Metabolism of xenobiotics

Seminar No. 8

Q. 2

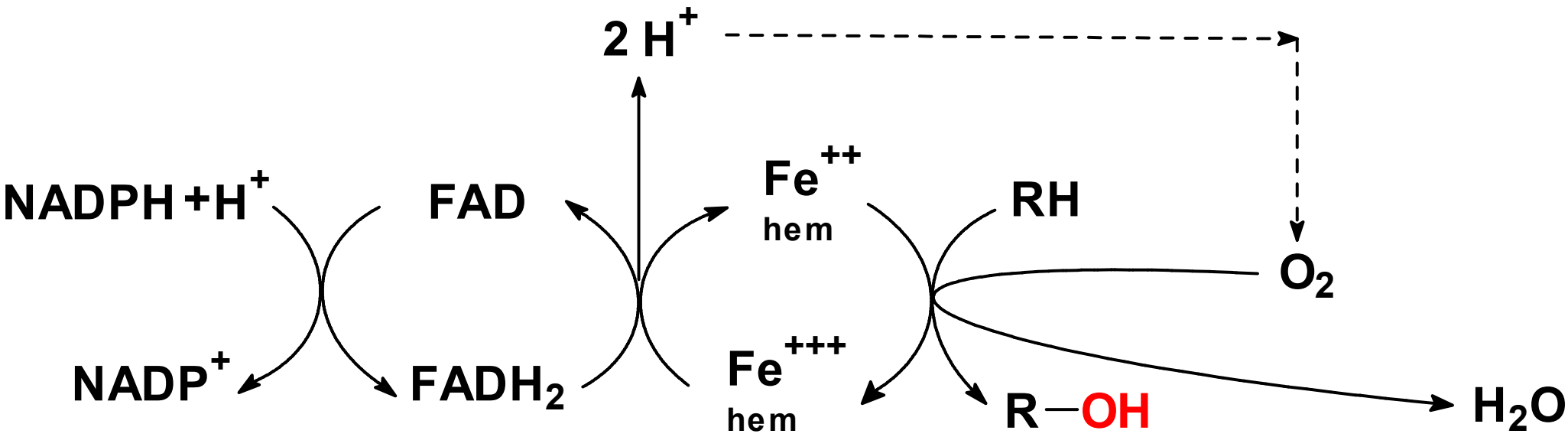
1. Phase of biotransformation = mainly oxidations

Reaction		Xenobiotic (example)
Hydroxylation	oxidations	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

Q. 3

A. 3



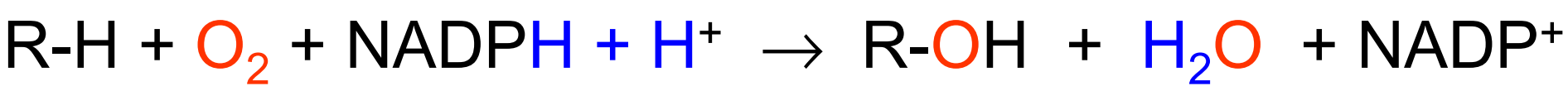
The system of cytochrome P-450 is composed from:

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

Q. 4 + 5

$R\text{-}H + O_2 +$

A. 4 + 5 Hydroxylation



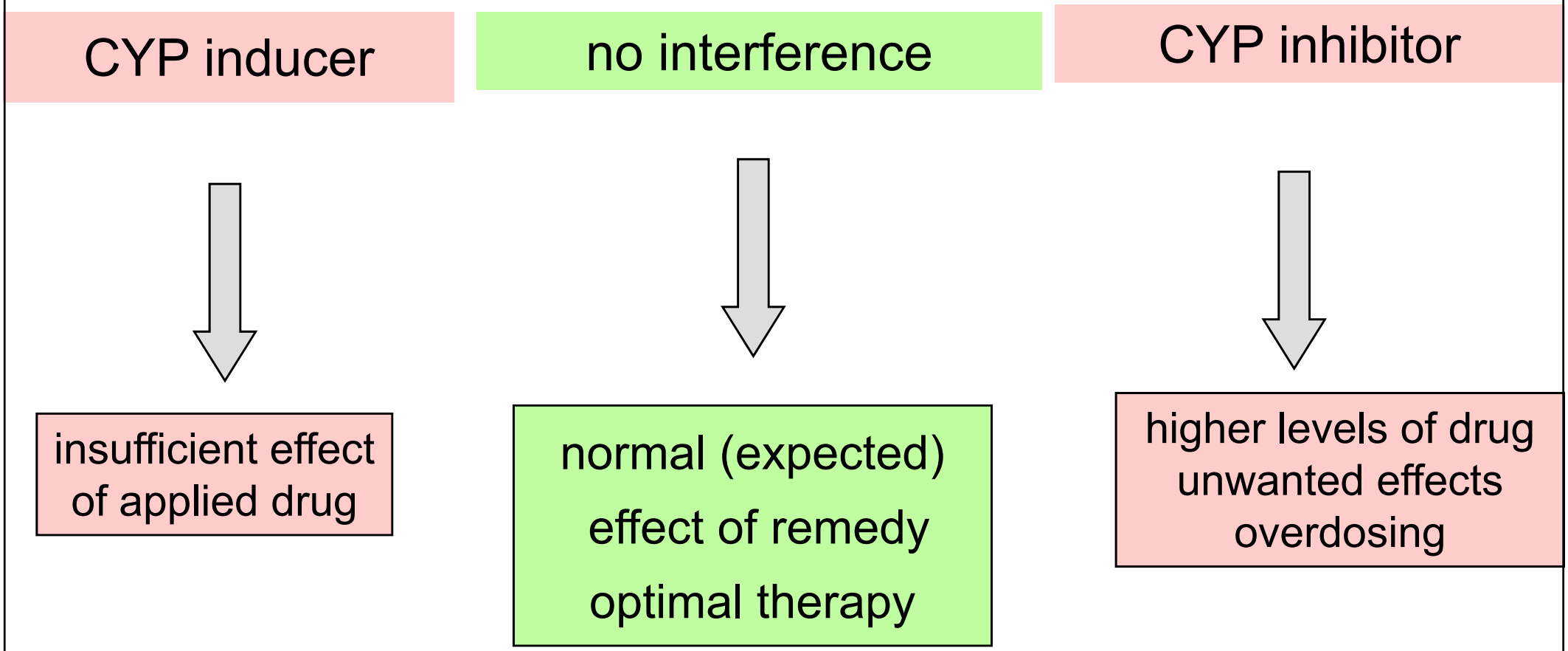
- substrate R-H reacts with O_2
- monooxygenase = from O_2 **one atom O** is inserted into substrate (between carbon and hydrogen atom)
- the second O atom makes H_2O, 2H come from NADPH+H^+
- dioxygen is reduced to -OH group and water

Q. 6

A. 6

- Inducer may act on several levels:
- Inducer in complex with intracellular receptor enters nucleus and binds to DNA ⇒ enhances the transcription of mRNA
- Decreases the degradation of mRNA and/or CYP
- Influences the poststranscription modifications of mRNA
- May cause the hypertrophy of ER

Influence of CYP inducers/inhibitors on the effect of drug (remedy)



Q. 7

A. 7

- if concurrently aplied inducer + medicament metabolized with the same CYP isoform ⇒ remedy is catabolized faster ⇒ is **less effective**
- **diclofenac is less effective**

Q. 8

A. 8

- if concurrently aplied inhibitor + medicament metabolized with the same CYP isoform ⇒ remedy is catabolized more slowly ⇒ higher concentration in blood ⇒ **adverse effects (overdosing)**

Q. 9

A. 9 - 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

A. 9 Overview of conjugation reactions

Conjugation	Reagent	Group in xenobiotic
Glucuronidation		-OH, -COOH, $-NH_2$
Sulfatation		-OH, $-NH_2$, -SH
Methylation		-OH, $-NH_2$
Acetylation		-OH, $-NH_2$
By GSH		Ar-halogen
By amino acid		-COOH

A. 9 Overview of conjugation reactions

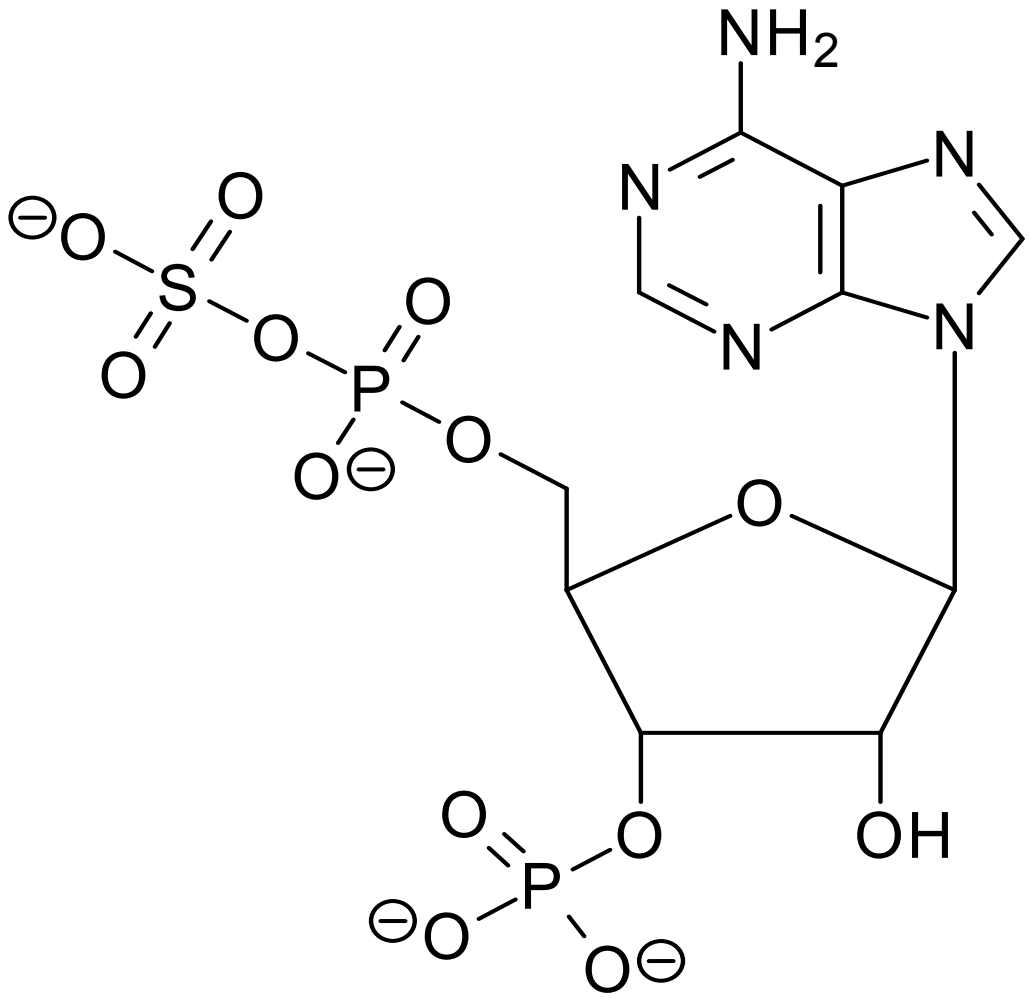
Conjugation	Reagent	Group in xenobiotic
Glucuronidation	UDP-glucuronate	-OH, -COOH, -NH_2
Sulfatation	PAPS	-OH, -NH_2, -SH
Methylation	SAM	-OH, -NH_2
Acetylation	acetyl-CoA	-OH, -NH_2
By GSH	glutathione	Ar-halogen
By amino acid	glycine, taurine	-COOH

GSH = glutathione, PAPS = phosphoadenosine phosphosulfate

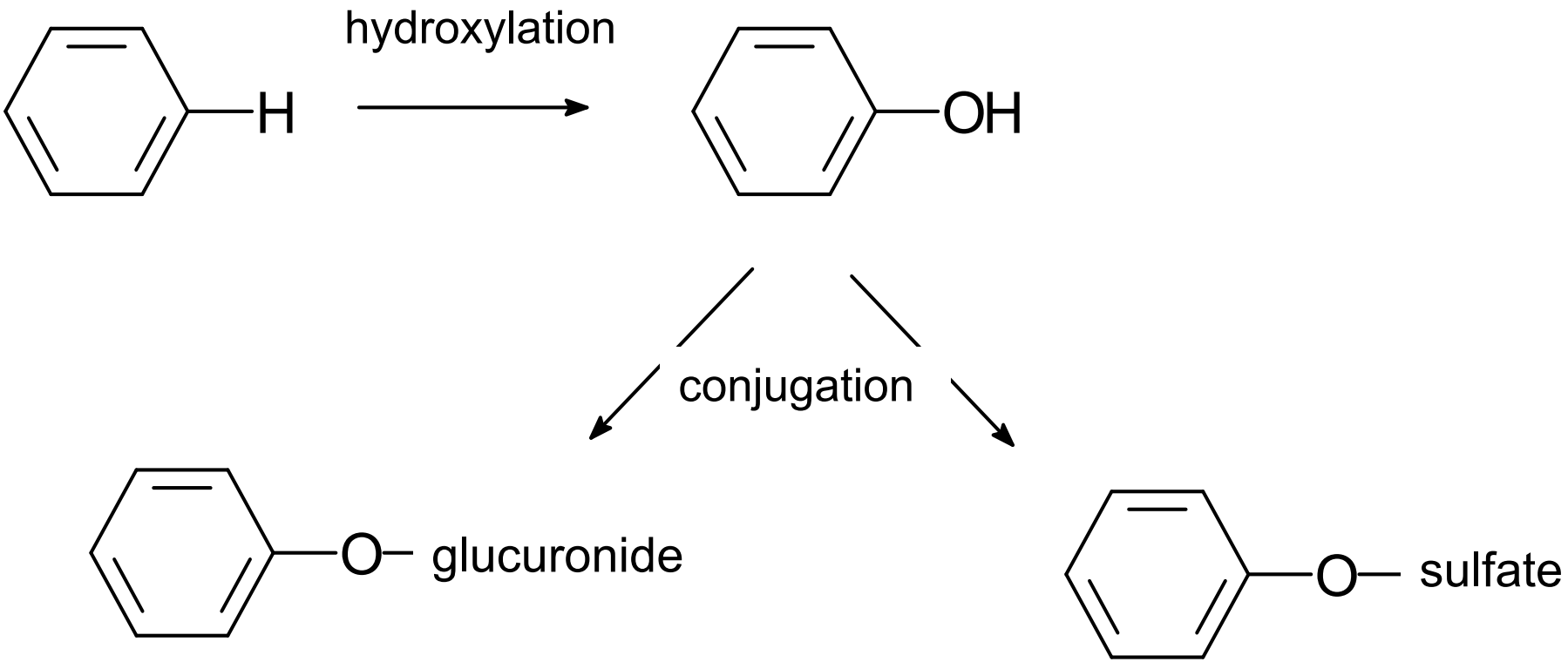
SAM = S-adenosyl methionine

PAPS is sulfatation reagent

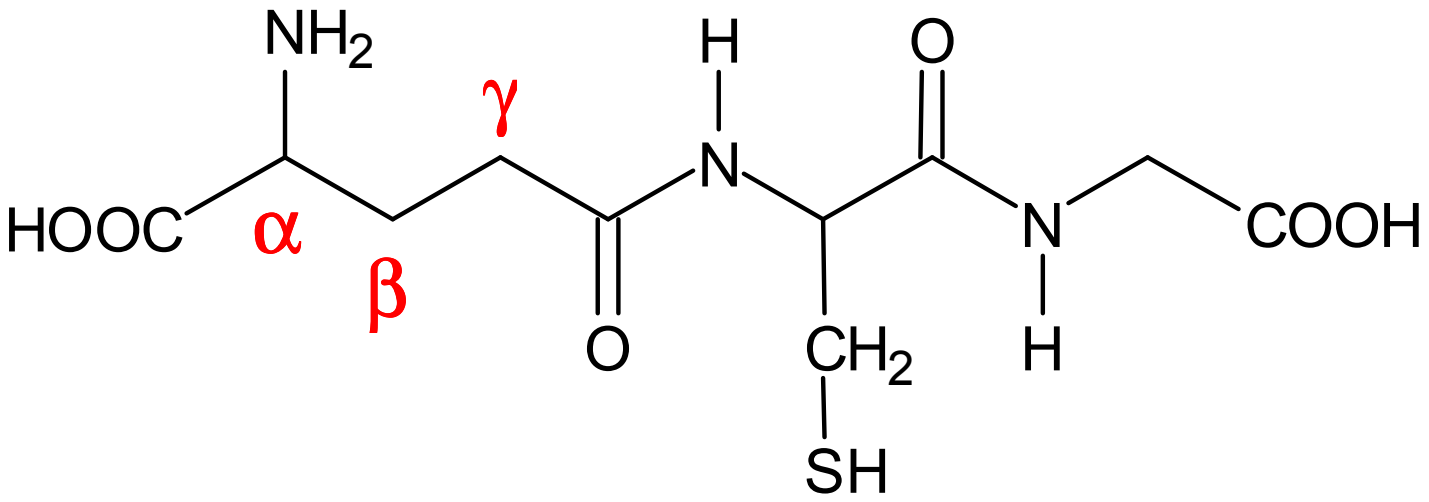
phospho adenosine phospho sulfate



The conjugation reactions of phenol



Glutathione (GSH)



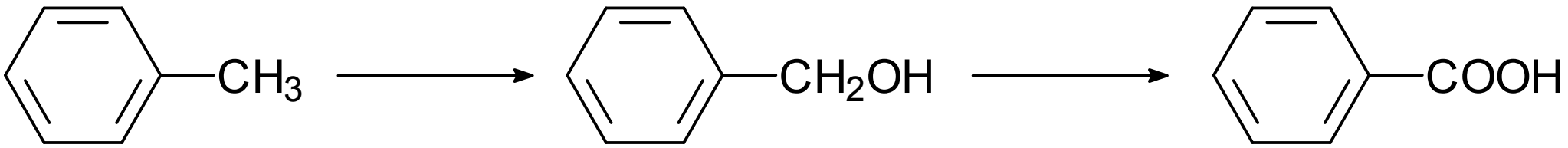
$$\text{R-X} + \text{GSH} \rightarrow \text{R-SG} + \text{XH}$$

R-X halogen alkanes (arenes)

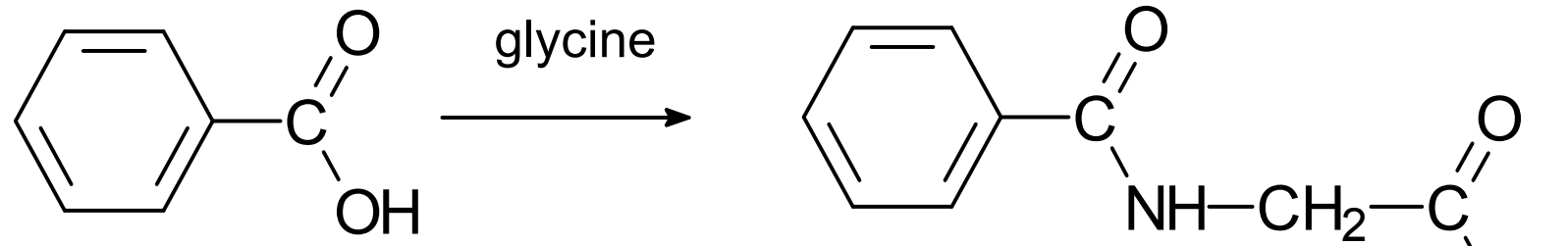
Conjugation with aminoacids

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

Biotransformation of toluene (sniffers)



toluene benzyl alcohol benzoic acid



benzoic acid

hippuric acid

(N-benzoylglycine)

Ethanol

How can you calculate the level of alcohol in blood?

Per milles of alcohol in blood

‰ = per mille = 1/1000

$$\text{alcohol in blood (‰)} = \frac{m_{alcohol}(\text{g})}{m_{body}(\text{kg}) \times f}$$

0.67 (males)
0.55 (females)

How do you calculate m_{alcohol} ?

m_{alcohol} is calculated from volume and density

$$m_{\text{alc}}\,(\text{g}) = V_{\text{alc}}\,(\text{ml}) \times 0.8\ (\text{g/ml})$$

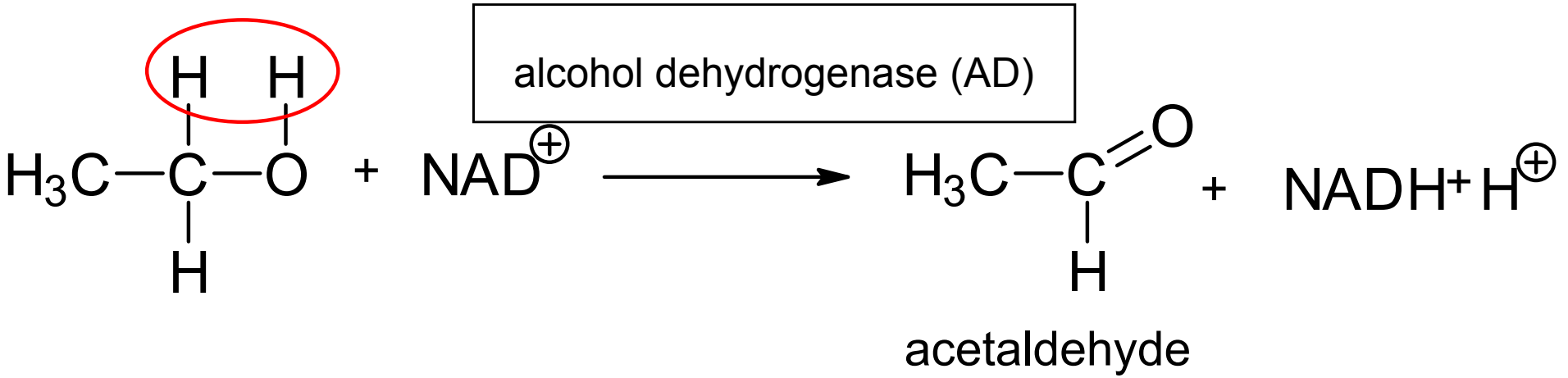
density

volume of pure alcohol is
calculated from volume fraction
beer 3-6 %
wine 6-12 %
liquors 40-50 %

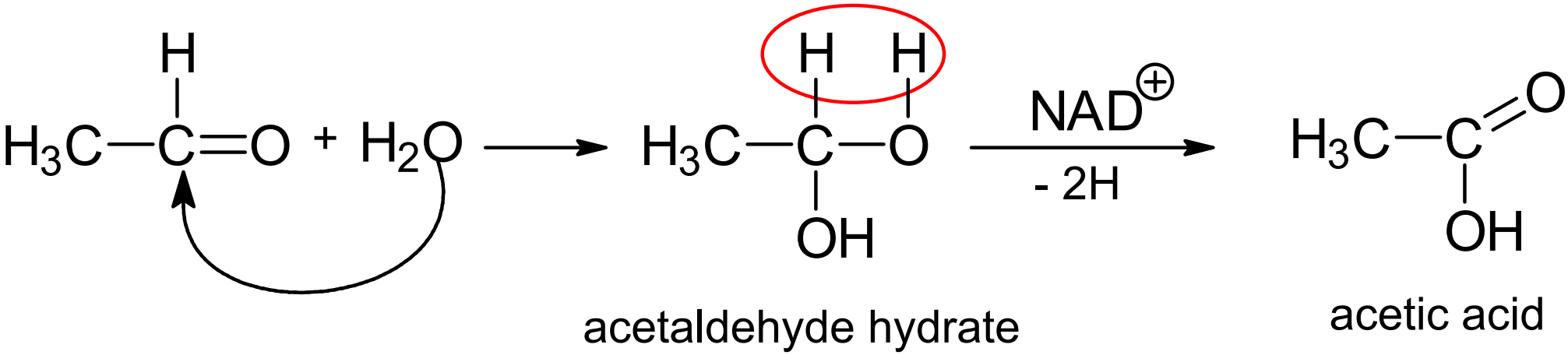
Metabolism of ethanol

Enzyme	Subcellular localization
Alcohol dehydrogenase (AD)	cytosol
MEOS	endoplasmic reticulum (ER)
Catalase (hem)	peroxisome
Acetaldehyde dehydrogenase	cytosol / mitochondria

Write AD and AcD reactions



acetaldehyde



acetaldehyde hydrate

acetic acid

Alternative pathway of alcohol biotransformation occurs in endoplasmic reticulum

MEOS (microsomal ethanol oxidizing system, CYP2E1)

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

activated at higher consumption of alcohol = higher blood level of alcohol (> 0.5 ‰) - chronic alcoholics

⇒ **increased production of acetaldehyde**

Q. 11

A. 11

Acetate is converted to acetyl-CoA

A) in liver $\Rightarrow$ synthesis of FA $\rightarrow$ TAG $\rightarrow$ VLDL

B) in other tissues $\Rightarrow$ CAC $\rightarrow$ CO_2 + energy

A. 13

$M_r = 46$ density = 0,8 g/ml

$$\frac{1\,(\text{mmol})}{1\,(\text{l})} = \frac{0{,}046\,(\text{g})}{1\,(\text{l})} = \frac{0{,}058\,(\text{ml})}{1\,(\text{l})} = 0{,}058 \times \frac{1\,\text{ml}}{1000\,\text{ml}}$$

$$\approx 0{,}06\ ‰$$

Q. 14

Metab. feature	Change	Explanation
$NADH/NAD^+$		
Lactate/pyruvate		
CAC		
Glycolysis		
Gluconeogenesis		

A. 14

Metab. feature	Change	Explanation
$NADH/NAD^+$	↑	NADH overproduction in AD/AcD reactions
Lactate/pyruvate	↑	NADH excess in cytosol in removed by LD reaction
CAC	↓	NADH is allost. inhibitor of ICDH and 2-OGDH
Glycolysis	↓	shortage of NAD^+
Gluconeogenesis	↓	shortage of pyruvate + OA (= predominate lactate + malate)

Q. 15

A. 15

A) Decreased gluconeogenesis due to lack of oxaloacetate – hypoglycemia **especially after fasting ingestion of alcohol** (+ usually poor dietary habits in chronic alcoholics)

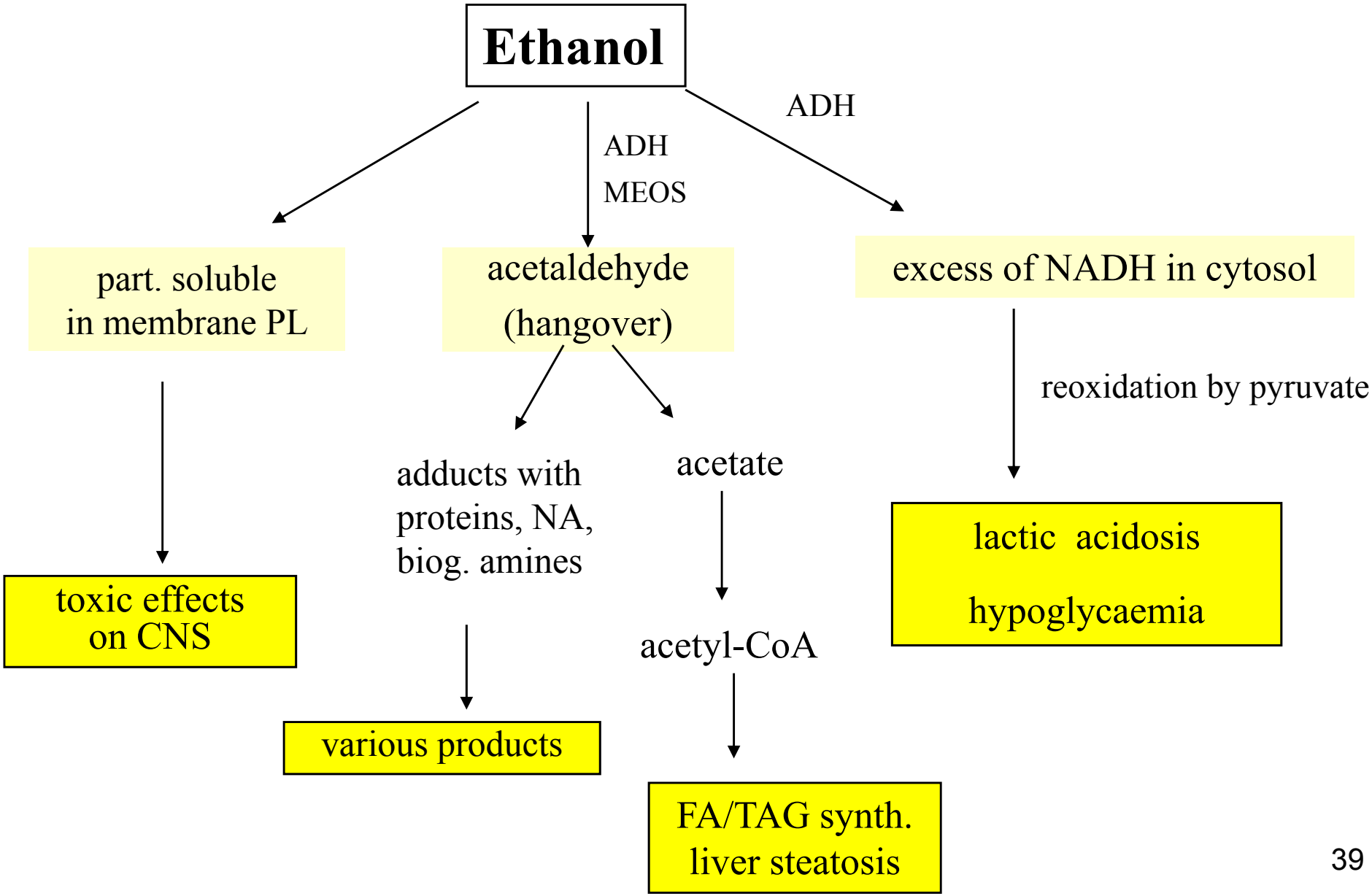
B) Excess of lactate in cytosol ⇒ increased lactate in blood plasma ⇒ lactic acidosis

C) Excess of acetyl-CoA ⇒ synthesis of FA +TAG ⇒ liver steatosis

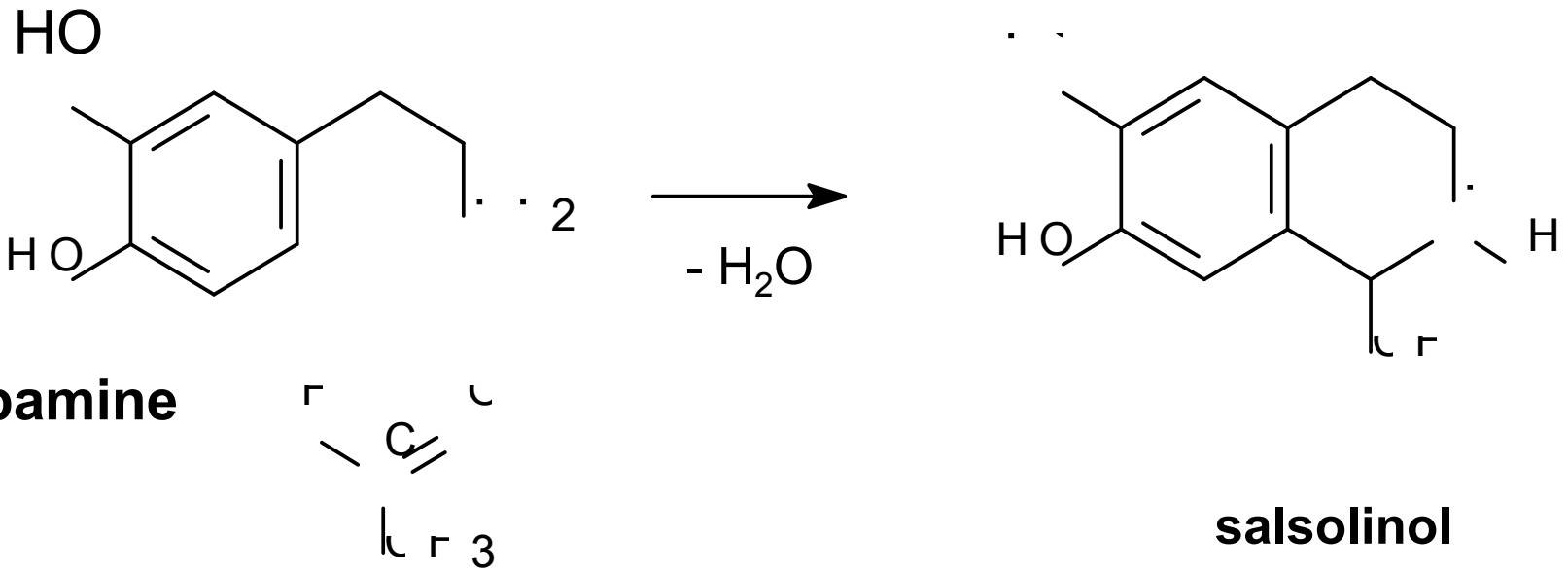
Consider that !

- ethanol is soluble both in polar water and non-polar lipids
- easily penetrates cell membranes
- goes through hydrophilic protein channels or pores
- as well as hydrophobic phospholipid bilayer

Metabolic consequences of EtOH biotransformation



Acetaldehyde reacts with biogenic amines to tetrahydroisoquinoline derivatives



dopamine

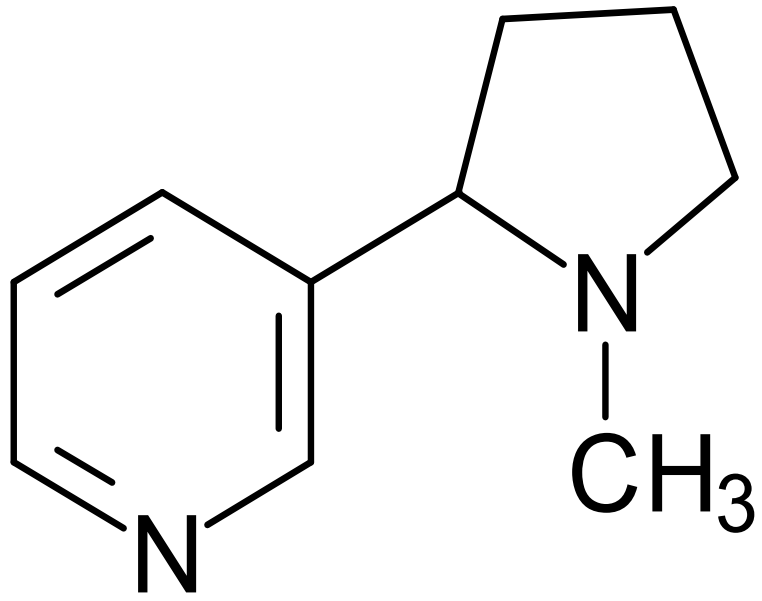
acetaldehyde

salsolinol

6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline

Neurotoxin ?

Nicotine - the main alkaloid of tobacco



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

On cigarette box:

Nicotine: 0.9 mg/cig.

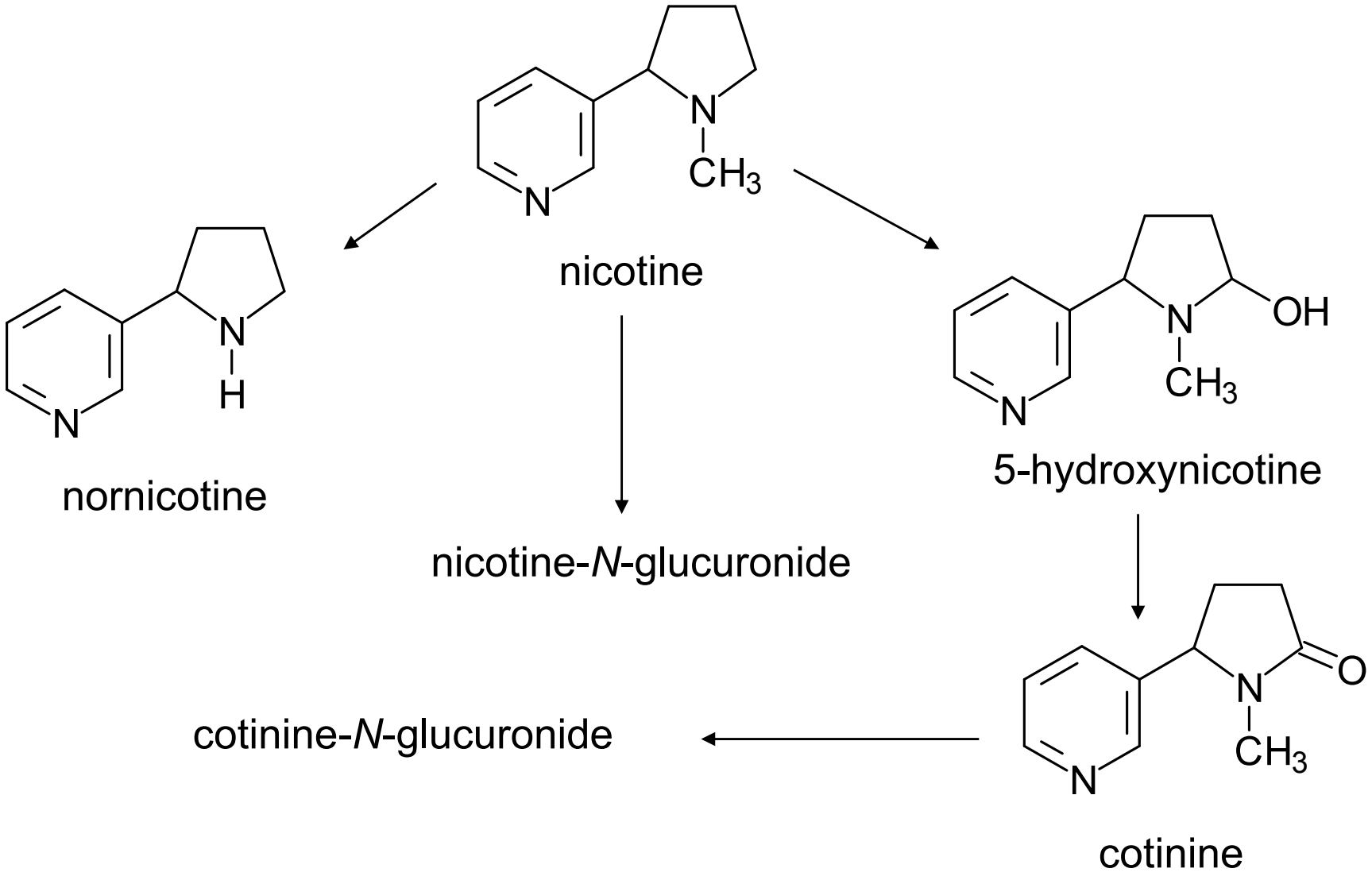
Tar: 11 mg/cig.

Cigarette smoke contains a number of different compounds

- **free nicotine** – binds to nicotine receptors in brain and other tissues
- **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



Biochemical markers of liver diseases

Liver function / condition	Biochemical marker
Integrity of hepatocyte membrane	
Necrosis of liver	
Bile excretion	
Proteosynthesis disorder	
Detoxification functions	
Disorder of AA metabolism	
Disorder of glucose metabolism	
Disorder of lipid metabolism	

Biochemical markers of liver diseases

Liver function / condition	Biochemical marker
Integrity of hepatocyte membrane	ALT, GMT, ALP
Necrosis of liver	AST, GMD
Bile excretion	ALP, bilirubin, bile ac., urobilinogen
Proteosynthesis disorder	(pre)albumin, CHS, coag. factors
Detoxification functions	caffeine test (p.o.) – metabolites in urine
Disorder of AA metabolism	urea, NH_4^+
Disorder of glucose metabolism	glucose
Disorder of lipid metabolism	TAG, HDL

Selected biochem. markers of liver damage (in serum)

Serum analyte	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	↑
Ammonia	5 - 50 µmol/l	↑
Urobilinogens (urine)	up to 17 µmol/l	↑
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Pseudocholinesterase	65 - 200 µkat/l	↓
Urea	3 - 8 mmol/l	↓
Albumin	35 - 53 g/l	↓