Semester I.
Seminar 4.

Dr. Balázs Varga
University of Debrecen, Faculty of Medicine,
Department of Pharmacology and Pharmacotherapy

Topics

7.

- Desensitization, tachyphylaxys and tolerance
- Indirectly acting parasympathomimetics
- Drugs used in the treatment of hyperlipidemias

8.

- The movement of drugs through biological membranes
- Structure-activity relationships demonstrated among sympathomimetics
- Drugs used for the treatment of congestive heart failure

PRESCRIPTION WRITING!

Desensitization, tachyphylaxis and tolerance – clarifying the terms

- Often, the effect of a drug gradually diminishes when it is given continuously or repeatedly (the biological response decreases)

- *Desensitisation and tachyphylaxis* are often used synonymously, however **desensitisation** is rather a **mechanism** of effect-decline, while **tachyphylaxys** is rather the name of a **phenomenon**:
- Tachyphylaxis is a sudden onset drug effect-decline, which often develops in the course of a few minutes, and which is not dose dependent

- The term *tolerance* is conventionally used to describe a longer-term decrease in responsiveness to a drug, taking days or weeks to develop

- *Drug resistance* is a term used to describe the loss of effectiveness of antimicrobial or antitumour drugs

Mechanisms of drug effect-decline

Many different mechanisms can give rise to this type of phenomenon. They include:

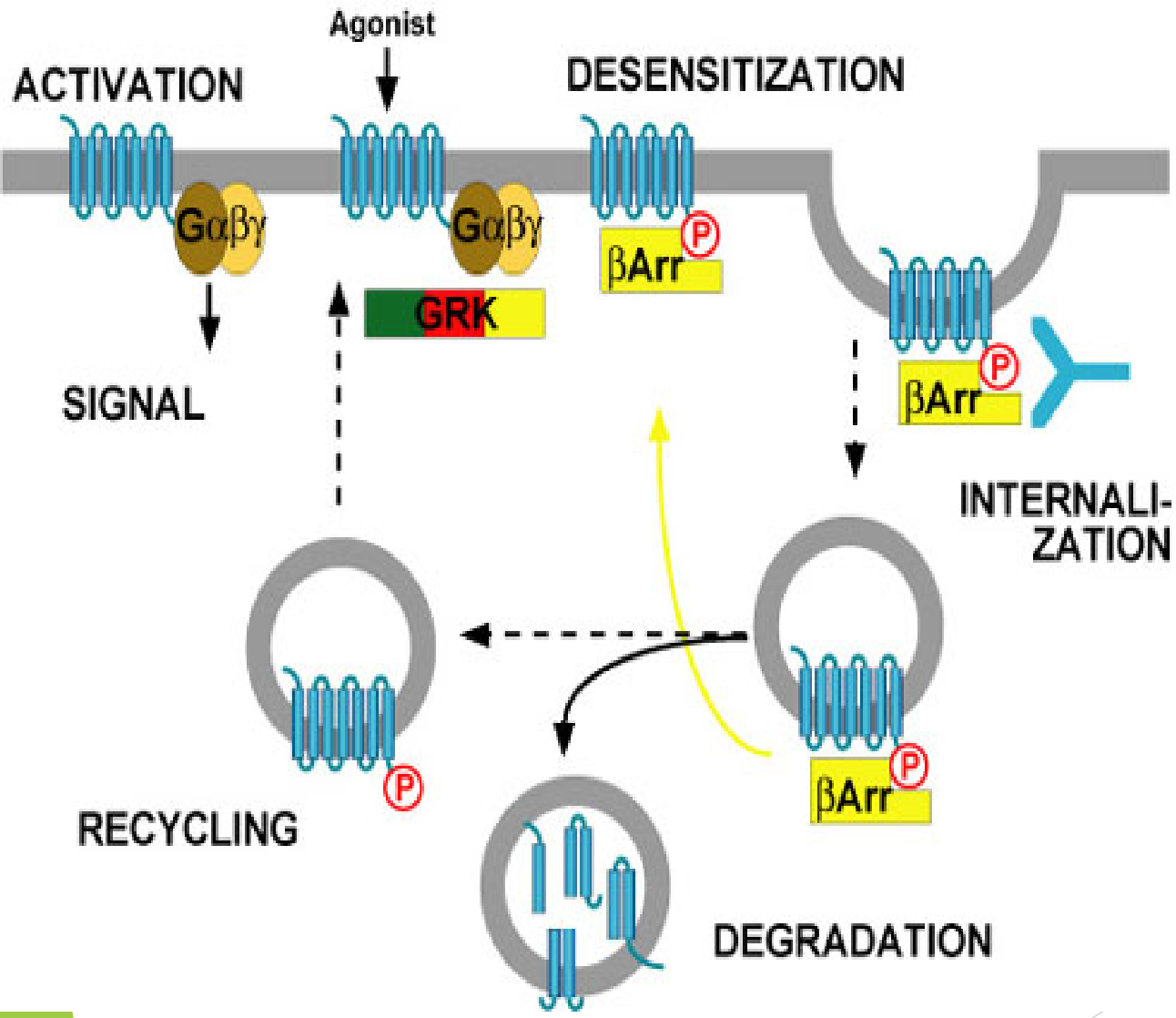
- • change in receptors
- • translocation of receptors
- • exhaustion of mediators
- • altered drug metabolism
- • physiological adaptation
- • active extrusion of drug from cells (mainly relevant in cancer chemotherapy)
- • formation of antibodies against the drug (e.g.: insuline)

Desensitization

change in receptors + translocation of receptors

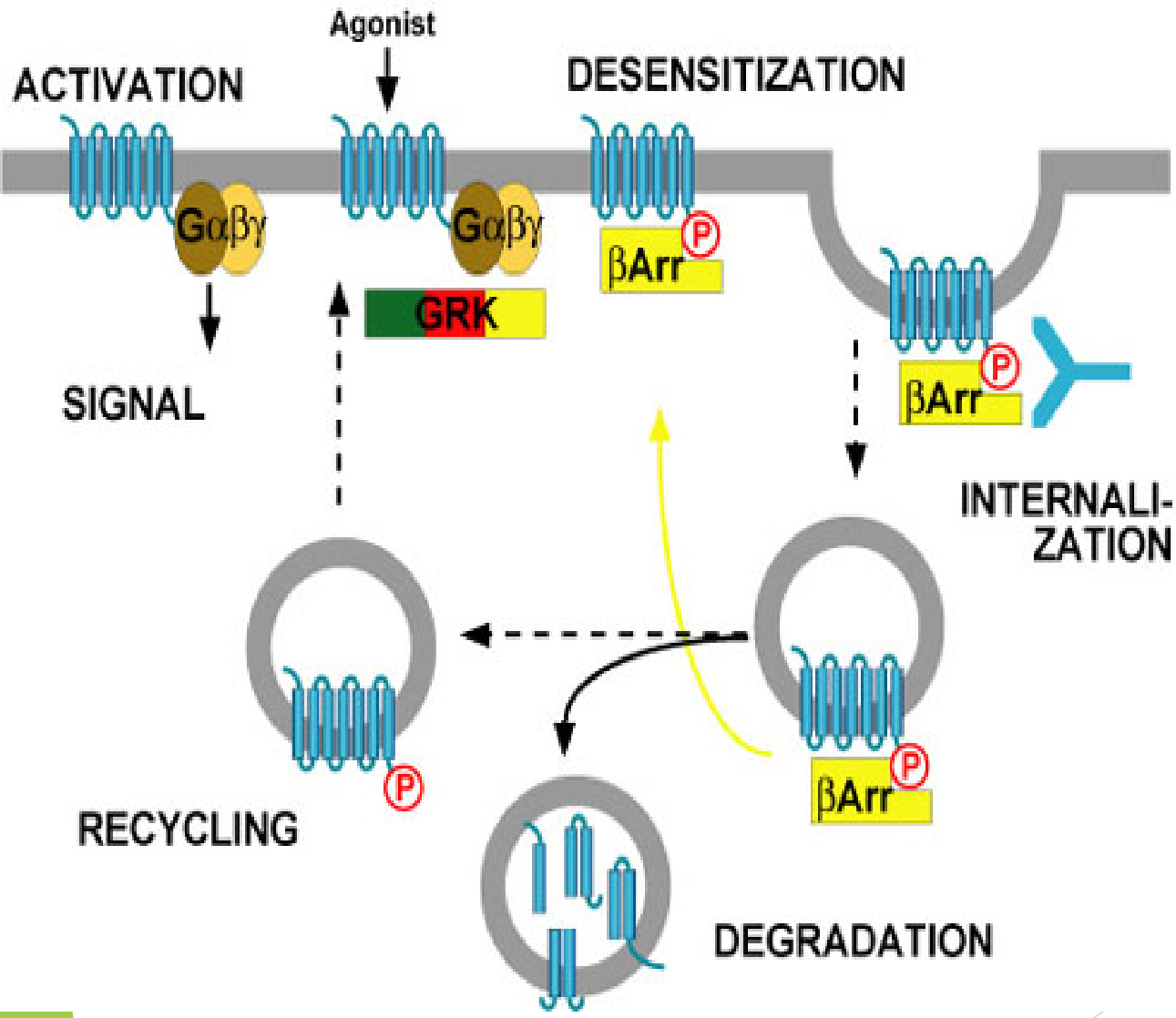
- A self-protecting mechanism against excessive stimuli
- The cell first cuts off the secondary messenger pathways ➔ uncoupling;
  later: receptor internalisation ➔ down-regulation
- The molecular mechanisms of this ‘uncoupling’ were first described in 1998 (*Lefkowitz*)
  - The receptor is still presented on the cell surface, but the function is lost
- Most common along G-protein-coupled receptors
  (they are targets for more than 30% of all prescription drugs)

Desensitisation – 1. Uncoupling



- Binding of an agonist to the receptor triggers a **signal**
- binding also converts the receptor into a **substrate for** a family of kinases, the G-protein-coupled receptor kinases (**GRK**s)
- these kinases **phosphorylate** only agonist-activated receptors
- the phosphorylated receptor becomes a binding partner for **arrestin**s
- This binding makes the receptor **inacessible** for G-proteins (the arrestin-bound receptor is desensitised)

Desensitisation – 2. Internalization (slower process)



- Arrestin-binding **target**s the receptor for internalisation as well
- This is because arrestins also bind components of **clathrin**-coated pits.
- Thus, arrestin-bound receptors move into clathrin-coated pits and are then internalised
- The internalised receptors are taken into the cell by **endocytosis** of patches of the membrane
- Receptors are later **degraded or recycled** to cell surface

Internalization

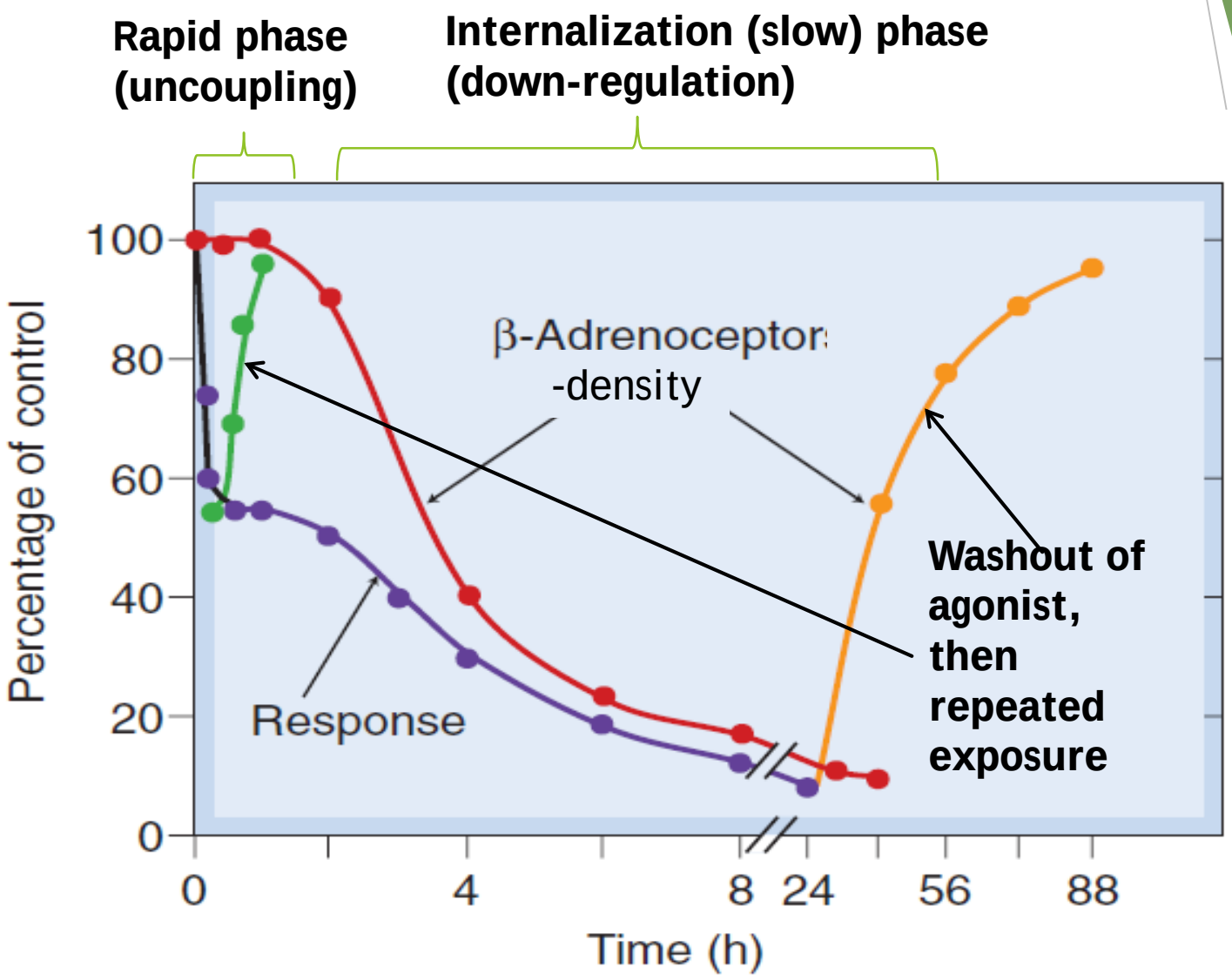
- This type of adaptation is common for beta-receptors and hormone receptors
- It is generally an unwanted complication when drugs are used clinically, but it can be exploited

- For example, **gonadotropin-releasing hormone (GnRH)** is used to treat endometriosis or prostatic cancer

because given continuously, this hormone paradoxically inhibits gonadotropin release

(in contrast to the normal stimulatory effect of the physiological secretion, which is pulsatile)

Desensitisation



Rang and Dale

Tachyphylaxis

- **Tachyphylaxis** (Greek *tachys*, "rapid", and *phylaxis*, "protection")
- is a term describing an acute (**sudden**) **decrease** in the response to a drug after its administration.
- It can occur after an initial dose or after a series of small doses

- Can caused by:
  - rapid desensitisation (see earlier)
  - Or rapid exhaustion of mediators (depletion)
    - depletion or marked reduction of the amount of neurotransmitter responsible for creating the drug's effect (e.g.: ephedrine, amphetamin)

Examples of tachyphylaxis

- Beta-2 agonists (asthma treatment)
  - Especially in case of long acting agonists (formoterol)
  - Both Tachyphylaxis and tolerance occur against bronchoprotective and bronchodilating effect
- Topical glucocorticoids are used often in dermatology.
  - *Intermittent* pulse therapy — treatment for several days or weeks alternating with treatment-free periods — may prevent development of tachyphylaxis
- Desmopressin (vasopressin V2-rec agonist)
  - used against bleeding by elevating vWF levels.
  - Clinical usage is limited because of tachyphylaxis and tolerance

Tolerance

- Drug tolerance is a pharmacological concept describing subjects' **reduced reaction** to a drug following its repeated use.
  - **Increasing the dosage** may **re-amplify** the drug's effects,
  - however this may accelerate tolerance
- taking days or weeks to develop (much **slower decrease**)

- characteristics of drug tolerance:
  - it is reversible,
  - the rate depends on
    - the particular drug and
    - dosage and
    - frequency of use,
  - for different effects of the same drug tolerance may develop differently (i.e. to varying degrees)

- Two main types:
  - Pharmacodynamic tolerance
  - Pharmacokinetic tolerance

Pharmacodynamic tolerance

- Pharmacodynamic tolerance occurs because of **reduced response** (cellularly) to a substance with repeated use.
- This may be caused by
  - Slow receptor desensitization,
    - a reduction in receptor density
  - Slow exhaustion of mediators/enzyme-cofactors (depletion)
- Important: blood levels of the drug do not change in case of pharmacodynamic tolerance
- Example: opioids (morphine)

Pharmacokinetic tolerance

- Pharmacokinetic tolerance occurs because of **a decreased quantity** of the substance reaching the site it affects.
- This may be caused by an increase in induction of the **metabolizing enzymes** required for degradation of the drug e.g. CYP450 enzymes
- This type of tolerance is **most evident with oral ingestion**, because other routes of drug administration bypass first-pass metabolism.
- Example: alcohol, carbamazepine – induce own metabolism

Examples – Nitrate tolerance

(exhaustion of mediators in the long term)

Repeated administration of nitrates to smooth muscle preparations results in diminished relaxation, possibly partly because of depletion of free –SH groups

↓

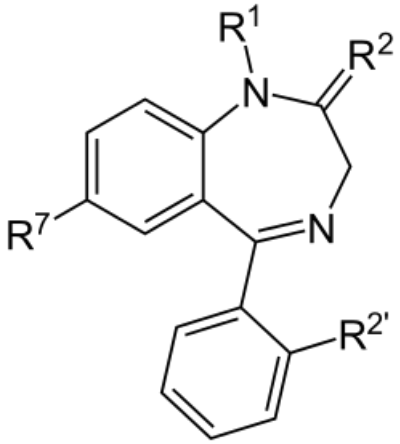
Tolerance to the antianginal effect of nitrates

- **does not occur** to a clinically important extent with ordinary formulations of **short-acting** drugs (e.g. glyceryl trinitrate),
- but **does occur with longer acting drugs** (e.g. isosorbide mononitrate)
- or when glyceryl trinitrate (**short-acting**) is administered by frequent application of **slow-release** transdermal patches

Examples – Tolerance to benzodiazepines

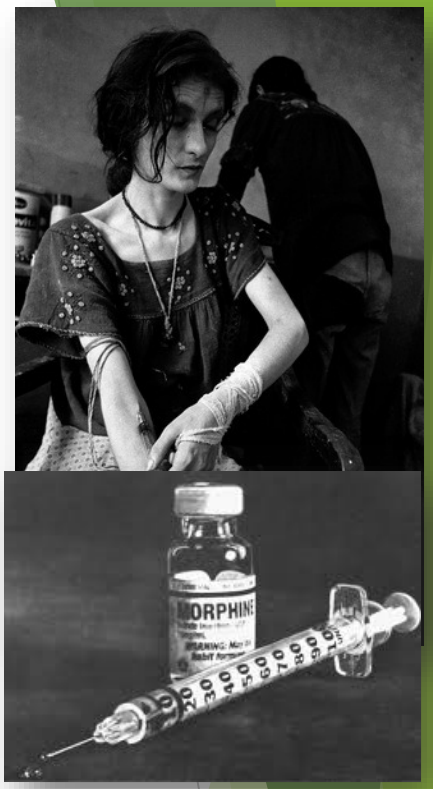
(differential tolerance)

- *A subject of debate is the development of tolerance to the anxiolytic effects of benzodiazepines.*
- *However, tolerance does develop to the muscle relaxant effects of these drugs*



Examples - Tolerance to opioids

(differential tolerance)



- Tolerance to many of the actions of opioids develops within a few days during repeated administration
- Including:
  - analgesia
  - emesis,
  - euphoria and
  - respiratory depression,
- but affects much less:
  - the constipating and
  - pupil-constricting actions.
- Therefore, addicts may take 50 times the normal analgesic dose of morphine with relatively little respiratory depression but marked constipation and pupillary constriction
- Cross-tolerance may occur between opioids acting at the same receptor
- In clinical settings, this is why the opioid **dose** required for effective pain relief **may increase** as a result of developing tolerance

Curiosity - Mithridatism

- **Mithridatism** is the practice of protecting oneself against a poison by gradually self-administering non-lethal amounts.
  - The word derives from Mithridates VI, the King of Pontus, who so feared of being poisoned that he regularly ingested small doses, aiming to develop ”immunity”
- There are only a few practical uses of mithridatism. It can be used by zoo handlers, researchers, and circus artists who deal closely with venomous animals.
- Mithridatism has been tried with success in Australia and Brazil and total immunity has been achieved even to multiple bites of extremely venomous cobras and pit vipers
- Mithridatism is used to treat peanut allergies.

THE MOVEMENT OF DRUG MOLECULES ACROSS CELL BARRIERS

Pharmacokinetics

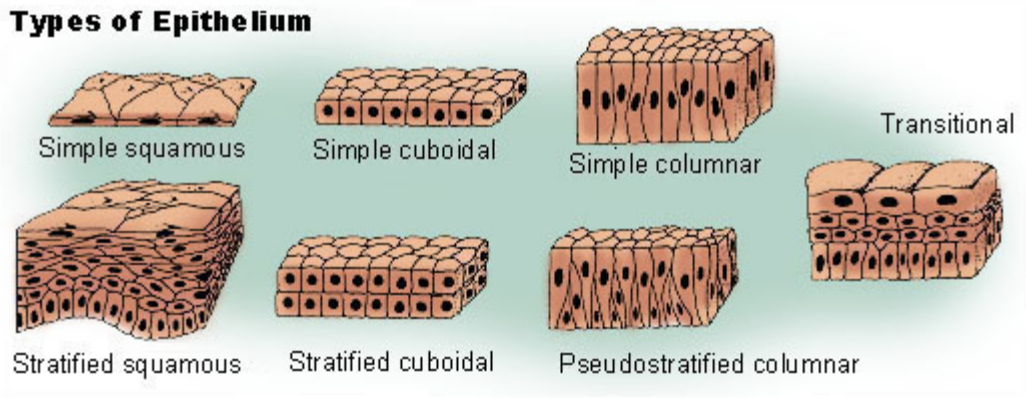
- Drug disposition is divided into 4 stages designated by the acronym ‘ADME’:

- • **Absorption from the site of administration**
- • **Distribution within the body**
- • Metabolism
- • **Excretion**

Movement of drugs through cell membranes are required

THE MOVEMENT OF DRUG MOLECULES ACROSS CELL BARRIERS

- Cell membranes = barriers between aqueous compartments in the body
- membrane separates extra- and intracellular compartments
- An epithelial barrier = a layer of cells tightly connected to each other
  e.g. GI mucosa or renal tubule
  ➔ molecules must traverse at least two cell membranes (inner and outer) to pass from one side to the other

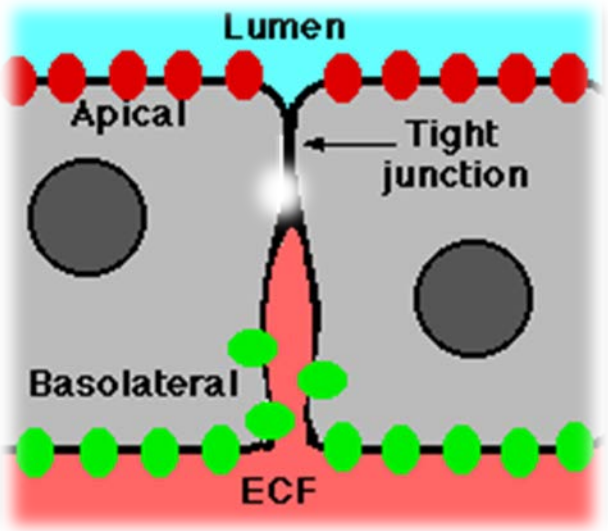


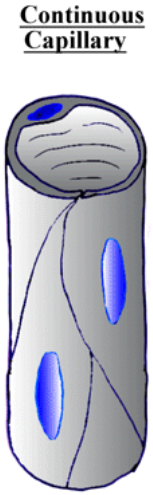
THE MOVEMENT OF DRUG MOLECULES ACROSS CELL BARRIERS

Vascular endothelium is more complicated, its anatomical disposition and permeability varying from one tissue to another

1. In some organs, especially the central nervous system (CNS) and the placenta, there are tight junctions between the cells

   These features prevent potentially harmful molecules from leaking from the blood into these organs





Continuous Capillary

Typical Locations

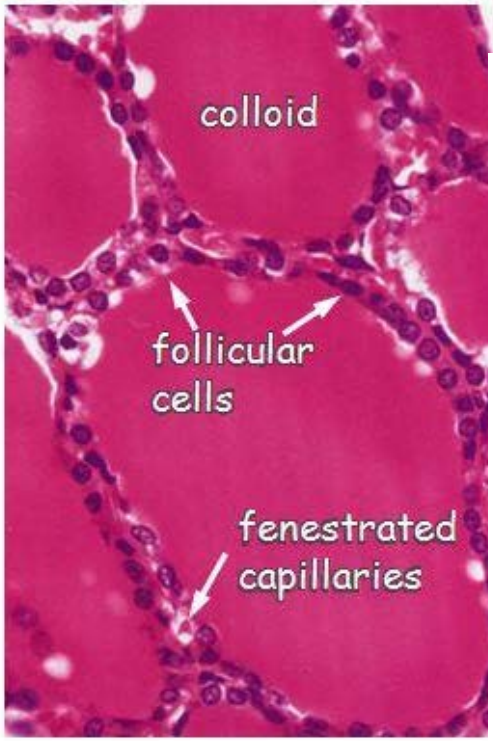
fat
muscle
nervous system

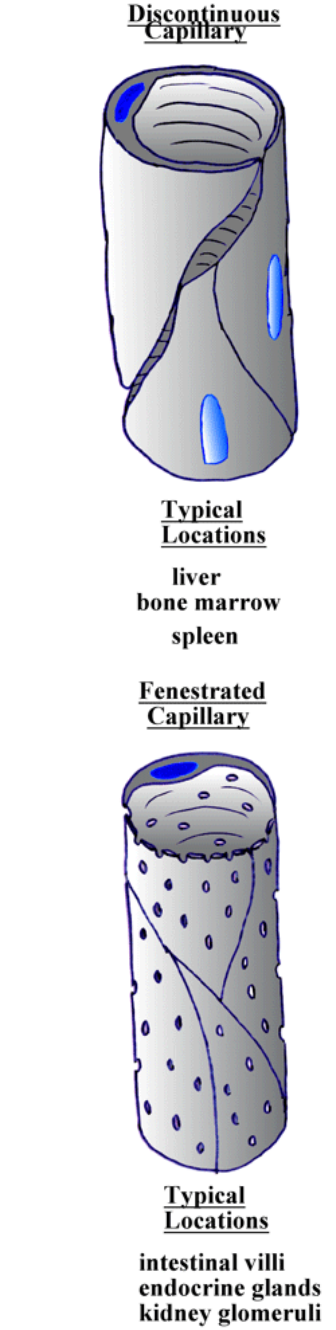
THE MOVEMENT OF DRUG MOLECULES ACROSS CELL BARRIERS

2. In other organs (e.g. the liver and spleen), endothelium is discontinuous, allowing free passage between cells.

   In the liver, hepatocytes form the barrier between intra- and extravascular compartments and take on several endothelial cell functions.

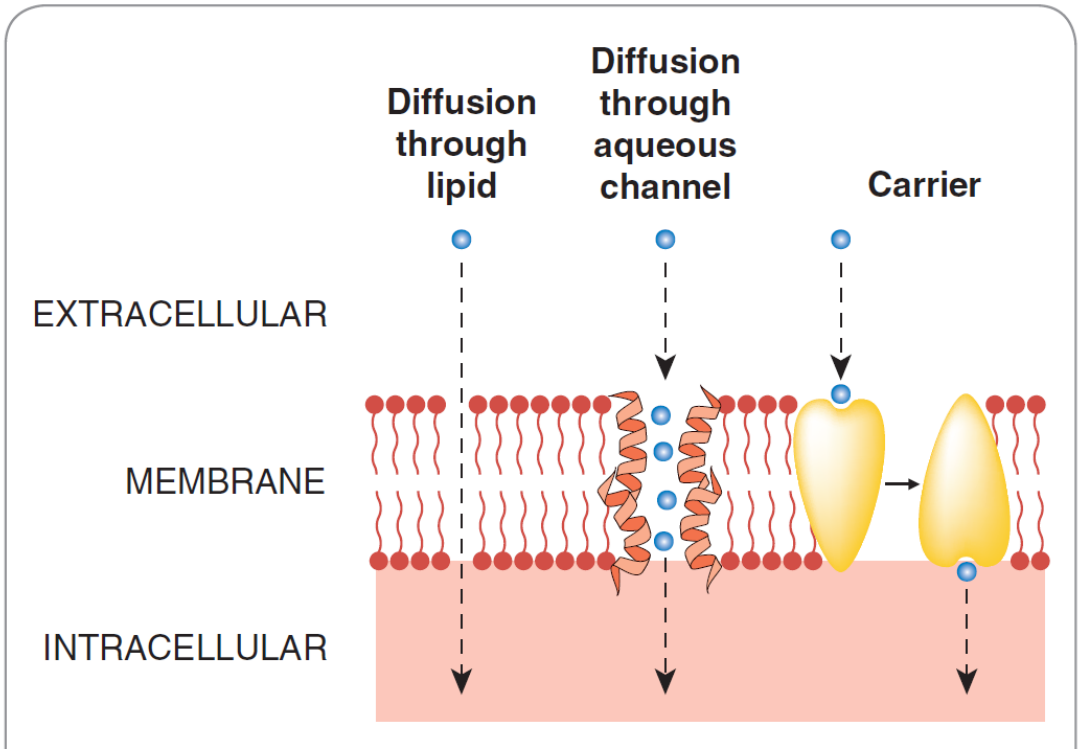
3. Fenestrated endothelium occurs in endocrine glands, facilitating transfer of hormones or other molecules to the bloodstream





Main ways

- There are four main ways by which small molecules cross cell membranes:
  - I. by diffusing directly through the lipid
  - II. by carrier-mediated transport
  - III. by diffusing through aqueous pores formed by special proteins (*aquaporins) that traverse the lipid*
  - IV. by *pinocytosis.*



I. DIFFUSION THROUGH LIPID

- Characteristic property of diffusion through lipid: the *permeability coefficient:*
  The number of molecules crossing the membrane per unit area in unit time.

- Non-polar molecules (in which electrons are uniformly distributed) dissolve freely in membrane lipids, and consequently diffuse readily across cell membranes.

Effect of lipid-solubility on diffusion

- Consequently, there is a close correlation between lipid solubility and the permeability of the cell membrane to different substances.
- lipid solubility is one of the most important determinants of the pharmacokinetic characteristics of a drug
- rate of absorption from the gut, penetration into different tissues and the extent of renal elimination **can be predicted from knowledge of a drug's lipid solubility**

Effect of pH on diffusion

- One important complicating factor in relation to membrane permeation is that many drugs are weak acids or bases,
  - and therefore exist in both unionised and ionised form.
  - the ratio of the two forms varies with pH

- the ionised species has very low lipid solubility ➔ virtually unable to permeate membranes
- The lipid solubility of the uncharged species depends on the chemical nature of the drug, but
- for many drugs, the uncharged species is sufficiently lipid soluble ➔ rapid membrane permeation

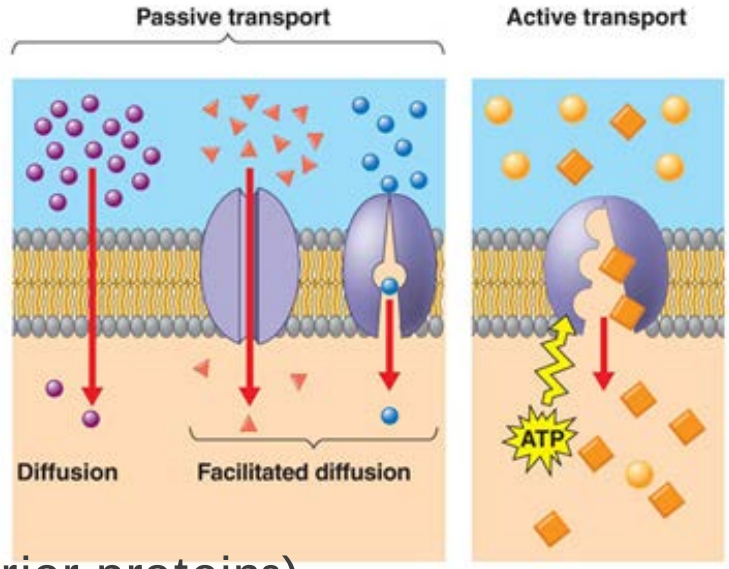
Important consequences of pH changing

- Urinary acidification accelerates excretion of weak bases and retards that of weak acids.
- Urinary alkalinisation has the opposite effects: it reduces excretion of weak bases and increases excretion of weak acids.

- Increasing plasma pH (= alkalizing e.g. by administration of sodium bicarbonate) causes weakly acidic drugs to be extracted from the CNS into the plasma.
- Conversely, reducing plasma pH (=acidifying e.g. by administration of a carbonic anhydrase inhibitor such as **acetazolamide)** causes weakly acidic drugs to become concentrated in the CNS
  ➔ their neurotoxicity increases

II. CARRIER-MEDIATED TRANSPORT

- Many cell membranes possess specialised transport mechanisms that regulate entry and exit of physiologically important molecules:
  - sugars,
  - amino acids,
  - neurotransmitters
  - and metal ions



Two main types are:

- active transport (requires ATP)
- passive (or facilitated diff., by carrier proteins)

Transporters

solute carrier (SLC) transporters

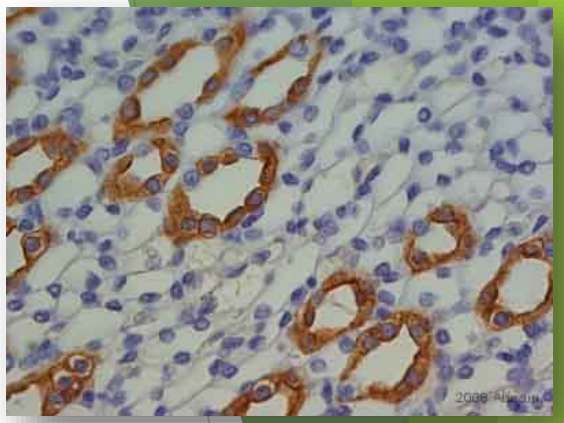
- mediate **passive** movement of solutes = **down their electrochemical gradient**
- The mechanism is called **facilitated diffusion** and the transporter is usually an ‘uniporter’ *

ATP-binding cassette (ABC) transporters

- are **active** pumps fuelled by **ATP**
- Most important: **P-glycoproteins** (P-gp)
  also known as **multidrug resistance protein 1**
  They play an important part in absorption, distribution and elimination of many drugs

* A group of SLC-s mediate secondary active transport and are „antiporters” e.g.: OAT-s, OCT-s

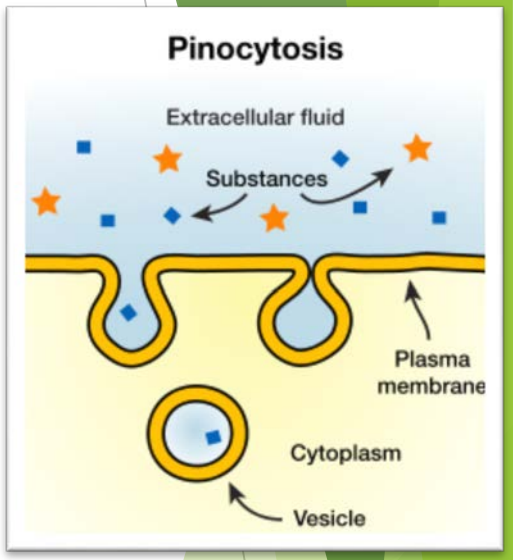
III. Aquaporins



- Aquaporins are "the plumbing system for cells”
  - (Peter Agre, 2003, Nobel-prize for discovery)
- Also known as **water channels,** aquaporins are integral membrane pore proteins
- Main function is water reabsorption in kidneys depending on ADH
  - They selectively conduct water molecules in and out of the cell, while preventing the passage of ions and other solutes.
- Diffusion through aquaporins is probably important in the transfer of **gases** such as carbon dioxide, but the **pores are too small in diameter** (about 0.4 nm) to allow most drug molecules (which usually exceed 1 nm in diameter) to pass through
- Aquaporins can be blocked by mercurial reagents (such as *para-chloromercurobenzene* sulfonate)

IV. Pinocytosis (pino = to drink)

- Pinocytosis ("fluid-endocytosis") involves invagination of part of the cell membrane and the trapping within the cell of a small vesicle containing extracellular constituents.



- The vesicle contents can then be released within the cell, or extruded from its other side.
- This mechanism is important for the transport of some macromolecules (e.g. **insulin crosses the blood-brain barrier by this process**),
- but not for small molecules such as conventional drugs
- The process is energy-dependent (ATP).
- This process occurs rarely.

Prescriptions for the exam

Practice

Undivided powders

- 4. Prescribe powder containing Aluminii oxydum hydricum and Magnesii oxidum leve

Prescribe powder containing Aluminii oxydum hydricum and Magnesii oxidum leve

- Aluminii oxydum hydricum
  - 40 g
- Magnesii oxydum leve
  - 10 g

Rp./

Aluminii oxydi hydrici
grammata quadraginta (g 40,0)
Magnesii oxydi levis
grammata decem (g 10,0)
Misce fiat pulvis
Detur ad scatulam
Signetur: Mix a half teaspoonful in water a.c.

Tablets (precompounded)

- 1. Prescribe Digoxin tablets

- 3. Prescribe Verapamil tablets
- 4. Prescribe Nitromint tablets
- 5. Prescribe Ulceran tablets

- 8. Prescribe Norvasc tablets
- 9. Prescribe Amilorid compositum tablets
- 10.Prescribe Minipress tablets

Prescribe Digoxin tablets

- Digoxin
  ↓
- Digoxin
  (Brand name)

Rp./

Tablettarum Digoxin 250ug 50x

scatulam originalem No. I (unam)

D.S: S.i.d.

Prescribe Verapamil tablets

- verapamil
  ↓
- Verapamil
  (brand name)

Rp./

Tablettarum Verapamil 40mg 50x

scatulas originales No. II (duas)

D.S.: T.i.d.

Usually pharmacists are kind to give the 2 boxes, but according to the rules prescription should contain:
'Supplied for 33 days'

(Packings: 40 mg 50x; 80mg 50x;
but 120-180-240mg retard version also exists with other brand name (e.g. Chinopamil))

Prescribe Nitromint tablets

- nitroglycerin
  ↓
- Nitromint

Rp./

Tablettarum Nitromint 0,5mg 50x

scatulam originalem No. I (unam)

D.S.: P.r.n. 1 tablet sublingually

Effect occurs in 1-2 minutes and lasts for 30-45 minutes. Can be repeated in 5 minutes (maximum 3 tablets)

(Packings:
0,5mg 50x; 2,6mg 60x retard (lattter is not good for acute myocardial infarct!))

Prescribe Ulceran tablets

ranitidine
↓
Ulceran

Rp./

Tablettarum Ulceran 300 mg 30x

scatulas originales No. III (tres)

D.S.: B.i.d

In this case 2(!) boxes are given out.
(lacking 'supplied for 45 days' sentence)

(Packings: 150mg 60x, 300mg 30x)

Prescribe Norvasc tablets

- amlodipin
  ↓
- Norvasc

Rp./

Tablettarum Norvasc 10mg 30x

scatulam originalem No. I (unam)

D.S.: S.i.d.

(Packings: 5mg 30x; 10mg 30x)

Prescribe Amilorid compositum tablets

- Amilorid+HCT
  ↓
- Amilorid compositum

Rp./

Tablettarum Amilorid Compositum

scatulam originalem No. I (unam)

D.S.: S.i.d.

(Packings: 5mg 30x; 10mg 30x)

Prescribe Minipress tablets

- prazosin
  ↓
- Minipress

Rp./

Tablettarum Minipress 2mg 30x

scatulam originalem No. I (unam)

D.S.: S.i.d.

(Packings: 1mg 30x, 2mg 30x)