BODY FLUIDS PHYSIOLOGY

Objectives after studying this chapter, you should be able to . . .

1. Know the composition of extracellular and intracellular body fluids.
2. Explain osmosis, osmolarity and osmotic pressure, and tonicity of the body fluids.
3. Know the forces producing movement of substances between compartments.
4. Describe the process of endocytosis and exocytosis.
5. Describe the primary factors (Starling forces) that determine fluid movement through the capillary membrane and the formation of interstitial fluid and lymph.
6. Describe the intake versus output of water.

Water is by far the most abundant components of the body, constituting about **60**% in young males and about **50**% in young women of the total body weight (TBW) (lower level in fat, upper level in thin, **80%** in infants). The percentage of body water varies inversely with the body's fat content and age.

The total body water in males is larger than in females because the latter have a somewhat larger amount of subcutaneous fat. In both sexes, the percentage of body water decreases with age, which can be attributed primarily to an increase of adipose tissue.

The body water is distributed into two major fluid compartments, **extracellular fluid (ECF)** (figure 2.1), which contains approximately **20%** of TBW, and **intracellular fluid (ICF)**, which contains approximately **40%** of the TBW.

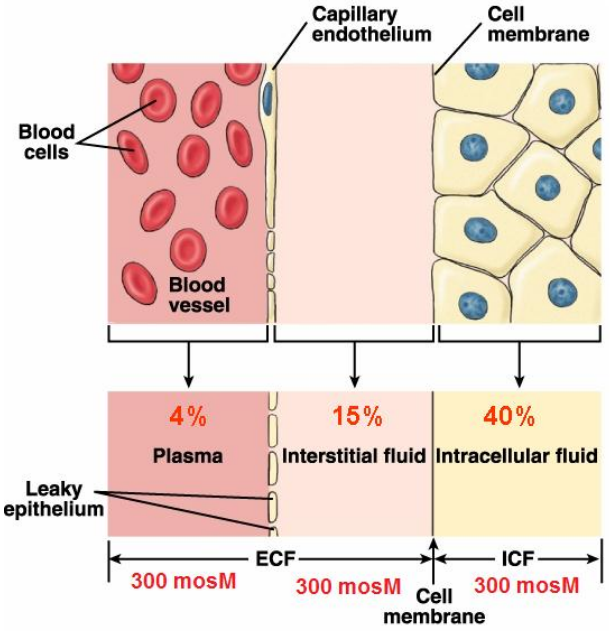


Figure 2.1: Water distribution between body compartments.

Extra cellular fluid (20% of the TBW):

1. The interstitial fluid (the fluids between cells and in lymphatic) which constitutes about **15%** of the TBW.

2. Plasma constitutes about **4%** of the TBW.

3. Transcellular fluids (include fluid in the gastrointestinal, biliary, and urinary tracts, the intraocular and cerebrospinal fluids, and fluid in the serosal spaces, such as the pleural, peritoneal, and pericardial fluid) constitutes about **1%** of the TBW.

Plasma and interstitial fluid: Have very similar composition, with **Na^+** as the predominant cation and **Cl^-** and **HCO_3^-** as the predominant anions (figure 2.2). However **an important difference between plasma and interstitial fluid is the larger concentration of proteins in the plasma.** This difference exists because the capillary endothelium is freely permeable to water and to small solutes (the so-called crystalloids), such as inorganic icons, glucose, and urea, but has limited permeability to larger solutes (colloidal particles), such as large proteins and lipids. Thus, interstitial fluid is an ultra filtrate of plasma.

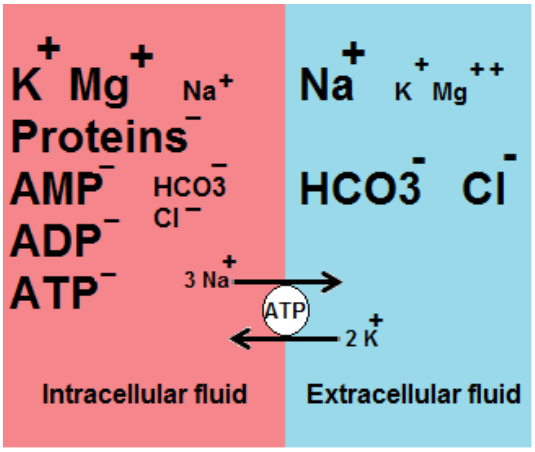


Figure 2.2: Relative concentration of various anions and cations between ECF and ICF.

In spite of the differences in ion concentrations and the total concentration of the similar charges, electrical neutrality is maintained within each compartment, i.e., the total number of cationic charges equals the total number of anionic charges.

Intracellular fluid (40% of the TBW): In contrast to ECF, the intracellular fluid (ICF) contains relatively low concentration of Na^+, Cl^-, and HCO_3^-. Instead, the predominant cation in ICF is **K^+** and **Mg^{++}**, while the predominant anions are **organic phosphates** (e.g. ATP, ADP, and AMP) and **proteins**. These striking composition differences between ICF and ECF can be attributed mainly to presence of the Na^+-K^+ ATPase pump in cell membranes which actively transports three sodium ions from and two potassium ions into cells, thereby accounting for the high sodium ion and low potassium ion concentrations in ECF and the opposite picture inside ICF.

Osmosis, osmotic pressure and osmolarity of the body fluids:

The **Osmosis** is the diffusion or flow of water (solvent) molecules across a semipermeable membrane (through channel proteins called **aquaporins**) into a region in which there is a high concentration of a solute to which the membrane is impermeable (figure 2.3). In another word; is the diffusion or flow of water molecules from region of high concentration of water molecules across a semipermeable membrane (through channel proteins called **aquaporins**) into a region of low concentration of water molecules.

Cells can regulate the rate of osmosis by adding aquaporins to the plasma membrane or removing them. Certain cells of the kidneys, for example, install or take away aquaporins to regulate the rate of water loss from the body in the urine

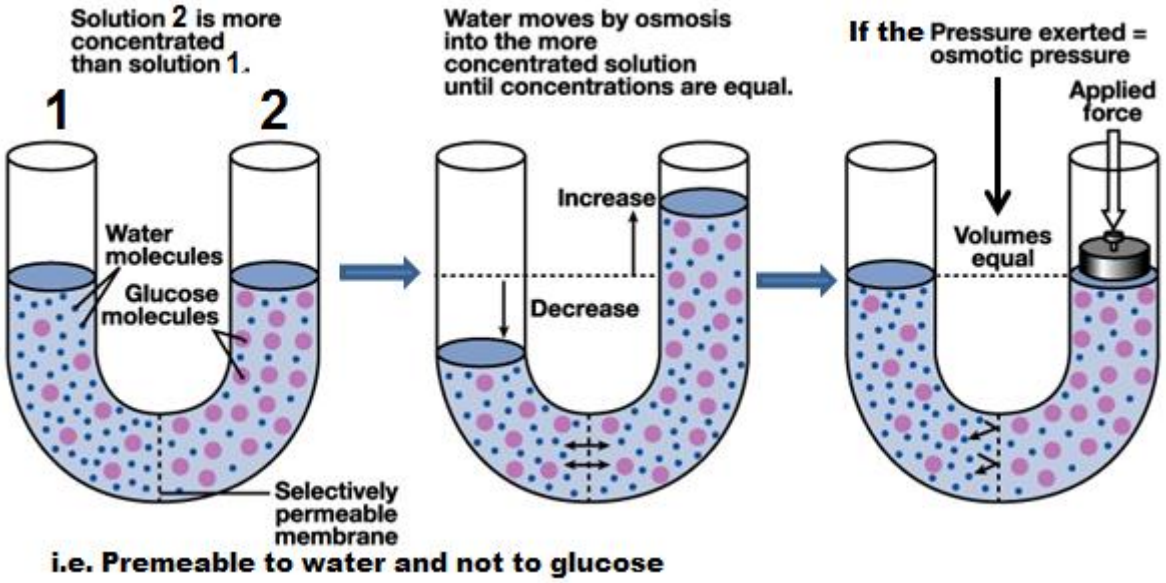


Figure 2.3: Osmosis through semipermeable membrane.

The **osmotic pressure** is an attractive force of the solute particles to water molecules that drives water molecules to move from solution 1 to solution 2 as a result of the presence of solute in solution 2. Consequently, the osmotic pressure difference across the membrane causes water to flow from solution 1 (which has no or less solute concentration and consequently lower osmotic pressure) to solution 2 (which has a higher solute concentration and a higher osmotic pressure) (figure 2.3). The number of the solute particles determines the magnitude of the osmotic pressure of the solution in which it is dissolved. **Oncotic pressure, or colloid osmotic pressure**, is a form of osmotic pressure exerted by large molecules such as **proteins** (notably albumin), in a blood vessel's plasma or interstitial fluid that usually tends to pull water toward these molecules.

➔ Each mole of a substance contains Avogadro's constant (6.022×10^{23}) of molecules.

➔**Molarity** of a substance is the concentration of non-dissociated substance (in moles) per one L (kg) of water.

➔ The **osmolarity** (osmole) is the concentration of osmotically active particles (the molecules or the particles which attract water to it) in one liter of a solution (**mol/L H_2O**).

➔The **osmolality** (osmole) is the concentration of osmotically active particles (the molecules or the particles which attract water to it) in one kg of a solution (**mol/Kg H_2O**).

➔ <u>The number of osmotically active particles in any fluid is determined by:</u> The number of moles per liter (or kg) of water **X** the numbers of osmotically active particles released into solution when the solute is dissolved.

Therefore;
One Avogadro's constant molecules of a substance/L H_2O = 1 Mole of substance/L H_2O (MOLARITY) = 1 Mole X the numbers of osmotically active particles released into solution when the solute is dissolved (OSMOLARITY, Osmole of substance /L H_2O).

Examples:
ʘ 5% of glucose in water has a molarity of 300 mMoles/L H_2O, and because the glucose molecules in water do not dissociated, it has an osmolarity of 300 mOsmol/L H_2O.
ʘ 0.9% NaCl in water has a molarity of 150 mMoles/L H_2O, and because each NaCl molecule in water is dissociated into two osmotically active particles (Na^+ and Cl^- ions); it has an osmolarity of 300 mOsmol/L H_2O.

Therefore, 5% of glucose in water and 0.9% NaCl in water have different molarity but identical osmolarity and consequently identical osmotic pressure.

Therefore, osmolality of the plasma and ECF is mainly due to sodium ions, chloride ions, bicarbonate ions, and to less extent due to other ions, urea, glucose, and proteins. The osmolality of the ICF is mainly due to potassium ions, magnesium ions, organic phosphates, proteins and other nitrogen containing solutes. In spite of the differences in composition, these fluids have essentially identical total osmolality.

In spite of the differences in composition of the body fluids, they have essentially identical total osmolalities of about of about 290-300 mOsmol/ kg H_2O. This is because the capillary endothelium and cell membranes are freely permeable to water, allowing the plasma, interstitial fluid, and ICF to be isomostic (iso-osmotic). It should be noted that the iso-osmolality principals applies primarily to the main body fluid compartments. Other body fluids can differ significantly from 290 mOsmol/kg H_2O, including the peritubular interstitial fluid of the renal medulla (as much as 1200 mOsmol / Kg H_2O) and certain transcellular fluids such as urine whose osmolality can vary from 70-1200 mOsmol/ Kg H_2O.

The term **tonicity** is used to describe the effective osmotic pressure of a solution relative to plasma in which the normal body cells can be placed without causing either swelling or shrinking. A solution with effective osmotic pressure as plasma is said to be **isotonic** which corresponds to 0.9 % solution of NaCl or a 5% glucose solution. Those solutions with greater pressure are **hypertonic** in which the normal body cells shrink when they are placed in it. Those solutions with lesser pressure are **hypotonic** in which the normal body cells swell when they are placed in it. Osmolarity and tonicity are related, but different concepts. The terms are different because osmolarity takes into account the total concentration of penetrating solutes (solutes that can diffuse through cell membrane easily such as urea) and non-penetrating solute (solutes that cannot diffuse through cell membranes such as NaCl); whereas tonicity takes into account the total concentration of **only** non-penetrating solutes. For example, if a solution had 150 mMol/L of NaCl and 100 mMol/L of Urea the tonicity of the solution would be 300 mOsmol/L for only the NaCl (non-penetrating solutes). The osmolarity of the solution, however, would be both NaCl and Urea and would therefore be 400 mOsmol/L. If we were to compare the osmolarity and tonicity of this solution to that of a typical cell (300 mOsmol/L), then it would be

isotonic (both have tonicities of 300 mOsmol/L) but hyperosmotic (the solution has an osmolarity of 400 mOsmol/L while the cell has an osmolarity of 300 mOsmol/L).

If water is added to the extracellular fluid by injection into the blood stream, by injection beneath the skin, or by ingesting water followed by absorption from gastrointestinal tract into the blood, the water dilutes the extracelluar fluids, causing it to become hypotonic with respect to the interstitial and to the intracellular fluids, osmosis begins immediately at the capillary and cells membranes, with large amounts of water passing to the interstitial and to the interiors of the cells, within a few minutes the water becomes distributed almost evenly amongst all the extracellular and intracellular fluid compartments, and all compartments will have the same osmolalities (i.e. the same tonicity). Excess water intake and dilution of extracellular fluid called **overhydration** or **water intoxication**.

If water is lost from the body by evaporation from the skin, lungs, or excretion of a very dilutes urine as in diabetes insipidus, or loss in feces as in diarrhea, the water will leave the extracellular fluid compartment causing this compartment to have a hypertonic fluid in respect to the fluids in the intracellular compartments. Osmosis begins immediately at the cell membrane with water passing to the interstitium and plasma and distributed uniformly between the three compartments, so that all the three compartments will have the same osmolalities. The overall effect is called **dehydration**.

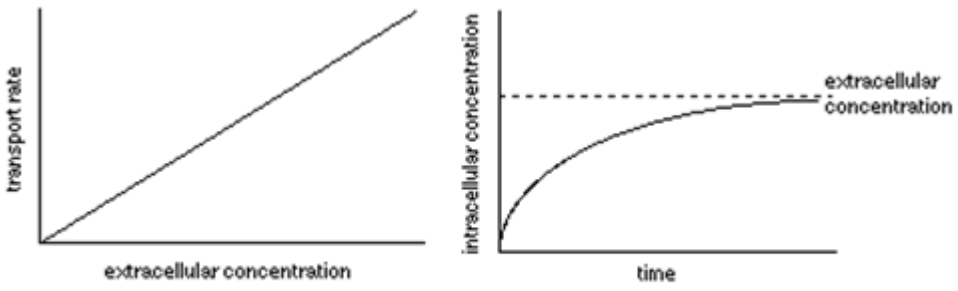
A principal laboratory test that indicates fluid deficit or excess is the urine **specific gravity**, which measures urine osmolarity. Normal range = 1.015-1.025. As fluid volume in the blood increases, the water excreted in the urine increases, making it more dilute and causing the specific gravity of the urine to decrease (below 1.015). Conversely, as the fluid volume in the blood decreases, as occurs in dehydration, the water excreted in the urine decreases, making it more concentrated and causing the specific gravity of the urine to increase (above 1.025).

Hematocrit levels also can indirectly indicate fluid volume in the blood. Since the test measures the number of blood cells per volume of blood, increased fluid in the blood, that is, hypervolemia will dilute the blood cells and cause the hematocrit level to decrease. Consequently, too little fluid in the blood, that is, hypovolemia, will cause hemoconcentration and result in a high hematocrit level. It is therefore important to consider the patient's hydration level when interpreting laboratory values.

The test for **serum osmolality** measures the concentration of osmotically active particles dissolved in blood. Sodium is a major contributor to osmolality in extracellular fluid. Serum osmolality generally ranges from 290 to 300 mOsmol/kg of H_2O. When fluid volume decreases, as in dehydration, serum osmolarity increases and vise versa.

Forces producing movement of substances between Compartments

[1] Simple diffusion: In which the molecules or ions tend to spread from regions to another regions according to electro-chemical gradients through the **cell membrane lipid bilayer** (for lipid soluble substances such as oxygen, nitrogen, CO_2, anesthetic gases, and alcohol) or through **cell membrane channel proteins** for water (aquaporin channels) or through water soluble substances (such as ion channels) until the concentration is uniform across the membrane. It does not require metabolic energy and therefore, it is passive. **Fick's law of diffusion** states that the net diffusion rate of a gas (Vgas) through a



kinetic behavior of simple diffusion

membrane is proportional to the tissue area (A) and the difference in partial pressure (P1-P 2) between the two sides, and is inversely proportional to the thickness (T). **Vgas = A x D x (Pi - P2)/T**, where D is a constant that depends on the solubility of the gas and inversely to the molecular weight and is given by D ∝ Solubility/Square root of molecular weight. Diffusion from one region to another is affected by:

(a) The concentration gradient. Diffusion of substances through cell membrane is directly proportional to the concentration gradient of that substance across the membrane.

(b) The electrical gradient. Whenever there is a potential difference between two regions, positively charged ions move along this electrical gradient to the more negative charged region, negatively charged ions move in the opposite direction.

(c) The thickness of the boundary. Diffusion of substances through cell membrane is inversely proportional to the thickness of the membrane

(d) The cross sectional area of the boundary across which diffusion is tacking place. Diffusion of substances through cell membrane is directly proportional to the cross sectional area of the boundary across which diffusion is taking place.

(e) Temperature. Diffusion is driven by the kinetic energy of the particles, and temperature is a measure of that kinetic energy. The warmer a substance is, the more rapidly its particles diffuse. This is why sugar diffuses more quickly through hot tea than through iced tea.

(f) Membrane permeability. Diffusion through a membrane depends on how permeable it is to the particles. Cells can adjust their permeability to such a substance by adding channel proteins to the membrane or taking them away. Kidney tubules, for example, do this as a way of controlling the amount of water eliminated from the body.

(g) The diffusion coefficient of the gas in the substance of the membrane.

[2] Filtration: Filtration is the process by which **water** and **water soluble substances** is forced through an **epithelial layer** or other barrier due to a <u>difference in hydrostatic pressure on the two sides</u>. Filtration is affected by

(a) The pressure gradient across the membrane,

(b) The surface area of the membrane,

(c) The diameter of the membrane pores,

(d) The size of the filtered molecules.

In physiology, the most important case of filtration is seen in the blood capillaries, where blood pressure forces fluid through gaps in the capillary wall. This is how water and water soluble substances (salts, nutrients, and other solutes) are transferred from the bloodstream to the tissue fluid and how the kidneys filter wastes from the blood.

[3] Osmosis.

[4] Carrier-mediated transport: It is the transport of substances across the cell membrane mediated by a carrier protein.

This type of transport is characterized by the following:

[i] Stereospecificity: For example, D-glucose is transported by facilitated diffusion, but the L-isomer is not.

[ii] Saturation: The transport rate increases as the concentration of the solute increases, until the carriers are saturated (the transport maximum, or T_m) then no more increase in transport in spite of increase in concentration of solute, for example, renal Tm of glucose..

[iii] Competition: Structurally related solutes compete for transport sites on carrier molecules. For example, galactose is a competitive inhibitor of glucose transport in the small intestine.

The direction of the carrier-mediated transport is:

[a] Uniport transport: If one transported substance is moving in one direction and without an associated transport of another substance (as in the facilitated diffusion of glucose).

[b] Co-transport or **symport:** If two or more of the transported substances are moving in the same direction (as Na^+-glucose co-transport, Na^+-amino acids co-transport, and Na^+-K^+-$2Cl^-$ co-transport).

[c] Counter-transport or **antiport:** If two or more of the transported substances are moving in the opposite direction (as Na^+-Ca^{2+} counter-transport and Na^+-H^+ counter transport).

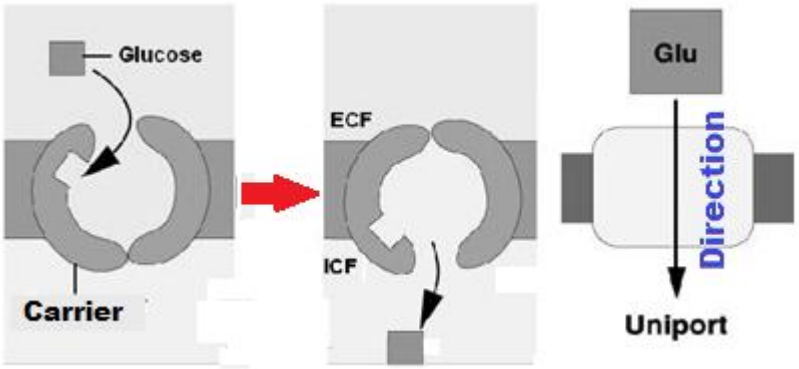
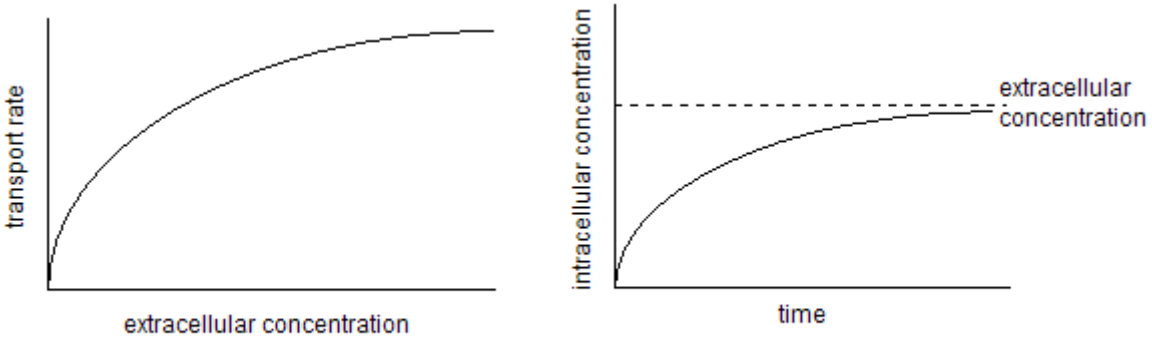


Figure 2.5: Facilitated diffusion.

Carrier-mediated transports are of two main types:

[A] Facilitated diffusion: When the carrier-mediated transport occurs according to electrochemical gradient and the **energy is not required** (passive)**,** the process is called facilitated diffusion (figure 2.5). It is also called a **uniport transport**. Example of facilitated diffusion is glucose transport in muscle and adipose cells is carrier-mediated, according to the electrochemical gradient, and is inhibited by sugars such as galactose. Therefore, it is categorized as facilitated diffusion. In diabetes mellitus, glucose uptake by the muscles and adipose cells is impaired because the carriers for facilitated diffusion of glucose require insulin.



kinetic behavior of facilitated diffusion

Simple diffusion	Facilitated diffusion
Diffusion according to electrochemical gradient	**Same**
Energy is not required	**Same**
Does not require a carrier protein	**Requires a carrier protein**
Simple diffusion is not saturable	**Have saturation limited (Tm)**
Bidirectional transport	**Uniport transport**

[B] Active transport: When the carrier-mediated transport occurs against electrochemical gradient, the process **requires energy** and is referred to as active transport. Active transport can be divided into two types:

(i) Primary active transport: In which high energy phosphate compound, ATP, **provides directly** the energy required for the transport process (figure 2.6). Such transports are Na^+-K^+-ATPase pump, H^+-ATPase pump (proton pump), H^+/ K^+-ATPases, and Ca^{2+}-ATPase pump.

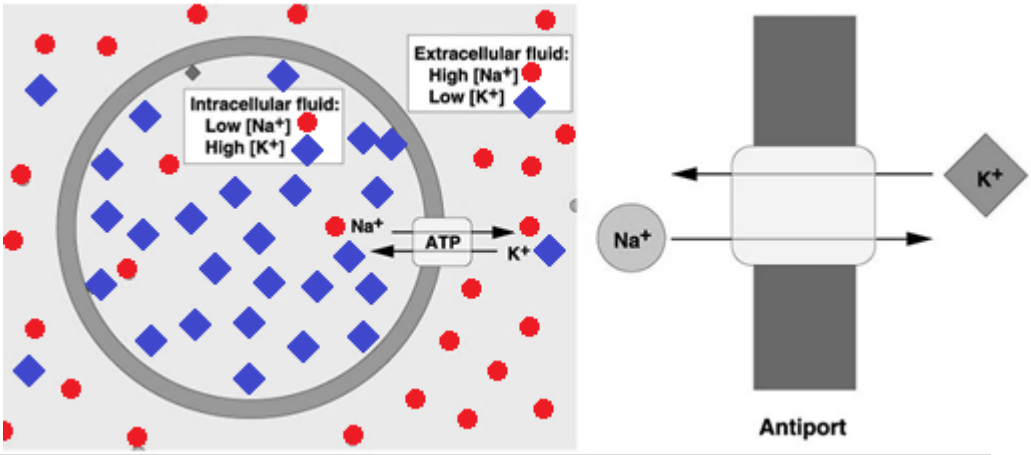


Figure 2.6: Primary active transport.

(ii) Secondary active transport: In which the transport of one substances (e.g. Na^+) accodrding to electrochemical gradient provides the energy to transport another substance against its electrochemical gradient (figure 2.7). The metabolic energy is **not provided directly**, but indirectly from the Na^+ gradient, which is maintained across cell membranes by Na^+-K^+ ATPase pump. Thus, inhibition of Na^+-K^+ ATPase

pump will decrease transport of Na^+ out of cell, decrease the transmembrane Na^+ gradient, and eventually inhibit secondary active transport.

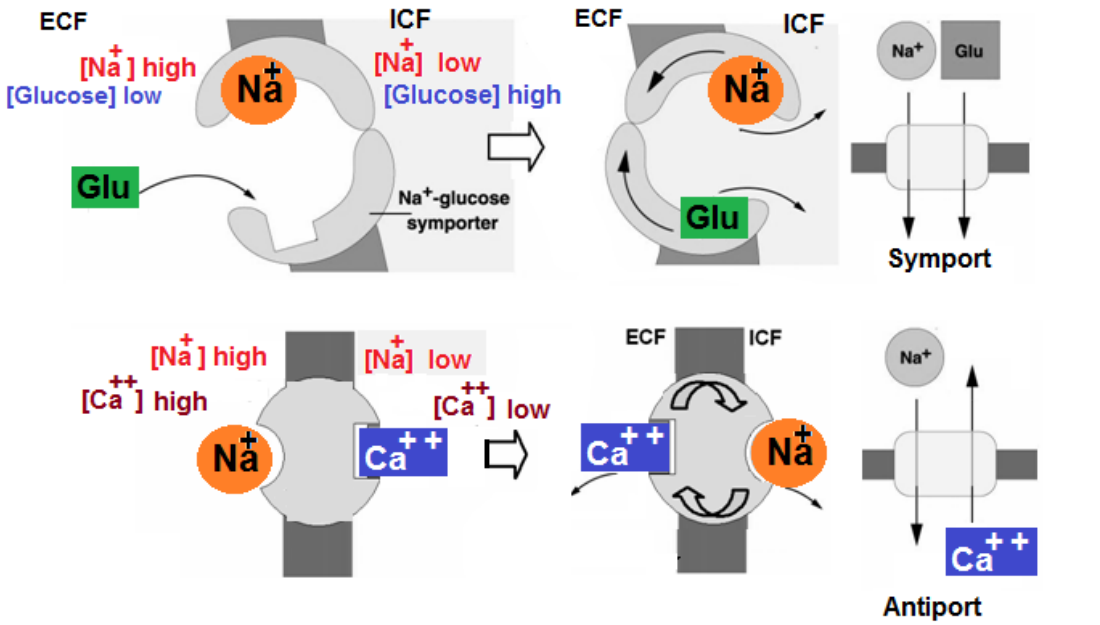
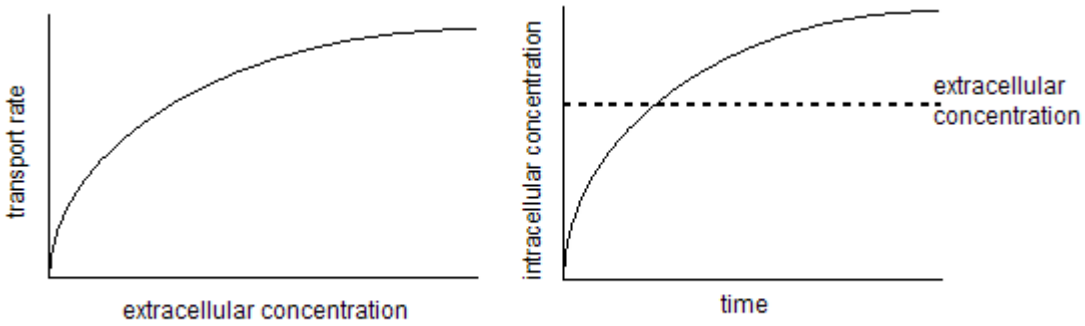


Figure 2.7: Secondary active transport.



kinetic behavior of Active transport

The following figure 2.8 summarizes the main types of transport of substances across the cell membrane:

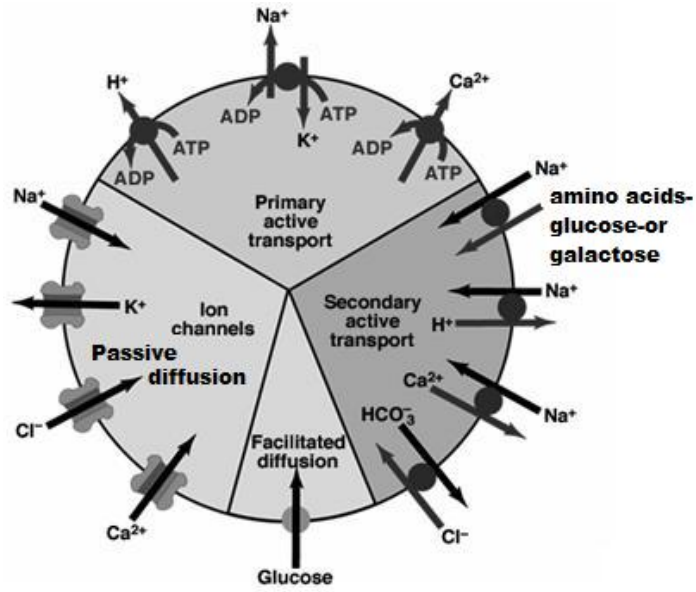


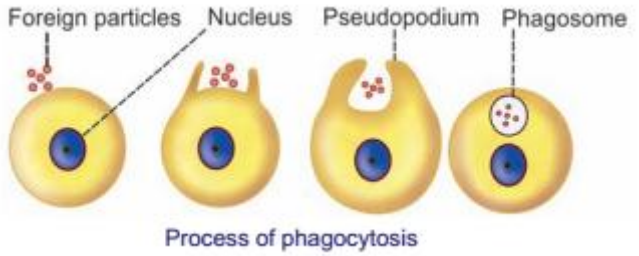
Figure 2.8: The main types of transport of substances across the cell membrane.

[5] Transport of proteins and other large molecules by cytosis: Cytosis is a completely different type of active transport involving the formation of membrane-bound vesicles with a diameter of 50–400 nm. **Vesicles** are either pinched off from the plasma membrane (**exocytosis**) or incorporated into it by invagination (**endocytosis**). Endocytosis and exocytosis are other forms of active transport but they do not involve membrane carriers. Cytosis in general has three characteristics:

1. It is associated with the expenditure of ATP (active processes).
2. All forms of cytosis involve the formation of protein-coated vesicles with some exceptions.
3. All are mediated by membrane receptors.

Endocytosis is of three types: Based on the nature and quantity of material taken up and the means of uptake, three types of endocytosis that use clathrin coated vesicles are recognized: phagocytosis, pinocytosis, and receptor-mediated endocytosis.

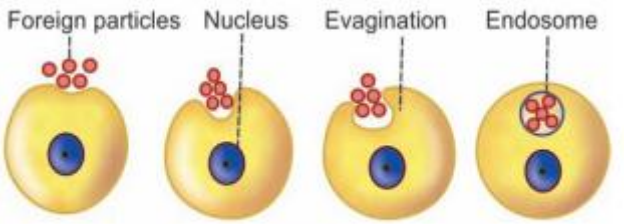
[A] Phagocytosis (cell eating) is the ingestion of large particles or microorganisms that occurs in specialized immune cells, and it is relatively nonselective. An important function of macrophages in humans is to remove invading bacteria. It occurs only after the extracellular particle has bound to the extracellular surface receptors. The particle is then enveloped by expansion of the cell membrane around it. This vesicle then fuses with many lysosomes, whereupon lysosomal enzymes digest its contents. Mechanism of phagocytosis involves the following steps:



Process of phagocytosis

i. When bacteria or foreign body enters the body, first the phagocytic cell sends cytoplasmic extension (pseudopodium) around bacteria or foreign body
ii. Then, these particles are engulfed and are converted into endosome like vacuole. Vacuole is very large and it is usually called the **phagosome**
iii. Phagosome travels into the interior of cell
iv. Primary lysosome fuses with this phagosome and forms secondary lysosome
v. Hydrolytic enzymes present in the secondary lysosome are activated resulting in digestion and degradation of the phagosomal contents

[B] Pinocytosis (cell drinking) is a general term for a nonspecific process in which a region of the plasma membrane is pinched off to form an endocytic vesicle inside the cell, it is also relatively nonselective. During vesicle formation, some fluid and dissolved solutes from the extracellular medium are trapped inside the vesicle and internalized by the cell. It occurs in almost all cells and it occurs continually and specific stimuli are not required. Mechanism of pinocytosis involves following events:



Process of pinocytosis

i. Macromolecules (in the form of droplets of fluid) bind to the outer surface of the cell membrane
ii. Now, the cell membrane evaginates around the droplets
iii. Droplets are engulfed by the membrane
iv. Engulfed droplets are converted into vesicles and vacuoles, which are called endosomes
v. Endosome travels into the interior of the cell
vi. Primary lysosome in the cytoplasm fuses with endosome and forms secondary lysosome

vii. Now, hydrolytic enzymes present in the secondary lysosome are activated resulting in digestion and degradation of the endosomal contents.

[C] Receptor-mediated endocytosis Receptor-mediated endocytosis is a highly selective mechanism with a minimum of unnecessary ECF fluid, by which cells take up a variety of important molecules, including hormones; growth factors; and serum transport proteins, such as transferrin (an iron carrier). Foreign substances, such as diphtheria toxin and certain viruses, also enter cells by this pathway. The receptors-mediated endocytosis also aids the cellular uptake of molecules present at low concentrations outside the cell. The surface receptors accumulate at specific depressions at the cell membrane where its cytosolic surface of it is covered with a coat of protein called **clathrin** (figure 2.9). This cell membrane depression is known as **coated pits**. The coated pits progressively encloses the substance to be taken into the cell and pinch off continually to form endocytic vesicles, providing the cell with a mechanism for rapid internalization of a large amount of a specific molecule without the need to endocytose large volumes of extracellular fluid.

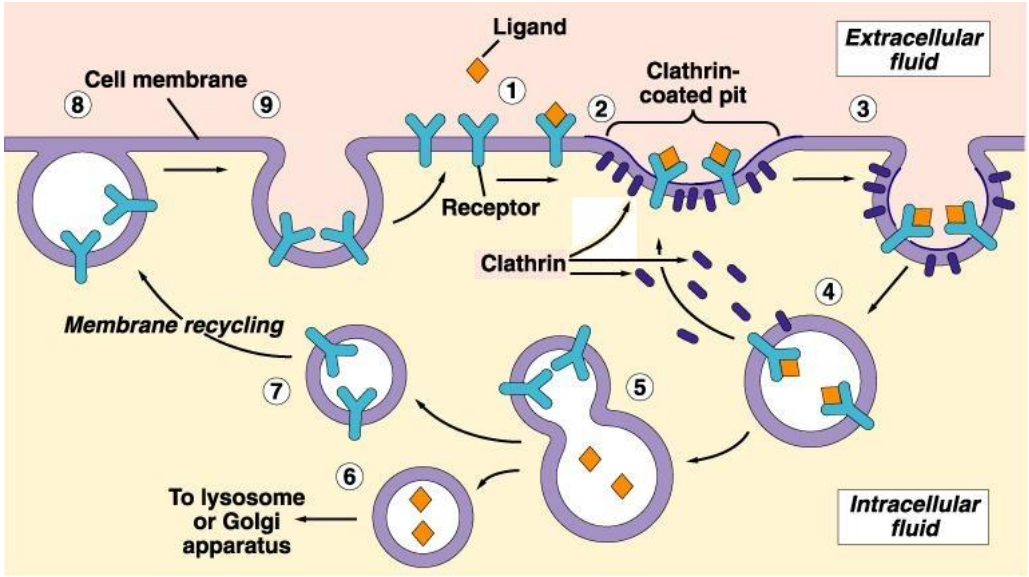


Figure 2.9: Receptor-mediated endocytosis.

Receptor-mediated endocytosis play an important role in the transport of several types of macromolecules into the cells, viz.

i. Hormones: Growth hormone, thyroid stimulating hormone, luteinizing hormone, prolactin, insulin, glucagon, calcitonin and catecholamines

ii. Lipids: Cholesterol and low-density lipoproteins (LDL)

iii. Growth factors (GF): Nerve GF, epidermal GF, platelet-derived GF, interferon

iv. Toxins and bacteria: Cholera toxin, diphtheria toxin, pseudomonas toxin

v. Some viruses

vi. Transport proteins: Transferrin and transcobalamine

vii. Antibodies: IgE, polymeric IgG and maternal IgG.

Some of the receptor-coated pits in cell membrane are coated with another protein called **caveolin** instead of clathrin. Caveolin-coated pits are concerned with the transport of vitamins into the cell.

Familial Hypercholesterolemia: The significance of LDL receptors and receptor-mediated endocytosis is illustrated by a hereditary disease called familial hypercholesterolemia. People with this disease have an abnormally low number of LDL receptors. Their cells therefore absorb less cholesterol than normal, and the cholesterol remains in the blood. Their blood cholesterol levels may be as high as

1,200 mg/dL, compared to a normal level of about 200 mg/dL. People who inherit the gene from both parents typically have heart attacks before the age of 20 (sometimes even in infancy) and seldom survive beyond the age of 30.

Exocytosis refers to a process reverse to endocytosis. Many cells synthesize important macromolecules that are exported from the cell. These molecules are synthesized in the endoplasmic reticulum, modified in the Golgi apparatus, and packed inside transport vesicles. The vesicles move to the cell surface, fuse with the cell membrane, and release their contents outside the cell. During exocytosis, the vesicular membrane is incorporated into the plasma membrane. In this way, cell membranes can be conserved and reused. An **increase in the intracellular Ca^{2+} concentration** (as a result of cell surface signal such as binding of a hormone to a membrane receptor or a change in membrane voltage) is a key event that triggers regulated exocytosis. Exocytosis accounts for hormone secretion, neurotransmitter release, mucus secretion, and in some cases, ejection of wastes.

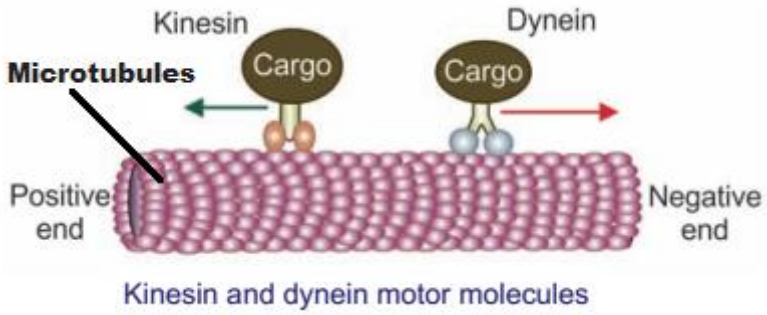
Molecular motors: Molecular motors are the protein-based molecular machines that perform intracellular movements in response to specific stimuli.

Functions of molecular motors

1. Transport of synaptic vesicles containing neurotransmitters from the nerve cell body to synaptic terminal
2. Role in cell division (mitosis and meiosis) by pulling the chromosomes
3. Transport of viruses and toxins to the interior of the cell for its own detriment.

Types of molecular motors: Molecular motors are classified into three super families:

1. Kinesin
2. Dynein
3. Myosin.



Kinesin and dynein motor molecules

1. Kinesin: Kinesin transports substances by moving over the microtubules. Each kinesin molecule has two heads and a tail portion. One of the heads hydrolyses ATP to obtain energy. By utilizing this energy, the other head swings continuously causing movement of the whole kinesin molecule. End portion of the tail carries the cargo (substances to be transported). Kinesin is responsible for anterograde (forward) transport (transport of substances towards the positive end of microtubule).

2. Dynein: Dynein is almost similar to kinesin and transports substances by moving over the microtubules. But it is responsible for retrograde (backward) transport (transport of substances towards the negative end of microtubule).

3. Myosin: Myosin transports substances by moving over microfilaments. Myosins are classified into 18 types according to the amino acid sequence. However, myosin II and V are functionally significant. Myosin II is involved in muscle contraction. Myosin V is involved in transport of vesicles.

The Na^+-K^+-ATPase pump: The Na^+-K^+-ATPase is a highly-conserved integral membrane protein that is expressed in virtually all cells of higher organisms. As one measure of their importance, it has been estimated that roughly 25% of all cytoplasmic ATP is hydrolyzed by sodium pumps in resting humans. In nerve cells, approximately 70% of the ATP is consumed to fuel sodium pumps.

The functions of Na^+/K^+-ATPase:

1. Helps in maintaining resting membrane potential: The sodium-potassium pump moves 3 sodium ions out and moves 2 potassium ions in, thus, in total, removing one positive charge carrier from the intracellular space. Decreasing the activity of Na^+/ K^+-ATPase leads to Na^+ enters the cell and K^+ leaves the cell, the concentration gradients across the cell membrane are dissipated and the cell membrane loses its polarized state.

2. Helps in transport of ions and substances through cell membrane: Export of sodium from the cell provides the driving force for several secondary active transporters membrane transport proteins, which import glucose, amino acids, and other nutrients into the cell by use of the sodium gradient. Therefore, Na^+-K^+ pump provides a Na^+ gradient that is used by certain carrier processes.

3. Regulate cellular volume: The sodium-potassium pump (Na^+/K^+-ATPase) maintains a low intracellular Na^+ concentration. A decrease in the activity of Na^+/K^+-ATPase leads to an accumulation of intracellular Na^+. The osmotic activity of the increased intracellular Na^+ pulls water into the cell, causing the cell to swell.

Regulation of Na^+/K^+-ATPase:

1. cAMP: The Na^+/K^+-ATPase is up-regulated by cAMP. Thus, substances causing an increase in cAMP up-regulate the Na^+/K^+-ATPase. In contrast, substances causing a decrease in cAMP down-regulate the Na^+/K^+-ATPase. Activation of protein kinase A by cAMP directly phosphorylates the pump and causes a conformational change of the pump and increases its affinity for intracellular sodium.

2. $[Na]_i$ and $[K]_o$: The activity of Na^+/ K^+-ATPase pump is stimulated by its own substrate, i.e. by increased intracellular sodium and extracellular potassium concentrations.

3. Exogenous substances: The Na^+-K^+-ATPase can be pharmacologically modified by administrating drugs exogenously. For instance, Na^+-K^+-ATPase found in the membrane of heart cells is an important target of cardiac glycosides (for example digoxin and ouabain), inotropic drugs used to improve heart performance by increasing its force of contraction. Muscle contraction is dependent on the Ca^{2+} release from the muscle cells' sarcoplasmic reticulum. Immediately after muscle contraction, intracellular Ca^{2+} is quickly returned to its normal concentration by a carrier enzyme in the plasma membrane, and a calcium pump in sarcoplasmic reticulum, causing the muscle to relax. Since this carrier enzyme (Na^+-Ca^{2+} counter-transport, exchanger) uses the Na gradient generated by the Na^+-K^+ pump to remove Ca^{2+} from the intracellular space, slowing down the Na^+-K^+ pump results in a permanently elevated Ca^{2+} level in the muscle, which may be the mechanism of the long-term inotropic effect of cardiac glycosides such as digoxin.

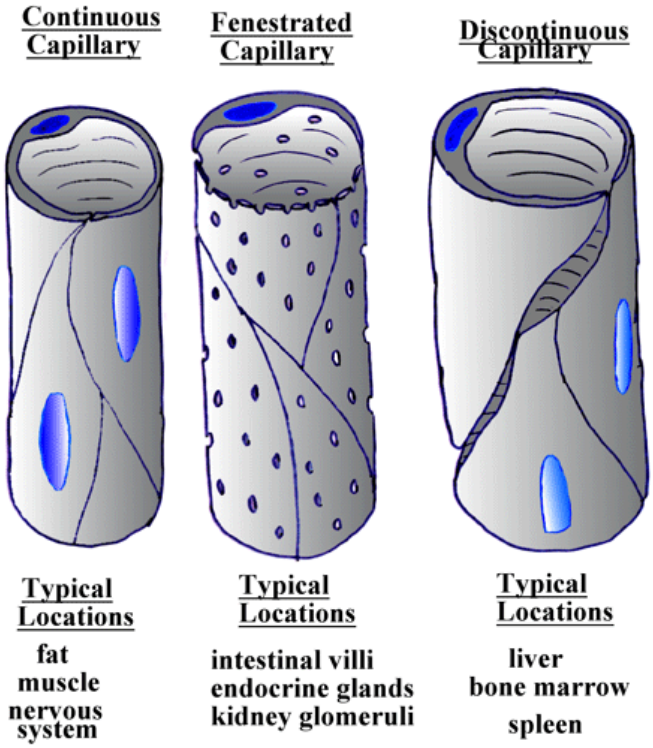
4. Muscle inactivity and low dietary potassium intake: Muscle inactivity and low dietary potassium intake decrease the abundance of pump in skeletal muscle, and vice versa. Accumulation of potassium in the interstitium of skeletal muscle during repetitive action potential depolarizes membrane potentials and contributes to muscle fatigue. Up-regulation of Na+, K+-ATPase will enhance muscle potassium uptake and reduce potassium accumulation in the interstitium during exercise, and explain why physical training increases exercise endurance. Up- and down-regulation of Na+, K+-ATPase are important in maintaining extracellular potassium homeostasis in response to high and low dietary potassium intake, respectively.

5. Hormones: Major hormonal controls over pump activity can be summarized as follows:

- **Thyroid hormones** appear to be a major player in maintaining steady-state numbers of pumps in most tissues. This effect appears to result from stimulation of gene transcription.
- **Aldosterone** is a steroid hormone with major effects on sodium homeostasis. It stimulates both rapid and sustained increases in pump numbers within several tissues. The sustained effect is due to enhanced transcription of the genes.
- **Catecholamines** have varied effects, depending on the specific hormone and tissue. For example, dopamine inhibits Na+-K+-ATPase activity in kidney, while epinephrine stimulates pump activity in skeletal muscle. These effects seem to be mediated via phosphorylation or dephosphorylation of the pumps.
- **Insulin** has multiple effects on sodium pump activity. Within minutes of elevated insulin secretion, pumps have increased affinity for sodium, up-regulation of pump activity through activation of adenylate cyclase to produce cAMP. In skeletal muscle, insulin may also recruit pumps stored in the cytoplasm or activate latent pumps already present in the membrane.

Formation of Interstitial Fluid and Lymph

Formation of interstitial fluid: Exchange of water and dissolved substances through capillary wall depends upon the type of capillary. In general, three types of capillaries have been described.



Type 1 or **Continuous capillaries:** These capillaries have uninterrupted membranes and they only allow smaller molecules, such as water and ions to pass through their intercellular clefts. However lipid-soluble molecules can passively diffuse through the endothelial cell membranes along concentration gradients. They occur in skin, muscle, pulmonary circulation, nervous system, connective and adipose tissue.

Type 2 or **Fenestrated capillaries:** These capillaries have fenestrated membranes. Fenestrations are being of the order of 0.1 micrometer. The extremely thin fenestral membrane confers a very high permeability to water and to small lipophobic solutes, and limited amounts of protein. They are found in tissues specialized for water exchange, including all the exocrine glands, e.g. salivary glands, pancreas. Typical sites are glomeruli of the kidneys and intestinal epithelium.

Type 3 or **Discontinuous (sinusoidal) capillaries:** Capillaries have discontinuous membranes. They are interrupted by large intercellular spaces through which fluids and cells can pass. These capillaries are found in the bone marrow, spleen and liver.

The primary factors (Starling forces) that determine fluid movement through the capillary membrane: Starling proposed that fluid exchange across the capillary wall between plasma and interstitial fluid was achieved by a balance between four forces, in addition to capillary permeability. These forces include (figure 2.10):

[1] The capillary hydrostatic pressure, which tends to move or to filter the fluid outward through capillary membrane. The capillary pressure at the arteriolar end is about **35 mm Hg** and it is about **15**

mm Hg at the venous side (except in glomerular capillaries, where it is higher and nearly constant of about 50-60 mm Hg). The filtration is greater at the arteriolar side than at the venous side.

[2] The interstitial fluid hydrostatic pressure, which tends to move fluid outward through the capillary membrane when interstitial fluid pressure is negative. It is about **– 4 to – 5 mm Hg.** The interstitial pressure can be affected by lymphatic drainage and by the tissue tension. If the lymphatic drainage is blocked, the interstitial fluid pressure is increased. Moreover, in palms of the hands and in the muscles, the interstitial fluid is less able to expand its volume because of the anatomical arrangement of its connective tissues and therefore is likely to cause an increase in the interstitial fluid pressure. On the other hand, the loose texture of the connective tissue below the eyes and behind the wrists is less resistance to an increase in volume of the interstitial fluid.

[3] The plasma colloid osmotic pressure, which tends to cause osmosis of fluid inward through the membrane. The colloid osmotic (oncotic) pressure of normal human plasma averages approximately **25 mm Hg** mainly is caused by the dissolved protein (6-8 g/dL). About 75% of the total colloid osmotic pressure of the plasma result from the albumin fraction, 25% from globulin, and almost non from the fibrinogen. This is because albumin has a smaller M.W. than others, so there are more molecules per gm of albumin than molecules per gm of globulin. Moreover, albumin is more dissociated in plasma than globulin.

[4] The interstitial fluid colloid osmotic pressure due to protein (about **2 g/dL**) which tends to cause osmosis of fluid outward through the membrane. It is about **6 mm Hg**. The protein concentration is influenced, in part, by the amount of fluid filtration into the interstitium. For example, increased capillary filtration into the interstitium decreases interstitial protein concentration and reduces the oncotic pressure.

[5] Capillary permeability: The capillary is completely permeable to small molecules and water, but is normally only slightly permeable to plasma protein. This permeability to colloids may be increased by a number of factors such as certain poisons, oxygen lack and bacterial toxins.

Therefore, the **Starling equation** is: $J_v = K_f [(Pc-Pi) – (\pi c – \pi i)]$.

J_v = Fluid movement (ml/min)

K_f = Capillary permeability (ml/min/mm Hg)

Pc = Capillary hydrostatic pressure (mm Hg)

Pi = Interstitial hydrostatic pressure

πc= Capillary oncotic pressure (mm Hg)

πi= Interstitial oncotic pressure.

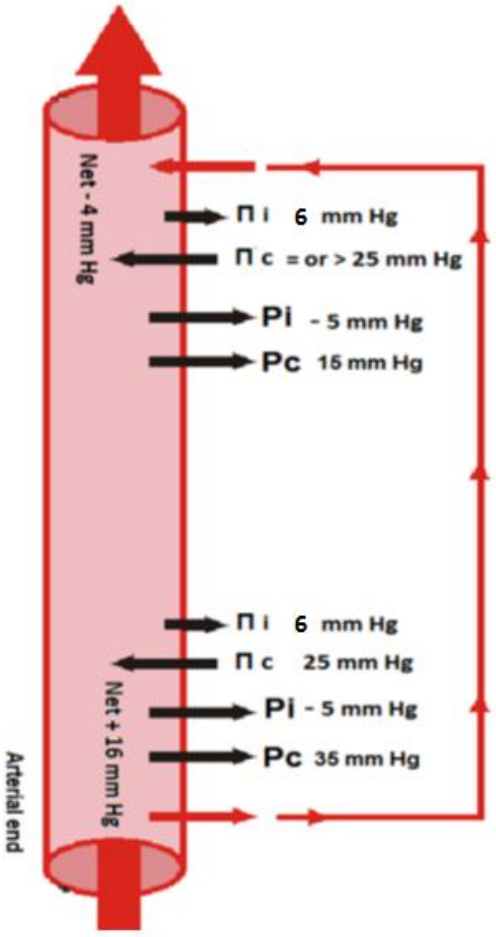


Figure 2.10: The primary factors (Starling forces) that determine fluid movement through the capillary membrane.

Example: At the arteriolar end of a capillary, Pc is 35 mm Hg, π c is 28 mm Hg, Pi is -4 mm Hg, and π i is 1 mm Hg. Will filtration or absorption occur?

Net pressure (Jv) (35 – [– 4]) – (28 – 6) = + 17 mm Hg. **Because the net pressure is positive, filtration will occur. If the net pressure is negative, absorption will occur**.

The hydrostatic pressure is greater at the arteriolar end than that at the venous end. The effect of the plasma proteins is to withdraw fluid from the more dilute

interstitial fluid into the highly concentrated plasma. Therefore, at the arteriolar end the hydrostatic pressure is greater than the osmotic pressure and water and salts pass from the plasma to the interstitial fluid, while at the venous end the hydrostatic pressure is lower than the osmotic pressure and water and salts pass from interstitial fluid to the plasma. In this way a dynamic equilibrium is set up by water passing from the plasma to the lymphatic.

Formation of lymph: Lymphatics form a closed system of tubes consisting of endothelial lining supported by fibrous tissue. Out of the filtered fluid from the capillary 10% enters in these lymphatics whereas 90% is reabsorbed at the venous end. Lymphatics are much more permeable to proteins than capillaries. The proteins leaked from plasma into interstitial space cannot return to capillary because of adverse concentration gradient. Their accumulation in interstitial space will upset starling equilibrium and proteins diffuse into the very permeable lymphatic capillaries together with large molecules produced by cells such (as hormones, enzymes, lipoproteins, chylomicrons). Large lymphatics have muscle fibers in their walls, lymphatic vessels possess numerous valves and the flow of lymph from periphery to thoracic duct and right lymphatic duct is brought about by muscular and respiratory movement in the same way as blood flows in the veins. Right lymphatic duct opens in right subclavian vein and thoracic duct opens in left subclavian vein. Approximately lymph flow via thoracic duct is 120 ml per hour. The lymphatics of intestine (lacteals) show rhythmic contraction which, because of the many valves propel lymph into the thoracic duct. This contractile activity is an intrinsic property of the lymphatics and is not coordinated by NS.

Lymph:

1. Has same concentration of salts as interstitial fluid and plasma.
2. Has lower concentration of proteins than plasma (except hepatic lymph which is higher in protein concentration) but has slightly higher concentration of proteins than interstitial fluid. This is because the lymph before reaching the blood lymph passes through at least one or more (usually 8-10) lymph nodes. During its passage through a lymph node the lymph is altered in composition by:
    i. Newly formed antibodies (immunoglobulins) are added.
    ii. Lymphocytes enter.
3. Has all clotting factors but low fibrinogen.
4. Rarely contains fat (except lymph from small bowel - high fat)

Main functions of Lymph:

1. Return of proteins to blood from tissue spaces.
2. Fat from intestine are mainly absorbed through lymph.
3. Maintain fluid distribution in body.

Edema: Disturbance of water balance in which there is an excess of fluid in tissue spaces and serous cavities of the body is called **edema.** Edema is detectable clinically only when the interstitial fluid volume is increased by at least 10%. The factors that cause edema are:

- Increase in capillary hydrostatic pressure (at arterial or venous side).
- Decrease in plasma oncotic pressure (due to **hypoproteinemia**) or an increase in interstitial oncotic pressure (due to an increase of capillaries permeability to proteins as in case of inflammation, toxins, or other conditions).
- Obstruction of lymphatic drainage (l**ymphedema, which** is an edema of a part of the body due to complete obstruction of lymphatic vessel draining from such part).

However, experimental and clinical observations indicate that edema does not occur until there is a relatively large change in one of these parameters. This is because the body has protective mechanisms against the development of edema.

Protection factors against edema:

- An increase in lymphatic flow: Lymphatic flow is able to increase as the interstitial fluid volume is increased, so that the excess filtrate can initially be carried away.
- As fluid initially moves into the interstitium from capillaries, the interstitial oncotic pressure will fall (both by dilution and by the lymphatic removal of interstitial proteins), thereby minimizing the gradient for further entry of water from capillaries into the interstitium.
- The increase in interstitial fluid volume will cause the interstitial hydrostatic pressure to rise; edema cannot occur until the normally negative value of interstitial space becomes positive.

The importance of these safety factors varies from organ to organ. In skeletal muscle, for example, all three contribute. In comparison, the hepatic sinusoids are relatively open and freely permeable to proteins. As a result, there is normally no oncotic pressure gradient across the sinusoids, since the plasma and interstitial oncotic pressures are roughly equal. Thus, the hydrostatic pressure gradient is unopposed, although the intrasinusoidal pressure is relatively low because most of the hepatic perfusion derives from the low-pressure portal venous system. In this setting, it is hepatic lymph flow that is primarily responsible for preventing the accumulation of excess interstitial fluid.

Clinical applications:

[A] Dehydration (volume contraction) states: They are of three types:

[1] Isomotic dehydration which is primarily caused by loss of isotonic fluid from ECF compartment. It can be caused by haemorrhage, plasma exudation through burned skin, and gastrointestinal fluid loss (as in vomiting and diarrhea). In this type of dehydration:

- ECF volume decreases while the osmolarity of the ECF is kept constant.
- Because osmolarity of ECF is unchanged, water does not shift between the ECF and ICF compartments.
- Therefore, the ICF volume and osmolarity do not change.
- The plasma protein concentration and haematocrit (Hct) are increased because the ECF volume is decreased.
- Arterial blood pressure is decreased.

[2] Hyperosmotic dehydration which is primarily caused by loss hypotonic fluid (water) from ECF compartment. It can be caused by diabetes insipidus, diabetes mellitus, alcoholism, administration of lithium salts (drugs), fever, and excessive evaporation from skin through heavy loss of sweat (which is hypotonic). In this type of dehydration:

- ECF volume decreases while the osmolarity of ECF is increased.
- Because osmolarity of ECF is decreased, water shifts from ICF to the ECF.
- As a result of this shift, ICF volume decreases while ICF osmolarity increases until it equals the ECF osmolarity.
- The plasma protein concentration is increased while the haematocrit (Hct) remains unchanged because water shifts out of the RBCs, decreasing their volume and offsetting the concentrating effect of the decreased ECF volume.

[3] Hyposmotic dehydration: This is primarily caused by loss of hypertonic fluid from ECF compartment. It can be caused by renal loss of NaCl because of adrenal insufficiency as in Addison's disease. In this type of dehydration:

- The osmolarity of ECF decreases.
- Consequently, water shifts from ECF to ICF.
- As a result of this shift, ECF volume is decreased while the ICF volume is increased with ICF osmolarity equals ECF osmolarity.
- Plasma protein concentration increases because of the decrease in ECF volume. Haematocrit (Hct) increases because of the decreased ECF volume and because of RBCs swell as a result of water entry.

[B] Overhydration (volume expansion) states: They are of three types:

[1] Isosmotic overhydration which is primarily caused by addition of isotonic fluid to the ECF compartment. It can be caused by any condition that is responsible to cause edema and also can be caused by oral or parenteral administration of large volume of isotonic NaCl (150 mmol/L). In this type of overhydration:

- The ECF volume is increased while the osmolality of the ECF is kept constant. Because osmolarity of ECF is unchanged, water does not shift between the ECF and ICF compartments.
- Therefore, the ICF volume and osmolarity do not change.
- The plasma protein concentration and haematocrit (Hct) are decreased because the ECF volume is increased.
- Arterial blood pressure is increased.

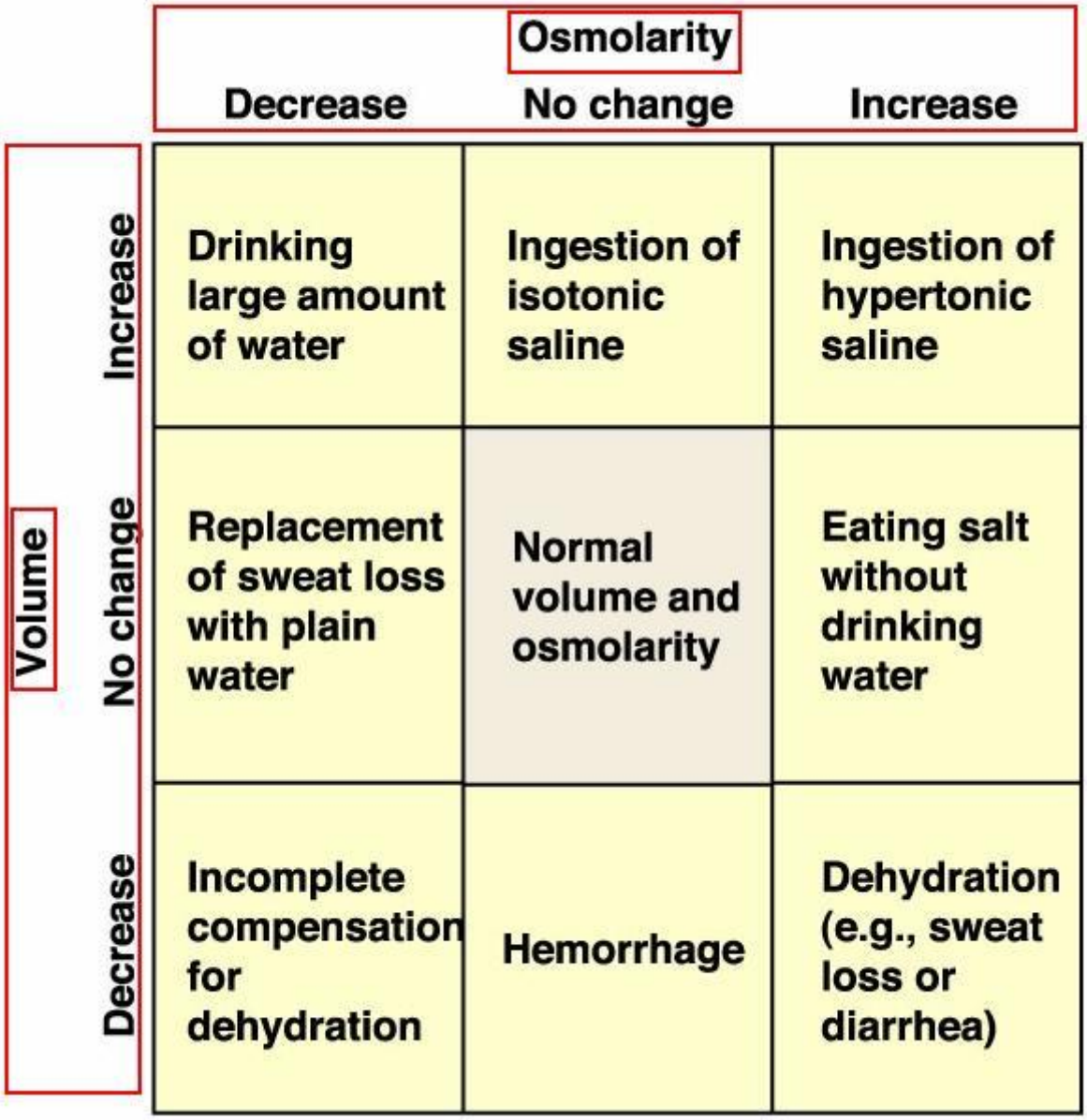
[2] Hyperosmotic overhydration which is primarily caused by addition of hypertonic fluid to the ECF compartment. It can be caused by oral or parenteral intake of large amounts of hypertonic fluid. In this type of overhydration:

- The ECF osmolarity is increased.
- Consequently, water shifts from ICF to ECF.
- As a result of this shift, ECF volume is increased while the ICF volume is decreased with ICF osmolarity equals ECF osmolarity.
- The plasma protein concentration and haematocrit (Hct) are decreased because of the increase in ECF volume.

[3] Hyposmotic overhydration which is primarily caused by addition of hypotonic fluid to the ECF compartment. It is caused by ingestion of a large volume of water or renal retention of water due to the syndrome of inappropriate antidiuretic hormone secretion (SIADH). In this type of overhydration:

- The volume of ECF increases because of water retention while ECF osmolarity is decreased.
- Consequently, water shifts from ECF to ICF.
- As a result of this shift, ICF volume increases and ICF osmolarity decreases until it equals ECF osmolarity.
- The plasma protein concentration decreases because of the increase in ECF volume. Haematocrit remains unchanged because water shifts into the RBCs, increasing their volume and offsetting the diluting effect of the gain of ECF volume.

Changes in volume and osmolarity of body fluids							
Type	**ECF volume**	**ECF osmolarity**	**ICF volume**	**ICF osmolarity**	**Plasma protein concentration**	**Hct**	**ECF [Na]**
Isosmotic dehydration	↓	=	=	=	↑	↑	=
Hyperosmotic dehydration	↓	↑	↓	↑	↑	=	↑
Hyposmotic dehydration	↓	↓	↑	↓	↑	↑	↓
Isosmotic overhydration	↑	=	=	=	↓	↓	=
Hyperosmotic overhydration	↑	↑	↓	↑	↓	↓	↑
Hyposmotic overhydration	↑	↓	↑	↓	↓	=	↓
= No change							


Osmolarity
Decrease
No change
Increase
Volume
Increase
No change
Decrease
Drinking large amount of water
Ingestion of isotonic saline
Ingestion of hypertonic saline
Replacement of sweat loss with plain water
Normal volume and osmolarity
Eating salt without drinking water
Incomplete compensation for dehydration
Hemorrhage
Dehydration (e.g., sweat loss or diarrhea)