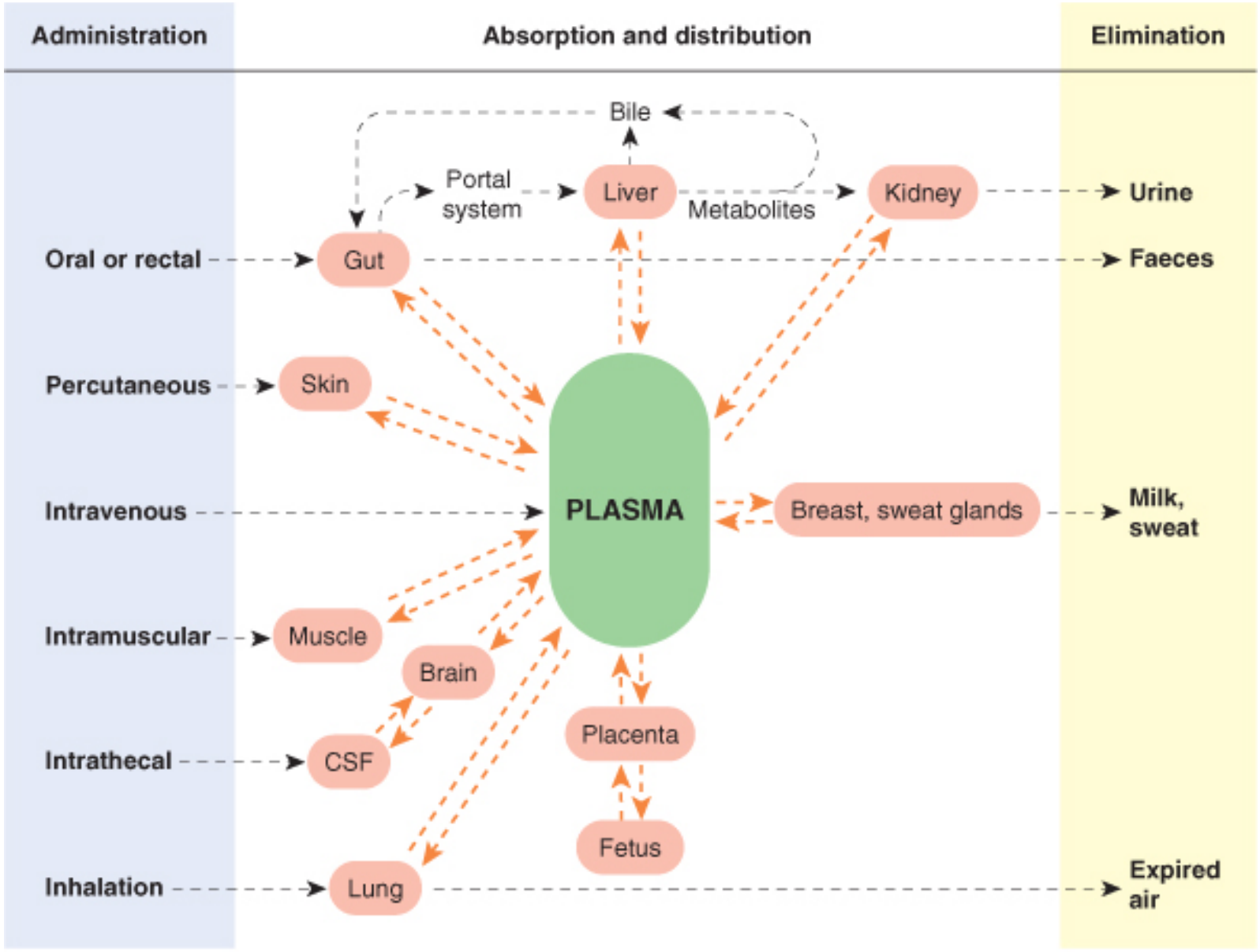
ADME

ADME

- Absorption
  - ➡ how do the drugs enter the body?
- Distribution
  - ➡ how are the drugs distributed in the body
- Metabolism
  - ➡ chemical modification of drugs (breakdown, increase of hydrophilicity to improve clearance)
- Excretion
  - ➡ how do the drugs leave the body

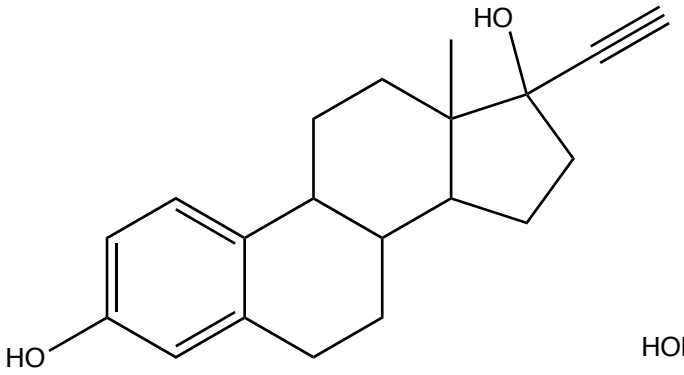


Drug formulation and uptake

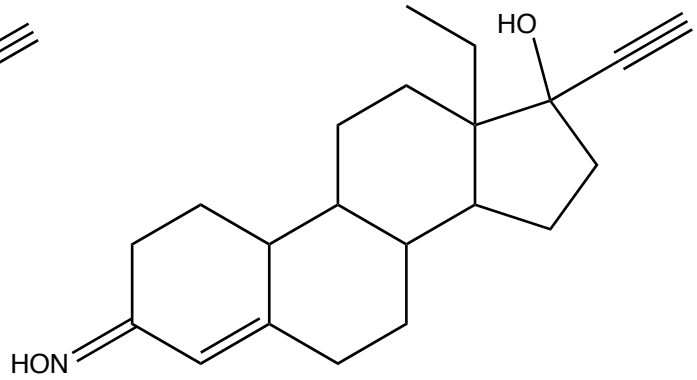
Routes of Drug Administration

- oral administration
- absorption through mucous membranes (mouth, or through the nose via spray)
- rectal (suppositories)
- topical (via skin, e.g. nicotine patches)
- inhalation (avoids digestive and metabolic enzymes of the GIT or the liver, anaesthetic gases, asthma drugs)
- injection: fast response, comparably easy dosing
  intravenous (fast), intramuscular, subcutaneous, intrathecal (into the spinal cord, compounds that do not pass the blood-brain barrier)

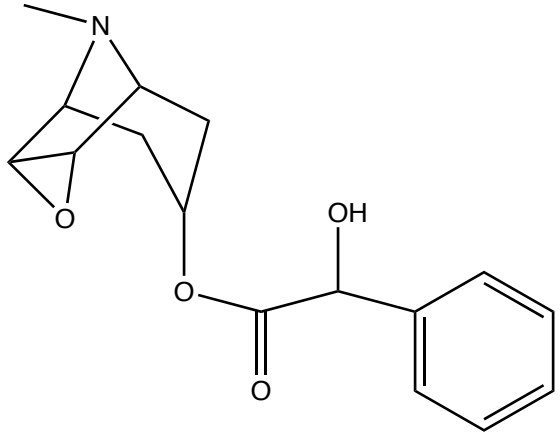
Drugs administered by a transdermal patch



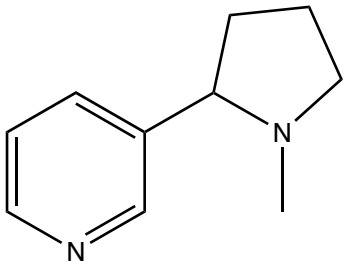
ethinyl estradiol
(contraceptive)



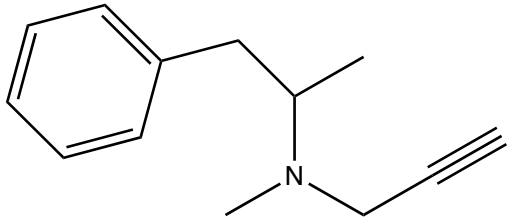
norelgestromin
(contraceptive)



scopolamine
(motion sickness)



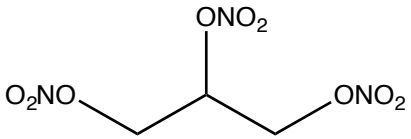
nicotine



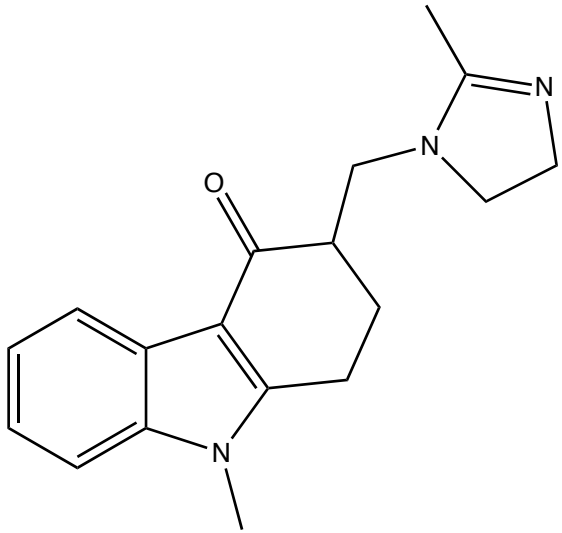
selegiline
(anti-depressant)

Sublingual Drugs

(kept under tongue)



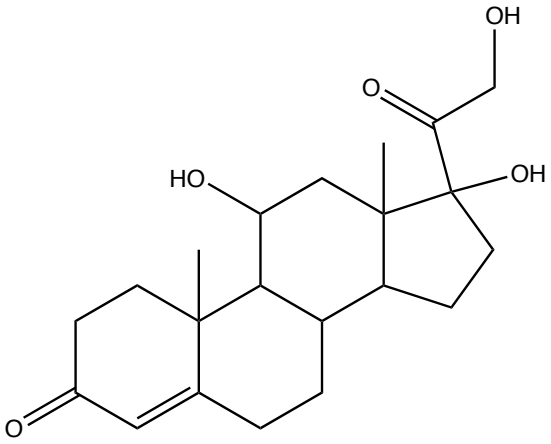
nitroglycerine
(angina)



ondansetron
(antinausea)

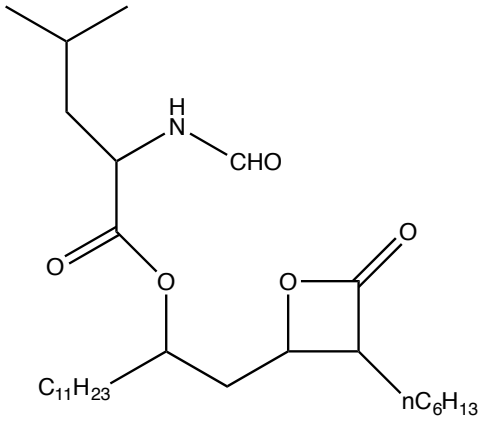
Topical Drugs

(directly applied at the place of action)



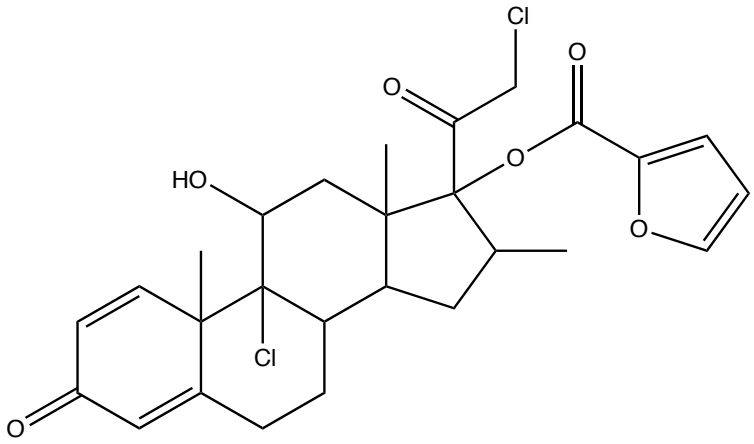
hydrocortison
(anti-inflammatory)

skin problems



orlistat
(weight loss)

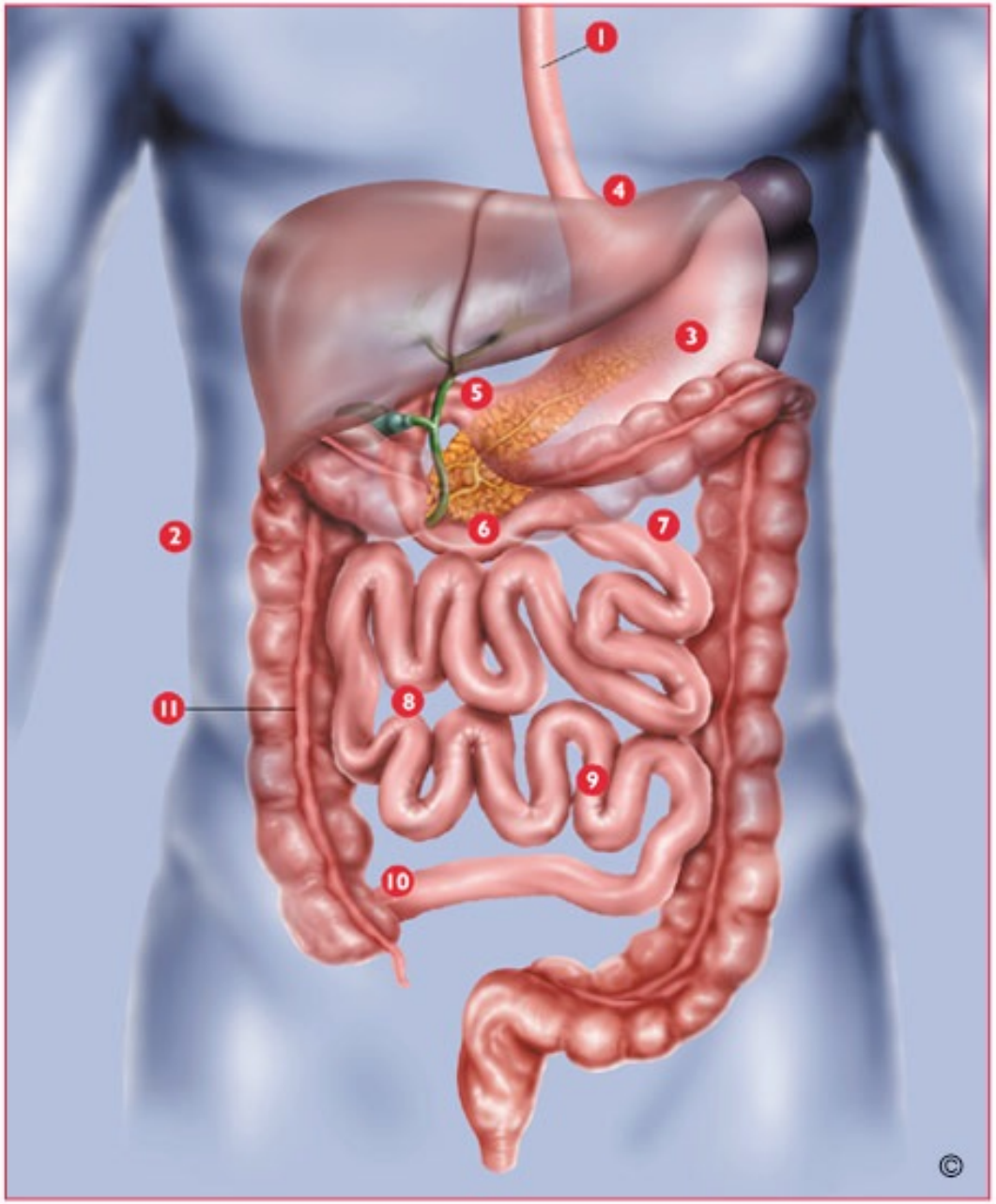
prevents pancreatic lipase to
hydrolyse triglycerides in
the intestine
oral pill that is not absorbed in the intestine



mometasone furoate
(seasonal allergies)

nasal spray

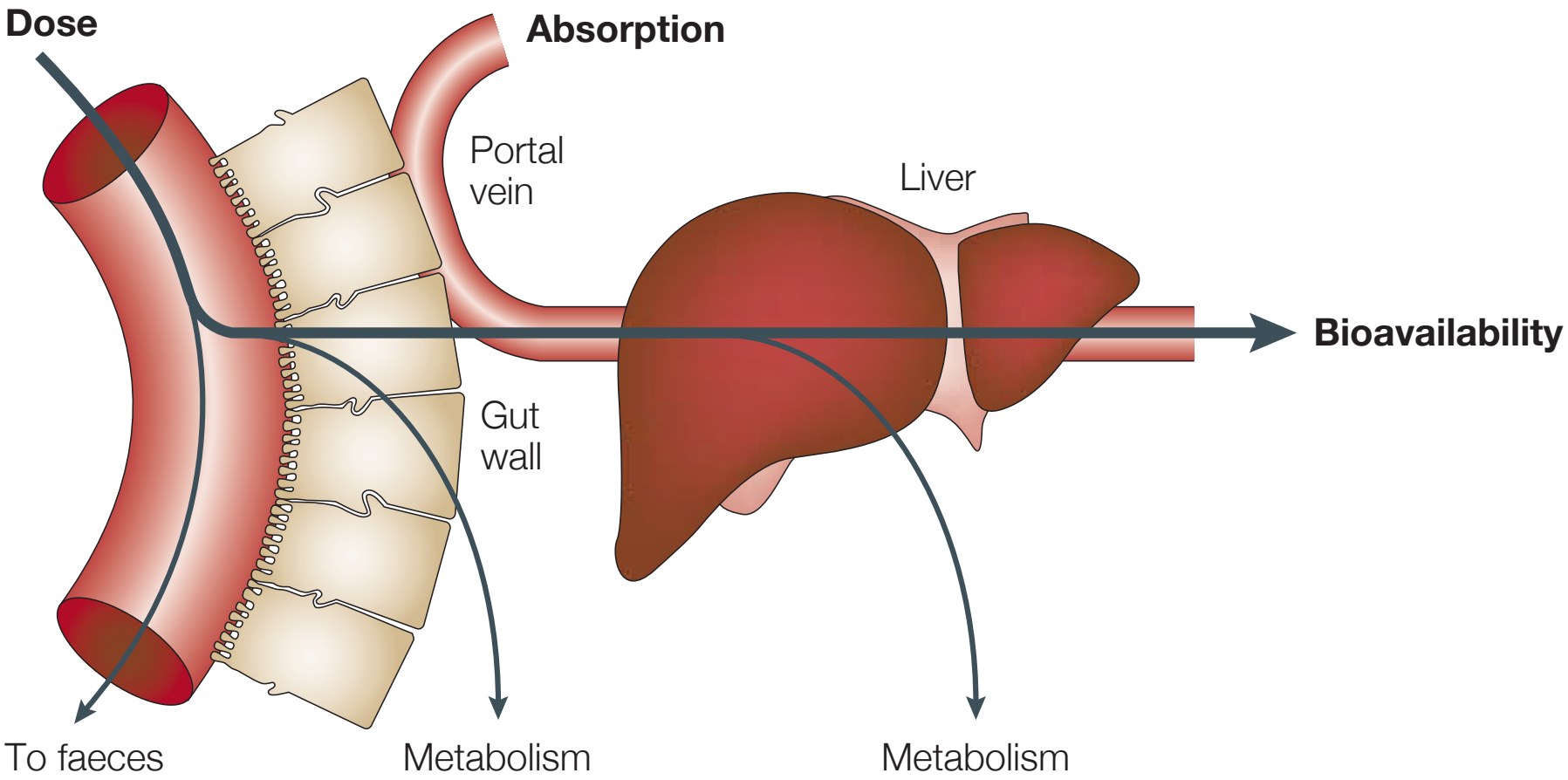
The gastointestinal tract



1. The esophagus is a long muscular tube, which moves food from the mouth to the stomach.
2. The abdomen contains all of the digestive organs.
3. The stomach, situated at the top of the abdomen, normally holds about 1500 ml of food from a single meal. Here the food is mixed with an acid that is produced to assist in digestion.
4. A valve at the entrance to the stomach from the esophagus allows the food to enter while keeping the acid-laden food from "refluxing" back into the esophagus, causing damage and pain.
5. The pylorus is a small round muscle and closes the stomach outlet while food is being digested into a smaller, more easily absorbed form.
6. The small intestine is about 4.5 to 6 meters and is where the majority of the absorption of the nutrients from food takes place. The small intestine is made up of three sections: the duodenum, the jejunum and the ileum.
7. The duodenum is the first section of the small intestine and is where the food is mixed with bile produced by the liver and with other juices from the pancreas.
8. The jejunum is the middle part of the small intestine; it is responsible for digestion.
9. The last segment of the intestine, the ileum, is where the absorption of fat-soluble vitamins A, D, E and K and other nutrients are absorbed.
10. Another valve separates the small and large intestines to keep bacteria-laden colon contents from coming back into the small intestine.
11. In the large intestines, excess fluids are absorbed and a firm stool is formed. The colon may absorb protein, when necessary.

from: http://www.genesishealth.com/services/bariatric_surgery/digestive_diagram.aspx

Bioavailability of Oral Drugs

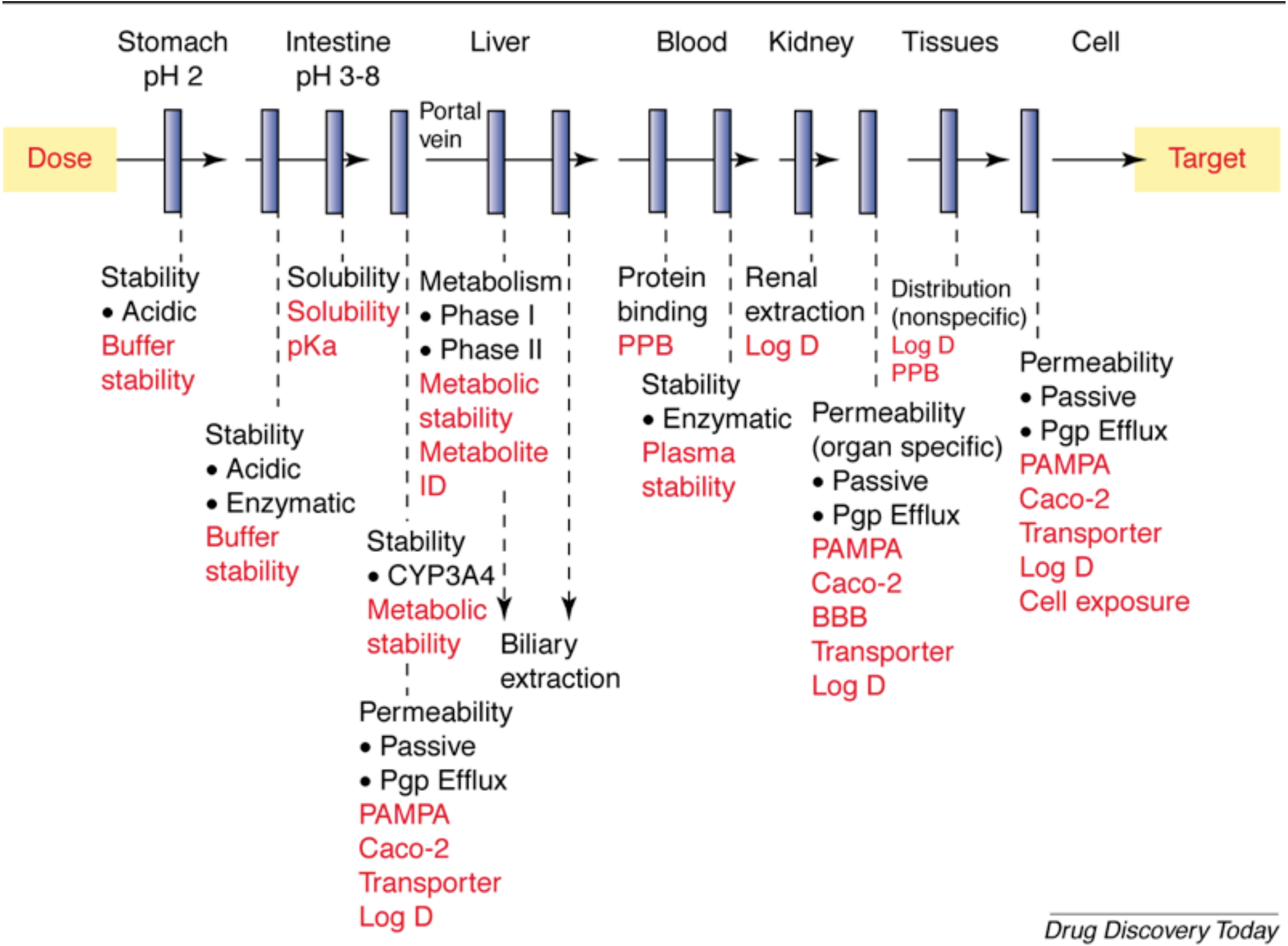


Blood:

46 % cells (45% erythrocytes)

54% plasma (46% water & electrolytes; 8 % proteins such as serum albumin, globulins, fibrinogen)

blood serum: residual liquid after whole blood is allowed to clot (or centrifugation)


Stomach
pH 2
Intestine
pH 3-8
Liver
Blood
Kidney
Tissues
Cell
Dose
Portal
vein
Target
Stability
• Acidic
Buffer
stability
Stability
• Acidic
• Enzymatic
Buffer
stability
Solubility
Solubility
pKa
Stability
• CYP3A4
Metabolic
stability
Metabolism
• Phase I
• Phase II
Metabolic
stability
Metabolite
ID
Biliary
extraction
Permeability
• Passive
• Pgp Efflux
PAMPA
Caco-2
Transporter
Log D
Protein
binding
PPB
Stability
• Enzymatic
Plasma
stability
Renal
extraction
Log D
Permeability
(organ specific)
• Passive
• Pgp Efflux
PAMPA
Caco-2
BBB
Transporter
Log D
Distribution
(nonspecific)
Log D
PPB
Permeability
• Passive
• Pgp Efflux
PAMPA
Caco-2
Transporter
Log D
Cell exposure

Body fluid compartments

- extracellular fluids
  - blood plasma (4.5% of b.w.)
  - interstitial fluid (16%)
  - lymph (1.2 %)
- intracellular fluids (30-40%)
- transcellular fluids

the concentration in the various compartments depends on the permeability across the tissue barriers, binding within the compartments, pH partitioning and fat:water partitioning

Volume of Distribution

V_d is a measure of the total volume of the body perfused by the drug (and therefore depends on where the drug accumulates and which compartments are accessible)

- The plasma volume is about 0.05L/kg b.w. All drugs that do not pass the capillary wall is confined to that volume

- The extracellular volume is about 0.2L/kg b.w. , and drugs that cannot pass cellular membranes are restricted to that volume

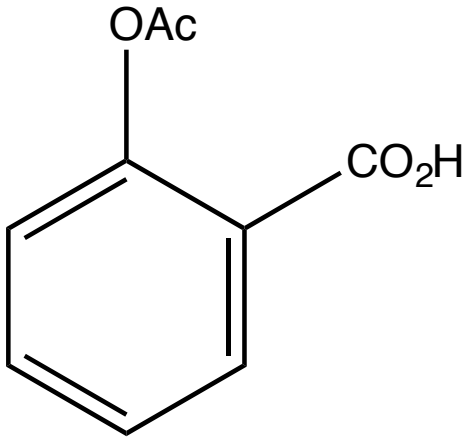
- The total body water is about 0.55L/kb b.w. It is the volume that is accessible to relatively lipid soluble drugs, that can pass the membranes. Partitioning into lipid will even increase V_d beyond total body water.

Drug Distribution

- bulk flow transfer (via cardiovascular system/blood stream).
- diffusional transfer, depends on lipophilicity (log P), molecular size.

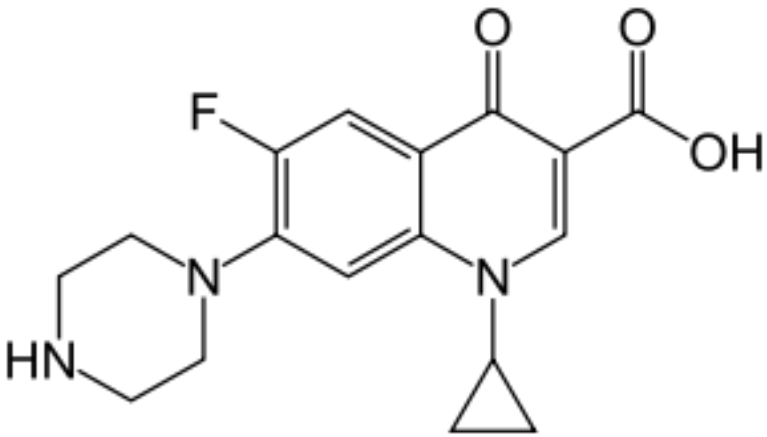
Questions:

Why is Aspirin absorbed in the stomach?

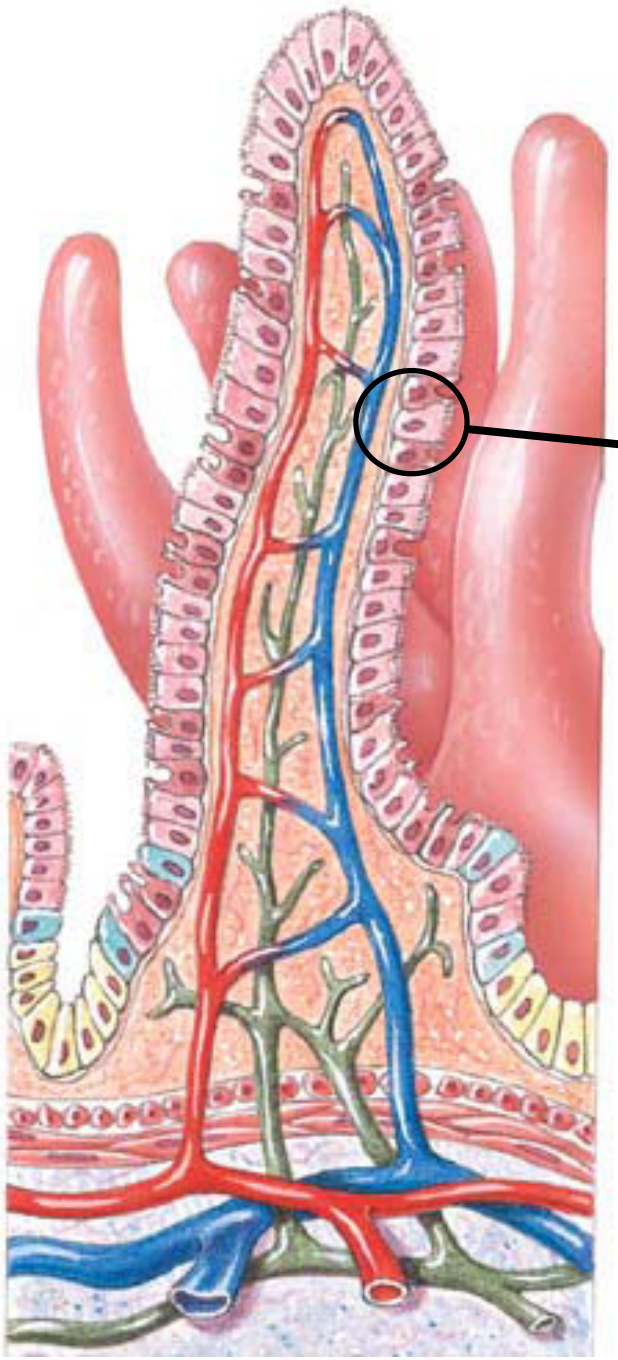


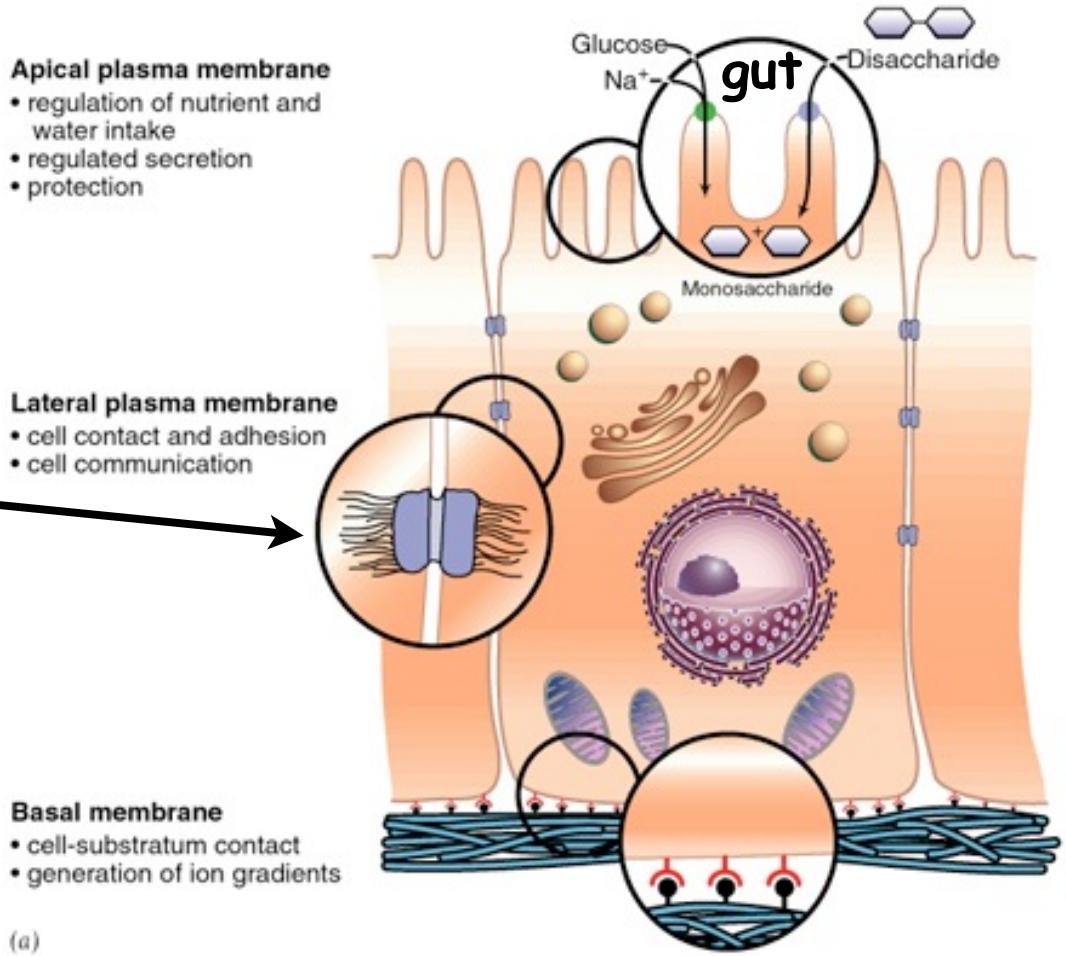
pKa=3.0

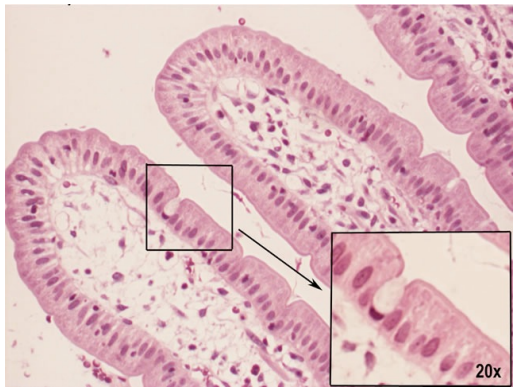
Why is Ciproflaxin NOT absorbed in the stomach?



Drug absorption in the duodenum

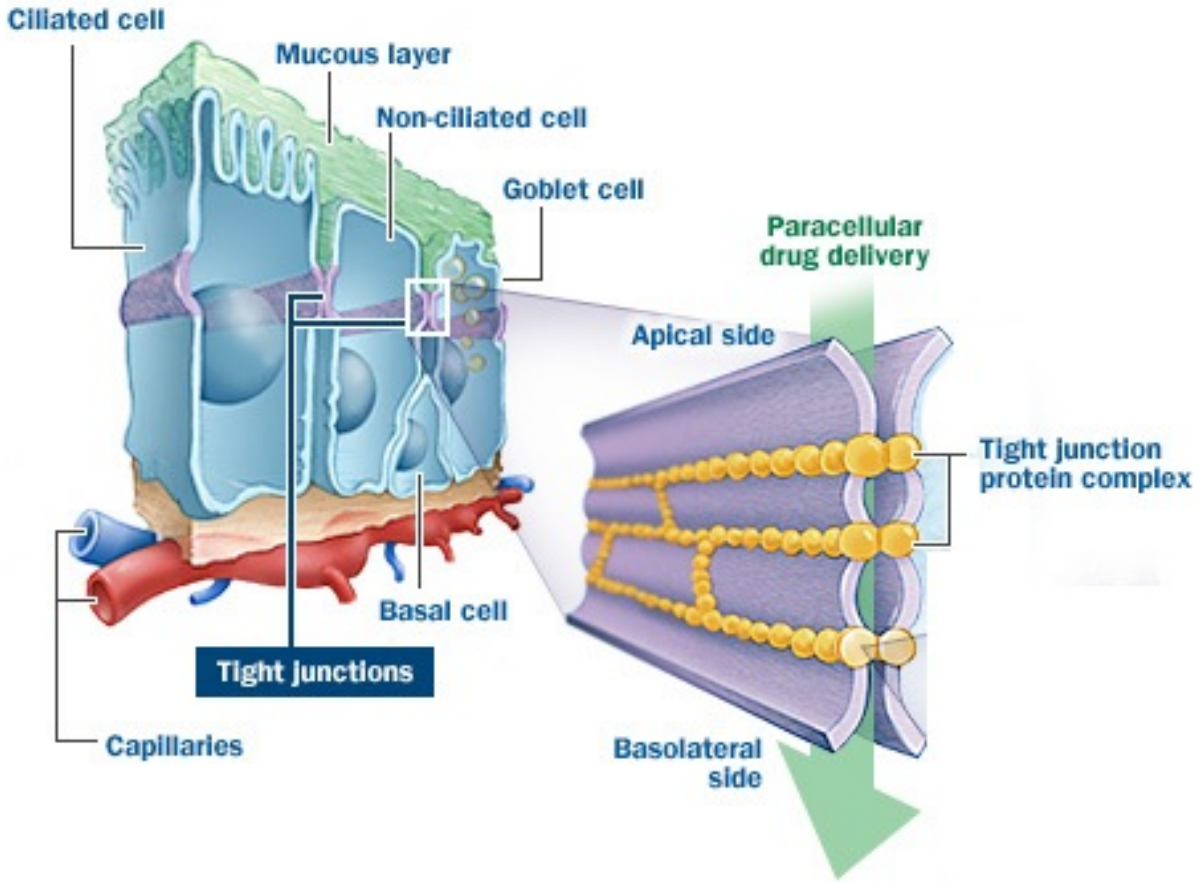






Epithelial Tissue

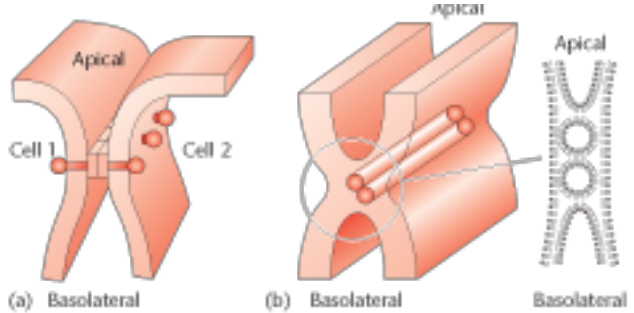
- Epithelial cell sheets are covering all cavities and free surfaces of the body (mouth, gut).
- Epithelial tissue is made of a monolayer of closely-packed cells arranged in flat sheets.
- The portion of the cell exposed to the lumen is called its apical surface. The rest of the cell (i.e., its sides and base) make up the basolateral surface.



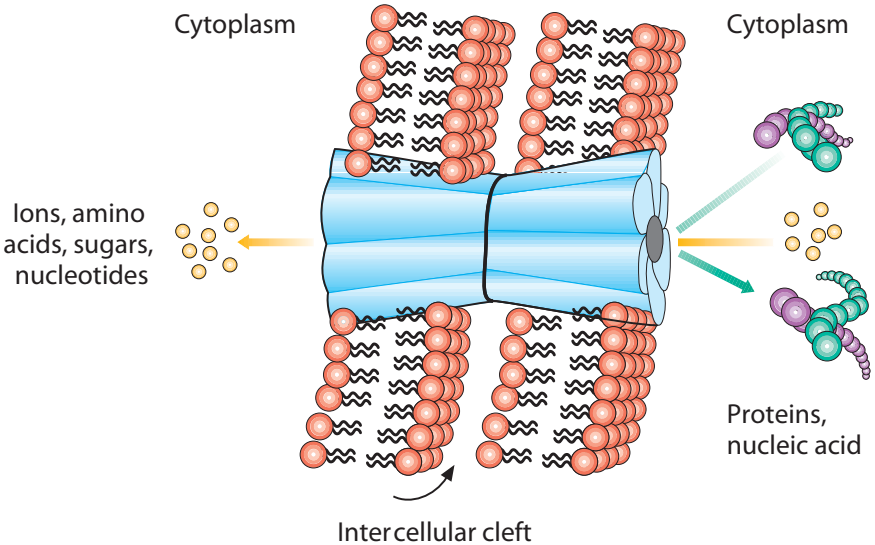
•nutrient and drugs diffuse into endothelial cells from the apical side (exposed to the gut) . They cannot pass the tight junctions. They can only pass via passive diffusion or with the help of transporters.

Epithelial Tissue (II)

- **Tight junctions** seal adjacent epithelial cells in a narrow band just beneath their apical surface. They prevent the passage of molecules and ions through the space between cells, so that materials must actually enter the cells (by diffusion or active transport) in order to pass through the tissue.

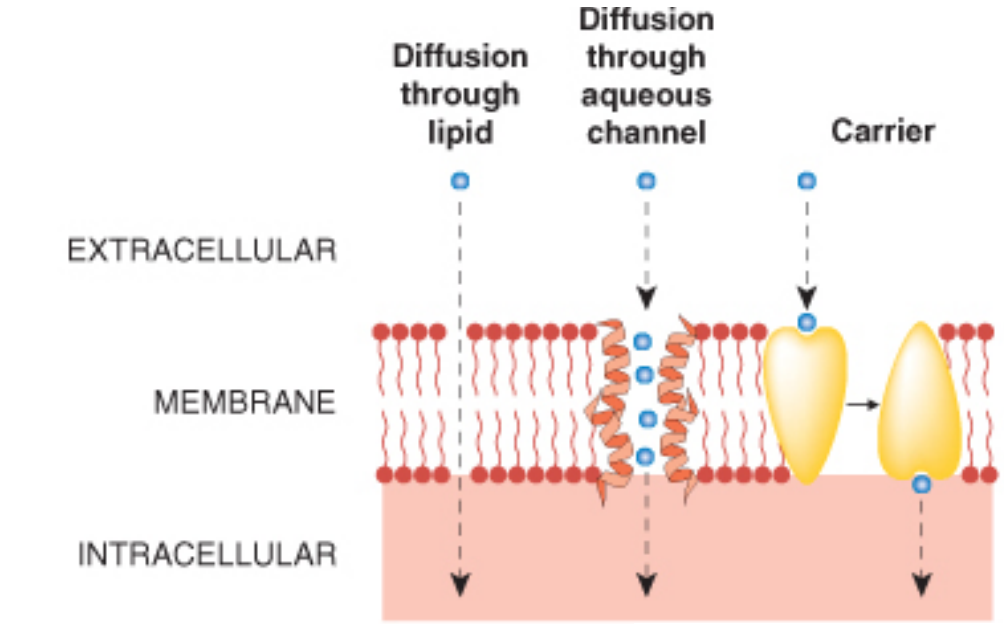


- Transport of molecules between endothelial cells might occur via **gap junctions**, formed by a group of transmembrane proteins called connexins.

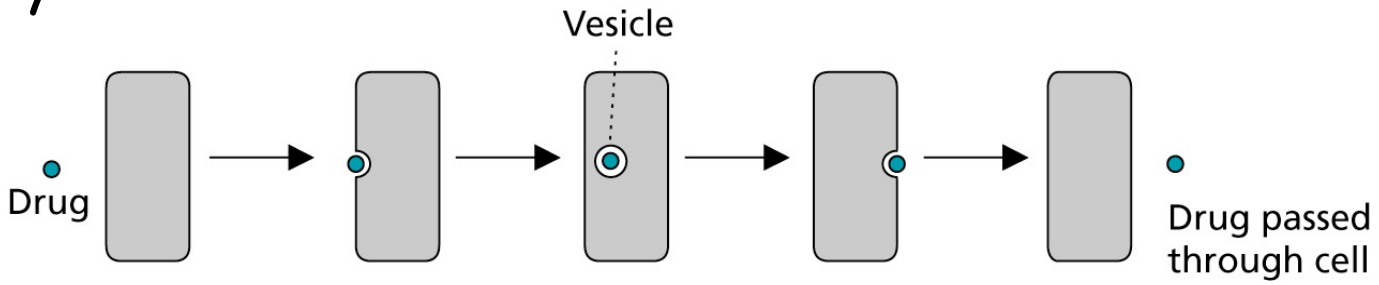


Transportation across membranes

- diffusion directly through the lipid
- diffusion through pores (aquaporins) formed by special proteins
- by help of a membrane carrier protein



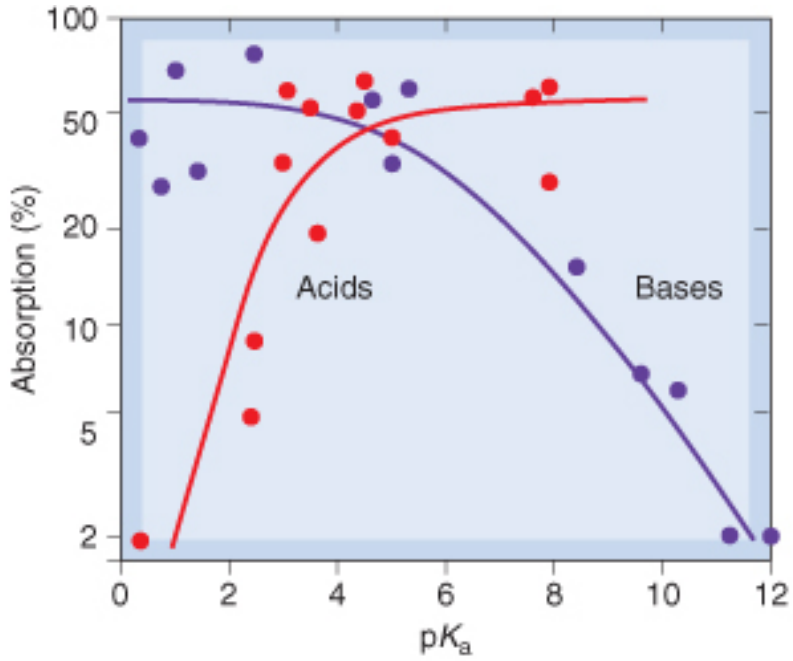
- by pinocytosis



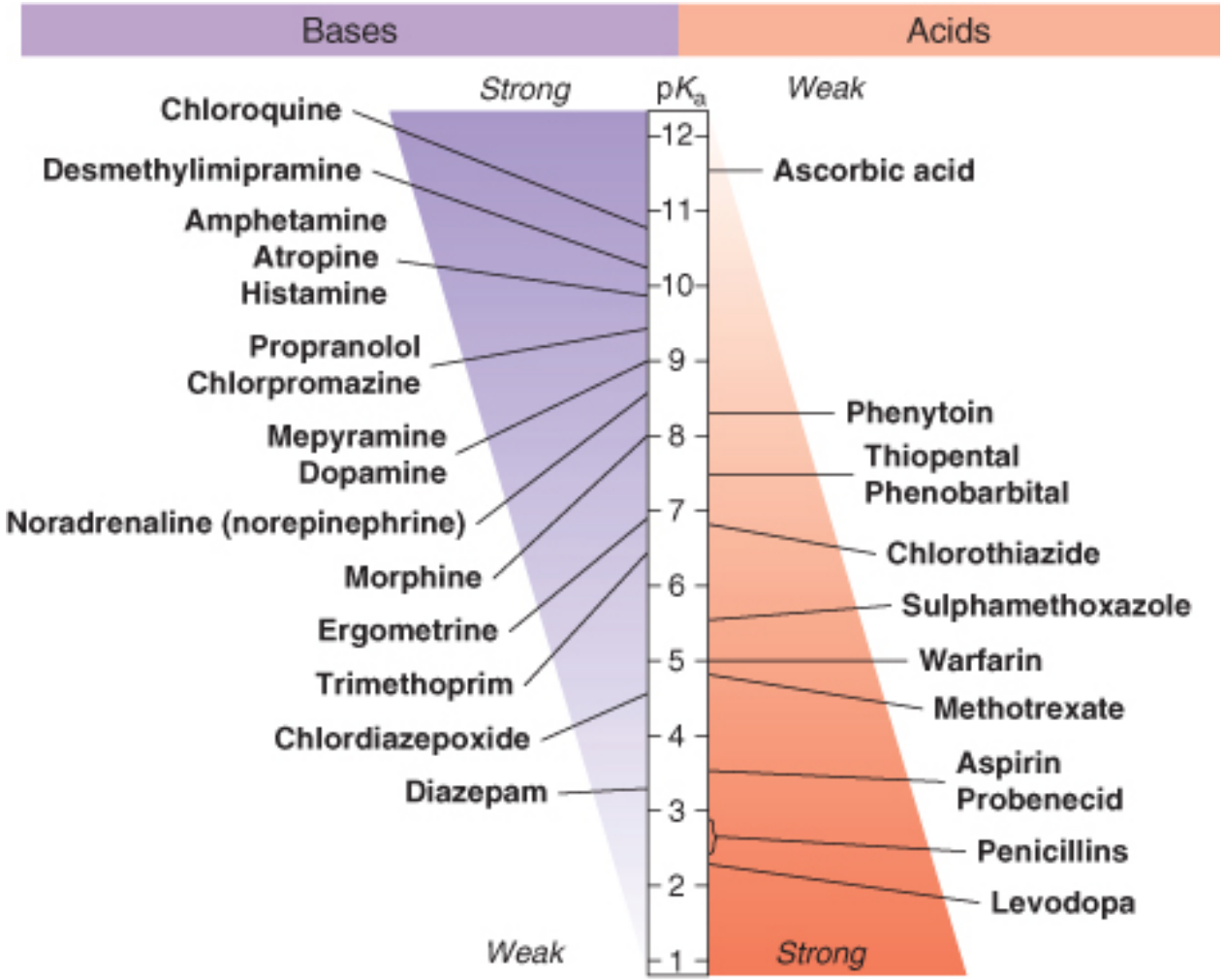
Movements across cellular barriers

- To traverse cellular barriers drugs have to cross lipid membranes
- they can do so by passive transfer or by carrier-mediated transfer
- the main factor influencing the passive diffusional rate is the drugs lipophilicity, much less important is its size
- from weak acids or basis only the non-charged form can pass through the membrane (pH partitioning)
- carrier-mediated transport is important for drugs chemically related to endogenous substances.

the rate of passive transport is determined by the **ionization** of the molecule and the lipid solubility. Strong bases or acids always remain ionized and hence are poorly absorbed, weak acids and bases are more likely to be uncharged and hence can more rapidly diffuse through the membranes:

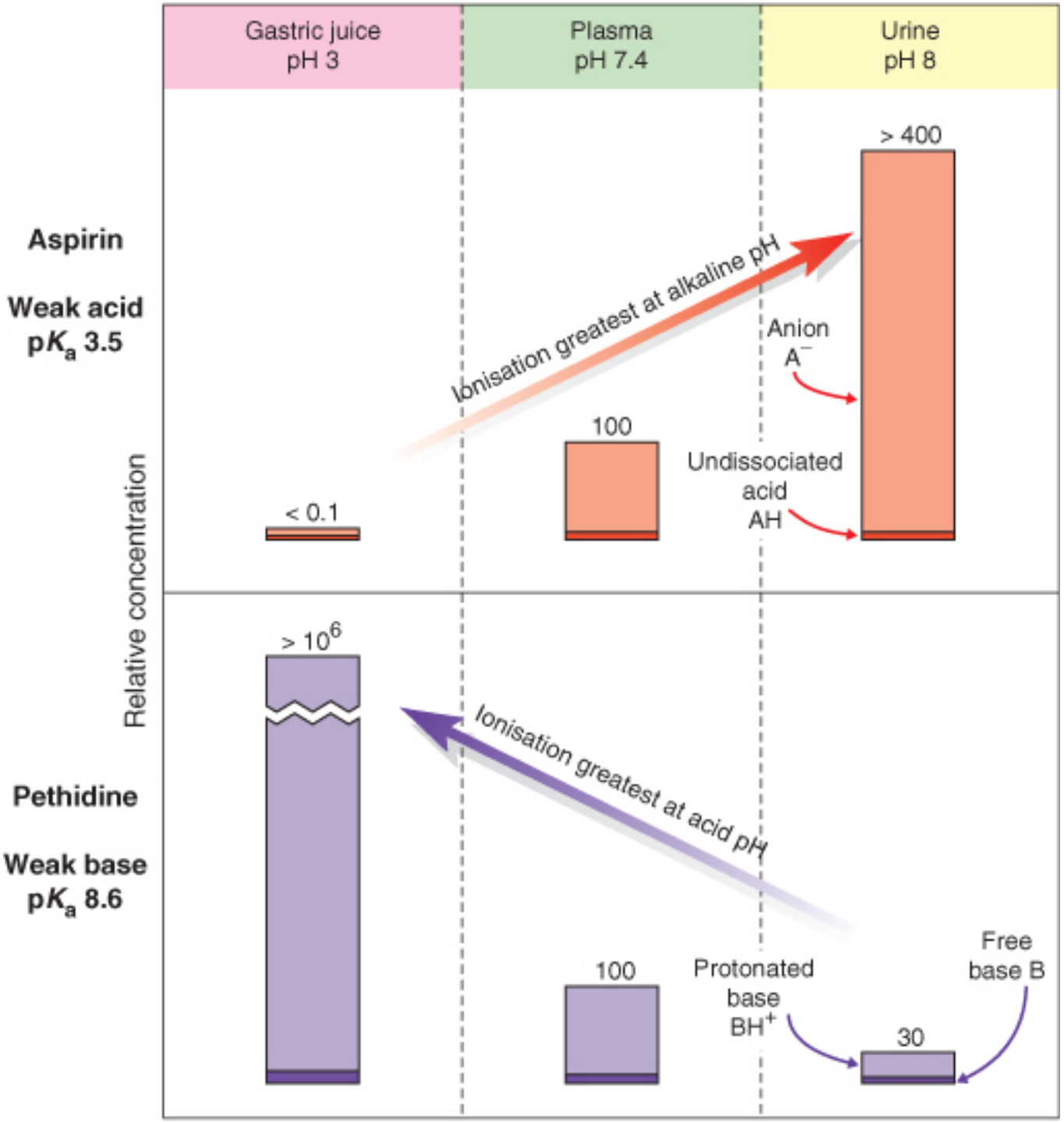


pH partitioning



pH in certain organs:

blood	7.4
Stomach	1-3
Duodenum	4.4-6.6
Jejenum	4.4-6.6
Illeum	6.8-8.0
Cebrospinal fluid:	7.4
Muscle	6.0
Urine	4.6-8.0



Blood-Brain Barrier

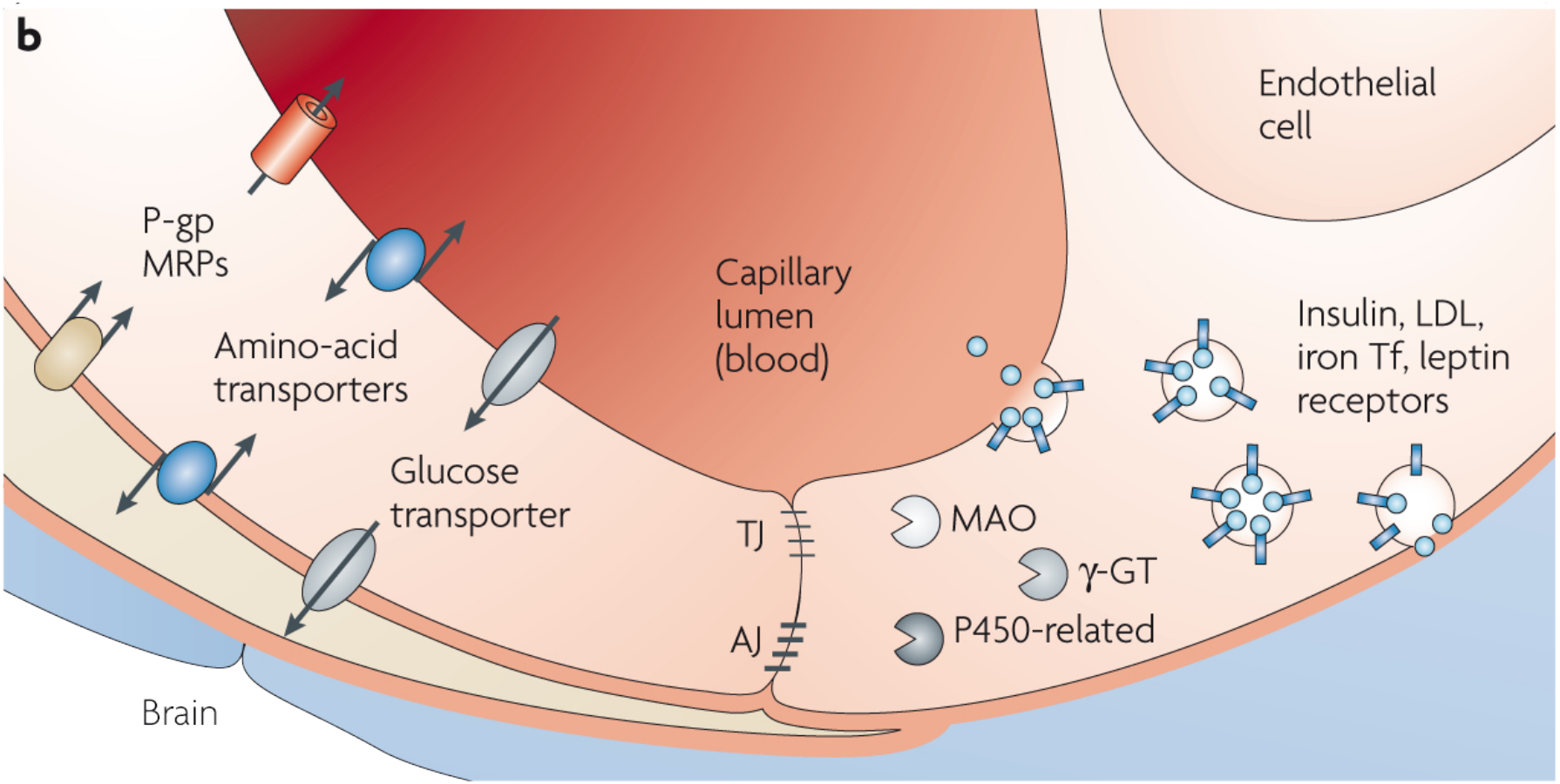
•a layer of endothelial cells joined by **tight junctions** and cell-cell adherens junctions. The tight junctions contain proteins such as occludin, claudins and the junctional adhesion molecules (JAMs).

•as a result **polar** substances do not penetrate the brain (e.g. anti-cancer drugs, antibiotics such as aminoglycosides).

•Inflammation disrupts the integrity of the blood-brain barrier.

•The BBB is also involved in neurodegenerative disorders such as Alzheimers disease and multiple sclerosis, stroke and traumatic brain injury, infectious processes and inflammatory pain.

Cecchelli et al., Nature Rev Drug Discov. **6** (2007), 650.



Schematic drawing of a cerebral endothelial cell, that form tight junction (TJ). Large molecules can also be transferred to the central compartment by receptor-mediated transcytosis, e.g. by receptors for low-density lipoprotein (LDL), iron transferrin (TF) and leptin. Other transporter are the P-gp (P-glycoprotein) or MRP (multidrug resistance-associated protein)

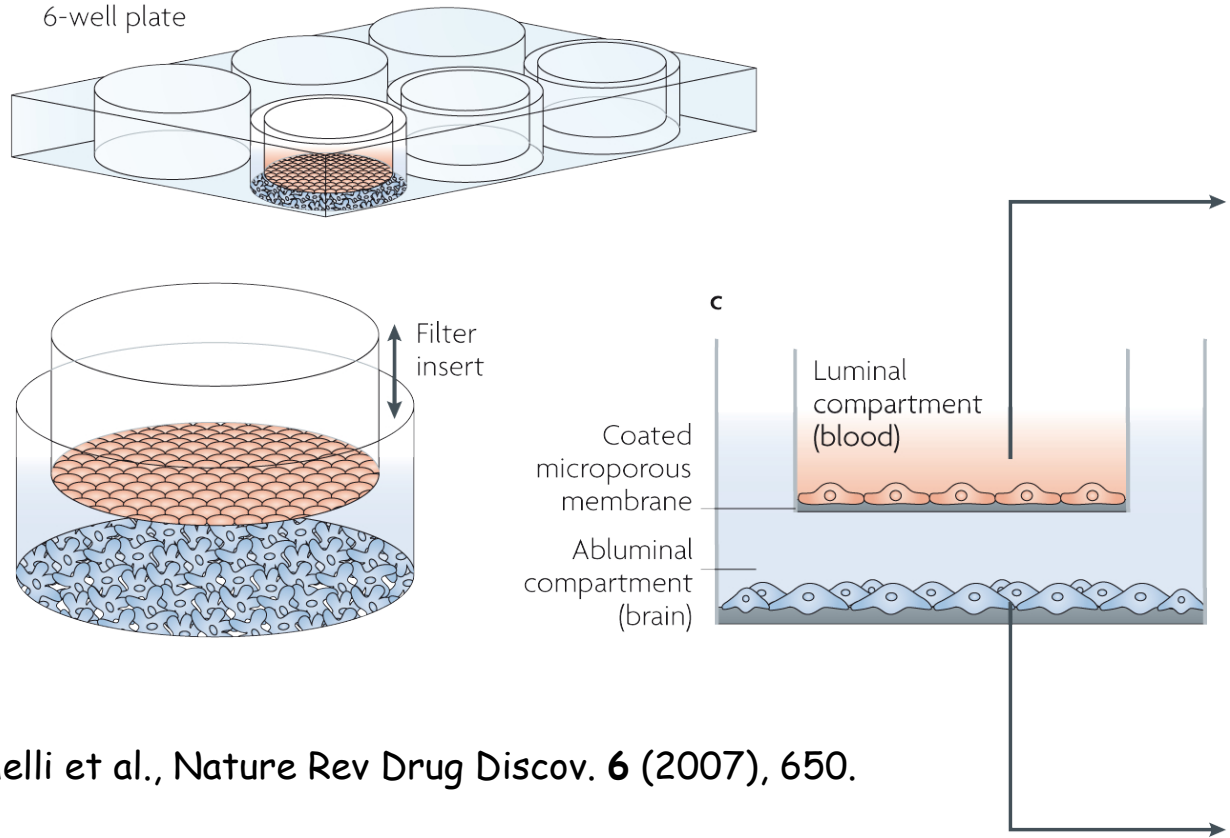
Cecchelli et al., Nature Rev Drug Discov. **6** (2007), 650.

BBB transporters

- amino acids
- glucose
- micronutrients
- electrolytes
- hormones and peptides
- eflux-pumps such as P-gp or MRP

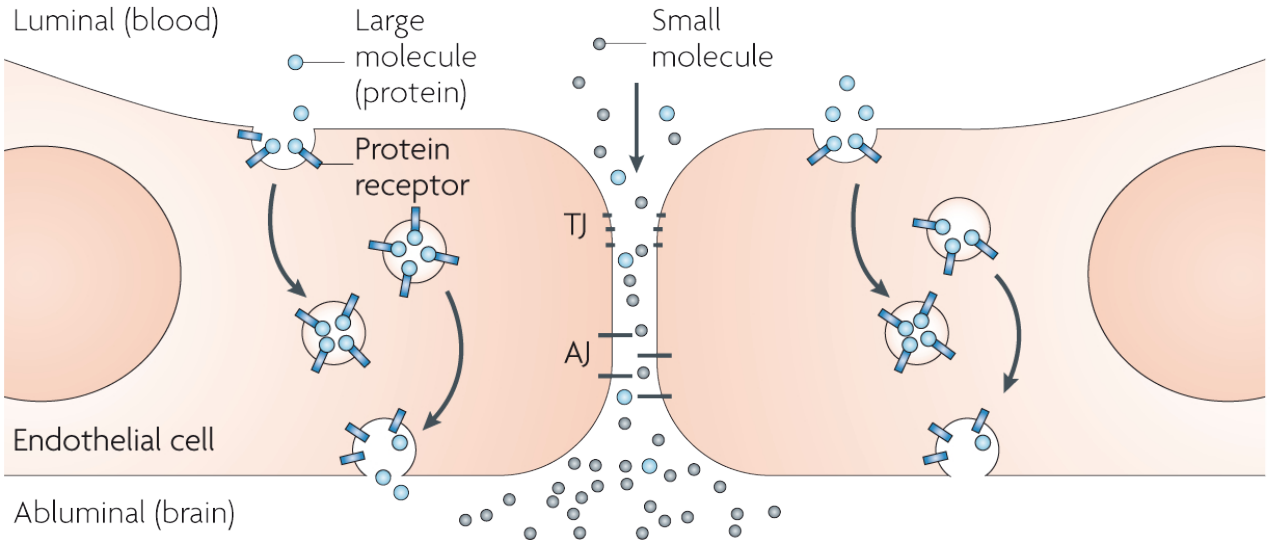
Test systems for BBB passage

- Isolated brain capillaries
- primary or low-passage brain capillary endothelial cell cultures
- immortalized endothelial cell lines
- cell lines of non-cerebral origin (Caco-2 (intestine), kindey (MDCK), carinome (ECV304)

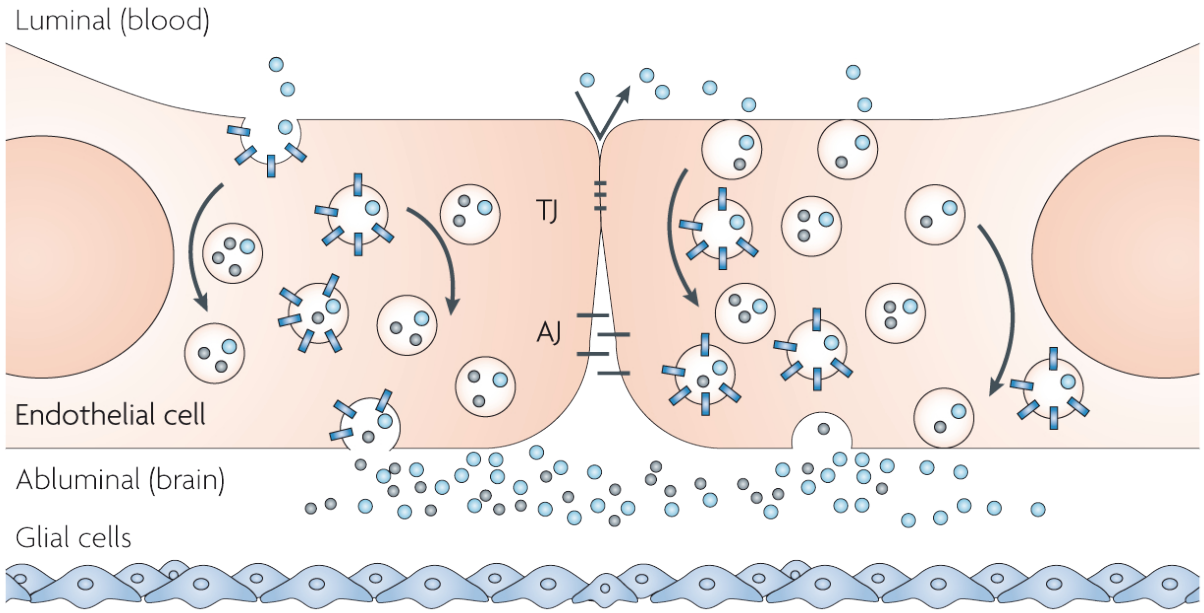


Cecchelli et al., Nature Rev Drug Discov. **6** (2007), 650.

Factors that compromise the BBB



- disrupted tight junctions



- up-regulation of non-specific transcytosis

Cecchelli et al., Nature Rev Drug Discov. **6** (2007), 650.

Carrier-mediated transport

- in the renal tubule
- in the biliary tract
- across the blood-brain barrier
- in the gastrointestinal tract

Example: **P-glycoprotein** (P-pg, a drug transporter believed to be responsible for multidrug resistance). The physiological role of P glycoprotein is to protect cells against environmental toxins. It acts as a "hydrophobic vacuum cleaner". It acts as an efflux pump, that alters pharmacokinetics of drugs and is involved in multidrug resistance. The expression of P-gp occurs on fully differentiated cells such that bidirectional transport is asymmetric.

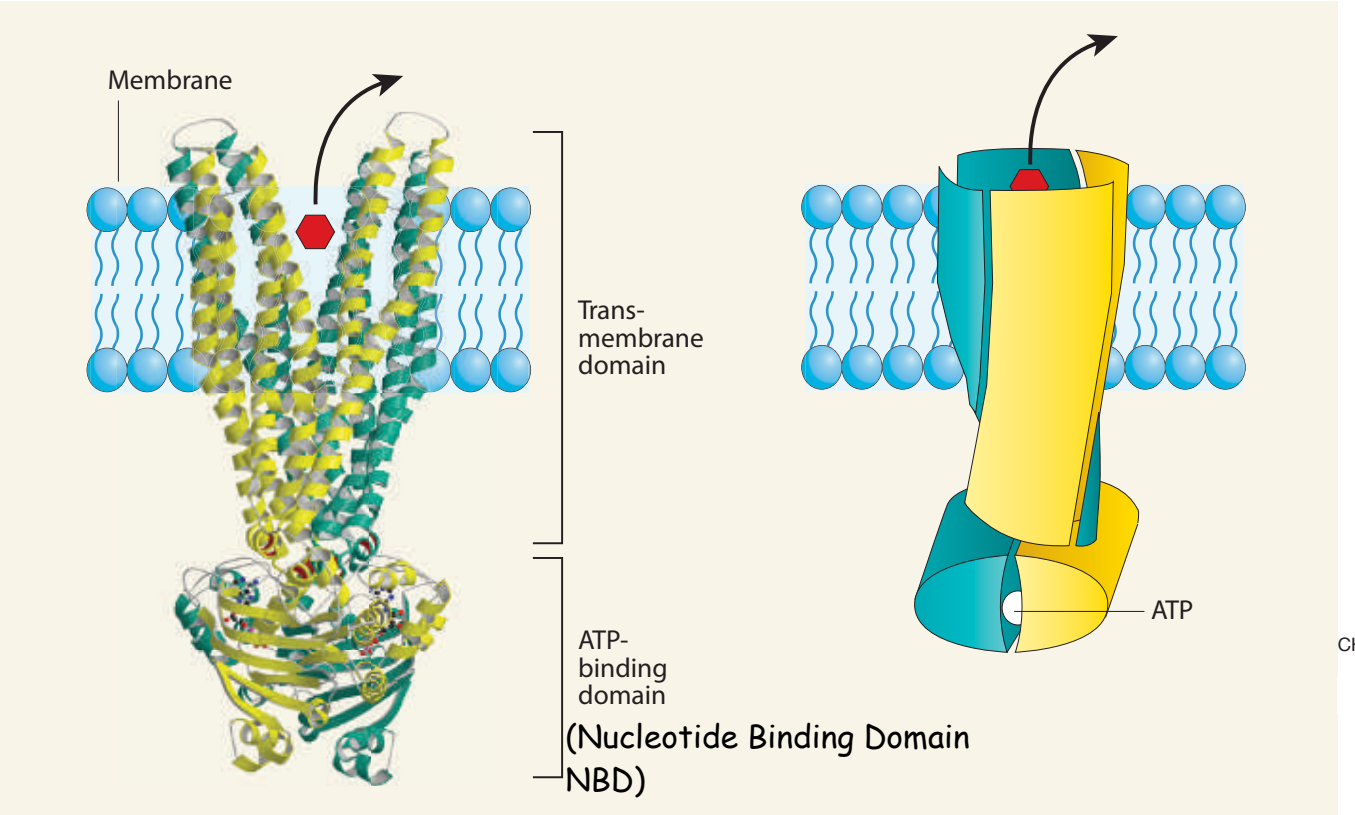
P-glycoprotein (P-gp)

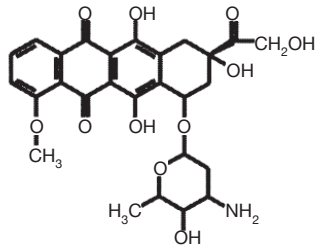
- expression levels are tissue-dependent, high in epithelial cells in the intestinal tract, levels of P-gp increase along the intestine
- it limits drug entry into systemic circulation from the intestine, pumps out hepatocytic drugs into the canalicular system, prevents distribution into vital organs and limits reabsorption into systemic circulation from renal tubules
- HIV protease inhibitor such as saquinavir or anti-cancer drugs like paclitaxel are substrates for P-gp. Bioavailability of paclitaxel increased from 11% to 35% in P-gp knock-out mice
- transport for molecules with good passive transport through membranes by P-gp is limited. Therefore, molecules not following Lipinki's rule of five are usually better substrates for P-gp.
- to reduce P-gp mediated efflux passive transport must be improved
- design of **inhibitors of P-gp** could greatly increase the therapeutic success. First successful candidates have been found. In addition, food/herbal extracts (e.g. from grapefruit juice, orange juice of St John's wort) contain P-gp inhibitors.

Substrates for P-gp

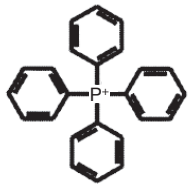
- anthracyclines (daunorubicin and doxorubicin)
- vinca alkaloids (vincristine and vinblastine)
- taxanes (paclitaxel and docetaxel)
- immunosuppressive agents (e.g. cyclosporin A)
- protease inhibitors (indinavir and saquinavir)
- antibiotics (e.g. erythromycin and actinomycin D)
- cardiac glycosides (e.g. digoxin)

The Structure of ABC Transporters

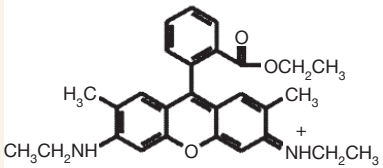




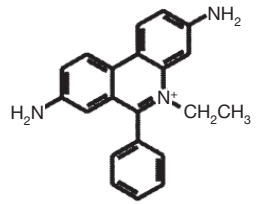
Doxorubicin



Tetraphenylphosphonium



Rhodamine 6G



Ethidium

Locher,
Nature **443** (2006), 180

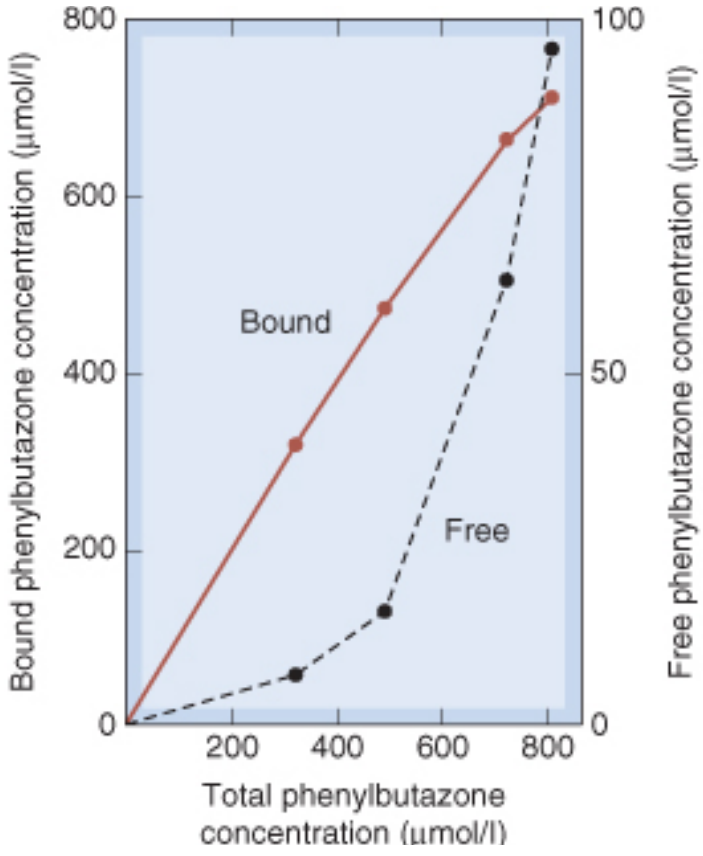
Schuldiner,
Nature **443** (2006), 156

- 12 TM membrane dimeric protein called **A**TP-**B**inding **C**assette (*ABC*) transporters
- consists of two transmembrane domains that provide a translocation pathway and two cytoplasmic nucleotide-binding domains (NBDs) that hydrolyze ATP or utilize the transmembrane proton gradients
- substrate-binding site is accessible from the cell interior

Binding to plasma proteins

(albumin, β-globulin, acid glycoprotein...)

- **albumin** binds **acidic drugs**, concentration is about 40 g/L!
  Its capacity corresponds to drug binding of 1.2mmol/L. Most drug concentrations in the blood are lower....
  Increasing the concentration above saturation may dramatically increase the concentration of free and active drug in a non-predictive manner! It also slows down drug elimination.
  Addition of drugs with better albumin binding affinity could replace other, bound drugs!



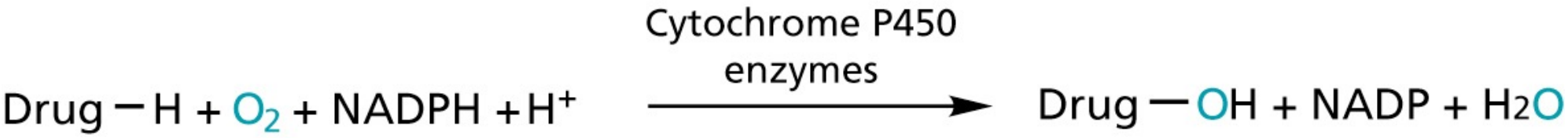
Rang, Pharmacology

Partitioning into body fat and other tissues

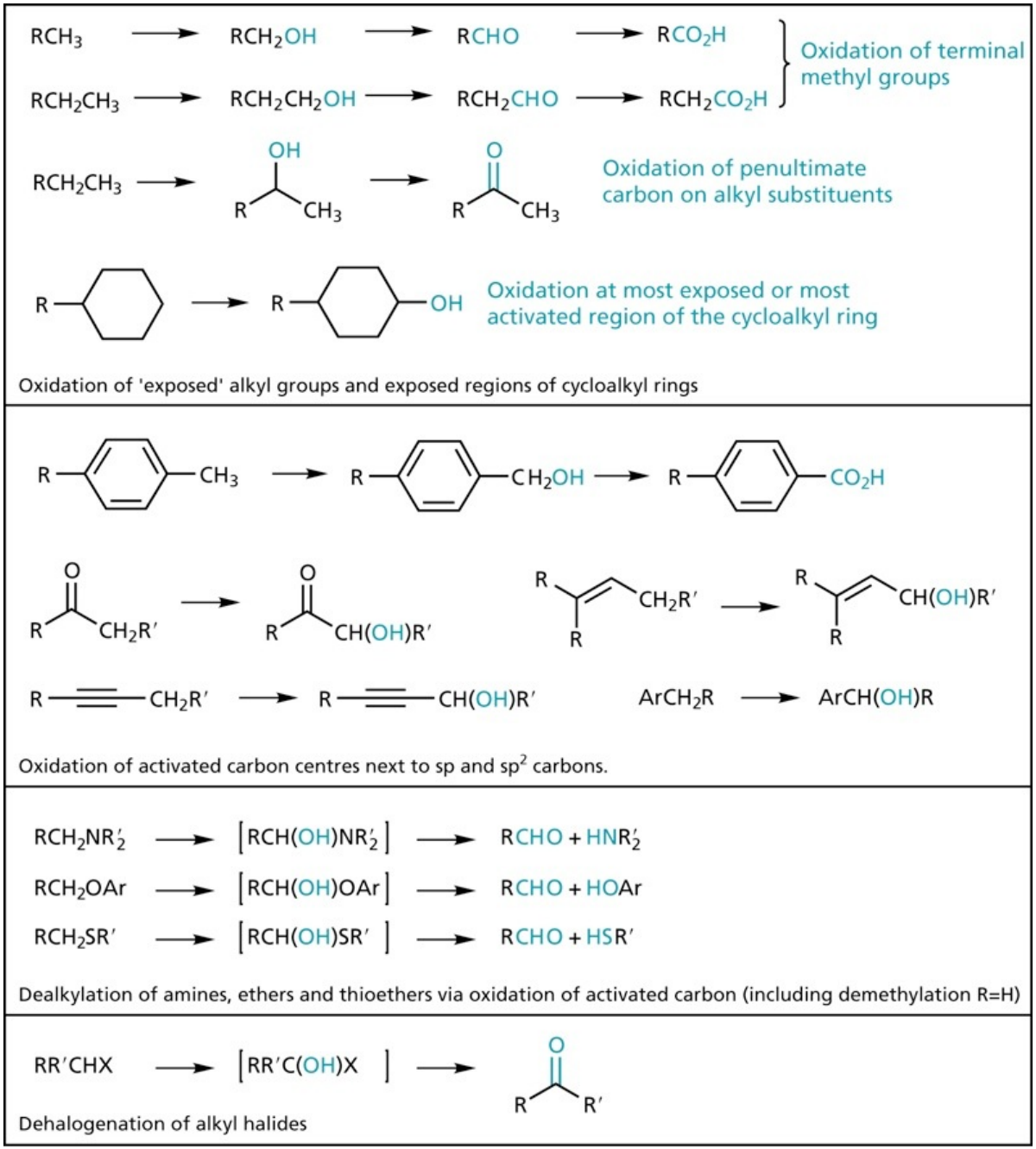
- depending on the fat-water partitioning coefficient (octanol partitioning) drugs may be more or less soluble in the body fat
- because blood supply to fat tissue is small, accumulation in the body fat is slow, delivery is therefore only fast for very lipid-soluble molecules (e.g. anaesthetics)
- highly lipophilic drugs tend to accumulate in the body fat over longer periods, and becomes a problem when the drugs are taken chronically..

Metabolic Phases: Phase I (first pass)

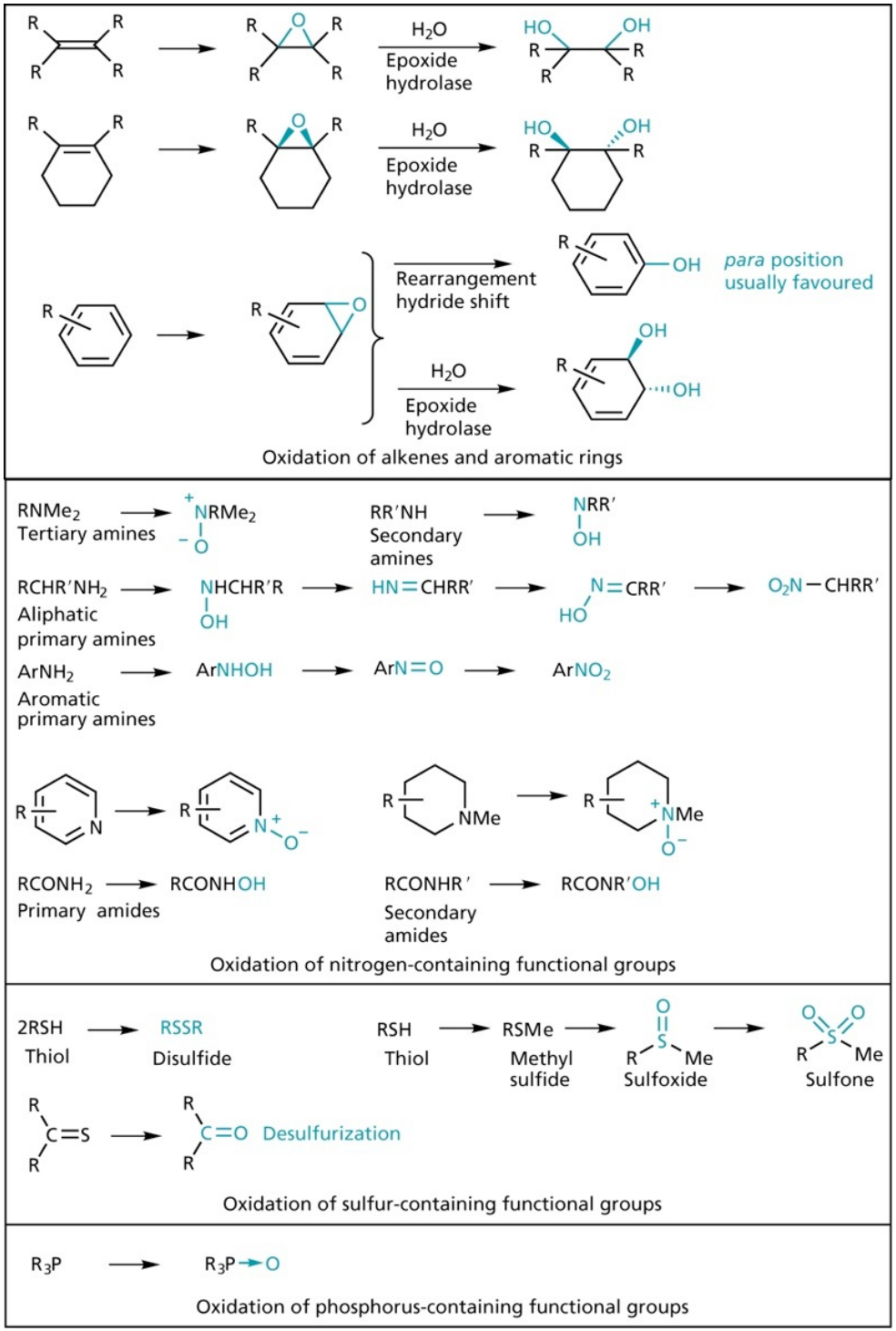
- mostly in the liver, some in the gut wall, blood plasma. Compounds unknown to the body (**xenobiotics**) are metabolized
- can be tested in liver microsomes
  - oxidations of N-methyl groups, aromatic rings, terminal positions of alkyl chain, least hindered positions of alicyclic rings
  - demethylations to yield masked hydroxy functions
  - reductions of nitro, azo and carbonyl groups
  - hydrolysis of amides or esters
  - the most important type of enzymes catalyzing oxidations are **monooxygenases** (esp. the **cytochrome-P450** enzymes or the flavin-type monooxygenases in the liver). Other enzymes are monamine oxidases, alcohol dehydrogenases and aldehyde dehydrogenases.



P450-catalyzed reactions (I)



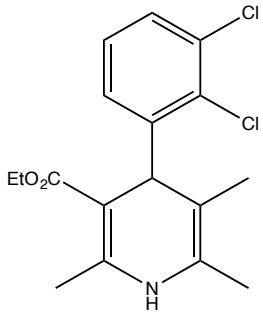
from: Patrick, Medicinal Chemistry



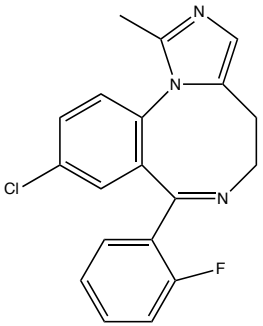
from: Patrick, Medicinal Chemistry

The grapefruit juice effect

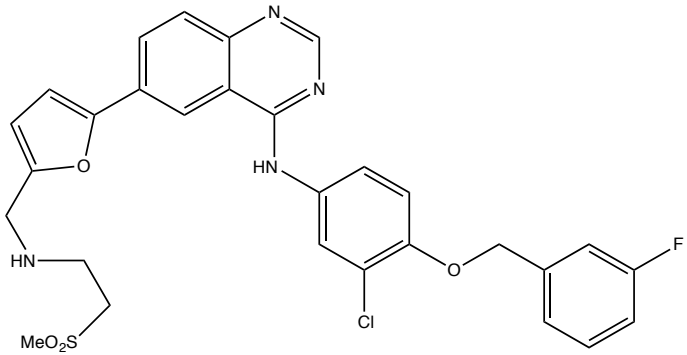
grapefruit juice is known to increase the amount of certain drugs
that reach the general circulatory system



felodipine
(antihypertensive)



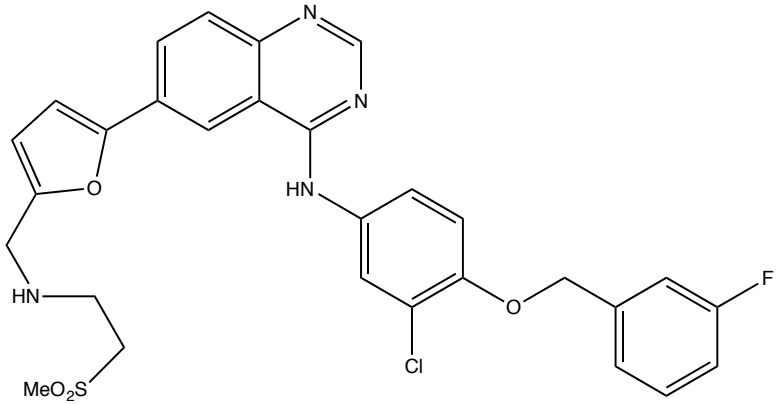
midazolam
(sedative)



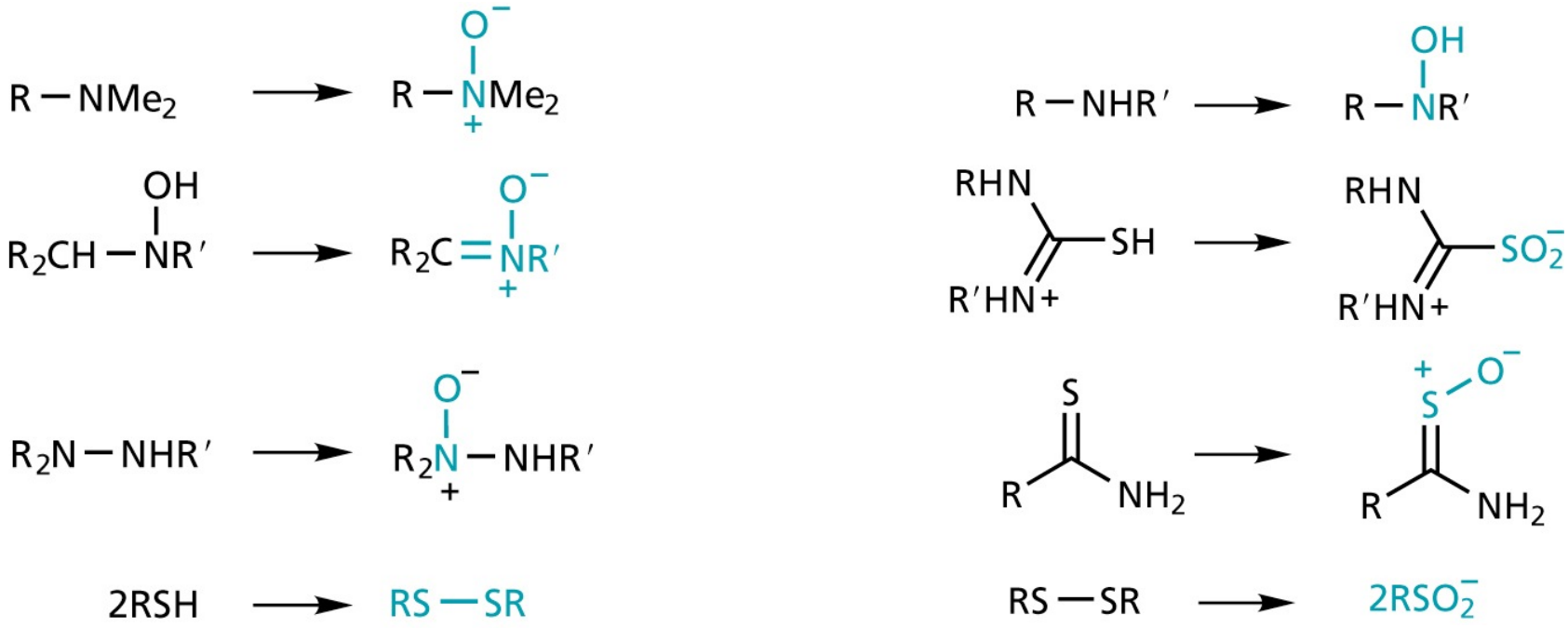
lapatinib
(anticancer)

grapefruit juice contains bergamottin that is an inhibitor of CYP3A4, an enzyme
that metabolites drugs in the liver and the small intestine

bergamottin (coumarin)

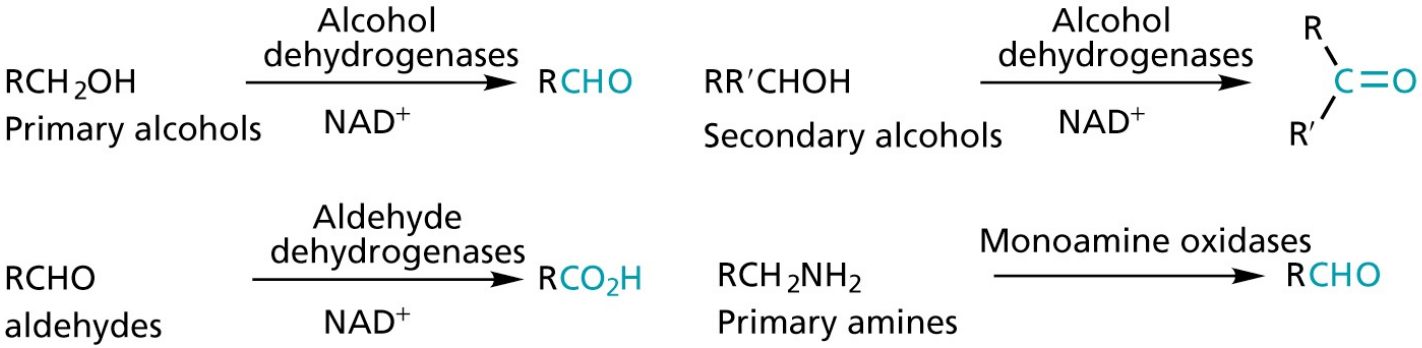


flavin-containing monooxygenases

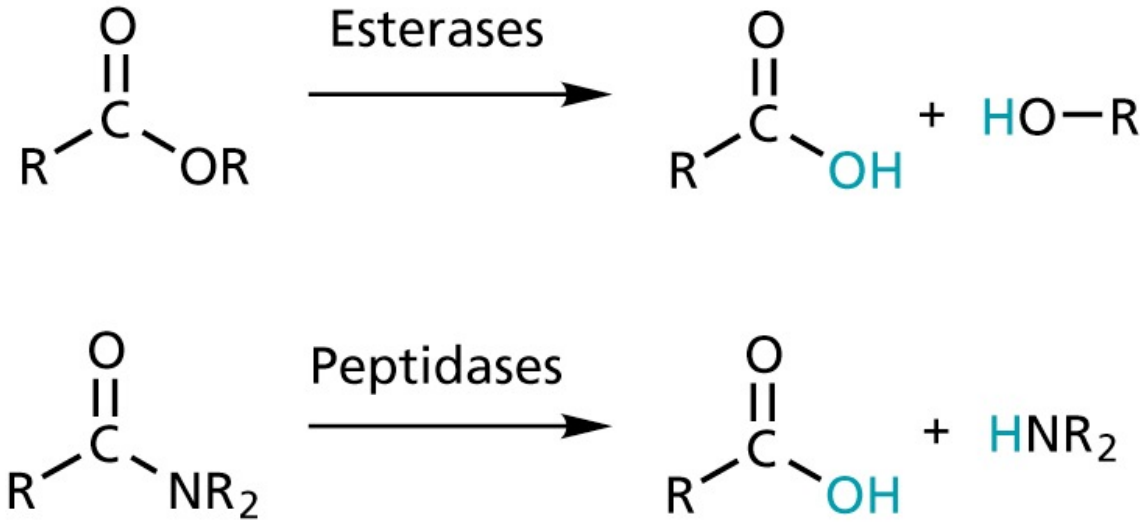


from: Patrick, Medicinal Chemistry

Oxidations

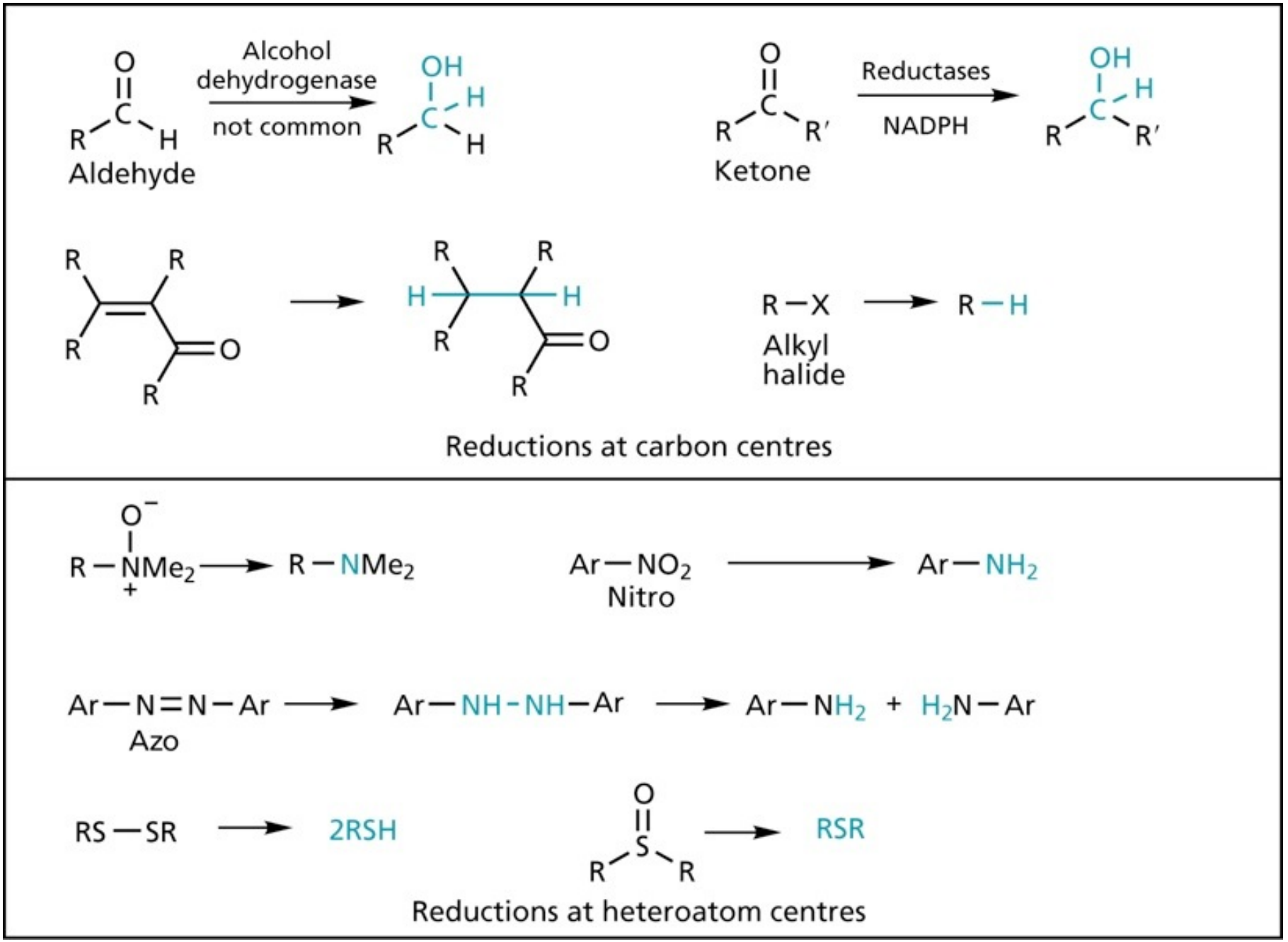


Hydrolysis



from: Patrick, Medicinal Chemistry

Reductions



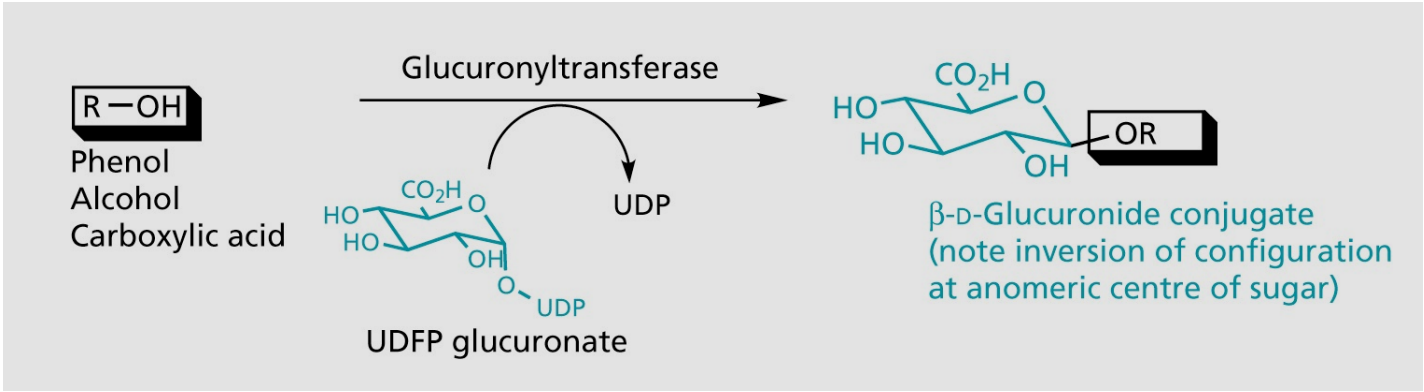
from: Patrick, Medicinal Chemistry

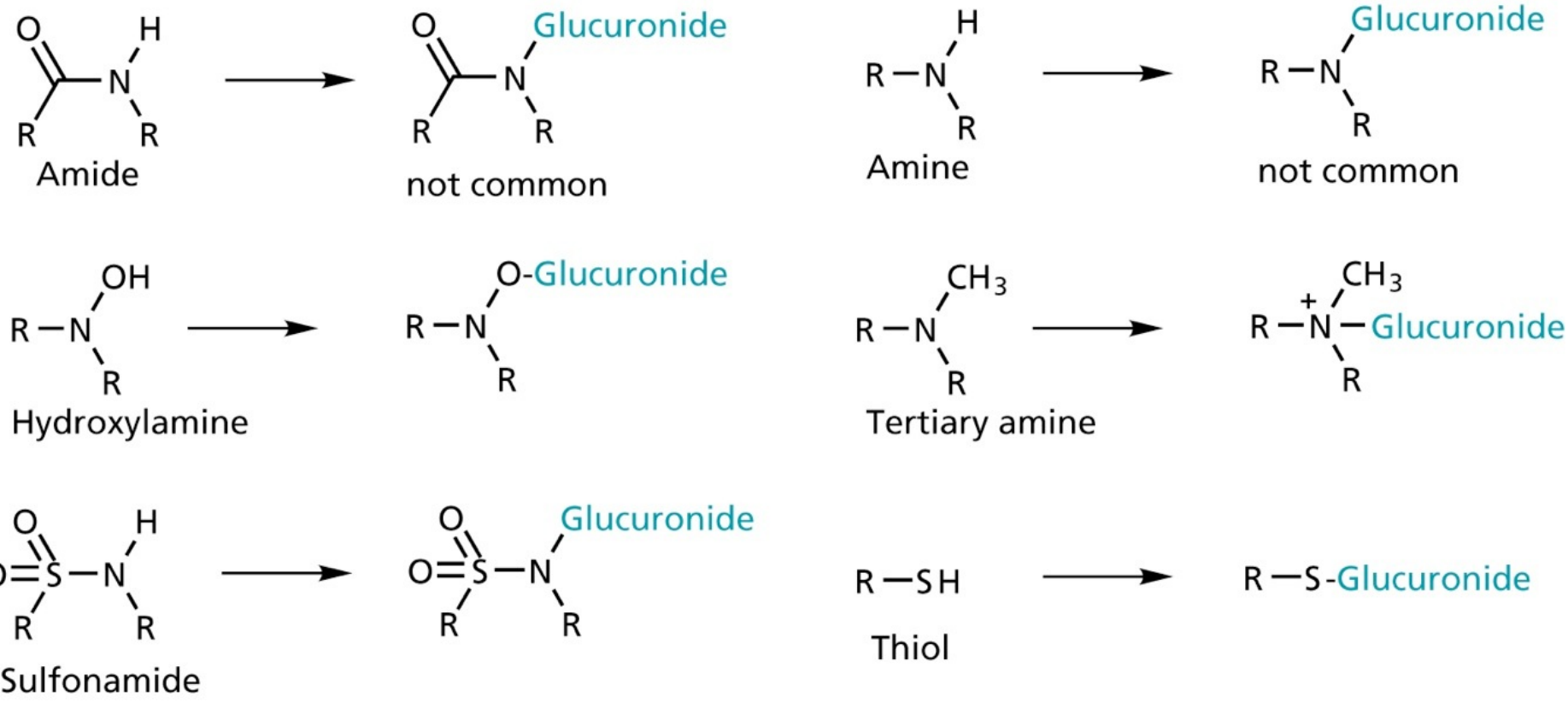
Phase II reactions

• conjugation reactions performed by transferase enzymes in the liver, but also kidney and lung

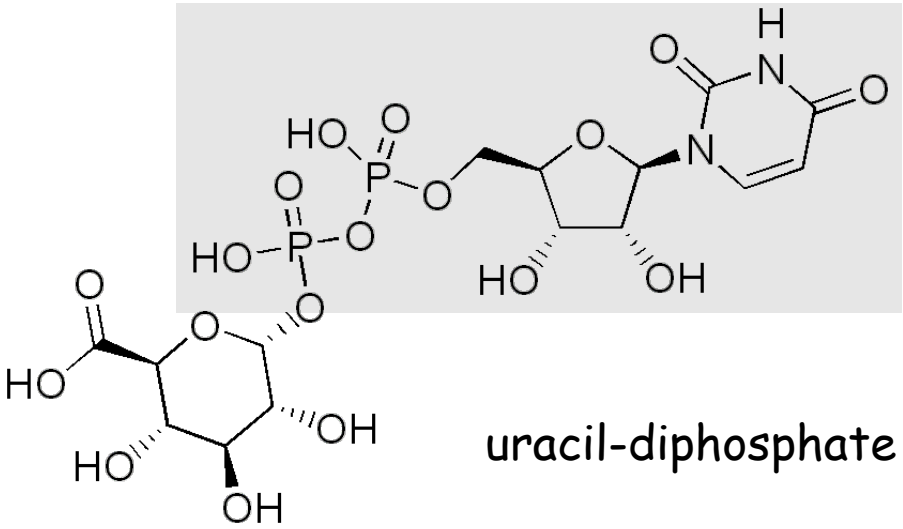
- **glucoronic acid addition** to phenols, alcohols, hydroxylamines and carboxylic acids to form O-glucoronides
- glucoronic acid addition to sulfonamides, amides or thiols to give N- or S-glucoronides
- **sulfate** added to phenols, alcohols, arylamines and N-hydroxy compounds
- conjugate is excreted in the urine (or in the bile, if MW > 300)
- carboxylic acids can be converted to peptides (predominantly with glutamine)
- electrophilic functional groups such as epoxides, alkyl halides, sulfonates, disulfides, and radical species can react with **glutathione**, these may then be further transformed into mercapturic acids.
- **methylation** or **acetylation** of phenols of phenols, amines and thiols.

Phase II



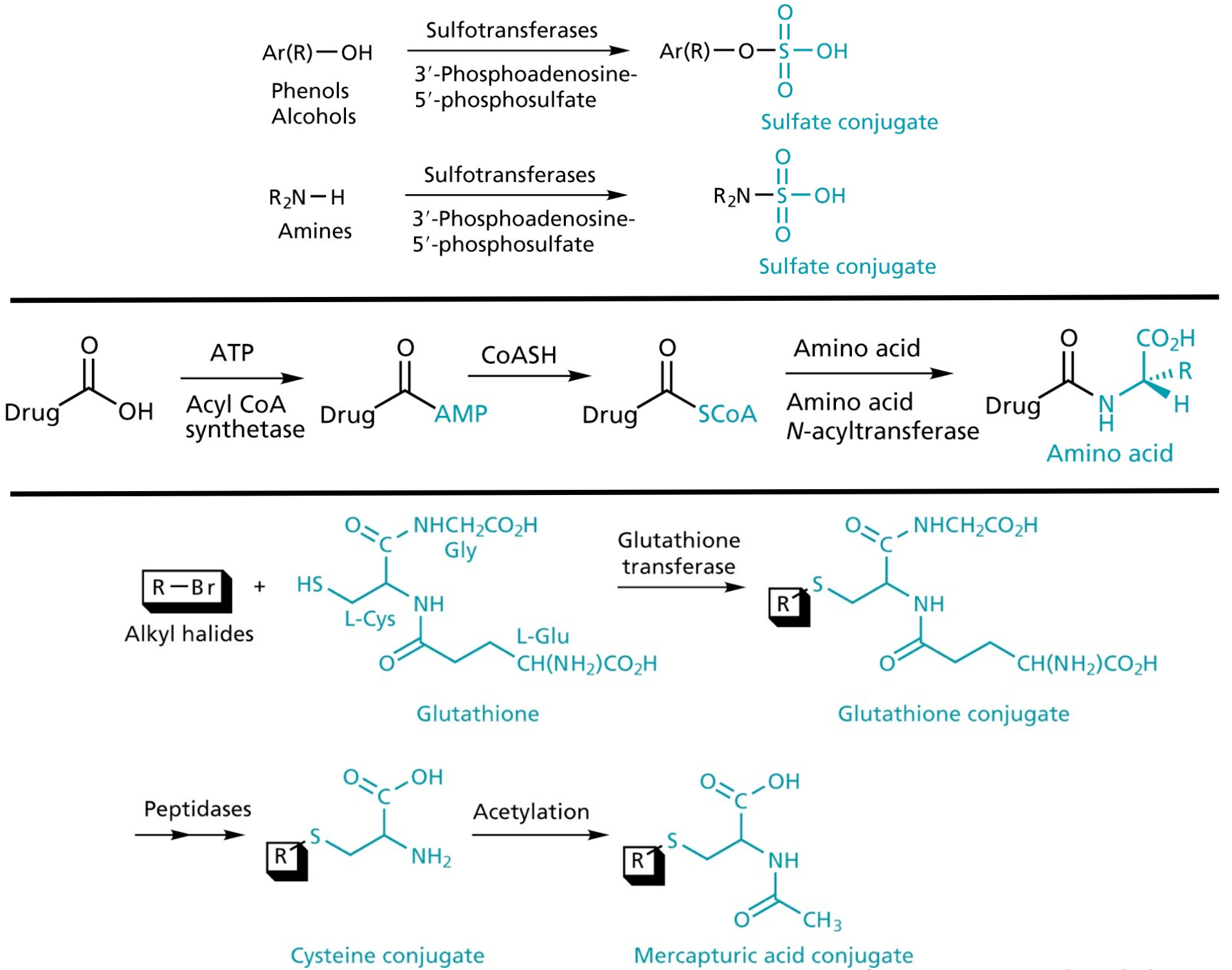


from: Patrick, Medicinal Chemistry

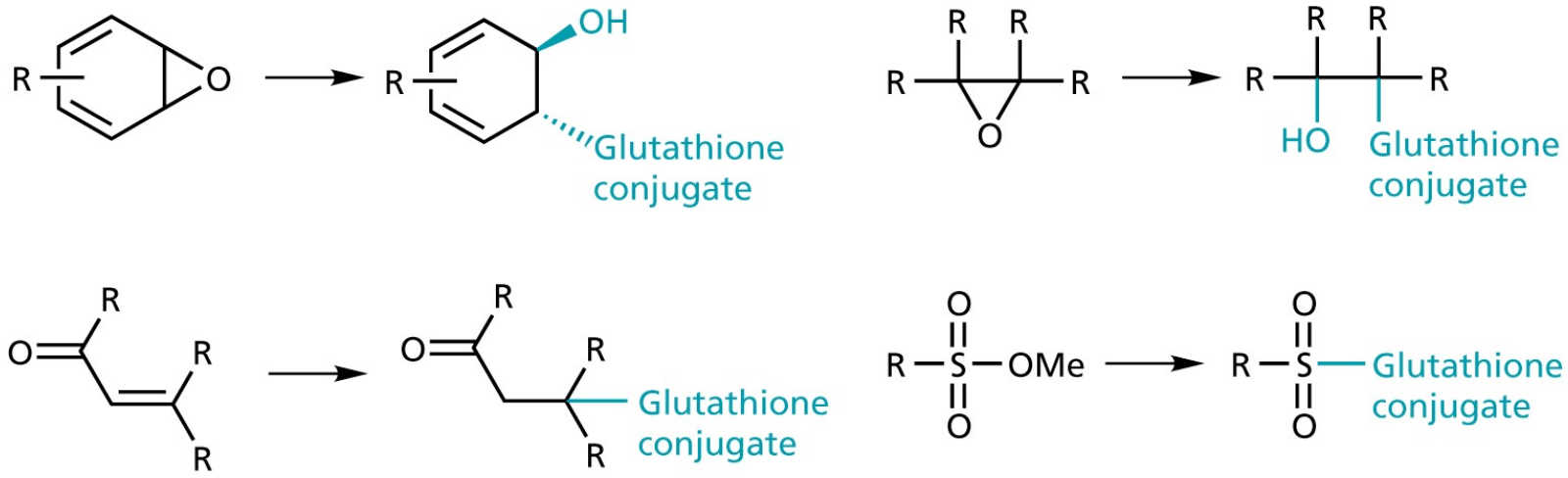


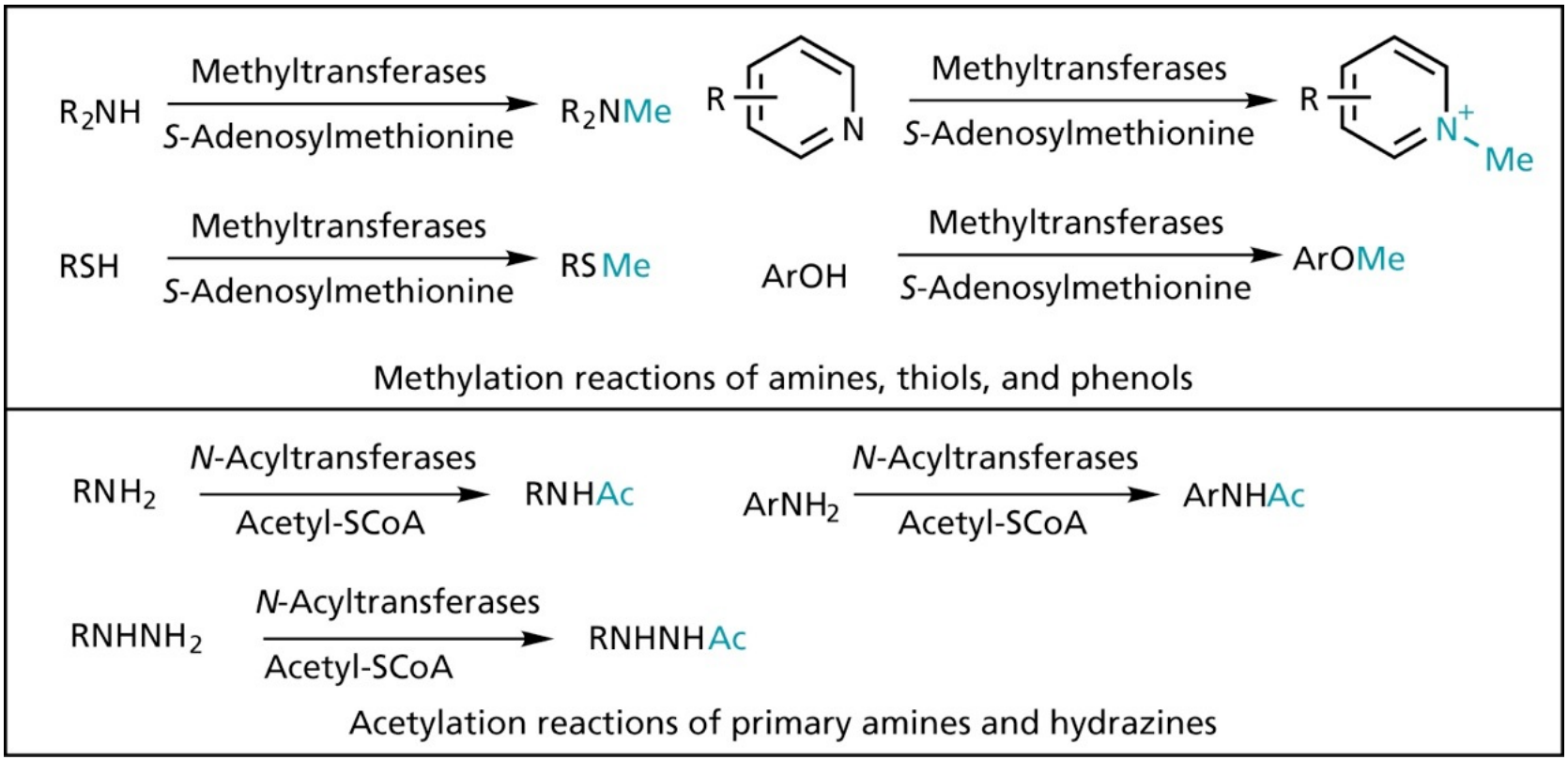
uracil-diphosphate

UDP sugars are activated forms of sugars that serve as substrates for glycosyltransferases



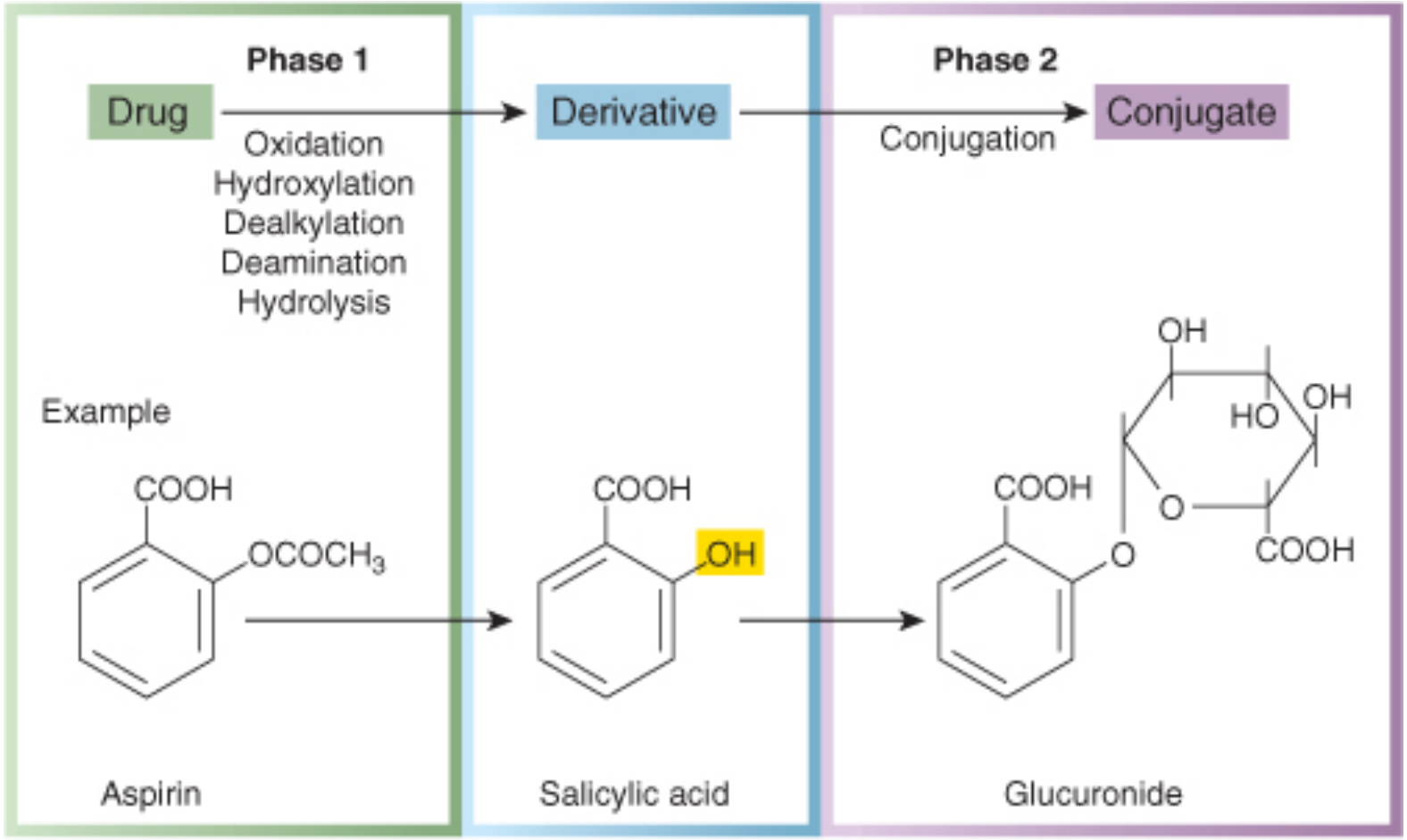
from: Patrick, Medicinal Chemistry



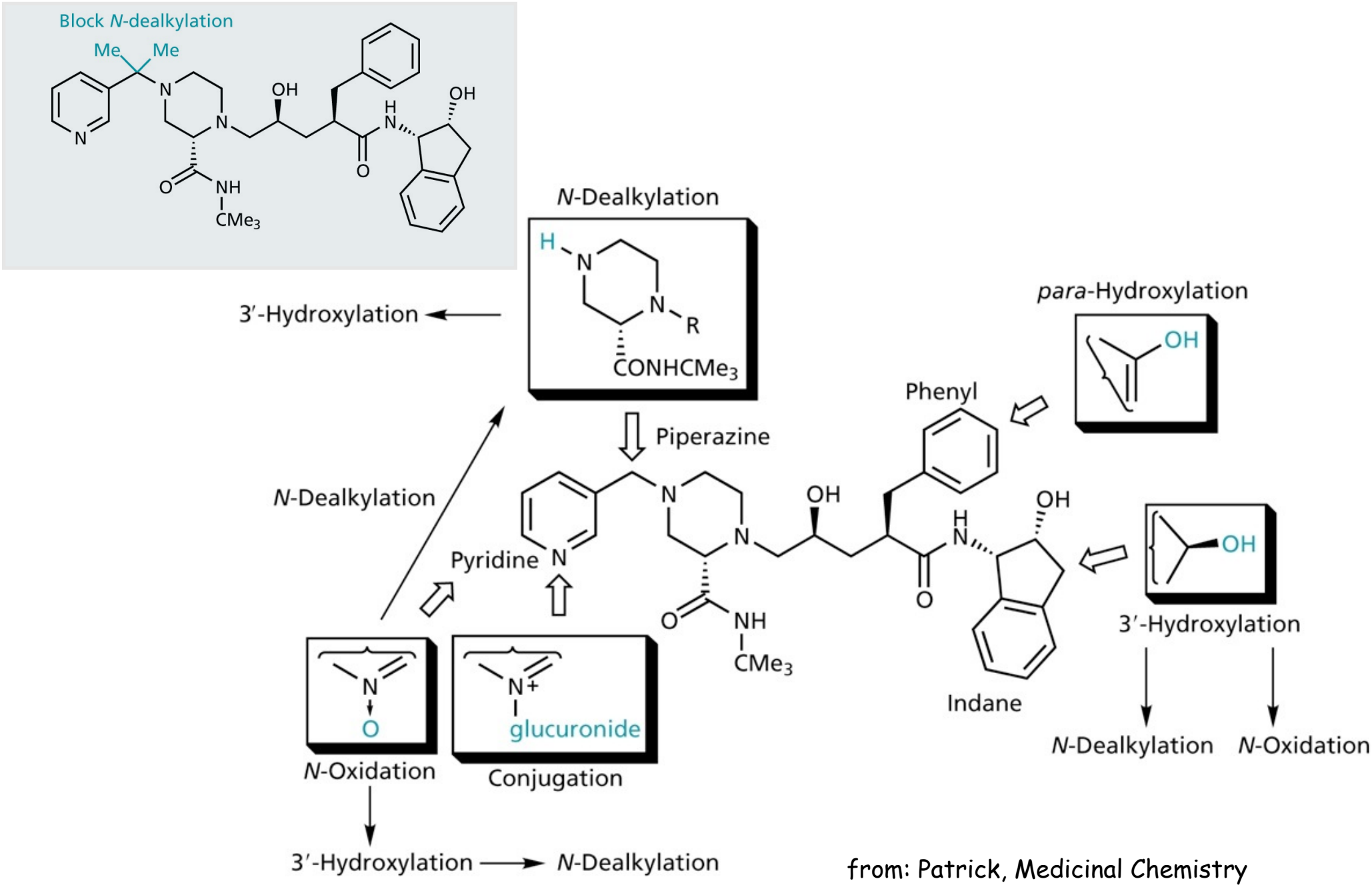


from: Patrick, Medicinal Chemistry

Metabolism of Aspirin

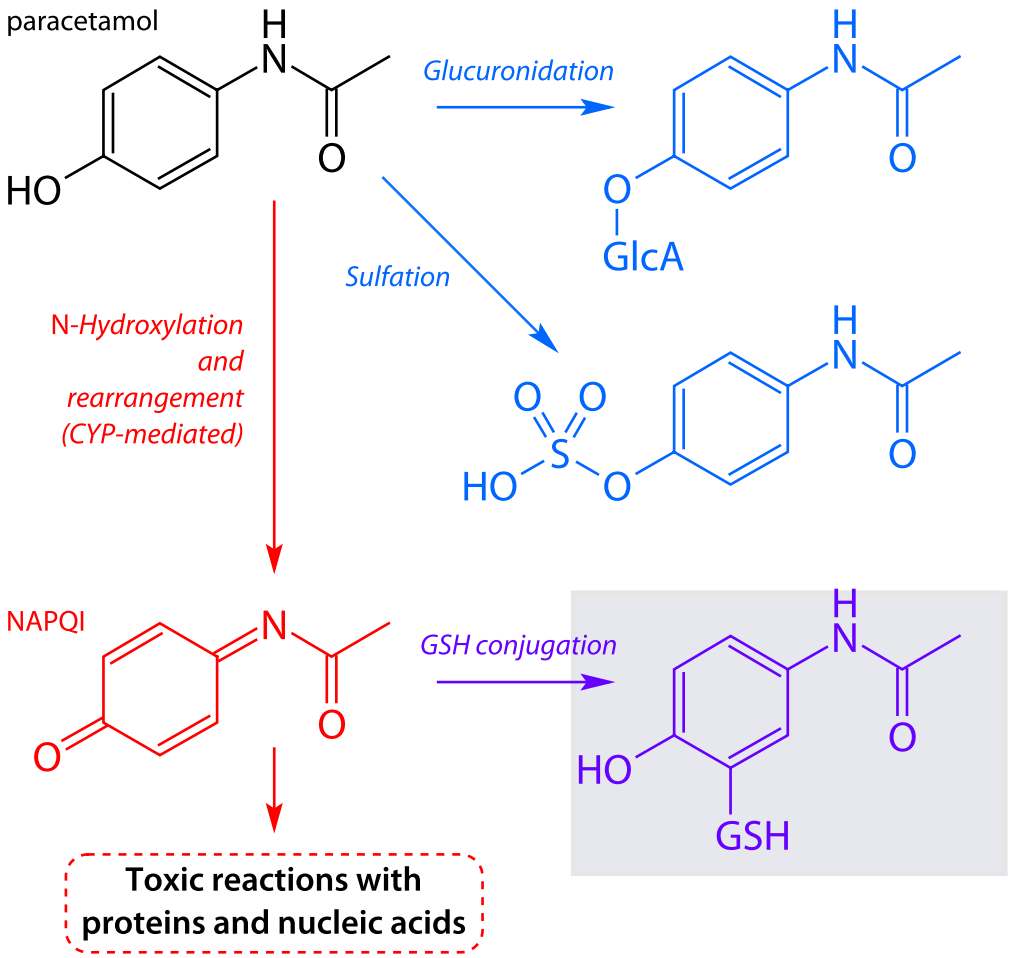


Metabolism of Indinavir



from: Patrick, Medicinal Chemistry

Metabolism of Paracetamol in the liver produces toxic metabolites



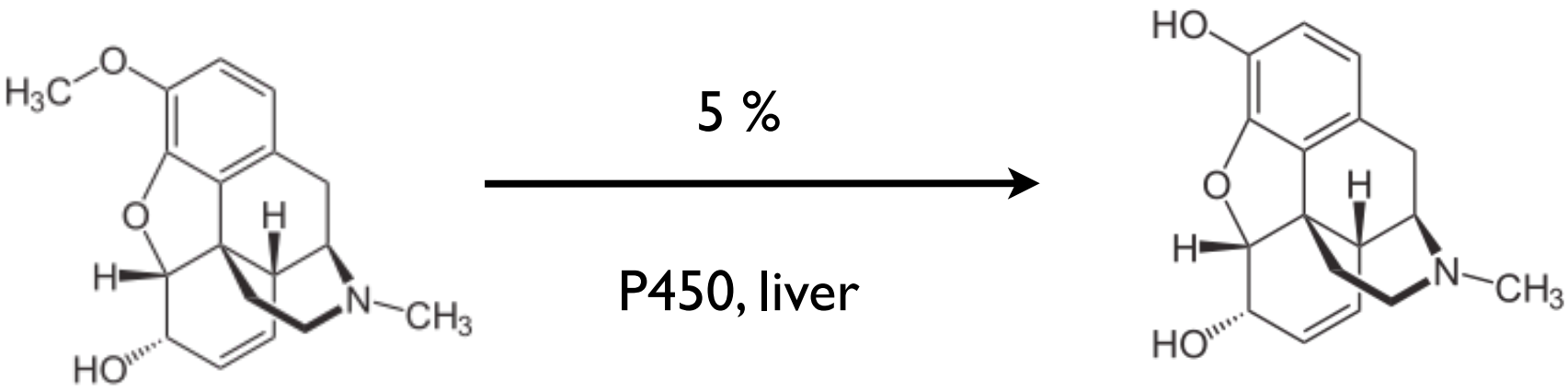
N-acetyl-p-benzo-quinone imine, abbreviated as NAPQI

Paracetamol overdose results in more calls to poison control centers in the US than overdose of any other pharmacological substance, accounting for more than 100,000 calls, as well as 56,000 emergency room visits, 2,600 hospitalizations, and 458 deaths due to acute liver failure per year

detoxification pathway at "normal levels"

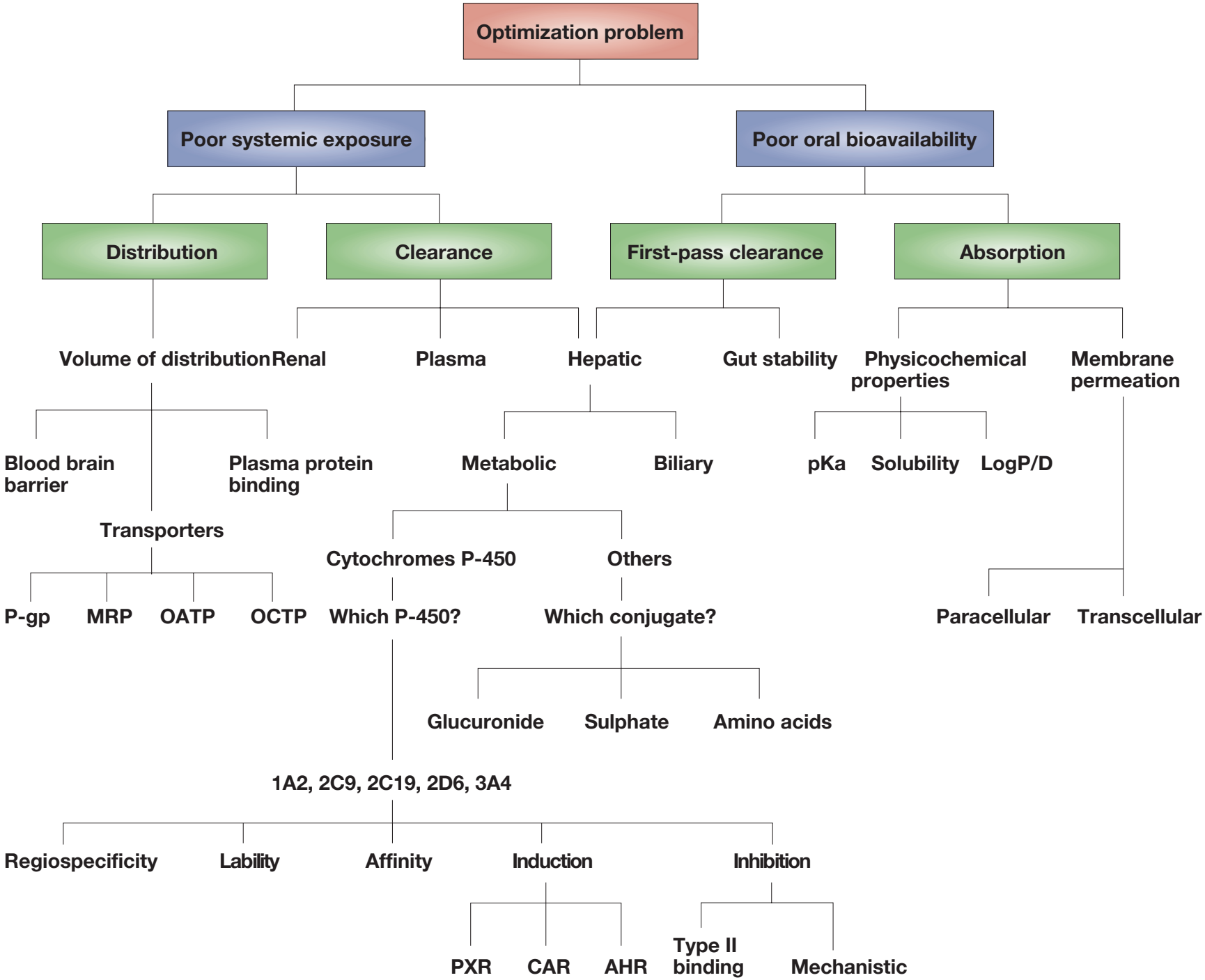
figure from Wikipedia

Metabolism of Codein to Morphine



codein

morphine



(Nature Rev. Drug Discov. 2002,2,192-204)

Elimination

- elimination via lungs (gaseous general anaesthetics)
- (small, MW < 300) lipophilic substances are eliminated via the bile
- 10-15% of drug may be lost through the skin in sweat
- kidneys are the primary route of excretion. They filter the blood, and the filtered waste is removed in the urine and enters the bladder. Larger entities (blood cells etc., MW > 20'000) are reintroduced into the circulation.
- However, much of the filtrate is reabsorbed into the blood supply. This can only occur for water (via aquaporins) or molecules that can pass the membranes of the nephrons. Hence **only lipophilic substances will be reabsorbed**. Metabolism makes substances more polar and hence is importance for their clearance.

- blood enters the kidneys via the renal artery.
- blood is then entering the capillaries. In the glomerulus it is forced under pressure through the capillary walls into the nephron. Any compounds **too large to pass** remain in the capillaries within the plasma and re-enters the circulation.

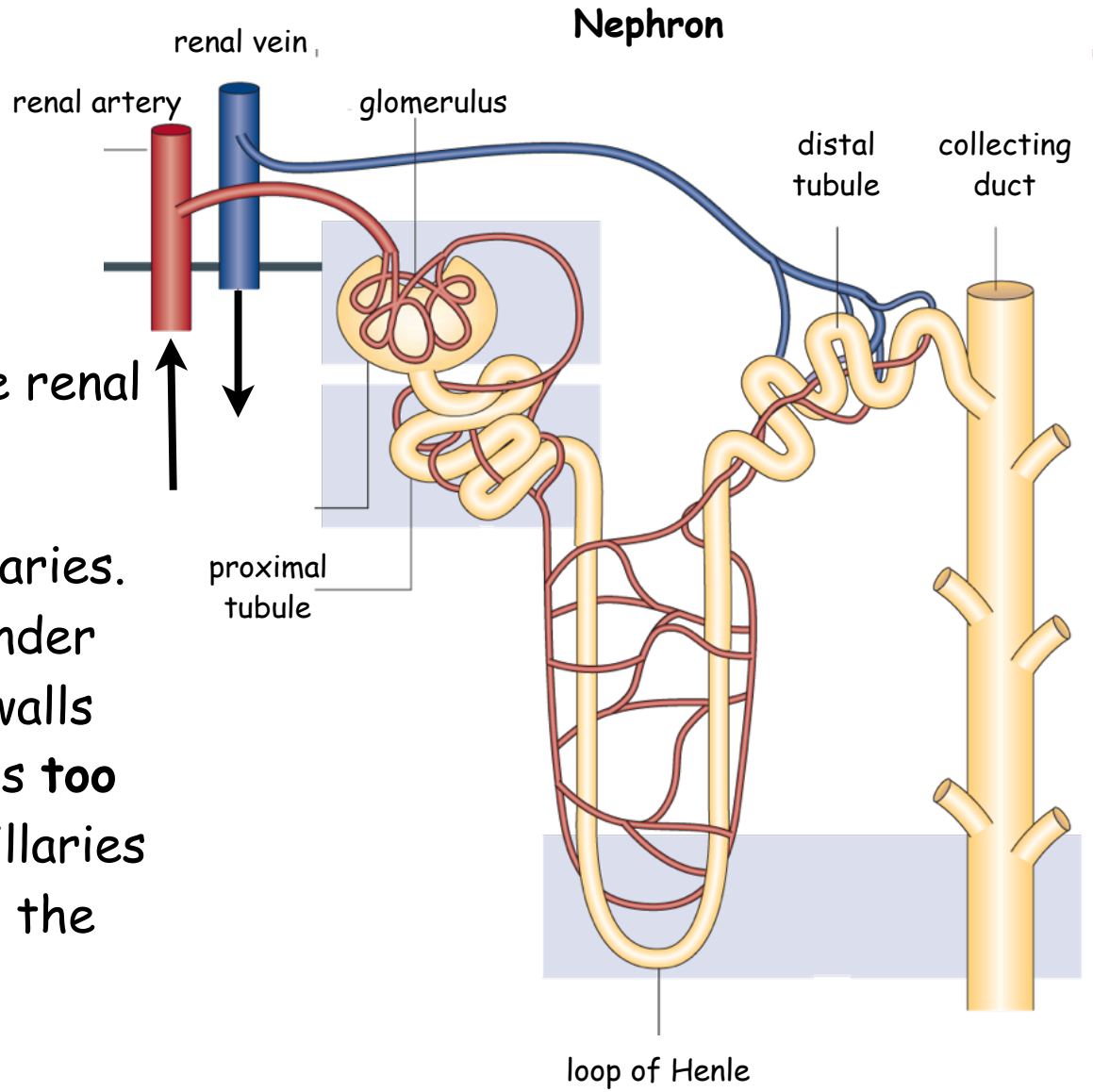
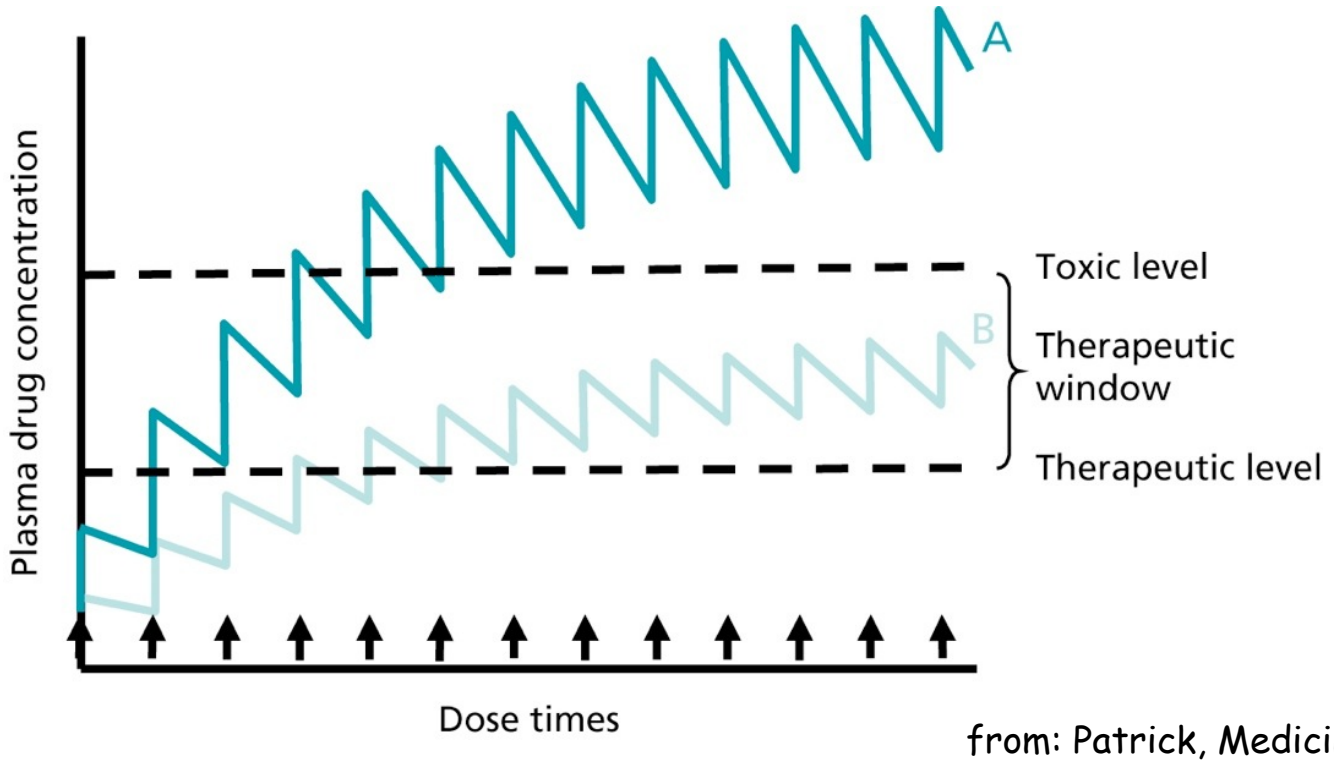


figure from: Nicholson et al, Nature Rev. Drug Discov. (**2**) 2002, 153

Clearance, also renal clearance or renal plasma clearance (when referring to the function of the kidney), of a substance is the inverse of the time constant that describes its removal rate from the body divided by its volume of distribution.

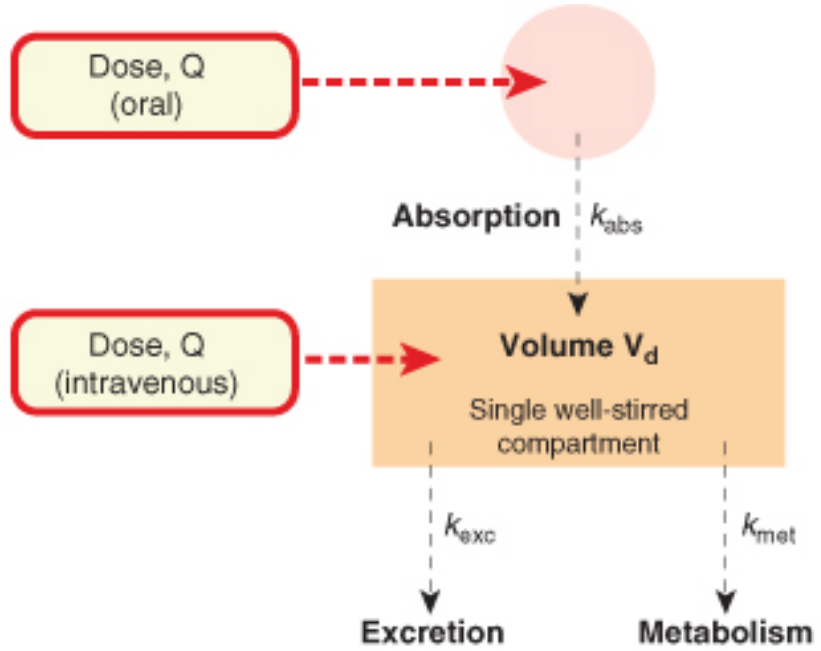
Dosing

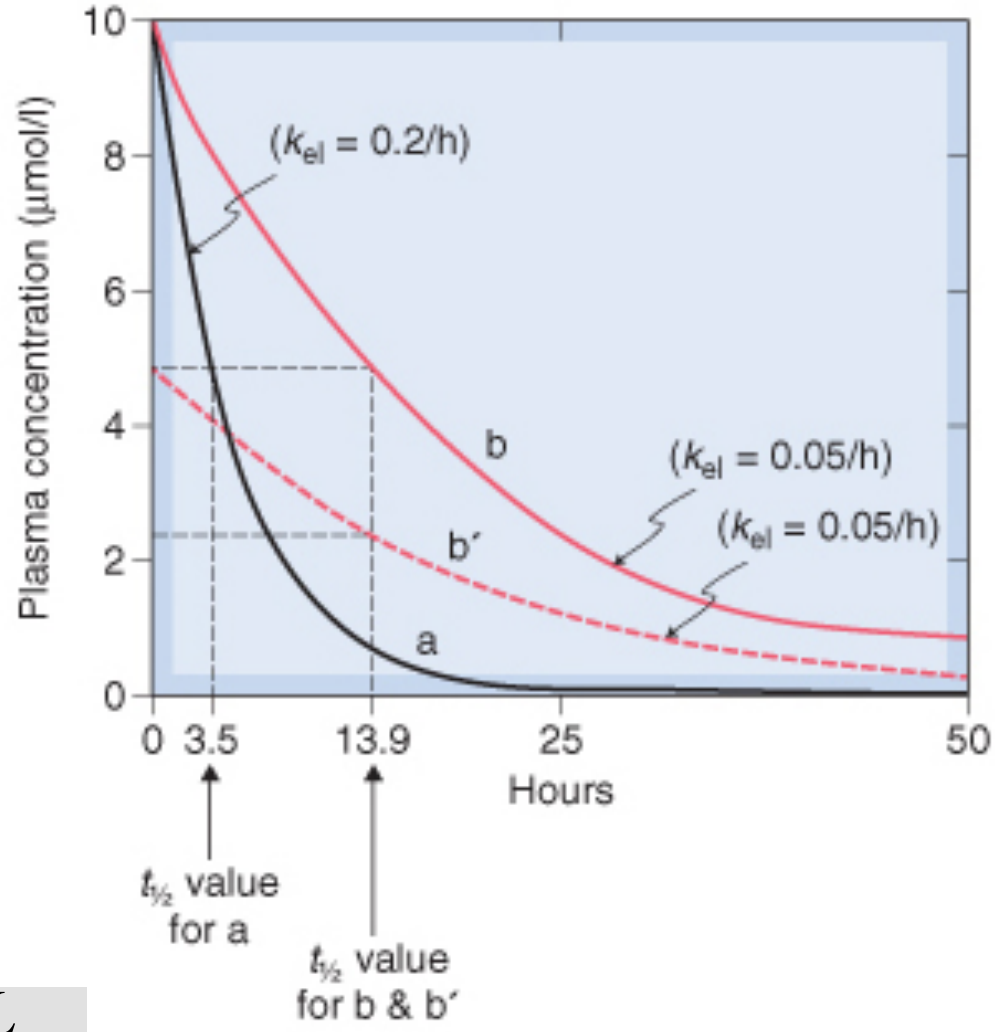
Pharmacokinetics: PK is describing the time-course of drug concentration in different regions of the body during and after dosing



from: Patrick, Medicinal Chemistry

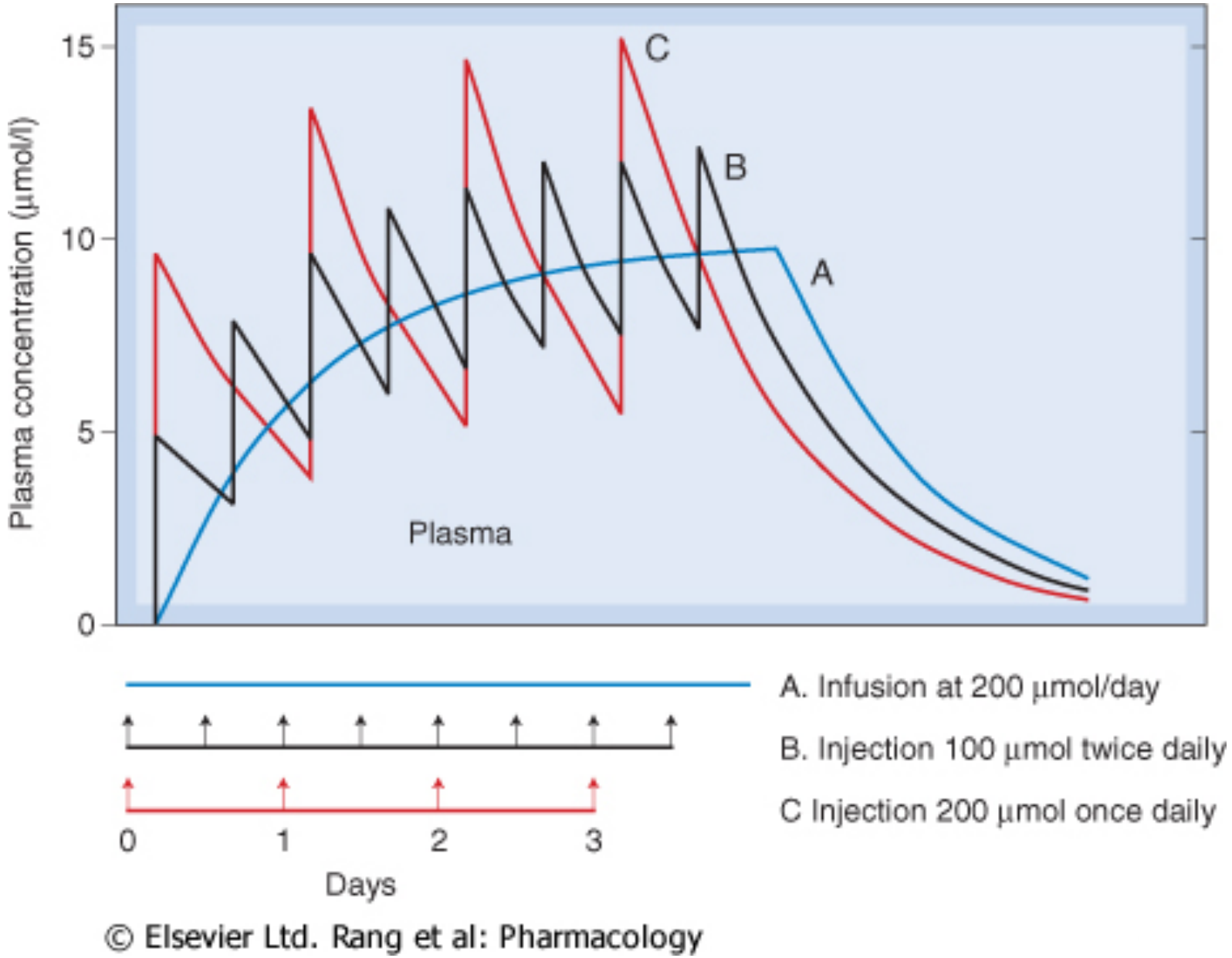
Single Compartment Model





$$C(t) = C(t_0)\exp\frac{-CL_s}{V_d}t$$

(CL_s total clearance)



Principle of Drug Toxicity

- metabolic activation: Drug becomes metabolized into a toxic compound (e.g. paracetamol)
- covalent binding of drug (metabolites) to receptors
- biological effects may be due to
  - cell death/ tissue injury (lethal?)
  - altered phenotype/ function (sub-lethal)
  - immunological hypersensitivity (down-regulation of the immune system)
  - stimulation of cancer growth

Mechanisms of Drug Toxicity

- **on-target**: modulation of the primary drug target, the receptor. Examples: Cerivastatin (Baycol™, Bayer). Rofecoxib (Vioxx, Merck™) was withdrwan because of increased risk of heart attack and stroke associated with long-term, high-dosage use (between 88,000 and 140,000 cases of serious heart disease)

- **hypersensitivity** (ranges from mild skin irritation to organ failure, allergic reactions)

- **off-target**: interactions with receptors other than the desired ones (e.g. the inhibition of cardiac ion (hERG) channels by terfenadine (Seldane™, Hoechst)). Seldane is a prodrug that is converted to the active form by a P450 variant. The matabolite is not toxic, but Seldane itself may lead to toxic effects on the heart's rhythm.

- **biological activation to toxic metabolites**: responsible for many tissue- and organ-specific toxicities

- **idiosyncratic toxicity**: rare toxicities related to genetic differences in human being, such that the effect becomes only visible in a larger population (and is therefore difficult to detect during clinical trials).

Drug Safety

- **drug overdosing:** How large is the safety margin between the required dose and the toxic dose (the **therapeutic index**) ? This determines how large the likeliness of mis-use (accidental or for suicide purpose) is. How are the effects of the drugs on ill people that suffer from compromised immune systems?

- **drug-enzyme interactions**: One problem is the inhibition of metabolising (P450) enzymes that lead to the accumulation of drugs in the body because clearance is reduced. Often, antibiotic or anti-fungal agents display P450-inhibitory potential.

- **adverse effects at therapeutic doses** can pose a substantial problem when they occur at lower frequency because then they might not be detected in preclinical or even in clinical trials.

Common Toxicities

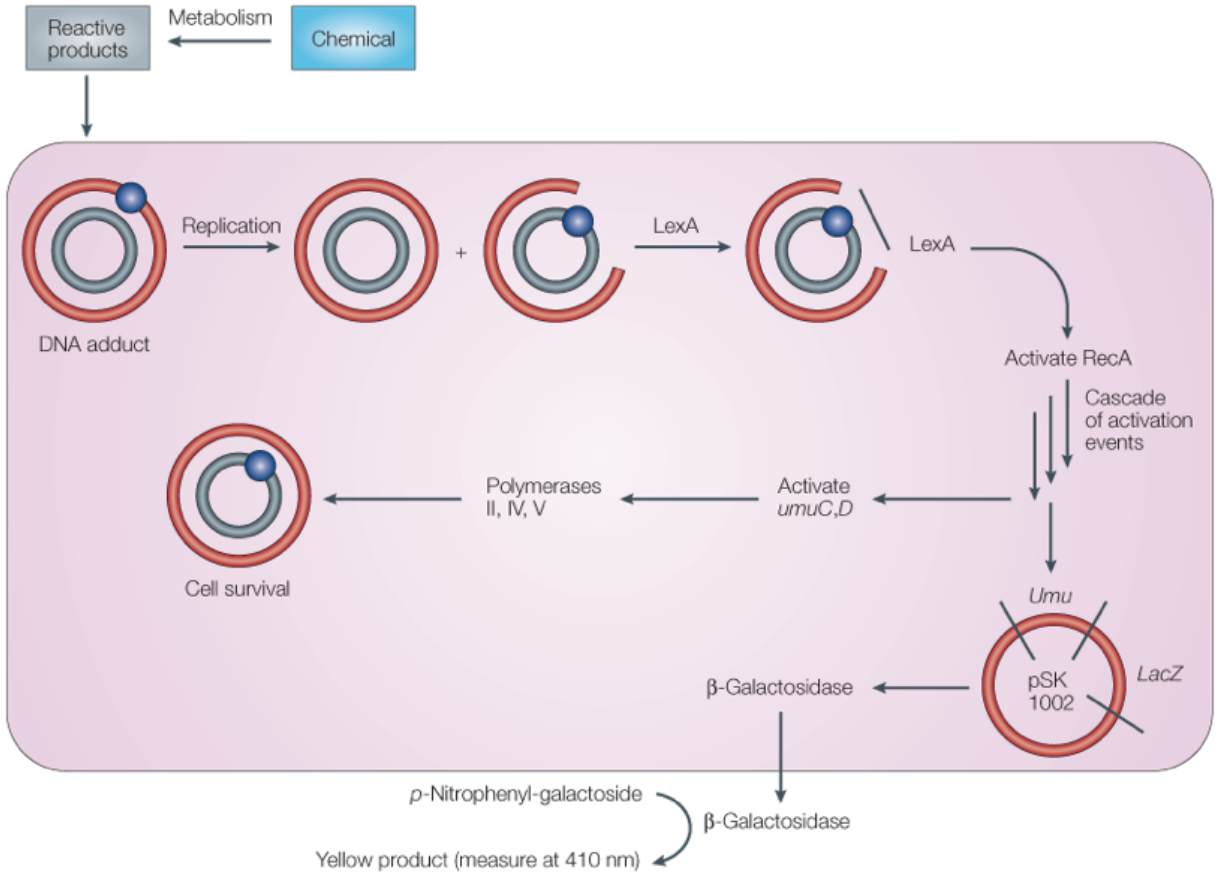
Type	Effect	Alert
Cardiovascular	blockade of the hERG potassium channel leading to prolongation of QT interval, arrhythmias and potential death	Binding assay and ion-channel electrophysiology
Hapatotoxicity	formation of glutathion adducts and irreversible P450 inhibition	in-vitro studies in hepatocytes
reactive metabolites	idosyncratic toxicity derived from mechanistic pathway/metabolites	metabolite screens
genetic	genotoxicity	Mini-Ames and in-vitro micronucleus test UMU test
phospholipidosis (access phospholipds in tissues)	lung and liver toxicity	in-vitro cellular assays, high V_d can be a warning
drug-enzyme interactions	P450 inhibition/induction	in vitro assays
CNS side effects	blood-brain barrier penetration, off-target pharmacology	broad CNS receptor and enzyme screening

Ganellin, Jefferis and Roberts, *Biological and Small Molecule Drug Research and Development*

Methods to study drug toxicity

- **in-vitro assays for overall drug toxicity**

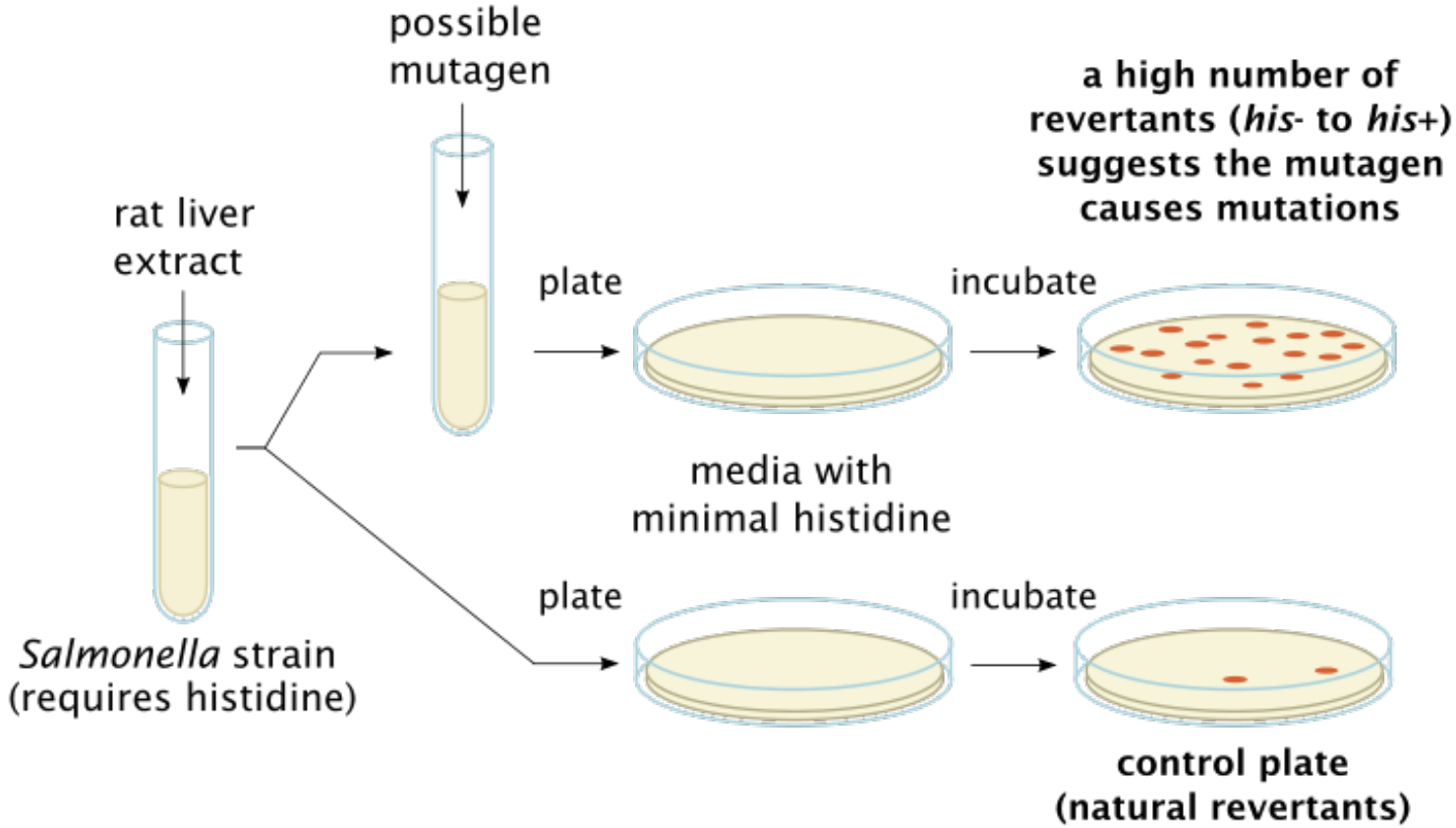
- **screens for genotoxicity (UMU test):** the compound under study is incubated with a metabolic system capable of converting it into a reactive product. The metabolic system may be **liver microsomes** or **recombinant P450 systems**. Binding to DNA results in regions to which complementary DNA cannot bind during replication, and hence which will be single-stranded. To these regions, the protease LexA binds to the plasmid, which after activation induces expression of a reporter plasmid (the umu gene), that in turn results in beta-galactosidase which can stimulate a color reaction:



Lieber et al, Nature Rev. Drug Discov. **4** (2005), 410-420

AMES test to study drug toxicity

Bruce N. Ames, William E. Durston, Edith Yamasaki, and Frank D. Lee (1973). "Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection". PNAS 70 (8): 2281–5.



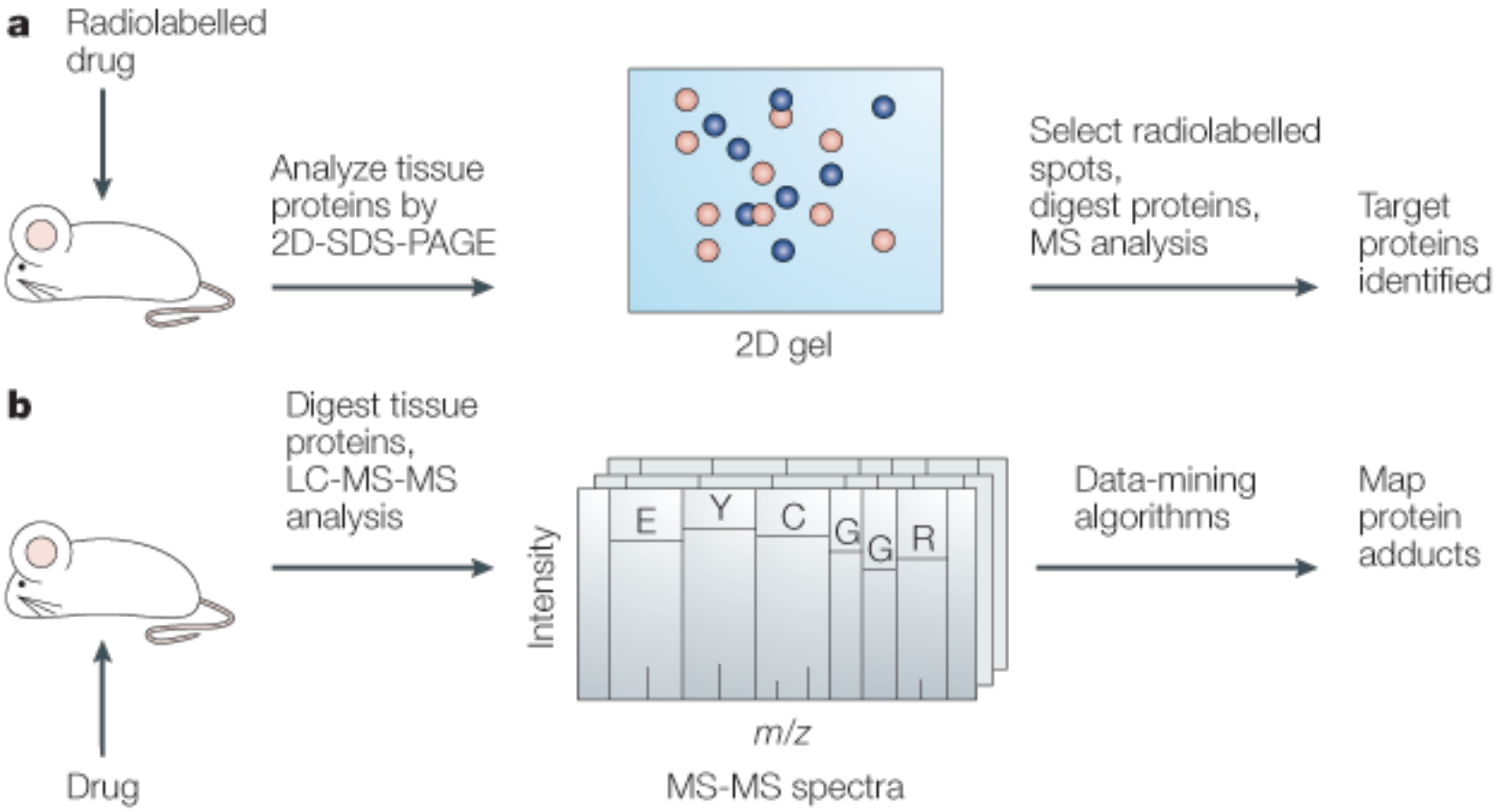
- **AMES test for genotoxicity:** the test uses several strains of the bacterium Salmonella typhimurium that carry mutations in genes involved in histidine synthesis. These strains are auxotrophic mutants, i.e. they require histidine for growth, but cannot produce it. The method tests the capability of the tested substance in creating mutations that result in a return to a "prototrophic" state, so that the cells can grow on a histidine-free medium.The tester strains are specially constructed to detect either frameshift (e.g. strains TA-1537 and TA-1538) or point (e.g. strain TA-1531) mutations in the genes required to synthesise histidine, so that mutagens acting via different mechanisms may be identified.

from Wikipedia

Methods to study drug toxicity (II)

- **covalent binding** to proteins is tested by synthesizing the drug in labelled (usually radio-labelled) form, possibly adducts are also directly detected by MS spectrometry.
- **transcriptomics**: Monitoring expression profiles (on the gene level) of cell model systems after the drug has been added. Can be for comparative reasons performed in micro-array fashion. A promising approach tries to use systems-biology techniques in combination with lower eukaryotes, for which experimental conditions can be tightly controlled. Studies in Saccharomyces Cerevisiae have revealed distinct changes in the transcriptome after addition of alkylating agents.
- **proteomics**: similar to transcriptomics, but expressed proteins are directly detected by e.g. 2D gels or by MS spectrometry following chromatographic separation.

Identification of Drug Targets in-vivo



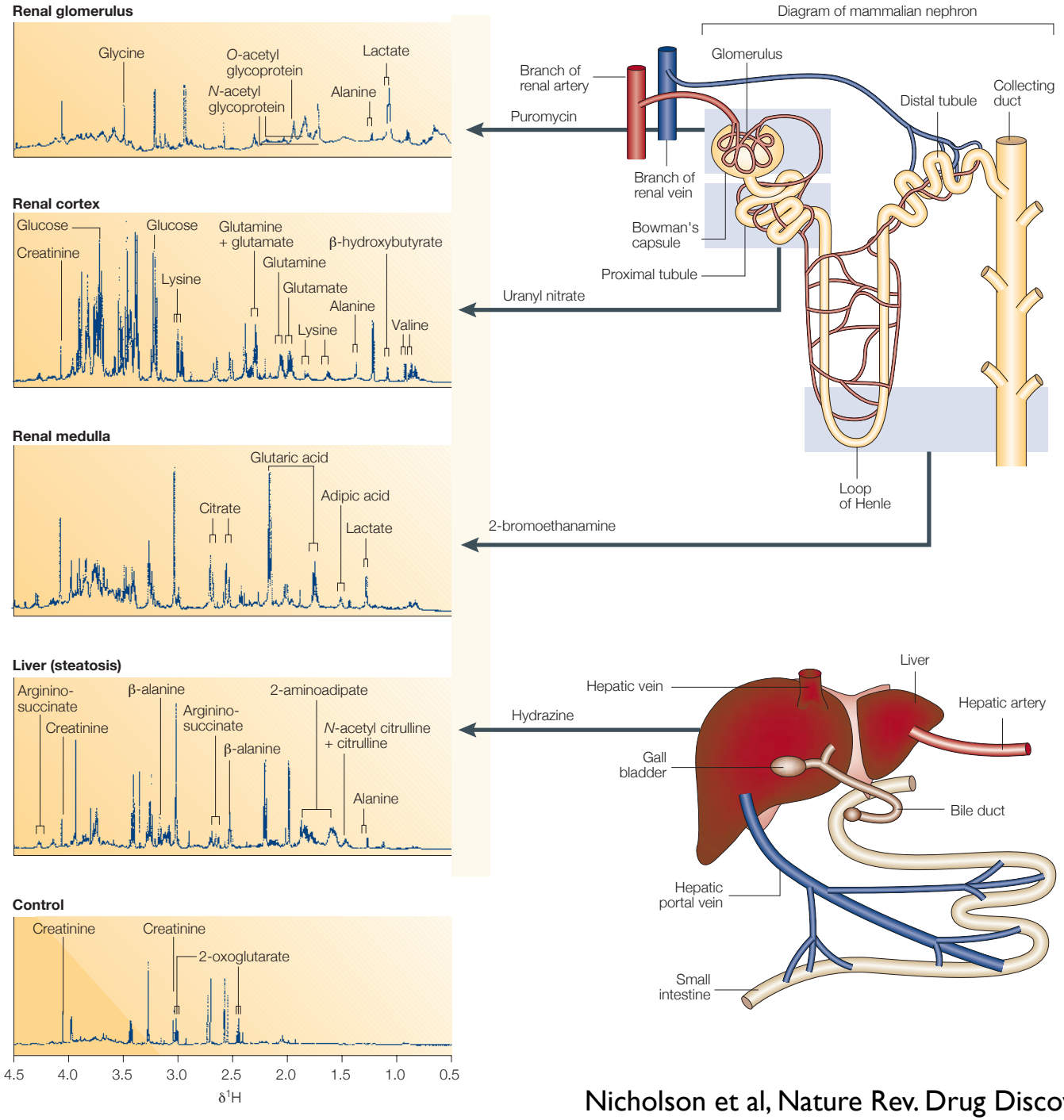
Lieber et al, Nature Rev. Drug Discov. **4** (2005), 410-420

Methods to study drug toxicity (III): Animal tests

- **Analysis of serum and other biofluids** to search for biomarkers of drug toxicity. In **metabonomic** studies, conducted with the help of NMR or MS, the metabolites are detected from the various body fluids, and are recognized by means of pattern recognition techniques. This can also be done with specific organ tissues, and thereby allow identifying organ-specific toxicity of certain drugs. It can also be used for kinetic studies if samples are taken at certain time intervals. Families of metabolites can be identified, allowing to connect metabolic pathways.

- **animals tests** in higher organisms (e.g. rodents, apes) and monitoring general body functions and organ performance

Metabonomics by NMR



Nicholson et al, Nature Rev. Drug Discov. (**2**)2002, 153

In-Vitro Safety Pharmacology Profiling

TABLE 1

A selection of cardiovascular targets included in the *in vitro* SPP assay panel used by Novartis

Targets	Possible ADRs[a]
Adenosine, Ad_1	Bradycardia, atrioventricular block. Renal vasoconstriction.
Adenosine, Ad_{2a}	Hypotension, coronary vasodilation. Facilitation of platelet aggregation.
Adenosine, Ad_3	Enhanced mediator release could exacerbate asthma and allergic conditions.
Adrenergic alpha, Al_{1a}	Hypertension and positive inotropic effect. Orthostatic hypotension.
Adrenergic alpha, Al_{1b}	Orthostatic hypotension.
Adrenergic alpha, Al_{2a}	Might inhibit insulin secretion, resulting in hyperglycemia. Hypertension exacerbates heart failure.
Adrenergic alpha, Al_{2b}	Hypertension, cardiac ischemia (block), vasoconstriction of arteries. Peripherally exacerbates heart failure, centrally reduces blood pressure.
Adrenergic alpha, Al_{2c}	Hypertension, cardiac ischemia. Increased muscular, skeletal blood flow.
Adrenergic beta, $Beta_1$	Positive inotropic and chronotropic effects, ventricular fibrillation. Facilitation of bronchospasm, impairs cardiovascular performance.
Adrenergic beta, $Beta_2$	Facilitates cardiac arrest, bronchodilation. Increased bronchospasm, impairs exercise stress cardiovascular performance.
Angiotensin II, AT_1	Increases blood pressure, cell proliferation and migration, tubular Na^+ resorption.
Bradykinin, B_1	Enhances nociception, inflammation, vasodilation and cough.
Bradykinin, B_2	Enhances nociception, inflammation, vasodilatation and cough.
Calcitonin gene-related peptide, CGRP	Hypocalcaemia and hypophosphatemia.
Ca channel type L, benzothiazepine	Hypotension.
Ca channel type L, phenylalkylamie	Hypotension.
Dopamine, D_1	Treatment of Parkinson's disease; induces dyskinesia, extreme arousal, locomotor activation, vasodilatation and hypotension. Schizophrenia, neurodegeneration, coordination disorders.
Endothelin, ET_a	Might cause vasoconstriction, positive inotropy, cell proliferation (e.g. smooth muscle and mesangial cells) and aldosterone secretion.
Endothelin ET_b	Causes initial vasodepression, vasoconstriction, bronchoconstriction and cell proliferation. Vasodilatation, platelet aggregation.

TABLE 1

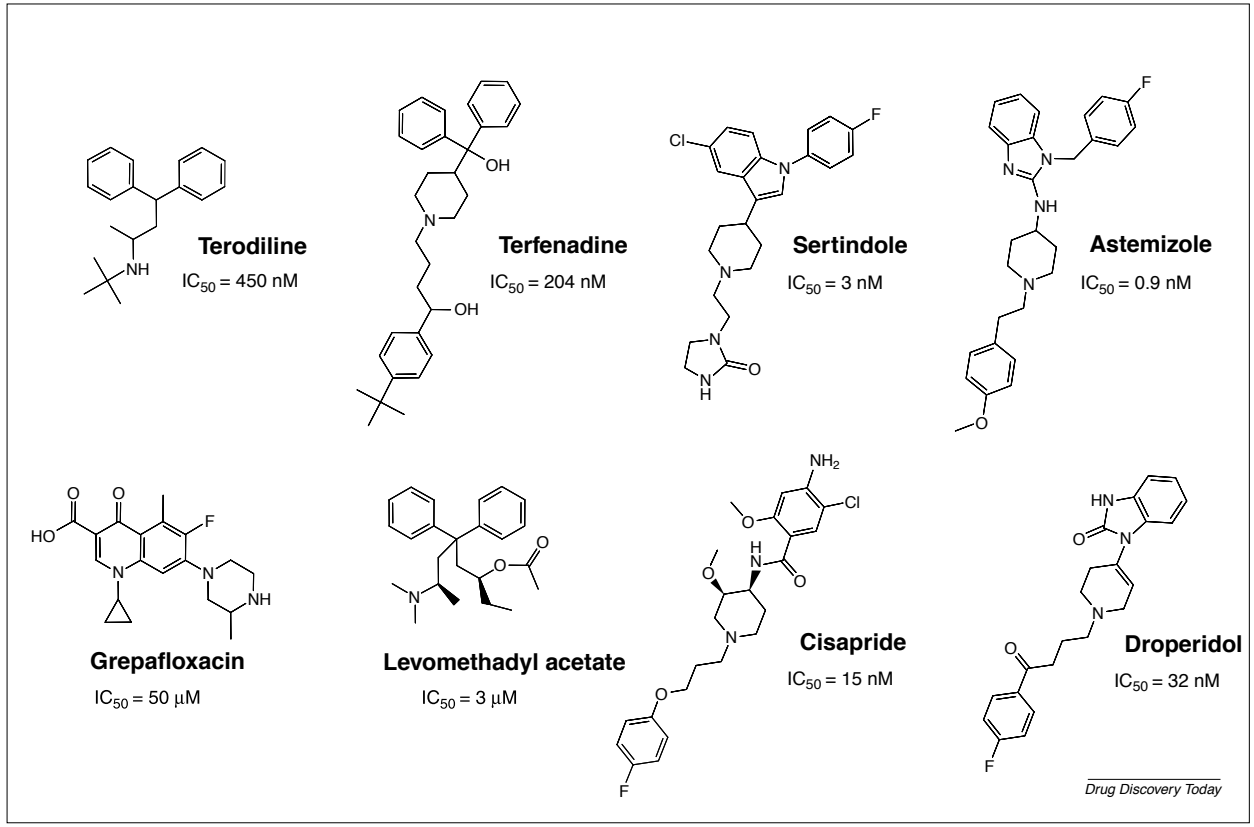
A selection of cardiovascular targets included in the *in vitro* SPP assay panel used by Novartis

Targets	Possible ADRs[a]
Ghrelin, GHS	Energy homeostasis, GH release, effects on glucose homeostasis, cardiovascular effects.
Histamine, H_3	Impairs memory, causes sedation, vasodilatation, bronchodilation, negative chronotropy and reduces gastrointestinal motility.
Muscarinic, M_1(h)	Vagal effects, blood pressure changes, secretory functions. Decreases gastric acid secretion.
Muscarinic, M_2	Vagal effects, blood pressure changes. Tachycardia.
Muscarinic, M_3	Vagal effects, blood pressure changes, salivation. Reduces incontinence, bronchoconstriction and gastrointestinal motility. Interferes with ocular accommodation, dry mouth.
Muscarinic, M_4	Vagal effects, blood pressure changes. Facilitation of D1 CNS stimulation.
NE transporter	Inhibitor increases adrenergic hyperactivity and facilitate a1 adrenergic activation.
Nicotinic acetylcholine	Stimulates autonomic cardiovascular, gastrointestinal functions. Palpitation, orthostatic hypotonia, nausea, sweating, muscle tremor, bronchial secretion. Effects on muscular and vegetative ganglionic functions.
NPY, Y_1	Antidepressant, causes vasoconstriction (venous), inhibits gut motility, gastric emptying, acid secretion, pancreatic exocrine secretions. Anxiogenic, inhibits ischemic brain injury.
Potassium Ch (hERG)	QT interval (electrocardiogram) prolongation.
Potassium Ch [ATP]	Hypotension. Hypoglycemia.
Serotonin, 5-HT_{2b}	Cardiac valvulopathy.
Serotonin, 5-HT_4	Facilitates gastrointestinal transit, mechanical intestinal allodynia. Useful in treatment of irritable bowel syndrome, cardiac arrhythmias.
Sodium Ch (site 2)	Antagonist causes cardiac arrhythmia.
Thromboxane a2 receptor, TP	Facilitates vascular, uterine and bronchial constriction, gastrointestinal spasm, allergic inflammation and platelet aggregation. Useful in treatment of chronic productive cough, thrombosis, atherosclerosis.
Vasopressin V_{1a}	Vasopressor.
Vasopressin V_{1b}	Vasopressor, anxiogenic.

[a]Possible ADRs or other physiological effects expected to occur when these targets are hit by compounds. Most of these targets are included in available *in vitro* assay sets provided by various CROs for early *in vitro* safety testing.

Receptors with much Promiscuity

- **hERG channel**: almost 50% of developed leads displayed affinity with an IC_{50} <30μM for the hERG receptor. The hERG receptor is targeted by a large diversity of structures. Inhibition of the hERG channel produces arrhythmias and can lead to death.

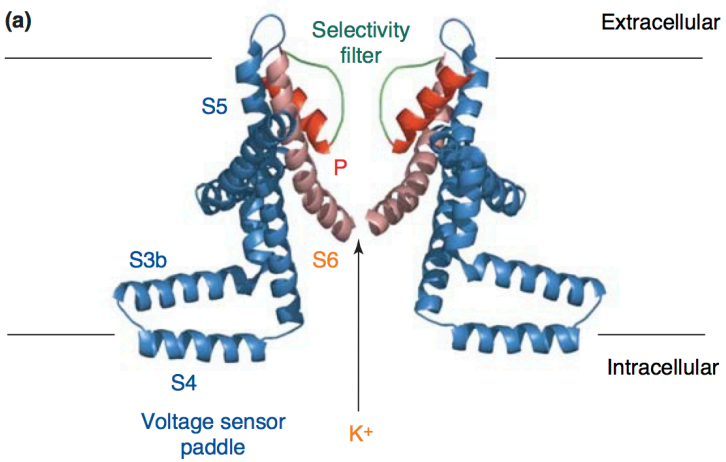


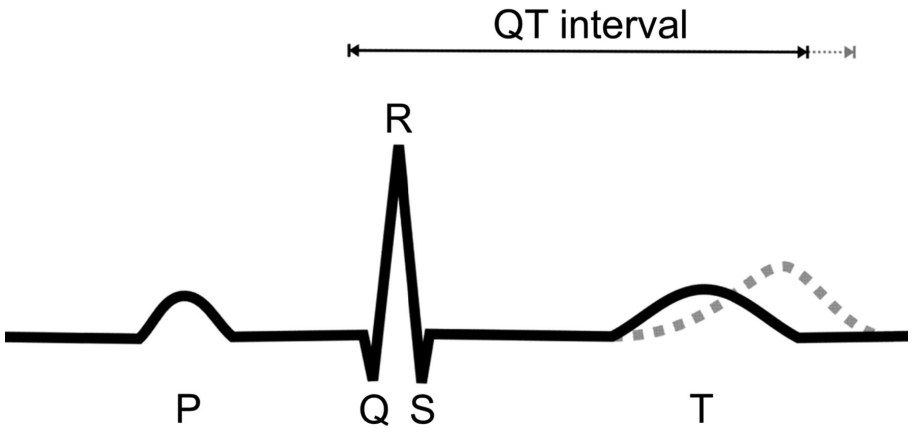
drugs withdrawn from the market because of hERG-related side-effects

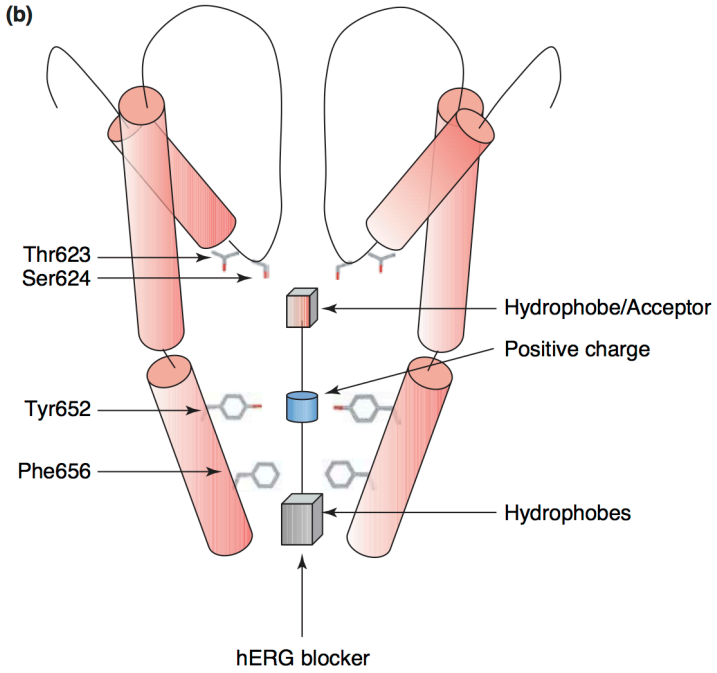
Whitebread et al, DDT ,**10** (2005), 1421

- **5-HT receptors**. Again, about 50% hit rate of leads against the $HT5_{2a}$ receptor, the serotonin receptor. This increases the probability of cardiovascular side-effects.

Blocking hERG channels





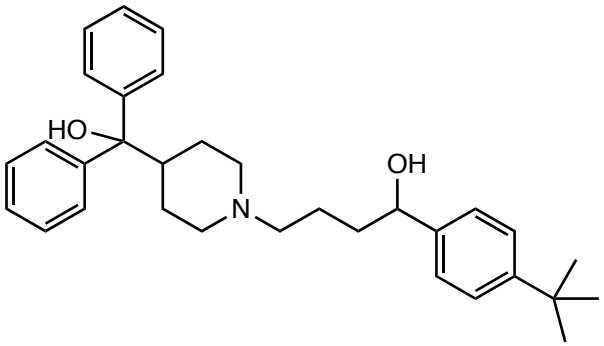


the QT interval of the ECG defines the interval of electrical pulses responsible for controlling the heart muscle cells. Delaying by 5-10 ms creates potentially dangerous effects, resulting in arrhythmias.

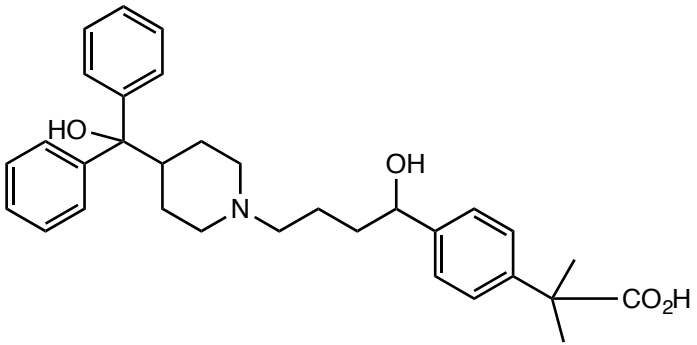
DDT ,**10** (2005), 149-155

Reducing side effects at hERG channels

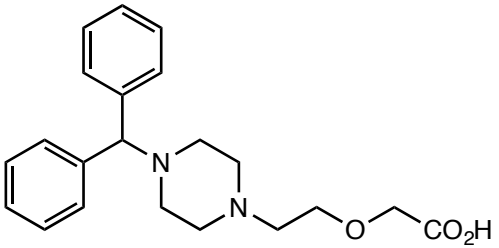
- remove or reposition or reduce basicity of amines
- add a carboxylic acid or other anionic group
- remove phenyl rings, replace with heterocyclic
- add e-withdrawing substituents to aryl rings
- rigidify or cyclise the molecule



Terfenadine
hERG IC_{50}=25 nM



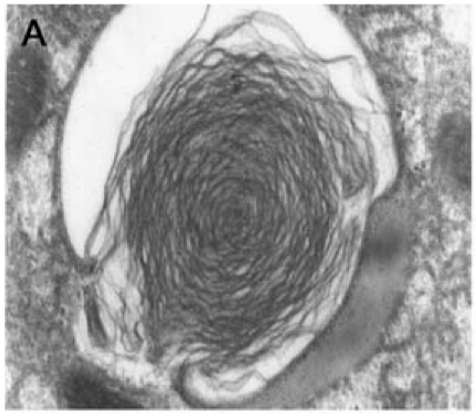
Fexofenadine
hERG IC_{50}> 30 uM

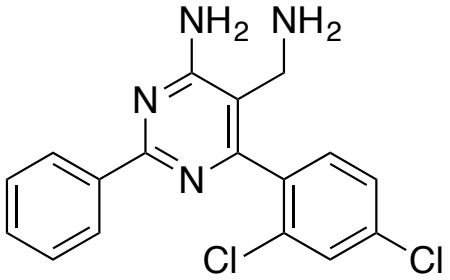


Cetrizine
no hERG activity
no QT prolongation

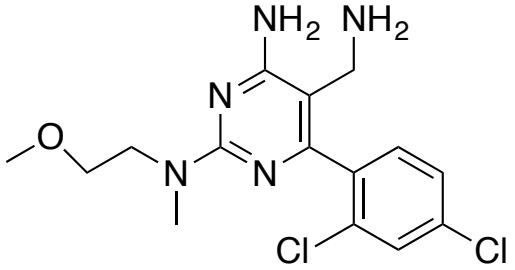
Drug-induced phospholipidosis

- Drug-induced phospholipidosis is a lysosomal storage disorder characterized by intracellular accumulation of phospholipids with **lamellar bodies**, most likely from an impaired phospholipid metabolism of the lysosome
- Organs affected by phospholipidosis exhibit inflammatory reactions and histopathological changes
- The mechanism involves trapping of drugs within the lysosomes followed by formation of drug-phospholipid complexes leading to a gradual accumulation of multi-lammetlar bodies.
- Affects amphiphilic, basic drugs





phospholipidosis
in fibroblasts



no phospholipidosis

reduction of lipophilicity
removal of basic sites

the case of Vioxx™ (Merck)

Rofecoxib

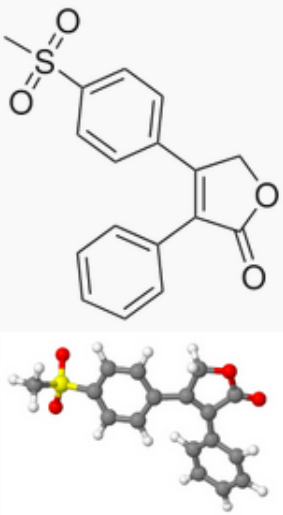
From Wikipedia, the free encyclopedia

Rofecoxib /ɹɒfɨˈkɒksɪb/ is a nonsteroidal anti-inflammatory drug (NSAID) that has now been withdrawn over safety concerns. It was marketed by Merck & Co. to treat osteoarthritis, acute pain conditions, and dysmenorrhea. Rofecoxib was approved by the Food and Drug Administration (FDA) on May 20, 1999, and was marketed under the brand names **Vioxx**, **Ceoxx**, and **Ceeoxx**.

Rofecoxib gained widespread acceptance among physicians treating patients with arthritis and other conditions causing chronic or acute pain. Worldwide, over 80 million people were prescribed rofecoxib at some time.[1]

On September 30, 2004, Merck withdrew rofecoxib from the market because of concerns about increased risk of heart attack and stroke associated with long-term, high-dosage use. Merck withdrew the drug after disclosures that it withheld information about rofecoxib's risks from doctors and patients for over five years, resulting in between 88,000 and 140,000 cases of serious heart disease.[2] Rofecoxib was one of the most widely used drugs ever to be withdrawn from the market. In the year before withdrawal, Merck had sales revenue of US$2.5 billion from Vioxx.[3] Merck reserved $970 million to pay for its Vioxx-related legal expenses through 2007, and have set aside $4.85bn for legal claims from US citizens.

Rofecoxib



Systematic (IUPAC) name