Solute Transport Across the Membranes

Transport

- **Movement of ions or molecules from one place to another, specifically across membranes**
- **Every living cell acquire from - raw materials for biosynthesis, for energy production**
- **Every living cell release to environment the byproducts of metabolism**
- **Proteins in plasma membranes recognize and carry into cell - sugar, amino acids and inorganic ions - tightly regulated, protein-mediated processes**
- **Components into the cell against a concentration gradient – “pumped” in**

Passive Transport (Energy-independent process)

- **Dependent on the permeability of the cell membrane, which is dependent on the organization and characteristics of the membrane lipids and proteins**

Characteristics:

1. **The substance moves from a higher chemical concentration to a lower concentration i.e. in response to a concentration gradient.**
2. **Movement of substance may also take place in response to an electrical gradient.**
3. **Passive transport does not require energy**

Simple Diffusion

- **Solutes move from the region of higher concentration to the region of lower concentration, when separated by a permeable divider**
- **Concentration gradient - The difference of concentration between the two areas**
- **Electrochemical gradient OR Electrochemical potential**
- **Diffusion will continue until gradient has been eliminated**
- **“Down the concentration gradient"**
- **“Against the concentration gradient"**

Simple Diffusion

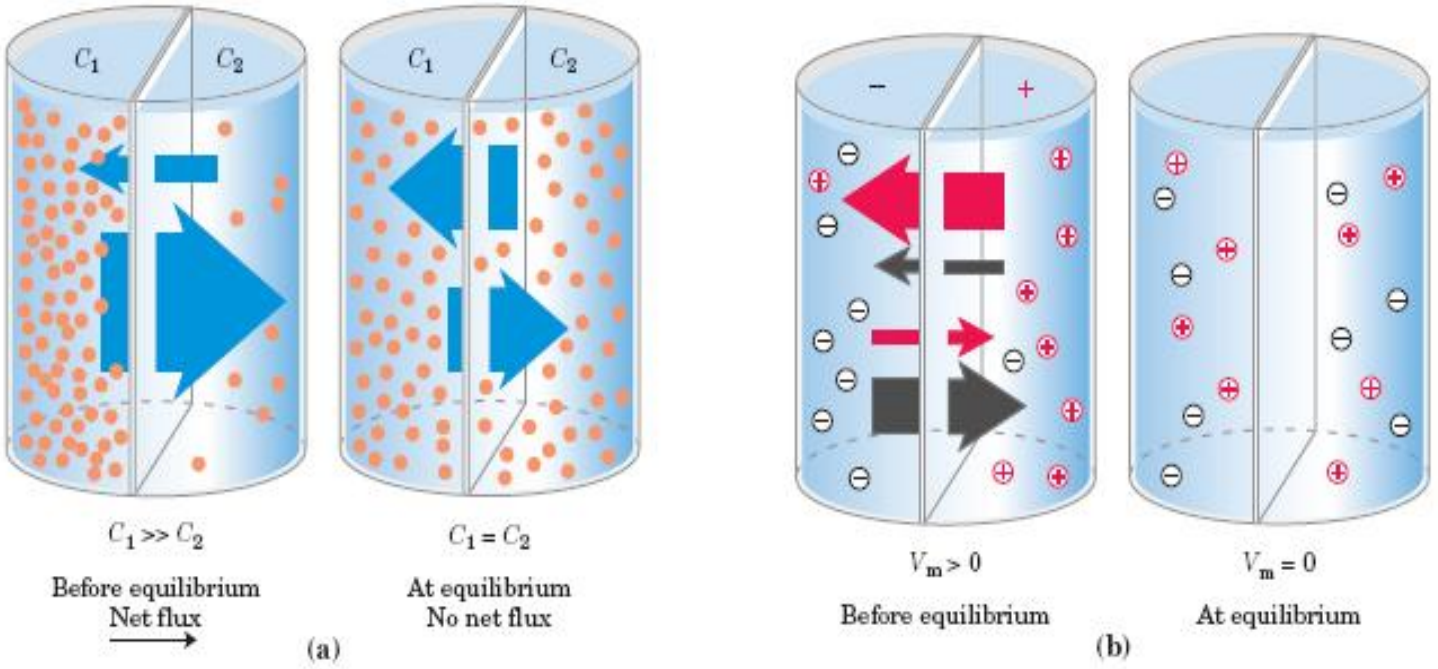


FIGURE 11-27 **Movement of solutes across a permeable membrane.** (a) Net movement of electrically neutral solutes is toward the side of lower solute concentration until equilibrium is achieved. The solute concentrations on the left and right sides of the membrane are designated C_1 and C_2. The rate of transmembrane movement (indicated by the large arrows) is proportional to the concentration gradient, C_1/C_2. (b) Net movement of electrically charged solutes is dictated by a combination of the electrical potential (V_m) and the chemical concentration difference across the membrane; net ion movement continues until this electrochemical potential reaches zero.

Simple Diffusion

- Simple diffusion impeded by selectively permeable barriers – the membranes
- To pass through the bilayer, a polar or charged solute must give up its hydration shell, then diffuse about 3nm through a solvent in which it is poorly soluble (the central region of lipid bilayer), before reaching the other side and regaining its water of hydration
- The energy used to strip away the hydration shell and move a polar compound from water into lipid is regained as the compound leaves the membrane on the other side and is rehydrated

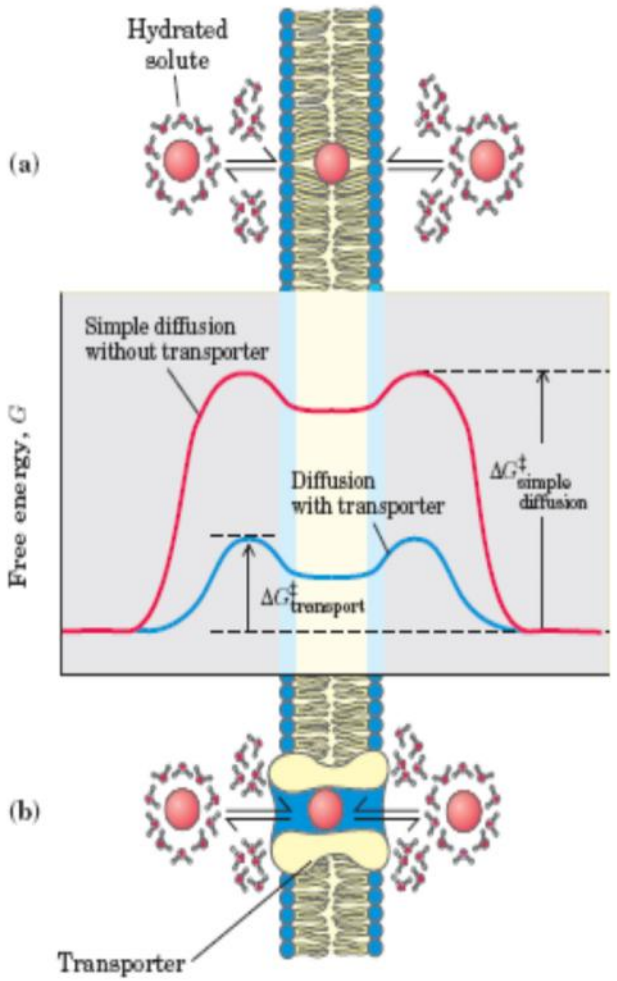


FIGURE 11-28 Energy changes accompanying passage of a hydrophilic solute through the lipid bilayer of a biological membrane. (a) In simple diffusion, removal of the hydration shell is highly endergonic, and the energy of activation ($\Delta G^{\ddagger}$) for diffusion through the bilayer is very high. (b) A transporter protein reduces the $\Delta G^{\ddagger}$ for transmembrane diffusion of the solute. It does this by forming noncovalent interactions with the dehydrated solute to replace the hydrogen bonding with water and by providing a hydrophilic transmembrane passageway.

Simple Diffusion

- **Pure lipid bilayers impermeable to polar and charged species – as large energy of activation for translocation of a polar solute across bilayer**

- **Molecular oxygen (O_2), nitrogen (N_2) and methane (CH_4) cross membranes by simple diffusion**

- **Water cross some biological membranes slowly by diffusion**

- **Aquaporins - specific integral proteins allow rapid transmembrane water movement in tissues (kidney)**

Facilitated Diffusion

- **Transmembrane passage of polar compounds and ions made - by membrane proteins**
- **Membrane proteins lower the activation energy for transport by providing an alternative path for specific solutes through the lipid bilayer**
- **These proteins not enzymes in the usual sense; their substrates are moved from one compartment to another, but are not chemically altered**
- **These membrane proteins called transporters or permeases**
- **Detailed structural information not yet available for most membrane transporters**

Facilitated Diffusion

- **These proteins are:**

1. **Difficult to purify and crystallize**
2. **Kinetics and specificity of transporters - analogous to that of enzymes**
3. **Like enzymes, transporters bind substrates through weak noncovalent interactions and with steriochemical specificity**
4. **Transporter proteins span the lipid bilayer at least once, and usually several times**

- **Form a transmembrane channel lined with hydrophilic amino acid side chains**
- **Channel provides an alternative path for substrates to move across the lipid bilayer, without having to dissolve in it**

Aquaporins

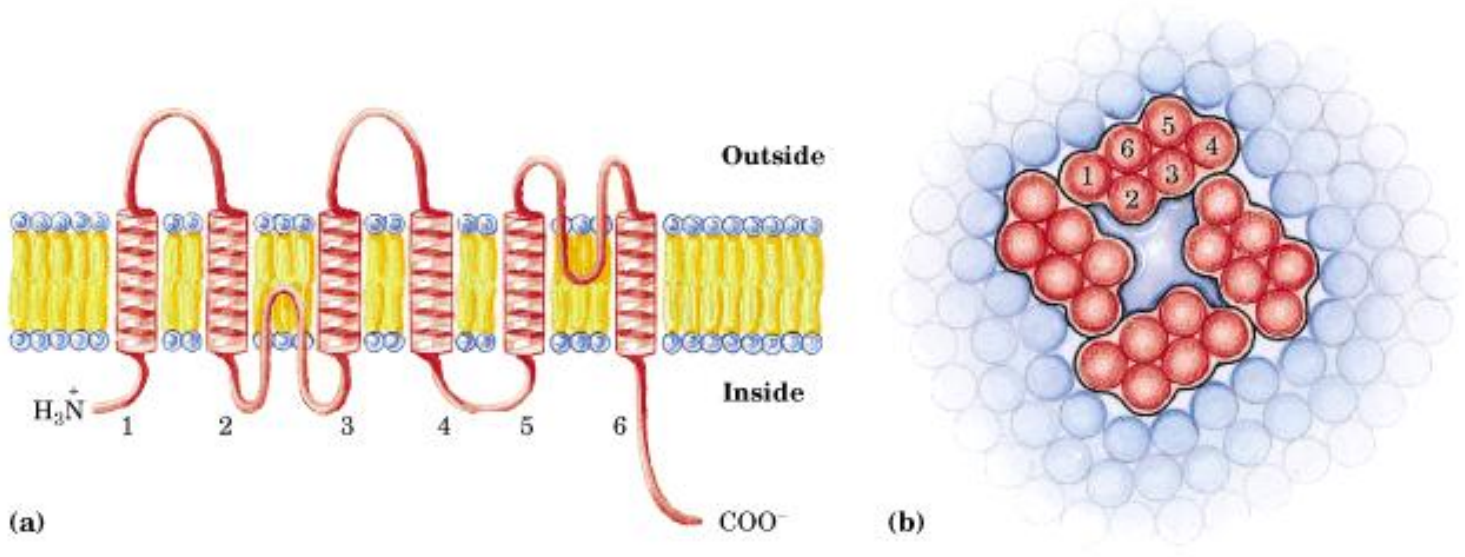
- **The Nobel Prize in Chemistry 2003 - Peter Agre for the discovery of water channels**
- **American physician, and molecular biologist at John Hopkins University**
- **Aquaporins - Hydrophilic Transmembrane Channels for the passage of water across plasma membranes**
- **Aquaporins - integral proteins**
- **Erythrocytes, which swell or shrink rapidly in response to abrupt changes in extracellular osmolarity as blood travels through the renal medulla, have a high density of aquaporins in their plasma membranes**
- **Plasma membranes of proximal renal tubule cells, which reabsorb water during urine formation**
- **Vacuolar membrane of plant cells, which require osmotic movement of water into the vacuole in order to maintain turgor pressure**

AQP-1

- **Four monomers form a tetrameric transmembrane channel lined with hydrophilic side chains**
- **Sufficient diameter to allow passage of water molecule at the rate of 5×10^8 molecules per second**
- **Water moves through the channels in a continuous stream**
- **Like most aquaporins, AQP-1 does not allow the passage of ions or other small solutes**

Aquaporins

All aquaporins are type lll integral proteins with six transmembrane helical segments.



Likely transmembrane topology of an aquaporin, AQP-1. This protein is also called CHIP-28. **(a)** each monomer consists of six transmembrane helices. **(b)** In a proposed structure for the aquaporin channel (viewed perpendicular or through the plane of the membrane), four AQP-1 monomers associate side by side, with their 24 transmembrane helices surrounding a central channel (shaded blue). The channel, through which water molecules diffuse one by one, is lined with hydrophillic side chains.

Aquaporins

TABLE 11–6 Aquaporins

Aquaporin	*Roles and/or location*
AQP-1	Fluid reabsorption in proximal renal tubule; secretion of aqueous humor in eye and cerebrospinal fluid in central nervous system; water homeostasis in lung
AQP-2	Water permeability in renal collecting duct (mutations produce nephrogenic diabetes insipidus)
AQP-3	Water retention in renal collecting duct
AQP-4	Cerebrospinal fluid reabsorption in central nervous system; regulation of brain edema
AQP-5	Fluid secretion in salivary glands, lachrymal glands, and alveolar epithelium of lung
AQP-6	Kidney
AQP-7	Renal proximal tubule, intestine
AQP-8	Liver, pancreas, colon, placenta
AQP-9	Liver, leukocytes
TIP	Regulation of turgor pressure in plant tonoplast
PIP	Plant plasma membrane
AQY	Yeast plasma membrane

TABLE 11–5 Permeability Characteristics and Predominant Distribution of Known Mammalian Aquaporins

Aquaporin	Permeant (permeability)	Tissue distribution	Subcellular distribution*
AQP-0	Water (low)	Lens	Plasma membrane
AQP-1	Water (high)	Erythrocyte, kidney, lung, vascular endothelium, brain, eye	Plasma membrane
AQP-2	Water (high)	Kidney, vas deferens	Apical plasma membrane, intracellular vesicles
AQP-3	Water (high), glycerol (high), urea (moderate)	Kidney, skin, lung, eye, colon	Basolateral plasma membrane
AQP-4	Water (high)	Brain, muscle, kidney, lung, stomach, small intestine	Basolateral plasma membrane
AQP-5	Water (high)	Salivary gland, lacrimal gland, sweat gland, lung, cornea	Apical plasma membrane
AQP-6	Water (low), anions ($NO_3^- > Cl^-$)	Kidney	Intracellular vesicles
AQP-7	Water (high), glycerol (high), urea (high), arsenite	Adipose tissue, kidney, testis	Plasma membrane
AQP-8†	Water (high)	Testis, kidney, liver, pancreas, small intestine, colon	Plasma membrane, intracellular vesicles
AQP-9	Water (low), glycerol (high), urea (high), arsenite	Liver, leukocyte, brain, testis	Plasma membrane
AQP-10	Water (low), glycerol (high), urea (high)	Small intestine	Intracellular vesicles

Source: Data from King, L.S., Kozono, D., & Agre, P. (2004) From structure to disease: the evolving tale of aquaporin biology. *Nat. Rev.* **5**, 688.

*Aquaporins that are present primarily in the apical or in the basolateral membrane are noted as localized in one of these membranes; those present in both membranes are described as localized in the plasma membrane.

†AQP-8 might also be permeated by urea.

The Glucose Permease of Erythrocytes GluT$_1$

- Energy-yielding metabolism in erythrocyte require constant supply of glucose
- In blood plasma, its concentration is maintained at about 5mM
- The glucose permease of erythrocytes mediates passive transport of glucose
- This integral membrane protein has 12 hydrophobic segments and probably spans the membrane 12 times

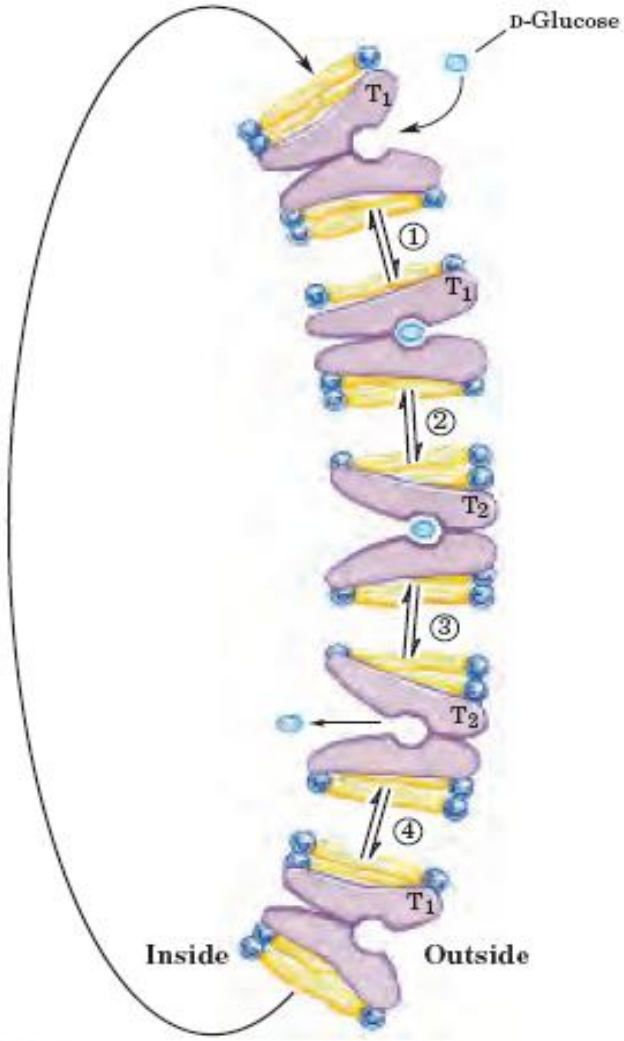


FIGURE 11-32 Model of glucose transport into erythrocytes by GLUT1. The transporter exists in two conformations: T_1, with the glucose-binding site exposed on the outer surface of the plasma membrane, and T_2, with the binding site exposed on the inner surface. Glucose transport occurs in four steps. (1) Glucose in blood plasma binds to a stereospecific site on T_1; this lowers the activation energy for (2) a conformational change from $S_{out} \cdot T_1$ to $S_{In} \cdot T_2$, effecting the transmembrane passage of the glucose. (3) Glucose is now released from T_2 into the cytoplasm, and (4) the transporter returns to the T_1 conformation, ready to transport another glucose molecule.

The Glucose Permease of Erythrocytes – $GluT_1$

- It is specific for D-glucose
- Allows glucose entry into the cell at a rate about 50,000 times greater than its unaided diffusion through a lipid bilayer
- As $[S]_{in} = [S]_{out}$, rate of entry and exit equals
- It simply achieves equilibrium of glucose across membranes, no net accumulation of glucose within the cell
- $GluT_2$ in liver – transports glucose out of hepatocytes
- $GluT_4$ present in muscles and adipose tissues

Chloride-Bicarbonate Exchanger

- **Facilitated diffusion system in Erythrocytes**
- **An anion exchanger, essential in CO_2 transport from tissues such as muscle and liver to the lungs**
- **Also called the anion exchange protein (AE), or band 3 (for historical reasons)**
- **Increases the permeability of erythrocyte membrane to HCO_3^- by a factor of more than a million**
- **An integral protein that probably spans the membrane 12 times**

Chloride-Bicarbonate Exchanger

- **Mediates a bidirectional exchange; for each HCO_3^- that moves in one direction, one chloride ion must move in opposite direction**
- **So, no net change in the charge or electrical potential across the erythrocyte membrane; the process is not electrogenic**
- **The coupling of Cl^- and HCO_3^- movement is obligatory; in the absence of chloride, bicarbonate transport stops**

Chloride-Bicarbonate Exchanger

- Waste CO_2 released from respiring tissues into the blood plasma enters the erythrocytes, where it is converted to bicarbonate (HCO_3^-) by the enzyme carbonic anhydrase
- The HCO_3^- reenters the blood plasma for transport to the lungs. Because HCO_3^- is much more soluble in blood plasma than is CO_2, this roundabout route increases the blood capacity to carry carbon dioxide from the tissues to the lungs
- In the lungs, HCO_3^- reenters the erythrocytes and is converted to CO_2, which is eventually exhaled

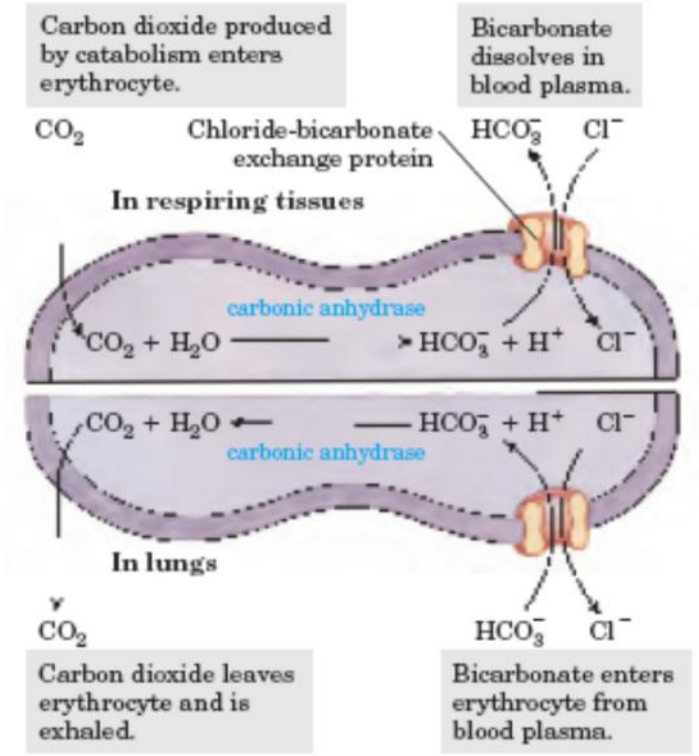


FIGURE 11-33 **Chloride-bicarbonate exchanger of the erythrocyte membrane.** This cotransport system allows the entry and exit of HCO_3^- without changes in the transmembrane electrical potential. Its role is to increase the CO_2-carrying capacity of the blood.

Transport Systems

- **Cotransport systems**
  systems that simultaneously carry two solutes across a membrane

- When the two substrates move in opposite directions, the process is **antipor**t **(Chloride-Bicarbonate Exchanger)**

- **Symport**, two substrates are moved simultaneously in the same direction

- **Uniport systems**, that carry only one substrate **(glucose permease)**

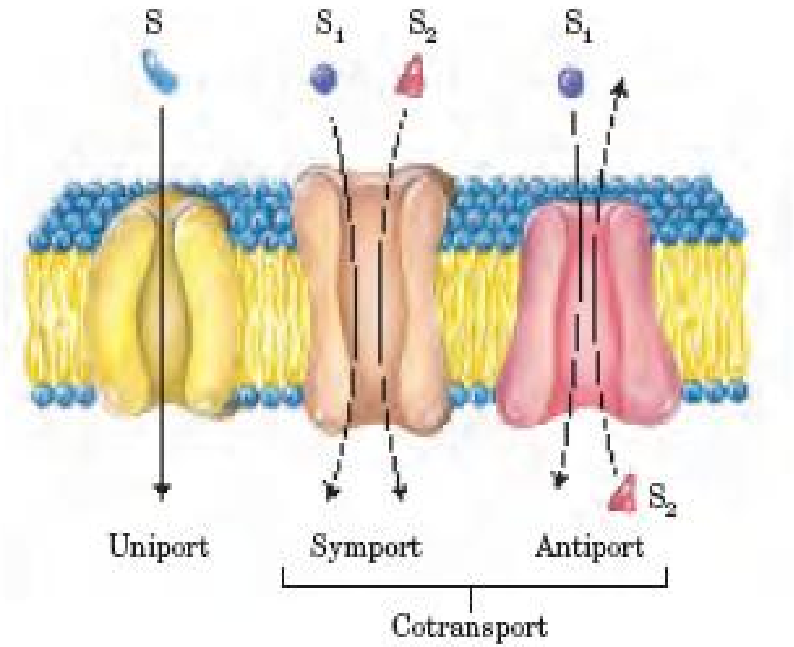


FIGURE 11-34 Three general classes of transport systems. Transporters differ in the number of solutes (substrates) transported and the direction in which each is transported. Examples of all three types of transporters are discussed in the text. Note that this classification tells us nothing about whether these are energy-requiring (active transport) or energy-independent (passive transport) processes.

Active Transport (Energy-dependent process)

- **Accumulation of a solute on one side of a membrane**
- **Thermodynamically unfavorable (endergonic)**
- **Occurs only when coupled (directly or indirectly) to an exergonic process such as**
  - **the absorption of sunlight**
  - **an oxidation reaction**
  - **the breakdown of ATP**
  - **the concomitant flow of some other chemical species down its concentration gradient**

Active Transport

- **Primary active transport**

  solute accumulation is coupled directly to an exergonic reaction (e.g., conversion of ATP to ADP + Pi)

- **Secondary active transport**

  occurs when endergonic (uphill) transport of one solute is coupled to the exergonic (downhill) flow of a different solute that was originally pumped uphill by primary active transport

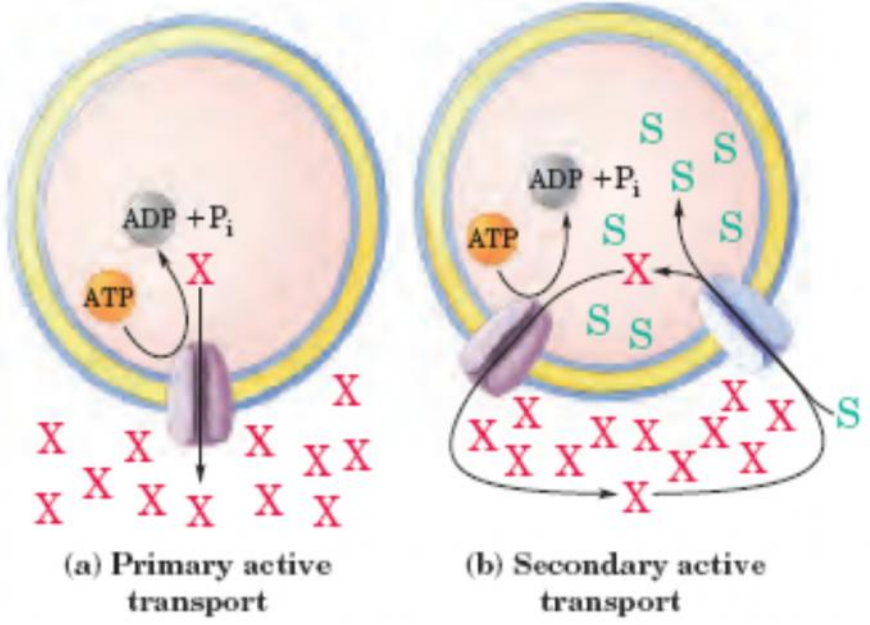


FIGURE 11-35 Two types of active transport. (a) In primary active transport, the energy released by ATP hydrolysis drives solute movement against an electrochemical gradient. (b) In secondary active transport, a gradient of ion X (often Na^+) has been established by primary active transport. Movement of X down its electrochemical gradient now provides the energy to drive cotransport of a second solute (S) against its electrochemical gradient.

Active Transport

- **Movement of a charged solute, without any accompanying counter-ion results in the endergonic separation of positive and negative charges**
- **The energetic cost of moving an ion therefore depends on the difference of**
  - **electrical potential across the membrane**
  - **chemical concentrations (the electrochemical potential)**
- **Most cells maintain ion gradients larger than ten-fold across their plasma or intracellular membranes**
- **The formation of ATP in mitochondria and chloroplasts occurs by a mechanism that is essentially ATP-driven ion transport operating in reverse**

Active Cotransport - Na^+K^+ATPase

- **Animal cell maintain**
  - **a lower concentration of Na^+ and**
  - **a higher concentration of K^+ than is found in its surrounding medium**
- **This imbalance established and maintained by a primary active transport system in plasma membrane, involving the enzyme Na^+K^+ATPase**
- **which couples the breakdown of ATP to the simultaneous movement of both Na^+ and K^+ against their concentration gradients**
- **For each molecule of ATP converted to ADP and Pi, this transporter moves two K^+ ions inward and three Na^+ ions outward, across the plasma membrane**

Active Cotransport - Na^+K^+ATPase

- The Na^+K^+ATPase - an integral membrane protein with two subunits, both of which span the membrane

- ATPase cycles between two confirmations:
  - confirmation ll, a phosphorylated form (designated P-Enz_{ll}) with high affinity for K^+ and low affinity for Na^+
  - confirmation l, a dephosphorylated form (Enz_l) with high affinity for Na^+ and low affinity for K^+

- Three Na^+ ions move outward for every two K^+ ions that move inward

- Process is electrogenic

Na^+K^+ATPase

1) Formation of phosphoenzyme:

$$ATP + Enz_I \longrightarrow ADP + P\text{-}Enz_{II}$$

2) Hydrolysis of phosphoenzyme:

$$P\text{-}Enz_{II} + H2O \longrightarrow Enz_I + Pi$$

Which sum to the hydrolysis of ATP:

$$ATP + H2O \longrightarrow ADP + Pi$$

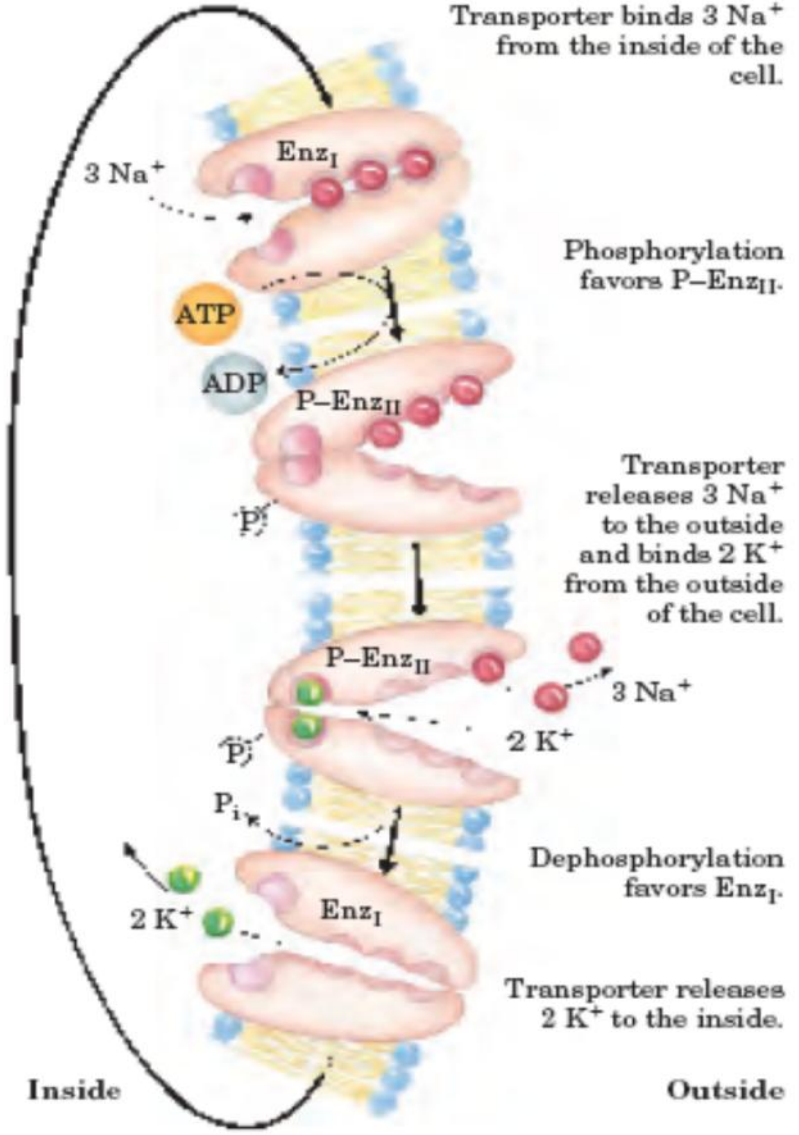


FIGURE 11-37 Postulated mechanism of Na^+ and K^+ transport by the Na^+K^+ ATPase.

Na^+K^+ATPase

- **Trans-membrane potential of – 50 to – 70 mV results**
- **Which is essential to the conduction of action potentials in neurons and nonneuronal animal cells**
- **Activity of Na^+K^+ATPase - an essential cell function**
- **About 25% of the energy-yielding metabolism of a human at rest goes to support the Na^+K^+ ATPase**

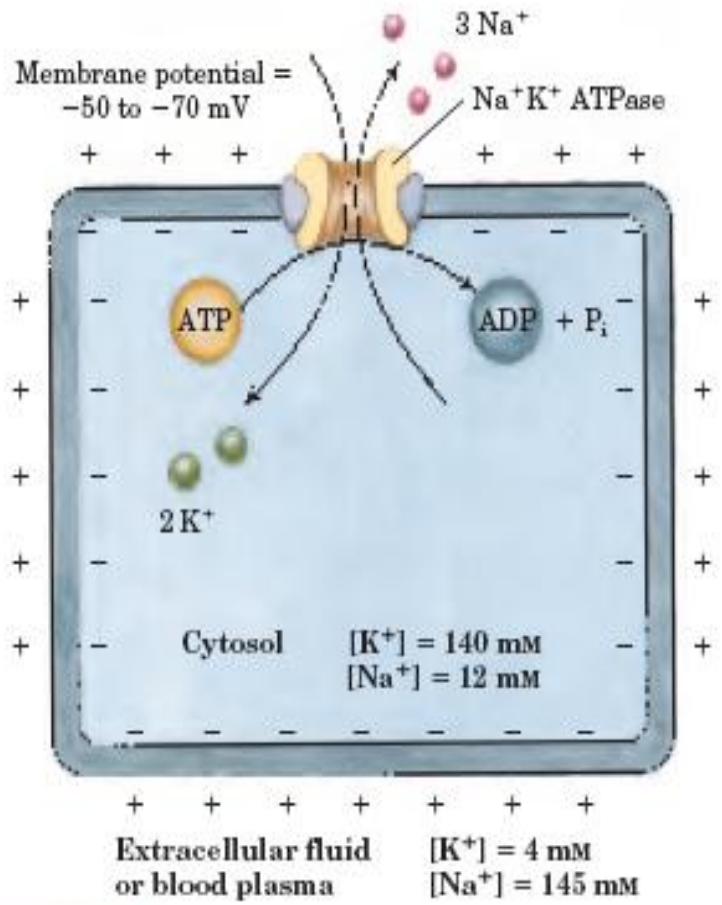


FIGURE 11-36 Na^+K^+ ATPase. In animal cells, this active transport system is primarily responsible for setting and maintaining the intracellular concentrations of Na^+ and K^+ and for generating the transmembrane electrical potential. It does this by moving three Na^+ out of the cell for every two K^+ it moves in. The electrical potential is central to electrical signaling in neurons, and the gradient of Na^+ is used to drive the uphill cotransport of solutes in many cell types.

Na^+K^+ATPase

- **Ouabain, a steroid derivative extracted from the seeds of an African shrub**
- **A potent and specific inhibitor of the Na^+K^+ ATPase**
- **Ouabain is a powerful poison used to tip hunting arrows; its name is derived from *waba yo*, meaning “arrow poison”**

Ion Gradient provide energy for Secondary Active Transport

- Galactoside permease of *E.Coli* allows accumulation of lactose to levels 100 times that in surrounding growth medium
- *E.Coli* has a proton gradient across its plasma membrane, produced by energy-yielding metabolism
- Protons tend to flow back into the cell, down this gradient
- Lipid bilayer impermeable to protons but the galactoside permease provides a route for proton reentry
- Lactose is carried into the cell on the symport protein (permease)

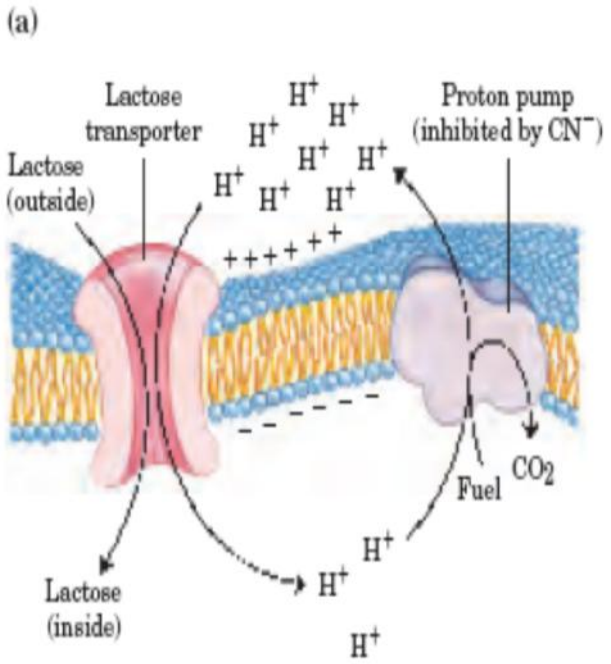


FIGURE 11-42 Lactose uptake in *E. coli*. (a) The primary transport of H^+ out of the cell, driven by the oxidation of a variety of fuels, establishes both a proton gradient and an electrical potential (inside negative) across the membrane. Secondary active transport of lactose into the cell involves symport of H^+ and lactose by the lactose transporter. The uptake of lactose against its concentration gradient is entirely dependent on this inflow of H^+, driven by the electrochemical gradient.

Ion Gradient provide energy for Secondary Active Transport

TABLE 11-5 Cotransport Systems Driven by Gradients of Na^+ or H^+

Organism/tissue/cell type	*Transported solute (moving against its gradient)*	*Cotransported solute (moving down its gradient)*	*Type of transport*
E. coli	Lactose	H^+	Symport
	Proline	H^+	Symport
	Dicarboxylic acids	H^+	Symport
Intestine, kidney (vertebrates)	Glucose	Na^+	Symport
	Amino acids	Na^+	Symport
Vertebrate cells (many types)	Ca^{2+}	Na^+	Antiport
Higher plants	K^+	H^+	Antiport
Fungi (*Neurospora*)	K^+	H^+	Antiport

Ion Gradient provide energy for Secondary Active Transport

- **Intestinal epithelial cells - glucose and certain amino acids are accumulated by symport using the Na+ gradient established by the Na^+K^+ ATPase**

- **Cells of vertebrate animals have antiport system that simultaneously pumps one Ca^{2+} out of a cell and allows three Na^+ ions in, so maintain low intracellular Ca^{2+} concentration required for normal function**

- **Role of Na^+ in symport and antiport systems requires the continued outward pumping of Na^{2+} to maintain the transmembrane Na^+ gradient**

- **Na^+K^+ ATPase is a central element in many cotransport processes**

Ion Gradient provide energy for Secondary Active Transport

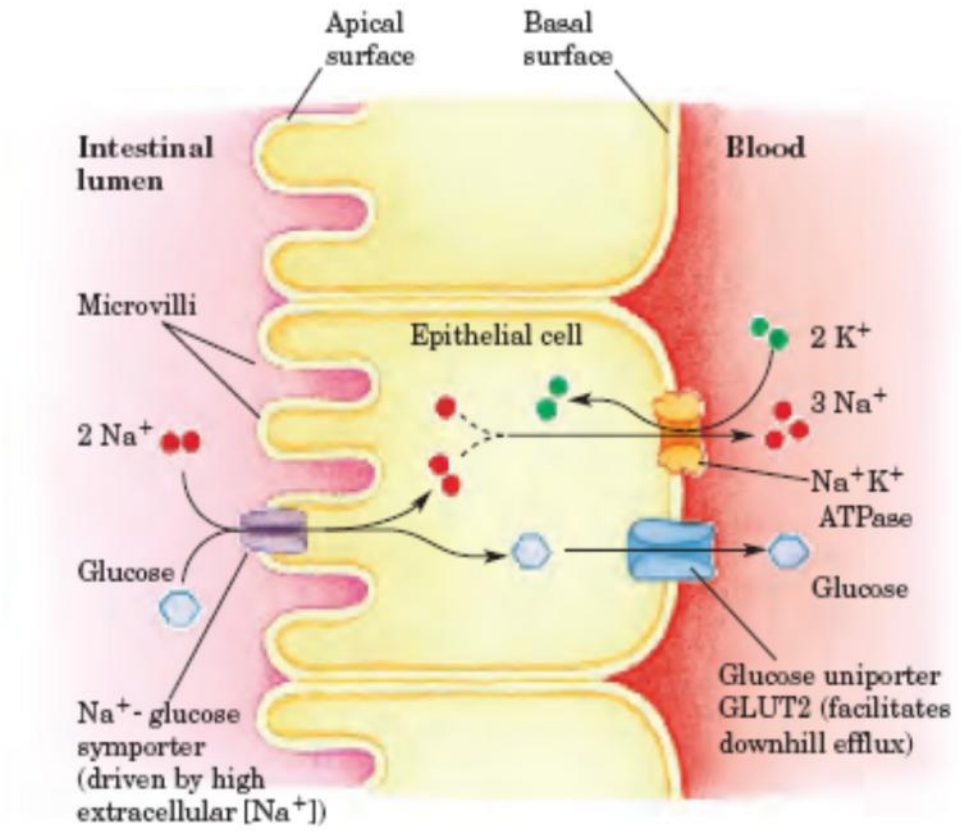


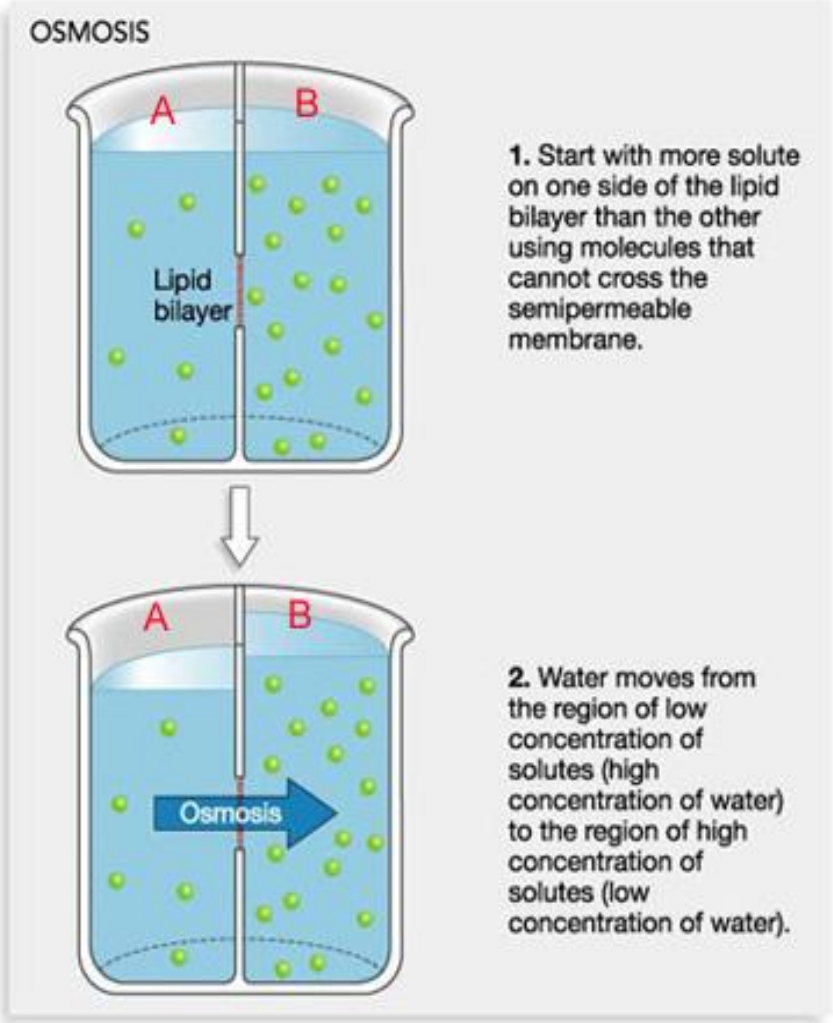
FIGURE 11-44 Glucose transport in intestinal epithelial cells. Glucose is cotransported with Na^+ across the apical plasma membrane into the epithelial cell. It moves through the cell to the basal surface, where it passes into the blood via GLUT2, a passive glucose transporter. The Na^+K^+ ATPase continues to pump Na^+ outward to maintain the Na^+ gradient that drives glucose uptake.

Osmosis

- **Diffusion of a solvent across a membrane to a region of higher solute concentration**
- **Diffusion of water plays important role in the biological functioning of any living being**
- **Water molecules diffusing from the region of higher water concentration to that of lower water concentration produce osmotic pressure**
- **Plasma membranes are more permeable to water than to most other small molecules, ions and macromolecules**
- **Water simply diffuse through the lipid bilayer**
- **Protein channels (aquaporins) selectively permit the passage of water**

Osmosis

- **<u>Osmotic pressure</u>: pressure require to stop osmosis completely**
- **<u>Osmole</u>: number of moles of a chemical compound that contribute to a solution's osmotic pressure**
- **<u>Osmolarity</u>: measure of osmoles of solute per liter of solution**



Osmosis

- **1 osmole = 22.4 atmosphere or 17024 mmHg**
- **In biology, milliosmole (1/1000th of osmole or 17 mm Hg) is used**
- **Osmotic pressure exerted by blood plasma, gastric juice, pancreatic juice, liver bile, cerebrospinal fluid = 280 – 295 milliosmoles/ liter**
- **Osmotic pressure proportional to**
  - **solute concentration (1% NaCl vs. 0.5%NaCl)**
  - **absolute temperature**
  - **for dilute solutions, osmotic pressure = CRT**

Osmosis

- **Osmotic pressure depends on**
  - **number of dissolved or dissociated particles per unit volume**

  e.g. Na ion [m.wt. 23], glucose molecule [M.wt. 180], serum albumin molecule [M.wt. 70,000] exert equal osmotic pressure

- **Different situation in case of electrolytes that split into more than one particle or ion**

  e.g. NaCl ionizes into Na^+ and Cl^- , so it exert double osmotic pressure than molar solution of a non-electrolyte

  Na_2SO_4 will exert three times osmotic pressure than molar solution of a non-electrolyte

Osmosis

- **Isotonic solutions**

  Solutions of equal osmolarity
- **Cell in isotonic solution, neither gains nor loses water**
- **In hypertonic solution with higher osmolarity than the cytosol, the cell shrinks as water flows out**
- **In hypotonic solution (of lower osmolarity), the cell swells and if unsupported by a cell wall, eventually bursts**

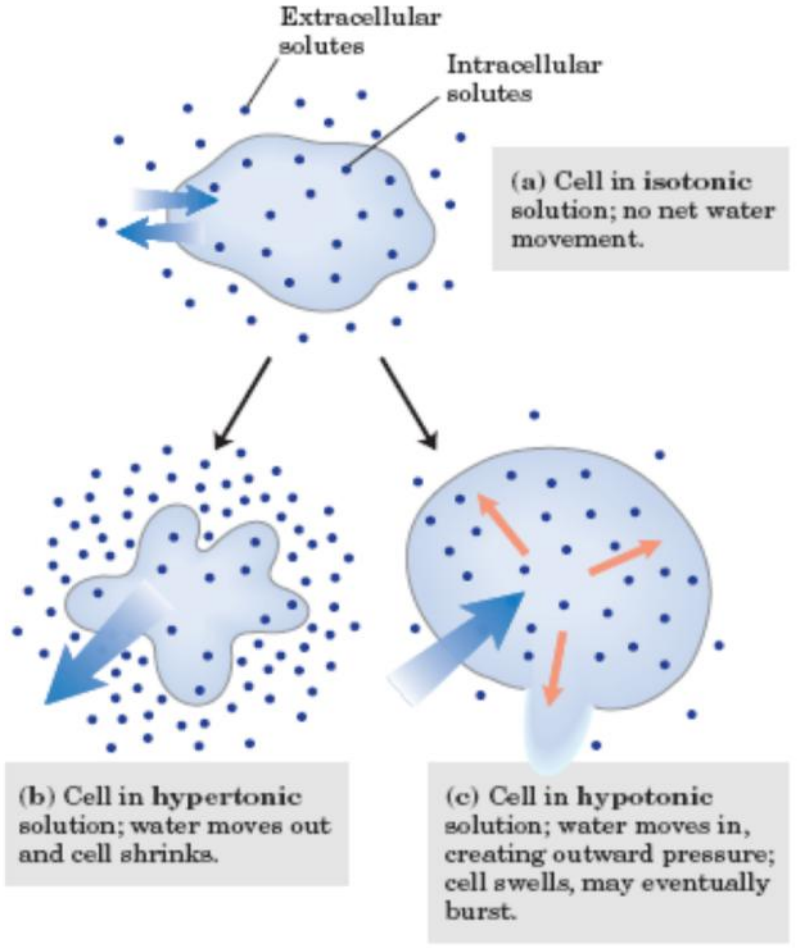


FIGURE 2–13 Effect of extracellular osmolarity on water movement across a plasma membrane. When a cell in osmotic balance with its surrounding medium (that is, in an isotonic medium) (a) is transferred into a hypertonic solution (b) or hypotonic solution (c), water moves across the plasma membrane in the direction that tends to equalize osmolarity outside and inside the cell.

Osmotic lysis

- **Osmotic pressure drive water into cells which generally contain higher concentration of biomolecules and ions than their surroundings**

- **If not counterbalanced, this inward movement of water would distend the plasma membrane and eventually cause explosion of the cell (osmotic lysis)**

Three mechanisms have evolved to prevent osmotic lysis:

- **Bacteria and plants - cell wall**

- **Fresh water protozoans - contractile vacuole**

- **Multicellular animals - albumin and other proteins in blood plasma contributes to osmolarity. Cells also actively pumps out ions such as Na^+ into the interstitial fluids to stay in osmotic balance with their surroundings**

Osmosis

- **Osmotic pressure gives mechanical rigidity in plants**
- **Turgor pressure**
- **Loss of water reduce turgor pressure, lettuce in salad wilts**
- **Dramatic alterations in turgor pressure produce movement of plant parts**
  - **touch-sensitive plants**

    Venus fly trap and mimosa

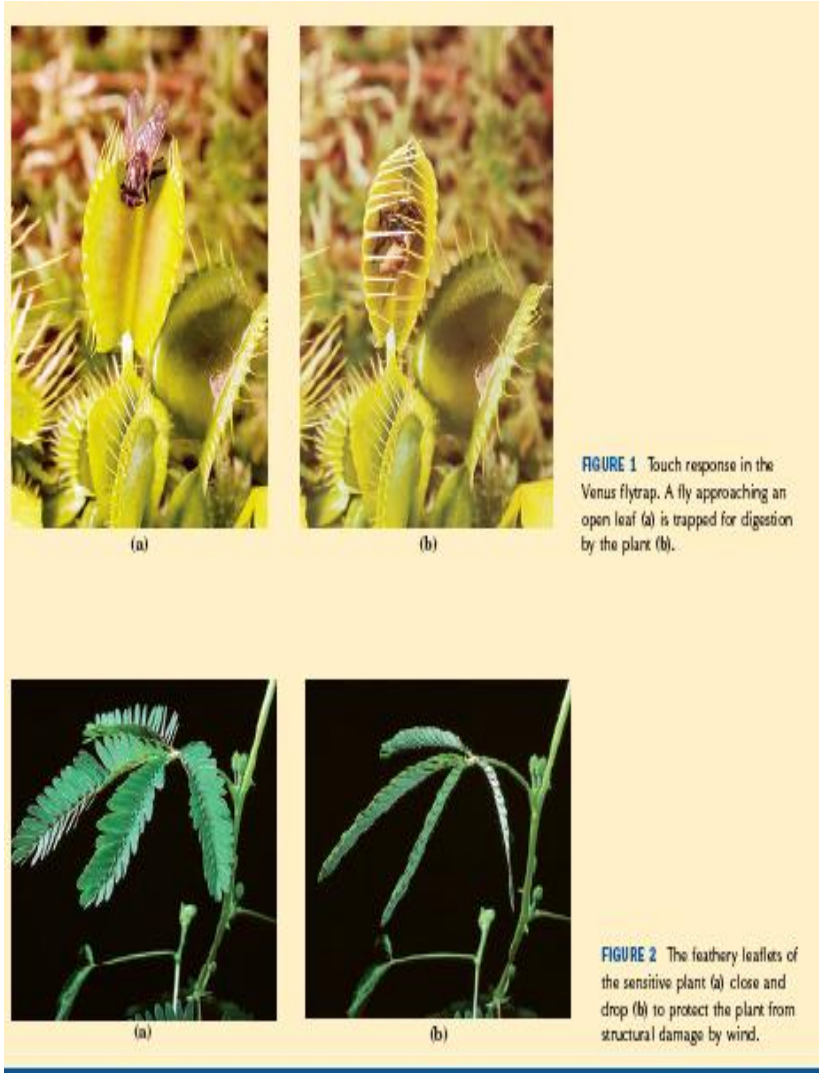


FIGURE 1 Touch response in the Venus flytrap. A fly approaching an open leaf (a) is trapped for digestion by the plant (b).

FIGURE 2 The feathery leaflets of the sensitive plant (a) close and drop (b) to protect the plant from structural damage by wind.

Osmosis and Laboratory Protocols

- **Cells placed in isotonic solutions to separate organelles**

- **Buffers used in cellular fractionations commonly contain**

  - **about 0.2 M of sucrose**

OR

  - **some other inert solute to perfect the organelles from osmotic - lysis**