Biotransformation of xenobiotics

Biochemistry II
Lecture 5

2008 (J.S.)

Xenobiotics are compounds present in the environment that cannot be used in normal biological processes – that are foreign to the body.

Humans are subjected to exposure to various xenobiotics continually. The principal classes of xenobiotics are **drugs**, **food additives**, **polycyclic aromatic hydrocarbons** (PAH) formed by incomplete combustion of organic compounds, or by smoking and roasting of food, various **pollutants** – products of chemical industry (halogen-derivatives of organic compounds, pesticides), and some **natural compounds of plant origin** that are strange for animals (e.g. alkaloids, spices).

They enter the body usually by ingestion, inhalation, or penetrate occasionally through the skin, sometimes inadvertently, or may be taken deliberately as drugs.

Most xenobiotics are **hydrophobic (lipophilic) compounds** and this property enables their nonspecific penetration across the phospholipid dilayer of plasmatic membranes.

The elimination of xenobiotics from the body depends on their transformation to more hydrophilic compounds.

The most hydrophobic xenobiotics, called **persistent organic pollutants**, once they are released into the environment remain intact for long periods of time. For example, polychlorinated biphenyls (PCBs), dioxins, insecticides DDT, and dieldrin accumulate in the adipose tissue of living organisms, cannot be excreted from the bodies, and are found at higher concentrations in the food chain.

The overall purpose of the biotransformation of xenobiotics is **to reduce their nonpolar character as far as possible**.
The products of transformation are more polar, many of them are soluble in water.
Their excretion from the body is thus facilitated.

Under certain conditions, some cell-types become resistant to drugs that were initially toxic to them. This phenomenon is called **multidrug resistance**, such cells are able to extrude drugs out of the cell before the drug can exert its effects.

Those cells express a membrane protein that acts as and ATP-dependent transporter of small molecules out of the cell. The protein is called **MDR protein** (multidrug resistance protein) and it belongs to the family of proteins that have two characteristic ATP-binding domains (ATP-binding cassettes, ABCs).

Excretion of xenobiotics from the body

After chemical modification, the more hydrophilic compounds are excreted into the urine, bile, sweat. They can also occur in the milk. Volatile products are breathed forth.

Under certain conditions, compounds excreted into the bile can undergo deconjugation and absorption (the enterohepatic circulation).

Biotransformation of xenobiotics

is located mostly **in the liver**

It proceeds in two phases:

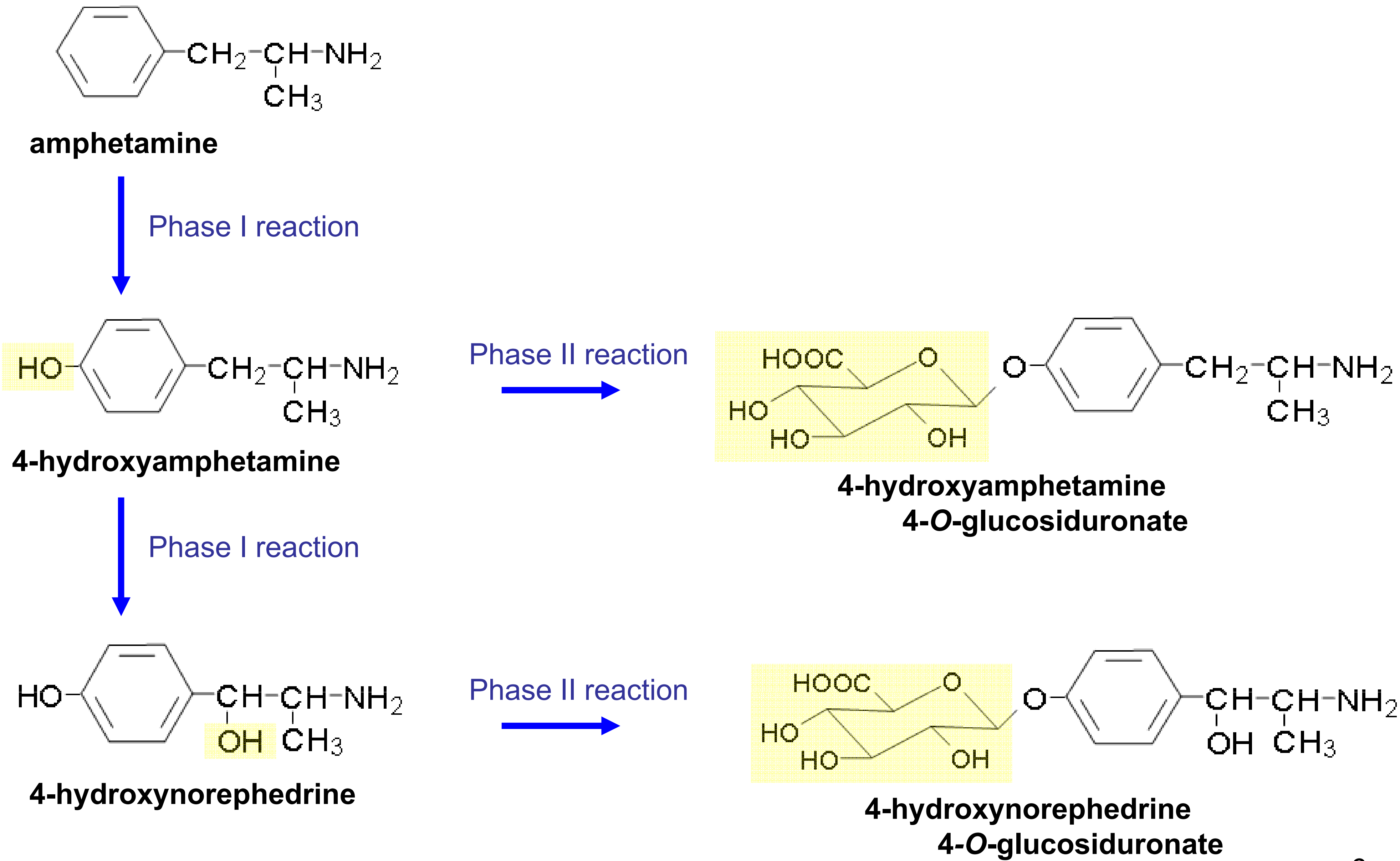
Phase I - the polarity of the compound is increased by **introducing a polar group** (hydroxylation is a typical reaction), increase in polarity by another way, or demasking a polar group (e.g., by hydrolysis of an ester or dealkylation of an amide or ether).
The reactions take place predominantly on the membranes of endoplasmic reticulum, some of them within the cytoplasm.

The first phase reactions may convert some xenobiotics to the compounds that are more biologically active than the xenobiotic itself.

Phase II – Cytoplasmic enzymes catalyze **conjugation** of the functional groups introduced in the first phase reactions **with a polar component** (glucuronate, sulfate, glycine, etc.). These products are mostly less biologically active than the substrate drug, **the xenobiotic is detoxified**.

Example:

Biotransformation of amphetamine



Reactions of biotransformation – phase I

Reaction	**Xenobiotic types**
Hydroxylation	aromatic systems (even heterocyclic)
Dehydrogenation	alcohols, aldehydes
Sulfooxidation	dialkyl sulfides (to sulfoxides))
Reduction	nitro compounds (to amines)
O- and *N*-dealkylation	ethers (to hemiacetals), sec. amines (to *N*-hemiacetals)
Hydrolysis	esters

and others.

The **liver microsomal monooxygenases,**

called also **hydroxylating monooxygenases**

or **mixed-function oxidases**

are prominent enzymes catalyzing reactions of the phase I.
They act on an infinite range of different molecular types because of having **low substrate specificity**.

There are two major groups of monooxygenases:

- **monooxygenases that contain cytochrome P450**, and
- **flavin monooxygenases.**

Flavin monooxygenases

are important in biotransformation of drugs containing sulfurous and nitrogenous groups on aromatic rings or heteroatoms (namely antidepressants and antihistaminics), and of alkaloids.
Typical products of the reactions catalyzed by flavin monooxygenases are **sulfoxides and nitroxides**.

Cytochrome P450 monooxygenases

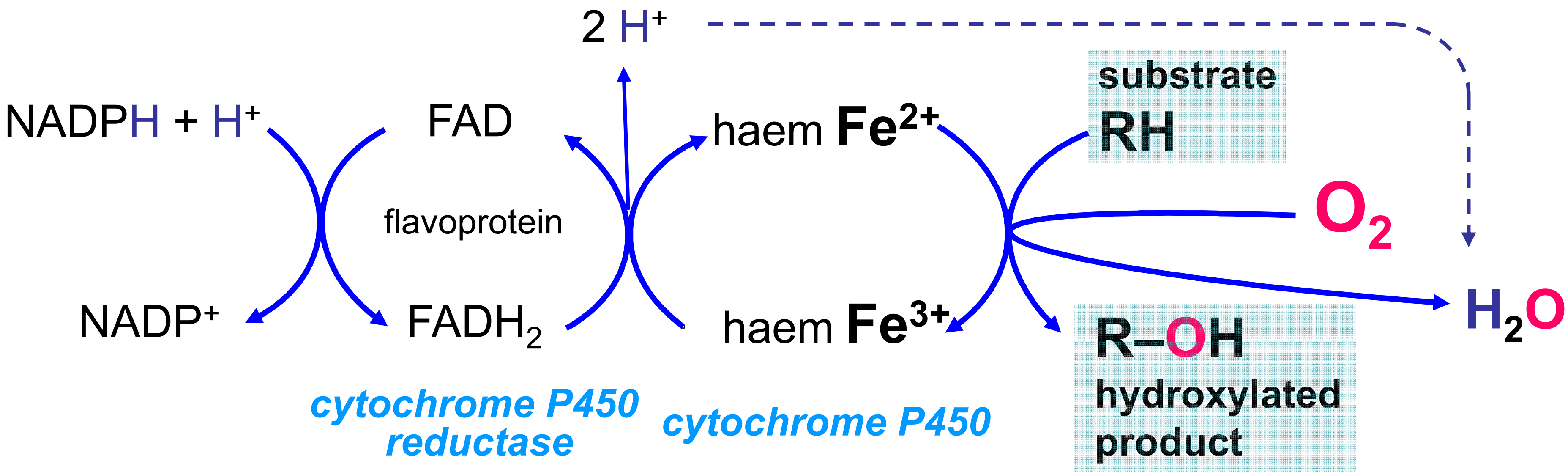
are the major monooxygenases of endoplasmic reticulum.

The abbreviation P450 is used because those enzymes can be recognized, if they bind carbon monoxide, as pigments that have a distinct band at 450 nm in their absorption spectra.

Approximately 400 isoforms of these enzymes have been found in the nature, over <u>30 isoforms in humans</u>.

These haemoproteins are the most versatile biocatalysts known.

In addition to their high activity in the liver cells, they occur in nearly all tissues, except for skeletal muscles and erythrocytes.



Cytochrome P450 monooxygenases transform also a large number of compounds that are **natural components** of the body.

Let us recall hydroxylation of cholesterol, calciols, steroid hormones, haemoxygenase in the haem catabolism, and also desaturation of fatty acids.

Many of cytochrome P450 monooxygenases are inducible.

The hepatic synthesis of cyt P450 monooxygenases is increased by certain drugs and other xenobiotic agents.

If another xenobiotic, which is metabolized by the same isoform of the enzyme and induces its synthesis, appears together with a needed drug in the body, the rate of phase I reactions transforming the needed drug can be many times higher during few days. Consequently, the biological effect of the drug is lower.

Some xenobiotics act as **inhibitors of cyt P450 monooxygenases**.

If an inhibitor is applied with a needed drug, the drug concentration in plasma is higher than the usual one. The patient may be overdosed or unwanted side effects can appear.

Genetic polymorphism of cyt P450 monooxygenases

Allelic variation that effects the catalytic activity of monooxygenases will also affect the pharmacologic activity of drugs.

Example of such polymorphism is that of the isoform CYP 2D6: there are

extensive metabolizers (most of normal population),
poor metabolizers (5 – 10 % of normal individuals), and
rapid metabolizers (individuals who rapidly metabolize debrisoquine as well as a significant number of other commonly used drugs).

In the group of rapid metabolizers – the plasma levels of drugs are higher than expected, unwanted side effects are oft.

In the group of rapid metabolizers – lower drug plasma levels than expected after usual doses, the treatment is ineffective. To obtain satisfactory results, the drug doses have to be higher than those used in extensive or poor metabolizers.

The most important human cyt P450 monooxygenases

Selected examples of substrates and effectors:

CYP	**Typical substrate**	**Inducer** – example	**Inhibitor –** example
CYP 1A2	theophylline	**tobacco smoke**	erythromycin
CYP 2A6	methoxyflurane	phenobarbital	-
CYP 2C9/19	ibuprofen	phenobarbital	sulfaphenazole
CYP 2D6	codeine	rifampicin	quinidine
CYP 2E1	alcohols, ethers	**ethanol**	disulfiram
CYP 3A4	diazepam	phenobarbital	furanocoumarins (in grapefruits)

Approximate fraction of total CYP activity:

CYP 2C9/19	10 %	
CYP 2D6	30 %	
CYP 3D4	50 %	(25 – 70 %)

Reactions of biotransformation – phase II

The reactions

- render xenobiotics even more water-soluble enabling excretion of them into the urine or bile,
- convert the biologically active products of phase I reactions into less active or inactive species.

Transferases (cytosolic or bound in membranes of ER) catalyze **conjugation**, **acetylation** or **methylation** of the polar groups in products of phase I reactions with another and mostly polar component.
The reactions are endergonic, one of the reactants have to be activated.

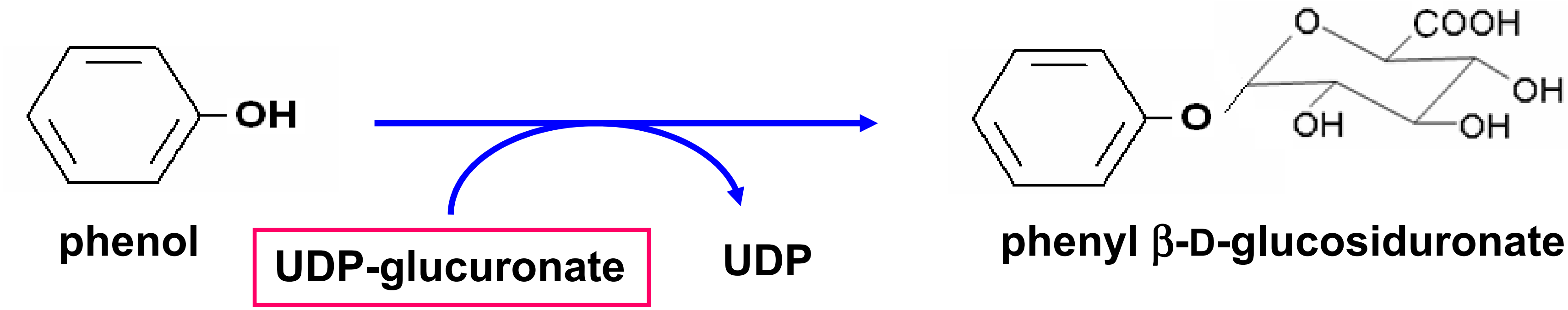
Reaction type	Reagent	Group of the xenobiotic	Bond type
Glucuronidation	UDP-glucuronate	-OH, -COOH, $-NH_2$, -SH	glycoside
Sulfation	PAPS	-OH, $-NH_2$	ester
Formation of sulfide	glutathione	electrophilic carbon	sulfide
Formation of amide	glycine, taurine	-COOH	amide
Methylation	*S*-AM	phenolic -OH	ether
Acetylation	acetyl-CoA	$-NH_2$	amide

● Glucuronidation

A variety of ***UDP-glucuronosyltransferases*** are present in both cytosol and membranes of endoplasmic reticulum.

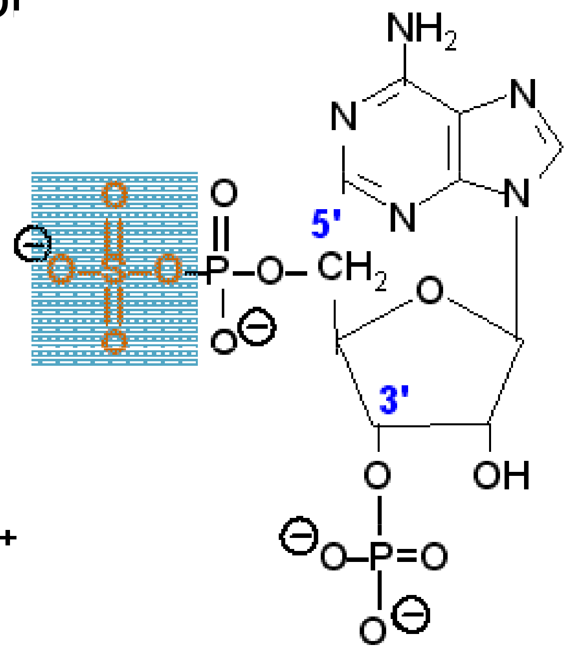
O-, *N*-, or *S*-**glycosides** are formed in the reaction of **UDP-glucuronate** with phenols, phenolic and benzoic acids, flavonoids, alcohols, amphetamines, primary aromatic amines, thiophenols, as well as endogenous bilirubin, many steroid compounds, catecholamines, etc.

Example:



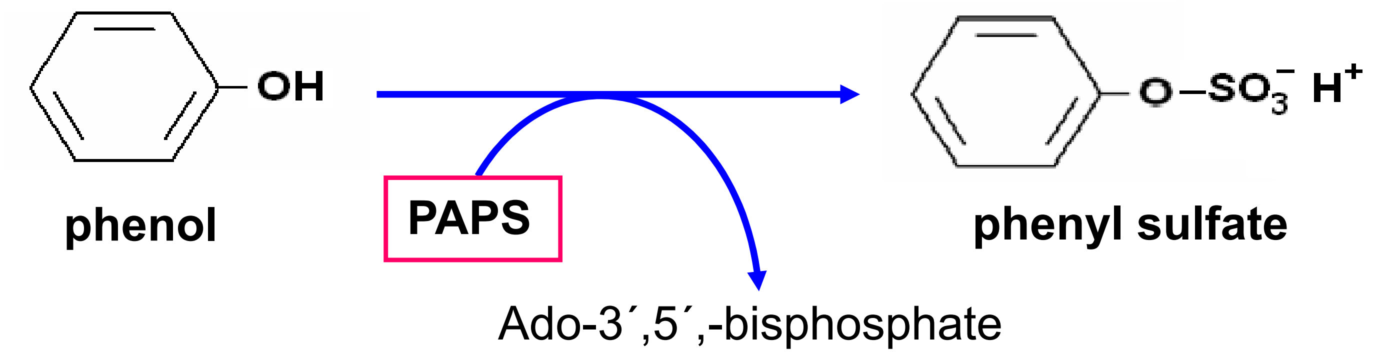
• Sulfation

Sulfotransferases bound in the membranes of endoplasmic reticulum transfer the sulfate group from the universal sulfate donor **3'-phosphoadenosyl-5'-phosphosulfate** (**PAPS**, "active sulfate") to all types of phenols forming so **sulfate esters** or to aryl amines forming so *N*-sulfates (**amides**). Steroid hormones and catecholamines are also inactivated by sulfation.



PAPS

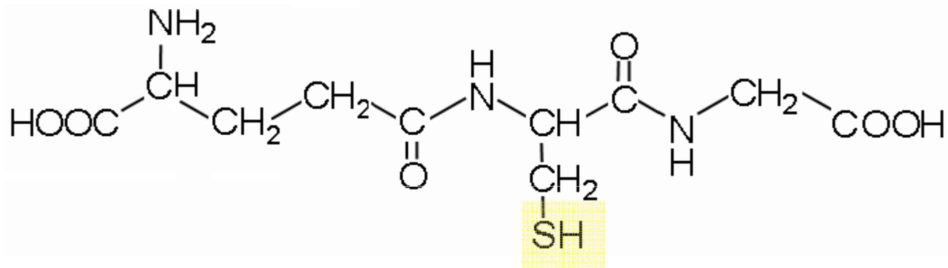
Example:



phenol **phenyl sulfate**

Conjugation with glutathione

Glutathione is an important intracellular reductant (antioxidant) and takes part in transfer of amino acids across plasmatic membranes.

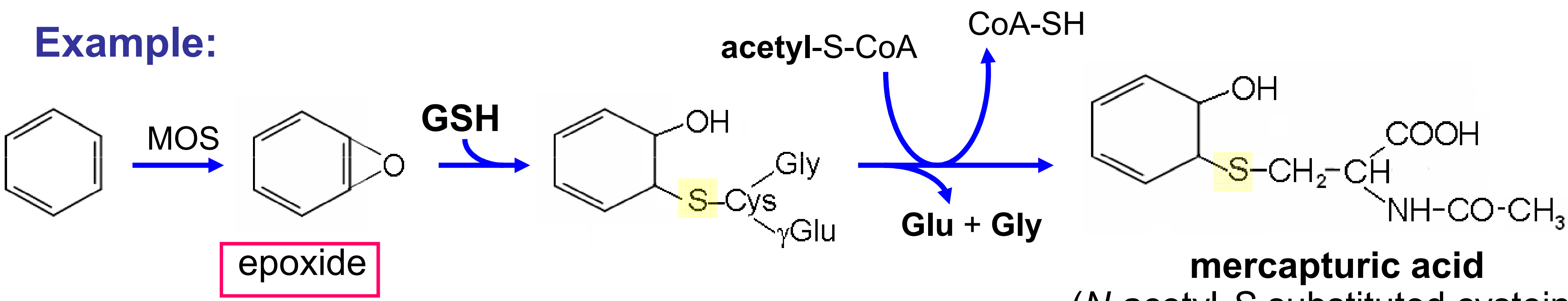


glutathione (**GSH**)
γ-glutamyl-cysteinyl-glycine

GSH-transferases catalyze the transfer of **glutathione** to a number xenobiotics (e.g. epoxides of aromatic hydrocarbons, aryl halides, electrophilic carcinogens), which results in formation of **aryl sulfides of glutathione**.

Glutamyl and glycyl residues are removed from these conjugates by hydrolysis, and the remaining **cysteinyls are *N*-acetylated**. The resulting conjugates of *N*-acetylcysteine called **mercapturic acids** are excreted into the urine.

Example:



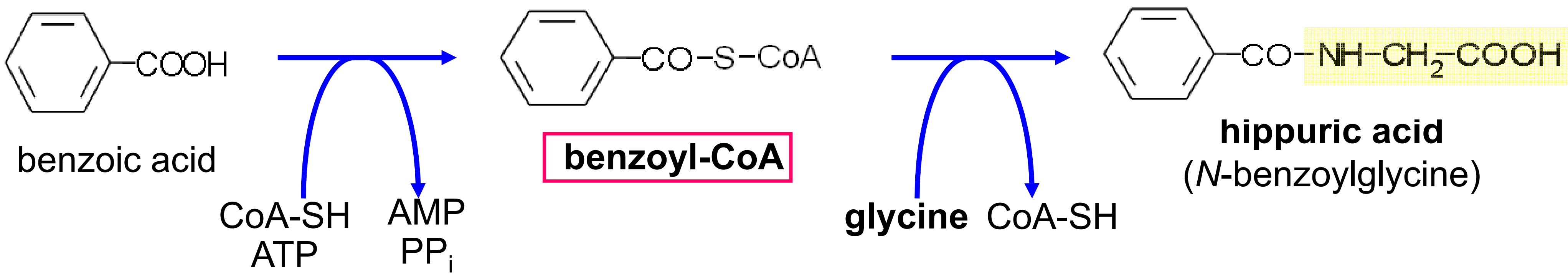
mercapturic acid
(*N*-acetyl-*S*-substituted cysteine)

● Conjugation with glycine

Arenecarboxylic acids, namely **substituted benzoic acids**, after activation to acyl-CoAs give **amides with glycine**. The reaction is catalyzed by cytosolic ***glycine-N-acyltransferases***.

N-benzoylglycines are called **hippuric acids**.

Unsubstituted hippuric acid is present in the urine of healthy individuals – benzoic acid is a normal constituent of vegetables and also an additive (fungicidal agent) to some foodstuffs. High urinary excretion of hippurate is a marker of exposition to toluene, which undergoes oxidation to benzoate.



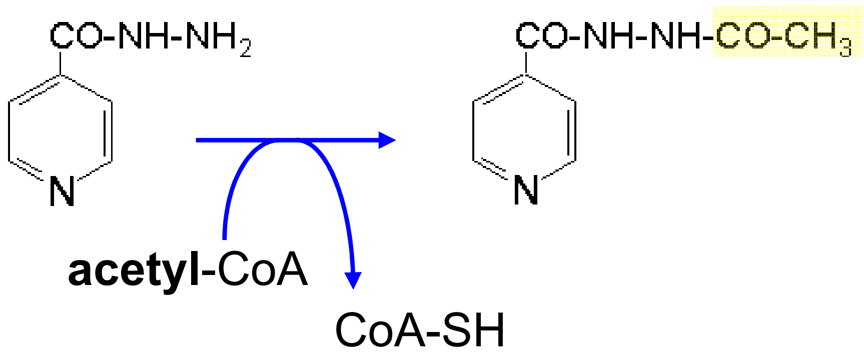
Bile acid, before secreted from the liver cells, are conjugated with glycine in the same way (**conjugated bile acids** – glycocholate, chenodeoxycholate, etc.)

Taurine $H_2N–CH_2-CH_2–SO_3^-$ may also serve in conjugation, however conjugation of bile acids with taurine is of minor importance in humans.

• Acetylation

is the reaction, by which the biological effects of **aromatic amines** and similar compounds are diminished. **Acetyl-CoA** is the donor of acetyl.

Example:



Isoniazid (INH, isonicotinic acid hydrazide) is an effective chemotherapeutic agent used in the treatment of tuberculosis).
The genetic disposition to acetylate this type of xenobiotics with different rates exists (slow and rapid acetylators).

• Methylation

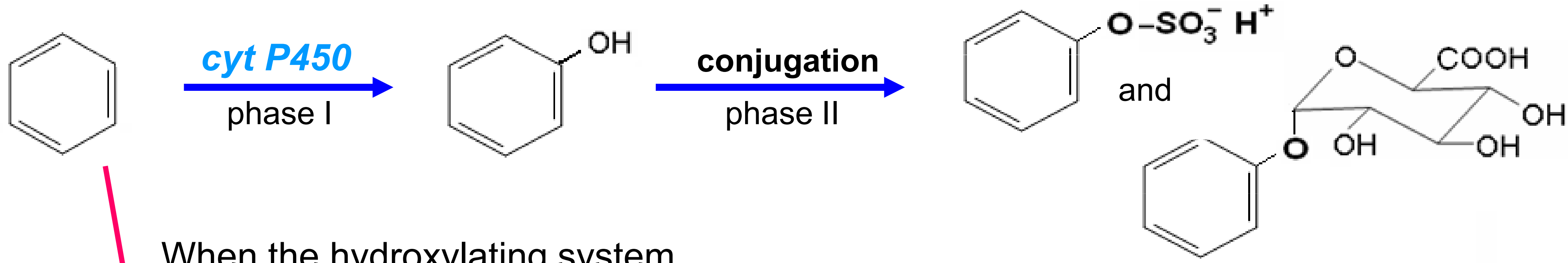
of phenolic groups occurs oft in phase II of biotransformation.
In spite a slight decrease in hydrophilicity of the products, the biological effects that depend on the phenolic groups are supressed in this way.

The donor of methyl group is ***S*-adenosyl methionine** (***S*-AM**), the reaction is catalyzed by ***O*-methyltransferases**.

Catecholamines and estrogens are inactivated by *O*-methylation.

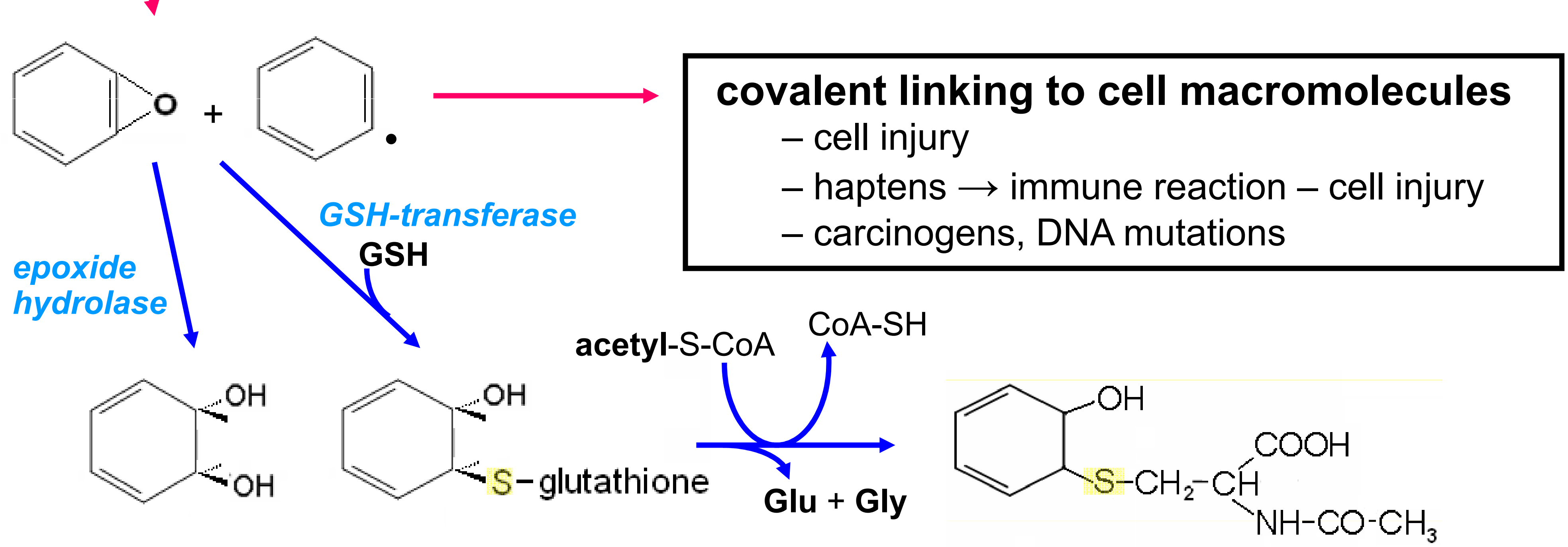
Biotransformation of selected compounds - examples

Benzene and other aromatic hydrocarbons



When the hydroxylating system is <u>overloaded</u>, increased amounts of **reactive metabolites** are formed:

High urinary excretion of phenol conjugates at high professional exposition to benzene.



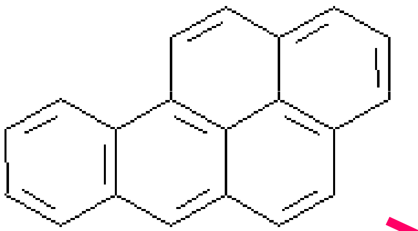
mercapturic acids

Polycyclic aromatic hydrocarbons (PAH)

Sources of PAH:

– industrial combustion of fossil fuels, production of coke, asphalt,
– combustion of wood (forest fires) and household rubbish,
– singed bread and pastry, smoking, grilling, barbecuing, and roasting of foodstuffs, overheated fats and oils,
– soot, tobacco smoke.

Biotransformation of PAH is similar to simple aromatic hydrocarbons, e.g.:



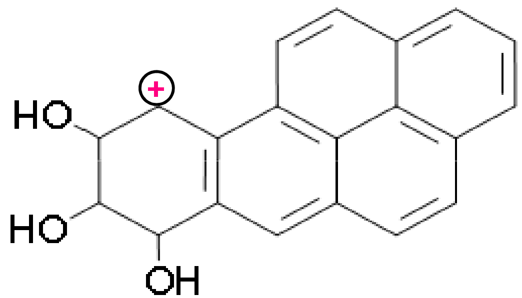
benzo[a]pyrene

hydroxylation

cyt P450

hydroxy derivatives that are mostly non-toxic and eliminated after conjugation in phase II reactions

epoxides that can give dihydrodiols and, after a further epoxidation, **carbanion ions i**nteracting with DNA – carcinogens.

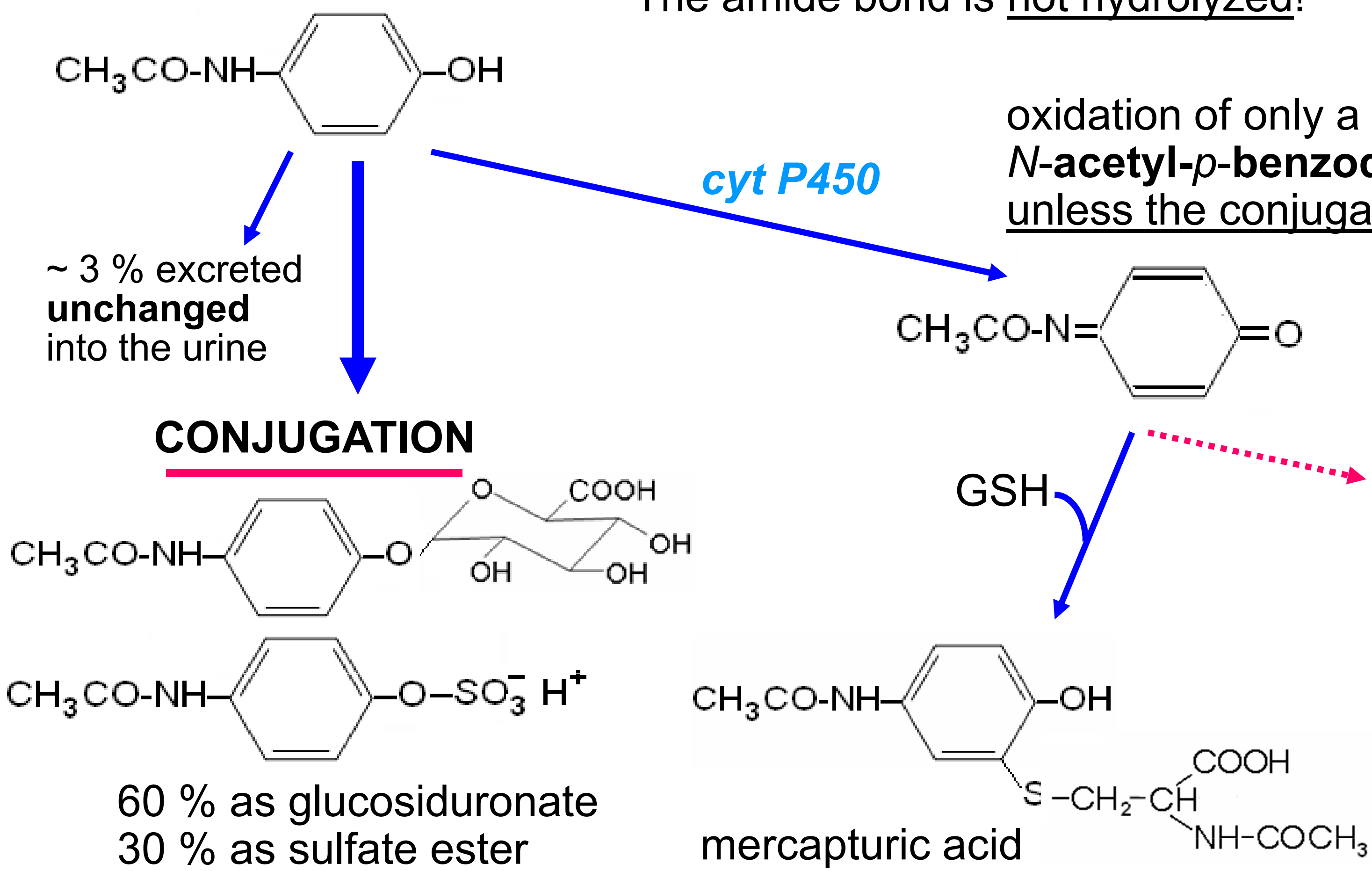


Acetaminophen (*p*-acetaminophenol, paracetamol)

was prepared in 1893. Since approx. 1975, when it turned out that acetylsalicylic acid may have some unwanted side-effects, serves acetaminophen as common **analgetic-antipyretic of the first choice**.

Biotransformation:

The amide bond is <u>not hydrolyzed</u>!



oxidation of only a **small part to** ***N*-acetyl-*p*-benzoquinoneimide** (**NAPQI**), <u>unless the conjugating capacity is exhausted</u>

<u>if conjugation capacity is limited,</u>

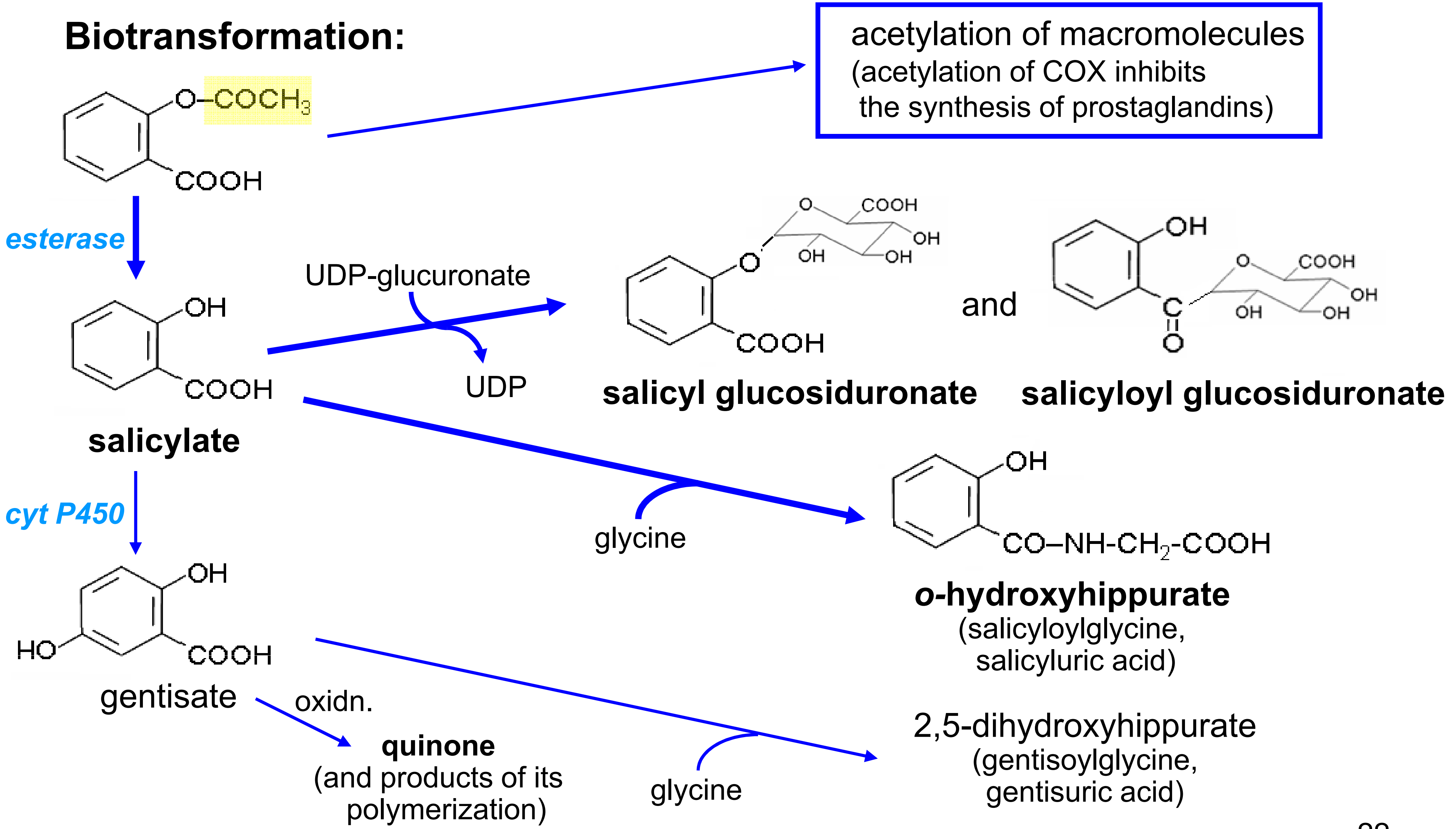
unwanted side effects:

- covalent bonding to proteins,
- oxidation of –SH groups in enzymes,
- depletion of GSH,
- hepatotoxicity at overdosing

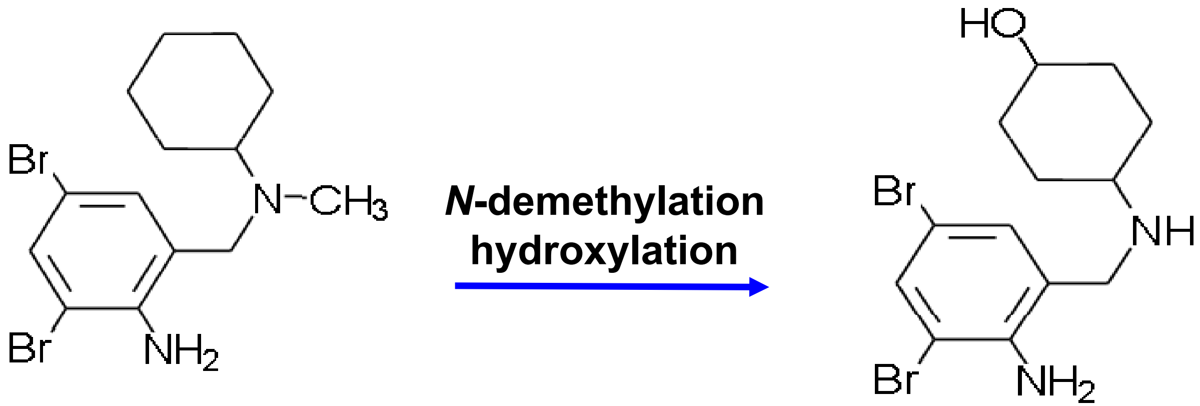
Acetylsalicylic acid (aspirin)

is an **analgetic-antipyretic** with antiinflammatory effect; minute doses inhibit aggregation of blood platelets.

Biotransformation:



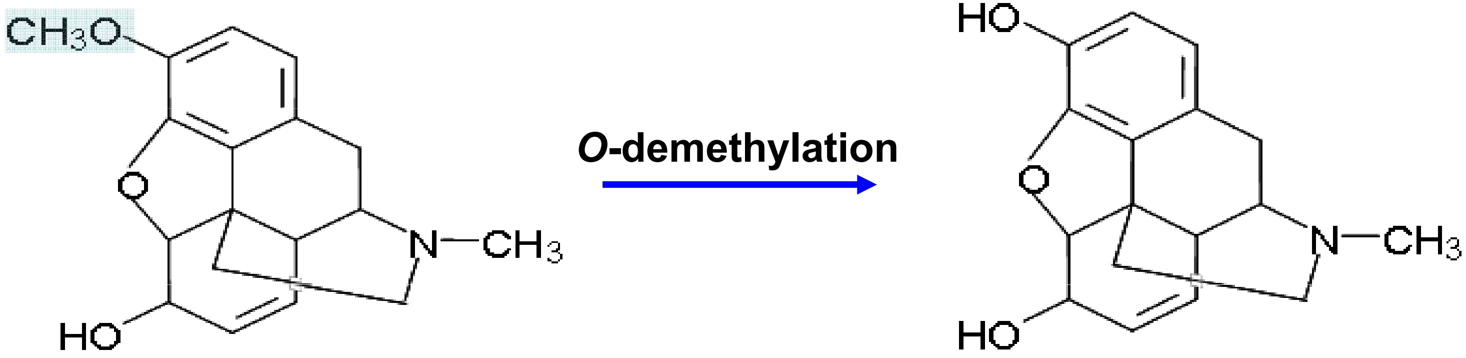
Bromohexin is the prodrug of an expectorant ambroxol:



bromohexin (**prodrug**)

ambroxol (expectorant)

Antitussic **codeine** (3-*O*-methylmorphine) is transformed in part and slowly into morphine:



codeine (antitussic)

morphine (analgesic, an addictive drug)

It is proper to avoid application of too many different remedies together, though their expected effects can be viewed as useful.

- Interactions between different drugs or their metabolites might cause enhancement or inhibition of pharmacological effects,
- the mixed type hydroxylases (cyt P450) are inducible, their activities may increase many times in several days, so that the remedies are less efficient,
- if the load of the detoxifying system is high, minor pathways of transformation can be utilized and produce unwanted side-effects due to the formation of toxic metabolites,
- intensive conjugation with glutathione might result in depletion of this important reductant in the cells, etc.

Biotransformation of ethanol

occurs mainly in the **liver**.

Ethanol is oxidized to acetaldehyde and then to acetic acid.

There are three reactions that give **acetaldehyde from ethanol**.

- Cytosolic NAD^+-dependent **alcohol dehydrogenase** is the most important, it functions even at low concentrations of ethanol (K_m = 2 mmol/l, i.e. 0,1 ‰):

$$CH_3\text{-}CH_2OH + NAD^+ \xrightarrow{\text{alcohol DH}} CH_3\text{-}CH{=}O + NADH + H^+$$

acetaldehyde

- **Microsomal ethanol oxidizing system** (**MEOS**, which contains CYP 2E1) is effective preferably at excess alcohol intake (at blood concentrations higher than 0.2 - 0.5 ‰; K_m = 10 mmol/l):

$$CH_3\text{-}CH_2OH + O_2 + NADPH + H^+ \longrightarrow CH_3\text{-}CH{=}O + 2\,H_2O + NADP^+$$

- In peroxisomes, **catalase** can catalyze oxidation of ethanol by hydrogen peroxide:

$$CH_3\text{-}CH_2OH + H_2O_2 \longrightarrow CH_3\text{-}CH{=}O + 2\,H_2O$$

Aldehyde dehydrogenase catalyzes oxidation of acetaldehyde to **acetic acid**:

$$\mathbf{CH_3\text{-}CH{=}O} + H_2O \rightarrow CH_3\text{-}CH(OH)_2 \xrightarrow[NAD^+ \;\rightarrow\; NADH + H^+]{\textit{aldehyde DH}} \mathbf{CH_3\text{-}COOH}$$

acetaldehyde — acetaldehyde hydrate — **acetate**

Acetate is activated to **acetyl-CoA**.

In excessive alcohol intake, **NAD^+ is spent for dehydrogenation of ethanol** preferentially so that excess lactate (from pyruvate) is formed.

In the liver cells lacking in NAD^+,

gluconeogenesis is decreased (resulting in hypoglycaemia),

β-oxidation of fatty acids inhibited (liver steatosis),

increased ketogenesis (from acetate), and

because the rate of acetaldehyde oxidation is reduced,

the **toxic effects of acetaldehyde** are more pronounced.

Consequences of drinking

ETHANOL

- → interpolates into membranes, increased membrane fluidity
  - → CNS
    - → **immediate toxic effects**
    - → **social consequences of chronic alcoholism**
- → (ADH / MEOS) → **acetaldehyde** (hangover)
  - → **various adducts** with proteins, nucleic acids. biogenic amines (alkaloids?)
  - → (AldDH) → acetate
    - → **acetyl-CoA**
      - → **fatty acid synthesis** (fatty liver)
- → (ADH + AldDH) → high **NADH**/NAD^+ ratio
  - → (reoxidation of NADH by pyruvate) → **lactacidaemia**, **hypoglycaemia** (inhibition of gluconeogenesis and β-oxidation of FA)

Tests for detection of ethanol intake

Elevated **blood levels** of ethanol decrease due to its oxidation, ethanol is eliminated from the body during several hours. **γ-Glutamyltransferase (γGT)** in serum is increased in chronic alcoholism oft, but this test is not specific.

New tests have been developed (unfortunately, they are not yet used commonly in routine laboratory practice), which are able to detect not only when a person drank last time, but also if the doses taken were moderate or excessive.

Ethyl glucosiduronate (EtG) increases in the blood synchronously with the decrease of blood ethanol and can be detected (in the urine, too) after few days, even up to 5 days.

Fatty acids ethyl esters (FAEE) appear in the blood in 12 – 18 h after drinking and can be detected even 24 h after alcohol in blood is no more increased. However, traces of FAEEs are deposited **in hair** for months and may serve as a measure of alcohol intake.

Phosphatidyl ethanol (PEth) is present in the blood of individuals, who have been drinking moderate ethanol doses daily, in even 3 weeks after the last drink.

Carbohydrate-deficient transferrin (CDT). In the saccharidic component of each transferrin molecules, there are 4 – 6 molecules of sialic acid. Drinking to excess disturbes the process of transferrin glycosylation so that less sialylated forms of transferrin (with only two or less sialyl residues per molecule, CDT) are detected in blood during approximately 4 weeks after substantial alcohol intake.