BSc Pharmacology and Translational Medicine

Module: Principles of Pharmacodynamics and Pharmacokinetics
Lecture: Transport of Drugs Across Biological Membranes
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Transport of Drugs Across Biological Membranes

Lecture Outline

- Recap on Drugs and Drug Therapy
- Aims of Drug Therapy
- Routes of Administration
- ADMET
- Recap on Structure of Biological Membranes
- Crossing Cell Membranes
- Diffusion
- Carrier-Mediated Transport
    - Facilitated Diffusion
    - Active Transport

What is a Drug?

Drugs and Medicines

- A *drug* can be defined as:

  "A chemical substance of known structure, other than a nutrient or an essential dietary ingredient, which, when administered to a living organism, produces a biological effect.

- Drugs may be synthetic chemicals, chemicals obtained from plants or animals, or products of genetic engineering.

- A *medicine* is a chemical preparation, which usually but not necessarily contains one or more drugs, administered with the intention of producing a therapeutic effect.

- Medicines usually contain other substances (excipients, stabilisers, solvents, etc.) besides the active drug, to make them more convenient to use.

- To count as a drug, the substance must be administered as such, rather than released by physiological mechanisms.

Aim of Drug Therapy

Drug Therapy

- The main aim of drug therapy is to use (bio)chemical agents to treat, prevent or control disease

- Understanding how drugs interact with the body is key to their use in a rational way and for discovering and developing new treatments

- The ideal is to deliver a pharmacologically effective dose to a target without causing (excessive) toxicity or unwanted off-target effects

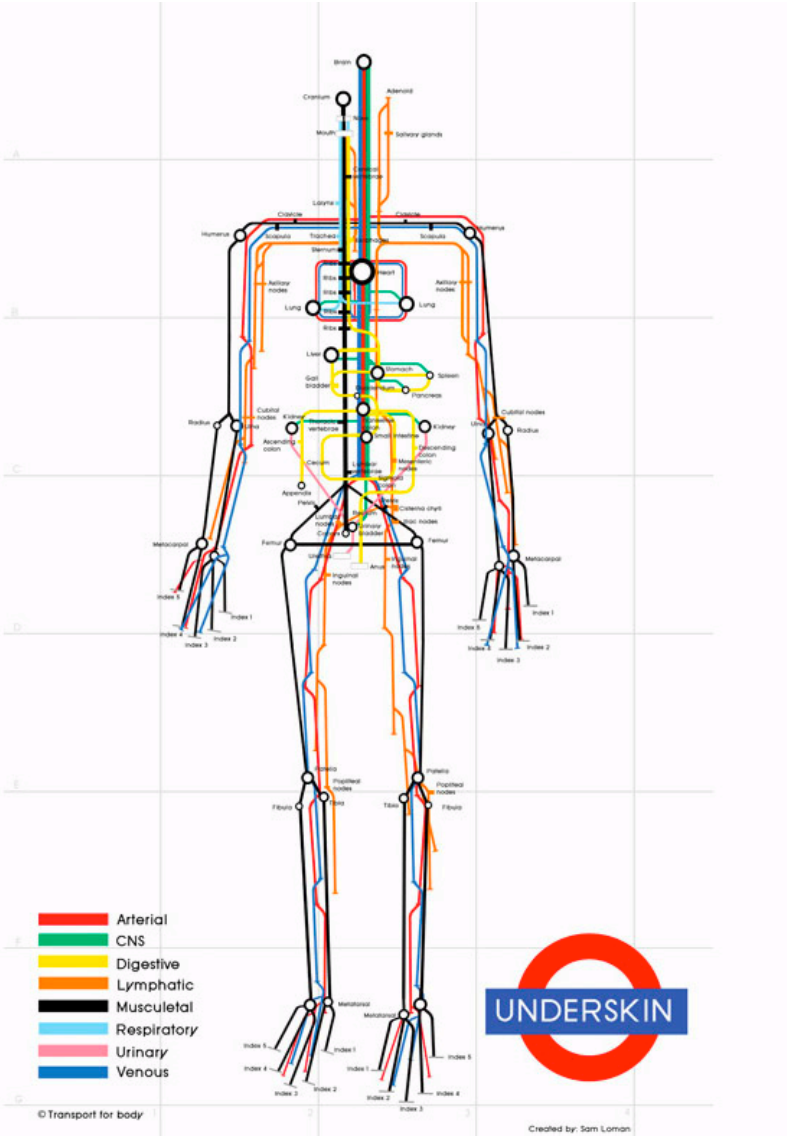
- A number of factors must be considered in relation to the administration of drugs in therapy:
  - *A*bsorption
  - *D*istribution
  - *M*etabolism
  - *E*limination
  - (*T*oxicity)

- ADME(T)

Routes of Administration

Routes of Administration

- Enteral (use of gastrointestinal (GI) tract)
  - Oral
  - Sublingual
  - Rectal
- Parenteral (not through the GI tract)
  - Intravascular
    - Intravenous (IV)
    - Intra-arterial (IA)
  - Intramuscular (IM)
  - Subcutaneous (SC)
- Other Routes
  - Inhalation
  - Intranasal
  - Intrathecal / Intraventricular
  - Topical
  - Transdermal



Routes of Administration

Absorption and Distribution

- Blood plasma is the main bulk carrier of drugs around the body and is therefore absorption into the plasma is the primary mechanism for subsequent distribution

- Absorption relates to the movement of a drug from the site of administration into the blood plasma (in most cases)

- The route of administration greatly influences the rate / profile of absorption
  - E.g. oral vs intravenous

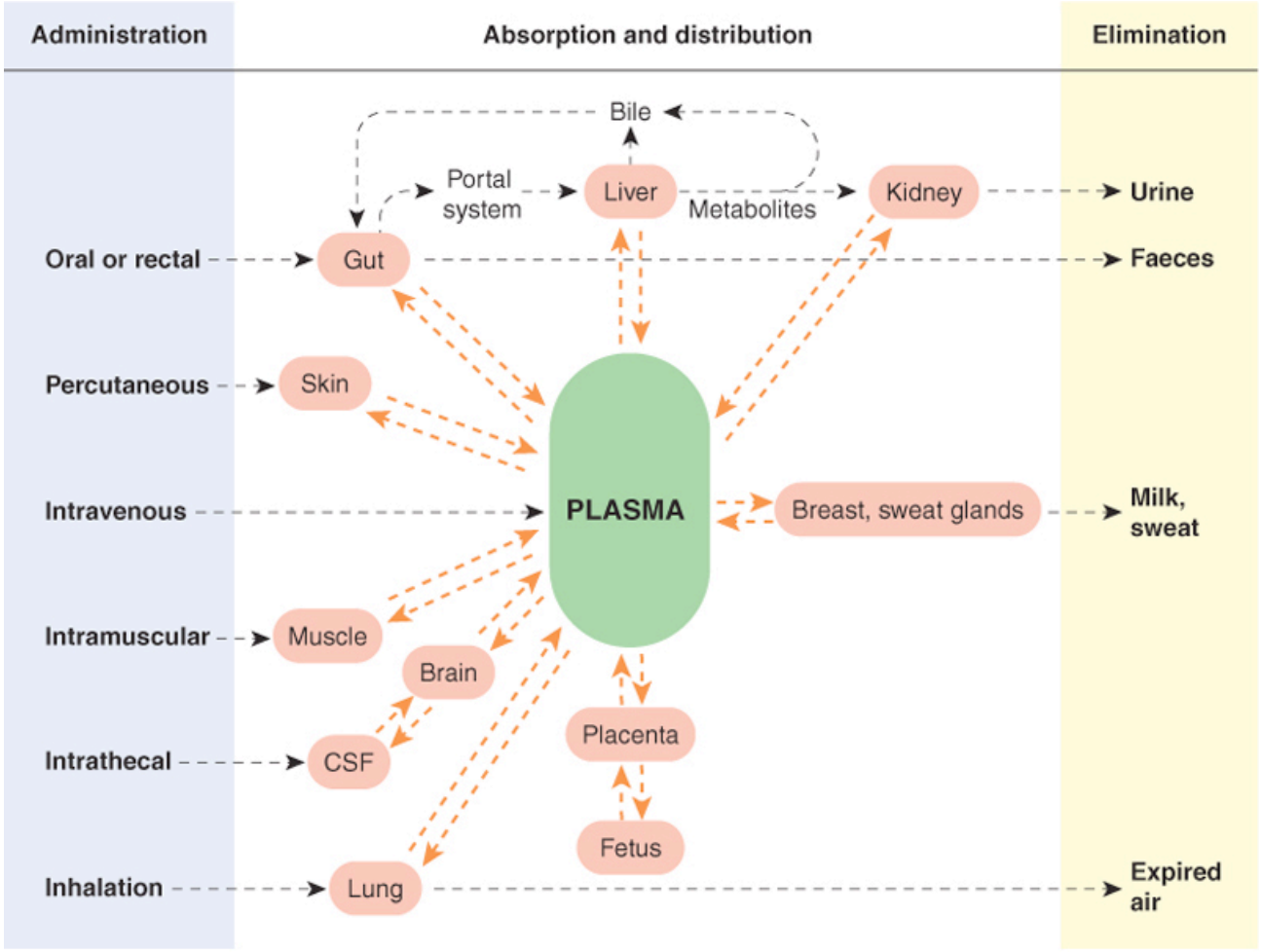
- Upon administration, a drug will need to travel to the target in order to bring about the desired therapeutic effect

- Transport between and within the different tissues and compartments is necessary

- Different tissues and compartments have different properties that influence drug transport and partitioning

ADMET

- A number of factors must be considered in relation to the administration of drugs in therapy:
  - *A*bsorption
  - *D*istribution
  - *M*etabolism
  - *E*limination
  - *T*oxicity
- Rates
- Routes

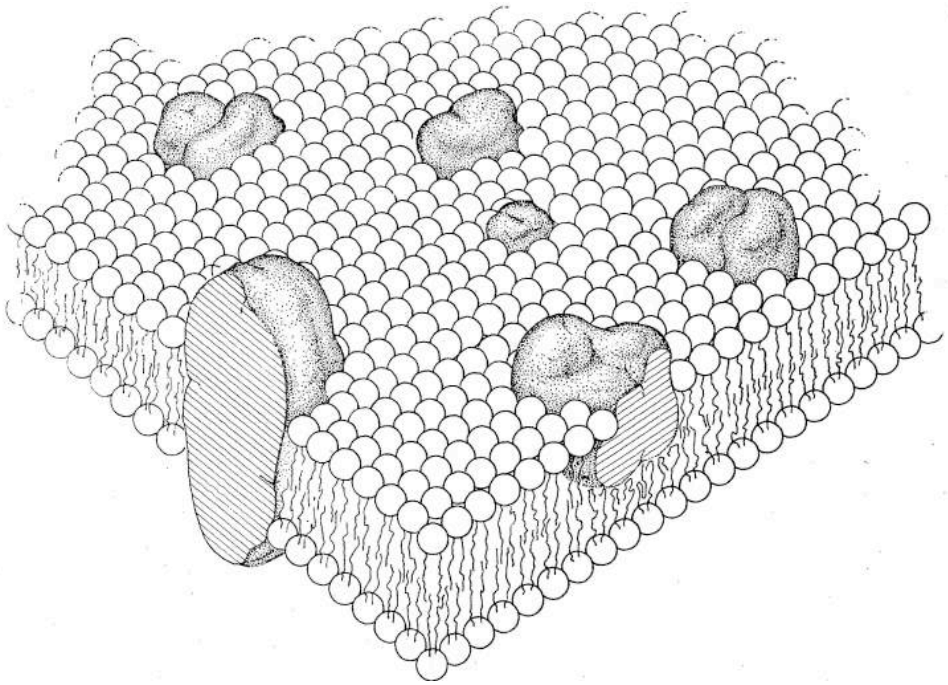


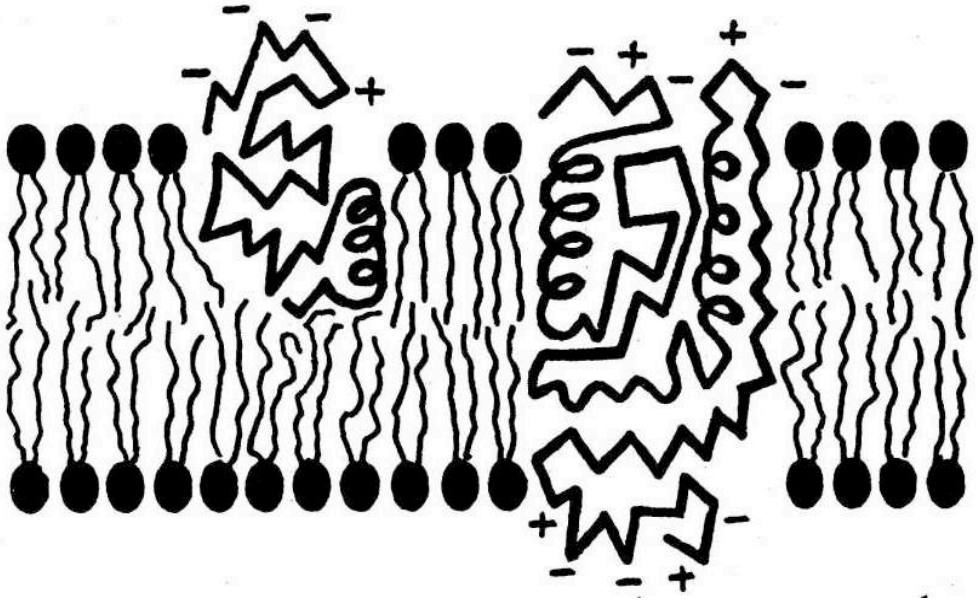
Structure of Biological Membranes

Structure of Biological Membranes

Fluid Mosaic Model

- Singer and Nicolson (1972)
- Fluid Mosaic model
- Currently accepted basic model for cellular membranes



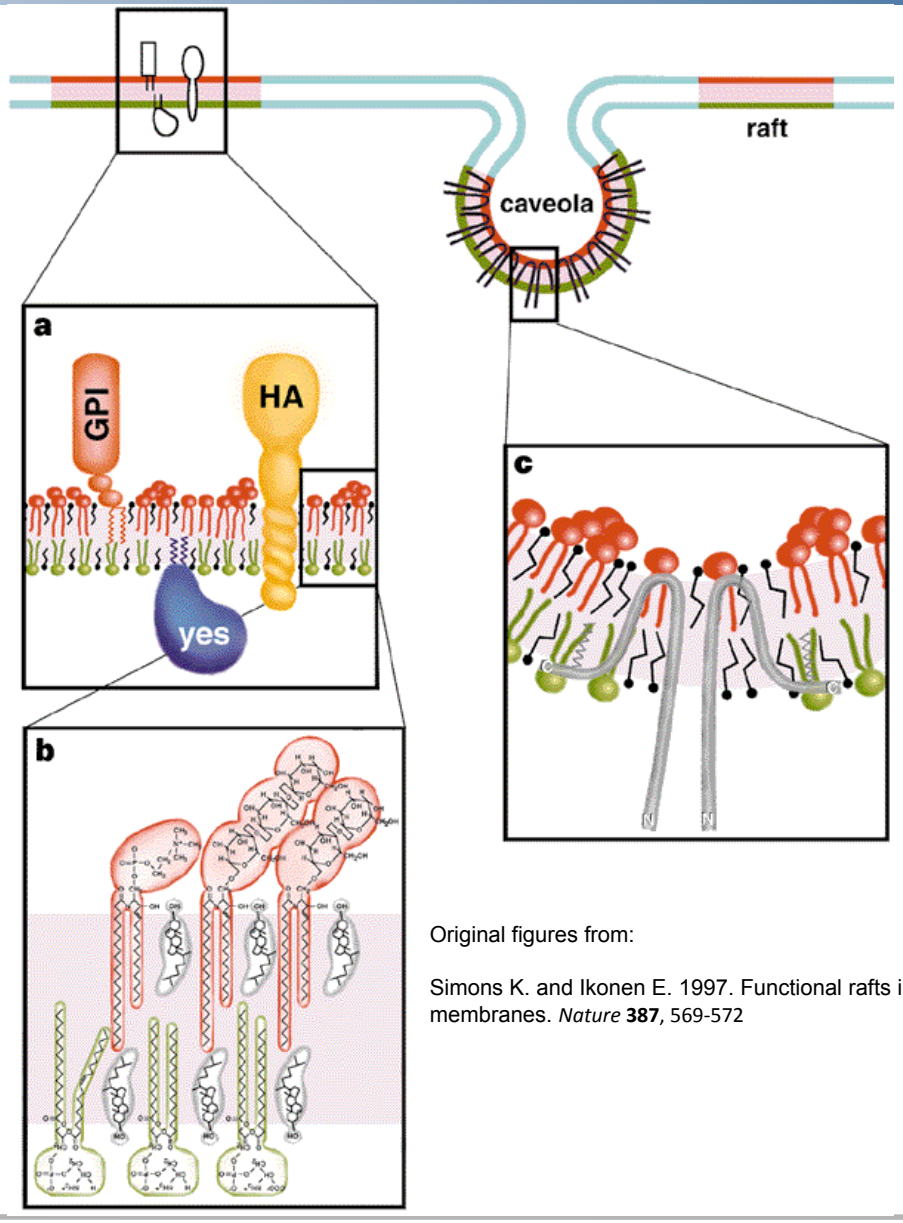


Original figures from: Singer, S.J. and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. Science. 175: 720-731

Structure of Biological Membranes

Lipid Raft Model

- Simons *et al.* (1997)
- Lipid raft model
- Lipids are asymmetrically distributed between the cytoplasmic and exoplasmic leaflets of the membrane. E.g.:
  - Exoplasmic leaflet enriched in sphingomyelin and glycosphingolipids
  - Cytoplasmic leaflet enriched in phosphatidylserine and phosphatidylethanolamine
- Lipids also exhibit a degree of lateral organisation in microdomains termed lipid rafts
- Proteins may be selectively recruited to rafts depending on their composition
- The function of lipid rafts in the cell membrane is currently a growing area of research



Original figures from:

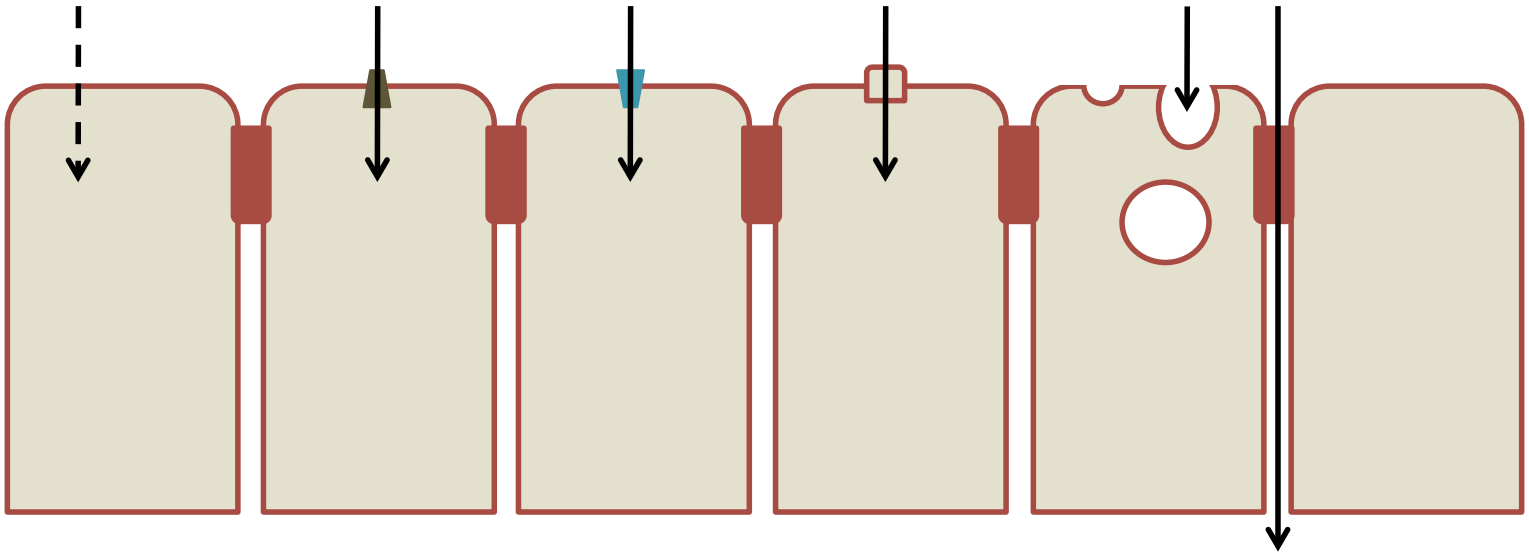
Simons K. and Ikonen E. 1997. Functional rafts in cell membranes. *Nature* **387**, 569-572

Crossing Cell Membranes

Crossing Cell Membranes

Types of Cell Membrane Transport

- There are six main ways for small molecules to cross cell membranes:
  - Passive diffusion through the phospholipid bilayer
  - Facilitated diffusion via transmembrane transport proteins
  - Active transport via transmembrane transport proteins
  - Filtration through aqueous pores in the phospholipid bilayer
  - Pinocytosis
  - Paracellular transport



Passive Diffusion

Diffusion Across Cellular Membranes

Passive Diffusion

- Non-polar substances can be readily dissolved in non-polar solvents
- Therefore they are able to freely diffuse across (and within) the phospholipid cell membrane
- The rate of diffusion across a membrane can be determined by
  - The permeability of the substance in the membrane
  - The concentration gradient across the membrane
- Rapid diffusion across the membrane requires permeant species to be present in the membrane in sufficient numbers and to be freely mobile

Diffusion Across Cellular Membranes

Membrane Permeability

- The permeability of a diffusing species in a cell membrane is largely related to its physicochemical properties, primarily:
  - The diffusivity of the species in the membrane, denoted by the diffusion coefficient.
  - The solubility of the species in the membrane, denoted by the partition coefficient.

- The diffusivity of different small molecules does not vary considerably, and therefore contributes little to the differences observed in membrane permeability between drugs

- Therefore the membrane (lipid) solubility of a molecule is the key factor in determining membrane permeability and rate of diffusion. This physicochemical property is therefore of considerable importance in understanding the transport of drugs

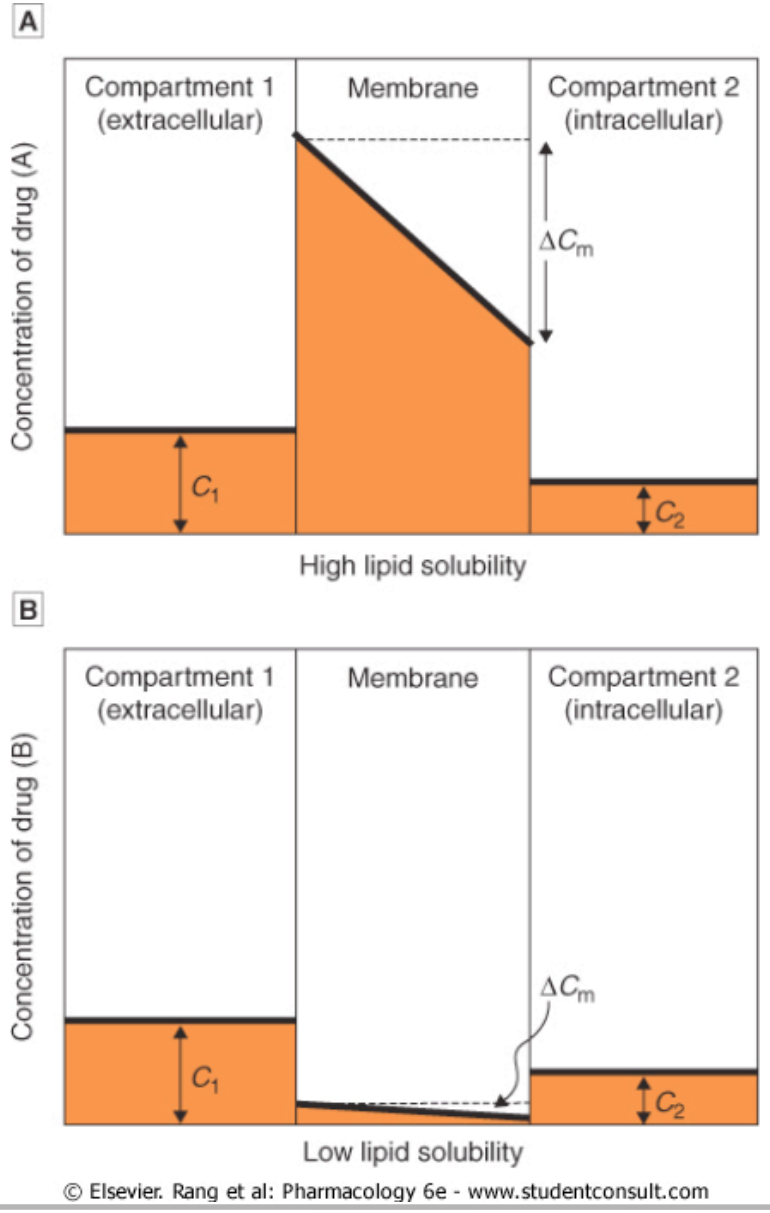
- The usual surrogate for estimating this is the logP octanol-water coefficient, which can be measured or predicted using computational chemistry methods

$$\log P_{oct/wat} = \log\left(\frac{[\text{drug}]_{\text{oct}}}{[\text{drug}]_{\text{wat}}}\right)$$

Diffusion Across Cellular Membranes

Membrane Permeability

- The transmembrane concentration gradient can be denoted ΔC_m
- ΔC_m increases with increasing partition coefficient (P)
- Therefore, the rate of diffusion increases with increasing P



Diffusion Across Cellular Membranes

Membrane Permeability – Molecular Weight

- The partition coefficient (P) is by far the greatest indicator of the rate of diffusion through a cell membranes. It contributes greatly to the overall permeability of a substance in a membrane.

- The diffusivity of different small molecules does not vary considerably, and therefore contributes little to the differences observed in membrane permeability between drugs

- However, the rate of diffusion of species with the same P value can vary to some degree

- Molecular weight is a good indicator of the relative diffusivity of a species, and provides an additional property with which to model diffusion in cell membranes

- Molecules up to a Mw of ~200 can diffuse into and with the membrane, with the rate of diffusion anticorrelated with Mw

- Larger molecules (e,g, sucrose; Mw 300) are excluded from the membrane and do not diffuse

Diffusion Across Cellular Membranes

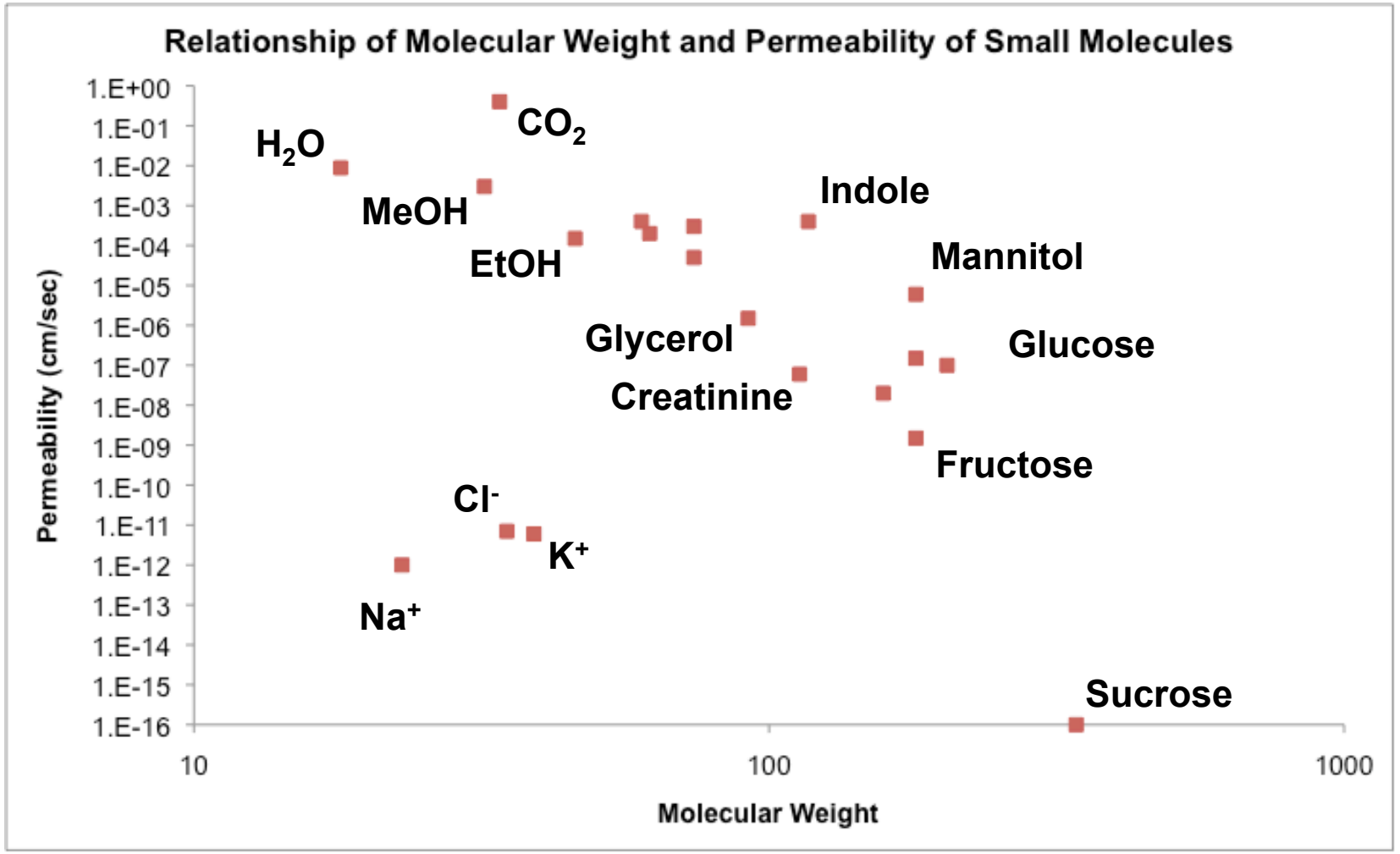
Lipids in Cell Membranes

Solute	Mw	Permeability (cm/s)
Carbon Dioxide	34	4.E-01
Water	18	9.E-03
Methanol	32	3.E-03
Urea	60	4.E-04
Butanol	74	3.E-04
Ethylene glycol	62	2.E-04
Ethanol	46	2.E-04
Methylurea	74	5.E-05
Glycerol	92	2.E-06
Creatinine	113	6.E-08
Indole	117	4.E-04
Uric Acid	158	2.E-08
Glucose	180	2.E-07
Fructose	180	2.E-09
Mannitol	180	6.E-06
Tryptophan	204	1.E-07
Sucrose	342	1.E-16
Na+	23	1.E-12
K+	39	6.E-12
Cl-	35	7.E-12

Adapted from Saltzman, W.M. Drug Delivery – Engineering Principles for Drug Therapy, OUP, 2001

Diffusion Across Cellular Membranes

Molecular Weight and Ionisation Affect Permeability



Membrane Components

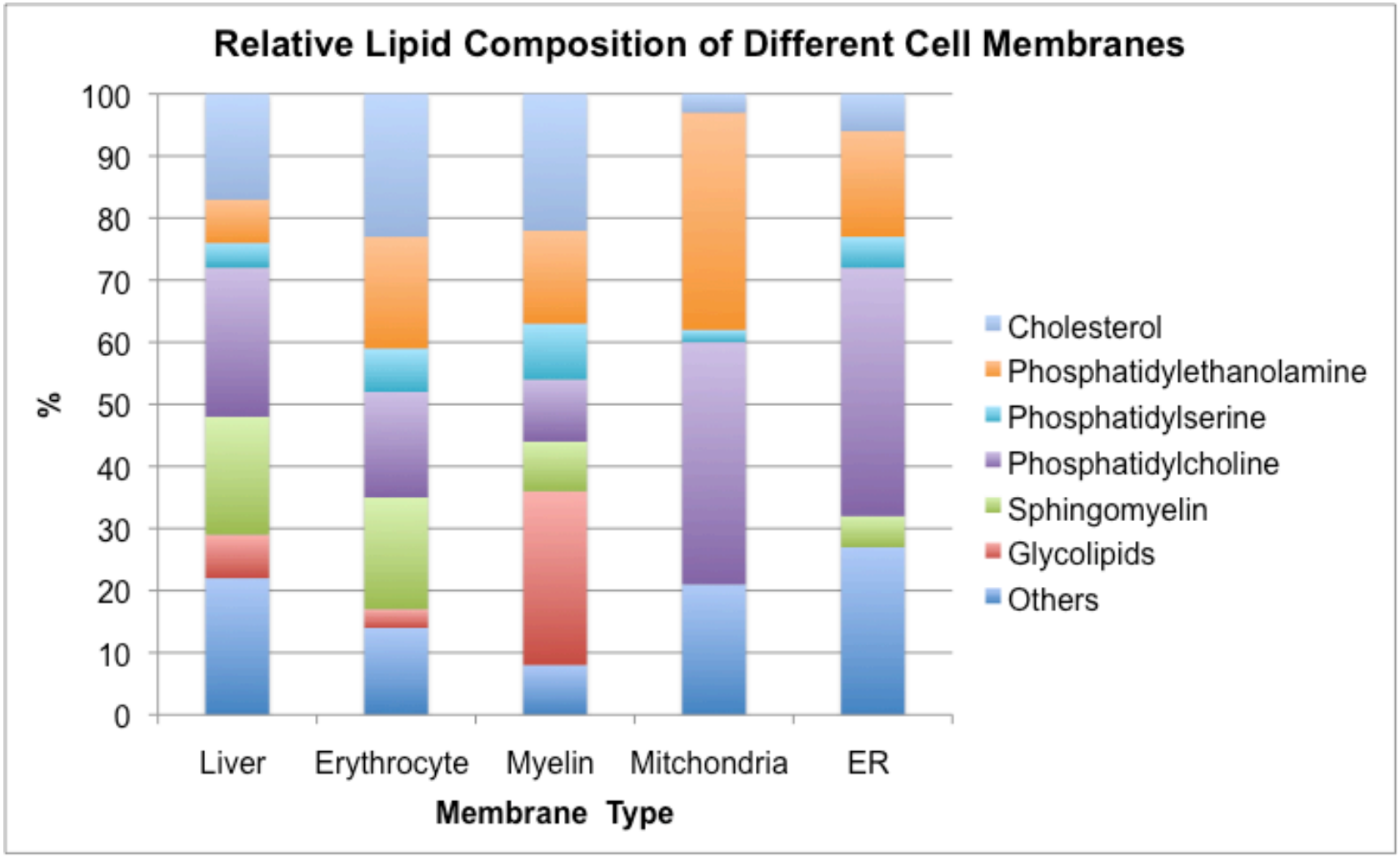
Lipids in Cell Membranes

	Liver	Erythrocyte	Myelin	Mitochondria	ER
Cholesterol	17	23	22	3	6
Phosphatidylethanolamine	7	18	15	35	17
Phosphatidylserine	4	7	9	2	5
Phosphatidylcholine	24	17	10	39	40
Sphingomyelin	19	18	8	0	5
Glycolipids	7	3	28	0	0
Others	22	14	8	21	27

Adapted from Frye, L. and Edidin. The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. Journal of Cell Science, 1970, **7**, 319-335

Membrane Components

Lipids in Cell Membranes



Data from Frye, L. and Edidin. The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. Journal of Cell Science, 1970, **7**, 319-335

pH and Ionisation

pH and Ionisation

Acids and Bases

- The Arrhenius theory of acid dissociation (an acid is a substance that dissociates, releasing H^+)

$HA <- -> H^+ + A^-$

- Brønsted-Lowry acid-base theory (generalised to proton exchange)

acid + base <- -> conjugate acid + conjugate base

- Acid dissociation constant (K_a) describes the equilibrium position of the dissociation and therefore the strength of the acid.

Svante Arrhenius
(1859 – 1927)

Thomas Martin Lowry
(1874 - 1936)

Johannes Nicolaus Brønsted
(1879 - 1947)

pH and Ionisation

Henderson-Hasselbalch Equation:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$\log_{10} K_a = \log_{10}\left(\frac{[H^+][A^-]}{[HA]}\right)$$

$$\log_{10} K_a = \log_{10}[H^+] + \log_{10}\left(\frac{[A^-]}{[HA]}\right)$$

$$-pK_a = -pH + \log_{10}\left(\frac{[A^-]}{[HA]}\right)$$

$$pH = pK_a + \log_{10}\left(\frac{[A^-]}{[HA]}\right)$$

if $[A^-] = [HA]$

$$pH = pK_a$$

pH and Ionisation

pH Affects Membrane Permeation

- The effect of pH and ionisation on diffusion through cell membranes is often considerable for drugs that are weak acids or bases

- The ionised form of will have a very low solutbility in lipid, and therefore exhibit very low diffusion/ permeation of the phospholipid bilayer

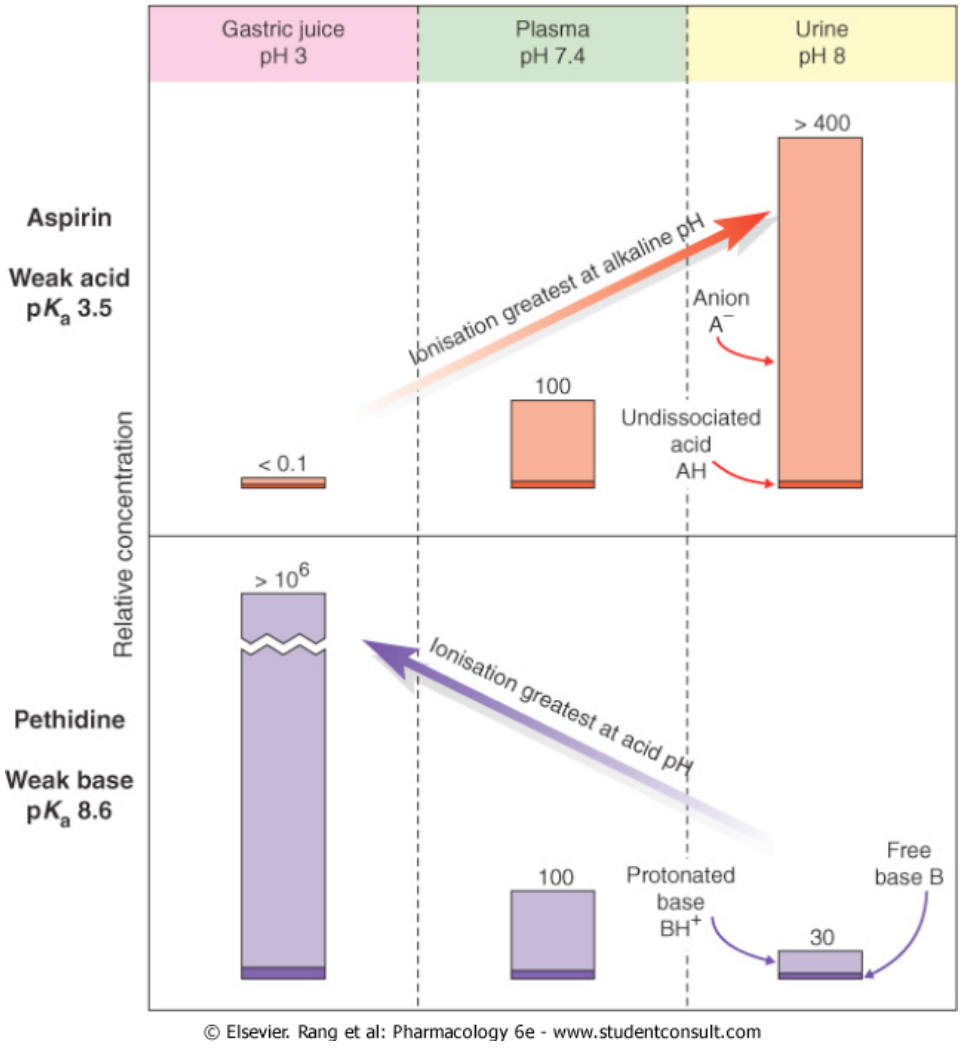
- In some cases the uncharged species is still insufficiently lipid soluble to diffuse
  - e.g. aminoglycosides

- This is usually because of the presence of groups that form hydrogen bonds
  - e.g. –OH

pH and Ionisation

pH Partitioning

- pH partitioning may result from ‘ion trapping’
- The differing pH of different biological tissues and fluids can result in partitioning where there are differences in the drug concentration
- The steady-state equilibrium position of charged and uncharged species will differ with pH for drugs that are weak acids and bases
- The ratio of the ionised and unionised species in each partition will differ as a result of the pH
- Therefore the total (ionised + unionised) drug concentration will differ
- The ideal (modelled) behavior of partitioning partially helps understand observed behavior



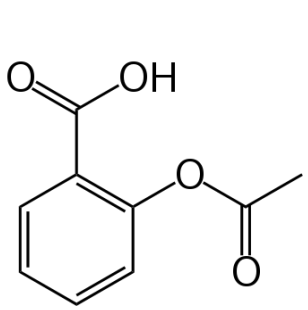
pH and Ionisation

pH Partitioning (2)

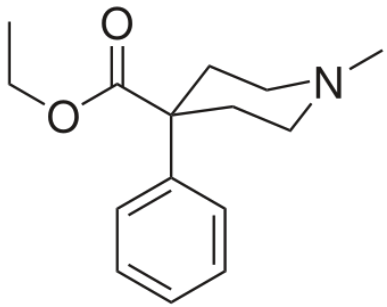
- Example:
  - aspirin (weak acid, pKa = 3.5)
  - pethidine (weak base, pKa = 8.6)

- Diffusion will result in a constant ratio of the unionised (diffusing) species *between* the partitions

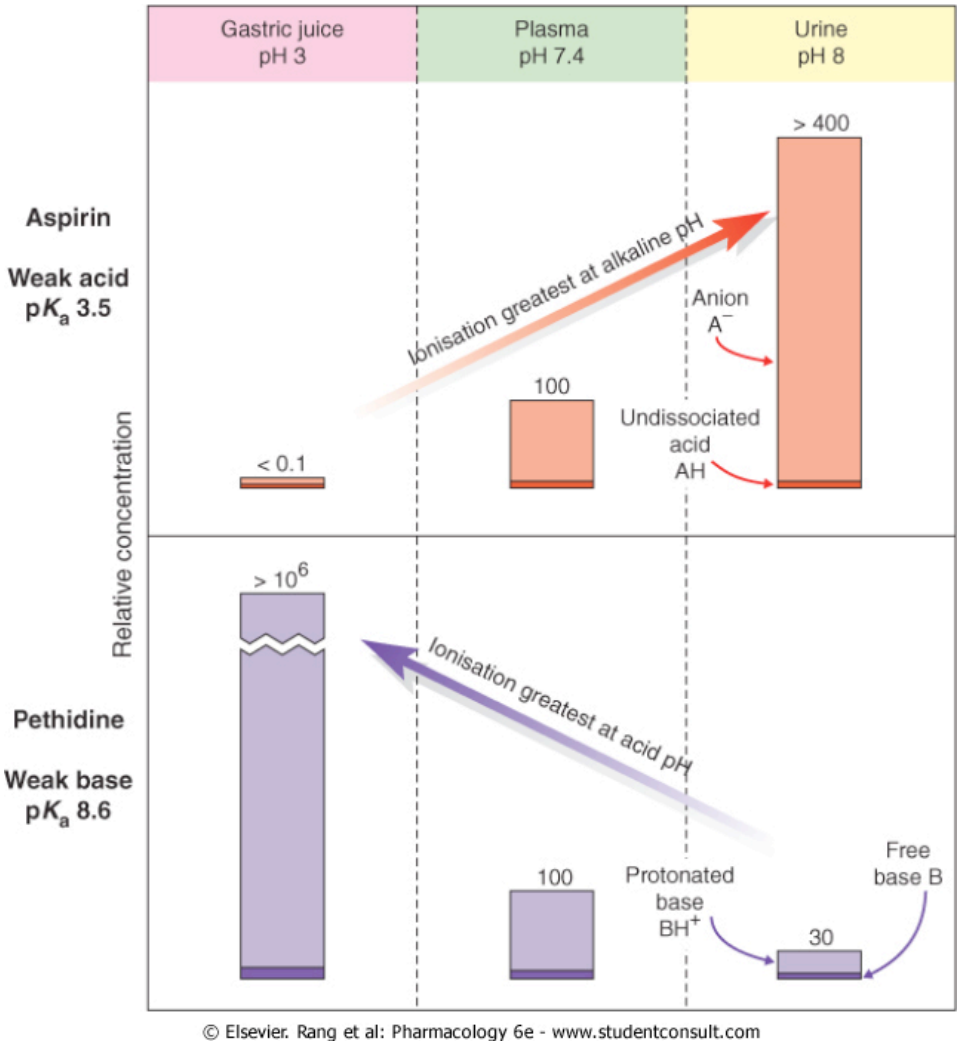
- The differing pKa of the two drugs results in a different distribution of *total* concentration



Aspirin

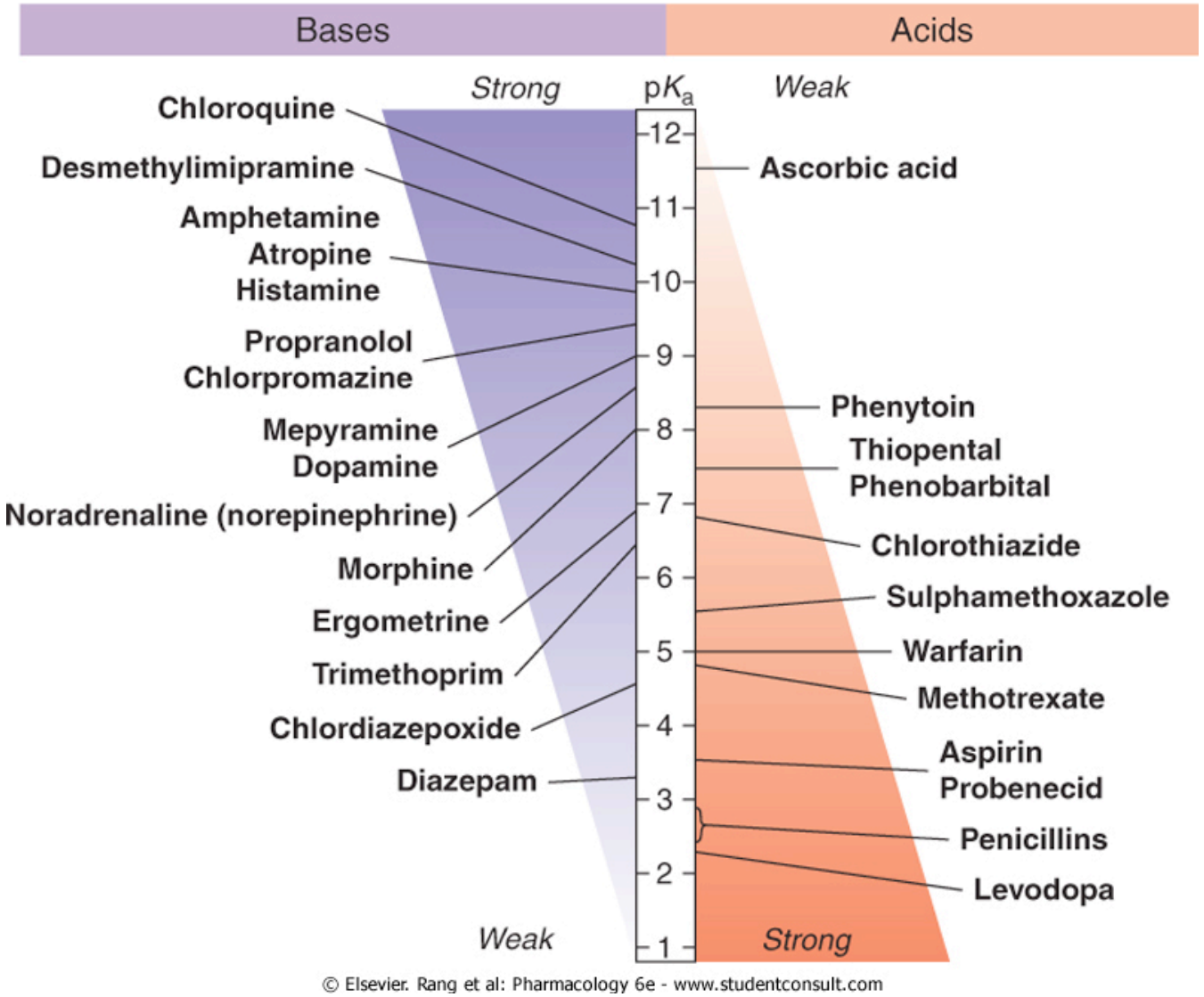


Pethidine



pH and Ionisation

pKa Values of Common Drugs



Carrier-Mediated Transport

Carrier-Mediated Transport

Introduction

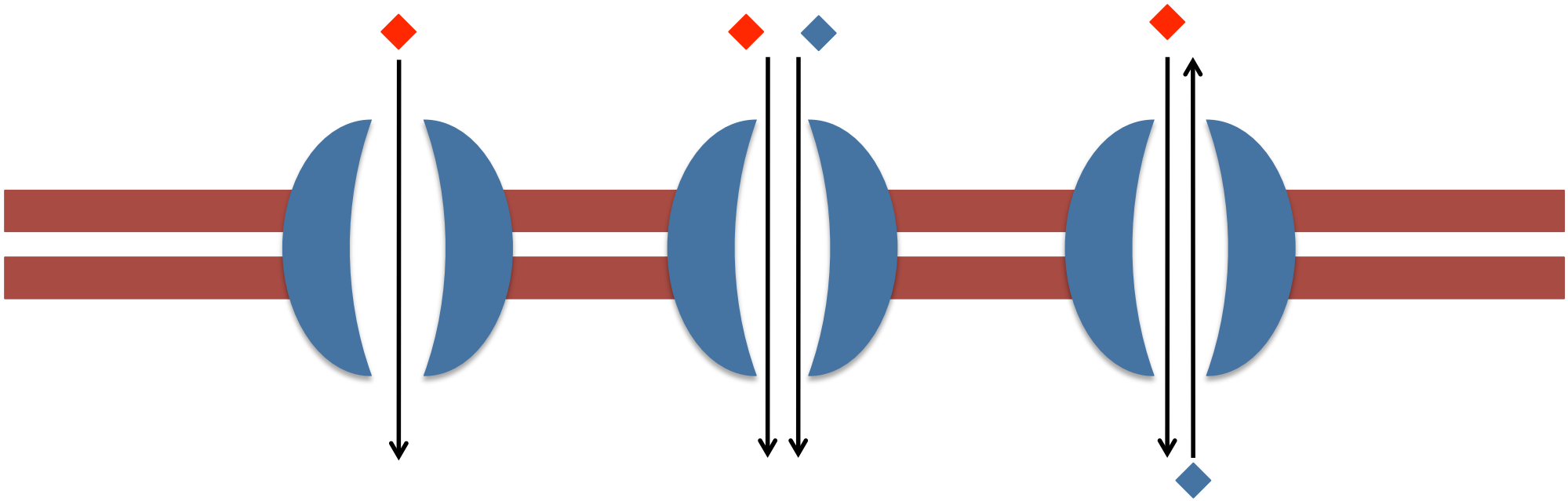
- In addition to diffusion, transport across membranes can occur by carrier-mediated processes:
  - Facilitated diffusion
  - Active transport

- Proteins in the membrane facilitate the transit of ions and molecules.
  - The range of molecules that undergo transport depends on the type of transporter
  - Some transporters are more substrate-specific than others

Carrier-Mediated Transport

Transport Processes

- It is useful to describe transporters according to the net translocation of substrates
  - Uniport – the movement of a single molecule at a time
  - Symport – the movement of two molecules in the same direction simultaneously
  - Antiport – the movement of two molecules in opposite directions simultaneously



Facilitated Diffusion

Carrier-Mediated Transport

Facilitated Diffusion

- The model of non-mediated transport by diffusion through cellular membranes does not appear to hold for some molecules and ions

- The permeability of some molecules (and ions) is much higher than would be expected

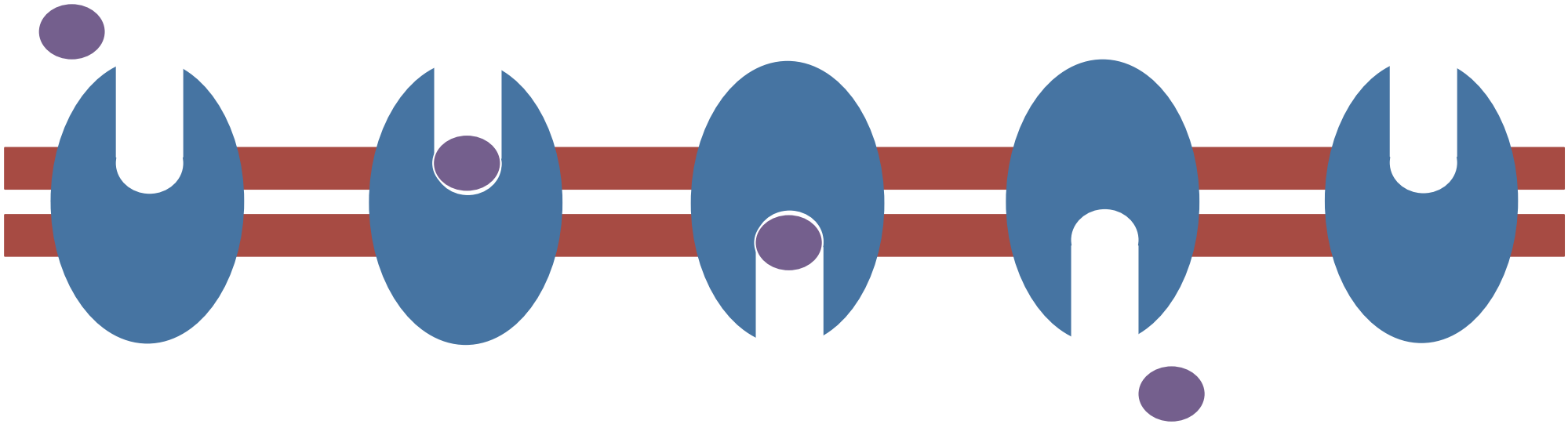
- Transport proteins in the membrane allow transport of molecules *via* different mechanisms and provide an alternative/additional route to diffusion through the membrane

- There are <u>three</u> main types of transport protein that facilitate passive diffusion:
  - Gated pores
  - Ligand-gated ion channels
  - Voltage-gated ion channels

Carrier-Mediated Transport

Facilitated Diffusion – Gated Pores

- Example: Glucose transporter
  - Part of the solute carrier (SLC) superfamily of membrane transport proteins
- Glucose binds to the transporter
- The binding of glucose causes a conformational change in the transporter
  - Closes the site of entry
  - Opens the site of exit
- Glucose dissociates from the transporter
- The transporter undergoes conformational change (recovery) to return to the original state

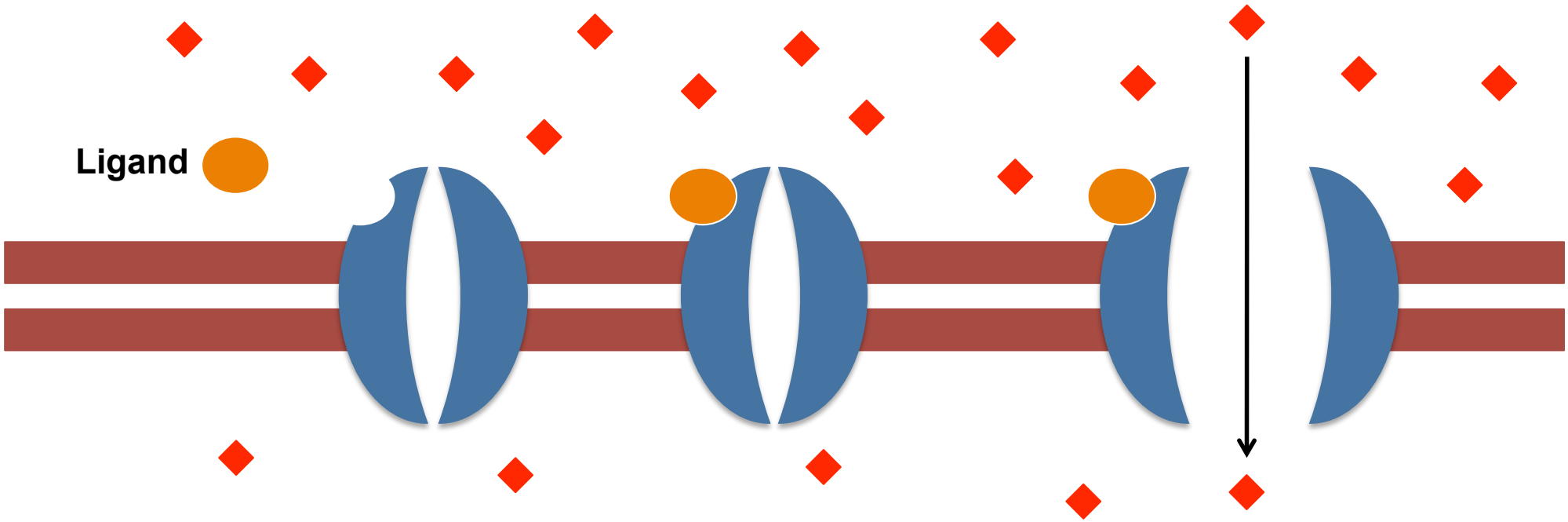


Carrier-Mediated Transport

Facilitated Diffusion – Ligand-Gated Ion Channel

- Some transmembrane proteins alter their conformation when bound with a ligand (usually a small molecule)

- On binding with an appropriate ligand, the protein opens up a channel that is pervious to aqueous species (e.g. ions) that would not normally diffuse through the membrane.



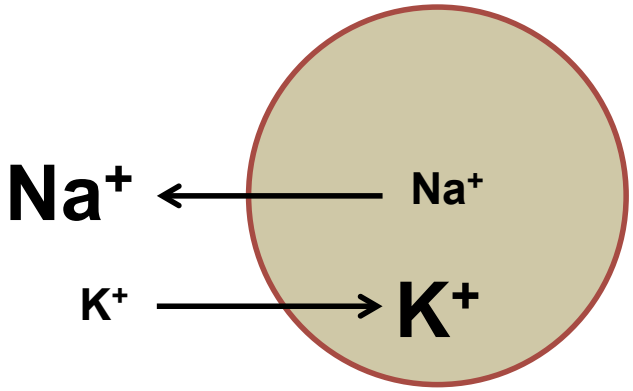
Carrier-Mediated Transport

Facilitated Diffusion – Voltage-Gated Ion Channel

- Some ion channels change confirmation depending on the potential across the plasma membrane

- Example: Neurons and nerve impulses

- In a resting neuron, the interior of the axon is maintained with:
  - a net negative charge
  - a lower concentration of sodium ions
  - a higher concentration of potassium ions



Carrier-Mediated Transport

Facilitated Diffusion – Voltage-Gated Ion Channel

- Sufficient depolarisation and opening of voltage-gated sodium ion channels in the membrane at a point causes adjacent voltage-gated sodium ion channels to open

- On opening, the channels allow influx of sodium ions down the electrochemical gradient to the interior

- This causes further local depolarisation of the membrane and the propagation of the impulse

- The net result of this is a 'wave' of depolarisation that travels along the cell (a nerve impulse)

- The mechanism results in a non-attenuated signal transmission along the neuron

- The resting state is restored (after ~1 ms) by proteins known as sodium/potassium ATPase
  - Pump two K^+ into the cell Pump three Na^+ out of the cell
  - Net result is restored polarisation of the membrane

Active Transport

Carrier-Mediated Transport

Active Transport

- Active transport of species across membranes requires the usage of high-energy

- Most typically the energy is provided by the hydrolysis of ATP

- *Indirect active transport* – the mechanism uses the chemical gradient that has already been acquired (by active transport of ions). The energy required to move a chemical species against its own chemical gradient is harnessed by the facilitated diffusion of the ions down their own gradient

- *Direct active transport* – transport proteins use ATP to move chemical species against the chemical gradient

Carrier-Mediated Transport

Indirect ATP-Driven Active Transport

- Indirect active transport occurs by symport of antiport proteins. Key examples are:

- Symport
  - Na^+ / glucose symport transporter (pump)
    - 1 Na^+ ion and 1 glucose molecule move across the membrane

- Antiport
  - Na^+ / Ca^{2+} exchanger (NCX)
    - 3 Na^+ ions in and 1 Ca^{2+} out

Carrier-Mediated Transport

Direct ATP-Driven Active Transport

- There main ATP-driven transporters are:

- Na^+ / K^+ ATPase
  - Uses 1 ATP to transport 3 Na^+ ions out and 2 K^+ ions in

- Ca^{2+} ATPase
  - Uses 1 ATP to transport 1 Ca^{2+} ion out of the cell

- H^+ / K^+ ATPase
  - Uses 1 ATP to transport 1 H^+ ion out and 1 K^+ ion in

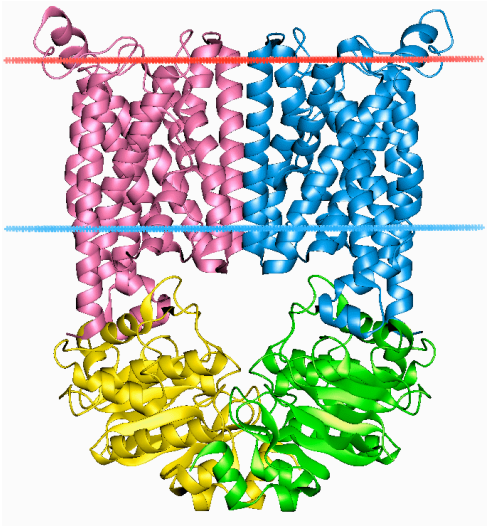
- ATP Binding Cassette transporters
  - Ligand transport (usually highly specific for each isoform)
  - Uses 1 ATP to transport a single ligand

Drug Transport

Drug Transporters

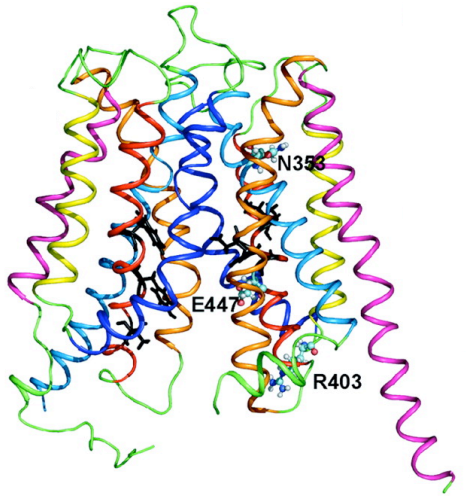
Superfamilies of Drug Transporters

- ABC Superfamily
  - *A*TP-*B*inding *C*assette superfamily



BtuCD

- SLC Superfamily
  - *O*rganic *A*nion *T*ransporters (OATs)
  - *O*rganic *C*ation *T*ransporters (OCTs)



OCT2

Drug Transporters

ABC Transporters

Transporter	Isoform	Typical Substrates	Sites in the Body
ABC Superfamily			
P-glycoprotein / MDR1[a]	ABCB1	Hydrophobic and cationic (basic) molecules; numerous drugs, including anticancer drugs	Apical surface of epithelial cell membranes. Liver, kidney, lung, intestine, BBB, testes, placenta,
MRP1[a]	ABCC1	Numerous molecules, including anticancer drugs, glucuronide and glutathione conjugates	Basolateral surface in most cell types. Kidney, lung, testes, blood:tissue barriers.
MRP2[a]	ABCC2	Numerous molecules, including anticancer drugs, glucuronide and glutathione conjugates	Apical surface of membranes. Liver, kidney, intestine
BRCP	ABCG2	Anticancer, antiviral drugs, fluoroquinolones, flavinoids	Apical surface of breast ducts and lobules. Intestine, colon, liver, placenta, BBB, lungs

From Waller D. G. et al. Medical Pharmacology and Therapeutics 3rd Edition, Saunders / Elsevier Ltd. 2010.

Drug Transporters

SLC Transporters

Transporter	Isoform	Typical Substrates	Sites in the Body
SLC Superfamily			
OAT1	SLC22A6	Numerous molecules, including PAH, NSAIDs, penicillins, diuretics and phase II drug metabolites	Kidney (BL), brain, placenta, smooth muscle
OAT2	SLC22A7	Salicylate, acetylsalicylate, PGE_2, dicarboxylates and PAH	Kidney (BL), liver
OAT3	SLC22A8	Similar to OAT1	Kidney (BL), liver, brain, smooth muscle
OAT4	SLC22A11	Steroid sulfate conjugates	Kidney (AP), placenta
OCT1	SLC22A1	Serotonin, noradrenaline, histamine, agmatine, aciclovir, ganciclovir	Liver, kidney, intestine, heart, skeletal muscle, placenta
OCT2	SLC22A2	Serotonin, noradrenaline, histamine, agmatine, amantidine, cimetidine	Kidney, placenta, adrenal gland, neurons, choroid plexus
OCT3	SLC22A3	Serotonin, noradrenaline, histamine, agmatine	Liver, kidney, intestine, skeletal and smooth muscle, heart, lung, spleen, neurons, placenta, choroid plexus

From Waller D. G. et al. Medical Pharmacology and Therapeutics 3rd Edition, Saunders / Elsevier Ltd. 2010.

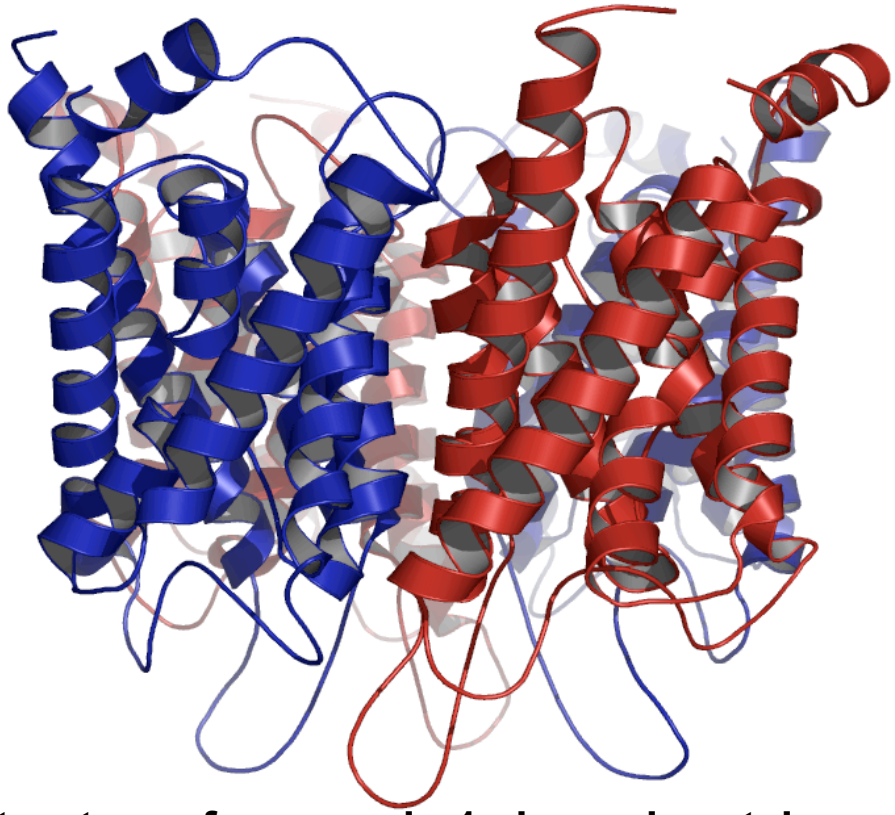
Crossing Cell Membranes

Filtration

Filtration

- The movement of water and solutes down a hydrostatic pressure gradient
  - E,g, pressure exerted on cardiovasular system

- Facilitated by aquaporins and aquaglyceroporins
  - Exhibit selectivity
  - Water and small solutes only



Structure of aquaporin 1 channel protein

Crossing Cell Membranes

Pinocytosis

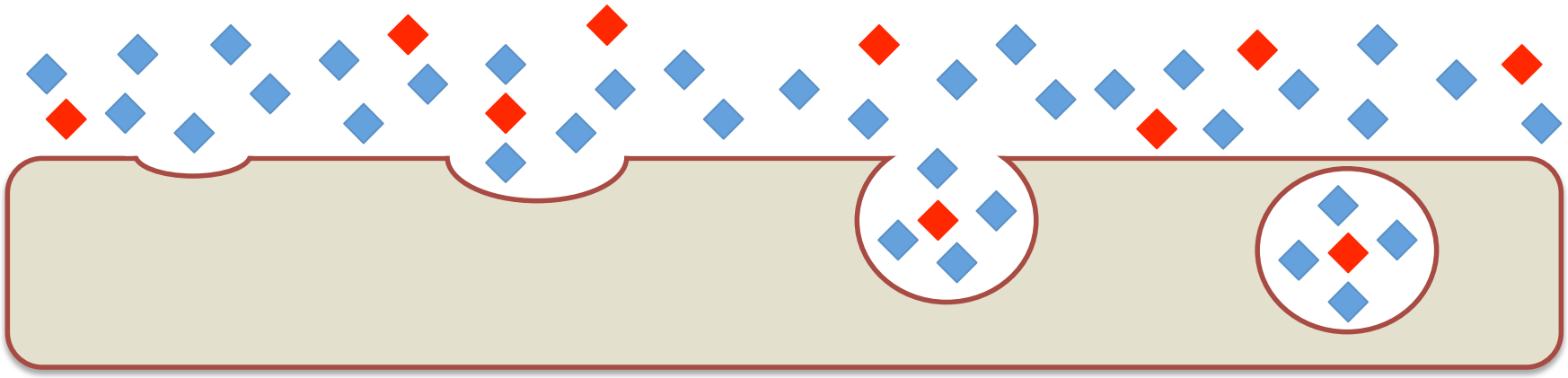
Pinocytosis

Transport by Pinocytosis

- A form of endocytosis that allows the non-specific entry of extracellular fluid and contents to the cell by way of the formation of vesicles. It requires a usage of ATP.

- The main stages in pinocytosis are:
  - Invagination of the cell membrane
  - Pinching/fusing of the invaginated area to form a vesicle
  - Movement of the vesicle away from the cell membrane

- It is not a major route of drug transport across cellular membranes apart from protein drugs or those with appropriate formulation

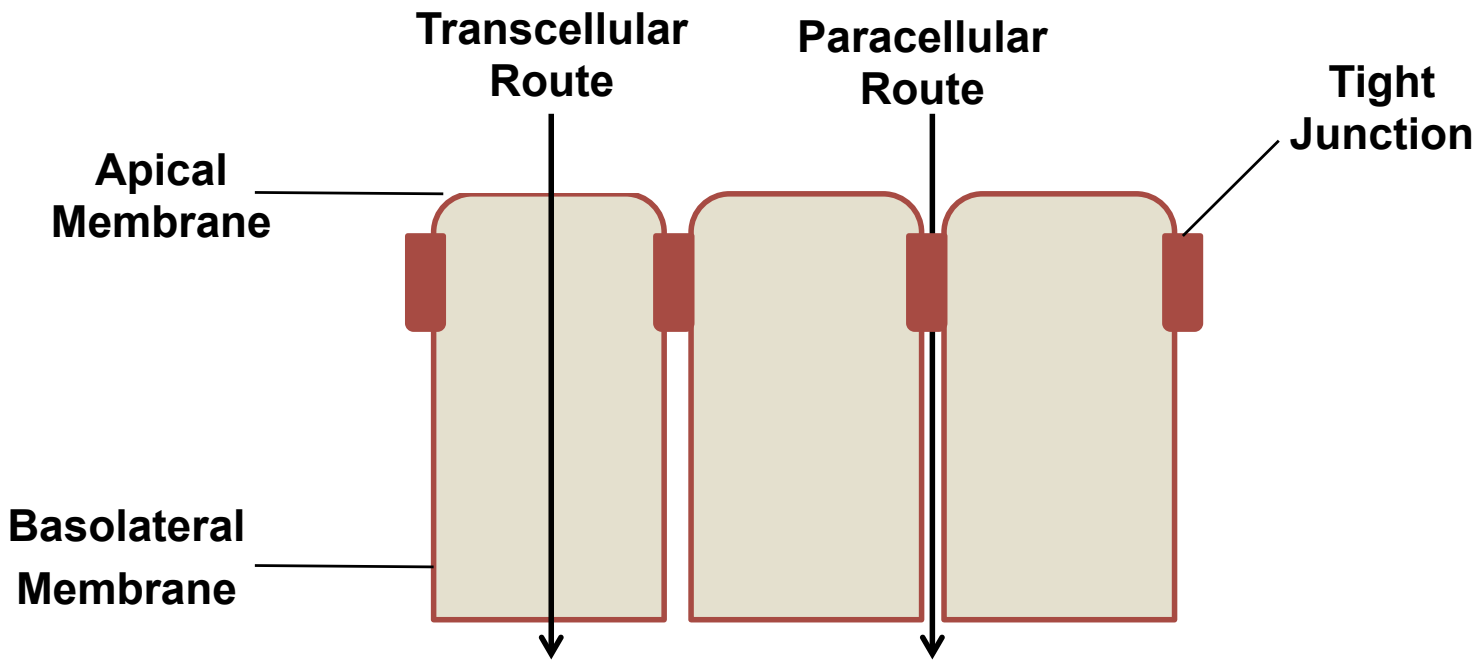


Paracellular Transport

Paracellular Transport

Transport by the Paracellular Route

- Movement of substances in between epithelial cells through the tight junctions that join them
- Most cells experience some paracellular transport
- Paracellular transport does not normally occur in the capillaries of the Blood Brain Barrier (BBB)
- Transport across the tight junctions is under cellular control / regulation



Lecture Summary

Main Points

- There are a variety of cellular membranes that are comprised largely of phospholipids and proteins

- Cell membranes consist of a phospholipid bilayer in which a variety of functional proteins can move freely (or in relation to microdomains with varying composition)

- Cell membranes serve as a barrier between adjacent aqueous compartments and control movement of electrolytes and solutes selectively or allow diffusion of molecules with appropriate physicochemical properties

- The ability of molecules to permeate the membrane by diffusion is a function of a number of factors including the relative proportions of the membrane lipid components, the physicochemical properties of the solute in question, and the transmembrane concentration gradient

- Cell membranes contain a variety of proteins that allow the passage of solutes by facilitated diffusion and active transport. Among these are ABC and SLC transporters that affect drug transport

- Other minor routes of transport such as pinocytosis and paracellular transport are also possible

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