Pharmacokinetics

Part 2: Drug Metabolism

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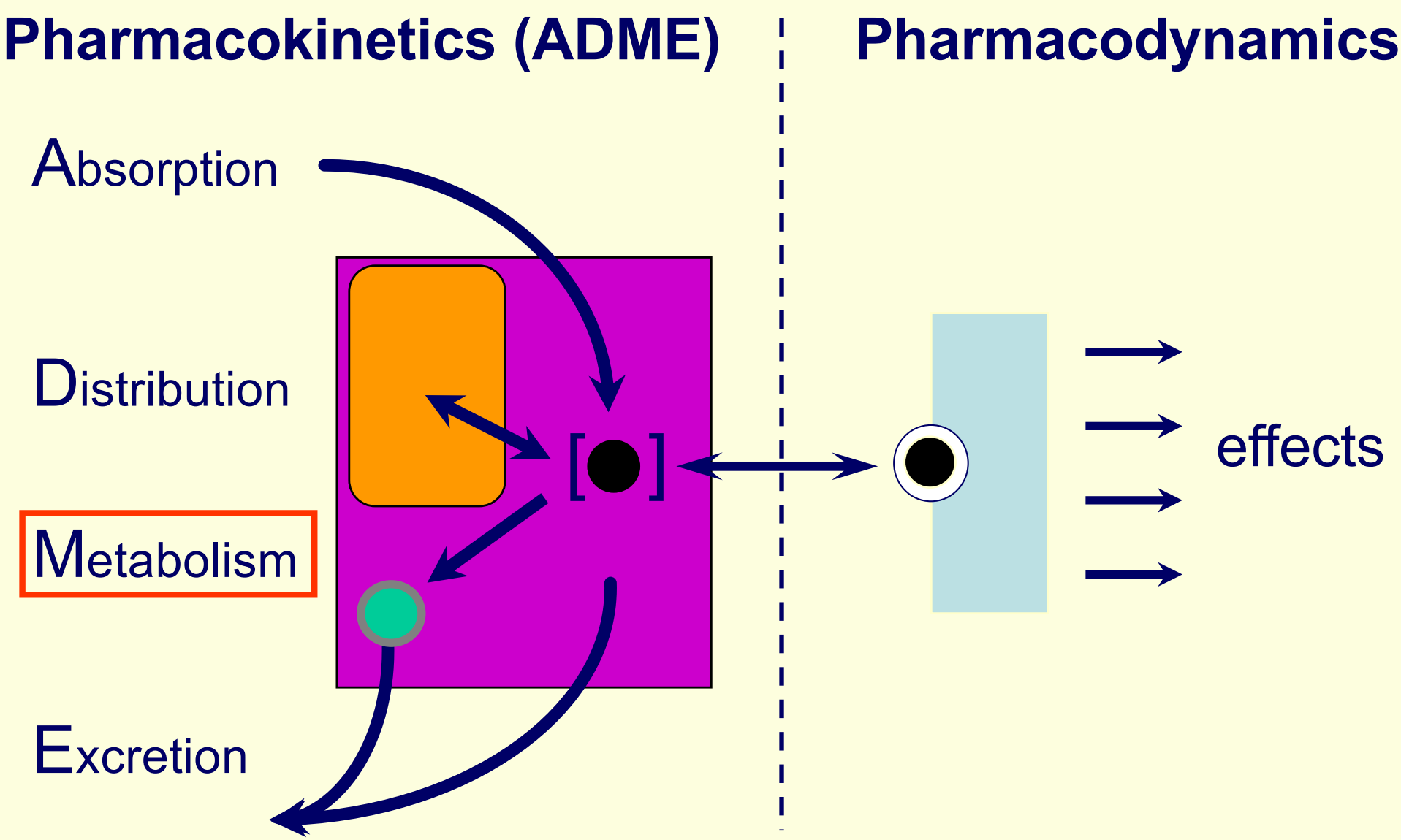
General Pharmacology



Drug Metabolism

- Drug = Xenobiotic

- Xenobiotics: Chemicals foreign to the body (manufactured or natural).
  - Drugs
  - Industrial chemicals
  - Pesticides
  - Pollutants
  - Pyrolysis products in grilled foods
  - Toxins produced by molds, plants, animals

- Biotransformation (metabolism) of xenobiotics = protection of organism

Tissue sources of metabolizing enzymes

- Liver is the richest source.
- Tissues associated with the major routes of exposure: gastrointestinal tract, skin, lung, nasal mucosa, eye.
- Others: kidney, adrenal, pancreas, spleen, heart, brain, testis, ovary, placenta, plasma, erythrocytes, platelets, lymphocytes, aorta.

General properties of xenobiotic-metabolizing enzymes

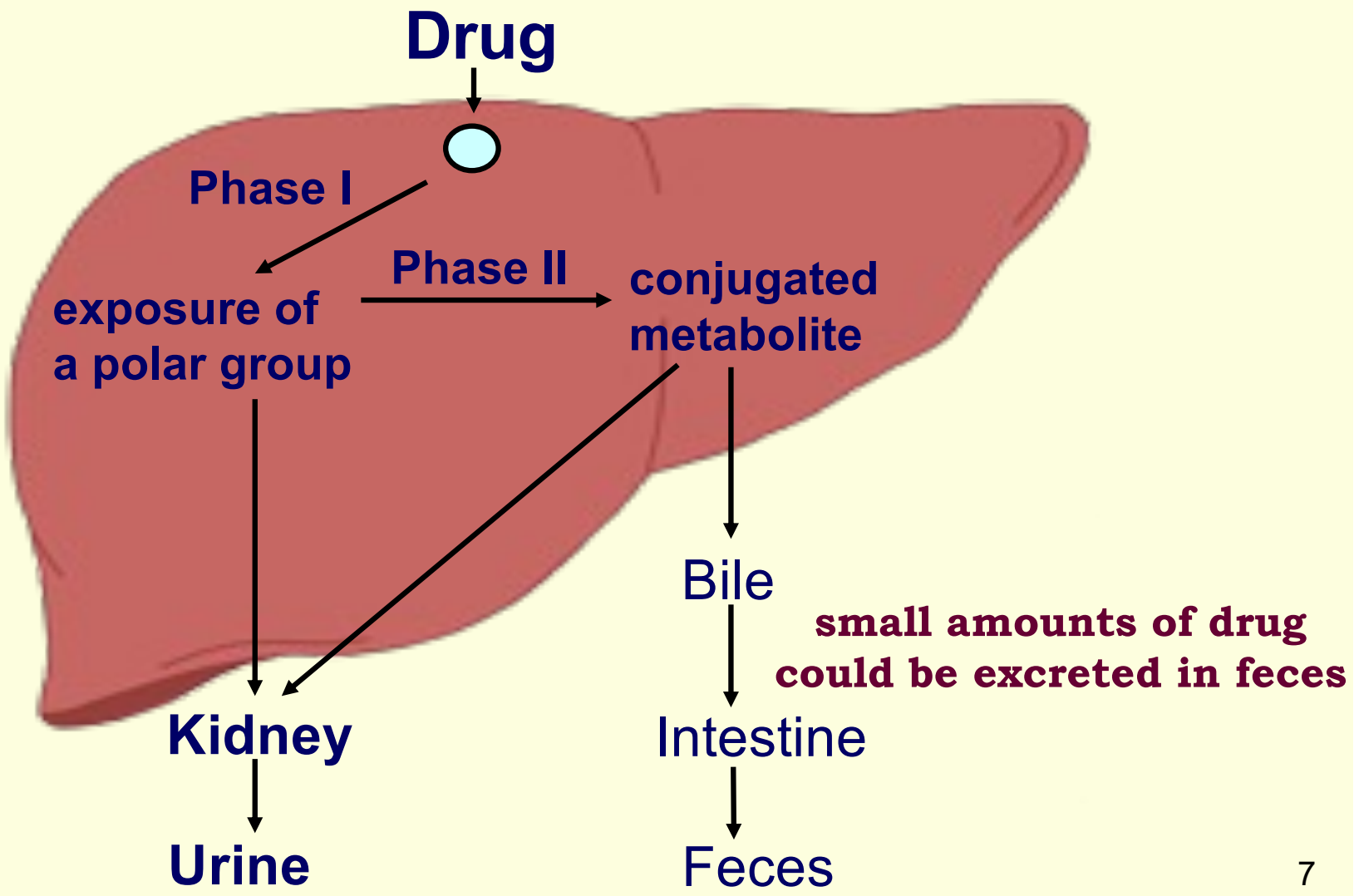
- Biotransformation is accomplished by a small number of enzymes with broad substrate specificity.
- Some xenobiotic metabolizing enzymes also metabolize endogenous compounds. Example: bile salts, bilirubin
- Metabolizing enzymes are either:
  - 1. Constitutive
  - 2. Inducible – enzyme activity is regulated by external stimuli.

Two phases of biotransformation reactions

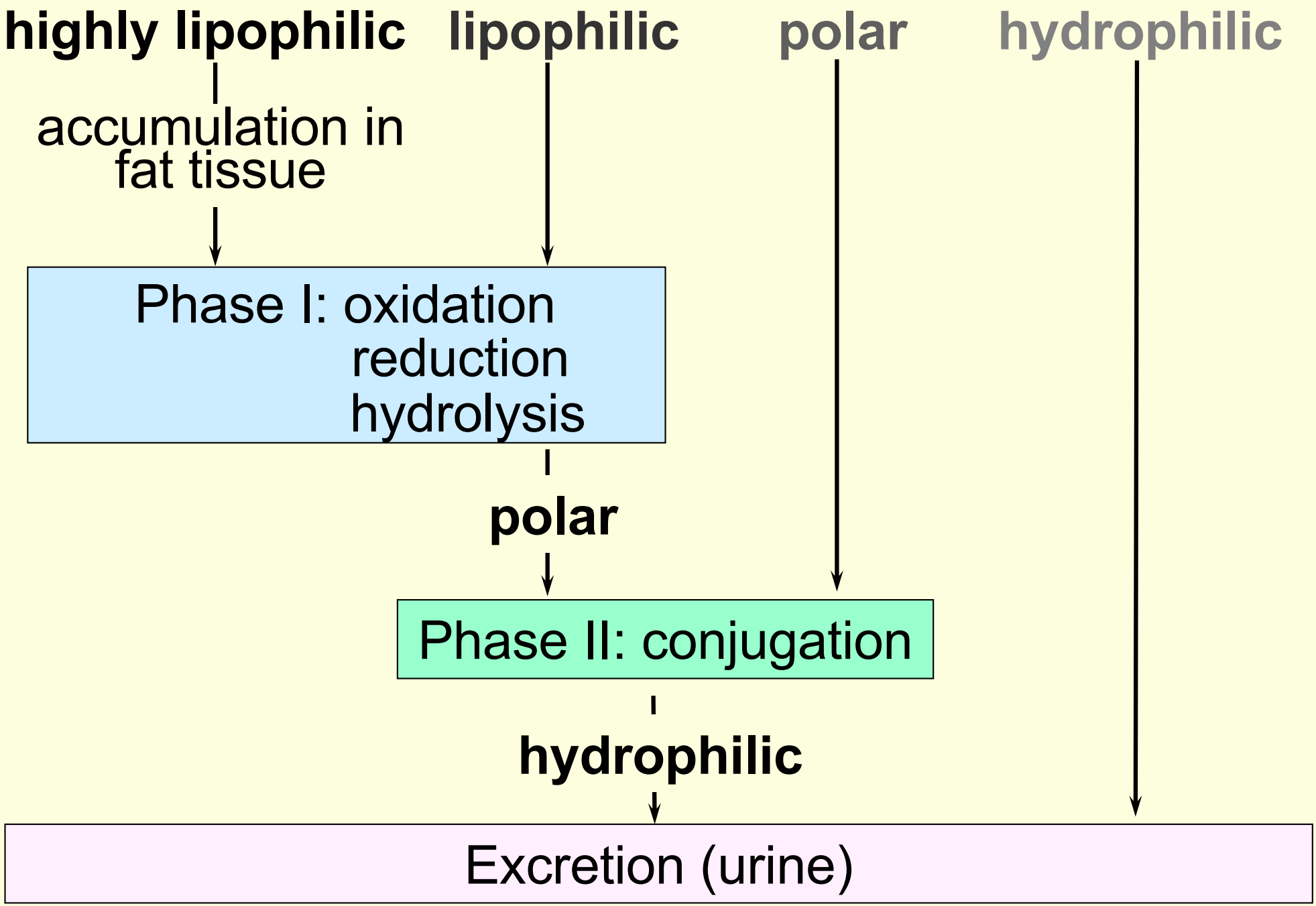
- Phase I: oxidation, reduction, hydrolysis
  - Reactions that expose or introduce a functional group (-OH, -NH2, -SH).
  - Imparts small increase in hydrophilicity.
- Phase II: conjugation
  - Covalent linkage between xenobiotic or one of its metabolites with a water-soluble endogenous compound, e.g., glutathione.
  - Imparts large increases in hydrophilicity.

Drug Metabolism

- Most drugs are metabolized in the liver and excreted by the kidney



Drug lipophilicity determines its metabolic pathway



Phase I reactions may occur by oxidation, reduction or hydrolysis reactions

Oxidation

- cytochrome P450 monoxygenase system
- flavin-containing monoxygenase system
- alcohol dehydrogenase and aldehyde dehydrogenase

Reduction

- NADPH-cytochrome reductase
- reduced (ferrous) cytochrome P450

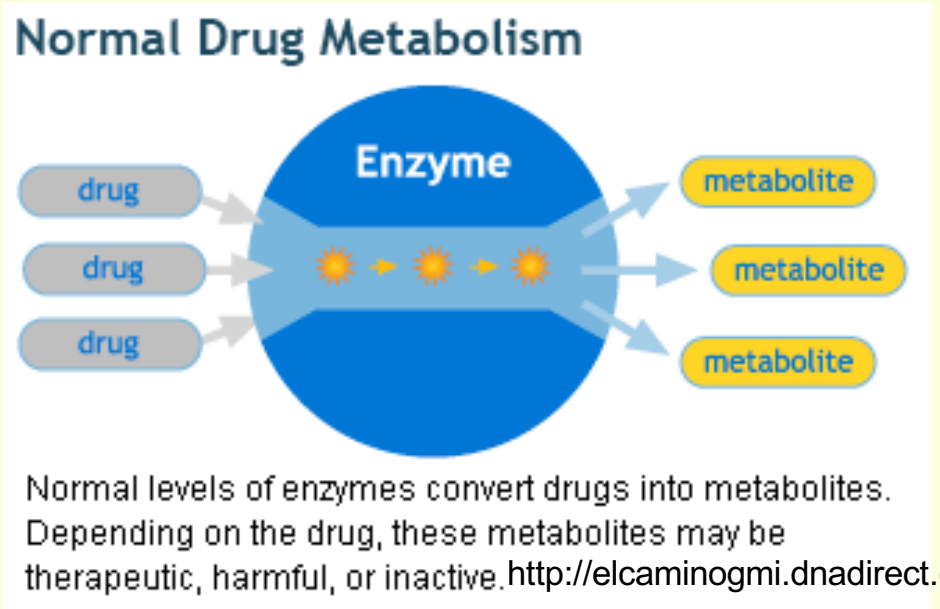
Hydrolysis

- esterases and amidases
- epoxide hydrolase

Subcellular localization of Phase I enzymes

Reaction	Enzyme	Localization
	Phase I	
Hydrolysis	Esterase	Microsomes, cytosol, lysosomes, blood
	Peptidase	Blood, lysosomes
	Epoxide hydrolase	Microsomes, cytosol
Reduction	Azo- and nitro-reduction	Microflora, microsomes, cytosol
	Carbonyl reduction	Cytosol, blood, microsomes
	Disulfide reduction	Cytosol
	Sulfoxide reduction	Cytosol
	Quinone reduction	Cytosol, microsomes
	Reductive dehalogenation	Microsomes
Oxidation	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Mitochondria, cytosol
	Aldehyde oxidase	Cytosol
	Xanthine oxidase	Cytosol
	Monoamine oxidase	Mitochondria
	Diamine oxidase	Cytosol
	Prostaglandin H synthase	Microsomes
	Flavin-monooxygenases	Microsomes
	Cytochrome P450	Microsomes

Properties of Phase I Metabolites



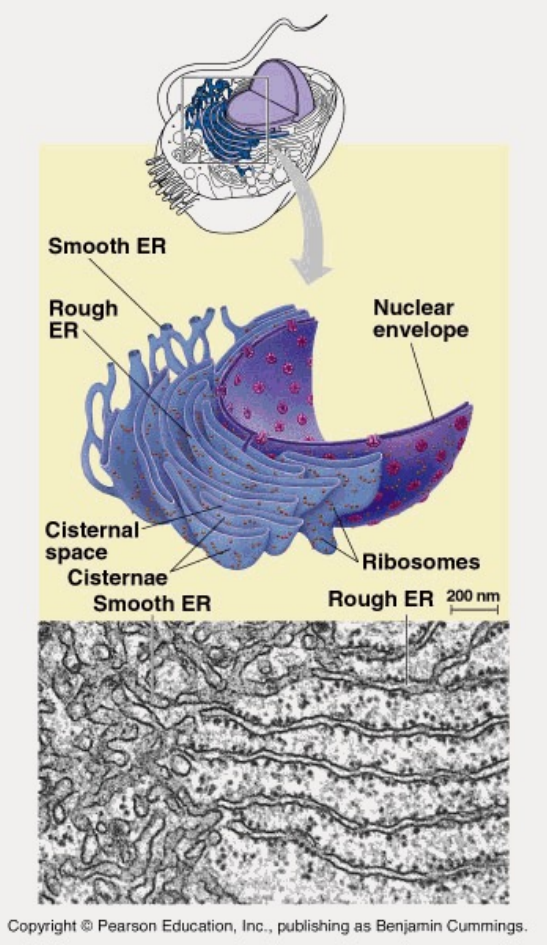
Normal levels of enzymes convert drugs into metabolites. Depending on the drug, these metabolites may be therapeutic, harmful, or inactive. http://elcaminogmi.dnadirect.com

EXAMPLES:

- ✓ Inactive
  e.g. many
- ✓ Equally Active
  e.g. fluoxetine (Prozac) → norfluoxetine
- ✓ More Active
  e.g. losartan → active metabolite E-3174
- ✓ Toxic
  e.g. acetaminophen → N-acetyl-p-benzoquinoneimine
- ✓ Activating of « prodrug »
  e.g. codeine → morphine

Phase I reactions – cytochrome P450

- cytochrome P450 are heme-containing mono-oxygenases that are responsible for ~75% of drug metabolic transformations
- cytochrome P450 enzymes are located in the ER (microsomes)
- highest concentration in the liver
- represent about 2.5% of total hepatic microsomal protein



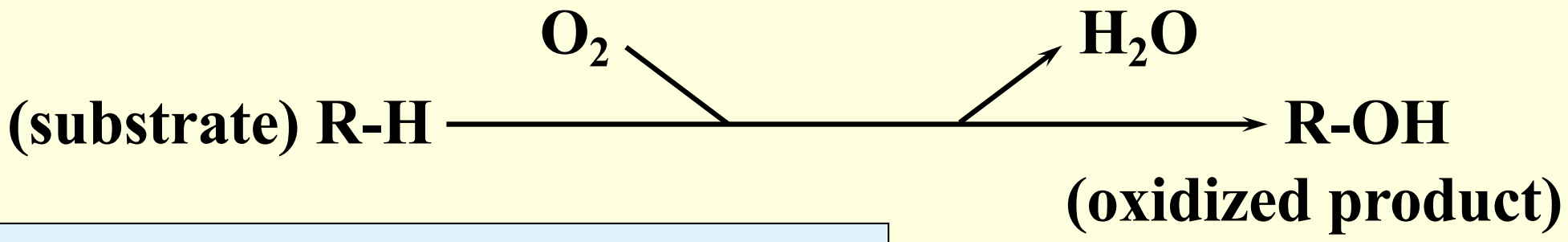
P450 enzymes - historical

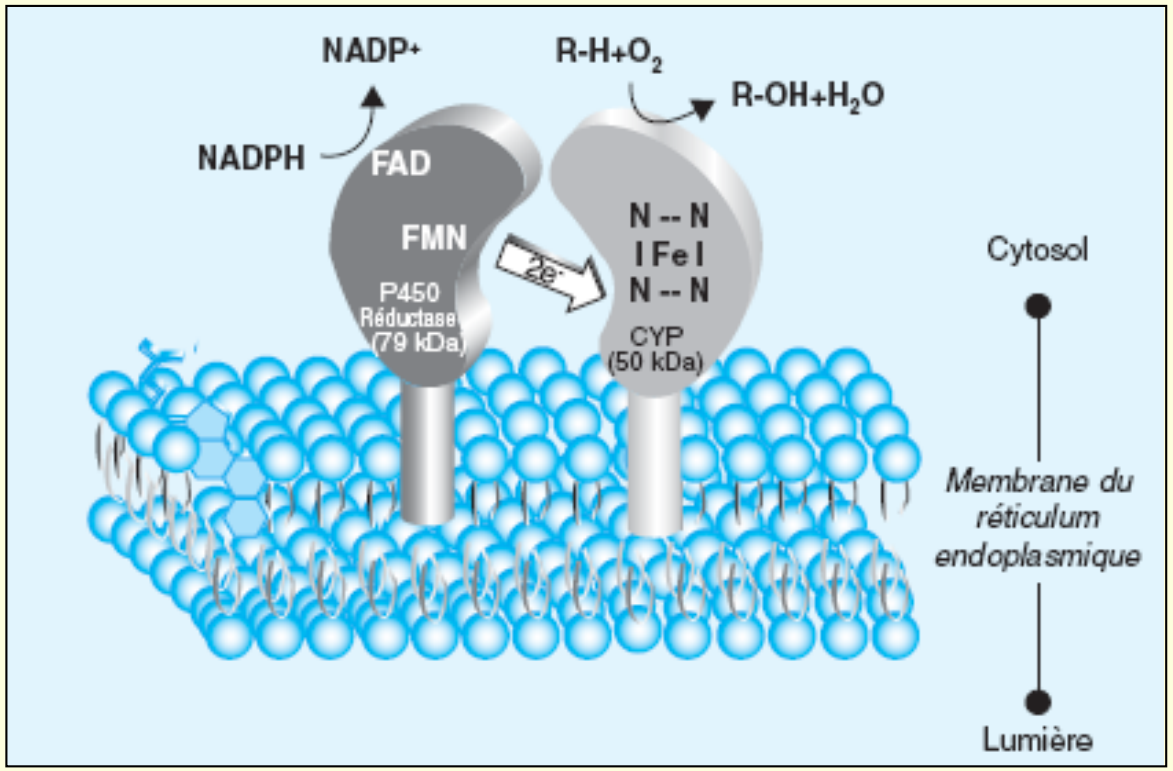
- The name P450 originates from the initial beliefs that these enzymes are similar to the mitochon-drial cytochromes and because under certain conditions they maximally absorb light at 450 nm.
- Cytochrome P450 gene family is believed to be the product of an ancestral gene formed about 3 billion years ago (existed in *Eubacteria* & *Archaea*).
- It is thought that P450s are the result of evolution of plants producing toxins and animals evolving enzymes to detoxify these chemicals.

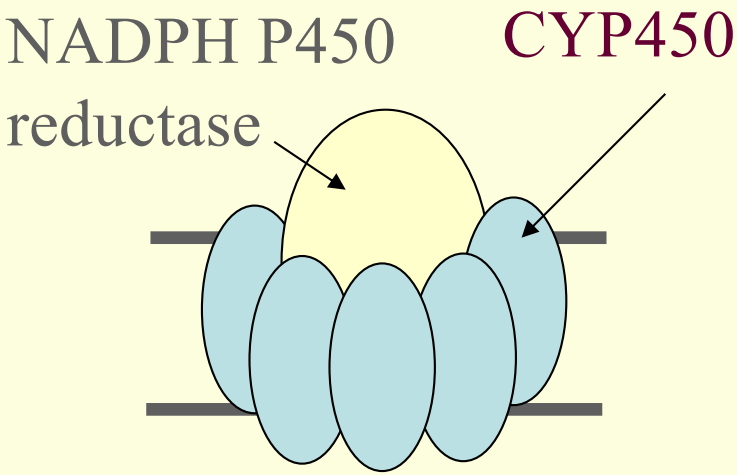
CYP450 reaction

Definition: - multienzymatic system (CYP450 + reductase)

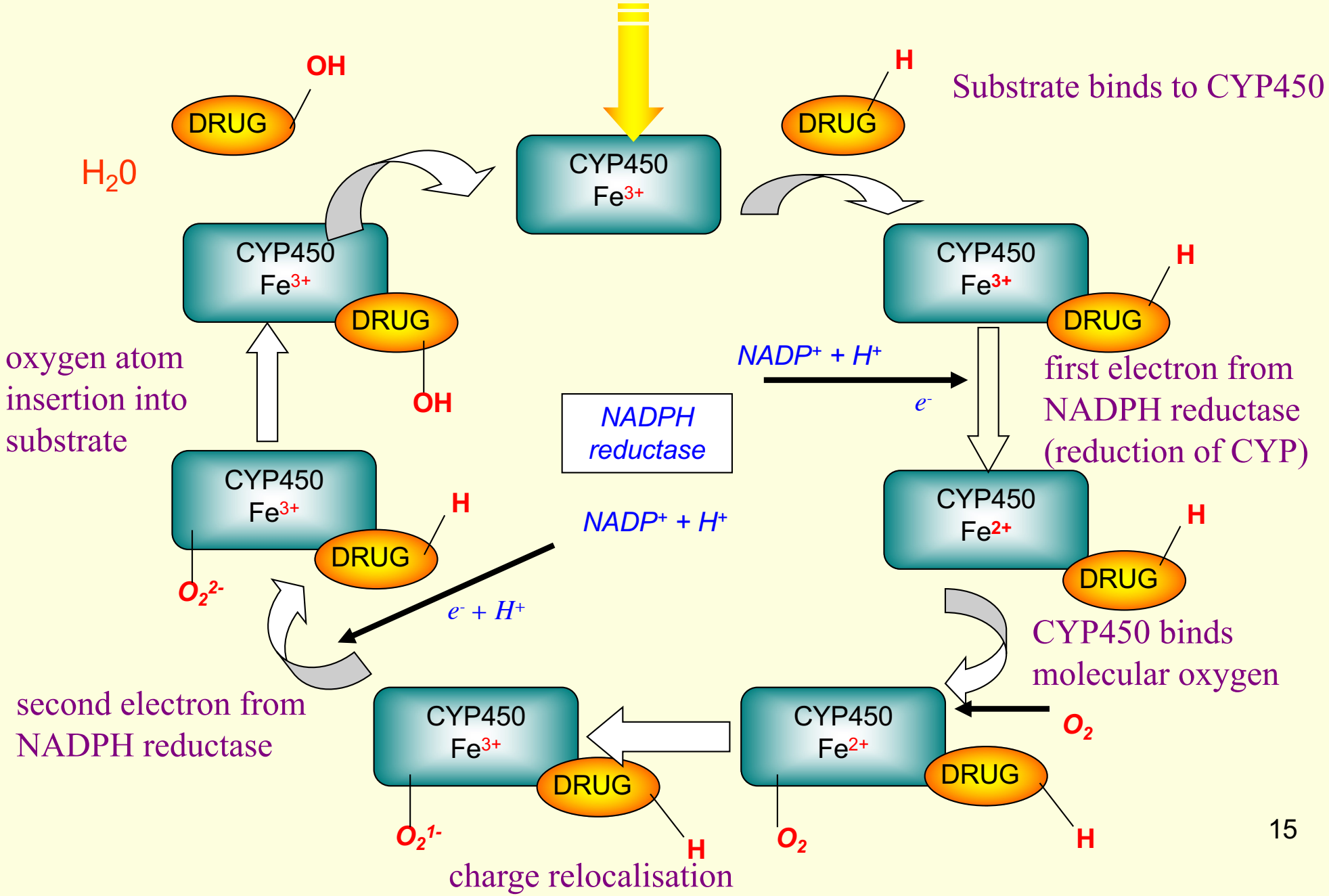
- transfer of 1 atom of oxygen from O_2 to substrate
- reduction of the second O in H_2O



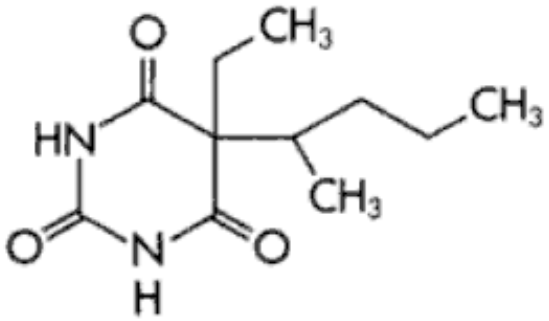
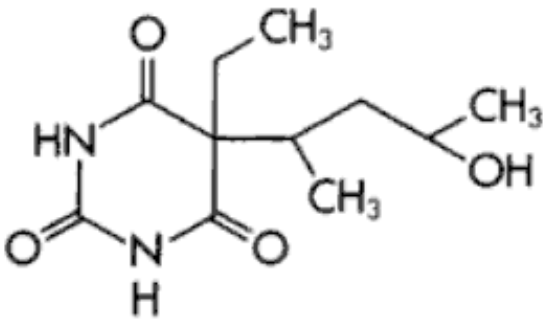
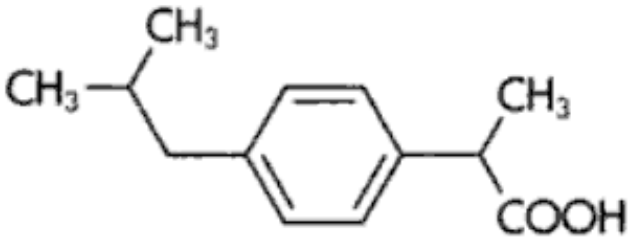
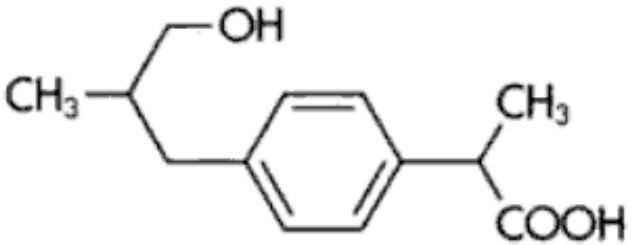
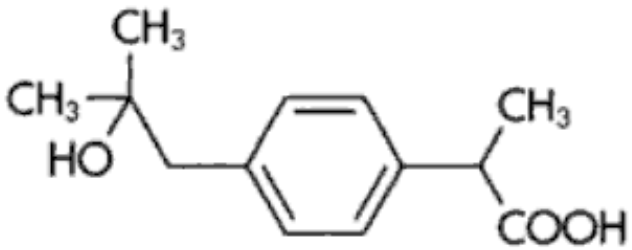




Phase I – CYP450 Reaction Sequence

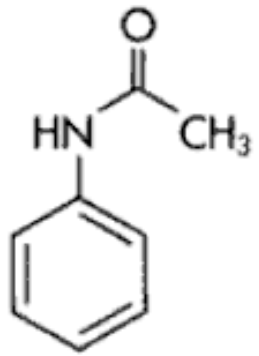


Phase I – Examples of oxidation reaction

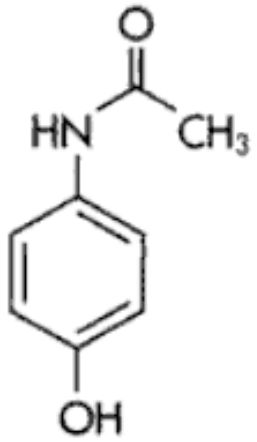
Substrate	*Product(s)*
1. Side-chain oxidation	
 Pentobarbital (sedative)	
 Ibuprofen (nonsteroidal anti-inflammatory)	
	

Phase I – Examples of oxidation reaction

2. Aromatic ring oxidation

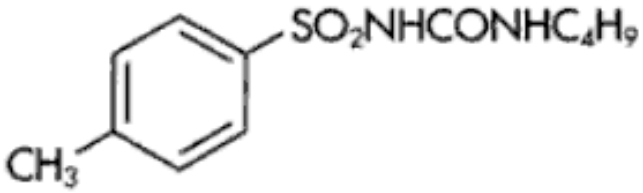


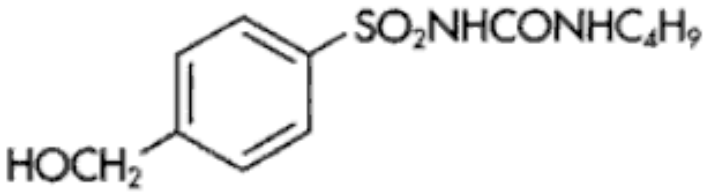
Acetanilide
(antipyretic and analgesic)



Paracetamol
(non-opioid antipyretic and analgesic)

3. Methyl oxidation



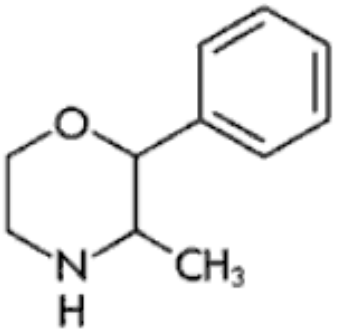


Tolbutamide (↑ insulin secretion)

Phase I – Examples of oxidation reaction

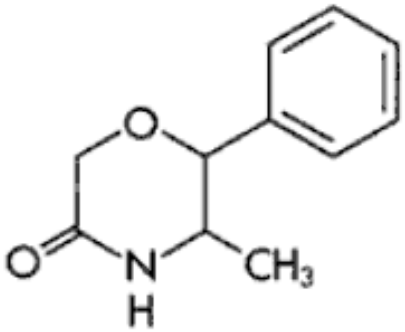
Substrate	*Product(s)*

4. Heterocyclic ring oxidation

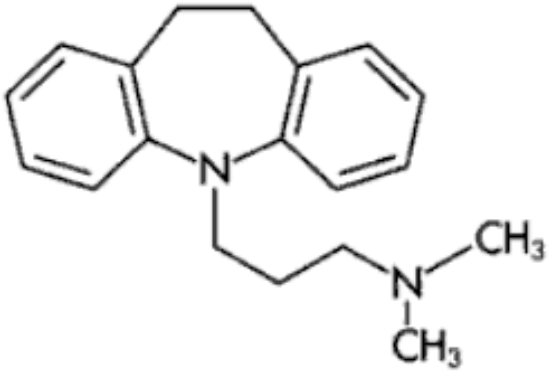


Phenmetrazine

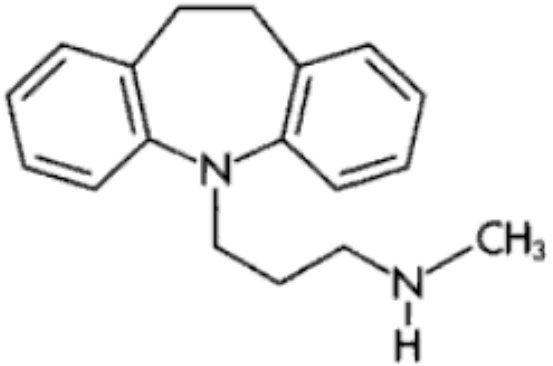
(appetite suppresant)



5. *N*-Dealkylation



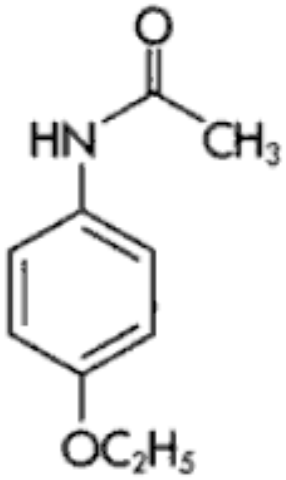
Imipramine (antidepressant)



Desipramine (antidepressant)

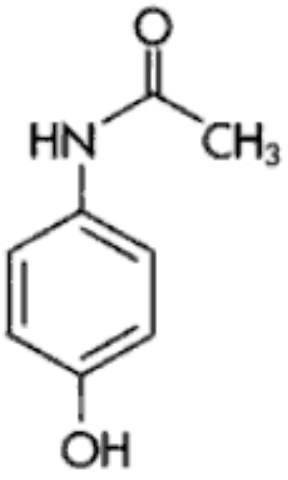
Phase I – Examples of oxidation reaction

6. *O*-Dealkylation



Phenacetin

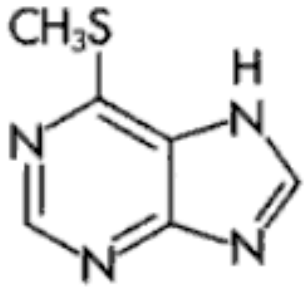
(non-opioid antipyretic and analgesic)



Paracetamol

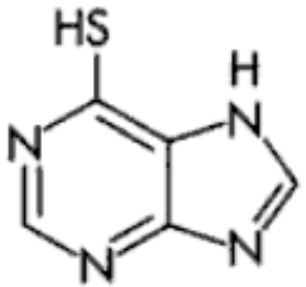
(non-opioid antipyretic and analgesic)

7. *S*-Dealkylation



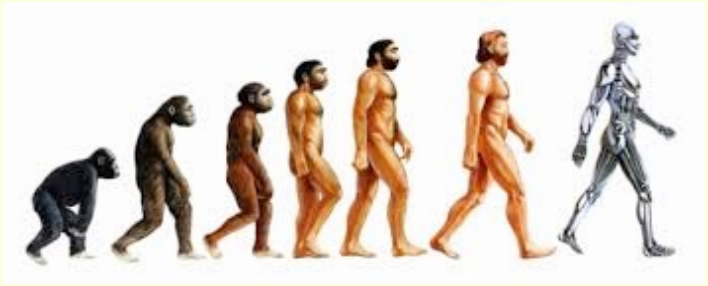
6-Methylmercaptopurine

(chemotherapy)



6-Mercaptopurine

Cytochrome P450 gene families



Human 18

Molluscs 1

CYP450

Plants 22

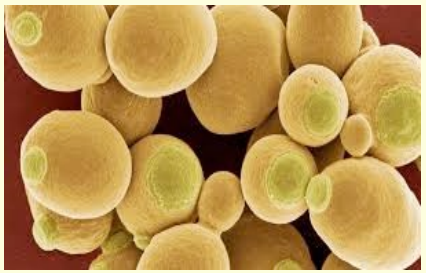
Insects 3

Bacteria 18

Fungi 11

Yeasts 2

Nematodes 3



CYP450 genes in human:

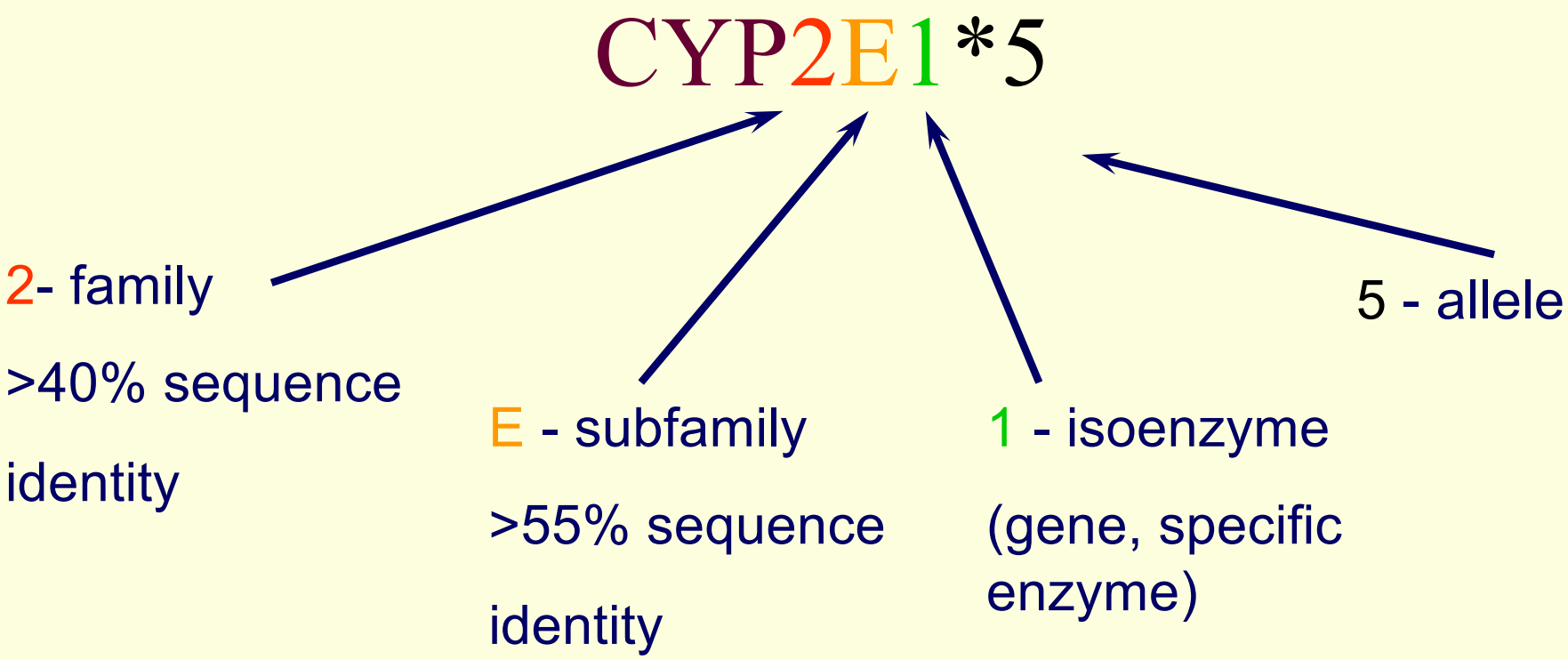
18 families, 41 subfamilies, 57 genes, 59 pseudogenes

Three families are involved in drug metabolism (CYP 1, 2 and 3)

Remaining 15 families are involved in normal physiological/homeostatic functions including the biosynthesis or degradation of :

Cholesterol
Bile acids
Steroid hormones
Vitamin D3
Arachidonic acid metabolites
other

CYP450 naming



Isoenzymes of drug-metabolizing cytochrome P450

CYP1A1

CYP1A2

CYP2A6

CYP2B6

CYP2C9

CYP2C19

CYP2D6

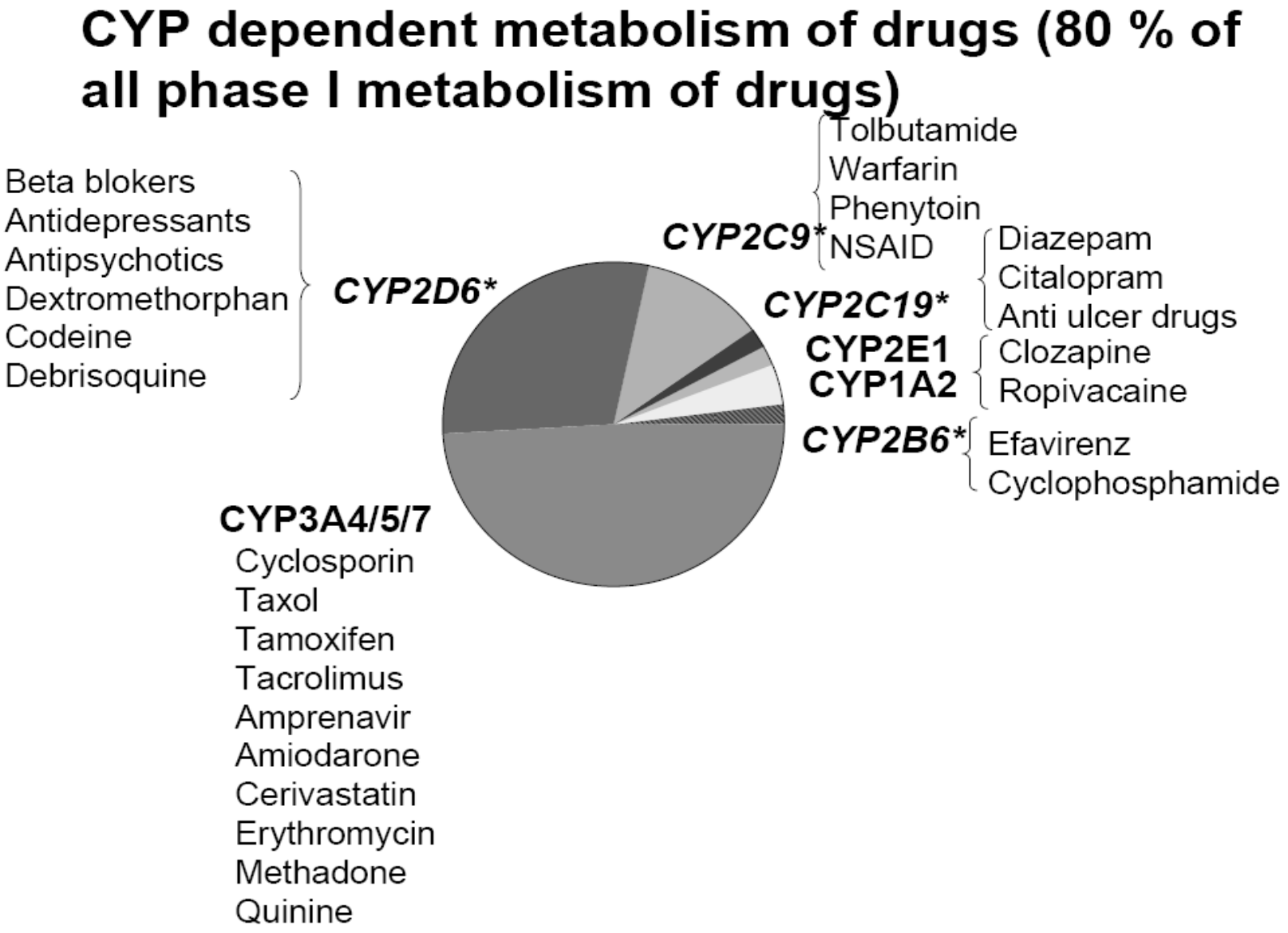
CYP2A1

CYP3A4

CYP3A5

CYP3A7

Relative importance of CYPs in drug metabolism



Other Phase I enzymes: hydrolysis

Carboxylesterases

Hydrolyze esters, amides, and thioesters

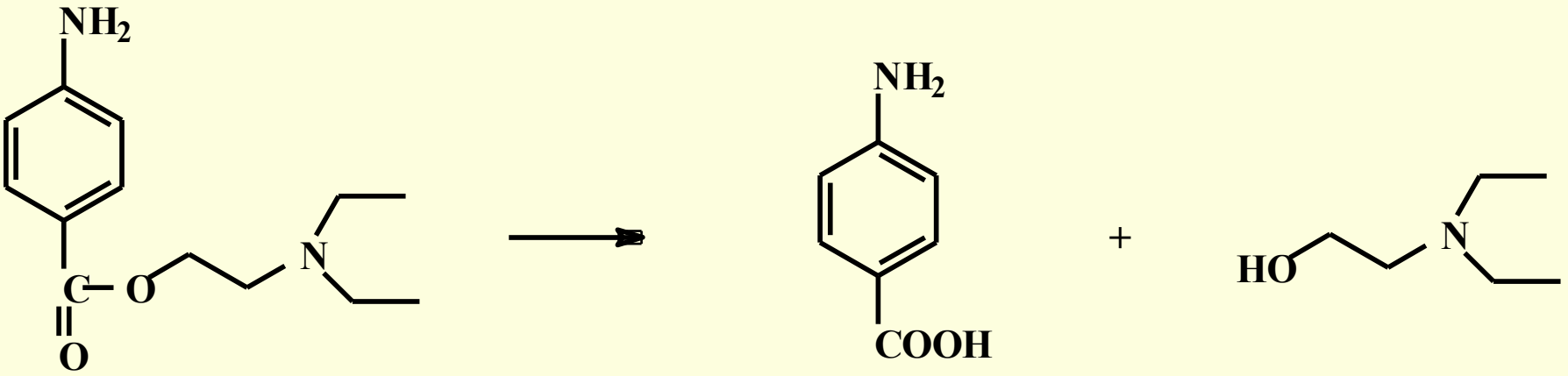
Organophosphatases

Hydrolyze phosphoric acid esters

- important for metabolism of some drugs and of insecticides/pesticides

Other Phase I enzymes: hydrolysis

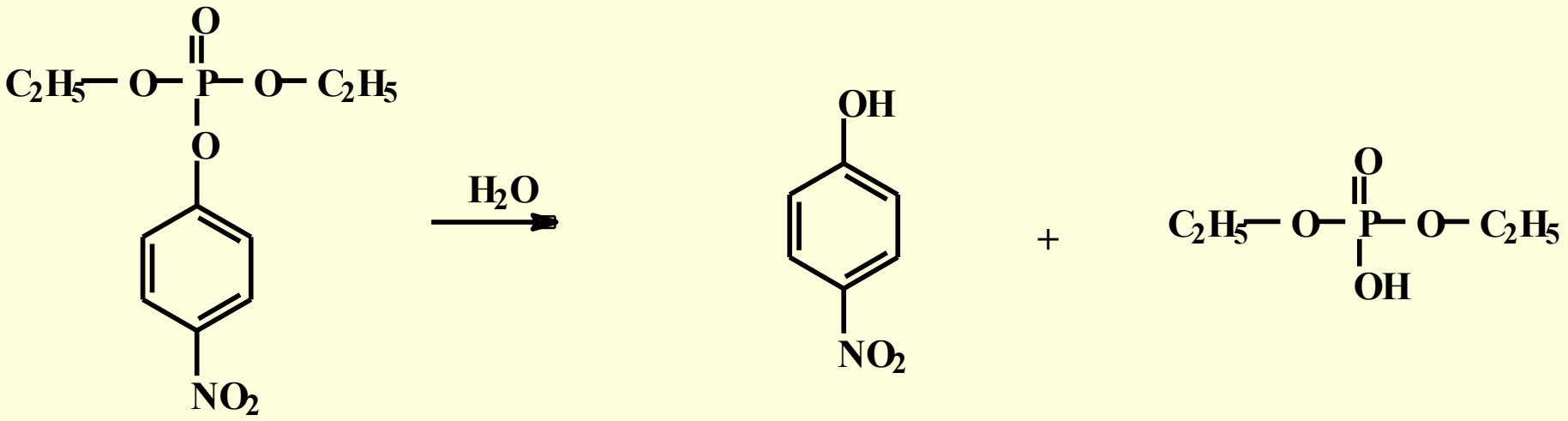
Procaine – local anesthetic



carboxylesterase

Other Phase I enzymes: hydrolysis

Paraoxon (parasympathomimetic)

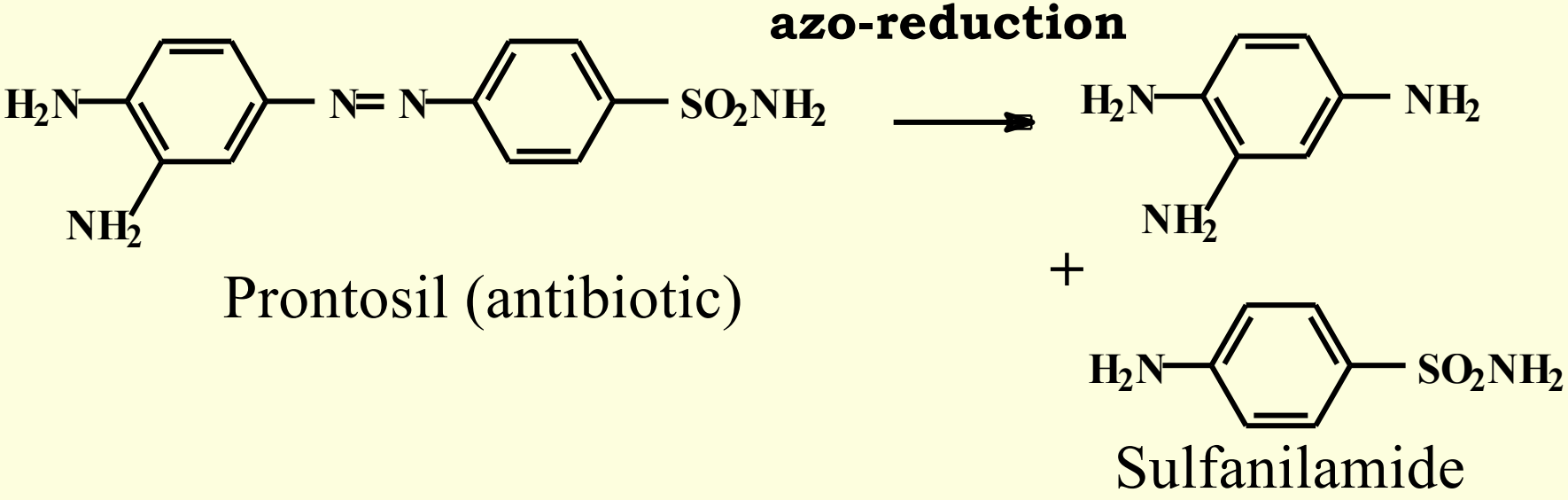


organophosphatase

Other Phase I enzymes: reduction

Reduction of alkenes, nitro- or azo-compounds can occur in the intestine by:

- The intrinsic enterocytes enzymatic system
- Bacterial (intestinal microflora) enzymatic system



Prontosil (antibiotic)

Sulfanilamide

Phase II reactions –

Drugs conjugation to a water soluble adduct using polar handles from Phase I reactions

Examples:

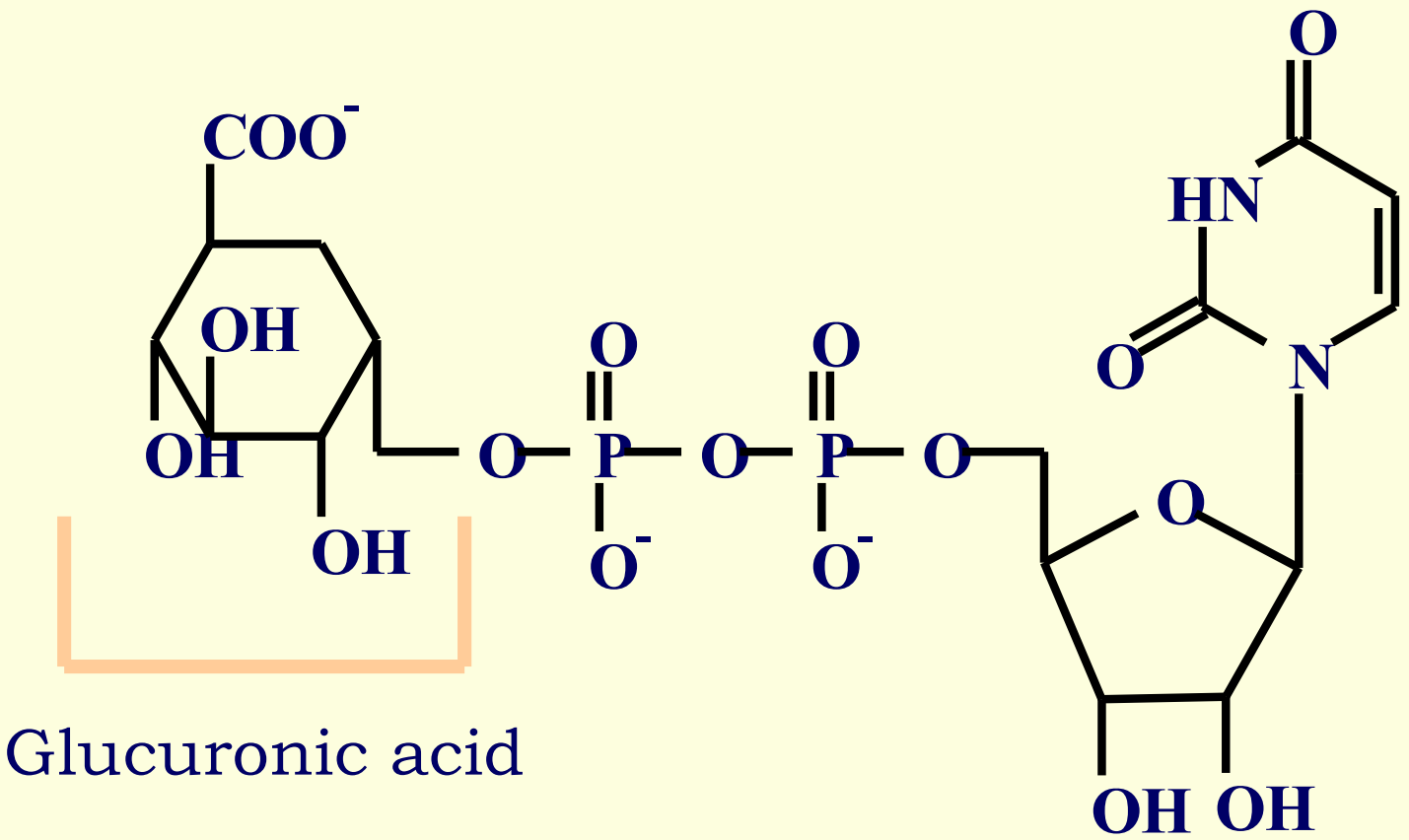
- glutathione-S-transferases (GST)
- UDP-glucuronosyltransferases (UGT)
- Sulfotransferases (SULT)
- N-acetyltransferases (NAT)
- Amino acid N-acetyl transferases

Cellular localization of Phase II enzymes

Phase II	
Glucuronide conjugation	Microsomes
Sulfate conjugation	Cytosol
Glutathione conjugation	Cytosol, microsomes
Amino acid conjugation	Mitochondria, microsomes
Acylation	Mitochondria, cytosol
Methylation	Cytosol, microsomes, blood

Phase II: Glucuronidation

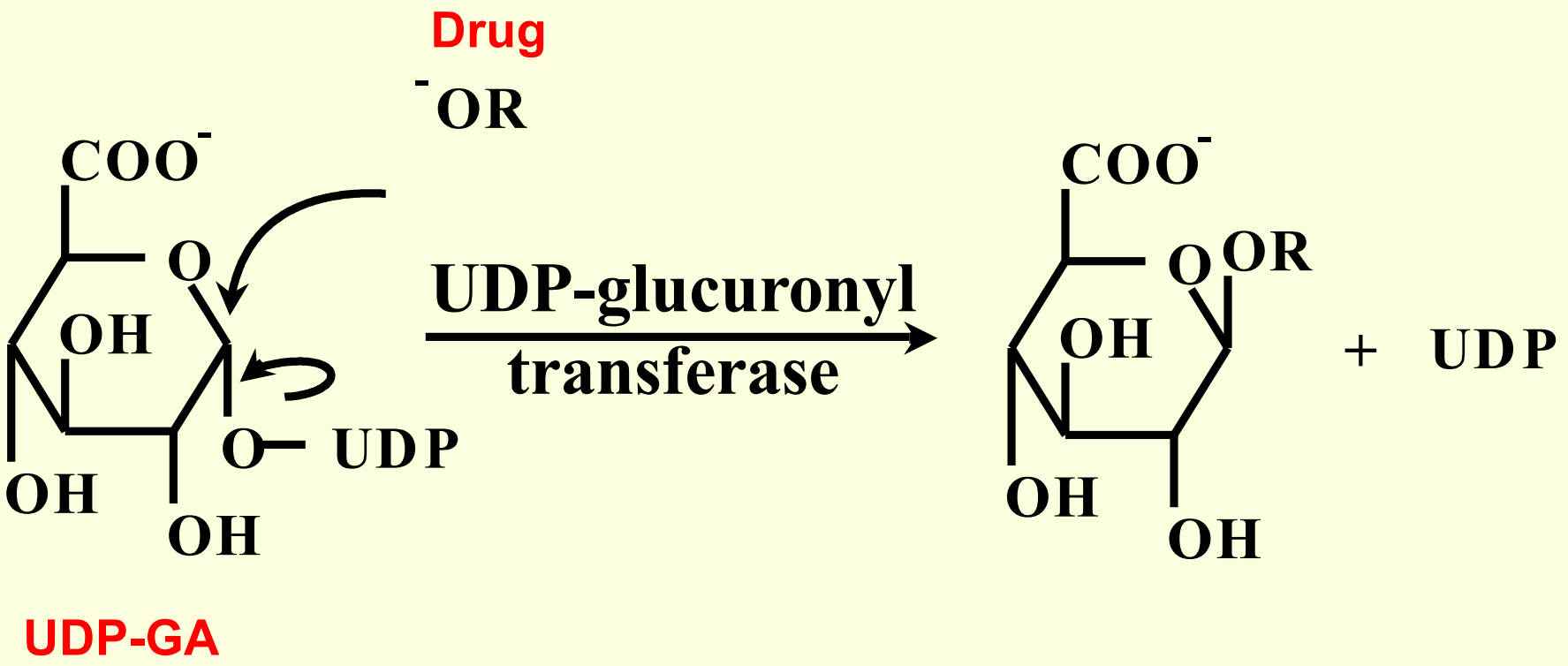
Required cofactor:
Uridine-5-diphospho-D-glucuronic acid (UDP-GA)



Glucuronic acid

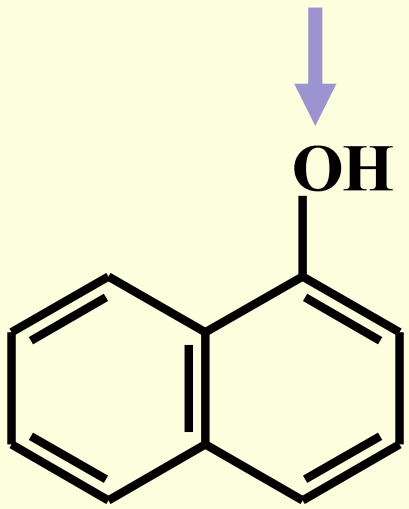
Glucuronidation reaction

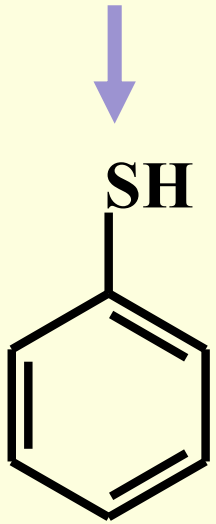
Required enzyme: UDP-glucuronyl transferase

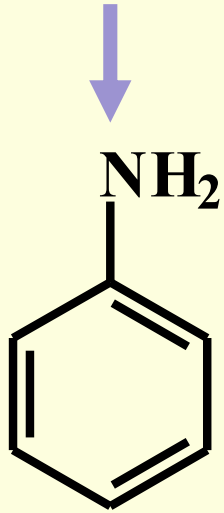


UDP-Glucuronyl transferase

- Conjugates glucuronic acid to alcohols, phenols, carboxylic acids, aromatic and aliphatic amines, and free sulfhydryl groups.
- In general, reaction occurs with electron rich nucleophilic heteroatoms (O, N, or S).



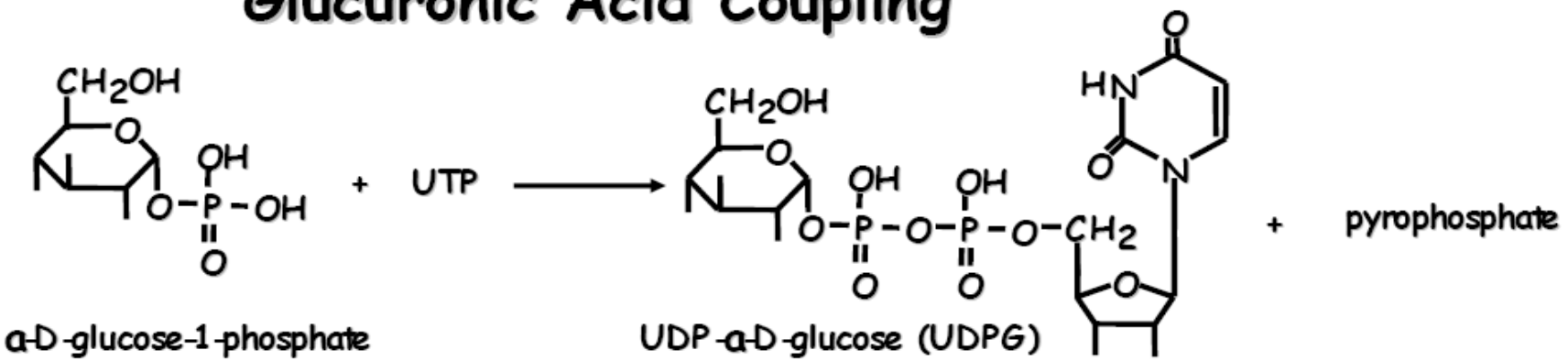




Glucuronidation reaction: paracetamol

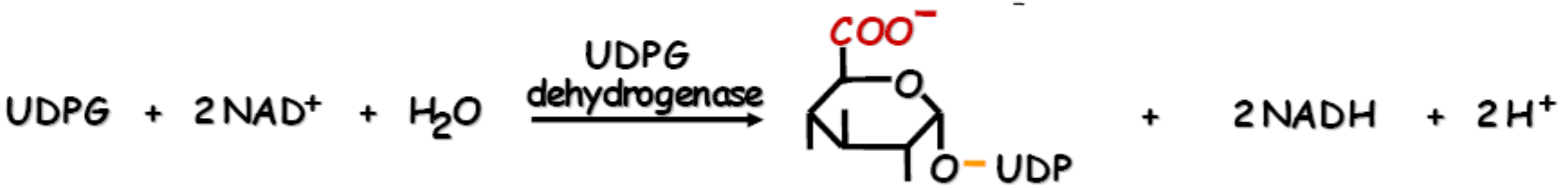
Phase II

Glucuronic Acid Coupling



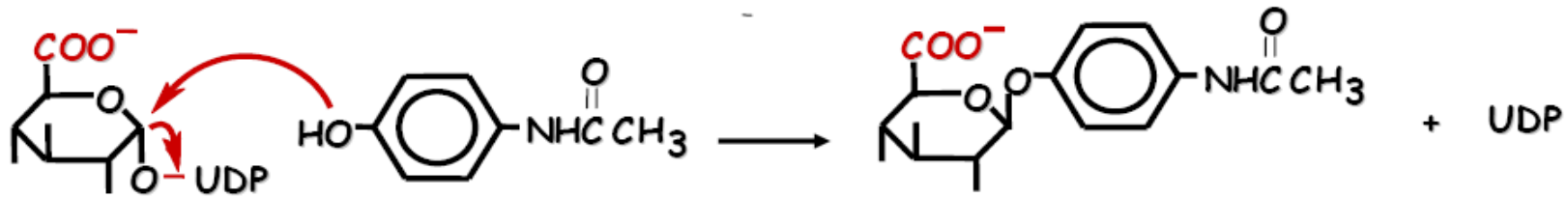
α-D-glucose-1-phosphate

UDP-α-D-glucose (UDPG)



UDP-α-D-glucuronic acid (UDPGA)

n.b.- cellular synthesis of UDPGA is an energy costly process

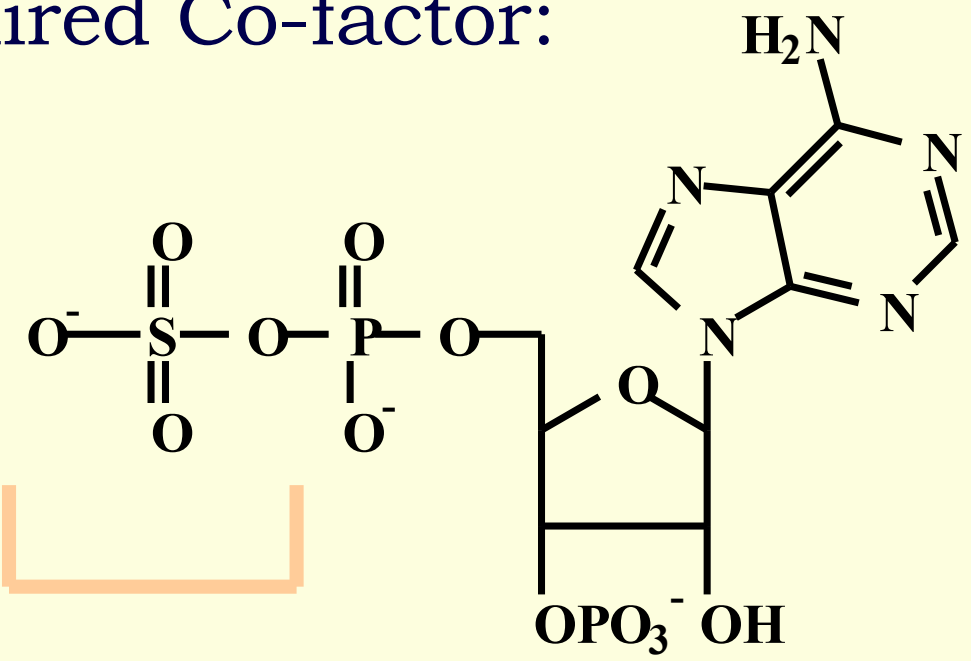


UDPGA

paracetamol

Phase II reactions: Sulfation

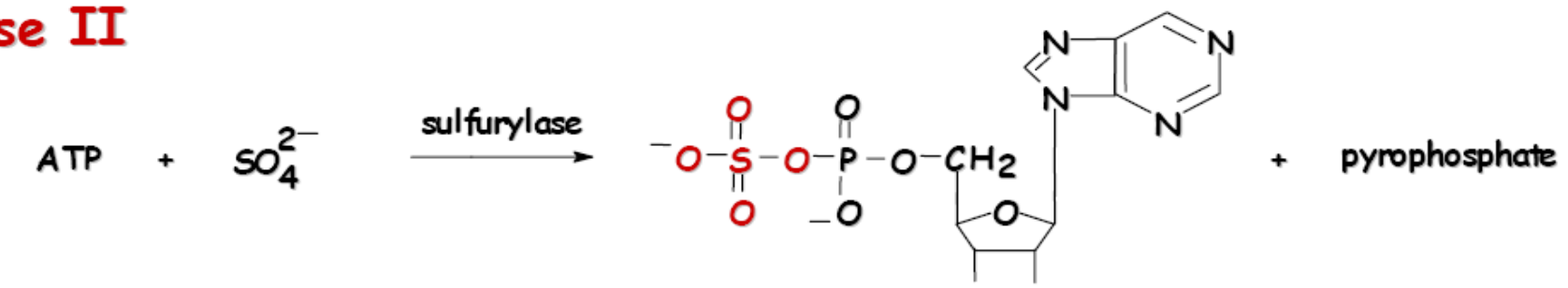
- Sulfation reactions occur for many of the same compounds that are glucuronidated.
- Enzyme: sulfotransferases (cytosolic)
  - Required Co-factor:



3’-phosphoadenosine-5’-phosphosulfate (PAPS)

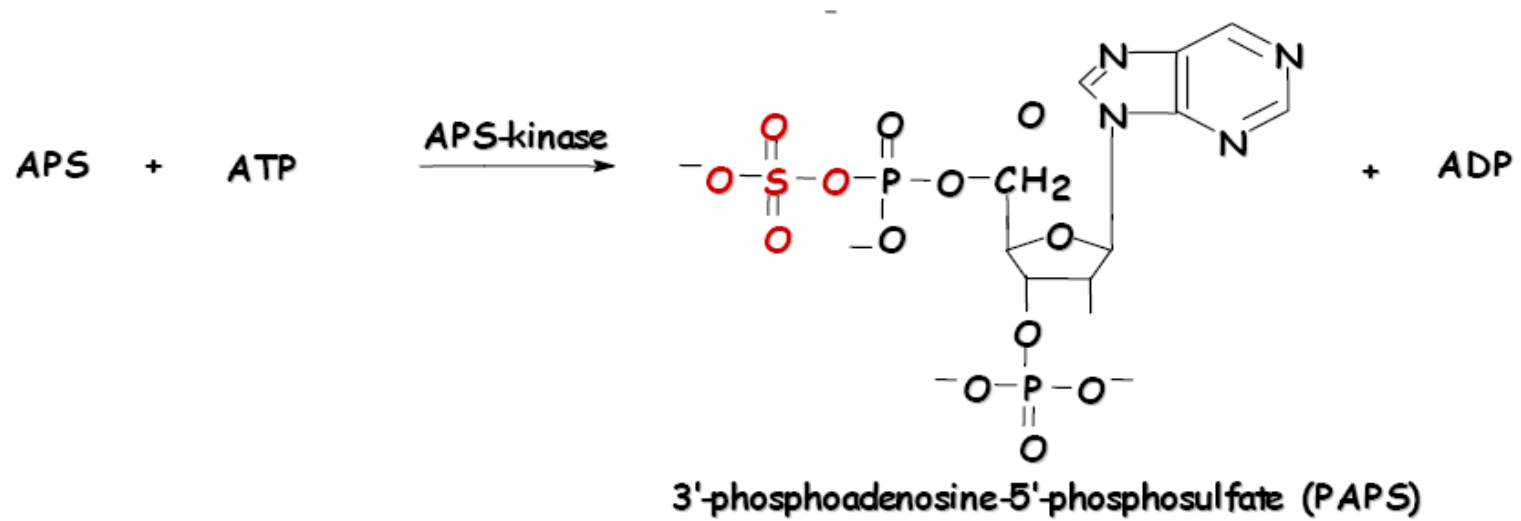
Sulfation reaction: paracetamol

Phase II



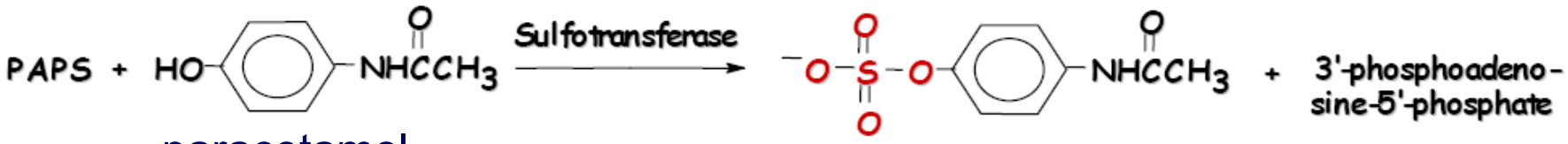
Sulfate Coupling

Adenosine-5'-phosphosulfate (APS)



3'-phosphoadenosine-5'-phosphosulfate (PAPS)

n.b.- cellular synthesis of PAPS is an energy costly process



paracetamol

Phase II: Glutathione Conjugation

- Enzyme: Glutathione-S-transferase (GST)
- Cofactor: Glutathione (GSH)
- Glutathione is a tripeptide comprised of glutamate, cysteine, and glycine.
- Conjugates GSH to electrophilic (positively charged) intermediates:
  - Electrophilic carbon atoms (ex: carbon in epoxides)
  - Electrophilic heteroatoms
  - Aryl halides
  - Polarized double bonds.

Glutathione Conjugation (cont.)

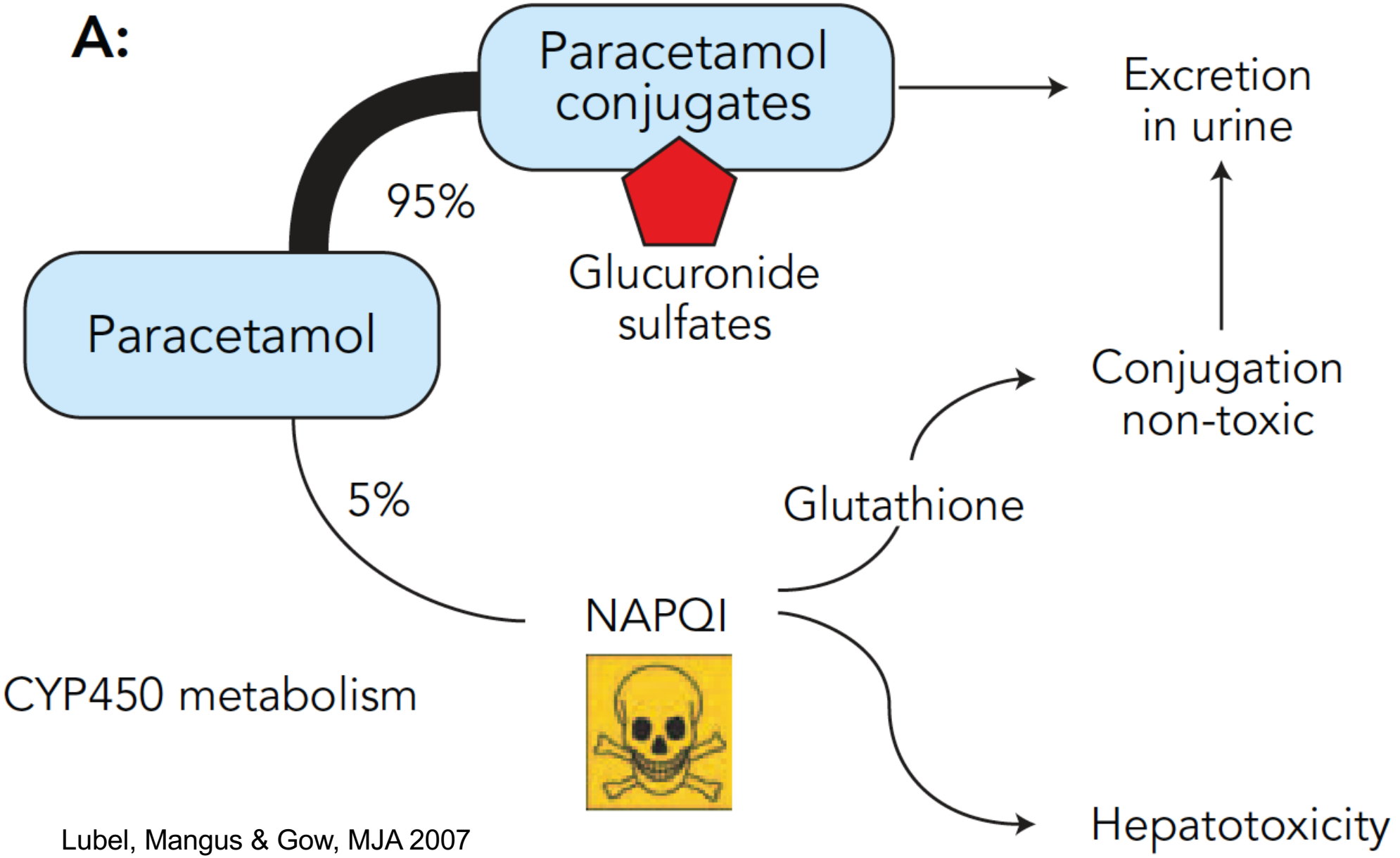
- Electrophilic metabolites (electron-poor; ex: molecules formed upon oxidative, inflammatory and metabolic stress) are intermediates capable of binding to nucleophilic (electron-rich) moieties on DNA or protein, and thus, are capable of causing protein dysfunction, DNA damage, and cell death.
- Glutathione competes for the binding, in turn protecting the cell from injury.

Example: N-acetyl-p-benzo-quinone imine (NAPQI) - a toxic byproduct produced upon metabolism of paracetamol (acetaminophen).

US statistics for paracetamol-caused injuries in 2006:

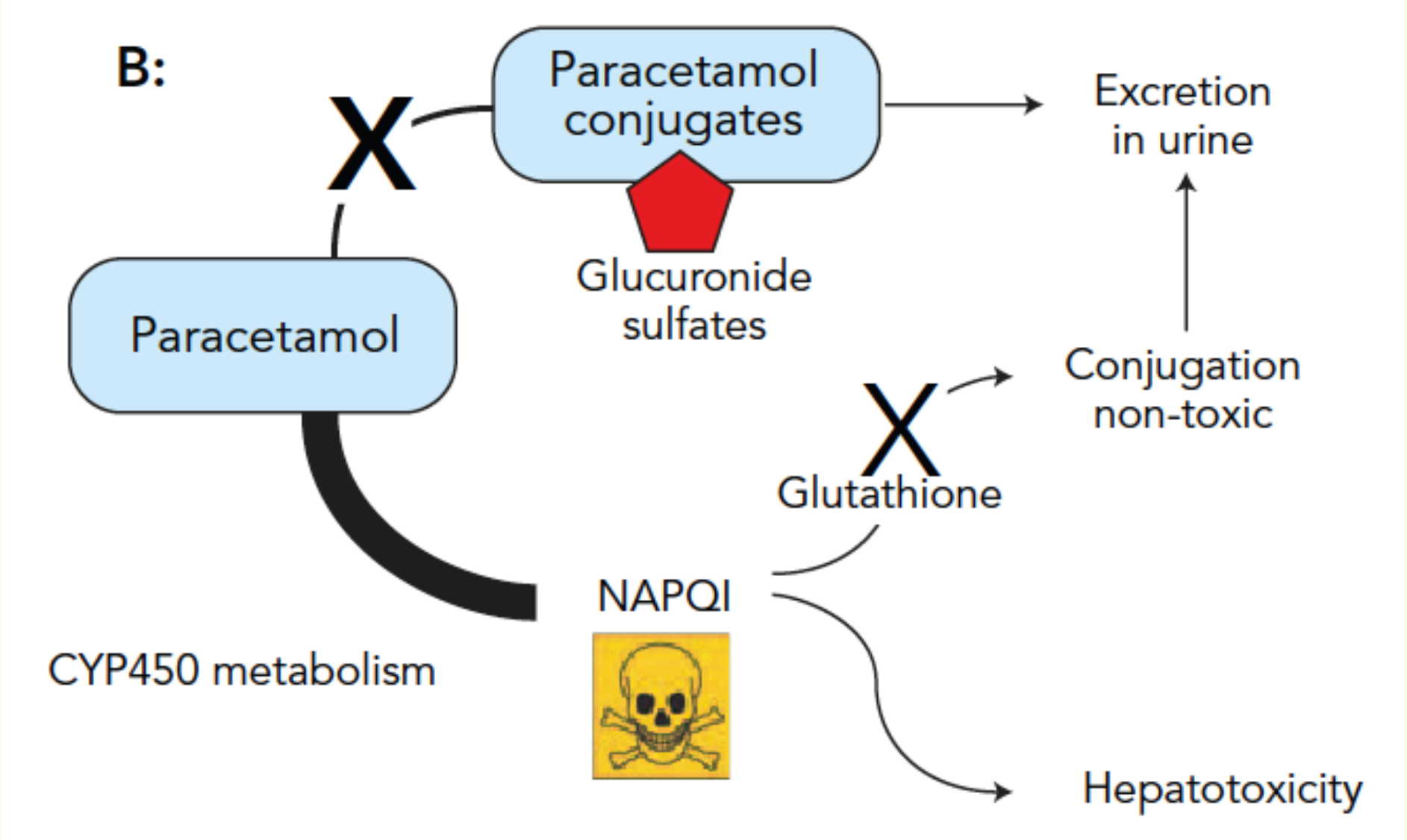
56'000 cases, 2'600 hospitalizations, 458 death

Glutathione conjugation: NAPQI toxicity



Lubel, Mangus & Gow, MJA 2007

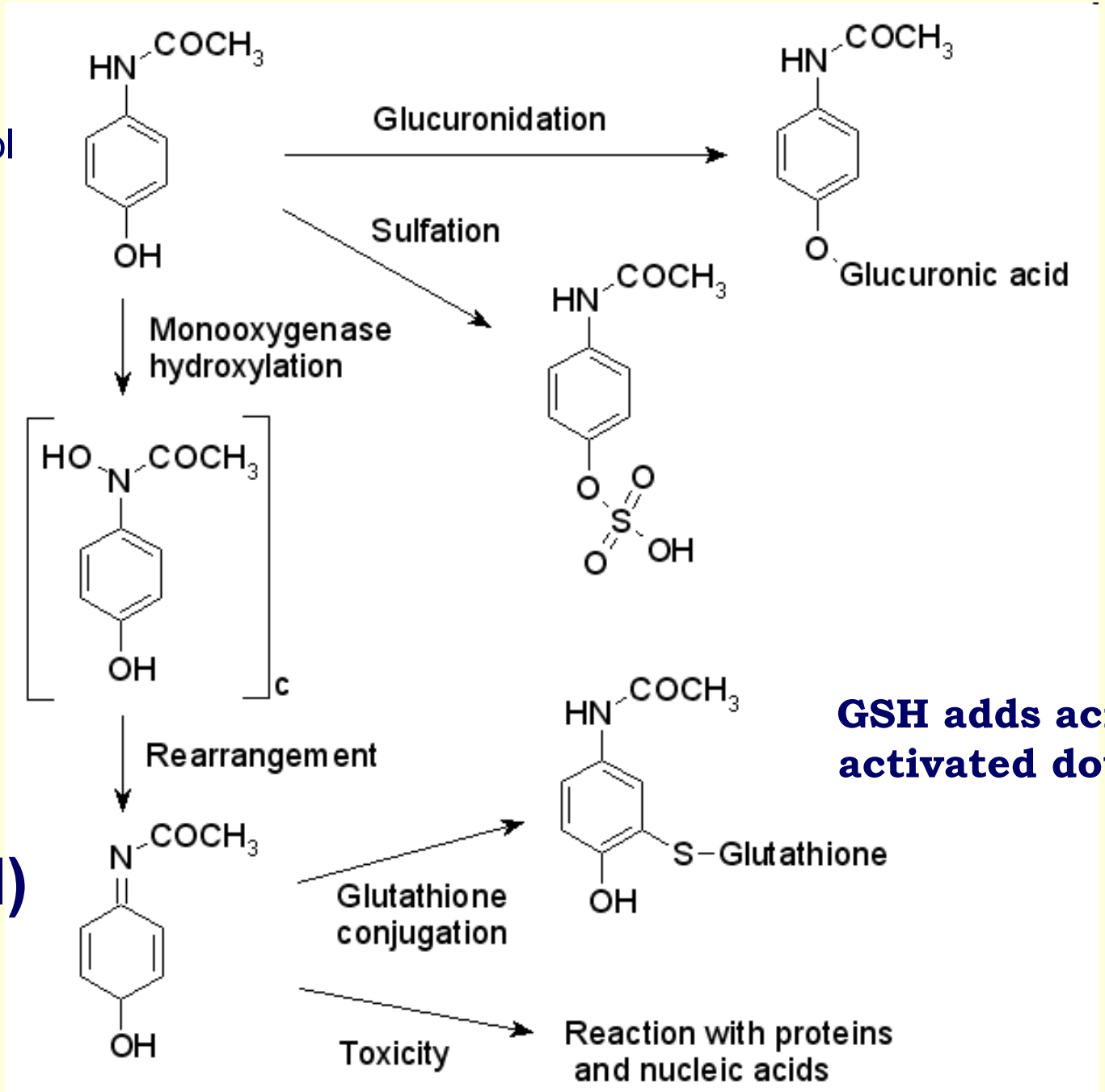
Glutathione conjugation: NAPQI toxicity: attention starvation



Lubel, Mangus & Gow, MJA 2007

Multiple possibilities for Type II reactions

paracetamol



(NAPQI)

GSH adds across an activated double bond

Kinetics of biotransformation reactions

- **Obey Michaelis-Menten kinetics**

$$\text{Rate of drug metabolism (V)} = \frac{Vmax \cdot C}{Km + C}$$

- **Mostly FIRST-ORDER kinetic – drug concentration is much less than Km**

$$V = \frac{Vmax \cdot C}{Km} \qquad \text{first-order kinetic}$$

- **Rarely metabolism is ZERO-ORDER, if drug dose is very large and its concentartion is much greater than Km (eg. aspirin, phenytoin, ethanol)**

$$V = Vmax \qquad \text{zero-order kinetic}$$

Phase I and II enzymes – important message

- a high sequence similarity between family members does not mean identity of function!
- Phase I enzymes from different species exhibit significantly different expression patterns

TABLE 3.—The distribution of CYPs and GSTs in the islet cells of the syrian hamster (SGH), nude mouse (NM), rat, rabbit, guinea pig, dog, monkey, and human.

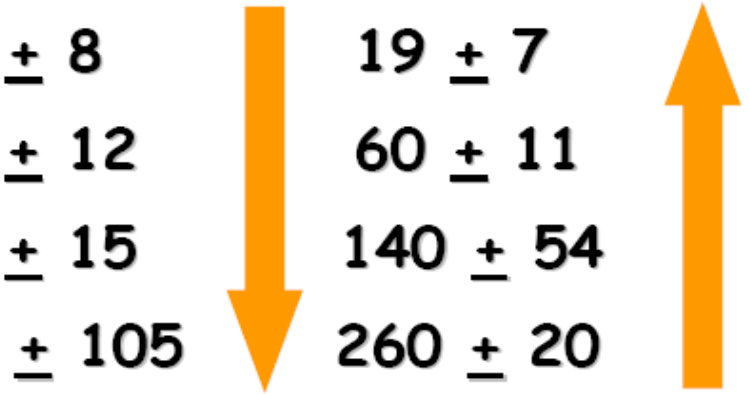
CYP	SGH	NM	Rat	Rabbit	Pig	Dog	Monkey	Human
1A1	++	+	++	+++[a]	+++	+	++	+/+++[b]
1A2	+++	+++[a]	+[a]	+++	–	–	–	+
2B6	+++[a]	+++	+++[a]	++[a]	+++[b]	–	+++[c]	–/+++[b,d]
2C8,9,19	+	+++	+++[a]	–	+++[b]	–	+	–/+++[b,e]
2D1	+	++	++	+	+	+	+	+++[f]
2E1	+++	++	+++	–	–	–	–	–/+[h]
3A1	+++[a]	++	+++	++	–	++[b]	+++	++[b]
3A2	+	+++[a]	+	+	+	+	+	++
3A4	+	++	+++	–	–	–	+	–/+++[k]
GST-π	+++	++[a]	+	+	–	++	++	–/+++[m]
GST-α	+++[a]	+	–	–	–	–	–	–/+[n]
GST-μ*	++	+	+	+++[a]	+[b]	–	++	++*

❖ reasons why the metabolism of new drug candidates has to be characterized in human

Ulrich et al, ToxPath 2002

Example: Species differences in metabolism of hexobarbital

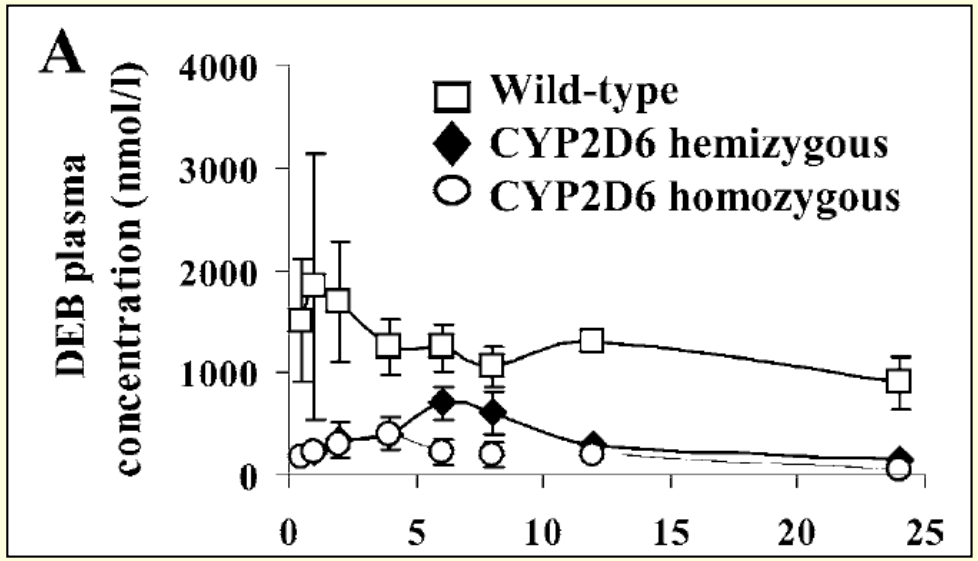
Species (n)	Sleeping time (min)	Hexobarbital half-life (min)	Enzyme Activity (mg/hr/g)
Mice (12)	12 ± 8	19 ± 7	598 ± 184
Rabbits (9)	49 ± 12	60 ± 11	196 ± 28
Rats (10)	90 ± 15	140 ± 54	134 ± 51
Dogs (8)	315 ± 105	260 ± 20	36 ± 30
Humans		≈360	

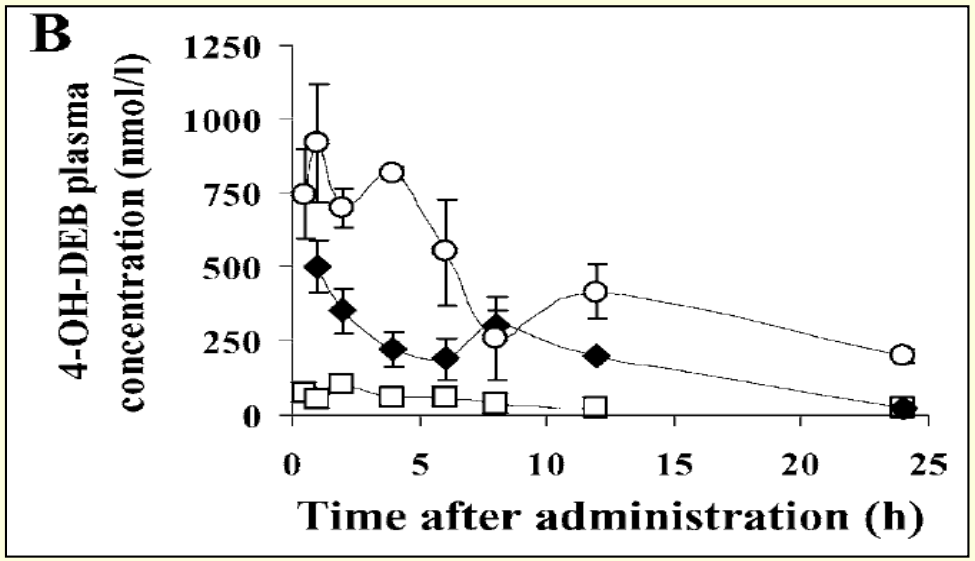


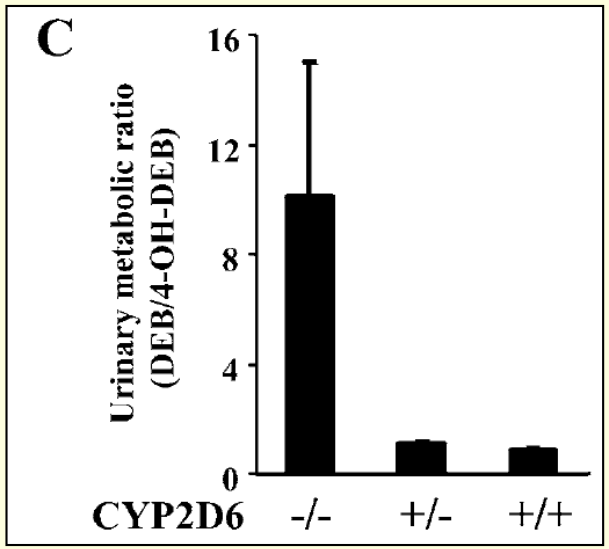
This is a pharmacokinetic difference

Kappas & Alvares SciAm1975

Functional characterization of CYP450 enzymes: *in vivo* studies – "humanization" of mouse models







Example: CYP2D6 humanized mice

Metabolism of debrisoquine (DEB) to 4-hydorxydebrisoquine (4-OH-DEB)

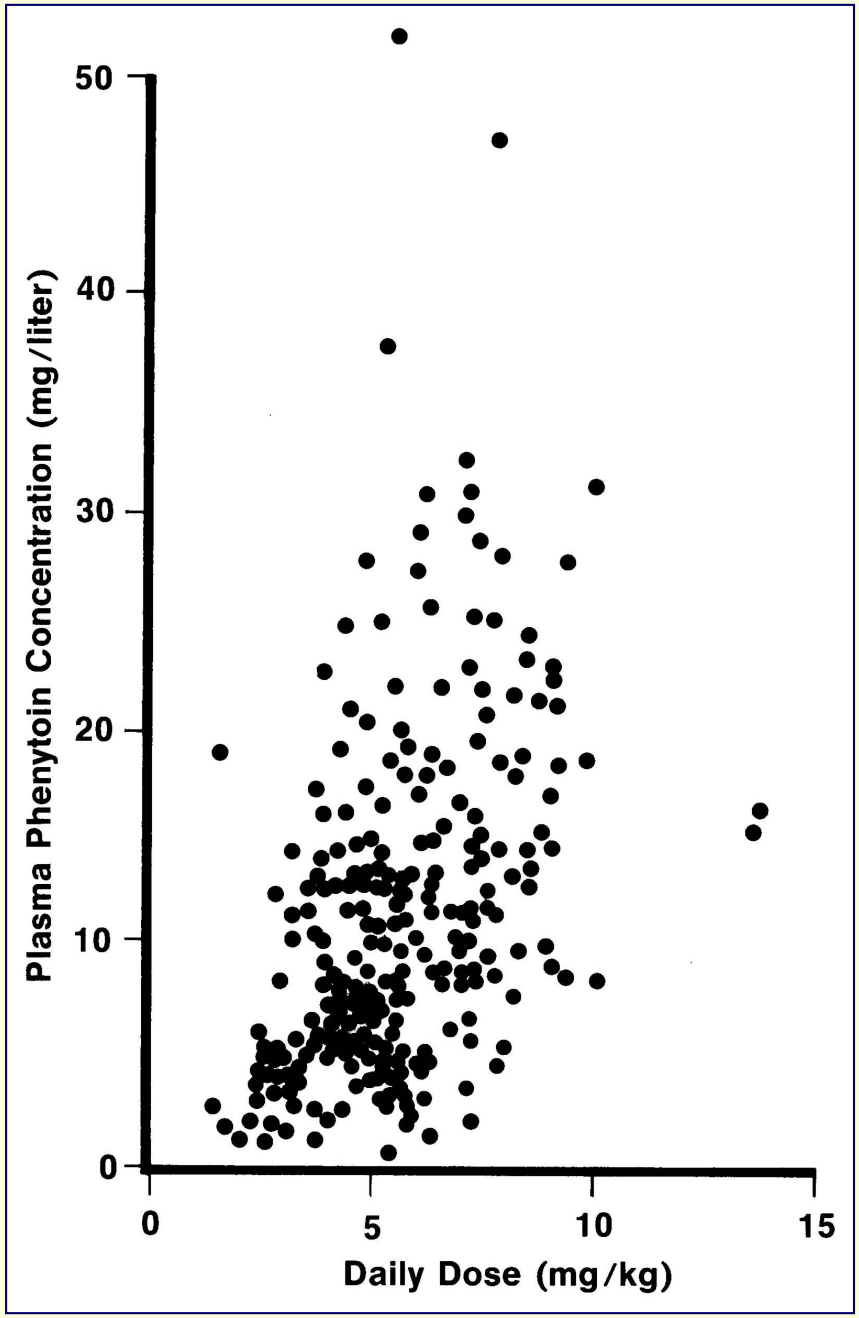
Corchero et al. MolPharm 2001

Factors affecting metabolism of xenobiotics

- age
- sex
- pathology
- food and nutrition status
- tobacco, alcohol, caffeine..
- environment
- interaction between different drugs
- pharmacogenetics
- chronopharmacology

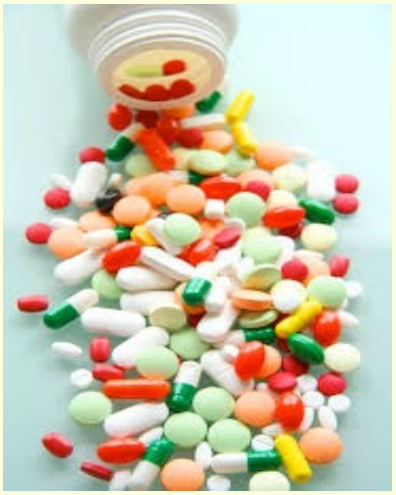
Factors affecting metabolism of xenobiotics

Example: individual variability in plasma phenytoin (antiepileptic) concentration.



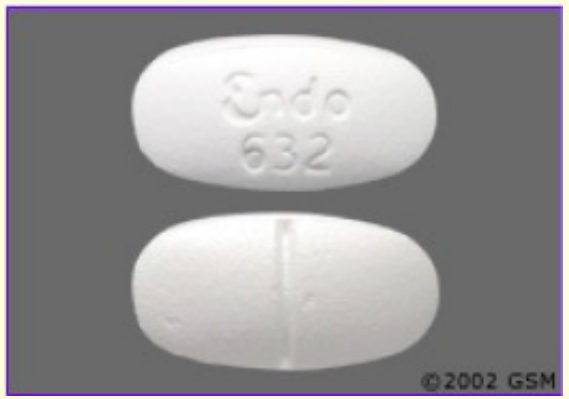
Inducers of drug metabolism

- Other drugs (rifampin, phenobarbital)
- Herbal remedies (St. John's wort) (millepertuis perforé)
- Chronic alcohol use
- Cigarette smoking
- Diet (cruciferous vegetables; garlic; citrus)
- Fasting (induces CYP2E1)



Inhibitors of drug metabolism

- Other drugs (cimetidine, ketoconazole)
- Herbal remedies (Dong Quai)
- Diet (grapefruit juice, garlic)
- Fasting (depletes cofactors necessary for Phase II drug metabolism)

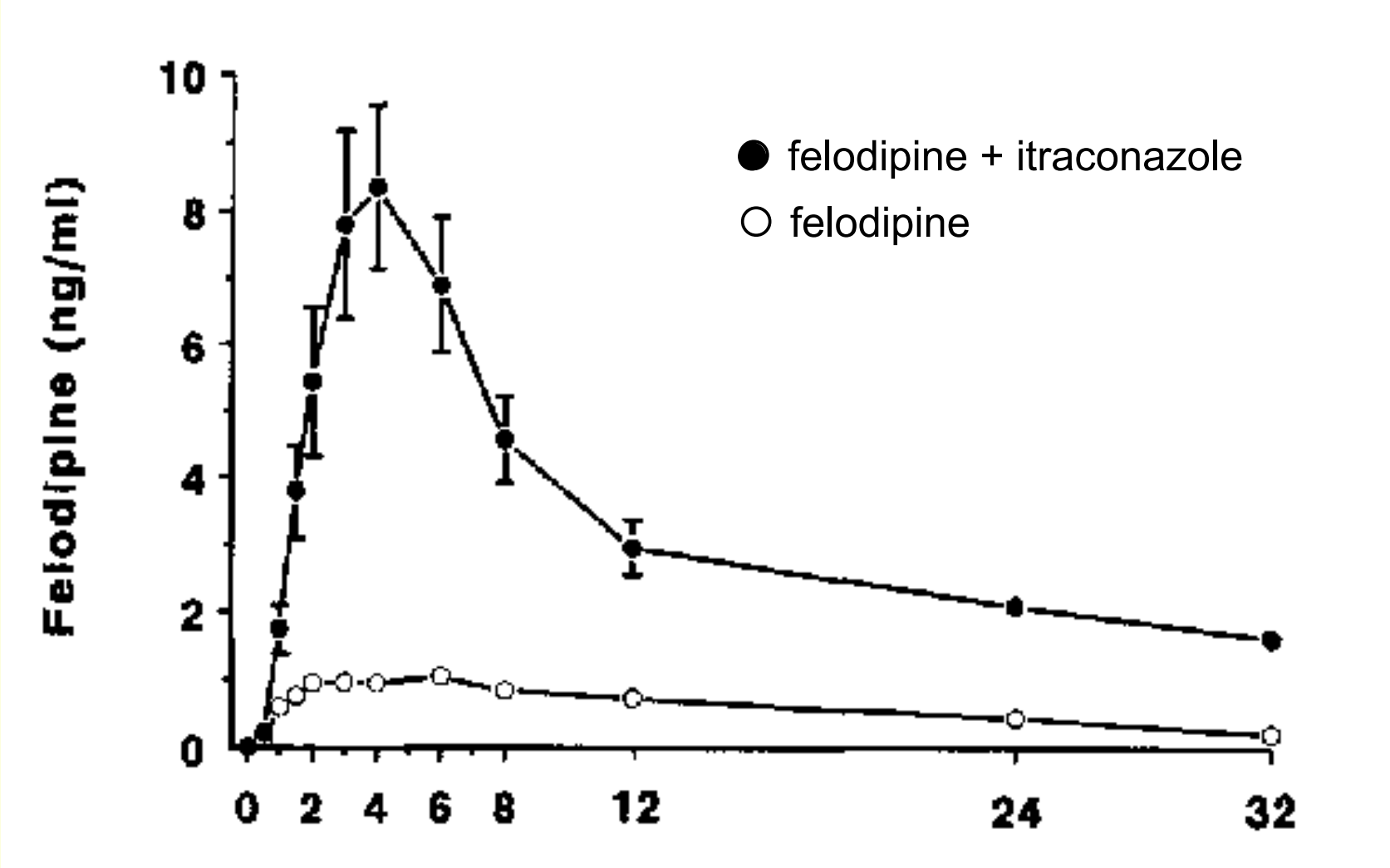


Drug-drug interaction affecting CYP450

Substrates

1A2	2C19	2C9	2D6	2E1	3A
Clozapine Cyclobenzaprine Fluvoxamine Haloperidol Imipramine Mexiletine Olanzapine Pentazocine Propranolol Tacrine Theophylline	Amitriptyline Citalopram Clomipramine Diazepam Imipramine Lansoprazole Nelfinavir Omeprazole Phenytoin	Celecoxib Diclofenac Flurbiprofen Ibuprofen Losartan Naproxen Phenytoin Piroxicam Torsemide Tolbutamide Warfarin	Amitriptyline Clomipramine Codeine Desipramine Dextromethorphan Imipramine Metoprolol Nortriptyline Oxycodone Paroxetine Propranolol Risperidone Thioridazine Timolol Venlafaxine	Acetaminophen Chlorzoxazone Dapsone Ethanol Enflurane Halothane Isoflurane	Alprazolam Astemizole Buspirone Calcium Channel Blockers Carbamazepine Cisapride Cyclosporine HIV Protease Inhibitors Lovastatin NOT pravastatin Simvastatin Midazolam Pimozide Tacrolimus Triazolam
INHIBITORS					
Cimetidine Ciprofloxacin Erythromycin Fluvoxamine Ofloxacin	Cimetidine Felbamate Fluoxetine Fluvoxamine Ketoconazole Lansoprazole Omeprazole Paroxetine Ticlopidine	Amiodarone Fluconazole Fluoxetine Fluvastatin Metronidazole Paroxetine Zafirlukast	Amiodarone Fluoxetine Haloperidol Indinavir Paroxetine Quinidine Sertraline Terbinafine Ticlopidine	Disulfiram	Amiodarone Cimetidine Grapefruit Juice HIV Protease Inhibitors Itraconazole Ketoconazole Macrolide Antibiotics (NOT Azithromycin) Nefazadone
INDUCERS					
Carbamazepine Rifampin Tobacco	Carbamazepine Norethindrone Rifampin	Phenobarbital Rifampin Secobarbital		Chronic Ethanol Isoniazid Tobacco	Carbamazepine Rifabutin Rifampin Ritonavir St. John's Wort

Drug-drug interaction: CYP3A4 inhibition by itraconazole (antifungal drug)



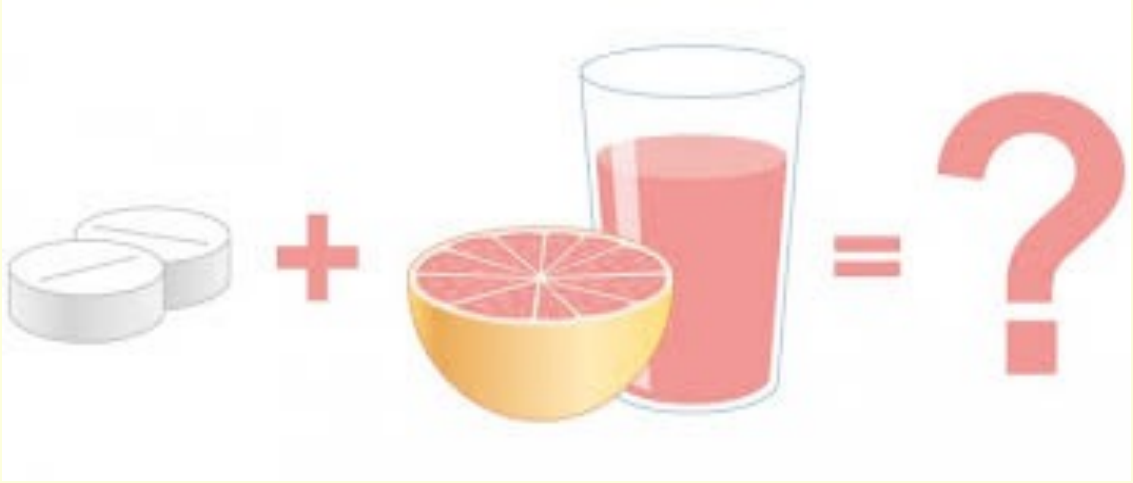
Jalava, 1997

Foods that affect CYP450

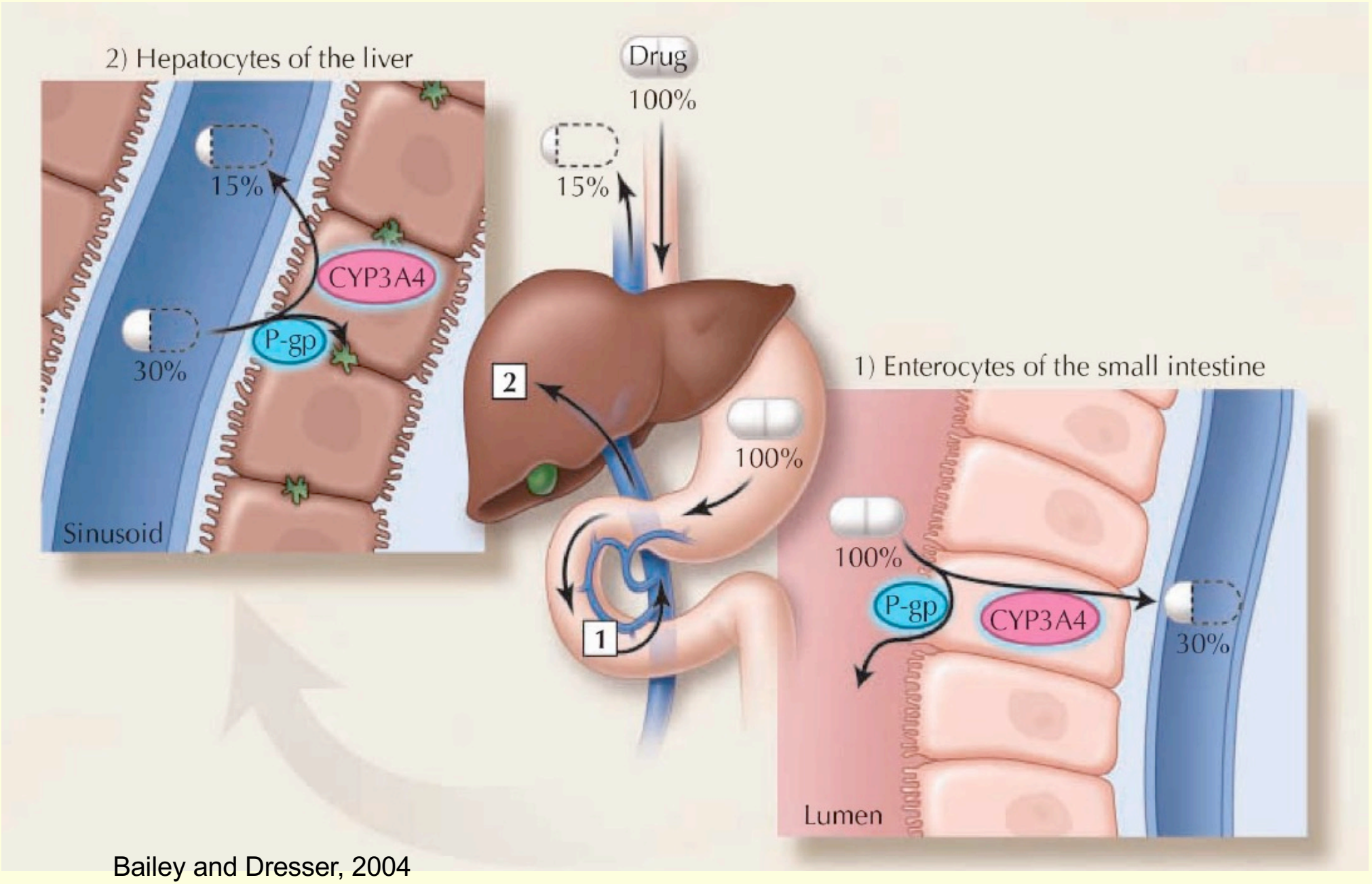
- Broccoli
- Cabbage
- Other cruciferous vegetables
- Spinach
- Leeks
- Onion
- Garlic
- Parsley
- Grapefruit
- Fried and charcoal broiled foods
- Smoked fish or meat
- Ham
- Sausage

Effect of grapefruit juice on drug metabolism

- ingestion of grapefruit juice inhibits CYP3A4 activity and reduces expression of CYP3A4
- CYP3A4 is responsible for metabolism of 60% of all drugs
- it comprises approximately 28% of hepatic cytochrome P450

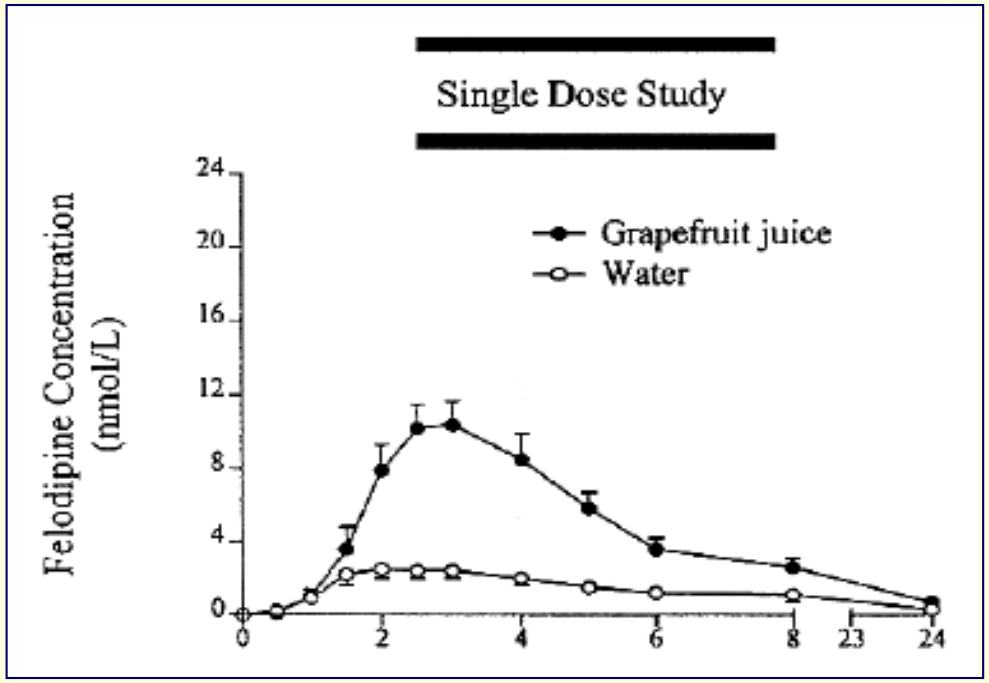


Expression and function of CYP3A4

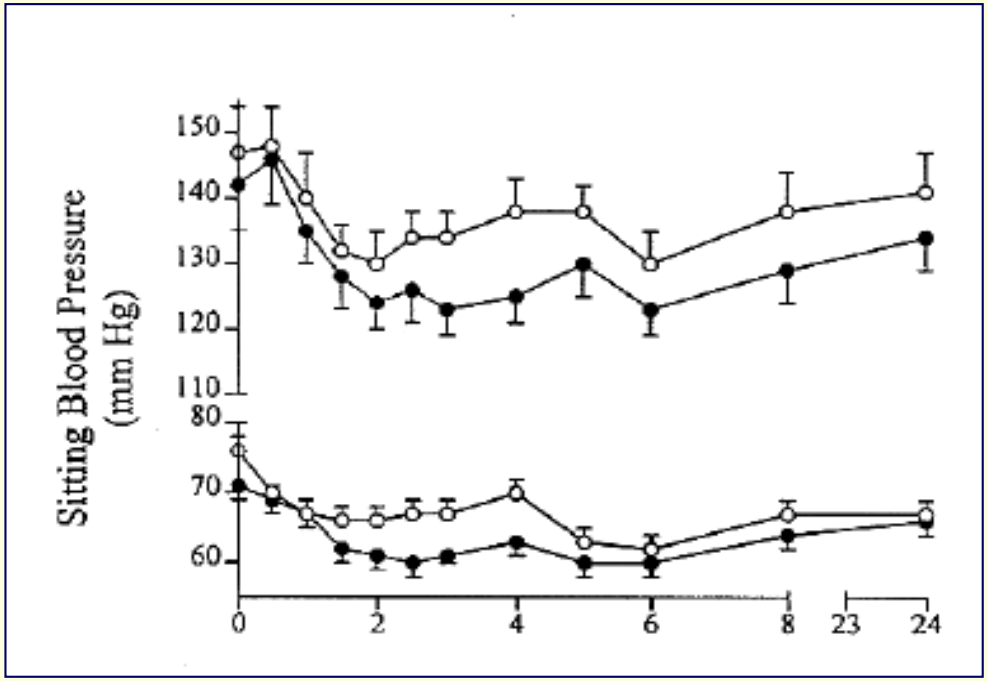


Bailey and Dresser, 2004

Grapefruit juice increases felodipine oral bioavailability and increases blood pressure-lowering effect of the drug



Hours after dosage



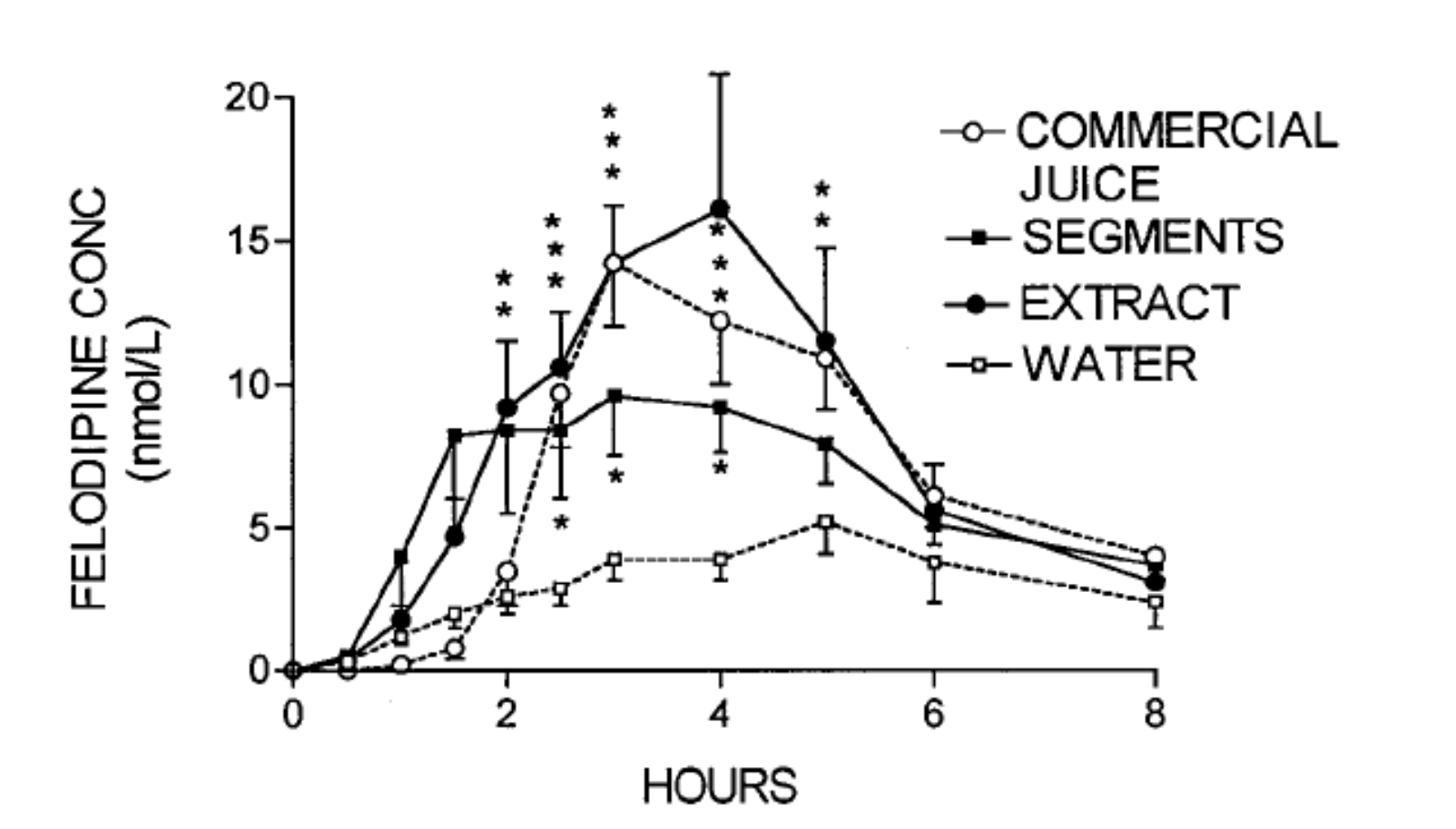
Hours after dosage

Dresser et al, 2004

Some drugs influenced by grapefruit juice

Drug	AUC increase
Felodipine	~ 3 fold
Cisapride	~ 1.4 fold
Cyclosporine	~ 1.5 fold
Saquinavir	~ 2 fold
Terfenadine	~ 2.5 fold
Buspirone	~ 9 fold
Lovastatin/simvastatin	~ 10 fold

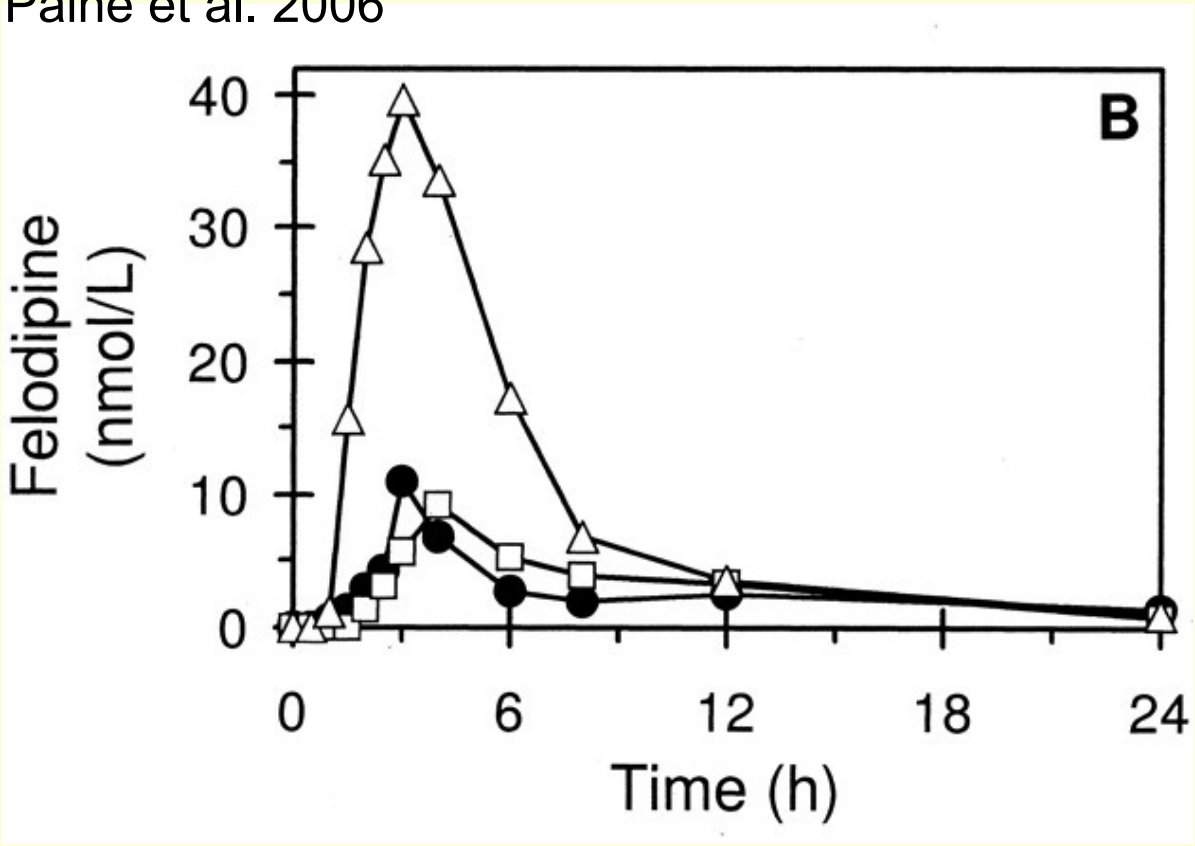
Grapefruit juice increases felodipine oral bioavailability: source-dependency



Bailey et al. 2000

Grapefruit furanocoumarins

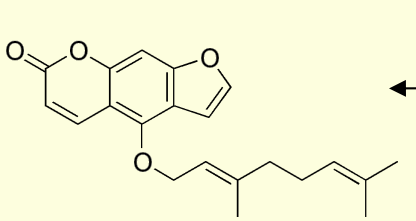
Paine et al. 2006



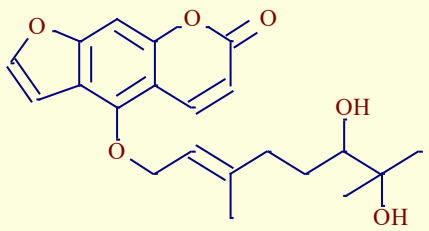
● - orange juice

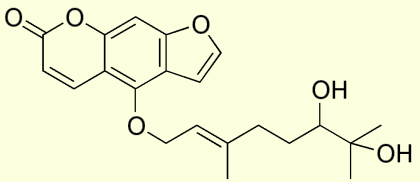
□ - grapefruit juice without furanocoumarins

Δ - grapefruit juice



bergamottin





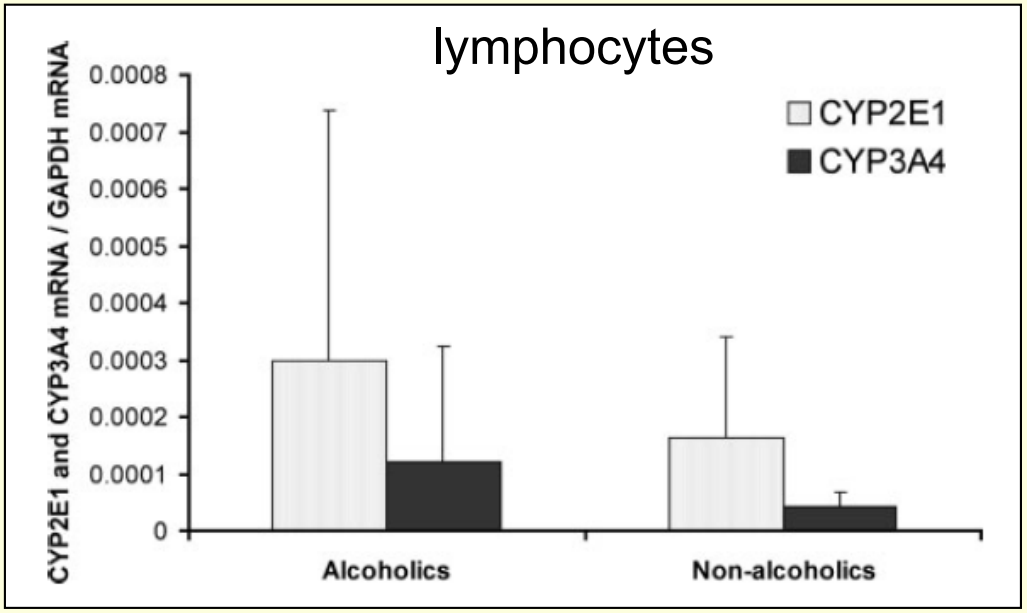
6', 7', - dihydroxybergamottin

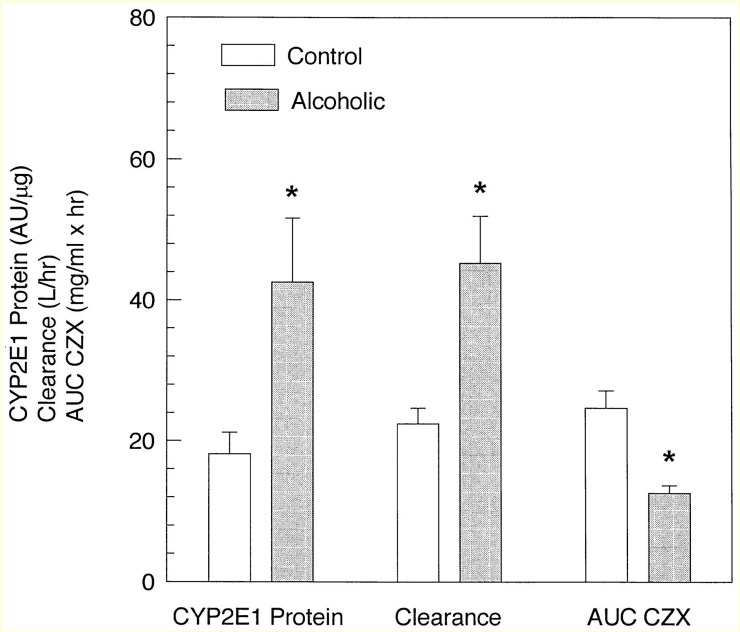
Smoking-drug interaction (CYP1A2 induction by polycyclic hydrocarbons)

Drug/Class	Mechanism of Interaction and Effects
Pharmacokinetic Interactions	
Alprazolam (Xanax)	▪ Conflicting data on significance of a PK interaction. Possible ↓ plasma concentrations (up to 50%); ↓ half-life (35%).
Bendamustine (Treanda)	▪ Metabolized by CYP1A2. Manufacturer recommends using with caution in smokers due to likely ↓ bendamustine concentrations, with ↑ concentrations of its two active metabolites.
Caffeine	▪ ↑ Metabolism (induction of CYP1A2); ↑ clearance (56%). ▪ Likely ↑ caffeine levels after cessation.
Chlorpromazine (Thorazine)	▪ ↓ Area under the curve (AUC) (36%) and serum concentrations (24%). ▪ ↓ Sedation and hypotension possible in smokers; smokers may need ↑ dosages.
Clozapine (Clozaril)	▪ ↑ Metabolism (induction of CYP1A2); ↓ plasma concentrations (18%). ▪ ↑ Levels upon cessation may occur; closely monitor drug levels and reduce dose as required to avoid toxicity.
Erlotinib (Tarceva)	▪ ↑ Clearance (24%); ↓ trough serum concentrations (2-fold).
Flecainide (Tambocor)	▪ ↑ Clearance (61%); ↓ trough serum concentrations (25%). ▪ Smokers may need ↑ dosages.
Fluvoxamine (Luvox)	▪ ↑ Metabolism (induction of CYP1A2); ↑ clearance (24%); ↓ AUC (31%); ↓ plasma concentrations (32%). ▪ Dosage modifications not routinely recommended but smokers may need ↑ dosages.
Haloperidol (Haldol)	▪ ↑ Clearance (44%); ↓ serum concentrations (70%).
Heparin	▪ Mechanism unknown but ↑ clearance and ↓ half-life are observed. Smoking has prothrombotic effects. ▪ Smokers may need ↑ dosages due to PK and PD interactions.
Insulin, subcutaneous	▪ Possible ↓ insulin absorption secondary to peripheral vasoconstriction; smoking may cause release of endogenous substances that cause insulin resistance. ▪ PK & PD interactions likely not clinically significant; smokers may need ↑ dosages.
Irinotecan (Camptosar)	▪ ↑ Clearance (18%); ↓ serum concentrations of active metabolite, SN-38 (~40%; via induction of glucuronidation); ↓ systemic exposure resulting in lower hematologic toxicity and may reduce efficacy. ▪ Smokers may need ↑ dosages.
Mexiletine (Mexitil)	▪ ↑ Clearance (25%; via oxidation and glucuronidation); ↓ half-life (36%).
Olanzapine (Zyprexa)	▪ ↑ Metabolism (induction of CYP1A2); ↑ clearance (98%); ↓ serum concentrations (12%). ▪ Dosage modifications not routinely recommended but smokers may require ↑ dosages.
Propranolol (Inderal)	▪ ↑ Clearance (77%; via side-chain oxidation and glucuronidation)
Ropinirole (Requip)	▪ ↓ Cmax (30%) and AUC (38%) in study with patients with restless legs syndrome. ▪ Smokers may need ↑ dosages.
Tacrine (Cognex)	▪ ↑ Metabolism (induction of CYP1A2); ↓ half-life (50%); serum concentrations three-fold lower. ▪ Smokers may need ↑ dosages.
Theophylline (Theo Dur, etc.)	▪ ↑ Metabolism (induction of CYP1A2); ↑ clearance (58–100%); ↓ half-life (63%). ▪ Levels should be monitored if smoking is initiated, discontinued, or changed. ▪ ↑ Clearance with second-hand smoke exposure. ▪ Maintenance doses are considerably higher in smokers.

Kroon JASHSP 2013

Alcohol-drugs interaction: CYP3A4 and CYP2E1 induction





Alcohol abuse increases CYP2E1 protein content and decreases the AUC for chlorzoxazone (muscle relaxant)

Liangounsakul et al, Hepatology 2005

Molecular mechanisms of CYP450 inhibition or induction

inhibition

- competitive inhibition or noncompetitive

  modulation of substrate binding

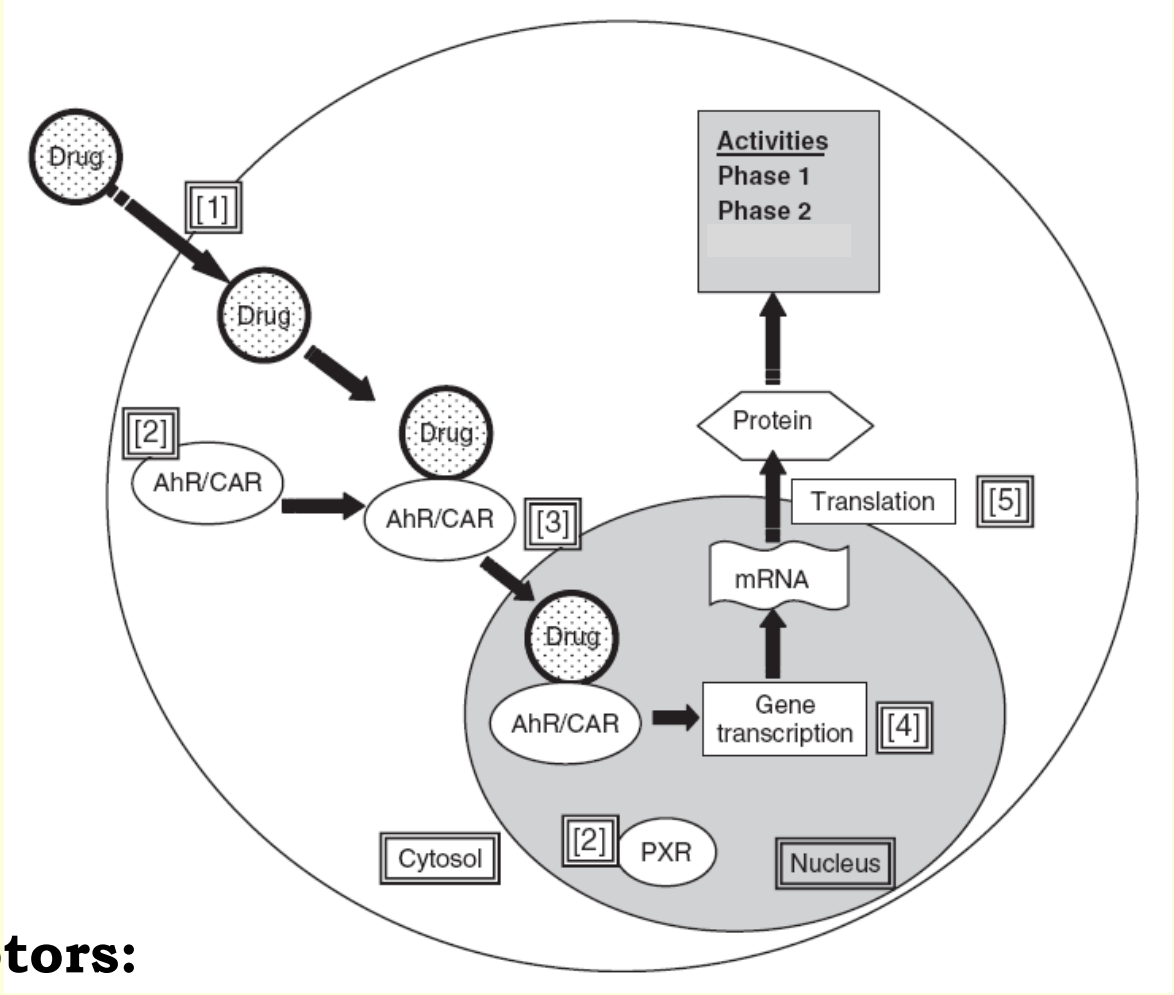
induction

- mRNA stabilization
- Protein stabilization

! Receptor-mediated transcriptional activation

- predominant mechanism
- via specific nuclear receptors

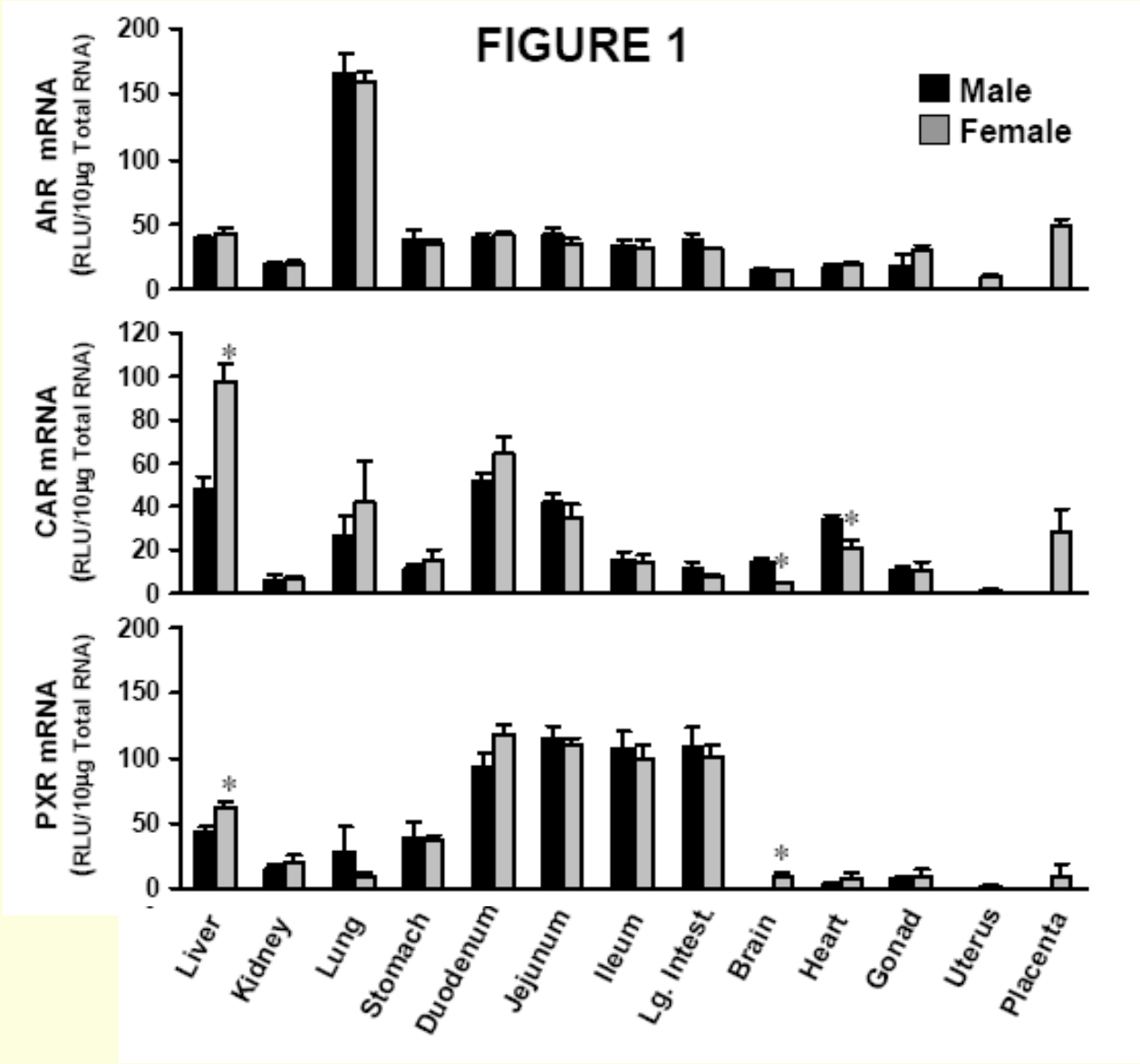
Molecular mechanisms of receptor-mediated induction of drug metabolizing enzymes



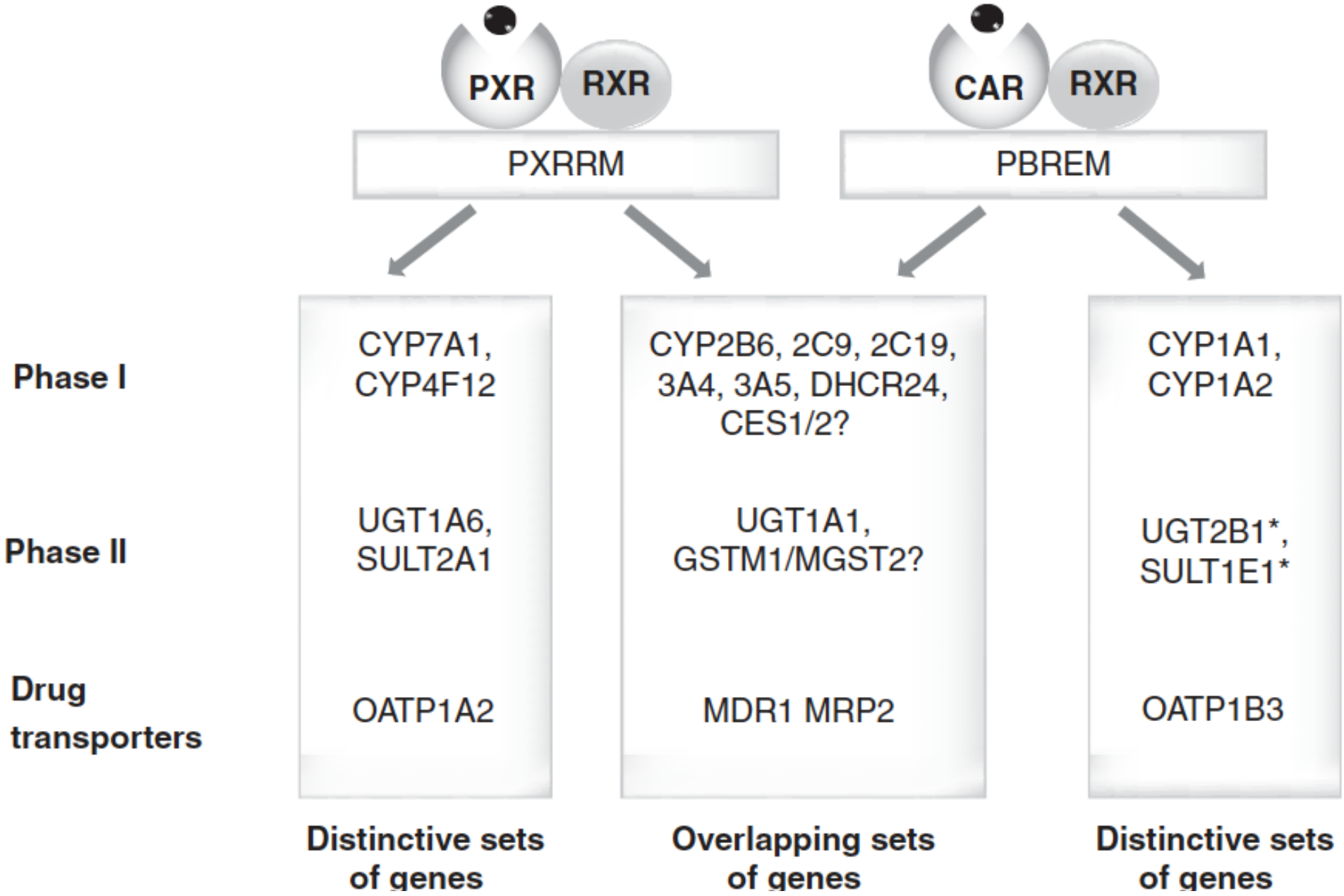
Nuclear receptors:

AhR – aryl hydrocarbon receptor; CAR – constitutive androstane receptor; PXR – pregnane X receptor; RXR – retinoid X receptor, PPAR – peroxisome proliferator activated receptor, ARNT – aryl hydrocarbon receptor nuclear translocator

Molecular mechanisms of receptor-mediated induction of drug metabolizing enzymes: tissue distribution of nuclear receptors



Molecular mechanisms of receptor-mediated induction of drug metabolizing enzymes

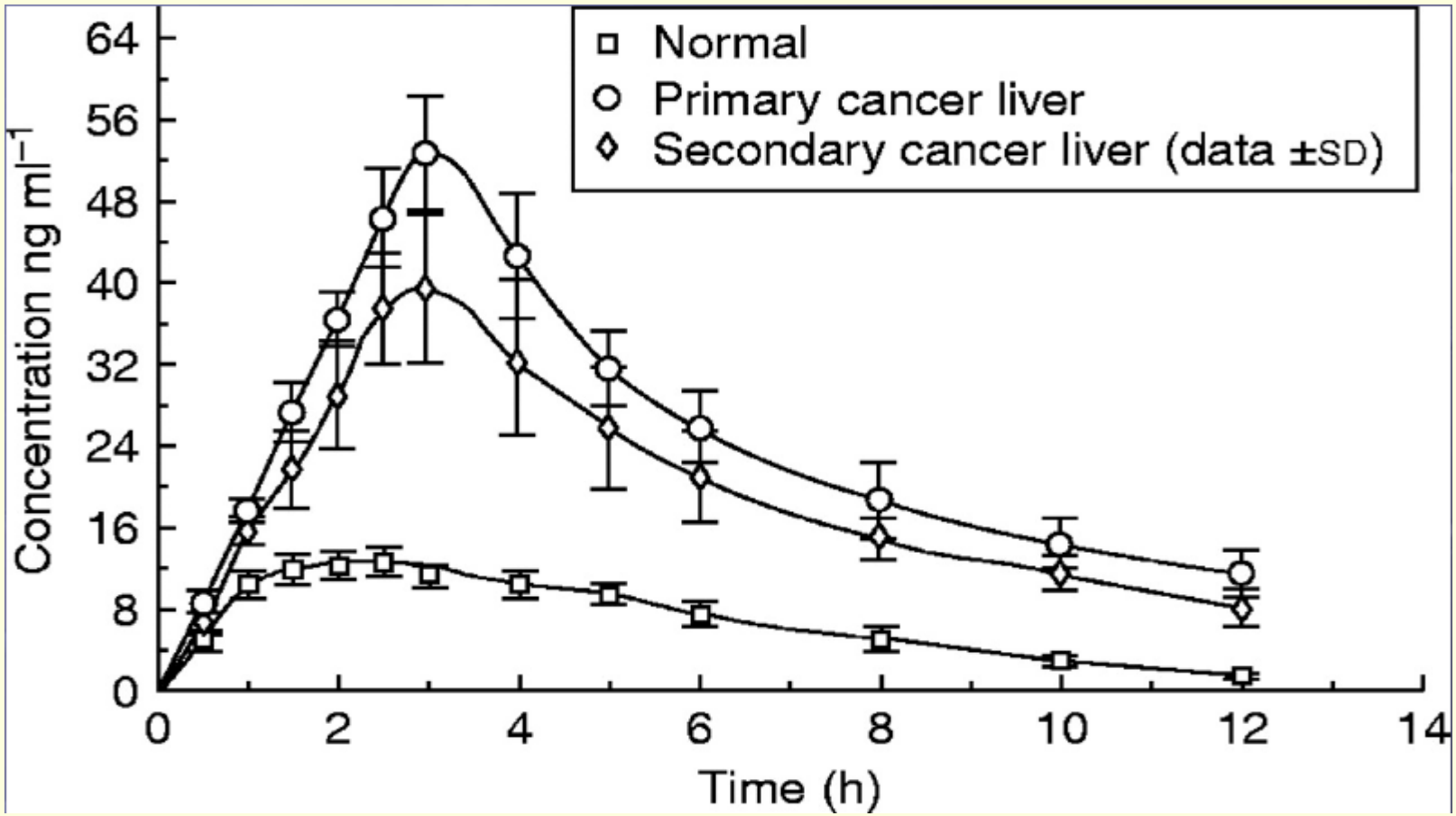


Molecular agonists of nuclear receptors

Facteurs de transcription	Agonistes potentiels	Exemples d'agonistes	Gènes cibles chez l'homme
PXR	Extraits de plantes	Hyperforine (millepertuis)	CYP2B6
	Statines	Lovastatine	CYP2C8
	Antibiotiques	Rifampicine	CYP2C89
	Antidiabétiques (TZD)	Troglitazone	CYP3A4
	Barbituriques	Phénobarbital	CYP3A7
	Acides biliaires	Acide ursodéoxycholique	GST
	Stéroïdes de synthèse	RU486, dexaméthasone	ST
	Stéroïdes naturels	Prégnénolone	UGT1A1
	Antihormones	Acétate de cyprotérone	MDR1
CAR	Analgésiques	Acétaminophène (paracétamol)	CYP2B6
	Barbituriques	Phénobarbital	CYP2C9
	Opiacés	Cocaïne	CYP2C19
PPARα	Hypolipidémiants (lactones)	Chlofibrate, fénofibrate	CYP1A
			CYP2A
			CYP2C
			CYP2E
			CYP4A
			UGT1A4
			UGT2B4
PPARγ	Antidiabétiques (TZD)	Rosiglitazone	CYP4A
AhR	Hydrocarbures polycycliques aromatiques	Dioxine	CYP1A1
	Benzo(a)pyrène	Fumée de cigarette	CYP1A2
	Antiulcéreux (benzimidazole)	Oméprazole	CYP1B1

Disease-induced changes in drug metabolism

Profile of morphine serum concentration after oral administration of morphine to normal, primary and secondary liver cancer patients:



Factors affecting metabolism of xenobiotics

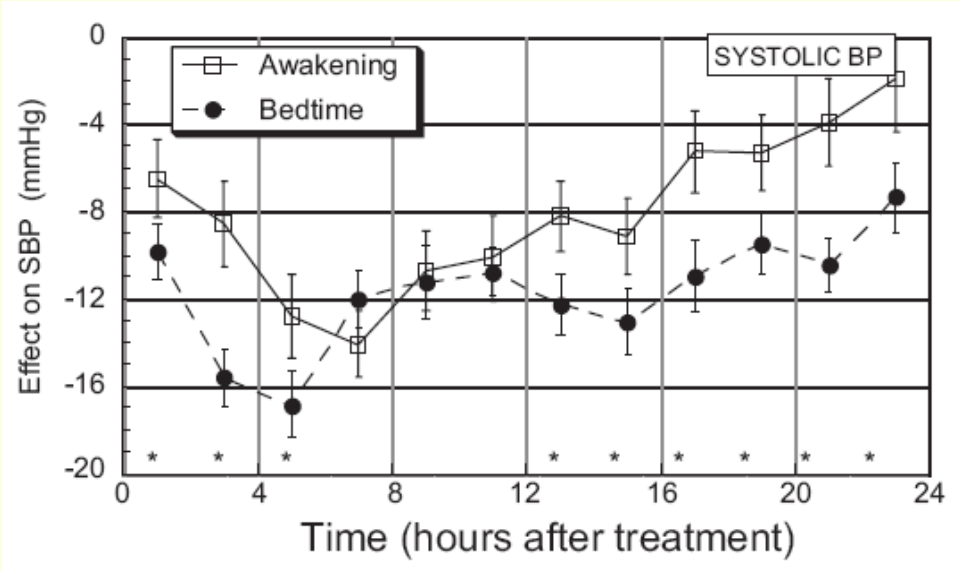
- Why does a bottle of acetaminophen have a warning label that “if you generally consume ≥ 3 alcoholic beverages a day, consult your doctor before using this product?”
- Have you ever heard patients advised NOT to drink grapefruit juice while taking certain medications?
- Do you know that if a woman is taking certain antibiotics, and is using oral contraceptives, that she should use another method of birth control while taking the antibiotics?

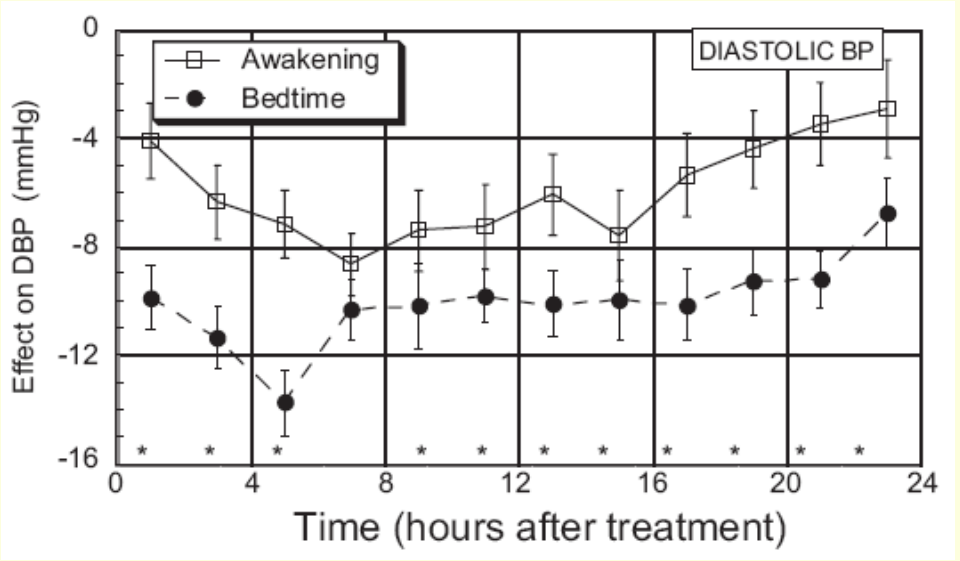
Factors affecting metabolism of xenobiotics

- age
- sex
- pathology
- food and nutrition status
- tobacco, alcohol, caffeine..
- environment
- interaction between different drugs
- pharmacogenetics
- chronopharmacology

Chronopharmacology – clinical observations

Changes in the pattern of SBP and DBP with ramipril (5 mg/day) ingested either in the morning or at bedtime

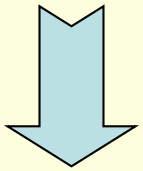




Hermida & Ayala 2009

Résumé of clinical observations and definition of chronopharmacology

- effect of many drugs varies significantly depending on the time of their dosage.
- this variability depends on biological rhythms (in many cases rhythms of ~ 24 hours).



Chronopharmacology - study of interaction between biological rhythms and time of drug administration.

Chronopharmacology

Chrono-

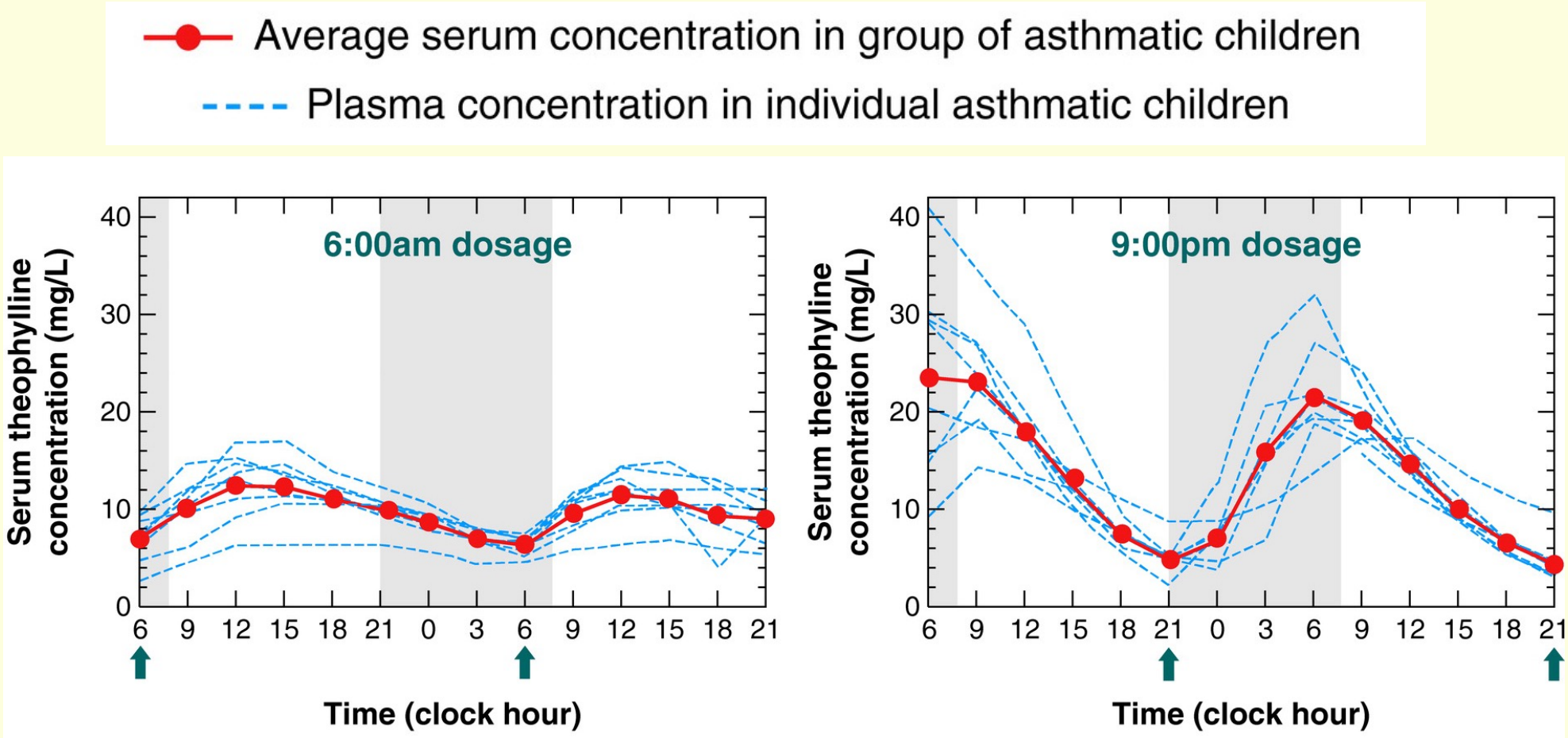
Pharmacokinetics

- **Absorption** 
- **Distribution** 
- **Metabolism** 
- **Excretion** 

Pharmacodynamic

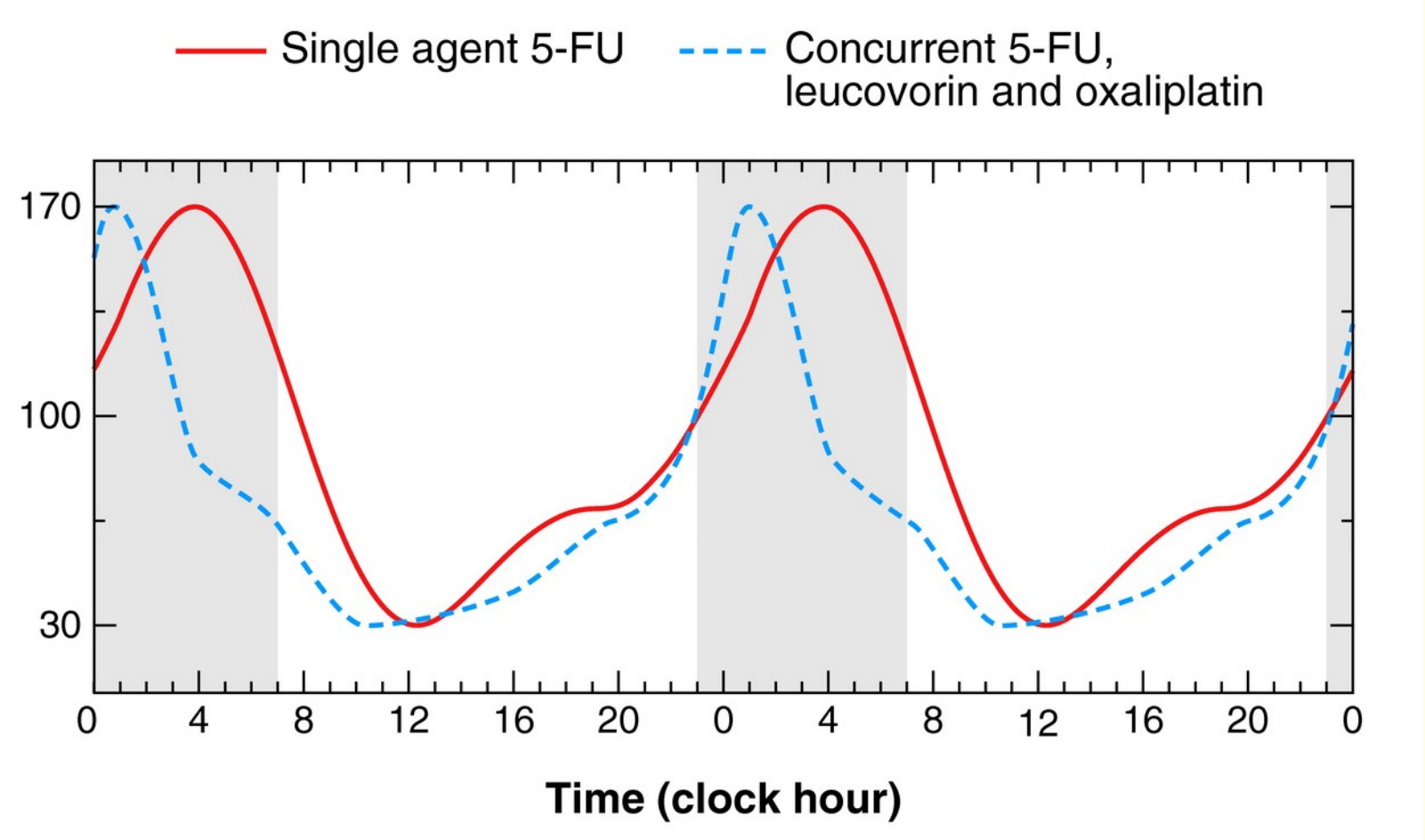
- **Density of R** 
- **Binding to R** 
- **Ionization of L** 
- **…….** 

Chronopharmacology – clinical observations



Levi F, Schibler U. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:593–628

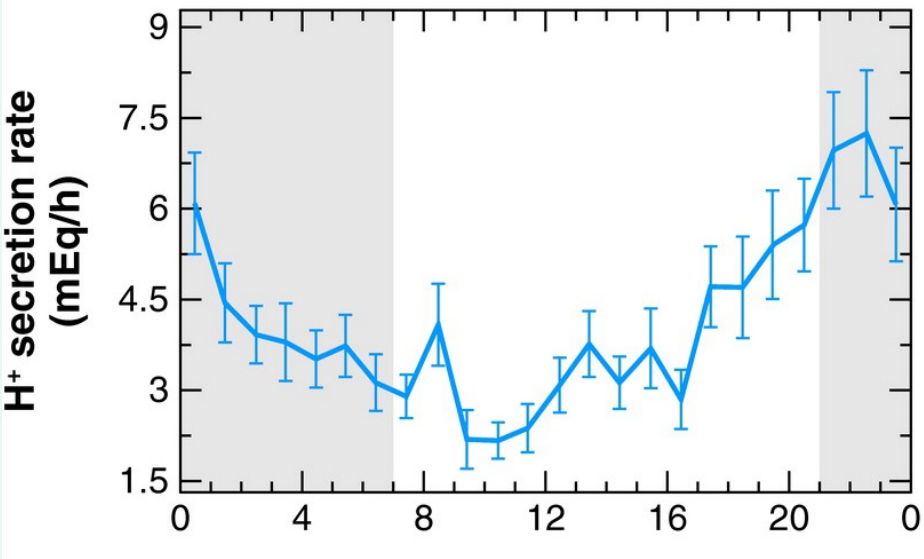
Chronopharmacology – clinical observations



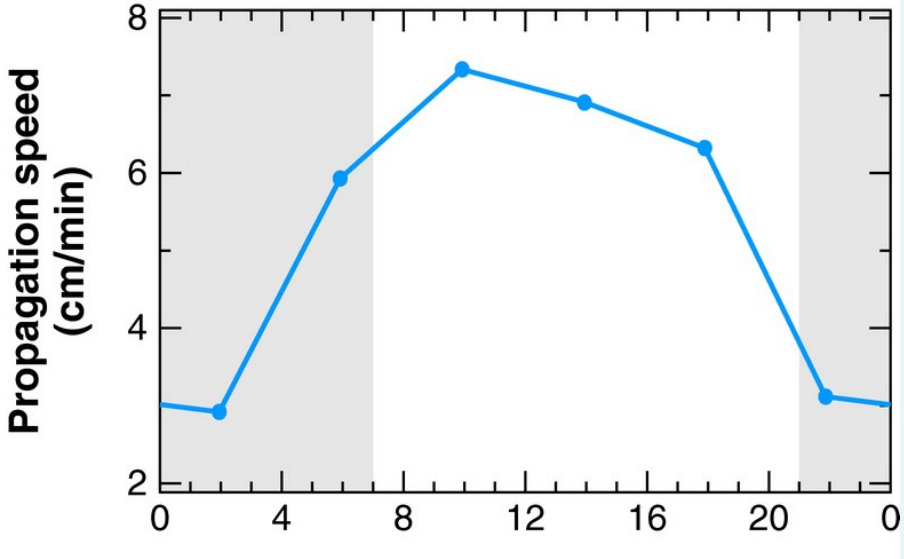
Levi F, Schibler U. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:593–628

Chronopharmacokinetics - Absorption

Gastric acid secretion



Small intestine motility

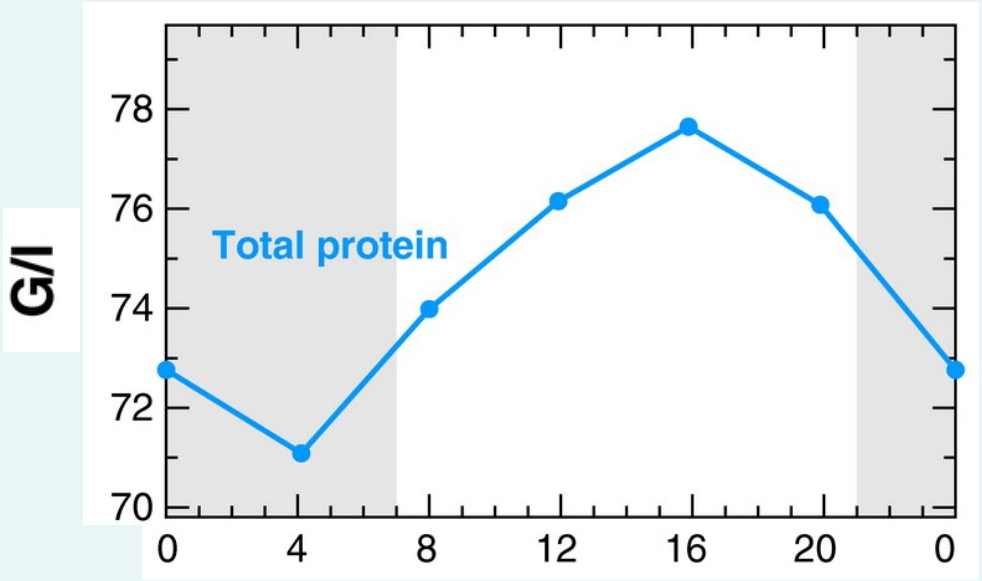


- Concerned drugs
  - benzodiazepines
  - blockers of Ca^{2+} channel
  - acetaminophen
  - antidepressants

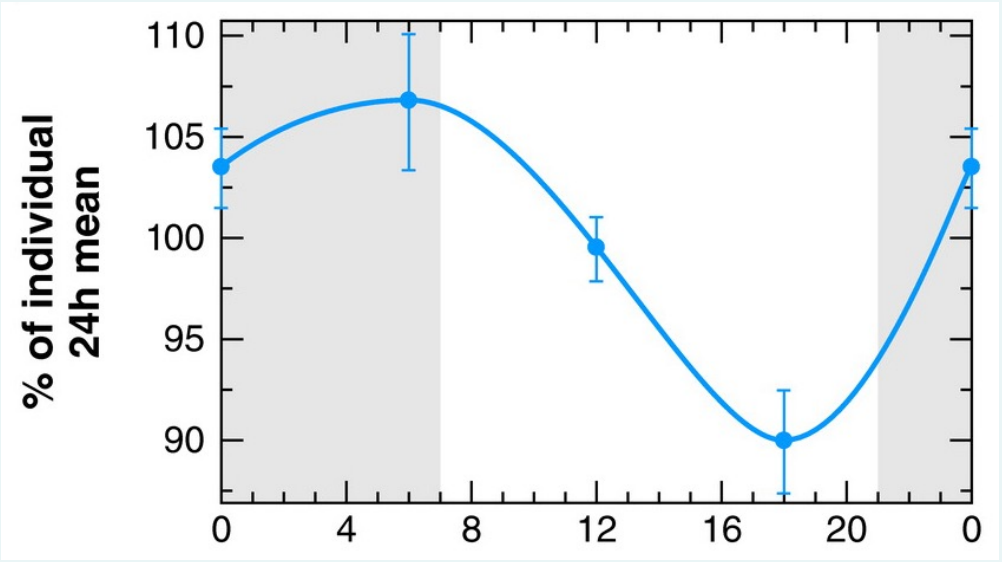
Levi F, Schibler U. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:593–628

Chronopharmacokinetics - Distribution

Plasma concentration of total proteins



Red blood cells microviscosity

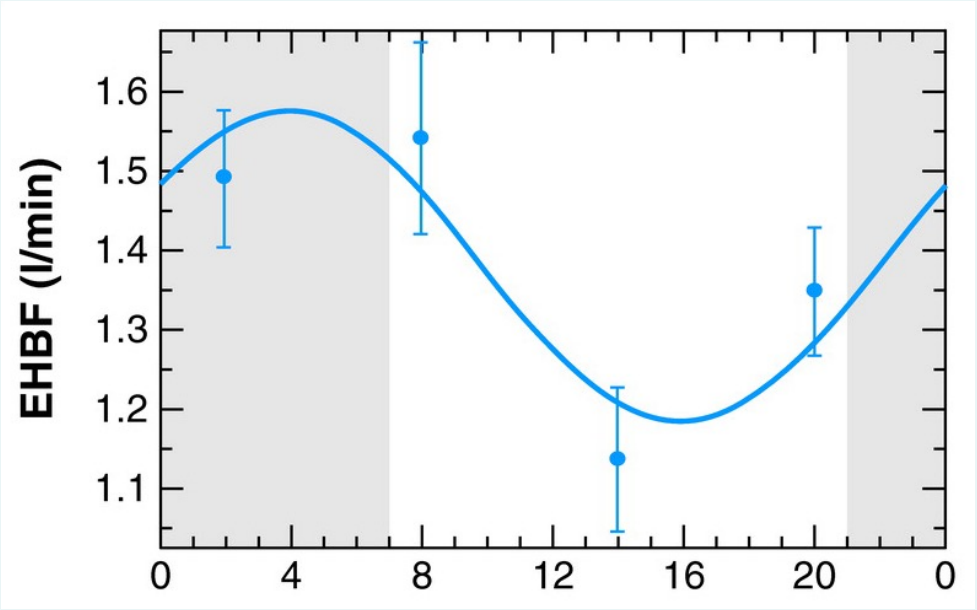


- Concerned drugs
  - steroids
  - indometacin
  - diazepam
  - .....

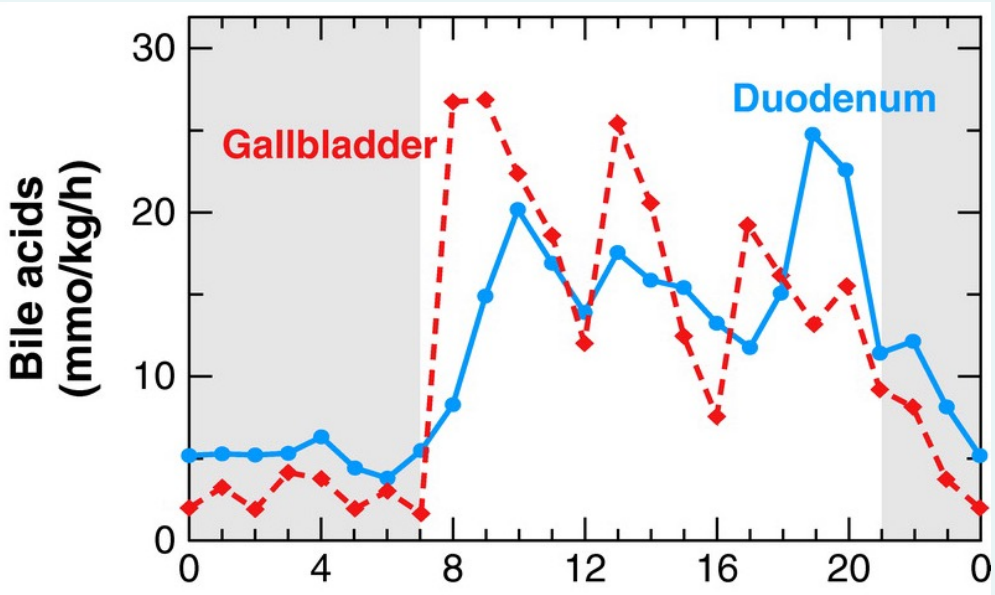
Levi F, Schibler U. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:593–628

Chronopharmacokinetics - Metabolism

Liver blood flow



Biliary secretion into the gallbladder and bile excretion into the duodenum

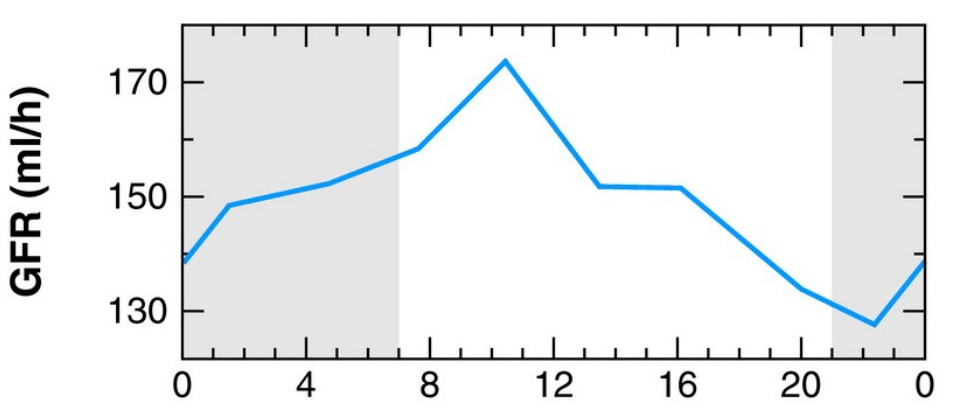


- Concerned drugs
  - most of drugs

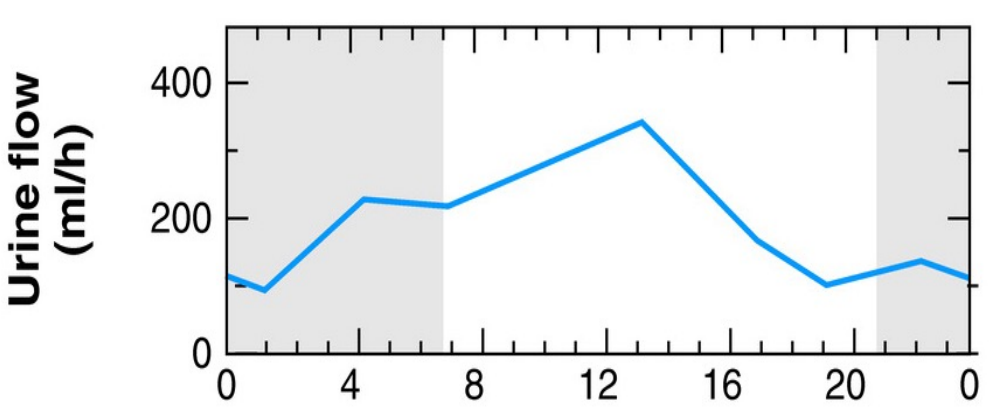
Levi F, Schibler U. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:593–628

Chronopharmacokinetics - Excretion

Glomerular Filtration Rate



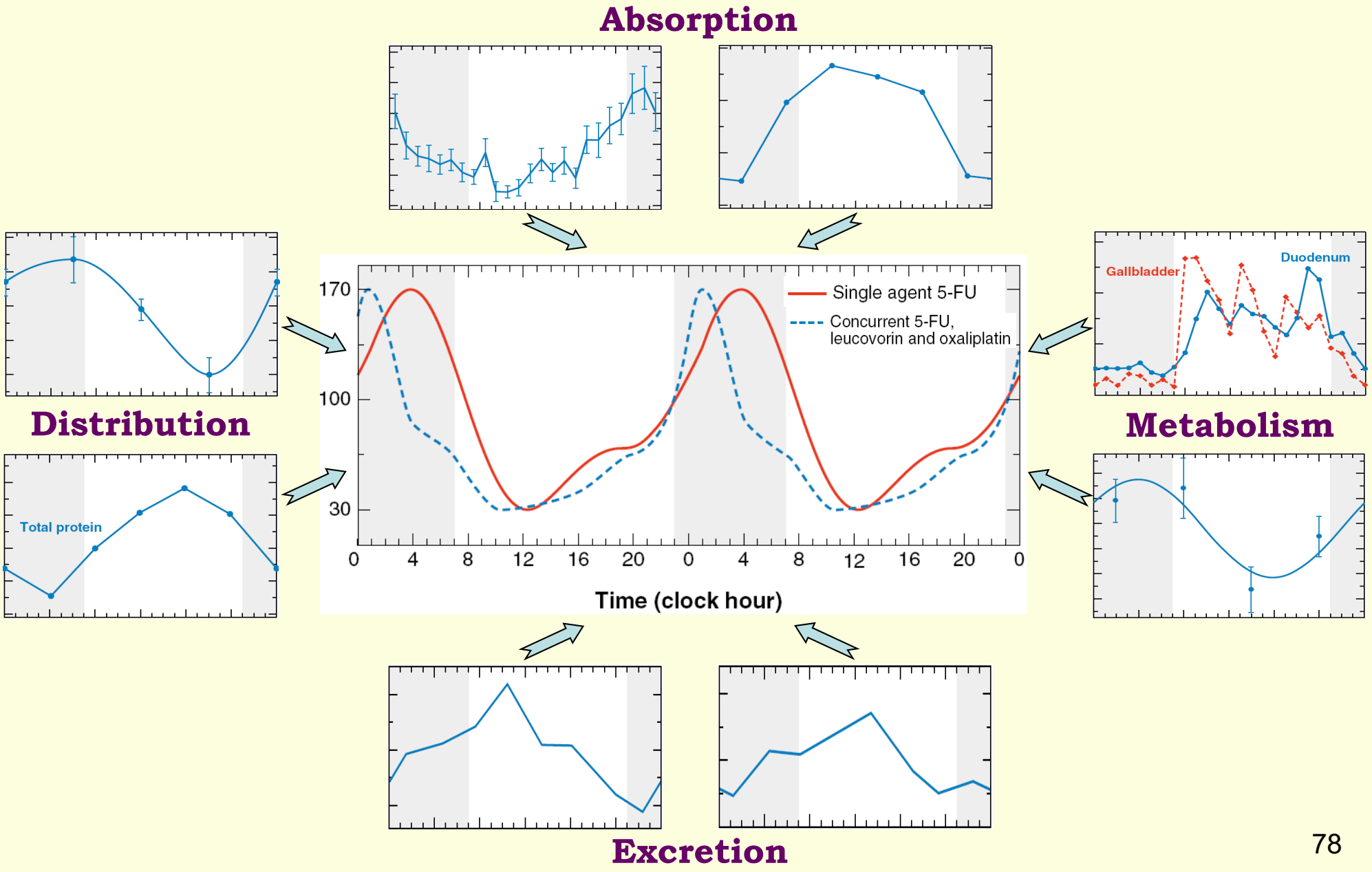
Urine flow



- Concerned drugs
  - most of drugs

Levi F, Schibler U. 2007.
Annu. Rev. Pharmacol. Toxicol. 47:593–628

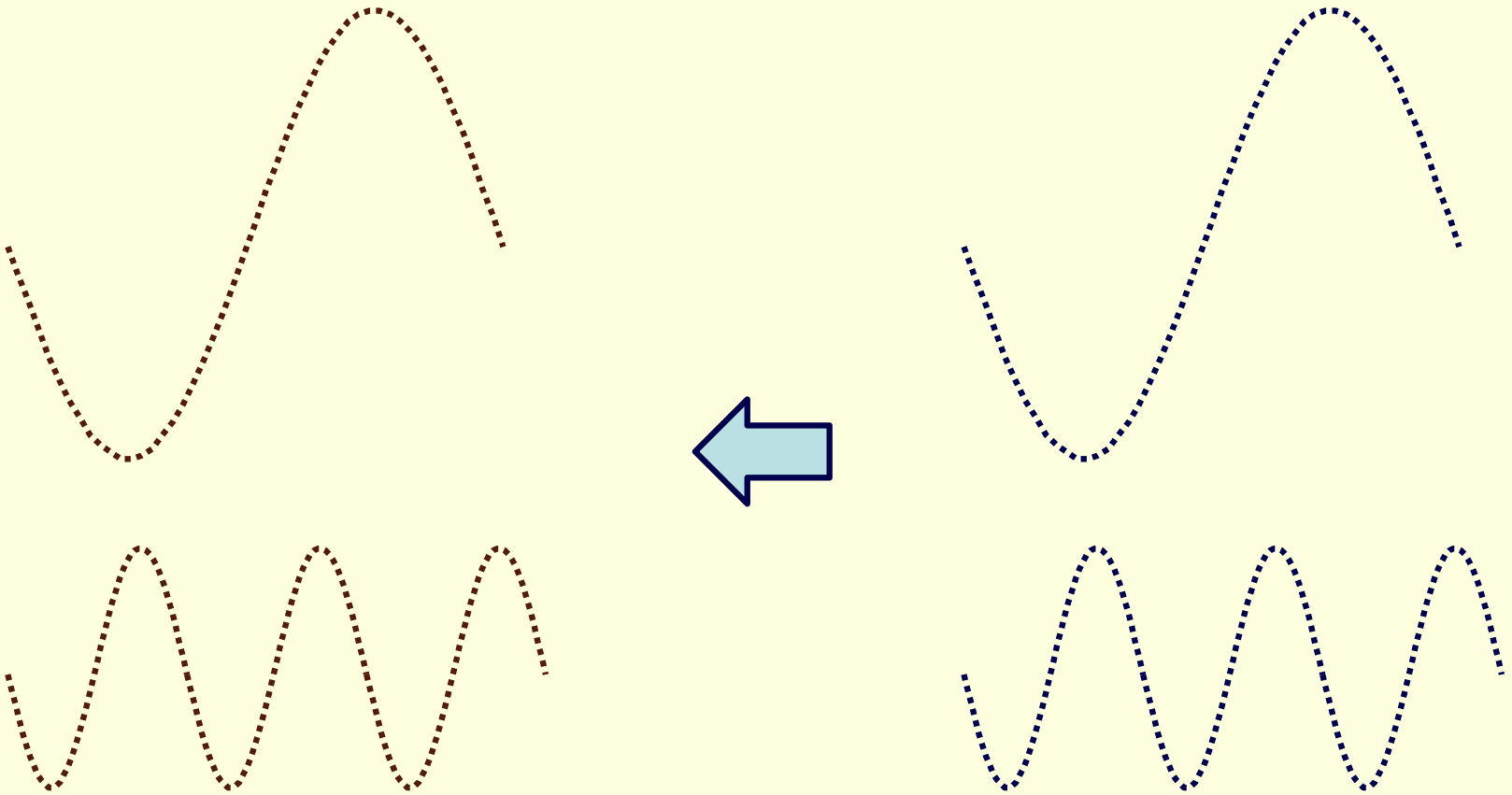
Chronopharmacokinetics: résumé (I)



Biological rhythms and the geophysical cycles

Biological rhythms

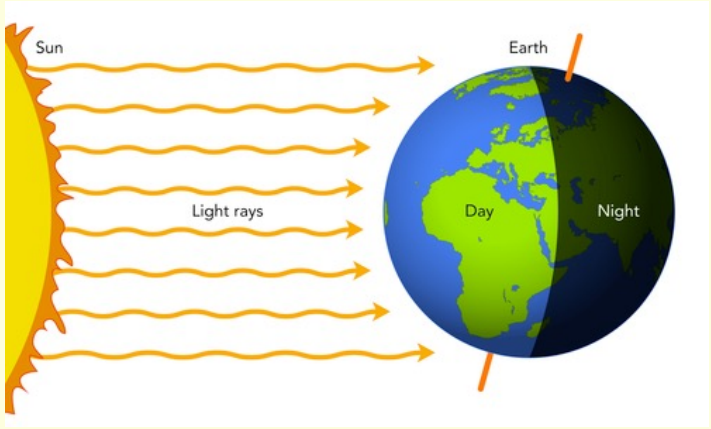
Geophysical cycles



Biological rhythms and the geophysical cycles

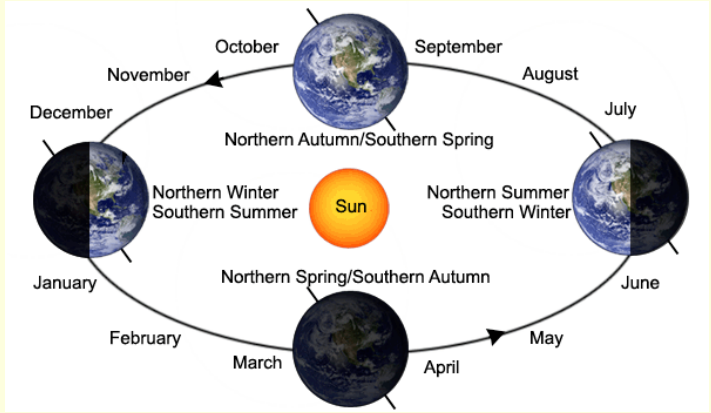
- The circadian cycles caused by the Earth's rotation around its axis

  24-hours cycles of: light, temperature, humidity; highly predicatble



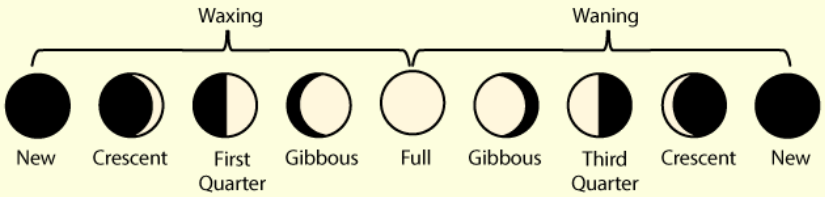
- The seasonal cycles are due to the tilting of the Earth on its axis

  365-days cycles of: light, temperature, humidity; highly predicatble



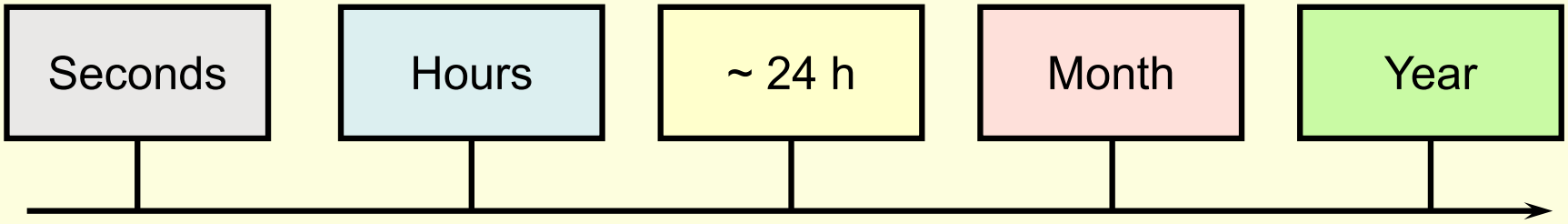
- The Moon cycles

  cycles of gravitational forces; highly predicatble

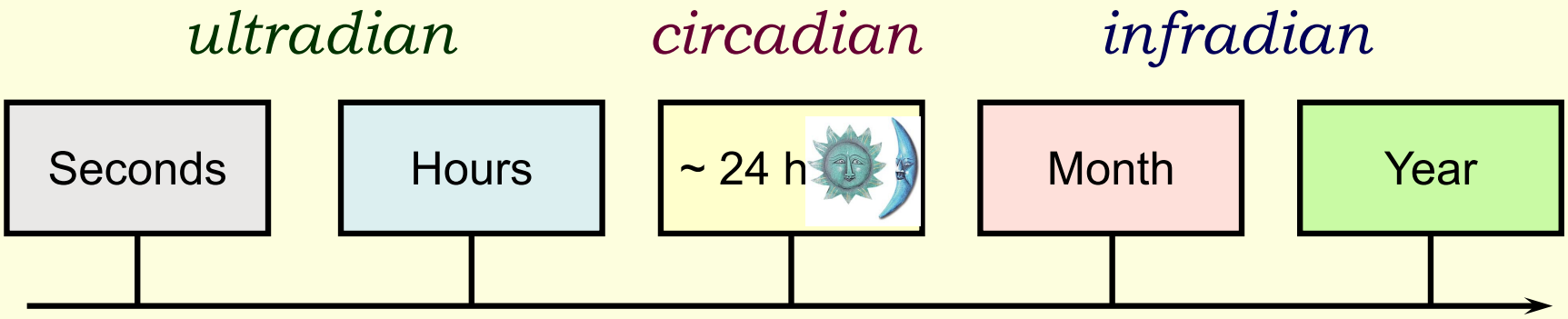


Biological rhythms

ultradian *circadian* *infradian*

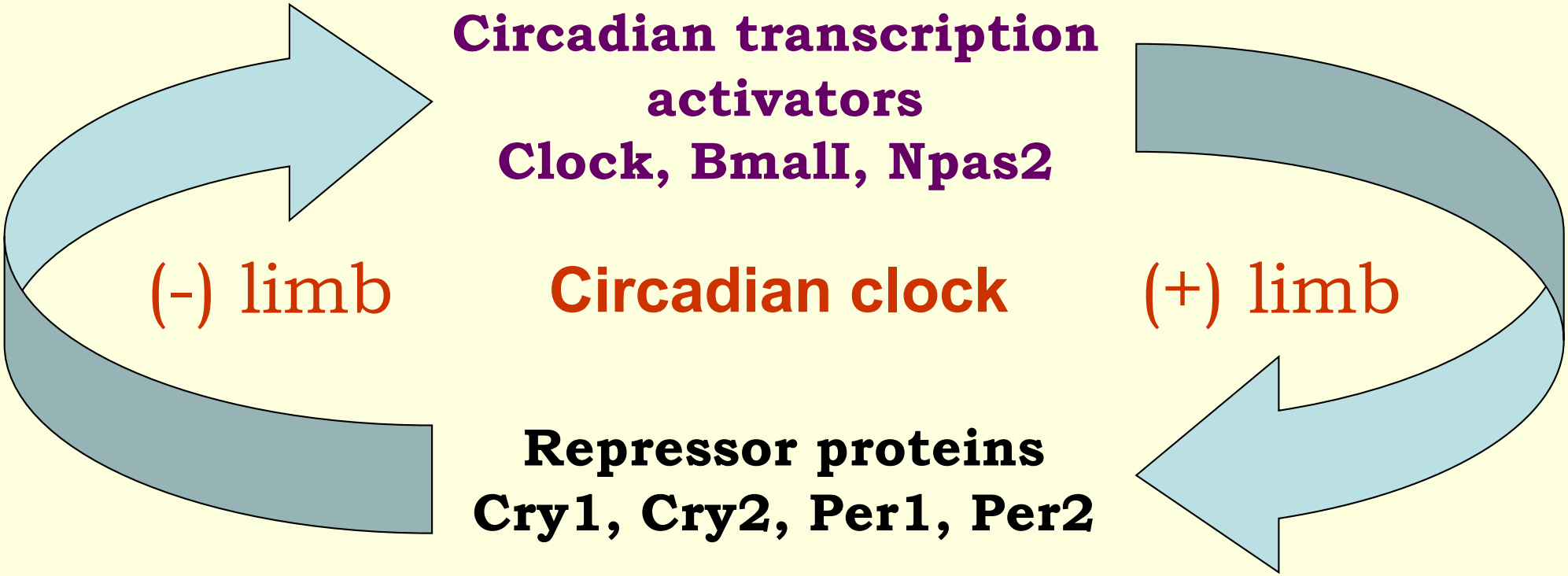


Biological rhythms



- *most of the functional circadian rhythms are self-sustained and continue to oscillate even in the absence of external time cues*
- *this suggests the existence of a molecular mechanism(s) that drives biological/functional oscillations in a self-sustained manner*

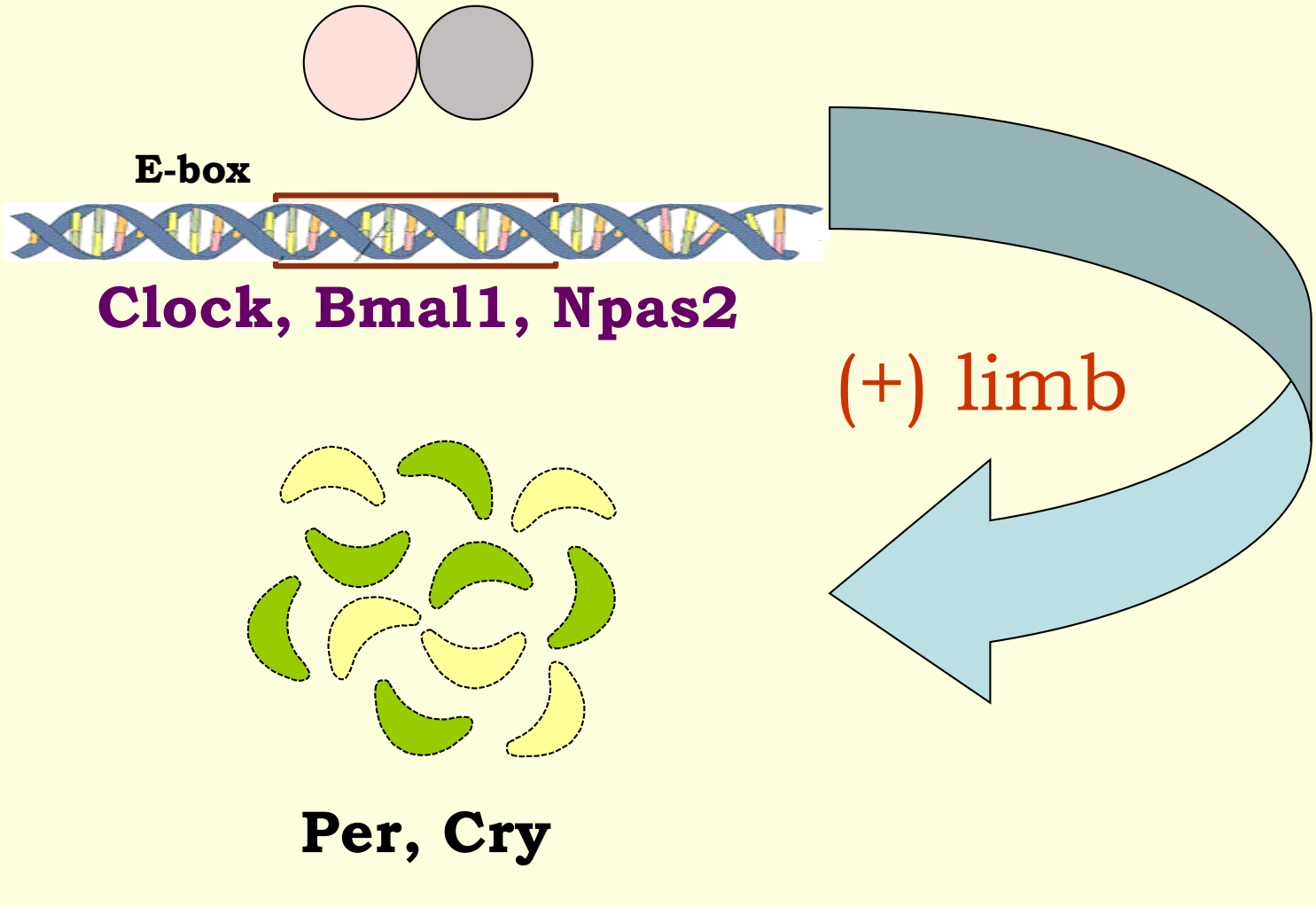
Circadian rhythms



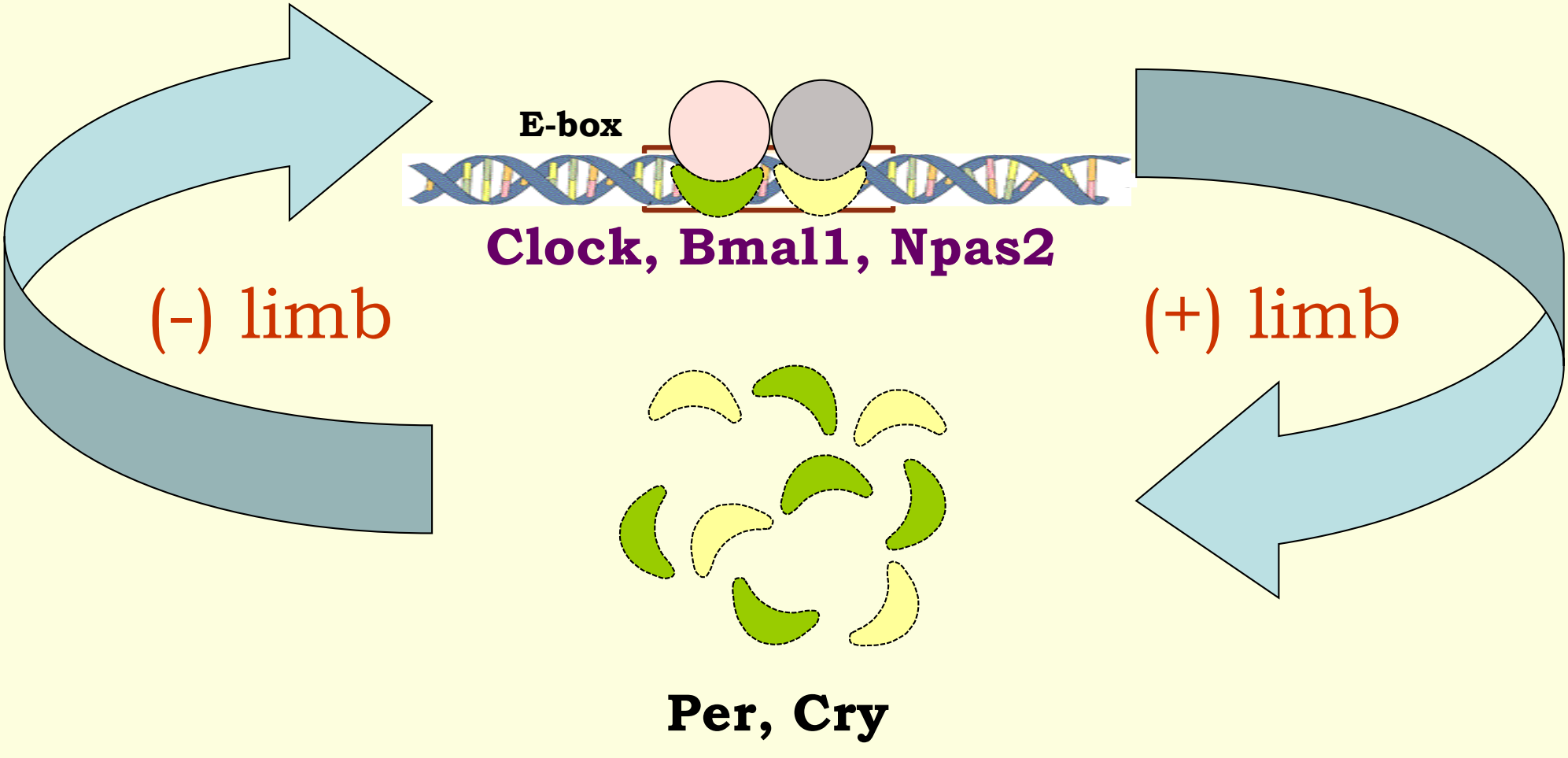
circadian (lat. *circa dies*) = about a day (~ 24 h)

Awarded by 2017 Nobel Prize in Medicine

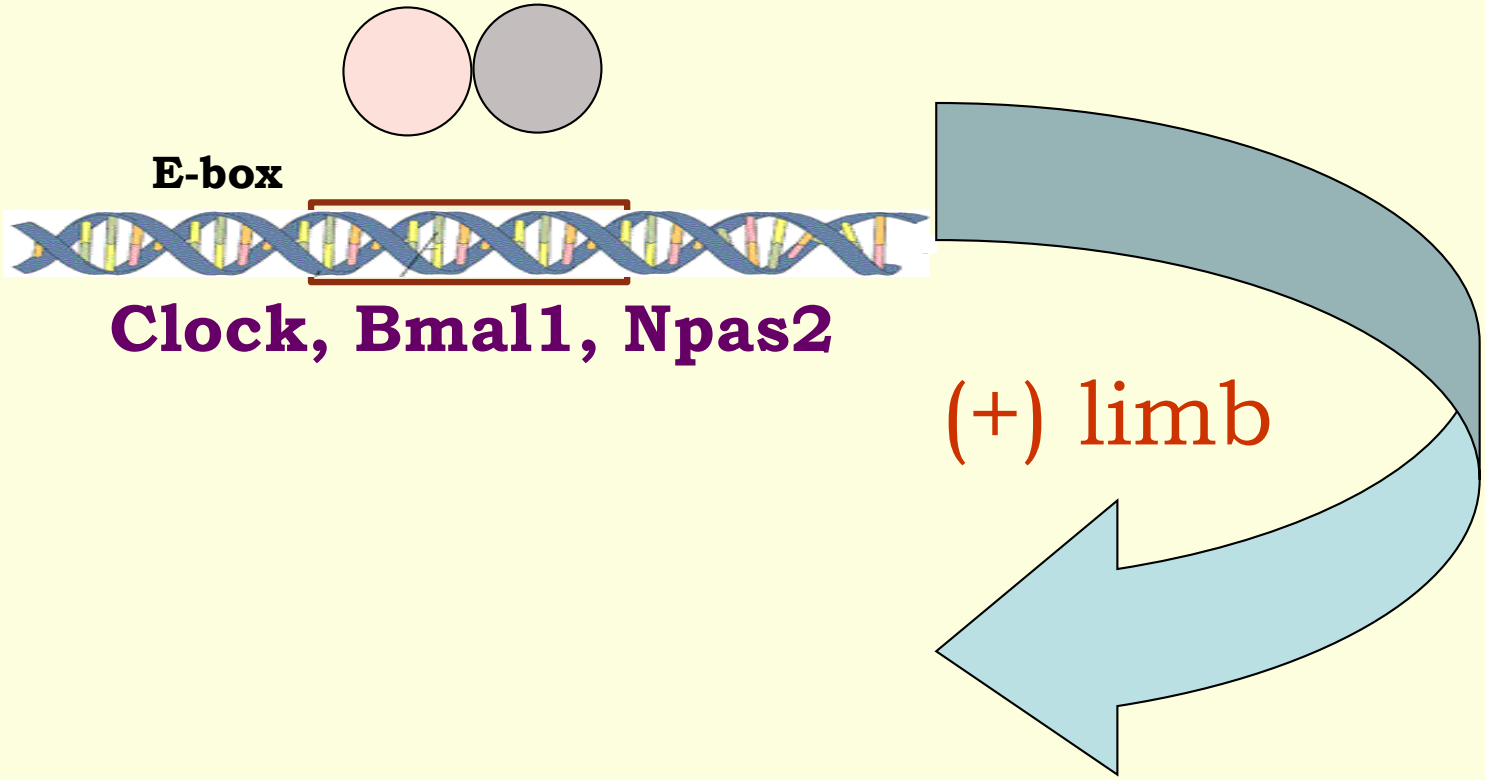
Circadian clock



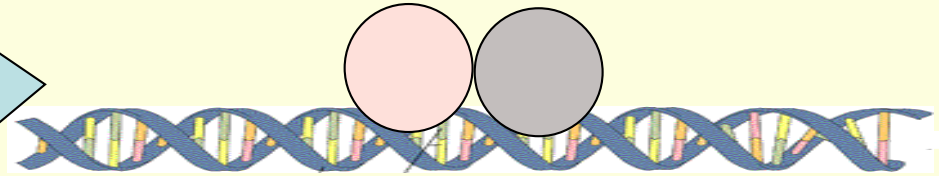
Circadian clock



Circadian clock



« Output genes »



Clock, Bmal1, Npas2

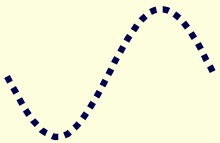
(-) limb

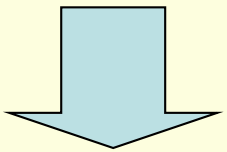
~ 24 hours

(+) limb

repressors
Cry1, Cry2, Per1, Per2

«output» genes
10-50% of transcriptome



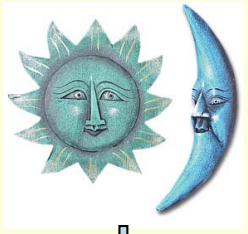


translation
posttranslational modifications

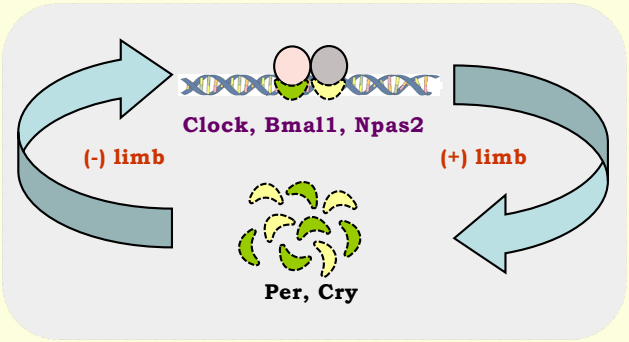
functional rhythms

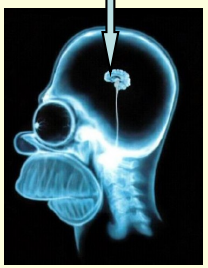
Circadian timing system

daily light/
dark cycle



~ 24 hours





resetting central oscillator
in the suprachiasmatic
nucleus (SCN)

humoral
signals

neuronal
signals

rest/
activity

feeding/
fasting

T°

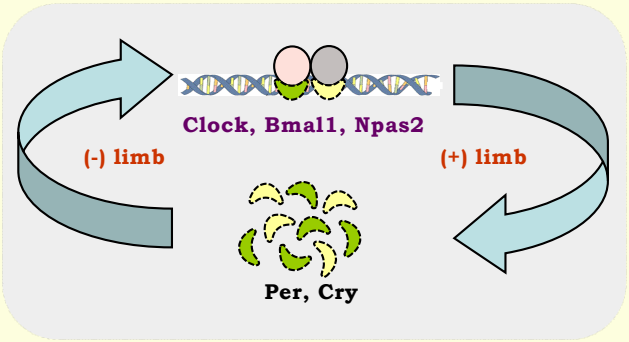
body
rhythms

resetting circadian oscillators in peripheral tissues

Circadian timing system

daily light/
dark cycle

~ 24 hours



resetting central oscillator
in the suprachiasmatic
nucleus (SCN)

humoral
signals

neuronal
signals

rest/
activity

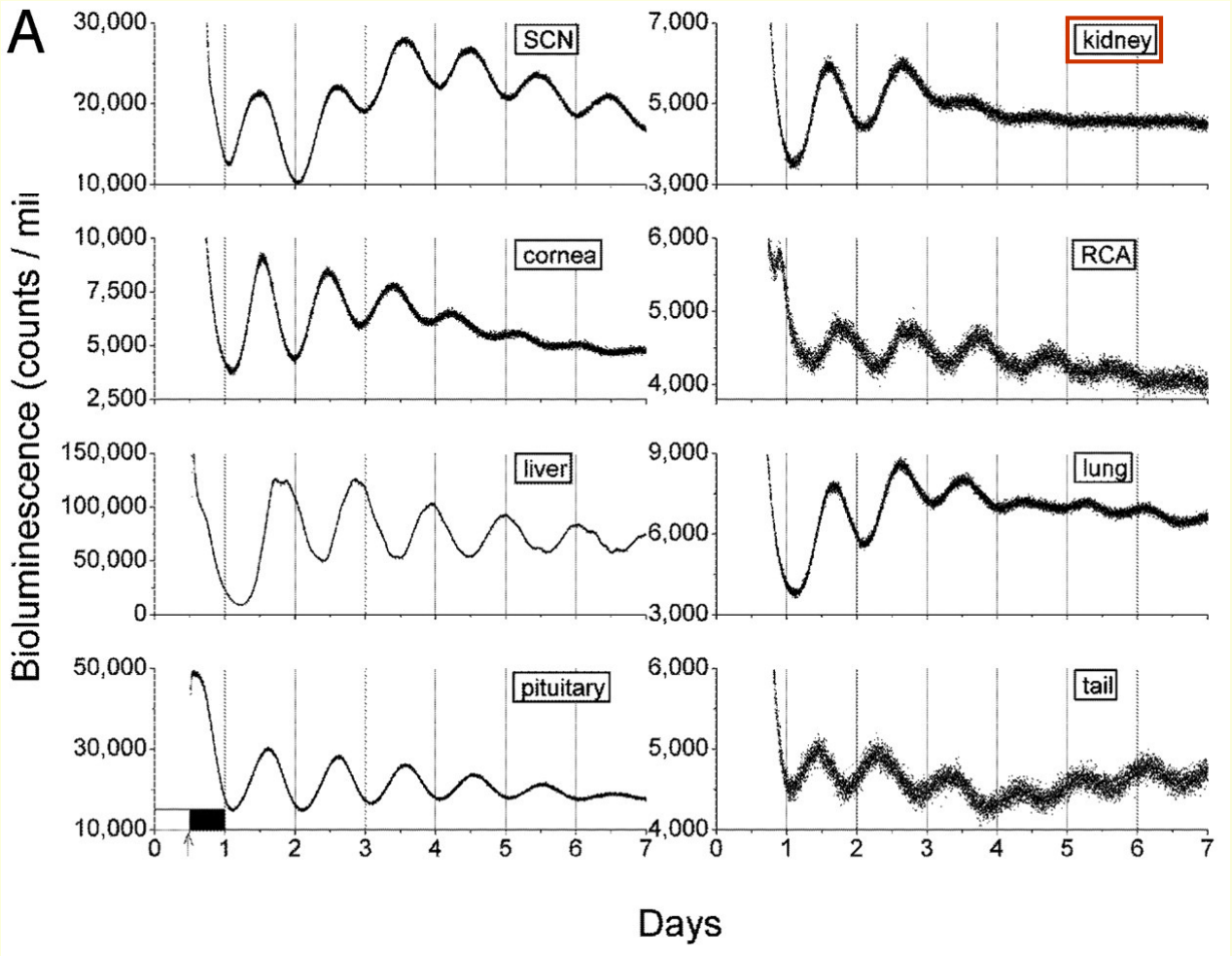
feeding/
fasting

T°

body
rhythms

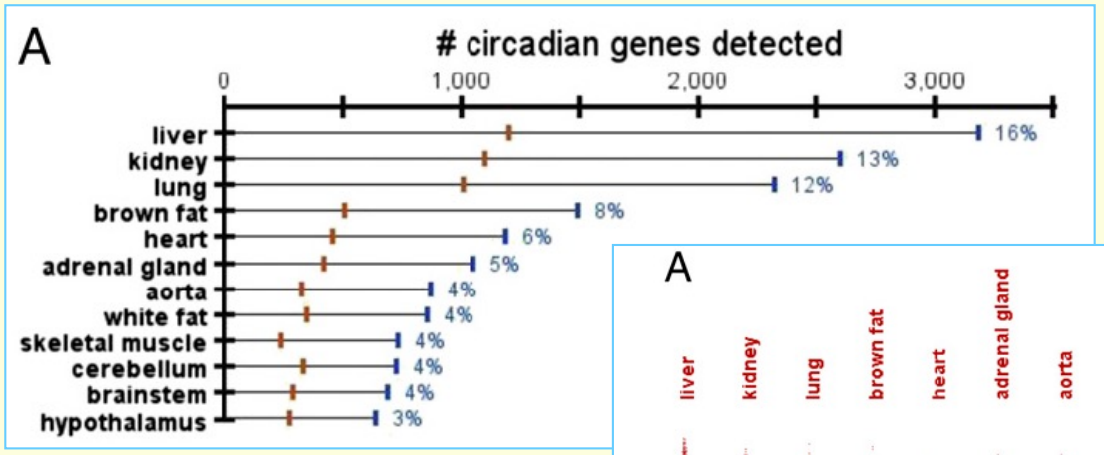
resetting circadian oscillators in peripheral tissues

Circadian rhythms in peripheral tissues are cell-autonomous and self-sustained

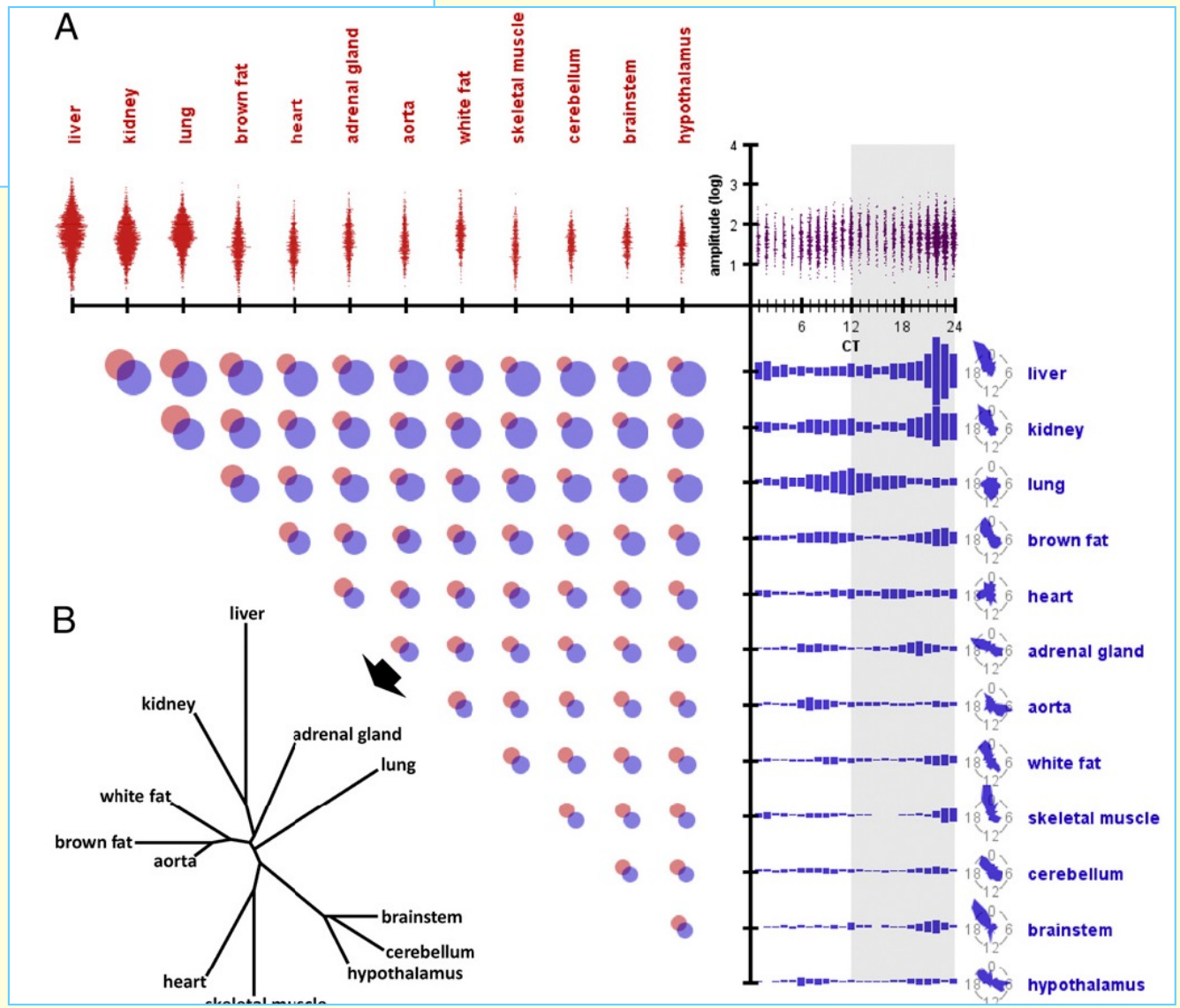


Real-time analysis of Per2-Luciferase expression in explanted peripheral tissues (Yoo et al. PNAS 2004)

Circadian genes in different tissues



Zhang et al, PNAS 2014



Many drugs are metabolized by or target proteins encoded by circadian genes

Table 1. Drugs of the top-100 best-seller list that target circadian genes and have half-life < 6h

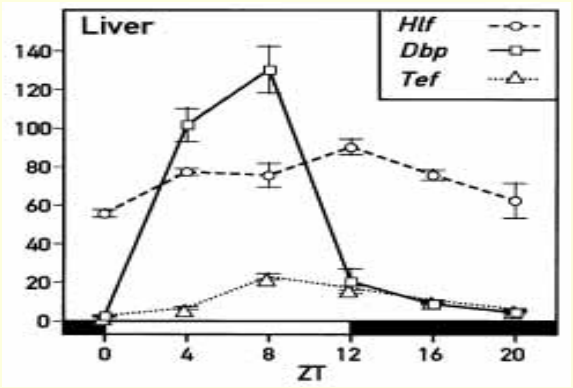
Rank	Sales, $	Trade name	Indications	Circadian-gene targets	Organs in which targets oscillate
2	1.46 b	Nexium	Gastritis, GERD, Esophagitis	*Atp4a*	L
5	1.28 b	Advair Diskus	Asthma, Chronic obstructive pulmonary di...	*Serpina6, Pgr, Nr3c2, Adrb2, Pla2g4a*	Lu, H, L, K, S, A
11	794 m	Rituxan	Rheumatoid arthritis, Non-Hodgkin's lymp...	*Fcgr2b, Ms4a1, Fcgr3*	L, K, S
20	538 m	Diovan	Hypertension, Heart failure	*Slc22a6, Agtr1a, Slco1b2, Car4, Kcnma...*	H, AG, L, K, S
27	431 m	Vyvanse	Attention deficit hyperactivity disorder	*Adra1b*	L
32	392 m	Tamiflu	Influenza	*Neu2, Neu1, Ces1g, Slc22a8, Slc15a1, ...*	Lu, L, BF, K, C
33	383 m	Ritalin	Attention deficit hyperactivity disorder	*Slc6a4*	AG, K
37	348 m	AndroGel	Hypogonadism	*Slc22a4, Slc22a3, Ar, Cyp1a1, Cyp2b10...*	Lu, H, BS, WF, AG...
38	346 m	Lidoderm	Pain	*Slc22a5, Cyp2b10, Egfr, Abcb1a*	Lu, H, AG, BF, L,...
44	304 m	Seroquel XR	Bipolar disorder, Major depressive disor...	*Htr2c, Htr1b, Htr2a, Chrm2, Drd4, Adr...*	Lu, H, BS, WF, AG...
45	289 m	Viagra	Erectile dysfunction	*Cyp1a1, Pde6g, Abcc5, Abcc10, Pde5a, ...*	Lu, H, BS, WF, AG...
47	281 m	Niaspan	Hyperlipidemia	*Slco2b1, Slc22a5, Qprt, Slc16a1*	Lu, H, BS, AG, WF...
48	279 m	Humalog	Diabetes mellitus T2	*Igf1r*	K
49	274 m	Alimta	Mesothelioma, Nonsmall cell lung cancer	*Tyms, Atic, Gart, Slc29a1*	Lu, H, BS, BF, L,...
54	267 m	Combivent	Asthma, Chronic obstructive pulmonary di...	*Slc22a5, Slc22a4, Chrm2, Adrb1, Adrb2*	Lu, H, BS, BF, K,...
56	262 m	ProAir HFA	Asthma, Chronic obstructive pulmonary di...	*Adrb1, Adrb2*	Lu, K, S
62	240 m	Janumet	Diabetes mellitus T2	*Slc47a1, Slc22a2, Prkab1, Abcb1a, Dpp4*	H, BS, AG, Hy, L,...
66	236 m	Toprol XL	Hypertension, Heart failure	*Slc22a2, Adrb1, Adrb2, Abcb1a*	Lu, H, AG, BF, L,...
71	220 m	Vytorin	Hyperlipidemia	*Hmgcr, Cyp2b10, Soat1, Abcc2, Anpep, ...*	Lu, H, BS, AG, BF...
78	209 m	Aciphex	Gastritis, GERD, Esophagitis	*Cyp1a1, Atp4a, Abcg2*	Lu, H, BS, WF, L,...
90	189 m	Lunesta	Insomnia	*Ptgs1, Tspo, Gabra3*	Lu, H, AG, K
98	173 m	Prilosec	Gastritis, GERD, Esophagitis	*Cyp1a1, Atp4a, Abcg2, Cyp1b1, Abcb1a*	Lu, H, BS, WF, AG...
99	171 m	Focalin XR	Attention deficit hyperactivity disorder	*Slc6a4*	AG, K

Rank and sales are based on USA 2013 Q1 data from Drugs.com. A, aorta; AG, adrenal gland; BF, brown fat; BS, brainstem; C, cerebellum; H, heart; Hy, hypothalamus; K, kidney; L, liver; Lu, lung; S, skeletal muscle; WF, white fat.

Zhang et al, PNAS 2014

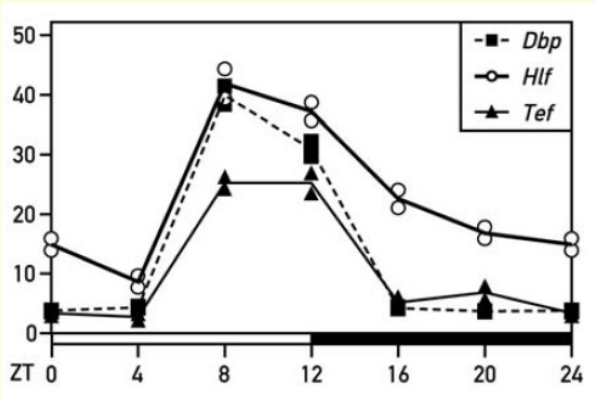
Chronopharmacokinetics: role of transcriptional factors DBP, HLF et TEF

Liver



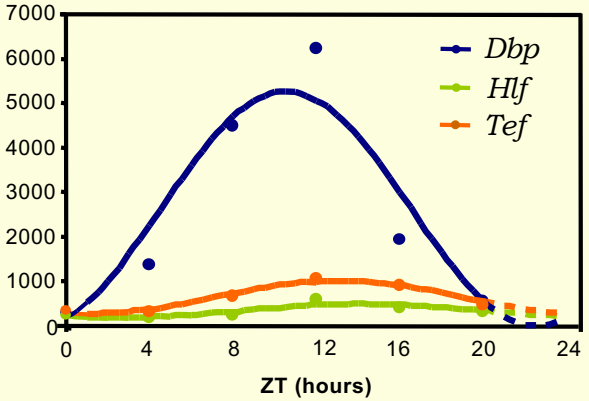
Gachon et al, 2004

Small Intestine

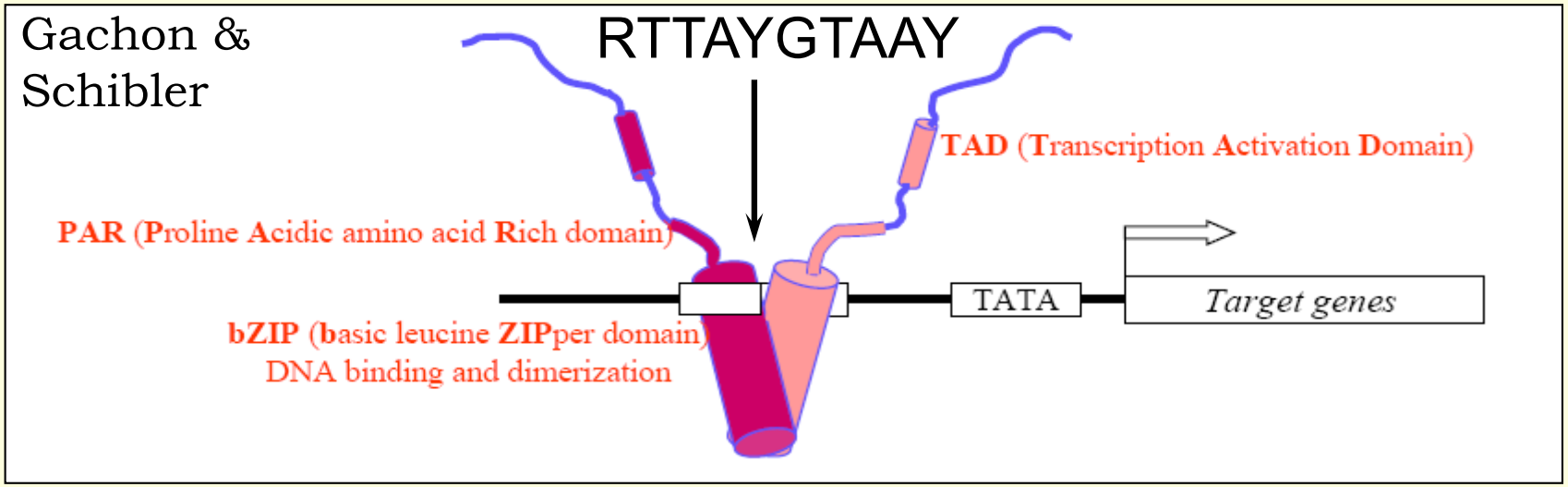


Gachon et al, 2006

Kidney



Mercier et al, 2009



The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification

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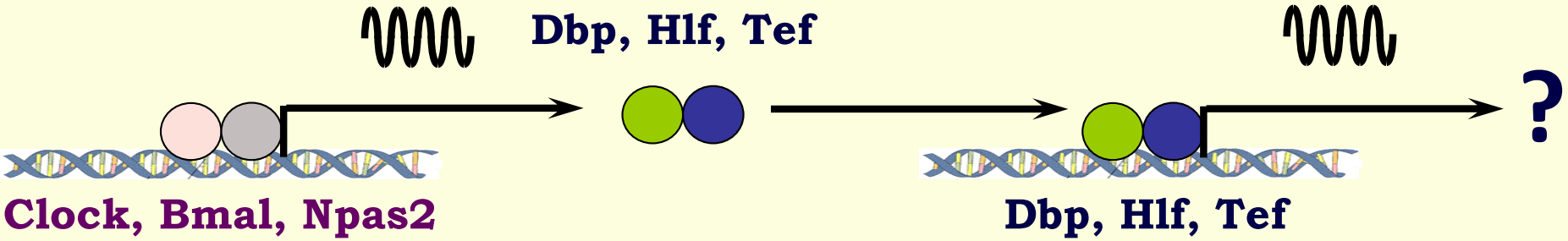
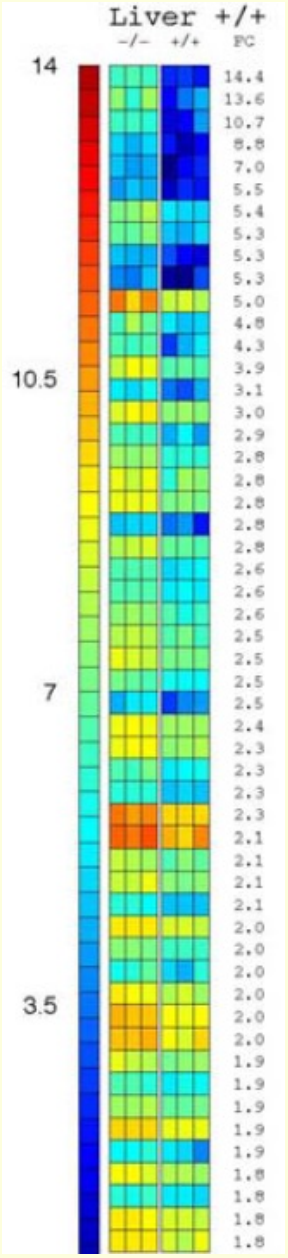


Genes differentially expressed in the liver of wt mice and *Dbp(-/-)/Hlf(-/-)/Tef(-/-)* mice



Phase I reactions:

Cyp2b, Cyp3a, Cyp2c, Cyp4a, Ces, Aldh1a1, Aldh1a7, Aldh3a2, Alas1, Por ...

Phase II reactions:

Sult1d1, Sult3a1, GSTt1, Gsta2, Gsta3, Ugt1a1, ...

Transporters of xenobiotics:

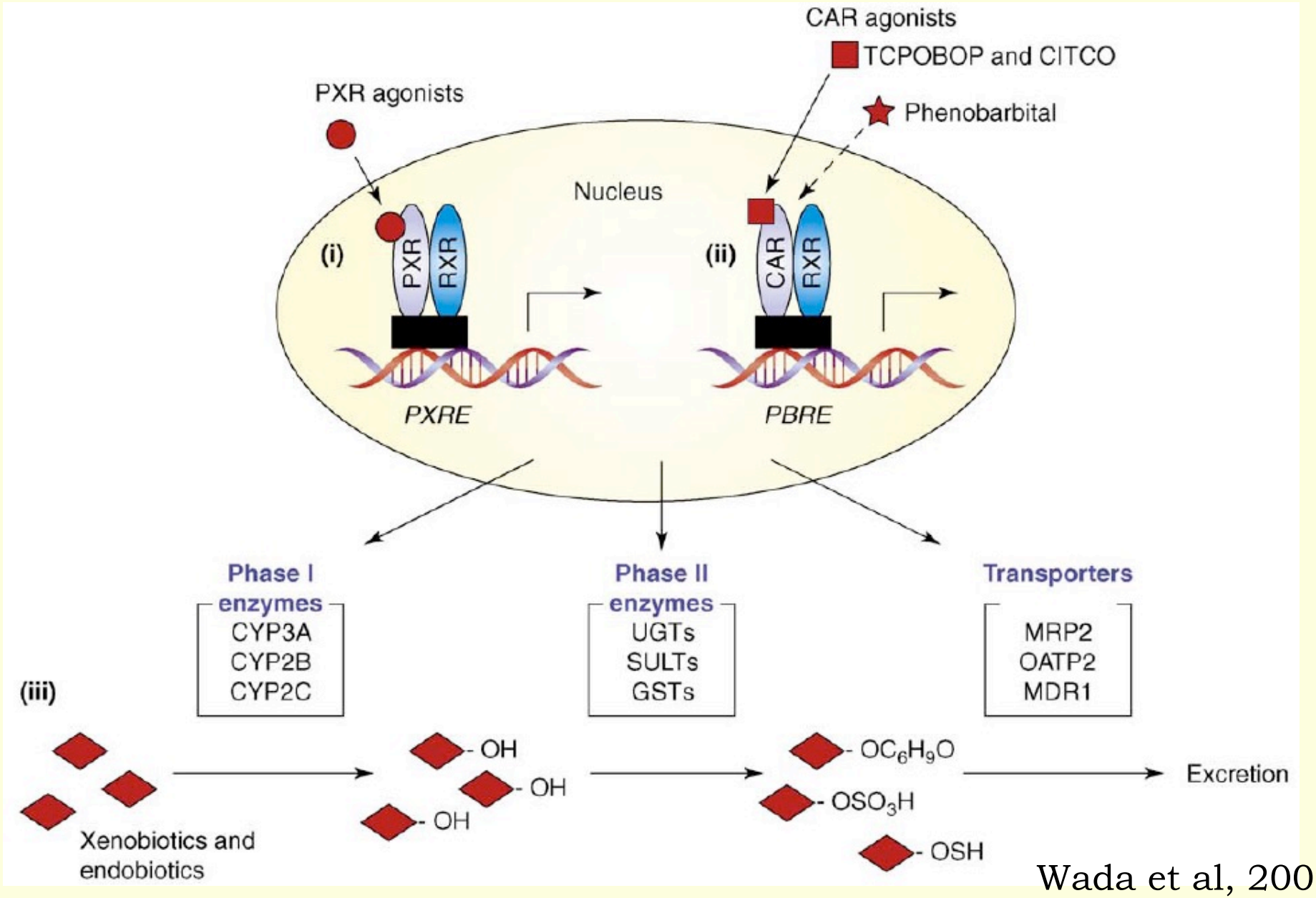
AbcG2, AbcC4, AbcA8a, AbcA6, AbcB1b, ...

Nuclear receptors controlling metabolism of xenobiotics:

Constitutive androstane receptor (CAR)

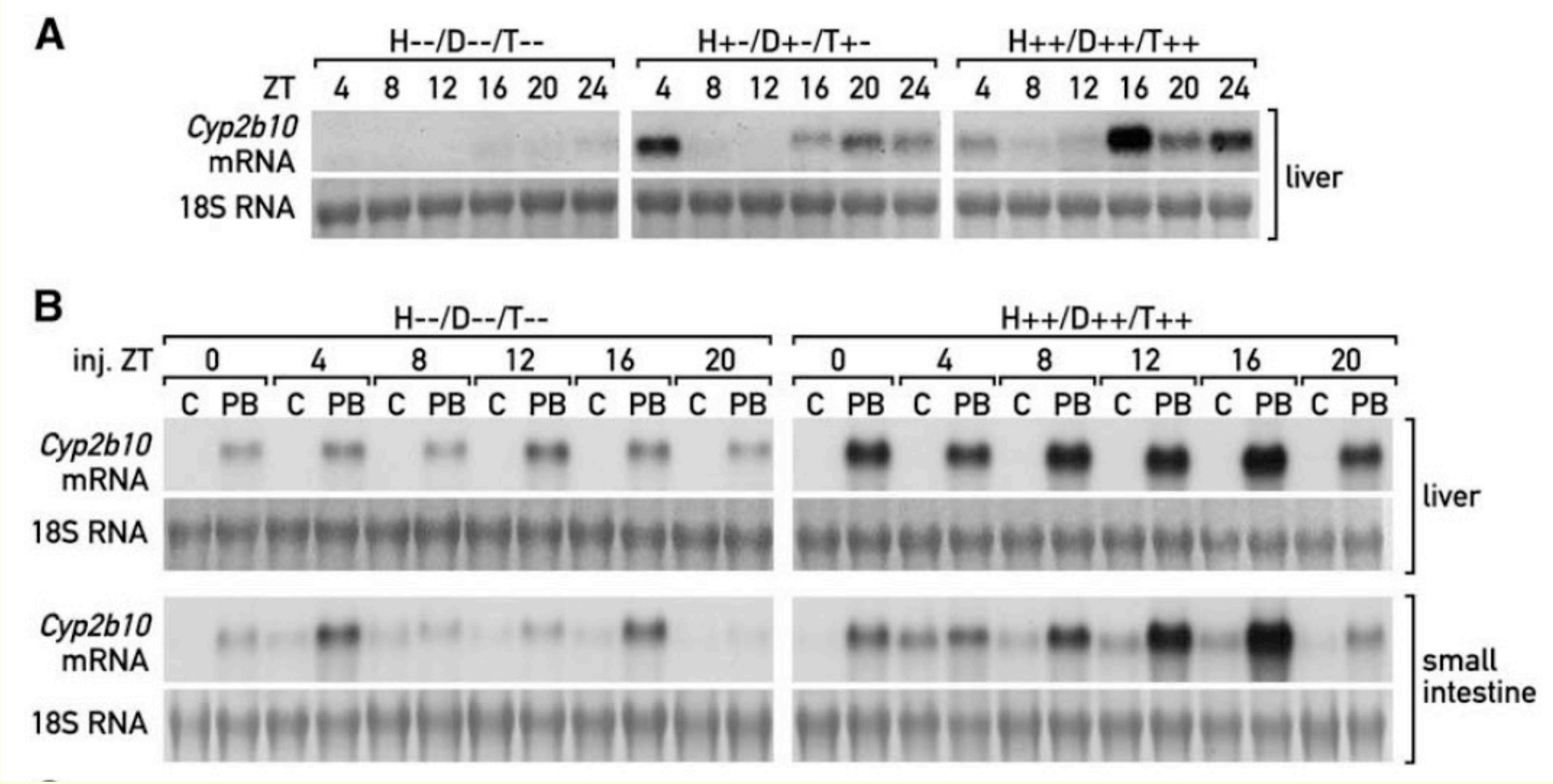
Gachon et al, 2006

Constitutive Androstane Receptor (CAR) – a reminder



Wada et al, 2009

Example: transcriptional control of Cyp2b10 expression by PAR bZip(s)



C – control PB – phenobarbital induction

Gachon et al, 2006

Substrates of Cyp2b10 (Cyp2b6 in human)

~ 8 % of drugs currently used in clinics

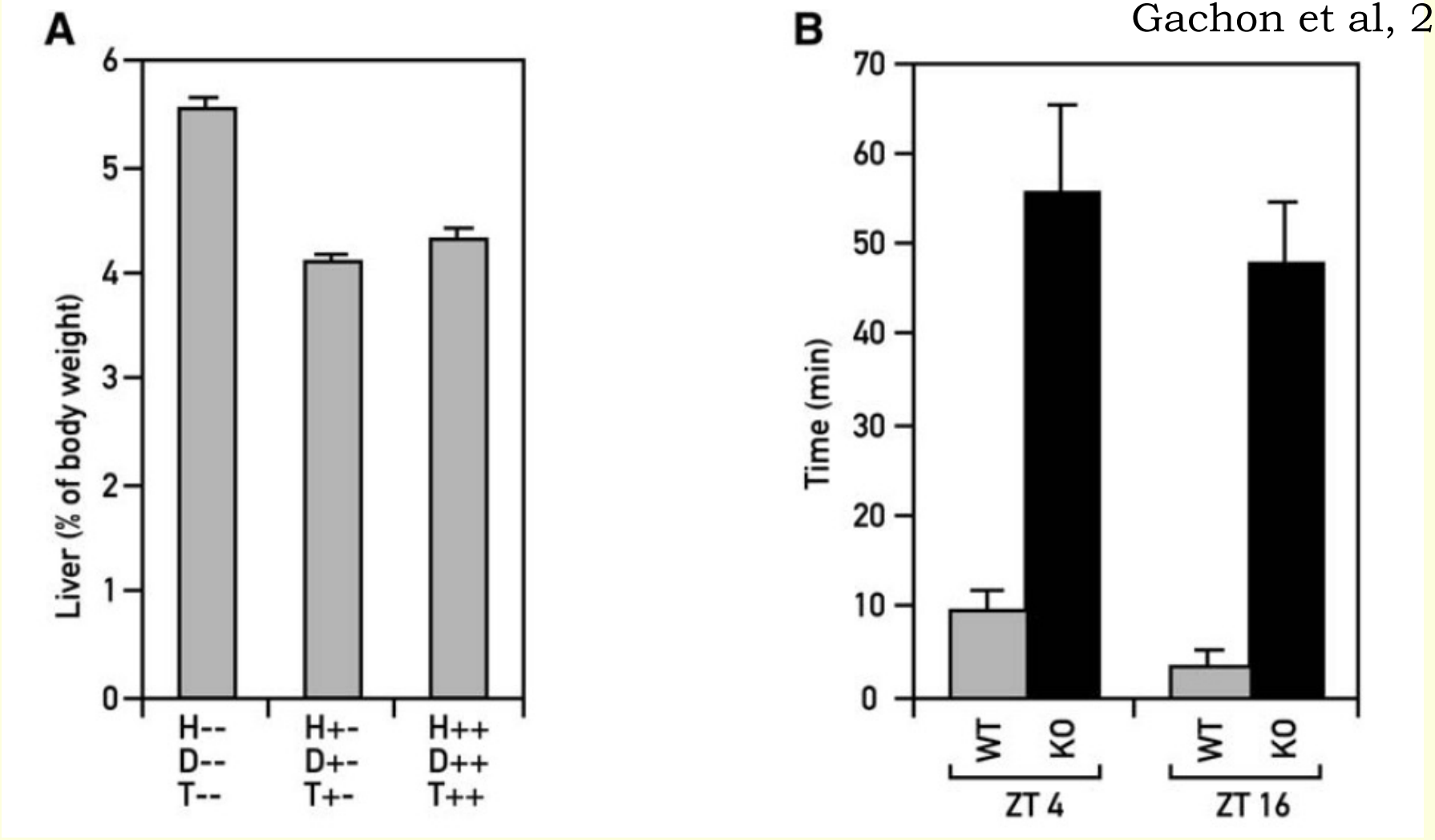
cyclophosphamide
ifosfamide
tamoxifen
ketamine
artemisinin
nevirapine
efavirenz
bupropion
sibutramine
propofol

<u>other substrates</u>:
arachidonic acid
lauric acid
17β-estradiol
estrone
ethinylestradiol
testosterone

Mo et al., Curr Drug Metab 2009

Increased liver mass (A) and decreased pentobarbital clearance (B) in mice *Dbp(-/-)/Hlf(-/-)/Tef(-/-)*

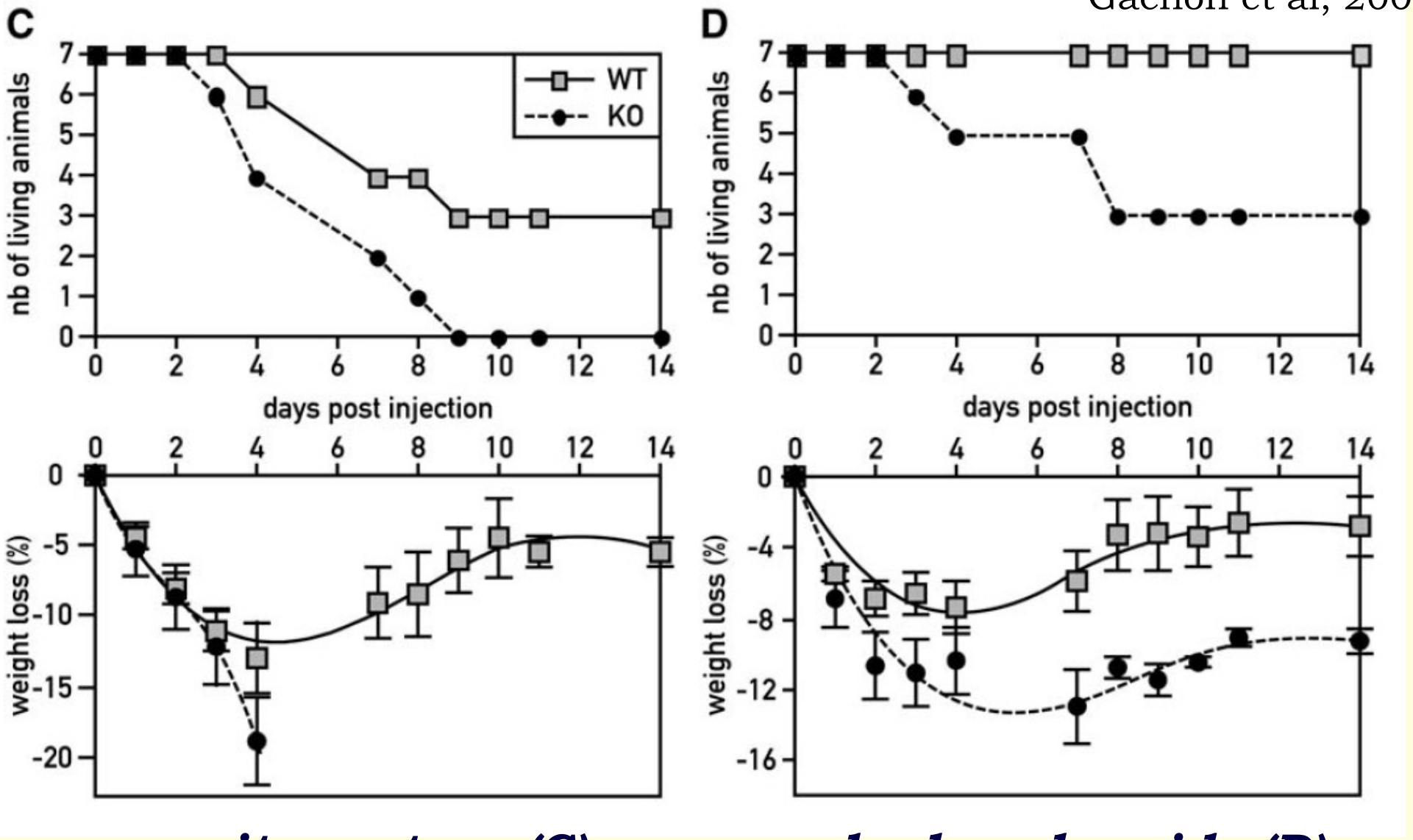
Gachon et al, 2006



Pentobarbithal-induced sleeping time is increased to 48 min in ko mice, from 3 min in wild-type mice!

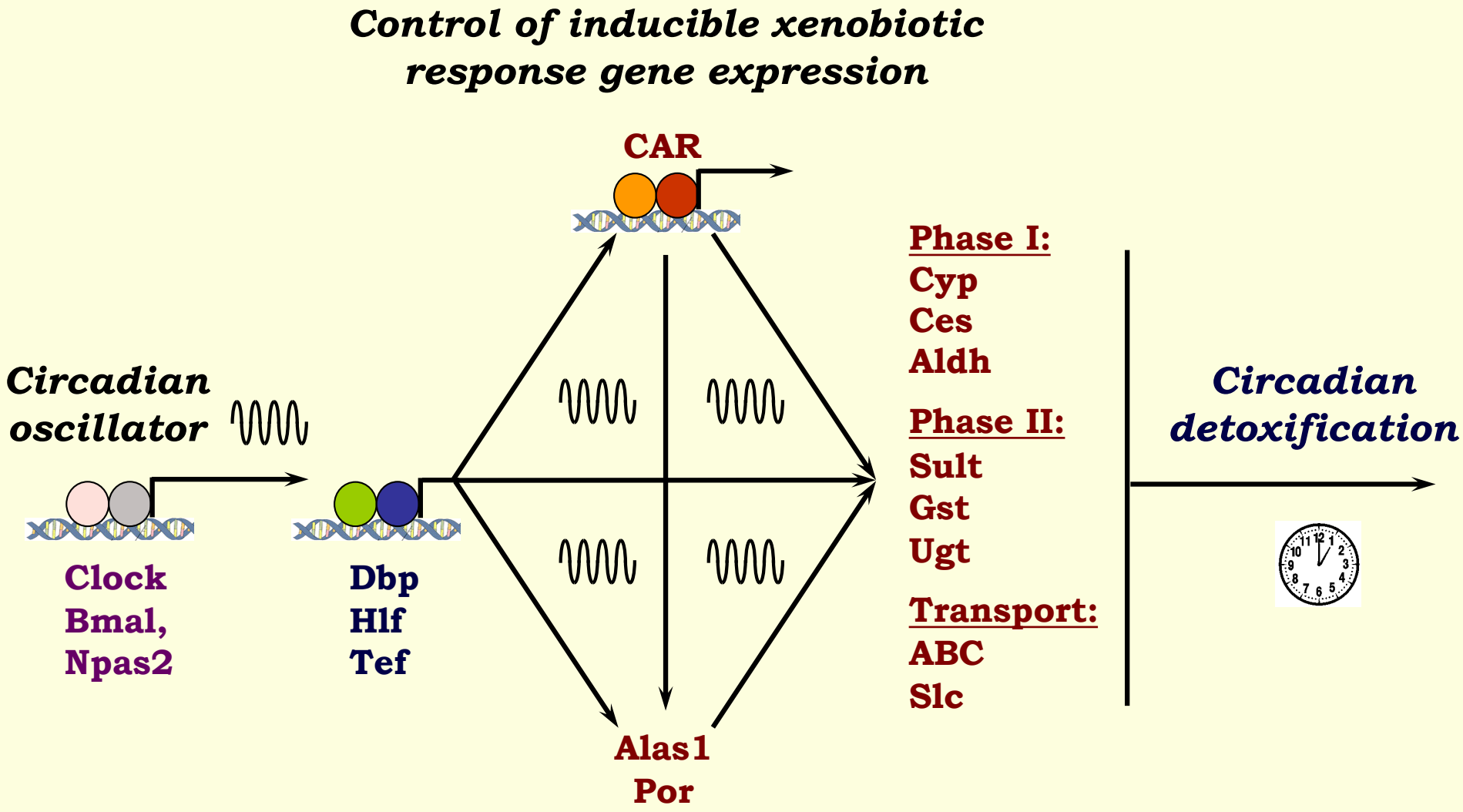
Increased toxicity of anticancer drugs in mice
Dbp(-/-)/Hlf(-/-)/Tef(-/-)

Gachon et al, 2006



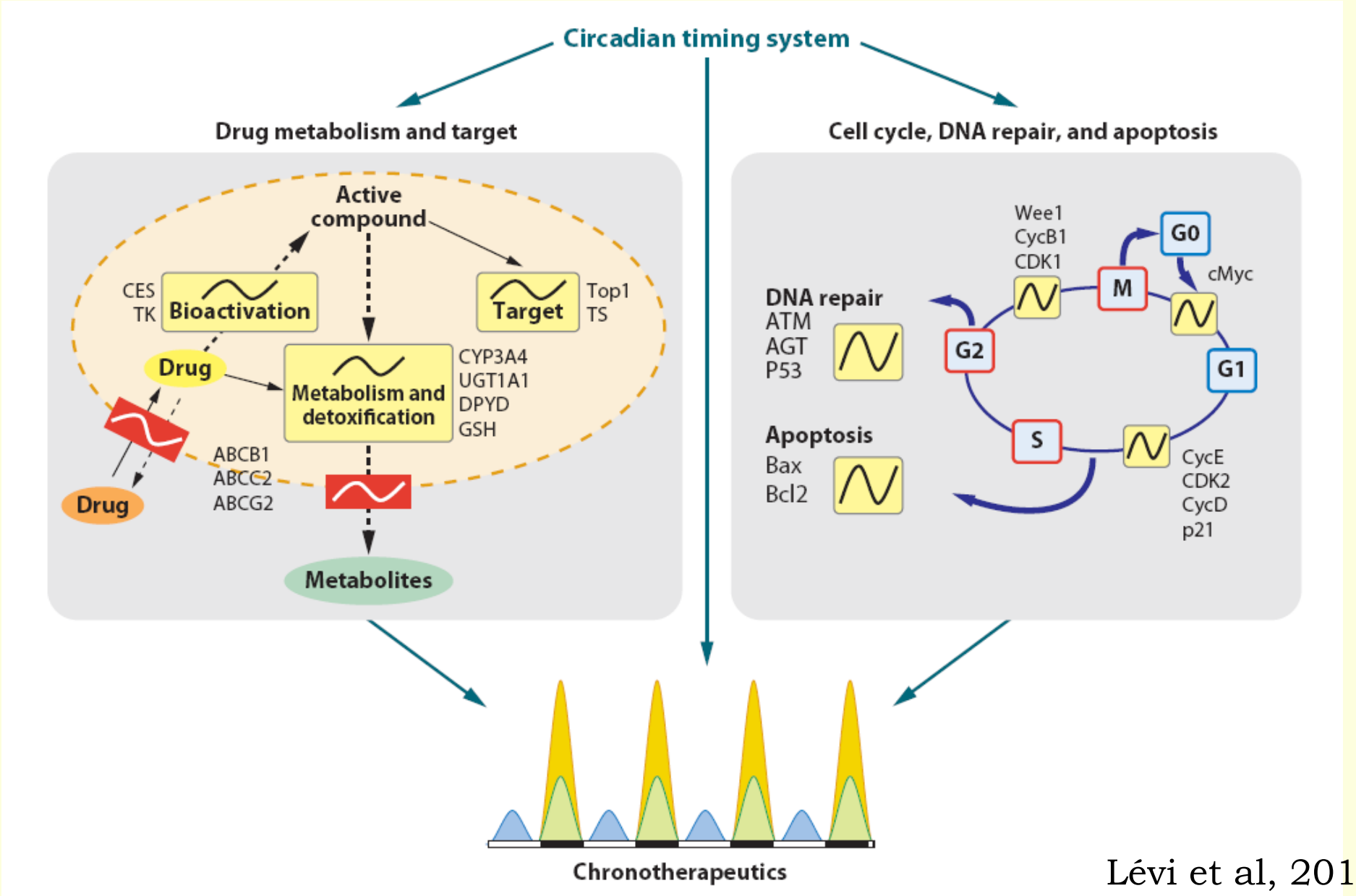
mitoxantron (C) ***cyclophosphamide (D)***

Chronopharmacokinetics: résumé (II)



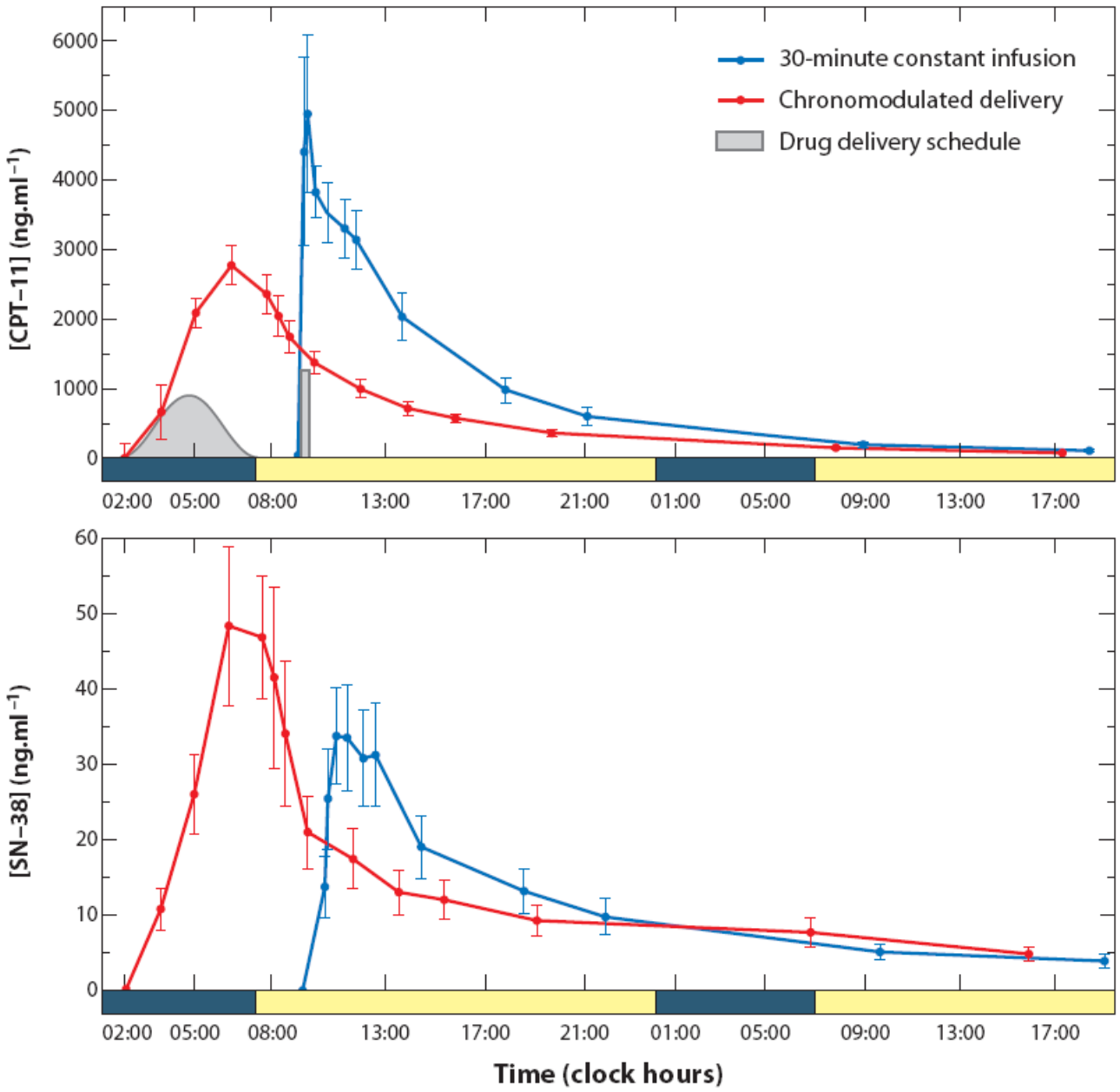
adapted from Gachon et al, 2006

Chronotherapy: cancer



Lévi et al, 2010

Chronotherapy: human



Example:

Chronomodulated irinotecan infusion in cancer patients:

- **↓ in mean C_{max}**
- **↓ coefficient of variation**
- **↑ metabolic ratio**
- **↓ incidence of severe diarrhea from 22,2% to 6%**
- **↓ incidence of asthenia from 44% to 23.5%**

Lévi et al, 2010