Davide Martelli

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Write me for any reason, I will try to assist you!

slides: virtuale.unibo.it

Important information about

ATTENDANCE REQUIREMENTS

The minimum attendance requirement to be admitted to the final exam is 60% of lessons. Since this learning activity is part of an Integrated Course (I.C. #84284-Signaling pathways in health and disease), the 60% attendance requirement refers to the total amount of I.C. lessons (14 CFUs=112 total hours). Students who fail to meet the minimum attendance requirement (i.e. 71 hours) will not be admitted to the final exam of this I.C., and will have to attend relevant classes again during the next academic year. Professors may authorise excused absences upon receipt of proper justifying documentation, in case of illness or serious reasons. Excused absences do not count against a student's attendance record to determine their minimum attendance requirement.

Students who regularly attended the course of Cell Signaling will be evaluated through a four-hour FINAL EXAM, a cumulative written test with both multiple-choice and open-ended response formats that include topics from all the teaching modules of the integrated course of Signaling Pathways in Health and Disease: Cell Signaling, Metabolic Biochemistry and Physiology. Final Grade Fractions: Cell Signaling, 9/32 pts; Metabolic Biochemistry 14/32 pts; Physiology, 9/32 pts. MAX GRADE: 30 cum laude.

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However, please also note that exam outcomes will not be disclosed to students as partial grades of each module, but as integrated final marks. Consequently, the passing value (18) can be reached with any possible mathematical combination of the hidden partial results from the three individual exam modules.

The final exam for the module of Physiology will be a multiple-choice question test

Please note that:
- There is only one correct answer
- Cleary write the letter of what you think is the correct answer in the corresponding box on the right
- Corrected or unclear answer will be considered as empty.
- Correct answer +1 / null or void answer +0 / wrong answer -0.25 (x9/16; max score you can get is 9)

***** EXAMPLE ****
1) What are the colours of Bologna FC1909 jersey?
a) Black and white
b) Green and yellow
c) Black and blue
d) Red and blue

D

physiology n. [from Lat. physiologĭa, Gr. φυσιολογία “science of nature”, from φυσιο- “physio-” and -λογία “-logy”]. –

- Science that studies the functions of living organisms, both animals and plants, and aims to understand the causes, the conditions, and the laws that determine and regulate vital phenomena.
- The functioning of [a cell], a tissue, an organ, or a system, and the way in which various organic processes or a specific process take place.

physiologist n. [from Late Latin physiolŏgus, Greek φυσιολόγος]. –

A scholar or expert in physiology, a student of physiology. Originally, φυσιολόγοι were what Aristotle called the Presocratic philosophers, who were mainly engaged in the study of φύσις, nature [the living and non-living world], rather than in the study of human beings.

Cellular Physiology

Cell: a biological system that maintains its characteristics unchanged thanks to:

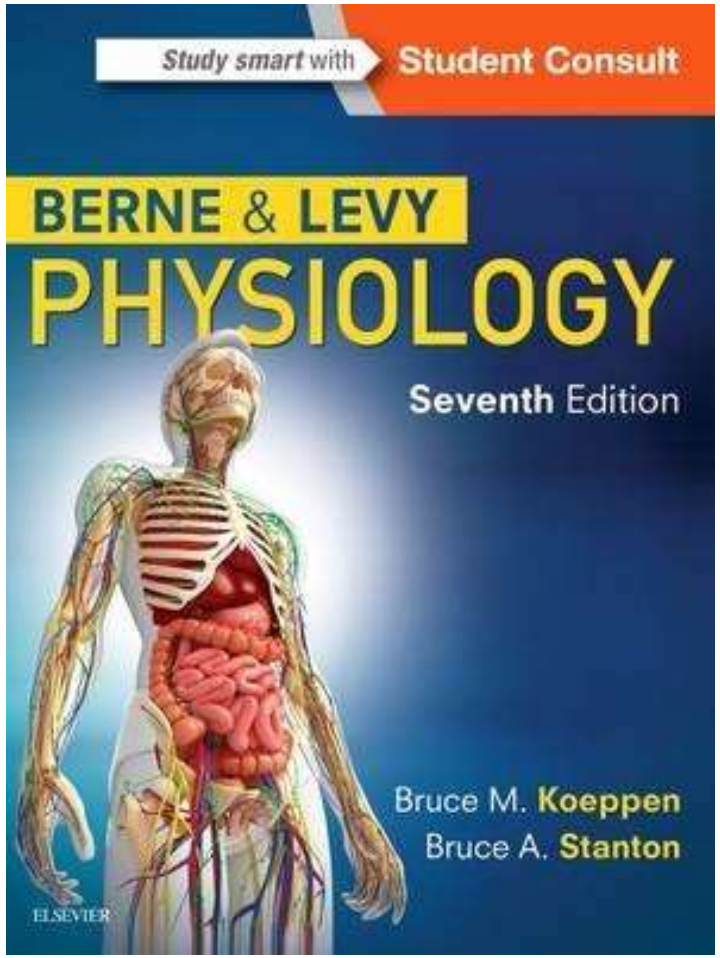
- interaction with the external environment
- renewal of its components
- regulation of its functions

Life!

Homeostasis: The maintenance within a “normal” range of the “fundamental” variables of the extracellular and intracellular compartments.

Multicellular organisms: cells differentiate and develop specific functions (mechanical, biochemical, information processing), leading to the development of systems; they present refined regulatory and interaction mechanisms with the external environment to obtain energy, preserve the internal environment, and respond to changes in the external environment (allostasis) → **Systems physiology**.

Transports



Chapter 1, 2 and 3

Eukariotic cells – Plasma Membrane

Eukariotic cells differ from prokariotic cells because of mb delimited nucleus

All eukariotic cells, apart human red blood cells and cells within the lens of the eye, have a nucleus and cytoplasm

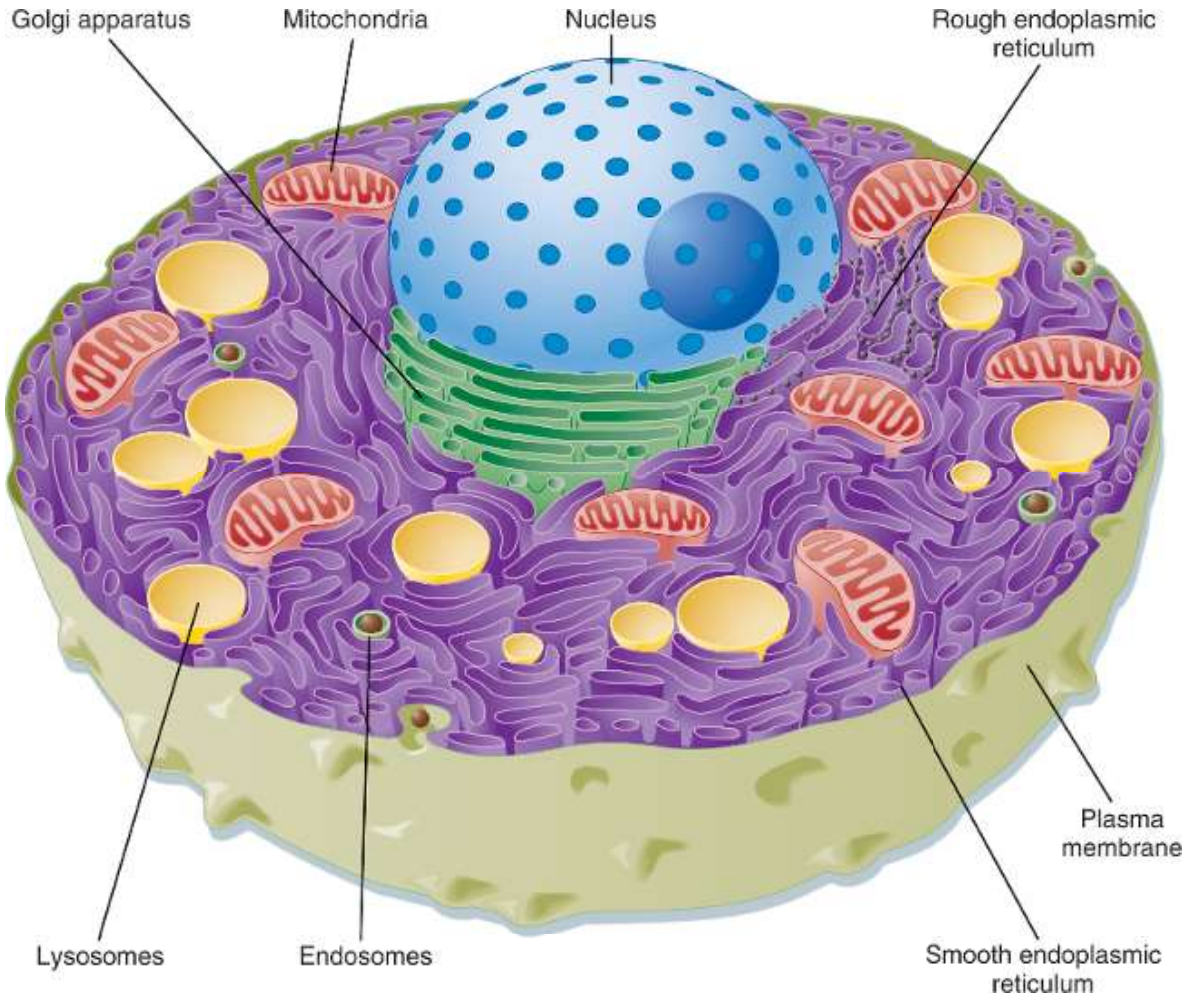


Fig. 1.1 Berne-Levy

Component	Primary Function	Component	Primary Function
Cytosol	Metabolism, protein synthesis (free ribosomes)	Endosome	Cellular uptake of cholesterol, removal of receptors from the plasma membrane, uptake of small molecules and water into the cell, internalization of large particles (e.g., bacteria, cell debris)
Cytoskeleton	Cell shape and movement, intracellular transport		
Nucleus	Genome (22 autosomes and 2 sex chromosomes), DNA and RNA synthesis	Golgi apparatus	Modification, sorting, and packaging of proteins and lipids for delivery to other organelles within the cell or for secretion out of the cell
Mitochondria	ATP synthesis by oxidative phosphorylation, Ca^{2+} storage		
Smooth endoplasmic reticulum	Synthesis of lipids, Ca^{2+} storage	Proteosome	Degradation of intracellular proteins
Free ribosomes	Translation of mRNA into cytosolic proteins	Peroxisome	Detoxification of substances
Rough endoplasmic reticulum	Translation of mRNA into membrane associated proteins or for secretion out of the cell		
Lysosome	Intracellular degradation		

ATP, adenosine triphosphate; mRNA, messenger RNA.

Table 1.1 Berne-Levy

Plasma membrane (**Mb**) separates intracellular from extracellular environment and its main functions are:

- Selective transport of molecules in and out of the cell (mb transport proteins)
- Cell recognition through the use of surface antigens
- Cell communication through neurotransmitter or hormone receptors and through signal transduction pathways
- Tissue organizaton, such as temporary or permanent cell junctions or interaction through extracellular matrix
- Mb dependent enzimatic activity
- Determination of cell shape by linkage with cytoskeleton

Plasma Mb – Structure and Composition

5nm-thick lipid bilayer with associated proteins integrated or loosely attached to the inner or outer surface

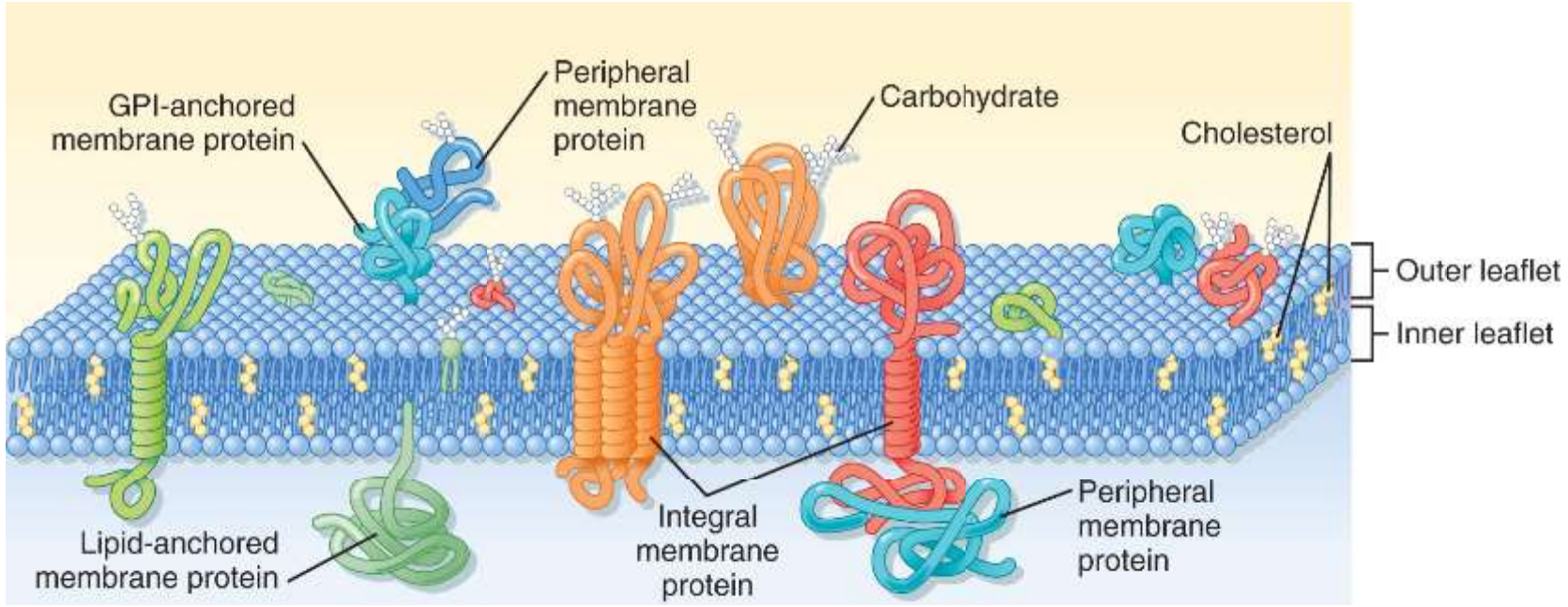


Fig. 1.2 Berne-Levy

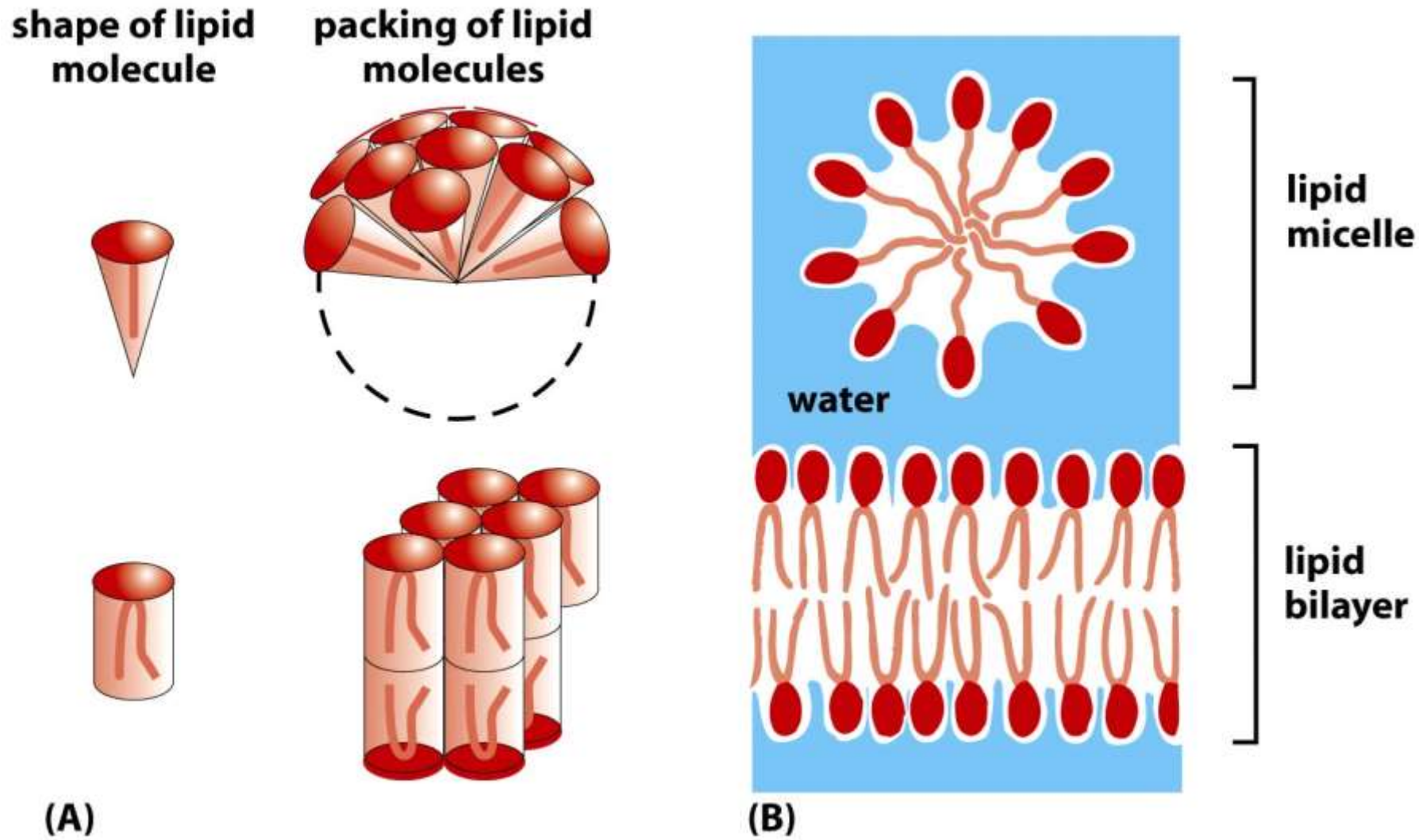


Figure 10-7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Mb lipids

The major part of lipids of the cell mb are phospholipids, and in particular glycerophospholipids (e.g. phosphatidylcholine).

Phospholipids are amphipatic molecules containing a polar hydrophilic head and two non polar hydrophobic fatty acyl chains.

Majority of phospholipids have a glycerol backbone to which are attached 2 fatty acyl chains (14-20C) and, via a phosphate group, an alcohol

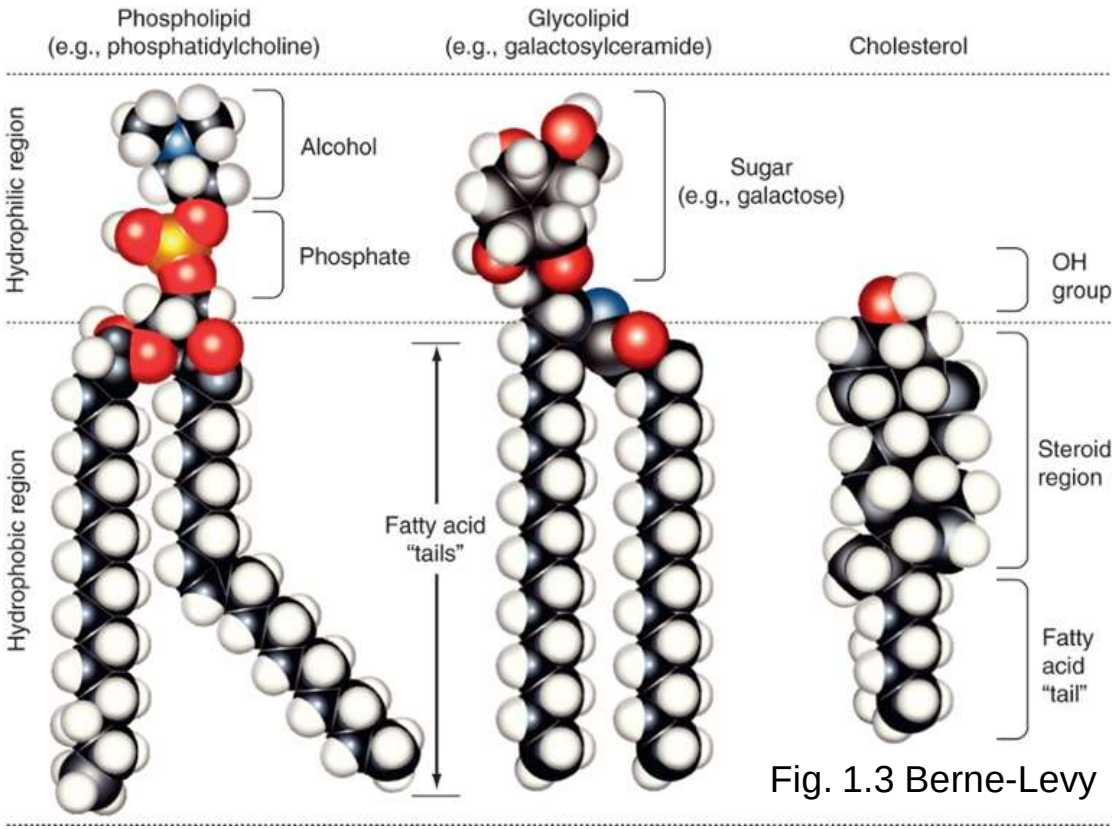
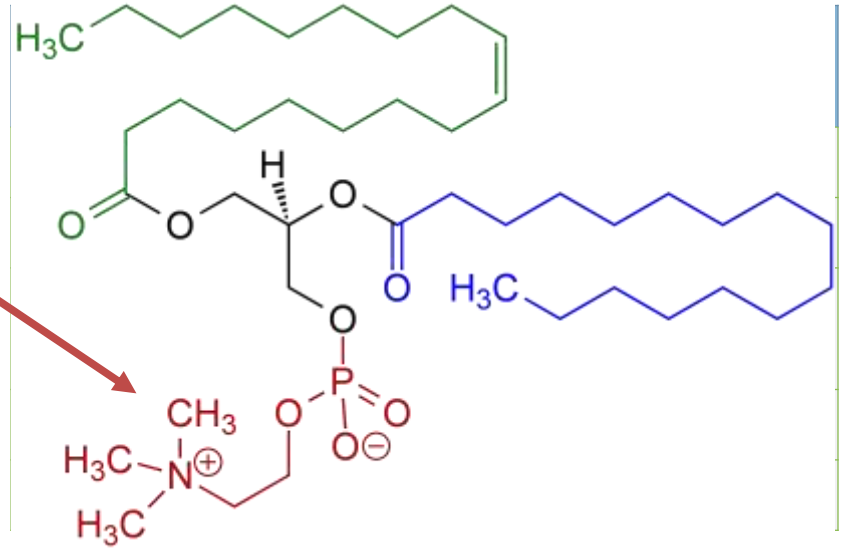


Fig. 1.3 Berne-Levy

Alcohol or quaternary ammonium compound



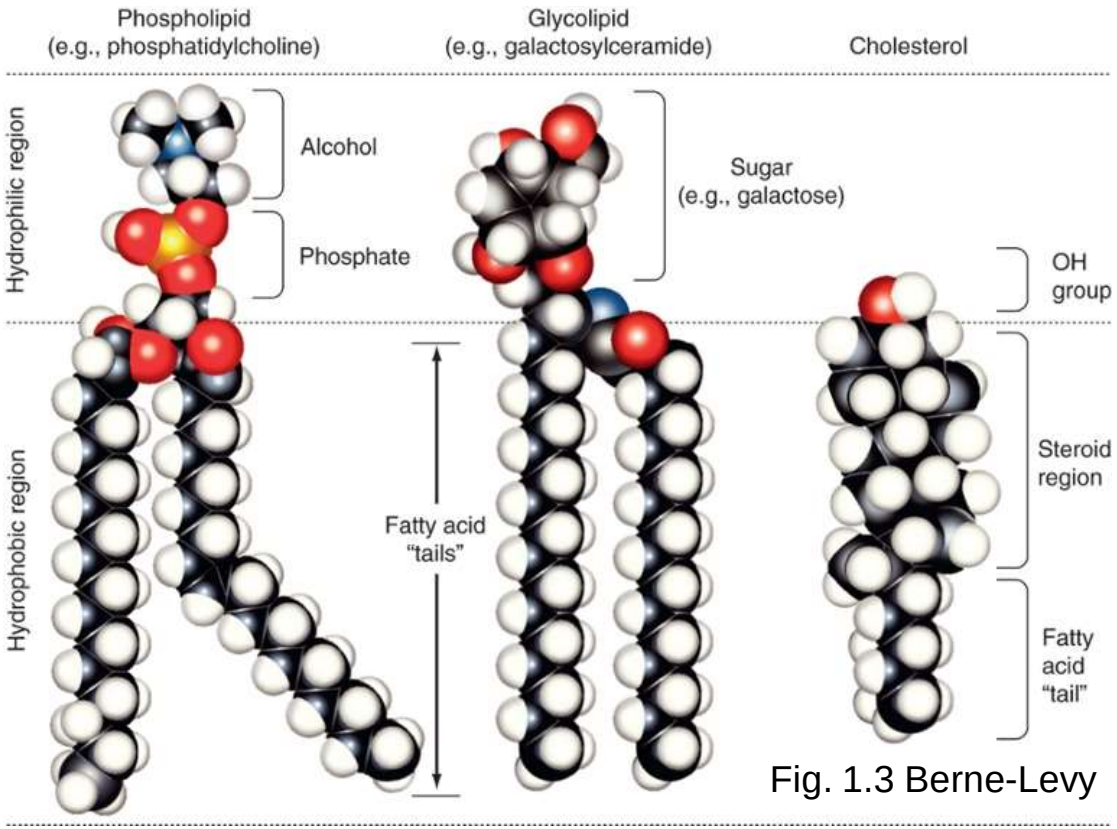


Fig. 1.3 Berne-Levy

Phospholipid	Primary Location in Membrane
Phosphatidylcholine	Outer leaflet
Sphingomyelin	Outer leaflet
Phospatidylethanolamine	Inner leaflet
Phosphatidylserine	Inner leaflet
Phosphatidylinositol*	Inner leaflet

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Phospholipids are amphipatic molecules containing a polar hydrophilic head and two non polar hydrophobic fatty acyl chains.

Majority of phospholipids have a glycerol backbone to which are attached 2 fatty acyl chains (14-20C) and, via a phosphate group, an alcohol

Sphingomyelin has the amino-alcohol sphingosine as its backbone instead of glycerol.

In one of the two fatty acyl chain there is a double bond (unsaturated fatty acyl chain) that produces a kink in the 3D structure of the molecule.

Glycolipids 2 apolar fatty acyl chains linked to a polar head that consists of a carbohydrate. Glycosylphosphatylinositol (GPI) anchors proteins to the outer leaflet of the mb.

Cholesterol is a critical component of the bilayer and serves to stabilize the mb structure at 37°C. 50% of the lipids found in mb can be cholesterol

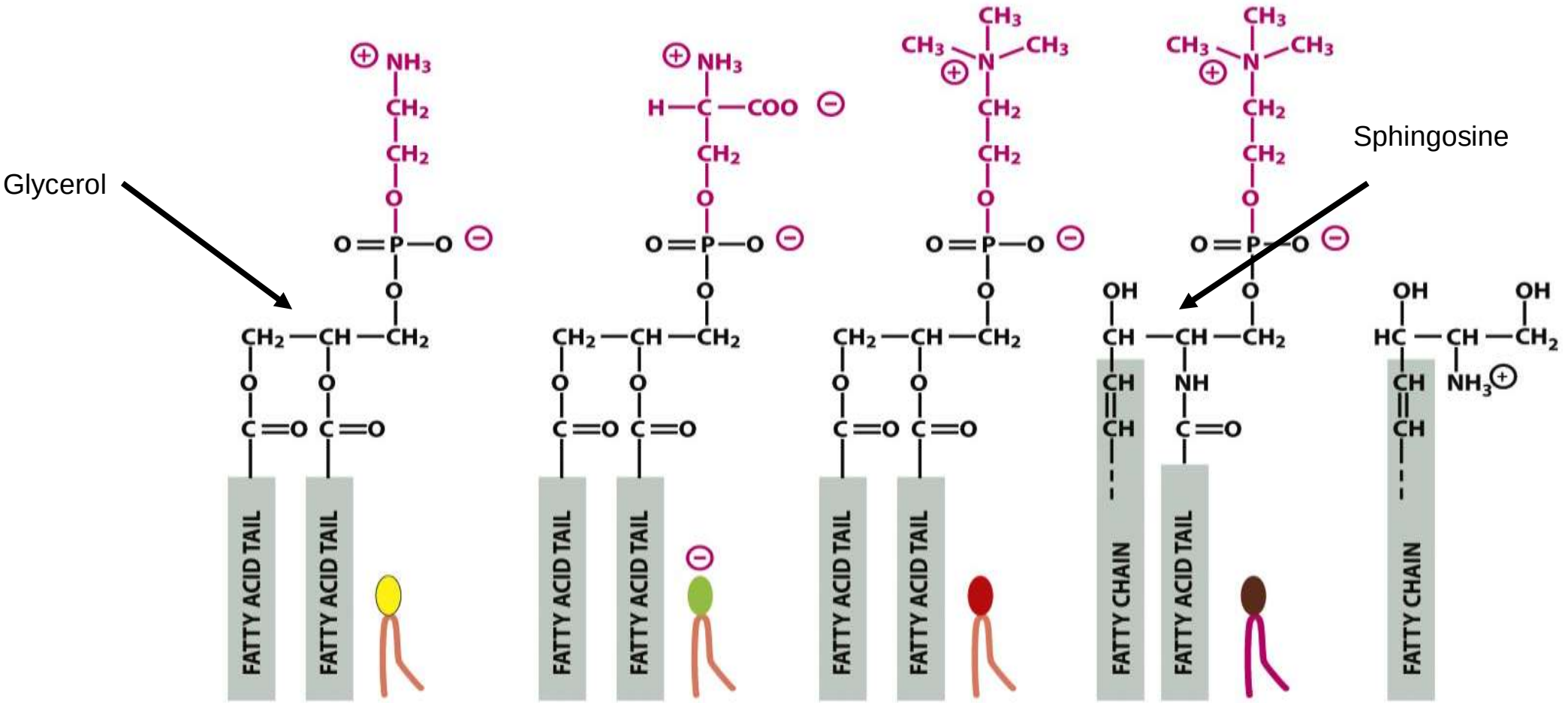
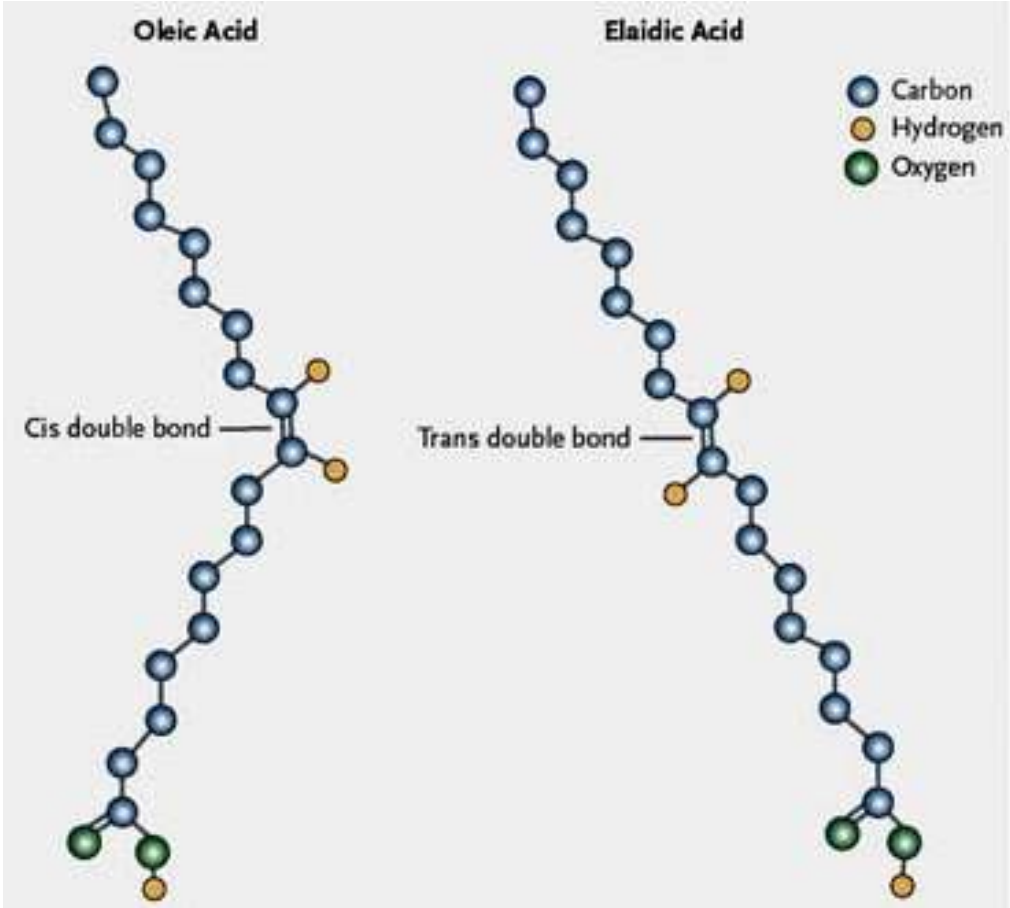


Figure 10-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)



In cis unsaturated fatty acid, the hydrogen atoms are on the same side, while in trans unsaturated fatty acid, the hydrogen atoms are on the different side. In cis unsaturated fatty acid, the cis double bond forms a sharp angle, while trans double bond forms a straight line which shows only a small kink at the double bond site (similar to a saturated fat)..

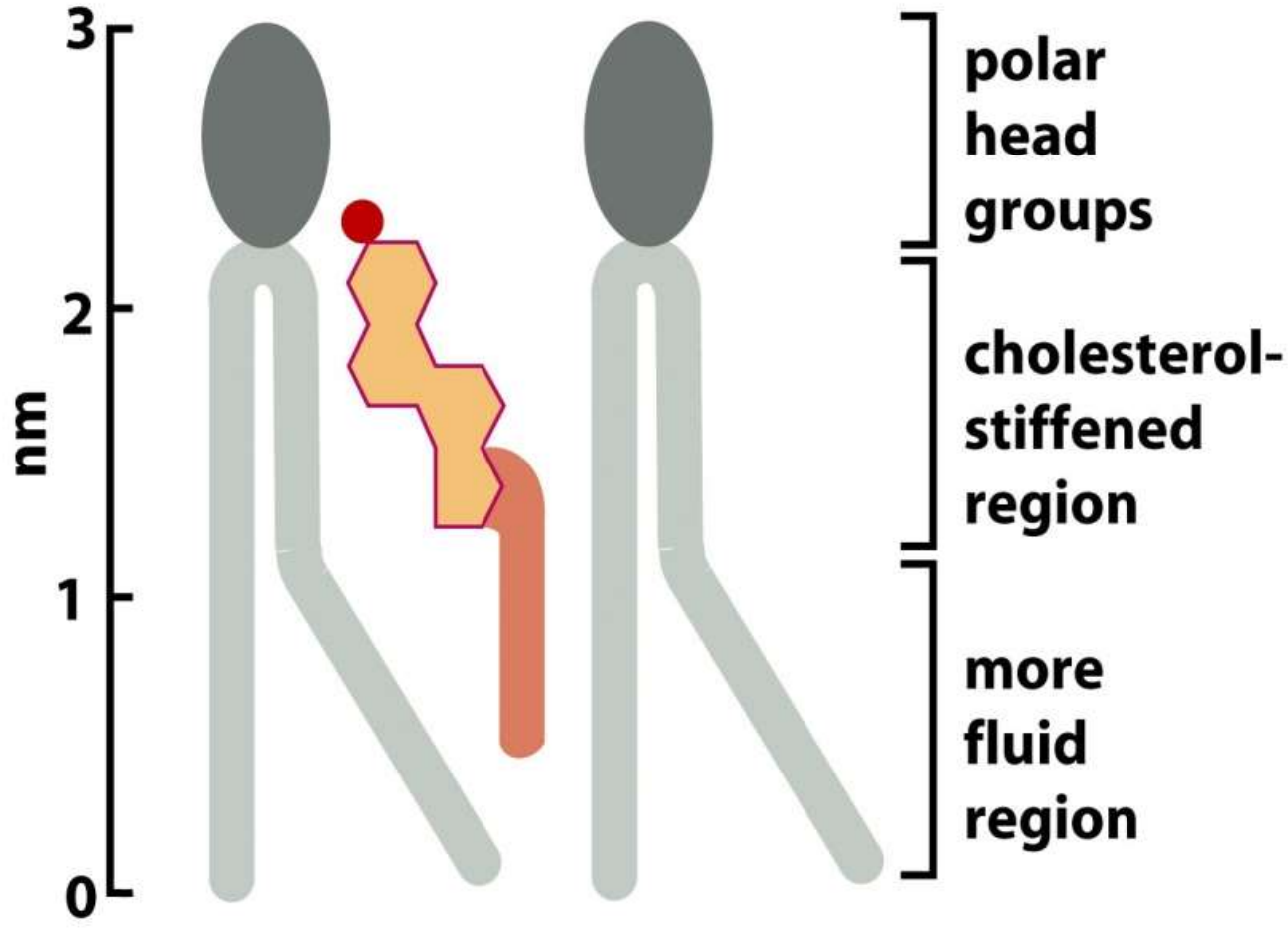


Figure 10-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Cholesterol increases the viscosity of the plasma membrane, which slows down and limits its movements

Membrane fluidity depends on: 1) the number of cis configuration; ↑
2) the number of cholesterol molecules. ↓

Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	*E. COLI* BACTERIUM
Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	28	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	44	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	13	8	23	27	30

Table 10-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

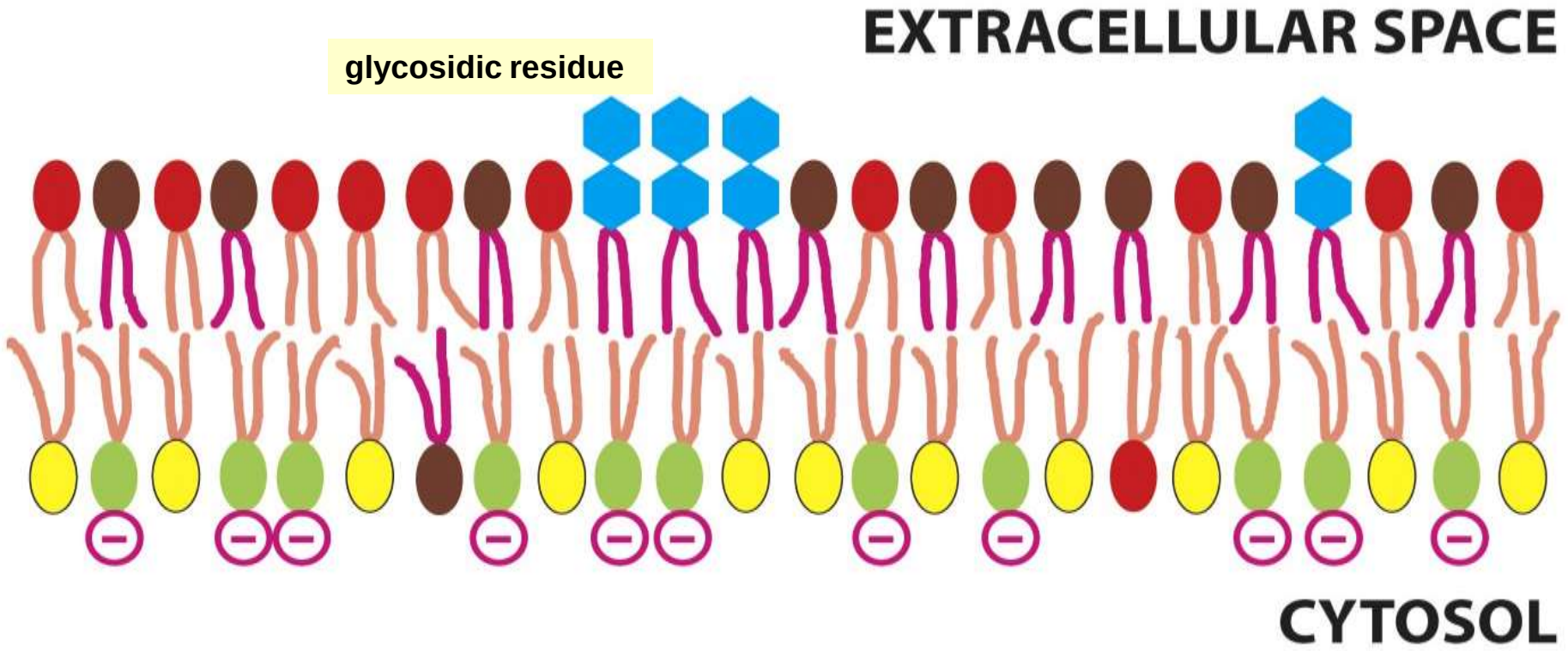


Figure 10-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The composition of the two lipid layers is different.

Some lipids in the outside layer are connected to glycosidic residue

- Phosphatidylethanolamine
- Phosphatidylserine
- Phosphatidylcholine
- Sphingomyelin

Mb is not a static structure but rather fluid even if movement of proteins can be constrained or limited

There are functional separated zones of the mb, e.g. tight junctions can separate (functionally) the plasma mb of epithelial cells in apical and basolateral mb that carry different roles in the transport of substances from one side of the epithelium to the opposite site

It is also possible to find regions of the mb where lipids like sphingomyelin and cholesterol aggregate into what are called lipid rafts. Important to segregate signaling molecules

Lipids can constantly move laterally. Such movement is temperature dependent. Since we can assume the temperature of the body as constant, the membrane composition is the key factor.

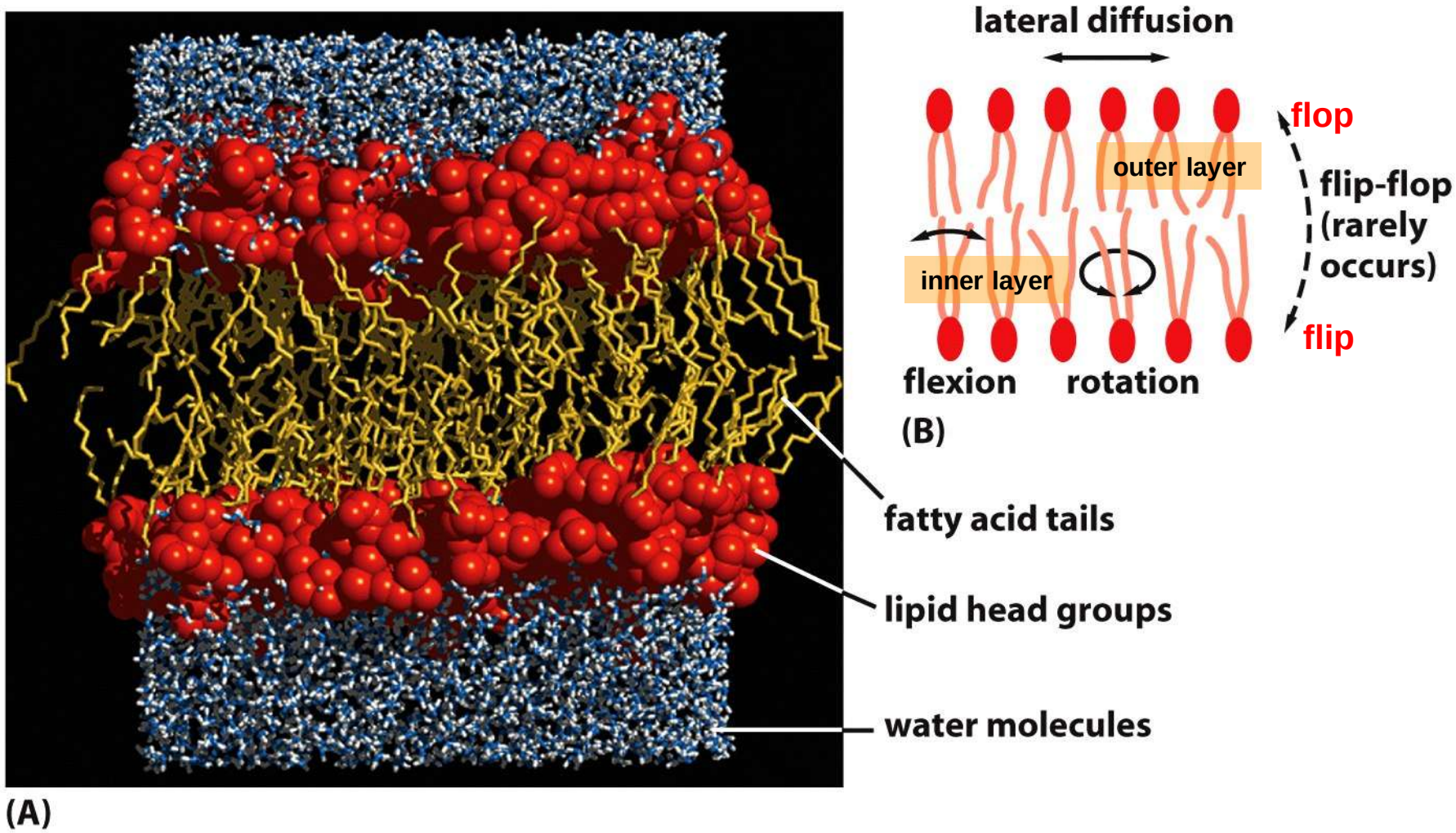


Figure 10-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)

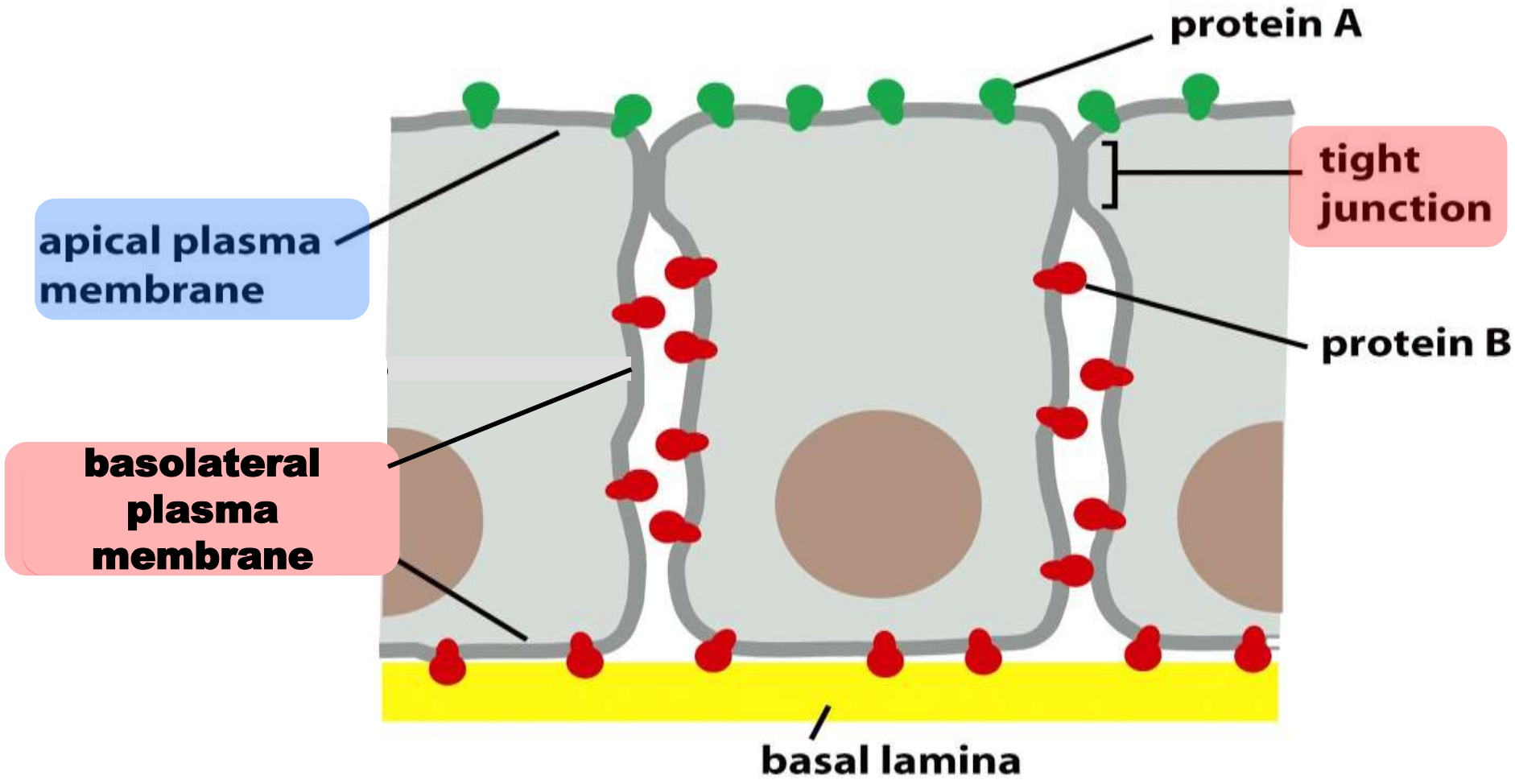


Figure 10-37 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The lateral movement of transmembrane proteins is limited by cellular junctions, especially by the tight junctions. Such compartmentalization of membrane proteins is at the base of the different functional role of the apical portion of the cell compared to the basolateral one.

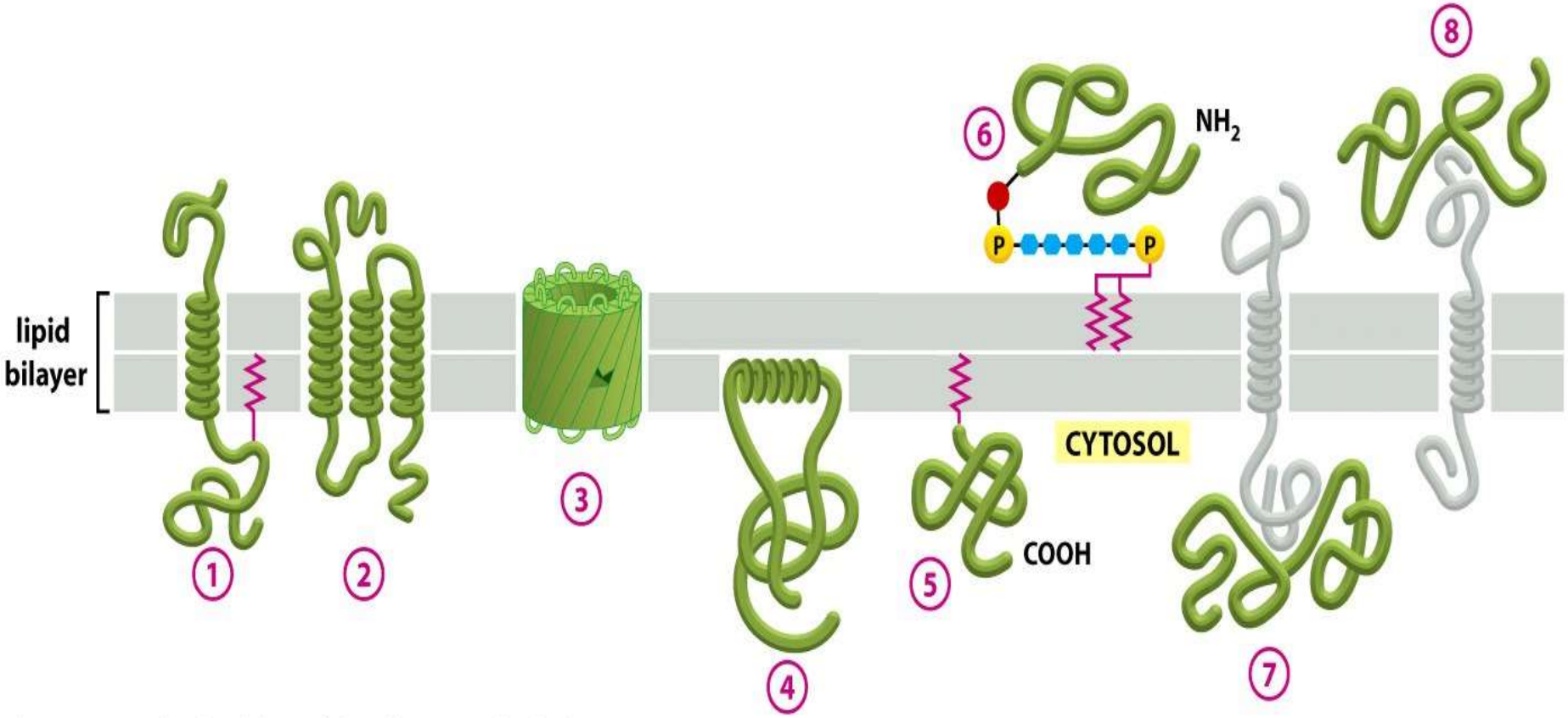


Figure 10-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Proteins interact with cellular membranes in different ways.

1, 2, 3, Transmembrane protein 4, Hydrophobic interaction. 5, 6 Covalent binding with the lipid chain, docking the protein to the membrane, or by binding to Phosphatidylinositol-bonded oligosaccharides; 7, 8, Non covalent binding with other proteins.

Integral mb proteins are embedded in the lipid bilayer, some of them spam the lipid bilayer and are termed transmembrane proteins. An example of those are the G protein coupled receptors that have 7α elixes domains.

Proteins can also be covalently attached to a lipid molecule (lipid anchors) but also to GPI. In many cells the outer leaflet lipids, as well as many proteins exposed to the outer surface of the mb, are glycosilated (oligosaccharides attached) that together form the glycocalyx. The glycocalyx is involved in cell recognition and formation of cell-cell interactions.

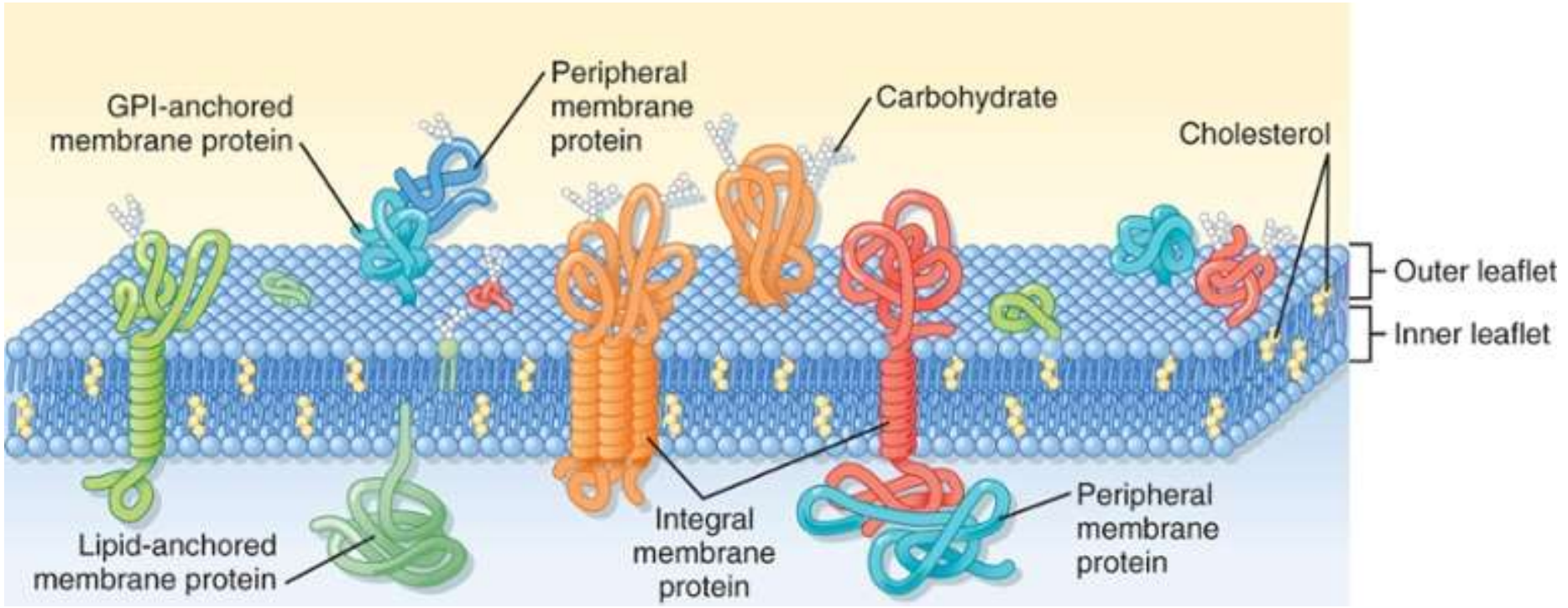
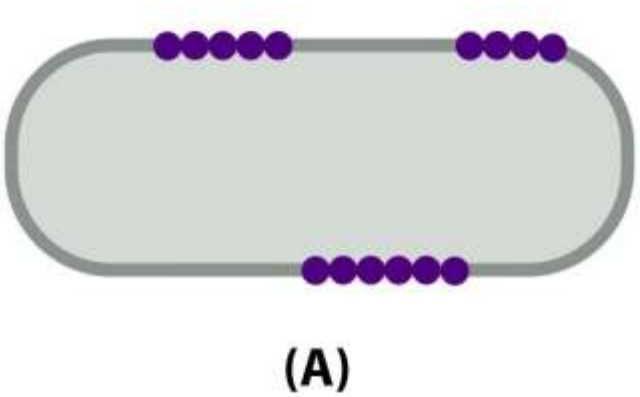
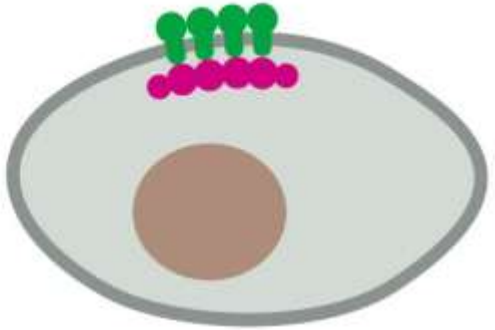


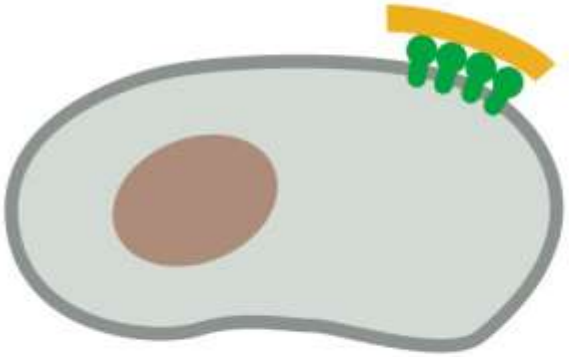
Fig. 1.2 Berne-Levy



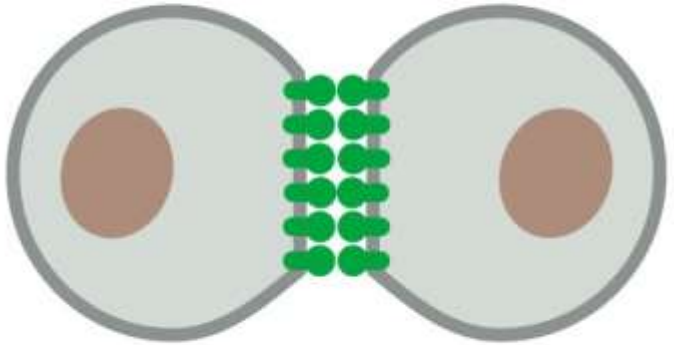
(A)



(C)



(B)



(D)

Transmembrane proteins can freely move along the lipid layers (lateral movement) or can regroup in clusters, less mobile because binded with:

A) Other transmembrane proteins;
B) Proteins of the extracellular matrix;
C) Protein of the cytoskeleton;
D) Other cell's proteins.

Many proteins are bonded to glycosidic residue

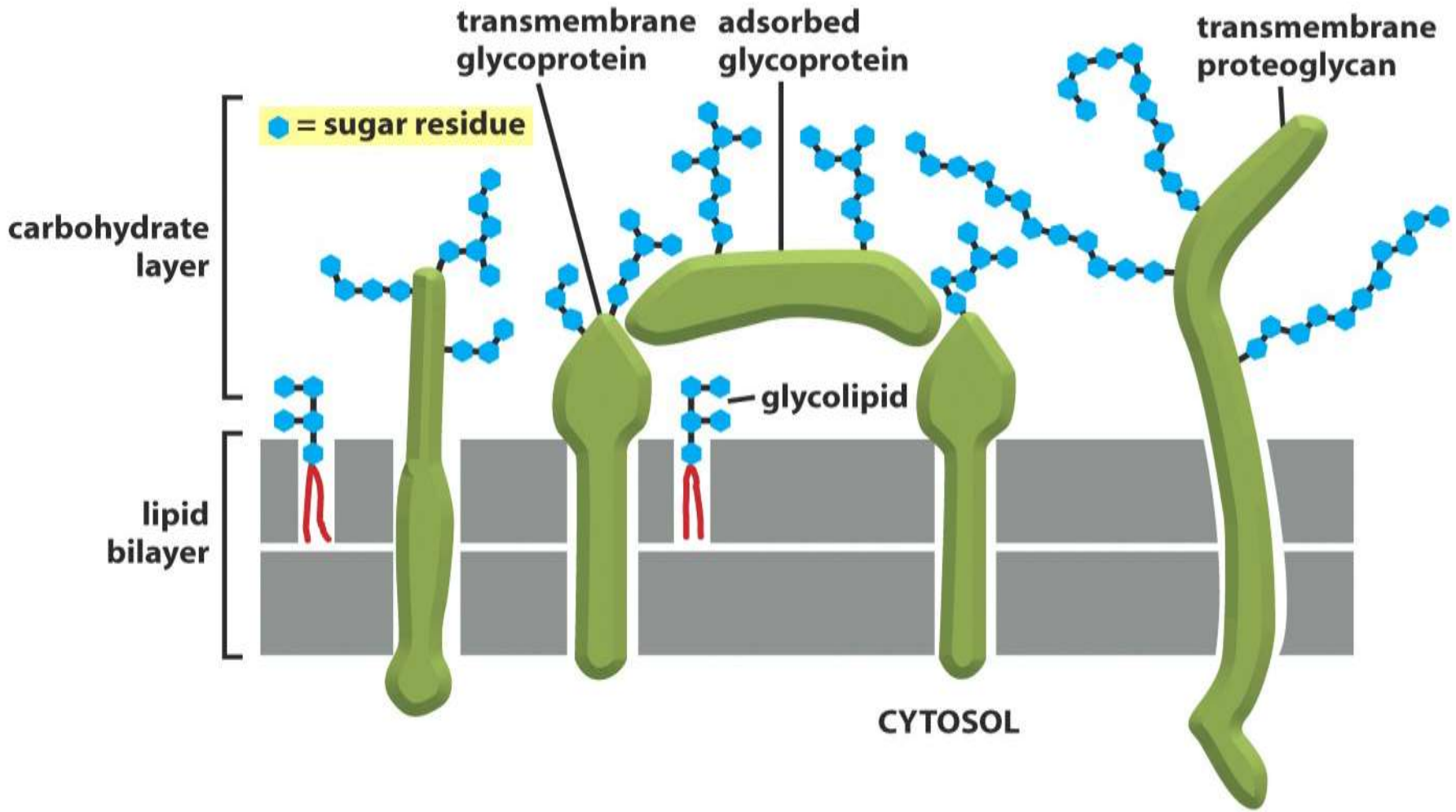
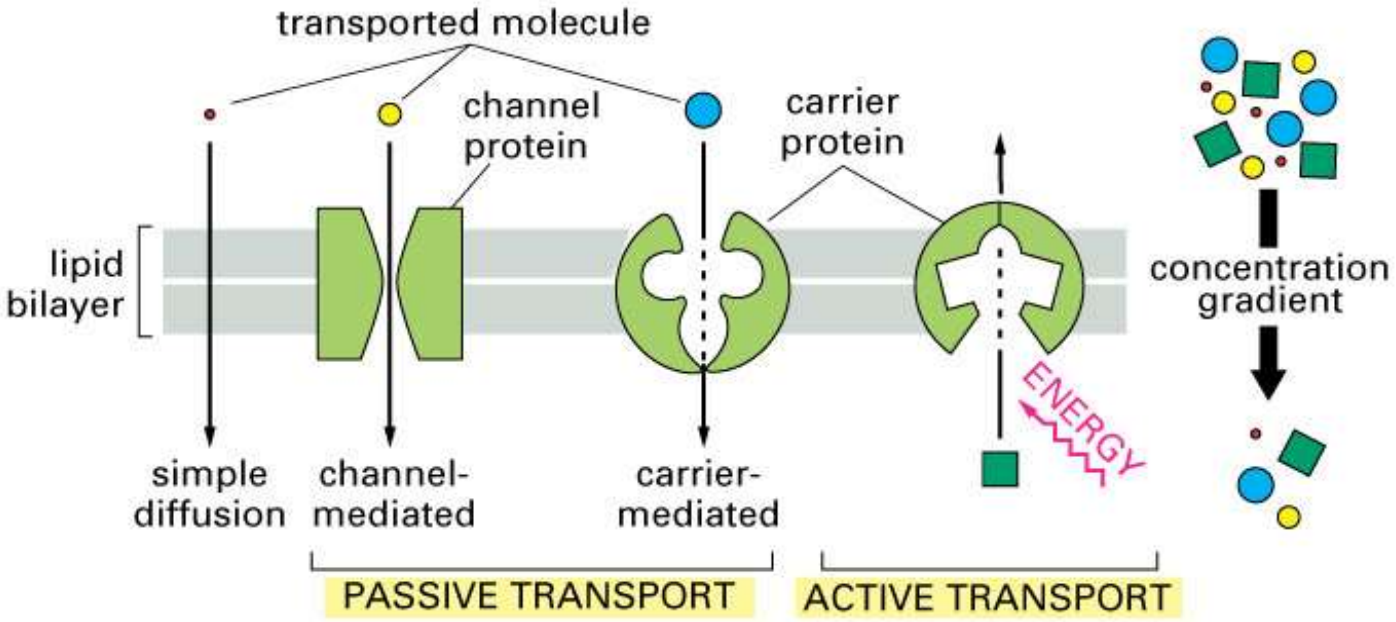


Figure 10-28b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Summary for Cell Transport

https://www.youtube.com/watch?v=BGeSDI03aaw



Transcellular passive transports move substances along a gradient by using a transmembrane protein (facilitated diffusion). Active transports (primary and secondary) move substances against a gradient.

Diffusion and solutes transport across cell membranes

Fluids and substances move by Convection.

Convection = the movement of molecules within fluids. The two most common examples in life science are:

i) Natural convection or Diffusion, due to the brownian motion of molecules (proportional to the temperature of the fluid).

ii) Forced convection or Advection, due to currents within the fluids (caused usually by pressure gradients)

Solutes (substances) and solvents (water) movement across cell membranes or epithelia is ruled by the same physics in living and non-living environments.

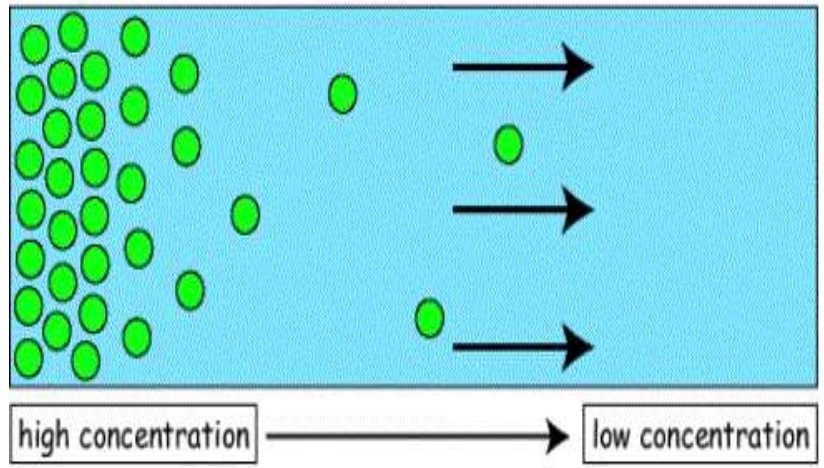
Life is able to shape the dynamics of those movements in space and time

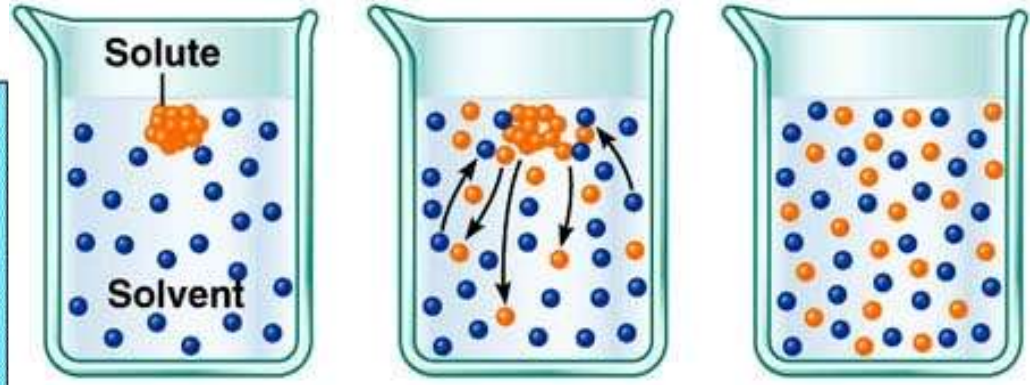
Diffusion

Diffusion is the process by which molecules move spontaneously from an area of high concentration to an area of low concentration until the gradient is dissipated.

Energy is necessary to maintain the gradient!

Diffusion





solute

Solute transport is from the left to the right; movement of the solutes is due to the concentration gradient (dC/dx).

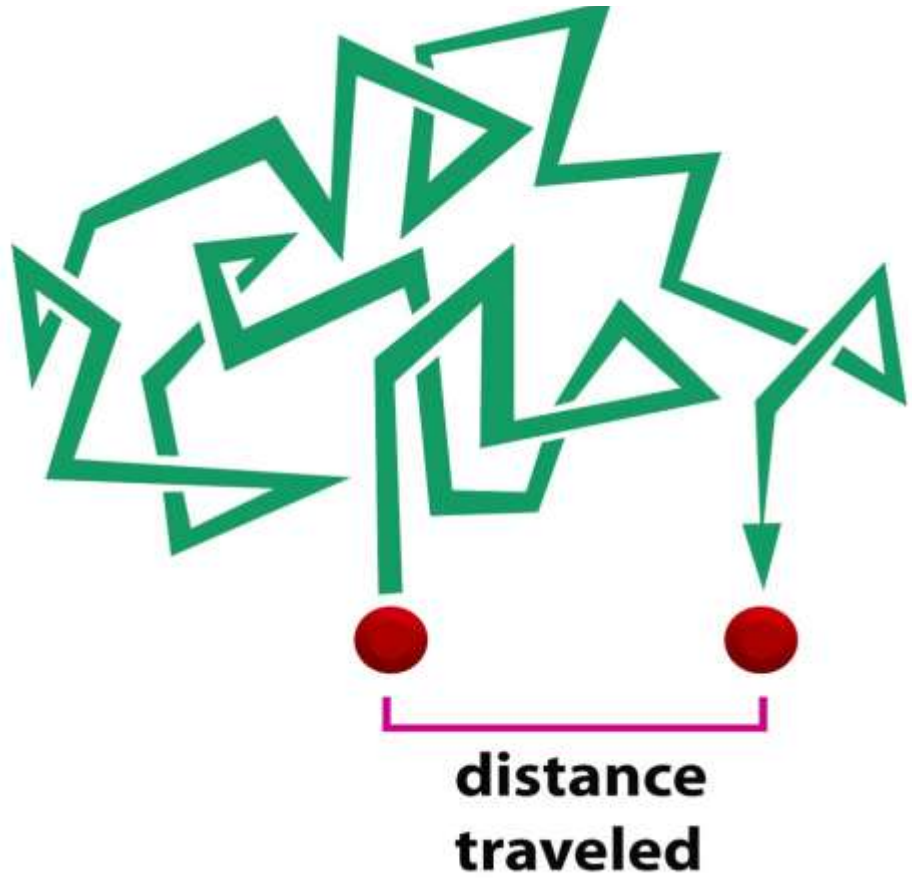


Figure 2-48 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Diffusion is described by the first Fick's laws of diffusion

The flux density (J, mol/(s cm^2)) is directly proportional to the concentration gradient across the membrane

J = number of moles that diffuses across a unit area of the membrane during an interval of time.

At the steady state, the concentration gradient is equal to the difference in the concentration (ΔC = Ca - Cb) of the solute on both sides of the membrane divided by the thickness of the membrane (Δx), so that

$$Js = -D (\Delta c / \Delta x)$$

It is assumed that the concentration gradient through the thin membrane is linear over the thickness (Δx)

The diffusion coefficient D (cm^2/s) indicates how much flux will occur for a given concentration gradient.

The diffusion coefficient (D) is a constant for a given substance and membrane under a given set of conditions.

In a free solutions, it depends upon the molecular size and shape of the substance.

For ions, the smaller the crystal radius (the radius of the unhydrated ion), the greater the charge density -> larger water shell.

In a liquid when spherical solute molecules are much larger than the solvent molecules we can use the Einstein-Stokes equation.

$$D=KT/6\pi r\eta$$

K= Boltzmann's constant = $1.38064852 \times 10^{-23}$ J/K

T = abs temperature,

η = viscosity

r = radius of the molecule

$$K=R/N_A$$

R= gas constant = 8.314 J/(mol K)

N_A = Avogadro constant = 6.022×10^{23} mol^{-1}

$$Js = -D\,(\Delta c / \Delta x)$$ **FICK'S EQUATION**

Let's now apply Fick's equation to molecules that diffuse through the lipid bilayer. In this case J does not only depend on D but also on β that is the partition coefficient.

If a molecule is apolar (dissolves and easily crosses a lipid bilayer) β is >1; if a molecule is hydrophilic β is <1.

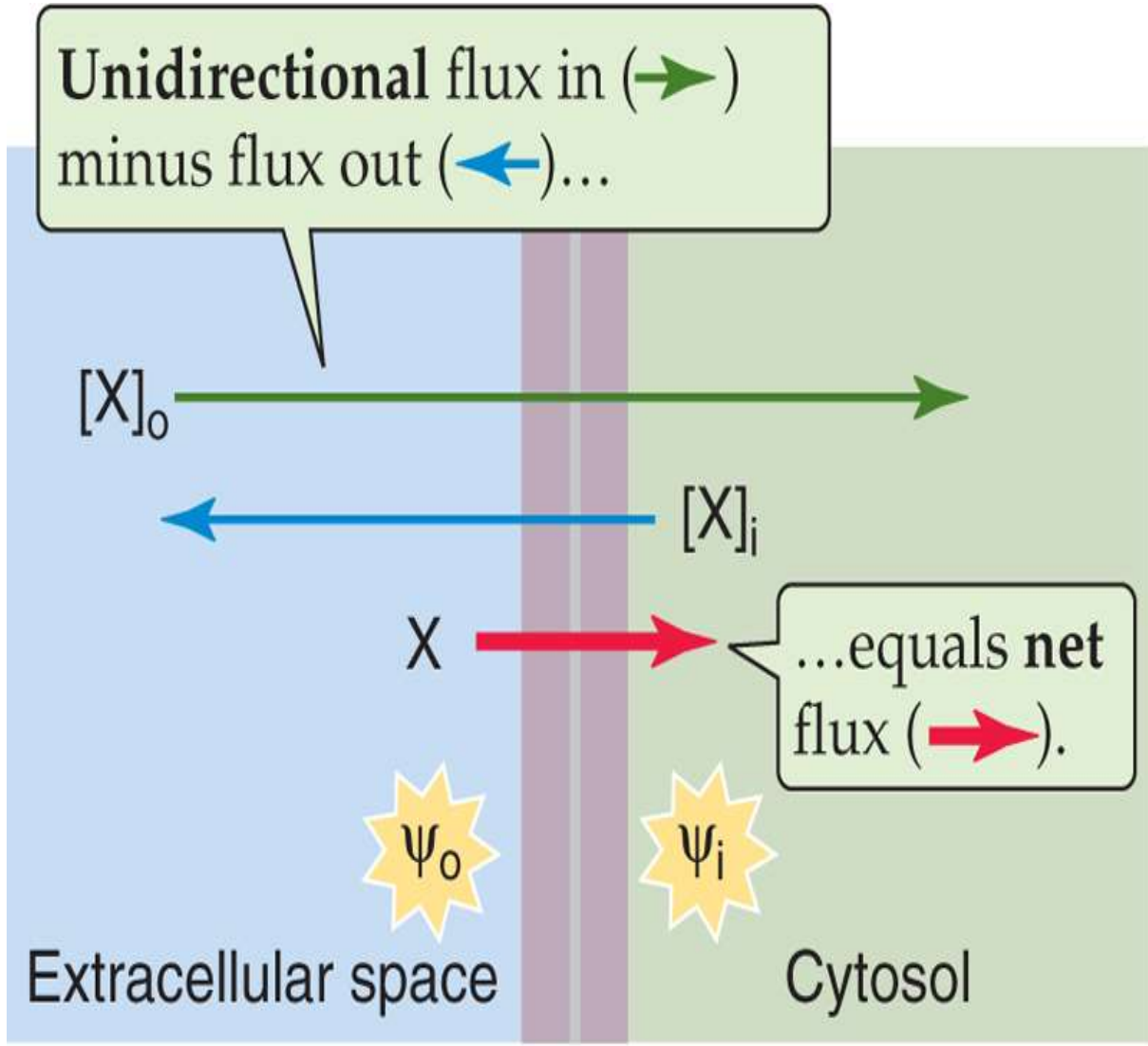
The permeability coefficient P reflects the properties of the pathway used to diffuse the solute

P is the product of the partition coefficient β and the diffusion coefficient D divided by the thickness Δx of the membrane

$$P = \beta D/\Delta x$$

Therefore, the flux density J

$$J = -P(Ci-Co)$$


Unidirectional flux in (→)
minus flux out (←)…
$[X]_o$
$[X]_i$
X
…equals **net**
flux (→).
ψ_o
ψ_i
Extracellular space
Cytosol

Electrochemical gradient

When we discuss about diffusion of electrocharged molecules (ions) the driving force is the electrochemical gradient.

$$\Delta\mu_x = RT\ln([x_i]/[x_o]) + z_xFV_m$$

Often measured as J/mol or KJ/mol.
In physiology it is expressed as an equivalent voltage, mV

R = Gas constant = 8.314 J/(mol K)
T = Temperature in K
Z_x = valence of charged molecules (Na^+ is 1, Ca^{++} is 2)
F = Faraday constant = electric charge per mole of electrons = 96485.332 C mol−1
$V_m = V_i - V_o$

For glucose only the first part of the equation is considered because it's not charged.

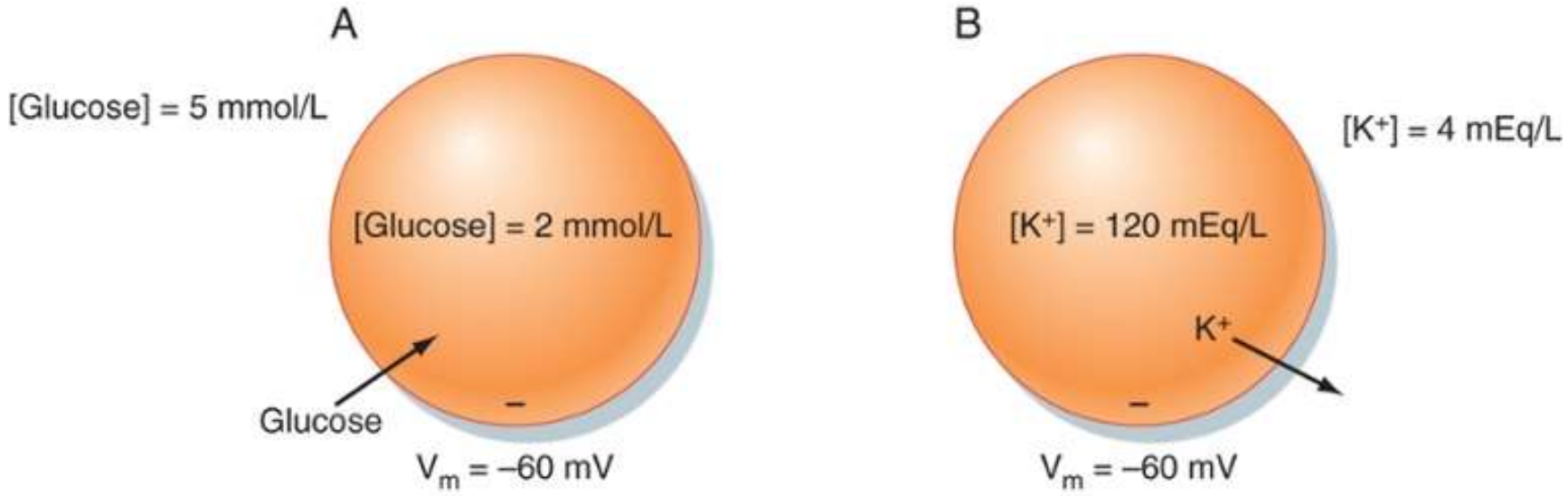


Fig. 1.6 Berne-Levy

Nernst equation and calculation for Nernst equilibrium potential for any ion (in this case K+ (Ek))

It is possible to deduce the Nernst equation because that is for the situation in which a molecule is at equilibrium

$\Delta\mu x = 0$

$$0 = RT \ln([Xi]/[Xo] + ZxFVm$$

$$-RT \ln([Xi]/[Xo]) = ZxFVm$$

$$Vm = -(RT/Zx) \ln([Xi]/[Xo])$$

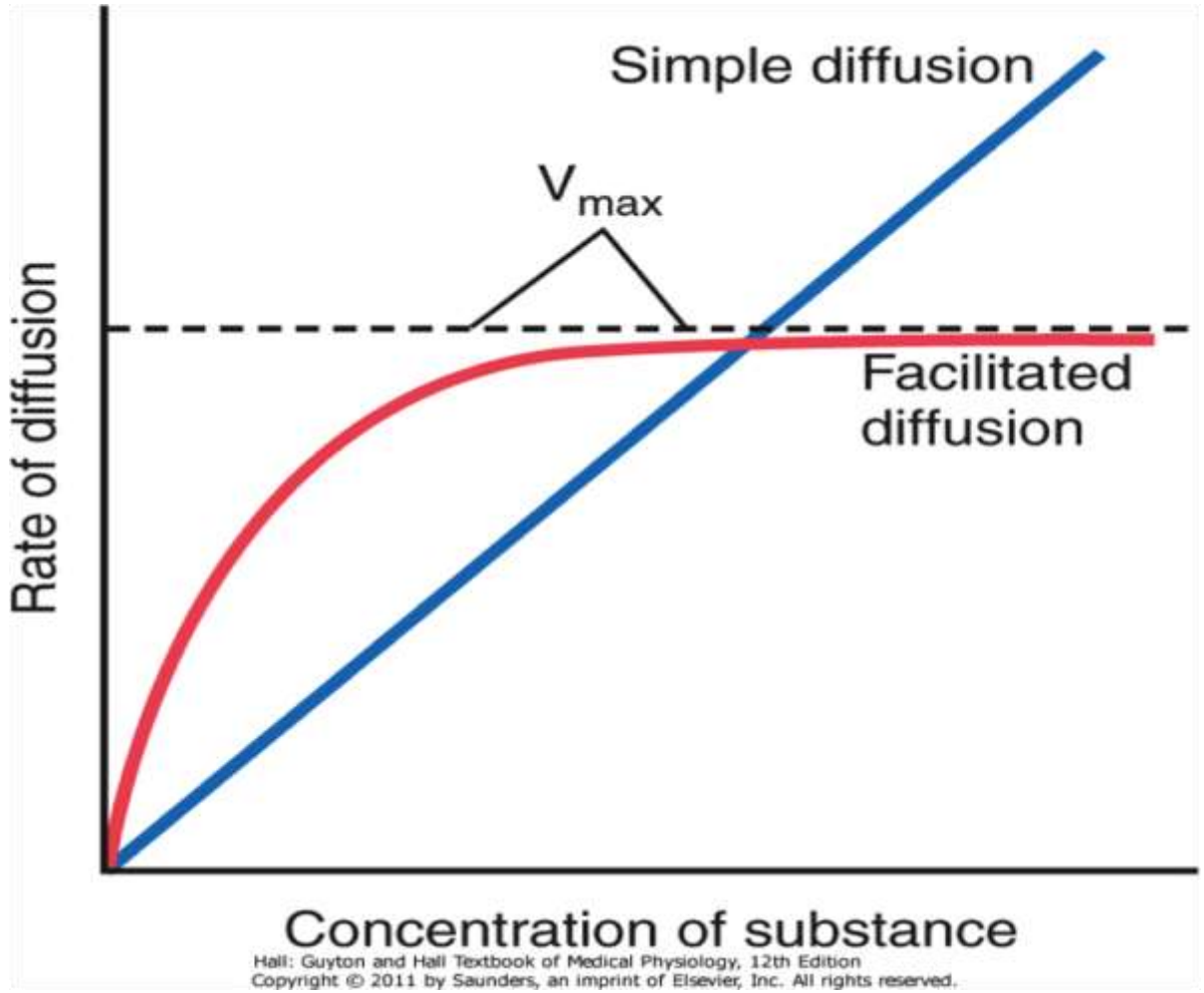
$$Vm = (RT/Zx) \ln([Xo]/[Xi])$$

At 37°C

$$Ex = (61.5mV/Zx) \log([Xo]/[Xi]$$

$$Ek = -90.8mV$$

Because Vm is -60mV net driving force is -60 – (-90.8) = 30.8mV, driving K out of the cell



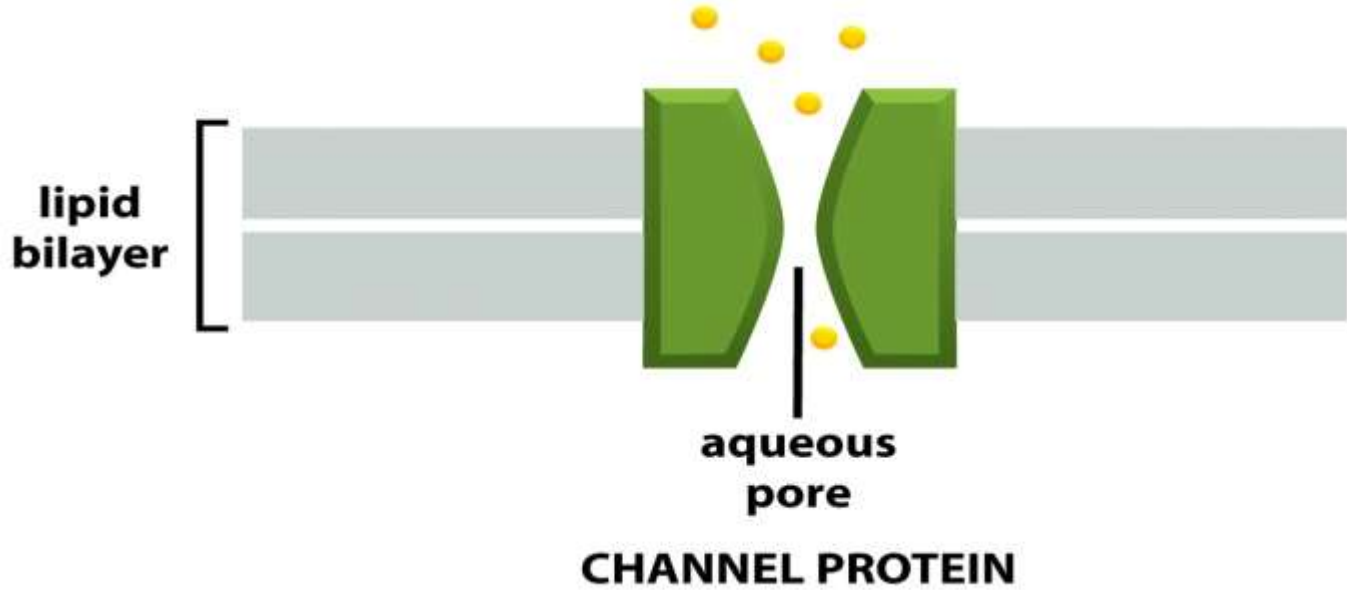


Figure 11-3b Molecular Biology of the Cell 5/e (© Garland Science 2008)

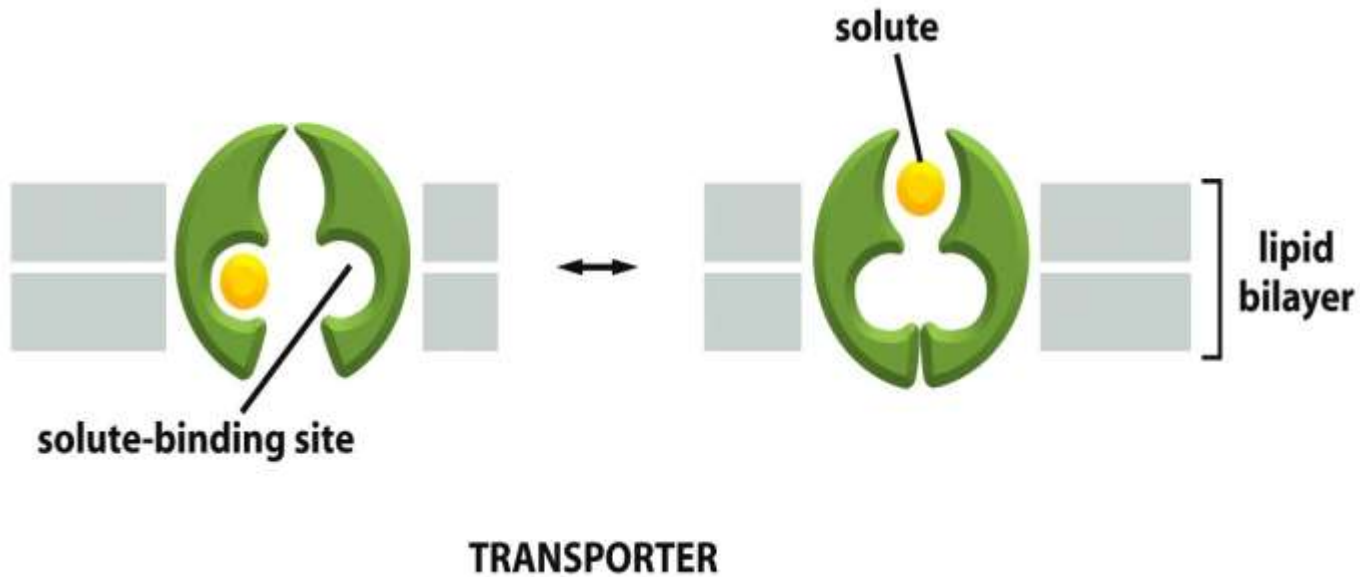


Figure 11-3a Molecular Biology of the Cell 5/e (© Garland Science 2008)

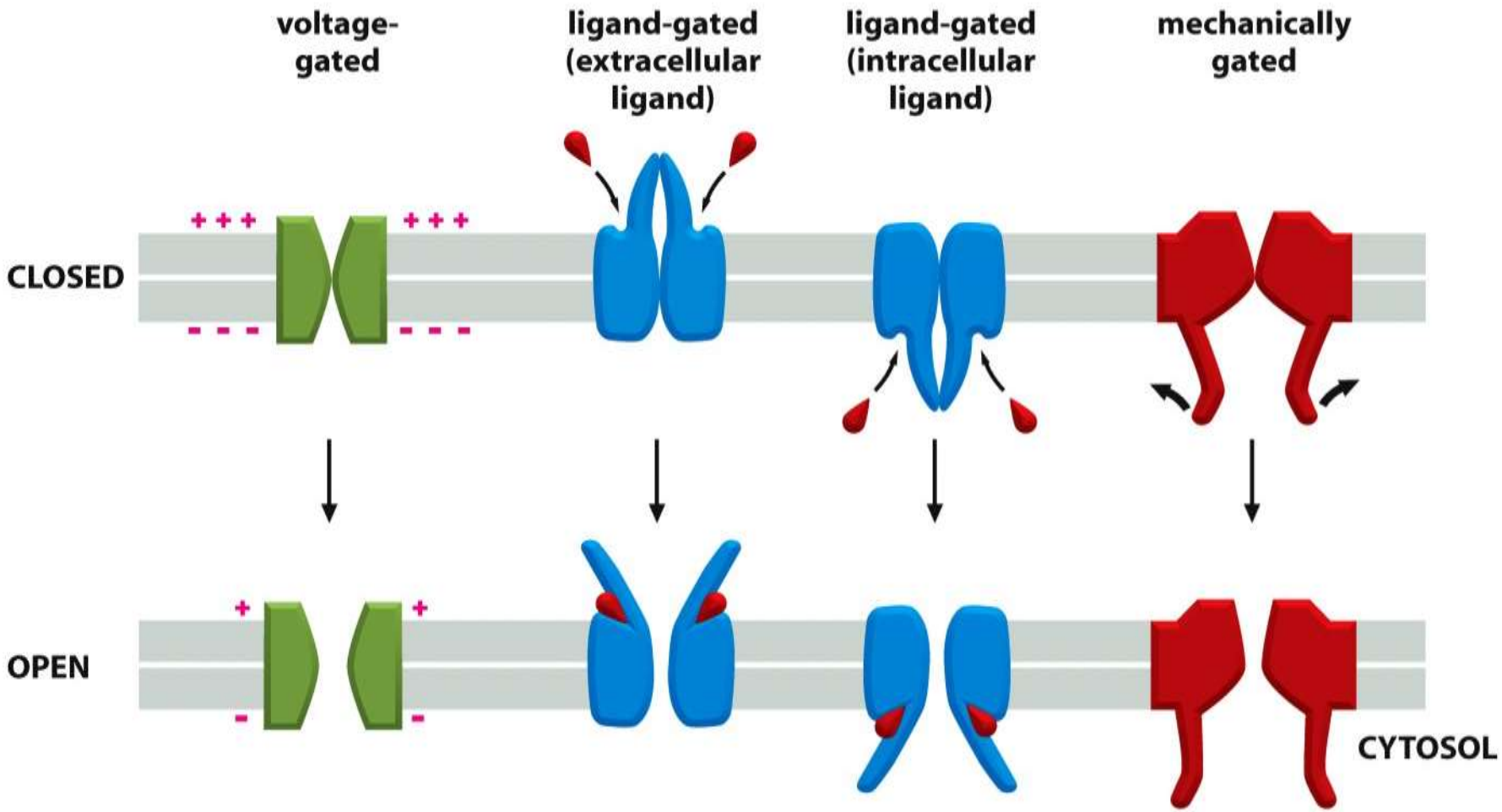


Figure 11-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Water channels

Also called aquaporins (AQPs) allow water to passively move in and out of the cell. They are widely distributed throughout the body and exist in different isoforms. For example, cells in the collecting duct of the kidney express AQP3 and AQP4 in the basolateral mb, while AQP2 in the apical mb. AQP2 abundance is regulated by ADH

Some AQPs allow also other molecules to move across the mb, like glycerol, urea, mannitol, purines, pyrimidines, Ca++, NH3. These channels are termed aquaglyceroporins

Each aquaporin consists of 6 domain transmembrane and a central pore. They usually form tetramers

Ion channels

Ion channels are found in every cell but are most important in excitable cells (neurons, muscle, cardiac cells)

Ion channels are classified by their:

- ***Selectivity***: Nature of the ions that pass through the channel

- ***Conductance***: number of ions that pass through a channel, typically expressed in pS (S=1/R). Sometimes a channel has larger conductance when ions are moving into the cell (or viceversa). These channels are termed inward rectifier (or outward)

- ***Gating***: Voltage, extracellular agonists or antagonists, intracellular messengers, mechanical stretch of plasma mb.

Solute carriers (SLCs)

As is typical of integral membrane proteins, SLCs contain hydrophobic transmembrane alpha helices connected to each other by hydrophilic intra- and extra-cellular loops. Transports do not need energy (ATP).

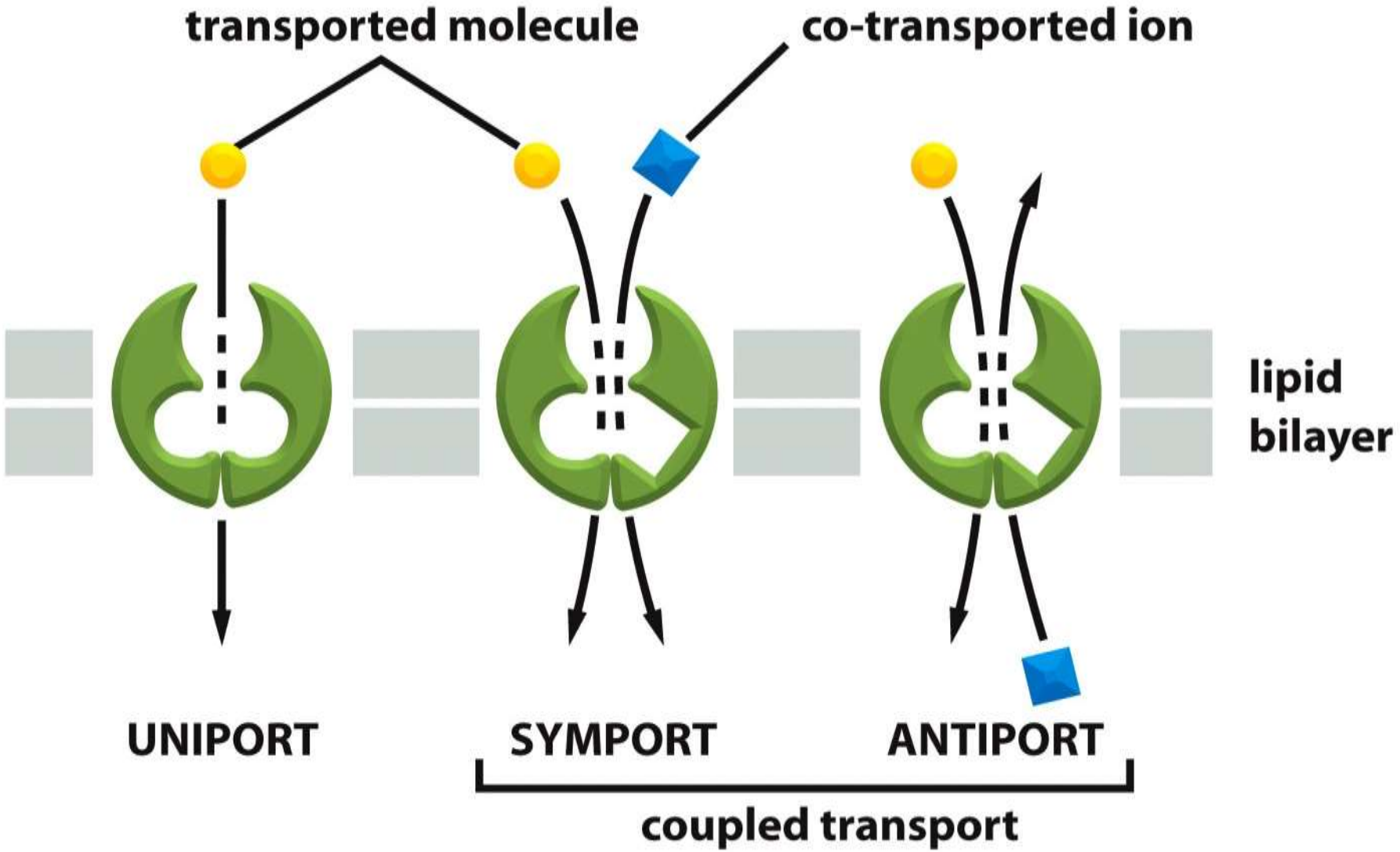
The SLC group include examples of transport proteins that are:
facilitative transporters (allow solutes to flow downhill with their electrochemical gradients)
secondary active transporters (allow solutes to flow uphill against their electrochemical gradient by coupling to transport of a second solute that flows downhill with its gradient such that the overall free energy change is still favorable)

These are subdivided in three groups:

- ***Uniporters***: Typical is GLUT1 that carries glucose into the cell

- ***Symporters***: Couple the transport of 2 or more ions or molecules across the mb. E.g. NKCC2 symporter found in the kidney

- ***Antiporters***: Work like symporters but the movement of molecules or ions is in the opposite direction. E.g. Na-H^+-antiporter (NHE-1) is found in all cells and is important to regulate intracellular pH.


transported molecule
co-transported ion
lipid bilayer
UNIPORT
SYMPORT
ANTIPORT
coupled transport
(passive transport)
(secondary active transport)

Active and passive transport

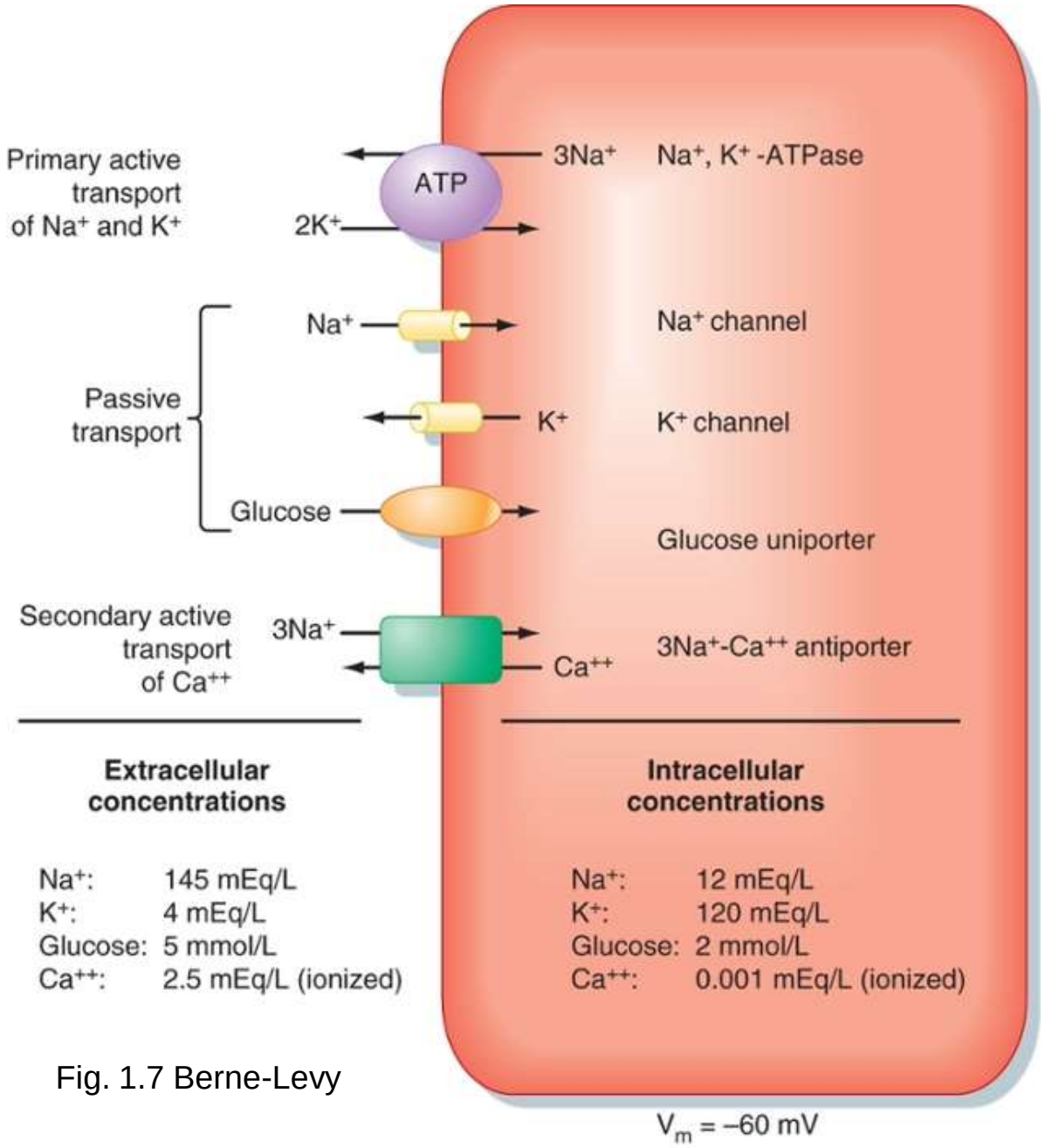


Fig. 1.7 Berne-Levy

When a molecule passes through the mb following the direction predicted by the $\Delta\mu x$ then we talk about passive transport otherwise active transport ⟶ it needs energy.

Movement of water through water channels, ions through channels or molecules through uniporters (e.g. glucose via GLUT1) is passive transport.

Transporters that use energy obtained hydrolizing ATP are what we termed primary active transports. Otherwise when a molecule is transported against $\Delta\mu x$ by coupling the movement of other molecules (predicted by $\Delta\mu x$) then we talk about secondary active transports (e.g. $1Ca^{++}/3Na^{+}$ antiporter Or SGLT-1 and 2)

	PASSIVE	Transport Mechanism	ACTIVE	
Property	**Passive Diffusion**	**Facilitated Diffusion**	**Primary active transport**	**Secondary active transport**
Requires specific protein	–	+	+	+
Solute transported against its gradient	–	–	+	+
Coupled to ATP hydrolysis	–	–	+	–
Driven by movement of a cotransported ion down its gradient	–	–	–	+
Examples of molecules transported	O_2, CO_2, steroid hormones, many drugs	Glucose and amino acids (uniporters); ions and water (channels)	Ions, small hydrophilic molecules, lipids (ATP-powered pumps)	Glucose and amino acids (symporters); various ions and sucrose (antiporters)

*Also called *secondary active transport*.

ATP - dependent transporters

These are subdivided in two groups:

- ATPase ion transporters: We know three families: The P-type, V-type and F-type ATPases. P-type are phosphorilated during the transport cycle. Typical is the Na+K+-ATPase that pumps 3 Na+ outside and 2 K+ inside the cell, against the electrochemical gradient using the energy from ATP hydrolysis. V-type are intracellular and often vacuolar (v stands for vacuolar). Typical is the V-type H+-ATPase (plays an important role in urinary acidification).

- ATP-binding cassette (ABC) transporters: Present in prokaryotic and eukaryotic cells. ABC are amino acid domains that bind ATP and allow, using the energy from ATP hydrolysis, to transport different ions or molecules (e.g. cholesterol).

Regulation of transport is achieved controlling number of transporters in the mb but also altering the kinetics (gating)

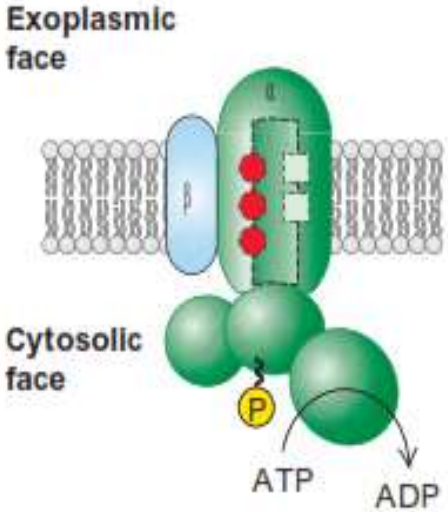
There are 4 types of membrane proteins that are responsible for the primary active transport

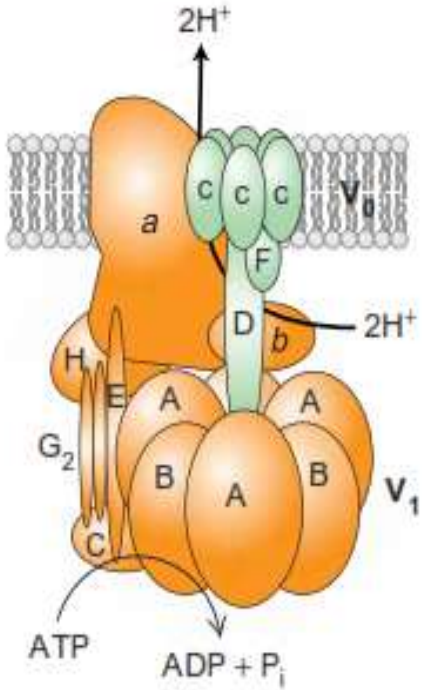
i) P, for ions (Na^+/K^+ pump, H^+ pump, Ca^{++} pump);

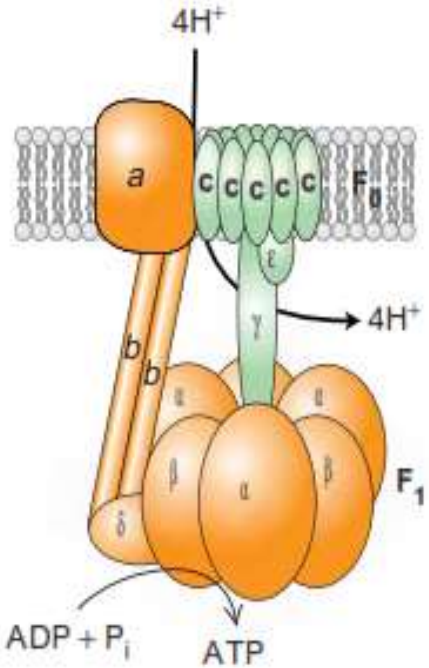
ii) V, Proton transporter;

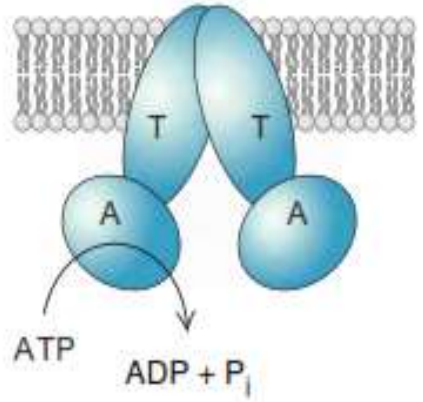
iii) F, Proton transporter (ATP synthase)

iv) ABC (ATP Binding Cassette). Ubiquitous for small molecules transports









P-class pumps

Plasma membrane of plants, fungi, bacteria (H^+ pump)

Plasma membrane of higher eukaryotes (Na^+/K^+ pump)

Apical plasma membrane of mammalian stomach (H^+/K^+ pump)

Plasma membrane of all eukaryotic cells (Ca^{2+} pump)

Sarcoplasmic reticulum membrane in muscle cells (Ca^{2+} pump)

V-class proton pumps

Vacuolar membranes in plants, yeast, other fungi

Endosomal and lysosmal membranes in animal cells

Plasma membrane of osteoclasts and some kidney tubule cells

F-class proton pumps

Bacterial plasma membrane

Inner mitochondrial membrane

Thylakoid membrane of chloroplast

ABC superfamily

Bacterial plasma membranes (amino acid, sugar, and peptide transporters)

Mammalian plasma membranes (transporters of phospholipids, small lipophilic drugs, cholesterol, other small molecules)

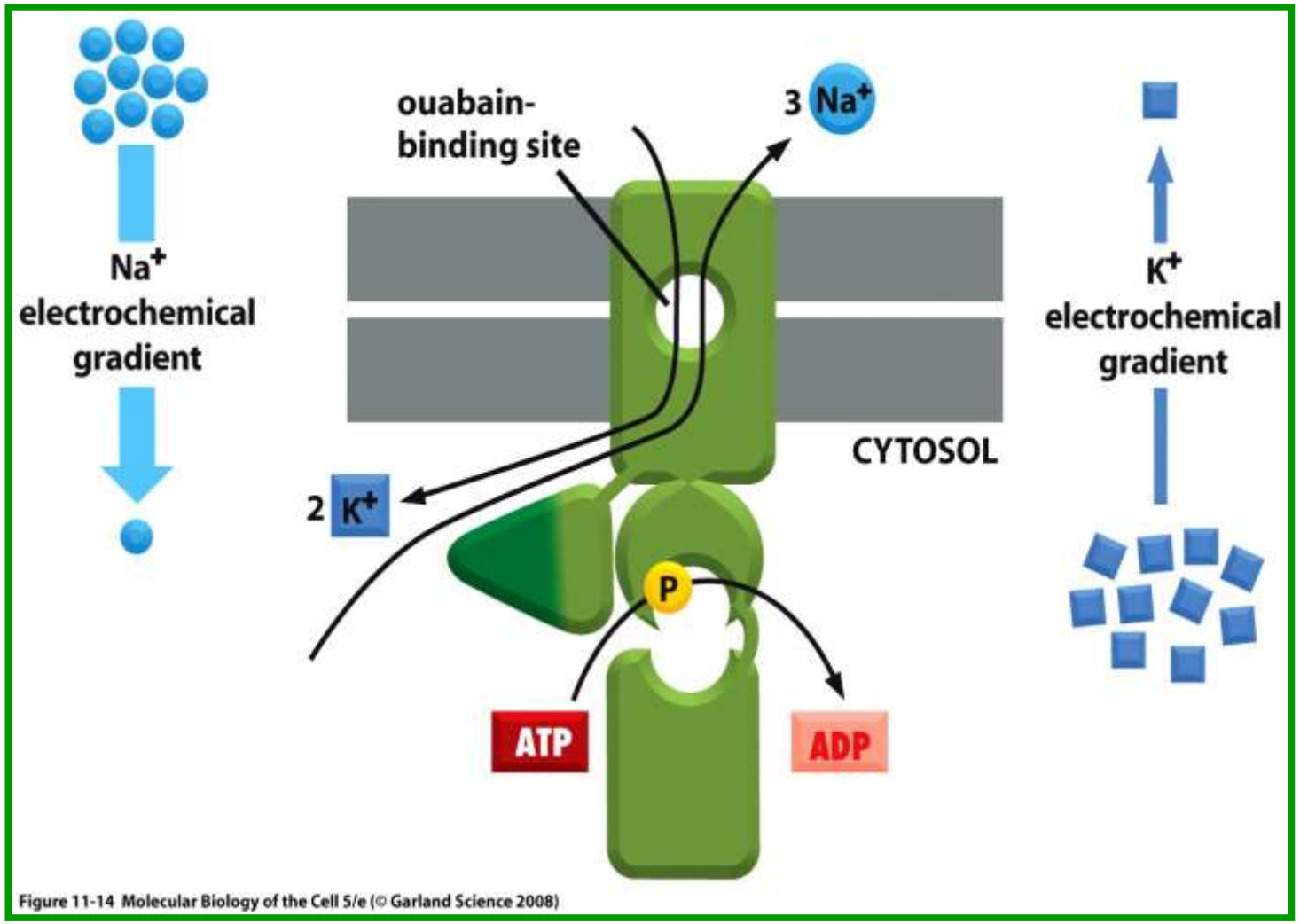
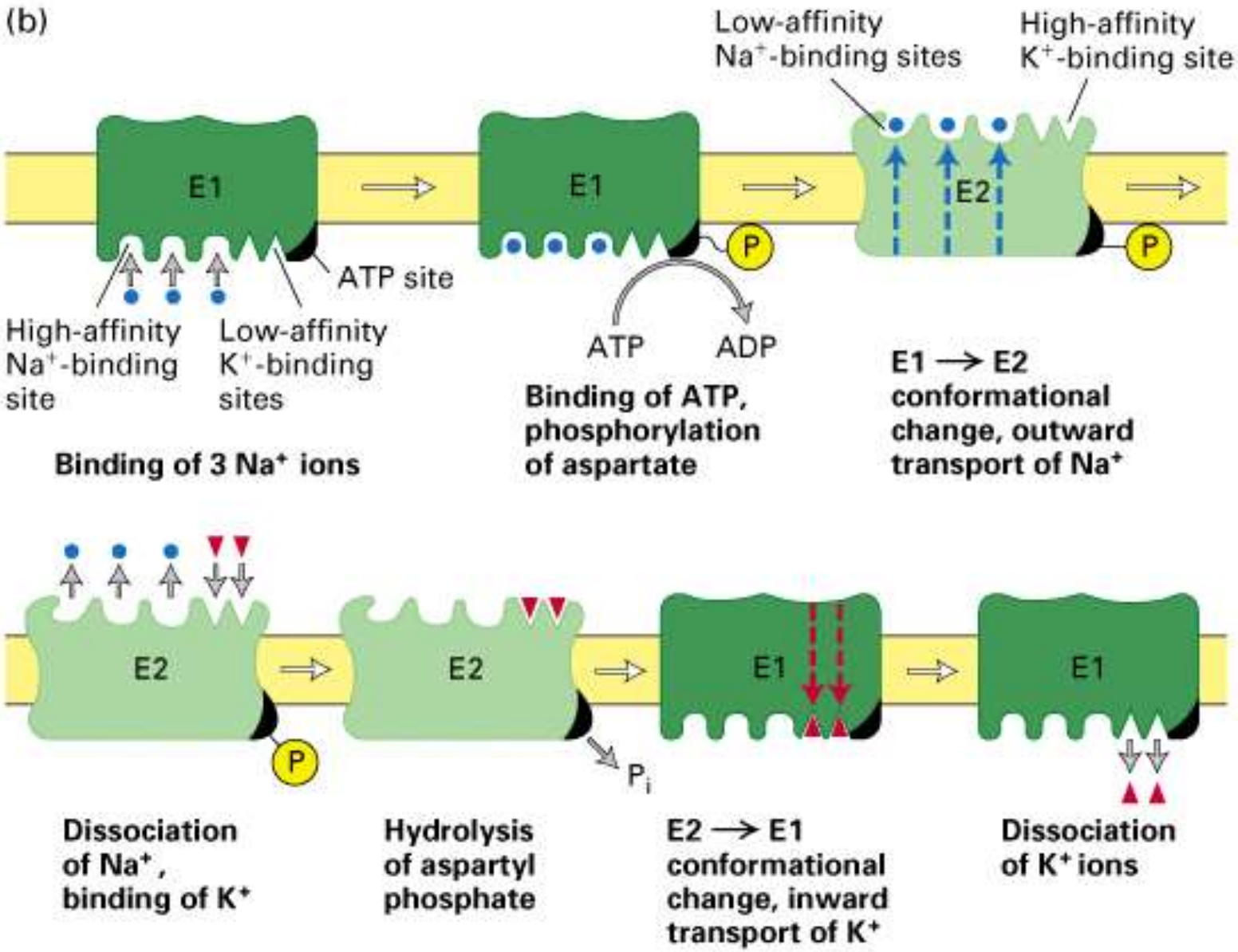


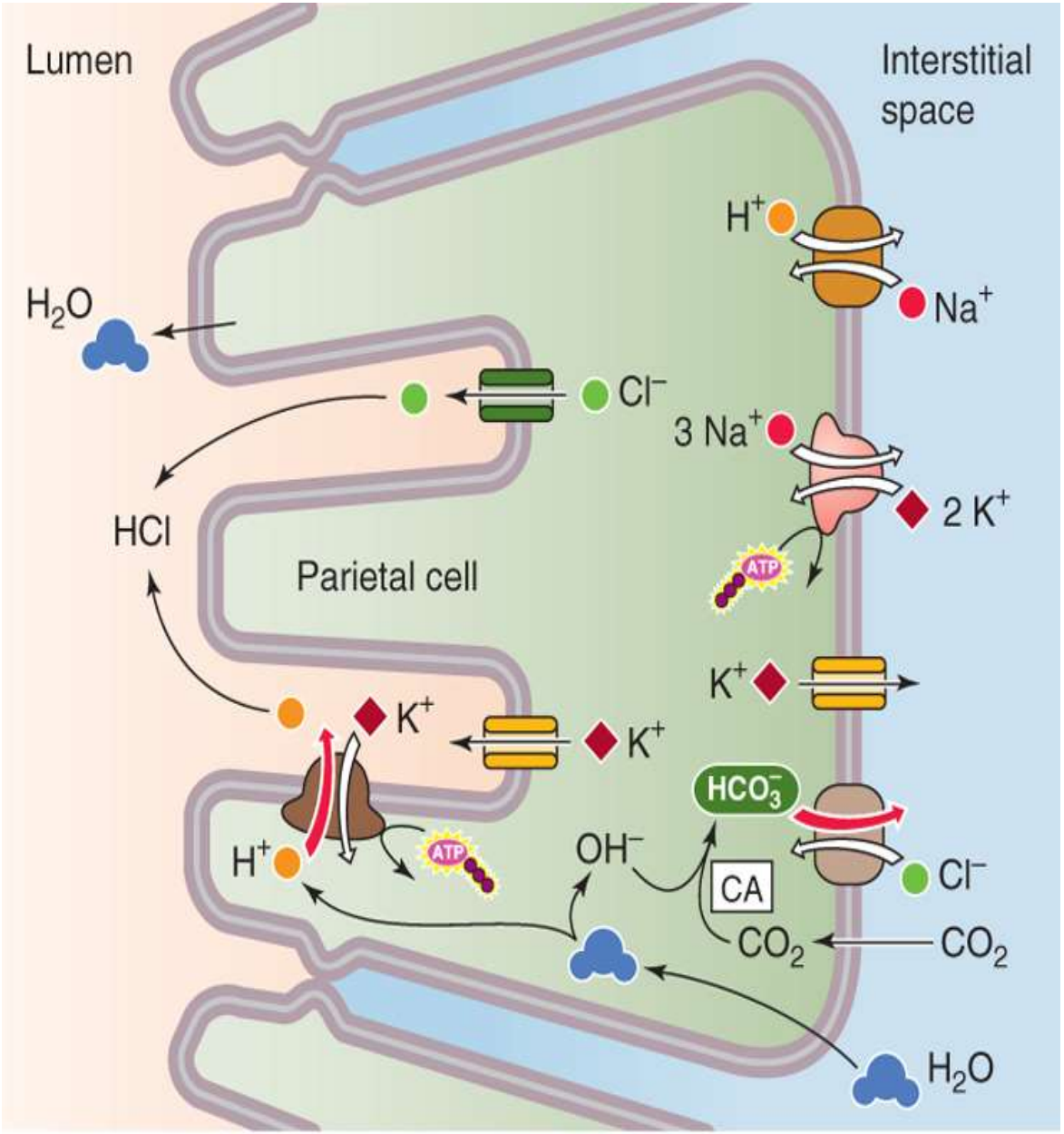
Figure 11-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

P-type pumps

Ouabain is medically used in very low concentration to treat hypotension and some arrhythmias



Ouabain blocks it here

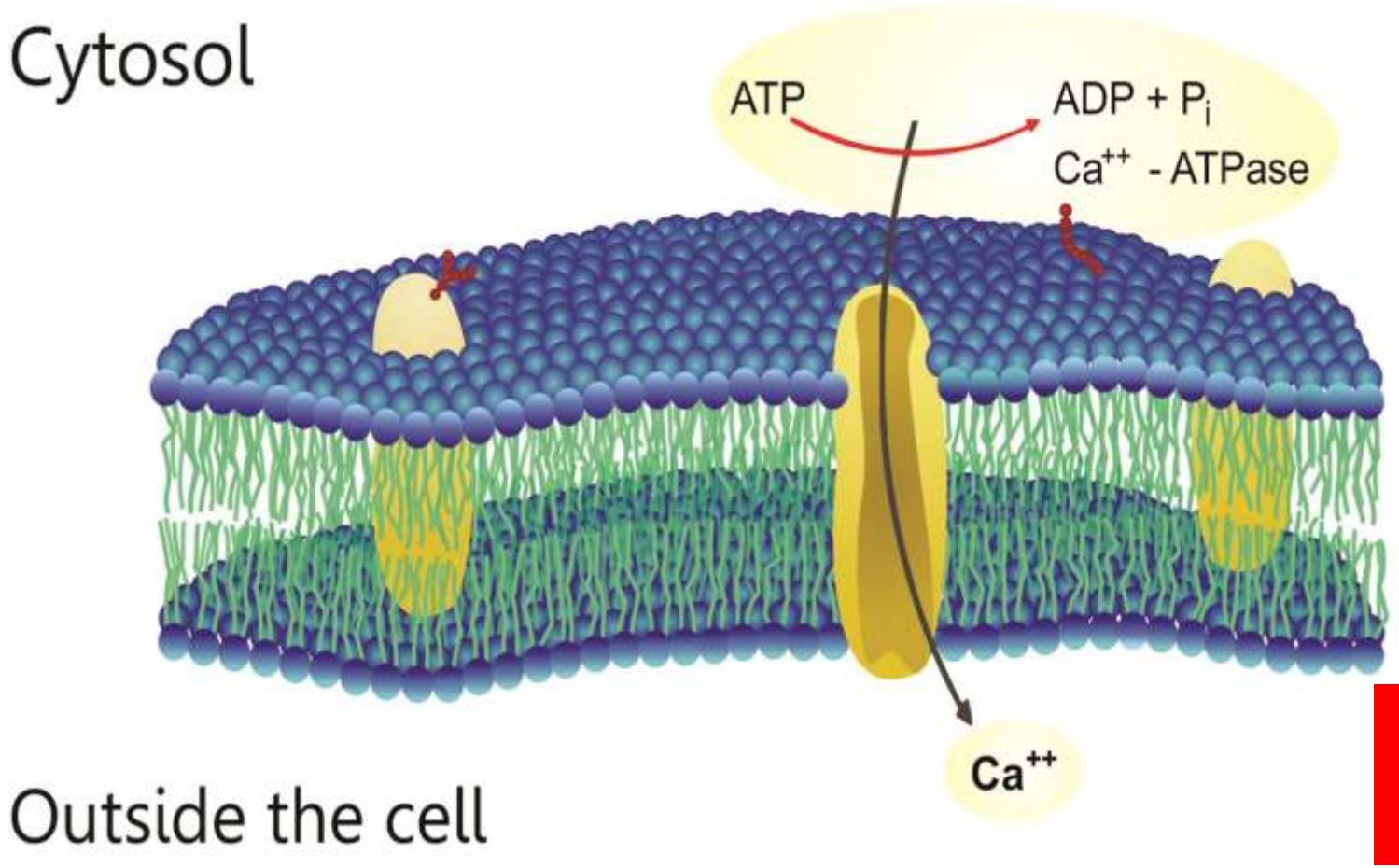


P-type pumps

Kidney, **stomach** and intestine 2H-2K

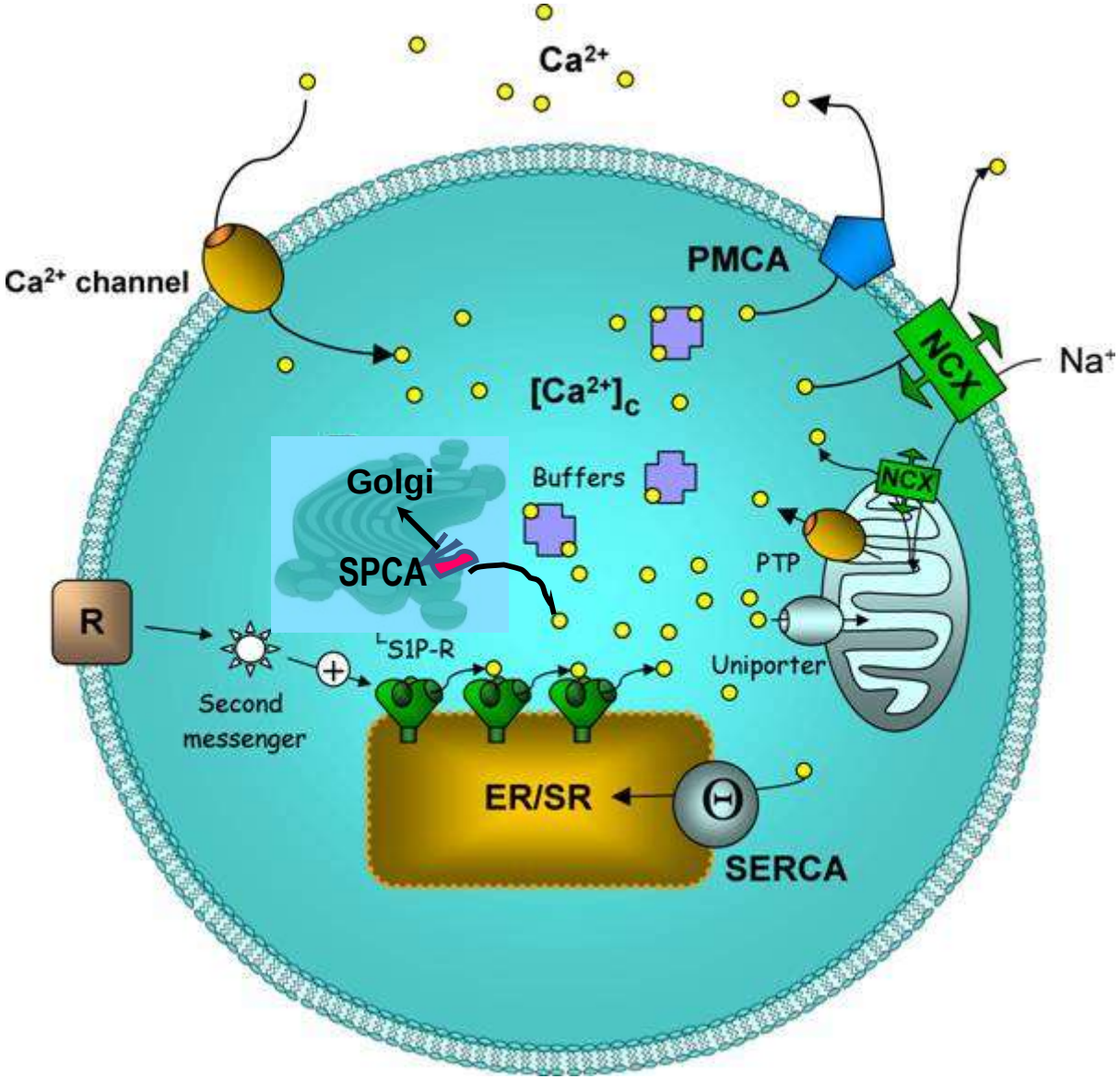
H^+/K^+ pump

All cells, is ubiquitous 1 Ca – 1 H.



P-type pumps

Ca^{++} pump in the cell memrbane



P-type pumps

Ca^{++} pump are divided into three major families:

i) SERCA (sarco / endoplasmic reticulum Ca2+ / ATPase);

ii) PMCA (plasma membrane Ca2+ / ATPase);

iii) SPCA (secretory pathway Ca2+ / ATPase)

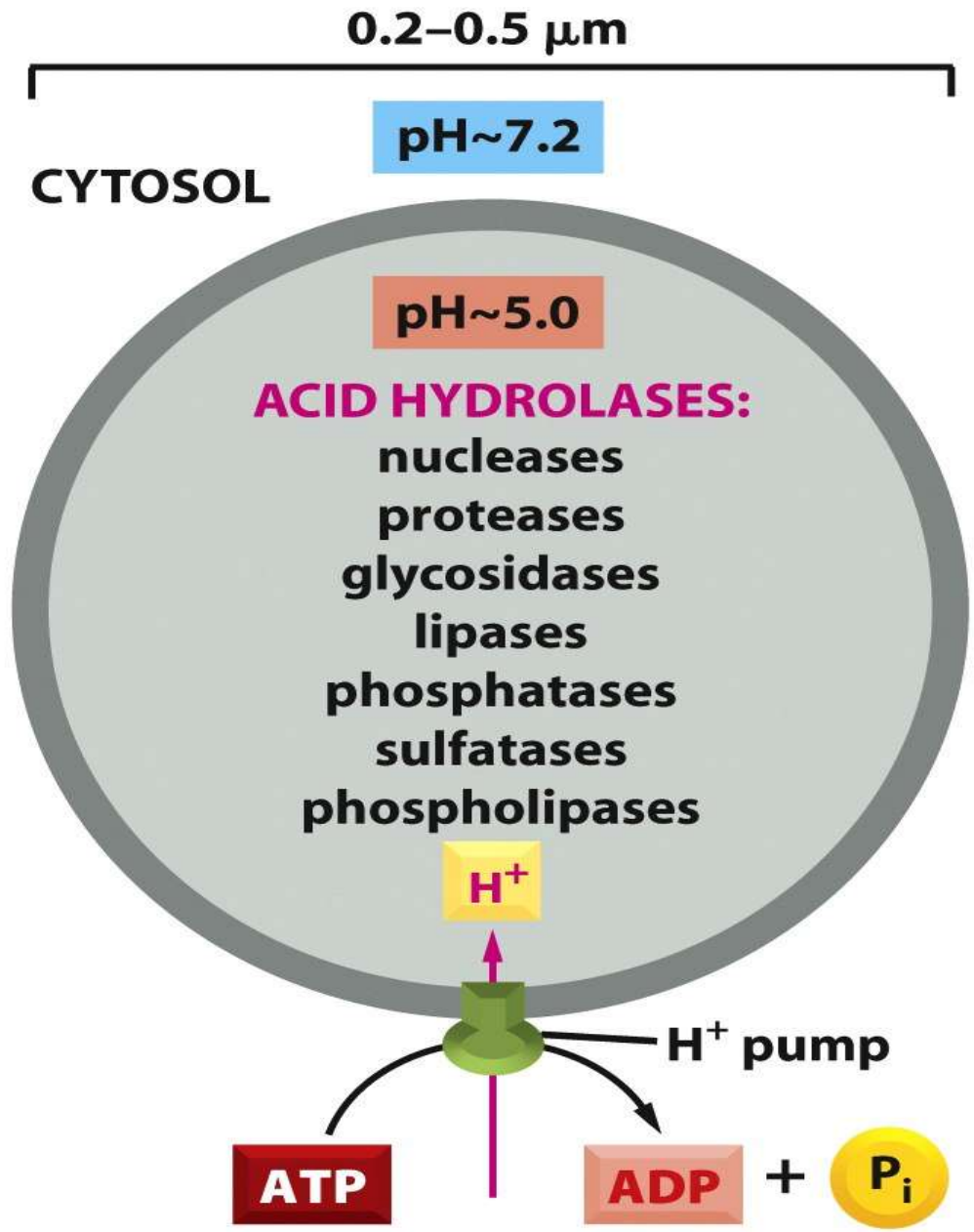


Figure 13-36 Molecular Biology of the Cell 5/e (© Garland Science 2008)

V-type proton pump

These are present on lysosomes, endosomes, Golgi apparatus.
H movement is not coupled to K movement

Lysosome acidification

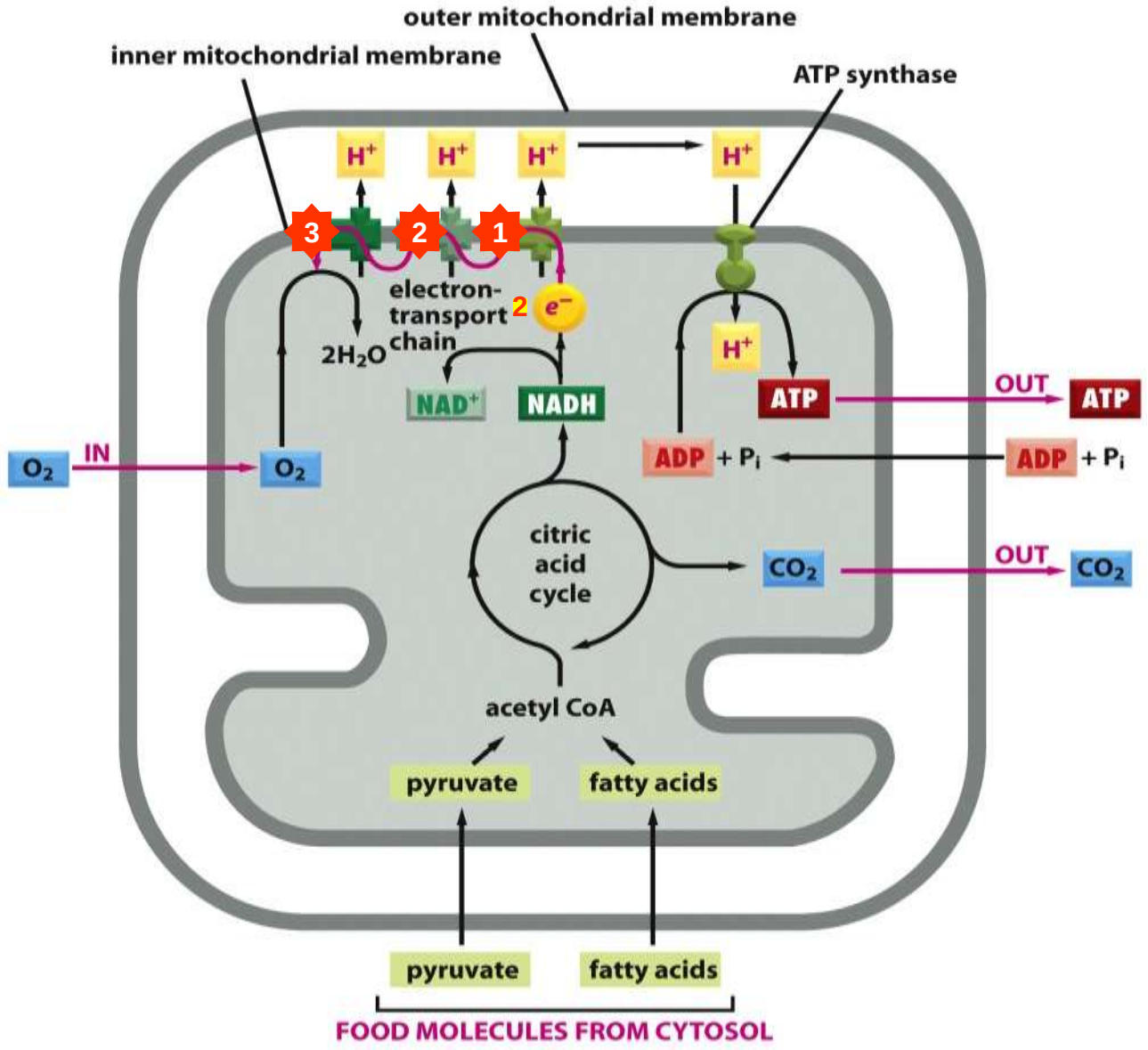


Figure 14-10 Molecular Biology of the Cell 5/e (© Garland Science 2008)

1. NADH dehydrogenase (complex I)
2. cytochrome bc1 (complex III)
3. cytochrome oxidase (complex IV)

F-type proton pump

ATP synthase

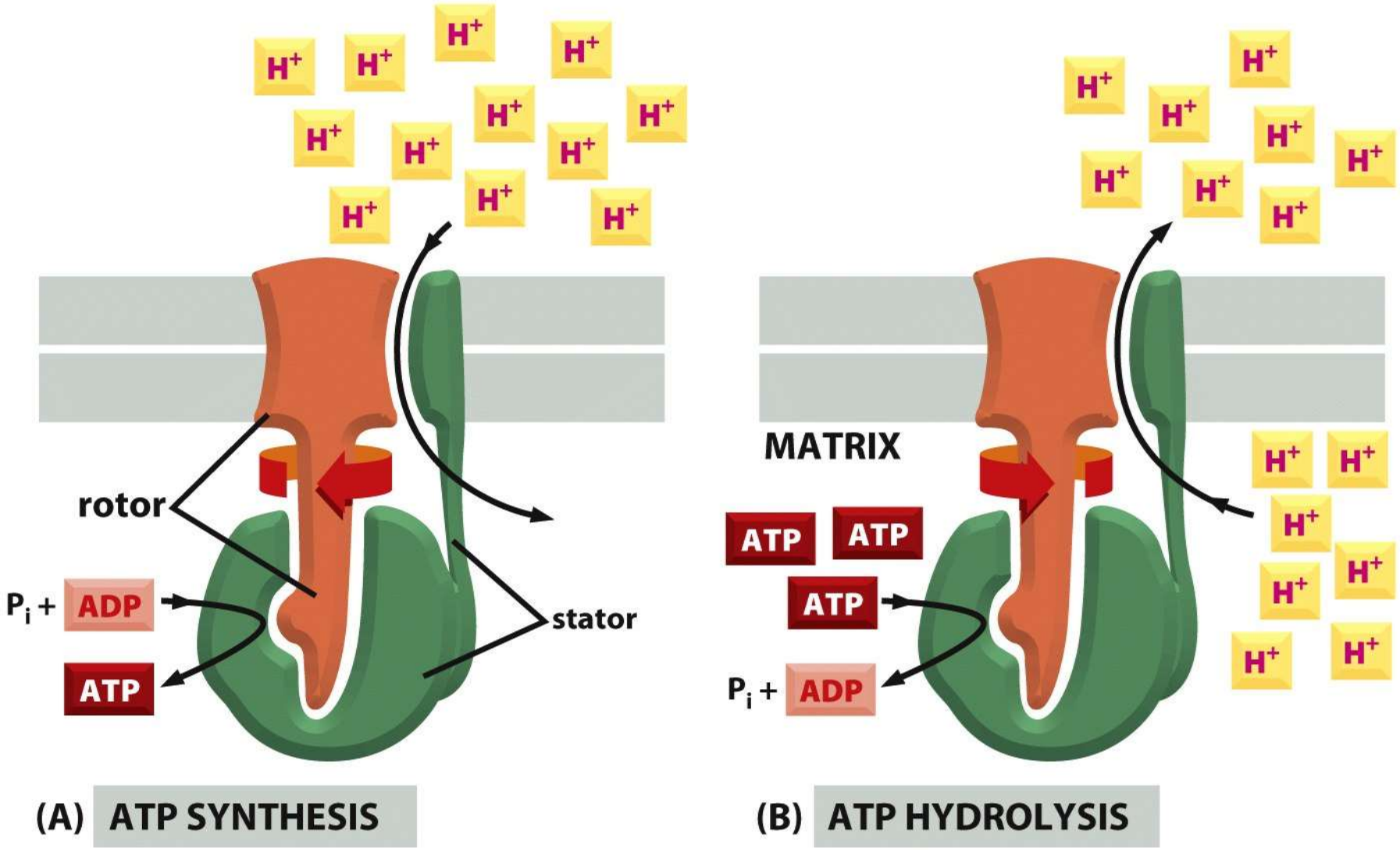


Figure 14-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

ATP synthase

ABC-transporters - 1

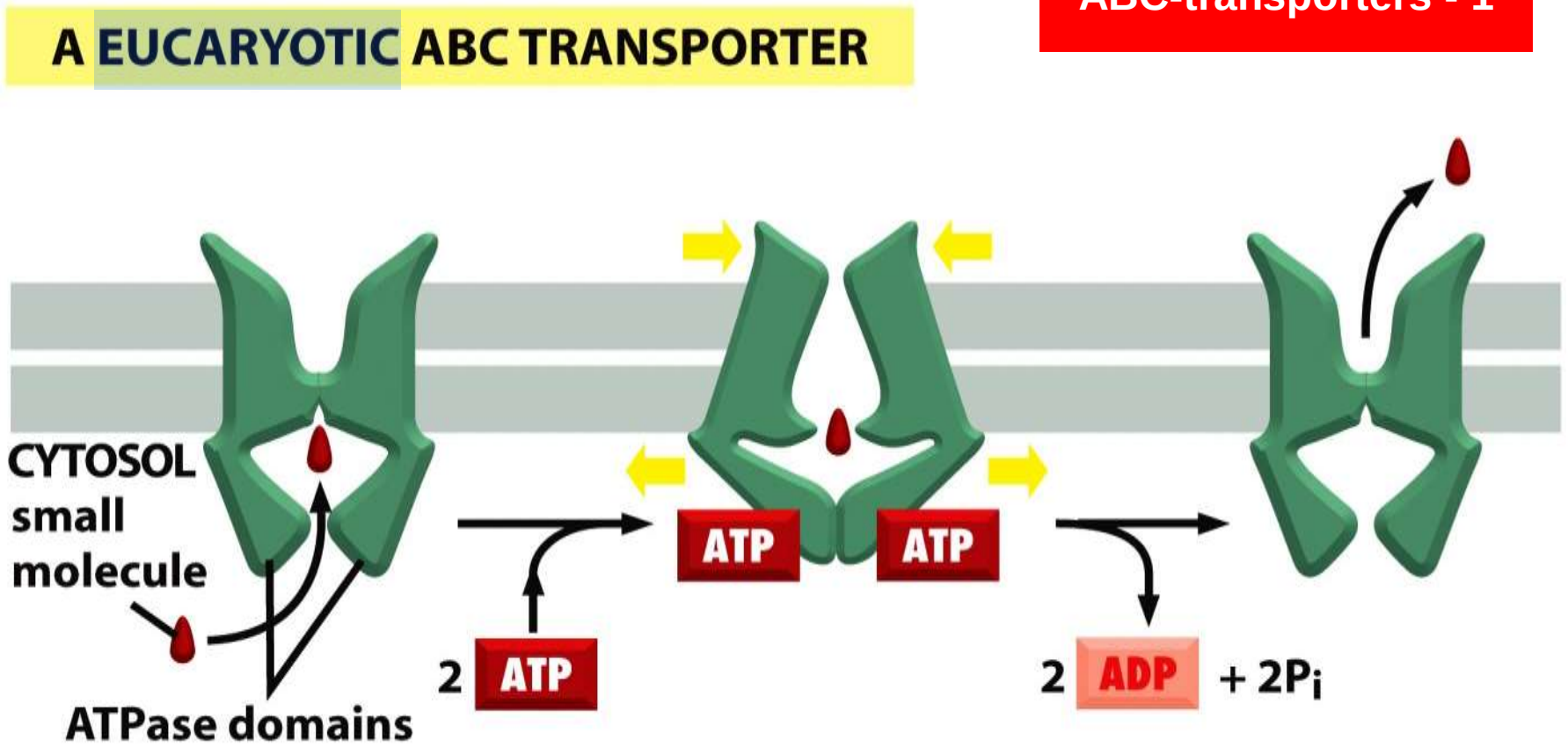


Figure 11-17b Molecular Biology of the Cell 5/e (© Garland Science 2008)

MDR (multidrug resistance) protein provides resistance to chemotherapy.

ABC-transporters - 2

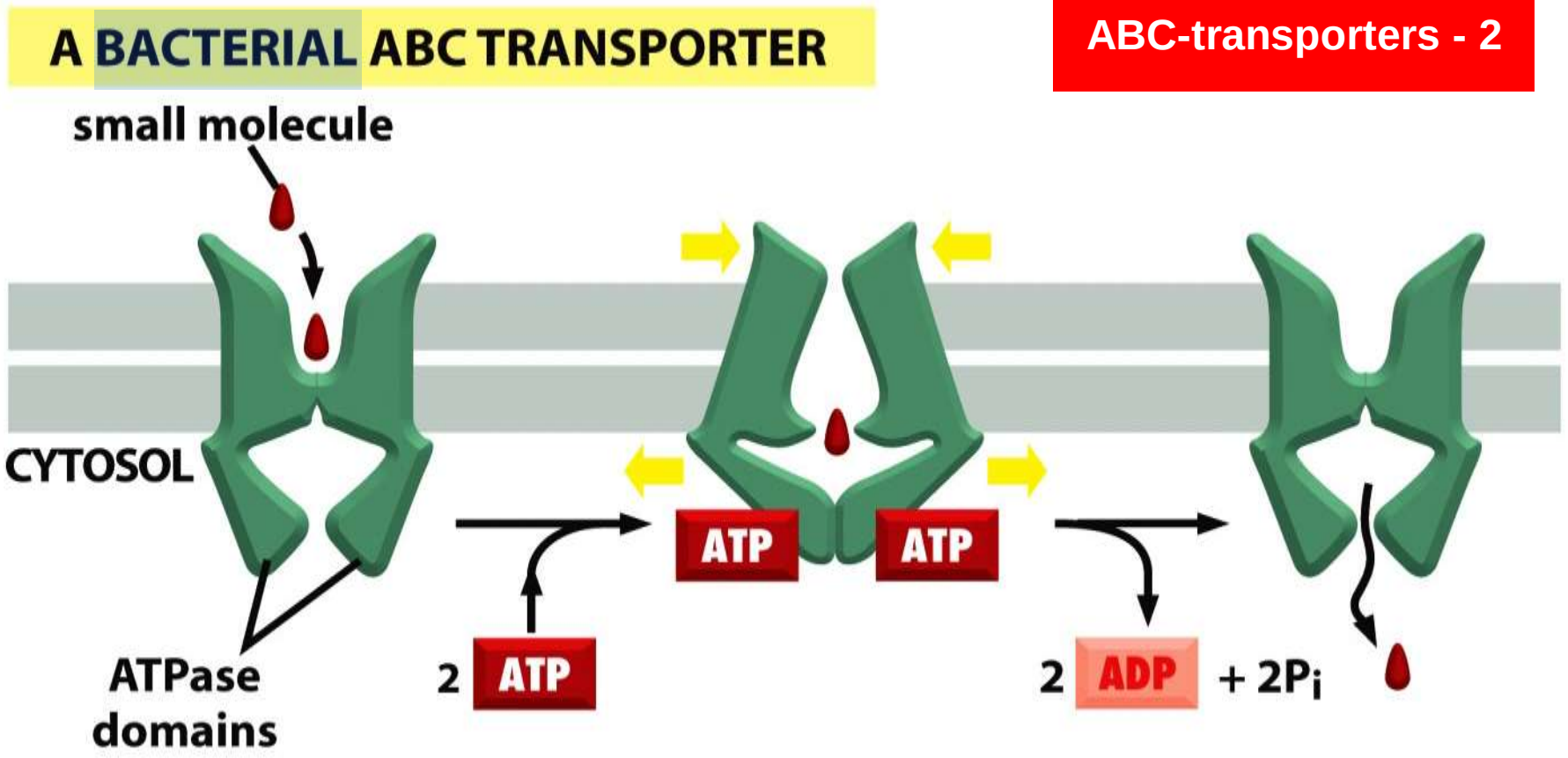
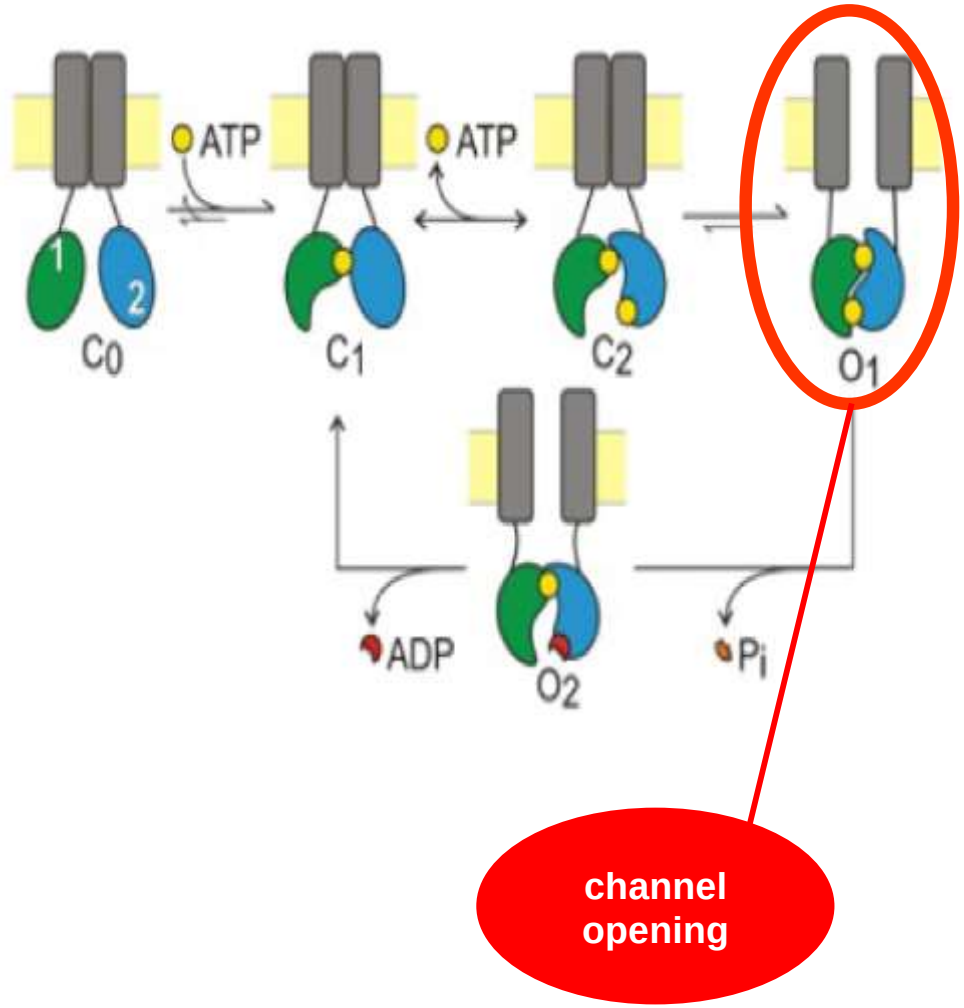


Figure 11-17a Molecular Biology of the Cell 5/e (© Garland Science 2008)

ABC-transporters – 3

CFTR is present on apical mb on epithelial cells.

Low conductance Cl channel



The cystic fibrosis transmembrane conductance regulator (CFTR) is a channel which transports chloride ions across a cell membrane. The disease cystic fibrosis is the result of a mutation which makes the CFTR chloride channel ineffective or unable to open.

When chloride cannot cross the cell membrane, anion and the accompanying water flow are decreased, causing the main symptoms of cystic fibrosis which is the thickening of mucus in the respiratory apparatus and in the pancreas. The result is an impairment of respiration and digestion.

CFTR is classified as ATP Binding Cassette transporter or ABC-transporter and it is an ion channel that uses ATP binding to open and close normally.

ATP binding to the 2 binding domains is crucial to the opening of the channel. Channel closure is determined by the hydrolysis of one of the bound ATP molecules and ADP release.

Vesicular transports (Bulk transport)

Solutes and water can be brought into the cell via endocytosis and out of the cell via exocytosis. When the two processes are contiguous we talk about transcytosis.

Endocytosis occurs in three mechanisms: pinocytosis, phagocytosis, and receptor-mediated endocytosis.

- ***Pinocytosis*** carries small molecules and water into the cell. Typical of endothelial cells

- ***Phagocytosis*** internalization of large particles. Typical of immune cells. Often it is receptor-mediated

- ***Rec-mediated endocytosis*** allows the uptake of specific molecules. Molecules bind to specific receptors on the cell surface. It needs a number of accessory proteins like adaptin, clathrin and GTPase dynamin

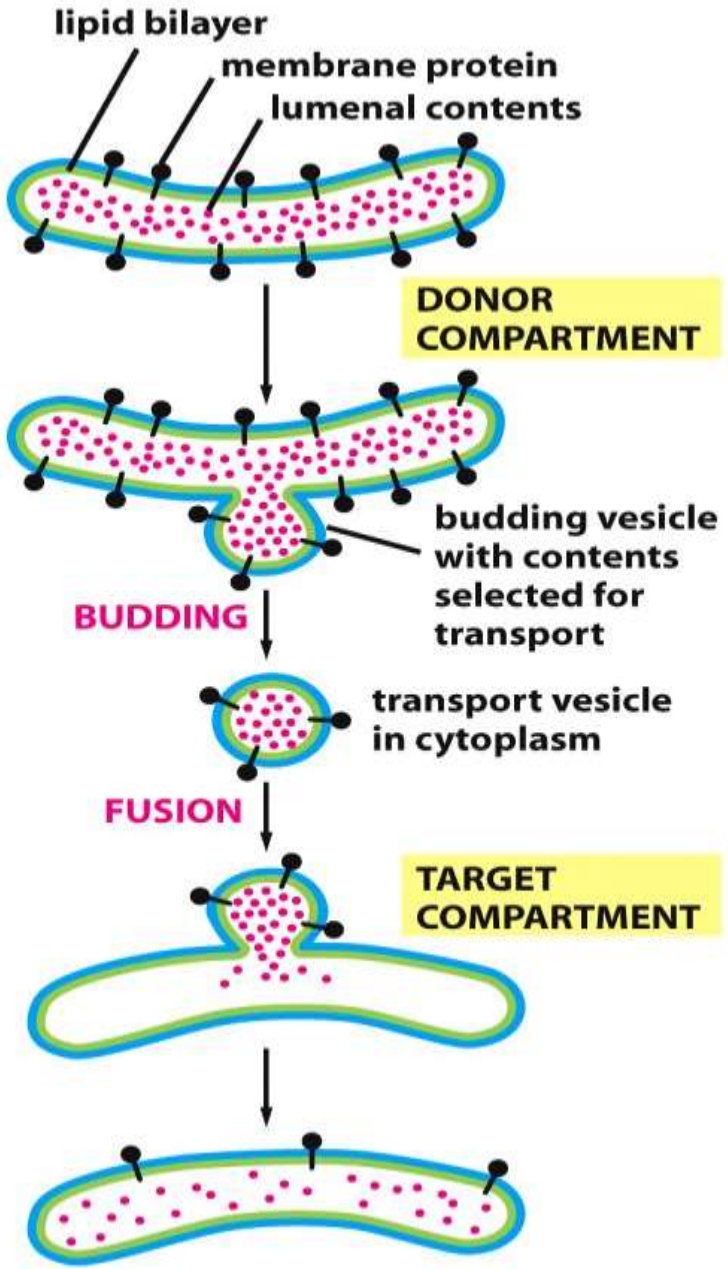
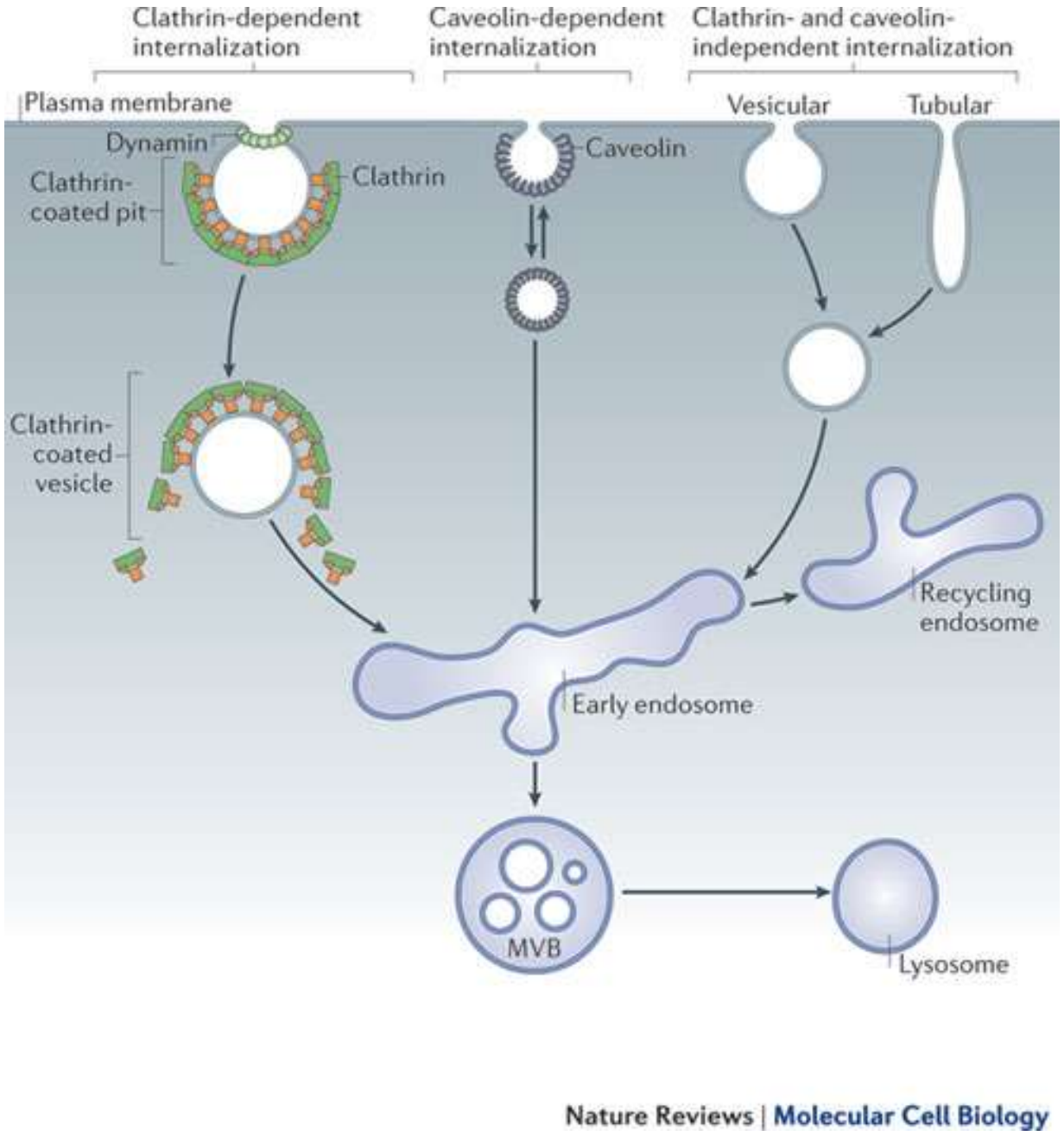


Figure 12-7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Vesicle formation



There are multiple pathways of endocytosis into cells; for example, clathrin-dependent, caveolin-dependent and clathrin- and caveolin-independent internalization.

Internalized cargo is trafficked into endosomes, where it is sorted either back to the surface of the cell or into other compartments (multivesicular bodies (MVBs) and lysosomes) for degradation.

Clathrin-mediated endocytosis is mediated by small vesicles that have a morphologically characteristic coat made up of a complex of proteins that are mainly associated with the cytosolic protein clathrin. Clathrin-coated vesicles are found in virtually all cells and form domains of the plasma membrane termed clathrin-coated pits.

Caveolae are the most common reported non-clathrin-coated plasma membrane buds, which exist on the surface of many, but not all cell types. They consist of the cholesterol-binding protein caveolin with a bilayer enriched in cholesterol and glycolipids. Caveolae are small flask-shape pits in the membrane. Uptake of extracellular molecules is also believed to be specifically mediated via receptors in caveolae.

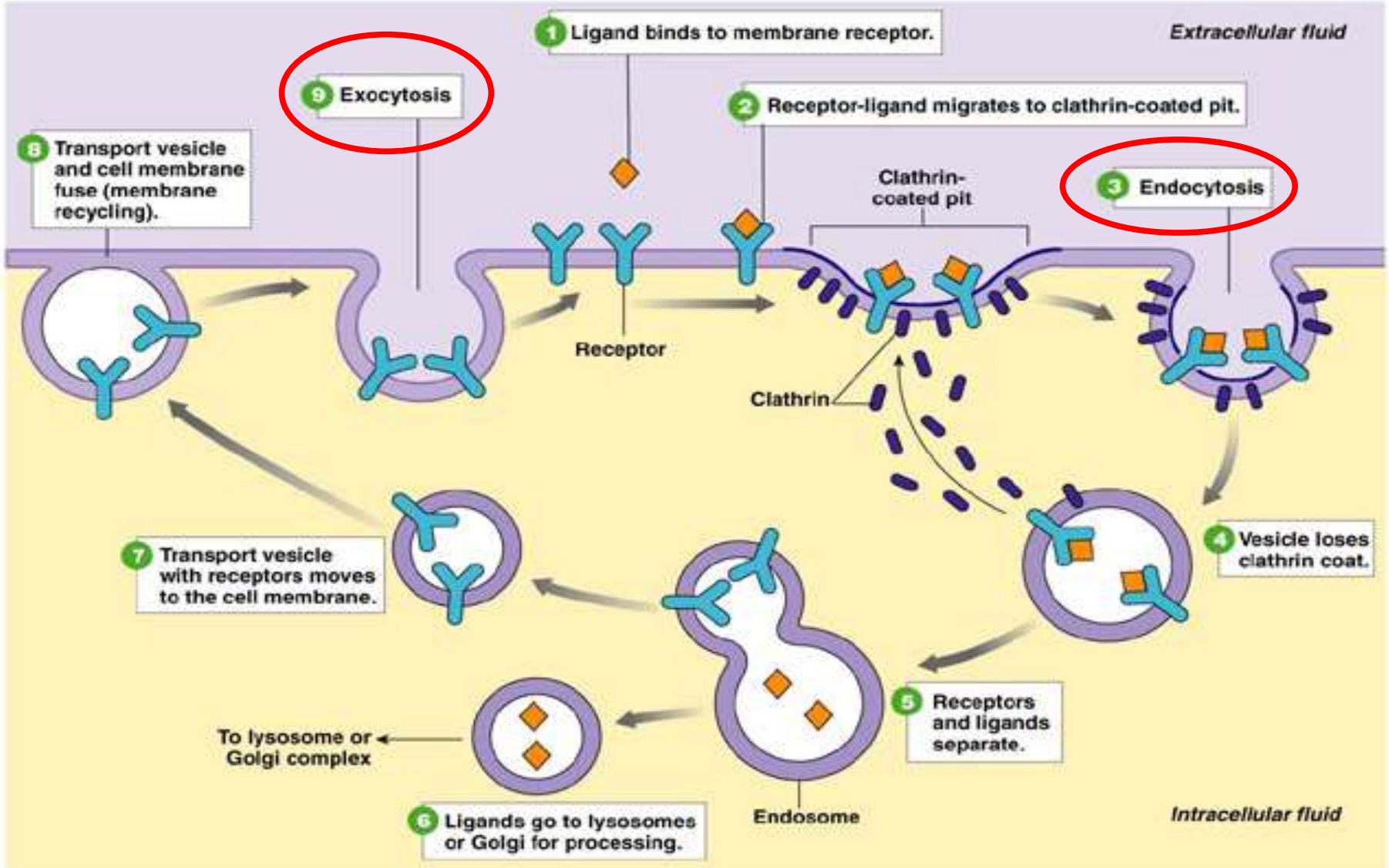


Fig. 5-24

Vesicular transports

Exocytosis can be constitutive or regulated. Constitutive for example in plasma cells releasing immunoglobulins or fibroblasts releasing collagen. Regulated secretion occurs in endocrine cells, neurons and exocrine glandular cells.

The secretory product is synthesized in the rough endoplasmic reticulum and Golgi, then stored in cytoplasmatic secretory granules.

Once the signal arrives, the mb of the granule fuses with plasma mb and the product is released

This process is often dependent by an increase in intracellular Ca++. 2 exceptions: renin secretion by juxtaglomerular cells of the kidney and parathyroid hormone by parathyroid glands are induced by decrease of intracellular Ca++.

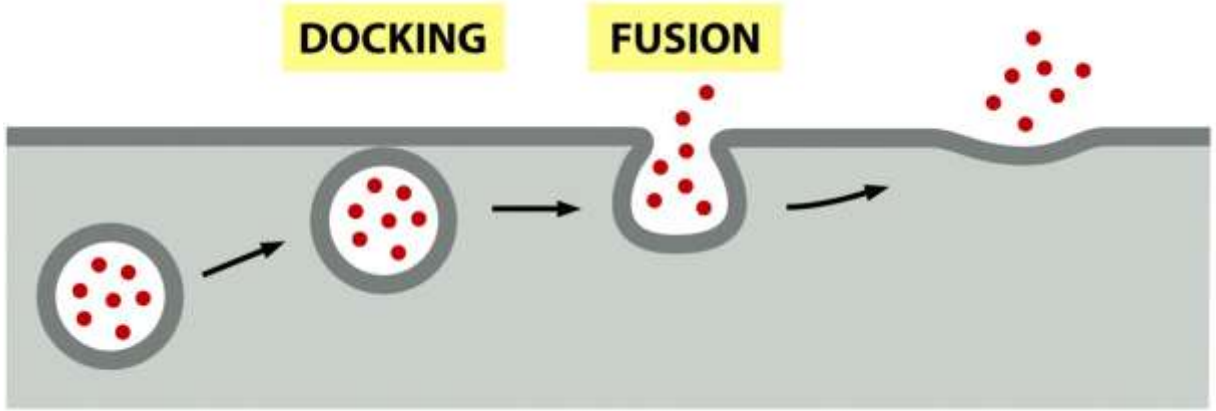


Figure 13-66a Molecular Biology of the Cell 5/e (© Garland Science 2008)

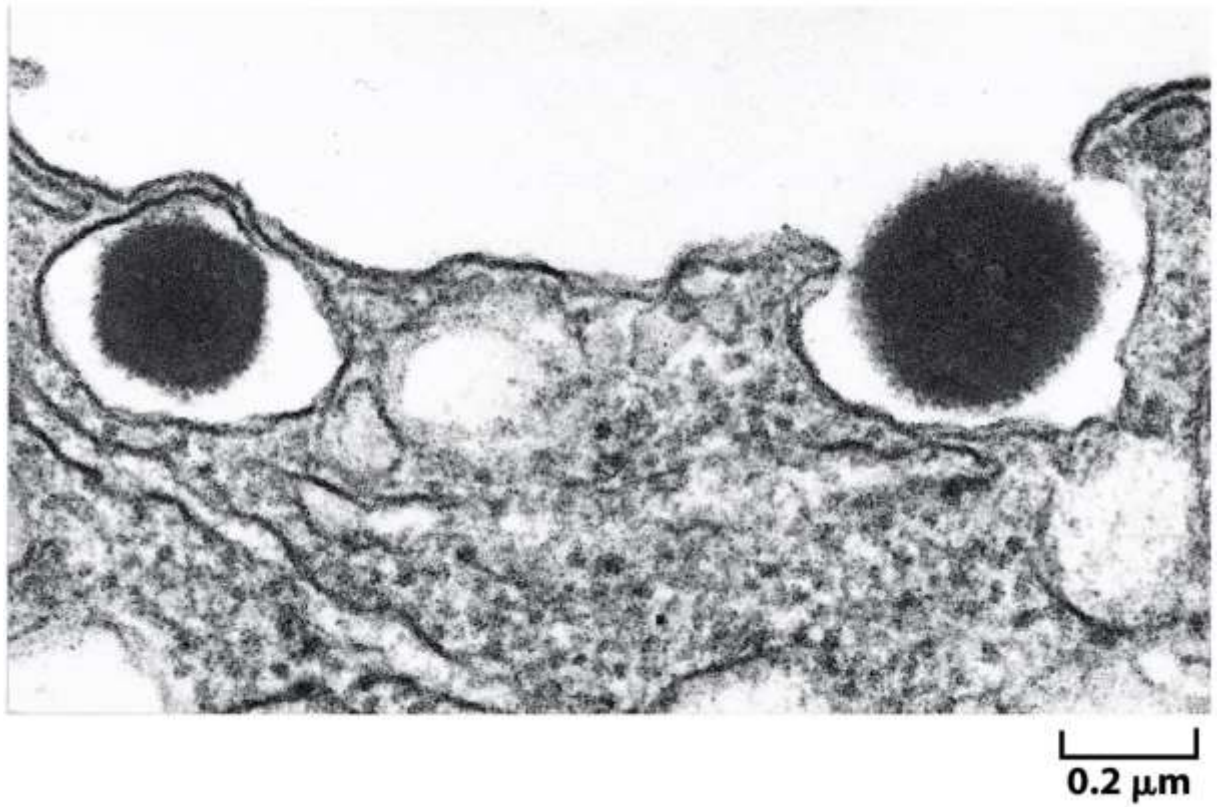


Figure 13-66b Molecular Biology of the Cell 5/e (© Garland Science 2008)

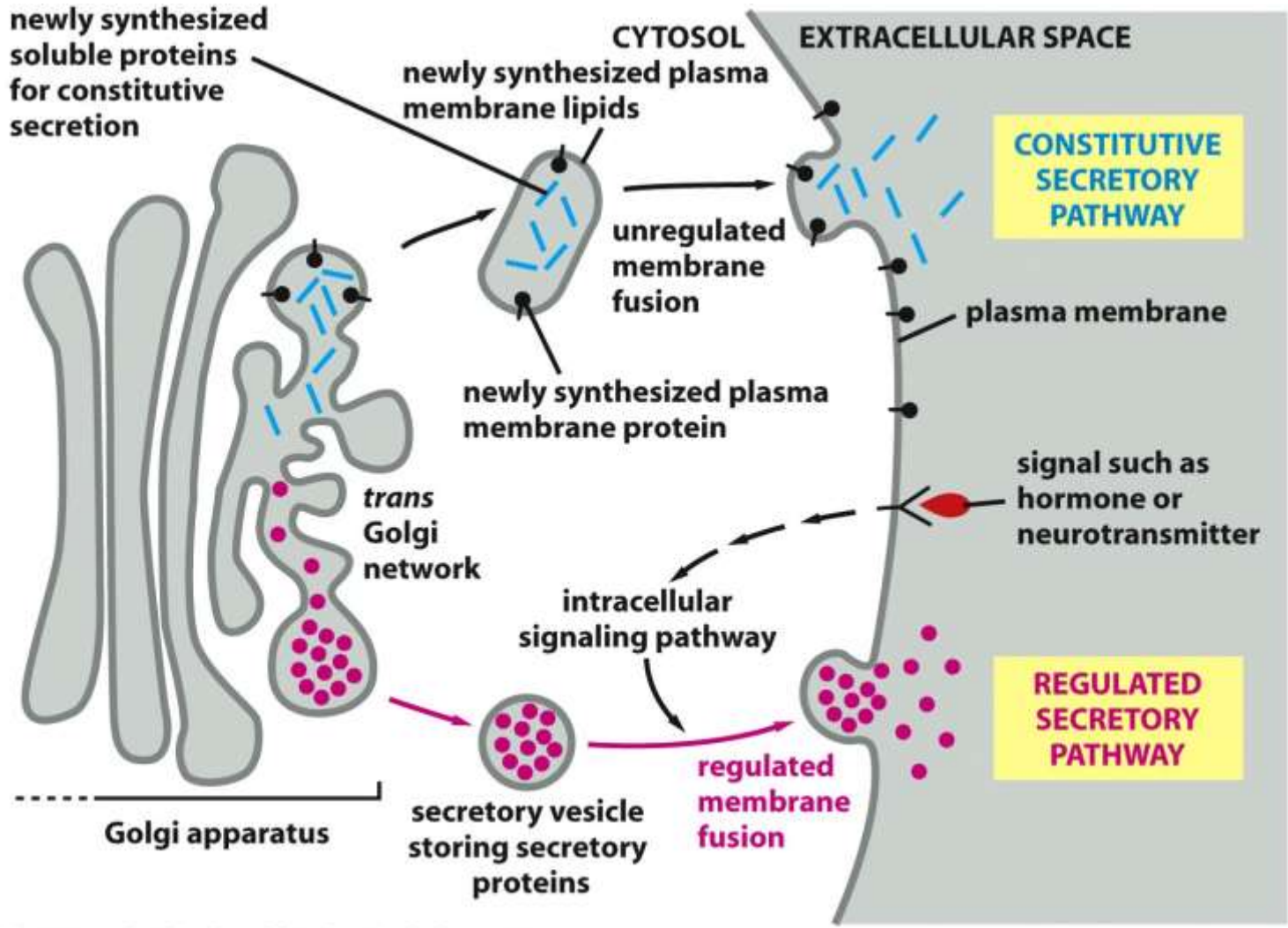


Figure 13-63 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Exocytosis

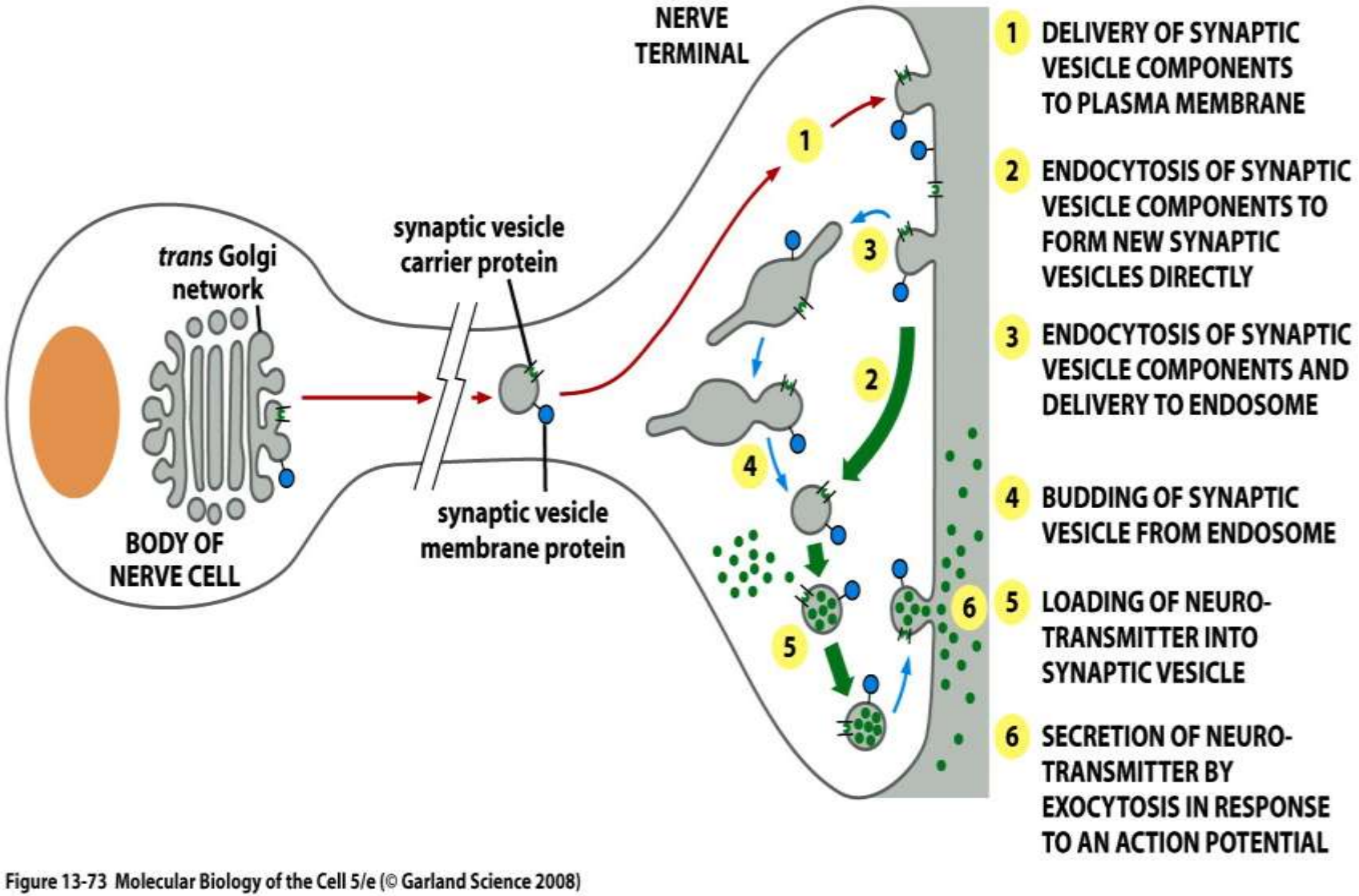


Figure 13-73 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Vesicular transport in a neuron

Summary for Cell Transport

https://www.youtube.com/watch?v=BGeSDI03aaw

Osmosis

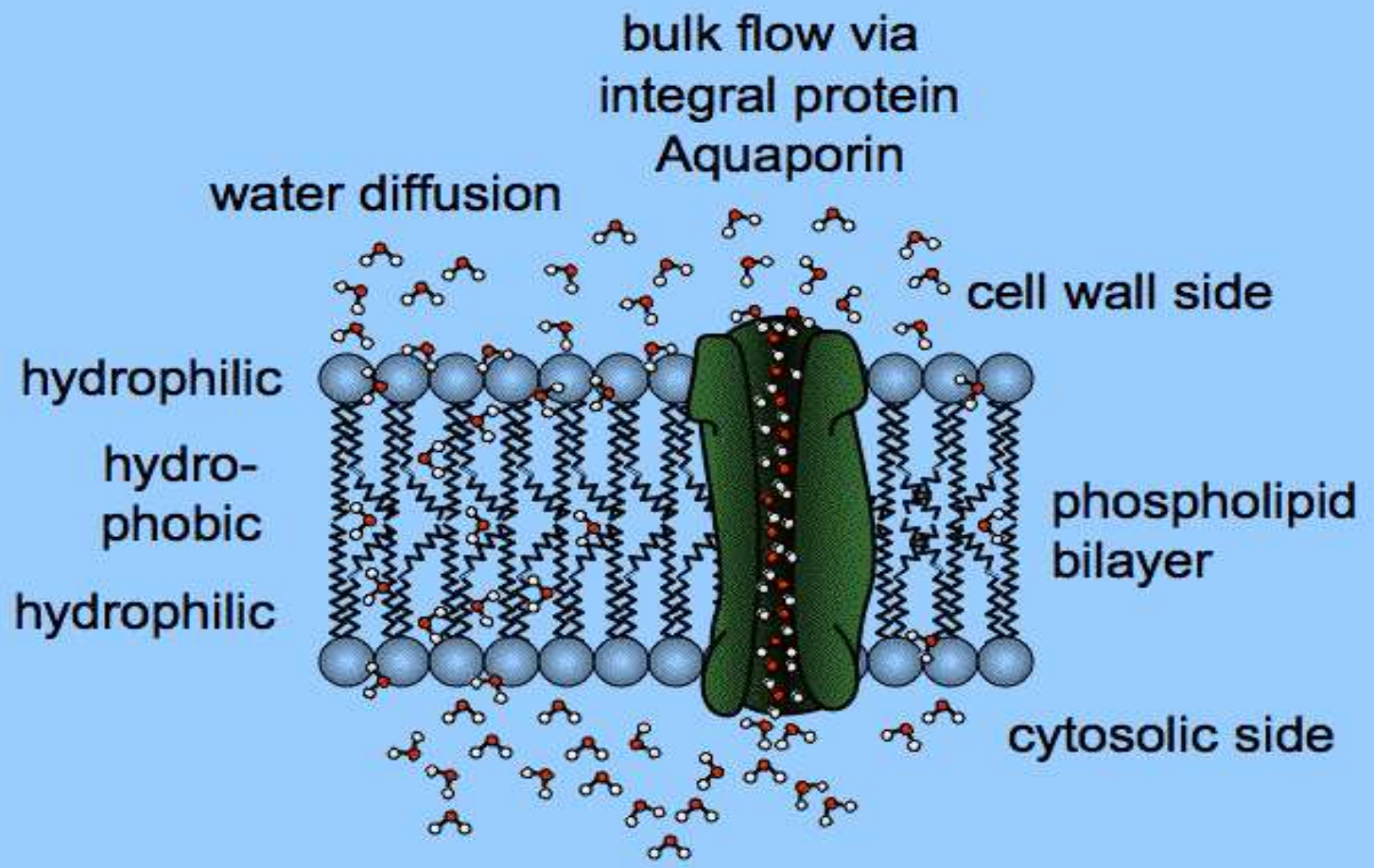
A peculiar case of diffusion is Osmosis

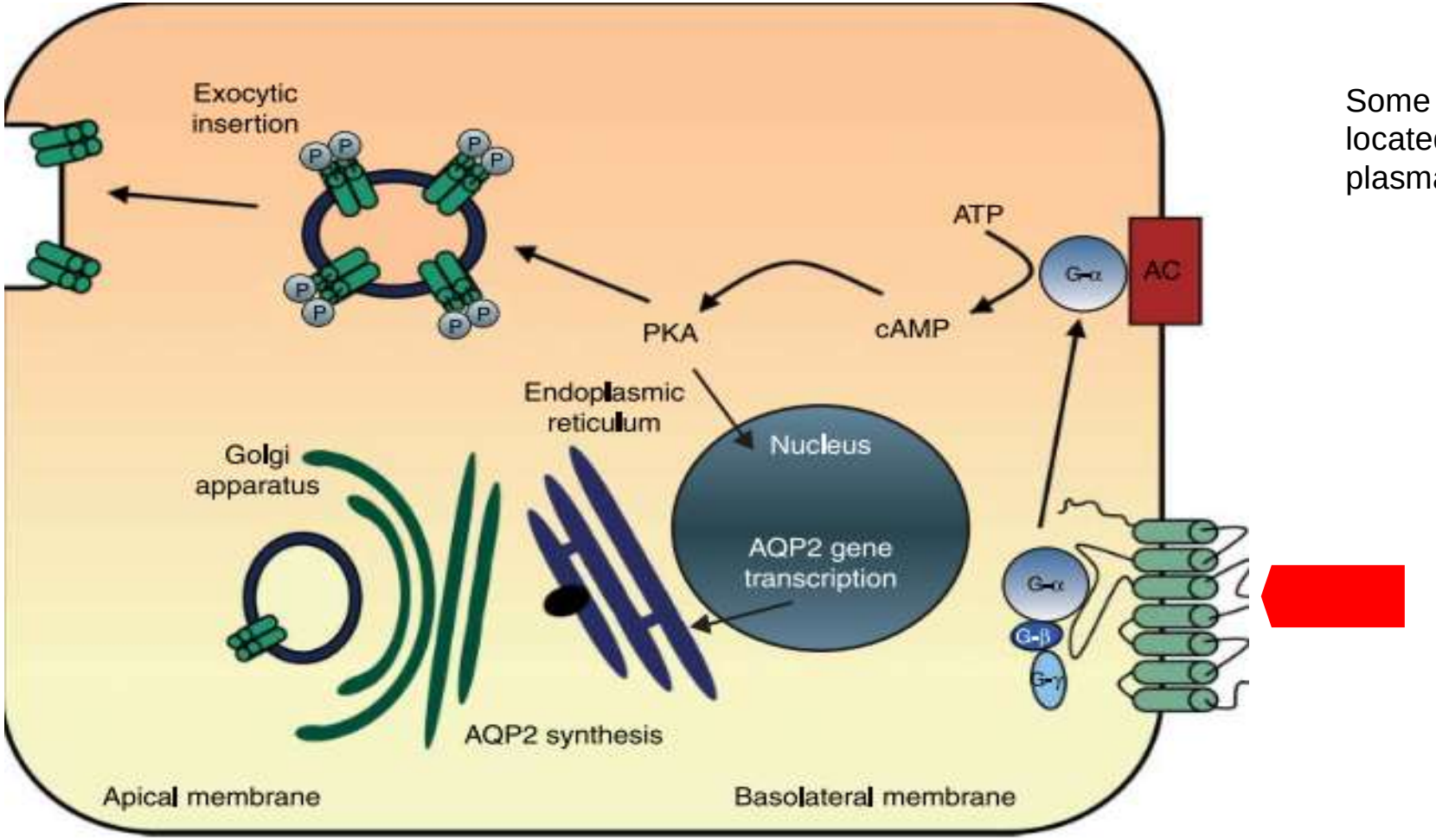
Osmosis is the movement of water molecules from a compartment with low concentration of substances to one with high concentration of substances, divided from the first by a membrane, permeable to the solvent, but not to solutes.

Specific channels, aquaporins, allow transcellular passage of water.

So, to the diffusion of water described by the first Fick equation, we can add a second modality: a diffusion of water that is helped by transmembrane protein. this is the facilitated diffusion.

Water movement across membrane

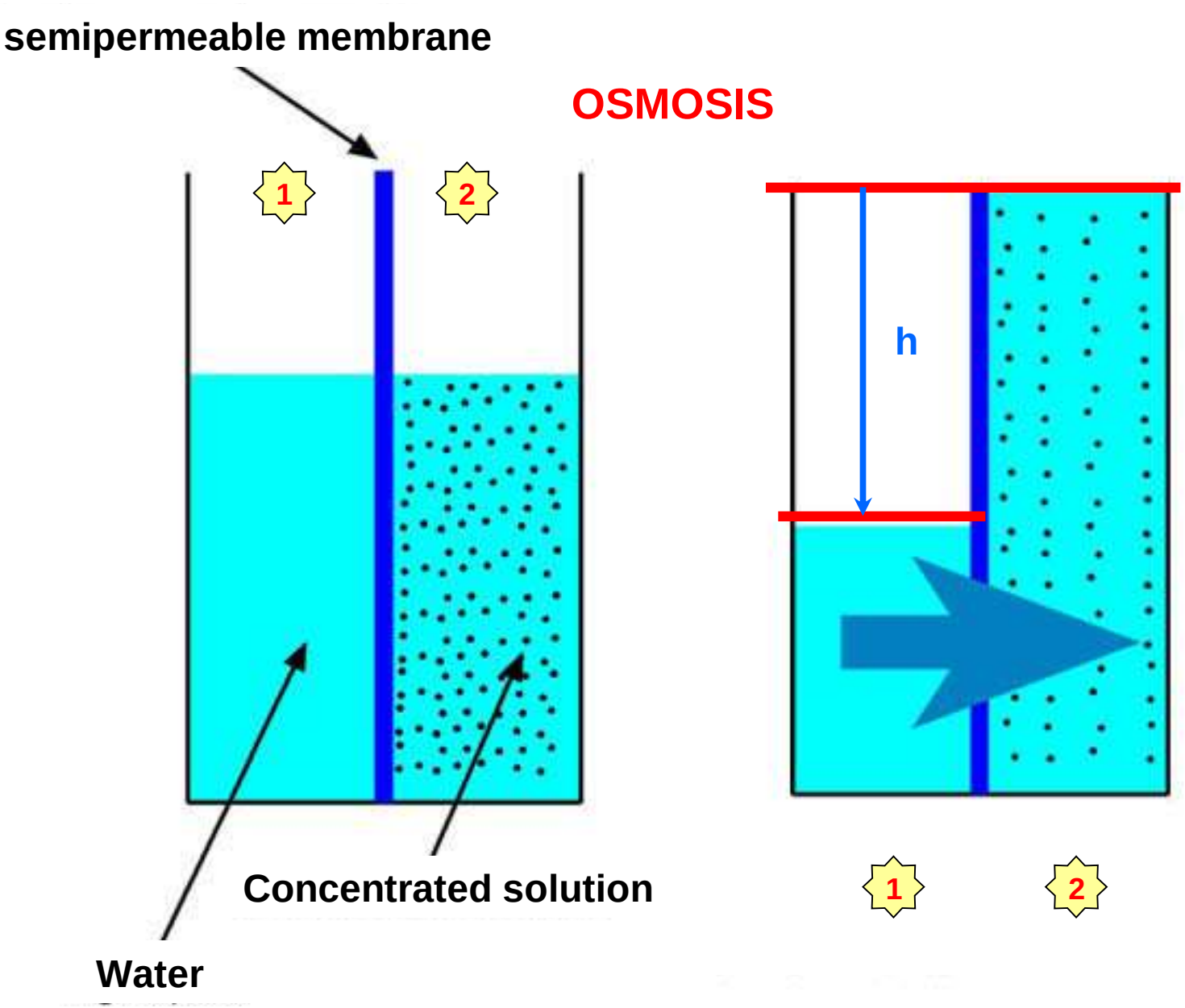




Some proteins are always located in a specific part of the plasma membrane

Aquaporins are regulated:

1) **on a short time-scale, by kinases phosphorylating cytoplasmic aquaporins.**
2) **on a longer time-scale by activating the transcription and expression of the aquaporin genes.**


semipermeable membrane
OSMOSIS
1
2
h
Concentrated solution
Water
1
2

The semipermeable membrane allows only water to pass across. This asymmetry generated a water movement from the first compartment to the second compartment. Such movement is not caused by a pressure gradient, but by the fact that water is more concentrated in the first compartment compared to the second compartment. There will therefore be a net movement of water towards the second compartment.

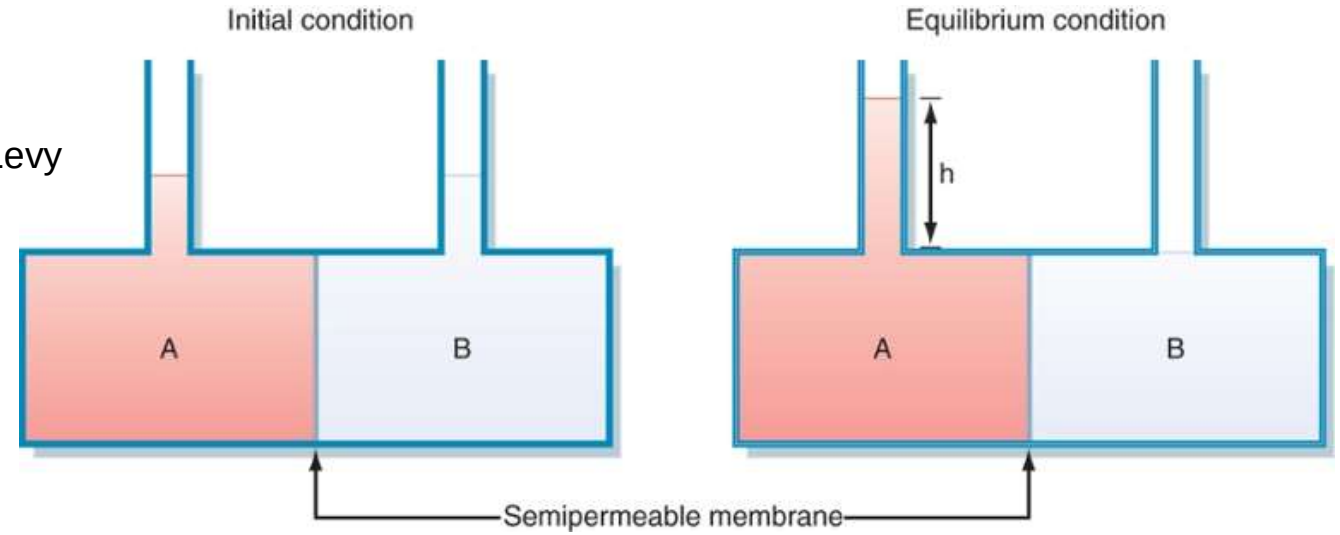
At the equilibrium, (net flux is zero), the volume of the second compartment will be increased.

Since, in our example, the wall is rigid, the difference in the water level between the two compartments, h, can be interpreted as a measure of pressure. The pressure that would be required to avoid any passage of water to the second compartment. This pressure is called osmotic pressure (Π)

Osmosis and osmotic pressure

The movement of water through acquaporins (passive transport) occurs by osmosis. Osmotic pressure is the driving force

Fig. 1.8 Berne-Levy



At equilibrium hydrostatic pressure exerted by the column of water h stops the net movement of water from B to A and is equal to the osmotic pressure exerted by the solute in A

Osmotic pressure is calculated by **Van't Hoff's law**

n = number of dissociable particles per molecules

C = total solute concentration

R = gas constant = 8.314Kj/mol K = 0.082 atm L/ K mol

T = temperature in K

$$\pi = nCRT$$

Glucose or urea (do not dissociate) solution of 1mmol/L

n=1; C=0.001mol/L; R=0.082; T=310K π=0.02542atm or 19.3mmHg

Alternatively the osmotic pressure is expressed in terms of osmolarity. 1mmol/L of urea or glucose has 1mOsm/L of osmotic pressure. NaCl has double because Na+ and Cl-

Much more common in physiology!

Osmolarity = C X n

Osmol is the unit of osmotic pressure. It is defined as the number of moles of particles of solute / Kg of solvent. If the solute does not dissociate in water, the number of osmol = the number of moles. If the solute does dissociate, n osmol = n mol of the undissociated substance * number of particles generated by the dissociation.

Definitions

Molarity = number of moles of solutes / L of solution
Molality = number of moles of solutes / Kg of solvent

Osmolarity = number of osmoles / L of solution
Osmolality = number of osmoles / Kg of solvent

Osmotic pressure is usually measured in osmole / Kg of solvent (osmolality); this measure does not depend on temperature.

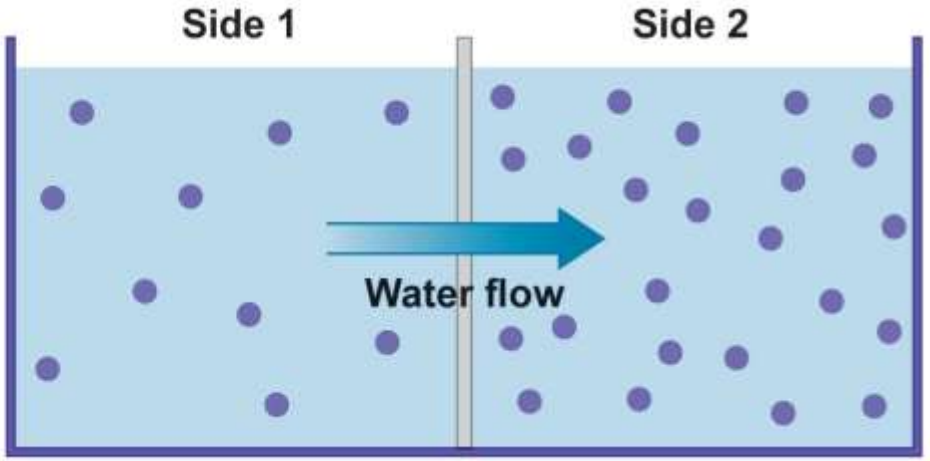
Osmolarity vs Osmolality

They are not the same.

Osmolarity: refers to osmotic pressure generated by dissolved solute molecules in 1 L of solvent.

Osmolality: number of molecules dissolved in 1Kg of solvent (mOsm/kg H_2O).

At 37°C for a dilute solution they are equivalent. Osmolarity is temperature dependent because V changes with T



Solute concentration:	300 mOsm (0.3 M)	500 mOsm (0.5 M)
Water concentration:	55.2 M	55.0 M
Osmotic pressure:	7.4 atm	12.3 atm

Osmotic pressure gradient ($\Delta\pi$): 12.3 atm – 7.4 atm = 4.9 atm

Using the VANT' HOFF Equation

At 25°C (298,15 K), $\Pi = n\,C\,RT$ (considering n = 1):

Side 1 = 0,3 X 0,0821 X 298,15 = 7,34 atm

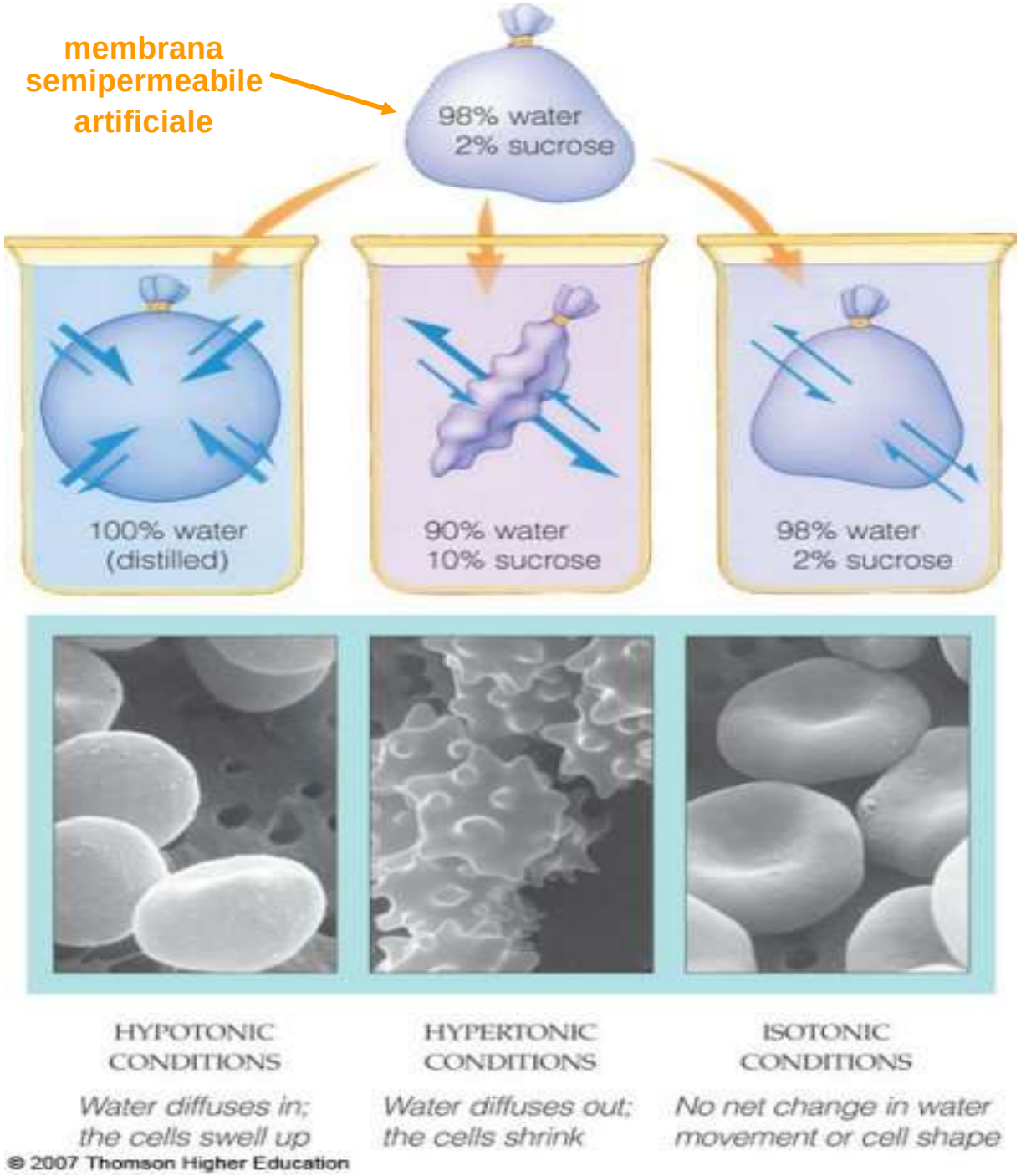
Side 2 = 0,5 X 0,0821 X 298,15 = 12,24 atm

Tonicity

Tonicity is a qualitative measure.

an isotonic solution does not change cell volume
a hypotonic solution will increase cell volume
a hypertonic solution will decrease cell volume.

Bodily fluid osmolality is about 290-295 mOsm / Kg.


membrana
semipermeabile
artificiale
98% water
2% sucrose
100% water
(distilled)
90% water
10% sucrose
98% water
2% sucrose
HYPOTONIC
CONDITIONS
Water diffuses in;
the cells swell up
HYPERTONIC
CONDITIONS
Water diffuses out;
the cells shrink
ISOTONIC
CONDITIONS
No net change in water
movement or cell shape
© 2007 Thomson Higher Education

Tonicity

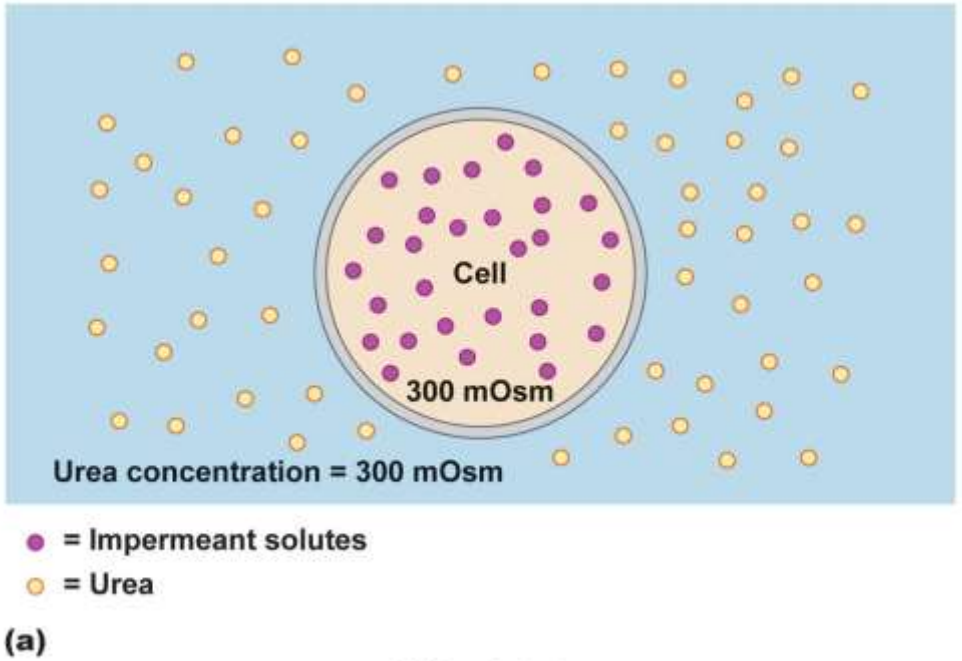
Tonicity relates to the effect of a solution on the volume of a cell.

Isotonic solutions do not change the volume of a cell, hypotonic induce swelling while hypertonic shrinking of a cell

Tonicity accounts for the ability of the molecules to cross the mb.

Example: 300mmol/L of sucrose and 300mmol/L of urea

Both solutions have osmolality of 300mOsm/Kg H_2O (they are isosmotic). However erithrocytes (intracellular osmolality 300mOsm/Kg H_2O) put in these 2 solutions behave differently:



In sucrose nothing happens (isotonic solution), in urea they swell and eventually blast (hypotonic solution) because urea can cross easily the mb by means of uniporters. The cell is permeable to urea but not to sucrose. To exert an osmotic pressure a molecule must not cross the mb. Sucrose is an effective osmole while urea is an ineffective osmole.

Ergo, the equation, Van't Hoff's law, becomes:

$$\pi = \sigma(nCRT)$$

σ = reflection coefficient (osmotic coefficient). It measures the relative ability of the molecule to cross the mb. σ = 0 for urea; σ = 1 for sucrose

Saline (saline solution) is the most commonly used isotonic infusion. It has 9 g NaCl / L.

NaCl molecular weight = 58.5 -> 9 g / L -> 9 / 58,5 mol =154 mmol / L. Since NaCl -> Na^+ + Cl^-, the osmolarity should be 2 * 154 = 308 mOsm / L.

But NaCl is only dissociated at 93%. Therefore:

308 X 0,93 = 290 mOsm / L

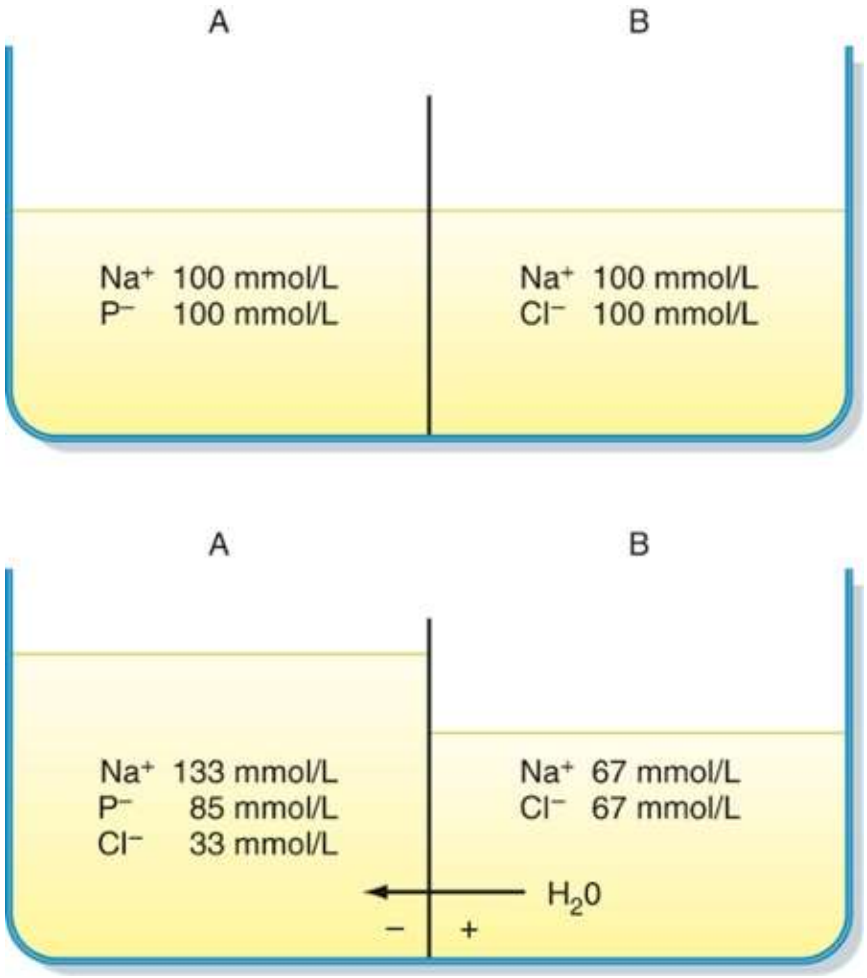
Regulation of cell volume

Changes in volume can lead to cell death. Cells have developed mechanisms to counteract changes of volume. Most cells are highly permeable to water because aquaporins. Even when a cell is placed in an isotonic solution, the maintainance of cell volume is an active process requiring the activity of Na-K-ATPase.

ISOTONIC CELL VOLUME REGULATION. The importance of Na-K-ATPase in maintainance of cell volume in isotonic solution can be appreciated when a solution of red blood cells is put in the cold and Na-K-ATPase is slowed down and red blood cells swell. This is due by the presence in ICF of proteins that induce ions to redistribute along the mb in a particular way ⟶ Gibbs-Donann effect

The Gibbs-Donnan effect

Fig. 2.6 Berne-Levy



Two solutions are separated by a membrane that is permeable by Na+, Cl−, and H2O but not permeable by protein (P−). The osmolality of solution A is identical to that of solution B.
Cl− diffuses from compartment B to compartment A down its concentration gradient. This causes compartment A to become electrically negative with regard to compartment B.
The membrane voltage then drives the diffusion of Na+ from compartment B to compartment A. The accumulation of additional Na+ and Cl− in compartment A increases its osmolality and causes water to flow from compartment B to compartment A (Note: the increase volume of compartment A results in a lower [P−]).
If the container containing the two solutions were sealed at the top so that water could not move from compartment B to compartment A, the pressure in compartment A would increase as the number of osmotically active particles increases in that compartment.

The Gibbs-Donnan effect occurs when a mb can be permeated by some but not all solutes. The same effect can be observed between plasma and interstitial fluid across capillary wall. Na-K-ATPase counteract the Gibbs-Donnan effect

Regulation of cell volume

Changes in volume can lead to cell death. Cells have developed mechanisms to counteract changes of volume. Most cells are highly permeable to water because aquaporins. Even when a cell is placed in an isotonic solution, the maintainance of cell volume is an active process requiring the activity of Na-K-ATPase.

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NON-ISOTONIC CELL VOLUME REGULATION. Most cells throughout the body are bathed with isotonic ECF but not all of them (medulla of the kidney, CSF). When ECF is not isotonic, cells may shrink or swell, but cells have mechanisms to contrast these effects. These are particularly important for neurons. These mechanisms consist of actively transporting osmolytes in or out of the cell to increase osmotic pressure. These osmolytes can be ions, organic molecules, polyols (sorbitol and myo-inositol), methyl amines and some amino acids. If the cell is exposed to non isotonic ECF for long period, the cell alters the ICF levels of organic osmolytes through metabolic processes.

We talk about regulatory volume increase (RVI) and decrease (RVD) when ECF is respectively hyper or hypotonic

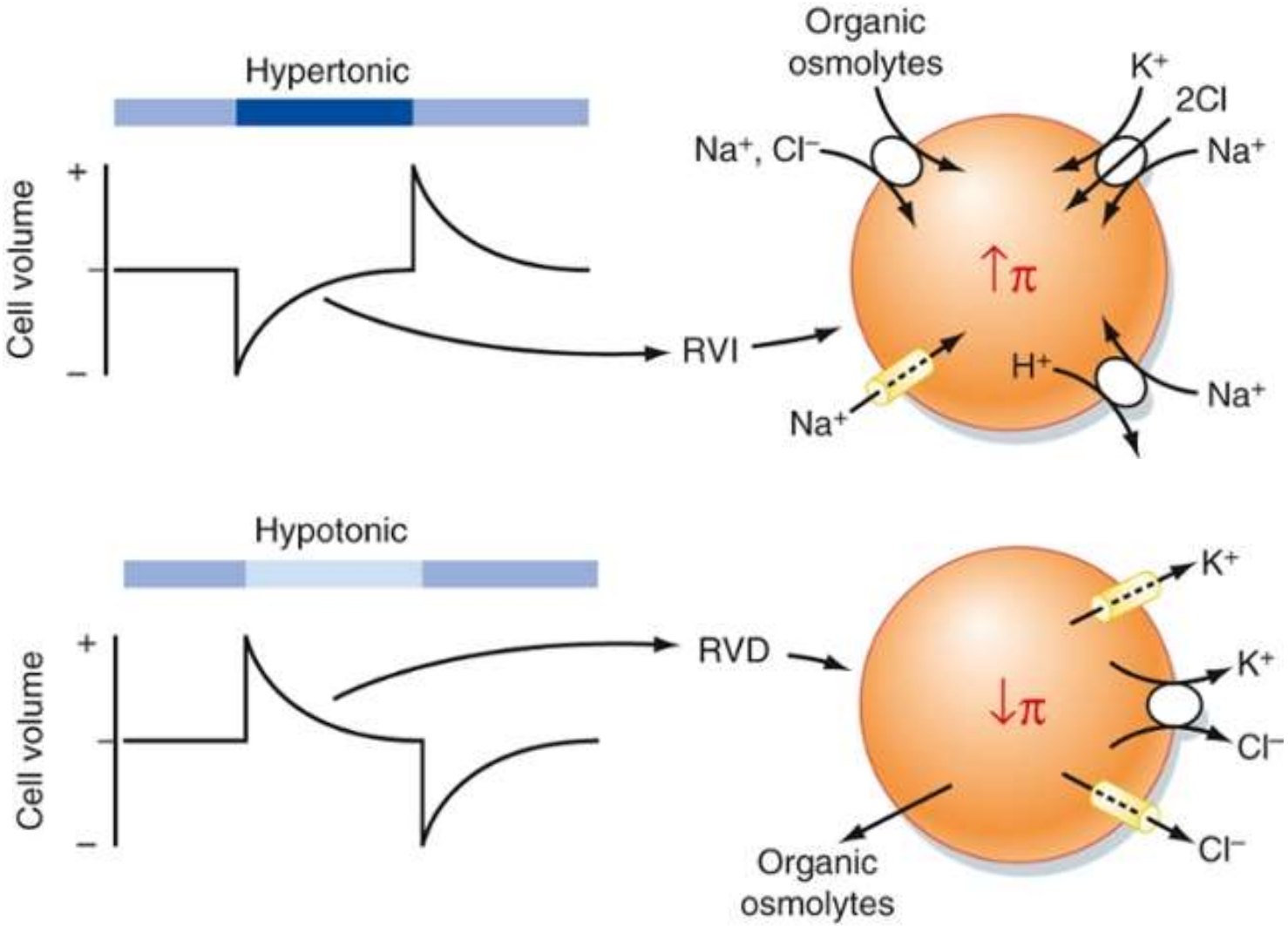


Fig. 2.7 Berne-Levy

Important for RVI are Na+, H+ antiporter (NHE-1), the 1Na+, 1K+, 2Cl- (NKCC-1) symporter and a number of cation selective channels that transport NaCl inside the cell. Then Na-K-ATPase activity is increased. In the end ICF net content of KCl is increased. Then other symporters (aa and organic molecules) use NaCl electrogradients.

RVD results in loss of KCl from ICF.

Changes in cell volume are monitored by: cytoskeleton, macromolecular crowding and ionic strength of cytoplasm, channels with gating that depends on stretch of the mb, second messengers.

Homeostasis: Volume and composition of body fluid compartments

Normal cellular function requires that intracellular environment remains constant (in a narrow range). This is accomplished by transport of many substances and water in and out of the cell. The maintenance of a constant environment (volume, temperature and composition) is termed Homeostasis. We talk of steady state balance.

Claude Bernard (1813-1877)

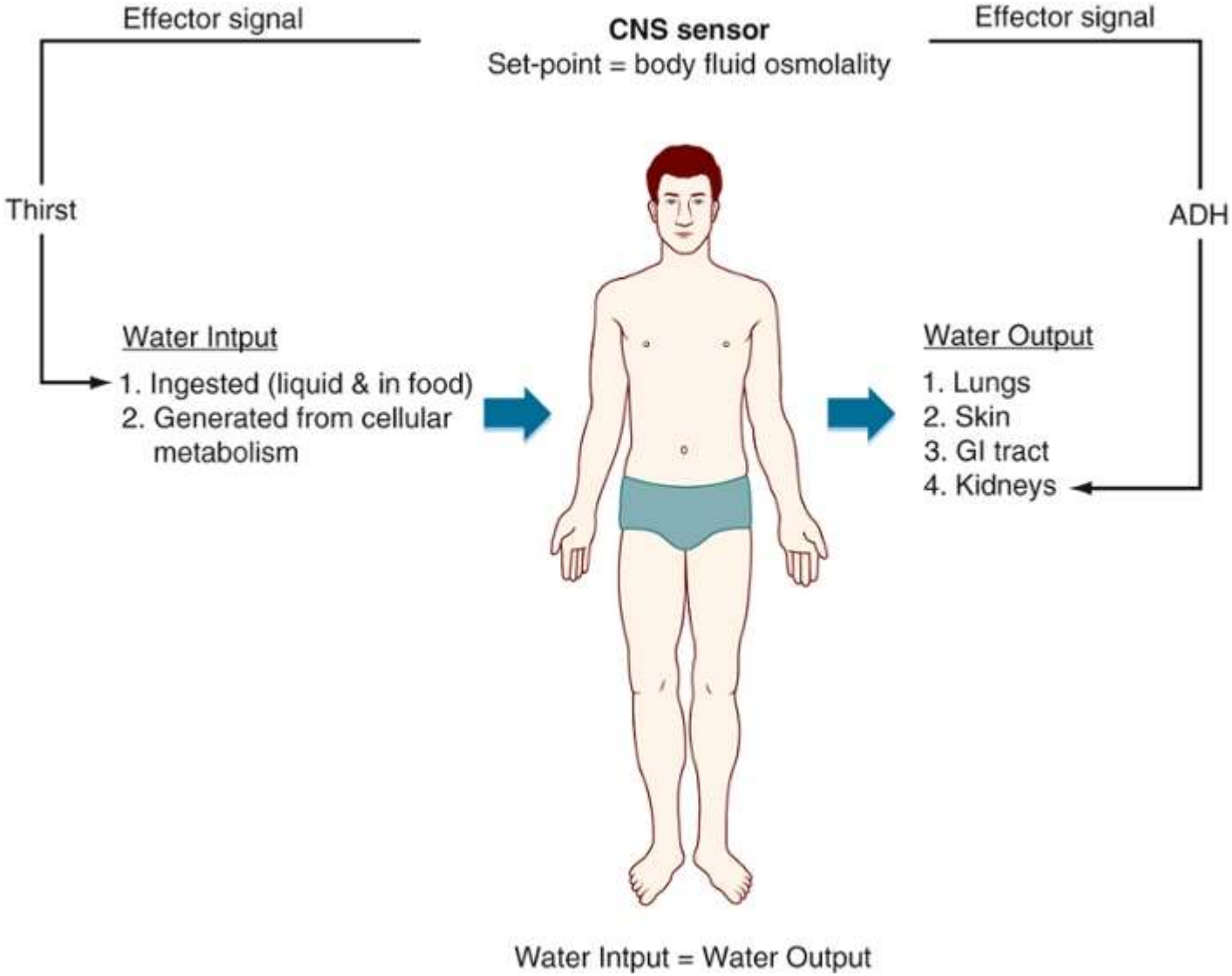
Claude Bernard and Walter Cannon are the fathers of modern physiology because they introduced the concept of homeostasis. Bernard recognized that the cells of our body cannot maintain the volume and composition through exchanges with the external environment, but through exchanges with the fluid of the internal environment that surrounds them, ergo the extracellular fluid that he called 'le milieu interieur'.

Characteristics of the concept of homeostasis are:

- There must be a 'set point' so that deviations from it can be monitored.
- Sensors must generate an 'effector signal' when the variable is different from the set point
- Effector organ must respond in the appropriate way to the effector signal
- The sensitivity of the system depends on several factors: the nature of the sensor, time necessary to generate effector signal and time necessary for effector organ to respond.

Walter B. Cannon (1871-1945)

Water balance homeostasis - Osmolality



Input > output ⟶ positive balance

Input < output ⟶ negative balance

Fig. 2.1 Berne-Levy

The physical and chemical characteristics of water, make it the key element in every bodily fluid

1) High specific heat (1 cal / (g X °C)) or (4,18 J / (g X °C))

2) High latent heat of evaporization (543 cal / g) or (2270 J / g)

3) High latent heat of fusion (80 cal / g) or (334 J / g)

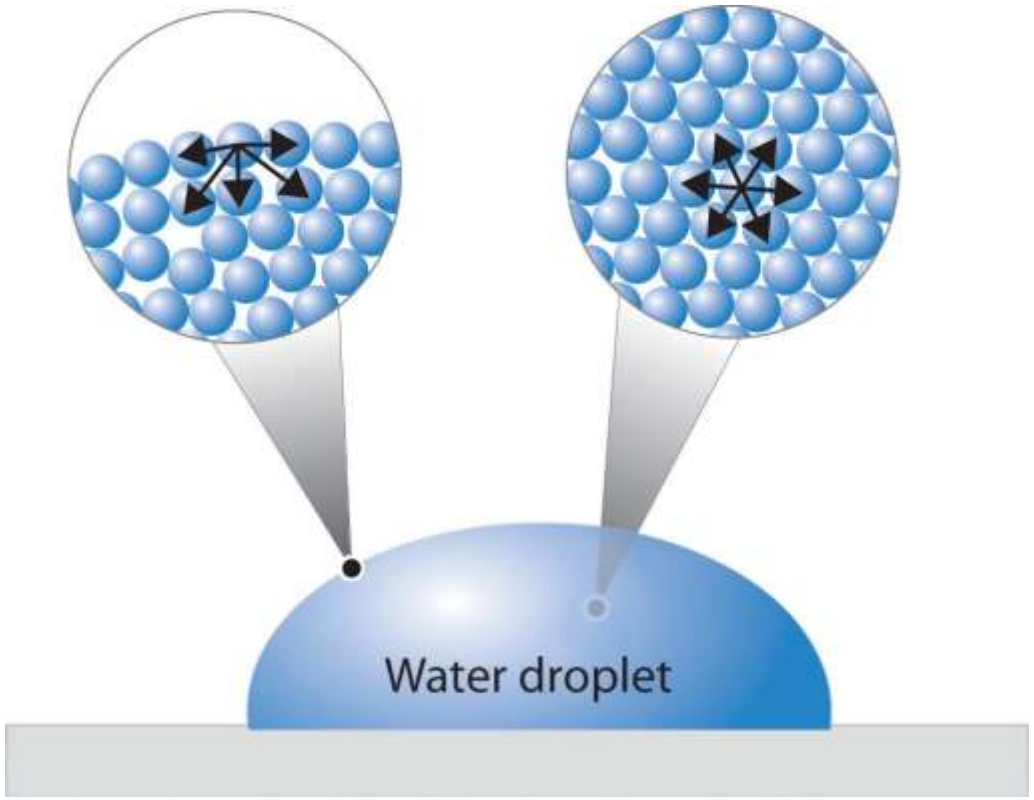
Specific Heat

The specific heat is the amount of heat per unit mass required to raise the temperature by one degree Celsius.

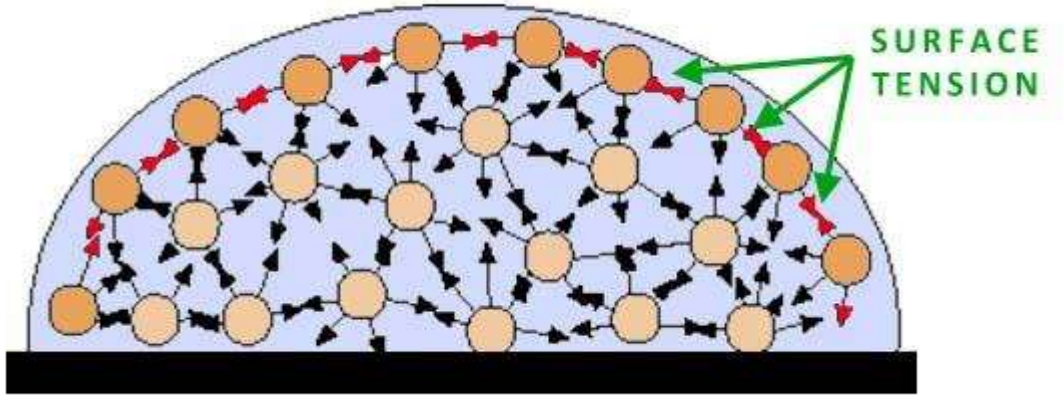
The specific heat of water is 1 calorie/gram °C = 4.186 joule/gram °C which is higher than any other common substance. As a result, water plays a very important role in temperature regulation.

Latent heat, energy absorbed or released by a substance during a change in its physical state (phase) that occurs without changing its temperature.

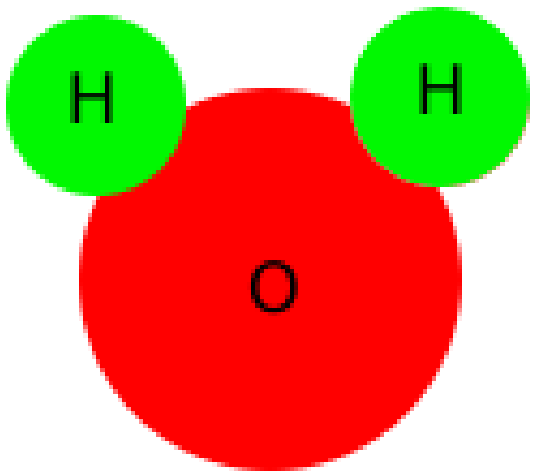
For example, when a pot of water is kept boiling, the temperature remains at 100 °C (212 °F) until the last drop evaporates, because all the heat being added to the liquid is absorbed as **latent heat of vaporization** and carried away by the escaping vapour molecules. Similarly, while ice melts, it remains at 0 °C (32 °F), and the liquid water that is formed with the **latent heat of fusion** is also at 0 °C. The **latent heat of fusion** for water at 0 °C is approximately **334 joules (79.7 calories) per gram**, and the heat of vaporization at 100 °C is about **2270 joules (533 calories) per gram**. Because the heat of vaporization is so large, steam carries a great deal of thermal energy that is released when it condenses, making water an excellent working fluid for heat engines.

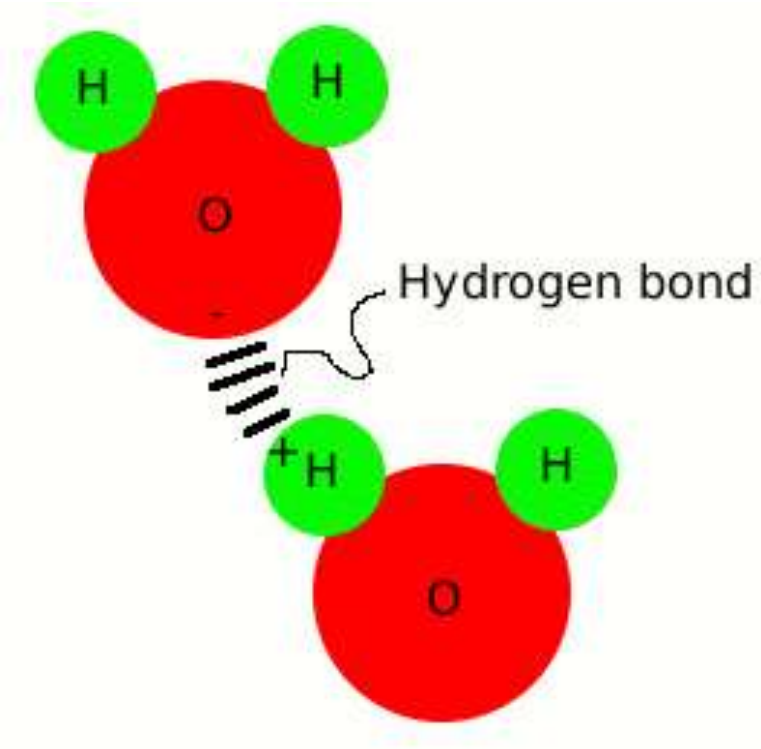


4) High surface tension



5) High solubilising capability





Water makes up approximately 60% of body weight (it varies with adipose tissue content). This changes with age (decreasing with age).

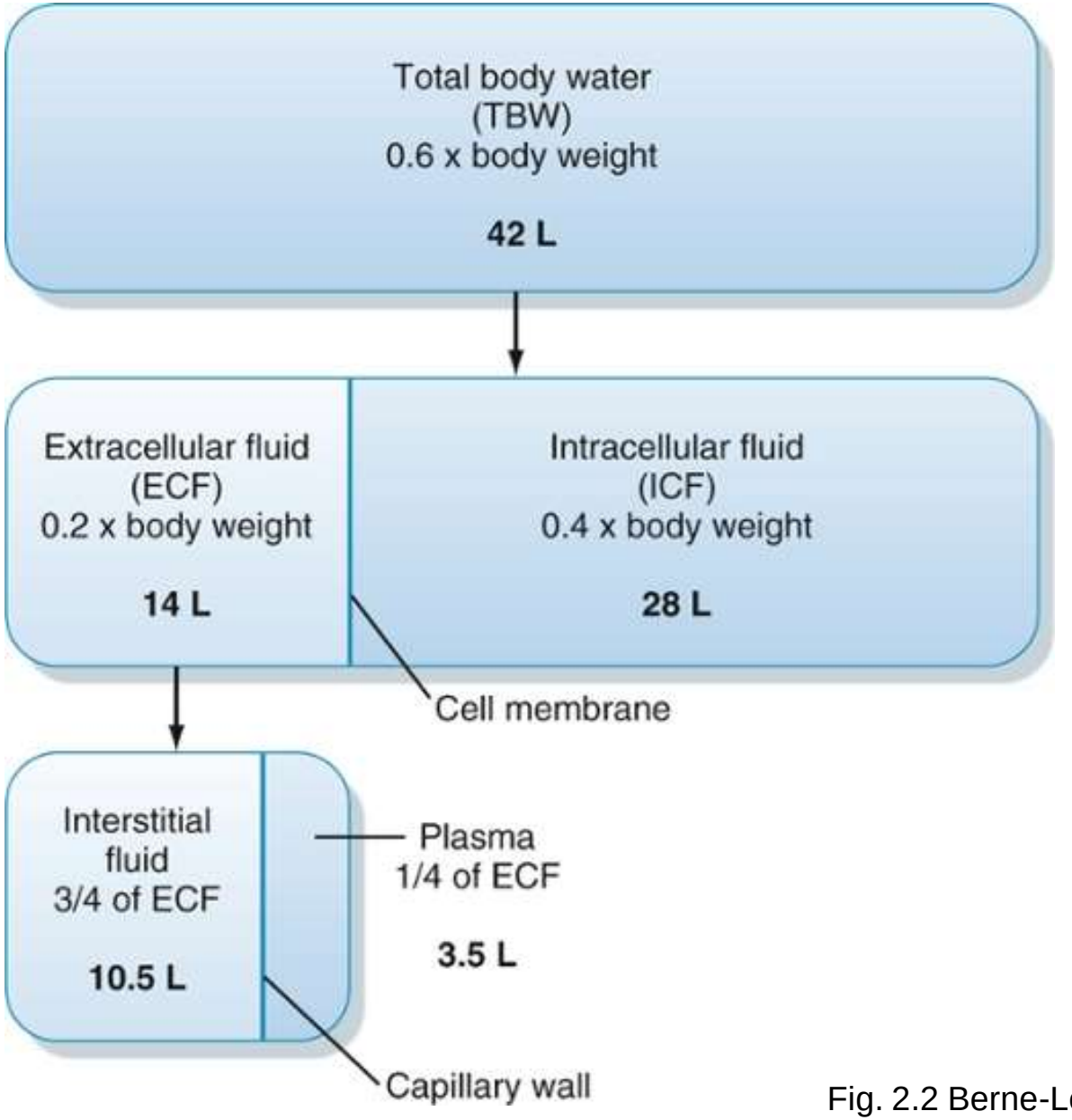
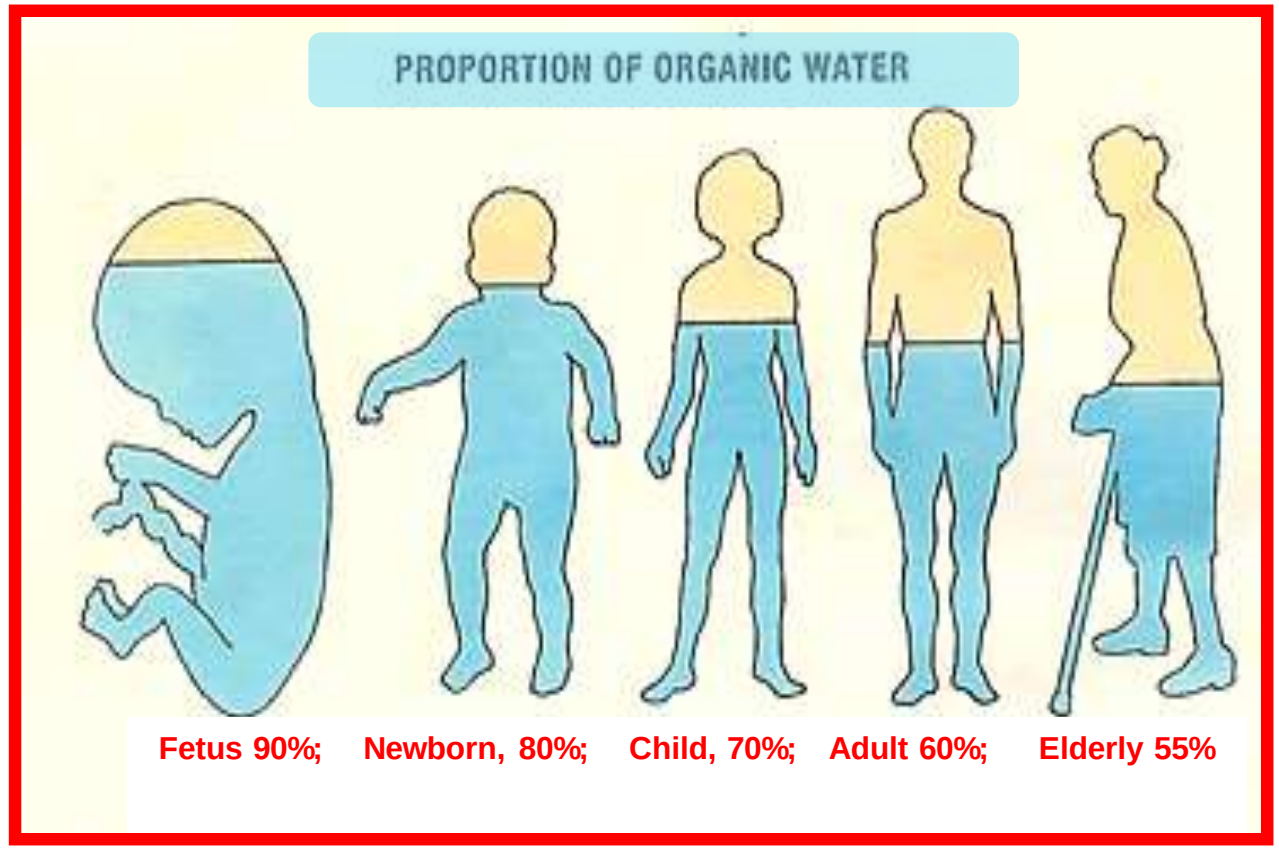
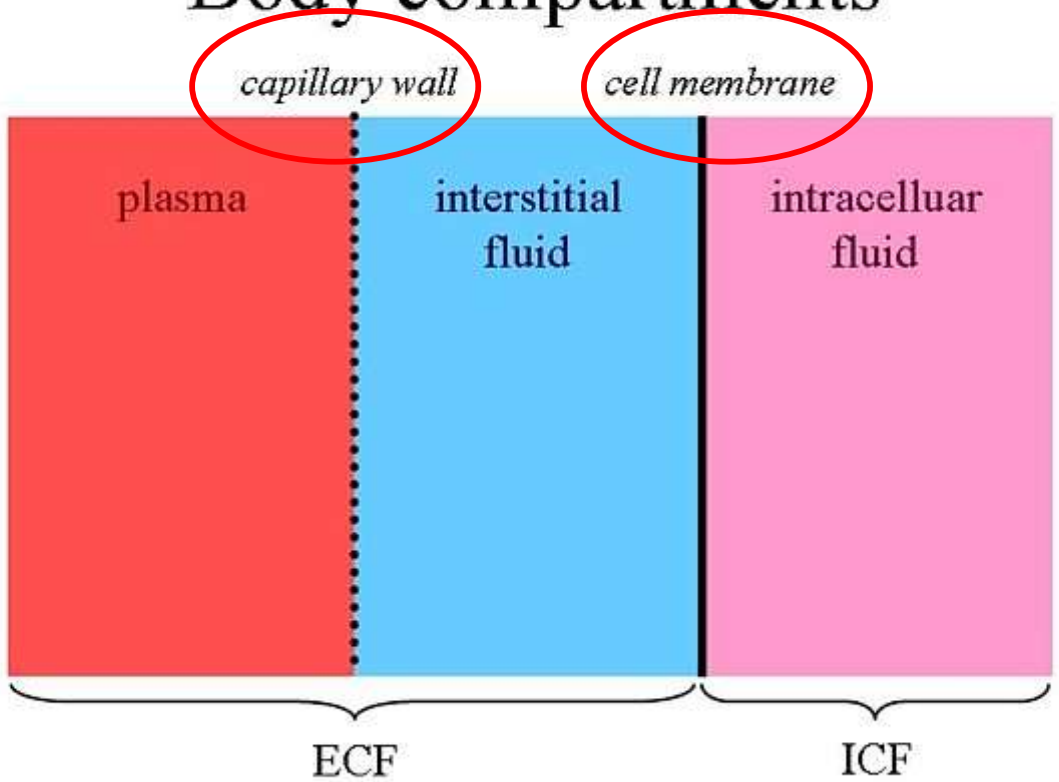


Fig. 2.2 Berne-Levy

Total body water is divided in extracellular fluid (ECF) and intracellular fluid (ICF). ECF is also subdivided in plasma and interstitial fluid. In some pathological conditions fluid could accumulate in third space (liver diseases – ascites)


PROPORTION OF ORGANIC WATER
Fetus 90%; Newborn, 80%; Child, 70%; Adult 60%; Elderly 55%

Body compartments



Movement of water between body fluid compartments

Water moves between ECF and ICF through cell mb and between interstitial fluid and vascular compartments across capillary wall.

Water moves through aquaporins and driving force is osmotic pressure. Osmotic pressure is determined by molecules/ions present in ECF and ICF. Osmotic pressure for both ECF and ICF is 280-295 mOsm/KgH2O. For ECF osmotic pressure contributed by protein is only 1-2 mOsm/KgH2O the rest is ions and low molecular weight molecules.

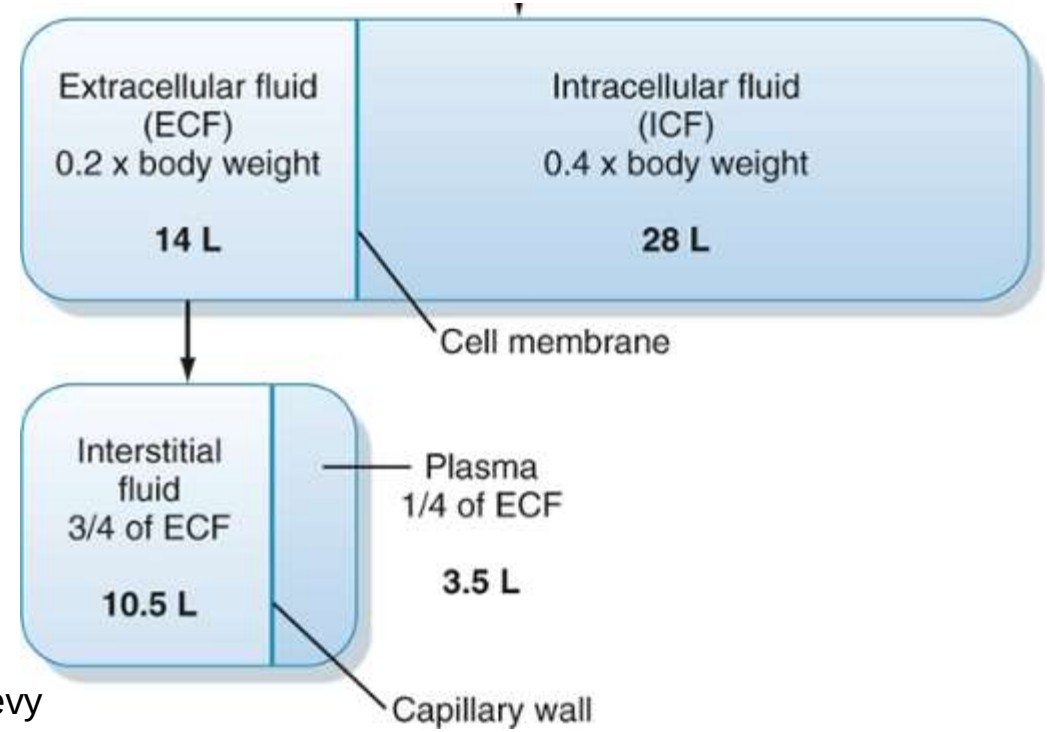
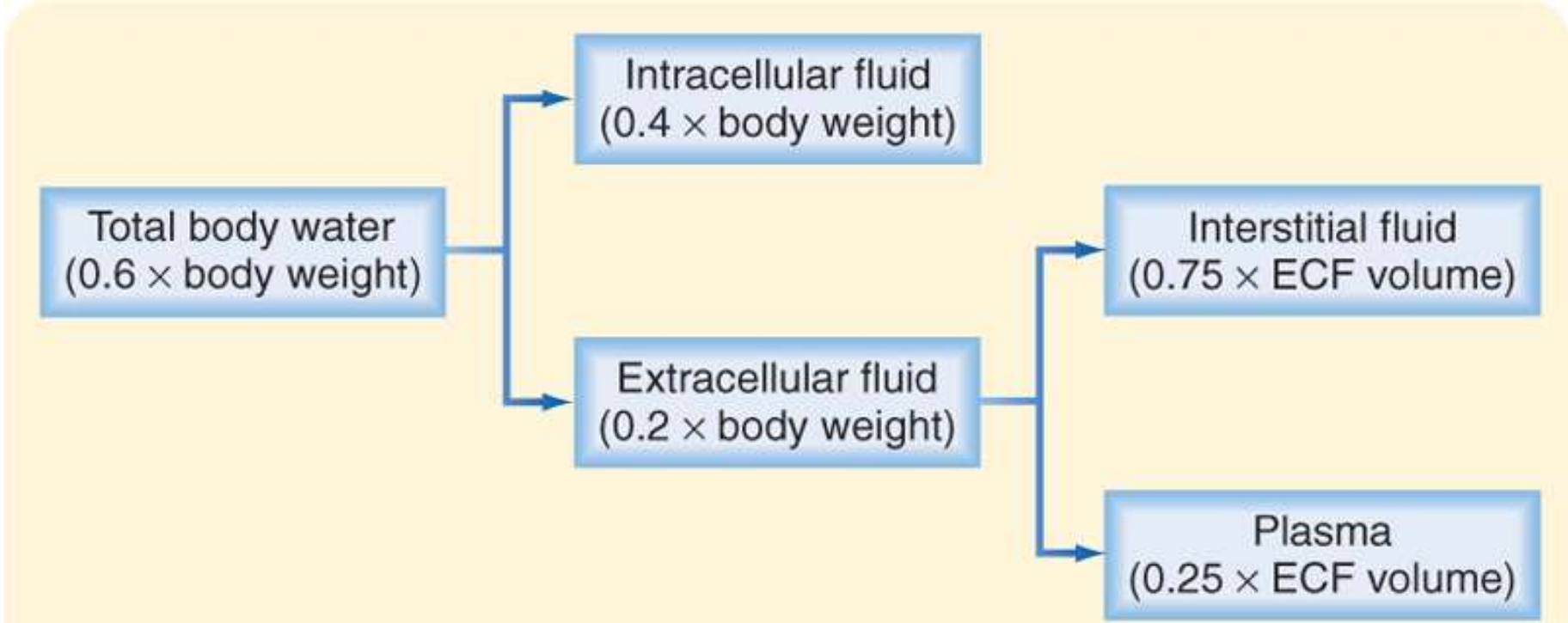


Fig. 2.2 Berne-Levy



1. All solutes and water that enter or leave the body do so via the ECF.
2. The ICF and ECF are in osmotic equilibrium. Water moves between these compartments only when an osmotic pressure gradient exists.
3. Equilibration of ICF and ECF osmolality occurs primarily by shifts in water and not shifts in solute.

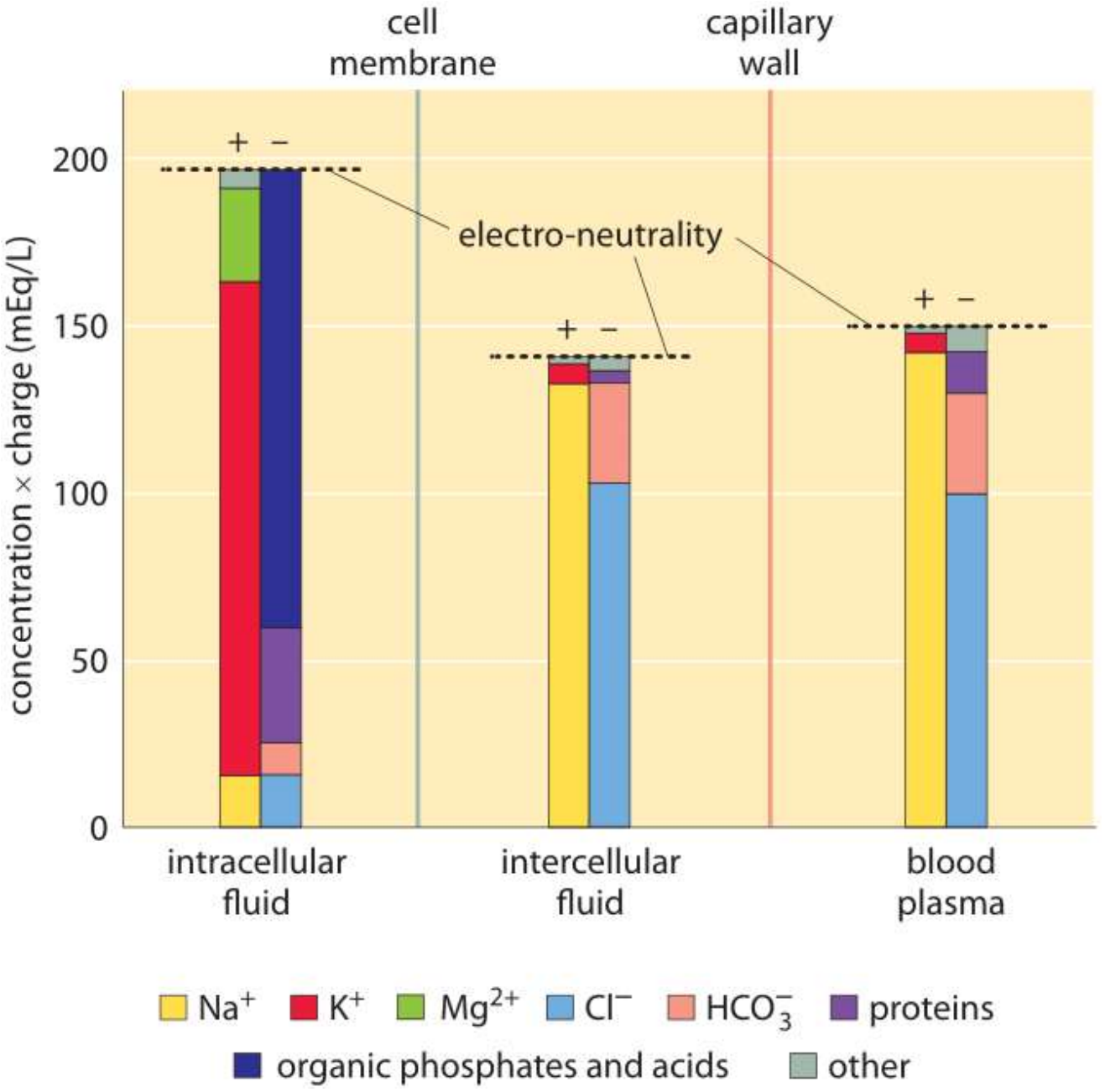
Any changes in osmolality of one compartment quickly causes water to redistribute across all compartments to bring them back in osmotic equilibrium. Plasma (or serum) osmolality is a good measure for osmolality of all compartments. Because of the high concentration of Na+ in the ECF (in respect to other solutes), Na+ (with its anions Cl- and HCO3-) is the major determinant of the osmolality of this compartment. Accordingly it is possible to obtain an appropriate estimate of ECF osmolality with this equation:

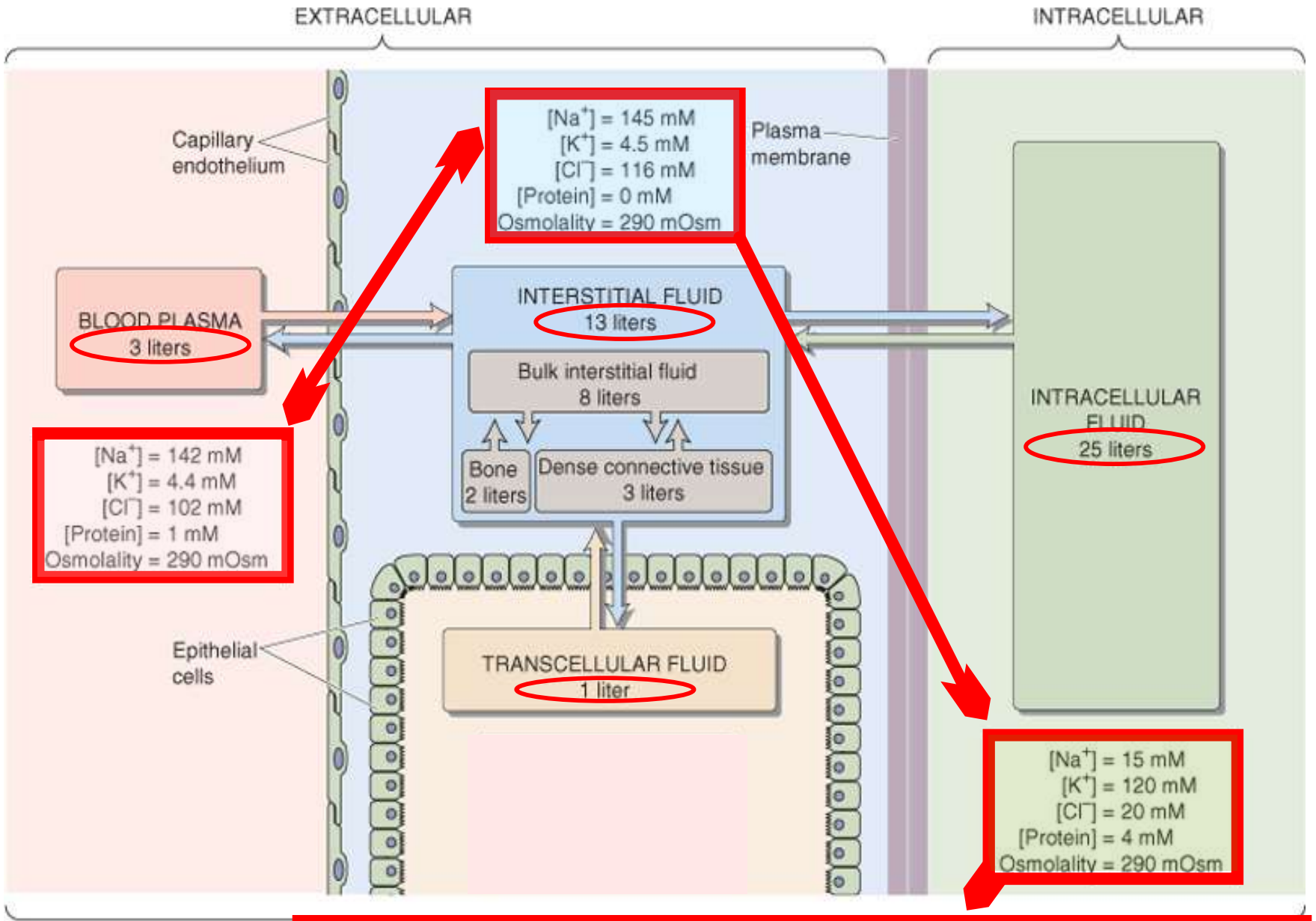
Plasma Osmolality = 2(serum [Na+]) = 290mOsm/KgH2O

There are some exceptions meaning that ECF is not consistent (constant) throughout the whole body. CSF has higher osmolality and ECF in the kidney can be higher or lower.

Composition of body fluid compartments

ICF has more macromolecules than ECF. Composition of ICF (the purpose of homeostasis is to defend ICF) is maintained stable by means of transporters like Na-K-ATPase that, by hydrolizing ATP, creates an electrochemical gradient. This can be used to transport other ions and molecules. Composition of interstitial fluid is similar to that of plasma but plasma contains more proteins.





ECF and ICF
are different solutions.

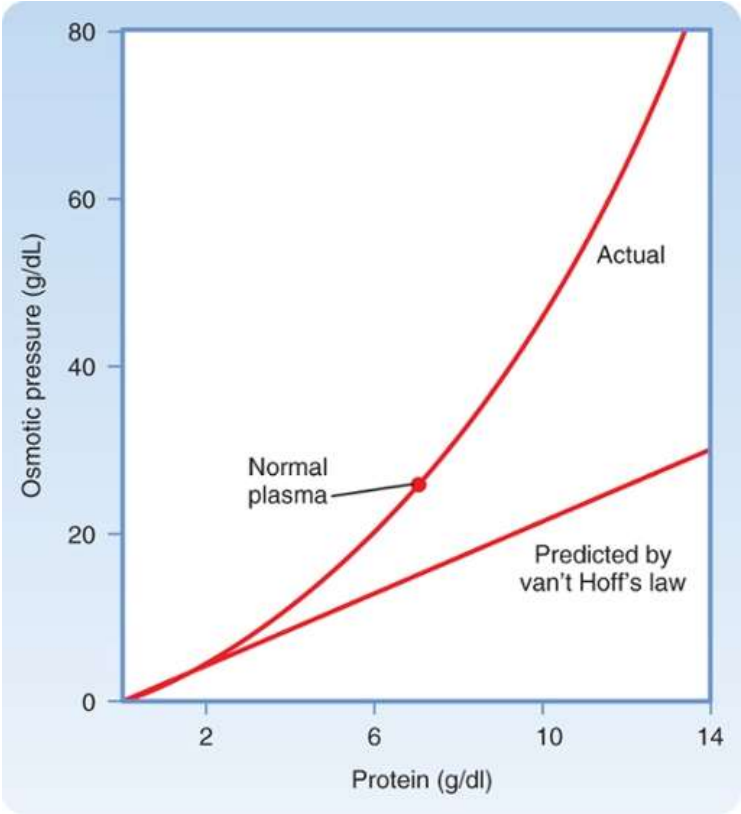
Approximate Solute Composition of Key Fluid Compartments

Solute	Plasma	ECF	Interstitium	Cell
Na^+ (mM)	142		145	15
K^+ (mM)	4.4		4.5	120
Ca^{2+} (mM)	1.2 (ionized) 2.4 (total)*		1.2 (ionized)	0.0001 (ionized)
Cl^- (mM)	102		116	20
Proteins	7 g/dL 1 mmol/L 14 mEq/L		1 g/dL	30 g/dL

1. All solutes and water that enter or leave the body do so via the ECF.
2. The ICF and ECF are in osmotic equilibrium. Water moves between these compartments only when an osmotic pressure gradient exists.
3. Equilibration of ICF and ECF osmolality occurs primarily by shifts in water and not shifts in solute.

Oncotic pressure

Is the osmotic pressure generated by large molecules (like proteins) in solution. The osmotic pressure developed does not conform to Van't Hoff's law



Oncotic pressure exerted by proteins in human plasma is approximatively 26/28 mmHg ≅ 1.4 mOsm/Kg H_2O. It is an important force involved in fluid movement across capillaries

Specific gravity

Total concentration of all molecules in a solution can also be measured by specific gravity.

Specific gravity is defined as the weight of a volume of solution divided by the weight of an equal volume of distilled water.

Specific gravity of normal human plasma is in the range 1.008-1.010

Principles of epithelial transport

Epithelial cells are arranged in sheets and provide the interface between the external world and internal environment (ECF). Functions:

- Barrier to microorganisms (lungs, GI tract and skin)
- Prevention of loss of water (skin)
- Maintenance of a constant internal environment (lungs, GI, kidneys)

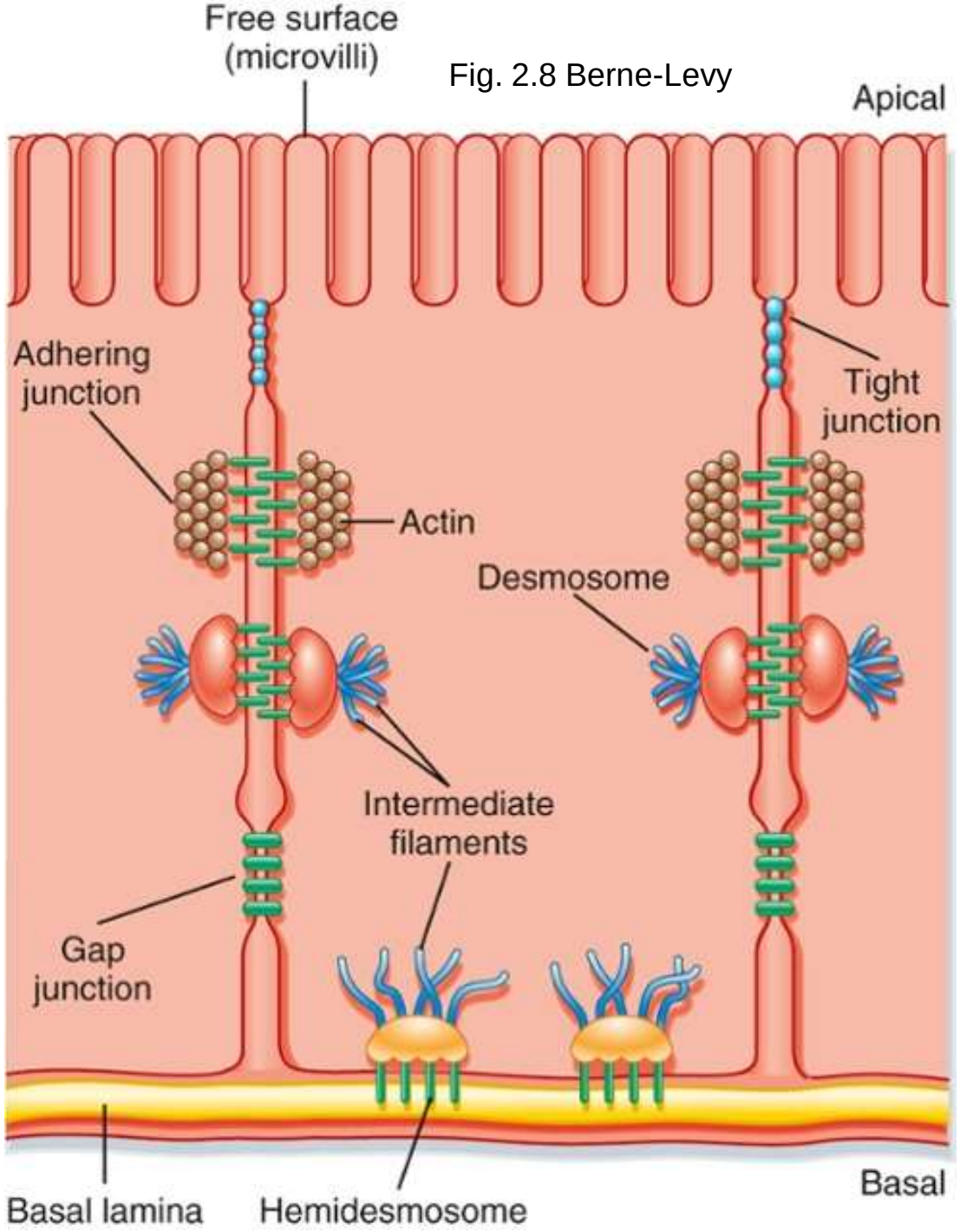


Fig. 2.8 Berne-Levy

Epithelial cells

The free surface is referred as the apical mb that is in contact with the external environment or the ECF.

Basal side rests on the basal lamina, secreted by epithelial cells and that is attached to underlying connective tissue.

Epithelial cells are connected to one another via different structures: adhering junctions, desmosomes, hemidesmosomes, gap junctions.

Gap junctions are low-resistance connections between cells. Their functional unit is the connexon, that is formed by 6 integral mb proteins called connexins. One connexon in one cell is aligned to another connexon in the next cell forming a channel. The channel may be gated.

There are tight junctions that separate apical from basolateral mb and restrict movements of lipids between these domains.

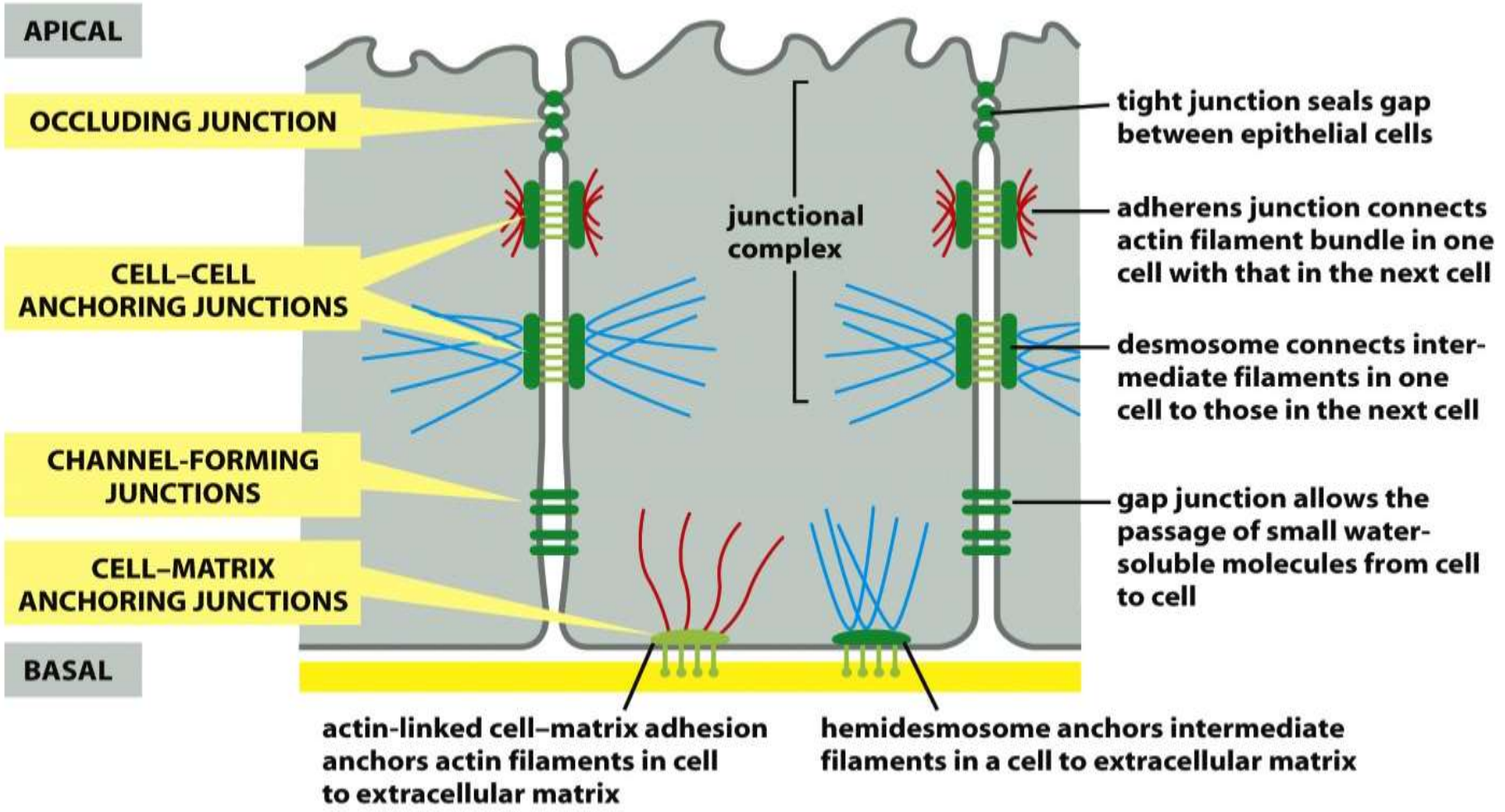


Figure 19-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The intercellular spaces between epithelial cells permit the transit of water and substances. It's the paracellular way.

Vectorial transport

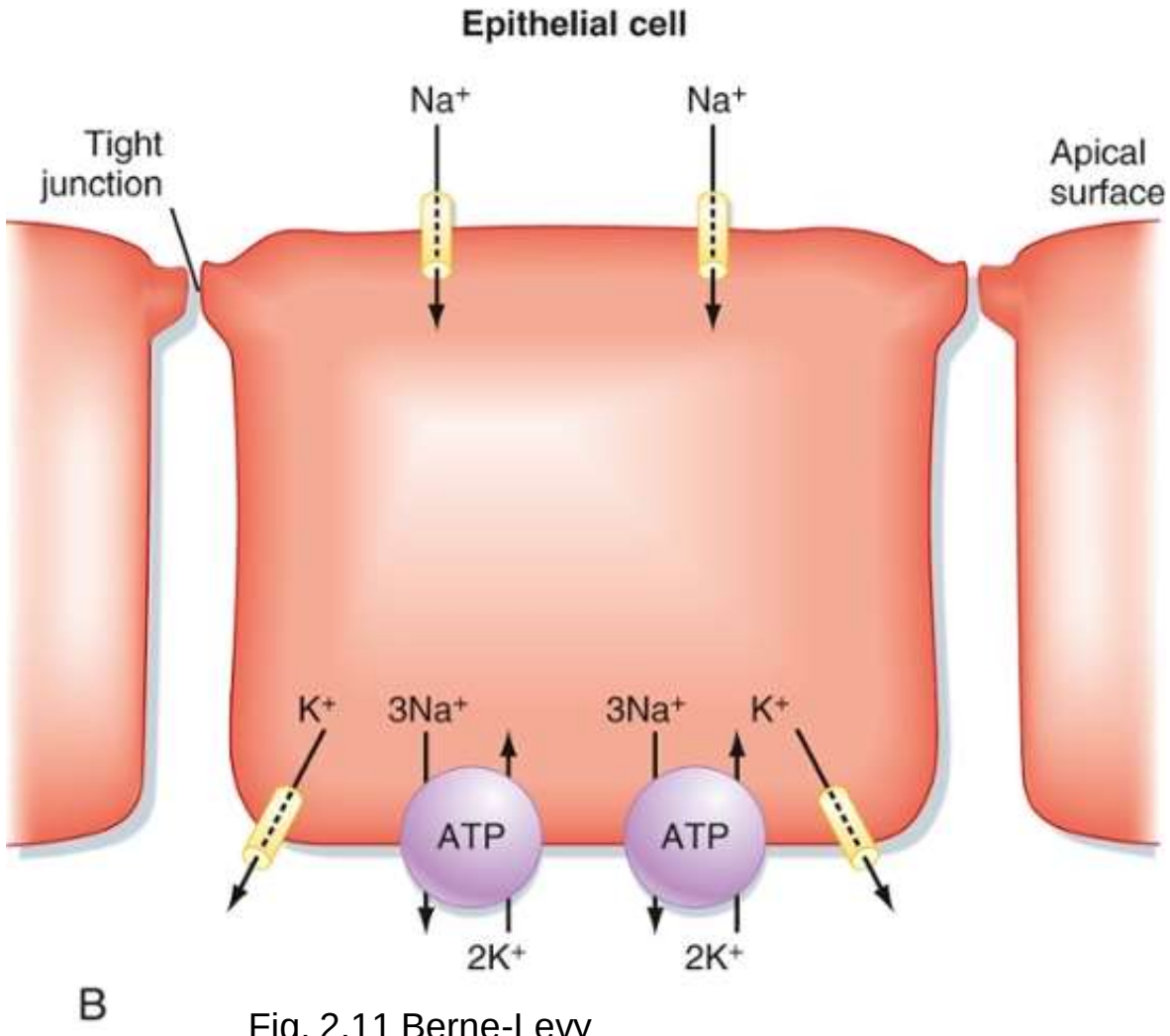
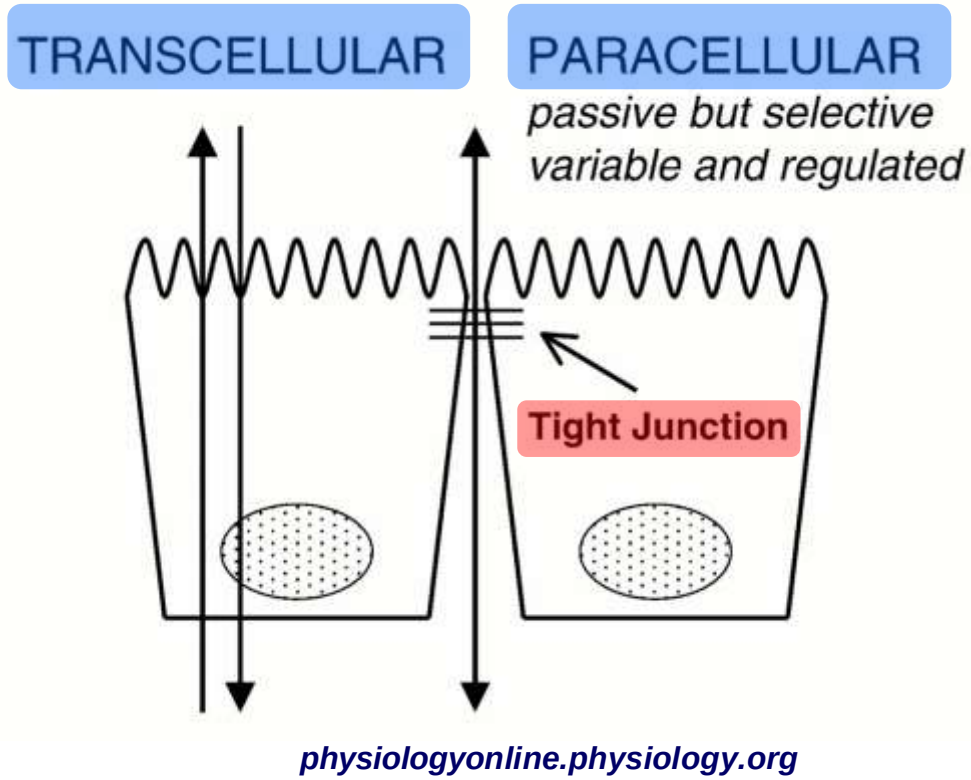


Fig. 2.11 Berne-Levy

Epithelial cells are able to perform vectorial transport because tight junctions separate apical from basolateral mb. Different tansport proteins are targeted in one or the other mb domain.

Transport from the apical side to the basolateral side of an epithelium is termed either absorption or reabsorption (e.g. nutrients from lumen of GI tract and water from lumen of nephron). Transport from the basolateral side to the apical side is called secretion.

In all epithelia, except choroid plexus and retinal pigment, the Na-K-ATPase is segregated in the basolateral mb.

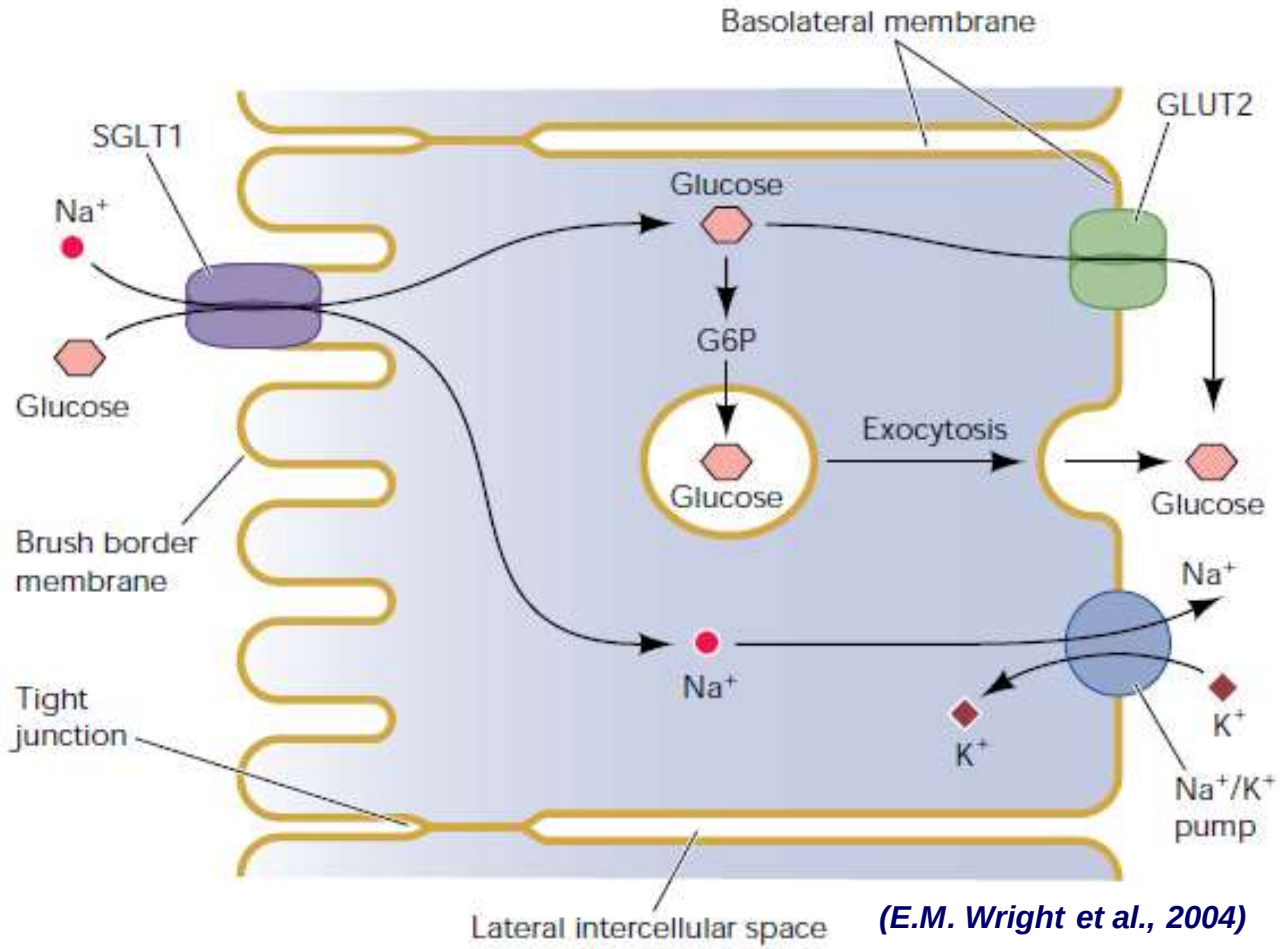


Transcellular transport is a two-step process. Generally, one of the step is passive and the other active.

Paracellular transport depends on permeability of tight junctions. Proximal tubules and early segments of the intestine have tight junctions with high permeability, while collecting duct of renal nephron, urinary bladder and terminal part of the colon have low permeability.

Tight junctions can be selective for certain solutes. Solute transport occurring through the paracellular pathway is passive. The two driving forces are transepithelial concentration gradient and transepithelial voltage. Polarity and magnitude of transepithelial voltage depends on specific mb transporters in apical and basolateral mb.

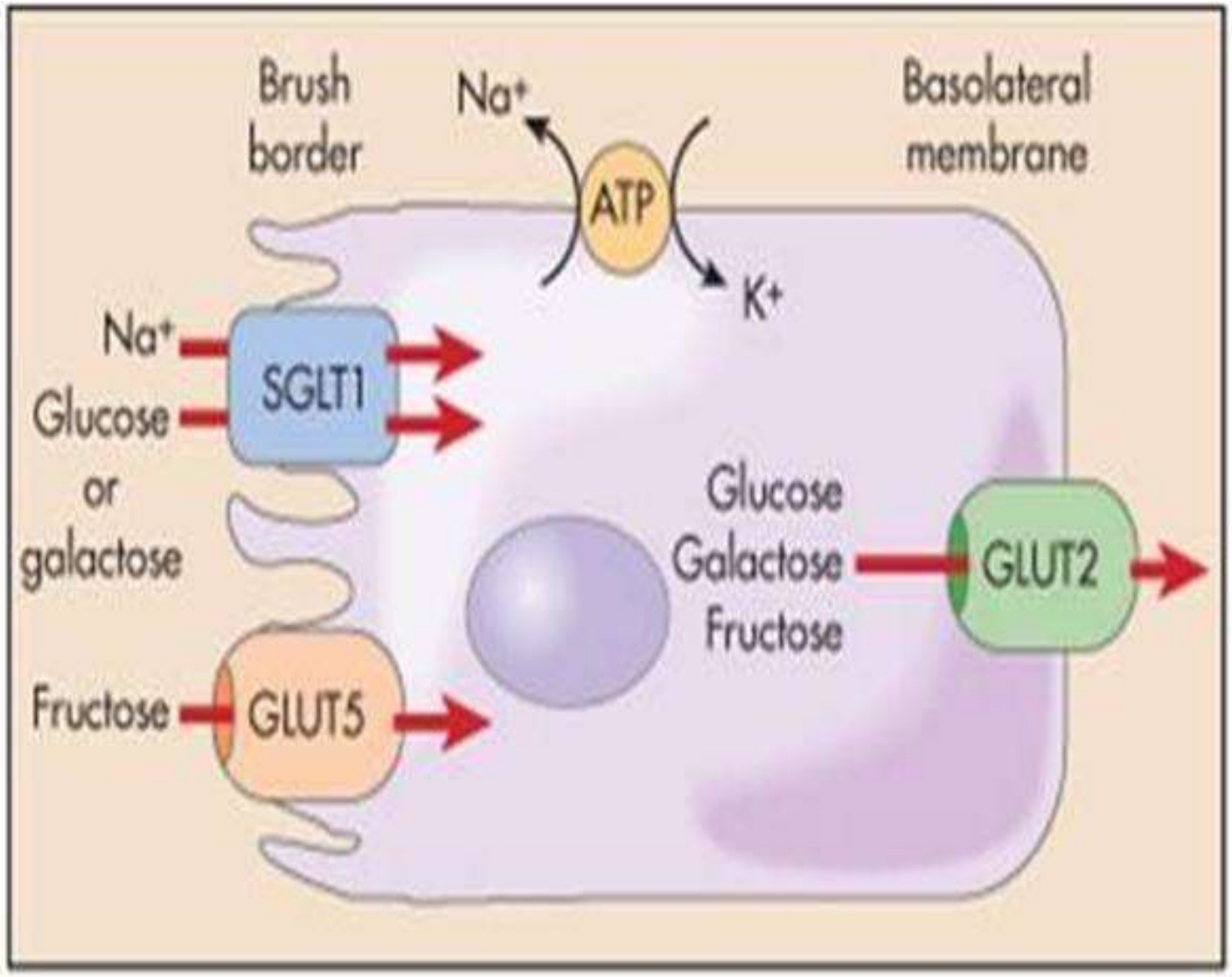
Transcellular transport creates the electrochemical gradient for paracellular transport.



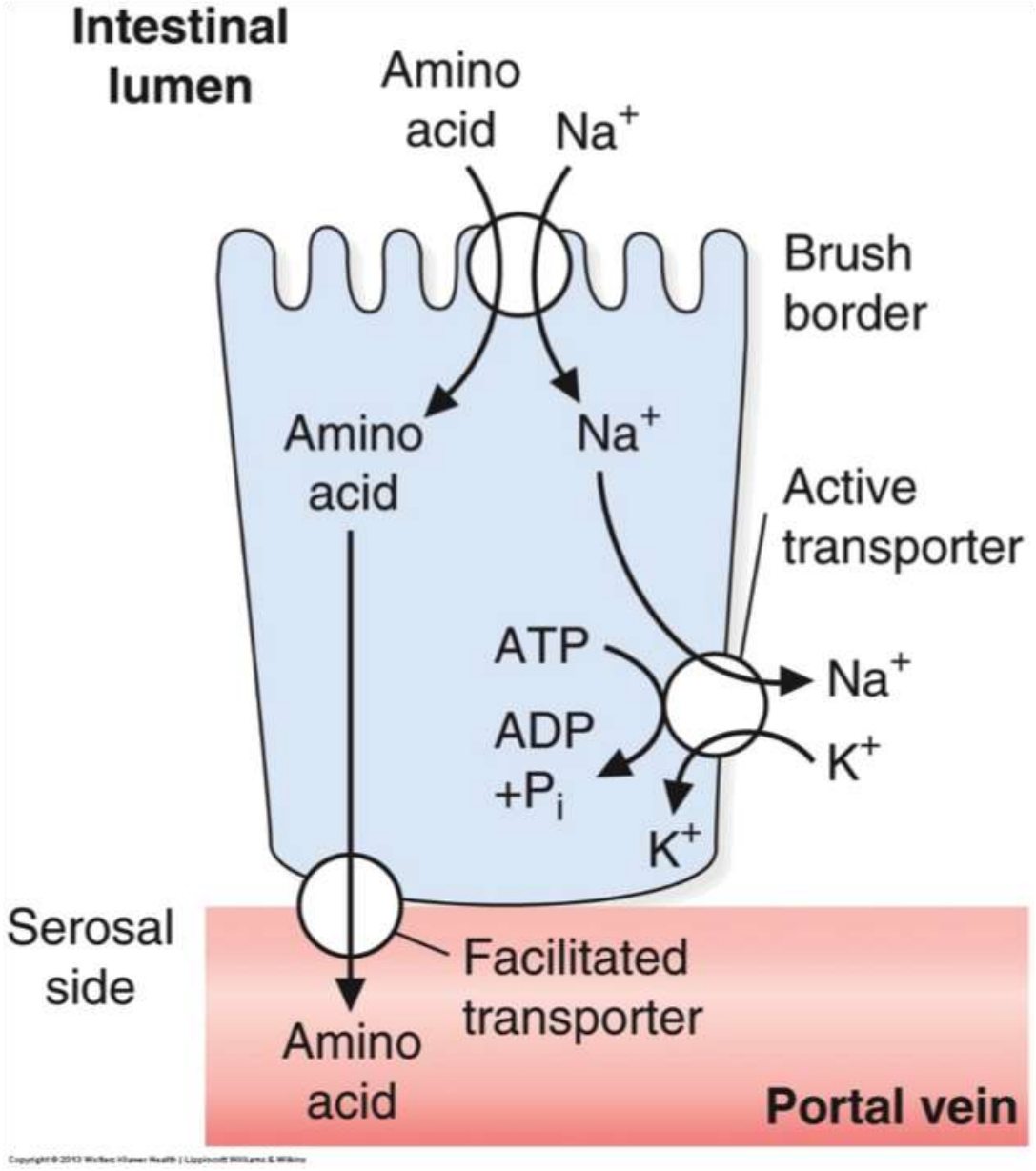
(E.M. Wright et al., 2004)

Na^+ / glucose transporter (SGLT (sodium glucose transporter); There are 6 known isoforms (from SGLT1 to SGLT6)

Glucose leave the cell by two mechanism:
1) Glucose uniport (known as GLUT, standing for Glucose Transporter). There are 12 known isoforms . GLUT2 is the most common.
2) Exocitosis



Galactose is absorbed in the intestine with the same mechanism used for glucose. Fructose uses GLUT5 for entering and GLUT2 for exiting.



Na^+/K^+ pump creates a gradient that can also be used to transport amino acid

Transepithelial water movement

Water transport through epithelia is passive and driven by transepithelial osmotic pressure gradients. It can occur via transcellular and paracellular pathways.

Movement of solutes creates transepithelial osmotic pressure gradient

In some epithelia the movement of water via the paracellular pathway can drive movement of additional solute. This process is called **solvent drag**.

Regulation of epithelial transport

Epithelial transport must be regulated to meet homeostatic needs. This involves hormonal or neural mechanisms.

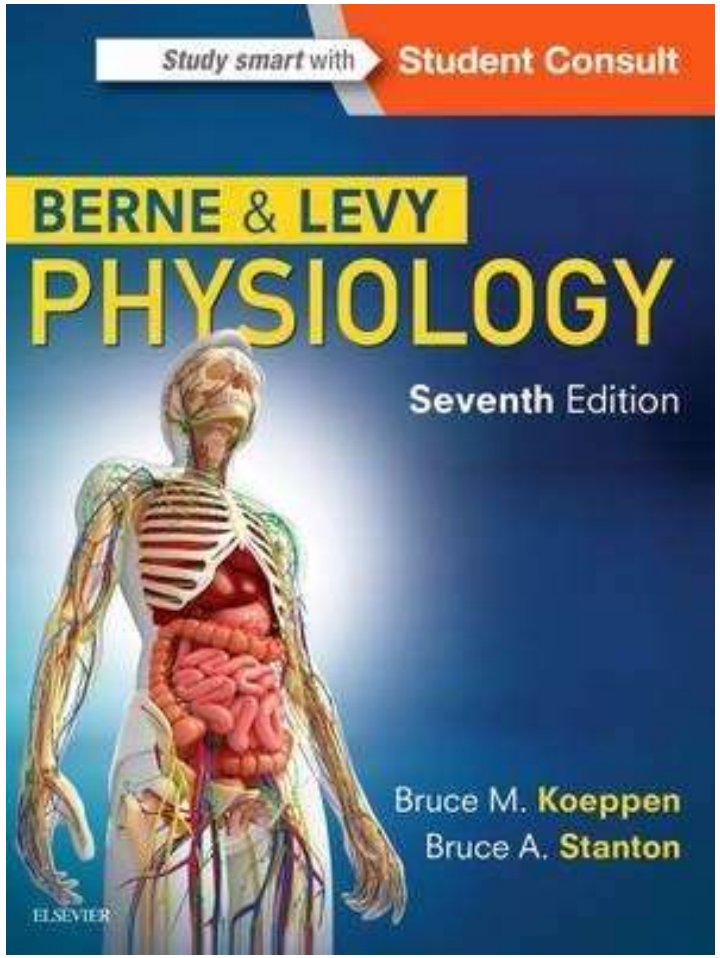
For example, the enteric nervous system regulates transport of water and solute from epithelial cells that line intestine and colon. Similarly, sympathetic nerves regulate transport by epithelial cells in the renal nephron. Aldosterone regulates transport of NaCl by epithelium in the colon, renal nephron and sweat ducts. There might also be paracrine regulation of transport like stimulation of HCl secretion by parietal cells of the stomach from histamine released by cells next to them.

Regulation of transport depends on:

- Retrieval of transporters from mb (endocytosis) or insertion in the mb (exocytosis)
- Change in activity of the transporter (gating)
- Sinthesis of specific transporters and insertion into the mb.

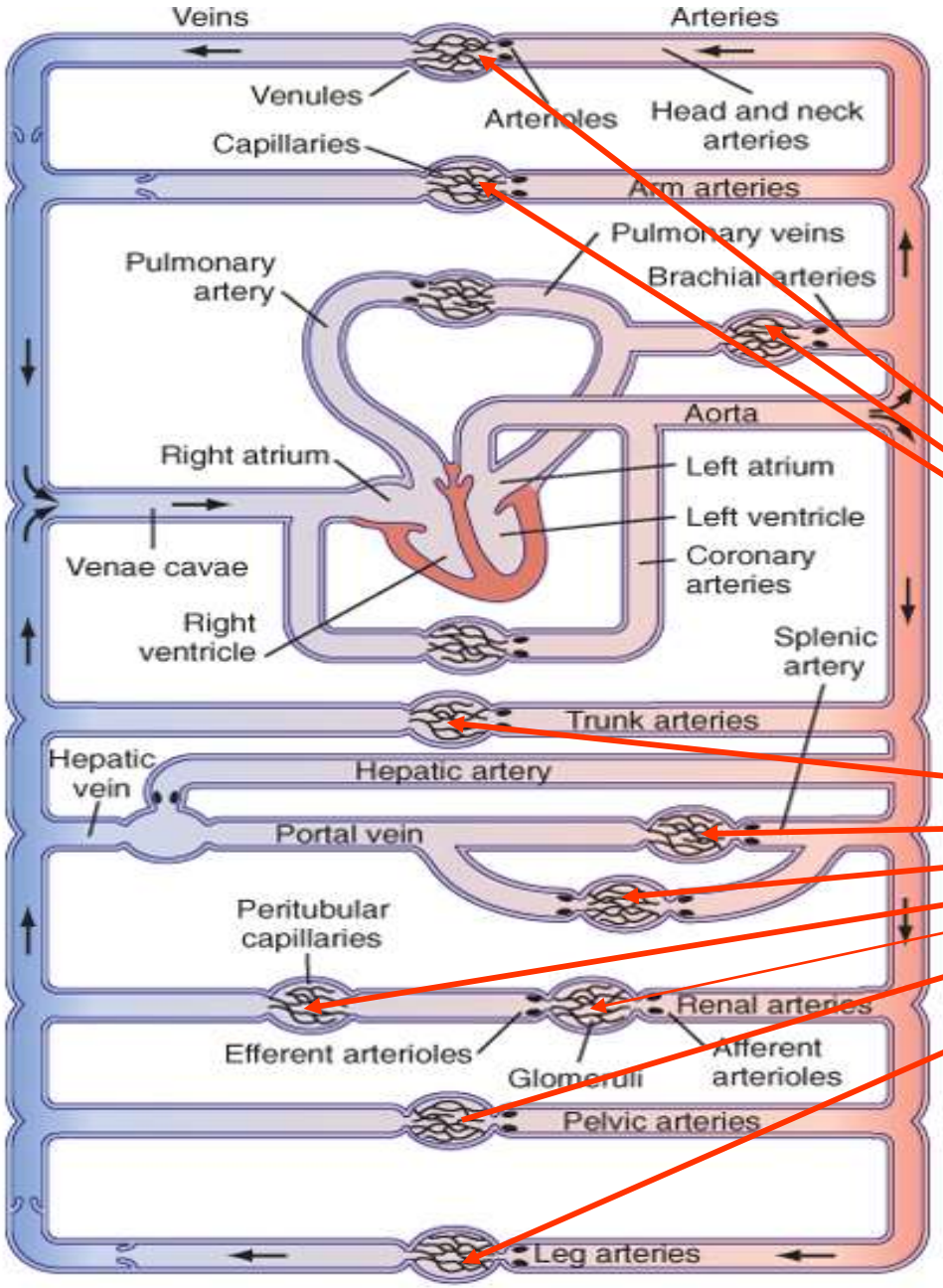
First two are rapid, last one is a slow process (minutes/hours/days)

Fluid exchange in the capillaries



Chapter 17

Fig. 15.1 Berne-Levy



systemic capillaries

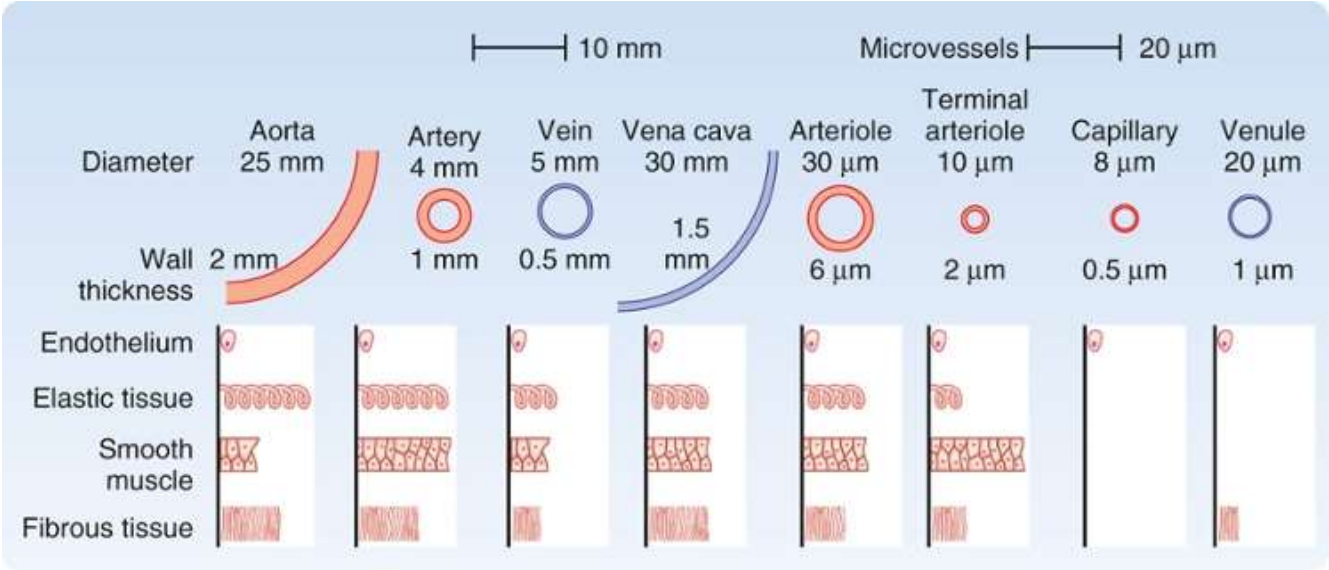


Fig. 15.2 Berne-Levy

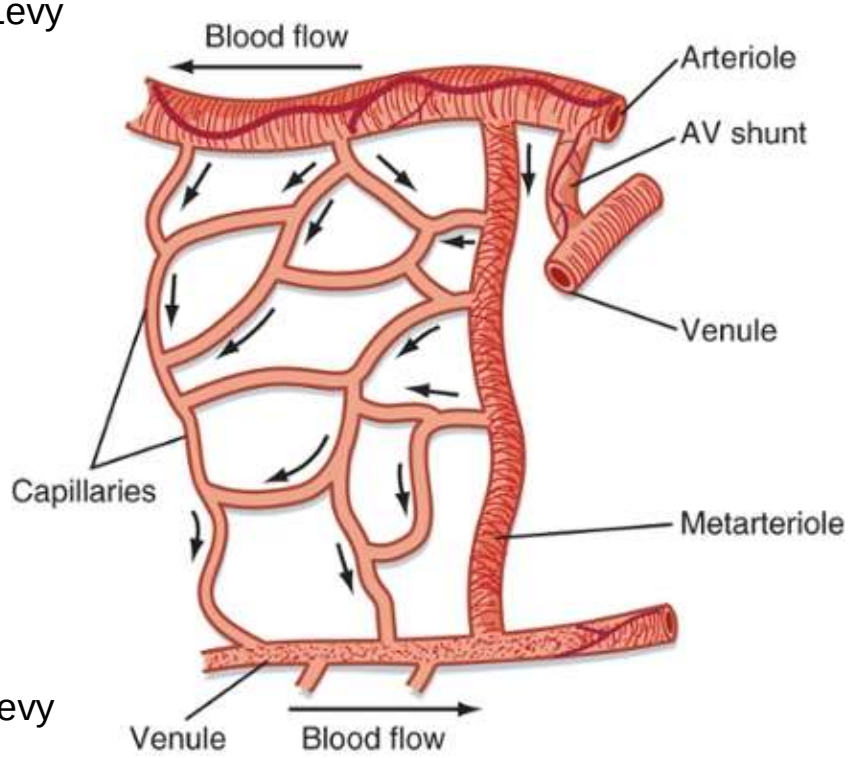
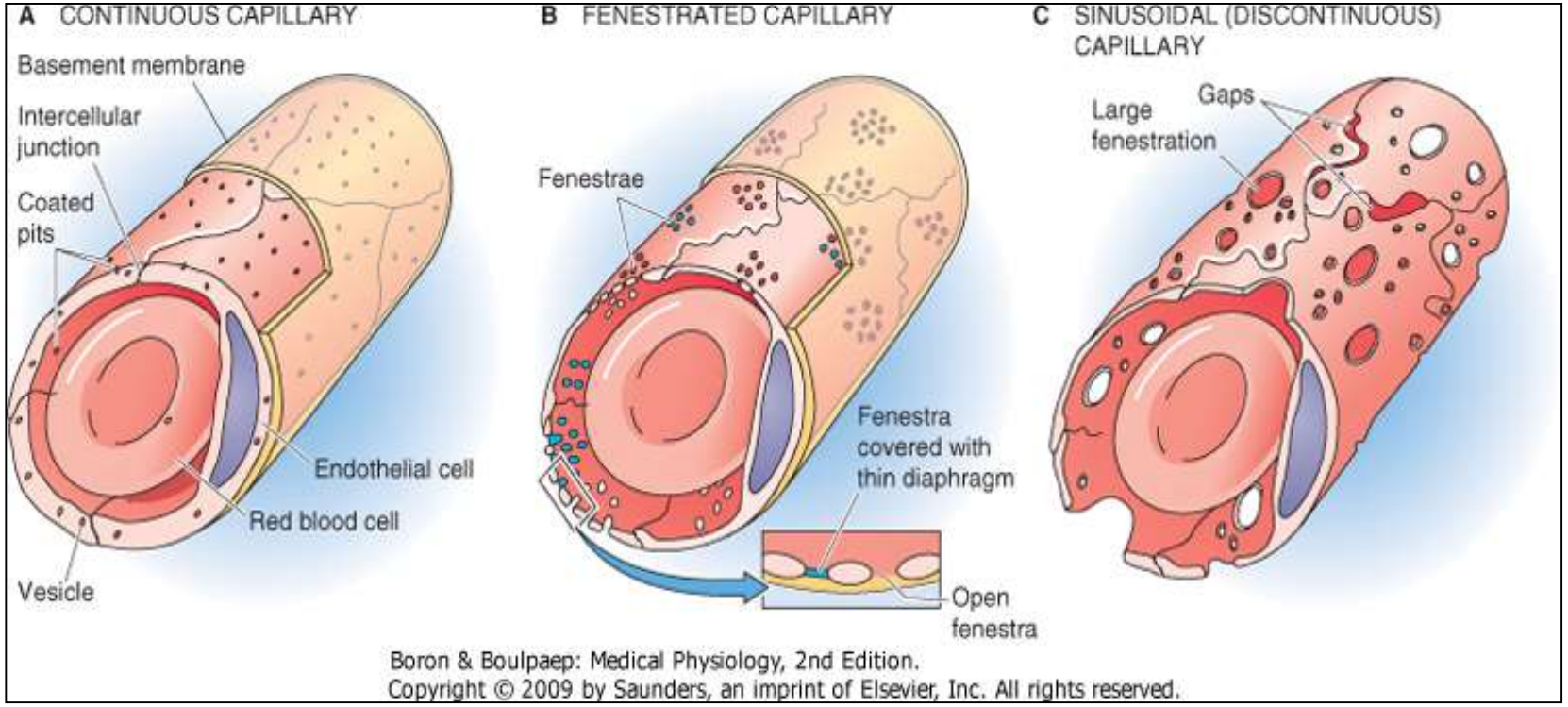


Fig. 17.22 Berne-Levy

Movement of water from interstitial fluid to intravascular environment (and viceversa) occurs across the capillary wall. Amount of water that moves and mechanism depend on type of capillary. E.g. capillary sinusoids of liver endothelial cells are separated by large gaps (fenestrated). Water and other components (sometimes even entire cells) can cross easily. For some other capillary, the endothelial cells form a tight barrier (like the blood brain barrier, BBB) and water and solutes can pass only if transported.

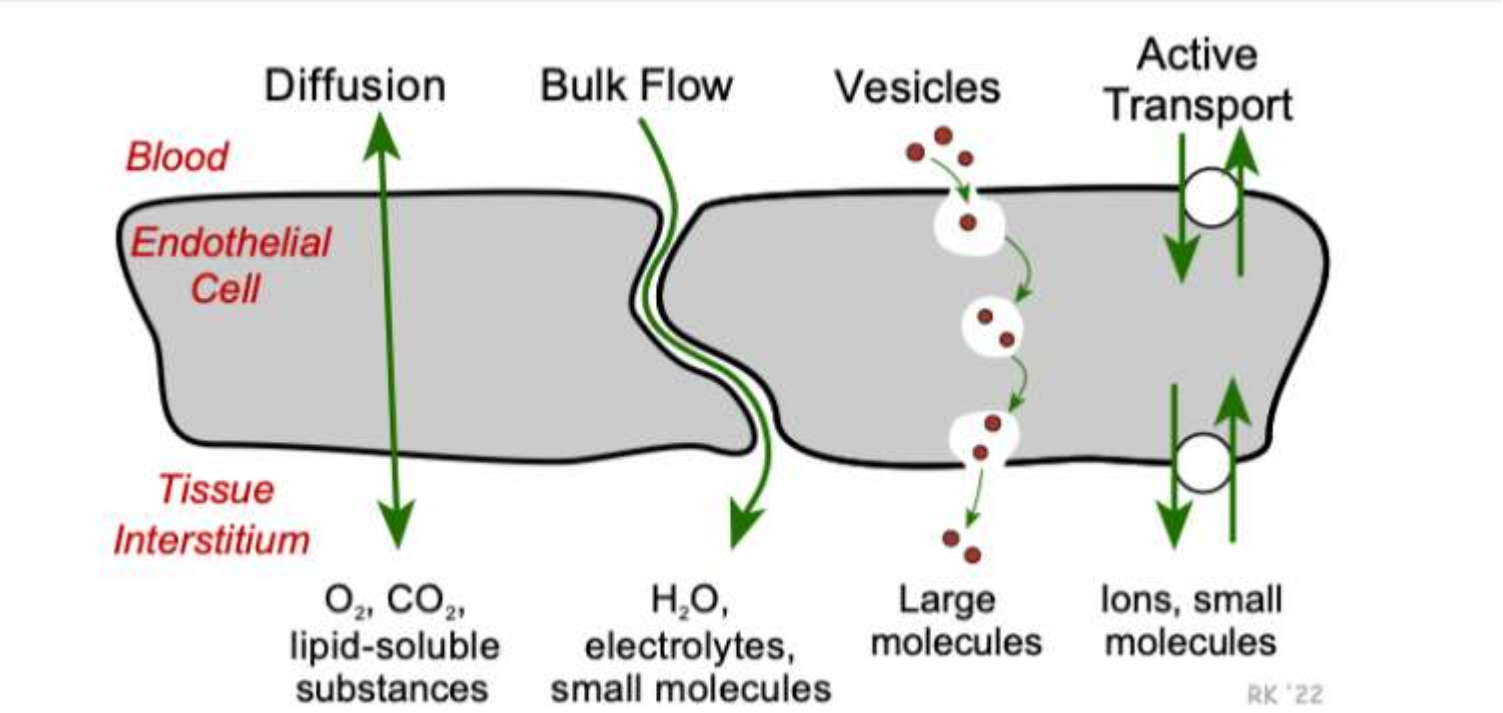


Following an infection, the BBB can become leaky

Capillary exchange

Solvent and solutes move across the capillary endothelial wall by three processes:

- Diffusion
- Bulk flow
- Vesicular transport
- Active transport



Diffusion is fundamental for exchanges of gases (O_2 and CO_2) and lipid-soluble substances (e.g., anesthetics); fluid and electrolytes are also exchanged, in part, by diffusional forces.

Fick's law can be expressed as:

$$J=-PS(Co-Ci)$$

P = capillary permeability

S = capillary surface area

PS product gives an index of available capillary surface area because the intrinsic permeability of the capillary is rarely altered. This changes in wounds, burns or bee stings

In capillary, diffusion of polar molecules is restricted to water filled channels or pores. Movement is complex and is influenced by interactions with solvent, with other molecules and charge of the solute in respect to charge of endothelial cells.

For small molecules such as H_2O, urea, NaCl and glucose the capillary walls offer low resistance to diffusion. Concentration gradient across the capillary wall is almost non existent. Diffusion becomes minimal when molecules approach the size of 60KDa. With small molecules the only limitation to transport across capillary wall is the flow ⟶ transport is flow limited

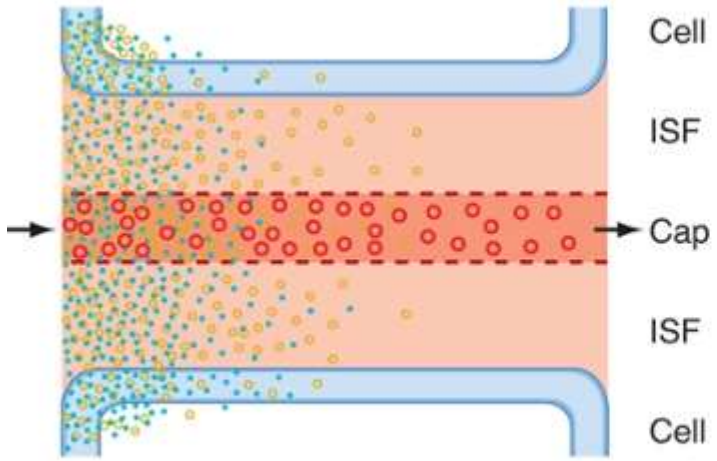


Fig. 17.25 Berne-Levy

A

Small molecules are subjected to flow limited transport
With large molecules, diffusion across capillaries becomes the limiting factor ⟶ diffusion limited transport

Lipid soluble molecules cross the mb, they don’t need pores or transporters

Measurements of O_2 partial pressure (PO_2) and O_2 saturation of blood in microvessels indicate that in many tissues O_2 saturation at the entrance of the capillary bed has already decreased to 80% ⟶ O_2 diffuses from arterioles and arteries. Also CO_2 loading, and resulting intravascular change in oxyhemoglobin dissociation curve occurrs at precapillary vessels

This is known also as counter-current exchange between adjacent arterioles and venules, or also arteries and veins. At low blood flow rates, the supply of O_2 to tissue will be low because of the precapillary counter-current exchange.

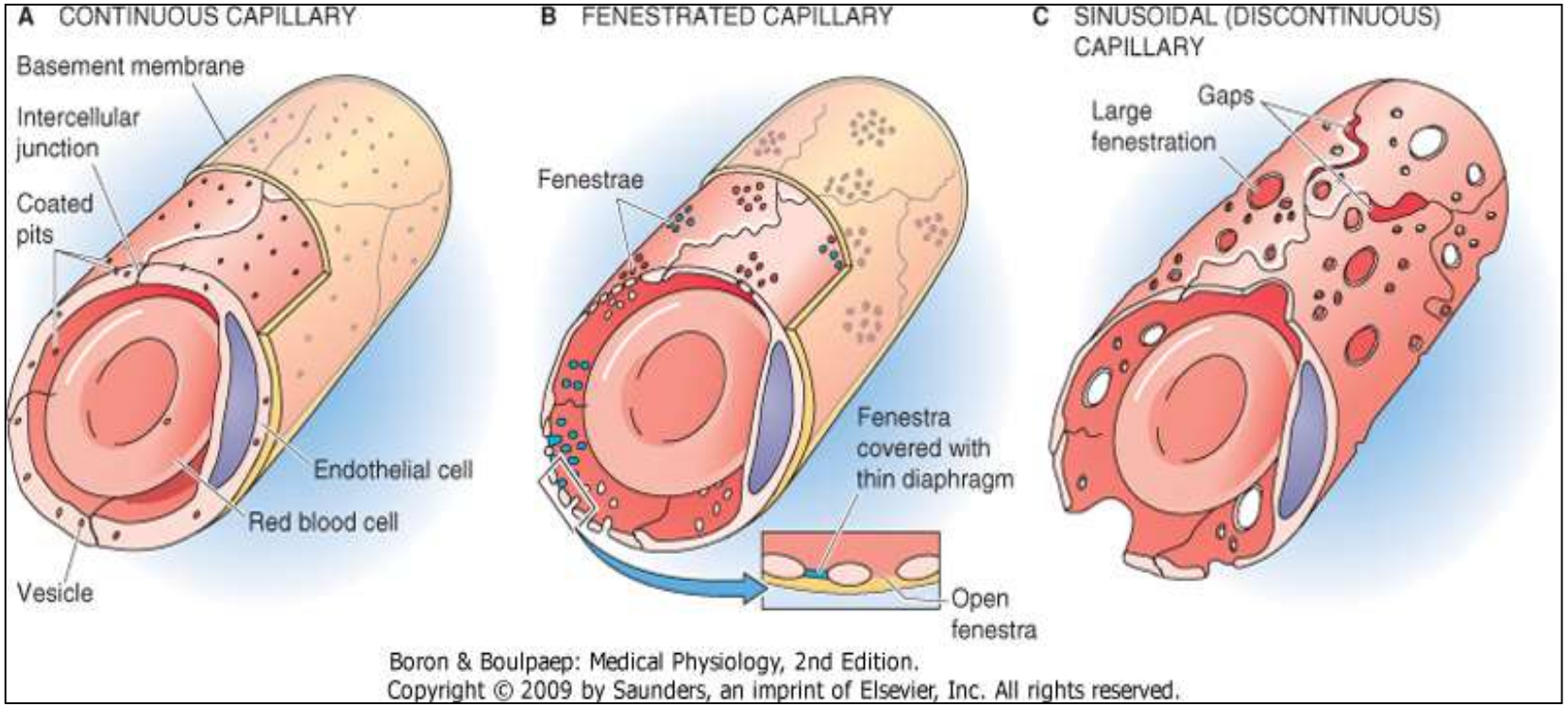
Capillary filtration

The permeability of the endothelial mb is not uniform. For example, in the liver capillaries are fenestrated and albumin can escape in the interstitial environment. Permeability is also not uniform in the length of the capillary. The venous end is more permeable than the arterial end.

Skeletal muscle or cardiac muscles have capillaries with smaller pores than in the liver (4nm). There are clefts between adjacent cells

Pores are absent in cerebral capillaries (BBB)

Also in kidney and intestine endothelium, capillaries can be fenestrated



The direction and magnitude of water movement across capillary wall can be estimated as algaebric sum between hydrostatic and osmotic pressures existing across the capillary wall.

Hydrostatic pressure within capillaries is not consistent but it depends on arterial and venous pressure and on arteriolar resistance but also venules resistance.

↑ Arterial and venous pressure → hydrostatic pressure in capillaries ↑

Resistance in arterioles → hydrostatic pressure in capillaries ↓

Resistance in venules → hydrostatic pressure in capillaries ↑

Pv changes weigh more than Pa changes. Approximately 80% of Pv changes is reflected on hydrostatic capillary pressure.

Hydrostatic pressure changes between body districts: at heart level in the skin is 32mm Hg at arteriolar end and 15mmHg at venules end. Pressure in the interstitium (that usually is 0) opposes the hydrostatic pressure for capillary filtration.

The key factor that restrains fluid loss from capillaries is the osmotic pressure developed by plasma proteins, especially albumin → oncotic pressure πp

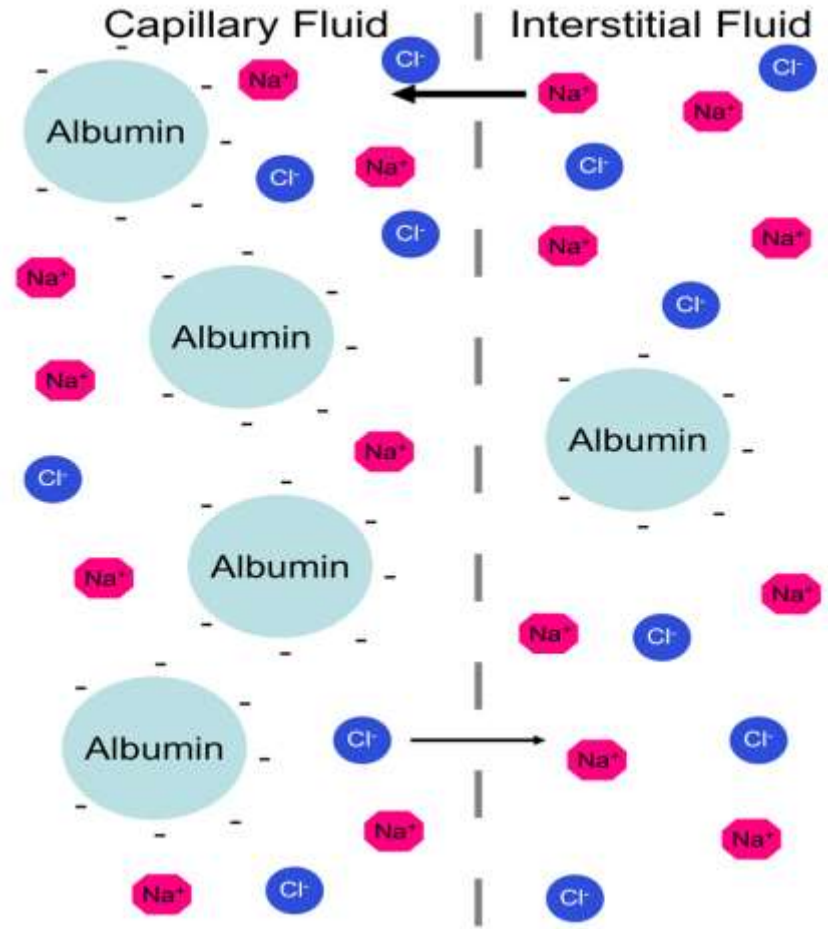
Total osmotic pressure of plasma is 6000mmHg (electrolites, small molecules and proteins) while πp is only 25mmHg. The reflection coefficient (σ), the relative impediment of a molecule to cross the mb, influences the osmotic pressure.

$0 < \sigma < 1$ with 1 totally impermeable (like albumin)

$\mathbf{\Pi = \sigma \Delta CRT}$ R=gas constant; T=absolute temperature; C=concentration.

Albumin is 69 Kda

Albumin exerts an osmotic force stronger than what is accounted solely for its concentration gradient. The reason for it is its negative charge at normal pH and consequent attraction and rejection of cations ⟶ Gibbs-Donnan effect.



From the first Fick's Equation we can elaborate and describe the passage of water caused by forced convection (pressure gradient ΔP) across the capillary wall (filtration),

$$Jv = Lp\ A\ \Delta P$$

Lp = water permeability
A = the area of the wall

if

$$Lp\ A = Kf$$ **(filtration coefficient)**

then

$$Jv = Kf\ \Delta P$$

Driving forces that move water across capillary wall are hydrostatic pressure (P) and oncotic pressure (π) ⟶ Starling Forces

$$\Delta P = [(Pc - Pis) - (\Pi c - \Pi is)]$$

we have to consider that the capillary wall is not completely protein permeable. So (Πc - Πis) has to be corrected by the appropriate coefficient. To determine such coefficient, we determine the ratio between the effective measured Πc (obs) and Πc theoretical, based on van't Hoff's equation.

$\Pi = n\ CRT.$

$$(\Pi c - \Pi is)teor = RT\ ([prot]c - [prot]is)$$

and then

$$(\Pi c - \Pi is)obs\ /\ (\Pi c - \Pi is)teor = \sigma$$

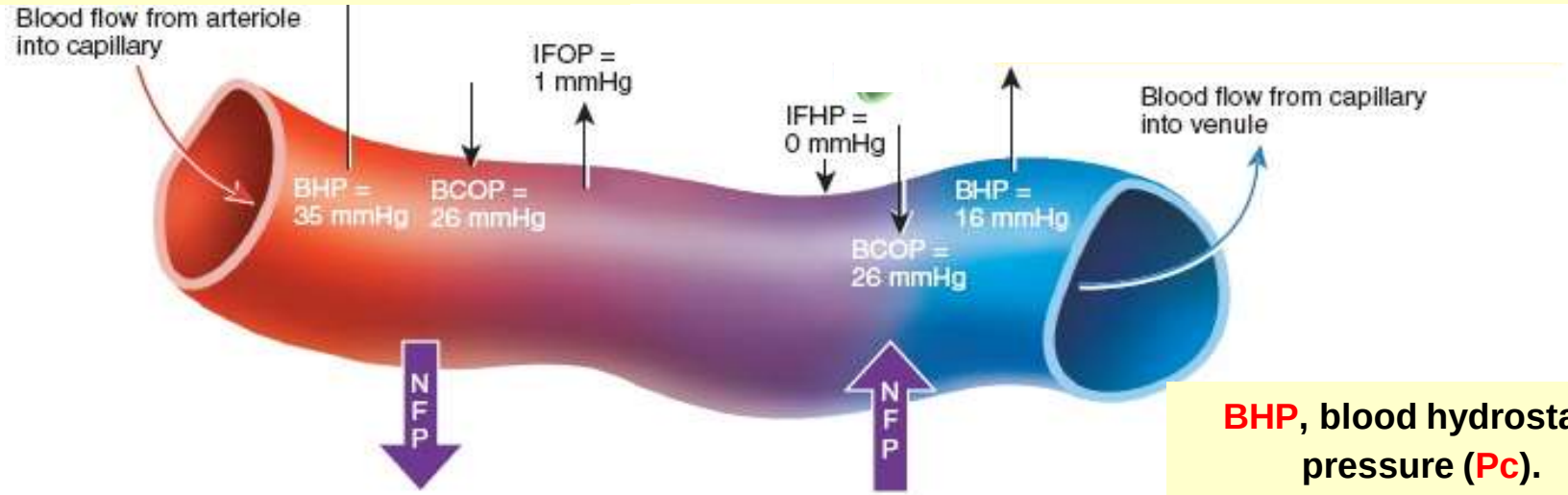
σ = the reflection coefficient. It ranges from 0 (total permeability) to 1 (no permeabilty)

$$\Delta P = [(Pc - Pis) - \sigma\ (\Pi c - \Pi is)]$$

Using then $Jv = Kf\ \Delta P$, and replacing ΔP, we can write the Starling equation

$$Jv = Kf\ [(Pc - Pis) - \sigma\ (\Pi c - \Pi is)]$$

	Capillary pressure (Pc)	Interstitial fluid pressure (Pis)	Capillary colloid osmotic pressure (Πc)	Interstitial fluid colloid osmotic pressure (Πis)	Net force (Pc + Πis) – (Pis + Πc)
Arteriolar end	+ 35	0	+ 26	+ 1	+ 10
Venular end	+ 16	0	+ 26	+ 1	- 9

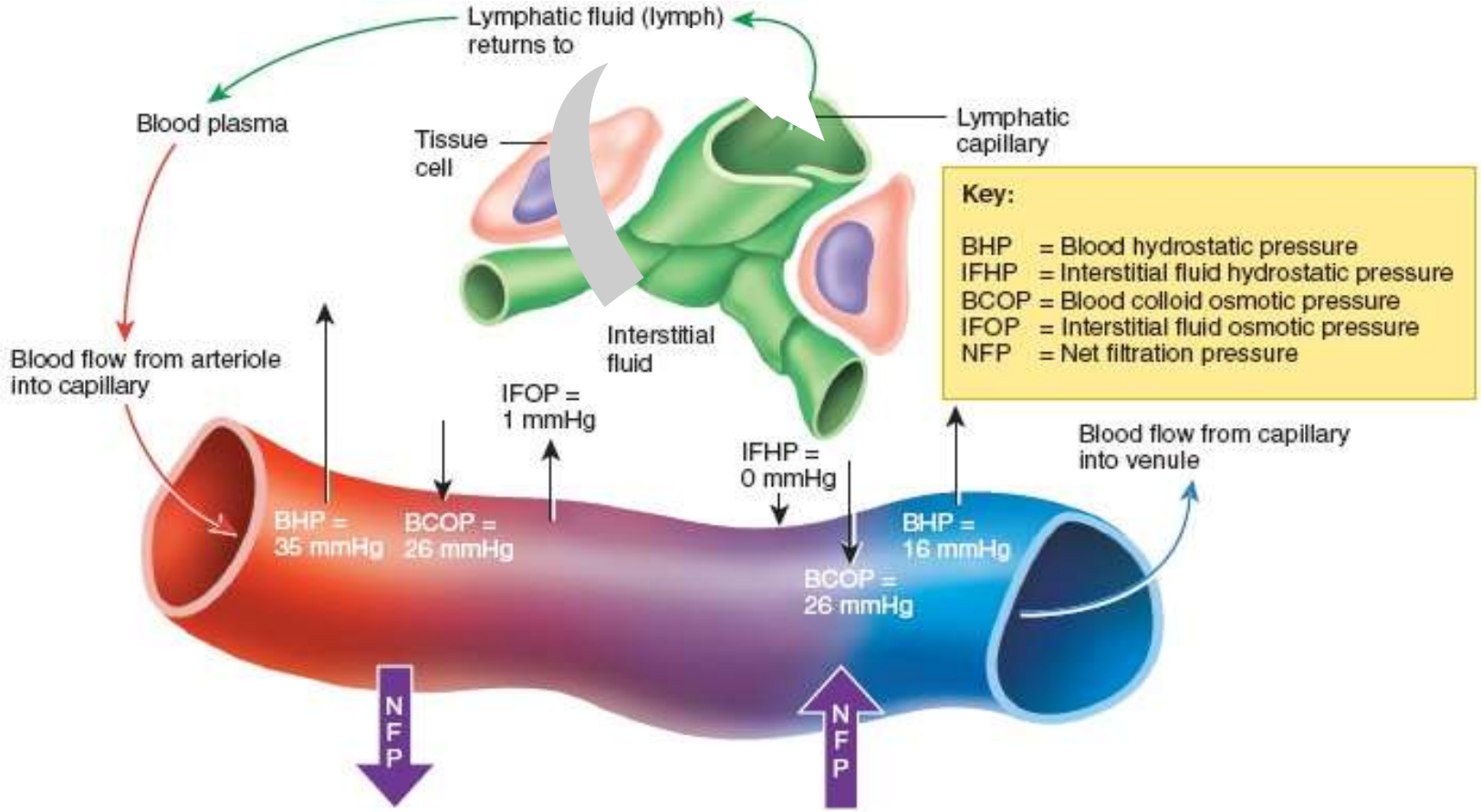


BHP, blood hydrostatic pressure (Pc).

BCOP, blood colloid osmotic pressure (Πc).

IFOP, interstitial fluid colloid osmotic pressure (Πis).

IFHP, interstitial fluid hydrostatic pressure (Pis).



Net filtration at arterial end of capillaries (20 liters per day)

Net reabsorption at venous end of capillaries (17 liters per day)

Net filtration pressure (NFP) = (BHP + IFOP) − (BCOP + IFHP)

Pressures promoting filtration

Pressures promoting reabsorption

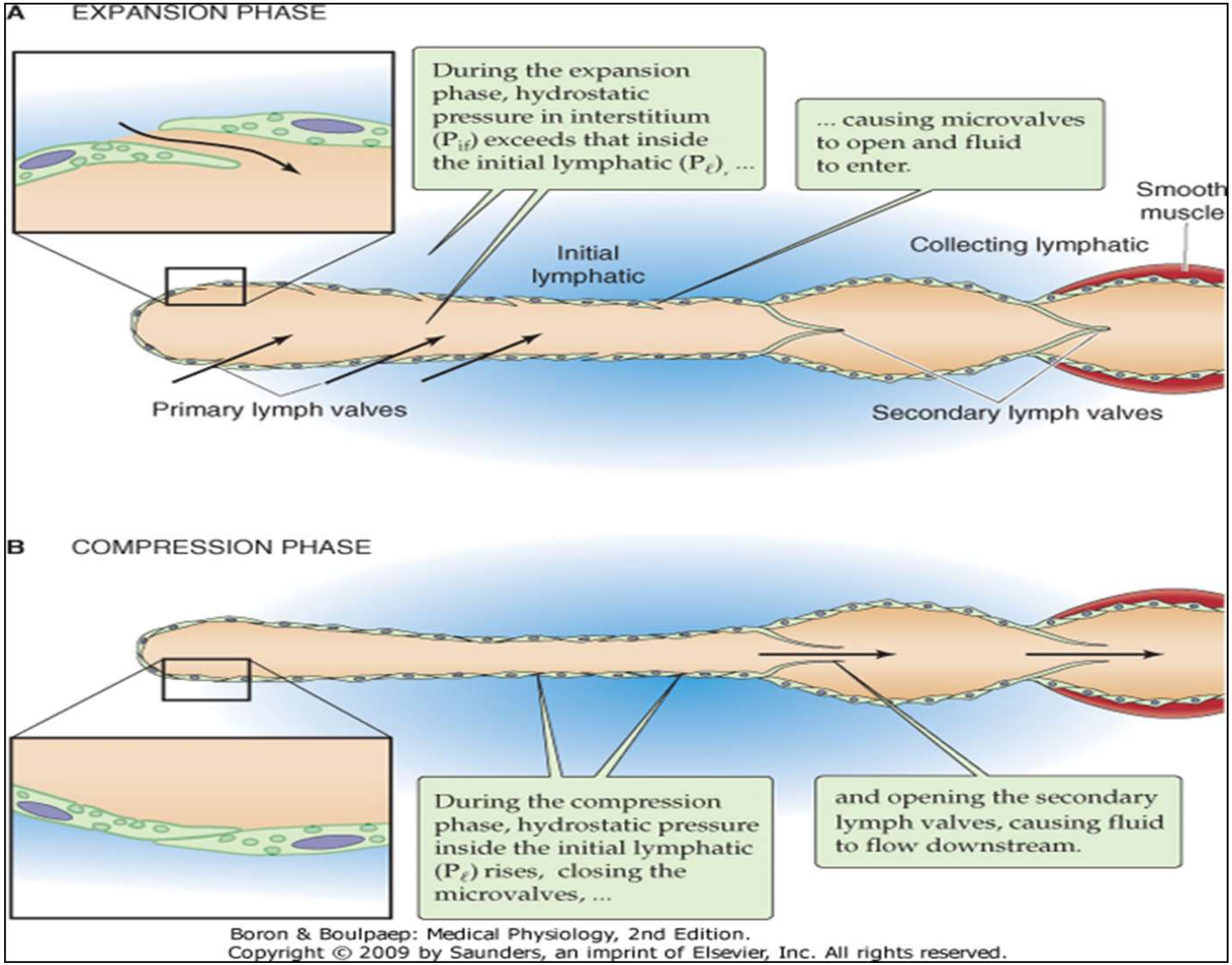
	Arterial end	Venous end
	NFP = (35 + 1) − (26 + 0) = 10 mmHg	**NFP** = (16 + 1) − (26 + 0) = −9 mmHg
Result	Net filtration	Net reabsorption

Lymphatic system

Lymphatic capillaries are a closed end network similar to vascular capillaries but they don’t have tight junctions between endothelial cells and there are fine filaments that anchor these lymphatic capillaries vessels to surrounding connective tissue.

With muscular contractions these strands pull the lymphatic wall and open large spaces between endothelial cells. Large proteins or other molecles, water included, can enter the lymphatic vessels.

Thanks to an extensive system of one-way valves the content is pushed through the circulating lymph.



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Thanks to an extensive system of one-way valves the content is pushed through the circulating lymph.

Lymphatic vessels resemble veins with thinner walls and contain only small amount of elastic tissue and smooth muscle.

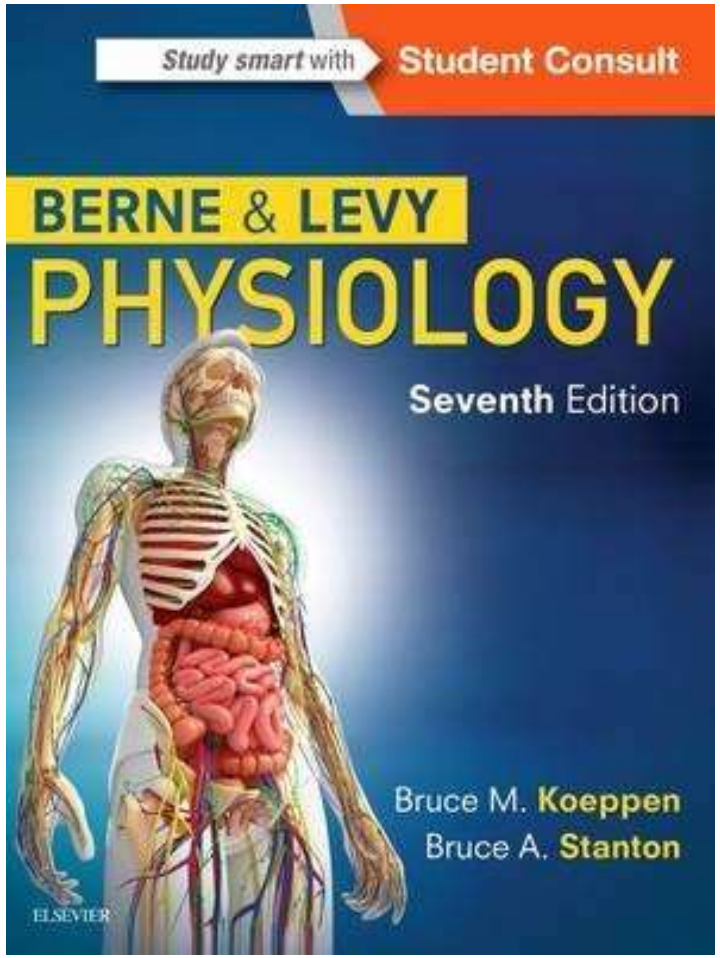
Lymphatic system is present everywhere except brain, bones, cartilages and epithelia.

Lymphatic capillaries drain into larger vessels that finally enter the right and left subclavian veins. The volume transported by lymphatic system in 24h equals the body's total plasma volume. Lymphatic vessels are important because they return all filtered proteins to the blood (if this doesn't happen edema or ascites).

Lymphatic system filters lymph at lymphnodes and remove foreign particles. It also carries substances absorbed by GI especially fat through chylomicrons.

Lymph flow in resting muscle is almost nil but it increases with exercise.

Oxygen and Carbon Dioxide Transport



Chapter 24

Oxygen and Carbon Dioxide Transport

https://www.khanacademy.org/science/health-and-medicine/advanced-hematologic-system/hematologic-system-introduction/v/hemoglobin-moves-o2-and-co2

https://www.khanacademy.org/science/health-and-medicine/advanced-hematologic-system/hematologic-system-introduction/v/bohr-effect-vs-haldane-effect

The respiratory and circulatory systems function together to transport oxygen (O_2) from the lungs to the tissues to sustain normal cellular activity and to transport carbon dioxide (CO_2) from the tissues to the lungs for expiration. Gas movement throughout the respiratory system occurs predominantly via diffusion. The rate of diffusion of a gas through a liquid is described by **Graham's law**

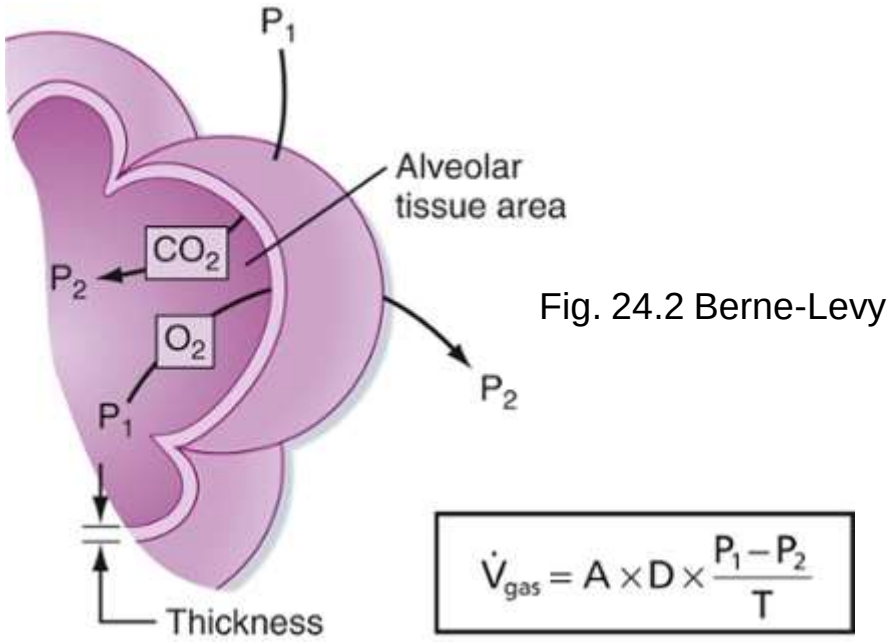


Fig. 24.2 Berne-Levy

$$\dot{V}_{gas} = A \times D \times \frac{P_1 - P_2}{T}$$

CO_2 and O_2 have relatively low solubility in the alveolar-capillary membrane but high solubility in blood because of their ability to bind to **Hemoglobin** (**Hgb**). However, their rate of equilibration is sufficiently rapid for complete equilibration to occur during the transit time of the red blood cell within the capillary. Equilibration for O_2 and CO_2 usually occurs within 0.25 seconds. Thus, O_2 and CO_2 transfer is normally **perfusion limited**.

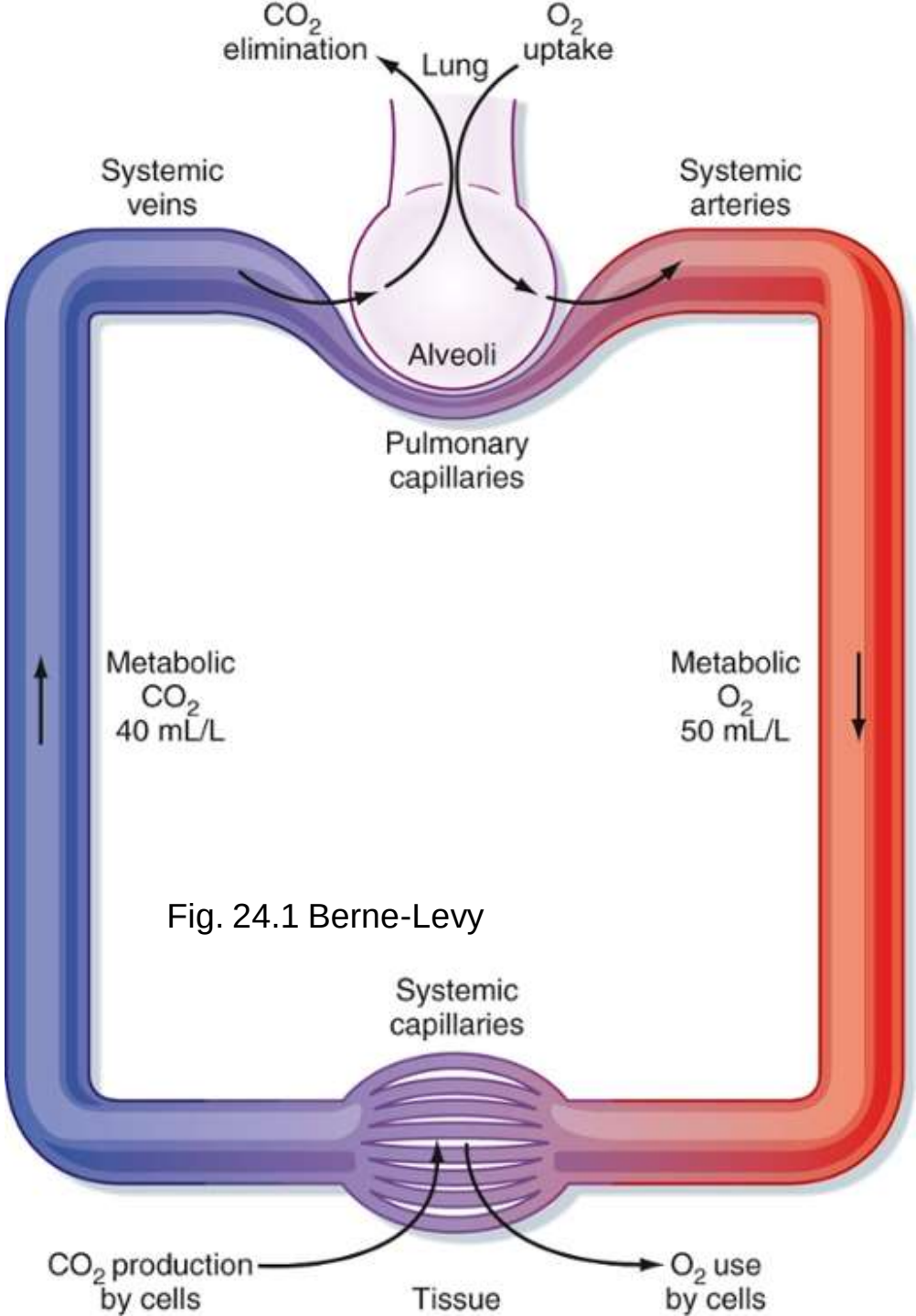
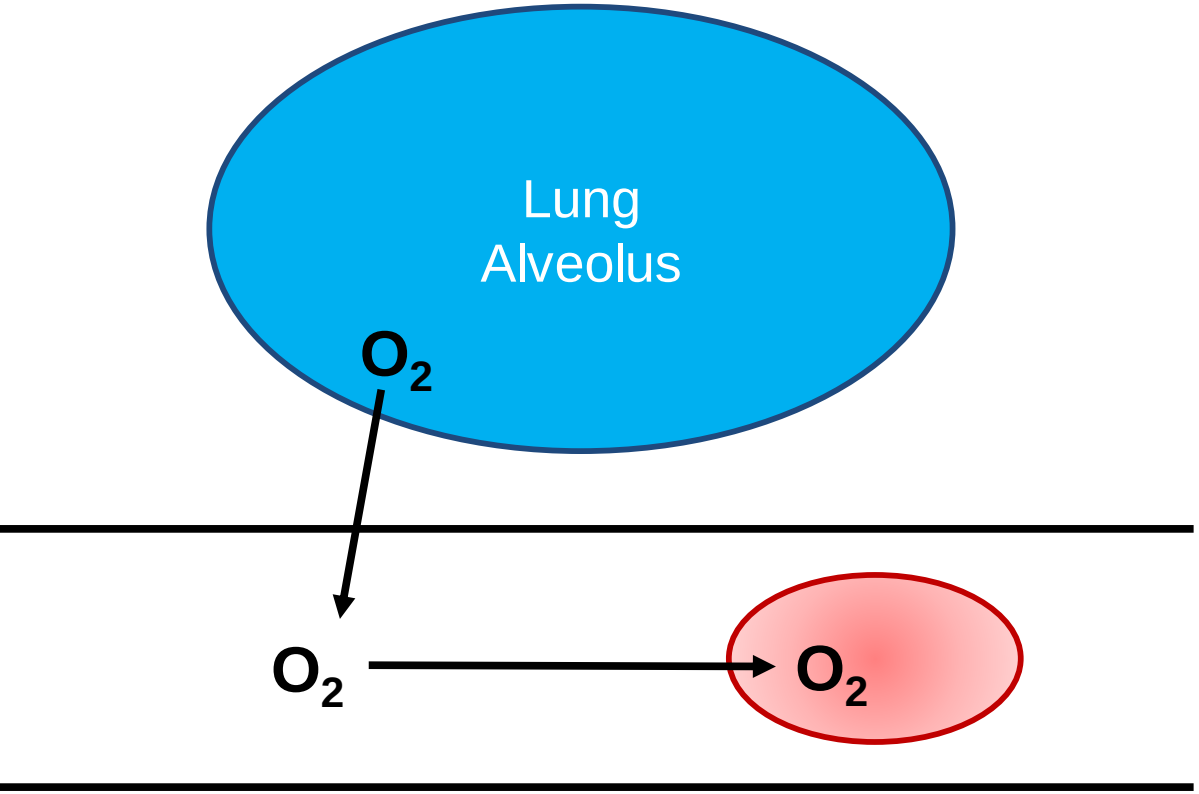


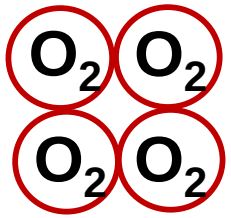
Fig. 24.1 Berne-Levy



1. O_2 dissolved in plasma
2. HbO_2 (Oxyhemoglobin)

Majority

Hemoglobin



The Hb molecule is a protein with two major components: four non-protein heme groups, each containing iron in the reduced ferric (Fe^{+++}) form, which is the site of O_2 binding, and a globin portion consisting of four polypeptide chains.

Oxygen Transport

Oxygen is carried in blood in two forms: dissolved O_2 and O_2 bound to Hgb. The dissolved form is measured clinically in an arterial blood gas sample as the partial pressure of arterial oxygen (PaO_2).
Binding of O_2 to Hgb to form **oxyhemoglobin** within red blood cells is the primary transport mechanism of O_2. Hgb not bound to O_2 is referred to as *deoxyhemoglobin.*

Binding and dissociation of O_2 with Hgb occur in milliseconds, thus facilitating O_2 transport because red blood cells spend only 0.75 seconds in the capillaries. There are approximately 280 million Hgb molecules per red blood cell, which provides an efficient mechanism to transport O_2.
In the alveoli, the majority of O_2 in plasma quickly diffuses into red blood cells and chemically binds to Hgb. This process is reversible, so that Hgb quickly gives up its O_2 to tissue through passive diffusion

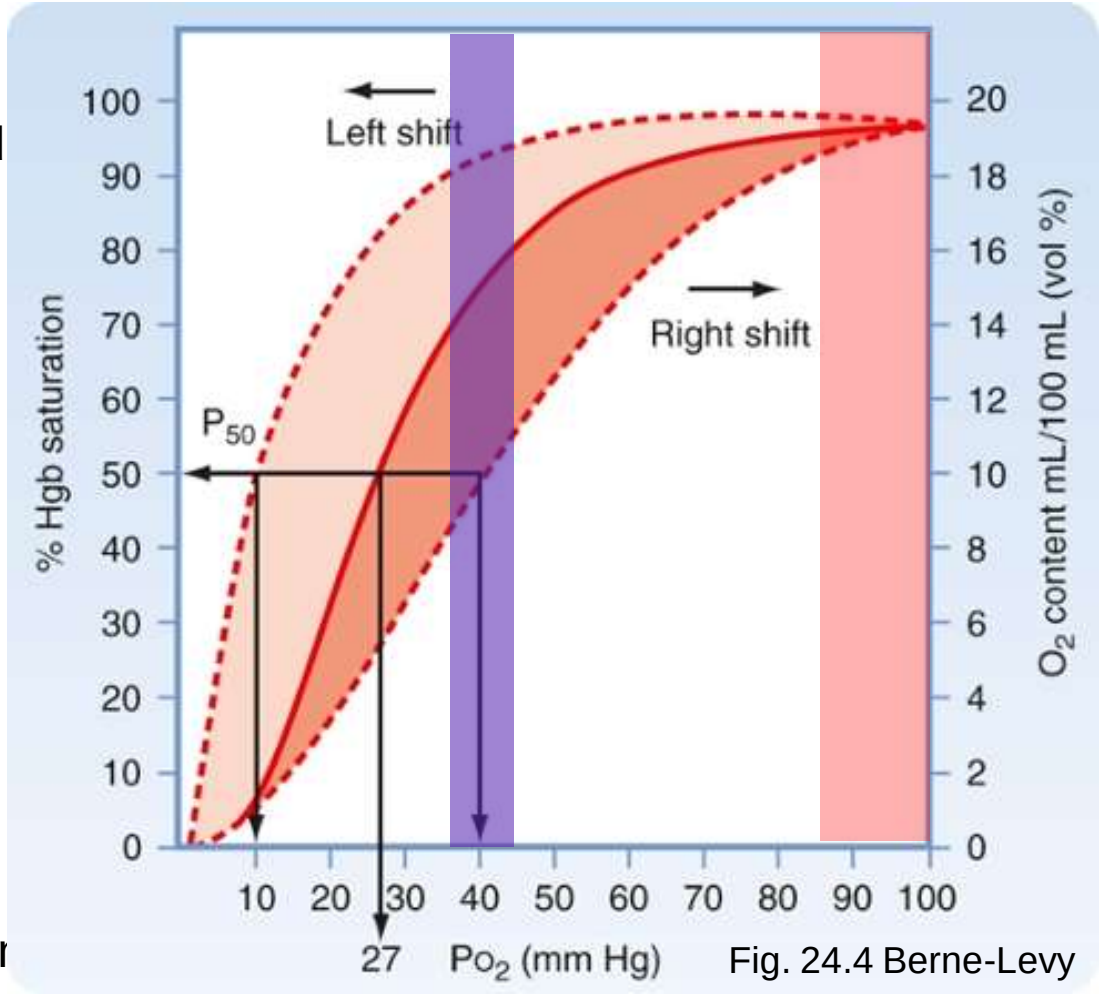


Fig. 24.4 Berne-Levy

The S shape of the curve demonstrates the dependence of Hgb saturation on PO_2, especially at partial pressures lower than 60 mm Hg.
The oxyhemoglobin dissociation curve can shift in numerous clinical conditions, either to the right or to the left: decrease in T, PCO_2, 2,3 DPG and increase in pH to the left. Dependence on PCO2 is the **Bohr effect**

Oxygen Transport

Each Hgb molecule can bind up to four O_2 atoms, and each gram of Hgb can bind up to 1.34 mL of O_2. We talk about **O_2 saturation** (SO_2) the amount of O_2 bound to Hgb in relation to the maximal amount of O_2

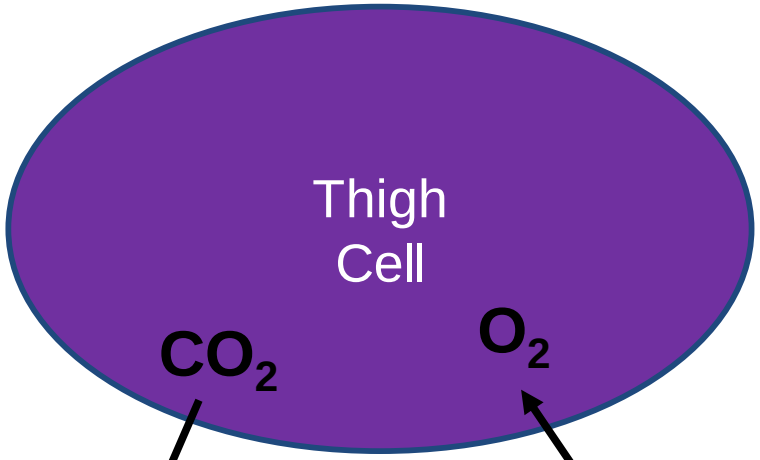
Binding of O_2 to each heme group increases the affinity of the Hgb molecule to bind additional O_2.

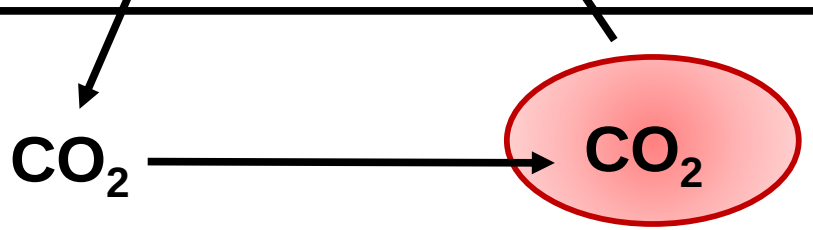
Oxygen delivery from the lungs to tissues is dependent on several factors, including cardiac output, the Hgb content of blood, and the ability of the lung to oxygenate the blood.

The actual O_2 extracted from blood by the tissue is the difference between the arterial O_2 content and the venous O_2 content, multiplied by cardiac output.

Hgb leaves the tissue 75% saturated with O_2, and only about 25% is actually used by tissues.

Tissue oxygenation depends on the concentration of Hgb and thus on the number of red blood cells available in the circulation. Red blood cell production **(erythropoiesis)** in the bone marrow is controlled by the hormone **erythropoietin,** which is synthesized in the kidneys by cortical interstitial cells.





Reasons for O_2 delivery

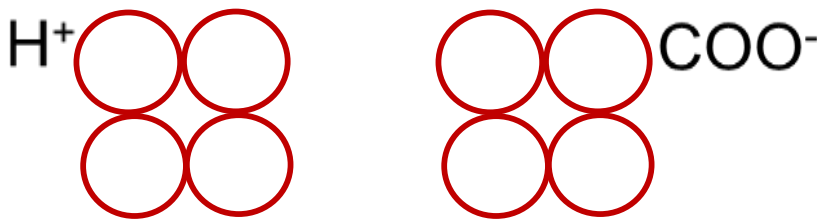
1. Low pO_2 (and high pCO_2)
2. H^+ compete with O_2 for Hb
3. CO_2 compete with O_2 for Hb

Carbonic anhydrase

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

$$CO_2 + HbO_2 \rightleftharpoons Hb\text{-}COO^- + H^+ + O_2$$

$$H^+ + HbO_2 \rightleftharpoons H^+Hb + O_2$$



1. $Hb\text{-}COO^-$
2. H^+Hb
3. CO_2 dissolved in plasma

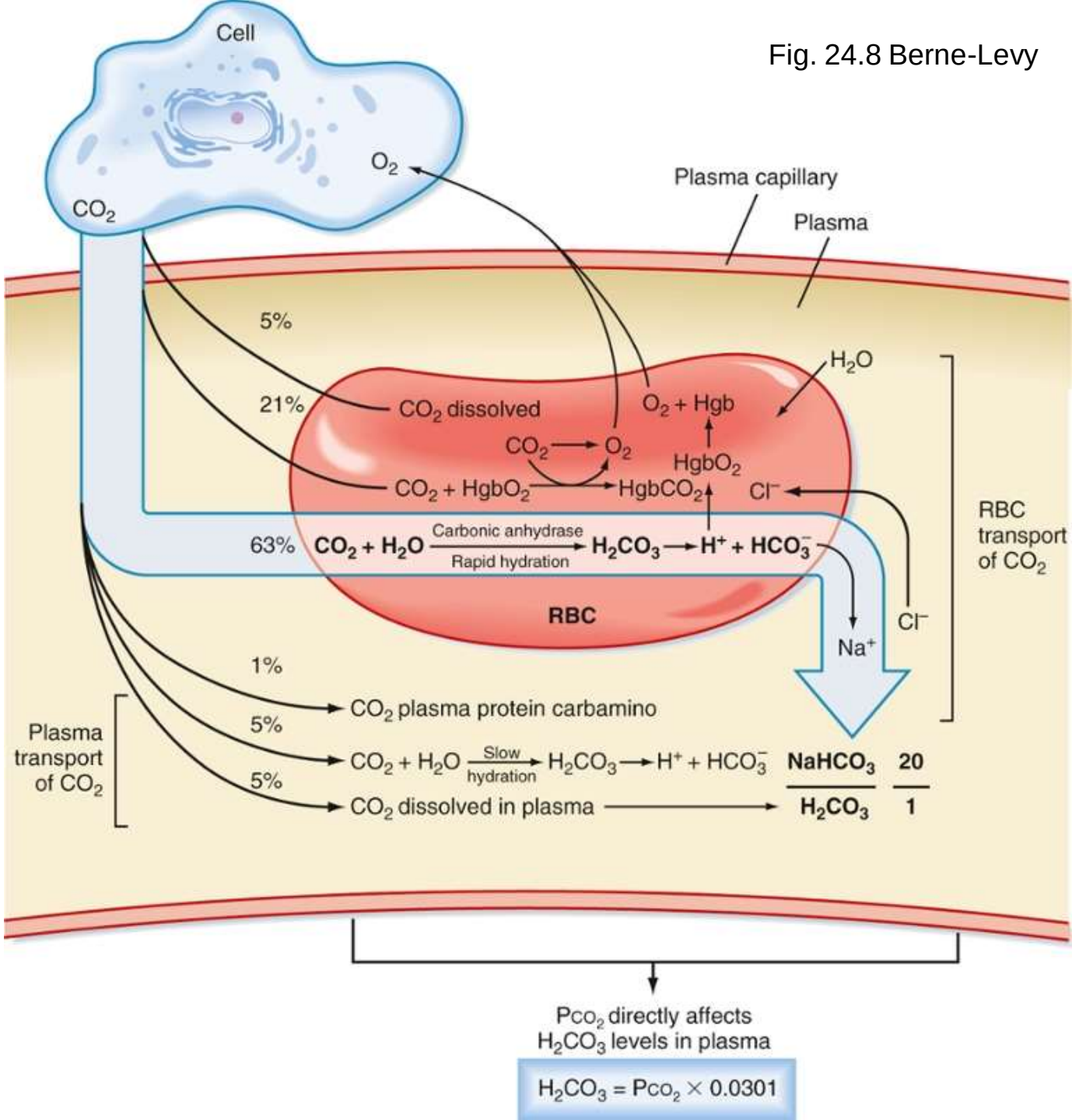
Carbon Dioxide Transport

CO_2 is produced at a rate of approximately 200 mL/minute under healthy conditions, and typically, 80 molecules of CO_2 are expired by the lung for every 100 molecules of O_2 that enter the capillary bed. The ratio of expired CO_2 to O_2 uptake is referred to as the **respiratory exchange ratio** and, under normal conditions, is 0.8.

Arterial PaCO2 is solely dependent on alveolar ventilation and CO2 production. There is an inverse relationship between alveolar ventilation and PaCO2.

The reaction of CO_2 with H_2O to form carbonic acid (H_2CO_3) is the major mechanism for the generation of HCO_3^- in red blood cells. The HCO_3^- diffuses out of the red blood cell in exchange for Cl^-, in a process known as the **chloride shift,** which helps the cell maintain its osmotic equilibrium.

Fig. 24.8 Berne-Levy



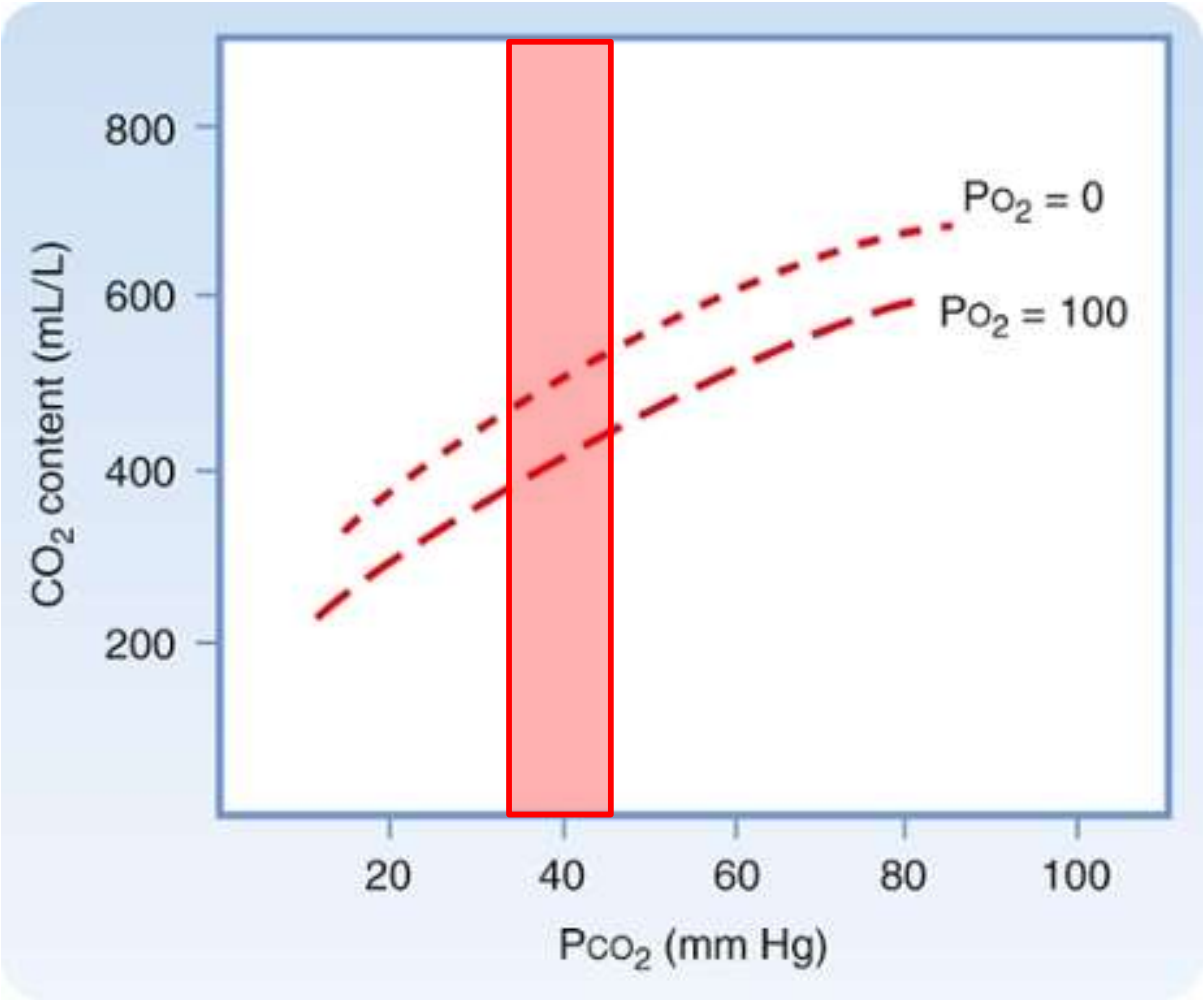
Carbon Dioxide Transport

In contrast to O2, the dissociation curve for CO2 in blood is linear and directly related to PCO2

Although O_2 and CO_2 bind to Hgb at different sites, deoxygenated Hgb has greater affinity for CO_2 than oxygenated Hgb. Thus deoxygenated blood (venous blood) freely takes up and transports more CO_2 than oxygenated arterial blood does.

The effect of changes in oxyhemoglobin saturation on the relationship of CO2 content to PCO2 is referred to as the **Haldane effect** and is reversed in the lungs when O2 is transported from the alveoli to red blood cells.

Haldane effect helps into delivering more CO2 from the red blood cells to the lung. Bohr effect helps into delivering more O2 from the red blood cells to the thigh



What Is the Bohr Effect and Why It Matters

Bohr Effect – Oxygen Delivery to Tissues

Key Concepts

•The **Bohr effect** describes how **carbon dioxide (CO_2) and pH influence hemoglobin's affinity for oxygen**.

•In tissues that are **metabolically active**, CO_2 builds up, lowering blood pH.

•Lower pH and higher CO_2 cause hemoglobin to **release oxygen more easily**.

Why It Matters

•Helps deliver more oxygen to cells that are working harder — e.g., exercising muscles.

What Is the Haldane Effect and Its Role

Haldane Effect – CO_2 Removal in the Lungs

Key Concepts

•The **Haldane effect** describes how **oxygenation of hemoglobin influences CO_2 carriage and release**.

•**Deoxygenated hemoglobin** (in tissues) can **carry more CO_2**.

•When blood reaches the **lungs and hemoglobin binds O_2**, it **releases CO_2** for exhalation.

Why It Matters

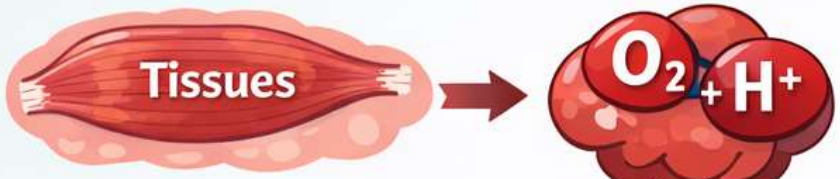
•Enhances **carbon dioxide removal** from the body and supports efficient gas exchange.

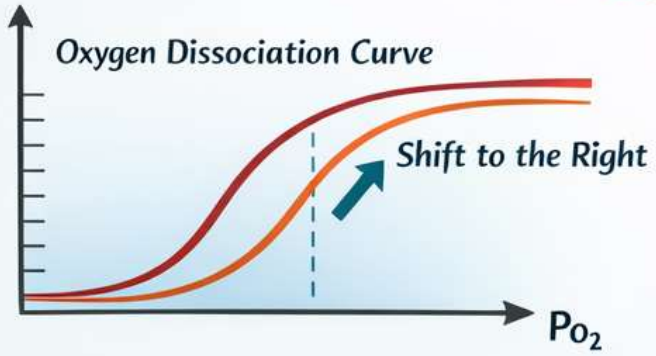
Bohr Effect vs Haldane Effect

Bohr Effect

Oxygen Release in Tissues

- ↑ CO_2 & ↓ pH
- ↓ Hemoglobin O_2 Affinity
- More O_2 Released

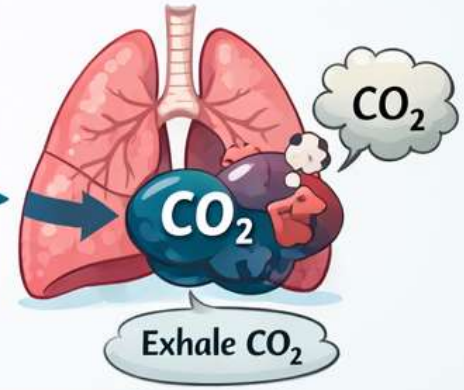




Haldane Effect

CO_2 Removal in Lungs

- ↑ O_2 in Lungs
- ↓ Hemoglobin CO_2 Binding
- More CO_2 Released



	Bohr Effect	Haldane Effect
Driver	CO_2 & pH	Oxygenation
Main Role	↓ O_2 in Tissues	↓ CO_2 in Lungs
Location	In Tissues	In Lungs