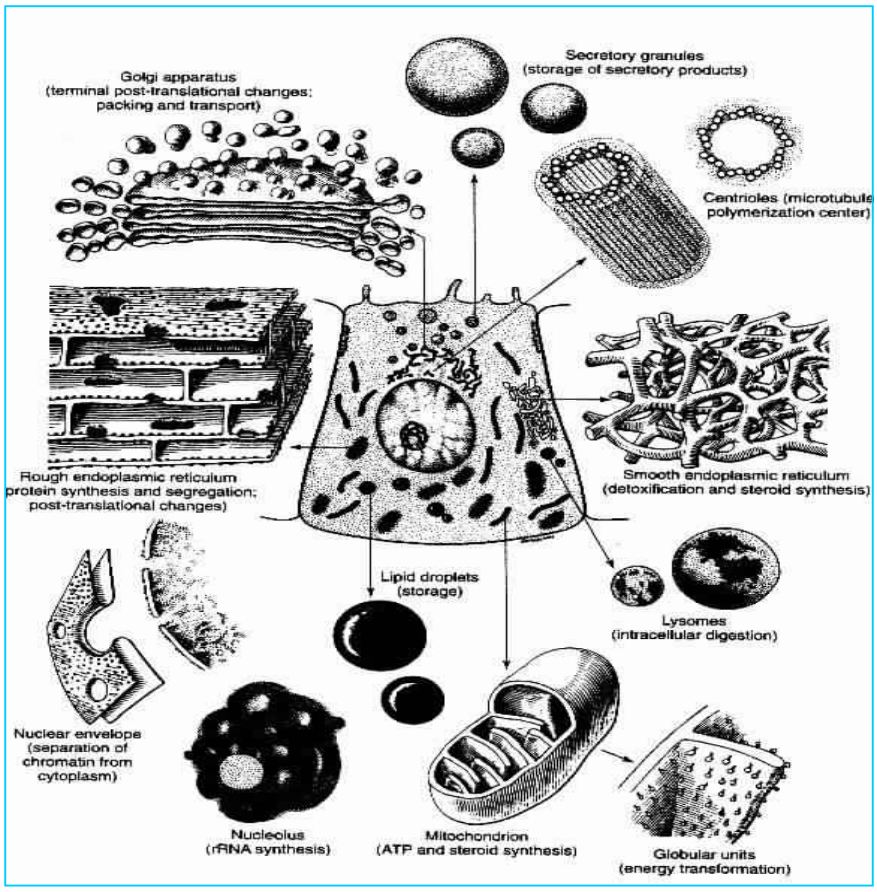
PHYSIOLOGY 2017: OPTO 5344

Lecture 1. Transport across the cell membrane Constanzo 1

I. Introduction

Fig. 1 Composition and size of cells and organelles (Ganong, 21st edition)



Water - 70-85% of cell mass

Electrolytes - (e.g. K^+, Na^+, Cl^-, Ca^{2+},Mg^{2+}, HCO^{3-}) <1% of cell mass

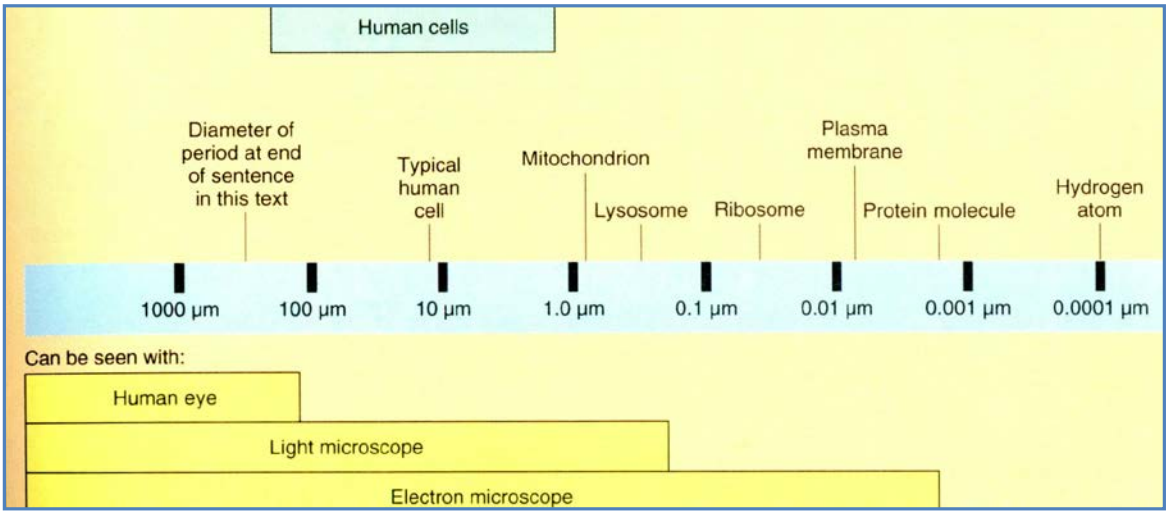
Proteins (amino acids) - 10-20% of cell mass

Lipids - 2-3% cell mass

Carbohydrates - 1%, mostly in combination with proteins & lipids as glycoproteins & glycolipids

Nucleic acids - nucleotides

Fig. 2 Size of cells and organelles (Vander Physiology)



Cytoskeleton

- Microfilament (mainly actin)
- Intermediate filament (tensile strength)
- Microtubule (tubulin: scaffold and tracks)

Plasma Membrane Structure

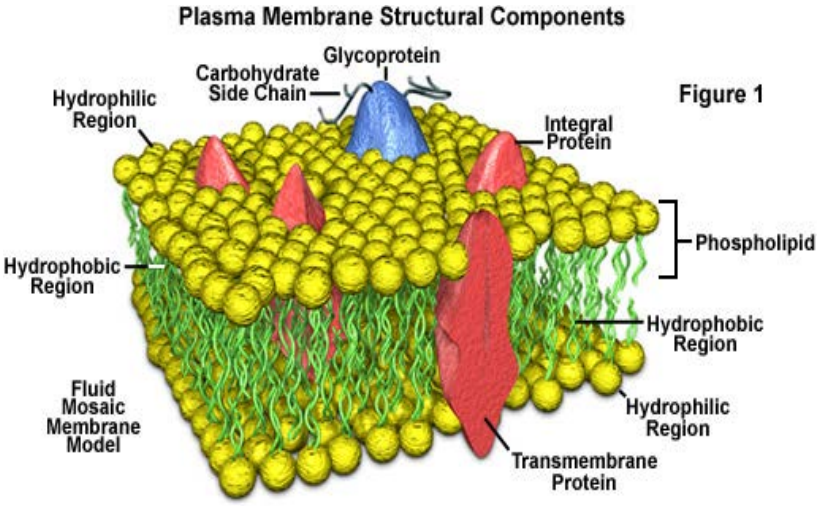
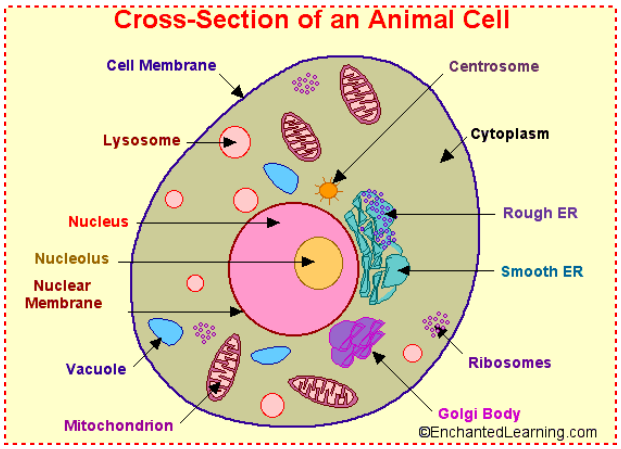


Figure 1



I. Structure of the cell membrane

A. Plasma membrane - the fluid-mosaic model (Fig. 3)

1. The cell membrane is generally thin, 7.5-10 nm, and elastic. Protein 55%, phospholipid, 25%, cholesterol 13%, other lipids 4%, carbohydrates, 3%.
2. The membrane is a phospholipid bilayer with proteins embedded and cholesterol woven in. The bilayer is responsible for passive permeability properties of the membrane
3. Lipids decrease movement of most molecules through the membrane, proteins provide pathways for selective entry.
   a. The phospholipids are amphipathic. They consist of a phosphorylated glycerol backbone (head) that is hydrophilic and two nonpolar fatty acid chains that are hydrophobic.
      1. The hydrophilic ends are oriented toward the exterior of the membrane, facing extracellular fluid (ECF), and intracellular fluid (ICF).
      2. The hydrophobic ends move away from water and attract each other; they are in the center of the membrane
   b. Integral and peripheral protein functions
      1. Integral proteins, embedded in the membrane, form receptors, ion (charged particle) channels, pumps and carriers, and enzymes. They are anchored to the membrane by hydrophobic interactions.

2. Peripheral proteins – loosely attached by electrostatic interactions with integral proteins, roles in cellular communication, e.g. regulatory protein subunits for ion channels and transmembrane receptors.

c. The cell membrane is fluid due to the lipids, and the proteins are globular masses that float in the lipids. Cholesterol is a "fluidity buffer" that diminishes lateral membrane mobility. It also reduces permeability to small water-soluble molecules.

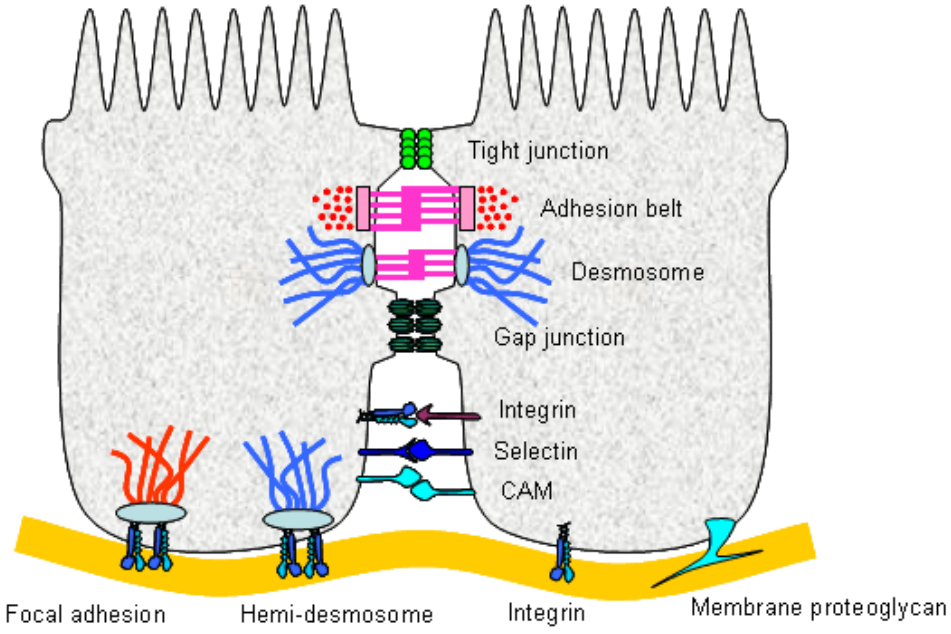
d. Lipid rafts: (cholesterol and sphingolipids) form micro (up to 200 nm) domains that are gel-like, called lipid rafts, where proteins are secured, and specific types of signaling will occur.

4. Carbohydrates join via covalent bonds with many of the integral proteins in the membrane to form glycoproteins, and with lipids to form glycolipids to form the glycocalyx – cell-cell contact, recognition (immune function) and communication.

B. Membrane junctions between cells (Fig. 4)

1. Tight junctions - fusion of the plasma membranes of two adjacent cells. A selective barrier - also called zonulae occludens.
2. Desmosomes - spot welds formed by protein filaments (intermediate). No transcellular communication - also true of zonulae adherens, and of hemidesmosomes, and focal adhesions that attach cells to basal lamina
3. Gap junctions - proteins-units (connexons) from two adjacent cells form channels forpassage of ions and other small molecules between the cells.

Fig. 4



C. Cell Adhesion Molecules (CAMS) – sticky proteins on the cell surface that bind with other cells or the extracellular matrix. They govern cell-to-cell interactions and are necessary for embryonic development, cell growth and differentiation, pathogen detection, inflammation, and wound repairs. CAMs bind to like molecules on other cells (homophylic binding), to other molecules (heterophylic) or to laminins in the extracellular matrix.

1. Calcium dependent CAMS
    a. Integrins – heterodimers that bind to various laminins, IgGs,
    b. Cadherins Ca^{2+} dependent molecules, cell to cell in homophylic relations
    c. Selectins (LEC-Cams) - lectin-like domains that bind to carbohydrates (mucins on WBC)
2. Calcium independent CAMs
    a. Ig superfamily of immunoglobins (later section on immunology)
    b. Lymphocyte homing receptors

II. Body fluid compartments

A. Fluid, or "total body water" is about 50-70% of body weight

1. Extracellular fluid (ECF) is 20% of body weight
    a) Interstitial 15% (extravascular - outside the vessels)
    b) Intravascular 5% (blood plasma)
2. Intracellular fluid (ICF) is 40% of body weight.

B. Electrolyte composition of intra- and extracellular fluids - (Table 1).

C. Solid components, 25%: protein (18%), minerals (7%) and fat tissue (15%)

Table I: Electrolyte composition of intra- and extracellular fluid (Costanzo)

TABLE 1.1 Approximate Compositions of Extracellular and Intracellular Fluids

Substance and Units	Extracellular Fluid	Intracellular Fluid[a]
Na^+ (mEq/L)	140	14
K^+ (mEq/L)	4	120
Ca^{2+}, ionized (mEq/L)	2.5[b]	1×10^{-4}
Cl^- (mEq/L)	105	10
HCO_3^- (mEq/L)	24	10
pH[c]	7.4	7.1
Osmolarity (mOsm/L)	290	290

[a]The major anions of intracellular fluid are proteins and organic phosphates.

[b]The corresponding total $[Ca^{2+}]$ in extracellular fluid is 5 mEq/L or 10 mg/dL.

[c]pH is $-\log_{10}$ of the $[H^+]$; pH 7.4 corresponds to $[H^+]$ of 40×10^{-9} Eq/L.

D, Total solute concentration (osmolarity) is the same in ECF and ICF

III. Functions of plasma membranes

A. Regulate the passage of substances into and out of the cell
    1. Across the membrane through channels, by diffusion or via carriers or pumps
    2. Endocytosis (into the cell by engulfing) and exocytosis (out of the cell – release of substances from vesicles that fuse to the membrane

B. Detect chemical messengers arriving at the cell surface (receptors)

C. Link adjacent cells together via membrane junctions and CAMs

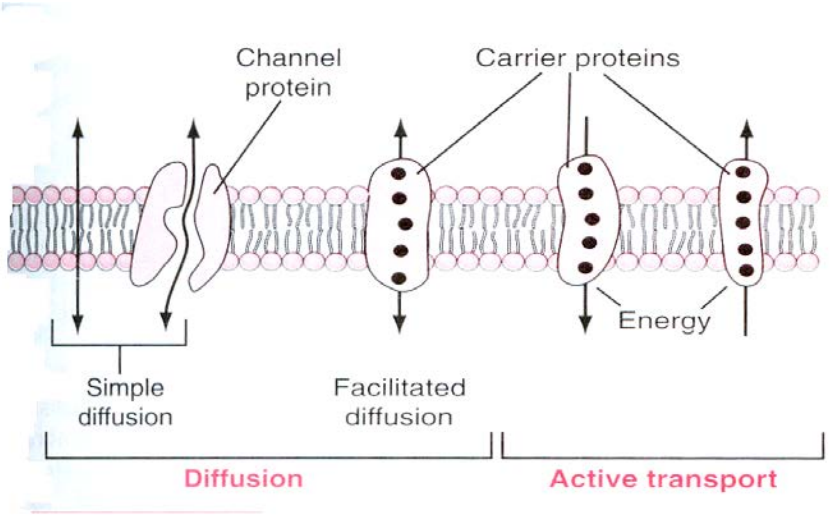
D. Anchor proteins

IV. Transport across (through) the cell membrane

A. Membrane Permeability - Introduction

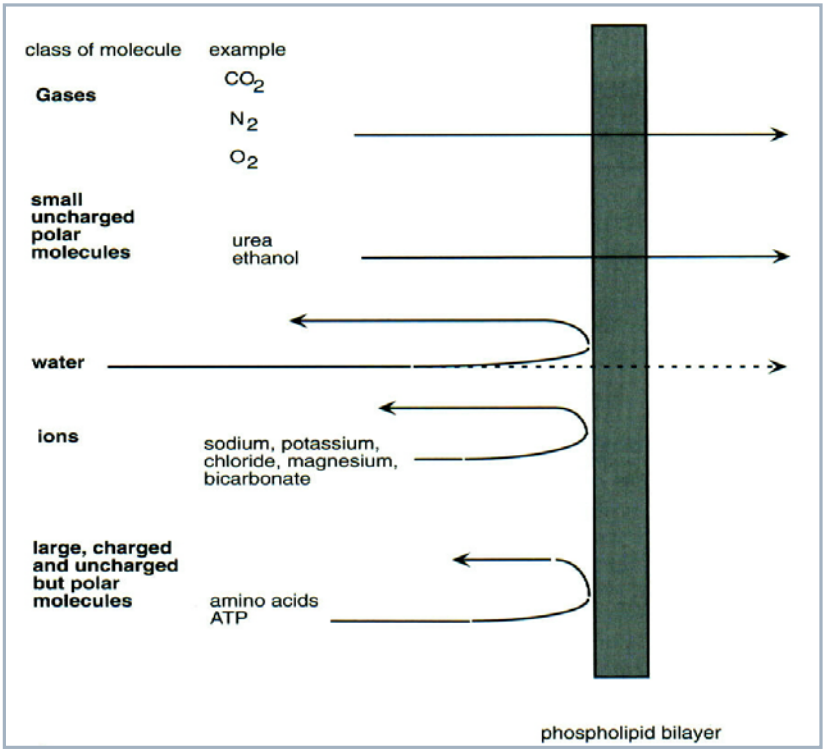
Membranes are "selectively" permeable - allowing some substances, but not others to pass through. There are several mechanisms by which substances can pass through membranes. These mechanisms fall in three major categories: diffusion (due to random thermal motion), osmosis (flow of water across a semipermeable membrane due to differences in solute concentration) and protein-mediated processes (channels, pumps, carriers). Water movement across membranes will occur either via movement between phospholipids or via water channels (Aquaporins).

Fig. 5A - Examples of channels and transporters



Transport pathways through the cell membrane and the basic mechanisms of transport.

Fig. 5B – Simple diffusion, in the absence of pumps and channels necessary for charged particles



Aquaporins – H_2O

Aquaglyceroporins
- gases
- glycerol
- small uncharged polar molecules

B. Diffusion - passive movement

A. Simple diffusion – When starting with a higher concentration of a substance in one of two compartments, net diffusion (net flux) will move the substance downhill from the higher to the lower concentration. Net rate of diffusion (J, moles or gm per sec)) is the difference between movement in both directions: J is proportional to the permeability (P) and the surface area of the membrane (A, cm^2). (C_A - C_B) is the difference in concentration on the two sides of the membrane (mole/cm^3).

NET FLUX = $J = PA(C_A - C_B)$ (Costanzo)

Permeability (P) includes the partition coefficient (lipid solubility, K), the diffusion coefficient within the membrane in cm^2/sec (D), and membrane thickness (cm, Δx),

$P = KD/\Delta x$ Note that the permeability and therefore, net flux, are inversely proportional to the thickness of the membrane that solute will cross.

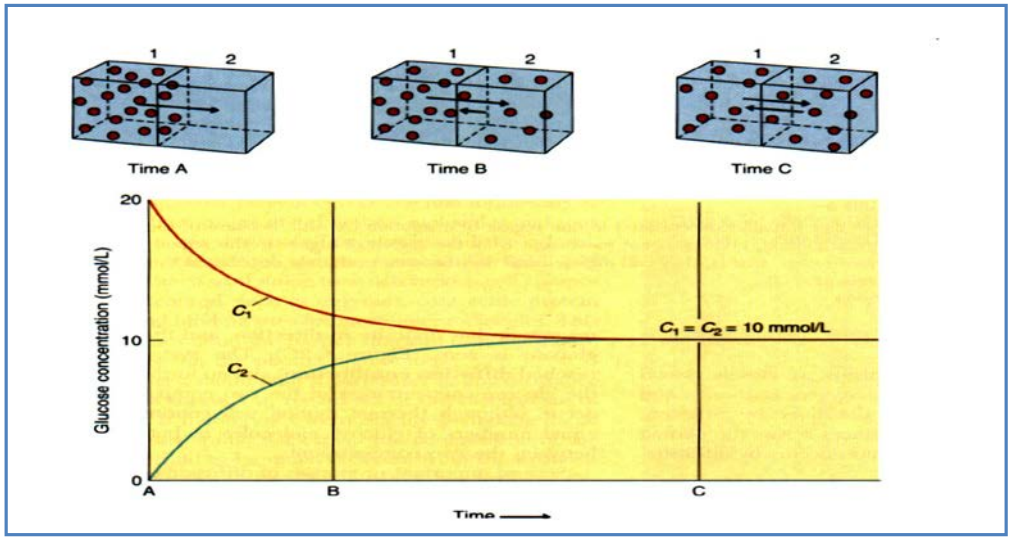
1. Factors that influence the diffusion coefficient (D) for a particular molecule:
   a. Size of the molecule - large molecules such as glucose do not pass through the membrane as easily as smaller molecules. D is inversely proportional to the molecular radius of the solute.
   b. Viscosity of the medium – D is inversely proportion to the viscosity of the medium
   c. Temperature - higher temperature leads to greater thermal motion of molecules and more net flux.
   d. Kinetic energy – Boltzman's constant for the solute

The description thus far assumed that the solute is a nonelectrolyte.

2. For charged ions (or electrolytes), an electric potential difference across the membrane also will cause diffusion of ions. Therefore it is important to consider both the concentration difference and the electrical difference. In addition, the diffusion generates a potential difference across a membrane, called a diffusion potential.

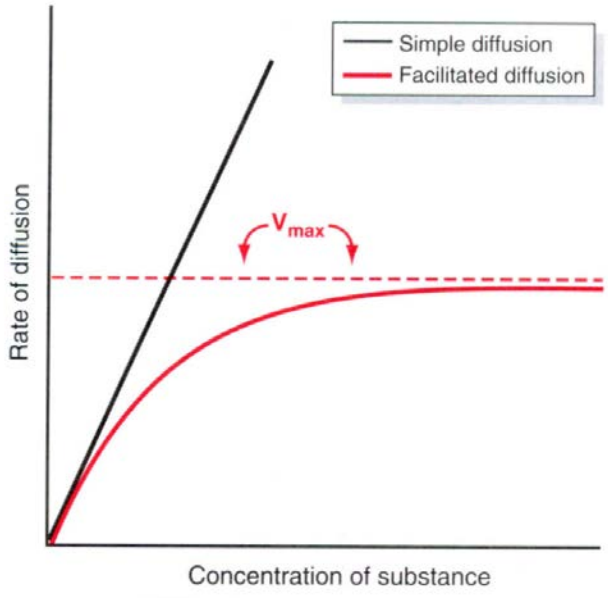
3. Presence of channels - charged ions such as Na^+, K^+ diffuse at faster rates than their low solubility in lipids would predict. These are four ways in which channels can be "gated" or "activated":
   a. Ligand or receptor-activated - the channel opens when a specific chemical binds to the channel protein outside the cell
   b. Second messenger-activated – gated by intracellular signaling molecule, e.g. cAMP.
   c. Voltage-activated - the channel opens when a "threshold voltage" is reached
   d. Mechano-sensitive - the channel opens when the channel protein is stretched or pressure is applied

Fig. 5 C. Artificial membrane between cells that allows passive diffusion of glucose shows that the concentration on the two sides of the membrane will equilibrate, but flux will continue.



C. Saturation

Facilitated diffusion (transport) (Fig. 6). A transport (carrier) protein facilitates (assists) diffusion of the substance across the membrane. The rate of facilitated transport will saturate, i.e. it will reach a maximal flux (Vmax), called transport maximum TM, when all of the carrier sites are occupied. For simple diffusion, there is no Vmax. The binding sites on transport proteins are stereospecific, but competition can occur when closely related substances bind to the sites and inhibit transport.



IV. Primary active transport - uses energy (ATP) to move (pump) ions across membranes (Fig. 7) and like facilitated diffusion, it is a form of mediated transport

A. Transport that is powered by phosphorylation of a transport protein
   1. Hydrolysis of ATP
   2. Uphill movement of ions against the concentration gradient (from low to high concentration)
   3. The Na^+-K^+ ATPase pump - maintains concentration gradients in virtually every cell. [Na^+] is high outside, [K^+] is high inside the cell. The pump is a protein that sends 3 Na^+ out for every 2 K^+ that it sends into the cell.
      a. The pump is electrogenic since there is a net change in charge which causes a flow of current.
   4. The Ca^{2+} ATPase: Ca^{2+} is kept very low inside the cell by plasma membrane ATPase (PMCA), also Ca^{2+} ATPase on endoplasmic reticulum and sarcoplasmic reticulum in skeletal muscle (SERCA).
   5. H^+-K^+ ATPase - transports H^+ out of cells (e.g. acid secretion in stomach)

B. H^+-ATPase that acidifies intracellular organelles, e.g. Golgi complex

C. H^+-ATPase on mitochondrial inner membrane - generally synthesizes ATP using energy of H^+ gradient across membrane

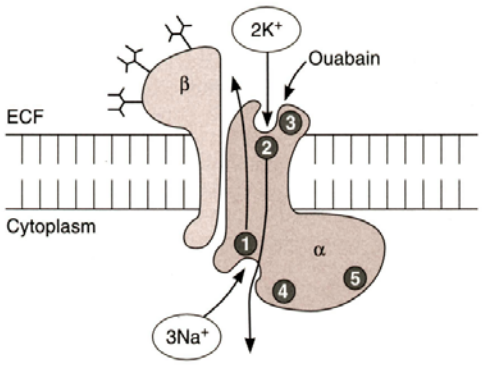
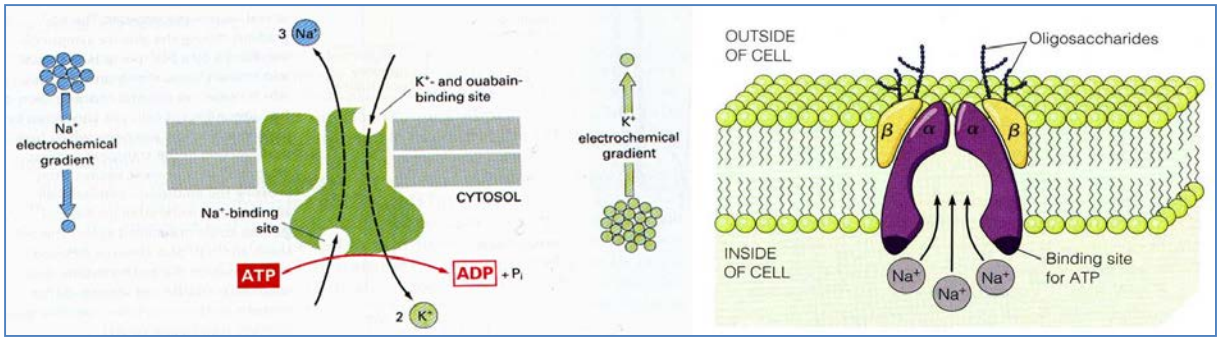


Fig. 7A. Na^+-K^+ ATPase (pump) In one cycle of the pump, in the E1 state, 3 Na^+ ions bind to the high affinity binding region inside the cell, and one ATP molecule is hydrolyzed to ADP + Pi releasing energy. A conformational change occurs, and the enzyme switches to E2 state in which affinity for Na^+ is low, and 3 Na^+ ions are released to the ECF. Then, 2 K^+ ions bind to high affinity binding sites for K^+ and Pi is released. The enzyme binds ATP, and 2 K^+ ions are released to the ICF. One charge (net) flows out of the cell for each pump cycle. (1) Na^+-binding site, (2) K^+-binding site, (3) ouabain (cardiac glycoside) binding site, (4) phosphorylation site, (5) ATP-binding site (Ganong, Fig. 1-32, 23rd edition).

Fig. 7B. Sodium and potassium are moved against electrochemical gradients



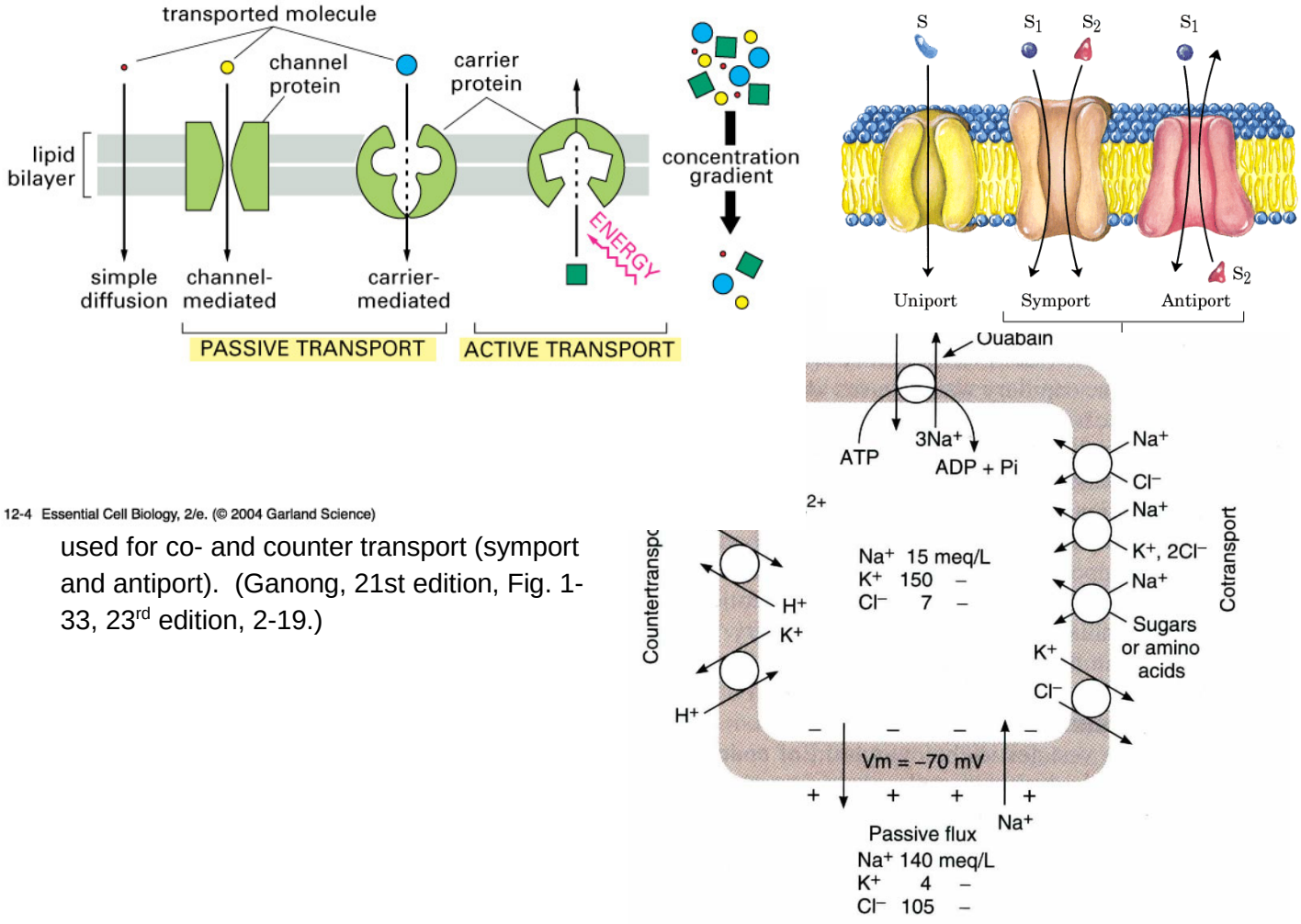
V. Secondary active transport - due to the <u>concentration gradient</u> (e.g. in Na^+) created by primary active transport, the ion flows across the membrane from the side with the high concentration of the ion to the side with the low concentration

A. Na^+ may allosterically modify affinity of a transport protein for its substrate.

B. Co-transport (symport) - Na^+ runs down its concentration gradient and goes into the cell, and it takes for example, Cl^-, sugar or amino acids into the cell - via a carrier in the membrane.

C. Counter transport (antiport or exchange) - Na^+ runs down its concentration gradient into the cell, and H^+ or Ca^{++} for example (see Figs. 8-9) is moved out of the cell.

Fig. 8



12-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

used for co- and counter transport (symport and antiport). (Ganong, 21st edition, Fig. 1-33, 23rd edition, 2-19.)

VI. Measures of solute concentration are:

A. Molarity - A one molar solution contains one mole (6×10^{23} molecules) of a substance. It is the molecular weight (in gms), placed in a liter of water.

B. Equivalent - takes the valence (charge) of ionized substances into account. One mole of Ca^{2+} = 2 equivalents of Ca^{2+}

C. Osmolarity - moles of solute particles per liter (<u>volume</u>) of solution. One mole of NaCl contains *about** 2 osmoles because there are both Na^+ and Cl^- particles. Therefore a 1 molar solution of NaCl is 2 osmolar. (*osmotic coefficient of NaCl about 0.93 - for accurate calculation, multiply 0.93 x 2 osmoles) that takes the osmotic coefficient into account (compound, concentration, temperature)

D. Osmolality - 1 mole per kg (based on mass). For plasma, osmolarity is 1-2% less than osmolality

E. Percent solution - Sometimes solutions are described according to the number of grams in 100 ml. A ten percent solution of glucose contains 10 gms in 100 ml total fluid.

Example:

NaCl - 154 mM (~308 mOSM) is equal to about a 0.9% solution (isotonic)

(molarity - molecular wt in gm/L)

- Na 23 gm
- Cl 35 gm
- NaCl 58 gm

To be worked out on the board in class

VII. Osmosis (movement of water)

The movement of solvent molecules (i.e. water) across a semipermeable membrane due to differences in solute concentration which creates osmotic pressure.

A. Osmotic pressure - the pressure that must be applied to stop osmosis when it is due to a solution containing nonpenetrating solutes on one side of a membrane separated by a semipermeable membrane from pure water on the other side. Osmotic pressure can be measured with an osmometer (freezing point.)

van't Hof equation: $\pi = gC\sigma RT$

$\pi =$ omotic pressure (atm or mm Hg)

g = number of particles per mole solution (Osm/mol)

C = concentration (mmol/L)

$\sigma =$ reflection coefficient (0-1) freely permeable to impermeable

R = gas constant (0.082 L- atm/mol - K)

T = absolute temperature (K)

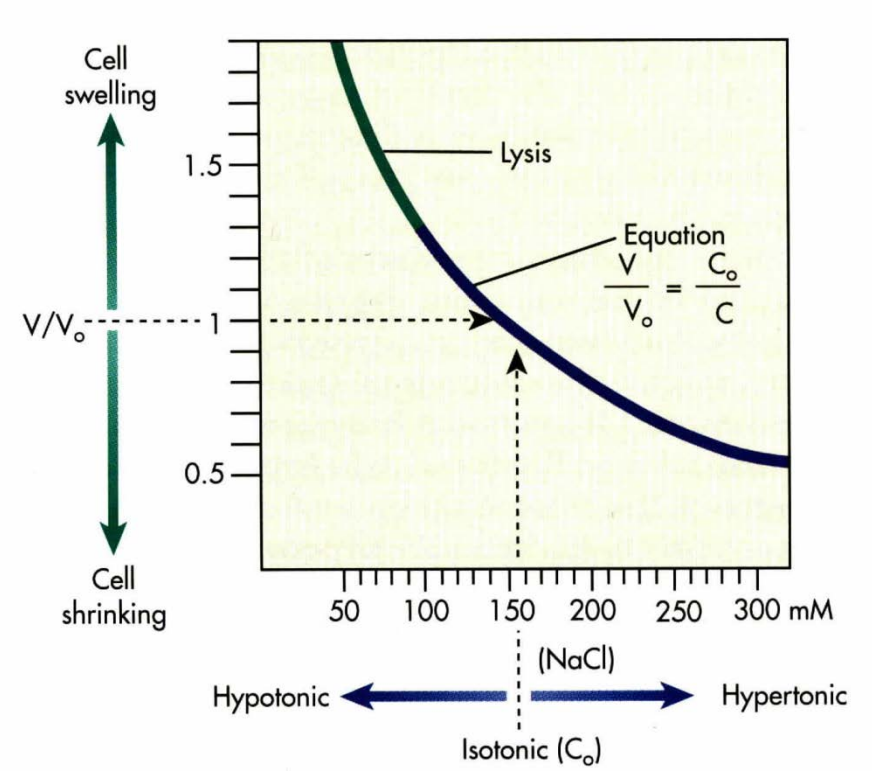
B. Isotonic solutions with respect to plasma - have the same osmolality as plasma (and extracellular fluid), which is almost 300 mOsM. Isotonic solutions are iso-osmotic with plasma.

1. Tonicity is based only on non-penetrating particles; it takes the properties of the membrane into account. Tonicity causes steady state volume changes in cells.

a. Hypertonic solution - cells shrink in a hypertonic extracellular solution when water leaves the cell
b. Hypotonic solution - cells swell (water enters the cell) in a hypotonic extracellular solution.
c. Isotonic solution - cell volume is unchanged in an isotonic solution

2. Osmolarity is based on all particles regardless of whether they are penetrating or non-penetrating. It therefore is independent of membrane characteristics.

Fig. 10 Osmotic behavior of human red blood cells that have a normal volume in isotonic solution (154 mM). (Berne & Levy, Fig. 1-8)



VIII. Endocytosis and exocytosis (Fig. 11)

A. Endocytosis - an active process in which plasma membranes form pockets that engulf extracellular fluid and other material that pinch off and enter the cell.

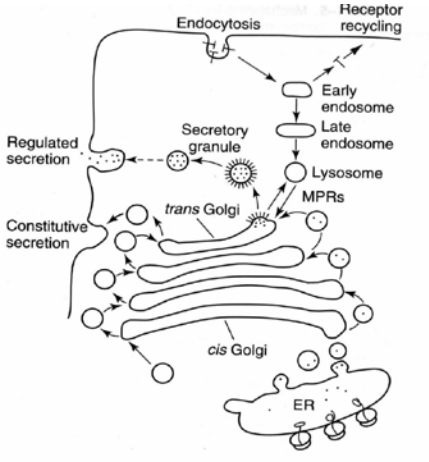
1. Pinocytosis - fluid adsorption
2. Phagocytosis - "cell-eating" Example: polymorphonuclear leukocytes in blood engulf bacteria (also see Fig. 12)
3. Clathrin-mediated (receptor-mediated) endocytosis

B. Exocytosis - opposite of endocytosis - pockets contain material that leaves the cell

1. Insert new plasma membrane
2. Release membrane-impermeant molecules synthesized by the cell.

Fig. 11. Protein processing by the Golgi apparatus, secretion by exocytosis and membrane recovery by endocytosis

(Ganong, Fig. 1-24)



Exocytosis and Endocytosis

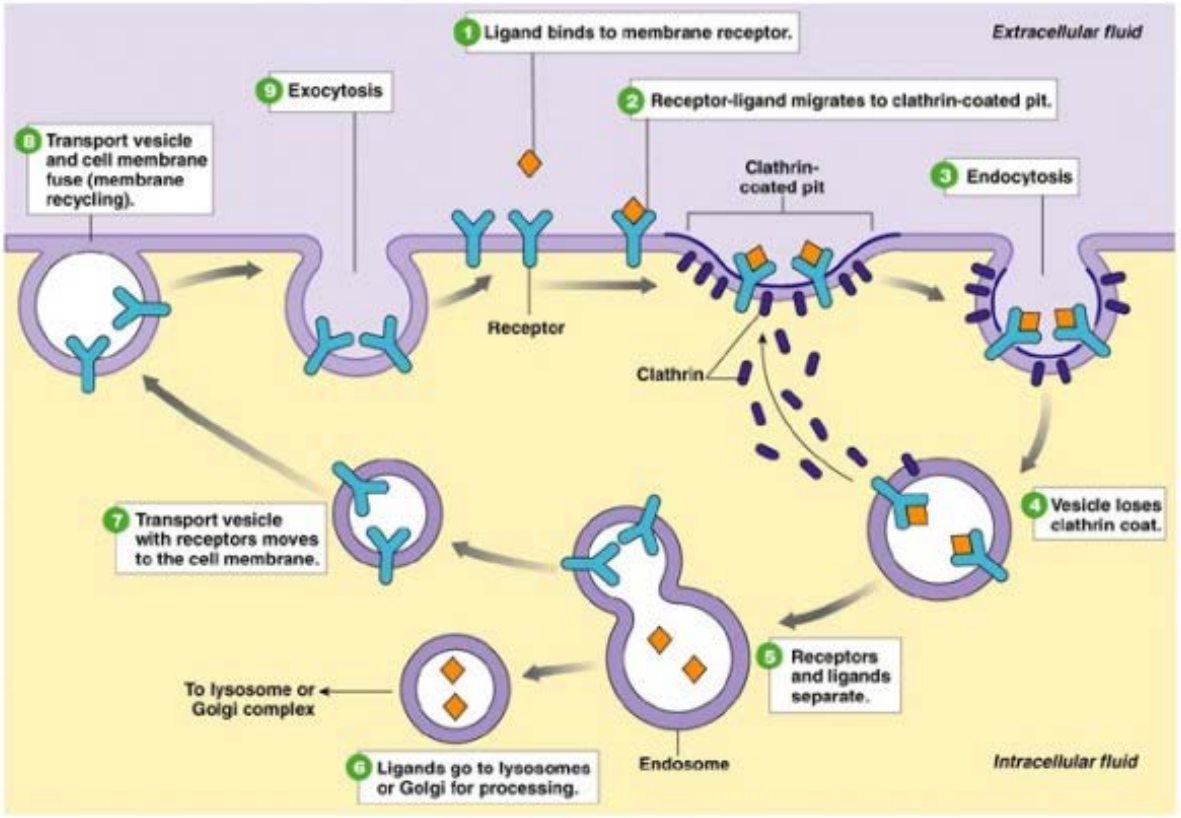


Fig. 5-24

Fig. 12. Example of phagocytosis in the eye by an epithelial cell: The rod and cone photoreceptors shed and are phagocytosed by the retinal pigment epithelium (RPE). The RPE is a monolayer of epithelial cells between the photoreceptors and the choroidal blood circulation behind it. The RPE forms the blood-retina barrier for that circulation.