NEUROPHARMACOLOGY

The course is composed of 3 parts

PART 1 (Prof. Chiara Florio): PHARMACOKINETIC (drug absorption, distribution, metabolism and excretion) and PHARMACODYNAMIC - THE AUTONOMIC NERVOUS SYSTEM

PART 2 (Prof. Chiara Florio): DRUGS OF THE CENTRAL NERVOUS SYSTEM (ENDOGENOUS OPIOIDS - ANTIDEPRESSANT DRUGS - ANTIPSYCHOTIC DRUGS - ANXIOLYTIC DRUGS - ANTI-EPILEPTIC DRUGS)

PART 3 (Prof. Gabriele Stocco): PHARMACOGENOMICS

AIM of the course is to provide the basic notions for the comprehension of the pharmacokinetic and pharmacodynamics properties of drugs and of their mechanism of action, with particular reference to drugs acting at the central nervous system in order to allow the students to:

1) to discuss clearly and with appropriate scientific terms pharmacological concepts
2) continue to enlarge autonomously and critically their knowledges
3) use the knowledges acquired for a proper use of drugs in experimental set-ups
4) apply knowledges for a critical consideration of experimental results

Students are provided by the slides used during the frontal lessons thought **Moodle (**Access code: 779SM)

Recommended text book:
Rang, Ritter, Flower, Henderson “Rang & Dale’s Pharmacology” Eighth Edition, Elsevier 2016

For further information, students are invited to contact dott. Florio by mail (florioc@units.it) using their institutional E-mail address

FINAL EXAMINATION

At the end of the course, students are required to take a final oral examination of 20-40 min consisting on three different topics covering the course program (1. Basic Pharmacology (pharmacokinetic and pharmacodynamics) or Autonomous nervous system, 2. Pharmacogenomics and 3. Drugs acting at the Central Nervous System)

The student should demonstrate to be able to link together different topics of the program and to communicate the acquired knowledges in a precise and efficacious manner.
The mark/30 must be equal or higher than 18.
The final mark/30 is the arithmetic mean of Neuroanatomy and Neuropharmacology

Phar·ma·col·o·gy

Etymology: Gk, pharmakon, drug + logos, science

The science that deals with the origin, nature, chemistry, effects, and uses of drugs; it includes pharmacognosy, pharmacokinetics, pharmacodynamics, pharmacotherapeutics, and toxicology (Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health)

pharmacokinetic

pharmacodynamic

Pharmacokinetic

How the drug comes and goes

Drugs need to achieve an adequate concentration in their target tissues to give the requested pharmacologic effect (➡️ pharmacodynamic)

The fundamental processes that determine the concentration of the drug at any moment and in any region of the body are:

1) Absorption from the site of administration

2) Distribution within the body

3) Biotransformation (drug metabolism)

4) Excretion

“ADME”

Pharmacokinetic

A: absorption

From its site of administration, drugs cross various barriers (membranes, capillaries, cell wall….) and reach the bloodstream (or lymphatic or cerebrospinal fluids)

D: distribution

The drug moves from the bloodstream (or lymphatic or cerebrospinal fluids) to its site of action (eg, the brain), again crossing various barriers

Distribution affects drug concentration at site of action (pharmacodynamic effect), drug site of excretion and biotransformation

Pharmacokinetic

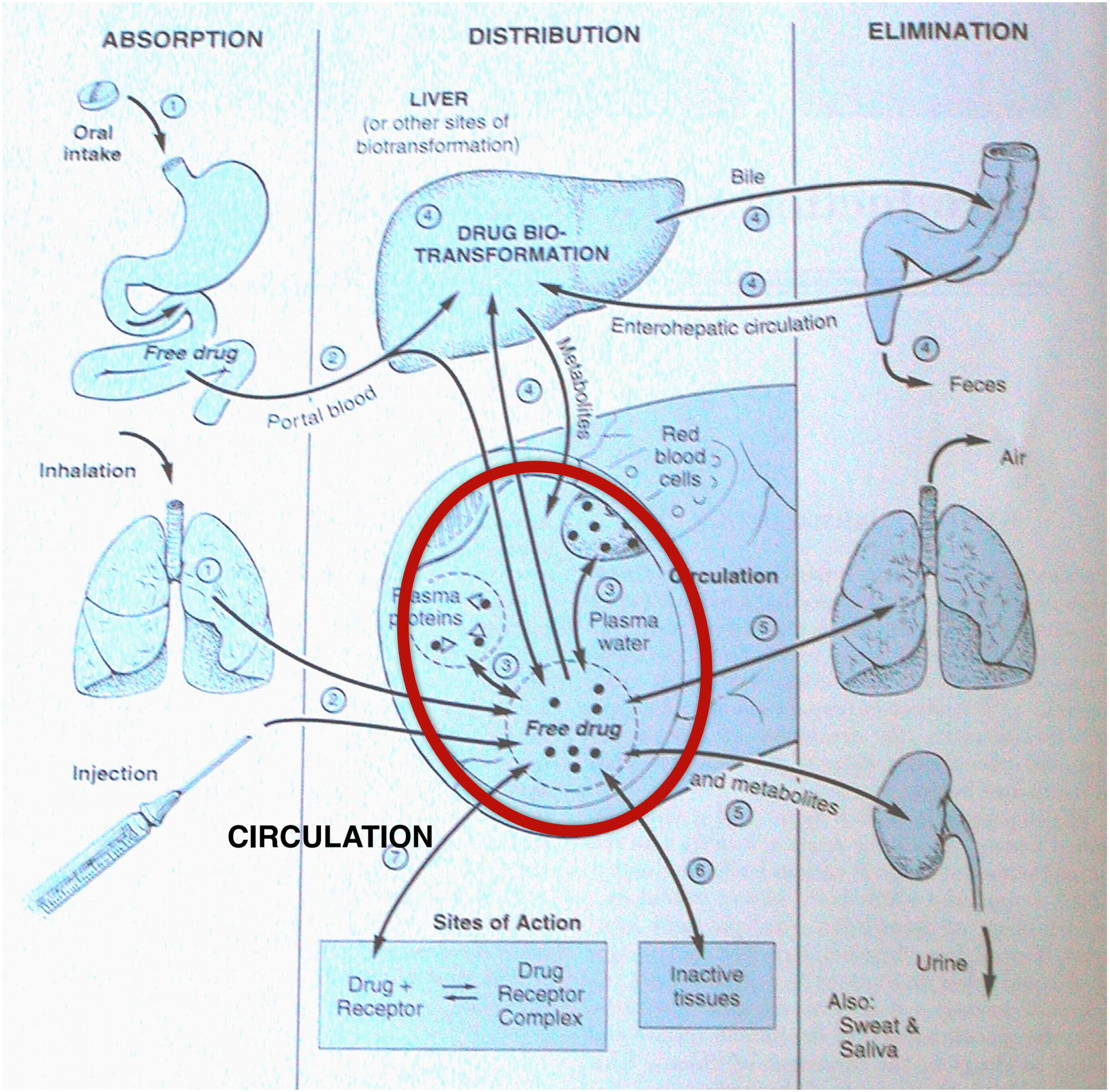
M:metabolism

Drugs are biotransformed into several different compounds by enzymes evolved to cope with natural compounds

Biotransformation may increase, decrease or change drug actions

E: excretion

Drugs are eliminated by excretion from the body through different pathways, e.g. renal


ABSORPTION
DISTRIBUTION
ELIMINATION
Oral intake
LIVER
(or other sites of biotransformation)
DRUG BIO-TRANSFORMATION
Bile
Enterohepatic circulation
Free drug
Portal blood
Metabolites
Feces
Inhalation
Red blood cells
Air
Plasma proteins
Plasma water
Circulation
Free drug
and metabolites
Injection
CIRCULATION
Sites of Action
Drug + Receptor
Drug Receptor Complex
Inactive tissues
Urine
Also: Sweat & Saliva

Drug Administration Routes

PARENTERAL

ENTERAL

Administration Routes: PARENTERAL

	ADVANTAGES	DISADVANTAGES
INTRAVENOUS	Rapid attainment of concentration; precise delivery of dosage; easy to titrate dose	High initial concentration (toxicity risk); risk of infection; requires skill
SUBCUTANEOUS INTRAMUSCULAR	Prompt absorption from aqueous medium; little training needed; avoid gastrointestinal environment	Cannot be used for large volume; potential pain or tissue damage; variable absorption

Administration Routes: PARENTERAL

	ADVANTAGES	DISADVANTAGES
PULMONARY	**Easy to titrate dose; rapid onset local effect; minimize toxic effects**	**Requires coordination; lung disease limits; variable delivery**
TOPICAL	**Minimize side effects; avoid first pass metabolism**	**Erratic absorption**

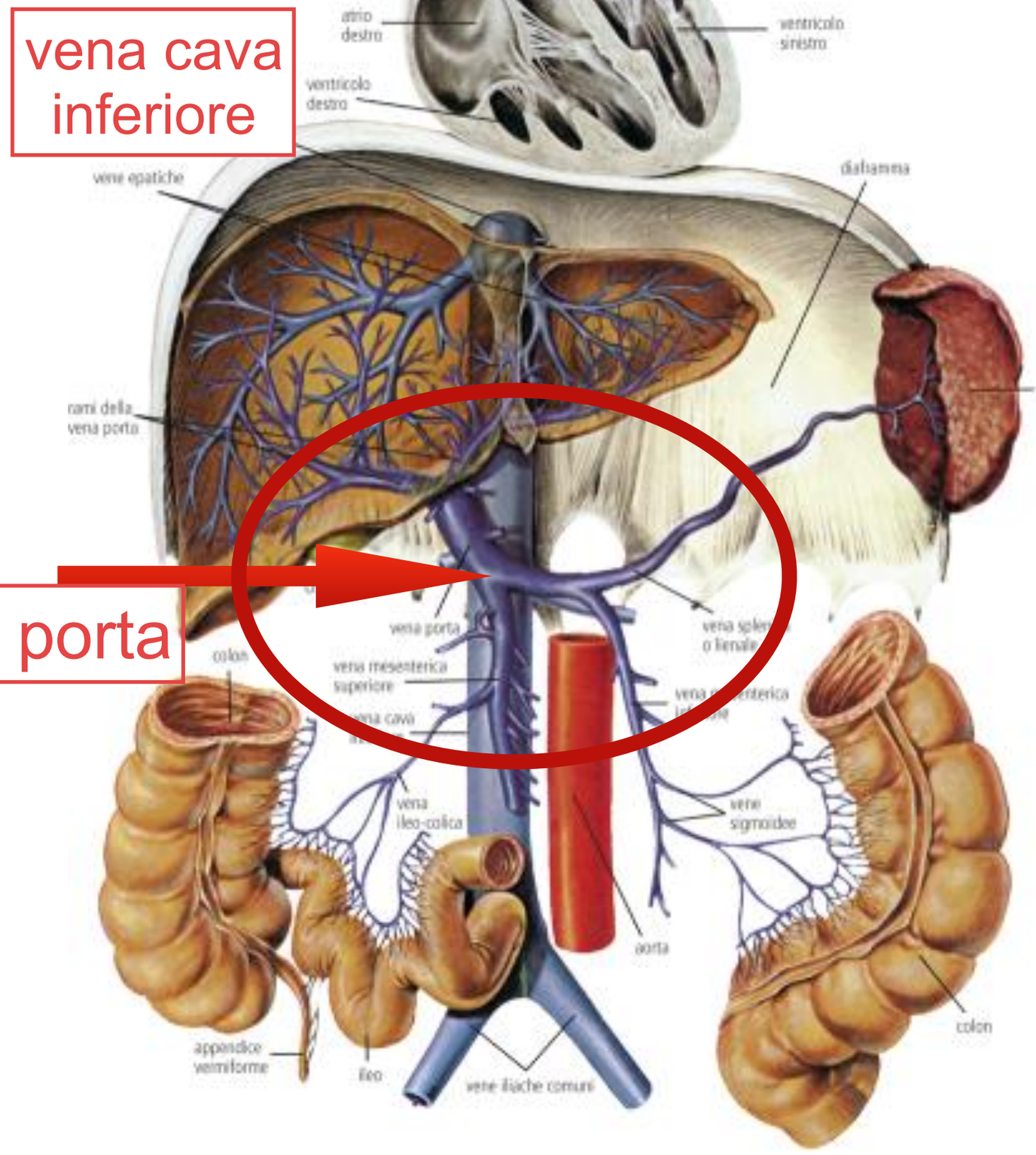
Administration Routes: ENTERAL

	ADVANTAGES	DISADVANTAGES
ORAL	Convenient (storage, portability); economical; non invasive; safe; requires no training	Delivery can be erratic or incomplete, depends on patient compliance; first pass effect
SUBLINGUAL	Rapid onset; avoid first passage	Few drugs adequately absorbed; patient must avoid swallowing; difficult compliance

Effetto di primo passaggio

Dopo assorbimento a livello GI, il farmaco viene trasportato tramite la vena porta al fegato e qui può essere metabolizzato (effetto di primo passaggio o presistemico)

L'effetto di primo passaggio riduce la quantità di farmaco che raggiunge il circolo sistemico (cioè la biodisponibilità).



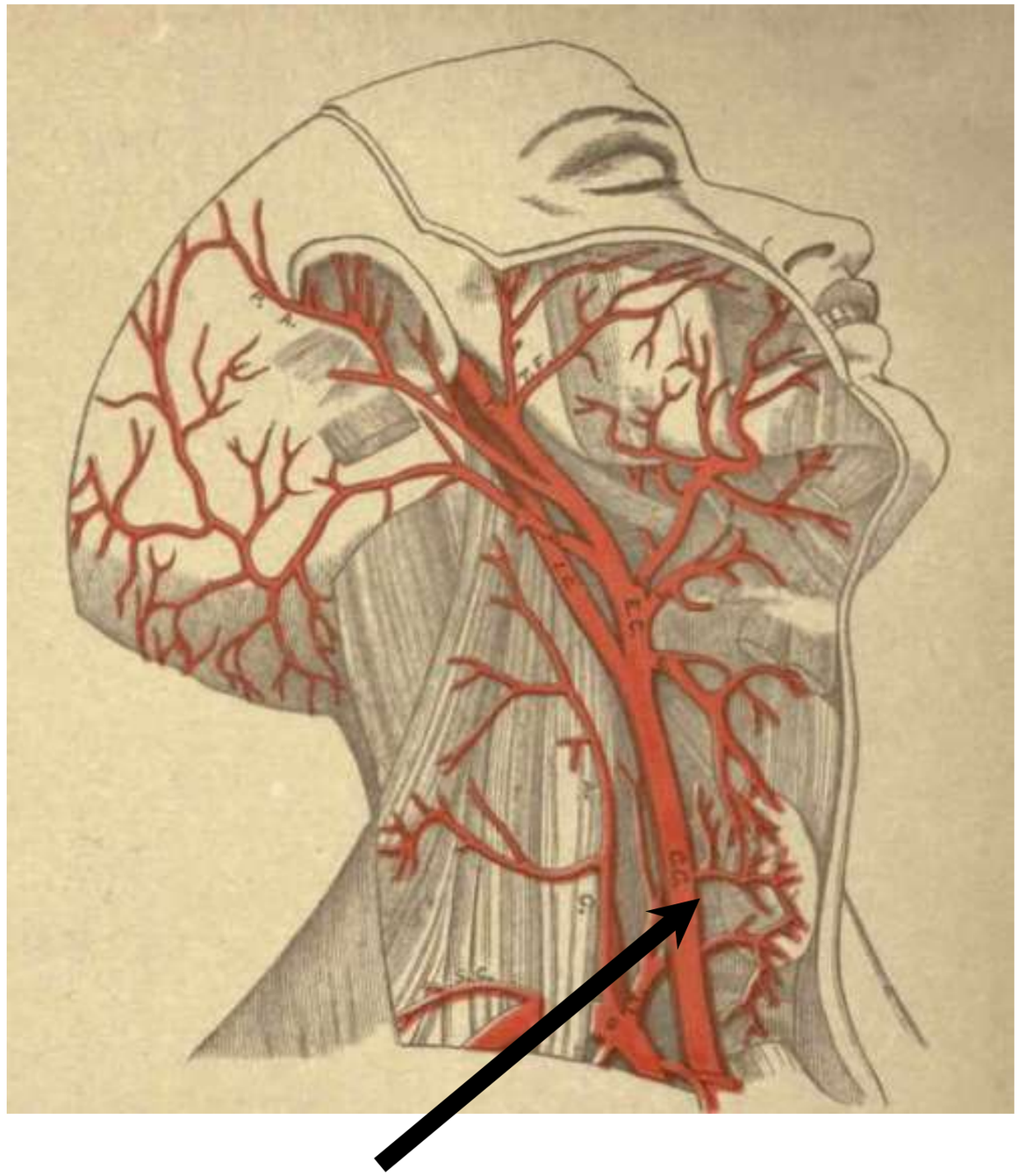
Via Sublinguale/Buccale

VANTAGGI:

1. Assorbimento rapido
2. Adatta a farmaci instabili al pH gastrico
3. Evita l'effetto di primo passaggio

SVANTAGGI

1. Scomoda
2. Solo piccoli volumi
3. Adatta a farmaci potenti
4. Sapore spiacevole in taluni casi



ADME: Absorption

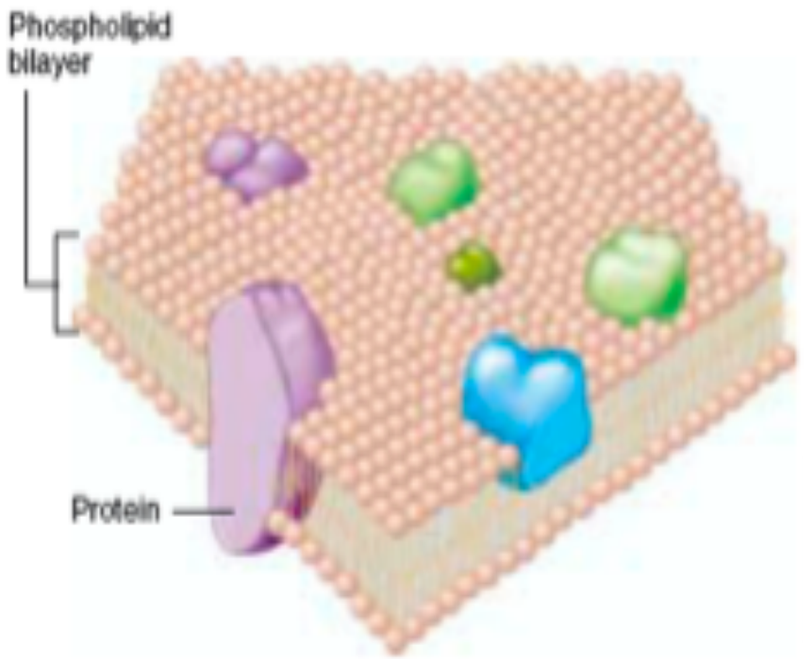
Absorption is the process by which a drug moves from its site of application and enters the bloodstream or the lymphatic system crossing cell barriers

The movement of drug molecules across cell barriers

Cell membranes form barriers between aqueous compartments in the body

The most universal function of cell membrane is to act as a selective barrier to the passage of molecules, allowing some molecules to cross while excluding others

Membranes and Absorption



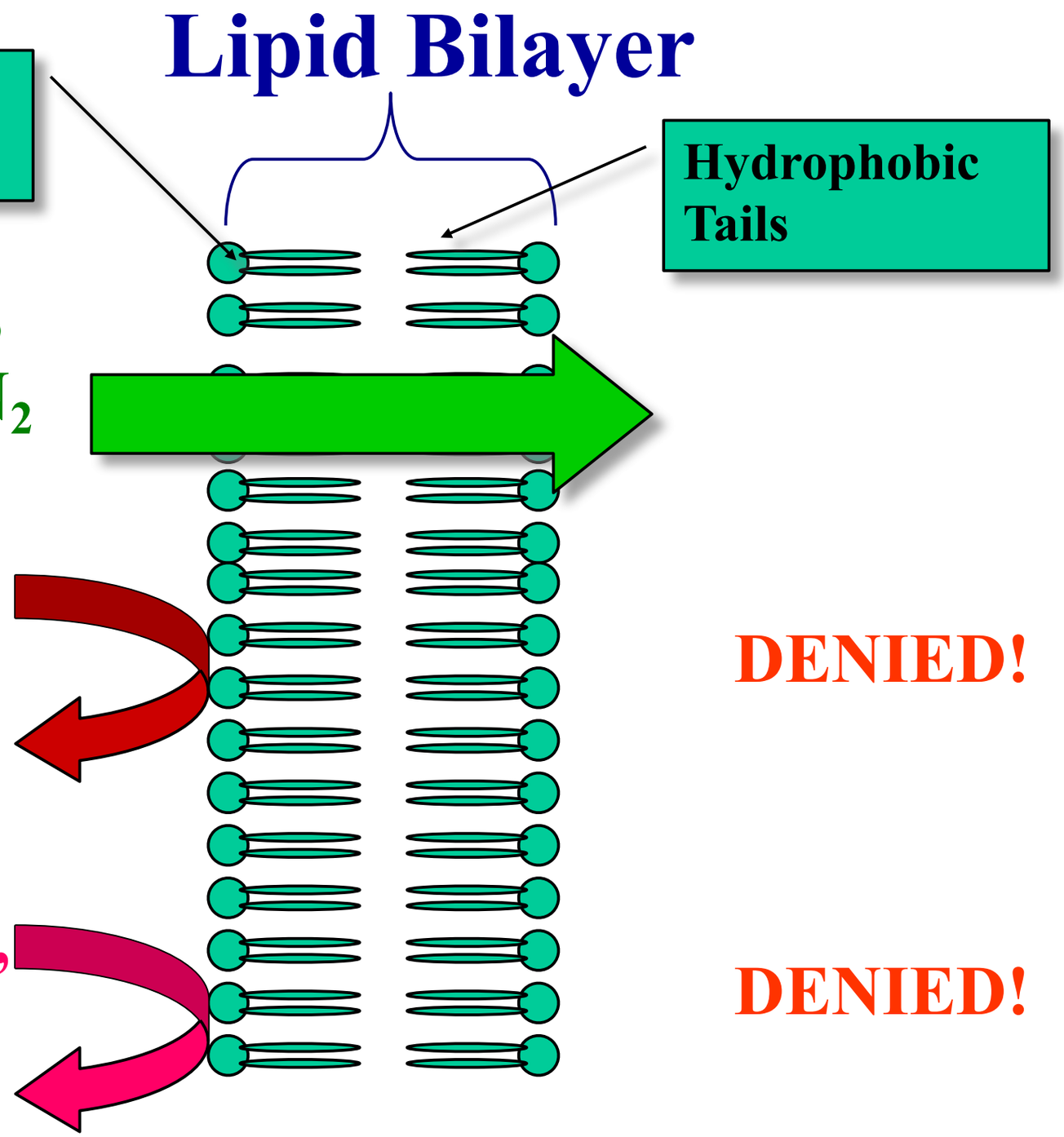
Small, uncharged

H_2O, urea, CO_2, O_2, N_2

Lipid Bilayer

Hydrophilic Heads

Hydrophobic Tails



Large, hydrophilic

Glucose
Sucrose

DENIED!

Small charged ions

H^+, Na^+, K^+, Ca^{2+}, Cl^-, HCO_3^-

DENIED!

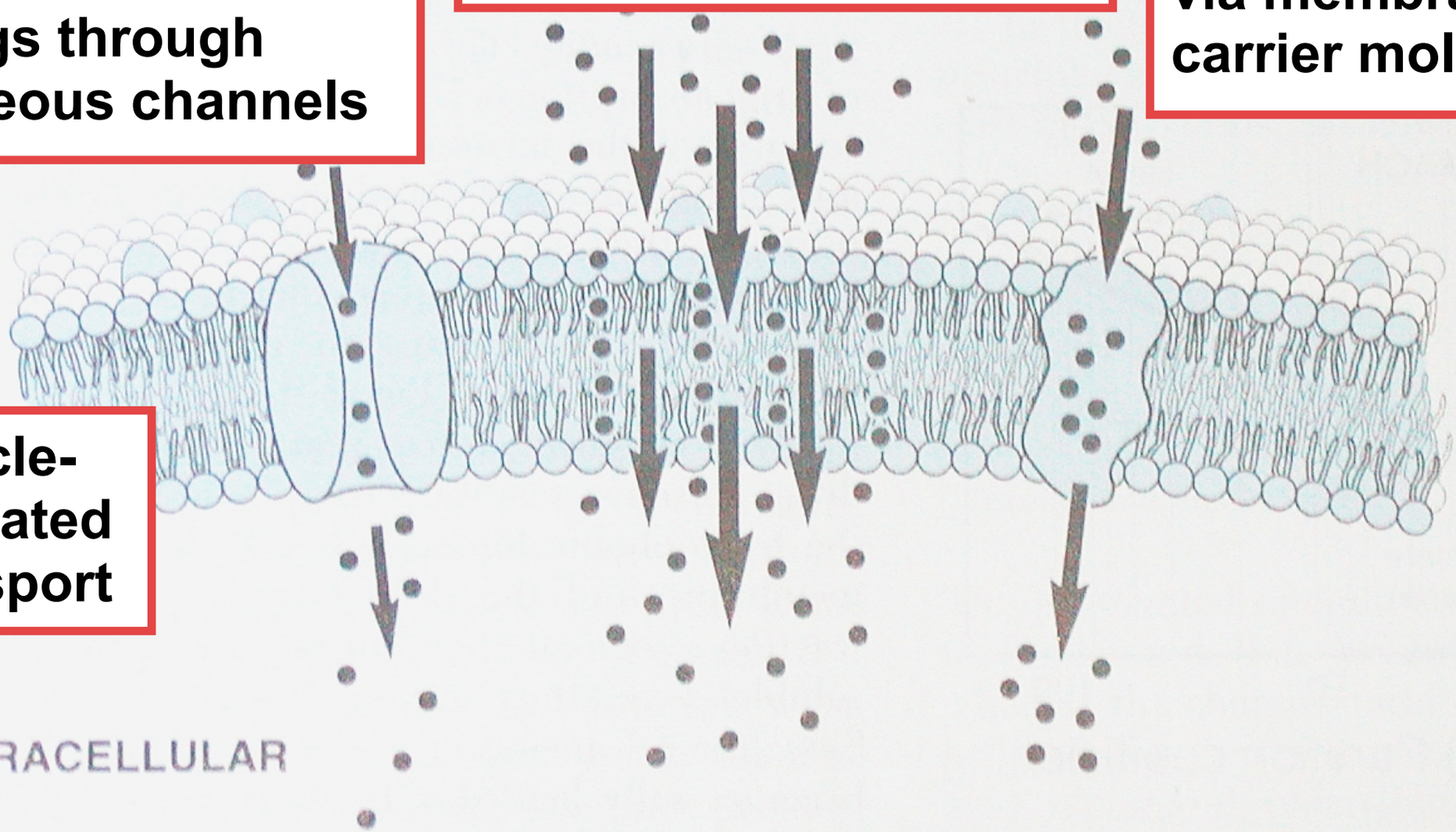
Mechanisms of Absorption

EXTRACELLULAR

Passive diffusion of water-soluble drugs through aqueous channels

Passive diffusion of lipid-soluble drugs via hydrophobic bonding with membrane lipids

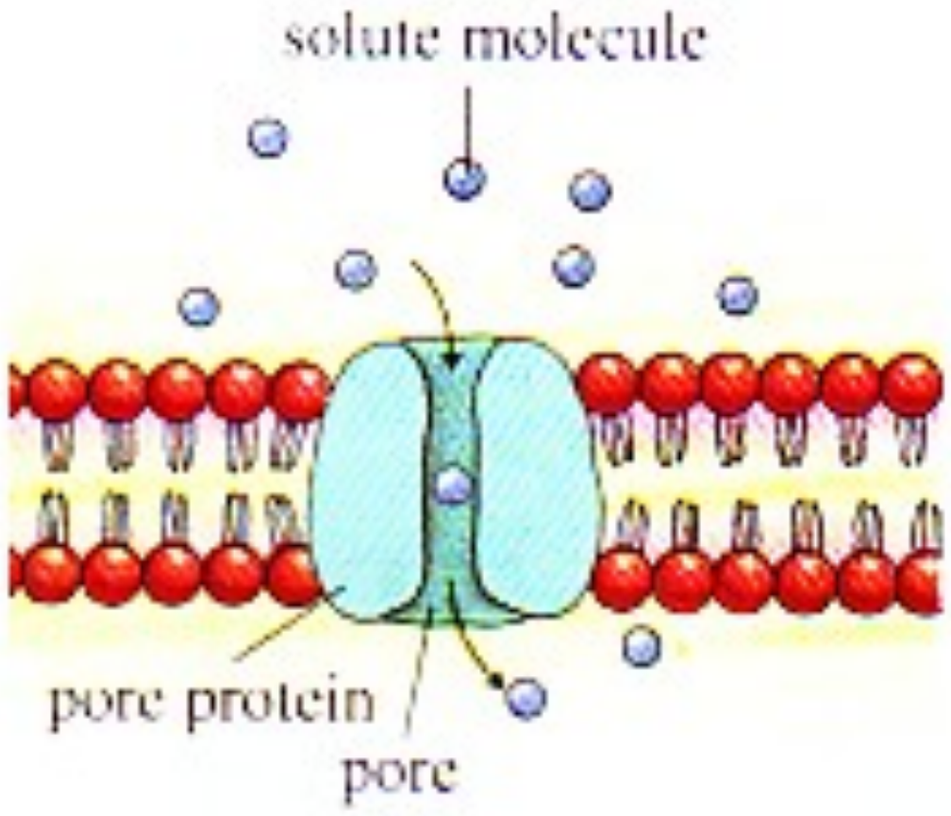
Active transport and facilitated diffusion via membrane carrier molecules



Vesicle-mediated transport

INTRACELLULAR

Passive diffusion of hydrophilic molecules trough acqueuos channels



Drug	Molecular weigth	Ripartition Coefficient
Caffein	194	0.17
Ascorbic acid	176	0.02
Ephedrin	165	1.6

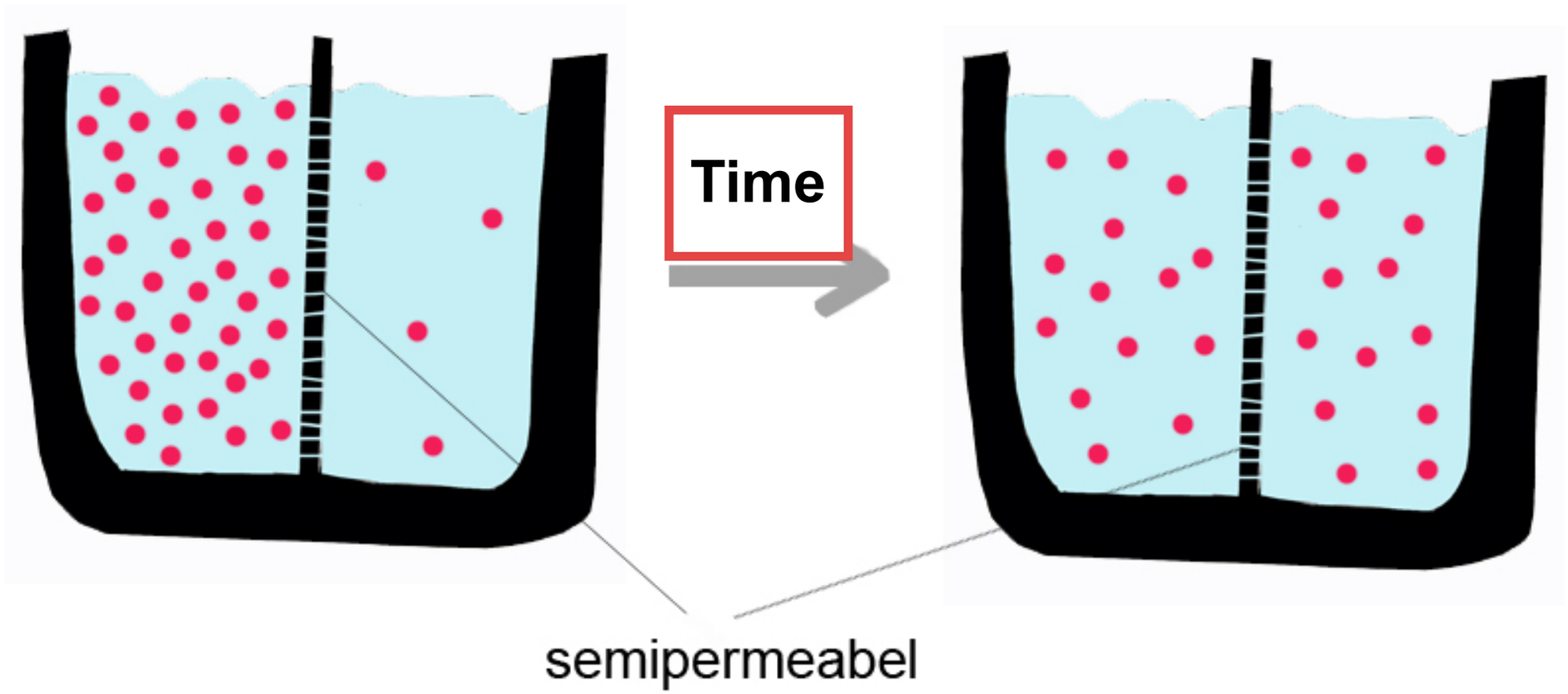
Passive Diffusion

The vast majority of drugs move through the body by this mechanism

Passive diffusion depends on:

1. **concentration gradient**
2. **lipid solubility**
3. **degree of ionization**
4. **thickness of membrane**
5. **surface area**

Passive Diffusion

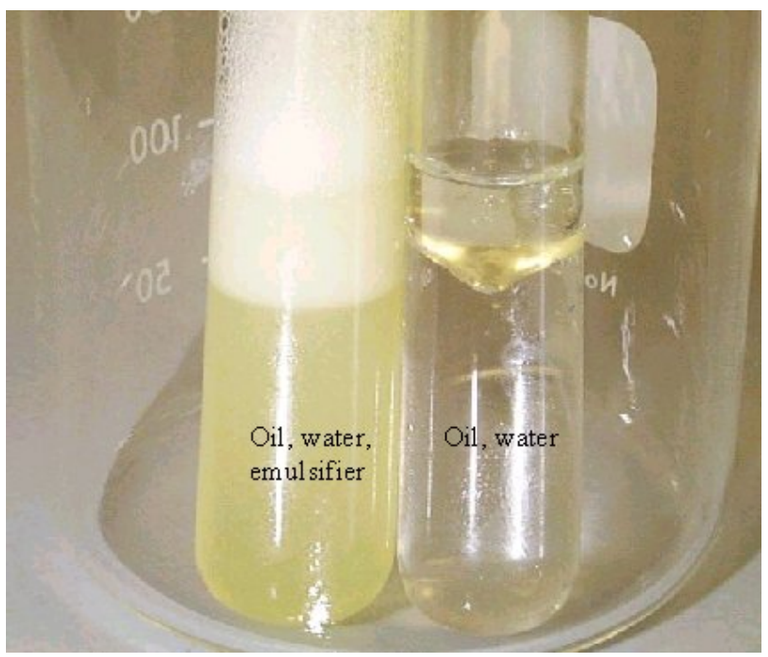


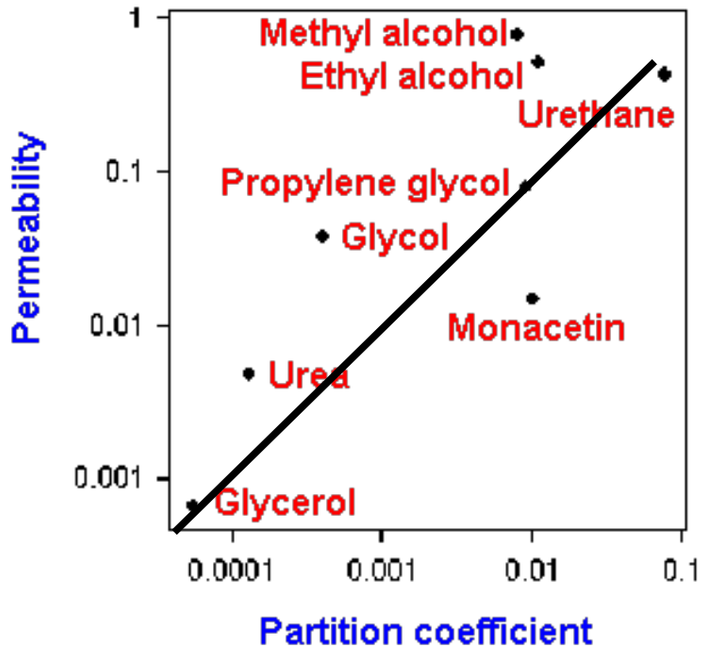
1) The concentration gradient is maintained by removal of the drug from the other side of the membrane

Passive Diffusion

2) Lipid solubility depends on the physiochemical properties of the drug

Is measured by the lipid/water partition coefficient (ratio of drug concentration in lipid phase and water phase when shaken in one immiscible lipid/ water system)





Passive Diffusion

3) **Degree of ionization (for week acidic or basic drugs)**

Acids **Release/Donate H^+**

$$HA \rightleftharpoons H^+ + A^-$$

Ionized form

Bases **Bind/Accept H^+**

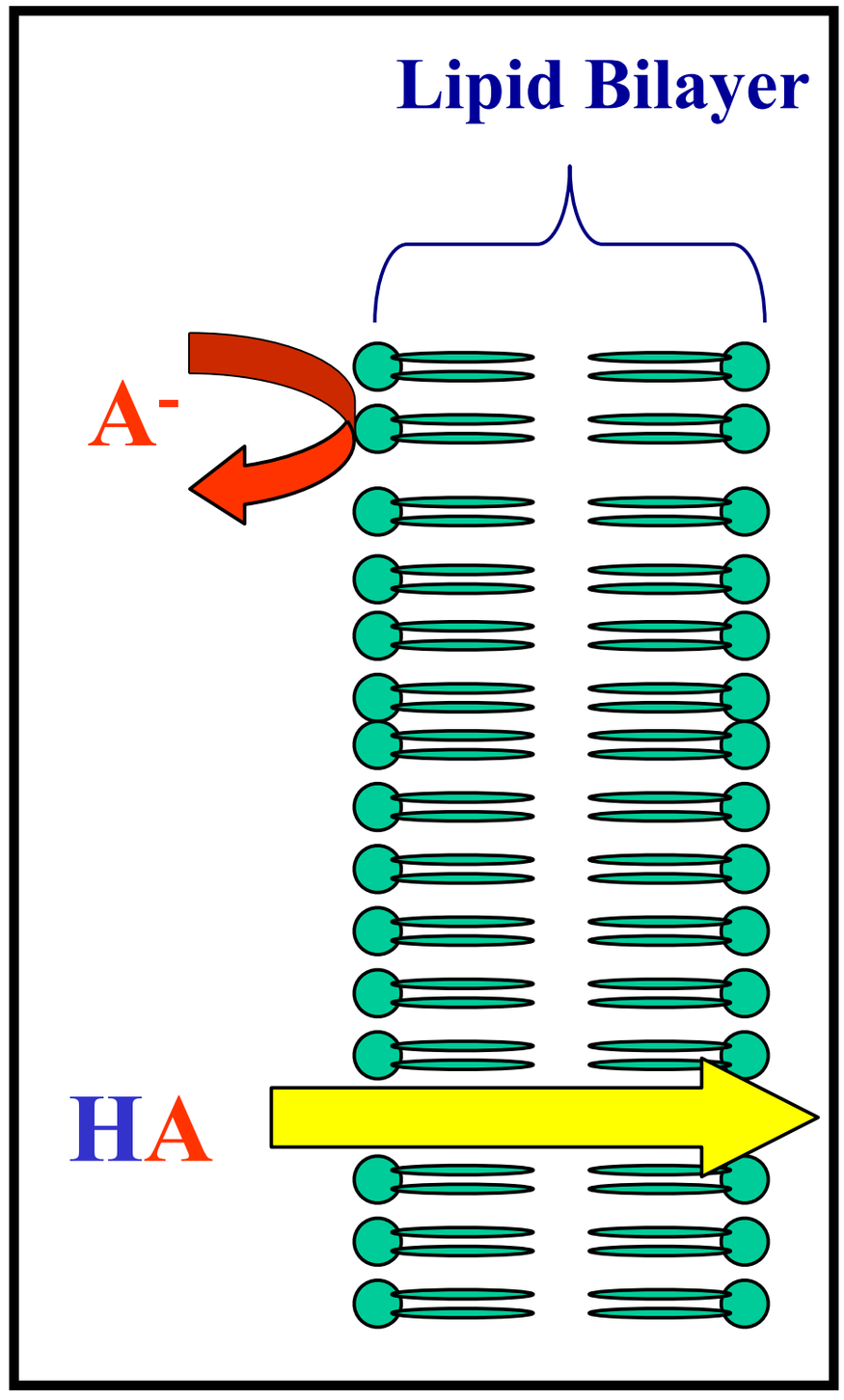
$$H^+ + B^- \rightleftharpoons HB$$

Non-ionized form

Environmental pH and Ionization

For an acidic drug, in an environment with low pH the non-ionized form will predominate

whereas in an environment with high pH the ionized form will predominate



As a consequence:

Acidic drugs are best absorbed from acidic environments
Basic drugs are best adsorbed from basic environments

And...

To increase absorption of an acidic drug acidify the environment
To reduce the absorption (or increase the elimination) of an acidic drug alkalinize the environment

The relative amount of charged and uncharged species for any drug molecule depends on the molecule's pKa and the pH of the medium

pKa

pH value at which the drug is 50% in the ionized form and 50% in the non-ionized form

pH	Acidic drug	% non ionized form	Basic drug	% non ionized form
1		99.9		0.1
2		99		1
3	HA	90	BH^+	10
4		***50***		***50***
5		10		90
6	A^-	1	B	99
7		0.1		99.9

pKa

Passive Diffusion

The vast majority of drugs move through the body by this mechanism

Passive diffusion depends on:

1. **concentration gradient**
2. **lipid solubility**
3. **degree of ionization**
4. **thickness of membrane**
5. **surface area**

Passive (or simple) Diffusion

Fick's Law

$$\frac{dQ}{dt} = \frac{PA}{h}(Cp - Ct)$$

dQ/dt = diffusion rate

P = oil/water partition coefficient

A = surface area

Ct = drug concentration in the tissue

Cp = drug concentration in the plasma

} **concentration gradient**

h = thickness of the membrane

Passive (or simple) Diffusion

- **Diffusion rate depends on the concentration gradient**
- **No energy or carrier is required**
- **It is not saturable**

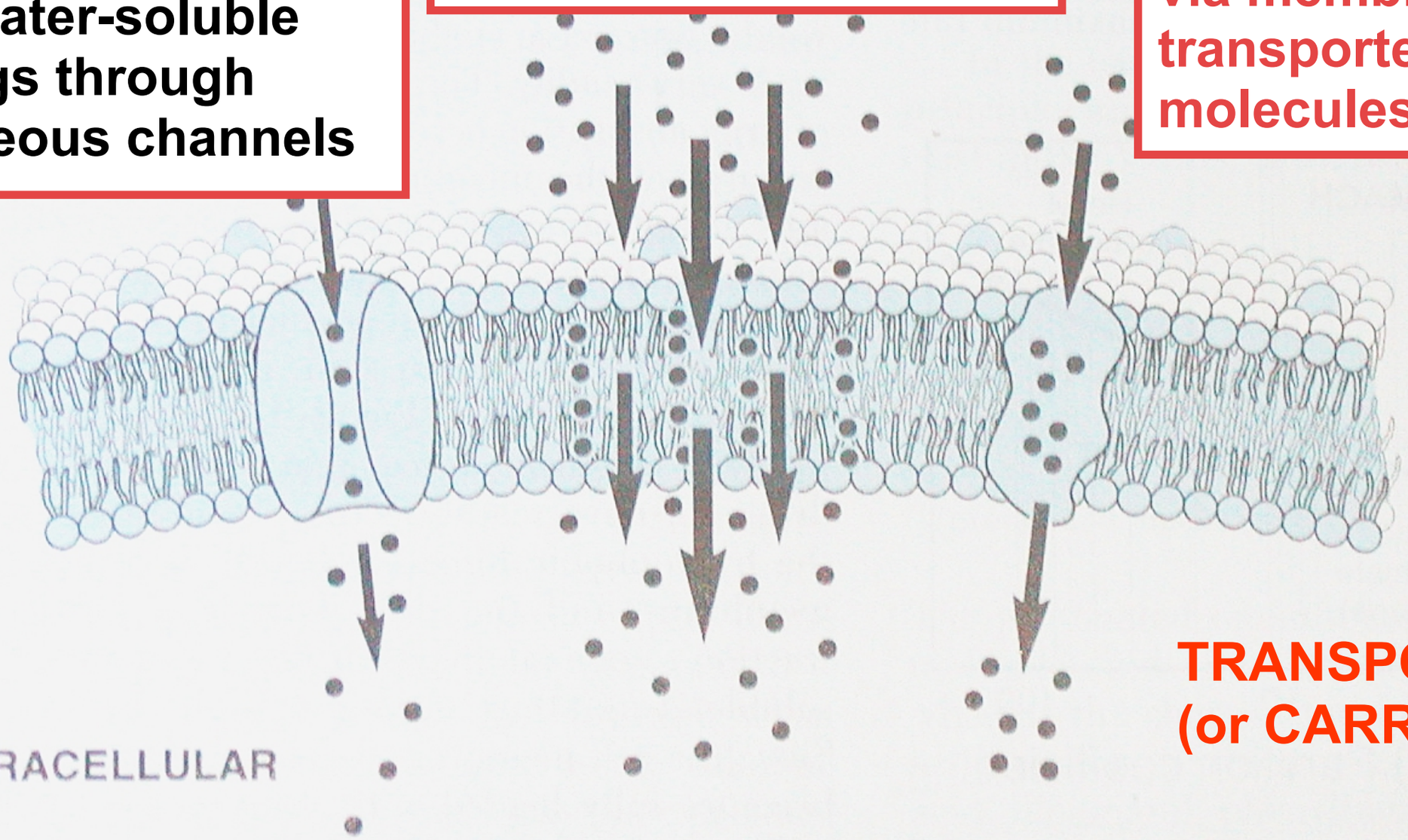
Mechanisms of Absorption

EXTRACELLULAR

Passive diffusion of water-soluble drugs through aqueous channels

Passive diffusion of lipid-soluble drugs via hydrophobic bonding with membrane lipids

Active transport and facilitated diffusion via membrane transporters molecules

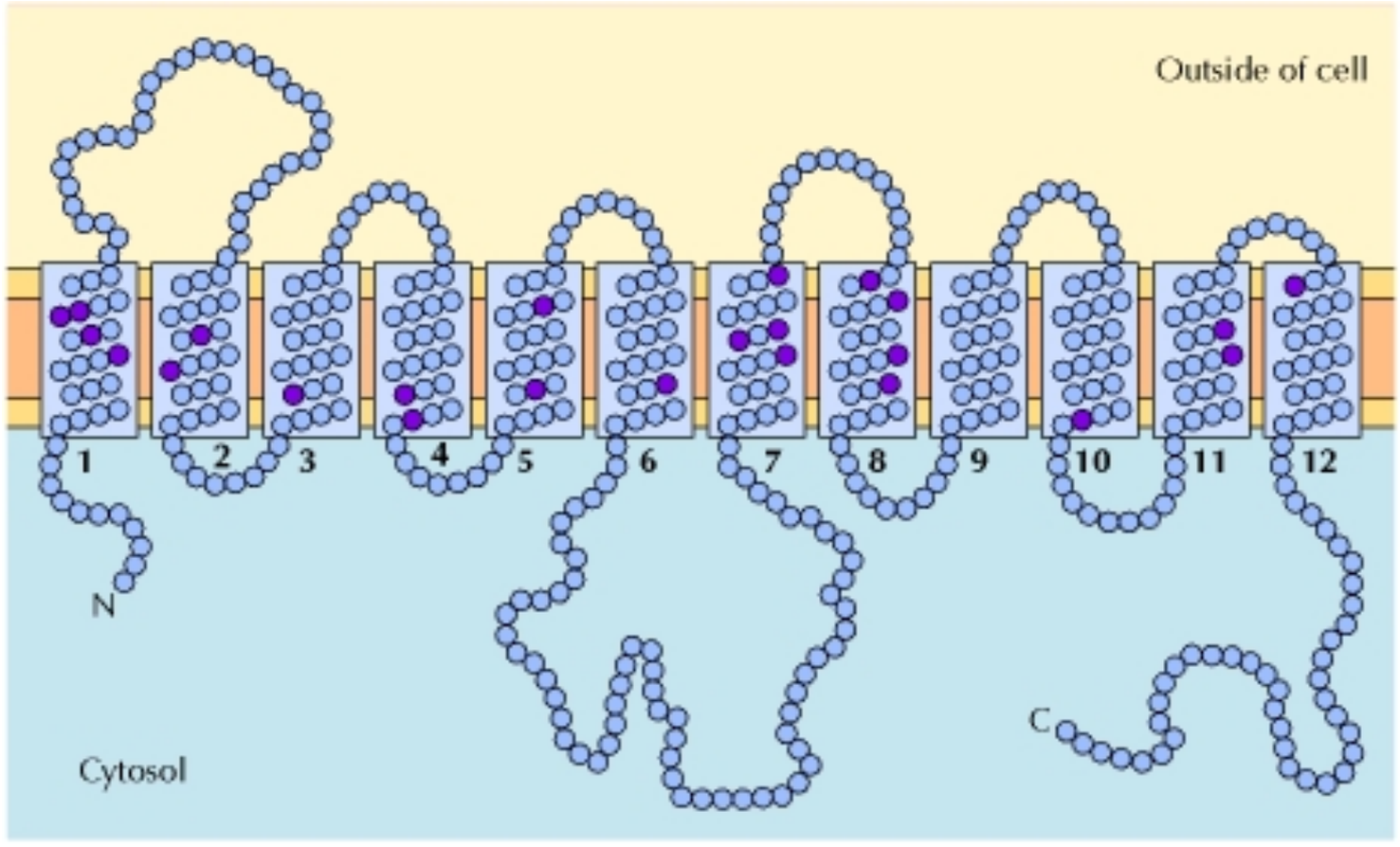


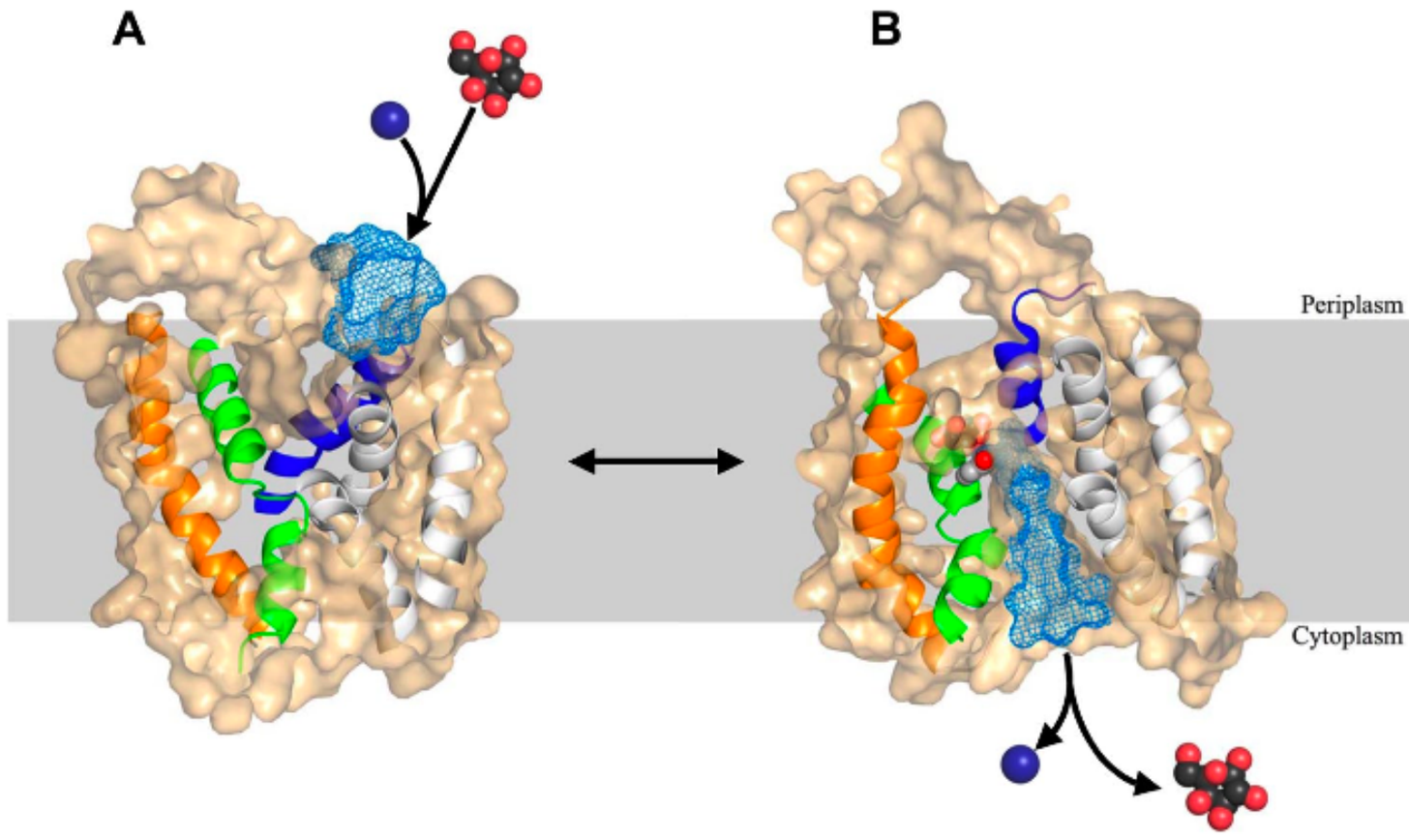
TRANSPORTERS (or CARRIERS)

INTRACELLULAR

TRANSPORTERS

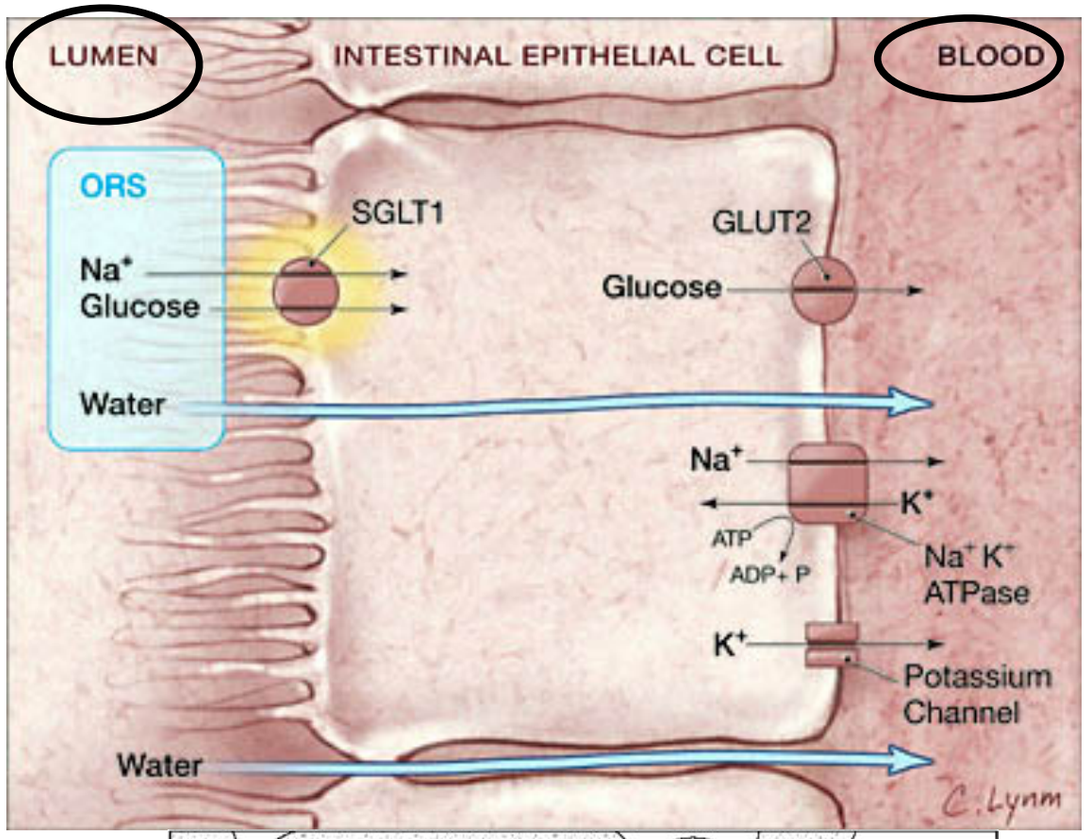
A transporter is a transmembrane protein which binds stereoselectively one or more molecules or ions, undergoes to a conformation change and releases them on the other side of the membrane





TRANSPORTERS

Epithelial cells (gut, kidney, lung)



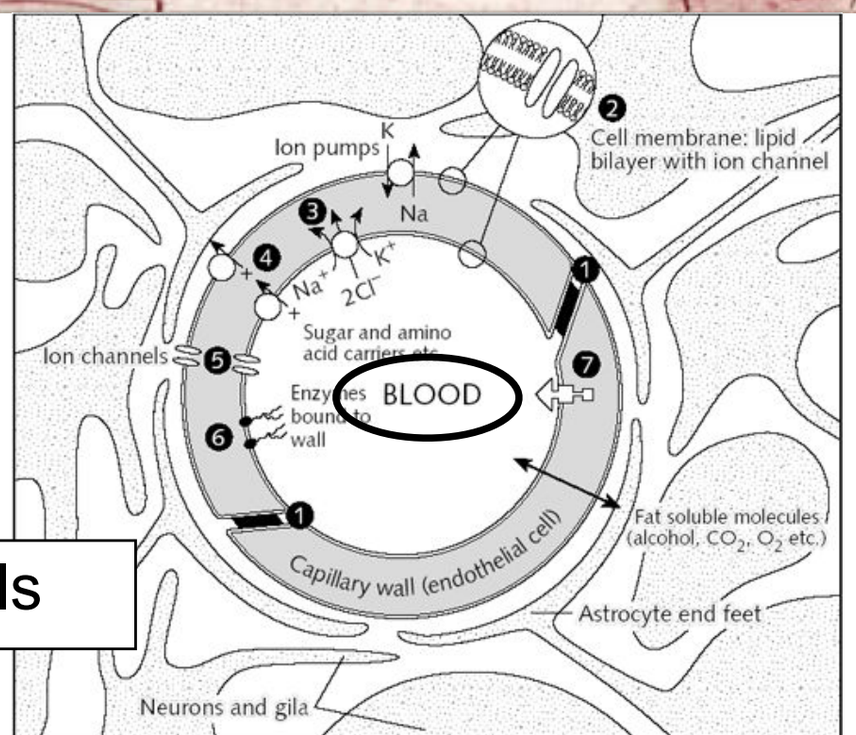
subcellular orientation:

apical (luminal) o
basolateral (abluminal)

Substrate direction:

Uptake (into the cell) or
efflux (outside the cell)

Endothelial cells

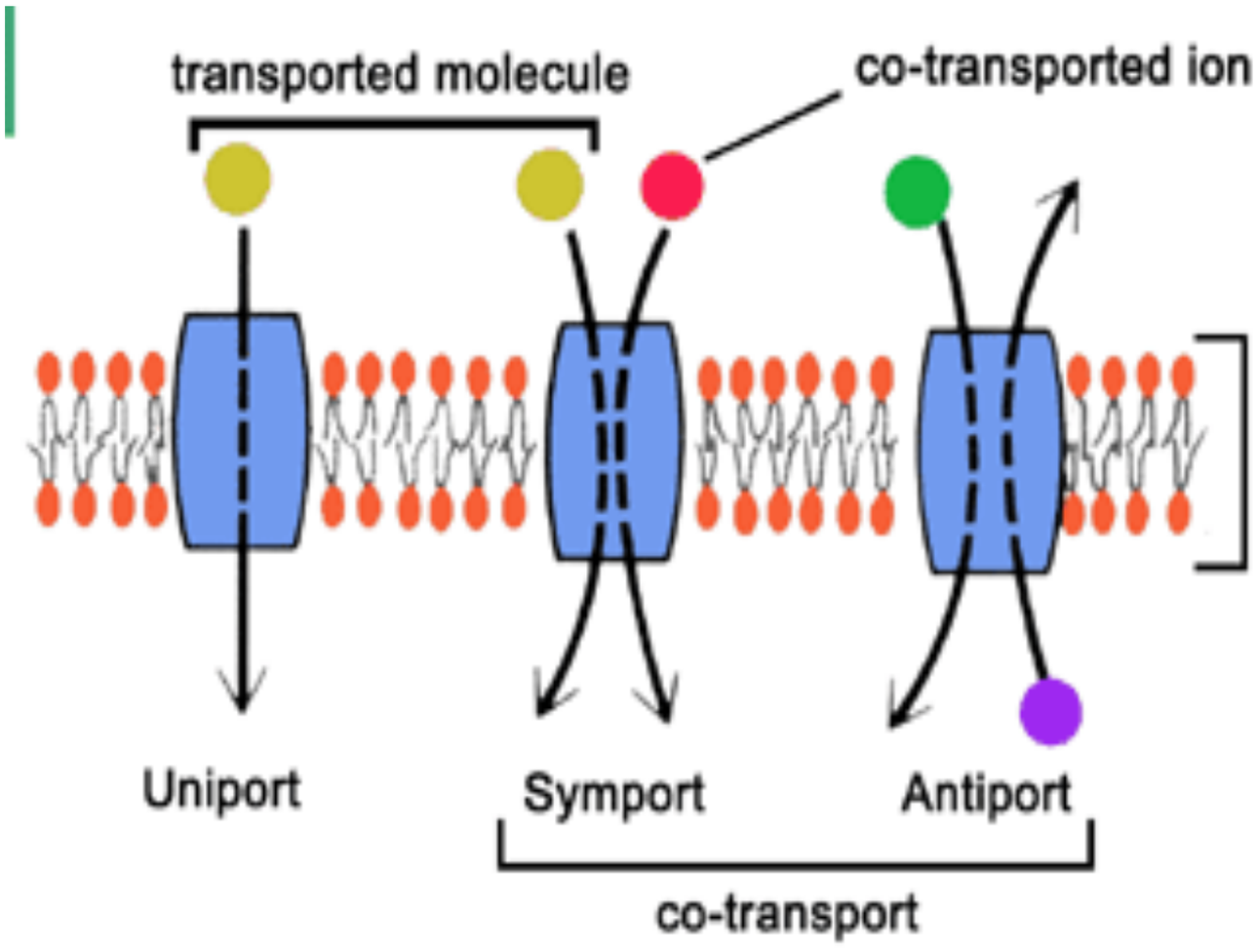


TRANSPORTERS

Facilitated diffusion	Active transport
Electrochemical potential-driven transporters (solute carrier, SLC)	Primary active transporters
Superfamily of SLC: 48 families 315 genes	Superfamily of ABC transporters: 7 families 49 genes

Facilitated Diffusion

Carrier molecules facilitate entry and exit of physiologically important polar and charged molecules molecules, such as sugars, amino acids, neurotransmitters and metal



- **No external energy source is needed**
- **Down concentration/ electrochemical gradient**
- **Transport is saturable (is mediated by a limited number of proteins) and selective**

Facilitated Diffusion

Neurotransmitter Transporters Family:

DAT
NET
5HTT

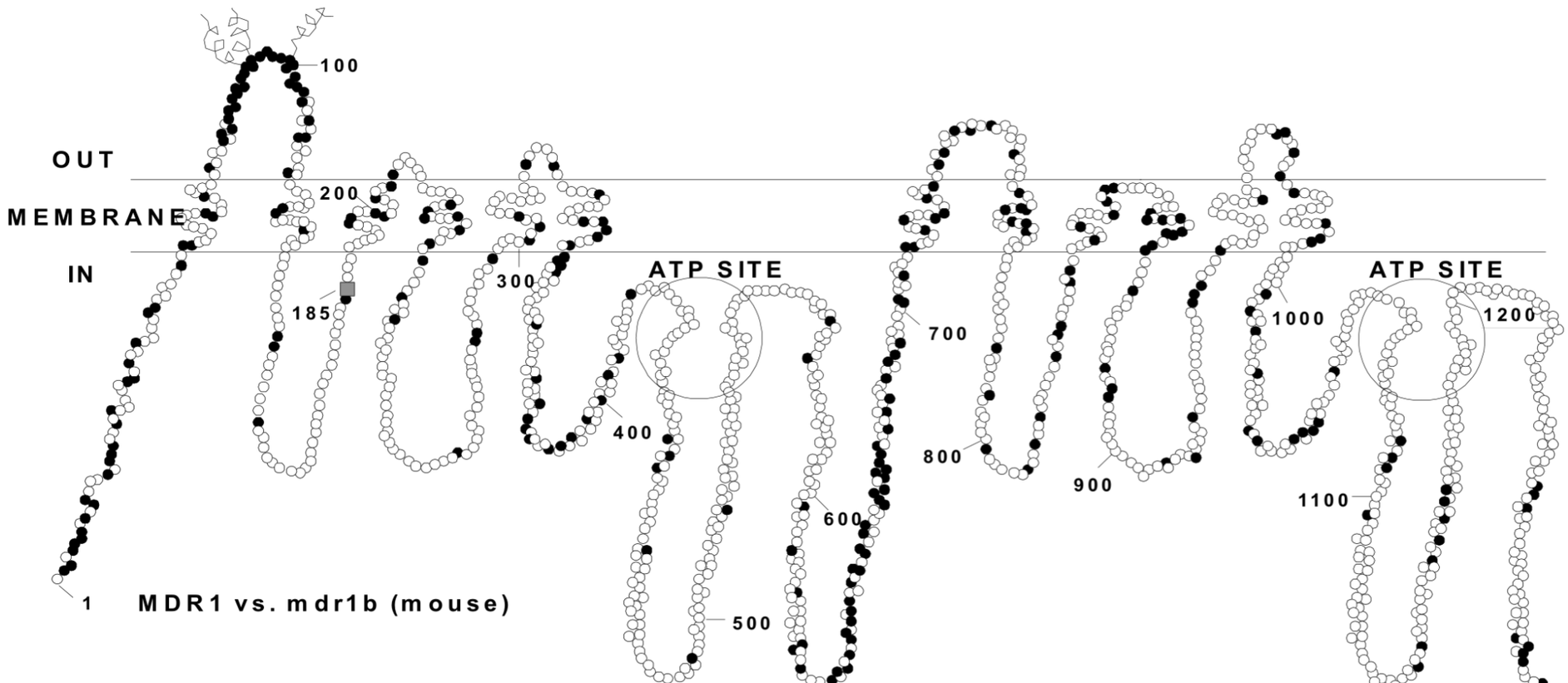
Major Facilitator Superfamily

ORGANIC CATION TRANSPORTER - OCT

ORGANIC ANION TRANSPORTERS – OAT

Active Transport

- **Directly coupled to energy source (ATPase)**
- **Against concentration gradient**
- **Transport is saturable (is mediated by a limited number of proteins) and selective**



Active Transport

P-ATPase Superfamily

SERCA

ATP Binding Cassette (ABC) Superfamily

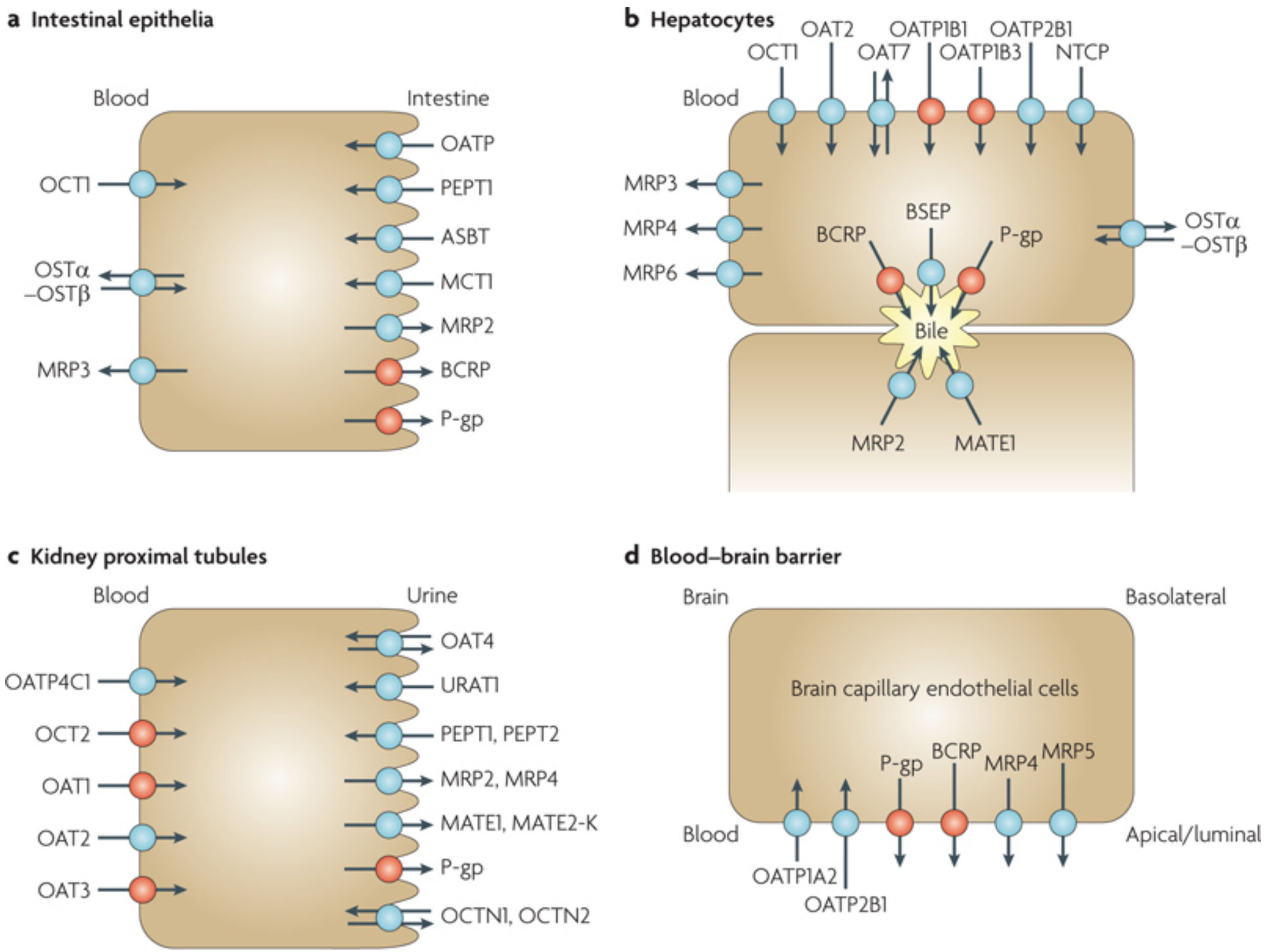
- **Large gene family**
- **Defined by sequence homology**
- **Critical for moving a wide range of substances**
- **Approximately 1000 ABC proteins have been identified, 48 in humans**

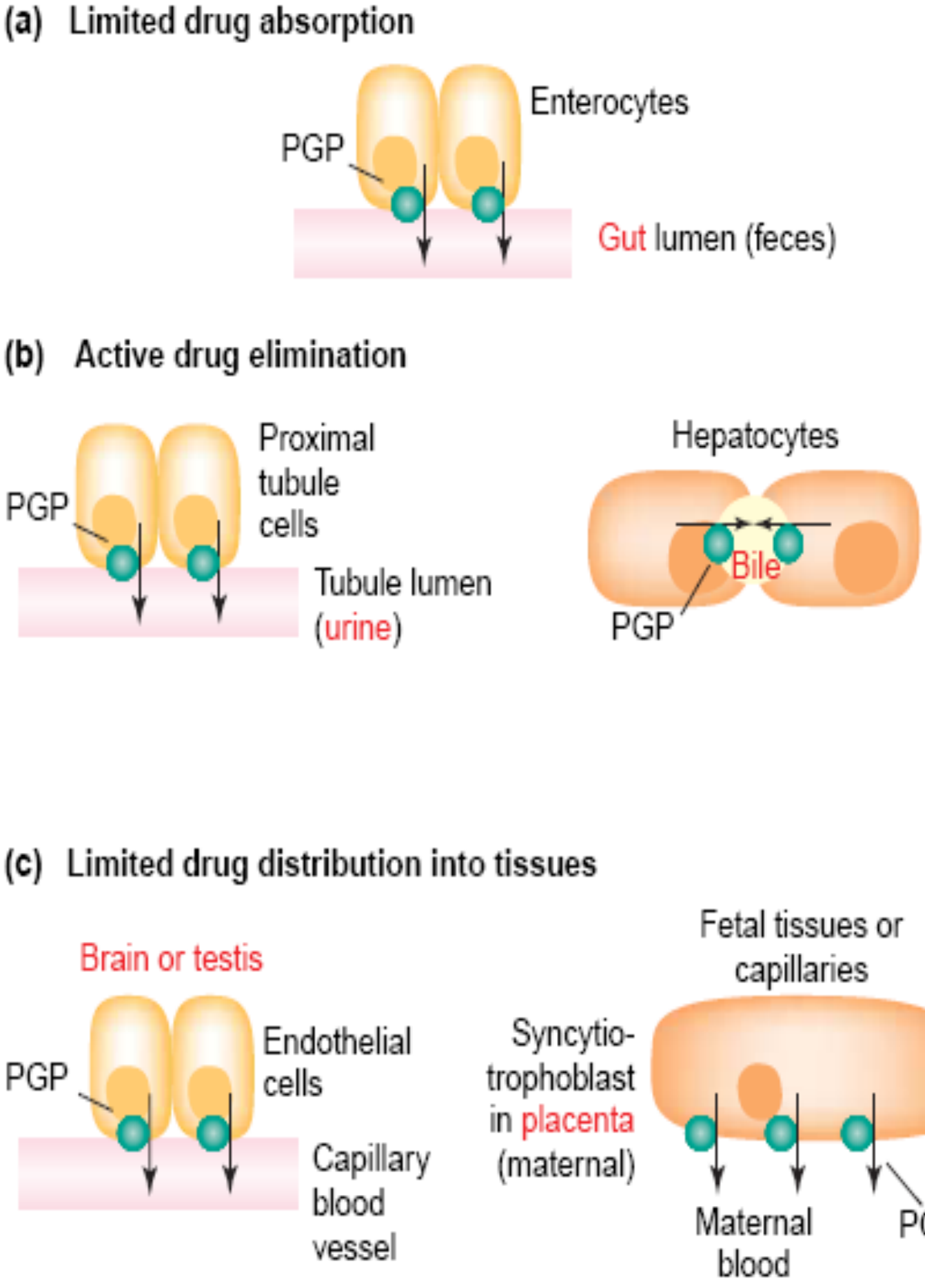
MDR (Multi Drug Resistance) family
e.g. P-glycoprotein

P-glycoprotein

- **Encoded by the MDR1 gene, is an efflux pump responsible for the resistance of tumor cells to multiple chemotherapeutic agents**
- **Expressed on the apical membrane of epithelial cells in the intestine, liver, kidney, testes, blood-brain barrier and adrenals**
- **Plays a role in the absorption, distribution and elimination of numerous drugs**

TRANSPORTERS LOCALIZATION

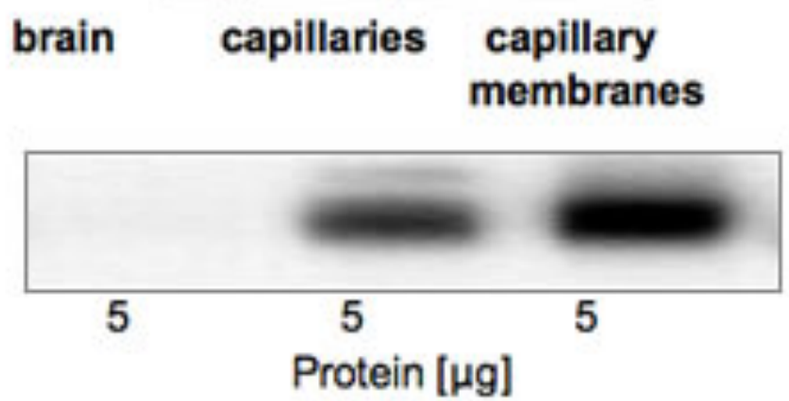




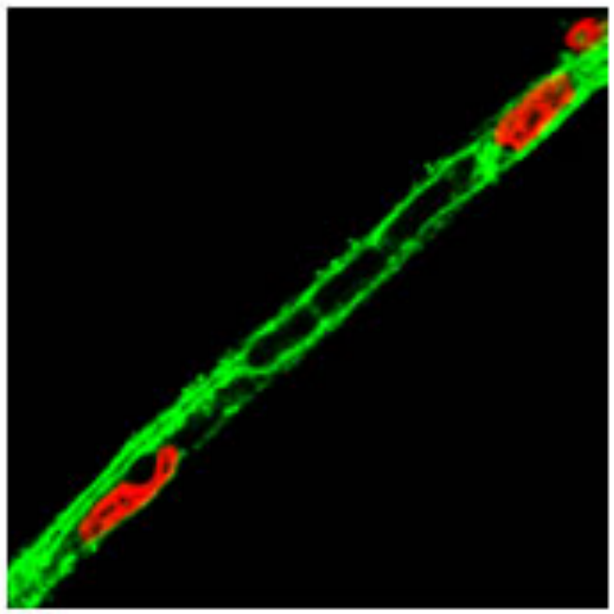
How P-glycoproteins expression affects ADME

Why is p-Glycoprotein the 800-lb Gorilla of the Blood-Brain Barrier?

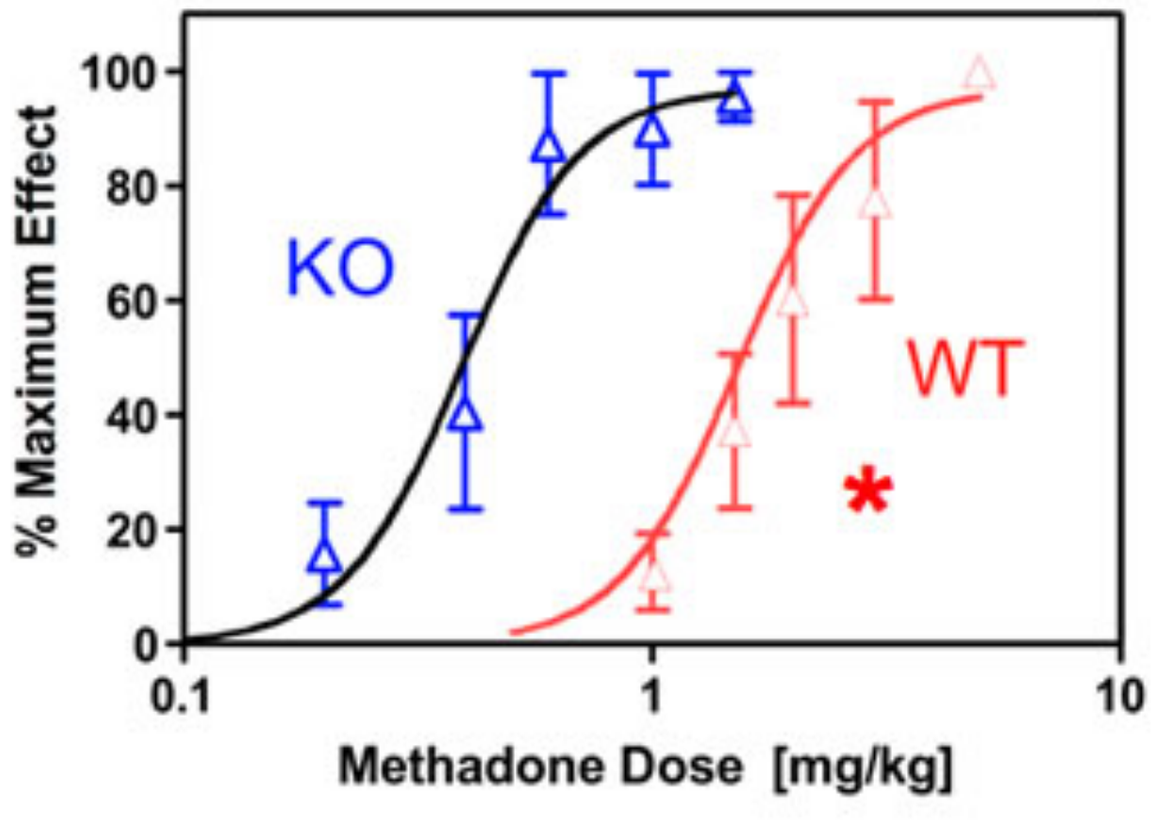
High Expression



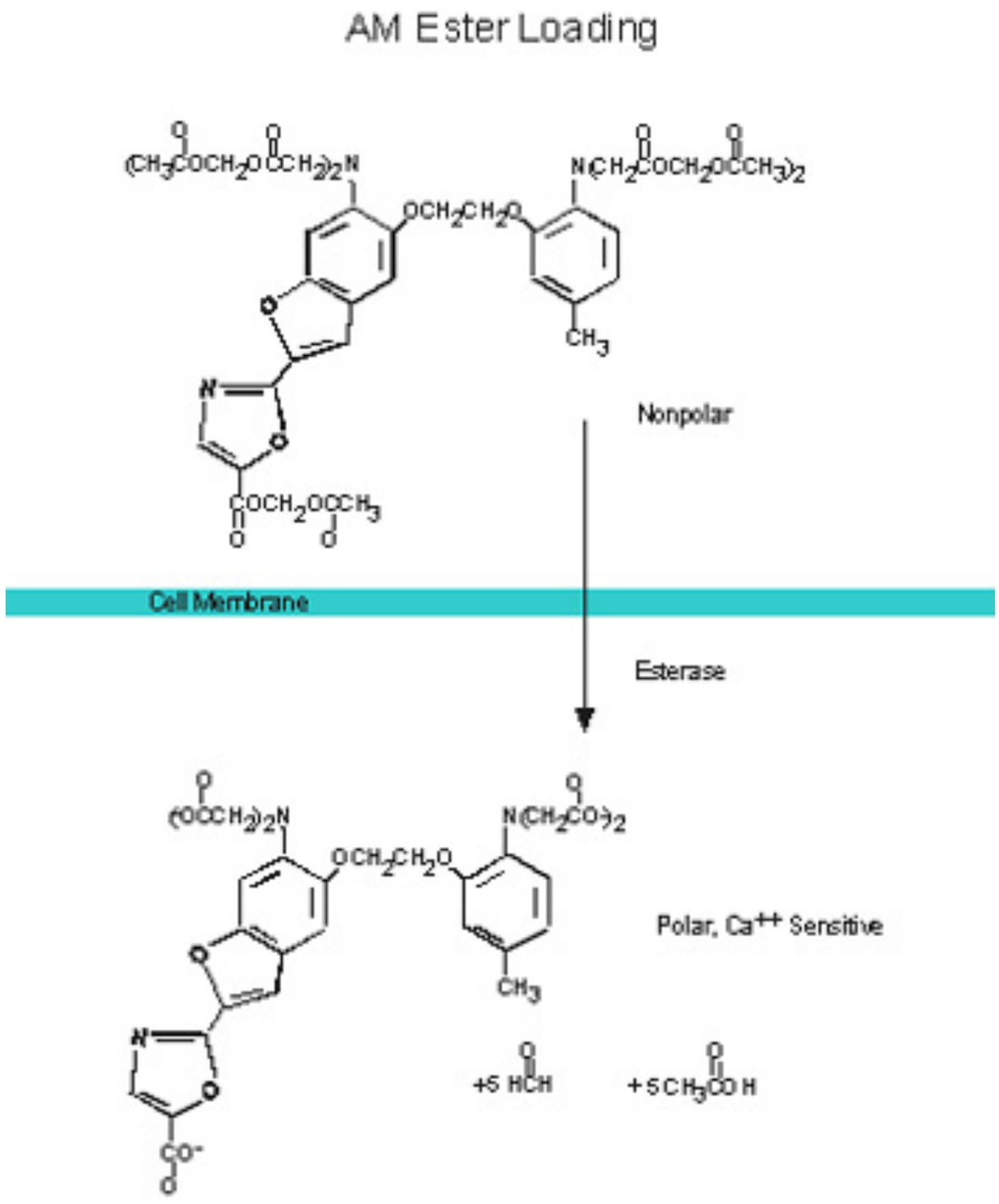
Right Location



KO for p-Gp

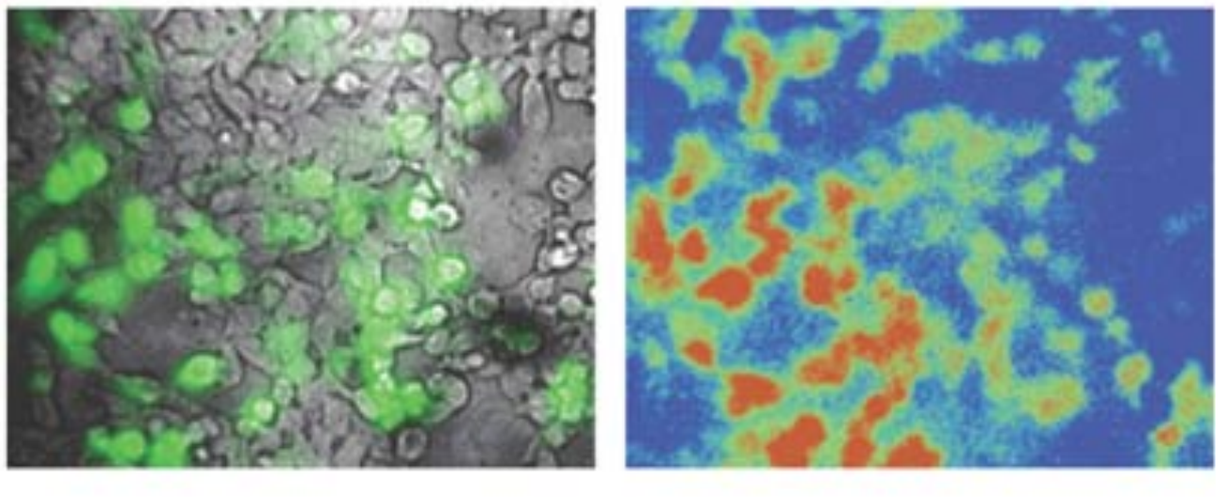


Fura-2

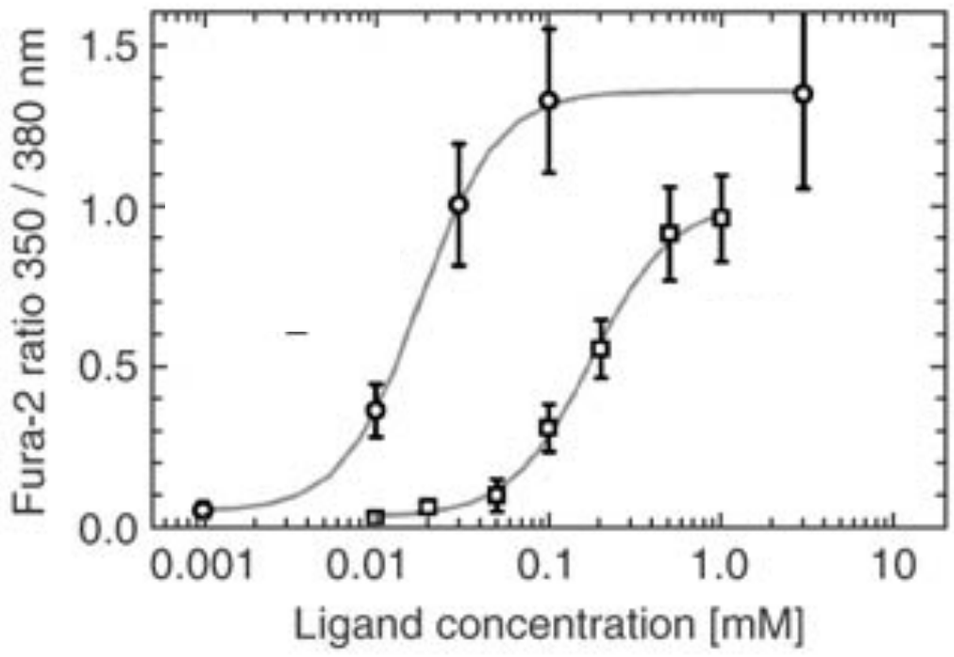


a no p-gp blocker (square)

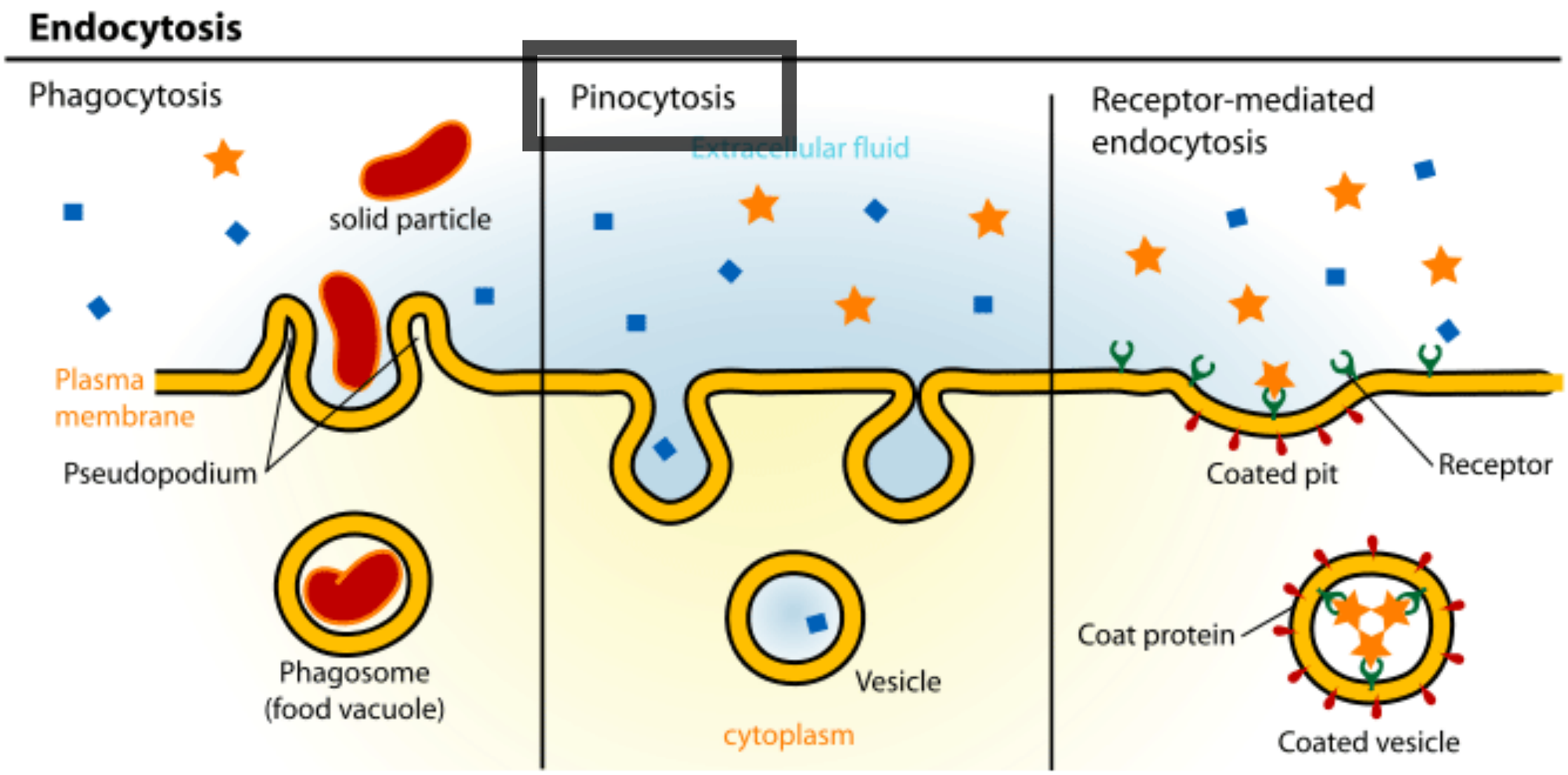
b plus p-gp blocker (circle)



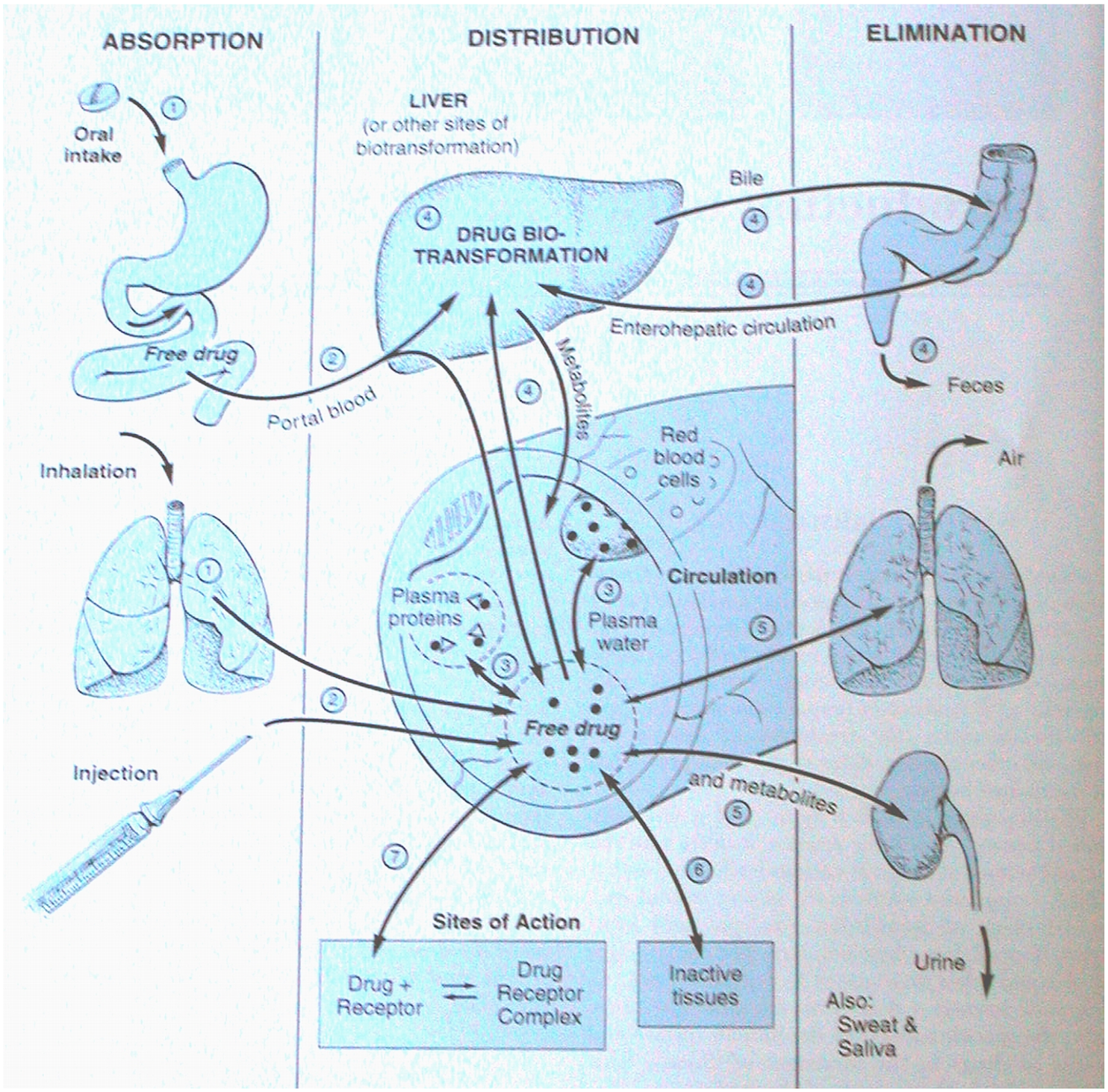
d



Vesicle-mediated transport



ADME: Distribution



Delivery of the drug from the blood to the tissues

It depends on:

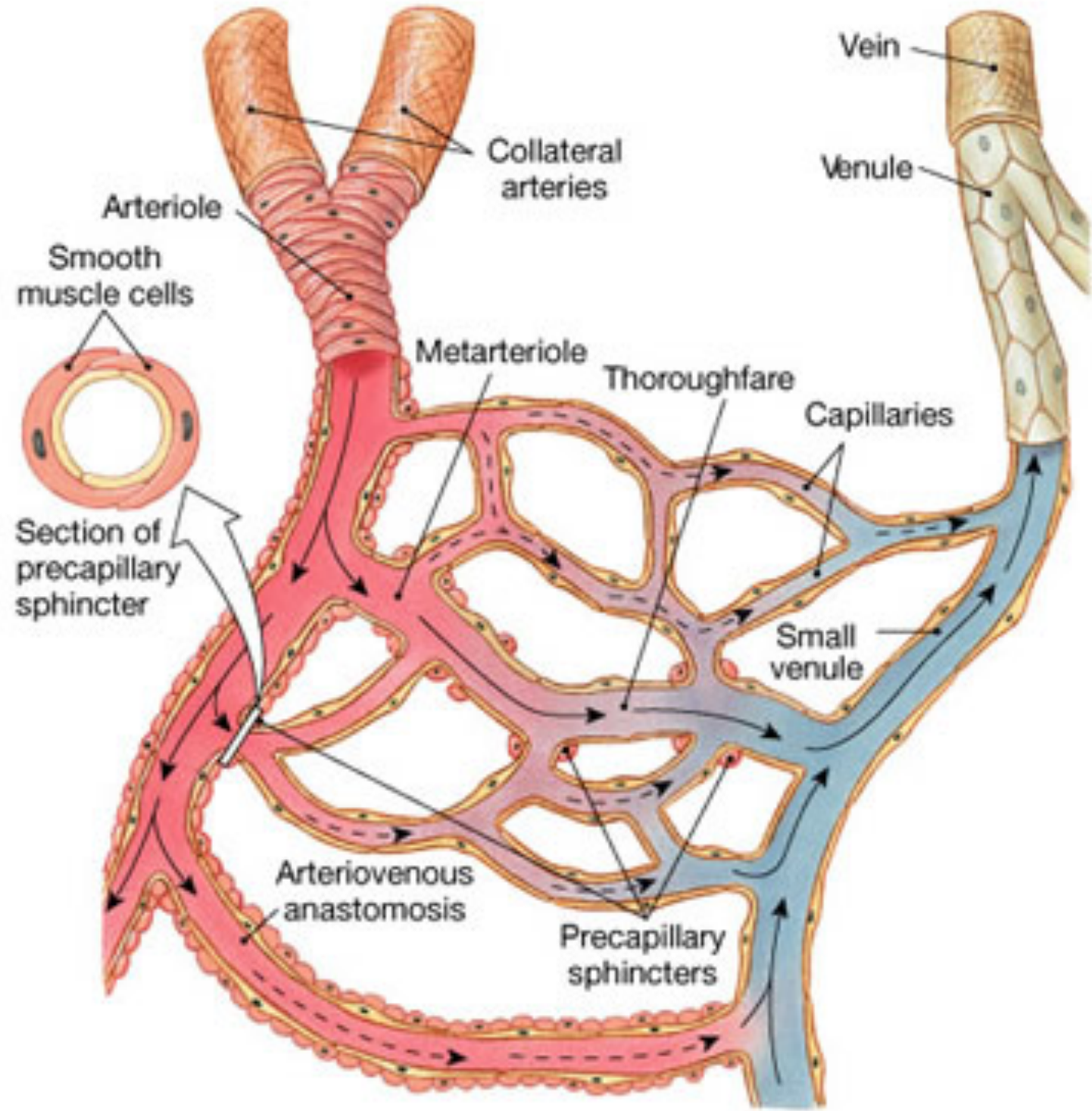
1. Tissue perfusion rate and type of capillaries

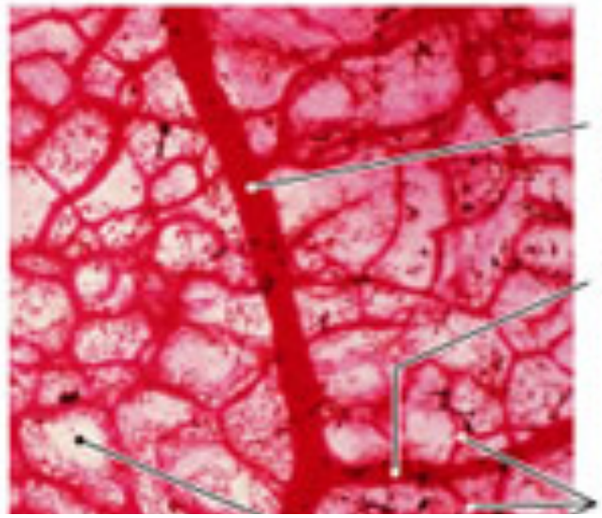
2. Plasma protein (albumin) binding

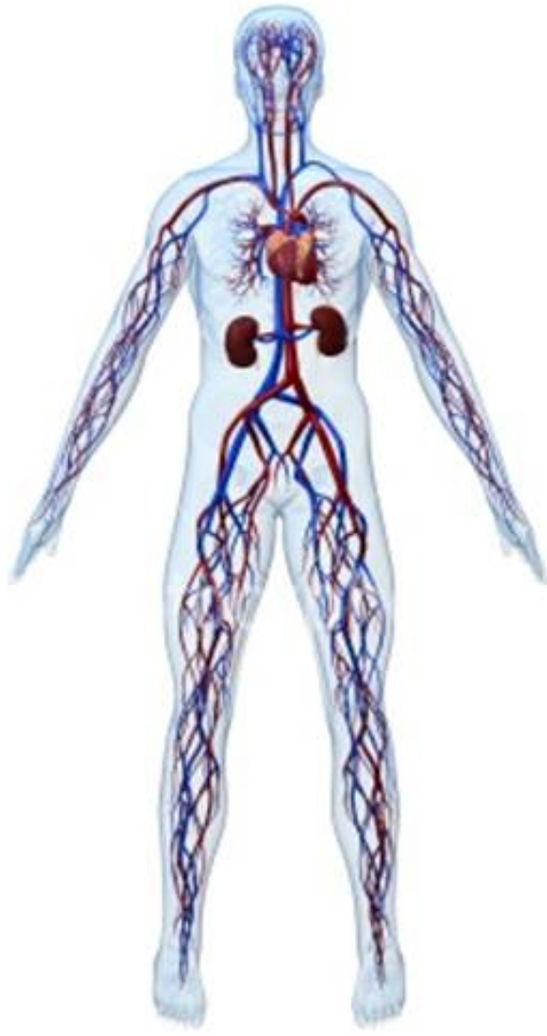
3. Accumulation in tissues

4. Presence of barriers

1. Rate of perfusion



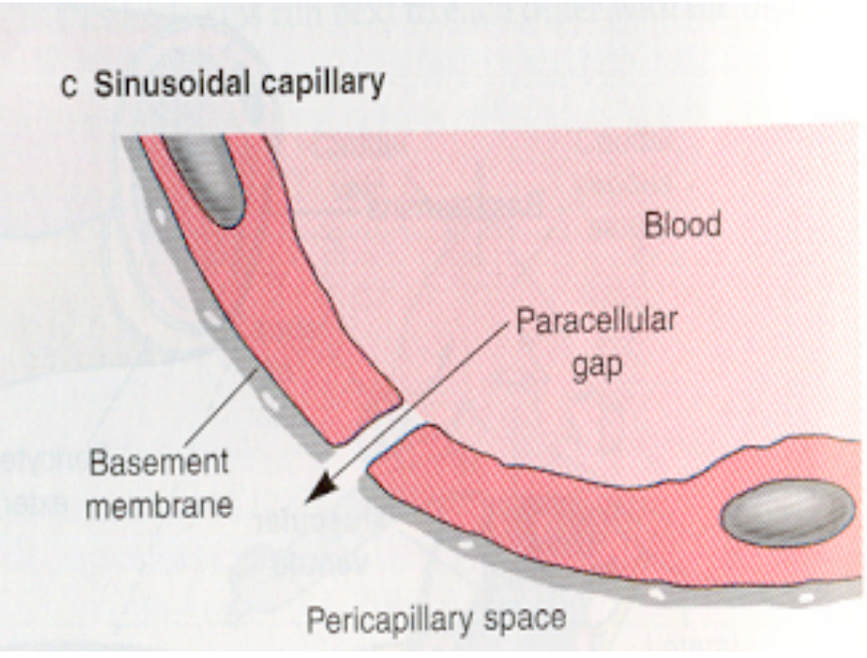


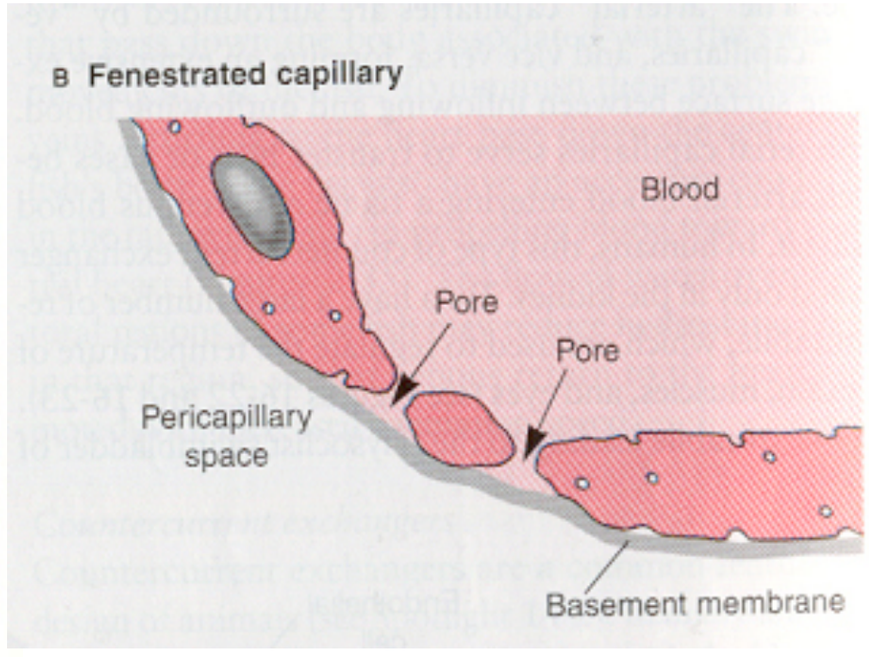


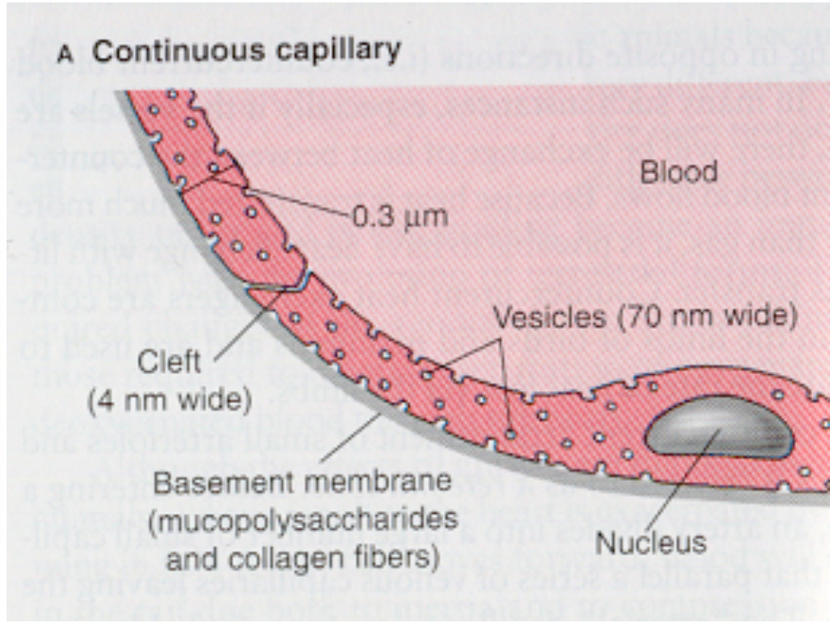
1. Rate of perfusion

ORGAN	PERFUSION RATE (ml/min)	% of cardiac output
Liver	**1350**	**27**
Kidneys	**1100**	**22**
Muscle	**750**	**15**
Brain	**700**	**14**
Skin	**300**	**6**
Heart	**300**	**6**
Bone	**250**	**5**
Fat	**200**	**4**

Different types of capillaries

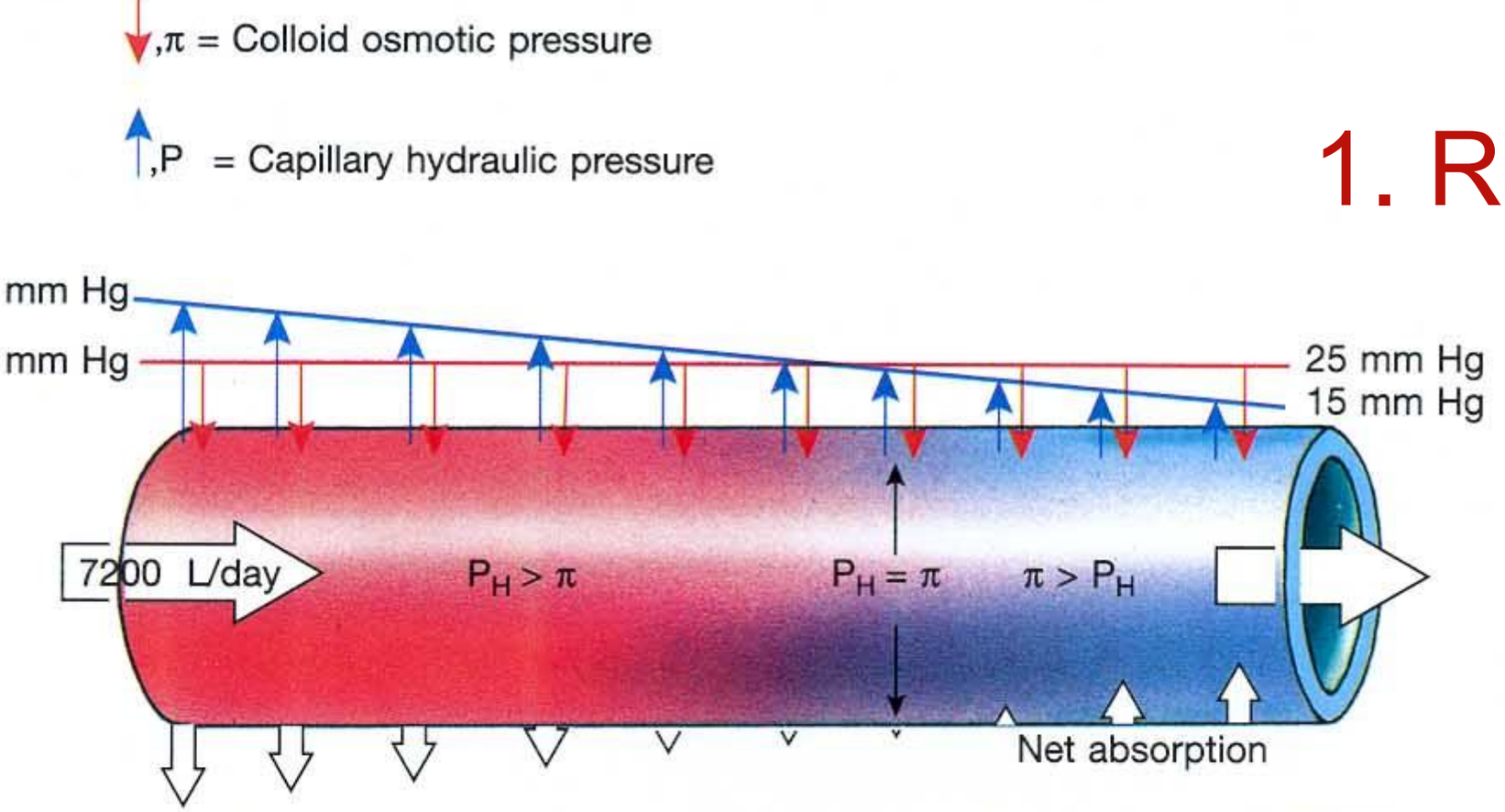


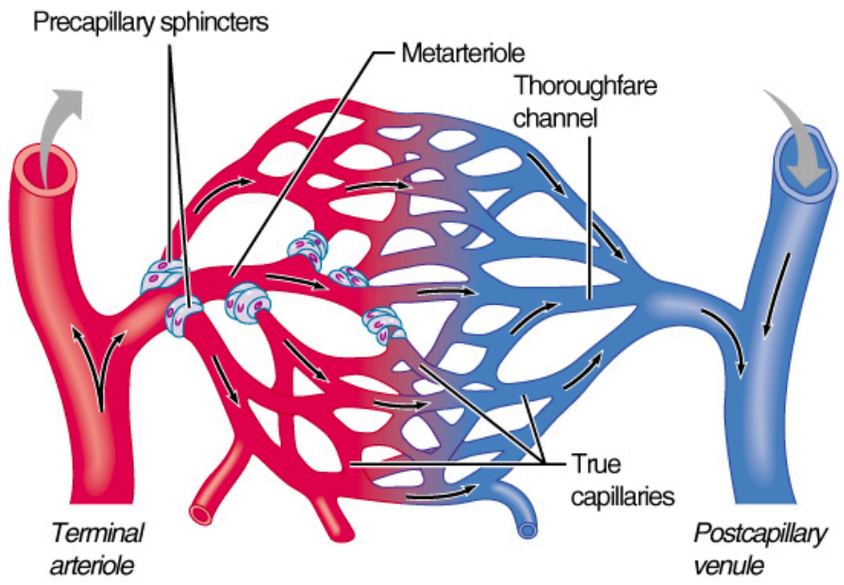




Sinusoids: **endothelium and basal membrane presents intercellular cleft**	***Fenestrated:*** **endothelium presents intercellular cleft, basal membrane is continous**	***Continous*****: endothelium and basal membran presents no intercellular cleft**
Localization**:**		
liver spleen Bone marrow limphonodes	**Gastro-intestinal mucosa kidney Endocrin glands**	**Skeletal and cardiac muscle Smooth muscle lung**
Permeability for hydrofilic molecules		
excellent	**good**	**scarce**

1. Rate of perfusion





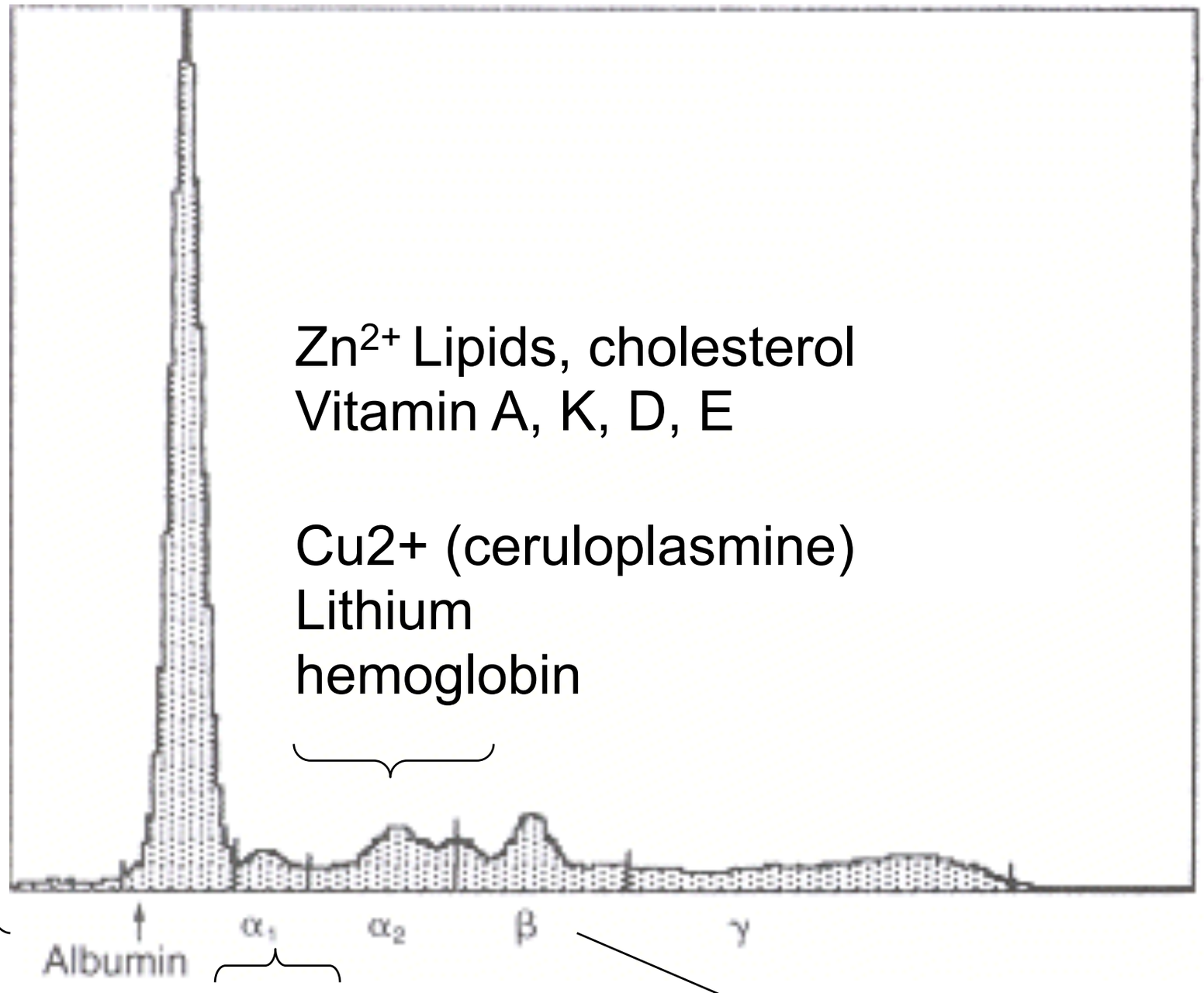
2. Plasma proteins binding

Bilirubin
Uric acid
Vitamin C
Adenosine
Tetracycline
Fatty Acids
Penicillin

Salicilates
Streptomicine
Histamine

Barbiturates

Ca^{2+}
Cu^{2+}
Zn^{2+}



Zn^{2+} Lipids, cholesterol
Vitamin A, K, D, E

Cu2+ (ceruloplasmine)
Lithium
hemoglobin

steroid hormones
(transcortine)
Vitamin B12
tiroxine

Fe^{2+}
(Transferrine)

2. Plasma proteins binding

drug-protein complex

Binding to plasma proteins is reversible → K_1 ⇅ K_2

inert, non-diffusible, not available for metabolism and excretion. It acts as a reservoir for drug

Free drug + protein

Sites of action of the drug:

Capillaries wall
Cell membranes
Intracellular sites

Sites of drug elimination:

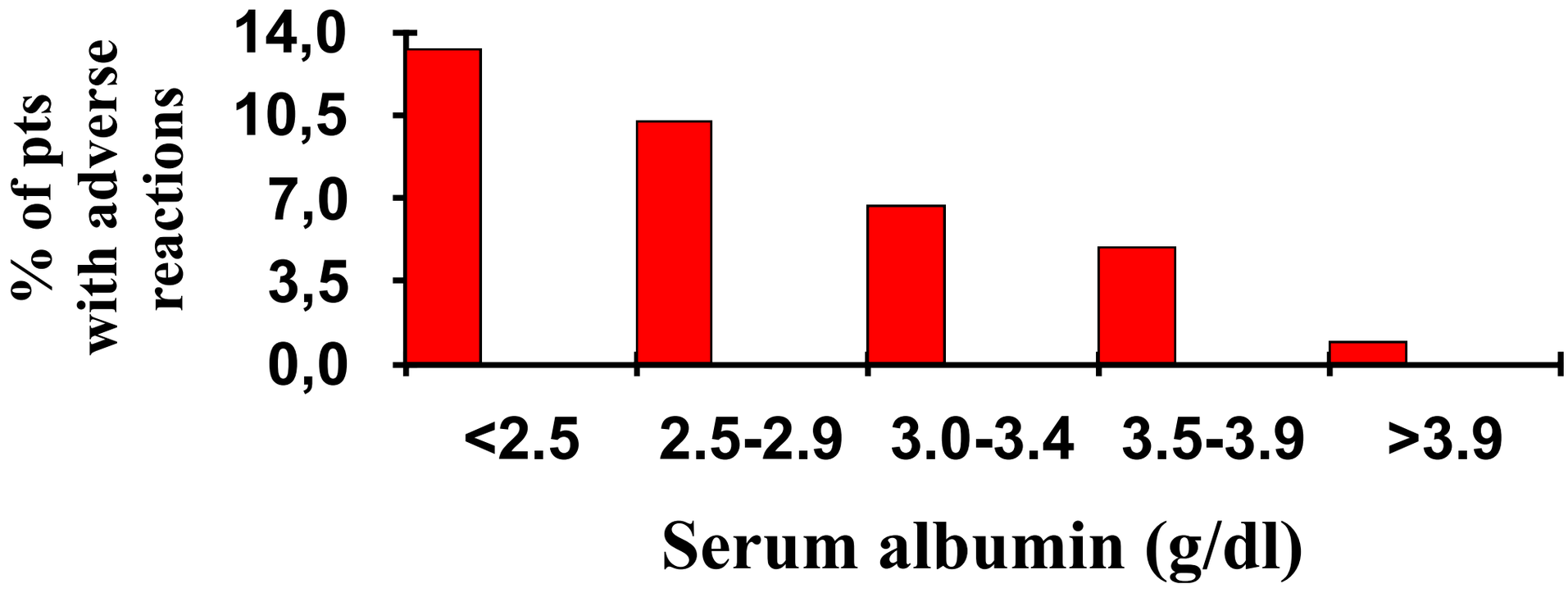
glomerular filtration
tubular secretion
biotrasformations
biliar secretion

2. Plasma proteins binding

The formation of drug-protein complex depends on:

- physiochemical properties of the drug
- drug concentration
- drug-protein affinity
- total proteins

Adverse Reactions to Phenytoin as a Function of Serum Albumin Concentration



2. Plasma proteins binding

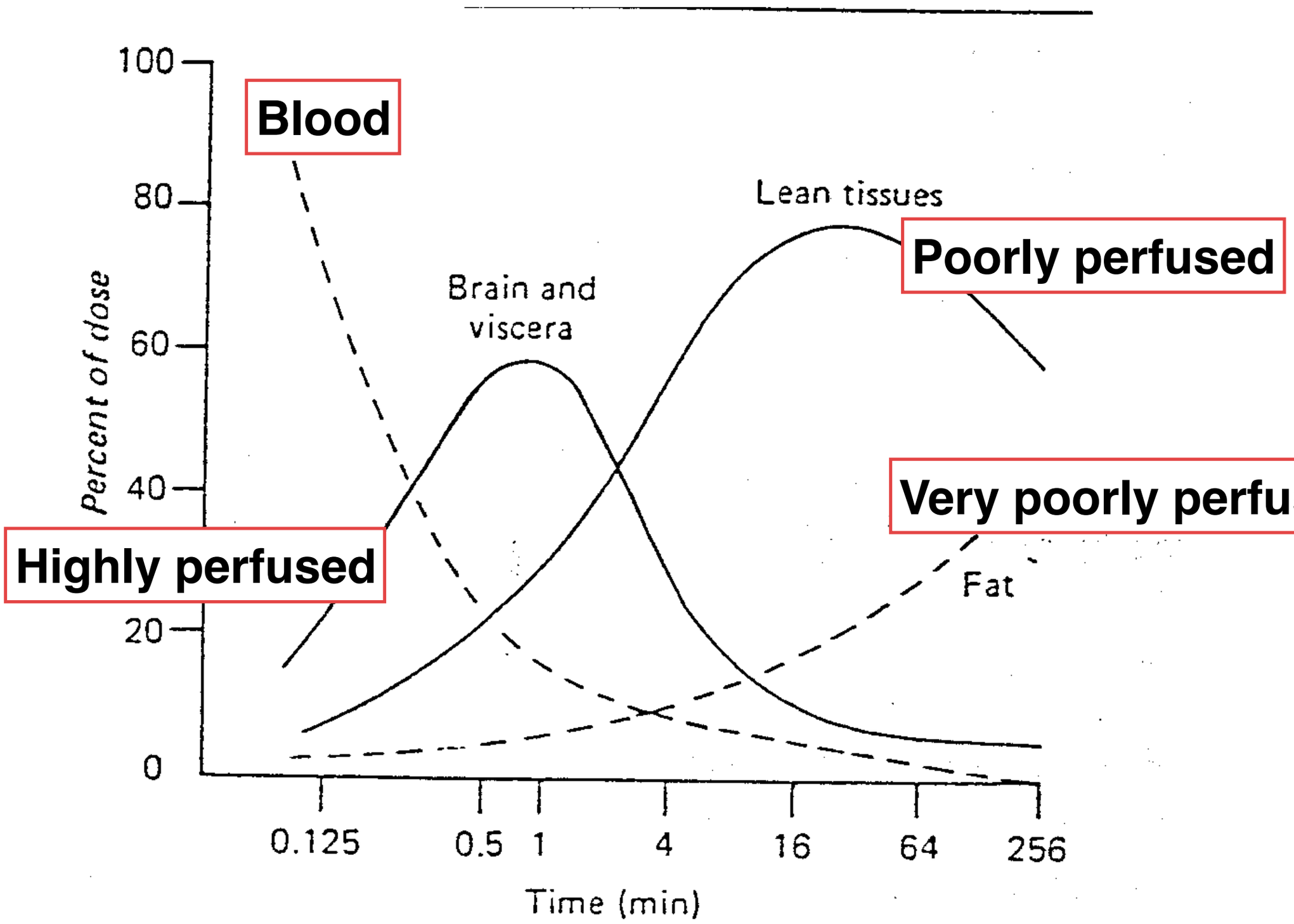
- **Drugs highly bound to plasma proteins generally persist in body longer than those less bound, have lower therapeutic activity and less efficient distribution**

- **Two drugs with affinity for plasma proteins compete with each other leading to displacement <u>drug interactions</u>**

Effect of the displacement of drugs highly bound to plasma protein

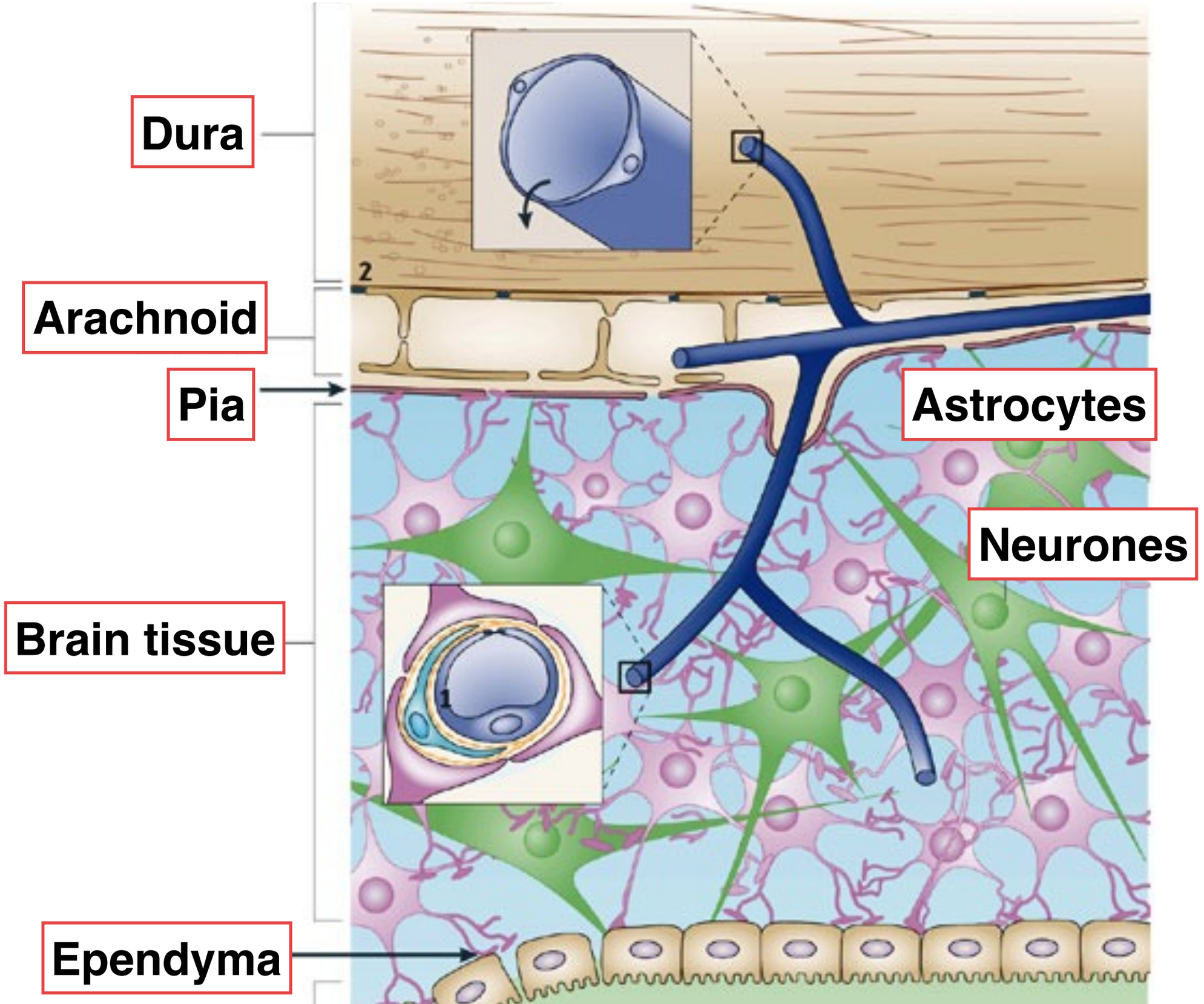
	% BEFORE DISPLACEMENT	% AFTER DISPLACEMENT	% INCREASE OF FREE DRUG
DRUG A			
% bound drug	95	90	
% free drug	5	10	+ 100
DRUG B			
% bound drud	50	45	
% free drug	50	55	+ 10

3. Accumulation in tissues

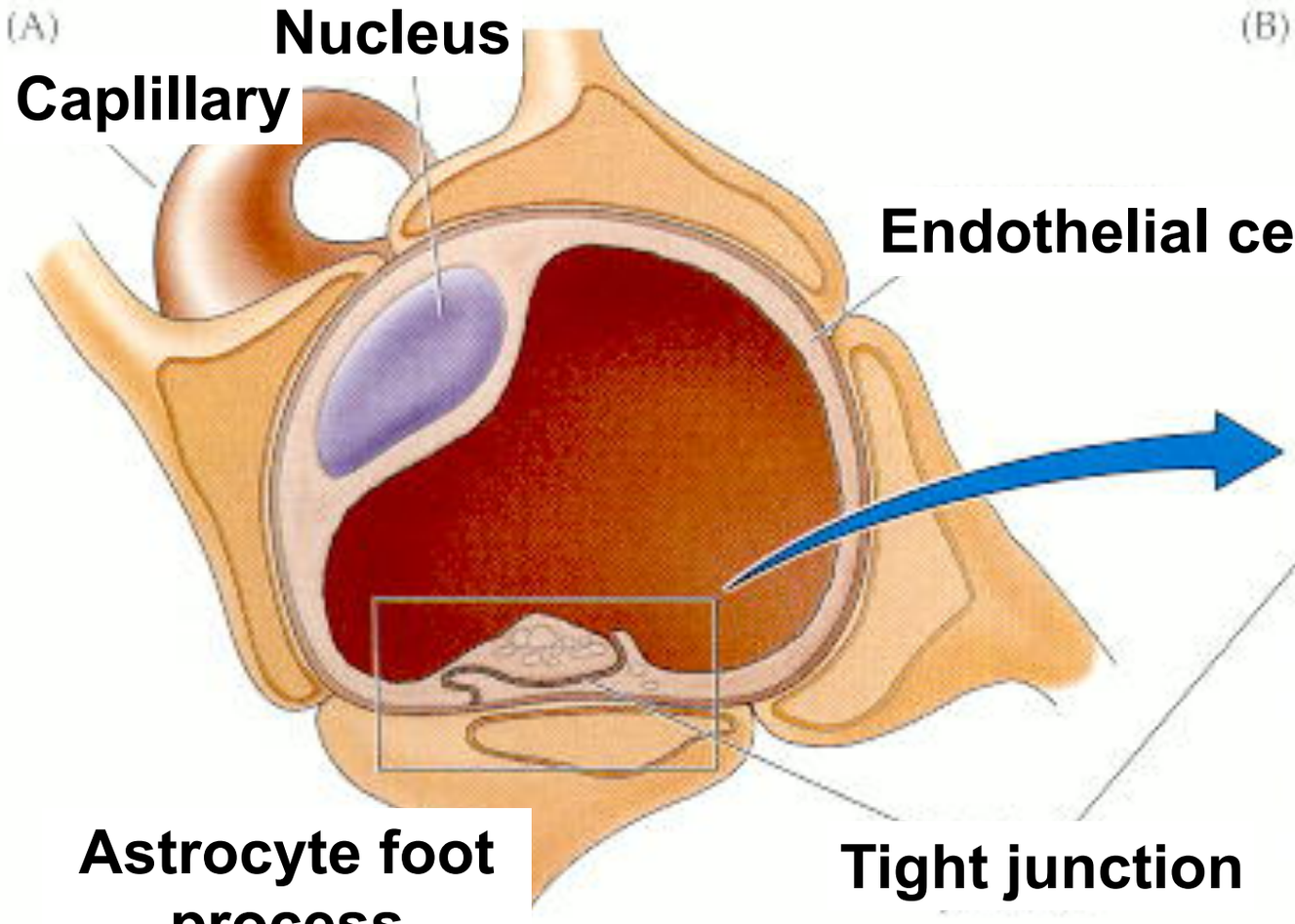


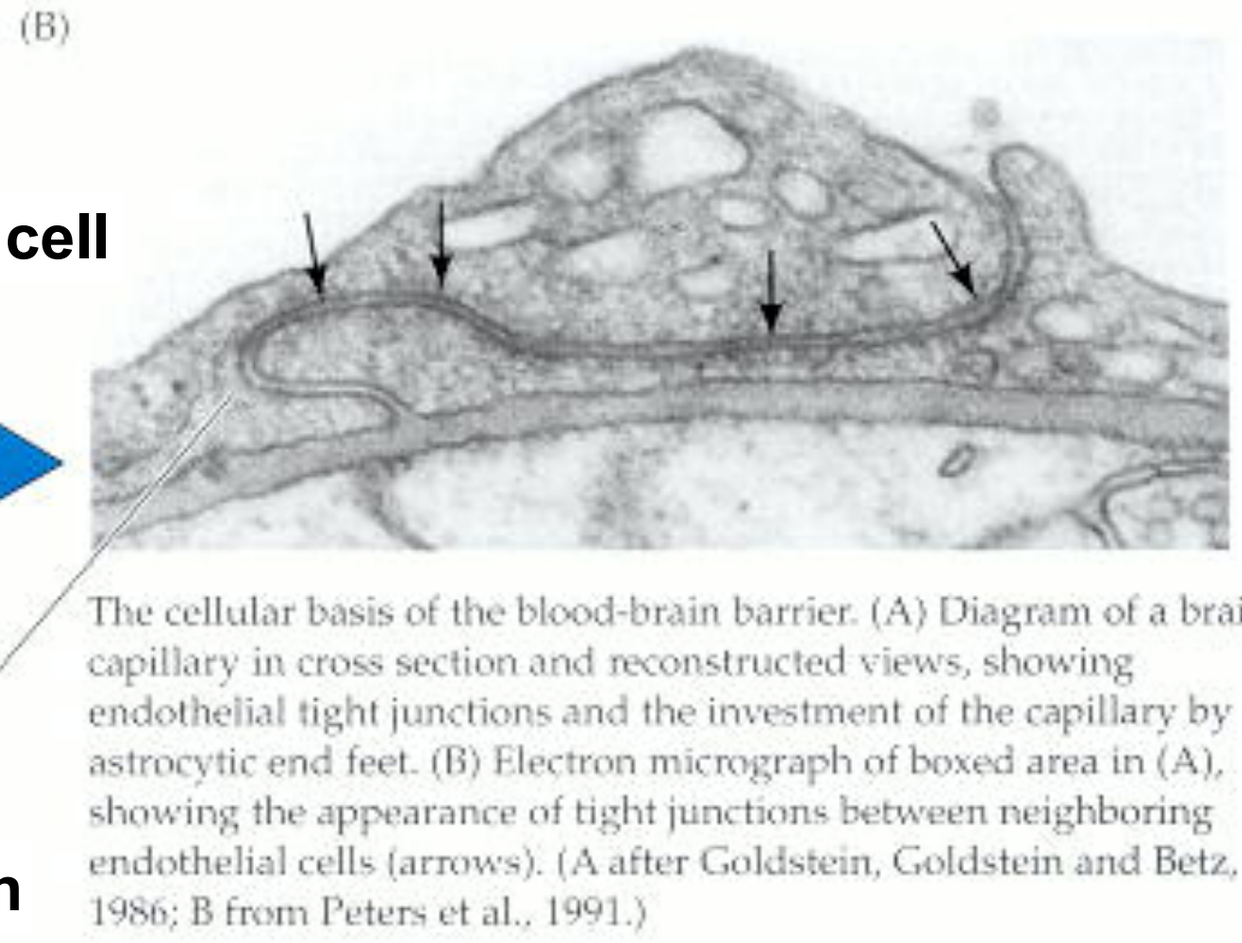
Time course of thiopental in blood and tissues after intravenous administration

4. Ability to cross barriers: the blood-brain barrier (BBB)



Blood Brain Barrier characteristics

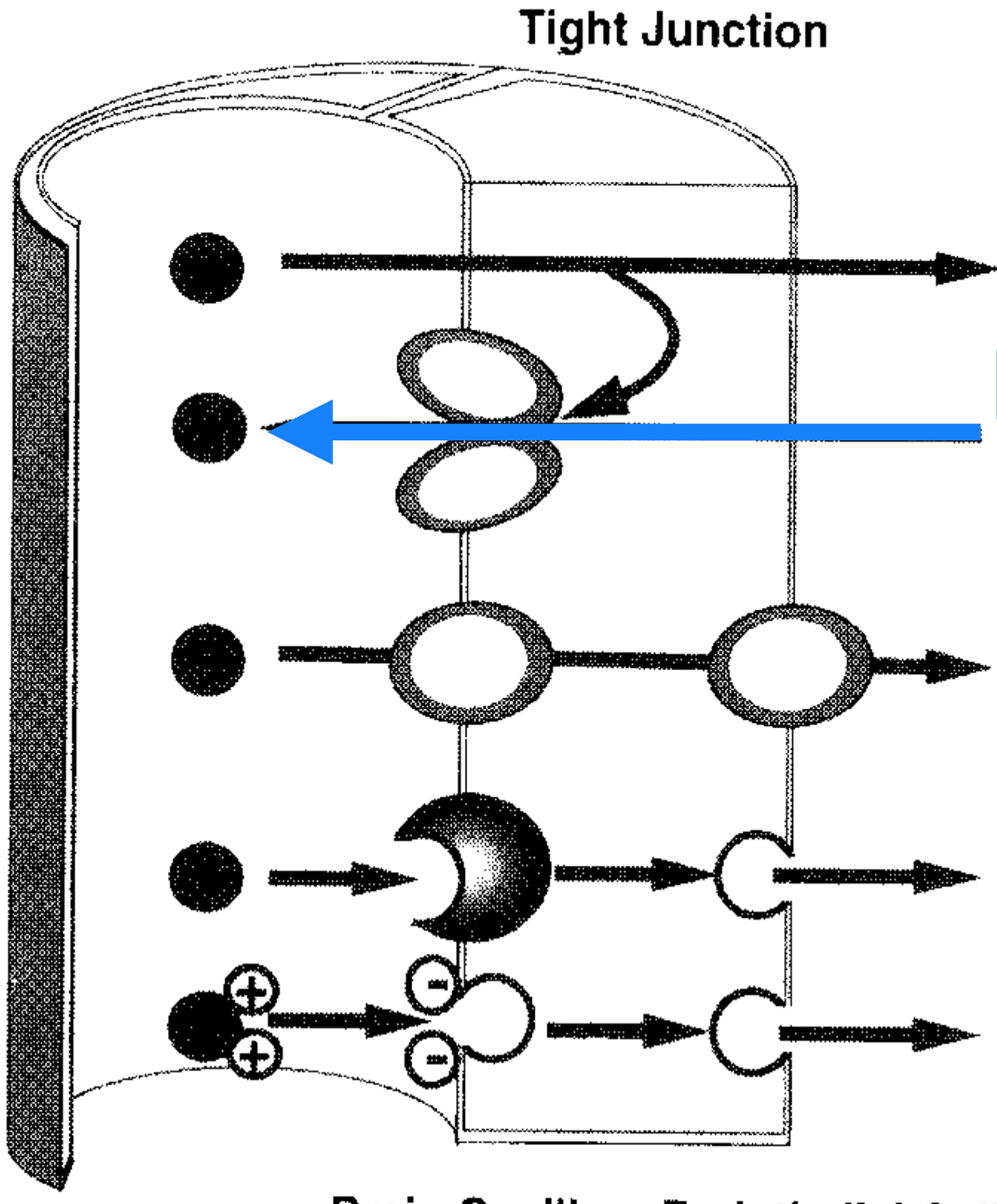




The cellular basis of the blood-brain barrier. (A) Diagram of a brain capillary in cross section and reconstructed views, showing endothelial tight junctions and the investment of the capillary by astrocytic end feet. (B) Electron micrograph of boxed area in (A), showing the appearance of tight junctions between neighboring endothelial cells (arrows). (A after Goldstein, Goldstein and Betz, 1986; B from Peters et al., 1991.)

1. No pores in endothelial membrane
2. Glial cells surround endothelial cells
3. Transporter in endothelial cells
4. Less protein concentration in interstitial fluid

Mechanisms of Blood-Brain Barrier Biotransport



Passive Diffusion

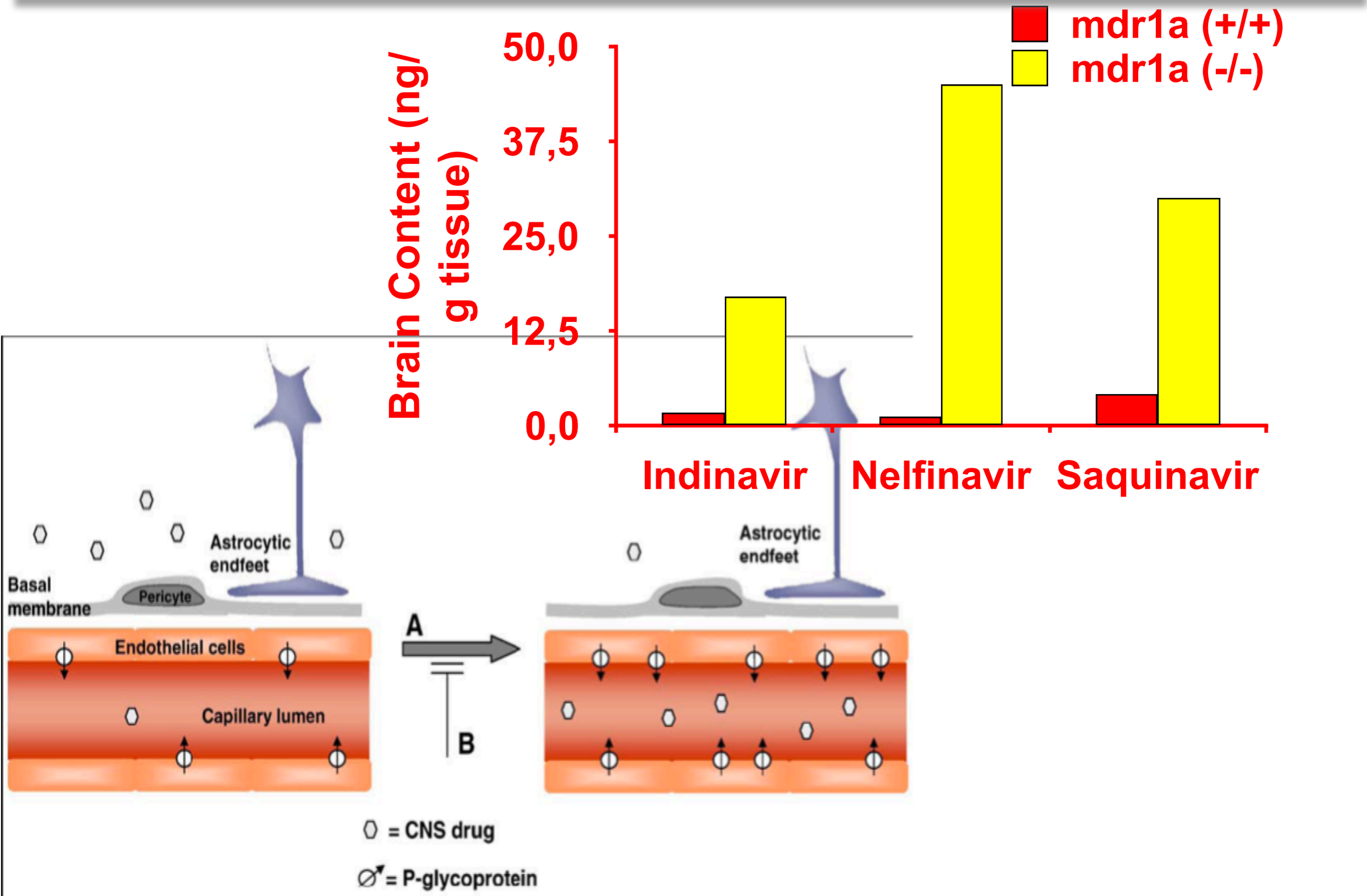
Carrier-Mediated Efflux Transport
ABC transporter (P-gp, MRP)
Transporters for organic anions,
CRH, anionic cyclic peptides,
cationic cyclic peptides, etc.

Carrier-Mediated Influx Transport
Transporters for monocarboxylates,
valproic acid, amines, amino acids,
hexose, nucleosides, glutathione,
small peptides, etc.

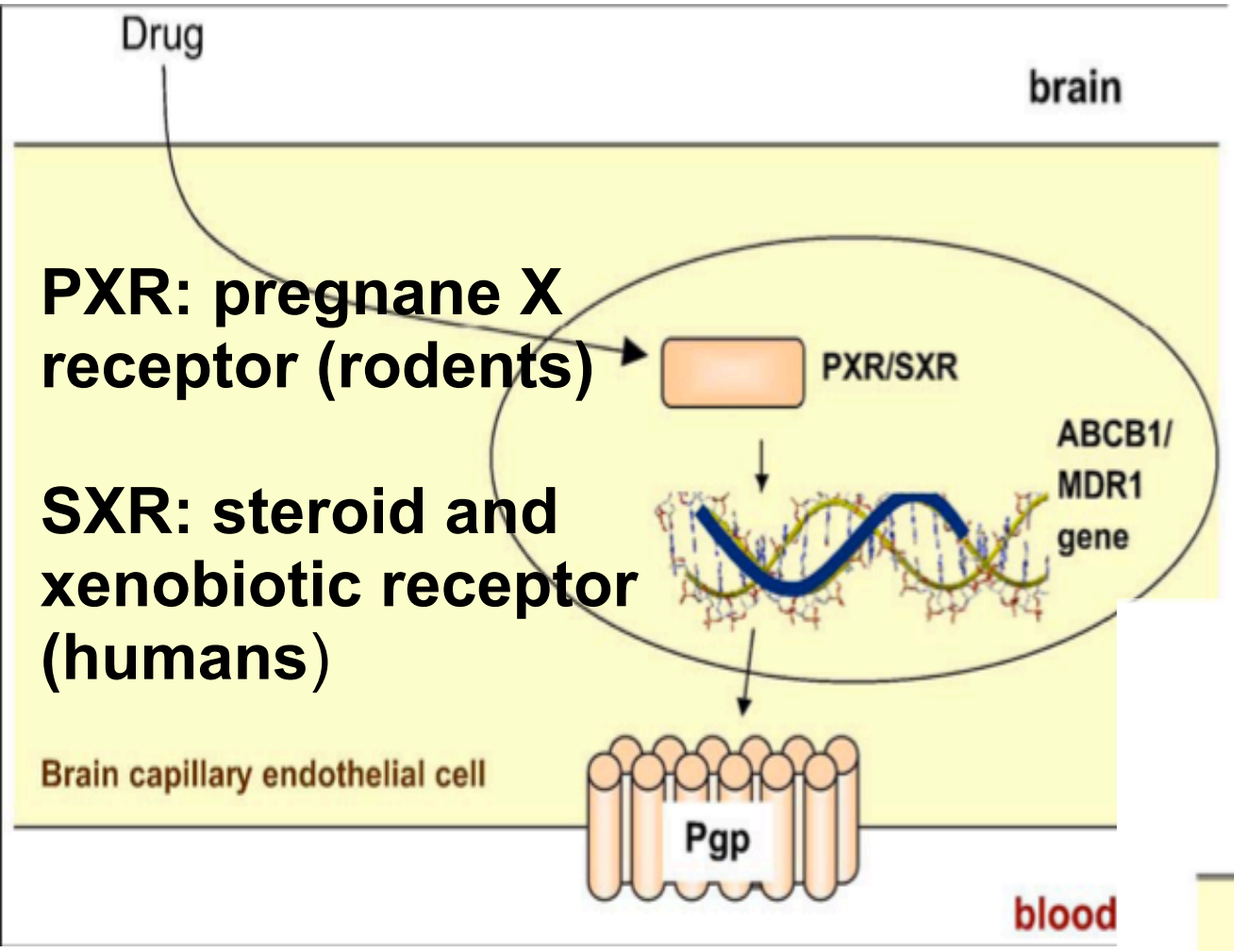
Receptor-Mediated Transcytosis
Transferrin, insulin

Adsorptive-Mediated Transcytosis
Histone, avidin, cationized albumin,
ebiratide, E-2078, cationic peptides

Role of P-glycoprotein determining brain content of protease inhibitors. Data from: Kim et al. *J Clin Invest* 101:289-294, 1998.



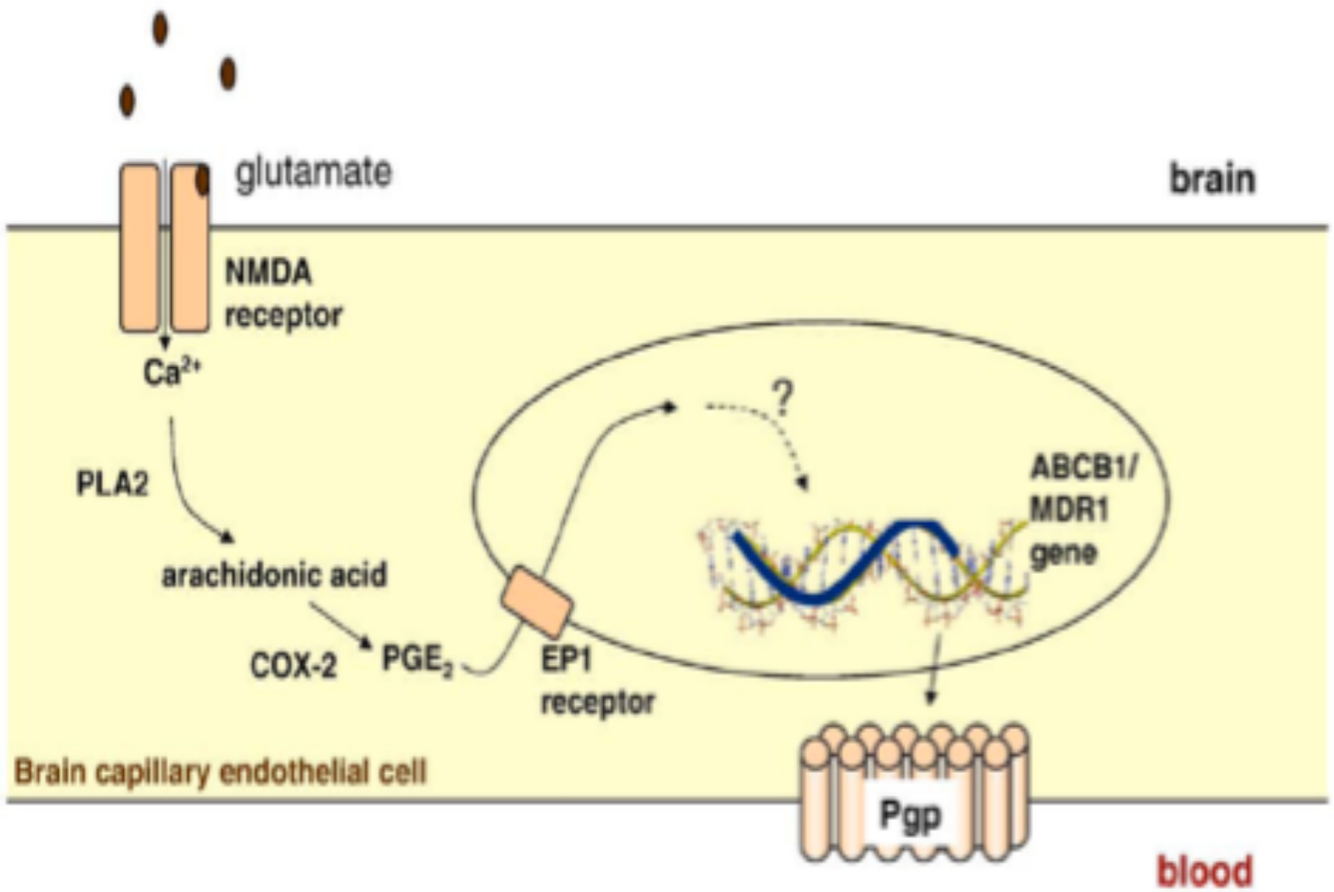
Role of drugs in the expression of P-glycoprotein at brain capillary endothelial cells



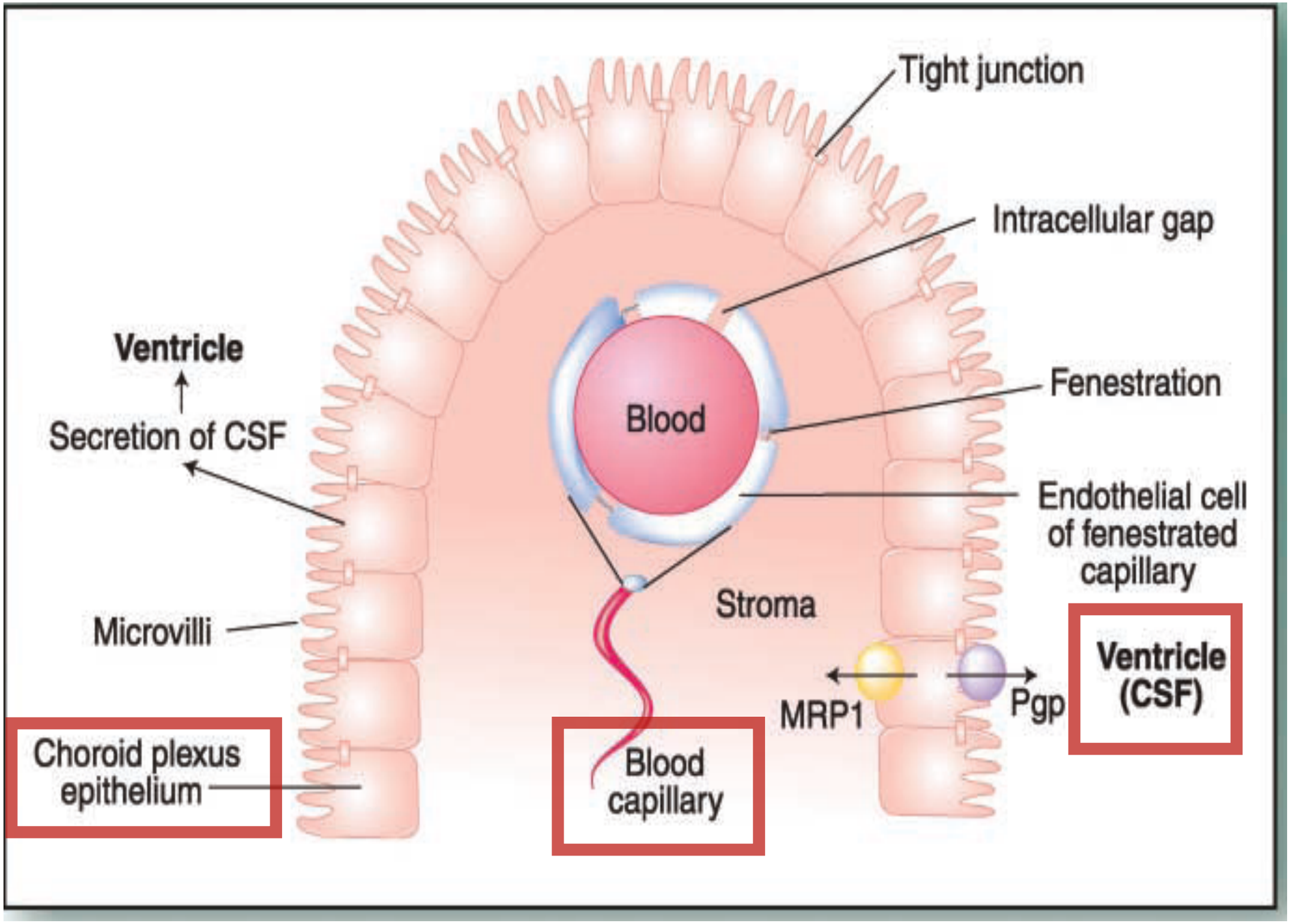
PXR: pregnane X receptor (rodents)

SXR: steroid and xenobiotic receptor (humans)

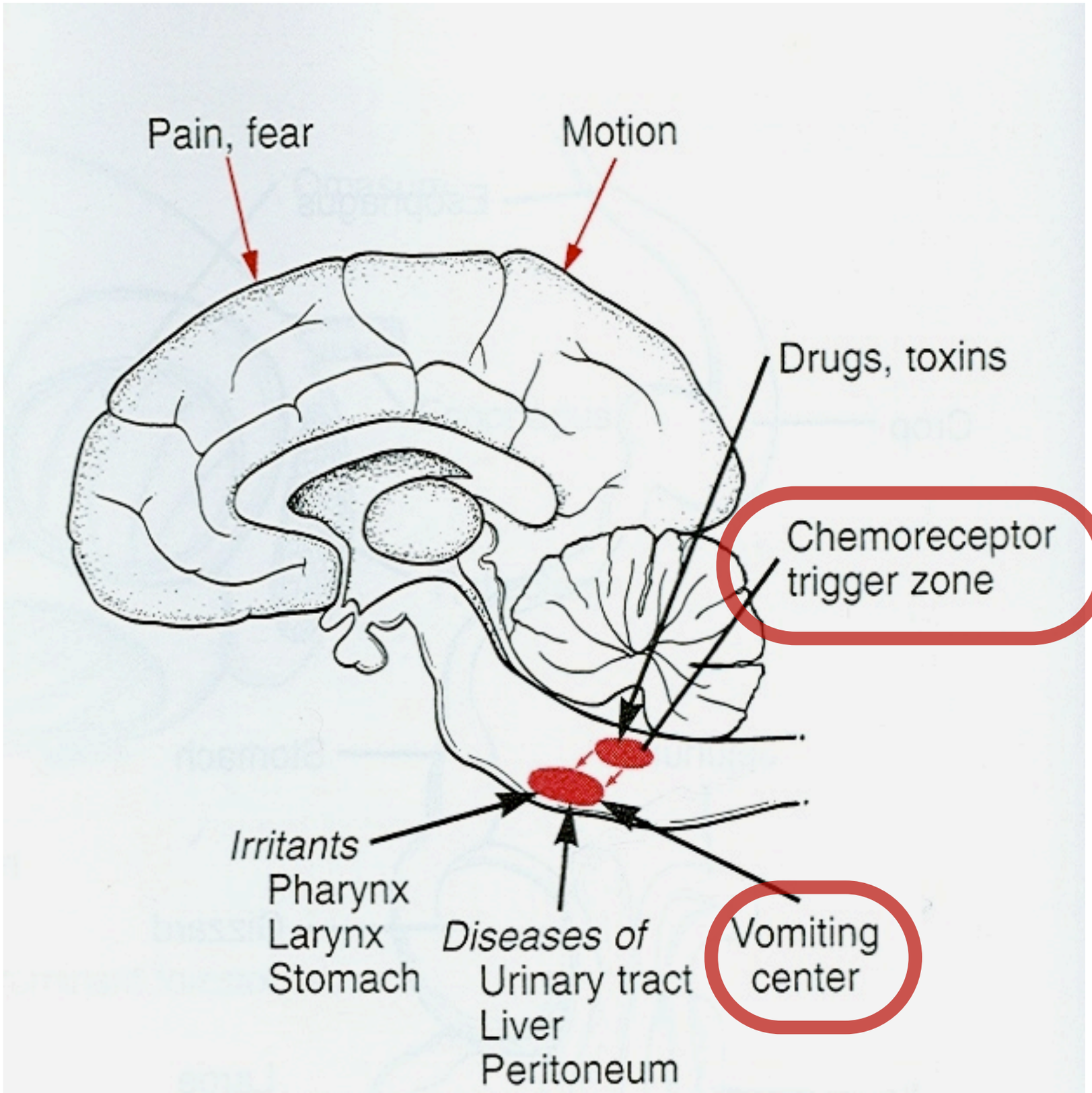
Epileptic seizure activity represents a strong trigger of transcriptional activation of P-glycoprotein



4. Ability to cross barriers: the blood-cerebrospinal barrier



The Chemoreceptor Trigger Zone (CTZ or Area Postrema)



The CTZ lies outside the BBB

Contains receptors for dopamine, serotonin, opioids, acetylcholine and substance P

Stimulation of these receptors activates the Vomiting center, leading to nausea and vomiting

Drug distribution

Four types of patterns:

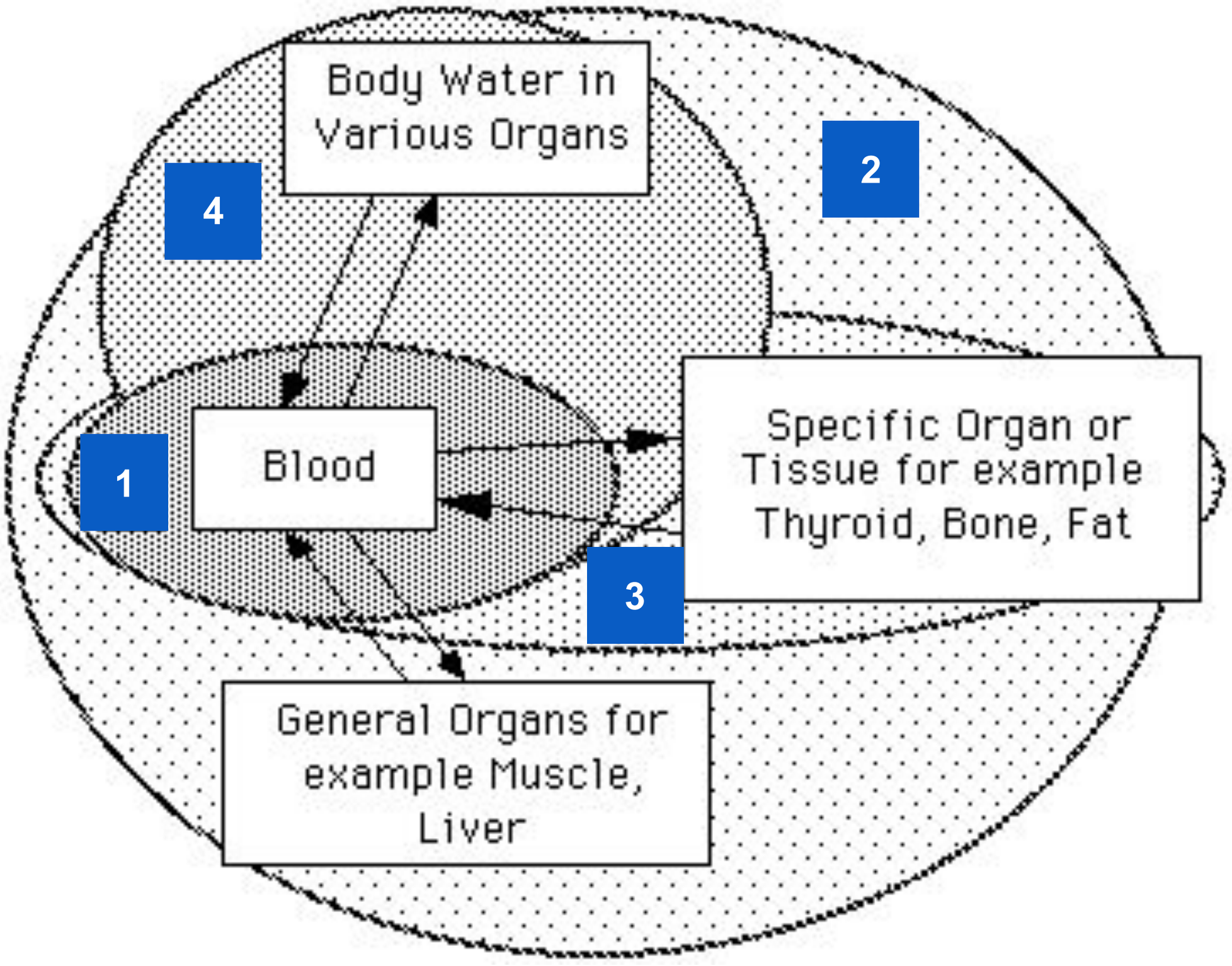
1) Some drugs may remain largely within the vascular system (eparin, drugs strongly bound to plasma protein)

2) Low molecular weight water soluble compounds (ethanol and a few sulfonamides) are uniformly distributed throughout the body water

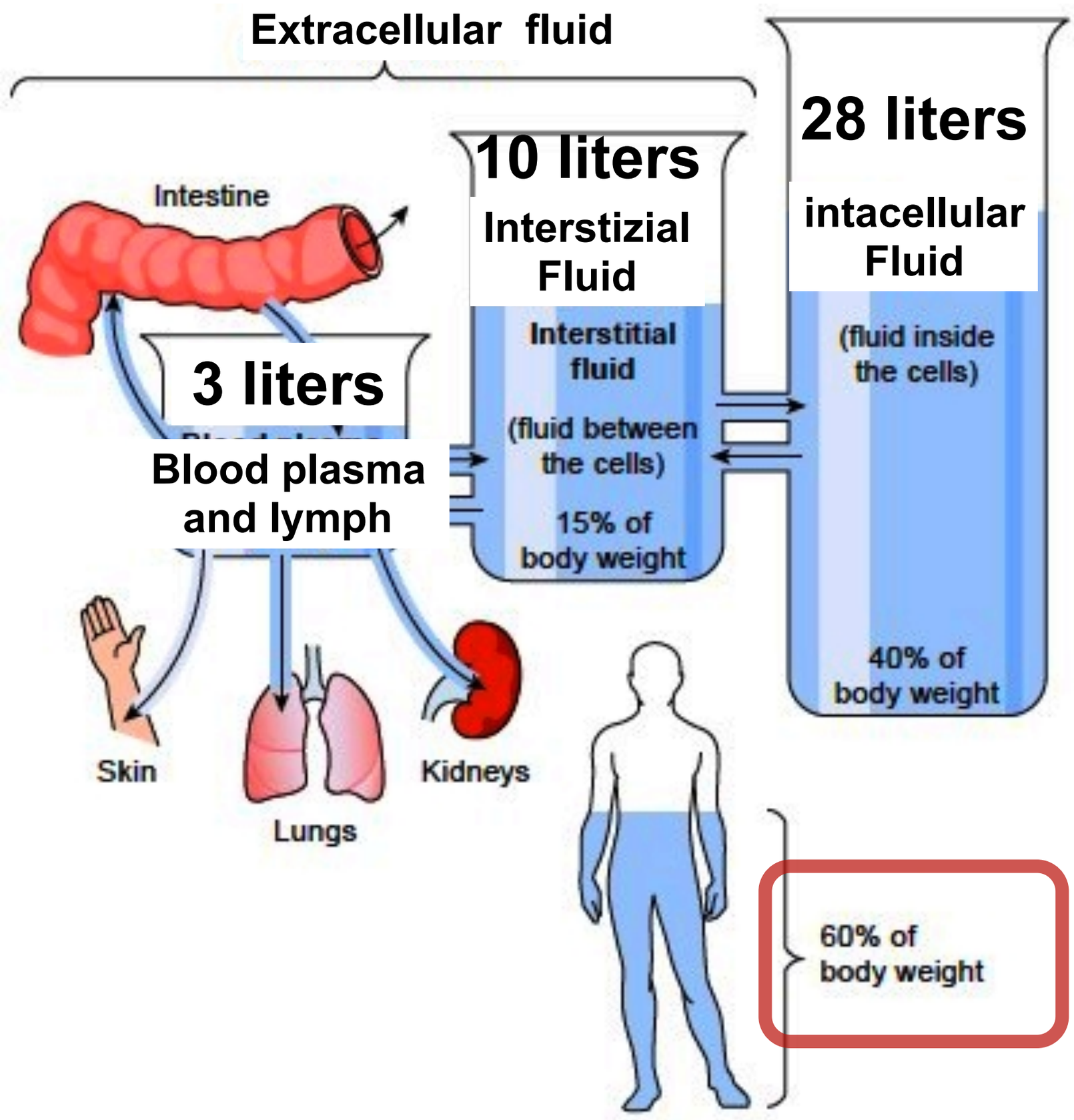
3) A few drugs are concentrated specifically in one or more tissues (iodine in the thyroid gland, chloroquine in the liver, tetracycline in bon and developing teeth, highly lipid soluble compounds in fat tissue)

4) **Most drugs exhibit a non-uniform distribution with variations that are largely determined by the ability to pass through membranes and their lipid/water solubility**

Drug distribution



Water compatments in the body



The Apparent Distribution Volume (Vd)

The Vd is an useful indicator of the type of the distribution pattern that characterizes a drug

Vd is the volume into which a drug *apparently* distributes to achieve a concentration equal to its (measurable) plasma concentration

In other words, Vd describes the relationship between the concentration of the drug in the blood and the amount of the drug in the body

$$Vd = \frac{\text{Amount of drug administered (mg)}}{\text{Drug concentration in plasma (mg/L)}}$$

The Apparent Distribution Volume (Vd)

The Vd is an useful indicator of the type of the distribution pattern that characterizes a drug

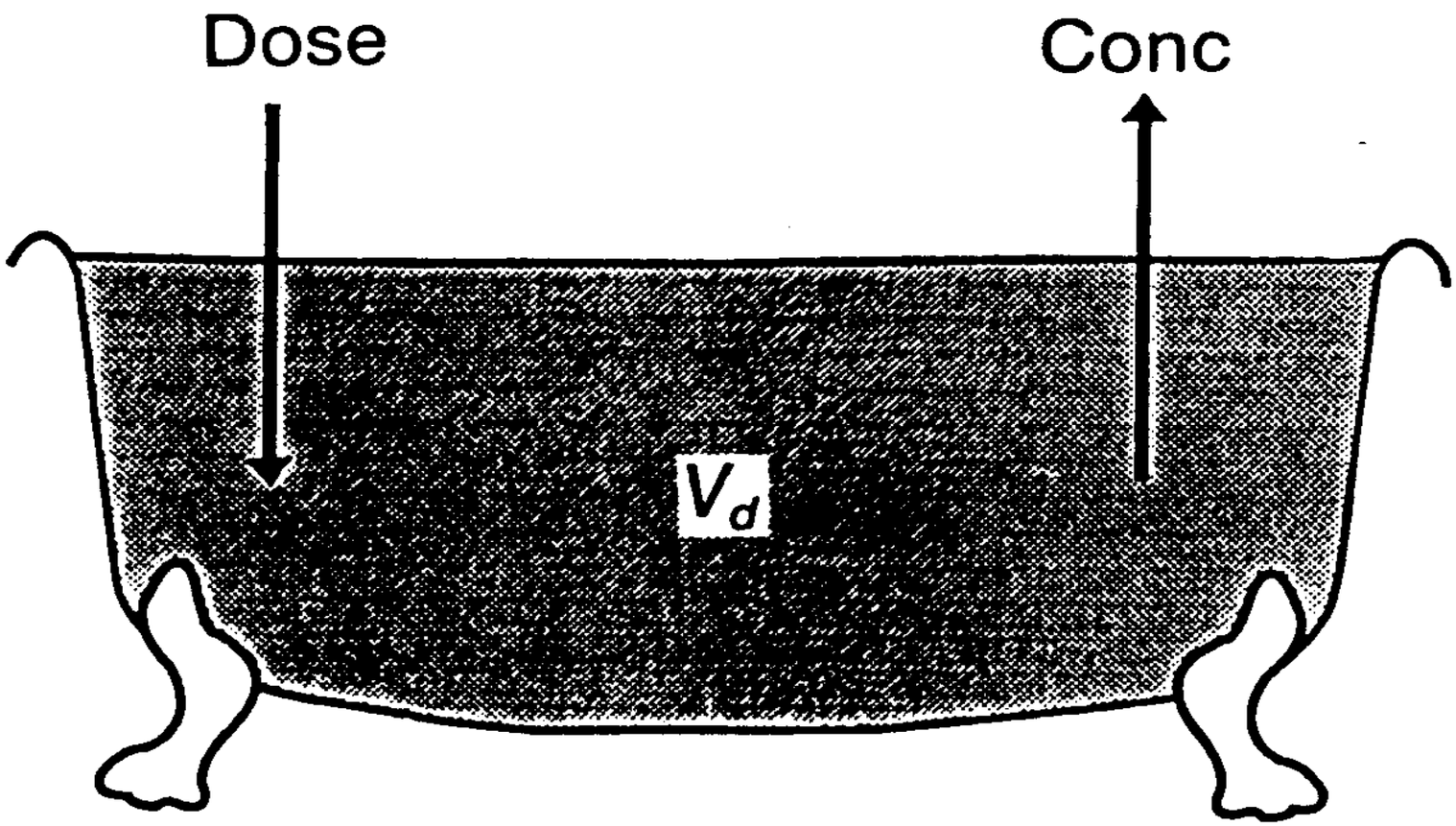
Vd describes the relationship between the concentration of the drug in the blood and the amount of the drug in the body

$$Vd = \frac{\text{Amount of drug administered (mg)}}{\text{Drug concentration in plasma (mg/L)}}$$

By definition, Vd is the volume into which a drug *apparently* distributes to achieve a concentration equal to its (measurable) plasma concentration

The Apparent Distribution Volume (Vd)

The bathtube model

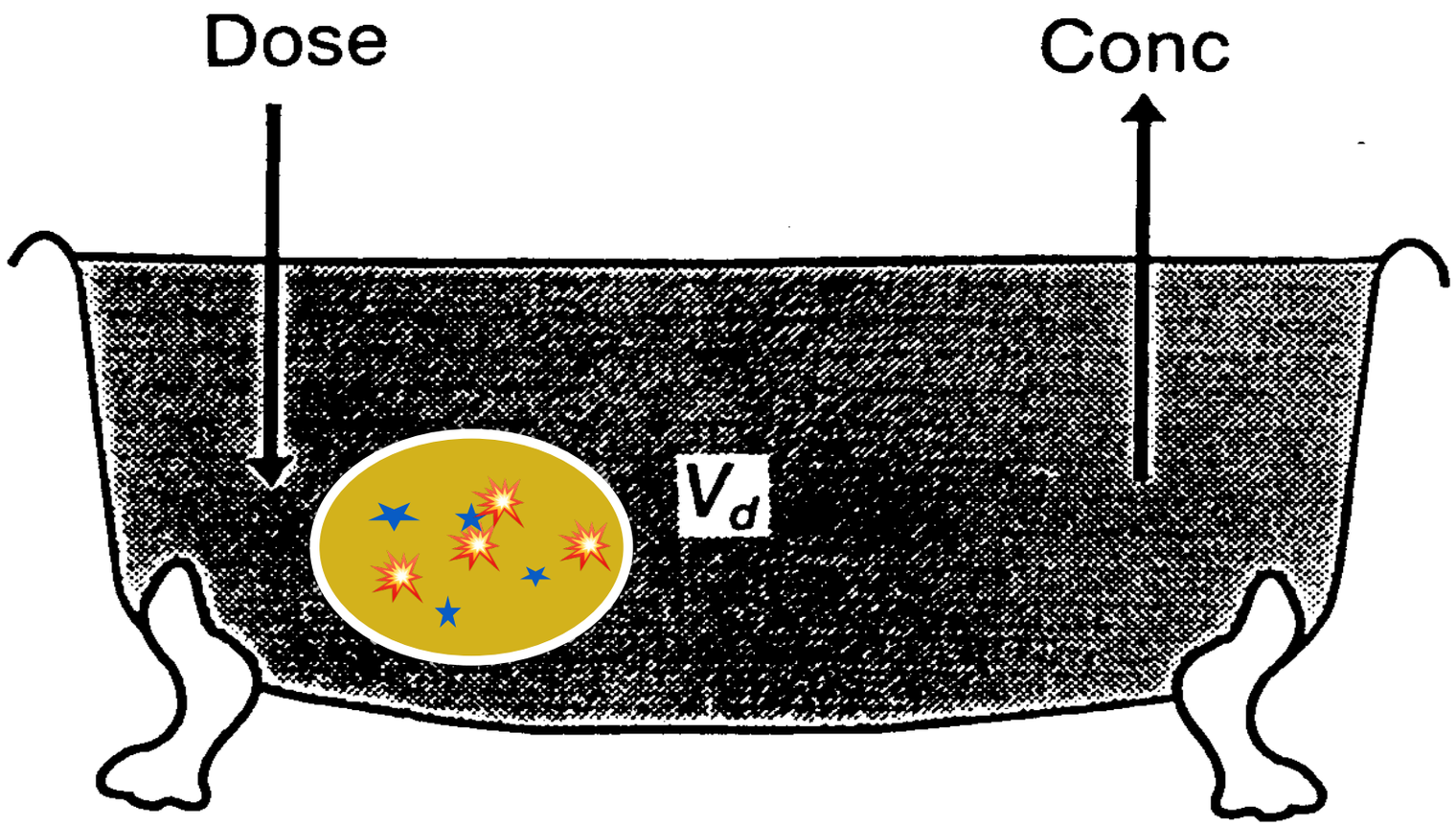


$$Vd = \frac{\text{amount of drug administered}}{\text{drug concentration in plasma}}$$

$$50\ L = \frac{500\ mg}{10\ mg/L}$$

The Apparent Distribution Volume (Vd)

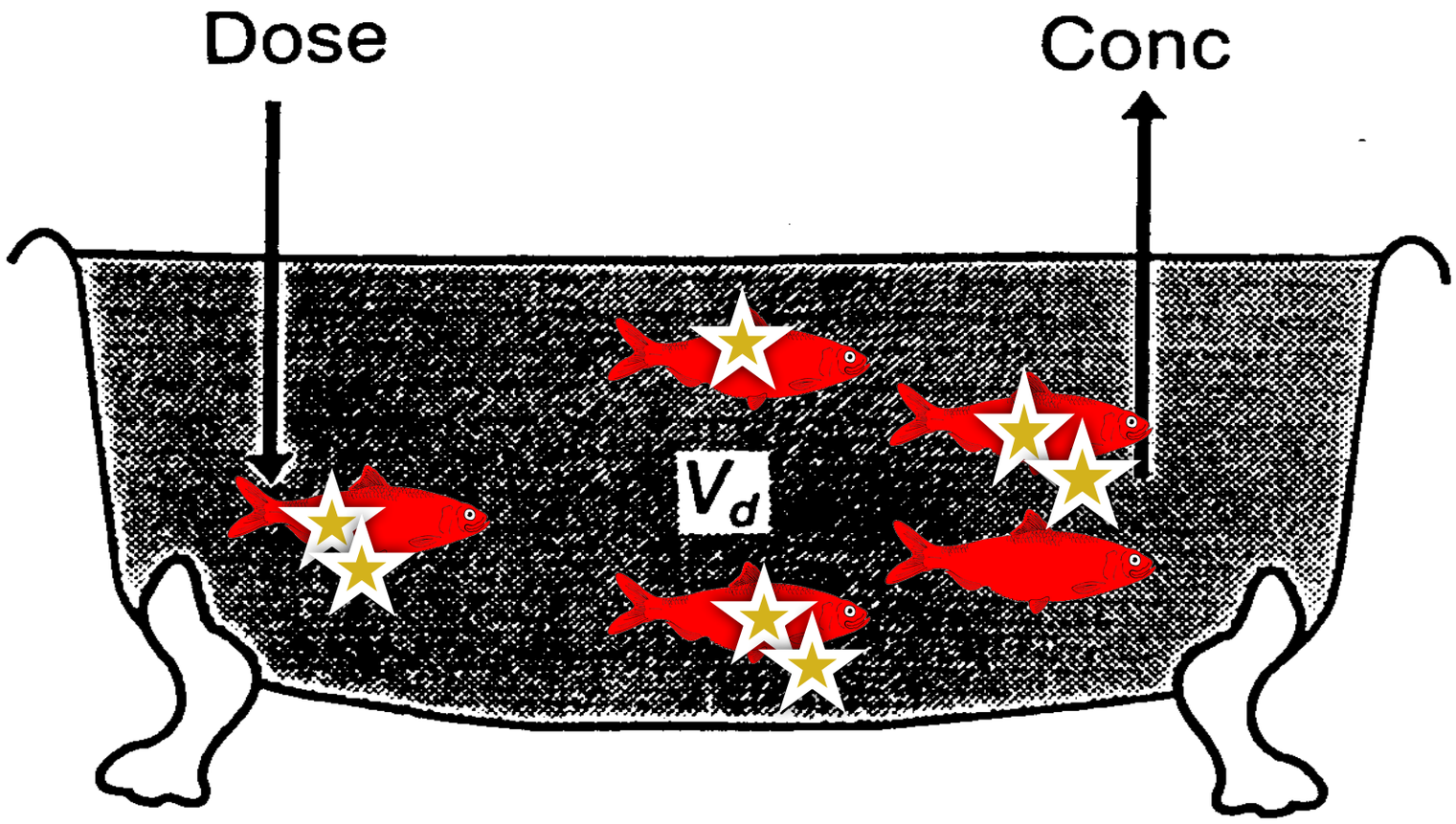
Tissue accumulation: the sponge model



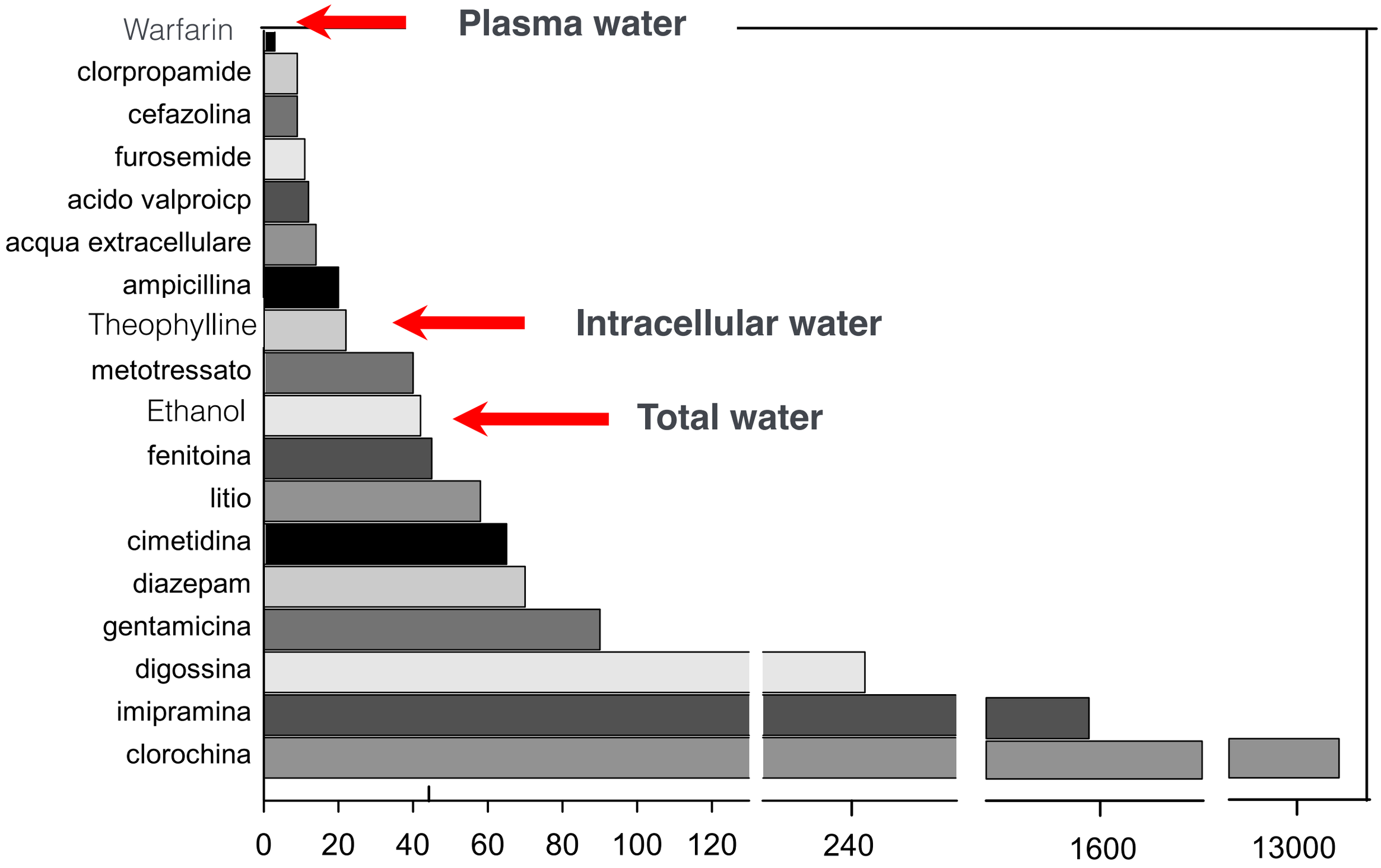
$$Vd = \frac{\text{amount of drug administered}}{\text{drug concentration in plasma}} \qquad 500\ L = \frac{500\ mg}{1\ mg/L}$$

The Apparent Distribution Volume (Vd)

Binding to plasma proteins: the red herring model



$$Vd = \frac{\text{amount of drug administered}}{\text{drug concentration in plasma}} \qquad 5\ L = \frac{500\ mg}{100\ mg/L}$$


Plasma water
Intracellular water
Total water
Warfarin
clorpropamide
cefazolina
furosemide
acido valproicp
acqua extracellulare
ampicillina
Theophylline
metotressato
Ethanol
fenitoina
litio
cimetidina
diazepam
gentamicina
digossina
imipramina
clorochina
0
20
40
60
80
100
120
240
1600
13000
Distribution Volume (L)