# Pharmacokinetics

## Part 3: Drug Excretion (Elimination)

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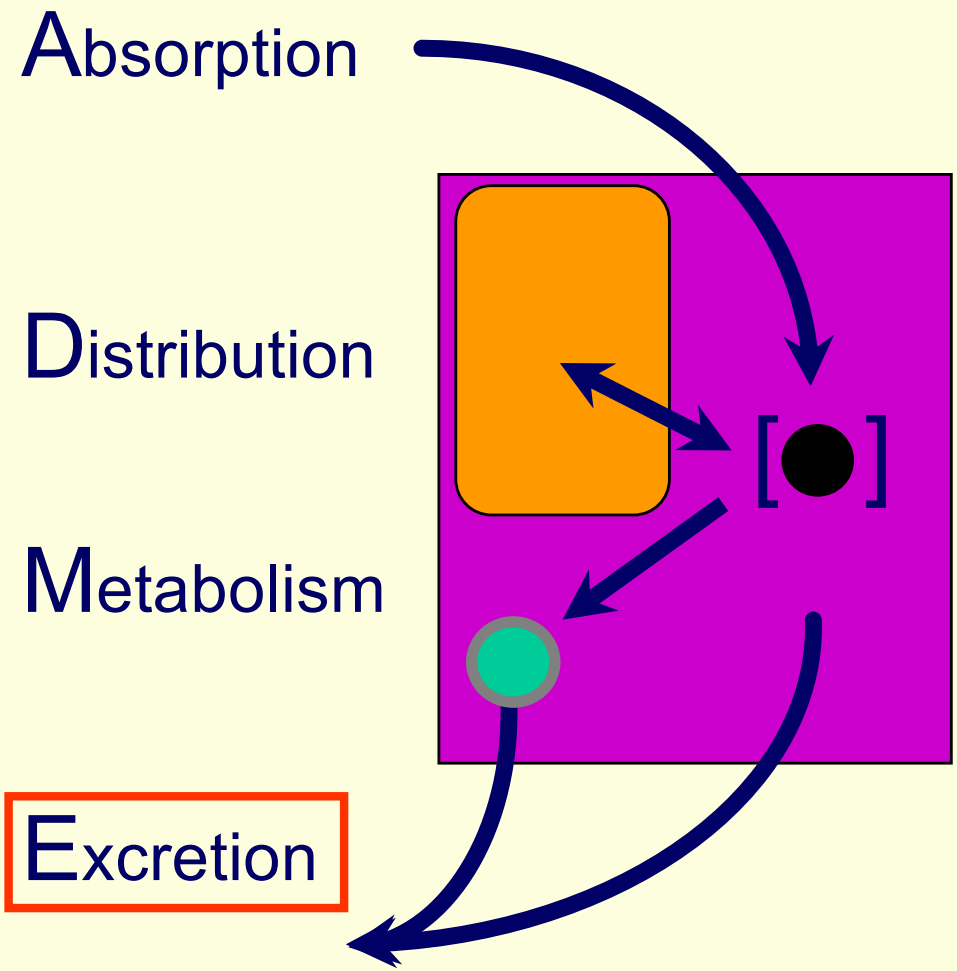
# General Pharmacology

## Pharmacokinetics (ADME)

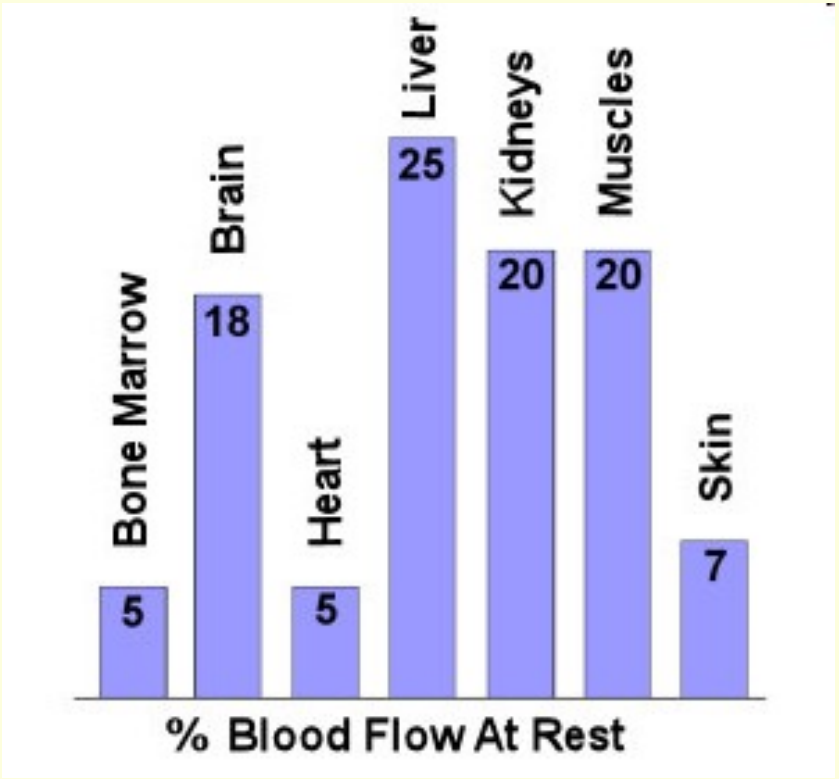


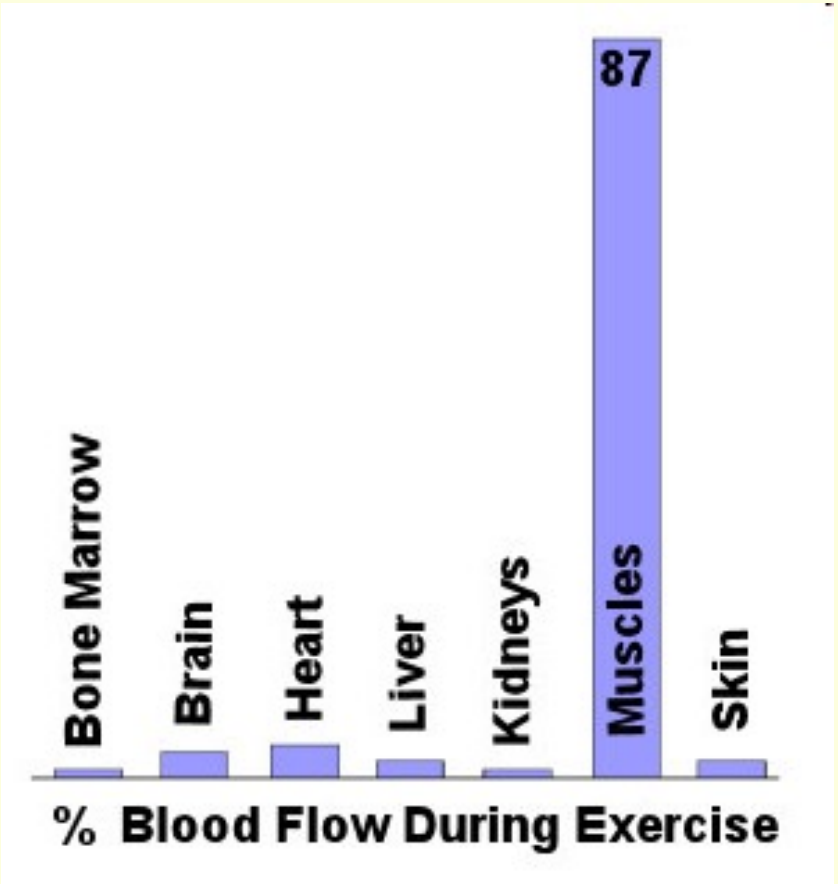

Excretion: The main process that body eliminates "unwanted" substances.

# Pathways of Excretion of Drugs and their Metabolites

- Urine (Kidneys): quantitatively most important route for excretion of hydrophilic substances (or substances that are transformed in hydrophilic substances upon hepatic or GIT metabolism (PhaseI/PhaseII reactions))
- Bile – feces (Liver - Intestine): excretion of all other nonvolatile substances
- Lung: elimination of volatile or gaseous substances
- Sweat, tears, reproductive fluids, milk, other excretory fluids - minor

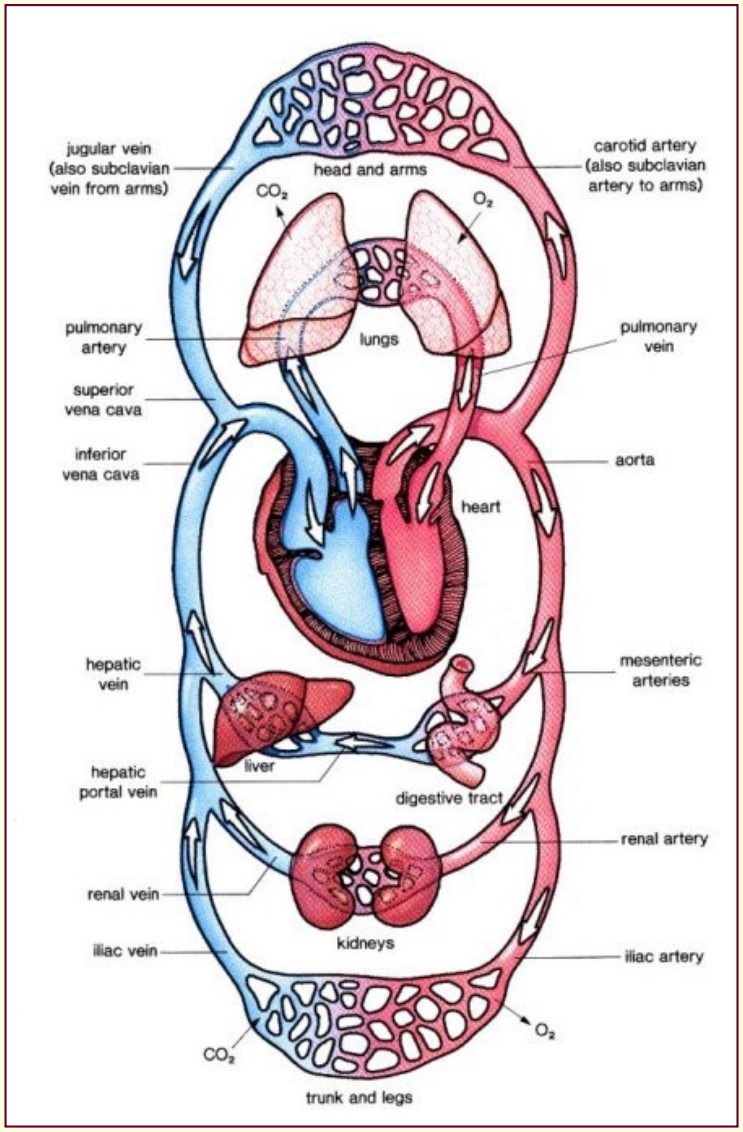
# Renal blood circulation

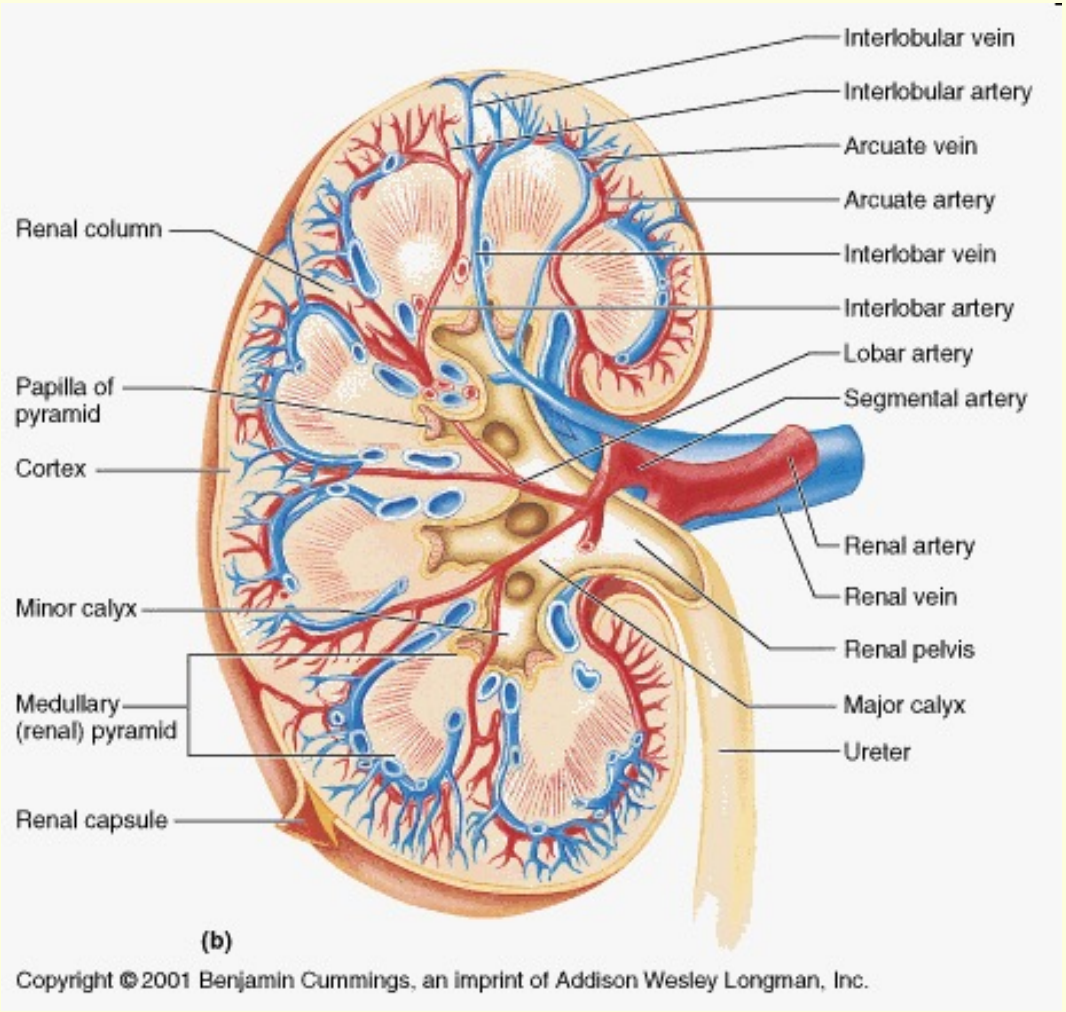


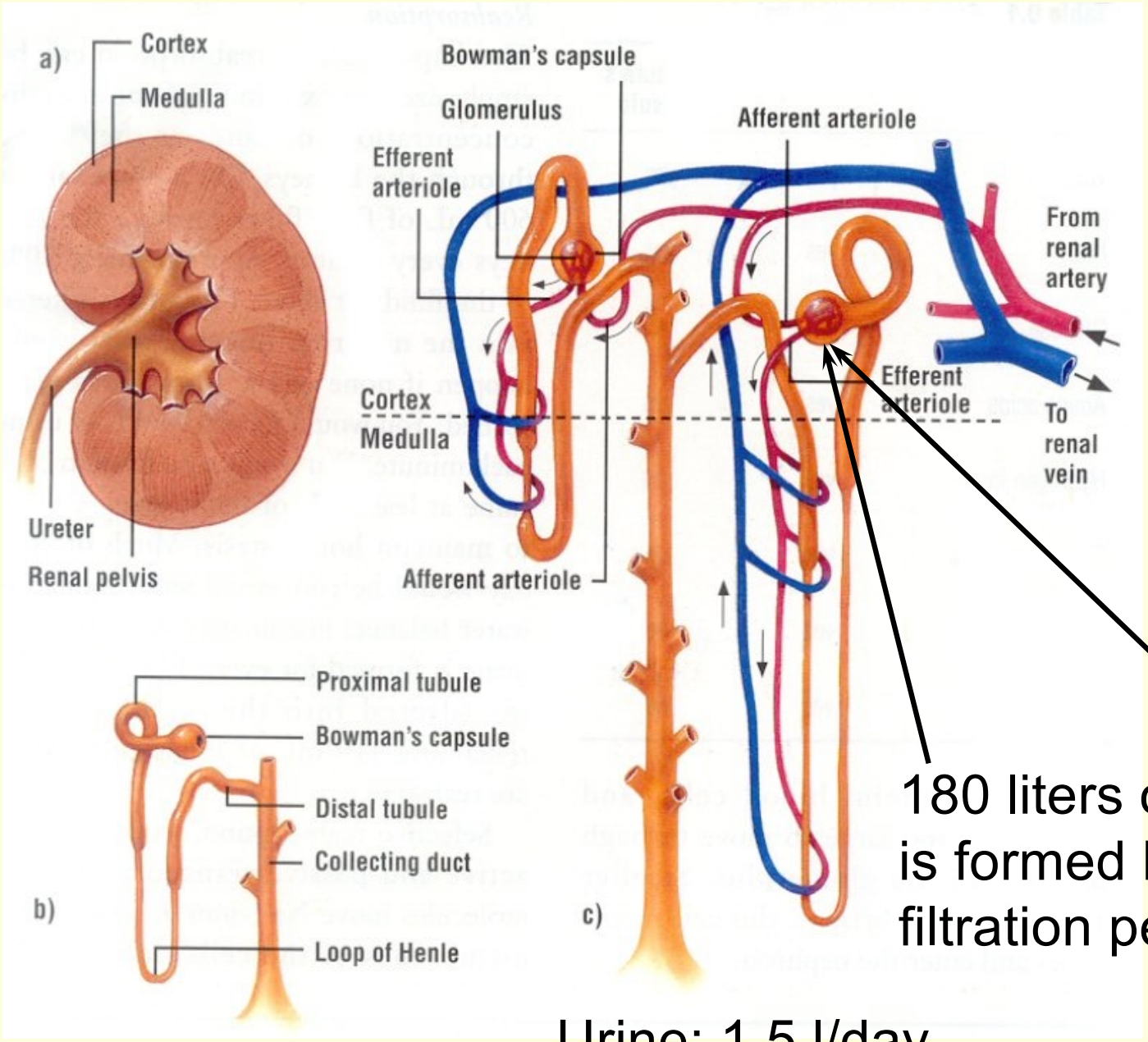

Kidney receives 20% of cardiac output (renal blood flow, RBF)

# Renal blood circulation



# Kidney: structure-function

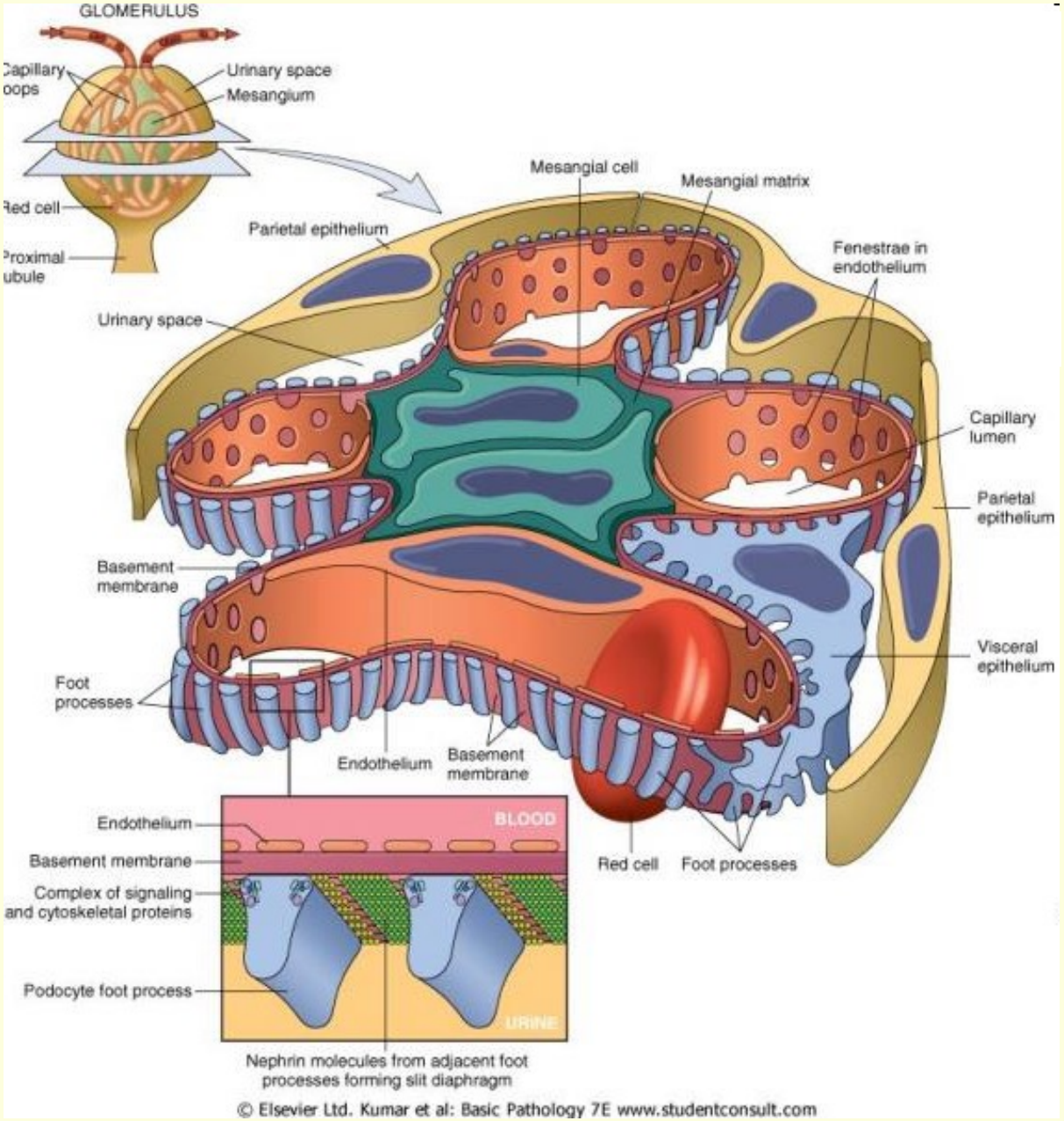



Human kidney is composed of ~500'000 nephrons

180 liters of primary urine is formed by glomerular filtration per day (24 hours)

Urine: 1.5 l/day

# Filtration : the glomerulus




**3 filtration barriers:**

**- fenestrations of endothelium**

**- basement membrane**

**- slits between pedicles of podocytes**

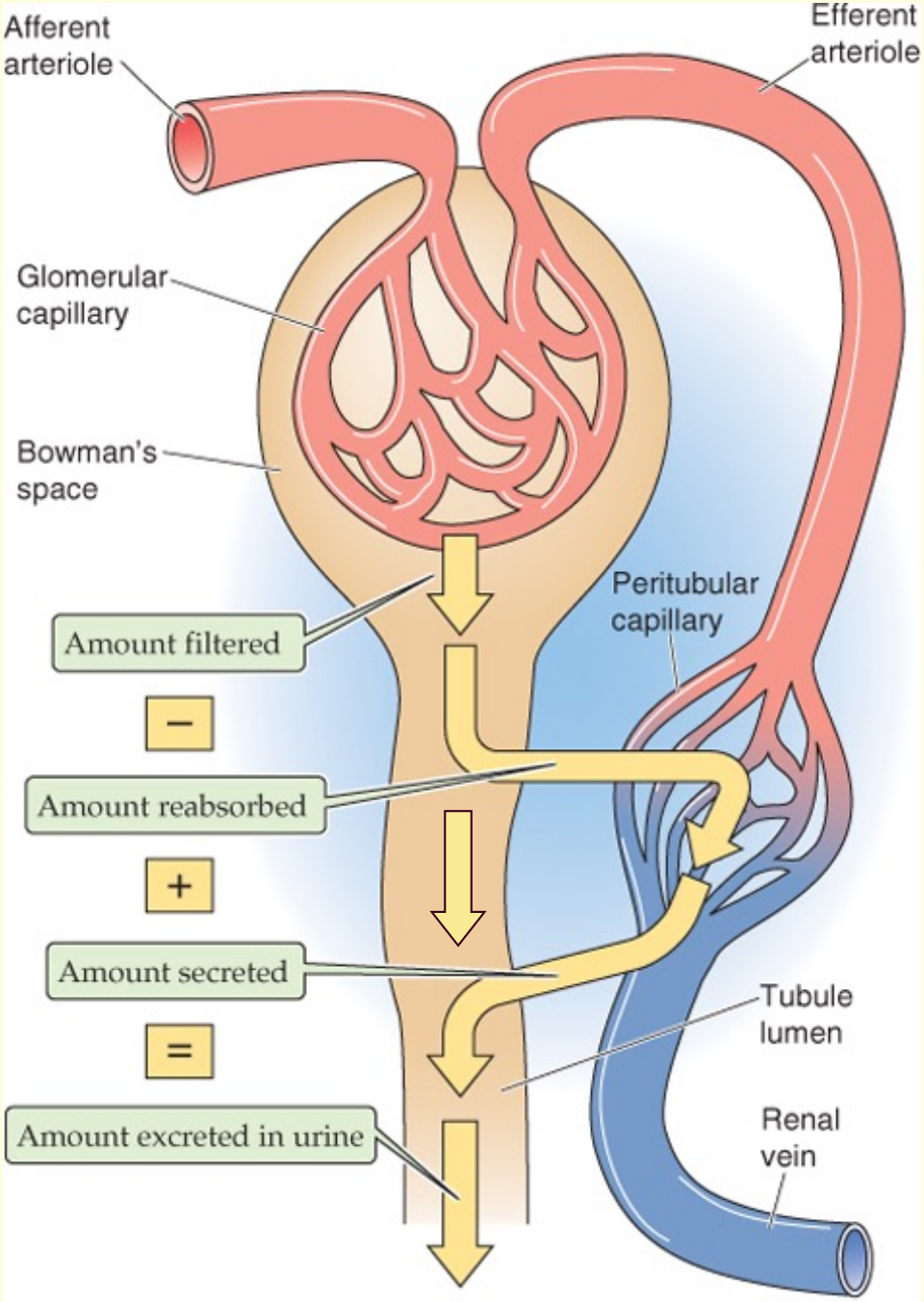
**<u>Excludes drugs bound to proteins!</u>**

**Drug eliminated by the kidney =**

**drug filtered**

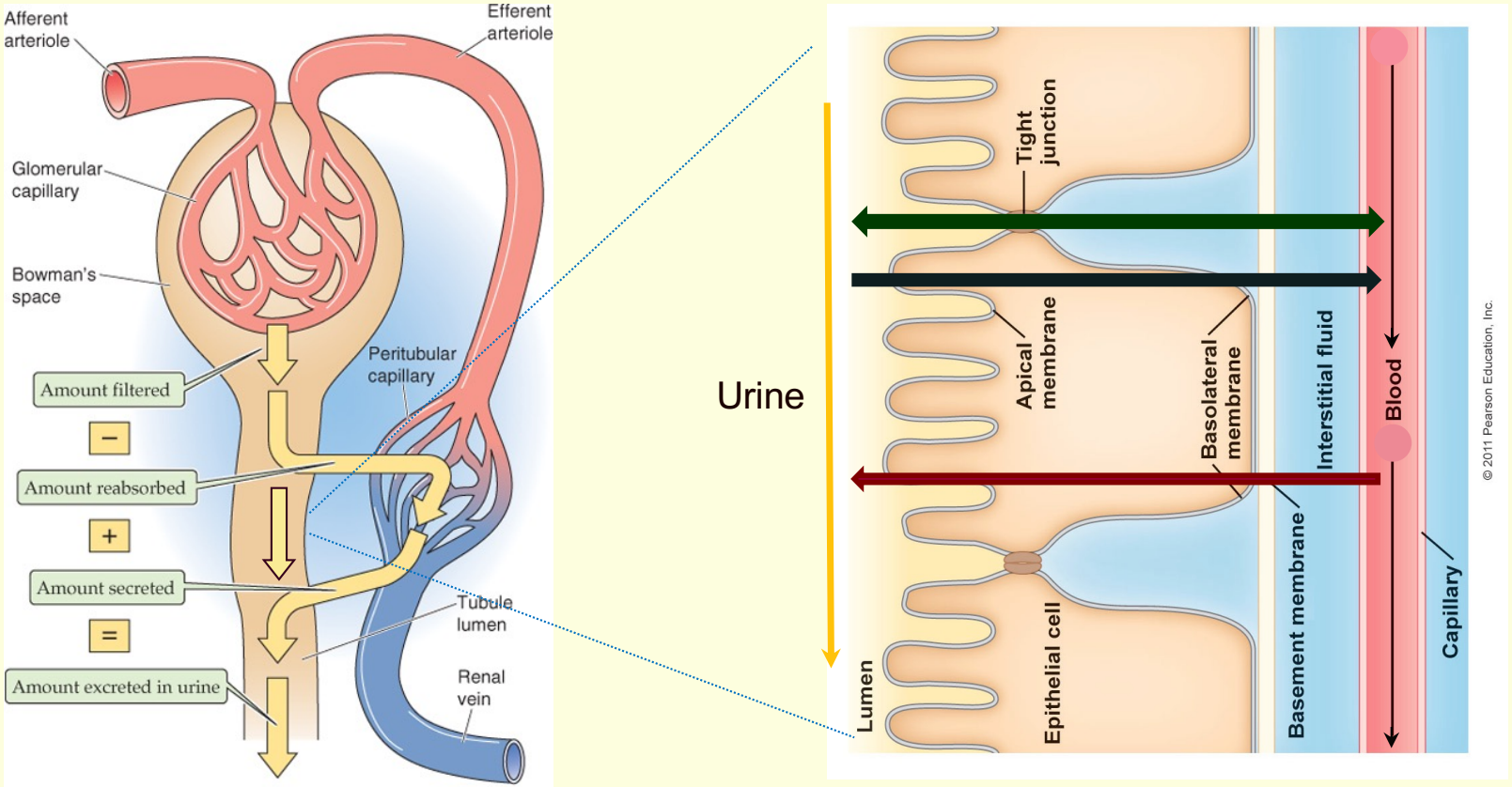
**- drug reabsorbed**

**+ drug secreted**

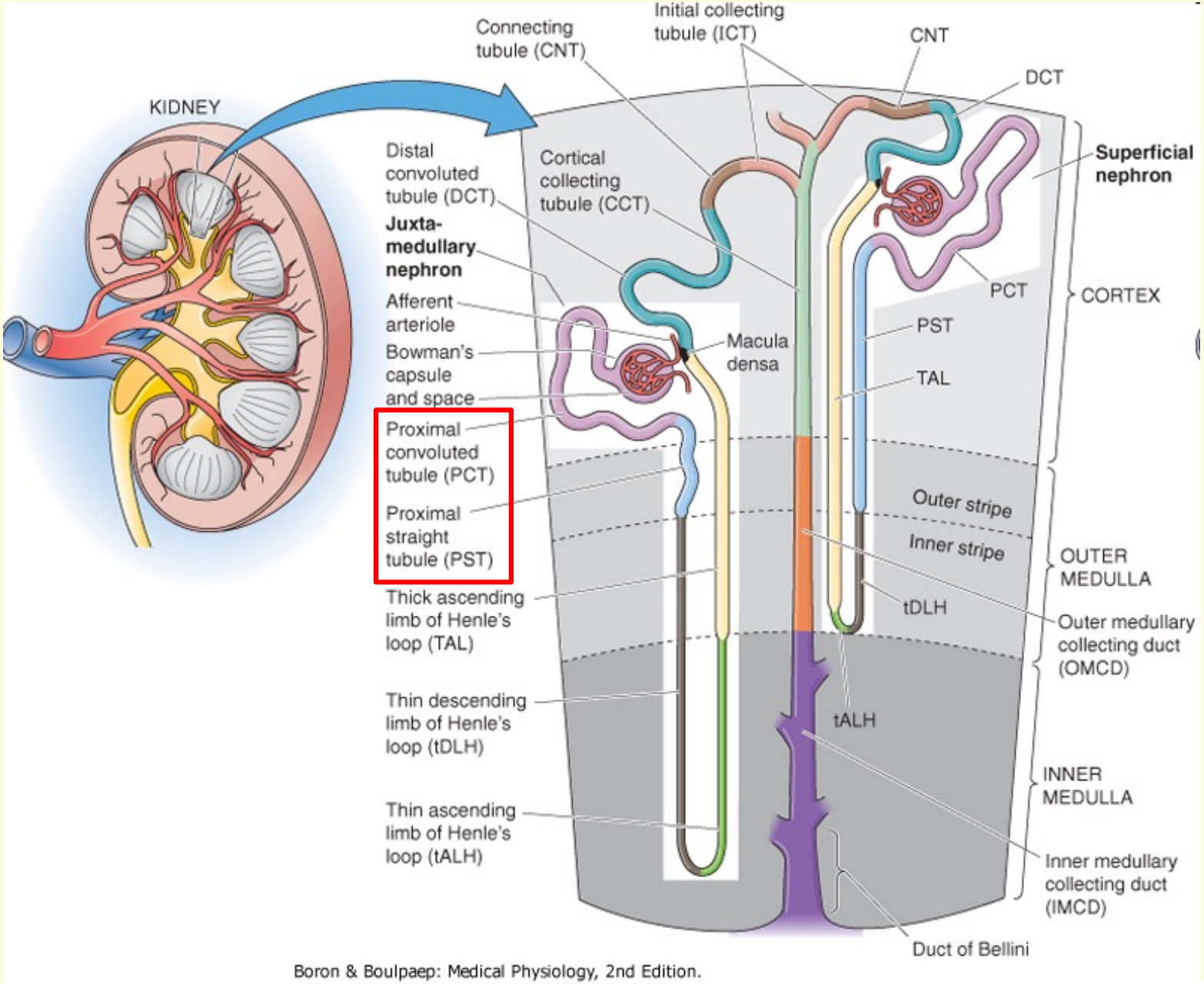


# Transepithelial transport of drugs and drug metabolites

- The reabsorption and secretion processes in the renal tubule (composed of epithelial cells) require passage of drugs and drug metabolites across biological membranes (transcellular) or cell-cell junctions (paracellular).



# Drugs excretion by the Kidney: main role of the proximal tubule

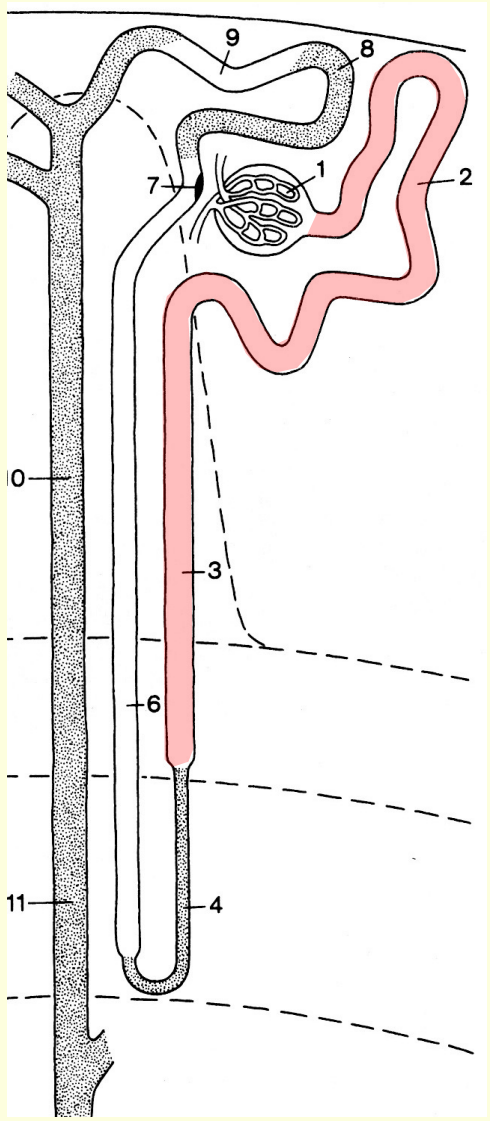



Boron & Boulpaep: Medical Physiology, 2nd Edition.

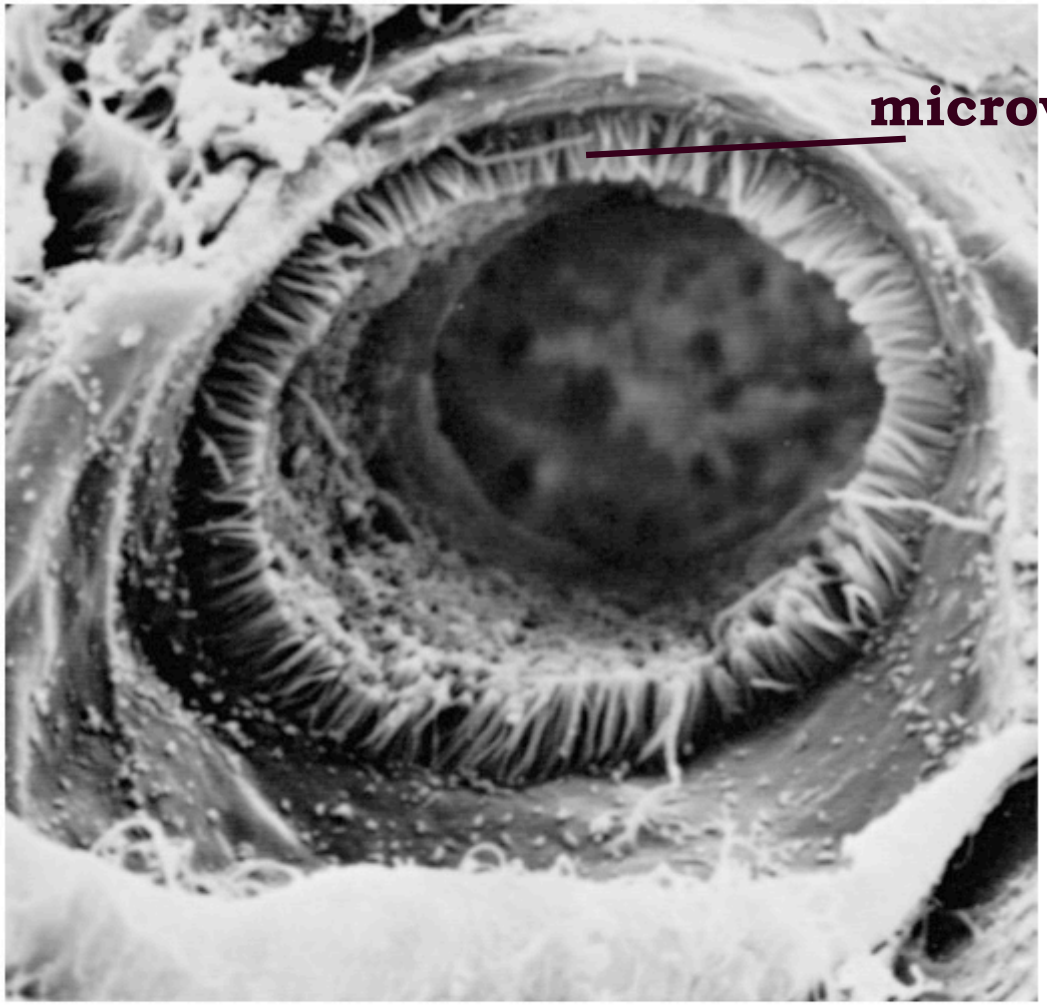
# Proximal tubule: main functions

- <u>reabsorption of</u>
- Na Cl and water, isotonic (~60% of the filtered amount)
- nearly complete reabsorption of glucose, amino acids, filtered peptides,
- bicarbonate, phosphate (~80%)
- some **drugs**

- <u>secretion of</u>
- protons
- numerous organic compounds (organic anions and cations)
- **many xenobiotics (drugs)**



# Proximal tubule – high reabsorption surface

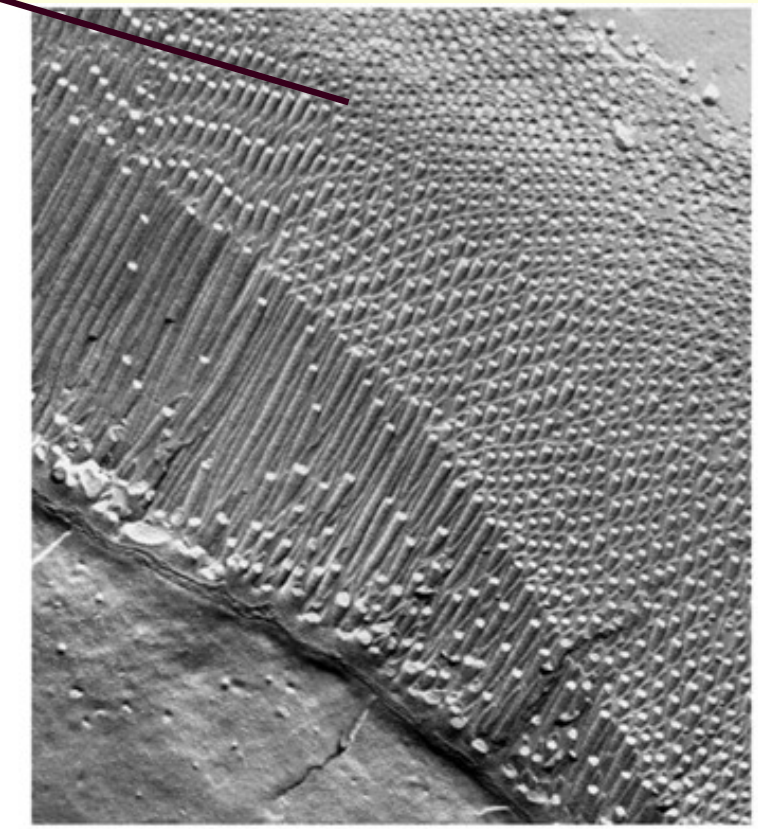


Scanning electron micrograph depicting the transition from the parietal epithelial cells of Bowman's capsule (*foreground*) to the proximal tubule cells, with their well-developed brush border, in the kidney of a rat. (Magnification, x3200.)



**microvilli**

**Brush border of proximal tubule**



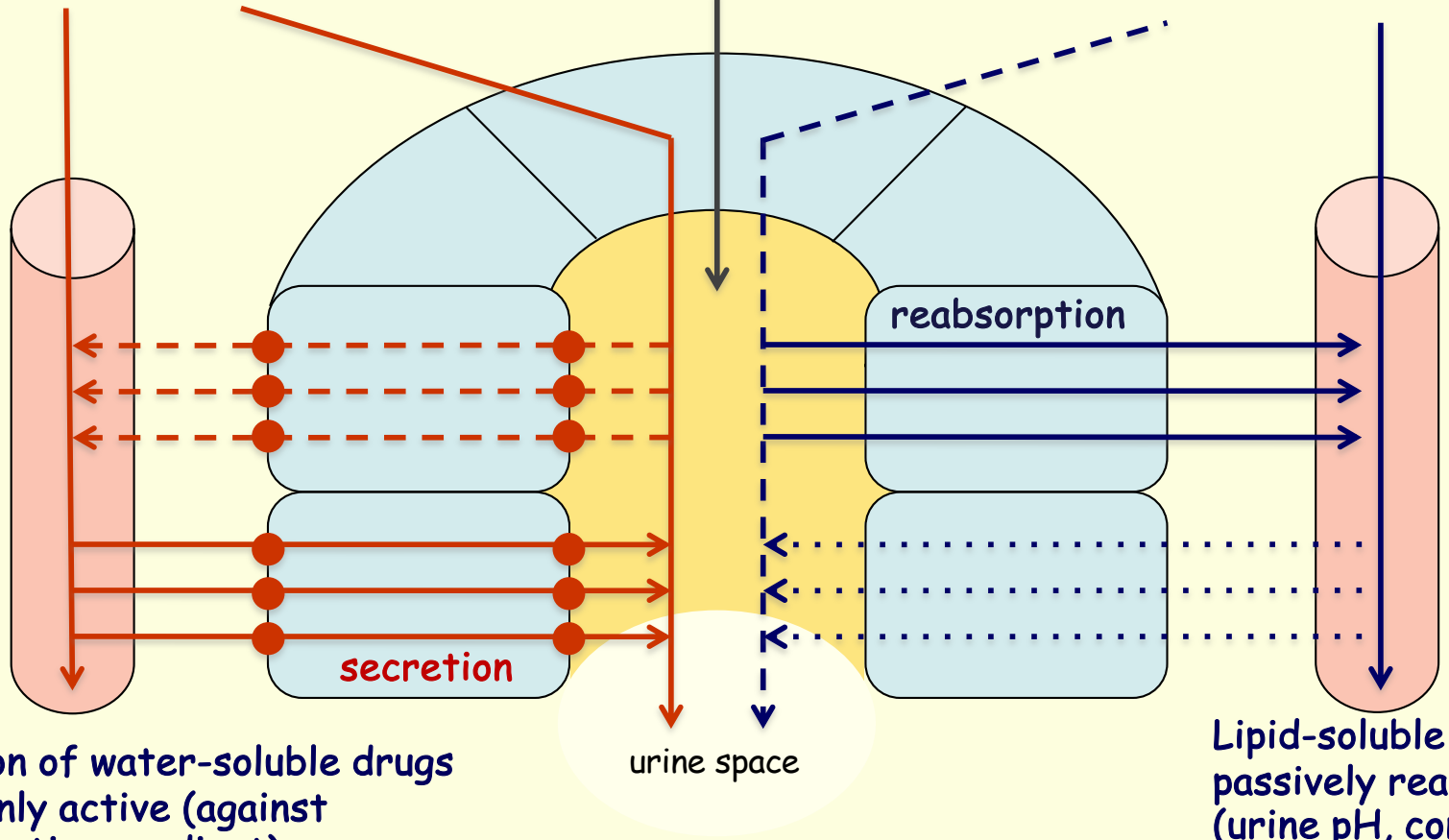
Nature Reviews | Molecular Cell Biology

# Drugs reabsorption/secretion along the nephron: facilitated vs passive diffusion

Water-soluble drugs
(or their metaboilites)

Glomerular filtrate

Lipid-soluble drugs
(partially protein-bound)



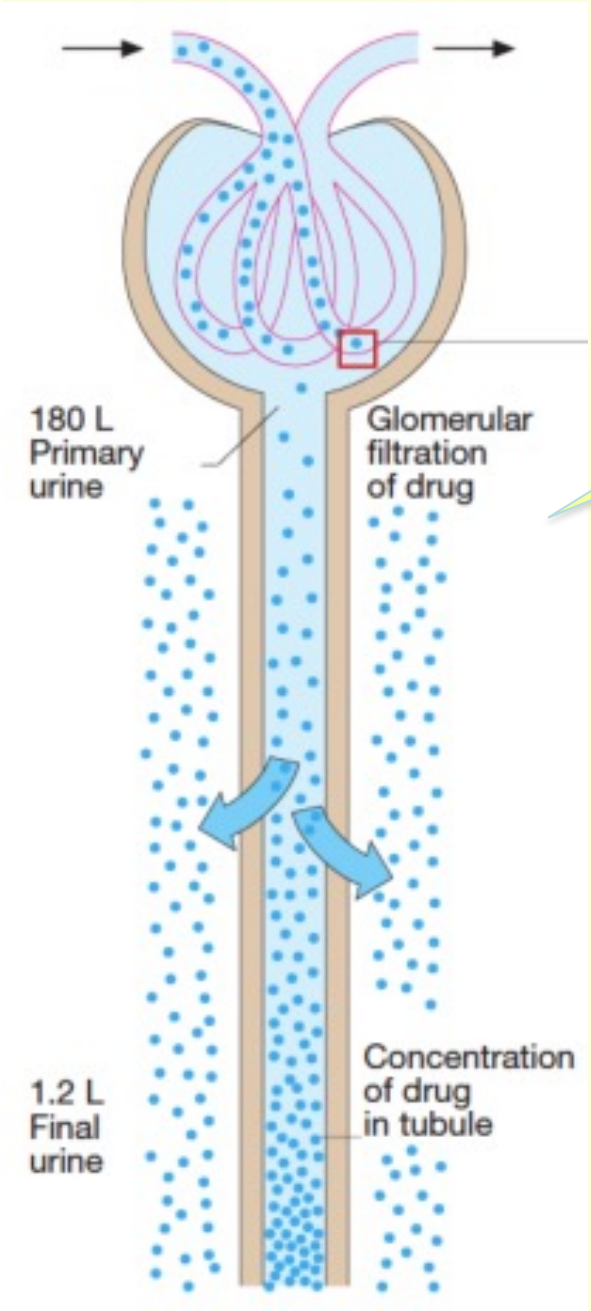

Secretion of water-soluble drugs can be only active (against concentration gradient)

Some water-soluble drugs can be partially reabsorbed (rare)

Lipid-soluble drugs are passively reabsorbed (urine pH, concentration gradient)

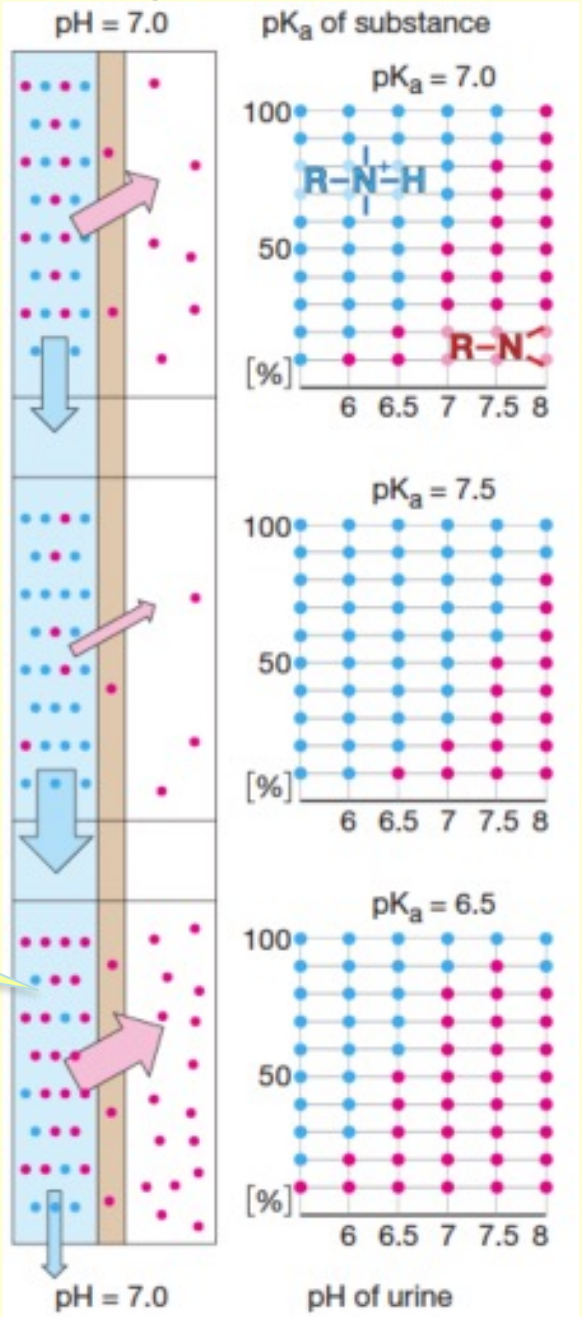
Secretion is rare (against concentration gradient, urine pH)

# Major factors influencing drugs elimination by the kidney

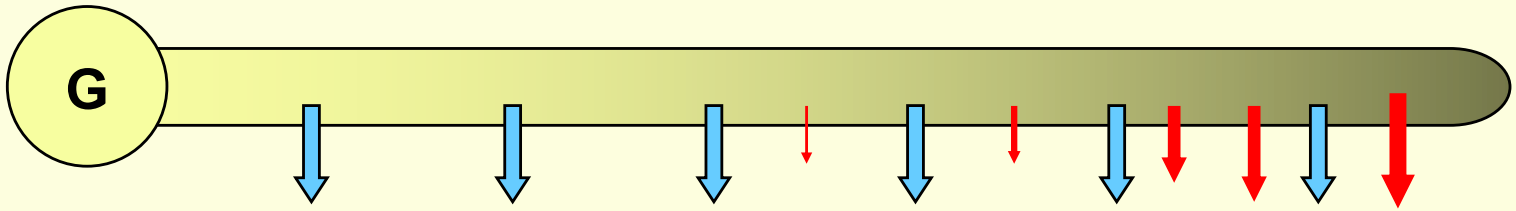



**Urine concentration along the nephron**

**Drug ionization influence of urine pH**



# Nonfacilitated tubular reabsorption

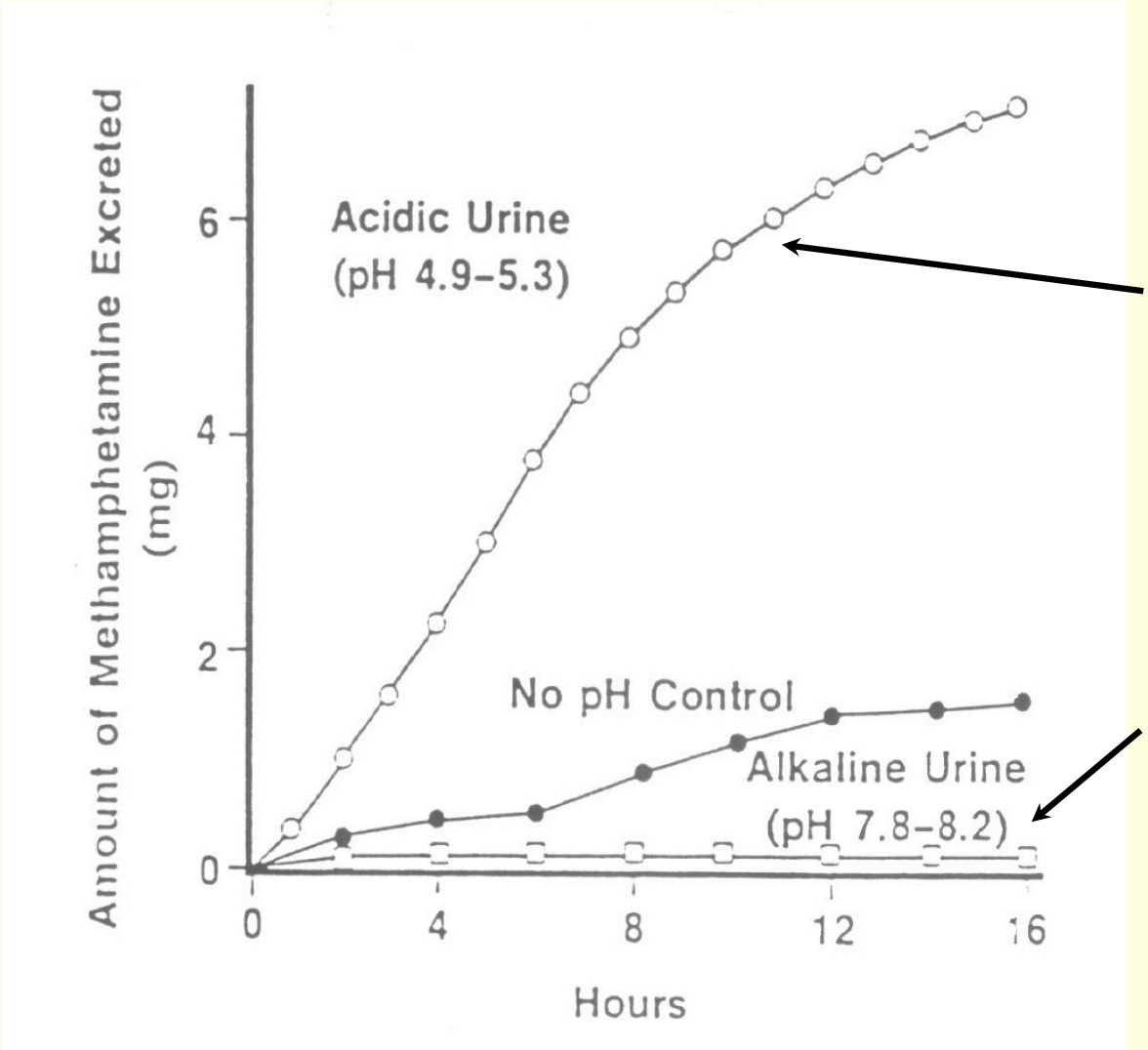



Nonfacilitated tubular reabsorption (diffusion) is a passive process driven by the concentration gradient between tubular lumen and intracellular compartment. This gradient results from water reabsorption along the nephron:

- depends on membrane permeability for the substance
- very efficient for lipophilic compounds with low molecular weight
- depends on urine flow (water reabsorption)
- for weak bases or acids depends on nonionic fraction (urinary pH)

# Tubular reabsorption – nonfacilitated diffusion, effect of urinary pH

- for weak acids: alkalinization of urine = more rapid excretion
- for weak bases: acidification of urine = more rapid excretion




Example: methamphetamine (weak base, pKa = 10.1)

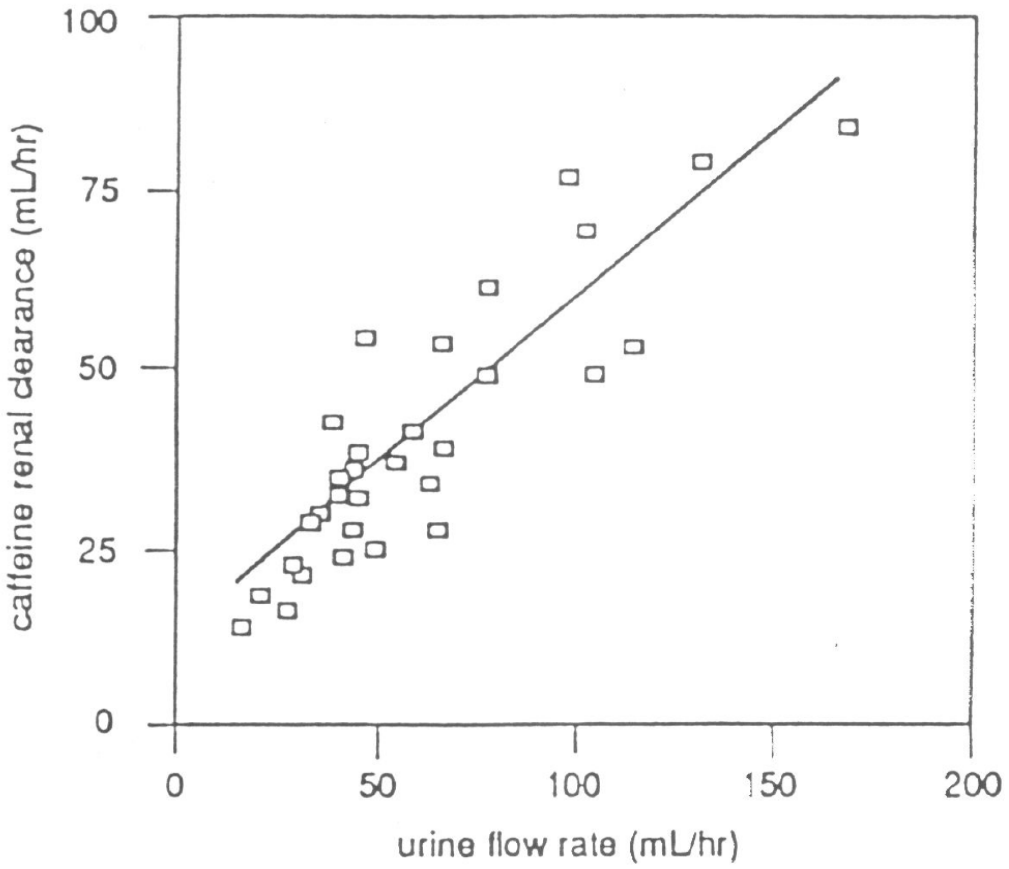
Treatment with ammonium chloride

($NH_3 + HCl \rightarrow NH_4Cl$) to make urine acidic, to increase excretion of amphetamine in overdose

Taking baking soda ($NaHCO_3$) to make the urine more alkaline, to decrease excretion of amphetamine & prolong the effect ??

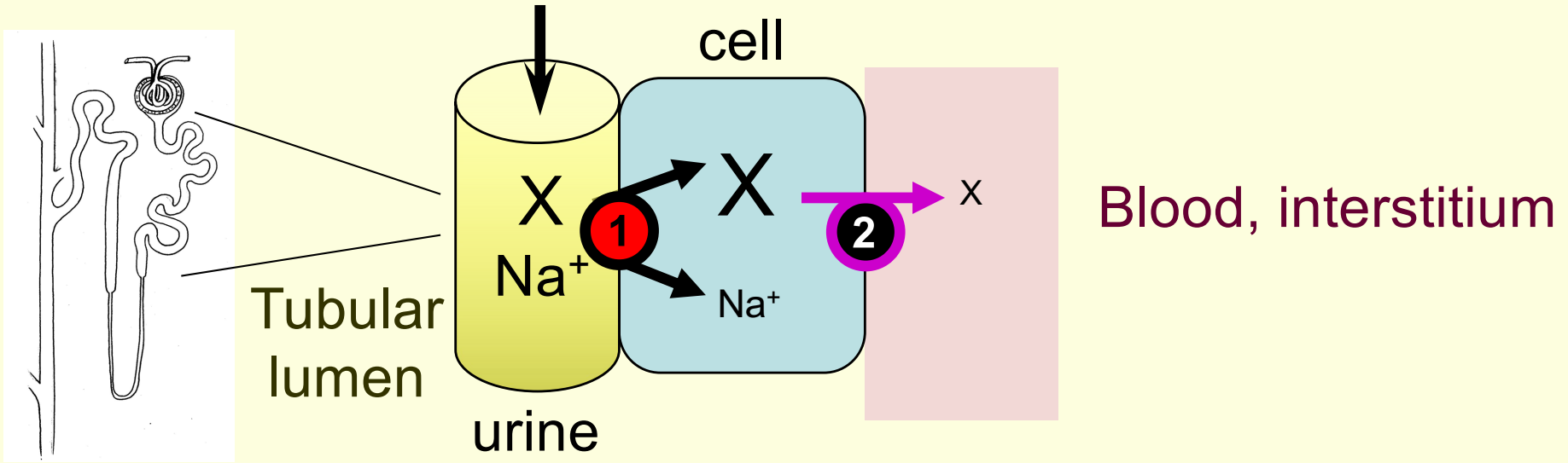
# Tubular reabsorption – nonfacilitated diffusion, influence of urine flow rate

- high urinary flow rate decreases drug concentration in the urine and, thus, decreases concentration gradient between tubular lumen and cytosolic space of tubular cells.




Example: effect of urine flow rate on caffeine clearance

# Facilitated tubular reabsorption



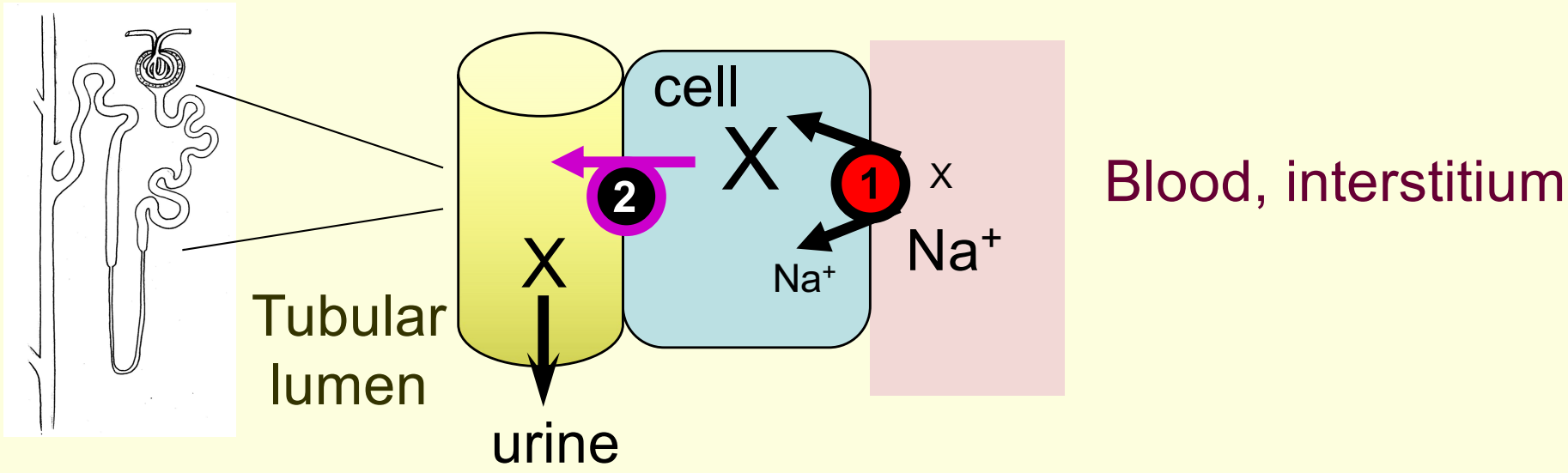

• Some ionized water-soluble drugs can be reabsorbed along the nephron (rare). In this case reabsorption is a two-step process:

1 – transport of substance from the lumen (primary urine) into the cell via the apical transport system (mainly co-transporters or exchangers)

2 – transport out of the cell into the interstitium and general circulation via the basolateral transport system

• Transport systems – thus, possibility of saturation, inhibition or competition

# Renal excretion of drugs and drug metabolites: facilitated tubular secretion of water-soluble ionized drugs




- A great number of substances are actively secreted in the proximal tubule. Renal secretion is a two-step process

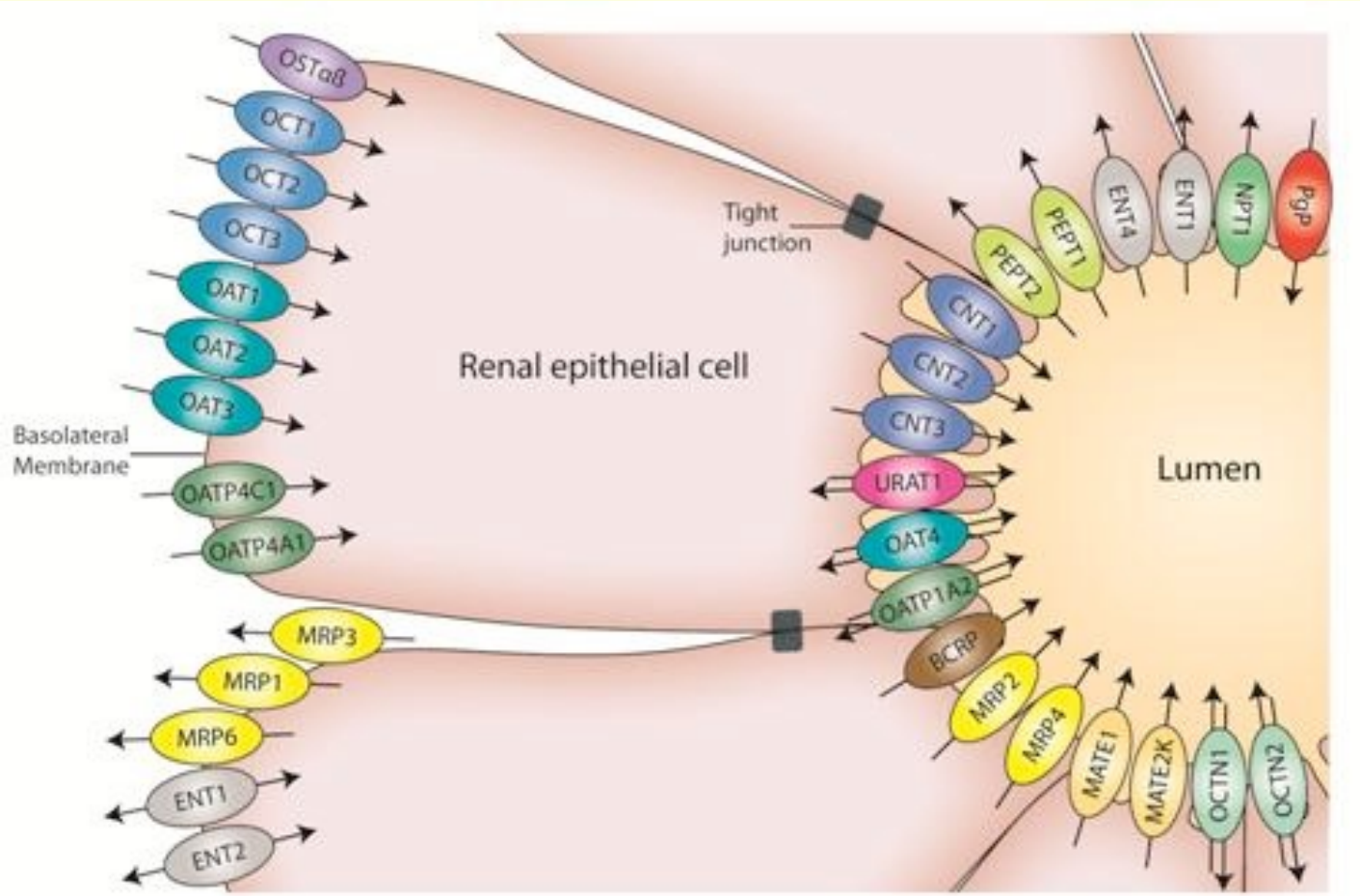
1 – absorption of substance into the cell via the basolateral transport system

2 – extrusion out of the cell into the tubular lumen via the apical transport system

# Facilitated tubular secretion and reabsorption: transporter systems

transport systems: exchangers, co-transporters, ABC proteins,

thus possibility of saturation, inhibition or competition, energy dependency, accepts drugs bound to proteins



# Factors influencing renal drug excretion

- **Gender**

  **female 80% renal function of males**

- **Age**

  **renal function decreases by 50% with age**

- **Pregnancy**

  **complex**

- **Disease**

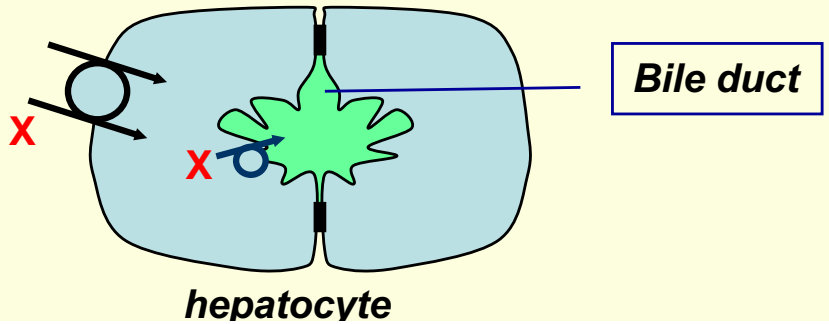
  **renal disease, heart failure**

# Excretion in bile

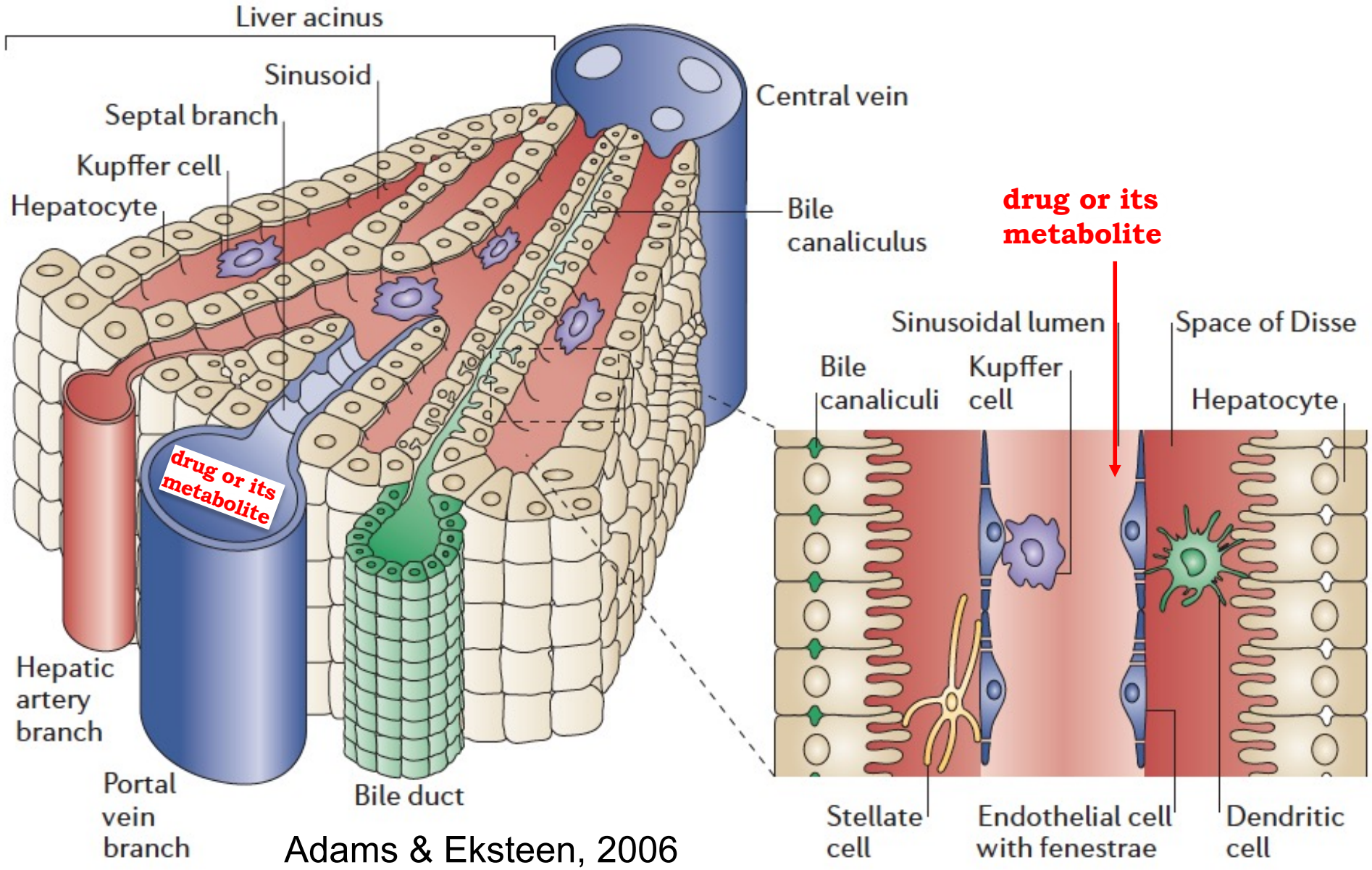
- two-step process: accumulation into hepatocytes via passive diffusion or via basolateral transport system (mainly SLC transporters, co-transport with sodium) and transport out of the cell into the bile duct and then in the intestine via the apical transport system (mainly ABC transporters)
- drugs excreted in bile are usually highly polar, ionized, with medium to high molecular weight (MW>300)

examples: digoxin/digitoxin - 10% i.v. dose in feces;

acebutolol - 6%

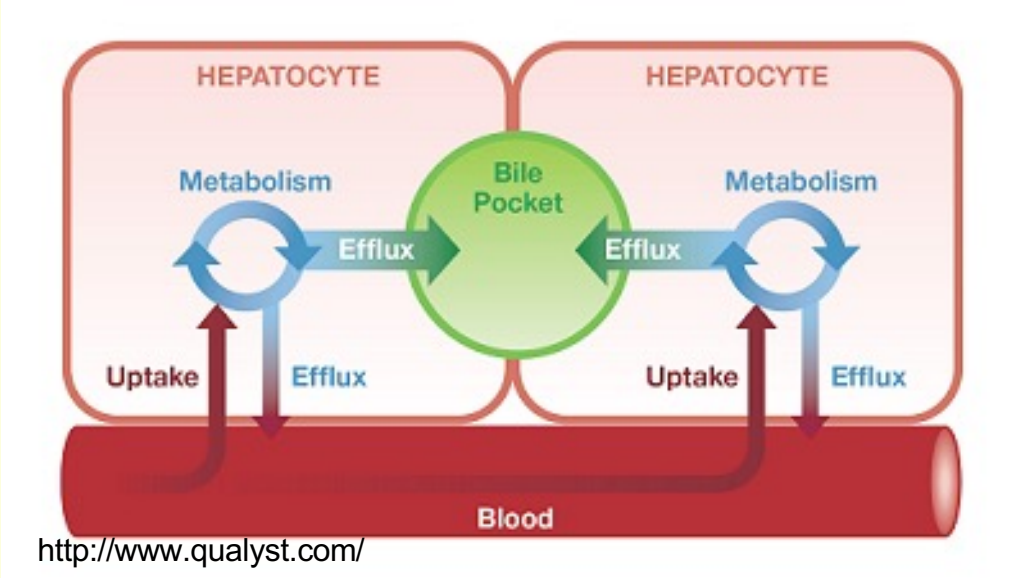


# The fate of drugs in the liver: a reminder



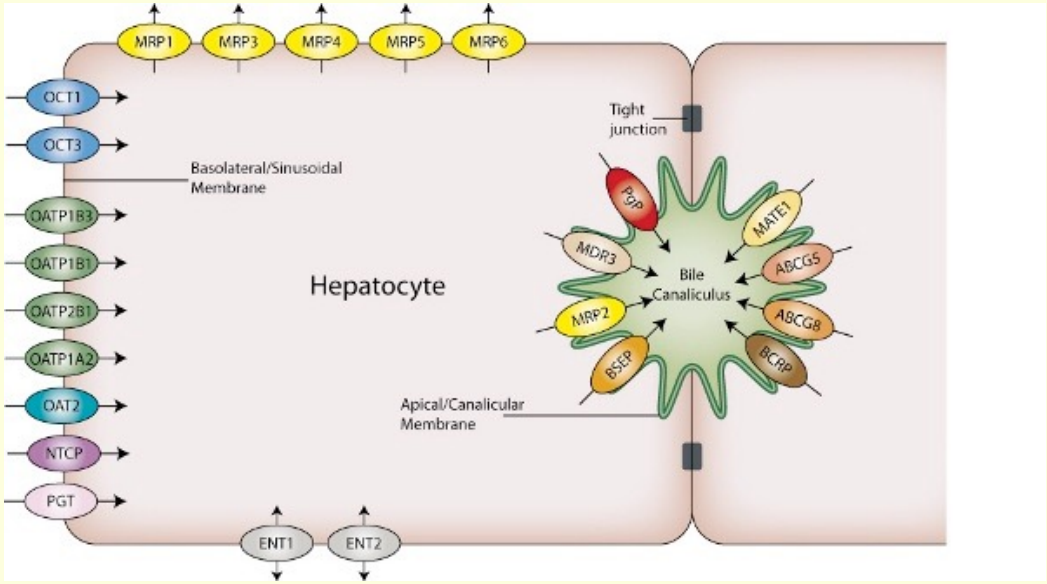

Adams & Eksteen, 2006

# Excretion in bile



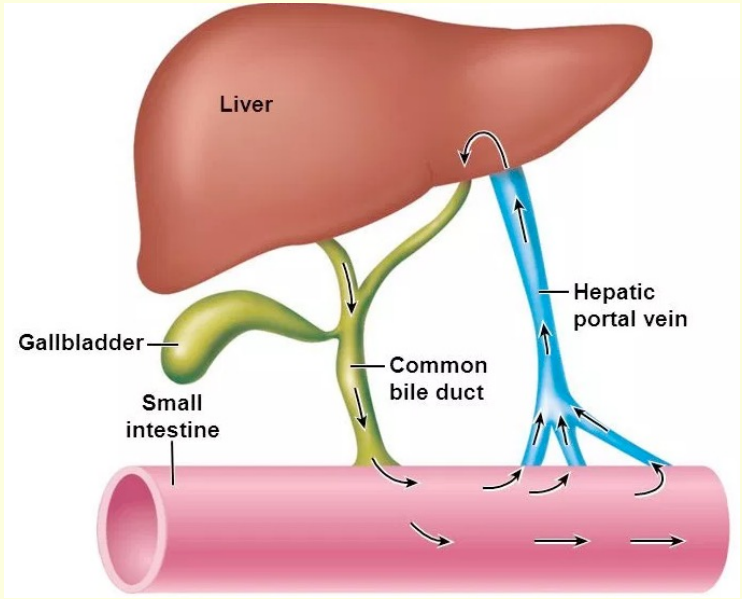

http://www.qualyst.com/

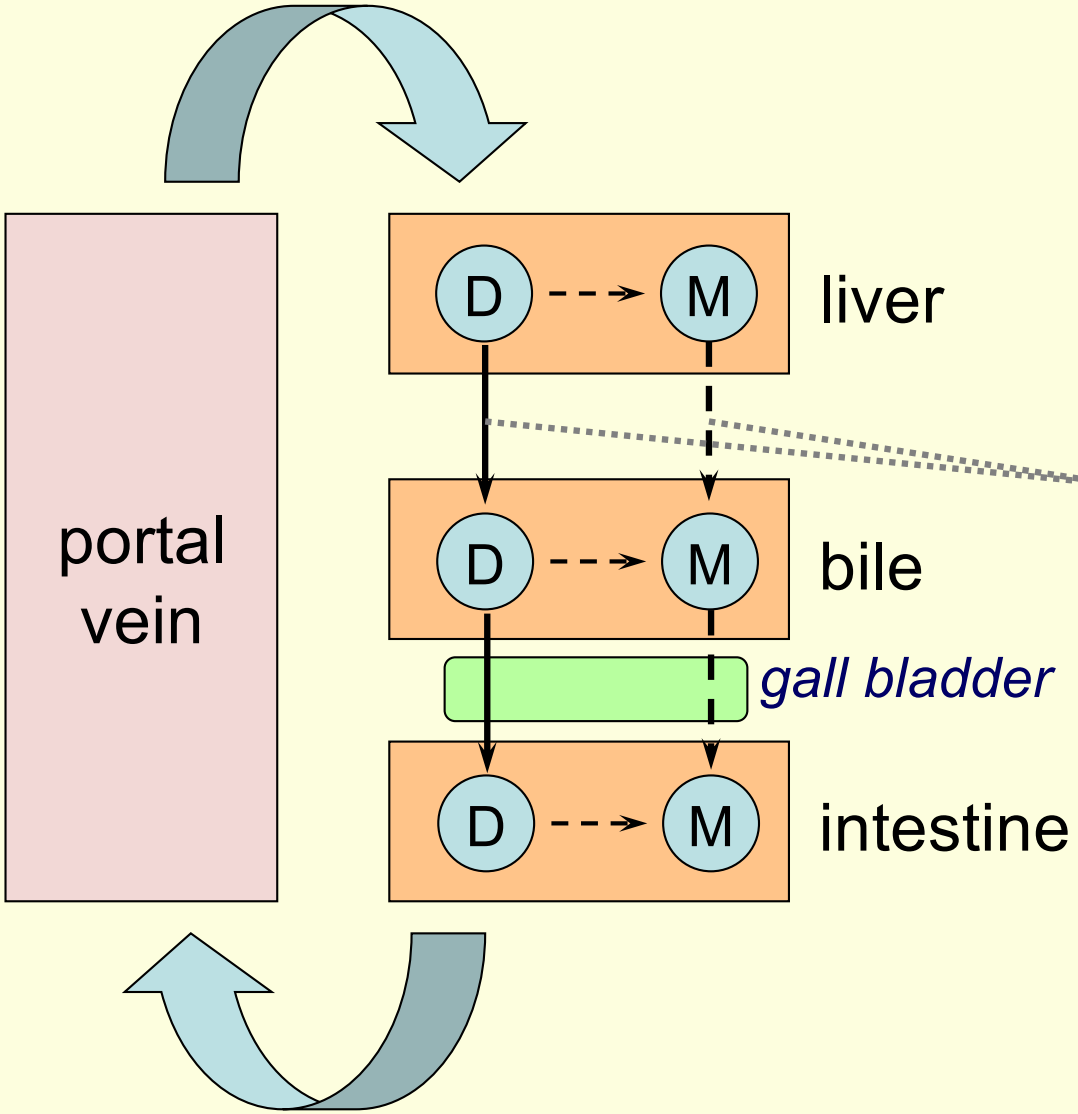
**Drugs or their metabolites are effluxed either apically into bile or basolaterally into blood stream**

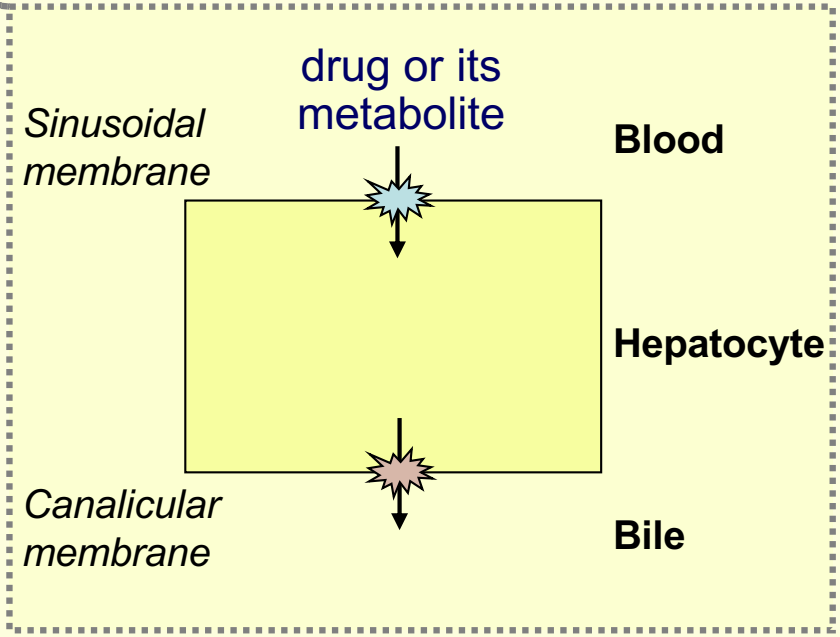



**Different transporter systems are involved**

# Excretion in bile: Enterohepatic Cycle

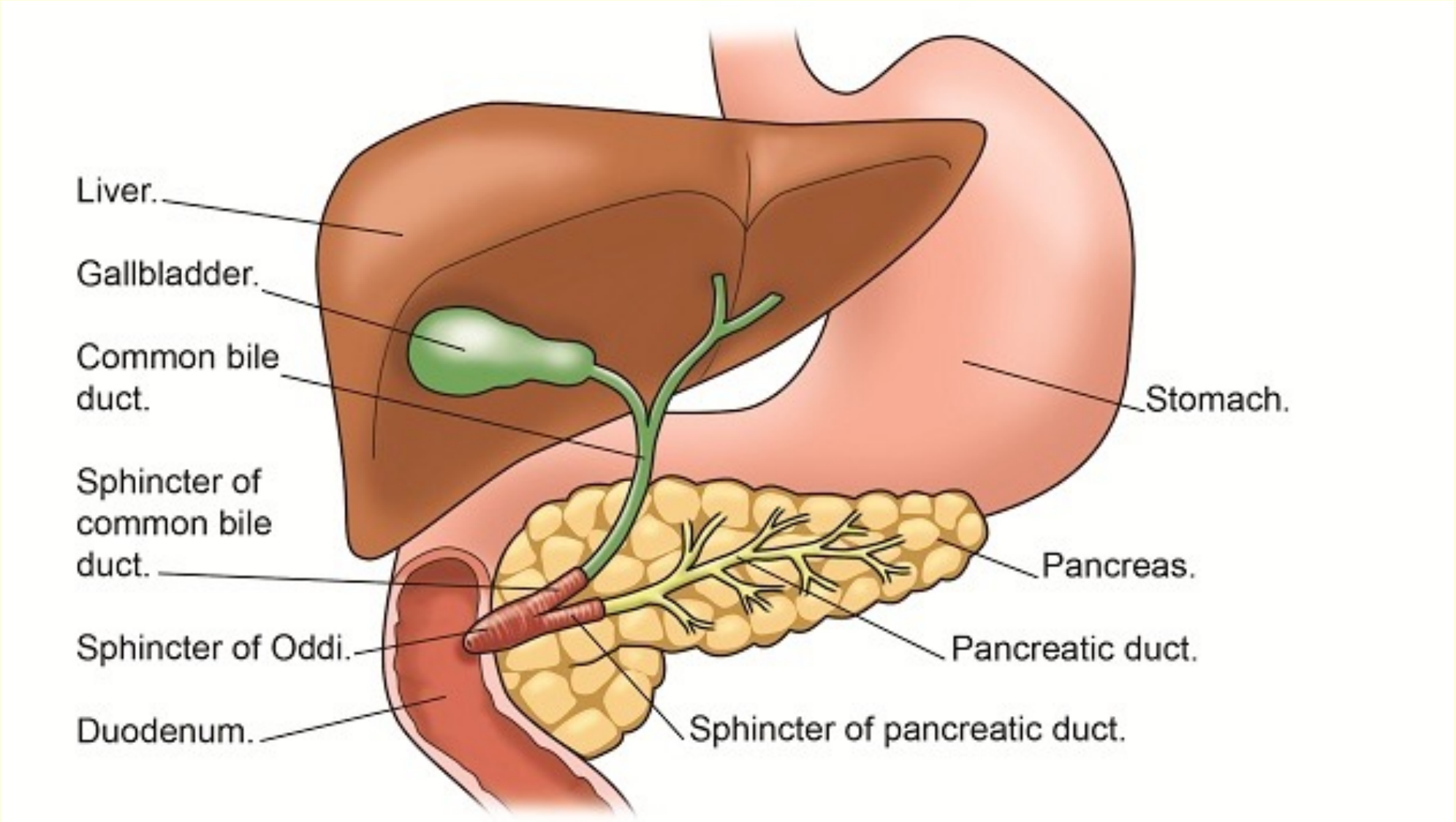



When Drug (D) or Metabolite (M) is absorbed from intestine, excreted in bile and reabsorbed from intestine it has undergone enterohepatic cycling

# Enterohepatic Cycling: sphincter of Oddi



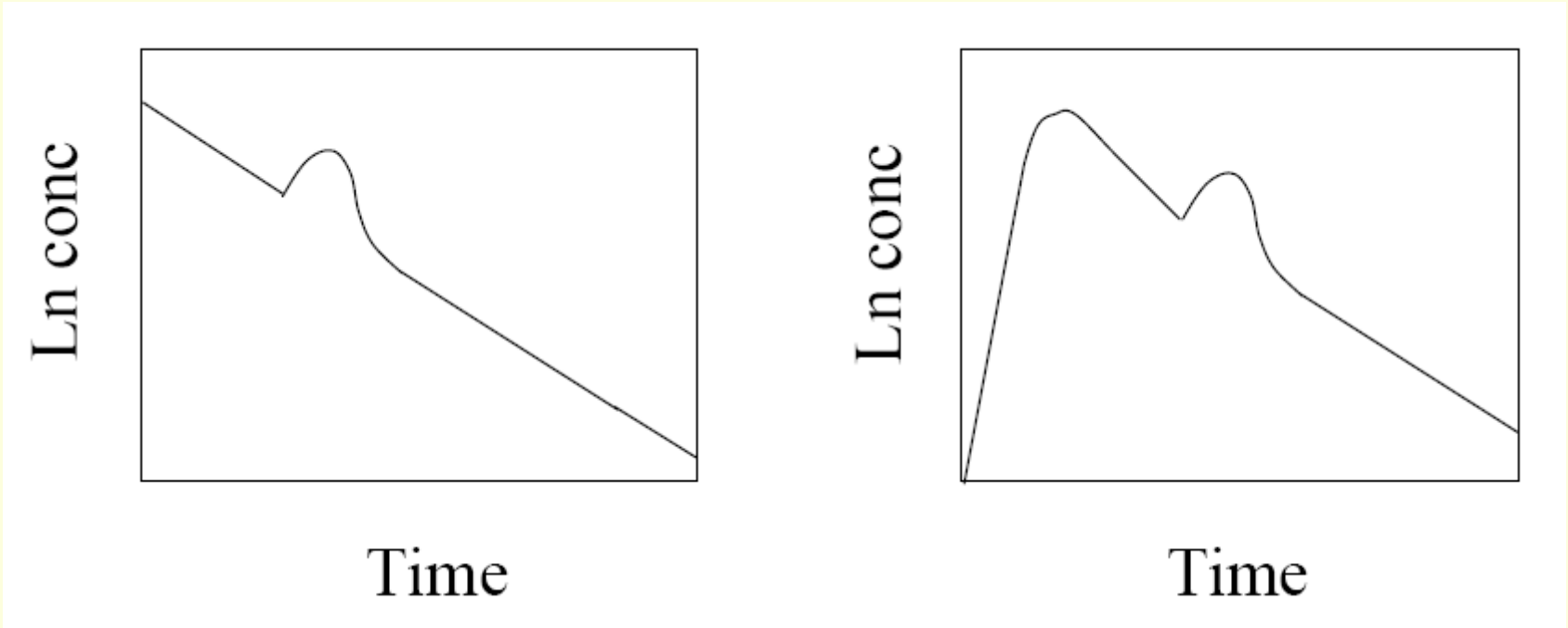

The sphincter of Oddi is a muscular valve that opens and closes. It allows bile to flow through the ducts from the liver to the small intestine.

# Excretion in bile: Enterohepatic Circulation

Bile secretion (openings of Oddi's sphincter) follows circadian pattern (circadian pattern of food intake)

This results in secondary peaks in plasma concentration time curves for bile-secreted drugs.

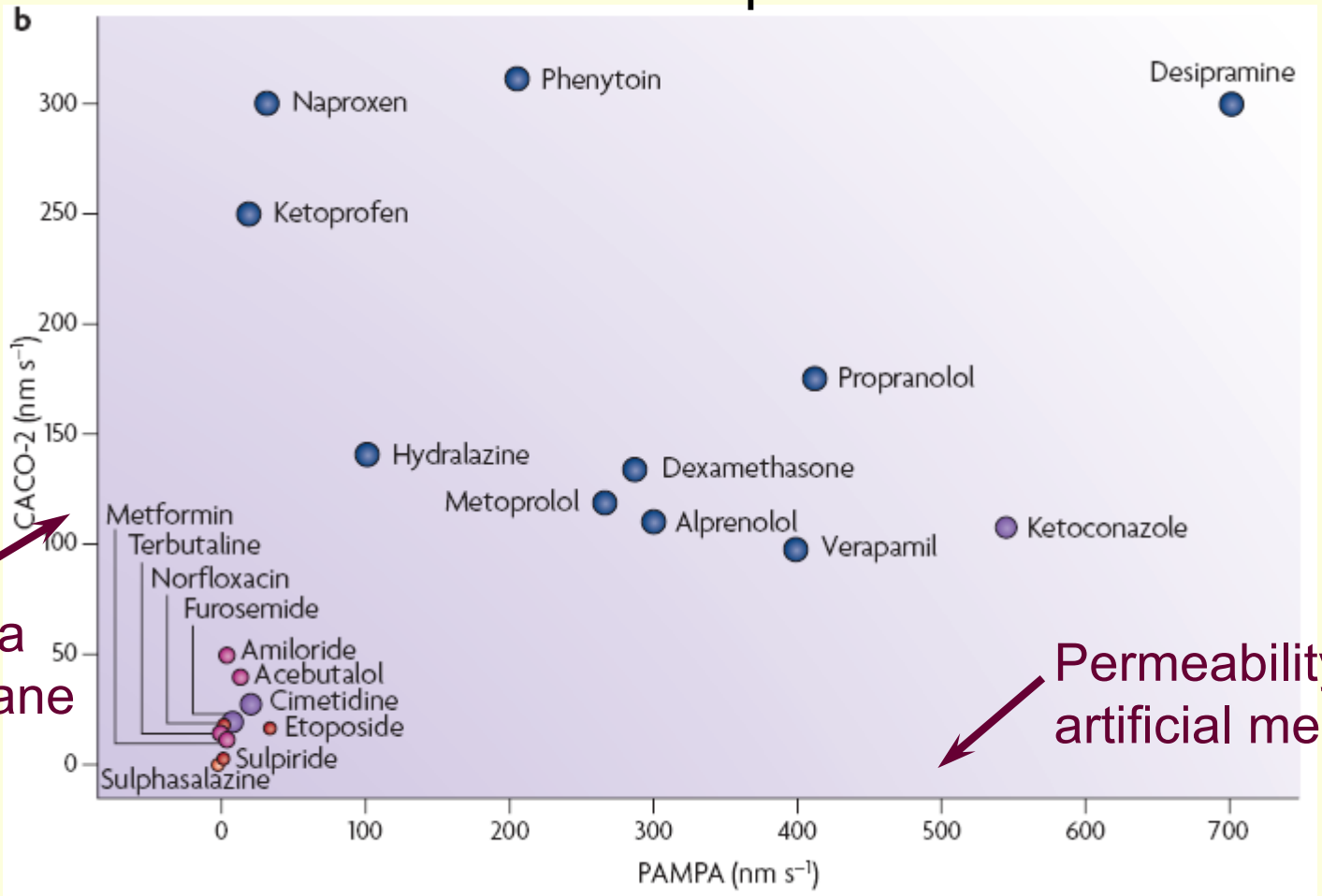
Enterohepatic cycle can significantly slowdown the excretion rate of a substance !



# Involvement of transporter systems in drug absorption and excretion

• a common rule: most drugs are lipophilic compounds that are absorbed and distributed via nonfacilitated diffusion

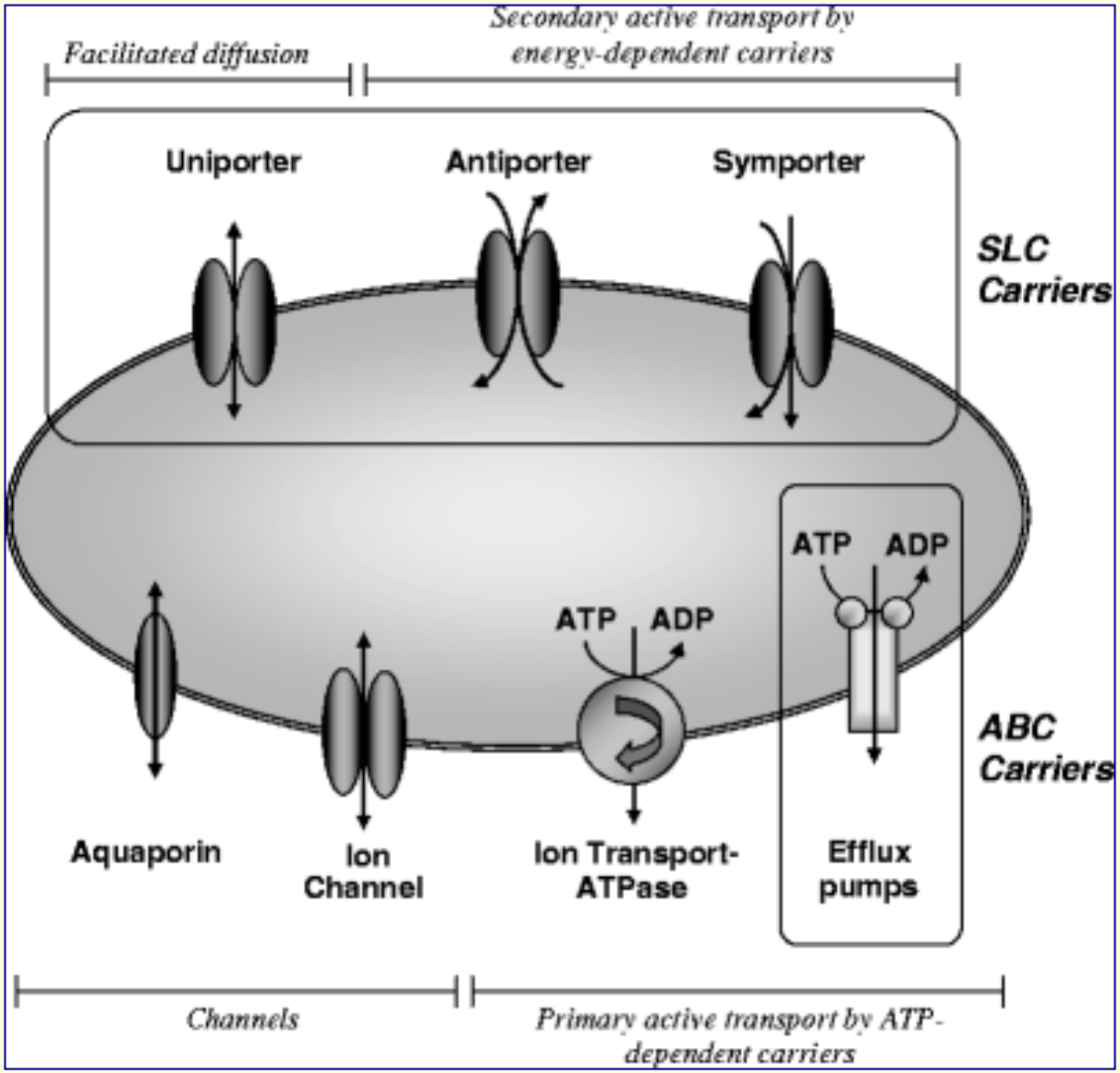
• however, for some drugs and many drug metabolites the carrier-mediated mechanisms are important:




Permeability of a natural membrane

Permeability of an artificial membrane

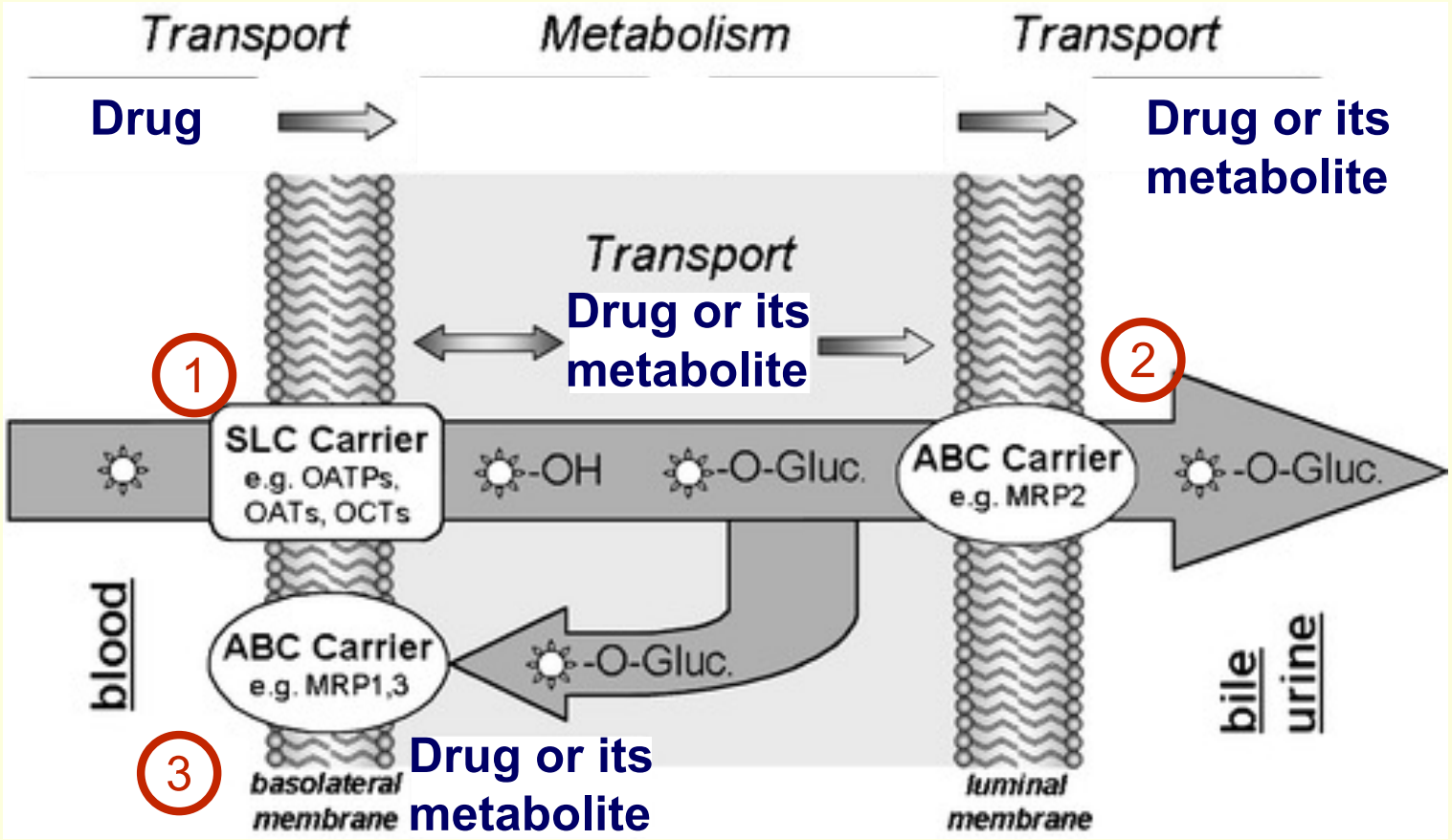
# Transporters involved in drug absorption and excretion




SLC transporters

ABC transporters

# Carrier-mediated (facilitated) drug transport



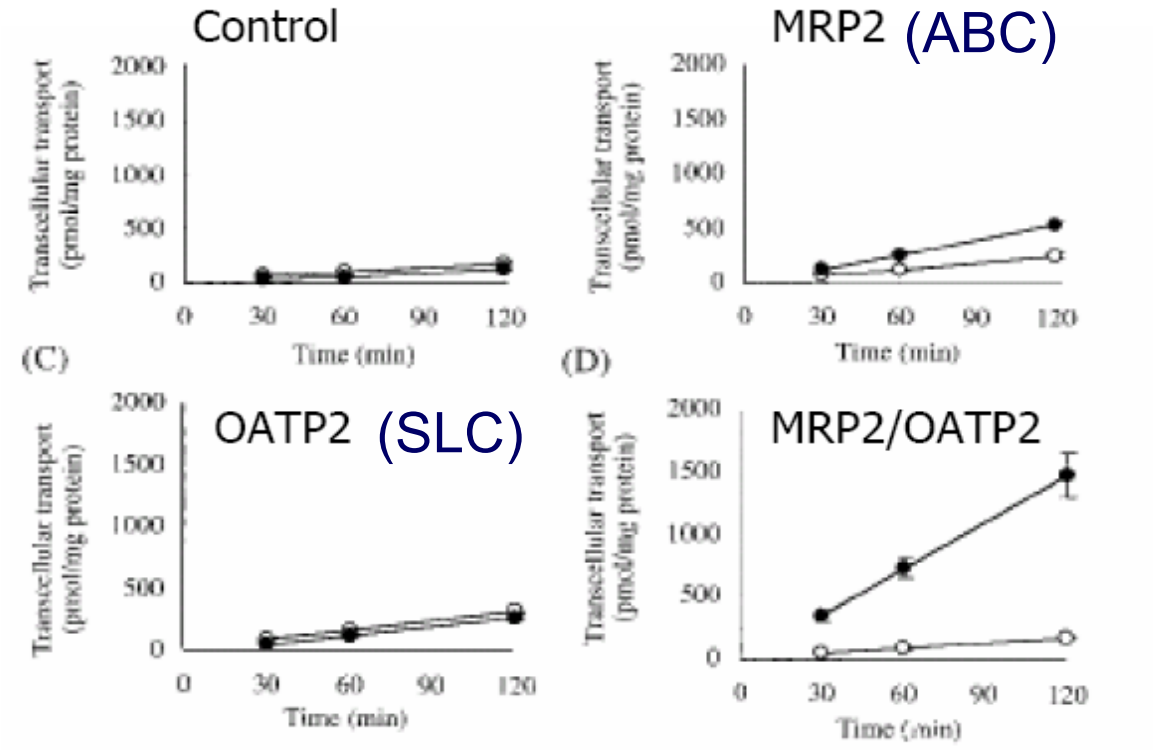

**Pathways of drug (or its metabolite) transport in a cell:**

**1 – uptake out of blood**

**2 – efflux into secreted fluids**

**3 – backward into blood**

# Example (experimental evidence): transcellular transport of estradiol 17 beta -D-glucuronide (E217G), a metabolite of estradiol, requires both SLC and ABC transporters




Cell are transfected with MRP2(ABC) and/or OATP2(SLC)

Sasaki et al JBC 2002

# Relevance of Drug Transporters

- Modulation of transporter function through inhibition or induction could result in changes in drug absorption, distribution and excretion: drug-drug interactions.
- A source of inter-individual variability in drug response - pharmacogenetics
- A source for nonlinear kinetics

# Major Drug Transporters

**ATP-Binding Cassette Transporters (ABC)**

- P-glycoprotein (P-gp or MDR1)
- Multidrug Resistance Associated Proteins (Mrps)
- Breast cancer resistant protein (BCRP)

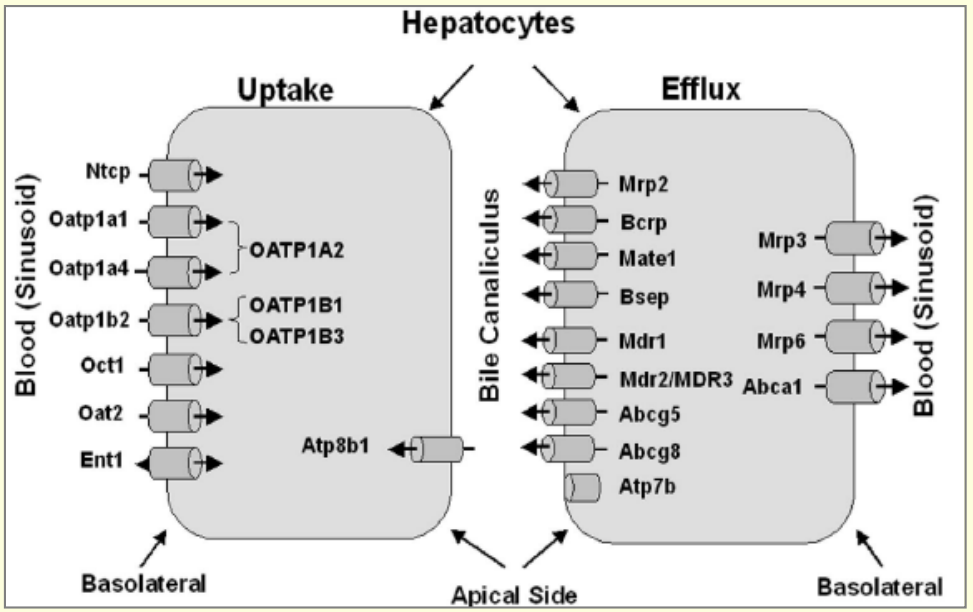
**Solute Carriers (SLC)**

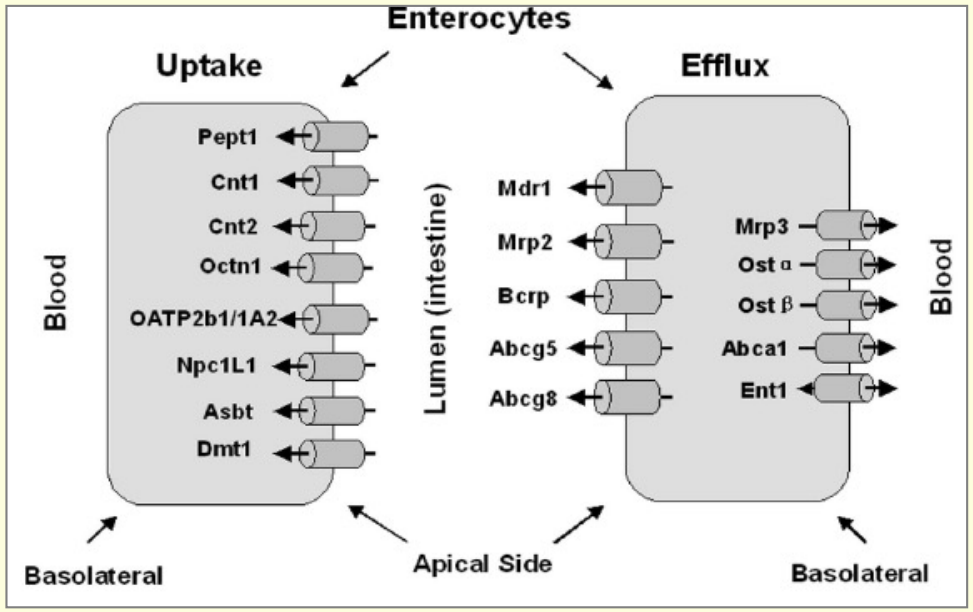
- Organic anion transporting polypeptides (OATPs)
- Organic anion transporters (OATs)
- Organic cation transporters (OCTs)
- Nucleoside transporters (ENTs, CNTs)
- Oligopeptide transporters (PepTs)
- Bile acid transporters (e.g. NTCPs)
- Monocarboxylate transporters (MCTs)

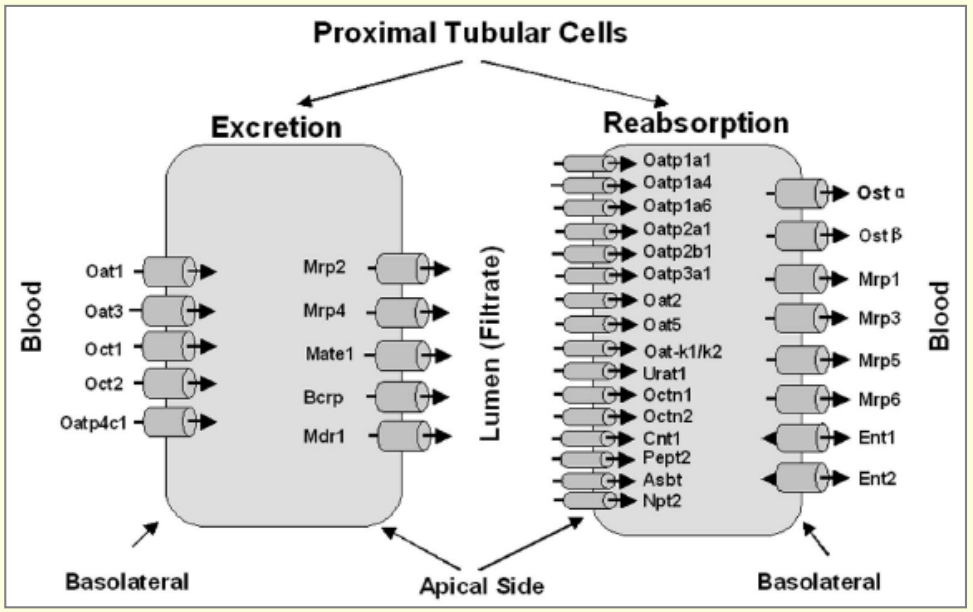
# The Challenge – each cell expresses its own set of SLC and ABC transporters

Examples from:

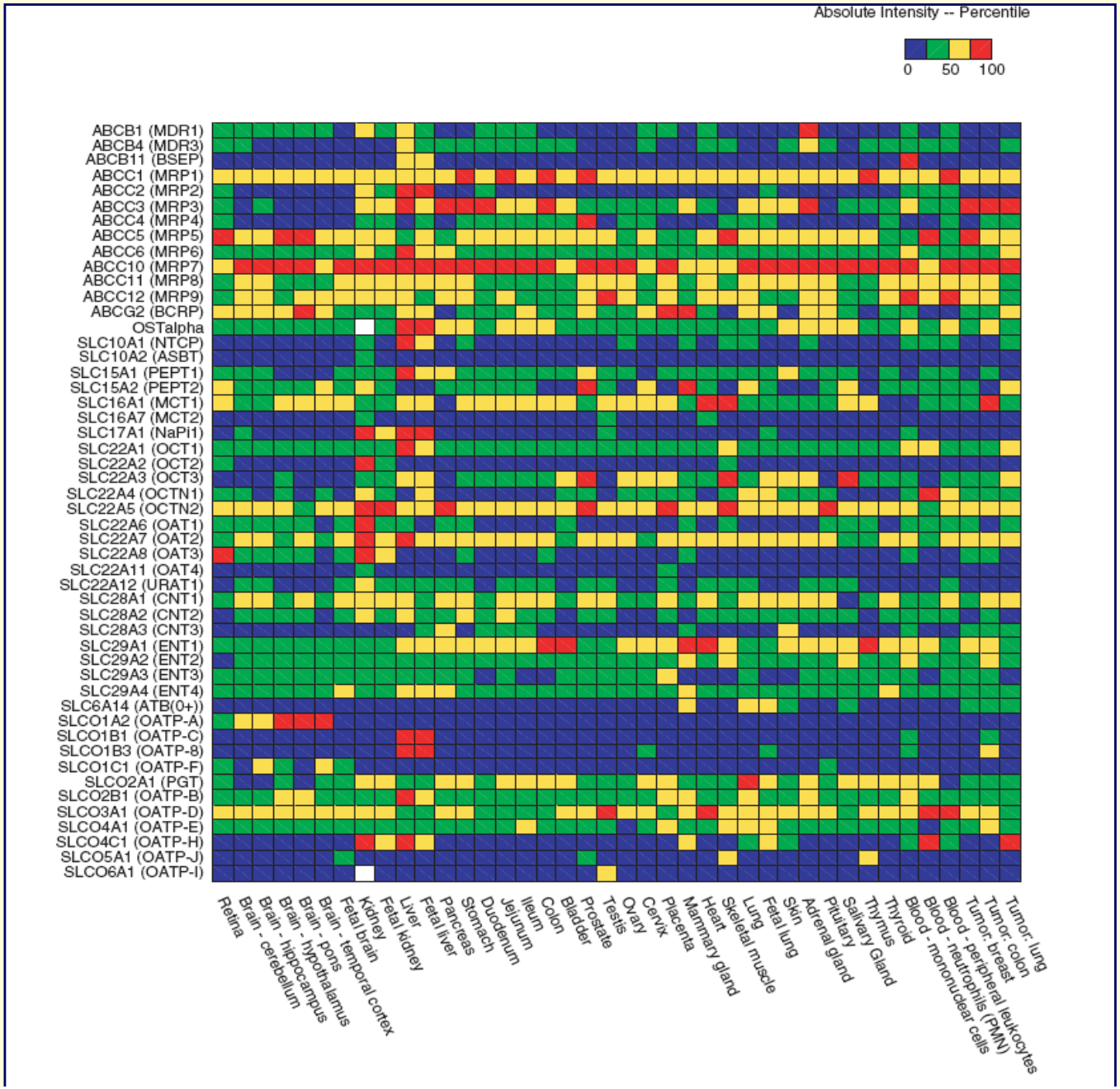
- hepatocytes
- enterocytes
- renal proximal tubule cells



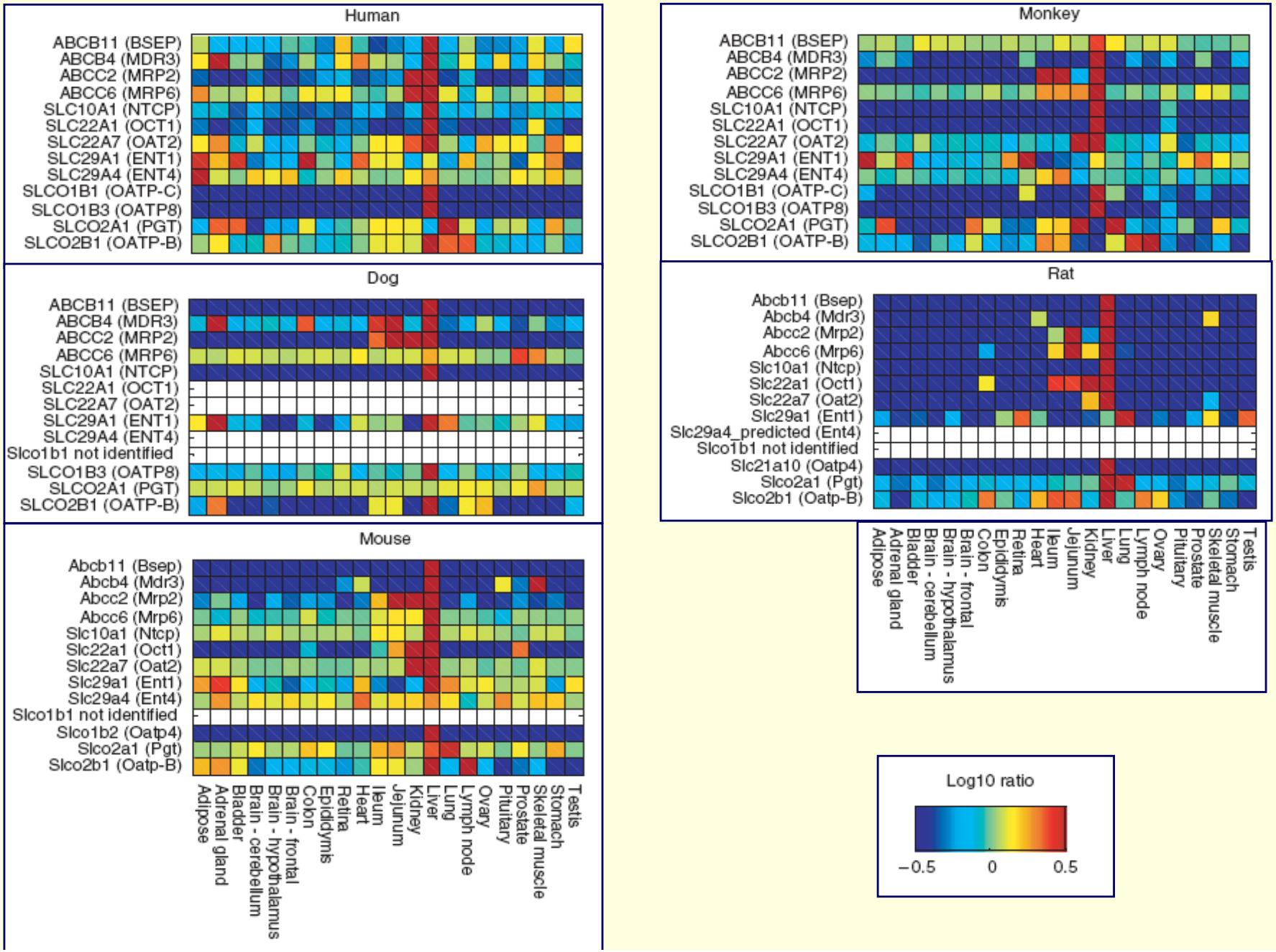



# Studies of tissue expression profiles for drug transporters - microarrays




Bleasby et al, 2006

# There is only low correlation for expression of drug transporters between different species!



# Functional characterization of drug transporters: *in vitro* assays

## In Vitro Transporter Assays

Uptake and Efflux Studies

- cells, membranes vesicles and isolated tissues

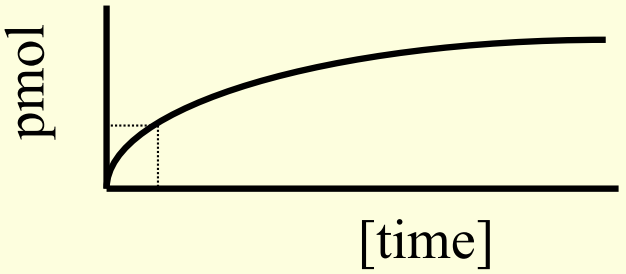
Monolayer Transport Studies

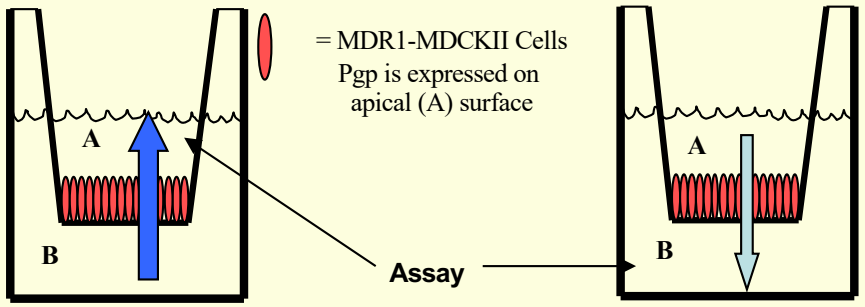
Inhibition Studies

- uptake, efflux, transport

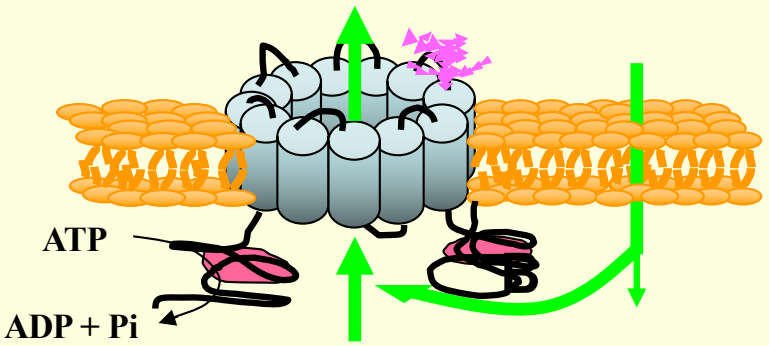
Surrogate Transporter Activity

- ATPase Studies (Pi)
- Co-factor utilization




**B-to-A/A-to-B = Efflux Ratio**

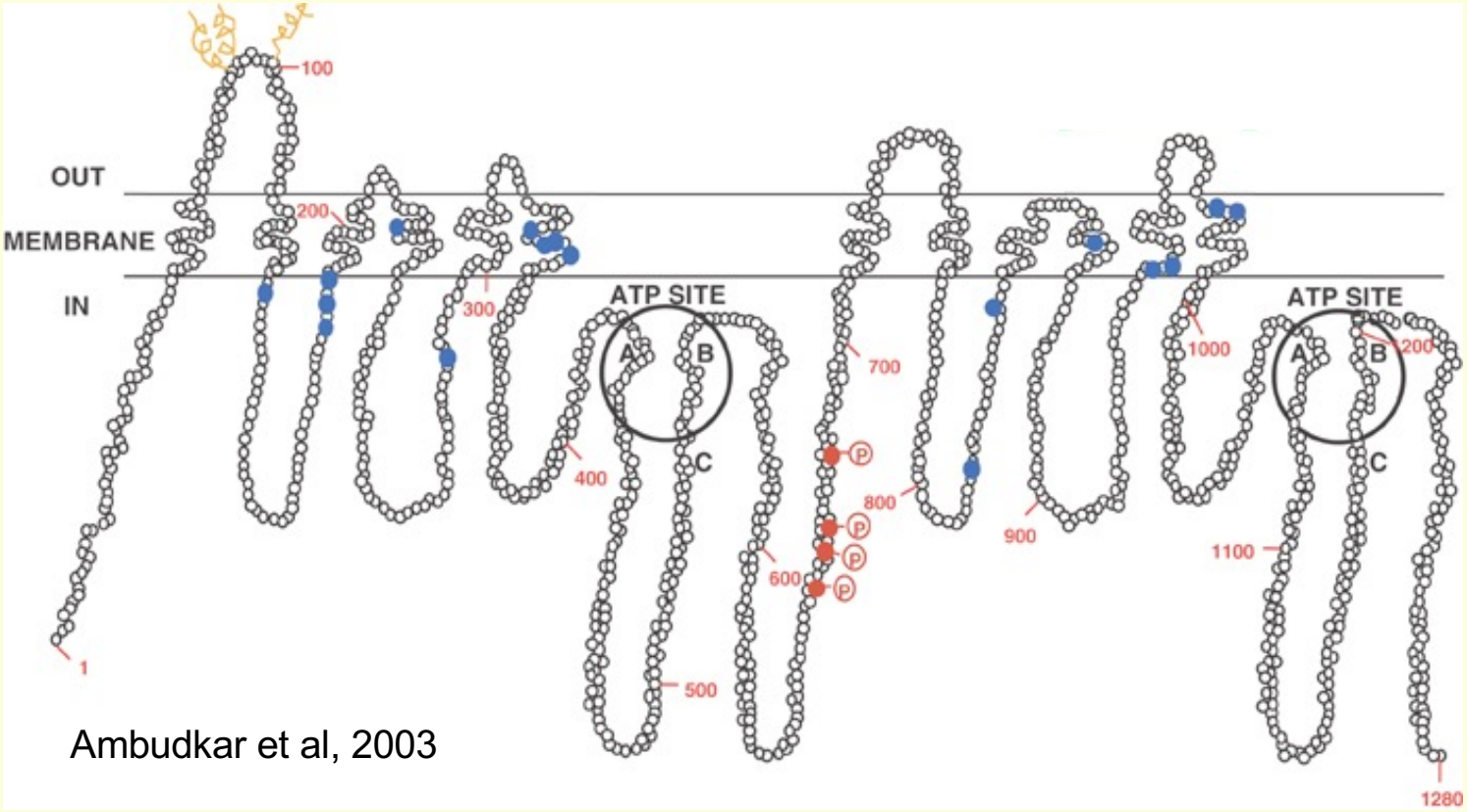


# Major drug transporters – ABC proteins

ABC – ATP Binding cassette family of transporters

- Efflux pumps
- Large gene family (49 members in human)
- Share sequence homology in several key areas
  - ❖ Walker A and B nucleotide binding motifs
  - ❖ C-region (LSGGQ signature sequence)
- Critical for movement a wide range of substances: nutrients, amino acids, sugars, lipids, ions, metabolites and xenobiotics.

# Example: ABCB1 (or MDR1, or P-glycoprotein)



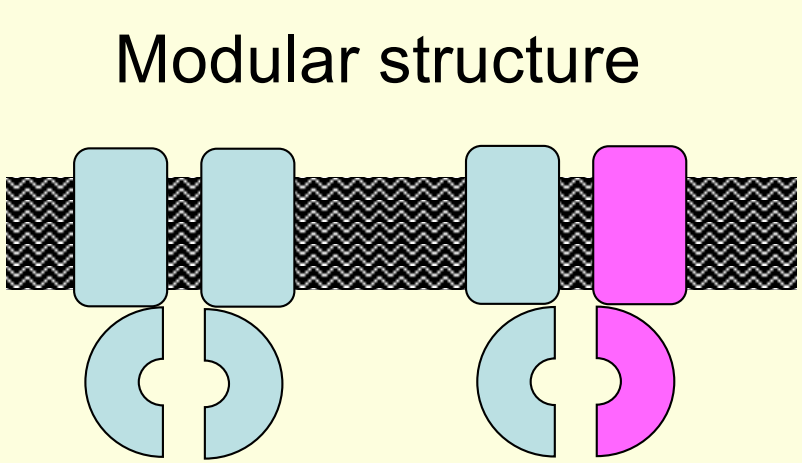

Ambudkar et al, 2003

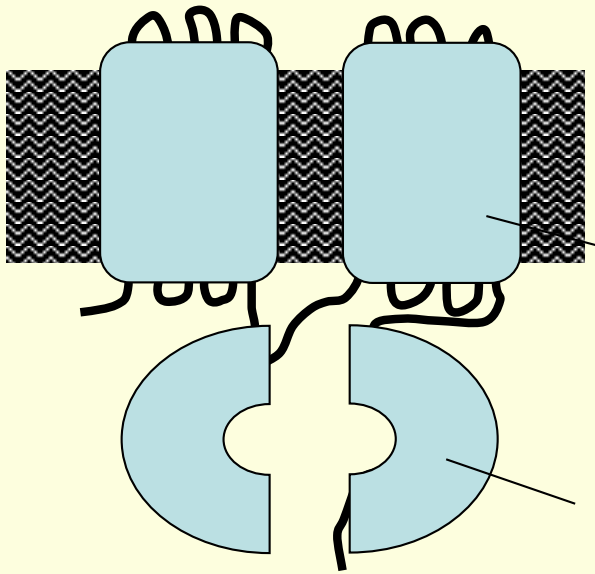
- 1280 amino acids, 12 membrane-spanning domains, 2 ATP-binding sites, N-glycosylated protein.

- was first discovered as a plasma membrane active efflux pump for a number of anticancer drugs; the upregulation of P-gp confers 'multidrug resistance' to tumor cells.

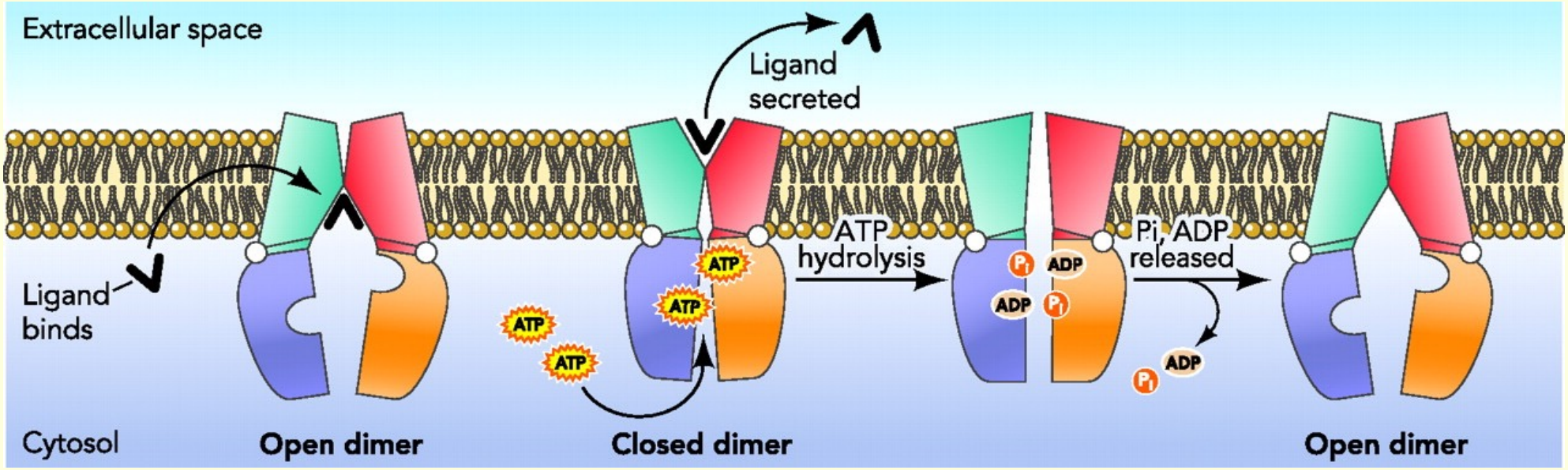
# ABC proteins: structure and function

Modular structure

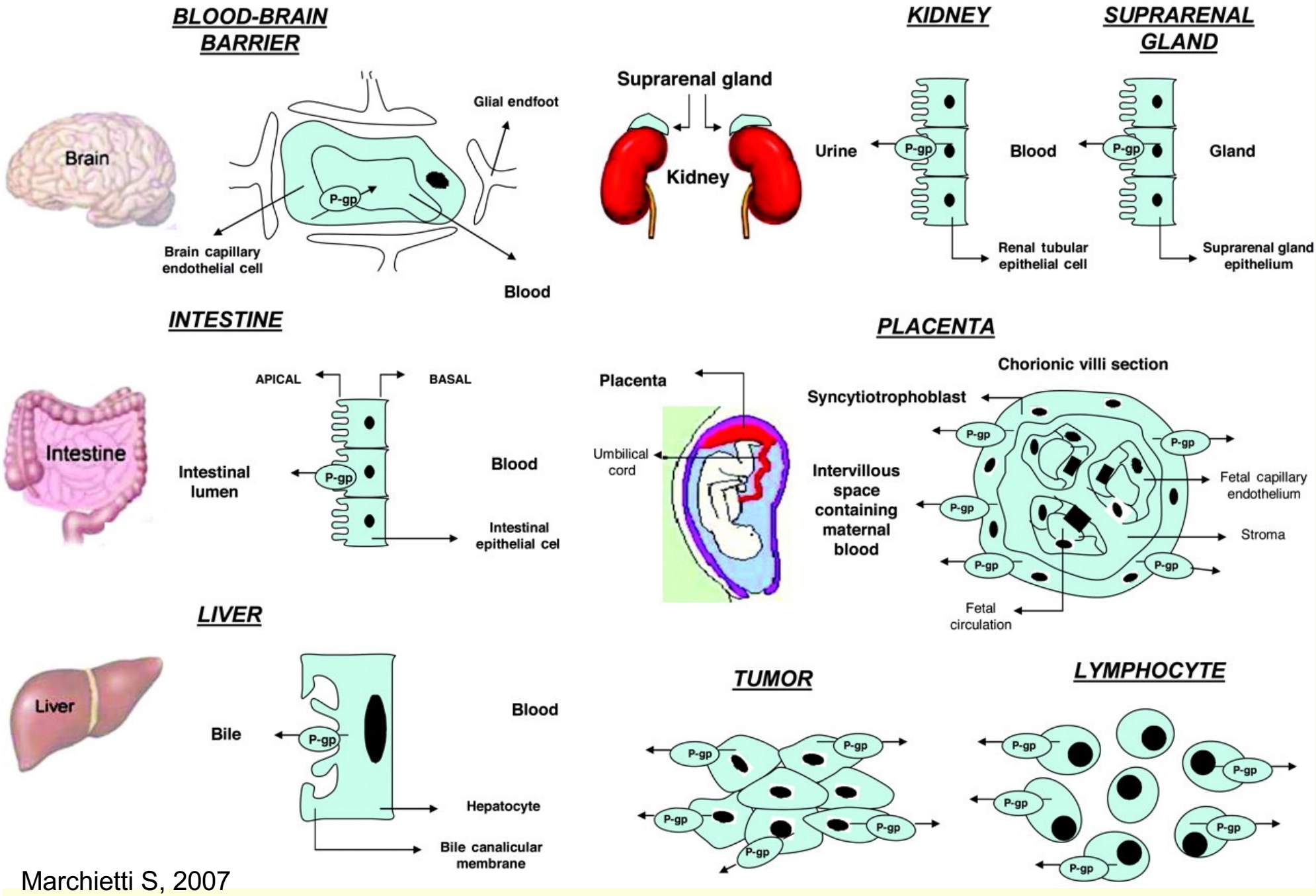





function



# ABCB1 (MDR1, P-gp) expression sites




Marchietti S, 2007

# List of ABCB1 Substrates and Inhibitors

## Substrates

***Anticancer Drugs***

Actinolycin
Dactinomycin
Daunorubicin
Docataxel
Doxorubicin
Ectoposide
Mitoxantrone
Mitomycin C
Paclitaxel
Teniposide
Topotecan
Vincristine
Vinblastine
VP16

***Aniconvulsants***

Carbamazepine
Phenytoin
Clozapine

***HIV Protease Inhibitors***

Indinavir
Saquininavir
Nelfinavir

***Others***

Atorvastatin
Celiprolol
Colchicine
Cortisol
Cyclosporine
Dexamethasone
Diltiazem
Domperidone
Digoxin
Erythromycin
Fexofenadine

## Inhibitors

FK506
Gramicidin
Haloperidol
Ivermectin
Loperamide
Morphine
Nifedipine
Odansetron
Progesterone
Rifa
Quinolone antibiotics
Terfenadine

***Antiarrythmics***

Amiodarone
Propafenone
Quinidine
Verapamil

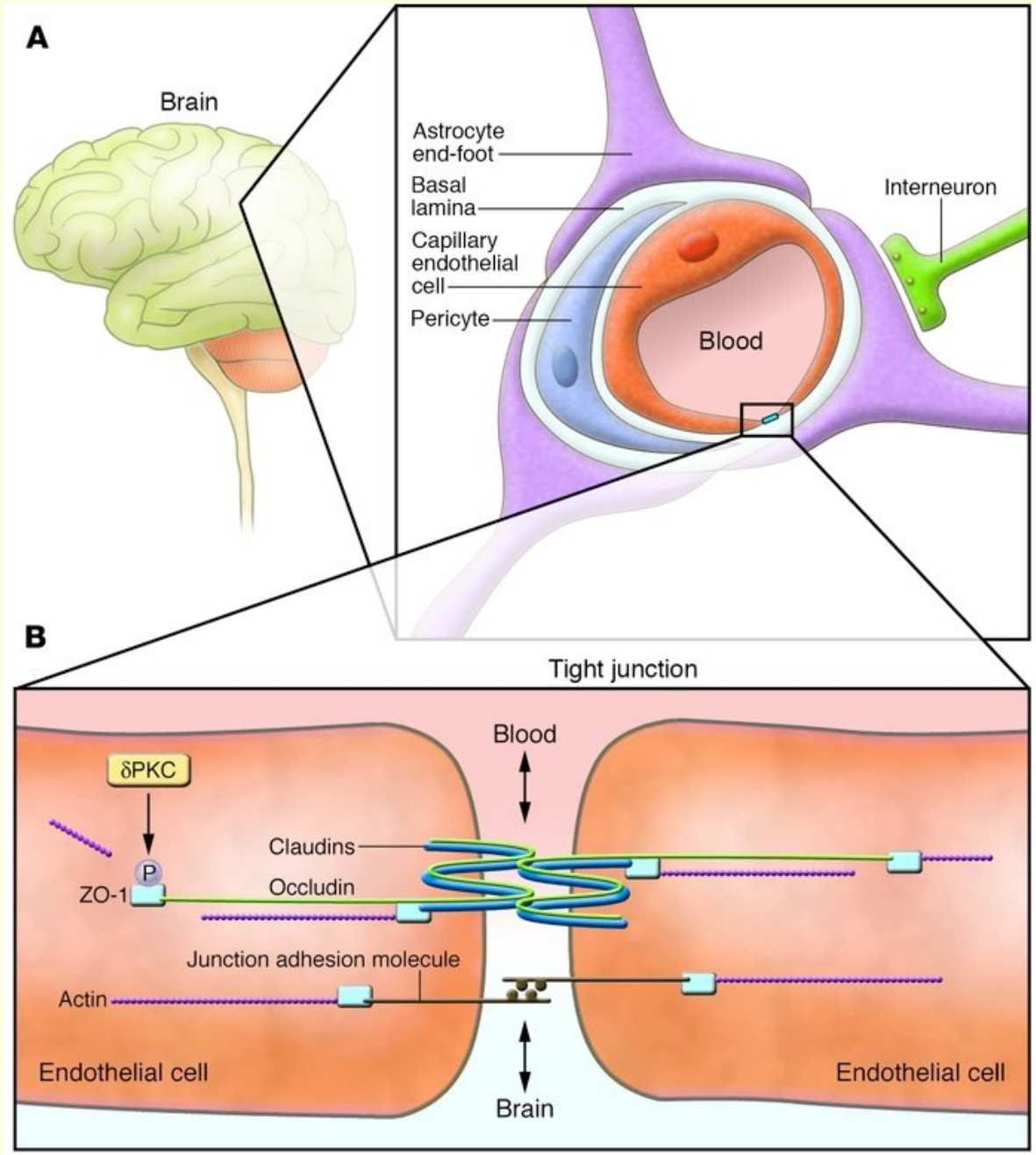
***Others***

Cremophor EL
Cyclosporine
FK506
Flupenthixol
Genistein
Kertoconazole
Mefloquine
Progesterone
Quinine
Quercetin
Rifampin
Rapamycin
Reserpine
Tamoxife
Trifluoperazine

***Pgp Modulators***

PSC-833
GF120918
LY335979

# Example: role of ABCB1 in blood-brain barrier (BBB)



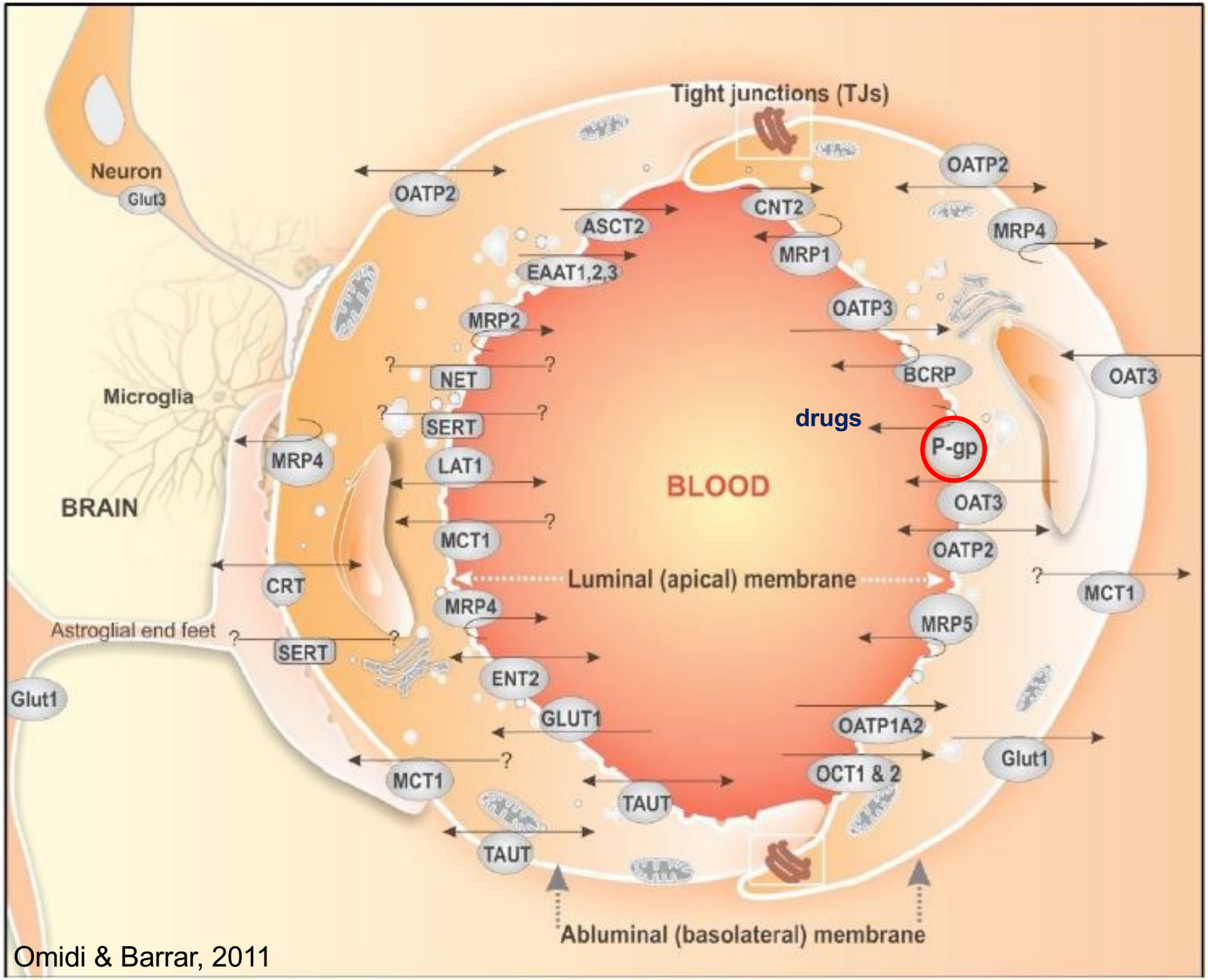

**Anatomical (physical) barrier** formed by the endothelial cells of brain capillary and the tight junctions between these cells

**Functional barrier**

- Formed by membrane transporters (efflux systems) – see next slide

Chou & Messing JCI 2008

# Role of ABCB1(P-gp) in BBB




Omidi & Barrar, 2011

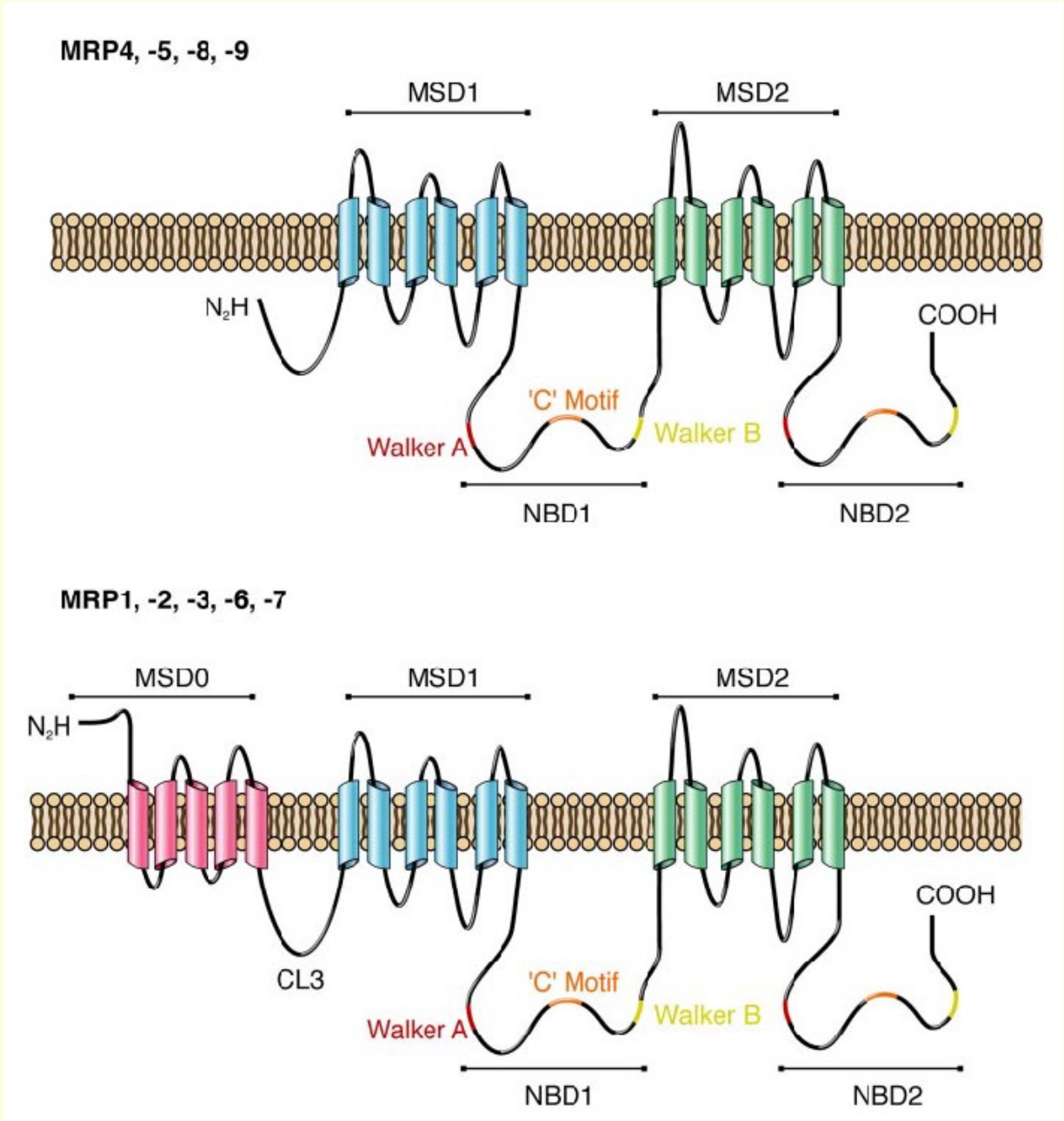
# Change of Drug Concentrations in ABCB1 Knockout Mice

| Drug | Plasma | Brain |
|---|---|---|
| Quinidine | 3.7-fold | 29-fold |
| Cyclosporine | 1.9-fold | 55-fold |
| Digoxin | 1.9-fold | 35-fold |
| Loperamide | 2-fold | 14-fold |

# MRPs: Multidrug Resistance-asssociated Proteins (ABCC family)

« Short » MRPs

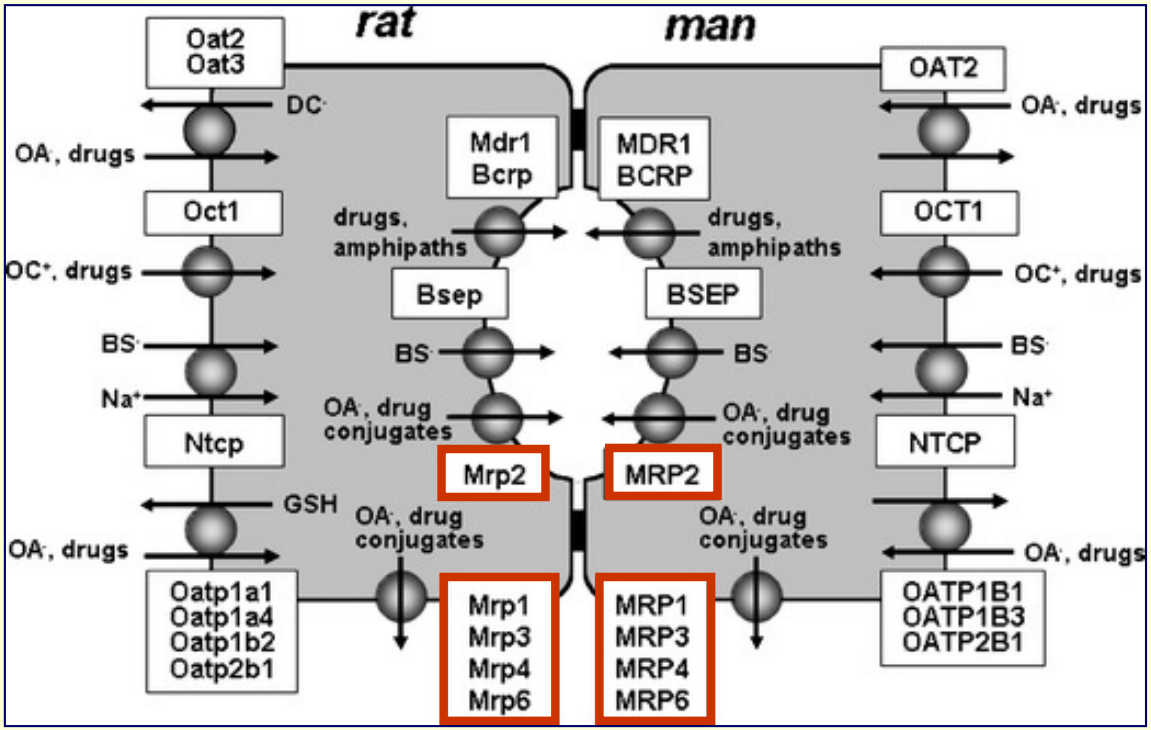
« Long » MRPs



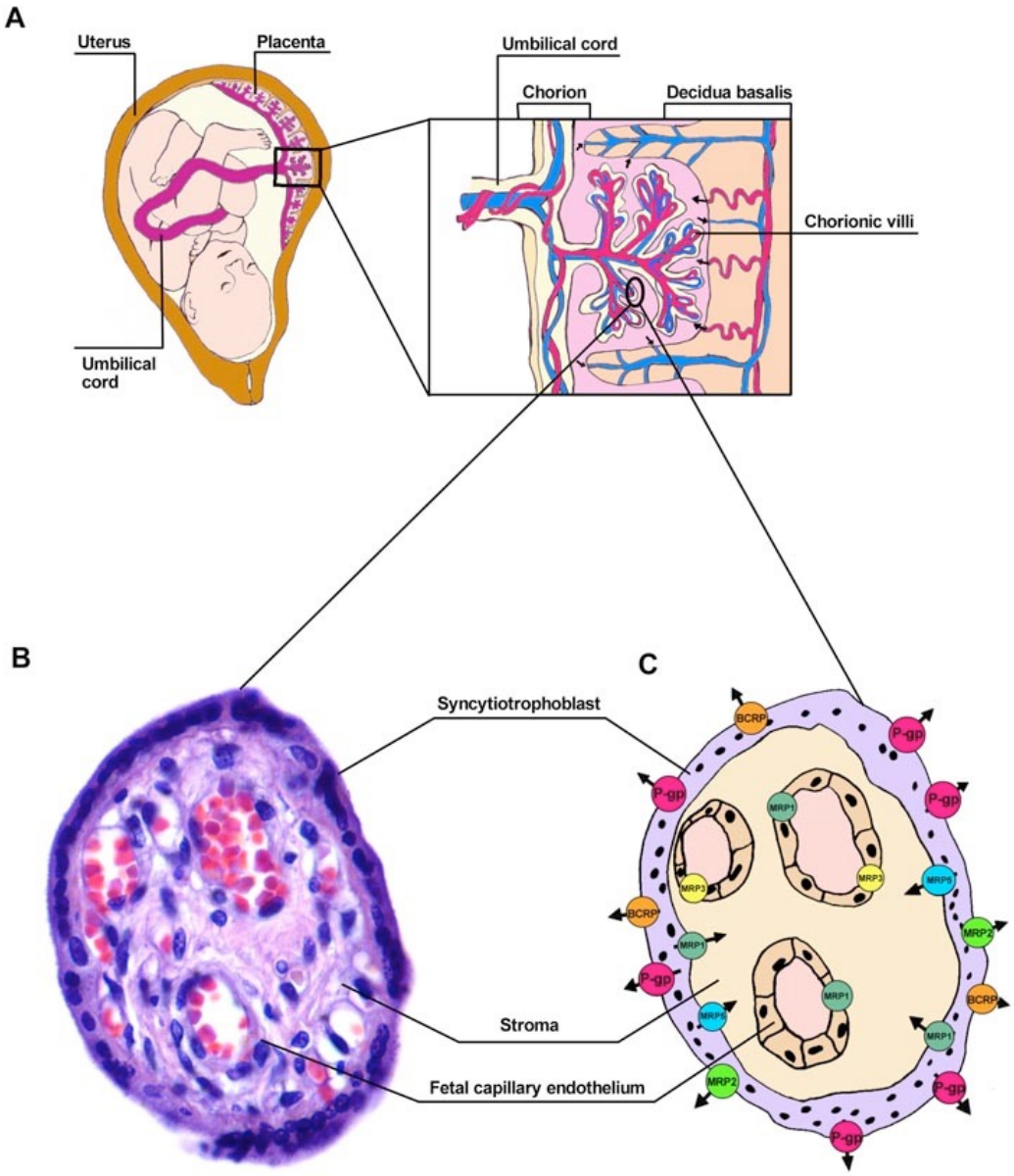

Deeley et al, 2006

# MRP expression in hepatocytes

- MRP2 is localized on the apical (canalicular) membrane and pumps drugs and drug conjugates into bile.
- MRP1, MRP3, MRP4 and MRP6 are localized on the basolateral (sinusoidal) side of the hepatocytes and transport drugs and drug conjugates from hepatocytes into blood.
- MRP substrates include anticancer drugs (vinca alkaloids, etoposide), HIV protease inhibitors, etc.
- drug conjugates (PhaseI/II metabolites): glutathione conjugates, glucuronide conjugates, sulfate conjugates



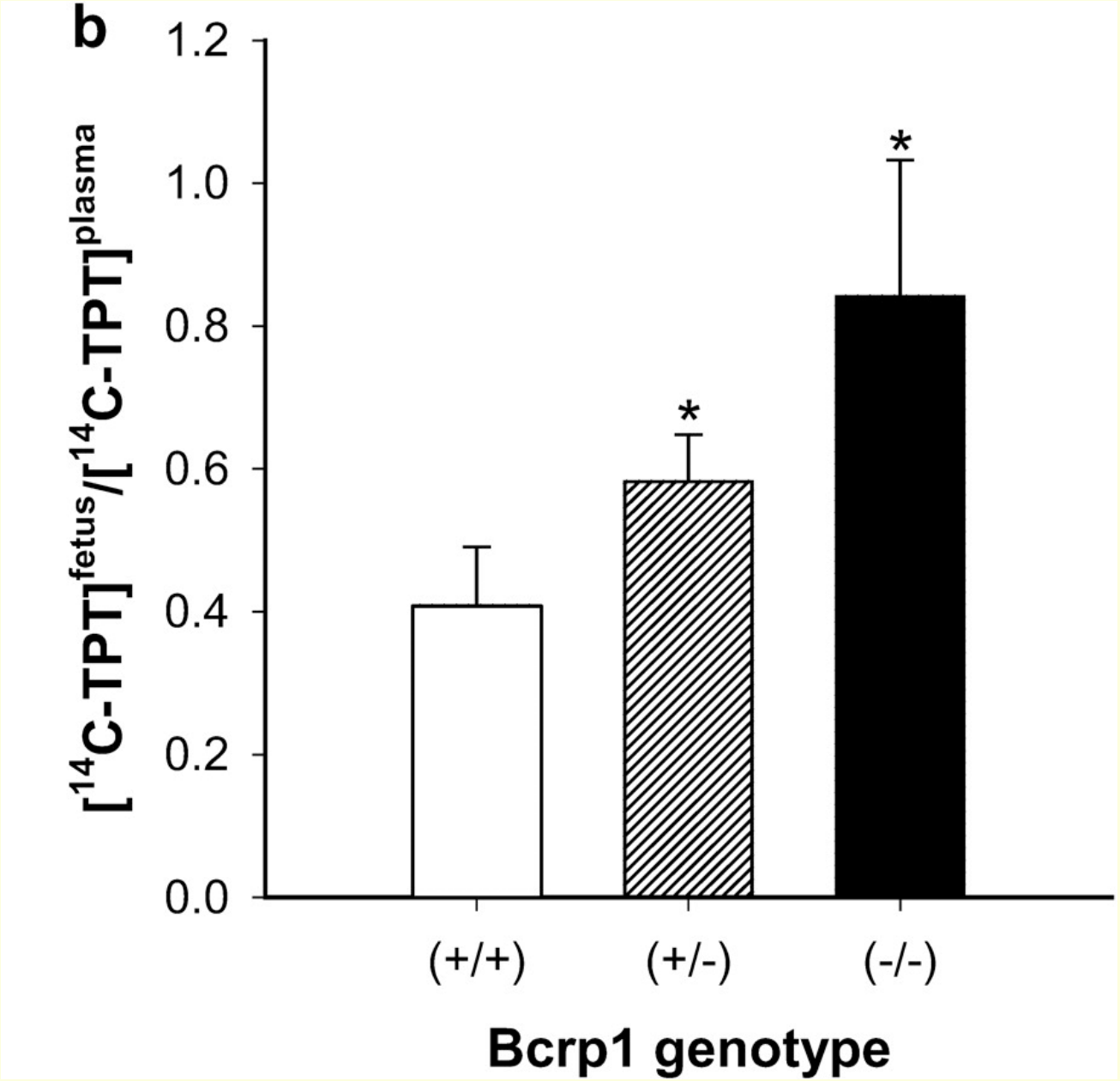
# Breast Cancer Resistance Protein – ABCG2 (BCRP, MXR, ABCP)



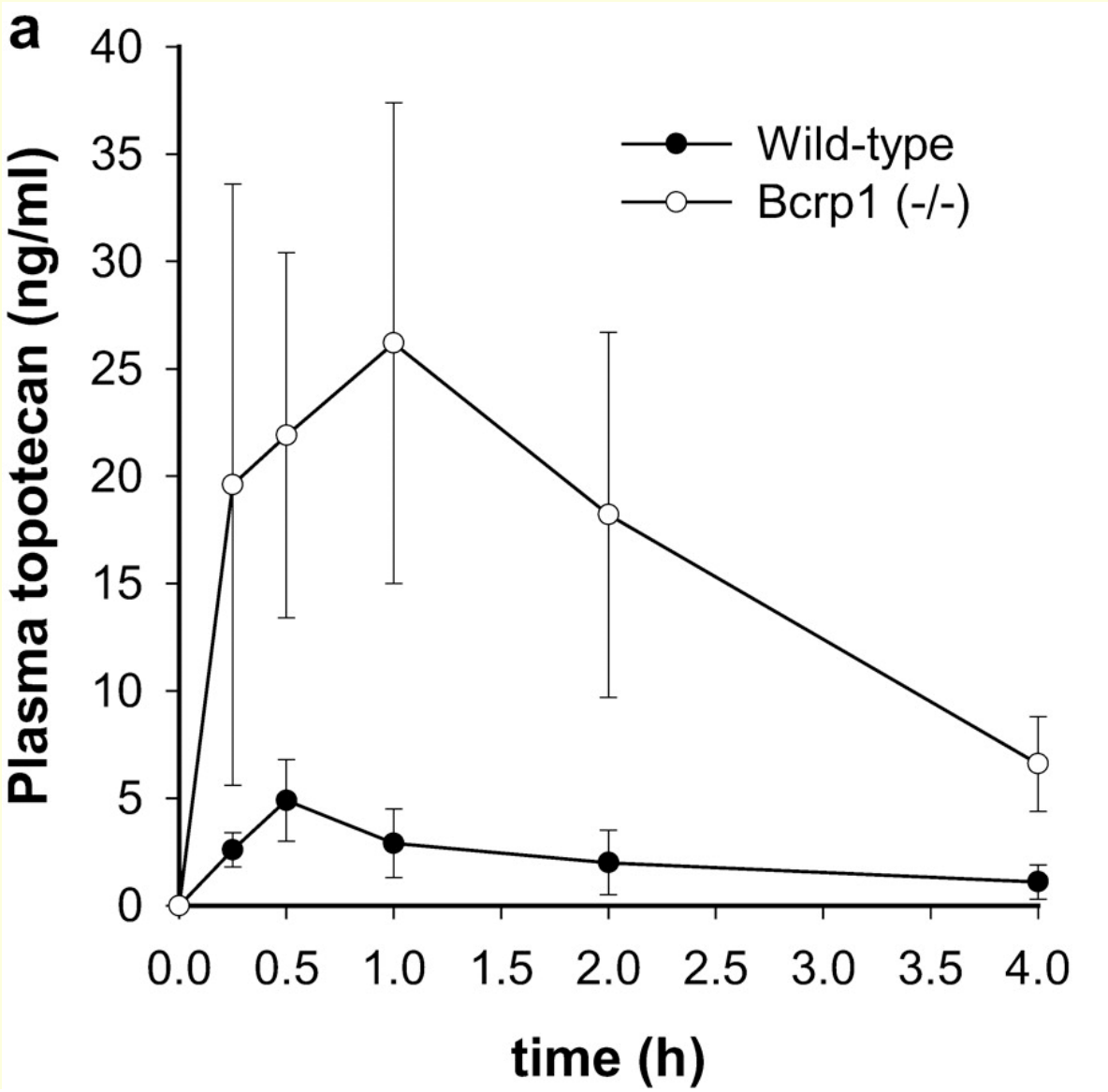

Ceckova-Novotna et al., *Reprod Toxicol*, 2006

- Expression in placenta, heart, ovary, kidney, liver, intestine, brain, colon
- Export pump –apical membrane localization
- Substrates
  - Anticancer: Mitoxantrone, Topotecan, Irinotecan, …
  - H2 antagonists: Cimetidine
  - Antiviral: Acyclovir
  - Antibiotics: Norfloxacin, Ofloxacin
  - Carcinogens: Phip, IQ…

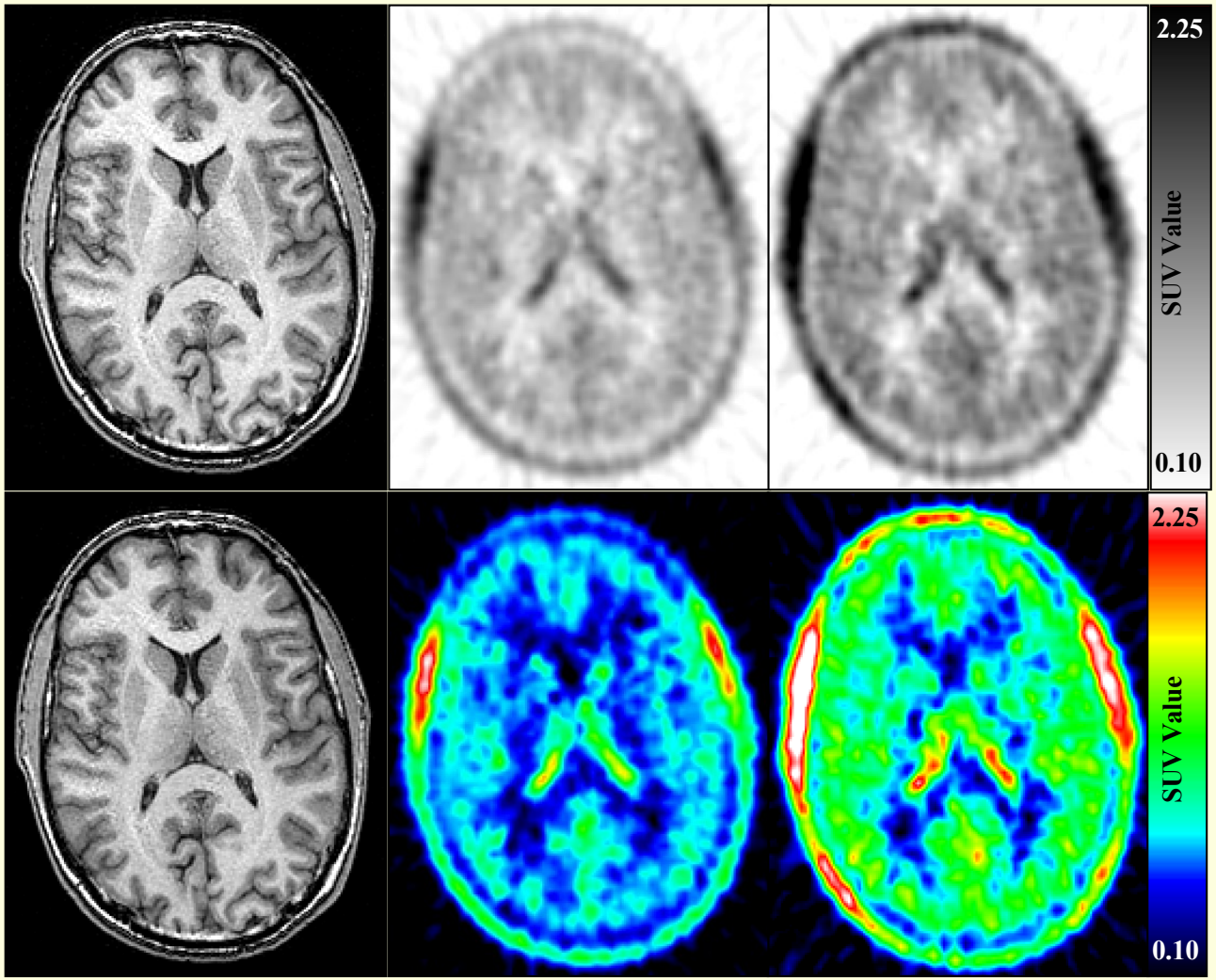
# ABCG2 limits fetal exposure to xenobitics (topotecan) – ABCG2 (BCRP1) ko mice



# Topotecan bioavailbaility in ABCG2 -/- mice (enteral administration, increased intestinal absorption)



# Competitive Inhibition of ABC transporters: example with ABCB1 (P-gp) in Drug Distribution into the Human Brain (drug-drug interaction)




Sasongko et al. Clin Pharmacol Ther. 77(6):503-14, 2005

# Drug-drug interaction via induction of ABC transporters

Example: Induction of intestinal ABCB1 by rifampin decreases enteral digoxin absorption

(Greiner et al., J. Clin. Invest. 104: 147-53, 1999).

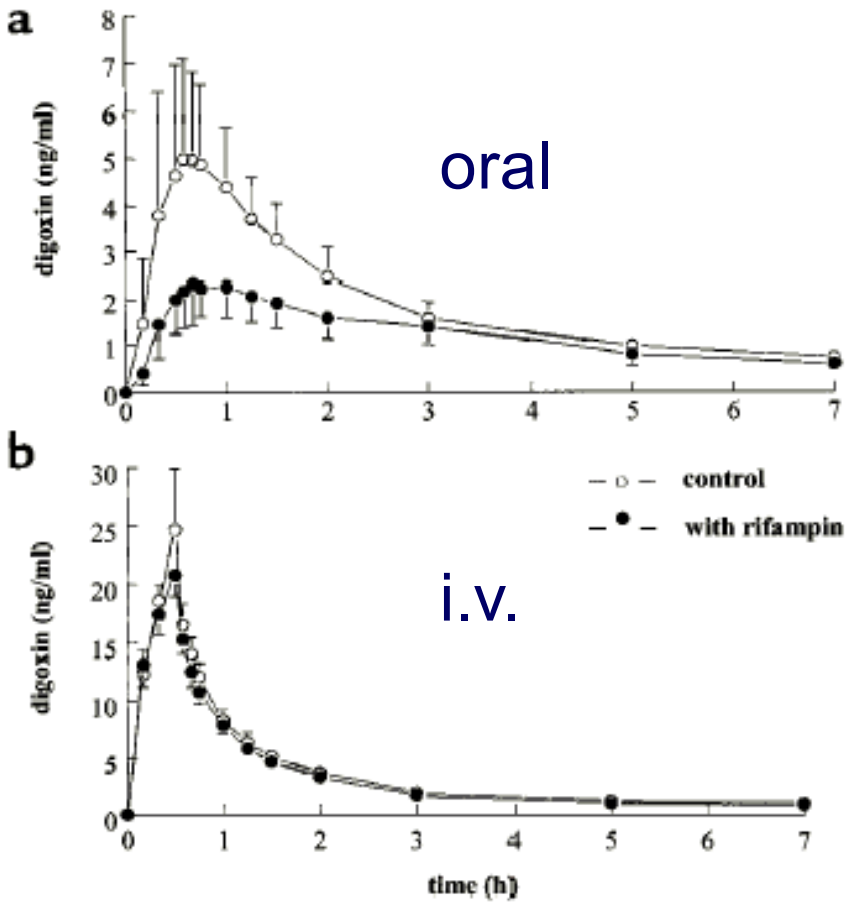
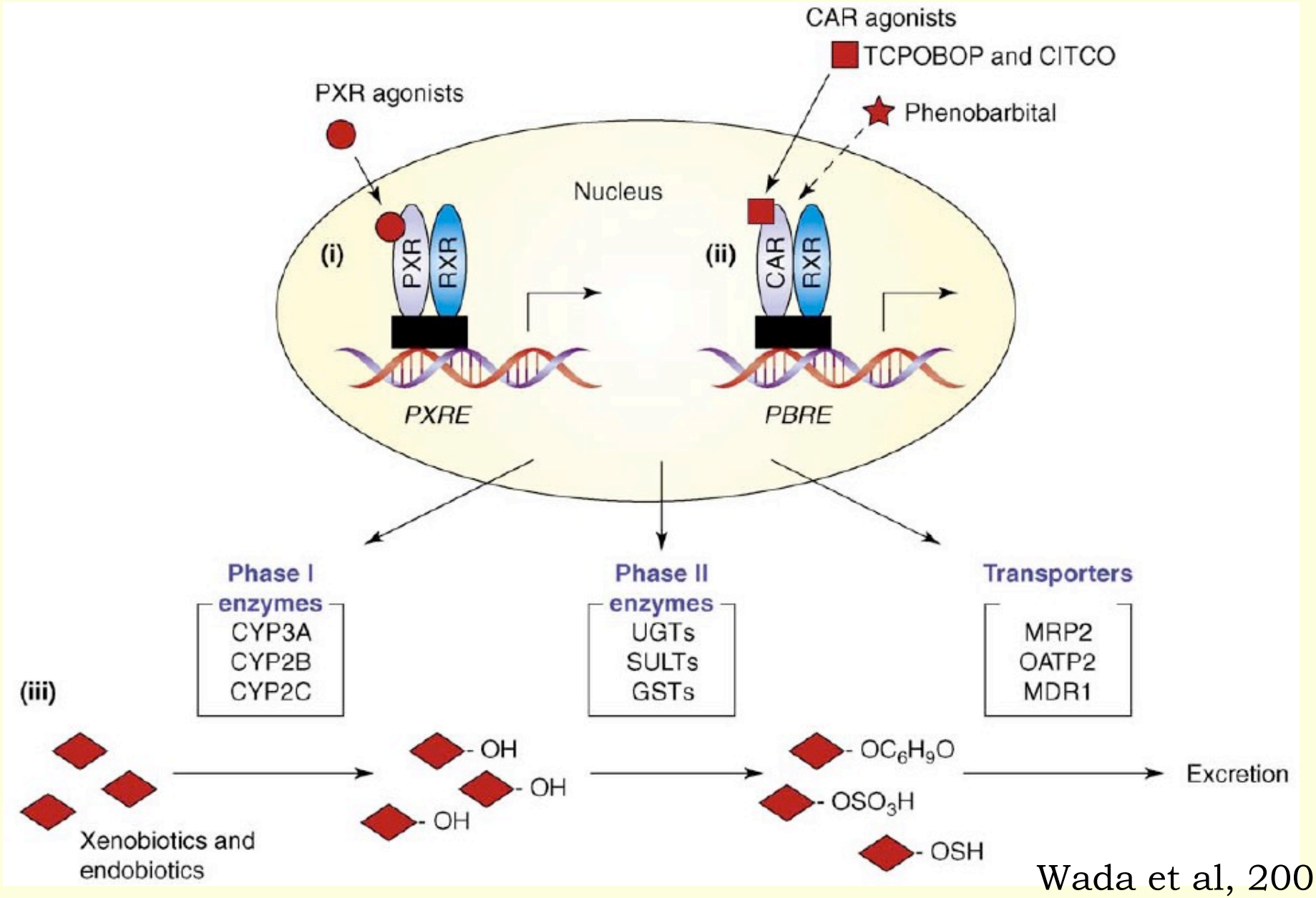



**Figure 1**

(**a**) Mean ($n$ = 8) plasma concentration (mean ± SD) time curves of orally administered digoxin (1 mg) before (open circles) and during (filled circles) coadministration of rifampin (600 mg). (**b**) Mean ($n$ = 8) plasma concentration (mean ± SD) time curves of intravenously administered digoxin (1 mg) before (open circles) and during (filled circles) coadministration of rifampin (600 mg).

# Induction mechanisms

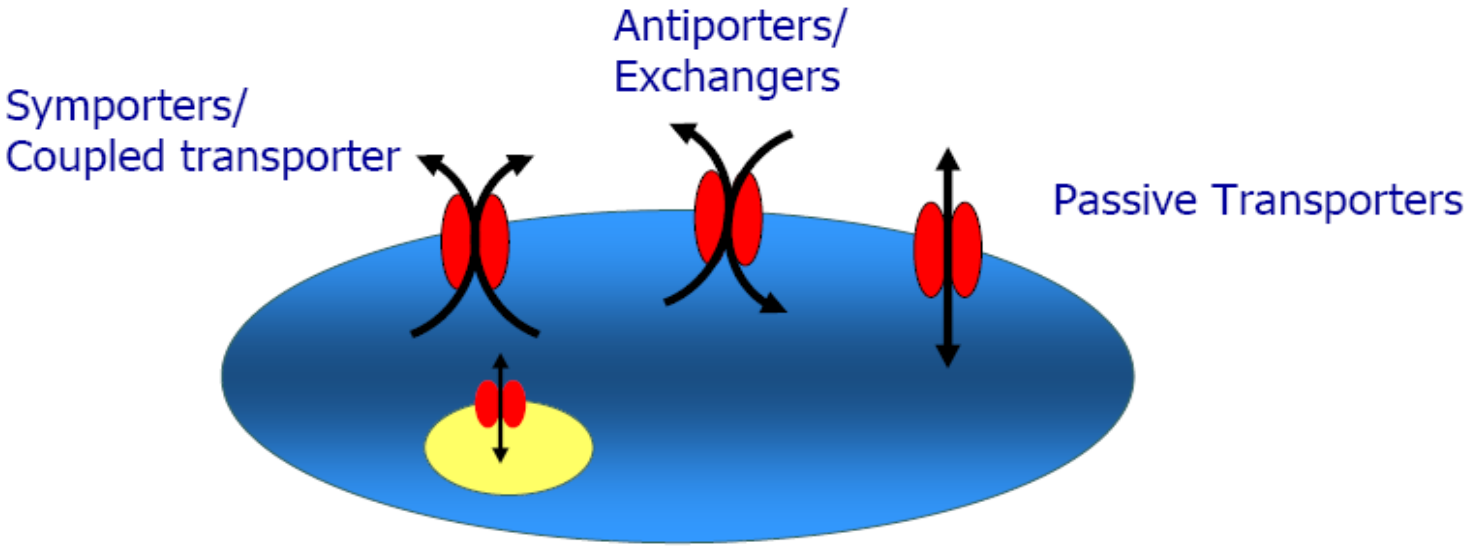
- same as for Phase I and Phase II enzymes




Wada et al, 2009

# SLC Transporters

- Solute carriers
  - Currently 43 gene families with approximately 298 transporter genes



# SLC Transporters: 43 Families, 298 Genes

SLC1: The high affinity glutamate and neutral amino acid transporters

SLC2: The facilitative GLUT transporters

SLC3: The heavy subunits of the heteromeric amino acid transporters

SLC4: The bicarbonate transporters

SLC5: The sodium glucose cotransporters

SLC6: The sodium- and chloride- dependent neurotransmitter transporters

SLC7: The cationic amino acid transporter/glycoprotein-associated

SLC8: The Na+/Ca2+ exchangers

SLC9: The Na+/H+ exchangers

SLC10: The sodium bile salt cotransporters

SLC11: The proton coupled metal ion transporters

SLC12: The electroneutral cation-Cl cotransporters

SLC13: The human Na+-sulfate/carboxylate cotransporters

SLC14: The urea transporters

SLC15: The proton oligopeptide cotransporters

SLC16: The monocarboxylate transporters

SLC17: The vesicular glutamate transporters

SLC18: The vesicular amine transporters

SLC19: The folate/thiamine transporters

SLC20: The type III Na+-phosphate cotransporters

SLC21/SLCO: The organic anion transportins

SLC22: The organic cation/anion/zwitterion transporters

SLC23: The Na+-dependent ascorbic acid transporters

SLC24: The Na+/(Ca2+-K+) exchangers

SLC25: The mitochondrial carriers

SLC26: The multifunctional anion exchangers

SLC27: The fatty acid transport proteins

SLC28: The Na+-coupled nucleoside transporters

SLC29: The facilitative nucleoside transporters

SLC30: The zinc efflux proteins

SLC31: The copper transporters

SLC32: The vesicular inhibitory amino acid transporter

SLC33: The Acety-CoA transporter

SLC34: The type II Na+-phosphate cotransporters

SLC35: The nucleoside-sugar transporters

SLC36: The proton-coupled amino acid transporters

SLC37: The sugar-phosphate/phosphate exchangers

SLC38: The System A & N, sodium-coupled neutral amino acid transporters

SLC39: The metal ion transporters

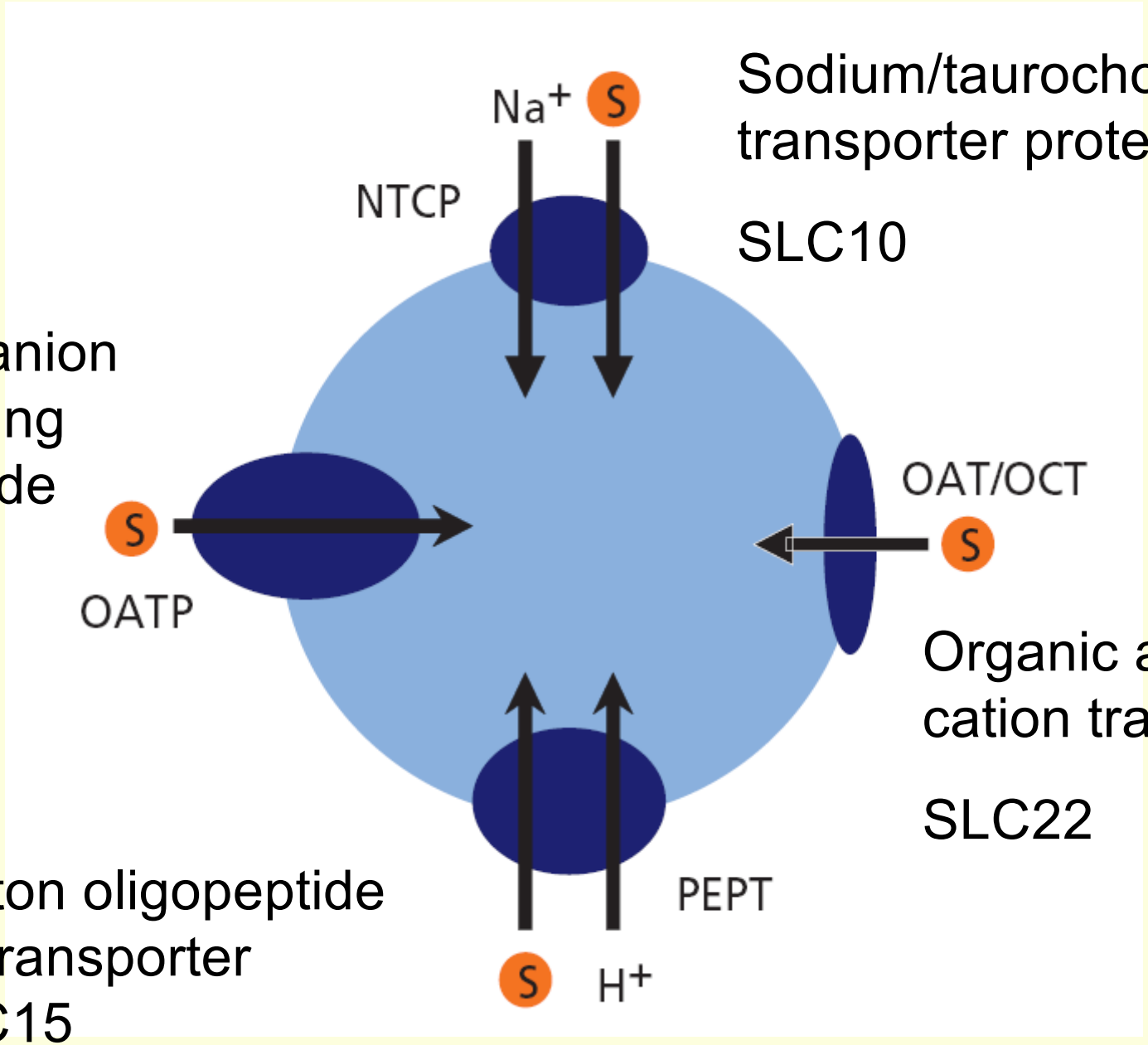
SLC40: The basolateral iron transporter

SLC41: The MgtE-like magnesium transporters

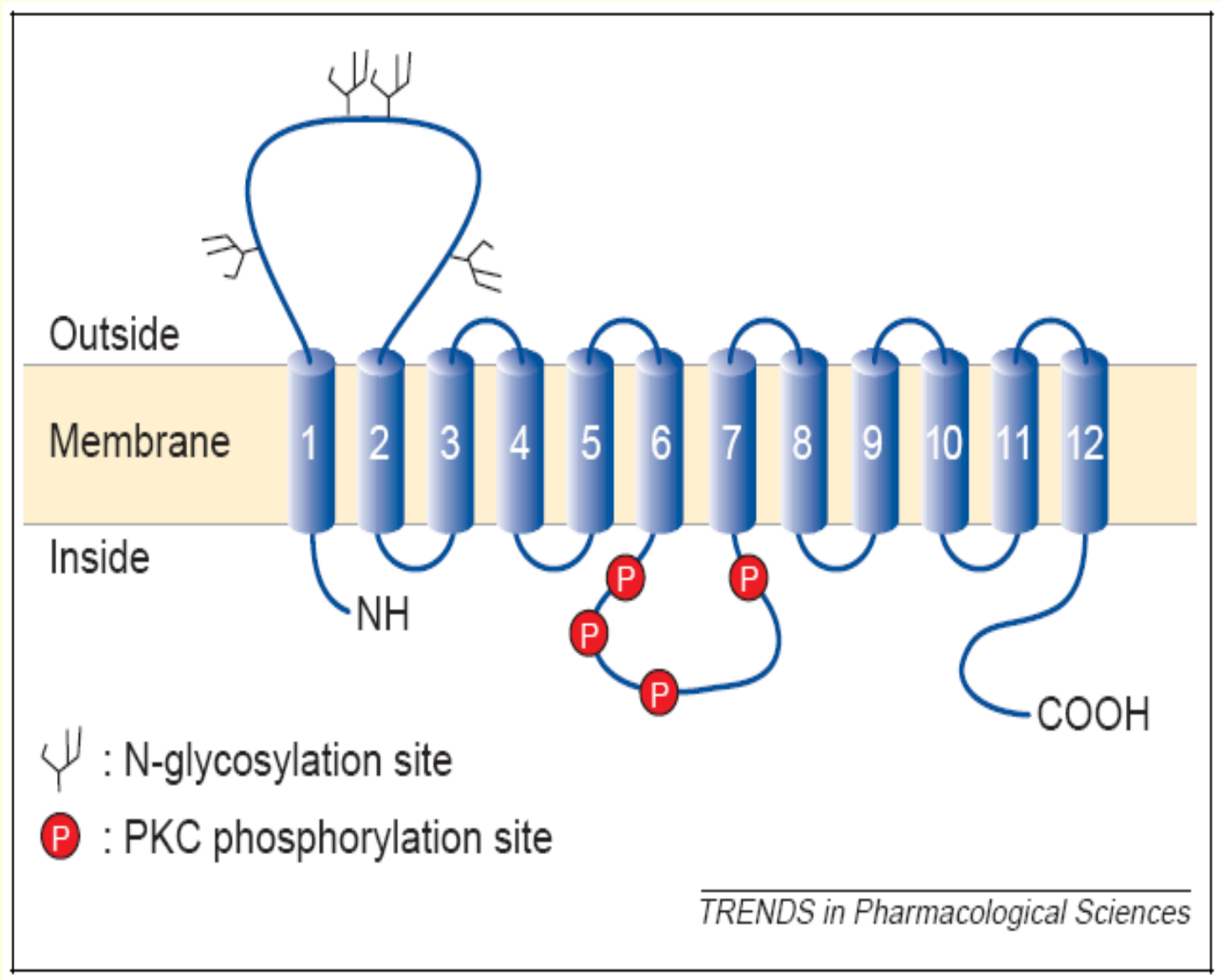
SLC42: The Rh ammonium transporters (pending) 3

SLC43: Na+-independent, system-L like amino acid transporters 3

# SLC's involved in drug transport



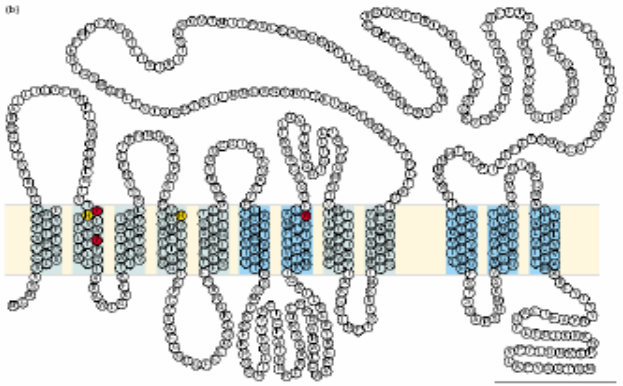
# Structure of SLC22A transporters (OAT, OCT, OCTN)



# OATP Substrates, Inhibitors and Inducers

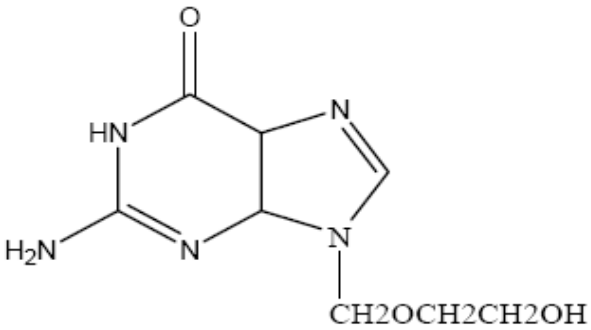
| Substrates | Substrates | Inhibitors |
|---|---|---|
| Taurocholate | Bilirubin | quinidine |
| cholate | mono/bi-glucuronosyl bilirubin | rifampicin |
| glycocholate | bromosulfophthalein | rifamycin |
| estradiol glucuronide | microcystin | digoxin |
| estrone sulphate | phalloidin | indocyanine green |
| prostaglandin E2 | CCK-8 | amiodarone |
| thromboxane B2 | deltorphin II | quinine |
| dihydroepiandosterone sulphate | digoxin | ibuprofen |
| LTC4 | ouabain | indomethacin |
| BQ-123 | rifampicin | verapamil |
| enkephalin | fexofenadine | cyclosporin A |
| benzylpenicillin | thyroid hormones | deoxycorticosterone |
| methotrexate | celiprolol | glibenclamide |
| pravastatin | | pravastatin |
| cerivastatin | | ritonavir |

# Peptide Transporters



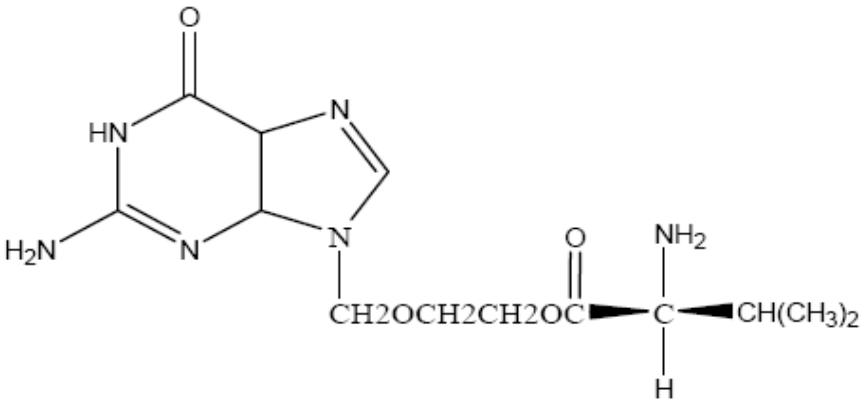
- PepT1 & PepT2 (SLC15 family)
- Transport di or tri peptides
  - Critical role in the absorption of protein digestion products
- Driving force: proton gradient
  - secondary active transporter
- Pharmacological role: mechanism for absorption of a variety of drugs
  - ß lactam antibiotics: Ampicillin, Amoxicillin, Cefadroxil, Cefadrine Cefixime, Ceftibuten Cefalexin
  - Angiotensin Converting Enzyme Inhibitors: Captopril, Enalapril, Fosinopril
  - Anticancer: Bestatin
  - Antiviral: Valacylovir, Valganciclovir,
  - Prodrugs: DOPA-amino-acids

# Peptide Transporter Can Be A Key Determinant of Bioavailability




Acyclovir
Bioavailability 10-20%

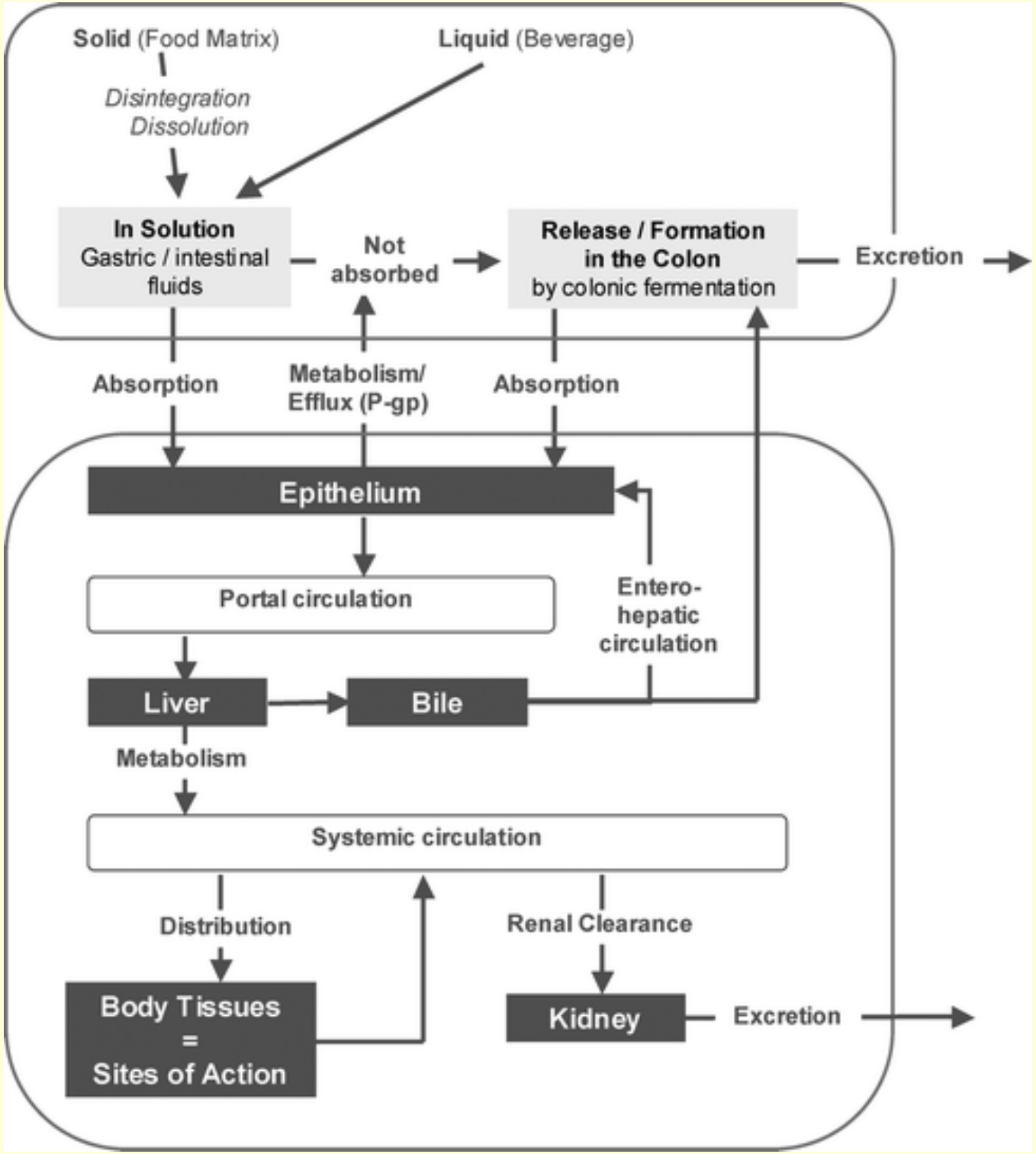
Not a substrate for Pept 1




Valacyclovir
Bioavailability 55%

Substrate for Pept 1

**R E M I N D E R**


Solid (Food Matrix)
Liquid (Beverage)
Disintegration
Dissolution
In Solution
Gastric / intestinal fluids
Not absorbed
Release / Formation in the Colon
by colonic fermentation
Excretion
Absorption
Metabolism/ Efflux (P-gp)
Absorption
Epithelium
Portal circulation
Entero-hepatic circulation
Liver
Bile
Metabolism
Systemic circulation
Distribution
Renal Clearance
Body Tissues = Sites of Action
Kidney
Excretion