Metabolic functions of liver (not in seminar book)

Catabolism of hem (Chapter 15)

Biotransformations of xenobiotics (not in seminar book)

Seminar No. 6

- Chapter 15 (partly) -

Glucose metabolism in liver

- **well-fed state (insulin):** glycogenesis, glycolysis
- **fasting (glucagon):** glycogenolysis, gluconeogenesis
- other pathways:

  pentose cycle (ribose, other pentoses, NADPH)

  the isomeration of glucose to galactose

  the conversion of fructose and galactose to glucose

  synth. of derivatives: glucuronic acid, glucosamine .....

Aminoacid metabolism in liver

- synthesis of most plasma proteins
- up-take and degradation of plasma proteins + peptide hormons
- catabolism of AA

  (transamination - ALT, deamination - GMD)
- synthesis of non-essential AA
- detoxication of ammonia (urea, glutamine)

Lipid metabolism in liver

- synthesis of FA and TAG
- synthesis of phospholipids
- synthesis of lipoproteins (VLDL, HDL)
- degradation of TAG/PL - CM remnants, IDL, LDL, HDL_2 (hepatic lipase, lysosome)
- β-oxidation of FA
- synthesis of KB – **for export only**
  succinyl-CoA:acetoacetate-CoA transferase (for activation of acetoacetate) is not in liver

Cholesterol metabolism in liver

- synthesis of cholesterol
- excretion of cholesterol into bile
- synthesis of bile acids
- conjugation of bile acids
- excretion of bile acids into bile

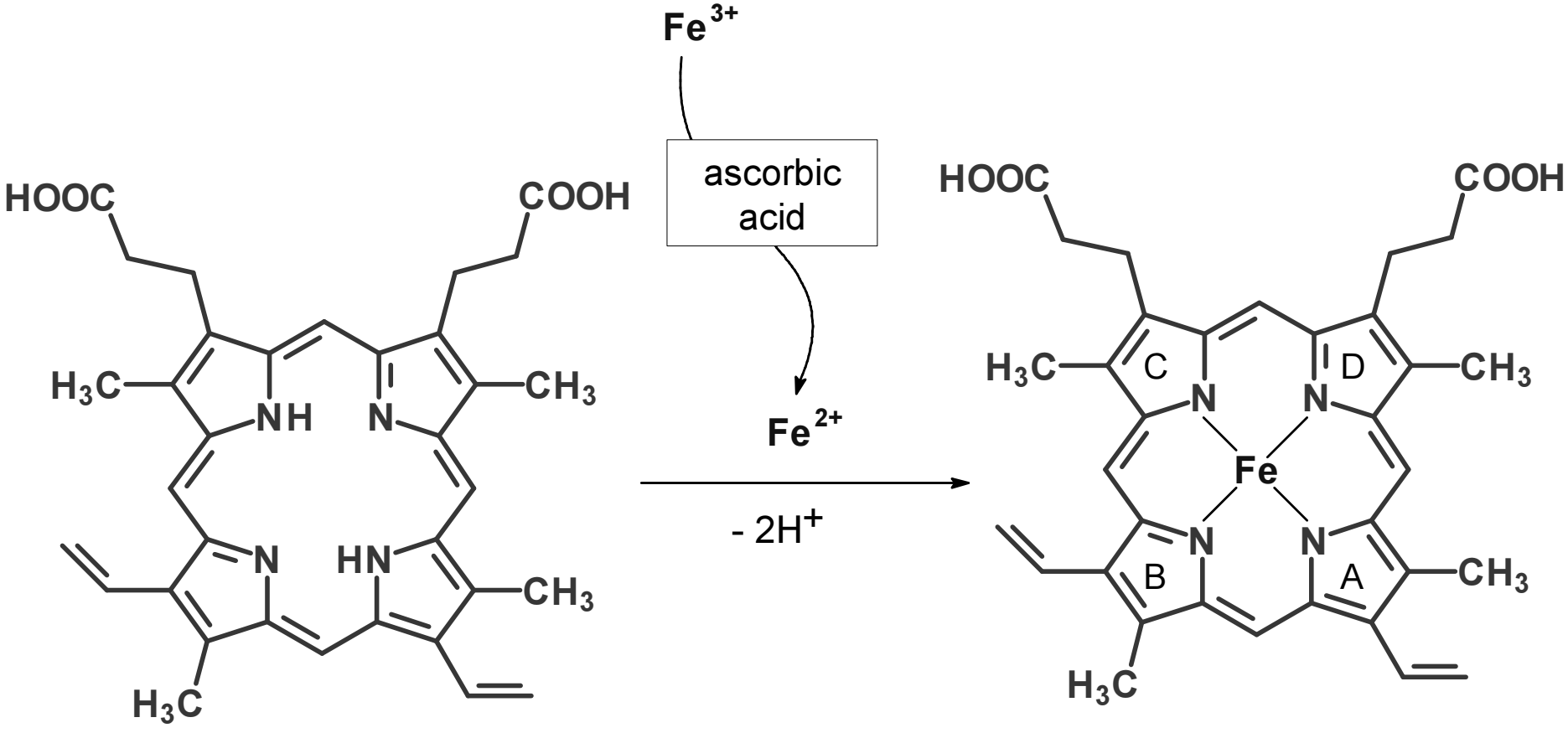
The localization of metabolic processes (see also p. 56)

Periportal hepatocytes	Perivenous hepatocytes
β-oxidation of FA	glycolysis
CAC	FA/TAG synthesis
gluconeogenesis	Gln synthesis (NH_3 detox.)
glycogen synthesis	
transamination of AA	**Biotransformation reactions:**
urea synthesis	hydroxylations (cyt P-450)
cholesterol synthesis	conjugations
ROS elimination	ethanol dehydrogenation

Catabolism of hem

p. 88

Hem is a chelate of protoporphyrin IX with Fe^{2+}



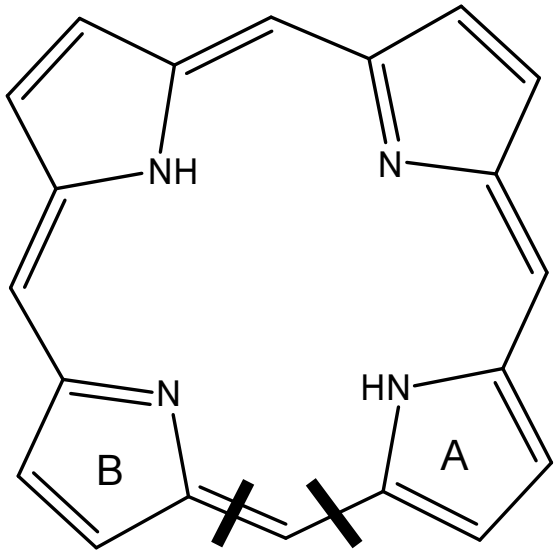
protoporfyrin IX

hem

Catabolism of hem

- occurs mainly in spleen, liver, bone marrow
- hemoxygenase (O_2, NADPH, cytochrome P-450)
- Fe^{2+} is released and oxidized to **Fe^{3+}**, bound to ferritin (store)
- **-**CH= between A/B rings is split off as **carbon monoxide (CO)**
- two O atoms are attached to the A+B pyrrole rings → **biliverdin**
- the central **-C=** bridge between C/D rings in biliverdin is then reduced to **-CH_2-** bridge → **bilirubin**

Hem degradation provides CO and bilirubin



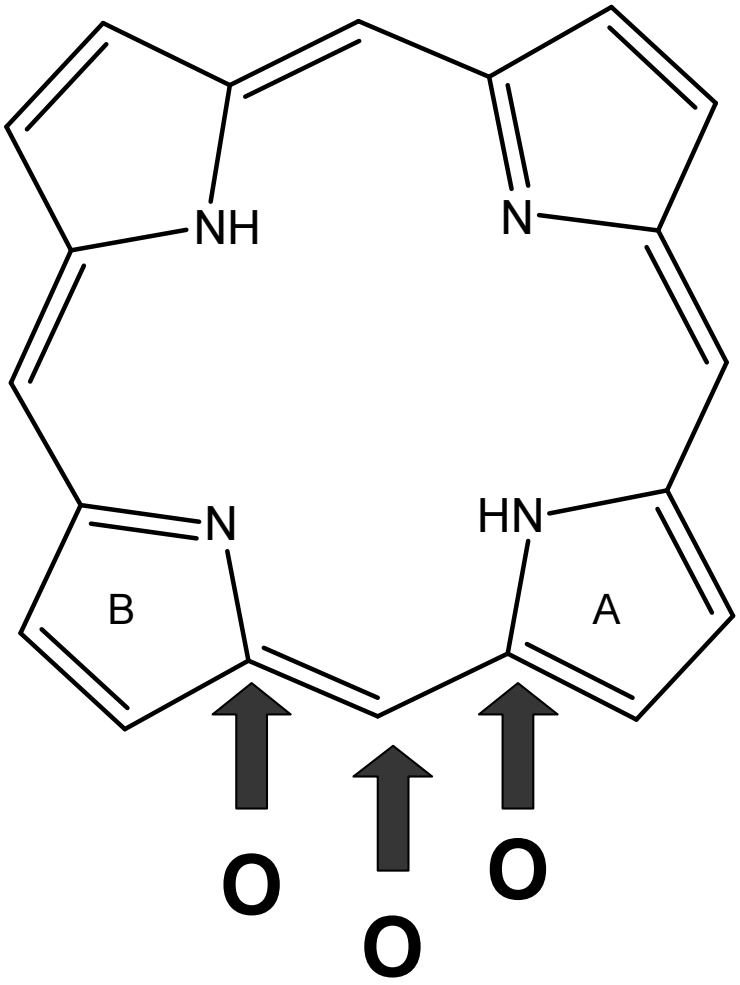
$3\ O_2 + 3\ NADPH+H^+$ | oxidative splitting

$CO + biliverdin + 3\ H_2O$

bilirubin

Three oxygen atoms attack protoporphyrin

hemoxygenase



one O is incorporated into CO, two O atoms are inserted into bilirubin

Q.

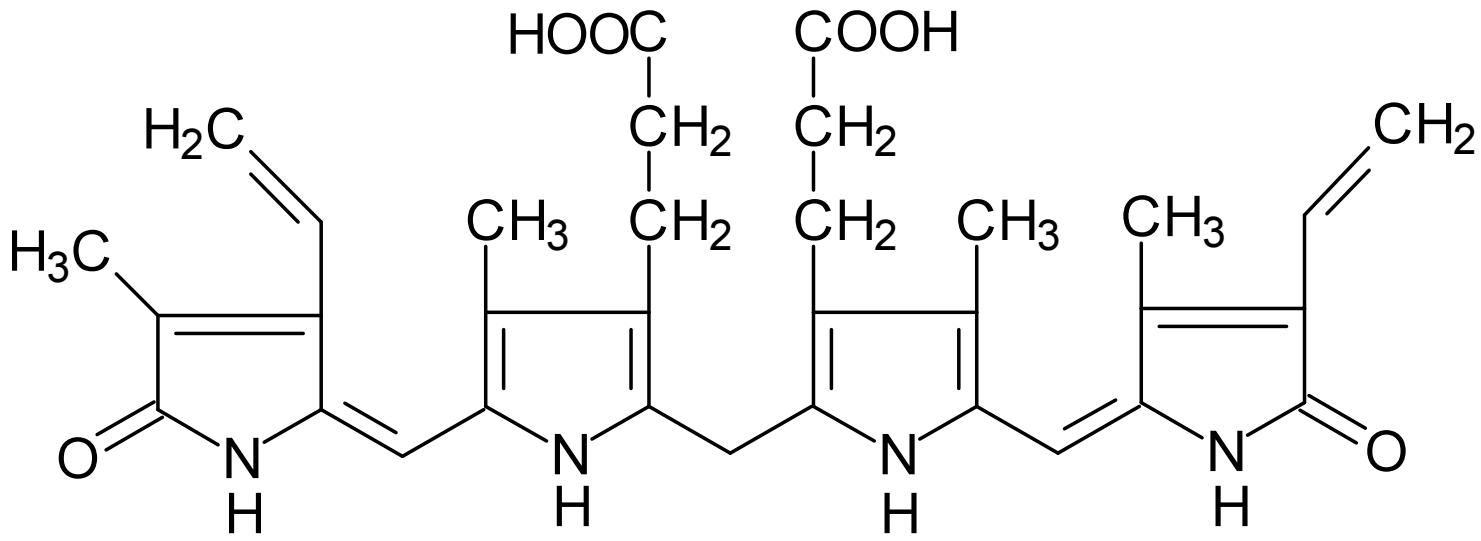
What happens with CO in human body?

Carbonylhemoglobin (CO-Hb) in blood

Subject / Situation	CO-Hb (%)*	
Newborns	0.4	Endogenous CO
Adults (rural areas)	1-2	
Adults (big cities)	4-5	Exogenous CO
Smokers	10-12	
Traffic policemen	12-15	
Poisoning	20-50	
Death	55-60	

* Percentage of total hemoglobin

Text-book structure of bilirubin



bilirubin has eight polar groups:

2 -COOH **2 C=O** **4 -NH-**

despite it bilirubine is **non-polar compound**

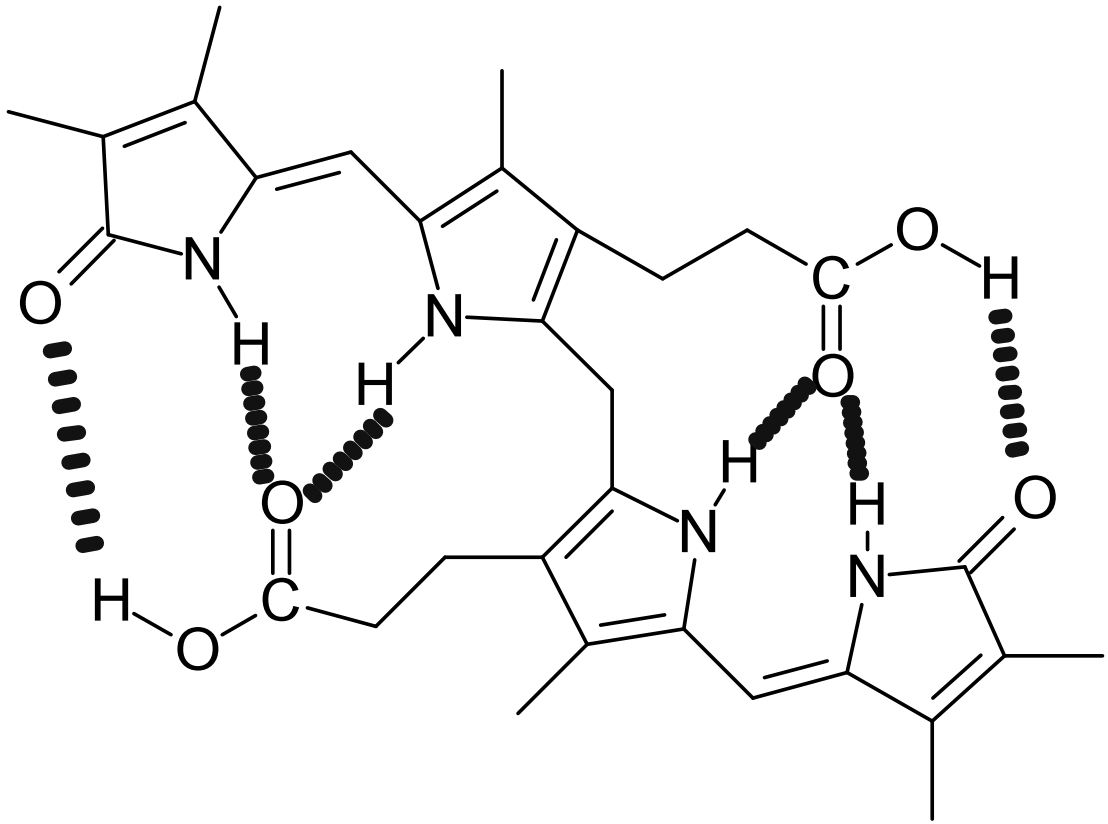
Q.

Why is bilirubin non-polar compound?

Properties of bilirubin

- linear tetrapyrrol system
- free rotation around central -CH_2- is possible
- non-linear conformation arises, stabilized by six intramolecular H-bonds
- **all polar groups are involved in H-bonds**
- consequence: free bilirubin is non-polar, insoluble in water, in plasma – **bound to albumin**

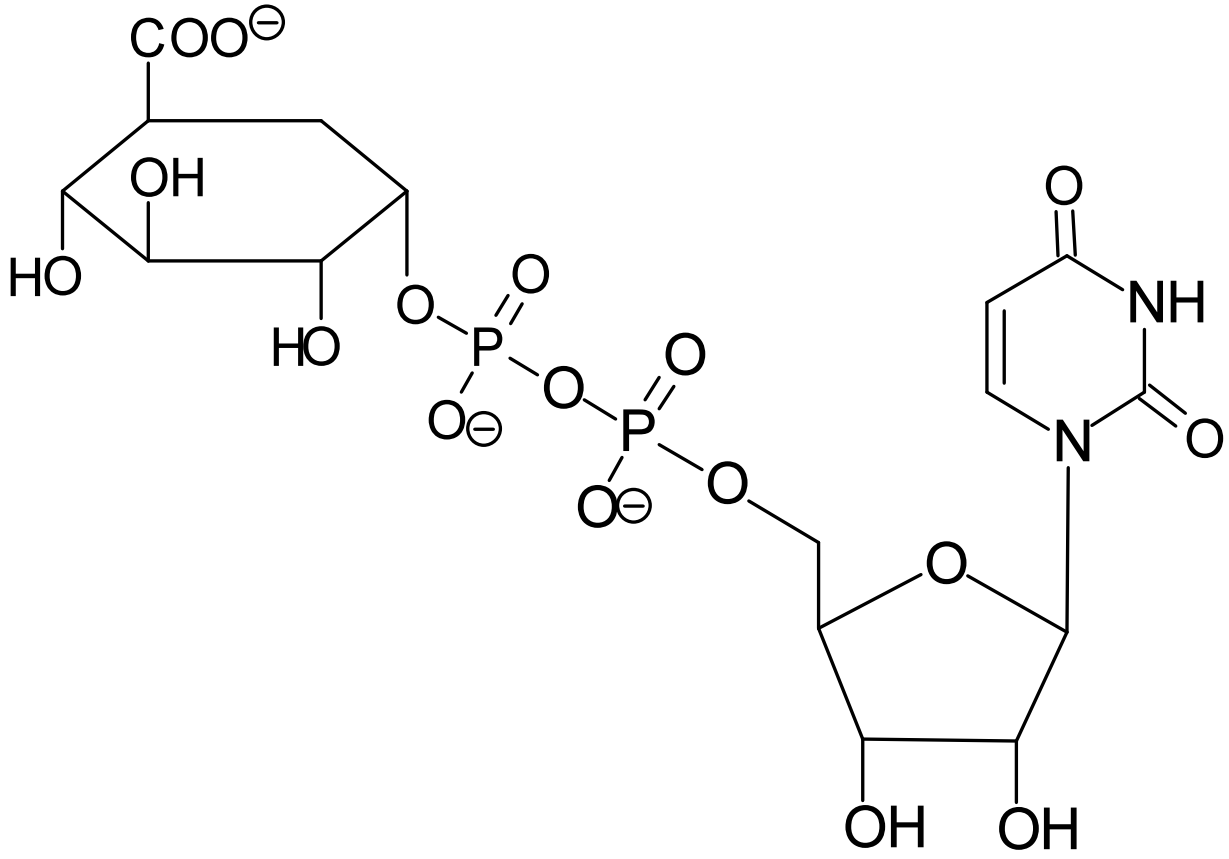
Real structure of bilirubin with six intramolecular H-bonds



Q.

What is UDP-GlcA?

Uridine diphoshoglucuronic acid



Conjugation of bilirubin in liver

- bilirubin reacts with two molecules of **UDP-glucuronate**
- two highly polar molecules of glucuronate are attached to bilirubin with glycosidic ester bond → **bilirubin bisglucuronide**
- conjugated bilirubin is soluble in water (bile, plasma, urine)
- conj. bilirubin is excreted with bile into intestine, where it is deconjugated and hydrogenated by microflora → **urobilinogens**, they are partially absorbed by v. portae and taken up by liver

Laboratory findings in three types of hyperbilirubinemia

Hyperbilirubinemia	Blood	Urine
Hemolytic	↑↑ unconjug.	-
Hepatic	↑↑ both types	↑ conjug.
Obstruction	↑↑ conjug.	↑ conjug.

Normal concentration of bilirubine in blood

total bilirubine:	5-20 μmol/l
unconjugated up to:	12 μmol/l
conjugated up to:	5 μmol/l

Biotransformation of xenobiotics

Greek word ξένος [xenos] means stranger

- Xenobiotics do not normally occur in human body
- **Chemical industry** – produces synthetic compounds which do not occur in nature (plastics, pesticides, pigments, food additives) and various pollutans (as side products)
- **Pharmaceutical industry** – produces drugs (medications) of synthetic origine or isolated from plants/animals/fungi/bacteria

Biotransformation of xenobiotics in cells

- two phases of biotransformations
- xenobiotics becomes more polar
- they are easily excreted from body (urine, bile - stool)

If not biotransformed very hydrophobic xenobiotics would persist indefinitely in body fat !!!

I. Phase of biotransformation

Reaction	**Xenobiotic (example)**
Hydroxylation	aromatic hydrocarbons
Sulfooxidation	disulfides (R-S-R)
Dehydrogenation	alcohols
Reduction	nitro compounds (R-NO_2)
Hydrolysis	esters

Reactions occur mainly in ER, some in cytosol

Enzymes of I. phase are rather non-specific

- **great advantage for human body !!**
- monooxygenases (cytochrome P-450)
- flavine monooxygenases
- peroxidases
- hydrolases
- alcoholdehydrogenases and other ...

Cytochrome P-450 (CYP)

- the group of **hem** enzymes (cca 150 isoforms)
- many of them are inducible
- occur in most tissues (except of muscles and RBC)
- **mainly in liver**

Abbreviation: P = pigment, 450 = wave lenght (nm), at which these enzymes exhibit intensive absorption after binding CO

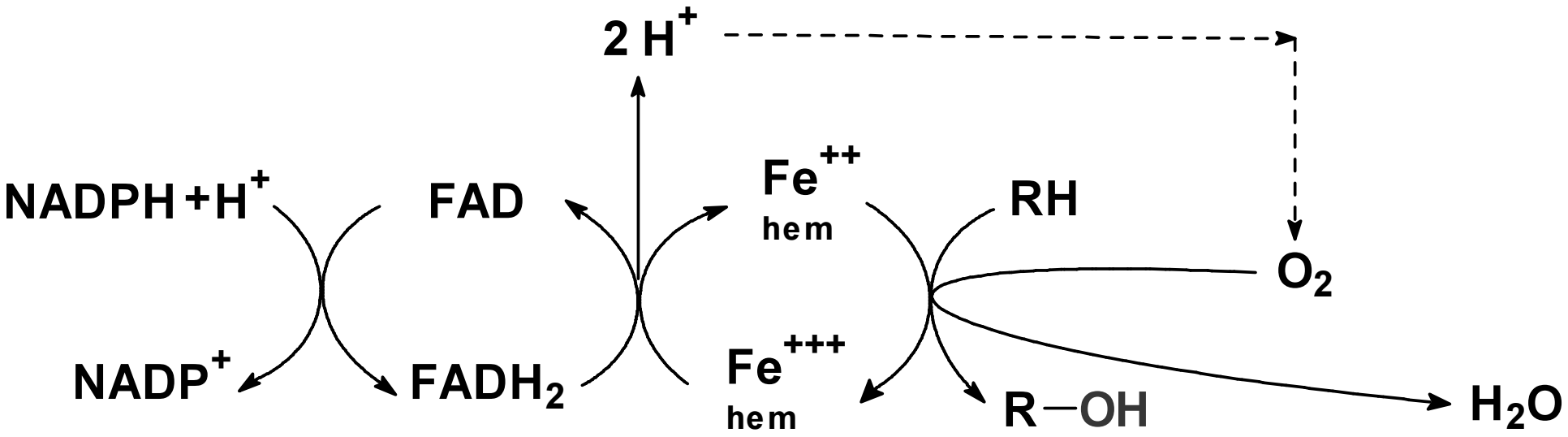
Mechanism of cytochrome reaction

- CYP catalyzes **hydroxylation** (R-H $\rightarrow$ R-OH)
- substrate reacts with O_2
- monooxygenase = from O_2 **one atom O** is inserted into substrate (between carbon and hydrogen atom)
- the second O atom makes H_2O, 2H come from $NADPH+H^+$
- dioxygen is reduced to -OH group and water

General scheme of hydroxylation

$$R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$$

A more detailed scheme of hydroxylation



The system of cytochrome P-450 is composed from:

- two enzymes (cytochrome reductase, cytochrome P-450)
- three cofactors (NADPH, FAD, hem)

Main isoforms of cytochrom P-450

CYP	Substrate	Inducer	Inhibitor
CYP1A2	theophylline	**cigarette smoke**	erythromycine
CYP2A6	methoxyflurane	phenobarbital	methoxsalem
CYP2C9	ibuprofen	phenobarbital	sulfaphenazole
CYP2C19	omeprazole	phenobarbital	teniposide
CYP2D6	codeine	rifampicine	quinidine
CYP2E1	halothane	**alcohol**	disulfiram
CYP3A4	diazepam*	phenobarbital	**grapefruit**

the most abundant isoform

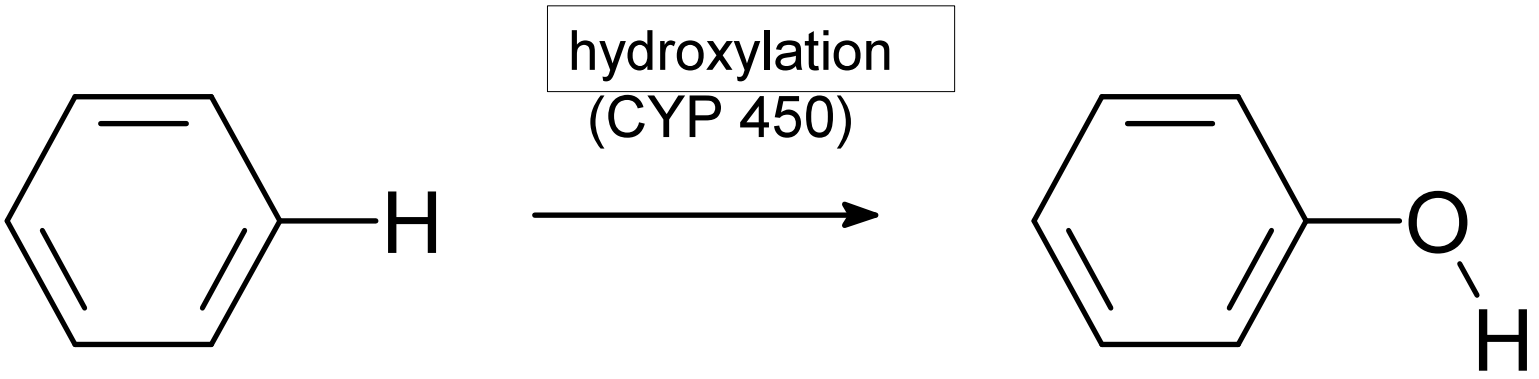
* and cca 120 other medicaments

Induction and inhibiton of CYP 450

- some xenobiotics trigger **induction of CYP synthesis** ⇒ metabolic capacity of CYP increases
- if concurrently aplied inducer + medicament metabolized with the same CYP isoform ⇒ remedy is catabolized faster ⇒ is **less effective**

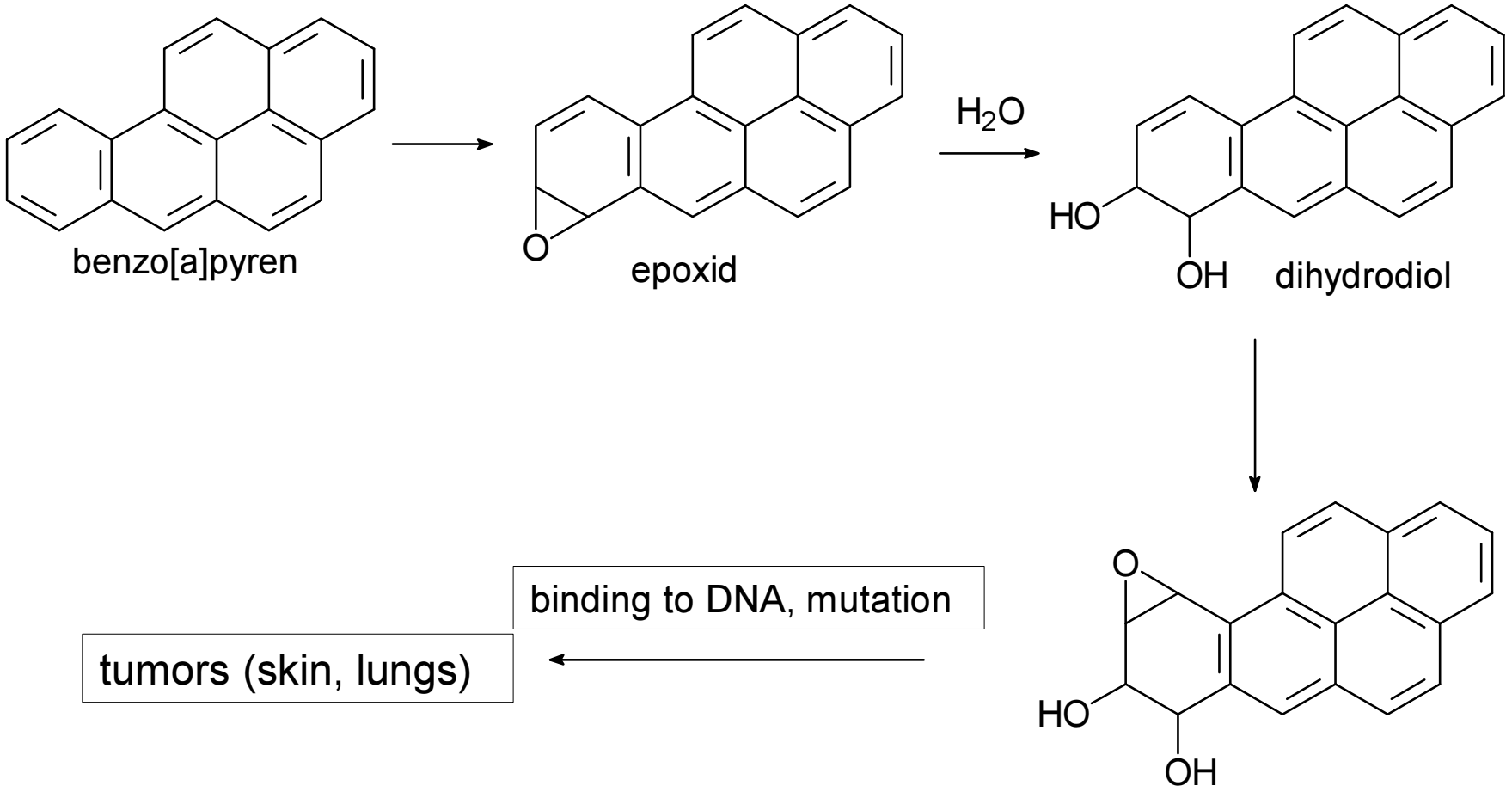
- some xenobiotics are **inhibitors of CYP**
- if concurrently aplied inhibitor + medicament metabolized with the same CYP isoform ⇒ remedy is catabolized more slowly ⇒ higher concentration in blood ⇒ **adverse effects/overdosing**

Biotransformation of benzene



Chronic benzene exposition can be proved by the detection of phenol in urine (workers in chemical industry, sniffers)

Biotransformation of polycyclic aromatic hydrocarbons (PAH)



II. Phase of biotransformation

- conjugation – synthetic character
- xenobiotic after I. phase reacts with conjugation reagent
- the product is more polar – easily excreated by urine
- conjugation reactions are endergonnic – they require energy
- reagent or xenobiotic has to be activated

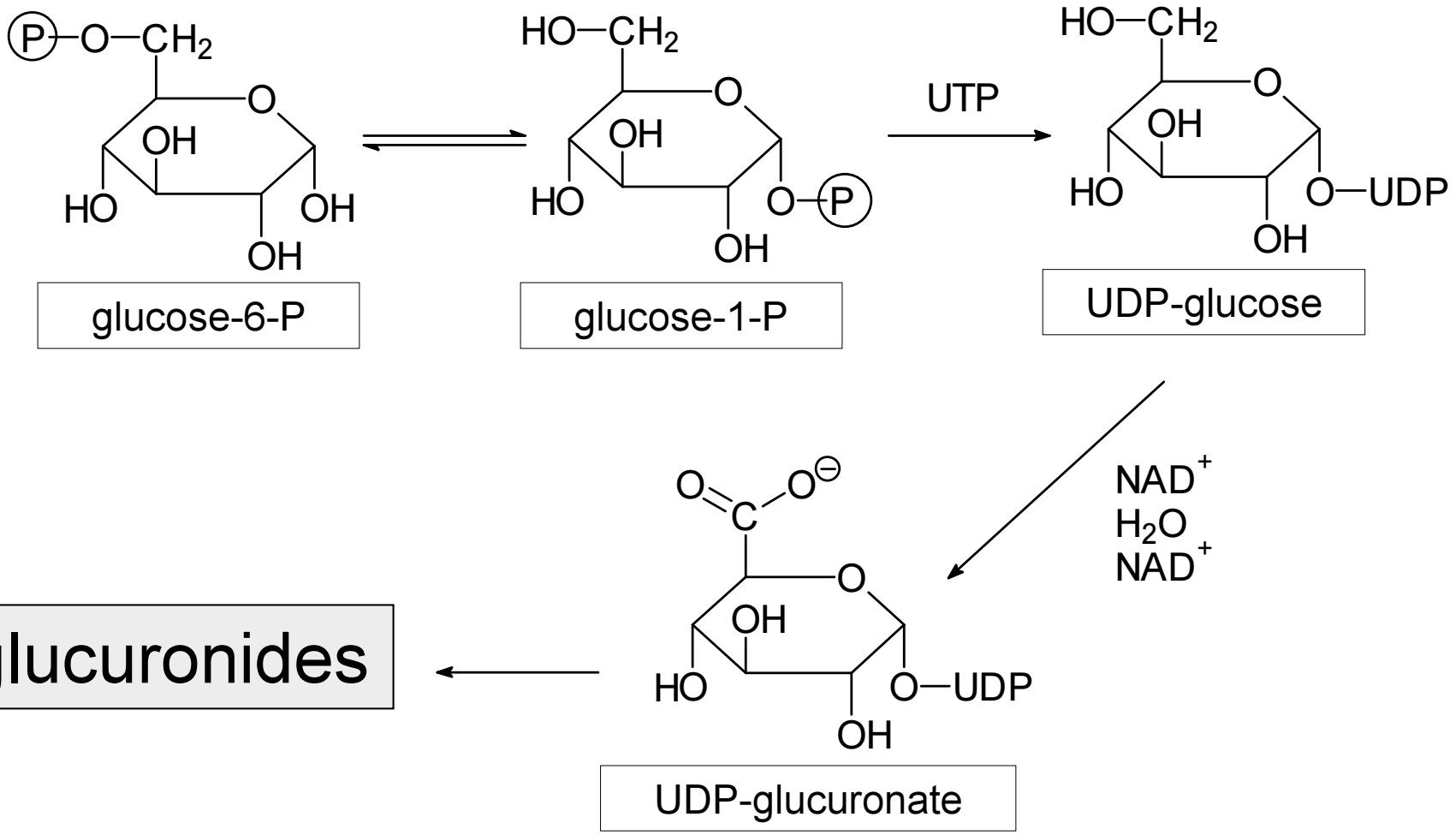
Overview of conjugation reactions

Conjugation	Reagent	Group in xenobiotic
Glucuronidation	UDP-glucuronate	-OH, -COOH, $-NH_2$
Sulfatation	PAPS	-OH, $-NH_2$, -SH
Methylation	SAM	-OH, $-NH_2$
Acetylation	acetyl-CoA	-OH, $-NH_2$
By GSH	glutathione	Ar-halogen
By aminoacid	glycine, taurine	-COOH

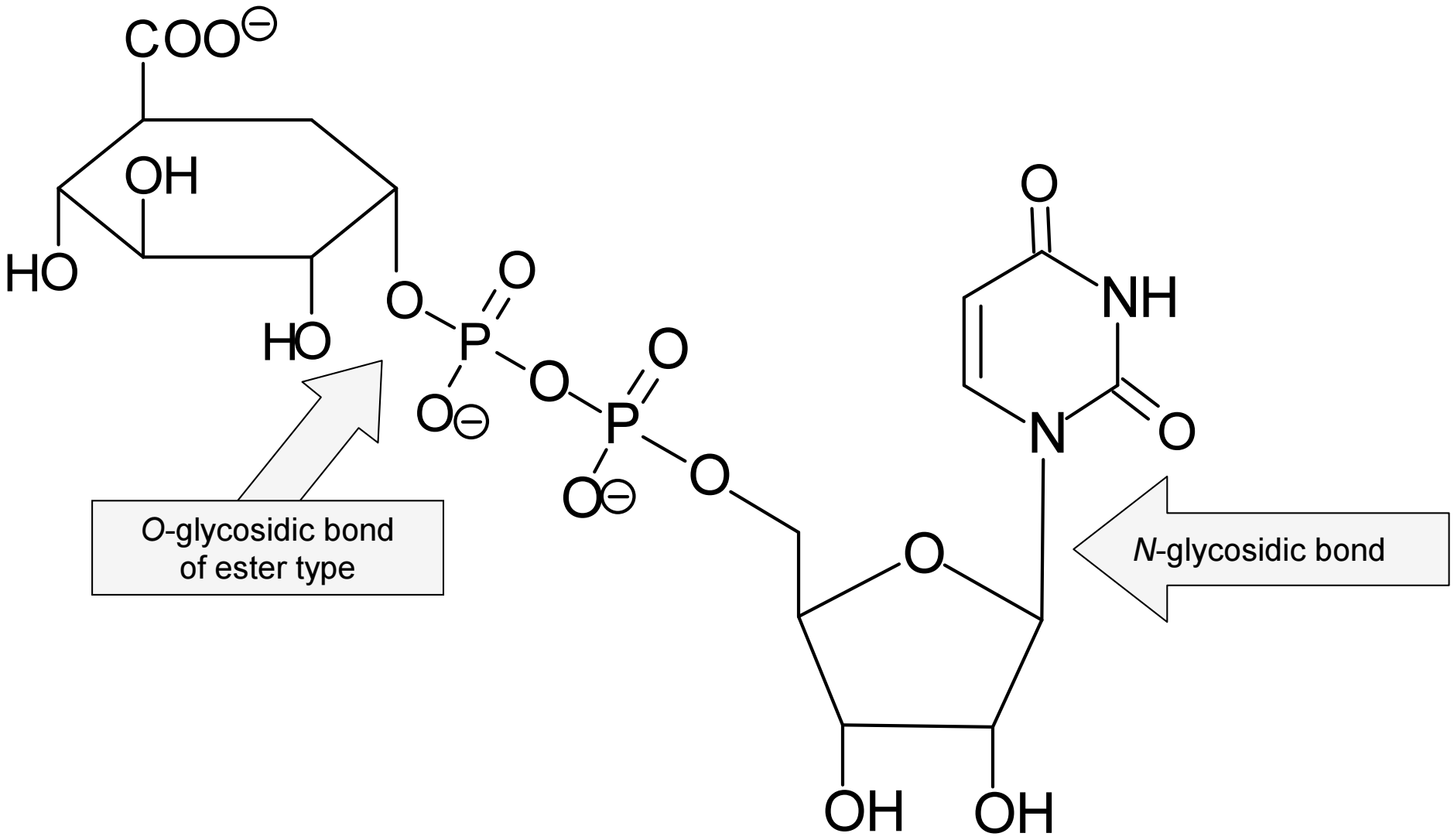
GSH = glutathione, PAPS = phosphoadenosine phosphosulfate

SAM = S-adenosyl methionine

Biosynthesis of UDP-glucuronate



The structure of UDP-glucuronate



Glucuronides are the most abundant conjugates

- ***O*-glucuronides**

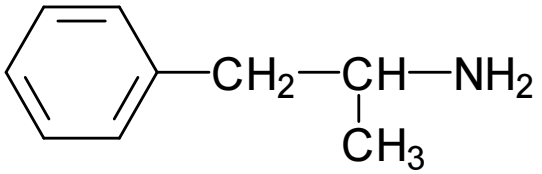
  ether type (Ar-O-glucuronide, R-O-glucuronide)

  ester type (Ar-COO-glucuronide)

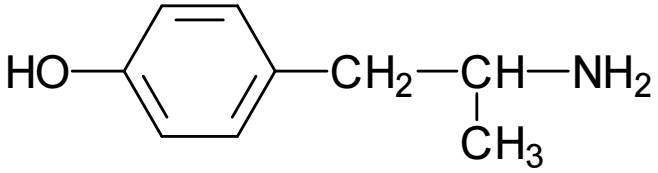
- ***N*-, *S*-glucuronides**
- exogen. substrates: arom. amines, amphetamines, salicylic acid, drugs, flavonoids …
- endogenous substrates: bilirubin, steroids

Example

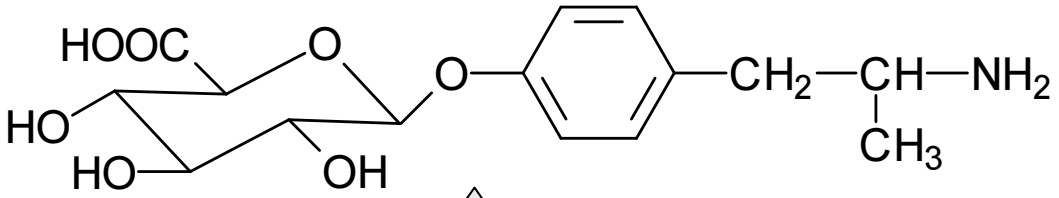
Biotransformation of amphetamine



I. phase - hydroxylation



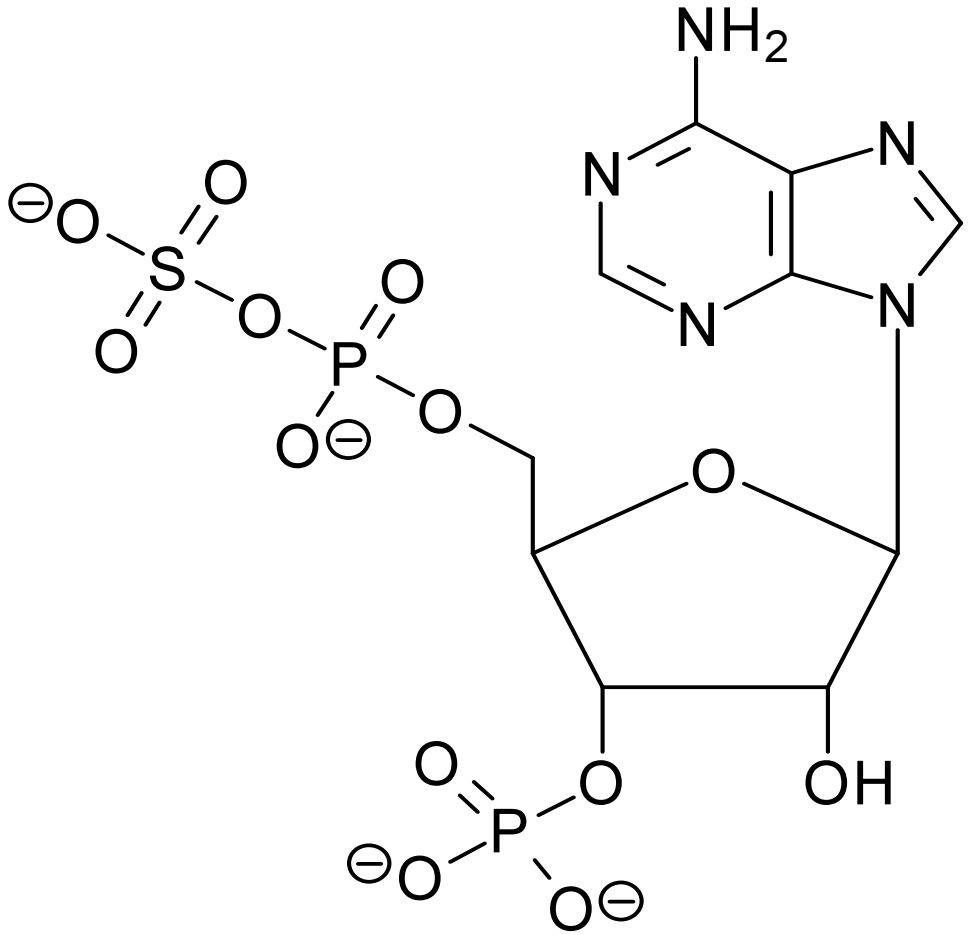
II. phase – conjugation with UDP-glucuronate



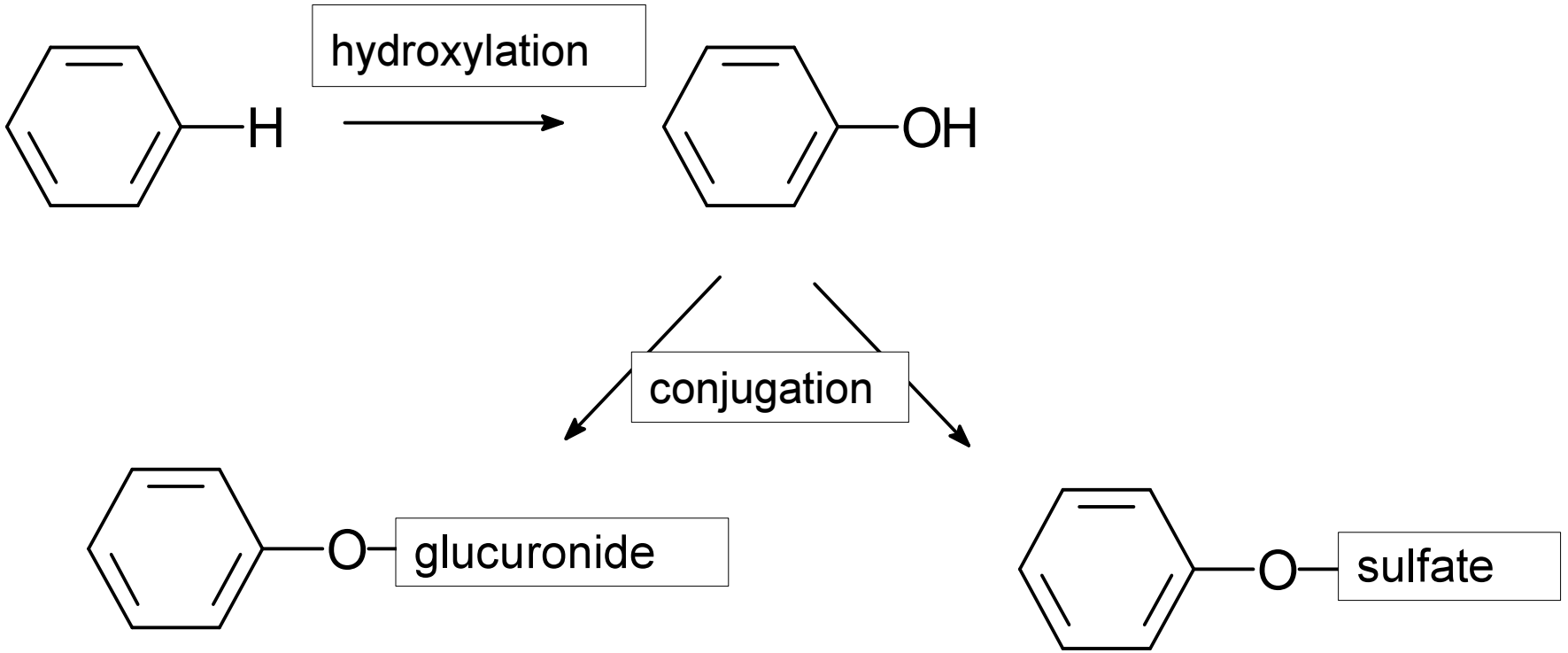
ether type glucuronide

PAPS is sulfatation reagent

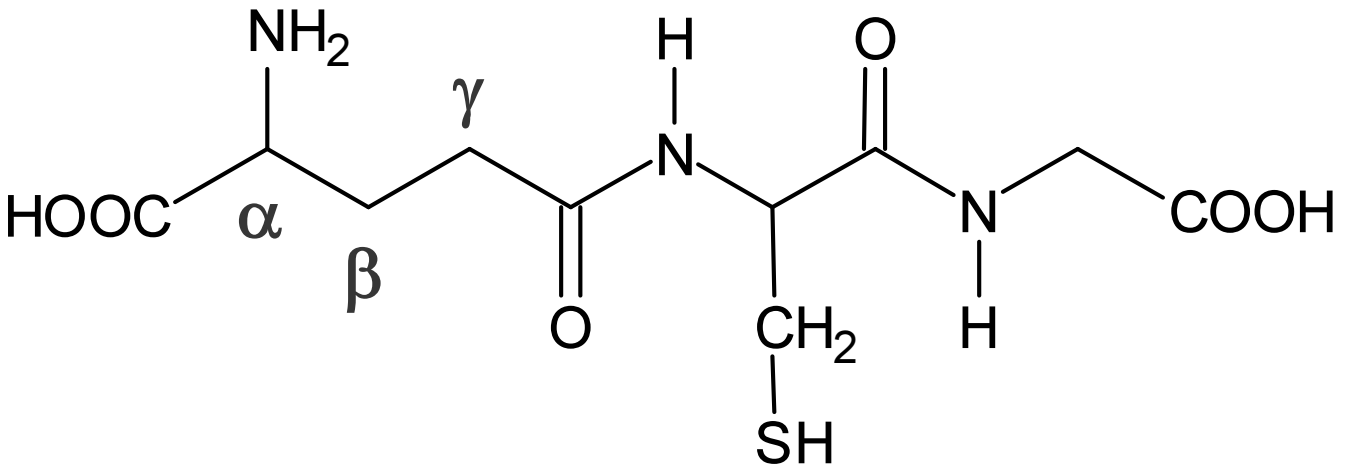
phospho adenosine phospho sulfate



The conjugation reactions of phenol



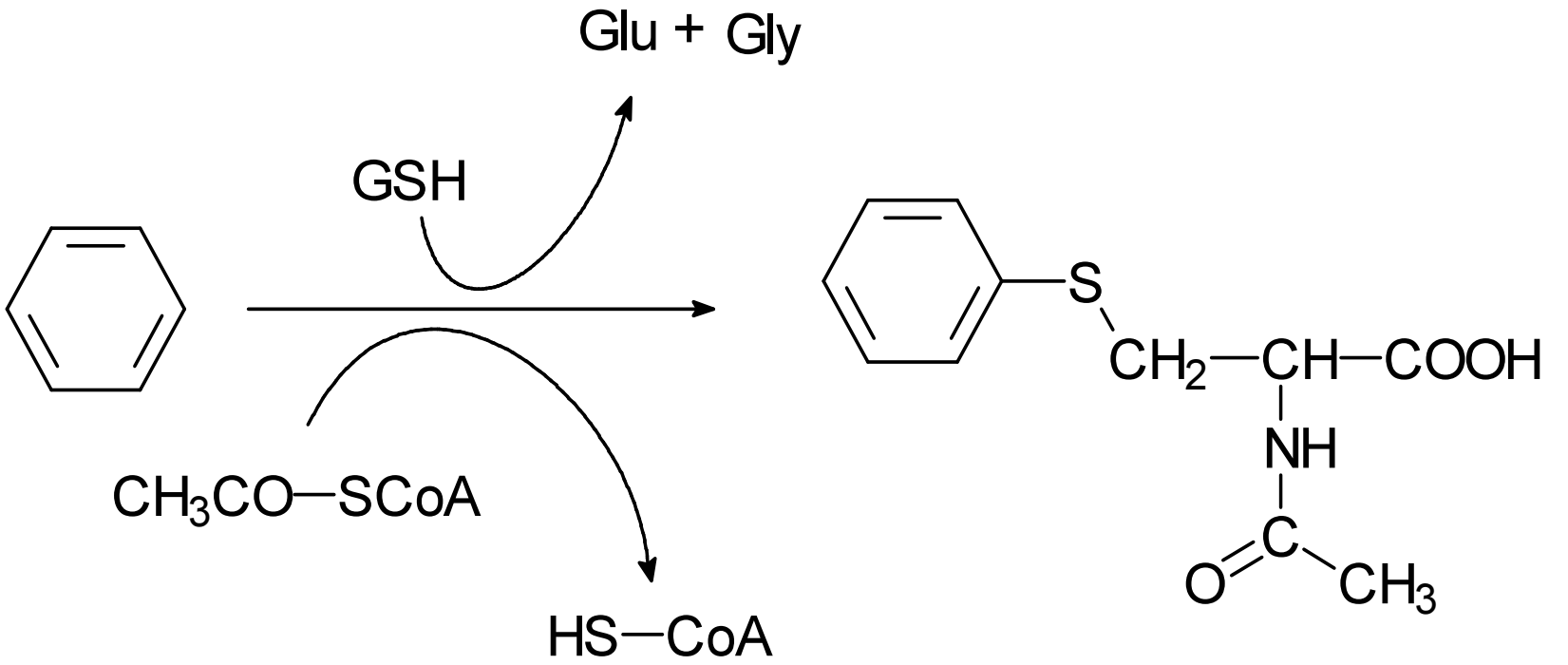
Glutathione (GSH)



$$\text{R-X} + \text{GSH} \rightarrow \text{R-SG} + \text{XH}$$

R-X halogen alkanes (arenes)

Mercapturic acids are final products of GSH conjugation



N-acetyl-*S*-phenylcystein

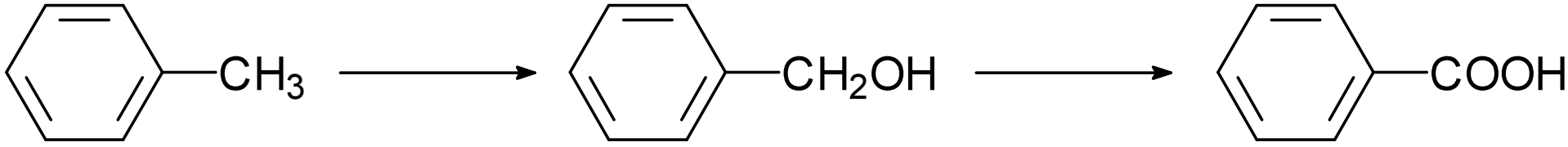
(mercapturic acid)

urine

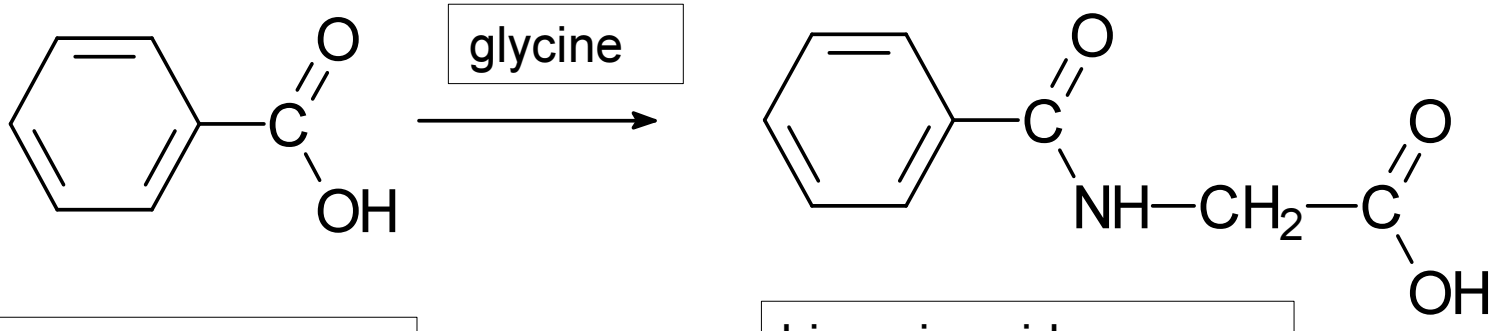
Conjugation with aminoacids

- glycine, taurine
- xenobiotics with -COOH groups
- the products of conjugation are **amides**
- endogenous substrates – bile acids

Biotransformation in toluene sniffers



toluene | benzyl alcohol | benzoic acid

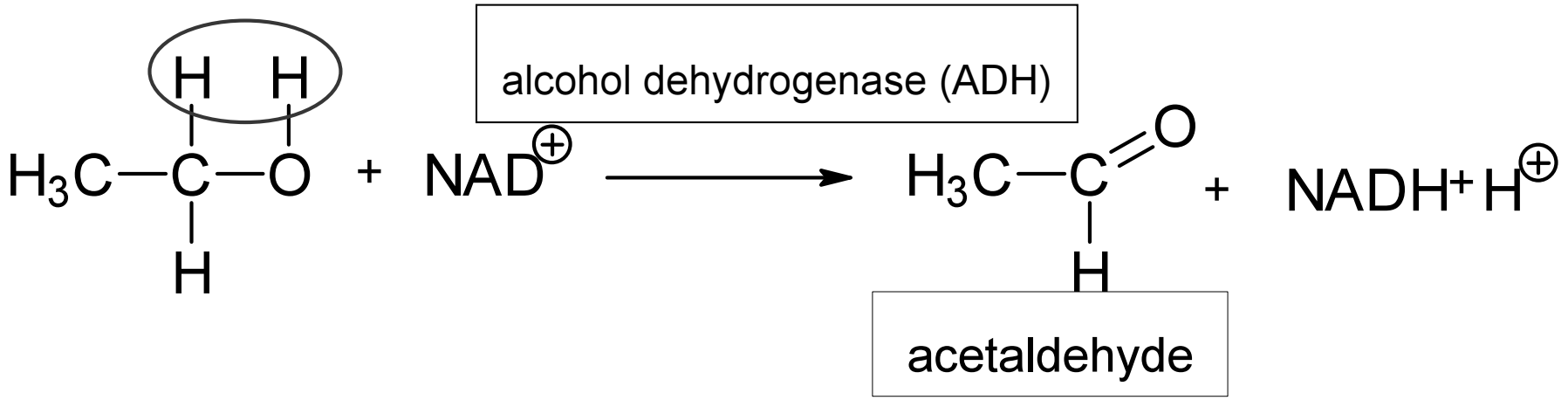


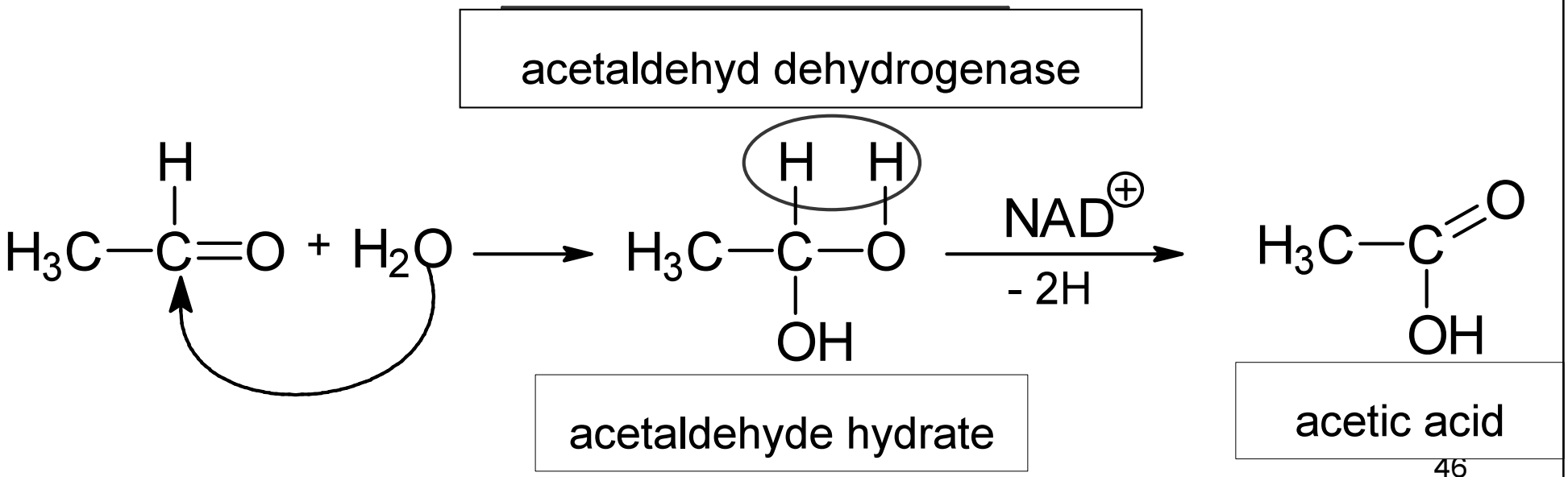
benzoic acid

hippuric acid

(N-benzoylglycine)

Main path of ethanol biotransformation occurs in liver cytosol





Alternative pathway of alcohol biotransformation occurs in endoplasmic reticulum

MEOS (microsomal ethanol oxidizing system, CYP2E1)

$CH_3\text{-}CH_2\text{-}OH + O_2 + NADPH+H^+ \rightarrow \mathbf{CH_3\text{-}CH=O} + 2\ H_2O + NADP^+$

activated at higher consumption of alcohol = higher blood level of alcohol (> 0,5 ‰) - chronic alcoholics

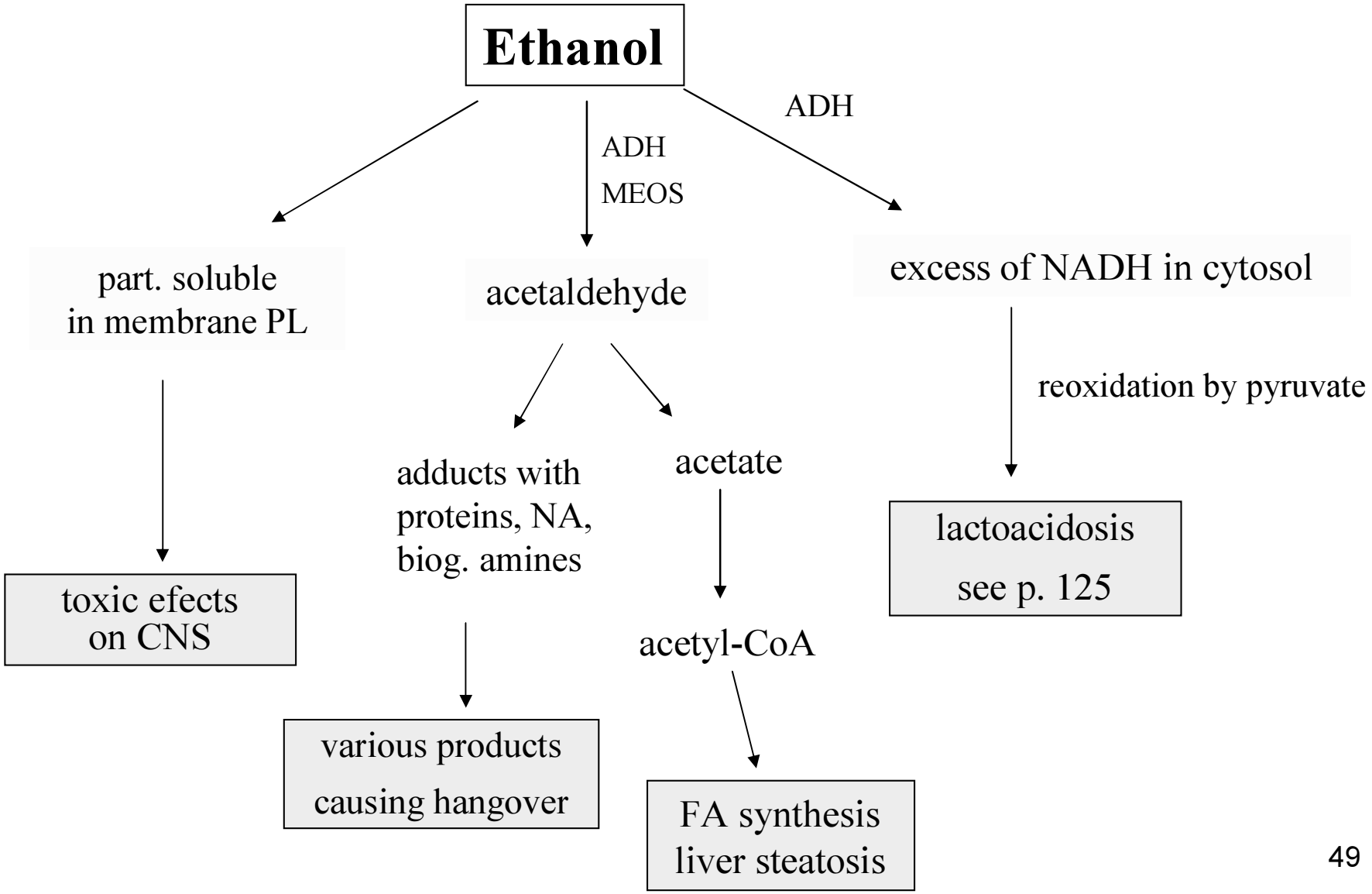
⇒ increased production of acetaldehyde

‰ = per mille = 1/1000

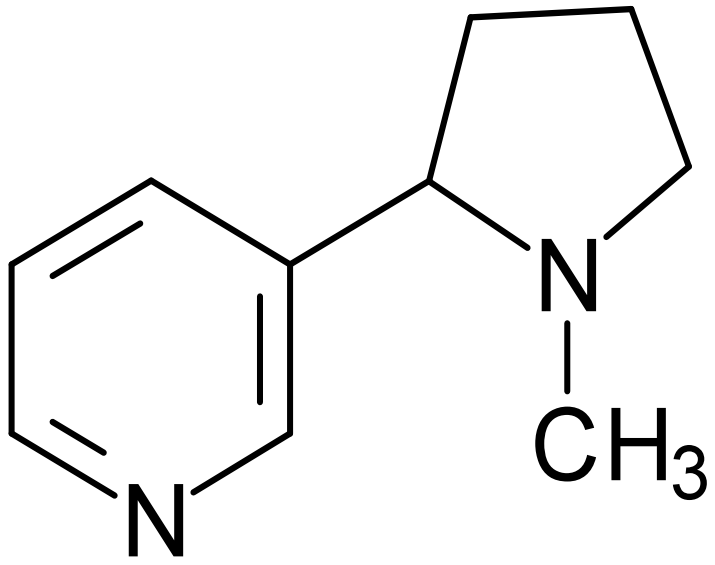
Q.

What are the main metabolic consequences of ethanol metabolism?

Metabolic consequences of EtOH biotransformation



Nicotine - the main alkaloid of tobacco



3-(1-methylpyrrolidin-2-yl)pyridine

On cigarette box:

Nicotine: 0.9 mg/cig.

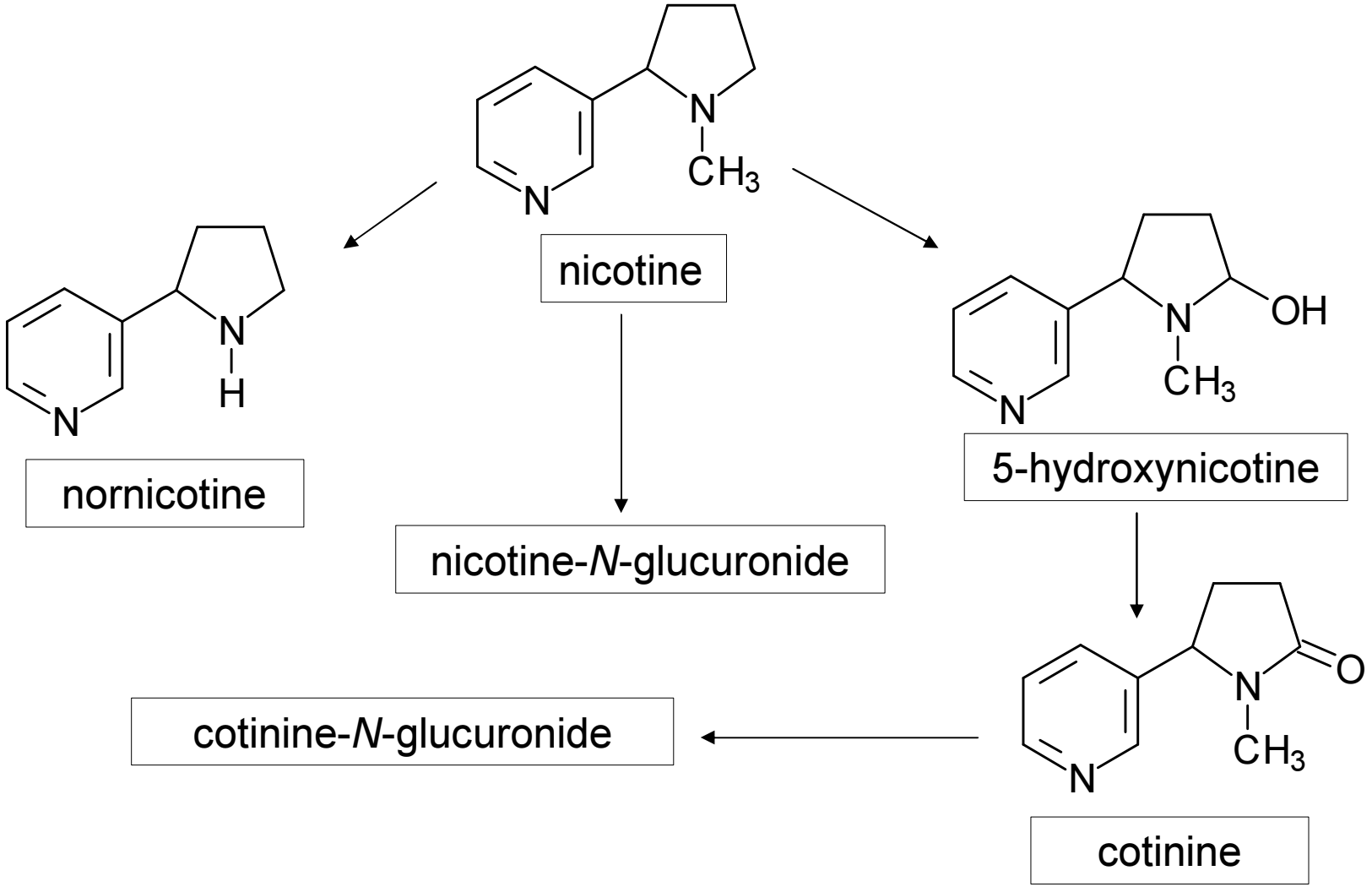
Tar: 11 mg/cig.

Cigarette smoke contains a number of different compounds

- **free nicotine** – binds to nicotine receptors in brain and other tissues (see page 135)
- **CO** – binds to hemoglobin → carbonylhemoglobin
- **nitrogen oxides** – can generate free radicals
- **polycyclic aromatic hydrocarbons (PAH)** (pyrene, chrysene), main components of **tar**, attack and damage DNA, carcinogens
- **other substances** (N_2, CO_2, HCN, CH_4, terpenes, esters …)

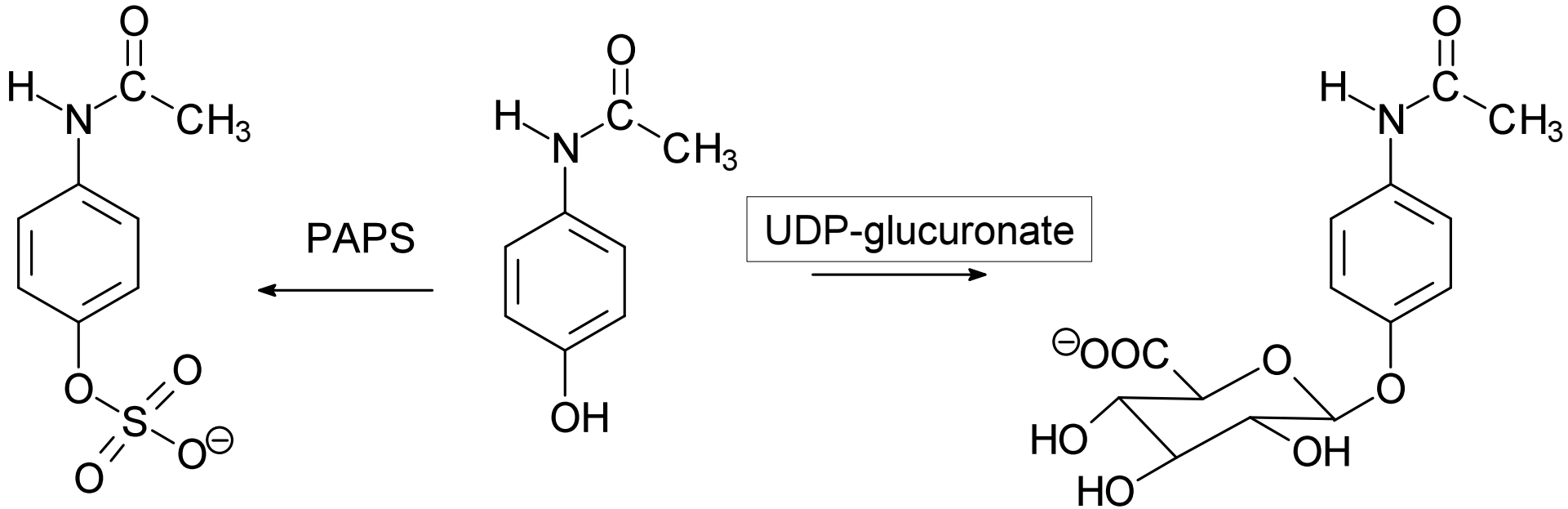
Example

Biotransformation of nicotine



Main pathways of paracetamol biotransformation

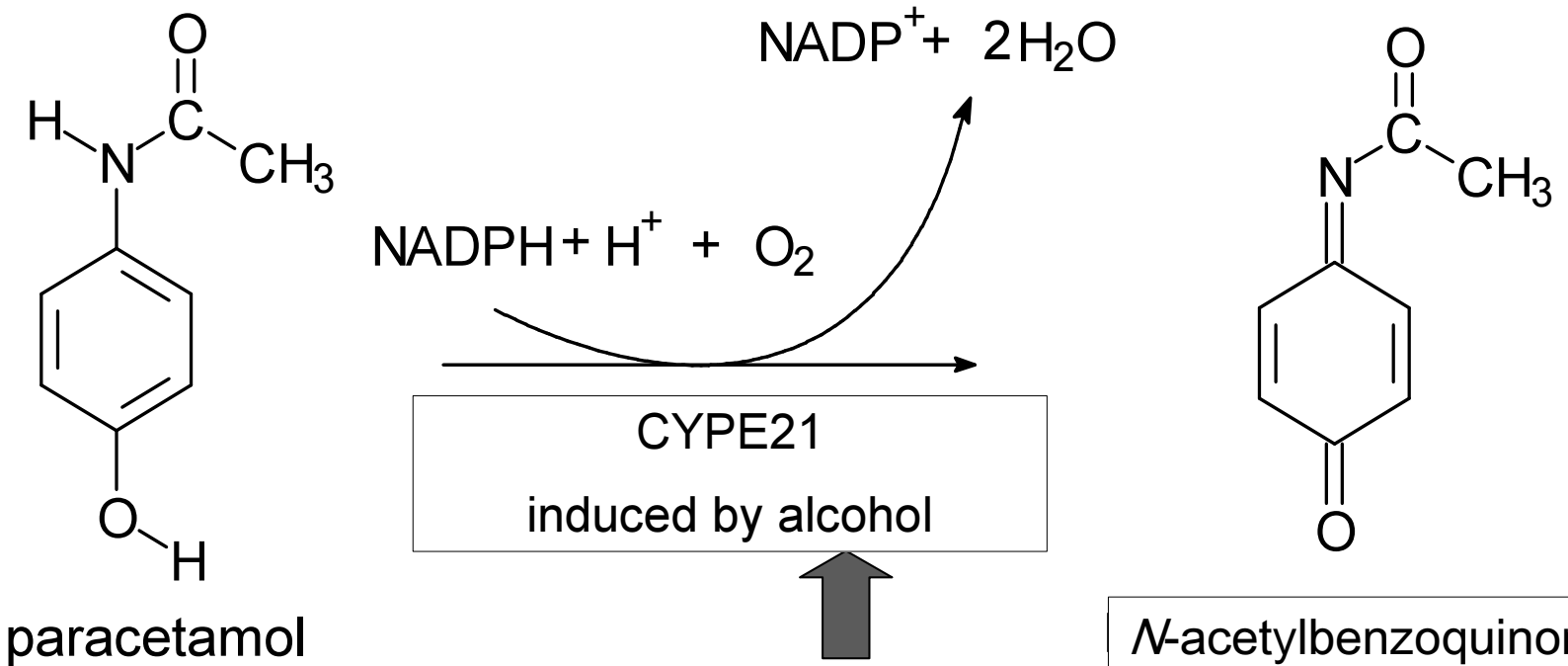
two types of conjugation



paracetamol
N-(4-hydroxyphenyl)acetamide

over the counter analgetic, antipyretic

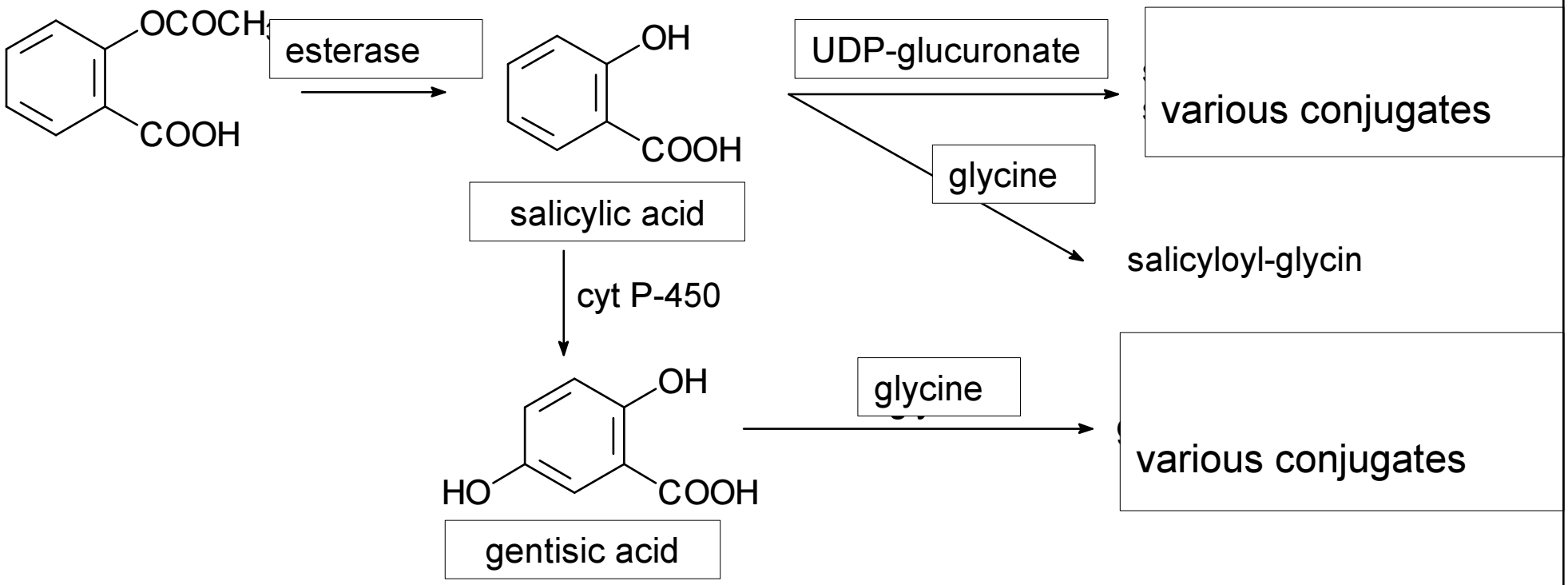
Side pathway of paracetamol biotransformation leads to hepatotoxic quinonimine



☠ danger upon overdosing

☠ danger in alcoholics

Biotransformation of acetylsalicylic acid



over the counter analgetic, antipyretic

Selected biochemical markers of liver damage

Analyt (serum)	Reference values	Change
ALT	0,1 - 0,8 μkat/l	⬆
GMD	0,1 - 0,7 μkat/l	⬆
GMT	0,1 - 0,7 μkat/l	⬆
Bilirubin	5 - 20 μmol/l	⬆
Urobilinogens (urine)	up to 17 μmol/l	⬆

Pseudocholinesterase	65 - 200 μkat/l	⬇
Urea	3 - 8 mmol/l	⬇
Albumin	35 - 53 g/l	⬇