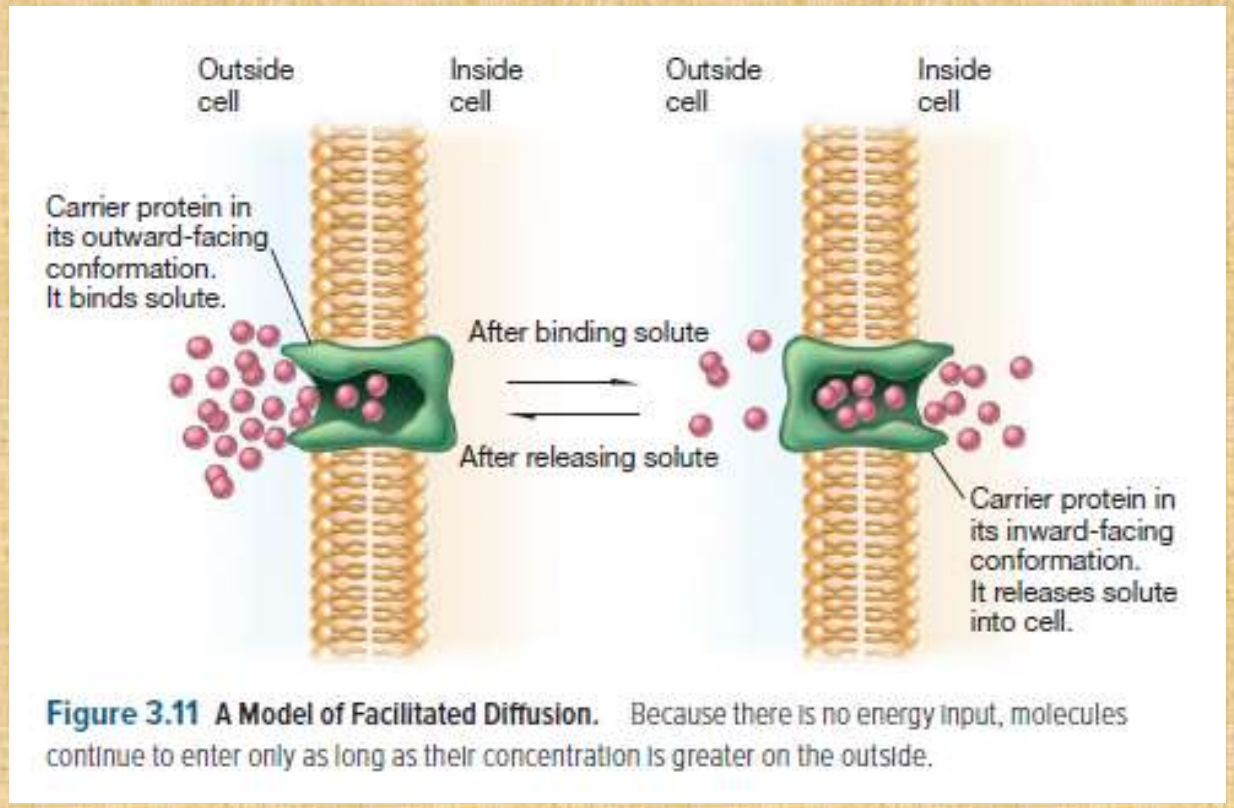


Figure 3.11 A Model of Facilitated Diffusion. Because there is no energy input, molecules continue to enter only as long as their concentration is greater on the outside.

3) Primary and Secondary Active Transport

Active transport is the transport of solute molecules to higher concentrations (i.e., **against a concentration gradient)** with the input of metabolic energy. Three types of active transport are observed in bacteria:

a) primary active transport,
b) secondary active transport, and
c) group translocation.

They differ in terms of the energy used to drive transport and on whether or not the transported molecule is modified as it enters.

Similarities and Distinguishing Features Between Active transport and Facilitated Diffusion:

Active transport resembles facilitated diffusion in that it involves carrier proteins. Active transport is also characterized by the carrier saturation effect at high solute concentrations (figure 3.10).

Nevertheless, active transport differs from facilitated diffusion because it uses metabolic energy and can concentrate substances. Metabolic inhibitors that block energy production inhibit active transport but do not immediately affect facilitated diffusion.

a) Primary active transport is mediated by carriers called primary active transporters. They use energy provided by ATP Hydrolysis to move substances against a concentration gradient without modifying them.

Primary active transporters are **uniporters;** that is, they move a single molecule across the membrane (**figure 3.12).** ***ATP-binding cassette transporters (ABC transporters)*** are important primary active transporters. ABC transporters that are used for import of substances. Most ABC transporters consist of two hydrophobic membrane-spanning domains associated on their cytoplasmic surfaces with two ATP-binding domains (**figure 3.13). The membrane-spanning** domains form a pore in the membrane, and the ATP-binding domains bind and hydrolyze ATP to drive uptake.

Most ABC transporters employ substrate-binding proteins to deliver the molecule to be transported to the transporter.

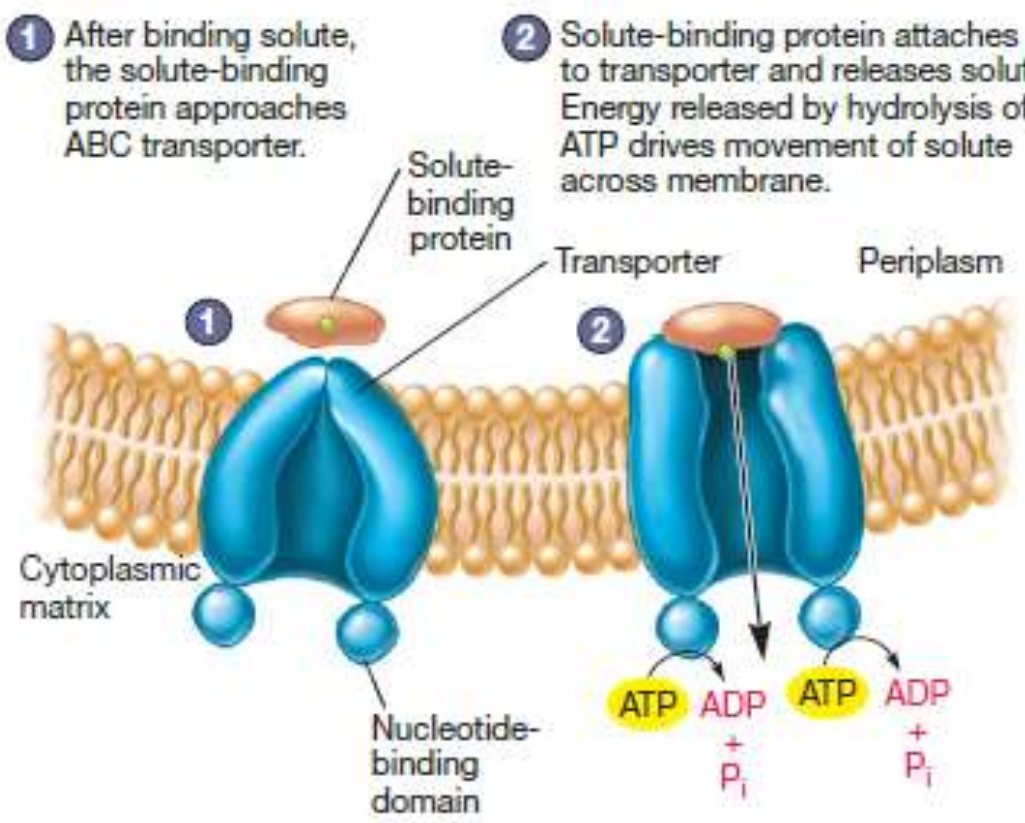


Figure 3.13 ABC Transporter Function. Shown here is a transporter that works with a substrate-binding protein free in the periplasm. Other substrate-binding proteins are associated with the plasma membrane, always associated with the transporter, or even fused to the transporter.

b) Secondary active transport couples the potential energy of ion gradients to transport of substances without modifying them.

Secondary active transporters are cotransporters (figure 3.12). They move two substances simultaneously: the ion whose gradient powers transport and the substance being moved across the membrane.

When the ion and other substance both move in the same direction, it is called **symport. When they move in opposite directions, it is** called **antiport.**

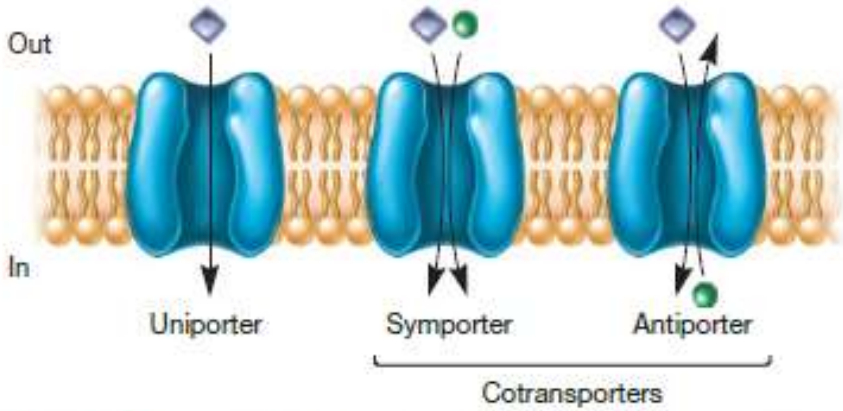


Figure 3.12 Carrier Proteins Can Be Uniporters or Cotransporters. Uniporters move a single substance into the cell. Cotransporters simultaneously move two substances across the membrane. When both substances move in the same direction, the carrier is a symporter. When the two substances move in opposite directions, the carrier is an antiporter.

The ion gradients used by secondary active transporters arise primarily in three ways:

1) The first results from bacterial metabolic activity. During energy-conserving processes, electron transport generates a proton gradient in which protons (H^+) are at a higher concentration outside the cell than inside. The proton gradient is used to do cellular work, including secondary active transport.

2)Some bacteria use the second method, in which an enzyme called a V-type ATPase hydrolyzes ATP and uses the energy released to create either a proton gradient (H^+) or a sodium gradient (Na^+) across the plasma membrane.

3) Finally, a proton gradient (H^+) can be used to create another ion gradient such as a sodium gradient (Na^+) . This is accomplished by an antiporter that brings protons in as sodium ions are moved out of the cell. The sodium gradient can then be used to drive uptake of nutrients by a symport mechanism.

The lactose permease of *E. coli* is a well-studied secondary active transporter. It is a single protein that transports a lactose molecule inward as a proton simultaneously enters the cell.
The proton is moving down a proton gradient, and the energy released drives solute transport. X-ray diffraction studies show that the carrier protein exists in outward- and inward-facing conformations.
When lactose and a proton bind to separate sites on the outward-facing conformation, the protein changes to its inward-facing conformation, and the sugar and proton are released into the cytoplasm.
Thus this is an example of symport. *E. coli* also uses proton symport to take up amino acids and some organic acids.

Bacteria often have more than one transport system for a nutrient, as can be seen with *E. coli*. This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes.

When several transport systems exist for the same substance, the systems differ in such properties as their energy source, their affinity for the solute transported, and the nature of their regulation. This diversity gives the bacterium an added competitive advantage in a variable environment.

4) Group Translocation

The distinguishing characteristic of **group translocation** is that a molecule is chemically modified as it is brought into the cell.

The best-known group translocation system is the **phosphoenolpyruvate : sugar phosphotransferase system (PTS),** which is observed in many bacteria.

The PTS transports a variety of sugars while phosphorylating them, using phosphoenolpyruvate (PEP) as the phosphate donor. PEP is an important intermediate of a biochemical pathway used by many bacteria to extract energy from organic energy sources.

PEP is a high-energy molecule that can be used to synthesize ATP, the cell's energy currency. However, when it is used in PTS reactions, the energy present in PEP is used to energize sugar uptake rather than ATP synthesis.

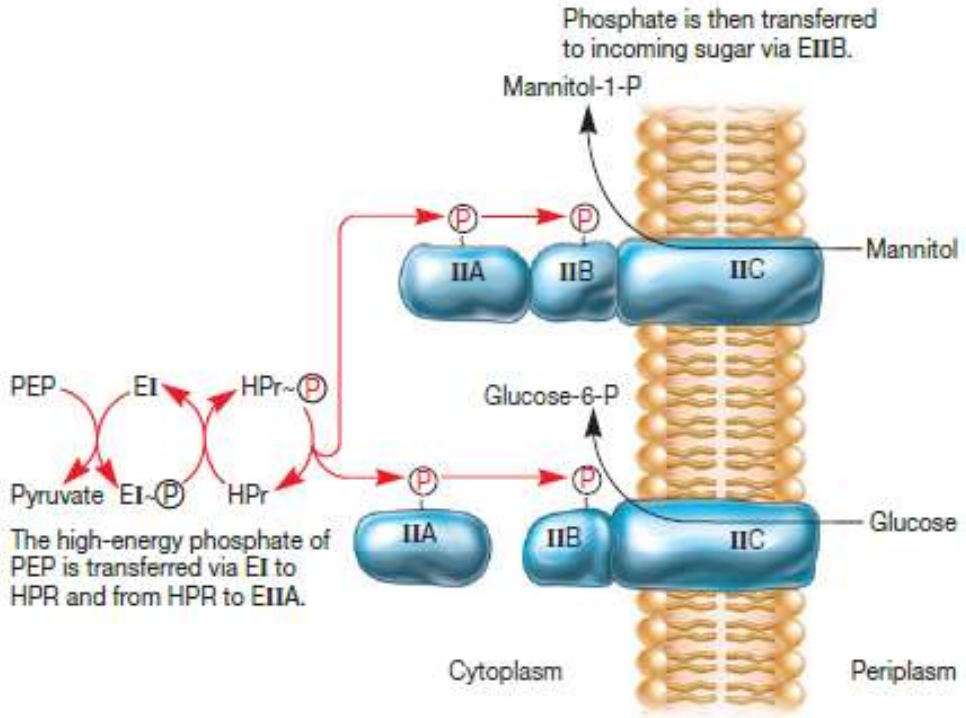


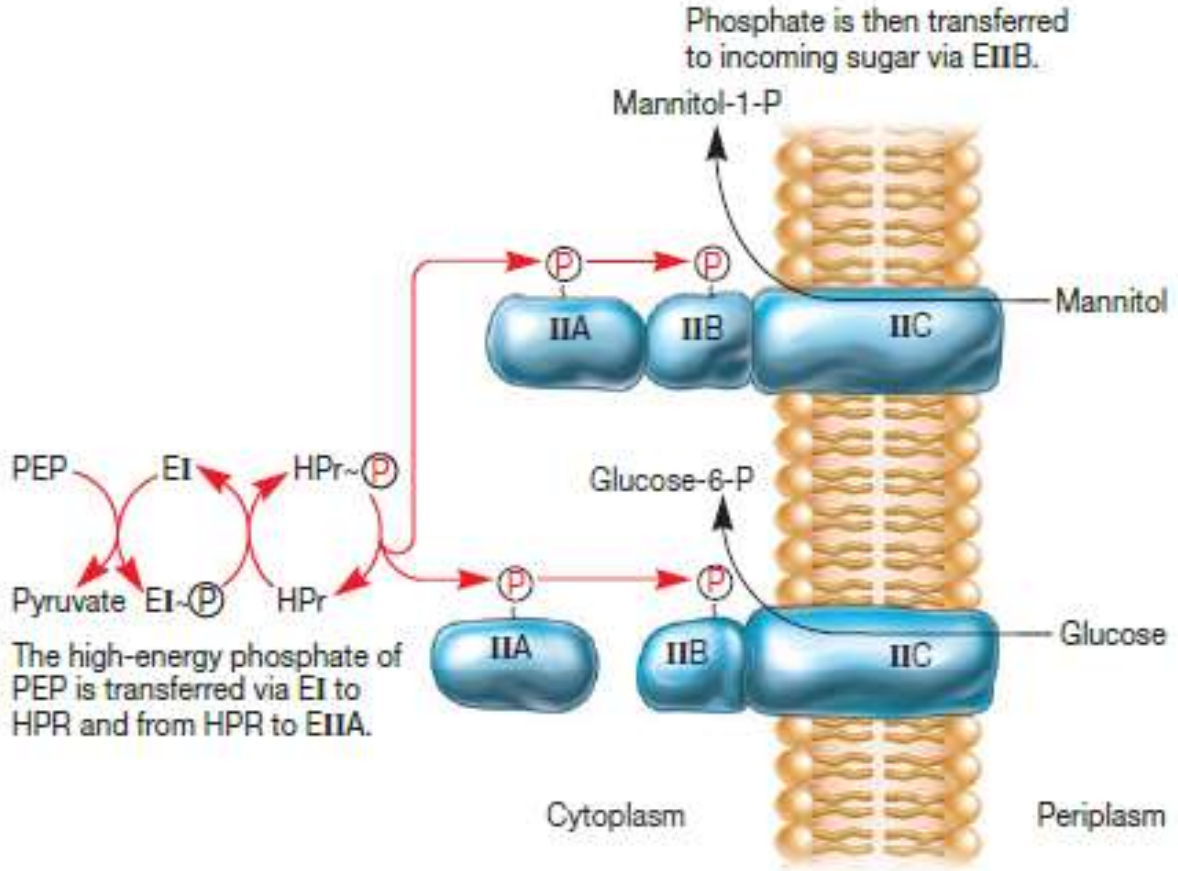
Figure 3.14 Group Translocation: Bacterial PTS Transport. Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system.

The transfer of phosphate from PEP to the incoming molecule involves several proteins and is an example of a **phosphorelay system.**

In ***E. coli*** **and** ***Salmonella,*** the PTS consists of two enzymes and a low molecular weight heat-stable protein (HPr). A phosphate is transferred from PEP to enzyme II with the aid of enzyme I and HPr (**figure 3.14).**

Enzyme II then phosphorylates the sugar molecule as it is carried across the membrane.

Many different PTSs exist, and they vary in terms of the sugars they transport. The specificity lies with the type of Enzyme II used in the PTS. Enzyme I and HPr are the same in all PTSs used by a bacterium.



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PTSs are widely distributed in bacteria, being found primarily among facultatively anaerobic bacteria (bacteria that grow in either the presence or absence of O_2); some obligately anaerobic bacteria (e.g., *Clostridium spp.)* also have PTSs.

However, most aerobic bacteria lack PTSs.

Many carbohydrates are transported by PTSs. *E. coli* takes up glucose, fructose, mannitol, sucrose, N-acetylglucosamine, cellobiose, and other carbohydrates by group translocation.

Besides their role in transport, PTSs function in numerous regulatory processes, including the regulation of carbon metabolism. One example is the role of PTSs in catabolite repression, a phenomenon in which the cell inhibits synthesis of degradative enzymes for some sugars so that it can catabolize a preferred sugar. PTS proteins also can bind chemical attractants, toward which bacteria move by the process of chemotaxis.

Iron Uptake

Almost all microorganisms require iron for building molecules important in energy-conserving processes (e.g., cytochromes), as well as for the function of many enzymes.

Iron uptake is made difficult by the **extreme insolubility of ferric iron (Fe^{3+}) and its derivatives, which leaves little free iron available for transport.**

Many bacteria have overcome this difficulty by **secreting siderophores** (Greek for iron bearers). Siderophores are low molecular weight organic molecules that bind ferric iron and supply it to the cell (**figure 3.15).**

Microorganisms secrete siderophores when iron is scarce in the medium. Once the iron-siderophore complex has reached the cell surface, it binds to a **siderophore-receptor protein**. **Then either the iron is released to enter the cell directly or the whole iron-siderophore complex is transported inside by an ABC transporter.**

Iron is so crucial to microorganisms that they may use more than one route of iron uptake to ensure an adequate supply.

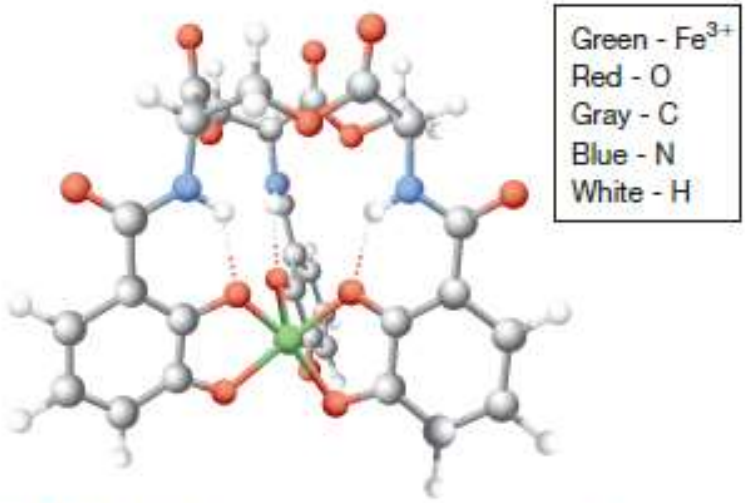

Figure 3.15 **Enterobactin: A Siderophore Produced by *E. coli*.** Ball-and-stick model of enterobactin complexed with Fe^{3+}.

Important Questions Prepared For Final Examination

Q1) On what basis are elements divided into macroelements and trace elements?
Q2) Distinguished between:
a) Passive Diffusion Vs. Facilitated diffusion
b) Active Transport Vs. Facilitated Diffusion
c) Active Transport Vs. Passive Diffusion
d) Symport, Uniport and Antiport
Q3) Describe facilitated diffusion, primary and secondary active transport, and group translocation in terms of their distinctive characteristics and mechanisms. What advantage does a bacterium gain by using active transport rather than facilitated diffusion?
Q4) What are uniport, symport, and antiport? Give Examples.
Q5) What are siderophores? Why are they important?
Q6) Write Short Note on (with proper diagram):
a) ABC Transporter
b) Group Translocation

THANK YOU